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United States Army
Medical Research Institute
of Infectious Diseases

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Fiscal Year 1986

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United States Army
Medical Research Institute
of Infectious Diseases
Fort Detrick, Maryland

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A report of progress on the research program of the U.S. Army Medical Research Institute of Infectious Diseases on Medical Defense Against Biological Agents(U) for fiscal year 1986 is presented.

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Low Molecular Weight Toxins
Small Molecular Weight Toxins
Recombinant DNA Technology
Rickettsial Diseases
Rapid Identification
Biological Warfare Defense

EDITOR'S NOTE

This FY 1986 Annual Progress Report is a general review of research activities of the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, conducted on Medical Defense Against Biological Warfare Agents (U) under Projects 3M161102BS12, 3M263763D807, 3M463750D809, 3S464758D847, 3M162770A870, 3M162770A871, and In-House Laboratory Independent Research, Project 3A161101A91C.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

FOREWORD

The research programs of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) are targeted to provide a medical defense against biological warfare (BW). Lieutenant General Leonard H. Perroots, in his Foreword to the Defense Intelligence Agency publication, Soviet Biological Warfare Threat, states, "In recent years, we have become increasingly concerned that this genre of weaponry will be developed by some nations including those of the Third World. We are gravely concerned that we will see BW programs underway in some countries within five years and limited production within a decade." In view of the Sverdlovsk incident in 1979, and the alleged uses of mycotoxins in SE Asia, there is no doubt that increased emphasis on medical defense against BW is needed. USAMRIID's response to this need is multifaceted; it includes drug and vaccine development, casualty management, and rapid identification and diagnosis.

New technologies, in particular recombinant DNA techniques, have brought new potential threats, and with the threats have come new public awareness, vigilance and sometimes fear. When planning a medical defense, it is now necessary to take into account the possibility of altered or drug-resistant microorganisms in addition to conventional agents. Obviously, defensive measures will lag behind a given biothreat; however, the same technologies that make these new potential threats possible will also help counter them. This report describes studies that use a wide range of those technologies. They encompass fermentation, a technique as old as winemaking, and the recombination of minute molecules of deoxyribonucleic acid, a technique whose potential and power is unimaginable.

In early 1985, recognition of both the new potential threats and defenses provided the impetus for the Vice Chief of Staff Army (VCSA) to mandate expansion and realignment of USAMRIID's programs. A brief overview of USAMRIID's contributions to Army readiness, its major scientific discoveries, and its management of people, money and facilities, will provide the reader with an appreciation of the challenges the Institute faces and the approaches being used to meet them.

There are now artificial intelligence applications for vaccine and antiviral drug development and laboratory instrumentation. The power of artificial intelligence can be illustrated by its application to drug development: a new genetic engineering software package makes it possible to predict, with existing DNA sequence and amino acid data, which segments of large proteins have a high potential for being antigenic. This approach may shorten the time by several months or even years, for discovery of alternative antigenic sites for drug intervention.

USAMRIID's leading role in drug development continues with various collaborative arrangements that include contracts with laboratories that perform specialized synthesis, testing and mechanism-of-action studies; collaborations with other Government

agencies involved in antiviral drug research and interferon and immunopotentiator studies; and cooperative agreements with pharmaceutical, biotechnology and chemical companies, academic institutions and nonprofit organizations. Under these and other agreements USAMRIID has increased its access to thousands of new chemical compounds for evaluation and testing. Selected compounds undergo initial tissue culture screening against a battery of 17 viruses. Promising compounds are tested in small mammals, and then in appropriate large mammalian species. Additional metabolism and formulation studies, FDA-approved toxicity and safety studies and pharmacokinetic evaluations are performed as required. This innovative program has placed USAMRIID at the forefront of antiviral research. The far-reaching aspects of the program became evident when the National Institutes of Health delegated responsibility for identification of new drugs for the treatment of AIDS patients to this Institute. In June of 1986, the NIH assigned a 1.3 million dollar annual grant to USAMRIID for the testing and development of new drugs that may be effective against AIDS.

A live, attenuated Junin viral vaccine has been developed to protect U.S. forces against highly infectious and frequently fatal Argentine hemorrhagic fever. At the request of the Argentine government, the vaccine is currently undergoing large-scale field tests in those areas in Argentina where the disease is endemic. This is an example of USAMRIID's ability to develop a new vaccine, from basic studies to characterize the pathogen, to identifying a means of gaining an immunogenetic response, to scaling the vaccine from laboratory to production quantities, and testing the vaccine for safety and efficacy, first in mice, then in larger laboratory mammals, primates, and finally in man. This vaccine program was completed in less than seven years, a significant achievement when compared to the norm of 10 to 15 years' development required for most successful vaccines.

The feasibility of using genetic engineering to develop an entirely new class of safer and more effective vaccines against Rift Valley fever (RVF) was highlighted in last year's report. To review, the gene coding for G-2 glycoprotein was inserted into a carrier, vaccinia virus. This recombinant vaccine was shown to protect mice against exposure to virulent RVF virus. This year, the vaccine's efficacy was tested in the most sensitive animal model system, pregnant sheep. The vaccine protected against RVF-induced abortion, clearly demonstrating vaccine efficacy. However, when the recombinant vaccine was prepared with a vaccinia strain more acceptable for human use, it was less effective, demonstrating that the carrier, vaccinia virus, plays an important role in the expression of the foreign gene inserts from RVF virus. While studies continue on that particular vaccinia virus carrier, several additional strains of vaccinia which should be acceptable for human use have been grown in certified cell substrates, purified, and are ready for RVF gene insertion.

USAMRIID researchers made an important contribution in demonstrating that soluble mediators, or leukotrienes, are major contributors to lethal arenavirus infections. Pathophysiological

studies, conducted in appropriate animal models, clearly revealed extensive cardiovascular derangement due to abnormally high levels of leukotiienes in the blood stream. Cardiac derangement occurred even in the absence of direct viral involvement in the myocardium. The leukotriene antagonist FPL-55712 improved the general health of infected guinea pigs and prolonged average survival times. This is the first evidence to indicate that effective treatment modalities can be developed for lethal arenavirus infections.

Another exciting development is the completion of a rapid diagnostic assay for Hantaan virus, the cause of epidemic hemorrhagic fever. The assay, a modified enzyme immunoassay which measures specific IgM antibody, uses safe, inactivated reagents and requires only 3-4 hours for completion. The assay system proved to be 100% specific in clinical assessments in Wuhan, China. This assay now increases to 27 the number of potential BW agents that can be rapidly identified within six hours. Most clinical laboratories require several weeks to identify common disease viruses, so quite obviously, the ability to identify 27 exotic viruses in a matter of hours represents a significant diagnostic capability. Most of these assays are too complex to be considered for field use at present, because they require special reagents, sophisticated equipment, and specially trained technicians. Considerable effort is being made to simplify these systems so that an Army field hospital can acquire some of these assay capabilities within the next year.

Clinical studies to evaluate the antiviral drug ribavirin were initiated in China, South Korea, and Argentina. Development of the drug was begun in 1979 at USAMRIID with studies on efficacy in realistic primate models for human disease. After its broad-range antiviral activity against numerous viral families was clearly established in a lengthy, prescribed series of pre-clinical studies, ribavirin was then targeted for clinical evaluation against three militarily important viruses: epidemic hemorrhagic fever with renal syndrome (HFRS), Argentine hemorrhagic fever (AHF), and dengue fever.

Ribavirin's success resulted in an invitation from the Government of the People's Republic of China, to conduct a large field trial in Wuhan, China, in collaboration with the Hubei Medical College. Initial results indicate a highly successful field trial, the human response paralleling that of laboratory animals and primates. Because available clinical laboratory facilities did not conform to standards expected by FDA, USAMRIID furnished the on-site laboratory with the required equipment and trained the local personnel in its operation and maintenance. Laboratory equipment, computers, reagents, and supplies all were shipped and installed in time for the start of the fall epidemic season. These efforts demonstrate that USAMRIID has successfully transitioned laboratory discoveries and developments for the treatment of life-threatening viral diseases into practical clinical applications. The ability to establish working relationships with medical scientists in foreign countries in order to evaluate drugs which will be used to treat U.S. military personnel, represents yet another extension of USAMRIID's capabilities and creative approach toward fulfillment of its mission.

Studies of classic aerosol technology showed that Ebola virus, the etiologic agent of hemorrhagic fever in Africa, is highly infectious as a small particle aerosol. Unfortunately, Ebola virus is probably one of the most virulent and lethal organisms known, and to date, prophylatic and therapeutic efforts have not been successful in reducing infection in experimental animals. Thus, in order to discover means of medical defense, a comprehensive, collaborative filovirus research program was organized with the Institut Pasteur and implemented in the Central African Republic. The innovative international program, a pioneering first, allows previously separate and independent laboratory and field efforts to be combined, closely integrated, and efficiently focused on Ebola and Marburg viruses. The effort has, through laboratory evaluation of field specimens, and epidemiological and ecological findings collected in remote areas, generated substantial and original evidence which indicates that filovirus infections are common and widely distributed throughout diverse African ecological zones. They seem to be, however, limited to a high-risk population. The means by which the filoviruses are maintained and transmitted in nature is unknown, but the finding that seropositive reactions are clustered in families strongly suggests person-to-person spread, and tends to corroborate the USAMRIID aerosol data in rhesus monkeys.

USAMRIID's innovative approaches for acquiring fresh insights on infectious diseases include using satellite remote-sensing imagery. Data from the advanced, very high resolution radiometer on board the National Oceanic and Atmospheric Administration's polar-orbiting meteorological satellites were used to infer ecological parameters associated with Rift Valley fever viral activity in Kenya, and to develop an indicator of potential viral activity. The high correlation between the satellite data and the ecological parameters associated with Rift Valley fever virus indicated that satellite data can serve as a forecasting tool for this disease in Kenya. The threat from Rift Valley fever virus to military personnel operating anywhere on the African continent can now be predicted. This technology can now be applied to many other diseases which decimate the world's population and are ecologically linked, either directly or through transmission vectors.

The In-house Laboratory Independent Research program has produced several major scientific advancements, one of which will be described here. In FY 1985, it was clearly demonstrated that an adversary could easily produce sufficient amounts of snake venom toxins to use as BW agents. This year an impressive number of genes and purified antibodies were prepared for efforts to develop a safe and effective vaccine against cobra (*Naja naja atra*) venom. The scientific implications of this study are certainly encouraging for the purposes of medical defense against BW, but they go beyond the military realm. A vaccine or antitoxin to cobra venom, a venom that kills over 10,000 people in Asia alone, could elevate successful results to the level of a world health breakthrough.

USAMRIID staff received a number of honors and awards over the past year; the following are among the highlights. The Department of

the Army awarded its "Research and Development Achievement Award" to Lieutenant Colonel Martin H. Crumrine in recognition of his contributions to the rapid identification of botulinal toxins and the development of antitoxins for therapeutic and diagnostic applications. William C. Patrick, Program Analysis Officer, was presented the "Barnet L. Cohen" award by the Maryland/District of Columbia Chapters of the American Society for Microbiology for sustained and significant contributions to the science of microbiology. Two scientific studies, presented at the Army Science Conference, West Point, N.Y., 17-20 June 1986, won second and third place honors. Second place was awarded to a scientific team headed by Dr. Peter G. Canonico whose presentation was entitled, "Ribavirin Prophylaxis of Sandfly Fever Sicilian Infection in Human Volunteers." Third place was awarded to the scientific team headed by Dr. Joel Dalrymple whose presentation was entitled "Evaluation of a Recombinant Vaccinia Virus Vaccine Candidate for Rift Valley Fever." Finally, USAMRIID received the "Award for Excellence in 1985" in "Laboratory of the Year" competition as outlined in AR672-305.

Another achievement that deserves recognition is the outstanding safety record of the Institute. In 1986, USAMRIID personnel worked 980,000 hours with some of the most lethal and poorly-understood diseases known, and for the second consecutive year, there were no man hours lost due to a work-related infection.

The Institute continues to make progress on medical defense against BW in an environment of negative publicity. Newspaper articles, TV commentaries, and a small but dedicated number of scientists in academia infer and frequently charge directly that USAMRIID could (or indeed, that it is) performing offensive BW research. USAMRIID management spends an inordinate amount of time in responding to this type of allegation. Certainly, the best method for combatting the allegations and the not-infrequent sensationalism is with facts. It is both timely and appropriate that a recently printed color brochure prepared by USAMRIID staff and originally intended primarily for recruiting for such positions as Army physicians, biogenetic engineers, and molecular biologists, will now also serve as an effective public relations tool. The brochure describes USAMRIID's approach to its mission and will undoubtedly help in educating the public and encouraging cooperation among the Institute, the local community, and other scientific laboratories.

USAMRIID's implementation of the program expansion mandated by VCSA, can be illustrated with the extramural contract program. In FY 1984, the Institute had 36 contracts which totaled 9 million dollars. During FY 1985, in the 8 months after the VCSA mandated expansion, the contract program increased to 68 contracts, totaling 14 million dollars. In FY 1986, the first full year of expansion, the number of contracts increased to 126 and a total of 19.3 million dollars. This rapid expansion of effort was aided by automating all possible contract functions with a Data Base III computer program (for contract and budget information), and with word processing stations. This approach has permitted a staff of only three to monitor and respond to the demands of an expanding extramural contract program efficiently.

In FY 1986, USAMRIID's professional staff of 107 investigators published an all-time high of 158 manuscripts in refereed and scientifically-recognized journals. The published manuscript represents an important element in the transfer of technology to other government agencies, educational institutions, and to private industry. The ratio of 1.45 manuscripts per scientist is an outstanding achievement in and of itself, particularly so considering program realignments that occurred in FY 85 and 86.

Physical security of the Institute was significantly improved by the installation of an automated key card system. The system progressively limits access to the research areas, acts as an anti-intrusion device, provides a record of who enters which areas, and correlates maximum containment laboratory entry to immunization status. This new, computerized system more efficiently controls the flow of Institute traffic, and prevents unauthorized entry into the maximum containment laboratory suites. A compromise in biological safety could pose grave consequences for USAMRIID personnel as well as for the surrounding community.

Over the past several years, a portion of the experimental containment ward was converted to offices to house an expanding professional staff. However, increasing emphasis on human testing of experimental products now requires that all of the experimental ward return to its original function. In response to USAMRIID's need for increased office area, the VCSA approved construction of modular units for an additional 6,900 square feet of administrative space. These new units became functional on 1 June 1986, and the experimental ward patient rooms have now been converted back to serve their original purpose.

USAMRIID continues to maintain a dynamic National Academy of Science/National Research Council postdoctoral training program. The program started a few years ago with just nine authorizations and expanded to 20 authorizations this year, two more than last year. Results of the yearly independent and thorough audit of USAMRIID scientists and research programs by the NAS are indicative of the high professional standards this Institute maintains.

The Institute is dedicated to providing its employees with the training necessary to keep them abreast of the information explosion in science and technology. Over 14,000 hours of university and in-house training were provided to 324 employees. The majority of the training was in biological sciences, computer and word processing, and mathematics. USAMRIID is particularly proud of its enlisted personnel: of a total population of 225, 77 are studying at local universities and colleges to complete requirements for M.S., B.S. and A.S. degrees.

The Institute continues to perform an in-depth, critical review and analysis of its programs in an annual three-day off-site meeting of key personnel. Plans, approaches, results, and goals are evaluated, and in some cases, reoriented so as to remain compatible with changing knowledge and program emphasis in support of USAMRIID's mission.

In conclusion, USAMRIID is more creative in its approaches to solving problems and more productive in achieving mission goals than in FY 1985. New vaccines, new treatment modalities, and new diagnostic assays for unusual diseases and toxins are all concrete evidence of the Institute's increased productivity. All of this information, although generated for exotic and dangerous agents with BW potential, has broad application to both military and civilian medicine. It may be concluded quite reasonably that USAMRIID has established itself as a center of excellence that fulfills a unique medical need by performing research on high hazard agents and toxins that cannot be studied anywhere else in the free world.

Questions or comments about this report are welcomed and may be addressed to:

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a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Middlebrook J M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7211
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Schmidt, J
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Trusal, L S

22. KEYWORDS (Precede EACH with Security Classification Code) (U) BW Defense; (U) Low Molecular Weight Toxins; (U)Microbial Toxins;(U) Biochemistry;(U) Therapy;(U) Lab Animals;(U) Guinea Pigs;(U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

23. (U) The technical objective of this work unit is to provide the research base for the development of protective modalities from toxins. Recent advances in molecular genetics have demonstrated that many toxins can be cloned and produced in mass quantities. We are studying several toxins of high potential for BW use with these new techniques.

24. (U) Our approach is to study any and all aspects of the toxin including detection, genetics, synthesis, elaboration, structure, composition, pharmacology, mechanism of action, pathogenesis, and sensitivity to drugs. We seek to develop novel means of protection from botulinum and marine toxins as well as mycotoxin. Some of these are synthetic vaccines, CRM-based vaccines, toxin-blocking drugs or toxin-reversing drugs.

25. (U) 8603 - 8609 Botulinum and tetanus toxins lower the level of cGMP in cells and synaptosomes. Degradation of cGMP was not accelerated by the two toxins; the cyclase was slightly activated. Studies were conducted to see if the biosynthesis of the precursor was inhibited by tetanus and botulinum toxins; preliminary results were consistent with the hypothesis, but further work is required. A much improved purification scheme was developed for types A, B, C and E botulinum neurotoxins. Sequence work on types C and E continued. Botulinum and tetanus toxins altered the metabolism of the second messenger phosphitidal inositols as did several snake toxins. Purification schemes were worked out for several snake toxins. Antisera was obtained against the toxins. In several instances, monoclonal antibodies were also obtained. Several snake toxins were fluorescently labeled in an attempt to measure binding to nerve cells. Some cytotoxicity of the snake toxins was observed with certain nerve cells. The binding of these toxins to nerve cells was also studied at the electron microscope level using gold-antibody conjugates.

PROJECT NO. 3M161102BS12: Science Base/Medical Defense Against BW

WORK UNIT NO. S12-AA-001: Basic Studies of Conventional Toxins of Biological Origin and Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. L. Middlebrook, Ph.D.

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L. Trusal, MAJ, Ph.D.

BACKGROUND

The BW potential of toxins has been recognized for a long time. The Army has had a research program directed towards the development of protective modalities, primarily vaccines. The program was driven by a combination of intelligence information and a knowledge of which toxins were potent enough and could be prepared in quantities sufficient for delivery. Basically, these considerations kept our list of candidate BW toxins rather short. With the emergence of molecular genetics as a real and practical technology, the number of potential threat toxins became enormous. It is clearly impractical to attempt to study each and every toxin. Rather, a program has been designed which is flexible enough to accommodate new threats rapidly, while developing information with representative toxins to build general principles for protection. At present, we are studying botulinum toxin, tetanus toxin, several snake phospholipase neurotoxins, several snake postsynaptic neurotoxins, ricin, snake myotoxins, and diphtheria toxin.

An important element in many cases is to obtain the gene for the toxin either by synthesizing it (based on known protein sequence data) or cloning. With the gene in a suitable expression system (no trivial task we know), we can produce the toxin in large enough quantities for use in basic research/drug development or to be toxoided and tested as a vaccine. The vaccine, in turn, can be utilized to produce antibody for clinical use or detection systems. Alternatively, the gene can be chemically altered to produce nontoxic, but immunologically cross-reacting, proteins (CRMs) and then these tested for possible efficacy as vaccines.

SUMMARY

Botulinum and tetanus toxins lower the level of cGMP in cells and synaptosomes. Degradation of cGMP was not accelerated by the two toxins; the cyclase was slightly elevated. We conducted studies to see if the biosynthesis of the precursor was inhibited by tetanus and botulinum toxins; preliminary results were consistent with the

hypothesis, but further work is required. A much improved purification scheme was developed for types A, B, C, and E botulinum neurotoxins; milligram-level amounts of the toxins are now available for department research purposes. Sequence work on types C and E continued. Botulinum and tetanus toxins altered the metabolism of the second messenger phosphotidal inositols, as did several snake toxins.

Purification schemes were worked out for several snake toxins. We obtained antisera prepared against the toxins. Several snake toxins were fluorescently labeled in an attempt to measure binding to nerve cells. Little binding was observed with a light microscope, but use of the much more sensitive fluorescence-activated cell sorter is planned. We observed some cytotoxicity of the snake toxins with certain nerve cells. The binding of these toxins to nerve cells was also studied at the electron microscope level with gold-antibody conjugates. We saw no distinct subcellular binding pattern.

MEGA Mechanism of Action of Bacterial Exotoxins. Studies on Botulinum Toxin.

PRINCIPAL INVESTIGATOR: J. L. Middlebrook, Ph.D.

In the last year, we have focused our studies on the cellular, subcellular, and biochemical events involved in the paralysis induced by botulinum toxin. Because of the many similarities between botulinum and tetanus neurotoxins, we have extended many of our experiments to include both toxins. Earlier, we reported that tetanus and botulinum toxins reduced the cGMP levels in synaptosomes and cells. We have attempted to determine the biochemical basis for that reduction.

First, we found that the rate of hydrolysis of cGMP was not increased by addition of the toxins to the target cells. Secondly, we found that the enzyme responsible for production of cGMP, guanylate cyclase, was actually stimulated (!!?) by the toxins. This was an observation that was extremely hard to understand; i.e., a stimulation of the enzyme which produced the chemical, no change in the enzymes which degraded the chemical, yet a large reduction in the concentration of said chemical. The only explanation we have for this paradox is that the substrate levels of precursor, GTP, might have been lowered by the action of the toxin.

We could demonstrate that the toxins were not GTPases, so a direct hydrolysis of precursor was ruled out. We then looked into the possibility that the biosynthesis of GTP was inhibited by tetanus and botulinum toxins. The biosynthesis pathway is very complex and progress has been slow. Thus far, we have obtained some indications that we may be on the right track, but it will be some time before all the variables can be investigated.

We have also begun a major project on the mechanisms of action of several snake toxins. During this reporting period, we developed new purification schemes for six to eight toxins. By using the purified toxins, we immunized rabbits and obtained antisera. Monoclonal

antibodies to several toxins have been developed under contract with Meloy Laboratories. One of these monoclones was found to be a very potent neutralizer of a neurotoxin. This material is being evaluated further for possible chemical utilization.

MEGB Protein Chemistry and Structure-Function Studies of *Clostridium botulinum* Neurotoxins

PRINCIPAL INVESTIGATOR: J. Schmidt, Ph.D.

Four partially purified preparations of type C toxin, obtained from Dr. Leonard Smith, showed many protein bands on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Methods for complete purification of type C toxin from these preparations were devised by using the FPLC system. About 5 to 6 mg of pure toxin was obtained. This represents a relatively high yield; that is, the type C strains used apparently produce less toxin than types A, B, or E.

Procedures for the individual purification of the heavy and light chains of type C were devised by using size exclusion chromatography in guanidine hydrochloride. Samples of type C heavy and light chains were sent to Dr. Randy Lewis at the University of Wyoming for sequence analysis. For the light chain, 13 of the first 15 residues were identified. No data were obtained for the heavy chain. Because the amount sent was adequate and highly pure, the reason for this is unknown. We made a decision to wait until our own gas-phase sequencer was set up and then to continue this effort in-house.

By using our own preparations, we improved and simplified the purification of type C toxin by adapting the procedure developed for type E. This has been successful; size-exclusion and precipitation steps (except for the first precipitation step) have been eliminated. It also appears to be likely that we will be able to substitute adsorption to an ion exchange or hydrophobic interaction resin for the first precipitation step. Work in this area continues.

Two preparations of type A toxin were obtained from Dr. Lynn Siegel. These had been acid-precipitated, treated with RNAse, and passed through a DE-52 column. A strategy was developed to purify this serotype completely, again based on that for type E. It can be done on the FPLC or, with user-prepared columns, as is the case for the methods I devised for types B, C, and E.

I think that, because of these efforts, milligram quantities of highly purified botulinum neurotoxins, types A, B, C, and E, are, for the first time, now available here at USAMRIID. Toxin has been supplied to in-house investigators and to Dr. John Robinson of Vanderbilt University.

Type E toxin was digested with trypsin and the resulting fragments were partially purified by HPLC. These were sent to Dr. Don Hunt at the University of Virginia for sequence analysis by mass spectrometry. About 15 to 20% of the total sequence was obtained, but analysis of the data is not yet complete.

Peptides based on the amino-terminal regions of the heavy chain of type E and the heavy and light chains of type B were obtained and cross-linked to carrier. In collaboration with Dr. Lynn Siegel, antibodies were raised in rabbits against these peptides. Antibodies are currently being evaluated by ELISA and also for possible neutralizing activity.

As a service to the Institute, amino acid d=sequence studies were done on samples submitted by other investigators. These included four samples from Dr. Leppla, two from V. Rivera (Dr. Middlebrook), and one from Dr. Dalrymple.

MEGC Morphological Studies of Binding and Internalization of Botulinum Toxin and T-2 Mycotoxin using Transmission Electron Microscopy Cytochemistry, Immuno-Cytochemistry, and Autoradiography-Mechanism of Action Studies on Botulinum Toxin.

PRINCIPAL INVESTIGATOR: L. R. Trusal, MAJ, Ph.D.

Fluorescent labeling of snake toxins (crotoxins, taipoxin) for the study of binding and internalization. We have labeled with rhodamine isothiocyanate the following snake toxin components: crotoxin antibodies, intact crotoxin, and intact taipoxin. We are using these components to visualize toxin binding to the cell surface of NG-108 and PC-12 cells. Results are preliminary. We still hope to use the fluorescence-activated cell sorter to study toxin-cell interactions.

Cytotoxicity of Snake Toxins We have examined the cytotoxicity of both crotoxin and taipoxin in PC-12 and NG-108 cells. We used release of lactate dehydrogenase (LDH) and vital staining with trypan blue as our assays. Results indicated that a crotoxin dose of 0.10 μg/ml caused little or no increase above controls in either cell type. At a higher dose of 10.0 μg/ml, PC-12 cells were more susceptible to the toxin than NG-108 cells. Not surprisingly, cytotoxicity appears directly related to the toxin dose. A similar experiment with taipoxin demonstrated release of both SGOT (AST) and LDH over a 3-h period. Both cell types appeared to show cytotoxicity at both doses but the effect was more dramatic in NG-108 cells. More studies are needed before real conclusions can be drawn. Results of vital staining with trypan blue were inconclusive, and we conclude that this method is not a reliable means of assaying cytotoxicity of these toxins.

Immunochemistry of Crotoxin Binding We have conducted preliminary studies of the binding of crotoxin to PC-12 and NG-108 cells by a toxin-antibody-protein-A-gold complex. Preliminary results demonstrate toxin binding, but in moderate amounts. Binding is random with no distinct pattern of binding on the cell surface. Studies with taipoxin binding will begin soon.

MEGE Genetics of Botulinum Toxin

PRINCIPAL INVESTIGATOR: L. Smith, Ph.D.

Eucaryotic cells possess three myoinositol-containing phosphatides (PI, PIP, PIP_2) which are metabolically highly active. In recent years, a wide variety of hormones, growth factors, neurotransmitters, and other biologically active substances have been shown to induce the turnover of phosphatidylinositols in their target cells. The products, inositol triphosphate and diacylglycerol, generated from receptor-activated phosphatide breakdown, serve as second messengers for numerous changes in the cell. Some of these changes include the immediate mobilization of calcium into the cell, release of arachidonate, increase in the concentration of cGMP, and the activation of protein kinase C. Since phosphatidylinositol turnover seems to be important for many cellular functions, including those of the nervous system, we studied the effect of different toxins on the uptake of [^{3}H]-myoinositol in guinea pig synaptosomes. We have observed that cardiotoxin, cobrotoxin, crotoxin, tetanus, and botulinum toxins inhibit the in vitro uptake of [^{3}H]myoinositol in guinea pig synaptosomes. These effects were concentration- and time-dependent. We have proceeded by placing cannulas in the brains of guinea pigs, injecting [^{3}H]myoinositol into the brains and determining the optimum incubation time for in vivo labelling of phosphatidylinositols in the synaptosomes. We also determined optimum parameters for the in vitro labelling of the phosphatidylinositols. One of the observations from the in vivo versus the in vitro labelling is that the phosphatidylinositols being labelled are in different compartments in the synaptosomes and appear to be hydrolyzed into the second messengers (IP_3 and DG) differently. We have begun to study the effects of crotoxin, botulinum, and tetanus toxins on the pre-labelled phosphatidylinositols. Preliminary experiments indicate that the toxins do affect the phosphatidylinositols in both compartments. At this stage, we only know the presence of the toxins drastically affects the levels of the phosphatidylinositols and second messengers compared to the controls. The toxins decrease these levels. Further investigation is required to explain these observations.

MEGF Cellular Mechanisms of Actions of Militarily-relevant Toxins

PRINCIPAL INVESTIGATOR: R. Crossland

Our objective is to elucidate the mechanisms of action of neurotoxins with a view to developing treatment for poisoning caused by these toxins. Initial studies will focus on presynaptic neurotoxins which have phospholipase activity as their salient feature (e.g., β-bungarotoxin, crotoxin, taipoxin, notexin) and on presynaptic neurotoxins which affect calcium channels (e.g., β-leptinotarsin-h, ω-conotoxin). Efforts to date have been directed toward procuring the equipment and laboratory facilities necessary to carry out the above objective.

MEFA Pathogenesis of Hemorrhagic Manifestations of Toxemias

PRINCIPAL INVESTIGATOR: T. M. Cosgriff, COL, M.D.

In the last year, studies on the hemostatic abnormalities induced by T-2 toxin in cynomolgus monkeys were completed and the findings published. These studies confirm the results previously noted in guinea pigs and point to inhibition of coagulation factor synthesis as an underlying mechanism.

In vitro studies on the effects of T-2 toxin on platelet function were also conducted during the last year by using human platelets. These studies revealed no in vitro effects at dose levels lethal in animals. Previous in vivo studies in guinea pigs revealed marked inhibition of platelet function in T-2-treated animals but no in vitro effects of the toxin. The discrepancy between the in vitro and in vivo results suggests that the platelet abnormality seen in T-2 toxicosis in guinea pigs is produced indirectly.

Studies on the effects of T-2 toxin on platelet function in cynomolgus monkeys are in progress.

PRESENTATIONS

1. Cosgriff, T. M. 1986. In vivo and in vitro effects of T-2 toxin on platelets. Presented at the Federation of the American Society for Experimental Biology Conference on Trichothecene Mycotoxins, Cooper Mountain, CO, July.

PUBLICATIONS

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6. **Schmidt, J. J., and L. Siegel.** 1986. Purification of Type E Botulinum neurotoxin by high performance ion exchange chromatography. *Anal. Biochem.* 156:213-219.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DAOG1522	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
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10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS12	AB	002
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Basic Studies on Conventional Agents of Biological Origin and Development of Medical Defensive Countermeasures

12. SUBJECT AREAS
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13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
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17. CONTRACT/GRANT
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b. CONTRACT/GRANT NUMBER
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e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	6.8	2256
87	7.0	2071

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Dalrymple, J M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2665
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Leppla, S H
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Schmaljohn C S

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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To elucidate antigenic composition, replicative strategies, and specific gene functions for selected togaviruses, flaviviruses, bunyaviruses, arenaviruses and anthrax. To evaluate specific protein and nucleic acid constituents and gene products as potential diagnostic antigens, probes and immunogens with prophylactic potential. To develop methodology and provide a technical base for an improved BW defense program.

24. (U) Characterize structural proteins and nucleic acid of selected pathogens by biophysical and biochemical techniques; identify the genome regions responsible for important antigenic determinants or diagnostic probes. Ultimately define the replication strategy for a better understanding of mechanisms for either deducing targets for chemotherapeutic intervention or inducing a protective immune response.

25. (U) 8603 - 8609 Studies on Crimean-Congo hemorrhagic fever (CCHF) virus were initiated and methods for propagation and assay were developed. Monoclonal antibodies, including one capable of neutralizing viral infectivity, have been defined, and the battery is being expanded. Additional antigenic determinants have been identified on RVFV and antibody to synthetic peptides generated. Recombinant vaccinia viruses expressing the envelope glycoprotein genes of RVFV clearly induce a protective humoral immune response in mice, generate a cytotoxic T cell response in mice, and protect pregnant sheep from RVFV-induced abortion. The complete nucleotide sequences for the M and S genome segments of Hantaan virus were determined revealing gene order and replication strategy. Sequential binding of anthrax toxin components to cellular receptors was detailed and precise mechanisms proposed. Aromatic amino acid-requiring mutants of the Sterne strain were developed as avirulent vaccine candidates.

PROJECT NO. 3M161102BS12: Science Base/Medical Defense Against BW

WORK UNIT NO. S12-AB-002: Basic Studies on Conventional Agents of Biological Origin and Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. M. Dalrymple, Ph.D.

ASSOCIATE INVESTIGATORS: C. S. Schmaljohn, Ph.D.
J. F. Smith, Ph.D.
M. A. Balady, MAJ, Ph.D.
S. H. Leppla, Ph.D.
A. M. Friedlander, COL, M.D.
G. B. Knudson, Ph.D.
B. E. Ivins, Ph.D.
J. R. Lowe, COL, Ph.D.
S. F. Little

BACKGROUND

Rift Valley fever Virus (RVFV) meets many of the requirements for military medical research interest because of the disease threat to troops operating in endemic areas and potential strategic use of RVFV as a BW agent. Although the existing RVFV vaccine has been shown safe and effective in man, the limitations for its widespread use have been detailed in previous reports and dictate the continued investigation of an improved product. This research has been directed specifically toward the application of recent techniques of biotechnology to viral vaccine development. The expected results will include both an improved vaccine for protection against RVFV and a well-defined, technical strategy for subsequent development of successful vaccines for other viral pathogens with similar characteristics.

Hantaan (HTN) virus is the presumed etiological agent of Korean hemorrhagic fever (KHF). It has a three-segmented, single-stranded RNA genome of negative polarity and serves as the prototype of a new, recently defined genus of Bunyaviridae, the Hantavirus genus. Viruses serologically related to HTN are believed to cause a variety of clinically similar diseases which are collectively termed hemorrhagic fever with renal syndrome (HFRS). In addition to viruses implicated in HFRS, many hantaviruses have now been isolated in geographic regions from rodents with no known associated disease. The ubiquity of these viruses in nature and their transmission via aerosolization rather than by arthropod vectors, impart a unique disease threat due to these agents. We have employed both molecular and epidemiological methods to examine the natural properties of these viruses with the intent of defining specific characteristics which will lead toward more effective diagnosis and disease control.

Crimean-Congo hemorrhagic fever virus has long been identified as a potential pathogen of military medical importance. This virus

recently became a candidate for intensified research efforts applying the experience obtained with other members of Bunyaviridae to the development of a safe and effective vaccine. We have approached the development of an improved vaccine against anthrax by detailed investigations of the toxin components as well as the examination of mutants from attenuated strains. The application of biotechnology to the study of the structure and function of the toxin components and analyses of the genes encoding these proteins should allow effective engineering of a means of improved protection.

SUMMARY

Initial studies of Crimean-Congo Hemorrhagic Fever virus have resulted in successful viral propagation in cell culture, and development of procedures to assay infectivity, neutralizing antibody, and viral antigens. Structural and nonstructural viral polypeptides have been identified by immunoprecipitation, and a small panel of lymphocyte hybridomas secreting monoclonal antibodies to viral structural components has been developed. We found only a single monoclonal antibody exhibiting virus-neutralizing activity, and attempts to expand the hybridoma battery are in progress.

Development of an improved RVFV vaccine has centered around the use of recombinant vaccinia virus expression of RVFV envelope glycoprotein genes and further definition of the important epitopes or antigenic determinants of the virus. The generation of additional monoclonal antibodies to RVFV resulted in the current panel of 66 defined antibodies recognizing a minimum of 22 epitopes on five virus-specified proteins. Some of these antibodies have been useful in constructing immunoaffinity columns for the purification of nonstructural proteins not otherwise easily isolated and purified. Synthetic peptides representing selected regions identified by monoclonal antibody reactivity have been used to prepare rabbit antisera for determination of the relative importance of these antigens in future vaccines. Recombinant vaccinia virus RVFV vaccines have been prepared in a mouse neurovirulent vaccinia parent as well as a derivative of the Wyeth human vaccine. Both recombinants protected mice from lethal challenge, but the recombinant resulting from the mouse neurovirulent strain was considerably more effective in protecting mid-term pregnant sheep from RVFV-induced abortion.

The complete nucleotide sequences of both the M and S genome segments of Hantaan virus were determined. The coding strategy of the S segment was found to be unique among known Bunyaviridae. A gene order with respect to messenger sense RNA of 5'-G1-G2-3' was determined for the M segment. Synthetic peptides corresponding to the predicted amino acid sequences of regions of G1 and G2 were reactive with authentic Hantaan proteins.

The role of cellular immune responses in protection against RVFV infection has not been clearly delineated. Cytotoxic T cells have been demonstrated in mice infected with either a strain of RVFV with reduced virulence for mice (Lunyo) or with recombinant vaccinia viruses expressing the RVFV envelope glycoproteins. The precise role

of these immunocompetent cells in modifying RVFV infection is currently under investigation.

Anthrax studies have emphasized the study of the three protein components of the toxin. Cell binding of lethal factor (LF) required the presence of protective antigen (PA), and this interaction was completed by edema factor (EF). Toxin receptors could be demonstrated on all cell lines examined to date. Expansion of the number of monoclonal antibodies against each of the three toxin components have identified at least 20 distinct antigenic sites and allowed the identification of the receptor-binding region at the C-terminal portion of PA. Transposon Tn*916* mutagenesis of the Sterne strain yielded mutants requiring for growth all three aromatic amino acids, and which are predicted to be avirulent and potential vaccine candidates.

MEIA Molecular and Biological Characterization of Nairoviruses

PRINCIPAL INVESTIGATOR: J. F. Smith, Ph.D.

Studies with Crimean-Congo Hemorrhagic Fever virus were recently initiated and have, as their initial objective, the identification and characterization of the antigens and antigenic determinants capable of inducing protective immunity. Future objectives will include cloning and sequencing the corresponding genes.

Basic serological and virological assays were not established previously for this virus, which characteristically replicates inefficiently in vitro. We have been successful in developing procedures for infectivity, enzyme-linked, immunosorbent assay (ELISA), radioimmunoassay (RIA), and neutralization assays, as well as methods to propagate the virus to moderate titers in cell culture. Structural and nonstructural virus-specific polypeptides have been identified by immunoprecipitation of lysates of infected cells, followed by polyacrylamide gel analysis. We also conducted hybridoma fusions, which have resulted in a small panel of hybridomas secreting monoclonal antibodies reactive with the viral nucleocapsid and envelope proteins. A single monoclone was found capable of in vitro virus neutralization, and we have used this antibody to construct immunoaffinity columns for isolating sufficient viral protein to initiate a larger fusion and a more extensive search for neutralizing monoclones.

MEIB Analysis of Experimental Rift Valley Fever Vaccines

PRINCIPAL INVESTIGATOR: J. M. Dalrymple, Ph.D.

ASSOCIATE INVESTIGATOR: J. F. Smith, Ph.D.

In an attempt to further define and characterize protective epitopes on RVFV viral proteins, additional monoclonal antibodies have been isolated which react with viral structural and nonstructural proteins. These additional monoclonal antibodies react with the viral glycoproteins (G1 or G2), the nucleocapsid polypeptide, a major

nonstructural protein (NSP-31), and a minor virion antigen which has not been identified with certainty, but which is distinct from the major structural and nonstructural proteins. With these additional reagents, the current panel of RVFV monoclones includes 66 defined antibodies recognizing a minimum of 22 epitopes on five virus-coded polypeptides.

Previous studies involving the passive immunization of mice with monoclones specific for G1, G2, or nucleocapsid polypeptide, have shown that only G2-specific, neutralizing monoclones were protective. Unexpectedly, our current studies have shown that some antibodies to the nucleocapsid polypeptide, which is presumed to be an internal component of the virus, as well as antibodies to the minor component, are also capable of providing protection. We are investigating the identity of the minor component and the mechanism by which nucleocapsid-specific antibodies protect. The existing antibodies to the nonstructural protein, NSP-31, are not protective. These antibodies have, however, been useful in the construction of immunoaffinity columns which have been successful in the purification of NSP-31 for N terminal sequencing and physical mapping studies.

We are further investigating the protective epitopes, which had previously been identified on G2 and mapped to domains on the N terminal third of the polypeptide, by synthesizing peptides corresponding to selected regions within these domains. Eleven synthetic peptides, ranging from 9 to 14 residues in length, have been synthesized, covalently coupled to keyhole limpet hemocyanin, and used to immunize rabbits. Several of these conjugates have elicited high-titer, peptide-specific responses, and we are currently evaluating these antisera for their reactivity with native proteins as well as virus-neutralization capacity.

We evaluated recombinant vaccinia viruses, containing the genes responsible for the envelope glycoproteins of RVFV, for their ability to protect otherwise susceptible mice from lethal challenge and mid-term pregnant sheep from RVFV-induced abortion. Recombinant vaccinia viruses from either the mouse neurovirulent WR vaccinia or a vaccinia strain derived from the currently licensed Wyeth vaccine were equally efficacious in protecting mice from lethal challenge. Although the WR vaccinia recombinant protected pregnant sheep from RVFV abortion, the recombinant vaccinia from the Wyeth derivative was less effective.

MEID Molecular and Biological Characterization of Hantaviruses

PRINCIPAL INVESTIGATOR: C. S. Schmaljohn, Ph.D.

Molecular and antigenic properties of viruses serologically related to Hantaan virus were identified and served as the basis for a proposal to establish a new genus of Bunyaviridae, the Hantavirus genus. The proposal was submitted to and accepted by the International Committee on the Taxonomy of Viruses in June 1986.

We determined the complete nucleotide sequence of the small (S) and medium (M) genome segments of Hantaan virus. The S segment

contained 1696 nucleotides and encoded a polypeptide with a molecular weight of 48,100, which was identified as the nucleocapsid protein. No other open reading frames capable of producing a polypeptide of 50 amino acids or greater were identified in any of the other five potential reading frames. Because all other Bunyaviridae examined to date encode both a nucleocapsid protein and a nonstructural (NS_S) protein in their S segment, this finding was unexpected. A small, 6-K polypeptide, which initiated at an AUG codon four nucleotides beyond the termination codon of, and in the same reading frame as, the nucleocapsid protein, was the only other potential gene product identified within the nucleotide sequence. A protein of this size is much smaller than any known Bunyaviridae NS_S and the existence of such a polypeptide in Hantaan-infected cells has not been demonstrated. Collectively, these data indicate that the S genome segment of Hantaan employs a different coding strategy than those reported for other members of the Bunyaviridae.

Hantaan M RNA was determined to be 3616 nucleotides long and encoded a single long open reading frame in the viral complementary sense RNA capable of producing a polypeptide with a molecular weight of 126,000. We determined amino terminal sequences of isolated G1 and G2 envelope glycoproteins, revealing a gene order with respect to message sense RNA of 5'-G1-G2-3'. Mature G1 begins 18 amino acids beyond the first AUG of the open reading frame, preceded by a short, hydrophobic leader sequence. G2 begins at the 649th amino acid of the open reading frame and also follows a hydrophobic sequence. We localized carboxy termini of G1 and G2 and verified gene order by immune-precipitation of Hantaan proteins with antisera to synthetic peptides generated by using amino acid sequences derived from the cDNA sequence. Unlike the phleboviruses, these data demonstrate that no nonstructural (NS_M) coding region precedes the amino terminus of the first mature glycoprotein encoded. Like RVFV, however, Hantaan appears to have a short (6 K), intergenic, polypeptide, coding region.

MEIB Cellular Immune Response of Rift Valley Fever With Vaccinia as a Vector

PRINCIPAL INVESTIGATOR: M. A. Balady, MAJ, Ph.D.

The cellular immune response to RVFV was tested in mice infected with RVFV strains and animals immunized with recombinant vaccinia vectors. The vaccinia vectors contained coding elements of the RVFV genome. Experiments showed that RVF viral antigen is produced and expressed on the surface of infected cells in both the natural infection and in animals immunized with recombinant vaccinia vectors. Expression of the cell surface antigen is dependent upon both the viral strain (Lunyo strain of RVFV produces more antigen than the ZH-501 strain) and cell type. The L-929 cell line and rat hepatocytes produced antigen on their cell surfaces, while the expression of antigen was undetectable in P815 and Vero cells (by fluorescent antibody visualization or complement-mediated lysis of infected cells).

We obtained cytotoxic T cells from Lunyo-infected mice and from mice immunized with vaccinia vectors containing RVFV genes via tail scarification and footpad inoculation. The cytotoxic T cells were obtained from spleen or lymph node cells. A primary cytotoxic T lymphocyte response, directed against Lunyo-infected target cells, peaked at day 6 post vaccination and waned to 50% of the peak response by day 13. We noted this in both the Lunyo-and recombinant vaccinia-infected mice. The cytotoxic T lymphocytes response was shown to be major histocompatibility complex(MHC)-restricted by the lack of alloreactive target lysis. We characterized the cells mediating the lysis of infected cells by selective depletion experiments utilizing antibody and complement. The cells responsible were anti-theta positive, Thy 1.2^+, $Lyt1^-$, 2^+, and 3^+, indicating a cytotoxic T cell response.

Rift Valley fever virus-neutralizing antibody titers were variable in all mice vaccinated with the vaccinia virus constructs and showed a twofold decline at 1 year post-vaccination. Five different vaccinia vector constructs were tested. The vaccinia vector containing a full length M segment of the RVFV genome gave the highest cytotoxic T lymphocyte response in those mice inoculated via the footpad, with lymph node cells as a source of cytotoxic T cells.

MEDA Basic Research Studies for Protection Against Anthrax

PRINCIPAL INVESTIGATOR: S. H. Leppla, Ph.D.

Effort in this research unit has focused on the structure and function of the three protein components of anthrax toxin, and analysis of the genes encoding these proteins.

Further analysis of the gene library created from the *Bacillus anthracis* plasmid pX01 DNA showed it to contain the genes for all three proteins. We sequenced the genes for protective antigen (PA) and for edema factor (EF). Computer searches did not reveal any sequence homology between these genes and other bacterial and eukaryotic proteins. We subcloned the gene for lethal factor (LF) into several plasmid vectors in preparation for sequencing.

Use of radiolabeled PA and LF demonstrated the existence of specific receptors on all cell lines examined. Binding of LF required the presence of PA, and was completed by EF. Measurement of component interactions by sedimentation equilibrium showed that LF and EF bind to the 65-kd fragment of PA, but not to intact PA. We concluded that PA is bound to receptors and cleaved by a trypsin-like enzyme in the membrane, so as to expose a site to which LF or EF binds. Amino acid sequencing of the 65-kd fragment showed that cleavage of PA in vitro by trypsin is at arginine-167, in a unique sequence of four contiguous basic amino acids. Consistent with this model is the finding that the 65-kd fragment substituted for PA in producing toxicity in combination with LF.

We obtained large numbers of monoclonal antibodies to all three toxin proteins. Antibodies to PA identified at least 20 distinct

antigenic sites. We showed that the monoclonal antibodies that neutralize PA bind to the C-terminal half of the protein, and prevent toxin binding to cells. This localizes the receptor-binding region to the C-terminal half of PA.

Transposon Tn*916* mutagenesis of the Sterne strain yielded mutants requiring all three aromatic amino acids for growth. We predict such mutants to be avirulent, and to prove to be safe live vaccines.

PUBLICATIONS

1. **Tappert, H. I., J. M. Meegan, J. M. Dalrymple, and C. J. Peters.** 1986. Monoclonal antibodies to Rift Valley fever virus: characterization and detection of antigenic variation in geographically different virus strains. Submitted to *J. Gen. Microbial.*

2. **Schmaljohn, C. S., S. E. Hasty, L. Rasmussen, and J. M. Dalrymple.** 1986. Hantaan virus replication: effects of monensin, tunicamycin and endoglycosidases on the structural glycoproteins. *J. Gen. Virol.* 67:707-717.

3. **Ihara, T., J. Smith, J. M. Dalrymple, and D. H. L. Bishop.** 1985. Complete sequences of the glycoproteins and M RNA of a Punto Toro Phlebovirus compared to those of Rift Valley fever virus. *Virology* 144:246-259.

4. **Schmaljohn, C. S., A. L. Schmaljohn, and J. M. Dalrymple.** 1986. Hantaan virus M RNA: coding strategy, nucleotide sequence, and gene order. Submitted to *Virology*.

5. **Schmaljohn, C. S., G. B. Jennings, J. Hay, and J. M. Dalrymple.** 1986. Coding strategy of the S genome segment of Hantaan virus. *Virology* 155:633-643.

6. **Leppla, S. H.** 1986. Anthrax toxin. Submitted to *Meth. Enzymol.*

PRESENTATIONS

1. **Hasty, S. E., A. L. Schmaljohn, D. S. Stec, and J. M. Dalrymple.** 1986. Comparison of geographic isolates of Sindbis virus. To be presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

2. **Smith, J. F., and M. C. Osterling.** 1986. Characterization of monoclonal antibodies specific for a Rift Valley fever virus nonstructural protein. Presented at the Annual Meeting of the American Society for Virology, Santa Barbara, CA, June.

3. **Dalrymple, J. M., J. C. Morrill, P. Sridhar, S. L. Hu, and M. S. Collett.** 1986. Evaluation of S recombinant vaccinia virus vaccine candidate of Rift Valley fever. Presented at the Annual Meeting of the American Society for Virology, Santa Barbara, CA, June.

4. **Schmaljohn, C. S.** 1986. The coding strategies of the M and S genome segments of Hantaan virus. Presented at the Annual Meeting of the American Society for Virology, University of California, Santa Barbara, CA, June.

5. **Battles, J. K., M. S. Collett, and J. M. Dalrymple.** 1986. Comparison of Rift Valley fever virus isolates and variants by nucleic acid sequencing of G2 envelope glycoprotein epitope genes. To be presented at the 35th Annual Meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

6. **Morrill, J. C., M. S. Collett, and J. M. Dalrymple.** 1986. Evaluation of experimental Rift Valley fever virus vaccines in pregnant sheep. To be presented at the 35th annual meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG1526	86 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS12	AC	003
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) Basic Studies Seeking Generic Medical Countermeasures Against Agents of Biological Origin

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	1.5	195
c. TYPE	d. AMOUNT	87	1.0	200
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Saviolakis, G A
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7181
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Bunner, D L
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Mice; (U) Therapy; (U) Mammalian Peptides; (U) Neurotransmitters; (U) Leukotrienes; (U) RAI; (U)Lab Animals

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To study the basic mechanisms of action and the physiological effects on host vital systems of mammalian low molecular weight peptides, such as neurohormones, known as putative peptide neurotransmitters, and leukokines. To develop therapeutic interventions for military personnel and to mitigate the adverse effects of these peptides, and to develop methods for their detection.

24. (U) I. - In vitro studies, using neural cell cultures, for characterization of peptide and non-peptide receptors, receptor regulation and interactions, post-receptor biochemical events, and evaluation of pharmacologic agonists and drugs. II. In vivo studies, using small animals, for evaluation of peptide CNS effects and pharmacokinetics.

25. (U) 8510 - 8609 I. - A human neural cell line, previously shown to have insulin receptors, was used for the detection of cholinergic and substance P receptors. Cholinergic receptors were found. Their pharmacological characterization indicated that they are of the muscarinic type. Muscarinic agonists increased cyclic GMP levels in these cells. Receptor concentration was decreased by muscarinic agonists and interleukin-1 and increased by dexamethasone and insulin. Substance P receptors were not found. II. - Brain stereotaxic surgery and push-pull perfusion of localized brain areas were established. The pyrogenic and metabolic effects of interleukin-1, instilled into the anterior hypothalamus and the ventral-septal area, were studied.

PROJECT NO. 3M161102BS12: Science Base/Medical Defense Against BW

WORK UNIT NO. S12-AC-003: Basic Studies Seeking Generic Medical Countermeasures Against Agents of Biological Origin

PRINCIPAL INVESTIGATOR: G. A. Saviolakis LTC, M.D.

ASSOCIATE INVESTIGATOR: D. L. Bunner, COL, M.D.

BACKGROUND

Low molecular weight endogenous peptides, such as neuropeptides and monokines, have major effects on physiological control of vital organ systems, such as cardio-respiratory, vascular, endocrine, and immune systems, and on behavior. Due to their demonstrated potency, many of these peptides may be important as potential biological warfare agents. The purposes, therefore, of this work unit are: (a) to assess the pathophysiological effects of selected groups of peptides, (b) to develop methods for their detection, and (c) to develop therapeutic interventions to mitigate their effects on military personnel.

SUMMARY

In vitro receptor studies - Human Y79 retinoblastoma cells, having properties expected of neural cells, were continued to be used for the detection and characterization of peptide and non-peptide neurotransmitter receptors. Substance P receptors were not detected. Cholinergic receptors were detected and pharmacologically characterized as muscarinic; nicotinic receptors were not found. Binding of a series of muscarinic antagonist and agonist drugs gave the expected potency order (antagonists > agonists). Agonists increased intracellular cyclic GMP levels, suggesting the presence of a functional muscarinic receptor. The receptor could be up-regulated by dexamethasone and insulin and down-regulated by agonist drugs and interleukin-1. This in vitro system is now being used to evaluate the effects of other peptides and may form the basis of a detection assay for substances interacting with an important CNS neurotransmitter system.

In vivo studies. - (A) With Dr. Ruwe, NRC Fellow, neurophysiological approaches (stereotactic surgery, push-pull perfusion) were used to study the direct CNS effects of interleukin-1 and endotoxin. A series of commercial interleukin-1 preparations and *E. coli* lipopolysaccharides were screened and found to have a direct pyrogenic activity, not only on the anterior hypothalamus, but on the ventral septal area as well, a region thought to be involved only in the suppression of fever. The question of the mediation of the acute phase hepatic reaction by the same CNS centers was also evaluated and samples are being analyzed.

In support of these studies, and also to expand the technical base of the bioregulator program, peptide separation techniques (chromatography, HPLC, PAGE, IEF) were established and used for the in-house purification of large quantities of interleukin-1 from crude LEM/EP preparations. (B) Nasopulmonary absorption of peptides, a study planned with Dr. Creasia, Inhalation Toxicologist, was suspended due to his commitments in other projects. Work will hopefully resume in the near future.

PUBLICATIONS

1. **Saviolakis, G. A., A. P. Kyritsis, and G. J. Chader.** 1986. Human Y-79 retinoblastoma cells exhibit specific insulin receptors. *J. Neurochem.* 47:70-76.

PRESENTATIONS

1. **Ruwe, W., and G. A. Saviolakis.** 1986. Identification and characterization of sites in the brain at which interleukin-1 initiates fever. Presented at the Congress on Research in Lymphokines and Other Cytokines, Boston, Massachusetts, August.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA302660	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
86 03 20	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AB	012
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Vaccine Development Studies on Rickettsia of Potential BW Threat

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	1.5	298
c. TYPE	d. AMOUNT	87	1.0	274
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Beveridge, J
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Vodkin, M H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Q Fever; U) Coxiella burnetii; (U) Vaccines; (U) RAI

23 TECHNICAL OBJECTIVE 24 APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) *Coxiella burnetii* is perceived to have significant potential as a BW agent. The currently available vaccine is reasonably protective, but highly reactogenic. An efficacious yet more safe vaccine needs to be developed and stockpiled to protect at-risk US troops. The objective is to proceed with evaluation of a chloroform-methanol extracted residue (CMR) vaccine to assess feasibility for use in humans. Simultaneously a new generation subunit vaccine is being sought, which can be produced readily without the requirement for high containment laboratories.

24. (U) Determine toxicity, safety, efficacy, and dose response to CMR vaccine. Extend animal model testing of CMR to identify and quantify humoral and cell-mediated immune responses. Proceed with human use evaluations if safety and efficacy are demonstrated. Define immunogenic subunits of *C. burnetii* to provide bases for development of a subunit vaccine.

25. (U) 8603 - 8609 - The ELISA detected antibodies earlier in *C. burnetii* infection than other conventional methods. Avirulent phase II (PhII) cells produced antibody only against PhII cells; PhI cells were virulent and induced high antibody titers and a marked anamnestic response. Foci of *C. burnetii* were demonstrated in wild mouse populations. An immune suppression complex was identified which induces suppression of lymphocyte proliferation. Chemical and SDS-PAGE analysis showed the PhI lipopolysaccharides (LPS) of *C. burnetii* were related, while PhII LPS exhibited marked differences. At least three endotoxic strengths of LPS were detected.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AB-012: Advanced Vaccine Development Studies on Rickettsia of Potential BW Threat

PRINCIPAL INVESTIGATOR: J. C. Williams, Ph.D.

ASSOCIATE INVESTIGATORS: J. Beveridge. M.S.
C. R. Bolt, M.A.
C. E. Snyder, Jr., Ph.D.
M. H. Vodkin, Ph.D.

BACKGROUND

Infection of laboratory animals and humans with virulent phase I (PhI) *Coxiella burnetii*, the etiologic agent of Q fever, leads to progressive but usually self-limiting disease, confirmed by serological measurement of anti-*C. burnetii* antibodies. The distribution of Q fever is worldwide. About 5% of cases are chronic, involving liver granuloma or cardiac tissue (endocarditis). In acute disease, the fever course of PhI infection is detected early (3-7 days). No fever response occurs after infection with Phase II (PhII) *C. burnetii*. The infection or vaccination of experimental laboratory animals with PhI *C. burnetii* induces temporal development of antibodies against avirulent PhII whole cells early (3-12 days), while antibodies to PhI whole cells are produced late (12-30 days). The early detection of anti-*C. burnetii* antibodies against whole-cell PhII antigen is generally measured by complement fixation assay (CFA), microagglutination assay (MAA), and indirect immunofluorescence assay (IFA) with trichloroacetic acid (TCA) -or meta-periodate (mPA)-treated PhI whole cells (which convert PhI antigens to PhII antigens). The sensitivity of specific antigenic determinants to each of these chemical treatments has not been evaluated. Therefore, the use of native formalin-inactivated PhI and PhII whole cells as antigen in the ELISA is preferable to chemically treated antigens.

The PhI whole cell vaccine has been replaced by chloroform-methanol-extracted residue (CMR) from PhI whole cells. The CMR vaccine has been shown to be efficacious and non-reactogenic, but it is still difficult to produce from infected yolk sacs. Thus, our objective is to prepare a subunit vaccine by cloning the DNA of virulent *C. burnetii* and screen the clones for production of immunogenic proteins. This approach complements the biochemical approach of purifying immunogen and has the advantage of not requiring infectious microorganisms.

SUMMARY

1) The ELISA detected antibodies earlier in *C. burnetii* infection and gave greater endpoint titrations than other conventional methods. Detection of antibody to the LPS-I antigen, a virulence

marker, was possible only with ELISA. Results of IFA, MAA, and CFA did not correlate with those of the ELISA, suggesting that different assays detect different epitopes.

2) In guinea pigs, the virulent PhI strain (CB9MIC7) and not the avirulent strain (CB9MIIC4) produced a fever response and infectious *C. burnetii* from days 4 to 8 after injection. Avirulent strain rickettsiae could not be isolated from the spleens of infected guinea pigs. Viable, avirulent, PhII cells produced antibody only against PhII cells; the extent and duration of the PhII antibody response was unexpected. This may be the result of an initial replicative burst of PhII *C. burnetii* followed by a brisk clearance. PhI cells were virulent and produced high antibody titers. Anamnestic antibody responses against LPS-I and PhI whole cells were markedly increased in animals previously infected with PhI cells; however, antibody titers to PhII cells were only slightly increased.

3) Mice indigenous to rural Frederick County, Maryland, were surveyed for seropositive reactions to *C. burnetii*. Three ecological habitats (barns, wooded fencelines, and open fields) were sampled. Three genera of mice were found within the habitats: *Mus, Peromyscus*, and *Microtus*. A high proportion (61-80%, inclusive) of seropositives existed among all three genera, regardless of habitat. There appeared to be no relationship between prevalence of Q-fever antibodies and the proximity of mice to large domestic animals. The results suggest endemic foci of the disease may be maintained within resident mouse populations.

4) An immune suppressive complex (ISC) was identified which induces suppression of lymphocyte proliferation in cells of T- and B-cell lineage. The *C. burnetii* components of the ISC can be separated into three separate fractions. Component 1 is not covalently linked to any of the remaining components and may shield the complex *in situ* from the effects of chemical and enzymatic degradation. Components 2 and 3 form an integral part of phase I CMR and may be linked by disulfide bonds. A reconstituted mixture of components 1 and 2 is required for biological suppressive activity.

5) Chemical compositional analysis of the LPSs revealed that the phase I LPSs of *C. burnetii* are related, while the purported phase II LPSs exhibited marked differences. The LPS from the biological phase II strain (CBAUSTII) appeared to be phase I LPS; CBAUSTII-LPS contained the GalNU-α(1-6)-GlcN, absent in the CB9MIIC4- and CBM44II-LPSs. Sodium dodecylsulfate-polyacrylamide gel electrophoresis showed the CB9MIIC4- and CBMIIC4-LPS major bands were the 2.5-kd components, while the CBAUSTII-LPS contained 2.5-and 10.5-kd bands as major components. The CB9MI514-LPS contained little KDO-like material and a marked increase in GlcN content. This LPS consists of a major band at 10.5 kd and a minor band at 23 kd and several bands between. Although no lower bands were detected, chemical analysis showed the presence of GalNU-α(1-6)-GlcN. Analysis of the neutral sugars in CB9MIIC4-, CBM44II, CBAUSTII-, and CB9MI514-LPSs also showed marked differences between the LPSs. The rough LPSs of CB9MIIC4 and CBM44II were devoid of virenose and dihydrohydroxystreptose, the semi-rough LPSs of

CBAUSTII and CB9MI514 only contained dihydrohydroxystreptose as well as GalNU α(1-6)-GlcN, while all of phase I LPSs contained both virenose and dihydrohydroyxstreptose plus GalNU-α(1-6)-GlcN.

6) We observed differences in the lethal toxicities of *Coxiella* LPSs. At least three endotoxic strengths were detected: a) LPSs from CBOI, CBHENI, and CBKAVI were the most endotoxic; b) LPSs from the CB9MI514 and CB9MIIC4 strains killed 100% at 10 μg, and 80 and 40% of the mice at 1 μg, respectively; c) LPSs from CBMIC7, CBM441, CBAUSTII, and CBKAVI killed 60 to 80% of the mice at 10 μg.

PUBLICATIONS

1. **Williams, J. C., L. A. Thomas, and M. G. Peacock.** 1986. Humoral response to Q fever: detection of antibodies against phase I and phase II antigens of *Coxiella burnetii* by microplate enzyme-linked immunosorbent assay. Submitted to *J. Clin. Microbiol.*

2. **Williams, J. C.** 1986. Role of the composition of *Coxiella burnetii* and immunity to Q fever, pp. *In* ( ), Biology of rickettsial diseases. (To be Published).

3. **Williams, J. C., L. A. Thomas, and M. G. Peacock.** 1986. Enzyme-linked immunosorbent assay antibody responses to *Coxiella burnetii*: demonstration of phase-specific antigenic fractions, nonimmune immunoglobulin binding, and suppression of phase II anamnestic responses. Submitted to *J. Infect. Dis.*

4. **Vodkin, M. H., and J. C. Williams.** 1986. Deletions of DNA *Coxiella burnetii*. *J. Gen. Microbiol.* (In Press)

5. **Amano, K.-I., K. Fukushi, and J. C. Williams.** 1986. Electron microscopic studies of lipopolysaccharides from phase I and phase II *Coxiella burnetii*. *J. Gen Microbiol.* 131:3127-3130.

6. **Williams, J. C., L. A. Thomas, and M. G. Peacock.** 1986. Humoral immune response to Q fever: enzyme-linked immunosorbent assay antibody response to *Coxiella burnetii* in experimentally infected guinea pigs. *J. Clin. Microbiol.* 24:935-939.

7. **Williams, J. C., L. A. Thomas, and M. G. Peacock.** 1986. Identification of phase-specific antigenic fractions of *Coxiella burnetii* by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* 24:929-934.

8. **Williams, J. C., T. A. Damrow, D. M. Waag, and K-I. Amano.** 1986. Characterization of a phase I *Coxiella burnetii* chloroform-methanol residue vaccine that induces active immunity against Q fever in C57BL/10 ScN mice. *Infect. Immun.* 51:851-858.

9. **Amano, K-I., J. C. Williams, S. R. Missler, and V. N. Reinhold.** 1986. Structural and biological relationships of *Coxiella burnetii* lipopolysaccharides: chemical composition and microheterogeneity of smooth, semi-rough, and rough LPS. Submitted to *J. Biol. Chem.*

PRESENTATIONS

1. **Williams, J. C., M. H. Vodkin, D. H. Waag, K. -I. Amano, and E. H. Stephenson.** 1986. Strategies for vaccine against Q fever: genetic and molecular basis of virulence and attenuation in *Coxiella burnetii*. Presented at the Army Science Conference, West Point, NY, June.

2. **Waag, D., J. Williams, K-I. Amano, M. England, and J. Beveridge.** 1986. Relationship between the ability to induce in vitro splenocyte hyporesponsiveness and the LPS phenotype of *Coxiella burnetii* strains. Presented at the 6th Annual Conference of the American Society for Rickettsiology, September.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA302662	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
86 04 07	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AC	013
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY-87 -01			

11. TITLE *(Precede with Security Classification Code)* (U) Advanced Vaccine Development Studies on Viruses of Potential BW Threat

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER		86	5.0	134
c. TYPE	d. AMOUNT	87	1.0	610
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS *(include zip code)* Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Lupton, H W
d. TELEPHONE NUMBER *(include area code)* 301-663-2833	d. TELEPHONE NUMBER *(include area code)* 301-663-2405
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)* Barrera Oro, J G
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR *(if available)* Cole, F E Jr.

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Military Medicine; (U) BW Defense; (U) Junin Virus (U) Vaccines; (U) Lab Animals; (U) Monkeys; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) Develop and test a live, attenuated Junin virus vaccine prepared against Argentine hemorrhagic fever for prophylactic treatment of at-risk personnel.

24. (U) Conduct preclinical tests to evaluate vaccine safety, immunogenicity, and stability. Conduct animal and in vitro studies to determine vaccine cross protection against heterologous Arenaviruses. Evaluate the vaccine constituents and production processes with goals of improved titer, stability, and immunogenicity. Design and conduct clinical trials to evaluate vaccine safety and efficacy.

25. (U) 8604 - 8609 Live, attenuated, Candid #1 Junin vaccine was evaluated in Phase I clinical trials to determine vaccine safety and immunogenicity. Three groups containing a total of 17 individuals were studied, while a fourth study group was initiated. Seronegative males and females were given 10^3 to 10^4 PFU of vaccine virus by subcutaneous or intramuscular routes, with only minor differences in antibody responses. No adverse local or systemic reactions were noted. Viremia was detectable in only one of 17 vaccine recipients. Fifteen of 17 (88%) vaccinees seroconverted after immunization. Studies have continued to evaluate vaccine stability. The freeze-dried product had no appreciable change in titer after 43 weeks of storage at -20°C, whereas, reconstituted vaccine was relatively stable for only 1 week at 5°C, 5 h at 25°C, and 4 h at 35°C.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AC-013: Advanced Vaccine Development Studies on Viruses of Potential BW Threat

PRINCIPAL INVESTIGATOR: H. W. Lupton, COL, Ph.D.

ASSOCIATE INVESTIGATORS: J. G. Barrera Oro, Ph.D.
F. E. Cole, Jr., Ph.D.

BACKGROUND

A United Nations Development Project jointly conducted by U.S. and Argentine investigators resulted in development of Candid #1 vaccine at USAMRIID. Master seed, production seed, and vaccine have been produced and preclinical testing has been completed in conformity with Good Laboratory Practices and Good Manufacturing Practices Regulations, and in compliance with vaccine requirements for both the United States and Argentina. Preclinical data were documented in an Investigational New Drug (IND) submission. Clinical protocols were approved and vaccine was administered to an initial group of research volunteers at USAMRIID in October 1985. Preliminary data from Phase I clinical studies indicate that the vaccine is safe and immunogenic. Field testing of the vaccine will be conducted at the Instituto Nacional de Estudios sobre Virosis Hemorragicas in Pergamino, Argentina, over the next 3.5 years. Adequate experimental design has necessitated a small safety trial in seropositive individuals, a larger-scale safety trial, and an efficacy trial. The efficacy trial will be a double-blind, placebo-controlled study in 3,500 individuals selected from a population with a disease incidence of 75 cases per 10,000 individuals over two endemic seasons. Thus, a threefold reduction in disease will demonstrate vaccine efficacy with 95% confidence and an 80% power. Concurrently, a case control study will be conducted to assist in selection of the high risk population, and a risk analysis study in the endemic region will determine Junin viral activity in rodents.

SUMMARY

Live, attenuated, Candid #1 Junin vaccine (IND 2257) was evaluated in Phase I clinical trials. Three cohorts containing a total of 17 individuals were studied and a fourth group was initiated by various vaccine doses (10^3 to 10^4 PFU) and routes of administration (s.c. or i.m.).

In cohort 1, four males were given 29,000 PFU of vaccine s.c. and monitored for 121 days, including 42 days as inpatients. Four unvaccinated individuals were monitored for 35 days to control clinical laboratory assays. No significant changes were observed in clinical or laboratory parameters. Three of four vaccinees seroconverted.

In cohort 2, 12 seronegative individuals were randomized (double-blinded) into three groups (Groups 1-3), and two additional subjects were used to control clinical laboratory assays: (Group 1) five subjects were given 58,000 PFU of vaccine i.m.; (Group 2) four were inoculated i.m. with 2,000 PFU of vaccine; and, (Group 3) three were given placebo (saline) i.m. Virus was isolated from one individual in Group 2 on postinoculation day 11. Eight of nine vaccinees seroconverted. No significant changes in clinical or laboratory parameters were noted, although minor clinical complaints, consistent with seasonal allergies or intercurrent viral infections, were noted in individuals from all groups.

In an additional group, four individuals were given 13,000 PFU of vaccine i.m. All individuals seroconverted without adverse clinical findings being observed.

Studies have continued to evaluate vaccine stability. The freeze-dried product had no appreciable change in titer after 43 weeks of storage at -20°C, whereas reconstituted vaccine was relatively stable for only 1 week at 5°C, 5 h at 25°C, and 4 h at 35°C.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA302664	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 85 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AD	014
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Drug Development Studies Against Agents of Biological Importance

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	2.5	788
c. TYPE	d. AMOUNT	87	3.0	957
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Canonico, P G
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2290
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Kende, M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Ussery, M A

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Antiviral Drugs; (U) Pharmacology; (U) Viral Diseases; (U) Lab Animals; (U) Mice; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Identify effective drugs against viruses that are potential threats to military personnel; obtain data on toxicology, pharmacology, and metabolism of antiviral drugs and conduct preclinical and clinical studies to assess safety and efficacy in compliance with FDA regulations. Conduct laboratory studies to develop novel applications of drug delivery systems for antiviral chemotherapy. Develop adjuvants for use with subunit viral vaccines.

24. (U) Assess efficacy of potential antivirals against viruses in tissue cultures and in rodent models for Rift Valley fever virus (RVFV) and VEE. Evaluate toxicity and pharmacology of promising compounds in preclinical protocols conducted in rodents and nonhuman primates. Provide technical support for clinical protocols. Evaluate state-of-the-art technologies for improved drug delivery, such as liposomes, topical application, implantable infusion pumps, and molecular carriers. Perform animal studies to assess efficacy of immunopotentiating compounds and drugs combinations as potential antivirals or vaccine adjuvants.

25. (U) 8510 - 8609 A new pyridinium prodrug of ribavirin was synthesized. The prodrug was significantly effective in prophylactically protecting Japanese encephalitis B-infected mice. In contrast, ribavirin or ribavirin triacetate were not protective. The immunomodulator, CL-246,738 was found to induce the production of interferon upon oral administration and infect up to 70% of mice with Rift Valley fever virus. Compound CL-259,763, also a known potent immunomodulator, did not induce interferon or demonstrate any antiviral activity. Prophylactic administrations of recombinant mouse gamma or alpha interferon were effective in protecting mice against Japanese encephalitis B infection.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AD-014: Advanced Drug Development Studies Against Agents of Biological Importance

PRINCIPAL INVESTIGATOR: P. G. Canonico, Ph.D.

ASSOCIATE INVESTIGATORS: M. Kende, Ph.D.
M. A. Ussery, MAJ, Ph.D.

BACKGROUND

Drug screening efforts at USAMRIID have identified a number of compounds with broad-spectrum antiviral activity against "exotic" RNA viruses. This research program addresses the continued evaluation of these lead compounds and their application as broad-spectrum antiviral agents.

Work on further development of immunomodulators has also continued. Particular attention has been given to compounds that are active by the oral route and prodrugs or delivery systems that target drugs to the brain. The interferon inducer, poly-(ICLC), has been evaluated further to determine the optimal prophylaxis and therapy schedule which could be used for human use. Similarly, the availability of recombinant alpha A and gamma interferon has, for the first time, made the use of interferon in the treatment of viral diseases a practical possibility. Hence, studies normally required prior to use of drugs in man were continued.

SUMMARY

Two known immunomodulators, CL-246,738 and CL-259,763, were evaluated for their prophylactic and therapeutic efficacy against Rift Valley fever (RVF) virus in CR mice. CL-246,738 was found to induce substantial amounts of interferon with oral administration and yielded up to 70% survivors upon prophylactic or therapeutic administration. Although, poly (ICLC) is somewhat more efficacious, only CL-246,738 is active orally, making this a potentially useful clinical compound.

Serological, virological, hematological, and clinical chemistry studies in the treatment of yellow fever viral infection with human recombinant α-2 interferon (Hα2-IFN) were conducted in African green monkeys. A dose of 5 x 10^5 IU/kg of Hα2-IFN was sufficient to clear viremia, followed by a rapid return of SGOT and HBDH enzyme titers to preinfection levels. This study demonstrates that the African green monkey is a valuable model to assess the antiviral efficacy of antiviral substances.

Pichinde virus-infected, strain 13 guinea pigs were treated with a combination of ribavirin and poly (ICLC). In a series of experiments, poly (ICLC) failed to protect against a lethal challenge

with Pichinde virus, even though treatment was initiated at the time of challenge, for 10 days, then on alternate days until day 20. Ribavirin administered on an identical schedule yielded a few survivors who also died within a few days after termination of treatment. A combined treatment with ribavirin and poly (ICLC) given i.p. or intranasally yielded 60 to 80% survivors. This is the highest survival rate ever achieved with any treatment regimen.

PUBLICATIONS

1. **Kende, M., D. J. Gangemi, W. Lange, D. A. Eppstein, J. Kreuter, and P. G. Canonico.** 1986. Carrier-mediated antiviral chemotherapy. To be published in Proceedings of the 5th International Conference on Comparative Virology by Academic Press, New York. <<Book Chapter>>

PRESENTATIONS

1. **Kende, M., J. Brown, J. Smith, A. Johnson, M. Ussery, and P. Canonico.** 1986. Treatment of yellow fever virus (YFV) infection in African green (AG) monkeys with human recombinant alpha-2 interferon (HuRaI). Presented at the IXth International Congress of Infections and Parasitic Diseases, Munich, FRG, July.

2. **Kende, M., and P. G. Canonico.** 1986. Design of carrier-mediated drug delivery for antiviral therapy. Presented at the 5th International Conference on Comparative Virology, Alberta, Canada, May.

3. **Kende, M., W. Rill, H. Levy, R. Williams, and P. Canonico.** 1986. Nonspecific and specific immunomodulation with poly(ICLC) for antiviral therapy. Presented at the International Symposium on Immunological Adjuvants and Modulators of Nonspecific Resistance to Microbial Infections, Columbia, MD, June (sponsored by the Office of Naval Research, USAMRDC, and the Air Force Office of Scientific Research).

4. **Kende, M., W. Rill, H. Levy, and P. Canonico** 1986. Antiviral effects of polyICLC in immunocompromised hosts. Presented at the 26th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, June.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DAOB6410	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
86 02 19	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AE	015
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U)Advanced Non-system Development Studies on Conventional Agents of Biological Origin for Development of Medical Defensive Countermeasures

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	1.0	312
c. TYPE	d. AMOUNT	87	1.0	189
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Scott, G H
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Lab Animals; (U) Bacillus anthracis; (U) Guinea Pigs; (U) Mice; (U) Hamsters; RAD I

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Prophylactic vaccines, therapeutic agents, and immunomodulators developed to protect US troops against potential BW agents must be effective when the microorganism is presented as an aerosol. The objective is to evaluate animal models of aerosol-induced infections and toxemias.

24. (U) Define and quantify the molecular aspects of clinical, pathological, and immunological changes that occur in vaccinated or treated animals when exposed to potential BW agents in aerosols.

25. (U) 8602-8609 - Antibody production was dose- and time-dependent in sensitive and resistant strains of mice infected with *Coxiella burnetii*. Similar antibody patterns to phase I and phase II antigens were observed. In both strains, 30 rickettsiae induced splenomegaly; the magnitude and duration of the response increased with dose. Effects on the liver were minimal. Concentrations of rickettsiae in the spleen were maximal at 4 to 7 days and continued to persist at low titers at 60 days after infection.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AE-015: Advanced Non-system Development Studies on Conventional Agents of Biological Origin for Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: J. C. Williams, Ph.D.

ASSOCIATE INVESTIGATOR: G. H. Scott, Ph.D.

BACKGROUND

Previous evaluations classified various strains of inbred mice according to their sensitivity to infection with phase I *Coxiella burnetii*. Strains exhibiting both morbidity and mortality after infection were classified as resistant. Those showing only morbidity were classified as intermediately sensitive, and strains showing no overt signs of the infection were considered resistant. Induction of splenomegaly and antibody production following infection were similar in sensitive and resistant strains; however, the dose of phase I *C. burnetii* required to kill 50% of the mice in resistant strains was about 1000 times more than that required for sensitive A/J mice. Similarly, moderate infecting doses induced suppression of splenic lymphocyte responsiveness in sensitive A/J mice, but the response of cells from a representative resistant strain (C57B/6J) was not significantly suppressed by doses lower than $10^{6.7}$ rickettsiae.

Coxiella burnetii was disseminated to all vital organs in sensitive A/J mice given a high infecting dose, and high titers of the microorganism persisted in the tissues. Several investigations have shown an inverse relationship between the replication of BCG in organs of infected animals and the size of the infecting dose; we interpreted this as a direct effect of specific anti-BCG immunity, which develops faster after a higher antigenic load. There also is evidence for a system of natural resistance to a number of other intracellular parasites (*Salmonella, Listeria, Leishmania*, and *Rickettsia*) in which the rate of bacterial replication in the preimmune phase of anti-bacterial response is responsible for the resulting level of resistance. Innate resistance also may exert a significant influence during a *C. burnetii* infection. The influence of dose and time on the replication and eventual clearance of *C. burnetii* from organs of sensitive and resistant strains of mice need to be determined.

SUMMARY

Strains of mice sensitive (A/J) and resistant (C3H/HeN) to *C. burnetii* were given varying doses of the phase I, 9 Mile isolate. Antibody production in both strains was dose- and time-dependent, plus similar antibody patterns against phase I and phase II antigens were observed. Phase II antibodies were detected by 7 days in mice

injected with $10^{5.5}$ rickettsiae. Significant phase I antibody titers were present at 21 days in mice infected with 300 or more rickettsiae. Antibody to lipopolysaccharide was not detected in A/J mice, and was detected only in C3H/HeN mice given $10^{5.5}$ organisms.

Changes in organ weight attributable to *C. burnetii* infection were similar in both mouse strains. A dose of 30 rickettsia induced splenomegaly which peaked at 21 days and was largely resolved within 60 days after infection. The magnitude of splenomegaly increased as the injected dose increased, and was not completely resolved after 2 months in mice given $10^{5.5}$ rickettsiae. Less dramatic changes in liver weights were associated with *C. burnetii* infections. Kidney weight did not appear to be affected by infection. Lung weight indices were erratic, especially for lungs measured before 96 h after injection; significant patterns of change could not be discerned.

The rickettsiae replicated equally well in the spleens of the sensitive and resistant strains. Injected organisms were detected at 12 h. The rate of increase and peak titers obtained were dose dependent. Maximum titers of $10^{5.3}$, $10^{6.8}$, and 10^{10} rickettsiae/spleen were obtained after injected doses of $10^{1.5}$, $10^{2.5}$, and $10^{5.5}$ rickettsiae, respectively. We observed peak titers 4 to 7 days post injection and titers were diminished by 21 days. However, viable *C. burnetii* were not completely cleared from the spleen after 2 months. Sixty days after injection, spleen titers were low and variable (6 to 1000 rickettsiae/spleen), but detectable.

PUBLICATIONS

1. **Williams, J. C., V. Sanchez, G. H. Scott, E. H. Stephenson, and P. H. Gibbs.** 1985. Variation in responsiveness of BALB/c sublines and congenic mice to phase I *Coxiella burnetii* infection and vaccination. *Curr. Topics Microbiol. Immunol.* 122:189-199.

2. **Scott, G. H., J. C. Williams, and E. H. Stephenson.** 1986. Animal models in Q fever: pathologic responses of inbred strains of mice to phase I *Coxiella burnetii* infections. Submitted to *Infect. Immun.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA302670	86 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
86 03 12	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AI	018
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE *(Precede with Security Classification Code)* Advanced Vaccine Development Studies on Toxins of Potential BW Threat

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0620 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER		86	1.0	130
c. TYPE	d. AMOUNT	87	1.0	20
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathology Division, USAMRIID
b. ADDRESS *(include zip code)* Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Siegel, L S
d. TELEPHONE NUMBER *(include area code)* 301-663-2833	d. TELEPHONE NUMBER *(include area code)* 301-663-7211
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Military Medicine; (U) BW Defense; (U) Microbial Toxins; (U) Vaccines; (U) Therapy; (U) Toxoids; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) Evaluation and testing of toxoids and antitoxins for protection against botulinal neurotoxins. These neurotoxins are considered to have significant biological warfare potential and our at-risk forces should be immunized against them.

24. (U) Obtain botulinum toxin serotypes A-G in partially or highly purified state. Evaluate toxoids prepared from such materials for protection of personnel against botulinum toxin poisoning. Simultaneously evaluate antitoxins produced against botulinal toxoids as suitable prophylactic or therapeutic agents for botulinal toxin poisoning.

25. (U) 8603 - 8609 - To continue to evaluate the efficacy of the current botulinum vaccine [botulinum pentavalent (ABCD) toxoid], personnel immunized with this product are being surveyed for neutralizing antibodies to types A and B botulinum toxins. With the current botulinum vaccine in a guinea pig model system, investigations are in progress to develop an immunization schedule to produce the highest titers of neutralizing antibody in the shortest possible time. Synthetic peptides, produced according to known amino acid sequences of the neurotoxin molecules, were coupled to carrier molecules and used to immunize rabbits. The animals produced high antibody titers; the antibody, however, neutralized only a small amount of toxin. Thus these particular peptides have limited potential as candidate vaccines. Additional peptides are being evaluated.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AI-018: Advanced Vaccine Development Studies on Toxins of Potential BW Threat

PRINCIPAL INVESTIGATOR: L. S. Siegel, Ph.D.

BACKGROUND

There are seven immunologically distinct neurotoxins (A-G) produced by the heterogeneous group of bacteria given the genus species designation *Clostridium botulinum*. Human botulism is associated with types A, B, E, and F. Although there have been few well-documented human cases reported, types C, D, and G have the potential of producing toxic effects in man. Toxoids (chemically inactivated but immunogenic toxins) for each serotype are used to elicit immunity to these toxins. The botulinum toxoid used for human immunization was prepared by treating types A, B, C, D and E toxins with formaldehyde and combining them to form a composite immunogen. This toxoid was made by the Michigan Department of Health under contract to the Army in the late 1960s. This toxoid produces sustained measurable antibody levels only after a series of four injections administered over a period of 1 year. Mild side effects, including tenderness, redness, heat, and swelling at the site of injection, are common. A new, improved toxoid prepared from more highly purified neurotoxins is required. In addition to the current pentavalent (A-E) toxoid, it should include types F and G.

SUMMARY

To evaluate the efficacy of the current botulinum vaccine [botulinum pentavalent (ABCDE) toxoid] and to establish a rational basis for reimmunization, personnel immunized with this product are being surveyed for neutralizing antibodies to types A and B botulinum toxins. (The antibody response to type A correlates well with the response to types C, D, and E. Typically, the response to type B is the poorest.) Blood is being drawn 14 days after the initial series of three immunizations (administered at 0, 2, and 14 weeks); just prior to the annual booster; and 14 days after the annual booster. Neutralization tests on sera, using a mouse bioassay with the World Health Organization Standard Antiserum as a standard, are being performed according to the standard neutralization procedure described by the Center for Disease Control. We have established an ELISA (using purified type neurotoxin) to assay for antibodies, and we are developing a data base to correlate ELISA values with neutralization titers, so that the ELISA may replace the cumbersome and time-consuming neutralization test.

With the current botulinum vaccine in a guinea pig model system, investigations are in progress to develop an immunization schedule to produce the highest titers of neutralizing antibody in the shortest possible time.

Studies are continuing to evaluate synthetic peptides, produced according to known amino acid sequences of the neurotoxin molecules, for their efficacy as vaccines. Peptides were coupled to a carrier molecule, keyhole limpet hemocyanin (KLH), and used to immunize rabbits. Sera from these animals were obtained and evaluated for antibody. Using the peptide coupled to a different carrier (bovine serum albumin) in an ELISA system, we demonstrated that the rabbits did produce high titers to the peptide moieties of the KLH conjugates. More importantly, these antibodies also reacted with purified native toxin in an ELISA. However, when assayed in the neutralization test, the antibodies neutralized only low amounts of toxin. Thus, these particular peptides have limited potential as candidate vaccines. Additional peptides are being evaluated.

MIGB Evaluation and Testing of Toxoiding Processes for the Toxins of *C. botulinum*.

A programmatic decision was made to table further development of botulinum toxoid. Two Requests for Proposals, one for the production of a hexavalent (ABCDEF) botulinum toxoid and a second for the development of a type G toxoid, were cancelled by the U.S. Army Medical Research and Development Command.

MIGC Evaluation, Testing and Field Testing of Antitoxins for Botulinum Toxins.

Research has not been conducted in this area during the reporting period.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA302668	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AG	019
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) Advanced Immunotherapy Studies Against Potential BW Viral Agents

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	0.0	235
c. TYPE	d. AMOUNT	87	2.0	265
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Jahrling, P B
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Lassa Virus; (U) Viral Diseases; (U) Lab Animals; (U) Monkeys; (U) Guinea Pigs; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

23. (U) To select, acquire, and test immune plasma and globulin fractions for protective efficacy and safety in prophylaxis and therapy of hemorrhagic fever virus infections that pose special problems for U.S. Forces sent to those areas where these diseases are endemic.

24. (U) Specific immune plasma is obtained by plasmapheresis from convalescent patients after naturally occurring infections with Lassa virus, Argentine hemorrhagic fever virus (Junin), and Ebola virus. Plasma units are tested by current blood bank procedures and for presence of protective (neutralizing) antibodies. Criteria are established for optimal therapeutic administration of the final products. Alternate strategies for acquiring high titered antibody are developed and tested.

25. (U) 8510 - 8609 Collection of Lassa virus (LV) convalescent plasma in Liberia (LIB) continued; among 497 plasma units collected from 86 donors, 407 (82%) had detectable LNI and 288 (58%) contained sufficient LNI to confer protection without further processing. From Sierra Leone (SL), no additional plasma was received to supplement the 191 low-titer units previously tested. All SL units and 407 LIB units were tested in a GLP-certified laboratory for HTLV-III antibodies; none were repeatedly positive. Six LIB and 3 SL donors were excluded for presence of hepatitis B surface antigen by RIA. Interpretation of indirect tests for non A-non B hepatitis is in progress. Efficacy testing of LIB IgG prepared by 5 chromatographic procedures was extended to in vivo testing in guinea pigs and monkeys. Minimal protective titers against LIB- and SL- LV strains were established. QAE chromatography yielded the most effective product. Removal of infectious HTLV-III by this process is being tested; if effective, the QAE process will be utilized to prepare a single lot of LIB IgG for human use. Parallel studies with Junin-immune plasma are in progress.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AG-019: Advanced Immunotherapy Studies Against Potential BW Viral Agents

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

BACKGROUND

Lassa fever is a viral disease of considerable public health importance in regions of West Africa, particularly Liberia, Sierra Leone, and Nigeria, where several thousand cases are believed to occur annually. While serological data suggest that subclinical cases may occur, the case-fatality ratios among hospitalized cases are still high, variously estimated at 14 to 22% in Sierra Leone and 13 to 14% in Liberia. To increase survival rates, passive immunization of acutely ill patients is employed frequently. One of many problems in evaluating plasma efficacy is the variable quality of the plasma infused. This study was designed to identify Lassa-convalescent patients to be recruited as plasma donors. Through this process, a pool of optimal donors was identified, and guidelines for identifying new donors were established. The availability of high-titer plasma has now facilitated the formal testing of passive immunization for treatment of Lassa fever.

SUMMARY

Collection of Lassa virus (LV)-convalescent plasma in Liberia (LIB) continued, and pilot lots of IgG were prepared from representative plasma units to determine the feasibility of this approach for treatment of Lassa fever. Among 497 plasma units collected from 86 donors, 407 (82%) contained detectable LNI and 288 (58%) contained sufficient LNI (>2.0) to confer protection without further processing. From Sierra Leone (SL), no additional plasma units were received to supplement the 191 low-titer units previously tested. All SL units and 407 of the LIB units were tested in a GLP-certified laboratory for HTLV-III antibodies; none was repeatedly positive. Six LIB and three SL donors were excluded because of the presence of hepatitis B surface antigen. Interpretation of indirect tests for non-A, non-B hepatitis (anti-core plus elevated SGPT) is in progress. IgG samples, prepared by five different procedures, were compared for protective efficacy and yield from 5 liters of LIB plasma. The method of choice appears to be aerosil precipitation followed by QAE chromatography. This process resulted in monomeric IgG in high yield (25 mg/ml, 79% efficiency). The LNI of the starting pool was 1.1 versus LIB-LV; the product contained IgG 1, 2, and 4, and the LNI titer versus LIB-LV was 3.2 and 2.3 versus SL-LV. Minimal protective doses of IgG were established in guinea pigs and confirmed in monkeys. In both animal models, 3.0 ml/kg were required to protect against LIB-LV and 6.0 ml/kg against SL-LV. Carboxymethylcellulose chromatography also yielded a product with similar LNI and protective

indexes; however, the yield was lower (48%), and no experience exists for safety of CM-products in human patients. Cohn-ethanol precipitation followed by pepsin disaggregation also yielded protective IgG, but in reduced yield; this method will be selected only if the aerosil/QAE method is shown to be inadequate for removal of adventitious agents. $F(ab')_2$ fragments were also prepared; this product retained no LNI and failed to protect guinea pigs treated with 12 ml/kg. Thus, despeciation of high-titer monkey plasma for treatment of human LF is not a viable option.

A survey of recently acquired Lassa viral isolates from Nigeria and plasma units from the same region confirm that the LNI are clearly higher when tested against the homologous LV strains; geographic matching of IgG with proposed study sites will be essential to treatment success. We are attempting to identify a source for reasonable quantities of LV-immune plasma from Nigeria for this purpose.

In comparative studies with Junin-immune plasma fractionated by QAE, CM, or treated to obtain $F(ab')_2$, both the QAE and CM products protected guinea pigs, while the $F(ab')_2$ did not, despite retaining neutralizing antibody activity. Thus, for Junin, elimination of infected cells may be more critical to recovery than neutralization of infectious virus.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA308926	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISS'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
86 03 20	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AL	020
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Development Studies on Immunomodulators/Enhancers

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 DEFENSE

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	3.0	322
87	1.0	569

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Anderson, A O
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Wood, O
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Friedlander, A M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Mice; (U)Vaccines;(U)Microorganisms;(U)Aerosols;(U)Lab. Animals;(U)Hamsters;(U)Guinea Pigs(U)RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Prophylactic vaccines, therapeutic agents, and immunomodulators developed to protect US troops against potential BW agents must be effective when the microorganism or toxin is presented in an aerosol. The objective is to determine the safety, efficacy, and dose response of prophylactics, therapeutics, and immunomodulators against an airborne challenge. Emphasis is on how effective products induce protection and why ineffective ones fail.

24. (U) Define and quantify the molecular aspects of clinical, pathological, and immunological changes that occur in vaccinated or treated animals when exposed to potential BW agents in aerosols. Apply advanced methodology to determine how a prophylactic or therapeutic induced protection; or, conversely to analyze why a product failed to instill protection.

25. (U) 8603 - 8609 The T_1 strain of Rift Valley fever virus (RVFV), isolated from mosquitoes, was used to immunize hamsters by intranasal and subcutaneous routes. Regardless of the route of administration, these hamsters were totally protected from lethal challenge with virulent RVFV. The immunomodulator, Avridine, produced modest (10-20% better survival than controls), nonspecific protection in C3H/HeJ mice when administered s.c. 21 days prior to challenge. However, single treatment with Avridine s.c. or intradermally significantly reduced mortality in the early stages of infection through day six postinfection. Avridine forms electrostatic interactions with protein antigens and inactivated vaccine virions when incorporated in lipid emulsions and liposomes. Seventy-five percent of all viruses were seen to be associated with Avridine-containing membranes in electron micrographs. During aerosol immunizations, Avridine appeared to be well tolerated by mice.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AL-020: Advanced Development Studies on Immunomodulators/Enhancers

PRINCIPAL INVESTIGATOR: A. O. Anderson, LTC, M.D.

ASSOCIATE INVESTIGATORS: O. Wood, Ph.D.
A. M. Friedlander, COL, M.D.

BACKGROUND

Prophylactic vaccines, therapeutic agents, and immunomodulators developed to protect U.S. troops against potential biological warfare agents must be effective when the microorganism or toxin is presented in an aerosol. Our objective is to determine the safety, efficacy, and dose response of prophylactics, therapeutics, and immunomodulators against an airborne challenge. The emphasis is on how effective products induce protection and why ineffective ones fail.

SUMMARY

Complete Freund's adjuvant, muramyl dipeptide in oil, and the lipoidal amine, Avridine, exhibit comparable adjuvant activities with regard to cellular and humoral immune responses to viral antigens when administered parenterally. These adjuvants increase lymphoid and mononuclear cell traffic in the local injection site and regional lymph nodes by effecting blood flow, transvascular chemotaxis, and angiogenesis of specialized, high-endothelial venules. These vascular alterations initiate the development of a lymphoid microenvironment in the local injection site by establishing lymphocyte recirculation and lodging of accessory cell types, such as Ia-antigen-positive, interdigitating, dendric cells and fibroblastic, reticular cells.

The mineral oil component (Pristane) of complete Freund's adjuvant is responsible for the spontaneous development of plasma cell tumors in most sublines of Balb/c mice when injected i.p. Balb/c Jax mice are resistant to plasma cell tumor development principally because the peritoneal lesions induced by Pristane are under immunoregulatory control by recirculating T-cells. Regression of inflammatory angiogenesis occurs concomitantly with differentation of high-endothelial venules in peritoneal granulomas of Balb/c Jax but not other Balb/c mice. Thus the induction of local lymphocyte recirculation provides strong regulatory signals which down-regulate tumor induction in addition to other favorable affects attributable to adjuvant activity.

The immunomodulator, Avridine, forms membrane-bound, lipid droplets and multi-lamellar liposomes when agitated with a 10% suspension of non-polar, soy bean lipids. The presence of Avridine in these structures favors the binding and surface display of protein

antigens and inactivated viral vaccine particles. In electron micrographs of vaccine/Avridine emulsions, > 75% of the intact virions were associated with Avridine-containing membranes. This association of antigenic proteins with Avridine may underlie its ability to potentiate specific immune responses. However, treatment of the emulsions with concentrations of hydrochloric acid normally found in the stomach resulted in dissociation of the viral particles from the lipid structures. This observation explains the inability of Avridine to potentiate responses after oral administration, while intraduodenal, intraperitoneal, or subcutaneous inoculations are effective.

In preparation for aerosol-challenge studies against a broad range of infectious agents of military relevance, the aerosol characteristics of the lipoidal amine immunomodulator, Avridine [N,N-dioctadecyl N',N'-bis (2-hydroxyethyl) propanediamine], were defined. No deleterious effects were noted in 80 C3H/HeJ mice, exposed to repeated doses of Avridine, upon subsequent aerosol infection by Rift Valley fever virus. These studies will provide important data on potential use of aerosol-disseminated immunomodulators as generic medical countermeasures against aerosol exposures to unrecognized or unrecognizable infectious agents.

PUBLICATIONS

1. **Gad, A. M., M. M. Hassan, S El Said, M. I. Moussa, and O. L. Wood.** 1986. Rift Valley fever virus transmission by different Egyptian mosquito species. Submitted to *Trans. Roy. Soc. Trop. Med. Hyg.*

2. **Rubin, D. H., M. A. Eaton, and A. O. Anderson.** 1986. Reovirus infection in adult mice: the virus hemagglutinin determines the site of intestinal disease. *Microbial. Pathogen.* 1:79-87.

PRESENTATIONS

1. **King, A. D., A. O. Anderson, and E. H. Stephenson.** 1986. Early pathogenesis of Venezuelan equine encephalitis virus: detection of specific adsorption of virus in brain tissue. Presented at the Army Science Conference, West Point, NY, June.

2. **Knauert, F, K., A. D. King, and B. D. Kelly.** 1986. An *in situ* hybridization assay detecting Rift Valley fever virus (RVFV) RNA in mouse liver sections. To be presented at the 35th annual meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA305650	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
86 03 20	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63763A	3M263763D807	AK	022
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Advanced Immunotherapy Studies Against Toxins

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	2.5	526
87	3.0	585

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Hewetson, J F
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7181
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Fricke, R F
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Mereish, K A

22. KEYWORDS (Precede EACH with Security Classification Code) (U) BW Defense; (U) Military Medicine; (U) Mycotoxins; (U) Lab Animals; (U) Mice; (U) Diagnosis; (U) RAI; (U) Monkeys

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop the ability to detect toxins in biologic fluids, to pursue studies on basic mechanism of action that directly relate to the development of potential therapy, and to develop prophylactic and/or post exposure therapy for soldiers exposed to toxins.

24. (U) Use immunoassays and develop alternate novel assays for detection of toxins and metabolites in biological specimens. Study basic toxicology and immunology including distribution, metabolism, and excretion in order to better develop therapeutic agents. Test a broad spectrum of agents both off the shelf and newly developed ones for prophylactic and/or post-exposure therapy, including drugs as well as vaccines and passive antibody transfer.

25. (U) 8603 - 8609 Low molecular weight toxins now being studied include saxitoxin, brevetoxin, and T-2. Good rabbit polyclonal antisera against saxitoxin is available and goat antisera is being produced. A suitable ELISA is in hand for saxitoxin and T-2, and an RIA for T-2 and brevetoxin. Metabolites of T-2 can be detected by immunoassay in organs of exposed animals. With radiolabeled brevetoxin, preliminary results indicate that most of the activity is excreted in the urine and feces. Purification and cross-reactivity studies indicate that this material does not cross react with anti-brevetoxin and therefore is probably metabolite. Steroidal, but not non-steroidal anti-inflammatory, agents were effective in decreasing the lethality of T-2 toxin. Glutathione prodrugs were assessed in an in vitro system for their ability to stimulate the synthesis of glutathione.

PROJECT NO. 3M263763D807: Industrial Base BW Vaccines/Drugs

WORK UNIT NO. 807-AK-022: Advanced Immunotherapy Studies Against Toxins

PRINCIPAL INVESTIGATOR: J. F. Hewetson, Ph.D.

ASSOCIATE INVESTIGATORS: R. F. Fricke, MAJ, Ph.D.
K. A. Mereish, CPT, Ph.D.
M. A. Poli, Ph.D.

BACKGROUND

Low molecular weight toxins which can lead to death or illness upon contact or ingestion are of interest to the Army because of their potential as biological warfare agents. The toxins that are being investigated include T-2 mycotoxin and its metabolites, saxitoxin and its derivatives, and brevetoxin. T-2 is a mycotoxin that occurs in moldy grain, causes alimentary toxic aleukia in humans and domestic animals, and has been implicated in biological warfare incidents in Southeast Asia and Afghanistan. Saxitoxin is a potent neurotoxin which acts on the sodium channel and has been responsible for many cases of paralytic shellfish poisoning. Brevetoxin is a polyether toxin which acts on the sodium channel at a different site than saxitoxin. It has been responsible for mass fish kills and human health problems. The need for rapid, reliable, and quantitative methods for detection of the low molecular weight toxins and the development of antidotes for treatment of the resulting toxicosis are well established. Because it does not use radioisotopes, methods available for detection include variations in the radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). The ELISA is most appropriate for field use. Both RIA and ELISA are available for T-2 and have been used for several studies. A saxitoxin ELISA is available but studies are continuing to improve and implement this assay for field use. An RIA, available for brevetoxin, has only limited use because of restricted availability of antisera. An ELISA for brevetoxin is not yet available. Possible methods of treatment which have been investigated for T-2 toxin are the glutathione prodrugs, anti-inflammatory glucocorticoids, intestinal adsorbing agents, anti-oxidants, and microsomal-inducing agents. Anti-T-2 antibodies have successfully reversed the toxic effects of T-2 intoxication. Likewise, anti-saxitoxin antibodies reverse the certain toxicity of saxitoxin. Adaptation of ELISA technology for toxin detection and defining effective therapies for treatment of toxicosis resulting from exposure are major goals of this work unit.

SUMMARY

Modifications of the ELISA for detection of saxitoxin are directed towards linking the toxin to solid matrices in different ways to increase the sensitivity. By using a polyacrylamide immunobead

with carboxylic functional groups, or a polystyrene bead with alkylamine functional groups, saxitoxin was efficiently coupled through bovine serum albumin (BSA) to the beads. This should produce an ELISA that will be more efficient for saxitoxin detection and for detection of antibody-producing clones.

Polyclonal antisera to saxitoxin was raised in rabbits and was effective for saxitoxin detection. This antisera will also be used for protection studies against saxitoxin exposure. Efforts to produce acceptable monoclones to saxitoxin have not yet been successful.

Studies on brevetoxin focused on detection of toxin or its metabolites in urine and feces of exposed rats by using radiolabeled toxin. Preliminary results show that 75% of the radioactivity was excreted in the feces and 14% in the urine over a 6-day period. Purification of this material on a silica gel 60 column or HPLC gave several peaks. Neither peak appeared to cross react with anti-brevetoxin serum, indicating that the peaks were probably metabolites. Toxicity studies, using death in Japanese madaka as an endpoint, gave an LD_{50} of 25 ng/ml for PbTx-2 and 75 ng/ml for PbTx-3. Subsequent investigations provided data showing that NaOH was the best method for brevetoxin decontamination in a laboratory setting. Incineration decomposed the toxin.

The efficacy of steroidal and non-steroidal anti-inflammatory agents on decreasing the lethality of T-2 toxin was evaluated in mice. Steroidal, but not non-steroidal, anti-inflammatory agents were all effective in decreasing the lethality of T-2 toxin. Dexamethasone was the most effective. In addition, when T-2 was given orally, followed immediately with activated charcoal, dexamethasone was 100% effective in preventing T-2 intoxication. As the time interval between exposure and charcoal treatment increased, the lethality from T-2 also increased. Glutathione prodrugs were assessed in an in vitro system for their ability to stimulate the synthesis of glutathione.

MIHA Exploratory Development of Detection and Treatment of Marine Toxin Poisoning

PRINCIPAL INVESTIGATOR: J. F. Hewetson, Ph.D.

Efforts to improve the methodology for detection of saxitoxin have focused on the production of monoclonal antibodies. These efforts have been largely unsuccessful, either because our assay for detection of antibody-producing clones is insensitive, or the number of antibody-producing clones is extremely low. Recent efforts were directed towards modifying the ELISA to detect saxitoxin linked in different ways to a solid matrix. Two kinds of derivatized beads were selected--a small (1μm), polyacrylamide immunobead with carboxylic functional groups and a large (3 mm) polystyrene bead with alkylamine functional groups. Saxitoxin has been coupled to each of these solid matrices alone and through a BSA link. With the BSA, there was excellent coupling efficiency to both kinds of beads. Current work is confirming the use of these beads for an ELISA. Success in this project should provide an assay that will be more sensitive for

detection of antibody-producing clones. In collaboration with Dr. Bert Ligderding, we are investigating protocols to maximize the immune response to saxitoxin.

Because of the lack of success in producing an acceptable monoclone for saxitoxin detection, efforts are underway to produce large amounts of affinity-purified, anti-saxitoxin antibodies which will satisfy the needs for multiple reproducible assays. Rabbits have been successfully immunized and are producing antibodies that can detect saxitoxin at levels of 10 ng/ml. Current studies will determine the cross reactivity among derivatives of saxitoxin. In addition, this antisera may provide a potential antidote for saxitoxin intoxication.

Efforts to repeat protection studies against T-2 intoxication with the 15H6 and 12C12 monoclonal antibodies have been unsuccessful. Studies on T-2 protection continue with the affinity-purification of goat polyclonal, anti-T-2 antibody. The elution of active antibody from an amino hexose sepharose column has presented a problem, since it is tightly bound; only high concentrations of 6 M guanidine will elute the antibody. A preparative column is now being prepared and this affinity-purified, anti-T-2 antibody will be used for protection studies.

MIHA Exploratory Development of Detection and Treatment of Marine Toxin Poisoning

ASSOCIATE INVESTIGATOR: R. F. Fricke, MAJ, Ph.D

The efficacy of steroidal and non-steroidal, anti-inflammatory agents on decreasing the lethality of T-2 toxin was evaluated in mice. Non-steroidal, anti-inflammatory agents: indomethacin (10 mg/kg, i.p.) and phenylbutazone (100 mg/kg, i.p.) potentiated (relative potency < 1.0) the lethality of T-2 toxin with relative potency values of 0.53 ($p<.001$) and 0.72 ($p<.001$), respectively. Acetylsalicylic acid (300 mg/kg, i.p.) was ineffective (relative potency = 1.09). At equivalent dosages for glucocorticoid activity, steroidal, anti-inflammatory agents: dexamethasone (10 mg/kg, i.p.), prednisolone (53 mg/kg, i.p.), and hydrocortisone (266 mg/kg, i.p.) all decreased the lethality of T-2 toxin, yielding relative potency values of 1.63 ($p<.001$), 1.44 ($p<.01$), and 1.25 ($p<.05$), respectively. When injected at the same time as T-2, dexamethasone, at 0, 0.125, 1.25, and 12.5 mg/kg, gave LD_{50} values of 2.46, 2.77, 3.09, and 4.04 mg/kg, respectively. In summary, steroidal, but not non-steroidal, anti-inflammatory agents were all effective in decreasing the lethality of T-2 toxin. Of the steroids tested, dexamethasone was the most effective.

Activated charcoal is effective in decreasing the lethality of oral T-2 toxin. Mice were given T-2 toxin by gavage (5 mg/kg) followed by oral administration of saline (control) or activated charcoal (7 g/kg) at the same time as toxin dosing or after a 1-h delay. Percent lethalities at different observation times were 50% (24 h), 80% (48 h), and 90% (72 h) for controls; 0% (24 h), 20% (48

h), and 30% (72 h) for 1 h post treatment. Mice treated immediately with charcoal had 0% lethality at all of the time points.

The efficacy of 2-substituted derivatives of thiazolidine-4-carboxylate (glutathione prodrugs) was assessed in vitro by measuring the ability of primary cultures of rat hepatocytes to synthesize glutathione. The order of effectiveness (% of control) was n-propyl- (252%), methyl- (224%), ethyl- (220%), 4-pyridyl- (217%), glucosyl- (212%), oxo- (204%), pentyl- (174%), ribose- (173%), xylosyl- (165%), galactosyl (164%), mannosyl- (163%), phenyl- (161%), glyceraldehyde (130%), lyxosyl- (130%), N-acetylcysteine (125%), and arabinosyl (121%). In the presence of buthione sulfoxime, a potent inhibitor of glutathione synthesis, glutathione levels for all of the compounds tested did not differ significantly from control values, indicating that the drugs were acting as precursors to glutathione.

MIBA Exploratory Development of Detection and Treatment of Marine Toxin Poisoning

ASSOCIATE INVESTIGATOR: M. A. Poli, Ph.D.

Research efforts during the past year have been primarily centered on the development of a radioimmunoassay to monitor the metabolism and excretion of the brevetoxins (PbTx-2 and PbTx-3), a unique class of polyether neurotoxins produced by the Florida red tide dinoflagellate *Ptychodiscus brevis*. The antiserum currently in use is a goat polyclonal raised against a PbTx-3-bovine serum albumin conjugate by a research group in Miami, Florida, in 1983. This antiserum was characterized and determined to contain a high-affinity class of antibody with an apparent Kd of 0.5 nM and binding capacity of 20 pmoles/ml. Assay conditions have been optimized and standard curves derived for PbTx-2 and PbTx-3. Both toxins are recognized equally by the antiserum, with a detection limit of approximately 150 pg/ml.

Metabolism and excretion of the brevetoxins are being investigated by using a rat model. Over a 6-day period, tritiated PbTx-3 administered i.v. was excreted primarily in the feces (75%), with a smaller amount (14%) excreted in the urine. Chromatographic analyses of fecal extracts showed multiple peaks of radioactivity which appeared to differ from native PbTx3. These peaks are currently being purified by HPLC for structural and immunological analysis.

PbTx-3 has been conjugated to keyhole limpet hemocyanin and is now being used as an immunogen to raise high-affinity antibodies in rabbits.

Finally, a fish model (Japanese madaka, *Oryzias latipes*) was used to develop an institutional protocol for detoxification and disposal of brevetoxins. For routine decontamination of glassware and benchtops, a 10-min exposure to 0.1 N NaOH is the method of choice. For the disposal of exposed animals, incineration at temperatures in excess of 500°C for 10 min completely destroys the toxin.

MIBB Toxins: Efficacy of Prophylactic Agents vs Aerosol Challenge

PRINCIPAL INVESTIGATOR: E. H. Stephenson, COL, DVM

No progress is reported under this APC.

PUBLICATIONS

1. Davio, S. R., and J. F. Hewetson. 1985. Development of antisaxitoxin antibodies in BALB/c mice: antigen preparation and antibody detection. *Fed. Proc.* 44:5.

2. Hewetson, J. F., J. G. Pace, and J. E. Beheler. 1986. T-2 mycotoxin detection and quantitation in organs of exposed rats by an immunoassay technique. Submitted to *J. Assoc. Off. Anal. Chem.*

PRESENTATIONS

1. Fricke, R. F. 1986. Effect of T-2 toxin on activities of trans-stilbene oxide-induced hepatic microsomal enzymes. Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March.

2. Frick, R. F., and J. Jorge. 1986. Effect of monoethyl glutathione ester (GEE) on total intracellular GSH levels of acetaminophen (ApAp) and buthione sulfoxime (BSO)-treated rat hepatocytes. Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March.

3. Thompson, W. L., and R. F. Fricke. 1986. The effect of cellular glutathione (GSH) levels on cytotoxicity of T-2 mycotoxin (T-2) in rat hepatocytes. Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March.

4. Fricke, R. F., and J. Jorge. 1986. Protective effect of ascorbic acid in decreasing T-2 toxin-induced lethality in mice. Presented at the Annual Meeting of the Federation of the American Society for Experimental Biology, St. Louis, MO, April.

5. Hewetson, J. F., and J. E. Beheler. 1986. Monoclonal antibodies against saxitoxin inhibiting binding of ^{3}H-saxitoxin to the sodium channel. Presented at the 1st Annual Meeting of the International Society of Toxinology, American Branch. Tempe, AZ, May.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA3)17	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 85 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AC	001
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY 87 -01			

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Q Fever

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 04	89 01	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE — EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	2.0	131
87	1.0	352

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Williams, J C
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meadors, G F
MILITARY/CIVILIAN APPLICATION H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Q Fever (U) Coxiella burnetti; (U) Vaccines; (U) Medical Defense; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) *Coxiella burnetii*, the causative agent of Q fever, is considered by most experts to have those properties which make it a potential candidate for BW. The existing vaccine to protect US troops against this threat is reasonably effective, but causes sterile abscesses in previously sensitized individuals. This institute has a broad program to improve the current vaccine.

24. (U) This work unit is dedicated to the transfer of research-level vaccine production technology to conditions for pilot-scale processing of the Q fever vaccine. Definition of the pilot-scale conditions will permit the orderly production of large volume lots of vaccine.

25. (U) 8510-8609 - A total of 72 volunteers were evaluated for humoral, cell-mediated, and delayed hypersensitivity immune responses prior to vaccination with IND 160 vaccine. Of these, 46% were considered immune and were not vaccinated; the remaining individuals were vaccinated and showed no adverse effects. The pilot lot of chemically extracted *C. burnetii* has been produced. Pre-clinical evaluation will commence this autumn.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-AC-001: Vaccine, Q Fever

PRINCIPAL INVESTIGATOR: J. C. Williams, Ph. D.

ASSOCIATE INVESTIGATOR: G. F. Meadors, MAJ, M.D.

BACKGROUND

Coxiella burnetii, the causative agent of Q fever, is considered by most experts to have those properties which make it a potential candidate for biological warfare. The existing formaldehyde-inactivated, phase I vaccine is reasonable effective, but does exhibit some undesirable effects in previously sensitized individuals. A broad program is ongoing to develop an improved vaccine.

SUMMARY

A total of 72 individuals at risk of acquiring Q fever in the laboratory were evaluated by measurement of humoral antibody titer (ELISA), cell-mediated response (in vitro lymphocyte proliferation), and delayed hypersensitivity (skin test) prior to administration of the formaldehyde-inactivated, phase I vaccine (IND 610). The criteria used were that individuals positive by at least two of the tests would not be vaccinated. Vaccine was not given to 46% of the tested individuals. Of those receiving vaccine, no adverse reactions were noted. The responses of 200 volunteers, before and after vaccination, are being assimilated, whereby the appropriate tests for the evaluation of protective immunity can be determined. At present, there appears little if any correlation between skin test data and either humoral or cell-mediated immunity.

The pilot lot of the chloroform-methanol residue (CMR) vaccine for *C. burnetii* has been produced. Sterility tests and composition evaluations are ongoing. Pre-clinical testing will commence this autumn.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA303505	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	EA	005
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) Rapid ID and Diagnosis System

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 03	88 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	0.2	50
87	1.0	426

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR LeDuc, J W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meegan, J
MILITARY/CIVILIAN APPLICATION. H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Nuzum, E

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Vaccines; (U) Medical Defense; (U) Viral Diseases; (U) Immunological Reagents; (U) Antigens; (U) Rapid Diagnosis; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25 PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop, standardize, then conduct field tests of developed, rapid diagnosis assays. To support extramural contracts in rapid diagnosis. To assess availability and suitability of commercial reagents for use in rapid detection assays for viruses affecting U.S. military personnel.

24. (U) Employ immunoassay methods to develop and evaluate rapid assays for diagnosis of viral diseases of natural or BW threat to the military. When possible, test these assays at field laboratories using epidemiologically relevant samples. Develop and supply for extramural rapid diagnosis projects standardized reagents, safety-tested and efficacy-tested.

25. (U) 8510-8609 - Rapid diagnosis assays for Junin virus (Argentine hemorrhagic fever) were successfully field-tested in Argentina on samples stored from previous epidemics. Assays for Hantaan virus (Korean hemorrhagic fever) were successfully field-tested during a collaborative study of an outbreak in the People's Republic of China. The efficacy of antiviral drug therapy was monitored with these assays during the China study. Throughout the year reagents were supplied to extramural contractors developing rapid diagnostic assays. Monoclonal antibodies developed by contractors are being characterized for their usefulness in rapid assays of West Nile, Chagres, and certain respiratory viral diseases. Assays for leptospirosis are under development.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-EA-005: Rapid Identification and Diagnosis System

PRINCIPAL INVESTIGATOR: J. W. LeDuc, LTC, Ph.D.

ASSOCIATE INVESTIGATOR: J. M. Meegan, CDR, USN, Ph.D.
T. G. Ksiazek, LTC, DVM
R. R. Graham, MAJ, DVM
E. O. Nuzum, CPT, DVM
C. A. Rossi (DA Intern)

BACKGROUND

The objectives of this study are to develop rapid diagnostic assays for agents of biological warfare potential or agents of geographic importance; to conduct field tests of developed rapid diagnostic assays; to determine if commercially available reagents can be used for rapid diagnosis of common respiratory viruses; and to provide technical base support and specialized reagents for extramural contracts for development of rapid diagnostic assays against these agents. The main technology utilized is the enzyme immunoassay. Field tests are conducted in areas of the world where the viruses in question naturally occur and select populations are known to be at risk of disease. The overall purpose of this work unit is to transfer assays developed in the laboratory or commercial sector to field use and evaluate their utility under realistic conditions.

Assays are developed, optimized, and tested under "real life" conditions. In previous years, this has included transport of assay kits to OCONUS field sites in lesser developed countries for evaluation with human samples obtained from patients experiencing local endemic or epidemic diseases. In general, tests have proved to be very durable, fast, reproducible, and simple. However, sensitivity of antigen-detection assays must still be improved. All rapid diagnostic systems tested were enzyme-linked immunosorbent assays (ELISA), which afford the advantages of simplicity, speed, reliability, and sensitivity. Our main goal is to detect antigen in clinical or environmental samples, and these assays can accomplish this in 2 to 3 h. However, we recognize that the first indication that a disease is present might be an infected serviceman. Consequently, for each agent, an attempt is made to include a rapid antibody assay which can establish a diagnosis, even if the patient is sampled after the viremic (antigenemic) phase, during the early convalescent stages when development of virus-specific IgM antibody is in progress.

In previous years, successful evaluation of DoD assays was conducted in Southwest and Southeast Asia by using epidemiologically relevant samples. Assays to detect rapidly virus-specific IgM were

successful in detecting Rift Valley fever (RVF), Crimean-Congo hemorrhagic fever (CCHF), and Chikungunya (CHIK) viral infections. Most, but not all commercial reagents functioned well in laboratory detection of respiratory viruses, including influenza, parainfluenza, adeno, and respiratory syncytial viruses. This facilitated preparation of an assay for use with collaborators at Brooks Air Force Base in a global surveillance program of respiratory diseases among Air Force personnel.

Support of extramural rapid diagnosis contracts includes providing specific antibodies, inactivated antigens, scientific direction, and advice for use in development of new technologies for rapid diagnosis. Reagents or assays produced under these contracts are evaluated for sensitivity and specificity.

SUMMARY

Rapid enzyme immunoassays to detect circulating virus and IgG and IgM antibodies were developed for Junin virus, cause of Argentine hemorrhagic fever, and were evaluated in Argentina, where stored samples from previous epidemics were available. Antibody tests functioned well, although, unlike other diseases, patients infected with Junin do not develop significant IgM antibody titers. This complicates rapid diagnosis of this disease and will necessitate more detailed study of assays to detect viremia. Detection of IgG antibody was reliable and correlated with established assays. The assay was useful in rapidly determining antibody levels in potentially therapeutic doses of immune plasma. Additionally, the assays were employed to evaluate the protective immune response induced by an experimental Junin vaccine developed at USAMRIID.

Rapid diagnostic assays were developed for Hantaan virus, cause of Korean hemorrhagic fever, and evaluated in the People's Republic of China. Antibody tests were successful in rapidly diagnosing disease and were employed in an efficacy study of the antiviral drug, ribavirin. Further modifications to the assays and additional field testing are in progress.

A strong technical base was maintained to support DoD extramural contracts in rapid diagnosis. Support was provided in a number of areas, especially by supplying quality reagents for contractors. Whenever possible, suitable reagents were sought from collaborators; however, many reagents were prepared in-house. Reagents were collected, expanded, purified if needed, tested for reactivity, and shipped to appropriate contractors. Stocks of reagents were inventoried, distributed into useful aliquots, and stored under optimal temperature conditions.

PUBLICATIONS

1. Binn, L. N., W. H. Bancroft, S. M. Lemon, R. H. Marchwicki, J. W. LeDuc, C. J. Trahan, E. C. Staley, and C. M. Keenan. 1986. Preparation of a prototype inactivated hepatitis A virus vaccine from infected cell cultures. *J. Infect. Dis.* 153:749-756.

2. **LeDuc, J. W., A. Antoniadis, and K. Siampoulus.** 1986. Epidemiological investigations following an outbreak of hemorrhagic fever with renal syndrome in Greece. *Am. J. Trop. Med. Hyg.* 35:654-659.

3. **LeDuc, J. W., G. A. Smith, J. E. Childs, F. P. Pinheiro, J. I. Maiztegui, B. Niklasson, A. Antoniadis, D. M. Robinson, M. Khin, K. F. Shortridge, M. T. Wooster, M. R. Elwell, P. L. T. Ilbery, D. Koech, E. T. Rosa, and L. Rosen.** 1986. Global survey of antibody to Hantaan related viruses among peridomestic rodents. *Bull. World Health Organ.* 64:139-144.

4. **Trahan, C. J., J. W. LeDuc, E. C. Staley, L. N. Binn, R. H. Marchwicki, S. M. Lemon, C. M. Keenan, and W. H. Bancroft.** 1986. Oral infection of the owl monkey (*Aotus trivirgatus*) with hepatitis A virus. *Lab. Animal Sci.* (In Press).

5. **LeDuc, J. W.** 1986. Epidemiology of hantaan and related viruses. *Lab. Animal Sci.* (In Press).

6. **LeDuc, J. W., and F. P. Pinheiro.** 1986. Oropouche fever. *In* T.P. Monath (Ed.), Epidemiology of arthropod-borne viral diseases, CRC Press. (In Press).

7. **Pinheiro, F. P., and J. W. LeDuc.** 1986. Mayaro fever. *In* T.P Monath (Ed.), Epidemiology of arthropod-borne viral diseases, CRC Press. (In Press).

8. **Niklasson, B., and J. W. LeDuc.** 1986. Epidemiology of nephropathia epidemica in Sweden. *J. Infect. Dis.* (In Press).

9. **Keenan, C. M., S. M. Lemon, L. N. Binn, and J. W. LeDuc.** 1986. Hepatitis A infection in the owl monkey (*Aotus trivirgatus*). (In Press).

10. **Shortridge, K. F., H. W. Lee, J. W. LeDuc, T. W. Wong, G. W. Chau, and L. Rosen.** 1986. Serological evidence of Hantaan-related viruses in Hong Kong. *Trans. R. Soc. Trop. Med. Hyg.* (In Press).

11. **Childs, J. E., G. W. Korch, G. E. Glass, J. W. LeDuc, and K. V. Shah.** 1986. Epizootiology of *Hantavirus* infections in Baltimore: isolation of a virus from Norway rats and characteristics of infected rat populations. *American J. Epidemiol.* (In Press).

12. **Antoniadis, A., J.W. LeDuc, and S. Daniel-Alexiou.** Clinical and epidemiological aspects of hemorrhagic fever with renal syndrome (HFRS) in Greece. Submitted to *European J. Epidemiol.*

13. **Glass, G. E., J. E. Childs, G. W. Korch, and J. W. LeDuc.** Ecology and social interactions of sylvatic and commensal Norway rats (*Rattus norvegicus*) populations in Baltimore, Maryland U.S.A. Submitted to *J. Zool.* (London).

14. **Wood, O. L., J. M. Meegan, J. Morrill, and E. Stephenson.** Rift Valley fever. *In* Z. Dinter and B. Morein (Ed.), Viral Infections of Ruminants, Elsevier, Amsterdam, to be published 1986.

15. **Meegan, J. M., and C. Bailey.** Rift Valley fever. *In* T. Monath (Ed.), Epidemiology of Arthropod-borne viruses. CRC Press, Boco Ratan, Florida, to be published in 1986/1987.

PRESENTATIONS

1. **LeDuc, J. W.** 1986. Recent advances in the epidemiology and diagnosis of Hantaan and related viruses. Presented at the Annual Meeting of the American Society of Microbiology, March.

2. **LeDuc, J. W., and A. Antoniadis.** 1985. Epidemiological studies of hemorrhagic fever with renal syndrome in Greece. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, November.

3. **Meegan, J. M., C. MacDonald, K. McKee, and C. J. Peters.** 1985. Rapid diagnosis of sandfly fever virus infection by detection of circulating antigen in human serum. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, December.

4. **Huggins, J. W., C. Hsiang, T. M. Cosgriff, Z. Wu, J. M. Meegan, J. W. LeDuc, Z. Zhen, J. I. Smith, S. Ge, M. Guan, C. Wang, T. Zhang, G. Yuan, and X. Gui.** 1986. Double-blind, placebo-controlled clinical trial of ribavirin therapeutic efficacy in the treatment of epidemic hemorrhagic fever: open phase for dose setting. Presented at the IXth International Congress of Infectious and Parasitic Diseases, Munich, FRG, July.

5. **LeDuc, J.** 1986. Epidemiology of epidemic hemorrhagic fever caused by Hantaan virus. Presented at the Annual Meeting Chinese Medical Society, Wuhan, People's Republic of China, June.

6. **Meegan, J. M.** 1986. Recent advances in rapid diagnosis of viral infections. Presented at the Annual Meeting Chinese Medical Society, Wuhan, People's Republic of China, June.

7. **Canonico, P. G., P. H. Gibbs, J. W. Huggins, C. D. Linden, C. MacDonald, K. T. McKee, J. M. Meegan, J. Morrill, D. D. Oland, C. J. Peters, and L. W. Reed.** 1986. Ribavirin prophylaxis of sandfly fever - Sicilian infection in human volunteers. Presented at the Army Science Conference, June.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA305651	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AN	002
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY 87 -01			

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Advanced Development

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	1.0	299
87	1.0	37

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Cosgriff, T M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2997
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meadors, G F
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Vaccines; (U) Volunteers; (U) High Containment Medical Care; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) (1) Monitor immunizations of persons where work places them at risk of exposure to vaccine agents; enter data on safety and immunogenicity of experimental and licensed vaccines into computer data base. (2) Maintain facilities to transport and treat patients under conditions of total (P-4) biohazard containment to prevent the possible spread of highly pathogenic microorganisms/toxins to medical personnel or the environment.

24. (U) (1) Reactions to immunizations are carefully monitored to assess safety in humans. Immune responses are measured utilizing conventional serologic assays, skin testing, and lymphocyte studies. (2) Maximum containment facilities, are maintained in a state of readiness at all times to enable transport and treatment of persons suspected or known to be ill from highly pathogenic microorganisms/toxins.

25.(U) 8510 - 8609 (1) The computer program for entry and monitoring of immunization of at-risk personnel with experimental vaccines is now fully functional. This has greatly facilitated data analysis and made it easier to meet deadlines for FDA reporting requirements. (2) The maximum containment facilities in the Medical Division have undergone extensive renovation, new equipment has been acquired, and further improvements have been made in the ongoing training program of personnel assigned to the facilities.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-AN-002: Vaccine, Advanced Development

PRINCIPAL INVESTIGATOR: T. M. Cosgriff, LTC, M.D.

ASSOCIATE INVESTIGATOR: G. F. Meadors III, LTC, M.D.

BACKGROUND

In 1981, the Department of Occupational Medicine was formed within the Medical Division to provide a formal and closely supervised approach to managing the USAMRIID Special Immunizations Program. A physician was placed in charge. The purpose of this program is to administer both investigational and licensed vaccines to personnel at risk of exposure to selected infectious agents and to monitor safety and serological responses.

SUMMARY

During the last several years, this program has been completely computerized to allow for the most efficient data entry and analysis. As a result, annual reports to the Food and Drug Administration on experimental vaccines can now be prepared very quickly. Assessment of vaccine safety and efficacy and monitoring of individual participants in the special immunizations program, has been greatly facilitated.

From a scientific standpoint, the special immunizations program provides a unique opportunity to monitor individuals over long periods of time with regard to responses to immunizations. As the individuals monitored are at risk of exposure to vaccine agents, the data provide important evidence for vaccine efficacy and the correlation of immune responses to protection afforded by the vaccines. Review of such data on the Rift Valley fever vaccine indicates that it is protective and has established a target antibody response level.

COOPERATIVE STUDIES CONDUCTED IN THE MEDICAL DIVISION IN FY86

The Medical Division conducted several studies in collaboration with WRAIR investigators during the last year.

Transmission of malaria by mosquitoes infected from cultured *P. falciparum*: In this study, 616 volunteers developed parasitemia after infected mosquitoes were allowed to feed on their forearms. Sporozoites were demonstrated in the salivary glands of these mosquitoes.

Phase I study of a candidate dengue 3 vaccine: Immunization of two yellow fever-immune volunteers produced an illness

indistinguishable from classic dengue fever. Given this degree of reactogenicity, we do not feel that further studies on this vaccine are warranted.

Comparative bioavailability of three oral formulations of the investigational antimalarial halofantrine HCl. Preliminary analysis of the data from this study reveals no significant differences in the bioavailability of capsule, tablet, and liquid suspension formulations.

Relative pathogenicity of piliated and non-piliated N-gonorrhea: This study is still in progress. Results to date support the hypothesis that gonorrhea is caused by piliated organisms.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA305993	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	BA	004
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Ribavirin

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	0.2	78
c. TYPE	d. AMOUNT	87	1.0	135
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Canonico, P G
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2290
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Huggins, J W
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Ribavirin; (U) Antiviral Drug; (U) Sandfly Fever; (U) Virus; (U) Prophylaxis; (U) Treatment; (U) RAI; (U) Volunteers

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop the drug ribavirin as an antiviral for treatment of viral diseases of military importance.

24. (U) Perform clinical trials to evaluate efficacy and toxicity in accordance with regulatory requirements.

25. (U) 8510 - 8609 A prospective prophylactic clinical study in volunteers, evaluating the efficacy of ribavirin against Sandfly fever virus, was completed. Data were analyzed, and a 13-volume final report was submitted to the Food and Drug Administration.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-BA-004: Ribavirin

PRINCIPAL INVESTIGATOR: P. G. Canonico, Ph.D.

ASSOCIATE INVESTIGATOR: J. W. Huggins, Ph.D.

BACKGROUND

Although most viral diseases are not associated with high mortality and are self-limiting, certain disease outbreaks, such as the influenza pandemic of 1918-19, can be associated with great loss of life. Exotic viral diseases about which we know very little and with which we are presently unable to cope, occur in many areas of the world. Many viruses with the potential for inducing illness of high morbidity and mortality remain endemic in certain areas of the world; notable examples are Rift Valley fever, Lassa fever and Ebola in Africa; Argentinian and Bolivian hemorrhagic fevers in South America; and Korean hemorrhagic fever in Asia. Numerous other examples can easily be cited. Broad-spectrum antiviral agents would be welcome insurance against the threat of a virus outbreak during military operations.

A number of compounds have recently been approved by the Food and Drug Administration for the prevention or treatment of virus-induced diseases. These compounds have extremely narrow spectra of activity. In contrast, the new antiviral drug, ribavirin, appears to provide broad-spectrum activity. It is the purpose of this research to evaluate in humans the potential of ribavirin to prevent sandfly fever.

SUMMARY

A prospective, prophylactic clinical study in volunteers evaluating efficacy of ribavirin against Sandfly fever virus was completed. Data were analyzed and a 13-volume final report was submitted to the Food and Drug Administration.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA310380	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 86 04 01	4. KIND OF SUMMARY H. TERM	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750A809	DB	006
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP FY87- 01			

11. TITLE (Precede with Security Classification Code) Antibody, Lassa Fever

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 03	86 09	DA	C. In House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	0.1	3
87*	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Jahrling, P B
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) RAI; (U) Military Medicine; (U) Lassa Fever; (U) Antibody; (U) Chromatography; (U) HTLV-III

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To determine whether plasma fractionation by the process of aerosol precipitation followed by QAE chromatography removes or inactivates infectious HTLV-III virus.

24. (U) Human plasma certified to be free of both HTLV-III virus and antibodies will be deliberately contaminated by addition of concentrated, infectious HTLV-III virus. This infectious plasma will then be fractionated by the procedure contemplated for Lassa-immune globulin preparation by using aerosol and QAE chromatography to yield monomeric IgG. Intermediate fractions and the final product will be tested for the presence of infectious virus, and for viral antigens (p25 and gp120) by using competitive radioimmunoassays.

25. (U) 8604 - 8609 High-titer HTLV-III virus was seeded into human plasma free of HTLV-III antibody. This deliberately contaminated plasma was then fractionated by QAE chromatography to prepare monomeric IgG. At each step of the fractionation process, samples were tested for infectious HTLV virus by inoculation and observation of MT-2 cells. (MT-2 cell assays were performed by collaborating scientists at the California State Health Laboratories, Berkeley, CA). Preliminary results indicate that the "spiked" plasma titered >7 log/ml; aerosol precipitation did not effectively reduce infectivity, but the second step in the preferred procedure (QAE) reduced infectivity to undetectable titers. Modifications may not be necessary to insure a safe product. These preliminary data require confirmation by independent assays (antigen ELISA's, RIA, dot-blot hybridizations) before acceptability of the QAE process can be ascertained.

*Only a one time effort.

PROJECT NO. 3M463750A809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO. 809-DB-006: Antibody, Lassa Fever

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

BACKGROUND

The feasibility of preparing IgG by various chromatographic procedures for intravenous treatment of human Lassa fever has been demonstrated. However, there is concern that adventitious agents present in the original plasma pool might not be removed by processing, and thus might contaminate the final product. The viruses causing AIDS (HTLV-III, LAV, or HIV), hepatitis A, B, and non-A, non-B are of principal concern. This work unit is designed to determine the efficiencies with which various plasma fractionation methods, proposed for preparation of monomeric IgG, remove these agents. Primary emphasis is on the viruses for which reliable infectivity assays exist.

SUMMARY

High-titer HTLV-IIIb virus was seeded into human plasma free of HTLV-III antibody. This deliberately contaminated plasma was then fractionated by aerosil precipitation followed by QAE chromatography. Aliquots of each step in the procedure were stored at -70°C, and all are being tested for infectivity by inoculation of serial dilutions into MT-2 cells. Inoculated cells are being observed daily for a period of 2 weeks for development of syncytia and fluorescent foci. These observations are being conducted by collaborating scientists at the Viral and Rickettsial Diseases Laboratory at the California State Health Laboratories in Berkeley, CA. Modifications of the preferred QAE procedure were also tested; these included heating the plasma prior to fractionation, and pepsin disaggregation of IgG. Infectivity assays of these samples from the modified procedures will be deferred unless it is established that the standard procedure is unacceptable.

Preliminary results suggest that the unmodified procedure does adequately remove all infectious HTLV-III virus. The "spiked" plasma had a titer of >7 log/ml. Aerosil precipitation did not significantly reduce infectivity, but the first eluate from the QAE column, (containing the monomeric IgG) was totally devoid of infectivity. Virus was apparently removed, not by physical inactivation, but by partitioning (ie. by adsorption to the column). Some infectious residual virus was recoverable by acid (pH 4.5) elution of the column.

These preliminary data require confirmation by independent assays, including antigen capture ELISA's, RIA, and dot-blot hybridizations, before acceptability of the QAE process for removal of HTLV-III can be stated by assurance. The suitability of this method for removal of other adventitious agents remains to be determined.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBO
	DA311563	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
	A. NEW	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D809	AK	007
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP FY	87- 01		

11. TITLE (Precede with Security Classification Code) Vaccine, Chikungunya

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	CONT	DA	C. In-House

17. CONTRACT/GRANT	
a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	0.0	00
87	1.0	146

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Lupton, H W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7241
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Cole, F E, JR
MILITARY/CIVILIAN APPLICATION H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Ramsburg, H H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Vaccines; (U) Volunteers; (U) Chikungunya; (U) Clinical Trials; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop and test a live, attenuated chikungunya vaccine for prophylactic treatment of at-risk military personnel.

24. (U) Conduct preclinical tests to evaluate vaccine safety and efficacy. Conduct animal and/or in vitro studies to demonstrate cross protection against heterologous viruses. Design and conduct clinical trials and laboratory tests to evaluate vaccine safety and efficacy. A literature search has been done.

25. 8510-8609 - (U) Preclinical testing of the attenuated chikungunya vaccine (CHIK 181/clone 25) was completed. An Investigational New Drug submission (IND-2426) was prepared and filed with the Office of Biological Research and Review of the Food and Drug Administration. Protocols for Phase I clinical evaluations of the CHIK vaccine were approved and scheduled to commence during first quarter FY87. Master and production seeds and pilot lots of vaccine were produced by using Rift Valley fever strain ZH-548, attenuated by 12 passages in the presence of a mutagen. A monkey neurovirulence test is in progress with preliminary data showing the attenuated virus to be well tolerated by monkeys, while non-attenuated virus caused lethal infection.

PROJECT NO. 3M463750D809: Development of Drugs and Vaccines Against Diseases of BW Importance

WORK UNIT NO: 809-AK-007: Vaccine, Chikungunya

PRINCIPAL INVESTIGATOR: H. W. Lupton, COL, Ph.D.

ASSOCIATE INVESTIGATORS: F. E. Cole, Jr., Ph.D.
H. H. Ramsburg

BACKGROUND

Chikungunya (CHIK), an arthropod-borne alphavirus, produces epidemics of a dengue-like illness throughout Africa, Southeast Asia, the Western Pacific, and India. Despite their widespread geographic distribution, individual strains of CHIK are antigenically related, thereby allowing a vaccine to provide broad-spectrum protection against heterotypic CHIK viral strains as well as other antigenically related viruses, including O'nyong nyong, Mayaro, and Ross River. Using CHIK strain 15561, a southeast Asian (human) isolate from a mild case of CHIK fever, we developed an attenuated vaccine seed strain (CHIK 181/clone 25) by a series of plaque-to-plaque passages in certified MRC-5 cells. Master and production seeds and vaccine have been produced and preclinically tested in compliance with both the Good Laboratory Practices Regulations and the Good Manufacturing Practices Regulations.

Chikungunya virus was confined to sub-Saharan Africa, rarely causing serious illness in man, until 1975, when deaths occurred in South Africa. In 1977, the virus spread to Egypt, where an increased incidence of lethal human disease made it evident that there was a need for a more effective vaccine. In 1982, USAMRIID and the University of Alabama, Birmingham, investigators received a grant from the U.S./Israel BARD Fund for initiation of a vaccine program at USAMRIID to develop a live, attenuated vaccine by serial passages of the virus in the presence of mutagenic chemicals. Preclinical testing has demonstrated that this viral strain is attenuated in multiple animals species.

SUMMARY

Live, attenuated chikungunya (CHIK) vaccine (CHIK 181/clone 25) has been produced at The Salk Institute in accordance with the Good Manufacturing Practices Regulations. Preclinical tests, including those for monkey neurovirulence and viral adventitious agents, have been completed in compliance with the Good Laboratory Practices Regulations. Preclinical data were documented in an Investigational New Drug submission (IND 2426). Phase I clinical evaluations of the vaccine will commence during first quarter FY87 in accordance with a protocol approved by The Surgeon General's HSRRB.

Strain ZH-548 RVF virus has been used as starting material to develop an attenuated vaccine by mutagenesis for potential use in livestock and humans. Preclinical testing demonstrated that the vaccine is attenuated for mice, hamsters, and rhesus monkeys; induces protection in sheep; and is not abortigenic. Master and production seeds and pilot lots of vaccine were produced at The Salk Institute. A monkey neurovirulence test is in progress with preliminary data showing the attenuated virus was tolerated by the monkeys, while non-attenuated virus caused lethal infection.

PUBLICATIONS

1. **Levitt, N. H., H. H. Ramsburg, S. E. Hasty, P. M. Repik, F. E. Cole, Jr., and H. W. Lupton.** 1986. Development of an attenuated strain of Chikungunya virus for use in vaccine production. *Vaccine* 4:157-162.

PRESENTATIONS

1. **Levitt, N. H., H. H. Ramsburg, S. E. Hasty, P. M. Repik, F. E. Cole, Jr., and H. W. Lupton.** 1986. Development, production, and preclinical testing of a live, attenuated Chikungunya virus vaccine. Presented at the 86th Annual Meeting of the American Society for Microbiology, Washington, DC, March.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA305652	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	64758A	3S464758D847	AN	002
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY-87 -01			

11. TITLE (Precede with Security Classification Code) (U) Vaccine, Advanced Development

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	1.0	350
87	1.0	1

19. RESPONSIBLE DOD ORGANIZATION

a. NAME USA Medical Research Institute of Infectious Diseases

b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011

c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L

d. TELEPHONE NUMBER (include area code) 301-663-2833

20. PERFORMING ORGANIZATION

a. NAME Medical Division, USAMRIID

b. ADDRESS Fort Detrick, MD 21701-5011

c. NAME OF PRINCIPAL INVESTIGATOR Cosgriff, T M

d. TELEPHONE NUMBER (include area code) 301-663-2997

f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meadors, G F

g. NAME OF ASSOCIATE INVESTIGATOR (if available)

21. GENERAL USE FINA

MILITARY/CIVILIAN APPLICATION. H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) RAI (U) Vaccines; (U) Volunteers; (U) Phase I, Phase II, and Phase III Clinical Trials

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Conduct Phase I (safety and tolerance), Phase II (efficacy), and Phase III (field trials) testing of experimental vaccines for prophylaxis of diseases of unique military importance, particularly those with potential as biological warfare threats.

24. (U) Experimental vaccines which have undergone immunogenicity and safety testing in preclinical studies will be studied in rigorous clinical trials after extensive scientific and ethical reviews.

25. (U) 8510 - 8609 Studies have been conducted on Rift Valley fever, Q-fever, and western equine encephalitis vaccines. Results in each case indicate that the vaccines are safe and immunogenic. A protocol for initial testing of a chikungunya vaccine has been approved. Studies will begin in October 1986.

PROJECT NO. 3S464758D847: Medical Defense Against Diseases of BW Importance

WORK UNIT NO. 847-AN-002: Vaccine, Advanced Development

PRINCIPAL INVESTIGATOR: T. M. Cosgriff, LTC, M.D.

ASSOCIATE INVESTIGATOR: G. F. Meadors, MAJ, M.D.

BACKGROUND

As part of the task of developing vaccines to meet the biowarfare threat, Phase I and Phase II clinical trials of candidate vaccines are undertaken by the Medical Division. If these trials demonstrate that a vaccine is safe and immunogenic, Phase III studies are conducted in larger numbers of volunteers, and when possible, in endemic areas.

SUMMARY

Rift Valley Fever Vaccine During fiscal year 1986, lots 19 and 20 of Rift Valley fever (RVF) vaccine, inactivated, freeze-dried, TSI-GSD-200, underwent initial safety and immunogenicity testing in 10 volunteers. Immunization resulted in no adverse reactions and all recipients developed titers which were judged protective. This study is complete except for gathering the final titers on the vaccinees.

An intralot comparison trial of the RVF vaccine was also begun in at-risk personnel. So far, a total of 26 volunteers have participated in this study, and eight lots have been tested. No meaningful comparisons are possible at present, but no adverse reactions have been observed, and all recipients have developed titers which were judged protective.

Q-fever vaccine During fiscal year 1986, Q-fever vaccine, inactivated, freeze-dried, NDBR 105, underwent evaluation in 72 volunteers under a new protocol. Immunization resulted in no adverse reactions. There appears to be no correlation between skin-test data and either humoral or cellular immunity, as measured by an ELISA assay and lymphocyte transformation.

Western equine encephalitis vaccine During fiscal year 1986, western equine encephalomyelitis vaccine, inactivated, freeze-dried, TSI-GSD-210, underwent initial evaluation in 25 volunteers. Two dosage regimens were used (two or three doses) on a randomized basis. Immunization resulted in no adverse reactions and all recipients developed titers which were judged protective.

Chikungunya vaccine During fiscal year 1986, a clinical protocol for the initial evaluation of chikungunya vaccine, live, attenuated, dried, TSI-GSD-218, was written and approved. The first four

volunteers were recruited, evaluated, and accepted into the study, which will begin in October 1986.

MB50 High Containment Facilities

PRINCIPAL INVESTIGATOR: T. M. Cosgriff, LTC, M.D.

ASSOCIATE INVESTIGATORS: G. F. Meadors III, LTC, M.D.
D. M. Driscoll, R.N.
K. E. Wilson, R.N., M.S.

During the last year, the high-containment clinical isolation unit was renovated and new equipment installed. This includes state-of-the-art patient-monitoring devices, as well as new anesthesia and operating room equipment. The capabilities of the High Containment Clinical Laboratory were also upgraded by the addition of new instrumentation. The medical staff assigned to operate the high-containment facilities continue to accrue valuable experience in functioning under the limitations imposed by high-containment conditions. Both regular training exercises and management of actual cases contribute to this experience.

The aeromedical isolation team has also become highly experienced during the last several years in the transport of patients suspected to have illness caused by highly dangerous pathogens. This team maintains its readiness by regularly scheduled training with the Air National Guard.

PUBLICATIONS

1. Meadors, G. F. III, P. H. Gibbs, and C. J. Peters. 1986. Evaluation of a new Rift Valley fever vaccine: safety and immunogenicity trials. *Vaccine* 4:179-184.

PRESENTATIONS

1. Driscoll, D. M., and J. D. Mims. 1985. High hazard disease containment and evaluation. Presented at the 8th Medical Symposium of the 102nd ARDOM, Kansas City, MO, October.

2. Driscoll, D. M., and J. D. Mims. 1985. Containment and transportation of patients with highly contagious infectious disease. Presented at the Annual Health Professionals Conference, Uniformed Services University of the Health Sciences, Bethesda, MD, October.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA308927	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	64758A	3S464758D847	BA	003
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY 87 -01			

11. TITLE (Precede with Security Classification Code) (U) Ribavirin

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 04	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	0.2	275
87	1.0	827

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Canonico, P G
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2290
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Huggins, J W
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Linden, C D

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Antiviral; (U) Ribavirin; (U) Chemotherapy; (U) Junin Virus; (U) Hantaan Virus; (U) Hemorrhagic Fever with Renal Syndrome; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Develop the drug ribavirin as an antiviral for the treatment of viral diseases of military importance.

24. (U) Conduct Phase III clinical trials appropriate for the development and FDA approval of ribavirin for the prophylaxis or treatment of serious viral infections. Establish liaison with medical authorities in appropriate areas to study the diseases. Design clinical trials and obtain appropriate U S and host country study clearances for conducting human trials. Conduct clinical trials to evaluate efficacy and toxicity in accordance with regulatory requirements.

25. (U) 8510 - 8609 A clinical trial of the efficacy of ribavirin in treating clinically ill patients with the Chinese variant of hemorrhagic fever with renal syndrome (HFRS) was initiated at Hubei Medical College, Wuhan, People's Republic of China. During the end of the 1985-86 season, sixty-nine patients were treated, of which 56 were serologically confirmed. Analysis of summary disease parameters by high-risk analysis shows treatment effects to be more pronounced in the more severe cases. Preparations for conducting a second season of the trial, to include 220 additional patients, were also completed.

PROJECT NO. 3S464758D847: Medical Defense Against Diseases of BW Importance

WORK UNIT NO. 847-BA-003: Ribavirin

PRINCIPAL INVESTIGATOR: P. G. Canonico, Ph.D.

ASSOCIATE INVESTIGATOR: J. W. Huggins, Ph.D.
C. D. Linden, Ph.D.

BACKGROUND

Ribavirin (1-β-D-ribofuranosyl-1,2,3-triazole-3-carboxamide), a nucleoside analogue with a close structural resemblance to guanosine, has been found to significantly inhibit a broad spectrum of both DNA and RNA viruses.

Ribavirin is the only antiviral agent which has been shown to have efficacy in the therapy of respiratory syncytial viral infection. Other clinical studies have shown that ribavirin is effective in the treatment of Lassa fever in man. In these studies, patients with an admission viremia of $\geq 10^{5.6}$ TCID/ml were found to have a $\geq$ 73% case fatality. Treatment with intravenous ribavirin within the first 6 days of illness reduced the mortality rate to 8%, compared to 43% in those treated after day 6.

This work unit expands these clinical trials by examining the efficacy of ribavirin in two other militarily relevant viral diseases, hemorrhagic fever with renal syndrome and Argentine hemorrhagic fever.

SUMMARY

Geographically diverse but clinically similar human diseases with the clinical "triad" of fever, hemorrhage, and renal damage are known collectively as hemorrhagic fever with renal syndrome (HFRS).

Because the antiviral drug ribavirin has demonstrated a significant antiviral activity against 13 viral isolates of HFRS in cell culture and is protective in a suckling mouse model, a field trial in Wuhan, People's Republic of China, was conducted jointly by USAMRIID and the Hubei Medical College.

Six patients were initially treated in an open pharmacokinetics phase to assess any disease-specific side effects in patients with renal insufficiency and to determine ribavirin blood levels. No unexpected drug-related side effects were seen. The only effect was the known anemia produced by ribavirin. The double-blind portion, designed for statistical reasons to treat 200 patients, was initiated late in the season. Sixty-nine patients were treated during this period; 56 were serologically confirmed. USAMRIID personnel participated in all areas of the study except direct patient care,

including extensive monitoring of laboratory tests and data collection. Patients were stratified by disease severity upon admission into two groups; 30 were mild and 26 more severe. All data were entered into a computer database and analysis of summary disease parameters by risk analysis showed that the treatment effect appeared to be more pronounced in the more severe group. The duration of proteinuria (spot urine and 24-h urine protein) was significantly reduced (p=.03) in ribavirin-treated patients, and odds ratio analysis strongly suggests that, with larger sample sizes, a positive treatment effect on duration of hypotension (both systolic and pulse pressure) and oliguria will be seen.

Preparations for conducting a second season of the trial, to include 220 additional patients, during October through January have been completed, including resupply of reagents and expendable. Analysis of the study is scheduled to occur in February 1987 to allow for filing of the data in support of an NDA.

PRESENTATIONS

1. **Huggins, J. W., C. Hsiang, T. M. Cosgriff, Z. Wu, J. M. Meegan, J. W. LeDuc, Z. Zhen, J. I. Smith, S. Ge, M. Guan, C. Wang, T. Zhang, G. Yuan, and X. Gui.** 1986. Double-blind, placebo-controlled clinical trial of ribavirin therapeutic efficacy in the treatment of epidemic hemorrhagic fever: open phase for dose setting. Presented at the IXth International Congress of Infections and Parasitic Diseases, Munich, FRG, July.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA302626	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
86 03 20	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AP	131
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Risk Assessment and Evaluation of Viral Agents and Their Vectors

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE — EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	1.0	140
87	1.0	100

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Bailey, C L
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2775
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Linthicum, K J
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Turell, M J

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases (U) Arthropod Transmission; (U) Entomology; (U) RAI; (U) Lab Animals (U)Gerbils

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Identify arthropods and vertebrates associated with the maintenance and transmission of medically important arboviruses to man and define ecologic and environmental factors influencing the ability of arthropods to transmit viruses. Information will allow for development of methods for predicting relative risk of military personnel to arbovirus infections and for designing specific vector control strategies.

24. (U) Ecologic and environmental factors relating to an arthropod's ability to transmit viruses are studied under natural and controlled environments.

25. (U) 8603 - 8609 Data from the advanced very high resolution radiometer on the National Oceanic and Atmospheric Administration's polar-orbiting meteorological satellites were correlated with a green vegetation index and used to predict Rift Valley fever (RVF) viral activity in Kenya. The high correlation between the satellite-derived green vegetation index and the ecological parameter of rainfall that is associated with RVF activity indicated that satellite data can serve as a forecasting tool for this disease in Kenya and, perhaps, in other areas of sub-Saharan Africa. The mosquito species, *Aedes lineatopennis*, which is believed to be the enzootic vector of this disease, was able to transmit this virus as early as 7 days after ingesting RVF virus from viremic animals in the laboratory.

U.S. G.P.O. 1984-421-646/17001

PROJECT NO. 3M162770A870: Risk Assessment, Prevention, and Treatment of Infectious Diseases

WORK UNIT NO. 870-AP-131: Risk Assessment and Evaluation of Viral Agents and Their Vectors

PRINCIPAL INVESTIGATOR: C. L. Bailey, COL, Ph.D

ASSOCIATE INVESTIGATORS: K. J. Linthicum, CPT, Ph.D
M. J. Turell, Ph.D

BACKGROUND

Previous research has incriminated floodwater *Aedes* mosquitoes, which breed in discrete, well-defined habitats known as dambos, as the endemic/enzootic vectors of Rift Valley fever virus (RVFV) in sub-Saharan Africa. Remote sensing technology has been suggested as a possible tool for predicting RVFV activity in areas where the virus is known to be endemic. Previous attempts to obtain information on the vector competence of the mosquitoes that are believed to be responsible for the disease have been hampered because of the unavailability of specimens.

SUMMARY

Data from the advanced very high resolution radiometer on board the National Oceanic and Atmospheric Administration's polar-orbiting, meteorological satellites have been used to infer ecological parameters associated with Rift Valley fever (RVF) viral activity in Kenya and to develop an indicator of potential viral activity. The indicator of potential viral activity was produced by weighting a green vegetation index derived from the satellite data for two different ecological regions in Kenya, where RVF is thought to be enzootic.

The satellite data were analyzed and correlated with rainfall, mosquito vector population levels, the flooding of mosquito vector breeding habitats, and isolation of virus from mosquito vectors from September 1985 to March 1985. The high correlation between the satellite-derived green vegetation index and the ecological parameters associated with RVFV indicated that satellite data can serve as a forecasting tool for RVF in Kenya and, perhaps, in other areas of sub-Saharan Africa.

Aedes lineatopennis is a known vector of RVFV in Kenya. Several thousand specimens of this species were collected as adults, assayed for presence of the virus, and tested for their ability to transmit virus. This species was able to transmit as early as 7 days after ingesting RVFV from a viremic hamster. The percentage of this species with a disseminated viral infection appeared to be relatively constant, regardless of the time since the infectious blood meal.

This is in contrast to results found for other species of *Aedes* tested, in which the percentage of mosquitoes with a disseminated viral infection increased with increasing extrinsic incubation.

PUBLICATIONS

1. **Turell, M. J., and C. L. Bailey.** 1986. Transmission studies in mosquitoes (Diptera:Culicidae) with disseminated Rift Valley fever virus infections. Submitted to *J. Med. Entomol.*

2. **Linthicum, K. J., F. G. Davies, A. Kairo, C. L. Bailey, H. F. Kaburia, and K. J. Lindquist.** 1986. Field ecological studies on Rift Valley fever virus. To be published in the *Proc. Ann. Mtg., Kenya Med. Res. Inst. and Kenya Trypanosomiasis Inst.*

3. **Turell, M. J., C. A. Rossi, and C. L. Bailey.** 1985. The effect of extrinsic incubation temperature on the ability of *Aedes taeniorhynchus* and *Culex pipiens* to transmit Rift Valley fever virus. *Am. J. Trop. Med. Hyg.* 34:1211-1218.

4. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and A. Kairo.** 1986. Observations on the dispersal and survival of a population of *Aedes lineatopennis* (Ludlow) (Diptera:Culicidae) in Kenya. *Bull. Entomol. Res.* 75:661-670.

5. **Turell, M. J., C. A. Rossi, R. F. Tamariello, Jr., and C. L. Bailey.** 1986. Reduced recovery of Rift Valley fever virus associated with assay of mosquito (Diptera:Culicidae) larval pools. *J. Med Entomol.* 23:416-422.

6. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker.** 1986. Prediction of Rift Valley fever virus activity in Kenya by satellite remote sensing imagery. Submitted to *Science*.

PRESENTATIONS

1. **Turell, M. J., and C. L. Bailey.** 1986. Effect of environmental temperature on the replication, dissemination, and transmission of Rift Valley fever virus by *Aedes fowleri*. To be presented at the 35th annual meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

2. **Turell, M. J., R. M. Tamariello, and C. L. Bailey.** 1986. Reduced recovery of Rift Valley fever virus associated with assay of larval pools. To be presented at the 35th annual meeting of The American Society for Tropical Medicine and Hygiene, Denver, CO, December.

3. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker.** 1986. Use of satellite remote sensing imagery to predict Rift Valley fever virus activity in Kenya. Presented at the Organization of African Unity Symposium on Viral Diseases in Africa, Nairobi, Kenya, June.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA302630	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 86 03 20	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AA	130
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Development Studies on Toxins of Biological origin for Development of Medical Defensive Countermeasures

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	9.0	350
c. TYPE	d. AMOUNT	87	2.0	559
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division. USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Friedlander A M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Young, H W
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Hines, H B

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Low Molecular Weight Toxins; (U) Rapid Detection; (U) Vaccines; (U) Lab Animals; (U)Mice; RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To define the respiratory toxicity of agents of potential BW importance; elucidate pathogenesis of intoxications induced by aerosols, to include determination of the sequence of events leading to protective immunity. Data obtained will provide the basis for evaluation of prophylactic and therapeutic regimens developed to protect deployed US forces.

24. (U) Develop animal models and define the clinical, pathological, and immunological changes during intoxications. Characterize immune defenses within the respiratory tract. Information is used to provide basis for efficacy of vaccination and therapy procedures.

25. (U) 8510-8609 - Research began on infrared spectrophotometry studies of saxitoxin, development of a solid phase extraction procedure for saxitoxin in urine, and HPLC analysis of brevetoxin metabolites. Final design of the nose-only aerosol exposure system was evaluated; performance goals were achieved, and a patent application was filed. To meet containment suite needs, the Collison nebulizer was modified for adaptation to a plastic globe, and a nonbreakable, plastic impinger was designed, fabricated, and characterized.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AA-130: Exploratory Development Studies on Toxins of Biological Origin for Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: A. M. Friedlander, COL, M.D.

ASSOCIATE INVESTIGATORS: J. L. Middlebrook, Ph.D.
H. B. Hines, Ph.D.
S. M. Naseem, Ph.D.
J. W. Dominik, B.S.
C. G. York, B.S.
H. W. Young

BACKGROUND

Any therapeutic drug or vaccine to protect against a potential BW agent must be effective when the agent is delivered by aerosol. Our investigations are designed to define the respiratory toxicity of potential agents, to identify commercially available equipment which will simplify the conduct of such studies, and to develop analytical chemistry methodologies for potential toxin threats.

SUMMARY

Research efforts have commenced on infrared spectrophotometry studies of saxitoxin, development of a solid-phase extraction procedure for saxitoxin in human urine, HPLC analyses of brevetoxin metabolites, and automated HPLC analyses.

The final design of the nose-only aerosol exposure system was received. An evaluation of the system, based on agreement between the experimental results and the initial characterization data, shows that the performance goals originally identified have been achieved. A patent application for the system has been filed through the office of the Judge Advocate General.

After appropriate modifications, the modified Hazelton H-1000 cabinet was used successfully in a P-4 containment suite. Performance of a collaborative study on aerosol transmissibility of Ebola virus was made possible by using the cabinet with a head-only monkey exposure system which had been designed in the Aerosol Engineering Technology Laboratory. Also, to meet the stringent safety requirements for working within a P-4 containment suite, the Collison nebulizer was modified for adaptation to a plastic globe, and a nonbreakable, plastic impinger was designed, fabricated, and characterized.

The compilation of an extensive data base on the performance of various available aerosol disseminator and sampling devices is ongoing; this will provide useful information in the continuing needs in support of airborne-induced toxemias.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA308918	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
86 03 20	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AD	131
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code)
(U) Exploratory Vaccine Development Studies on Conventional Agents of Potential BW Threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE — EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	2.0	455
87	8.0	2548

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Jahrling, P B
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Kenyon, R H
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Crumrine, M H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases; (U) Vaccines; (U) Lab Animals; (U) Guinea Pigs; (U) Monkeys; (U) RAI;(U)Rodents

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To isolate, study, and characterize agents of potential BW threat. To obtain immunogens that elicit protective immunity and to devise effective regimens to protect US military personnel in the field.

24. (U) Naturally occurring and laboratory-derived strains are molecularly and biologically characterized and assessed for virulence or attenuation in susceptible animal models. Attenuated strains are tested for ability to induce cross-reactions with virulent strains. Protective immunity elicited by inactivated antigens is determined.

25. (U) 8603 - 8609 Lassa virus (LV) RNA was prepared for cloning and sequencing, to facilitate construction of synthetic peptide and vaccinia-vectored LV glycoproteins for use as vaccines. Studies of the cellular basis for cross protection among Old-World arenaviruses were expanded to include additional viruses in guinea pigs and monkeys. For Junin virus, a lymphocyte transformation assay was developed which predicted protection more accurately than conventional serology. Priming of mucosal immunity was demonstrated to be important in protection of Rift Valley fever viral infections in C3H/HeJ mice. In anthrax studies, the protective antigen (PA) gene was cloned into *B. subtilis*; the clones effectively immunized rats and guinea pigs against challenge. Morphometric, electron microscopic analysis of *C. burnetti* phase 1 variants suggested that small variants differentiate to become large cell variants which are less resistant to physical stress; the basis is being investigated. Meloy Laboratories, Inc., continues to provide molecular biology and immunology support to this work unit on a task order, protocol-directed basis.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AD-131: Exploratory Vaccine Development Studies on Conventional Agents of Potential BW Threat

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

ASSOCIATE INVESTIGATORS: R. H. Kenyon, Ph.D.
M. H. Crumrine, LTC, Ph.D.
A. M. Friedlander, COL, M.D.
H. W. Lupton, COL, D.V.M.
A. O. Anderson, LTC, M.D.

BACKGROUND

Basic studies on the pathophysiology and immunology of conventional agents of potential BW threat are essential to the systematic development of vaccines to afford protection. Among the arenaviruses, four are significant human pathogens. Lassa virus causes severe, often fatal Lassa fever in tens of thousands of patients in West Africa annually. Junin and Machupo viruses are associated with Argentine and Bolivian hemorrhagic fevers, respectively, and have the documented potential to cause devastating outbreaks in South America. Lymphocytic choriomeningitis virus (LCMV) has a world-wide distribution, and has caused significant morbidity in human populations naturally exposed to aerosols from infected animals. All these viruses are highly infectious via the aerosol route and have the demonstrated potential to cause explosive outbreaks under artificial conditions. The aerosol potentials for anthrax and Q-fever (caused by *Coxiella burnetii*) are also well documented. An understanding of the protective immune mechanisms, especially mucosal and cellular immune responses responsible for recovery and protection against acute disease, are prerequisite to development of effective vaccines and therapeutic measures, and are the focal points for this research unit.

SUMMARY

Successful immunization of guinea pigs and monkeys against Lassa virus challenge was related to the eliciting of critical cellular immune responses. Cross protection among Old-world arenaviruses depended more on cross-reactive cytotoxic spleen cells than on humoral antibodies. Synthetic peptides, prepared as candidate vaccines for LCMV, (as a model for Lassa), elicited humoral immune responses but failed to elicit cell-mediated immunity (CMI) or protection. Efficacy testing of vaccinia constructs expressing LCMV structural proteins is in progress, and will be related to eliciting CMI responses. Lassa viral RNA has been prepared to facilitate construction of analogous synthetic peptides and Lassa-vaccinia hybrids as vaccine candidates. Characterization of newly isolated, naturally attenuated Lassa viral isolates is continuing. For Junin virus, cause of Argentine

hemorrhagic fever, a lymphocyte transformation (LT) assay was developed; preliminary data suggest that the assay is more sensitive than the neutralization test, and the LT assay is now routinely included in all Junin vaccine trials at USAMRIID. A critical role for antibody-dependent, cell-mediated cytolysis was also demonstrated as a determinant for Junin strain virulence in guinea pigs. Priming of mucosal immunity was shown to be important in protection against Rift Valley fever virus in C3H/HeJ mice; the mechanism is under investigation. Inadequate mucosal priming reduced or prevented aerosol-acquired encephalitis, but did not prevent hepatitis. In anthrax studies, the protective antigen (PA) gene was cloned into *Bacillus subtilis* BGSC1S53 with pUB110 as the vector. The clone effectively immunized rats against anthrax toxin challenge and guinea pigs against virulent anthrax spore challenge. Protection against anthrax was partially mediated by serum antibody, as determined by passive protection of recipient guinea pigs by serum from animals immunized with candidate anthrax vaccines. Morphometric, electron microscopic analysis of *C. burnetii* phase 1 variants suggested that small variants differentiate to become large variants which are less resistant to physical stress; the basis for this is being investigated. Meloy Laboratories, Inc., continues to provide molecular biology and immunology support on a task order, protocol-directed basis. Activities have included molecular cloning, DNA sequencing, plasmid DNA preparation, peptide synthesis, antigen conjugation, hybridoma development, and monoclonal antibody production.

MGLA Exploratory development of killed and attenuated Lassa virus vaccines

PRINCIPAL INVESTIGATOR: P. B. Jahrling, Ph.D.

The cross-protective relationships among Old-world arenaviruses were extended to include two additional, serologically distinct LCMV strains (UBC and ARM), plus two newly described viruses (Skukuza, from South African rodents, and IPPY, which may be identical with Mobala virus from the Central African Republic). Guinea pigs and monkeys immunized with any of these naturally attenuated strains resisted challenge with virulent reference Lassa strains from Liberia, Nigeria, and Sierra Leone; however, pre-challenge sera from these animals failed to neutralize challenge virus in vitro, and, in critical guinea pig tests, did not confer resistance to adaptively immunized animals. In contrast, immune spleen cells from donor guinea pigs immunized 14 to 21 days previously did confer protection to recipients, while immune cells obtained later did not. Cytotoxicity assays with infected ^{51}Cr-labeled, guinea pig kidney cells as targets and spleen cells from immunized guinea pigs confirmed the qualitative and temporal cross-protective relationships observed in the intact animals. Thus, generation of cytotoxic spleen cells appears to be critical to successful immunization against Lassa viral challenge.

The abilities of synthetic peptide vaccines to elicit protection and CMI responses in guinea pigs were explored by utilizing peptides, selected from potentially immunogenic regions of LCMV structural

proteins, mixed with adjuvant keyhold limpet hemocyanin. All immunized guinea pigs responded with antibody to the peptide immunogens, as detected in both ELISA and western blot assays, and to intact virus. However, to date, none has responded with cytotoxic spleen cells, and none has resisted challenge with LCMV strain WE. More combinations of peptides and adjuvants will be investigated, as will protective efficacies of genetically engineered vaccinia constructs containing coding sequences for LCMV structural proteins. In anticipation of success using these approaches, we have prepared Lassa RNA from a reference Liberian strain for the purpose of cloning and sequencing to facilitate design of synthetic peptides and vaccinia constructs in studies analogous to the LCMV studies described above.

MGLD Characteristics of virulent and attenuated Junin infections

PRINCIPAL INVESTIGATOR: R. H. Kenyon, Ph.D.

We are studying pathogenesis of Junin virus infection (Argentine hemorrhagic fever) and are evaluating treatments for the disease by using a guinea pig model. There are reports from Argentine clinicians that severe human AHF is accompanied by high-circulating interferon levels, possibly suggesting that interferon exacerbates clinical signs of the disease. We infected guinea pigs with a highly virulent or a moderately virulent strain of Junin and treated the infected animals with high doses of recombinant human alpha interferon for 14 or 21 days. There was no significant change in mortality, incubation period, or time-to-death between the treated and untreated animals. These data suggest that the high levels of interferon in the severe human cases probably is a response to a greater viral replication rather than a cause of more severe disease.

Accumulative experience in our laboratory suggests that cytolysis of infected cells (rather than or in concert with virus neutralization) may be the critical event in controlling Junin viral infection. For instance, we can detect ADCC (antibody dependent, cell-mediated cytolysis) as early as day 6 postinfection, but only in guinea pigs that abort infection. Complement (in the presence of antibody) may also play a role in elimination of infected cells. Cells infected with a virulent strain of Junin virus and then reacted with antibody appeared to be less susceptible to sensitization with complement than did cells infected with attenuated strains. This suggests that such a failure to sensitize cells infected with certain strains of virus adequately may contribute to their virulence.

Early human trials with the newly developed Junin vaccine suggested that neutralization assays and ELISA may not present an accurate picture of immune status. We developed a sensitive human lymphocyte transformation assay for Junin virus. Preliminary results showed that our test detected immunity before neutralization assays and as early as or before the ELISA. The LT procedure is now included with all Junin vaccine trials at the Institute.

We examined purified fractions of Junin-immune plasma as a possible method of standardizing treatment of human AHF. The $IgG_{1,2,4}$

and the $IgG_{1,2,3,4}$ fractions both protected infected guinea pigs. The $F(ab')_2$ fraction neutralized virus in vitro but failed to protect in vivo, supporting our hypothesis that elimination of infected cells is more critical in recovery than neutralization of virus.

MGBA Mucosal Immunity: Response to Aerosol Challenge with Toxins, Microbes, and Other Biologic Agents; Immunomodulators Mucosal Immunity

PRINCIPAL INVESTIGATOR: A. O. Anderson, LTC, M.D.

ASSOCIATE INVESTIGATOR: A. King, Ph.D.

Parenteral and/or enteric vaccination of mice with a mixture of inactivated Rift Valley fever vaccine and Avridine in liposomes provided significant protective efficacy against parenteral or aerosol challenge with virulent Rift Valley fever virus (RVFV) (ZH 501) 21 days after vaccination. Enteric vaccination completely prevented the development of encephalitis after aerosol challenge, while parenteral vaccination protected against the hepatic form of the disease. These studies have been useful in defining potential complications of high-dose aerosol exposure to RVFV, and provide some insight into prophylactic measures which should prevent serious encephalitis sequelae.

Mice were passively protected from both subcutaneous (98% survivors) and aerosol RVFV challenge (62% survivors) by a single intraperitoneal inoculation with monoclonal IgG_1 antibodies (MAb) which were specific for the G_2 capsular polypeptide of RVFV. Since only 10^{15} antibody molecules were administered per mouse 24 h prior to challenge, it is probable that protection was a result of combined passive and active immunity. When active systemic immunity was suppressed by mucosal tolerization, survival from challenge was less than what was seen when antibody was used alone.

Because mucosal priming in the gastrointestinal tract results in protection of mucosal sites, such as the lungs and conjunctiva, the traffic of "mucosa-committed" lymphoid cells is essential for the dissemination of protective immunity from the sites of initial antigenic exposure. We developed an in vitro assay of lymphoid cells "homing" to mucosal sites, using hybridoma cells expressing specific immunoglobulin isotypes and frozen sections of mucosal epithelium. IgA-expressing hybridoma cells selectively adhered to mucosal epithelial cells while hybridoma cells expressing other immunoglobulin isotypes either failed to adhere or adhered to nonmucosal structures. It is likely that these in vitro studies will be helpful in defining the cellular and molecular mechanisms which result in mucosal accumulation of s-IgA-secreting cells. Such information may be used in developing products which will hasten development of protective immunity of the conjunctiva, upper respiratory tract, and lungs through hastening the accumulation of IgA-committed cells at those sites.

MGDA Exploratory research for protection against anthrax

PRINCIPAL INVESTIGATOR: M. H. Crumrine, LTC, Ph.D.

ASSOCIATE INVESTIGATORS: J. W. Ezzell, Ph.D.
B. E. Ivins, Ph.D.

As previously reported by Dr. Ezzell, a 93-kd extractable protein (EA1) is the principal protein antigen recognized in guinea pigs vaccinated with live Sterne strain veterinary anthrax vaccines. A second protein, termed EA2 (62 kd), has been identified, and, like EA1, is not recognized by guinea pigs vaccinated with the human vaccine. EA1 appears to be a major surface protein and, to date, has been found to be soluble only in urea, guanidine-HCl, and sodium dodecyl sulfate, thereby making its purification difficult. The EA1 protein is not associated with either of the two *B. anthracis* plasmids, whereas EA2 is only detected in strains possessing the pX01 toxin plasmid. In collaborative studies with Dr. A. P. Phillips (from Porton Down, United Kingdom), antibody to EA1 has been shown to be of significant diagnostic value for identifying *B. anthracis* vegetative cells.

Immuno-electrophoretic analyses have indicated that protective antigen (PA) may be integrally associated with the cell surface. This finding has prompted studies to determine if the presentation of PA on the cell surface may be important in eliciting a protective immune response in animals vaccinated with live spore vaccines.

The galactose-N-acetylglucosamine polysaccharide of *B. anthracis* cell walls has been conjugated to bovine serum albumin. In vaccine trials, the animals developed titers to the polysaccharide, but were not protected against lethal challenge.

The following work has been carried out by Dr. Bruce Ivins in cooperation with Susan Welkos, Steve Little, John Ezzell, and Greg Knudson of the Bacteriology Division. The *B. anthracis* PA gene was cloned into *B. subtilis* BGSC 1S53 by using pUB110 as the vector. The two clones, PA1 and PA2, produce PA at levels equivalent to or greater than that produced by the parent *B. anthracis*, and can protectively immunize rats against anthrax toxin challenge and guinea pigs against virulent anthrax spore challenge, even when the spores come from a "vaccine-resistant" strain. These two clones constitute our first prototype, "new generation," live vaccines against anthrax.

Transposon Tn*916* mutagenesis was used to generate mutants of *B. anthracis* which are unable to synthesize either phenylalanine, or tyrosine, tryptophan and phenylalanine. These mutants will be tested as candidate live vaccines. We found that *B. anthracis* can donate (in a filter-mating system) Tn*916* to several other bacterial strains, suggesting that the transposon may be useful in generating mutants for other candidate bacterial vaccines.

Passive administration of serum from guinea pigs immunized with PA1, PA2, the Sterne veterinary vaccine, or the human chemical vaccine

protected or extended time to death in guinea pigs challenged 3 days later with virulent anthrax spores. These data strongly suggest that protection against anthrax is at least partially mediated by serum antibody.

MGBI *Coxiella burnetii* genetic and cellular aspects of pathogenesis and immunogenesis

PRINCIPAL INVESTIGATOR: A. M. Friedlander, COL, M.D.

ASSOCIATE INVESTIGATOR: T. McCaul, Ph.D.

Immunoelectron microscopy of monospecific and polyclonal antibodies against whole cells, chloroform-methanol residue (CMR), and lipopolysaccharide antigens and *Coxiella burnetii* phase I and II cells have been performed successfully. We have attempted use of colloidal gold as immunolabel and pre- and post-embedding with hydrophilic or hydrophobic media. We are investigating these various factors to provide the best possible technique for revealing surface determinants. We will attempt "fracture-label," based on fracturing the cell membranes along the apolar matrix into two monolayered structures that comprise the peripheral membrane proteins which are adsorbed at either surface. Preliminary studies have shown that all cell types harbour integral membrane particles. Freeze-fracture also can reveal the internal structure of the spore coat. Our preliminary studies indicate that the spore coat harbours similar membrane particles. Our future work will involve labeling these fractured membranes with poly- and monoclonal antibodies.

Because any population of *C. burnetii* contains morphologically variant cells, we performed electron microscopy on phase I cells cultivated in chicken yolk sacs and purified by two cycles of isopycnic renograffin gradient centrifugation. We used morphometric analysis with the MOP-VIDEOPLAN Image Analysis System to determine statistically the differences in the various sizes among each variant. The analytical data provided further evidence that the resistant, small cells retain their integrity during exposure under extreme environmental conditions which destroy larger cells. The analysis also shows that the large cell variants are derived from the small cell variants, and that once the small variants initiate differentiation, they become susceptible to physical stress. We have attempted to isolate cell walls and peptidoglycan protein complexes from each cell variant. Colloidal gold label and post-embedding techniques have revealed that all cells, regardless of the cell type, will cross-react with immunoglobulins raised against components of each cell type. These results reinforce the hypothesis that the large cell variants are differentiated from small cell variants.

MGIL Meloy Contract

PRINCIPAL INVESTIGATOR: H. W. Lupton, COL, D.V.M.

During the past year, Meloy Laboratories, Inc., has provided collaborative molecular biology and immunology research support for

USAMRIID investigators on a task-order, protocol-directed basis. Activities have included molecular cloning, DNA sequencing, plasmid DNA preparation, peptide synthesis, antigen conjugation, hybridoma development, and monoclonal antibody production.

Significant molecular biology support has been provided through DNA sequence data derived for *B. anthracis* PA (4.2Kb) and edema factor (3.5Kb), *C. burnetii* (1.9Kb), and two segments from Hantaan virus (2.9 and 2.6Kb). Further support has been provided by synthesis of Rift Valley fever virus peptides for use as diagnostic probes and for identification of essential antigenic epitopes. Extension of these studies has included attachment of peptides to carriers and immunization of rabbits to produce polyclonal antibodies. Additionally, polyclonal antibodies have been produced in rabbits against various botulinum toxins.

Hybridoma services have provided fusion experiments to produce hybridomas against cardiotoxin, bungarotoxin, taipoxin, diphtheria toxin, and notexin. Cell lines producing monoclonal antibodies have been cloned and mouse ascitic fluid produced and characterized.

PUBLICATIONS

1. **Kenyon, R. H., P. G. Canonico, D. E. Green, and C. J. Peters.** 1986. Effect of ribavirin and tributylribavirin on Argentine hemorrhagic fever (Junin virus) in guinea pigs. *Antimicrob. Agents. Chemother.* 29:521-523.

2. **Kenyon, R. H., and C. J. Peters.** 1986. Cytolysis of Junin infected target cells by immune guinea pig spleen cells. *Microb. Pathogen.* 1:453-464.

3. **Kenyon, R. H., K. T. McKee, J. I. Maiztegui, D. E. Green, and C. J. Peters.** 1986. Heterogeneity of Junin virus strains. *Med. Microbiol. Immunol.* 175:169-172.

4. **Liu, C. T., P. B. Jahrling, and C. J. Peters.** 1986. Evidence for the involvement of sulfidopeptide leukotriences in the pathogenesis of Pichinde virus infection in strain 13 guinea pigs. To be submitted to *Prostaglandins, Leukotriences Med.* (In Press).

5. **Kenyon, R. H., D. E. Green, G. A. Eddy, and C. J. Peters.** 1986. Treatment of Junin virus-infected guinea pigs with immune serum: development of late neurological disease. *J. Med. Virol.* 20:207-218.

6. **Peters, C. J., P. B. Jahrling, C. T. Liu, R. H. Kenyon, K. T. McKee, and J. G. Barrera Oro.** 1986. Experimental studies of arenaviral hemorrhagic fevers. To be published in *Curr. Topics Microbiol. Immunol.*

7. **Jahrling, P. B., and C. J. Peters.** 1986. Serology and virulence diversity among Old-World arenaviruses, and the relevance to vaccine development. *Med. Microbiol. Immunol.* 175:165-167.

8. **Liu, C. T., and B. S. Lowery.** 1986. Observations of the *in situ* contracting heart of guinea pigs infected with Pichinde virus. Submitted to *Milit. Med.*

PRESENTATIONS

1. **Kenyon, R. H., D. E. Green, and C. J. Peters.** 1986. Differences in Junin virus (JV) strains. Presented at the Annual Meeting of the American Society for Virology, Santa Barbara, CA, June.

2. **Kenyon, R. H., and C. J. Peters.** 1986. Immune response of guinea pigs to Junin virus (JV). To be presented at the 2nd Annual Congress of Virology, Cordoba, Argentina, October.

3. **Kenyon, R. H.** 1986. Complement in Junin virus infections. Presented at the Professional Staff Conference, USAMRIID, April.

4. **Kenyon, R. H., and C. J. Peters.** 1986. Actions of complement on Junin virus (JV) and on JV-infected cells. To be presented at the 2nd Annual Congress of Virology, Cordoba, Argentina October.

5. **Liu, C. T., R. P. Sanders, R. S. Dixon, and C. J. Peters.** 1985. Treatment with IV infusion of lactated Ringer's or human albumin solution in Pichinde virus-infected guinea pigs. Presented at the Annual Meeting of the American Physiology Society, Niagara Falls, NY, October.

6. **Liu, C. T., P. B. Jahrling, and C. J. Peters.** 1986. Evidence for the involvement of leukotriene in the pathogenesis of Pichinde virus infection. Presented at the Annual Meeting of the Federation of the American Society for Experimental Biology, St. Louis, MO, April.

7. **Liu, C. T.** 1985. Alterations of ECG in guinea pigs infected with Pichinde virus. Presented at the 30th International Congress of Physiological Sciences, Vancouver, Canada, November.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA302646	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AE	134
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Vaccine Development Studies on Toxins of Potential BW Threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	2.0	360
c. TYPE	d. AMOUNT	87	1.0	96
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Anderson, A D
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Meade, B
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense;(U, Vaccines (U) Cardiotoxin; (U) Lab Animals; (U) Rabbits; (U) Cobrotoxin; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To define the toxicity potential of natural toxins of BW importance and to develop vaccines, leading to protective immunity, that protect deployed military personnel from intoxication by natural toxins.

24. (U) Develop vaccines by chemical inactivation (toxoiding) or synthesize non-toxic peptides that mimic the protective antigenic determinants on the toxin molecule.

25. (U) 8602-8609 - Procedures were developed to purify cobrotoxin, erabutoxin b, four cardiotoxins, and the acidic phospholipase A_2 of *Naja naja atra*. Polyclonal rabbit antibodies to all toxins have been prepared and considerable progress has been made in developing an immunoassay for cardiotoxin detection in biological samples. Preliminary toxoiding studies indicate that formalin inactivation may be useful in vaccine development for some, but not all, cobrotoxins.

U.S. G.P.O. 1984-421-646/17001

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AE-134: Exploratory Vaccine Development Studies on Toxins of Potential BW Threat

PRINCIPAL INVESTIGATOR: A. O. Anderson, LTC, M.D.

ASSOCIATE INVESTIGATOR: B. Meade, Ph.D.

BACKGROUND

The goal of our research is the development of vaccines that protect from intoxications by natural toxins. The toxins of current interest are those from lapid snakes, specifically the cardiotoxins, the phospholipases A_2, and the post-synaptic neurotoxins, cobrotoxin of *Naja naja atra* and erabutoxin b of *Laticauda semifasciata*. Two complementary approaches are being taken. The first is the development of vaccines by the traditional approach of chemical inactivation, or toxoiding; the second is the chemical synthesis of non-toxic peptides that mimic protective antigenic determinates on the toxin molecule.

SUMMARY

The first phase required reagent preparation and assay development. We have developed or adapted procedures to purify cobrotoxin, erabutoxin b, and the four cardiotoxins and the acidic phospholipase A_2 of *N. n atra*. We have also prepared polyclonal antibodies to all the toxins and are preparing, with contract support, monoclonal antibodies to cobrotoxin, erabutoxin b, and cardiotoxin. Antibodies to these toxins can be detected in solid-phase immunoassays, and considerable progress has been made in developing an immunoassay to detect cardiotoxin and cobrotoxin in biological samples

Conditions have been determined under which cobrotoxin and cardiotoxin are inactivated by formalin. The resulting toxoids are immunogenic in mice, and lead to the development of antibodies that recognize native toxin in solid-phase immunoassay. Animals immunized with cobrotoxoid showed a moderate degree of resistance to challenge with a lethal dose of cobrotoxin; however, very few of the mice immunized with cardiotoxoid, including those with high antibody titers, survived a challenge with cardiotoxin. This suggests either that formalin destroyed important antigenic determinants or simply that insufficient antibodies were available at the challenge site to neutralize the toxin.

The post-synaptic neurotoxins, cobrotoxin and erabutoxin b, are being used as a model system to study the feasibility of synthetic peptide vaccines. Many algorithms have been proposed for predicting

antigenic sites in proteins; many require a knowledge of protein three-dimensional structure. Because the structure has been solved for erabutoxin b, the predictive procedures have been applied to this toxin, and peptides corresponding to the predicted antigenic determinants are being synthesized. Analogous peptides are being prepared for cobrotoxin, a similar toxin for which the crystal structure is not known. Experimental animals will be immunized with these peptides, and sera from these animals will be assayed to determine if antibodies have been produced which will react with and neutralize native toxin.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA302650	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 85 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AF	135
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code)
(U) Exploratory Immunotherapy Studies on Toxins of Potential BW Threat

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE | EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	7.0	2100
87	7.0	2071

19. RESPONSIBLE DOD ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L
d. TELEPHONE NUMBER (include area code) 301-663-2833

20. PERFORMING ORGANIZATION
a. NAME Pathophysiology Division, USAMRIID
b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF PRINCIPAL INVESTIGATOR Wannemacher, R W, JR
d. TELEPHONE NUMBER (include area code) 301-663-7181
f. NAME OF ASSOCIATE INVESTIGATOR (if available) Siegel, L S
g. NAME OF ASSOCIATE INVESTIGATOR (if available) Pace, J A

21. GENERAL USE FIC
MILITARY/CIVILIAN APPLICATION M

22. KEYWORDS (Precede EACH with Security Classification Code) (U)Military Medicine;(U)Low Molecular Weight; RAI Toxins;(U)T-2 Toxin;(U)Saxitoxin;(U)Rapid Detection;(U)Lab Animals;(U) Mice;(U)Therapy

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop the ability to detect toxins in biological samples; to study the mechanisms of action; and to develop and evaluate biologics and selected compounds for prevention and treatment of diseases induced by toxins of military importance.

24 (U) Develop new technology for fermenter-type production of sufficient toxin for isolation, purification, alteration, and detection studies; Use HPLC and mass spectrometry (MS) plus immunology to detect agents and their metabolites in biological fluids. Immunogenicity of various antigens will be utilized to make experimental toxoids/vaccines.

25. (U) 8510 - 8609 In vivo models have been developed for assessing toxicity, absorption, and pattern of excretion of T-2 metabolites after skin exposure. T-2 tetraol is the major metabolite in urine following dermal exposure. Aerosol exposure to T-2 mycotoxin in mice and rats results in a 1-2 log greater toxicity than by systemic administration. A poor correlation exists between in vitro inhibition of protein synthesis by T-2 metabolites and their toxicity in a mouse model. 3,4-Diaminopyridine significantly prolonged the survival of mice poisoned in type A botulinal toxin, but had no effect on survival from types B, C, E or F.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AF-135: Exploratory Immunotherapy Studies on Toxins of Potential BW Threat

PRINCIPAL INVESTIGATOR: R. W. Wannemacher Jr., Ph.D.

ASSOCIATE INVESTIGATORS: D. L. Bunner, COL, M.D.
C. B. Templeton, CPT, DVM, Ph.D.
D. A. Creasia, Ph.D.
J. G. Pace, Ph.D.
L. S. Siegel, Ph.D.
E. C. Hauer, M.S.
W. L. Thompson, M.S.

BACKGROUND

A number of low molecular weight toxins are potential biological warfare agents. In order to develop a medical defense against these toxins, we must study their toxicities, pathophysiologies, and pharmacokinetics. This information will be utilized to develop rational therapy and means of detection. Initial studies were done on a component of "yellow rain," namely the trichothecene mycotoxins. Many of the other potential agents are neurotoxins, such as saxitoxin, which blocks the sodium channel. Botulinum toxins block the release of the chemical neurotransmitter, acetylcholine, at the neuromuscular junction and thereby produces paralysis. Chemotherapy agents known to increase neurotransmission release, such as 3,4-diaminopyridine, may be useful in relieving toxin-induced paralysis.

SUMMARY

An efficient, in vivo, jacketed-barrier model has been developed in the rat, guinea pig, and rabbit for studies on rates of absorption, pharmacokinetics, and distribution of T-2 mycotoxin after dermal exposure. Toxicity and rate of penetration of this toxin through skin is a function of the delivery solvent (such as dimethylsulfoxide), area of exposed skin, and concentration of T-2 mycotoxin. T-2 mycotoxin is rapidly metabolized to hydroxylated derivatives or glucuronide conjugates, with T-2 tetraol being the major metabolite detected in urine.

Following aerosol exposure, T-2 mycotoxin is extremely toxic, with an LC_{50} comparable to that seen for many of the chemical agents. This toxin is 10 to 50 times more potent when inhaled as compared to systemic exposure. Inhalation of the T-2 mycotoxin does not effect respiratory function and is apparently rapidly absorbed through lung tissue into the circulation.

When injected systemically into the rat, T-2 mycotoxin and cycloheximide resulted in an early similar decrease in protein and a

DNA synthesis, which returned to near normal rates before the rats showed signs of terminal shock. In vitro studies in L-6 cells also documented the very rapid onset of inhibition of protein synthesis and uptake of amino acids, calcium, and 2-deoxyglucose. A poor correlation existed between the ability of a number of trichothecenes to inhibit in vitro rates of protein synthesis and their lethal effects in a mouse model. All of this raised a question as to whether inhibition of protein synthesis is the major mechanism of action responsible for the extreme toxicity of T-2 mycotoxin. Further, studies are continuing to evaluate the use of an in vitro cell model for screening potential therapeutic agents. New computer programs are being developed to help in evaluating these agents.

Treatment with 3,4-diaminopyridine did not significantly increase survival times of mice injected with type C botulinum toxin. The observation that 3,4-diaminopyridine is efficacious against type A botulinum toxicity but not against types B, C, E, or F suggests that the neurotoxins have different pharmacological activities.

MGHB Detection and Treatment of Mycotoxin Poisoning

PRINCIPAL INVESTIGATOR: R. W. Wannemacher, Jr., Ph.D.

ASSOCIATE INVESTIGATORS: D. L. Bunner, COL, M.D.
C. B. Templeton, CPT, DVM, Ph.D.
D. A. Creasia, Ph.D.
E. C. Hauer, M.S.

A jacketed-barrier model has been developed for in vivo exposure to toxins by the dermal route in rats, guinea pigs, and rabbits. By use of the jacketed-barrier model, 99% of a nonabsorbed dose of a radioactive chemical is maintained at the application site. We established that the rate of absorption of T-2 mycotoxin in dimethylsulfoxide from the skin is a function of both the area of exposure and the concentration of toxin.

We have completed studies on the acute response of mice and rats when exposed for 10 min to an aerosol of various concentrations of T-2 mycotoxin. The aerosol LC_{50} for T-2 mycotoxin was 350 and 200 mg $min^{-1}m^{-3}$, respectively, for mice and rats. Thus, the aerosol toxicity of T-2 mycotoxin is equivalent to that for nerve agents and a log more toxic than aerosolized mustard. From measurements of retained dose, we calculated that, by inhalation, T-2 mycotoxin is at least 10 times more toxic in the mouse and 50 to 100 times more toxic in the rat than if it were administered systemically. Mice cannulated via the larnyx so as to completely by-pass the upper respiratory tract are about twice as susceptible to the toxicity from inhaling T-2 aerosol as are the noncannulated mice, therefore eliminating deposition of the T-2 aerosol in the upper respiratory tract as a major component of the enhanced aerosol toxicity of T-2 mycotoxin. Rats exposed to T-2 toxin via aerosol did develop metabolic acidosis but had a normal blood PCO_2 and elevated PO_2. Thus, we concluded that inhaled toxin rapidly passed through the respiratory tract into the general circulation and did not reflect respiratory function.

In L-60 cells, T-2 mycotoxin had an EP_{50} of 4 ng/ml for protein synthesis inhibition (PSI). The effect on PSI was noted in less than a minute, as was reduced uptake of amino acids, calcium, and 2-deoxyglucose.

A number of computer programs have been written for the IBM Personal Computer, which will be of value in literature surveys, data acquisition and storage, and visual aids production.

MGGC Role of Microbial Toxins in Human Disease-Development of Growth and Purification Processes for the toxins of *Clostridium botulinum*

PRINCIPAL INVESTIGATOR: L. S. Siegel, Ph.D.

The effect of treatment with a candidate drug, 3,4-diaminopyridine (3,4-DAP), on the survival times of mice poisoned with type C botulinum toxin was evaluated. (We have previously demonstrated that therapy with 3,4-DAP significantly prolonged the survival of mice poisoned with type A botulinum toxin, but was not effective in the treatment of mice poisoned with types B, E, or F botulinum toxin).

At zero time, a group of mice (30 or more) were injected i.p. with 10, 20, or 40 LD_{50} of type C botulinum toxin. After 3 h, when the mice showed signs characteristic of botulism (ruffling of the fur and labored abdominal breathing), half of each group of mice were treated with 3,4-DAP. The drug was administered i.p. at a dose of 8 mg/kg, beginning at 3 h, and at hourly intervals thereafter. Mice were examined at 15-min intervals for survival. (Groups of mice receiving 3,4-DAP only were not affected by the injection of the drug). The pattern of survival of untreated versus treated groups was compared by using a computer statistical program.

Treatment with 3,4-DAP did not significantly increase the survival time of mice injected with type C botulinum toxin, at the concentrations of drug and toxin tested. The observation that 3,4-DAP is efficacious against type A botulinum toxin, but not against types B, C, E or F suggests that the neurotoxins have different pharmacological activities. These results also emphasize that extrapolating information obtained with one type of botulinum toxin to the other six types is unwarranted.

MGHG Pathogenesis of Low Molecular Weight Toxins

PRINCIPAL INVESTIGATOR: J. G. Pace, Ph.D.

ASSOCIATE INVESTIGATOR: W. L. Thompson, M.S.

In vivo distribution and metabolism studies of T-2 in dermally exposed guinea pigs, rats, and rabbits showed that dimethylsulfoxide as a delivery solvent increases the rate of penetration of the toxin. T-2 was rapidly metabolized to hydroxylated derivatives and glucuronide conjugates. T-2 tetraol was the major metabolite detected

in urine up to 14 days postexposure. In vitro skin-penetration studies (guinea pig) were in excellent agreement with in vivo data.

In vivo rat studies showed that T-2 and cycloheximide cause early, similar decreases in protein and DNA synthesis, followed by death many hours later. We observed recovery of protein and DNA synthesis with sublethal doses of mycotoxin.

T-2 non-specifically inhibits mitochondrial protein synthesis, a prokaryotic-like system, at levels slightly higher than the ED_{50} dose required to inhibit eukaryotic protein synthesis.

Metabolism of labeled T-2 was studied in Vero cells, rat spleen lymphocytes, chick embryo heart cells, rat intestinal segments, and rat hepatocytes. Metabolism of labeled verrucarol and verrucarin A was studied in rat hepatocytes.

In vitro liver perfusion studies showed that T-2 was rapidly and efficiently metabolized and cleared by the liver. Increased clearance was solely dependent on the rate of blood flow to this organ. Enzymes inducers had little effect on toxin clearance.

Glutathione stimulators and suppressors had no effect on T-2 mycotoxin cytotoxicity in rat hepatocytes.

An HPLC method for the detection and quantitation of mycotoxins in biological fluids was developed. The protein synthesis inhibition assay is being used to compare the antibody neutralization capability of several antibody preparations to their potency in a standard immunoassay.

PUBLICATIONS

1. Schmidt, J. J., and L. S. Siegel, 1986. Purification of type E botulinum neurotoxin by high-performance ion exchange chromatography. *Anal. Chem.* 156:213-219.

2. Thurman, J. D., D. A. Creasia, J. L. Quance, and A. J. Johnson. 1986. Adrenal cortical necrosis caused by T-2 mycotoxicosis in female, but not male, mice. *Am. J. Vet. Res.* 47:1122-1124.

3. Thompson, W. L., and R. W. Wannemacher, Jr. 1986. Structure-function relationships of 12,13-epoxytrichothecene mycotoxins in cell culture: comparison to whole-animal lethality. Submitted to *Toxicon*.

4. Cosgriff, T. M., D. L. Bunner, R. W. Wannemacher, Jr., L. A. Hodgson, and R. E. Dinterman. 1986. The hemostatic derangement produced by T-2 toxin in cynomolgus monkeys. *Toxicol. Appl. Pharmacol.* 82:532-539.

5. Pace, J. G. 1986. Metabolism and clearance of T-2 mycotoxin in perfused rat livers. *Fund. Appl. Toxicol.* 7:424-433.

6. Bunner, D. L., R. W. Wannemacher, H. A. Neufeld, C. R. Hassler, G. W. Parker, T. M. Cosgriff, and R. E. Dinterman. 1985. Pathophysiology of acute T-2 intoxication in the cynomolgus monkey and rat models, pp. 411-421. *In* J. Lacey (ed.), *Trichothecenes and other mycotoxins*, Chapter 37. John Wiley & Sons, Ltd., NY.

7. Wannemacher, R. W. Jr., D. L. Bunner, J. G. Pace, H. A. Neufeld, L. H. Brennecke, and R. E. Dinterman. 1985. Dermal toxicity of T-2 toxin in guinea pigs, rats, and cynomolgus monkeys, pp. 423-431. *In* J. Lacey (ed.), *Trichothecenes and other mycotoxins*. Chapter 38. John Wiley & Sons, Ltd., NY.

8. Siegel, L. S., A. D. Johnson-Winegar, and L. C. Sellin. 1986. Effect of 3,4-diaminopyridine on the survival of mice injected with botulinum neurotoxin type A, B, E, or F. *Toxicol. Appl. Pharmacol.* 84:255-263.

9. Creasia, D. A. 1986. Acute inhalation toxicity of T-2 mycotoxin in mice. To be submitted to *Toxicol. Appl. Pharmacol.* (In Press).

10. Kemppainen, B. W., R. T. Riley, J. G. Pace, and F. G. Hoerr. 1986. Effects of skin storage conditions and concentration of applied dose on [^{3}H]T-2 toxin penetration through excised human and monkey skin. *Fd. Chem. Toxic.* 24:221-227.

11. Creasia, D. A., M. L. Neally, L. J. Jones, III, C. G. York, R. W. Wannemacher, Jr., and D. L. Bunner. 1986. Acute inhalation toxicity of a saline suspension of T-2 mycotoxin in mice. Submitted to *Toxicol. Appl. Pharmacol.*

PRESENTATIONS

1. Hewetson, J. F., and J. E. Beheler. 1986. Monoclonal antibodies against saxitoxin binding of ^{3}H-saxitoxin to the sodium channel. Presented at the Second American Symposium on Animal, Plant and Microbial Toxins, Tempe, AZ, May.

2. Wannemacher, R. W., Jr., D. L. Bunner, J. G. Pace, and R. E. Dinterman. 1986. Efficacy of a non-occlusive barrier (B) model to study toxin pharmacokinetics after dermal (D) exposure (E). Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March.

3. Pace, J. G, W. J. Canterbury, and C. Matson. 1986. Fate and distribution [^{3}H]T-2 toxin in topically exposed guinea pigs. Presented at the Annual Meeting of the Society for Toxicology, Atlanta GA, March.

4. Creasia, D. A., R. W. Wannemacher, Jr., and D. L. Bunner. 1986. Acute inhalation toxicity of aerosols of T-2 toxin in solution and as a suspension. Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March.

5. **Wannemacher, R. W., Jr., D. L. Bunner, and R. E. Dinterman.** 1986. The relationship between concentration and exposed area on absorption and excretion of T-2 mycotoxin through rabbit skin. Presented at the Annual Meeting of the Federation of the American Society for Experimental Biology, St. Louis, MO, April.

6. **Creasia, D. A., J. D. Thurman, R. W. Wannemacher, Jr., and D. L. Bunner.** 1986. Acute inhalation toxicity of T-2 toxin in the rat and mouse. Presented at the Annual Meeting of the Federation of the American Society for Experimental Biology, St. Louis, MO, April.

7. **Bunner, D. L.** 1985. Pathophysiology and treatment of low molecular weight toxin exposure. Presented at a joint USAMRDC-Israeli Symposium on T-2 Toxin. Tel Aviv, Israel, December.

8. **Bunner, D. L., E. R. Morris, J. G. Pace, and C. F. Matson.** 1986. Effect of T-2 mycotoxin on amino acid uptake in L-6 myoblasts. To be presented at the Second American Symposium on Animal, Plant and Microbial Toxins, Tempe, AZ, May.

9. **Thompson, W. L., M. B. Allen, and R. W. Wannemacher, Jr.** 1986. Comparison of in vivo and in vitro protein and DNA synthesis inhibition in response to T-2 mycotoxin. Presented at the FASEB Summer Conference, Copper Mountain, CO, July.

10. **Thompson, W. L., and J. G. Pace.** 1986. Studies of in vitro protein synthesis inhibition and metabolism of the 12,13-epoxytrichothecenes by tissue culture and primary cell lines. Presented at the Federation for the Society for Experimental Biology and Medicine Summer Conference, Copper Mountain, CO, July.

11. **Bunner, D. L., E. R. Morris, J. G. Pace, and C. F. Matson.** 1986. Effect of T-2 mycotoxin on amino acid uptake in L-6 myoblasts. Presented at the first annual meeting of the International Society of Toxinology, American Branch, Tempe, AZ, May.

12. **Templeton, C. B., and D. A. Creasia.** 1986. Blood gas changes following aerosol T-2 exposure in rats. Presented at the Summer Conference of the Federation of American Societies for Experimental Biology, Copper Mountain, CO, July.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DAOG3811	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 85 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AK	139
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Development Studies Seeking Rapid Diagnostic Procedures to Detect Agents of Biological Origin in Clinical Specimens

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT		18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	EXPIRATION	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		86	2.0	158
c. TYPE	d. AMOUNT	87	2.0	538
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR LeDuc, J W
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Knauert, F K
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Ezzell, J W

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Viral Diseases; (U) Immunology; (U) Lab Animals; (U) Rats; (U) Biotechnology; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To develop technology for rapid diagnosis and identification of BW agents in the military clinical and environmental sphere. Field diagnosis will enhance the medical protection of US military personnel.

24. (U) To develop and refine state-of-the-art nucleic acid probes and other methods for virus detection and identification.

25. (U) 8510 - 8609 Nucleic acid filter hybridization was used to detect complementary Rift Valley fever virus (RVFV)-RNA in clinically and epidemiologically relevant samples including human and sheep sera, tissue homogenates from aborted fetuses of RVFV-infected ewes, and homogenates of infected mosquitoes. Several modifications and alternate formats were tried to improve sensitivity. We are recloning RVFV M-segment RNA sequences, along with portions of the segment, into a vector that will enable us to produce RNA probes that are reportedly more sensitive than DNA probes. These reagents will also be used to determine whether we can use nucleic acid technology to distinguish various geographical isolates of RVFV as well as RVFV from other members of the genus *Phlebovirus*, and the family, Bunyaviridae. We have started to develop an *in situ* hybridization assay to study virus morphogenesis and pathogenesis, and a combined immunological nucleic acid hybridization assay to detect immune complexes.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AK-139: Exploratory Development Studies Seeking Rapid Diagnostic Procedures to Detect Agents of Biological Origin in Clinical Specimens.

PRINCIPAL INVESTIGATOR: J. W. LeDuc, LTC, Ph.D.

ASSOCIATE INVESTIGATORS: F. K. Knauert, Ph.D.
J. W. Ezzell, Ph.D.

BACKGROUND

Recent technological advancements have made nucleic acid hybridization an attractive alternate method for detecting viruses and other pathogens in clinical and environmental samples. We have used a cloned sequence complementary to the M segment RNA of Rift Valley fever virus (RVFV) to develop such an assay to evaluate the usefulness of this methodology compared to currently used procedures for detecting and identifying pathogens. In the model system we developed using infected Vero cells and infected cell media, we are routinely able to detect between $5X10^3$ to $1X10^4$ PFU of virus, depending on the source. With the standard assay, we are also able to detect RVFV RNA sequences in inactivated vaccine samples and RVFV-containing aerosolized samples. When we attempted to transfer this technology to the detection of RVFV in clinically and epidemiologically relevant samples, including human and sheep sera, tissue homogenates from aborted fetuses of RVFV-infected sheep, and tissue homogenates of infected mosquitoes, we found that, while we were able to detect successfully RVFV-RNA in these samples, we could not achieve the overall sensitivity realized in our model system.

The primary problem in these samples is the presence of excessive amounts of cellular proteins and nucleic acids which nonspecifically adsorb to the nitrocellulose filters, competing with the viral RNA for limited binding-sites, eventually clogging the filter, and contributing to nonspecific binding with the DNA probe. We are trying modifications to the model procedure to attempt to eliminate this problem and to increase the sensitivity and specificity of this assay.

SUMMARY

To eliminate the problem associated with excessive cellular constituents in samples that nonspecifically adsorb to the nitrocellulose filter, and interfere with the hybridization assay, we investigated a number of ways of modifying the standard sample preparation procedure.

We found that pretreatment with the nuclease, DNase I, or the protease, proteinase K, could in some circumstances improve the

filterability of the samples and increase the sensitivity of the assay. Based on this limited success with the predigestion modification, we tried a number of other general proteases by themselves and in combination with proteinase K to determine if we could find a better method for eliminating the protein contaminant in these samples. Although some of these proteases were able to improve sample filterability, none, by themselves or in combination with proteinase K, resulted in an increase in sensitivity. One problem is that the proteases themselves contribute to the filtration problem and only limited amounts can be added to the sample before they start to block filtration and decrease the sensitivity of the assay. In addition, these protease preparations are a mixture of enzymes, including RNases, which are deleterious to sample preparations. We also tried prefiltering the samples through 0.2- and 0.45-µm filters in an effort to eliminate fine particulate material not removed by routine centrifugation. This step improved the filterability of the samples, and, in combination with proteinase and/or DNase I digestion, improved sensitivity over the standard procedure. However, we still have not achieved the sensitivity realized in the model system. To increase sensitivity, we are trying a number of approaches. Under a contract with Meloy Laboratories, Inc., we are recloning the entire M-segment RNA sequence, along with portions of the segment that, in total, represents the entire sequence, with a vector which will enable us to make RNA probes, instead of DNA probes (since the RNA probes reportedly have increased sensitivity). In addition, we plan to use the partial sequences to determine if we can find a segment that will enable us to distinguish different geographical isolates of RVFV from each other, as well as RVFV from other members of the genus *Phlebovirus*, and the family, Bunyaviridae. We are also testing alternate hybridization formats to determine if they can eliminate some of the problems associated with the filtration format.

In addition to the filter hybridization assay for detecting complementary sequences in clinical and environmental samples, we developed two other assays using nucleic acid hybridization principles.

The first is an *in situ* hybridization assay for following both viral morphogenesis and pathogenesis. Experiments were initiated in collaboration with Airborne Diseases Division. We detected complementary sequences in fresh frozen liver sections of RVFV-infected mice. The system employs a biotinylated probe. Hybridization was monitored by reaction of the hybridized probe with avidin-horseradish peroxidase complexes followed by addition of substrate. Successful hybridizations resulted in deposition of a brownish precipitate in cells. We tried the same procedure with fresh-frozen brain sections from infected hamsters or mice and were not able to generate a detectable signal, even though there was histological evidence of pathology. We are currently working on procedures to intensify the peroxidase-generated signal in these tissues. We are also trying to adopt these procedures to detect viral RNA in parafin-embedded sections.

A second assay under development is one to detect immune complexes in serum from infected animals. The system combines both immunological and nucleic acid hybridization principles. Briefly, we plan to remove immune complexes from serum by using appropriate immunological reagents and to identify the pathogens trapped in these complexes with nucleic acid hybridization.

MGLP Technology development for rapid diagnosis and identification of BW agents

A model nucleic acid hybridization assay has been developed for detecting RVFV-complementary RNA in infected Vero cells and infected cell media. When this procedure was used to detect viral sequences in clinically and environmentally relevant samples, a filtration problem was encountered which decreased the sensitivity of the assay compared to the model system. A number of modifications to the standard procedure have resulted in an increase in sensitivity, but we still have not reached the level of sensitivity realized in the model system. We are testing RNA probes and alternate hybridization formats to determine if we can improve the system. We are also developing nucleic acid hybridization assays for other purposes. One procedure under development is an *in situ* hybridization assay for following viral morphogenesis and pathogenesis. The second is a combined immunological/nucleic acid hybridization assay for detecting immune complexes.

MGDB Rapid Identification of Bacterial Agents and Diagnosis of Bacterial Infections

PRINCIPAL INVESTIGATOR: J. W. Ezzell, Ph.D.

Electrophoretic immunotransblots, EITB (Western blots), were established to confirm suspect exposure of individuals to anthrax. The technique was invaluable in our recent serology support to the Centers for Disease Control (CDC), Atlanta, GA in their investigations of an outbreak of cutaneous anthrax which occurred among Lengua Indians in rural Paraguay during February, 1986. Twenty-five cases were identified by Dr. Lee Harrison of CDC, including two deaths. The EITB was used to analyze 29 sera with antibody to the protective antigen component of the anthrax toxin. Based on clinical symptoms, the EITB correctly diagnosed anthrax in 11 of 12 cases in a blind study. However, none of the 17 negative controls included in the study was false-positive. These studies clearly demonstrated that our EITB is a sensitive and specific test for anthrax and does not present problems with non-specific reactions previously encountered with the ELISA procedure in similar studies of sera from Thailand. Using the EITB procedure, we are developing immunoassays for detecting antibody to *Francisella tularensis, Brucella sp.*, and *Borrelia sp.*

Preliminary investigations show that the EITB procedure can be used to detect protective antigen and its breakdown products in the urine of terminally ill guinea pigs. Attempts are being made to develop an EITB procedure for early detection of anthrax toxin components in infected individuals.

The EITB analyses revealed a 91-kd protein, termed EA1, in whole cell extracts of *B. anthracis*. Dr. Anthony Phillips, Porton Down, U.K., who has been working in my laboratory during the past year, has performed a series of indirect fluorescent antibody studies using specific antisera to EA1. He has confirmed our suspicions that EA1 is a major surface protein of *B. anthracis* vegetative cells and has found that sera to EA1, which has been adsorbed with certain *Bacillus thuringiensis* strains, are specific for the anthrax bacillus and therefore are of diagnostic value.

PUBLICATIONS

1. **Ezzell, J. W., and T. G. Abshire.** 1986. Immunological analysis of cell-associated antigens of *Bacillus anthracis*. Submitted to *Infection and Immunity* in September 1986.

2. **Burnett, A. M., J. W. Ezzell, Jr., J. Singh, G. F. Zipperle, Jr., and R. J. Doyle.** 1986. Induced release of *Bacillus* spores from sporangia by sodium sulphate. *J. Appl. Bacteriol.* 60:337-339.

3. **Ivins, B. E., J. W. Ezzell, Jr., J. Jemski, K. W. Hedlund, J. D. Ristroph, and S. H. Leppla.** 1986. Immunization studies with attenuated strains of *Bacillus anthracis*. *Infect. Immun.* 52:454-458.

PRESENTATIONS

1. **Burnett, A. M., R. J. Doyle, and J. W. Ezzell.** 1986. Release of *Bacillus* spores from sporangia by sodium sulfate. *Abstr. Annu. Meet. Am. Soc. Microbiol.* J6:189.

2. **Doyle, R. J., T Beveridge, and J. Ezzell.** 1986. Evidence for a surface array of *Bacillus anthracis*. *Abstr. Annu. Meet. Am. Soc. Microbiol.* J18:191.

3. **Harrison, L. H., J. W. Ezzell, T. Abshire, and A. F. Kaufmann.** 1986. Application of an electrophoretic-immunotransblot method for serologic diagnosis of anthrax. *Abstr. 26th Intersci. Conf. Antimicrob. Agent. Chemother.*

4. **Ezzell, J. W.** 1986. Electrophoretic immunotransblots for the rapid diagnosis of anthrax and other infectious agents. Seminar presented at the Centers for Disease Control, Atlanta, GA, May.

5. **Knauert, F. K., J. C. Morrill, and M. J. Turell.** 1985. A nucleic acid hybridization assay for detecting Rift Valley fever virus. Presented at the 34th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Miami, FL, November.

6. **Knauert, F. K.** 1986. Use of a DNA probe to detect Rift Valley fever virus RNA in epidemiologically relevant specimens. Presented at the American Society for Virology 1986 Annual Meeting, Santa Barbara, CL, June.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

Field	Value
1. AGENCY ACCESSION	DAOG3815
2. DATE OF SUMMARY	86 10 01
REPORT CONTROL SYMBOL	DD-DR&B(AR) 636
3. DATE PREV SUM'RY	86 03 20
4. KIND OF SUMMARY	D. CHANGE
5. SUMMARY SCTY	U
6. WORK SECURITY	U
7. REGRADING	
8. DISB'N INSTR'N	CX
9. LEVEL OF SUM	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AH	146
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Development Studies Seeking Generic Medical Defensive Countermeasures Against Agents of Biological Origin

12. SUBJECT AREAS 1503 Defense; 0605 Clinical Medicine; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT	
a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	3.0	546
87	2.0	648

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Virology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Canonico, P G
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-2290
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Ussery, M A
MILITARY/CIVILIAN APPLICATION M	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Pifat, D Y

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U)Antiviral Drugs; (U)Pharmacology; (U)Viral Diseases; (U)Lab Animals;(U) Mice;(U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Conduct laboratory studies to develop novel antiviral drugs by identifying potential targets for pharmacologic intervention. These drugs are needed to treat soldiers who may become sick with viral infections.

24. (U) Describe mechanisms of action and metabolism of new drugs and provide analytical support for drug analysis. Perform structure-activity analyses to identify new analogs for synthesis. Apply approaches of molecular virology and cell biology to define the molecular basis of virus binding, uptake, uncoating, replication, and maturation.

25. (U) 8503 - 8609 Institute-based drug evaluation antiviral screen has been reformatted to include dengue hemorrhagic fever. Seventy-four compounds from Burroughs Wellcome Laboratories were tested against dengue viruses *in vitro*. Twenty of these have shown evidence of activity. The antiviral effects of three related carboxamides, ribavirin, tiazofurin and selenazofurin, were tested against five bunyaviruses of the California serogroup. These studies established that ribavirin is a potent antiviral agent against California serogroup viruses.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AH-146: Exploratory Development Studies Seeking Generic Medical Defensive Countermeasures Against Agents of Biological Origin

PRINCIPAL INVESTIGATOR: P. G. Canonico, Ph.D.

ASSOCIATE INVESTIGATOR: M. A. Ussery, MAJ, Ph.D.
D. Y. Pifat, Ph.D.
J. W. Huggins, Ph.D.
M. Kende, Ph.D.

BACKGROUND

This research program has focused on the discovery and development of agents or procedures which will be effective against a broad range of "exotic" RNA viruses.

The program maintains an extensive antiviral drug screening effort. Initial testing consists of *in vitro* assays against a battery of RNA viruses as well as rodent models. Another approach is the targeting of antiviral agents to specific tissue sites. This approach involves the synthesis of compounds which have an inherent capacity to concentrate in specific organs; also the capability of antivirals to couple to carriers which will promote the site-specific delivery of the drug.

Research activity is also directed at combination chemotherapy. Combinations of certain antivirals have been shown to exhibit significant synergy. The synergy of drugs, which enhance the immune system, is also of special interest. Finally, the number of natural products which nonspecifically activate the immune system, resulting in enhanced host resistance to viral infections, has grown significantly due to the efforts of the National Institutes of Health. USAMRIID's program is evaluating these natural products as antiviral agents, as well as exploiting approaches for the specific targeted delivery of these immunomodulators, given either alone or in combination with other antivirals.

SUMMARY

The overall antiviral activity of three related carboxamides, ribavirin, tiazofurin and selenazofurin, against five bunyaviruses of the California serogroup: California encephalitis, snowshoe hare, La Crosse, and two wild field isolates, are very similar. All three compounds had ED_{50}s between 150 and 200 μM. Only ribavirin, however, yielded ED_{90} values within non-cytotoxic concentrations. Preliminary data indicate that the viral proteins are synthesized, even in the presence of high concentrations of ribavirin. Further molecular studies on the antiviral mode of action of ribavirin are warranted.

Seventy-four compounds have been tested for activity against dengue 1-4 viruses in vitro. Of these, 20 have shown evidence of activity. Additional studies to confirm and define this activity, and describe generic mechanisms of action are in progress.

The lipophilic kanamycin derivatives showed activity against Venezuelan equine encephalitis (VEE), yellow fever, Rift Valley fever (RVFV) and vericular stomatitis virus in vitro. We observed no in vivo activity in either of the VEE or RVFV mouse models.

The in vitro antiviral activity of nucleoside dialdehyde derivatives was found to correlate with the inhibition of S-adenosylhomocysteine hydrolase, an important enzyme in mRNA methylation and capping.

Progress in the area of model development includes a Japanese encephalitis virus (JEV) peripheral lethal model in weanling mice. Infection of C57 black mice (i.p.) with 10 LD_{50} of Peking strain (JEV) resulted in 100% mortality with a mean time to death of 10 days.

PUBLICATIONS

1. Wood, S. G., K. G. Upadhya, N. K. Dalley, P. A. McKernan, P. G. Canonico, R. K. Robins, and G. R. Revankar. 1985. Synthesis and biological activity of 5-thiobredinin and certain related 5-substituted imidazole-4-carboxamide ribonucleosides. *J. Medicinal Chem.* 28:1198-1203.

2. Huggins, J. W., G. R. Kim, O. M. Brand, and K. T. McKee, Jr. 1986. Ribavirin therapy for Hantaan virus infection in suckling mice. *J. Infect. Dis.* 153:489-497.

3. Kenyon, R. H., P. G. Canonico, D. E. Green, and C. J. Peters. 1986. Effect of ribavirin and tributylribavirin on Argentine hemorrhagic fever (Junin virus) in guinea pigs. *Antimicrob. Agents Chemother.* 29:521-523.

PRESENTATIONS

1. Ussery, M. A., R. M. Bunte, and P. G. Canonico. 1986. Kinetics of peripheral Japanese encephalitis virus (JEV) infection in C57 black mice. Presented at the XIth International Congress of Infections and Parasitic Diseases, Munich, FRG, July.

2. Ussery, M. A., Y. Mine, T. Takaya, and P. G. Canonico. 1986. Broad spectrum antiviral activity of kanamycin A derivatives with lipophilic acyl groups at the N-1 position. Presented at the Annual Meeting of the American Society for Virology, Santa Barbara, CA, June.

3. Ussery, M. A., M. A. Balady, P. G. Canonico, and C. W. Czarniecki. 1986. Prophylactic and therapeutic efficacy of recombinant interferons alpha and gamma in Japanese encephalitis virus-infected C57 black mice. Presented at 26th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, September.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DAOG3810
2. DATE OF SUMMARY: 86 10 01
REPORT CONTROL SYMBOL: DD-DR&E(AR) 636

3. DATE PREV SUM'RY: 85 10 01
4. KIND OF SUMMARY: D. CHANGE
5. SUMMARY SCTY: U
6. WORK SECURITY: U
7. REGRADING:
8. DISB'N INSTR'N: CX
9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AB	150
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP, FY87- 01			

11. TITLE (Precede with Security Classification Code) (U) Exploratory Development Studies of Conventional Agents of Biological origin for Development of Medical Defensive Countermeasures

12. SUBJECT AREAS
0613 Microbiology; 0605 Clinical Medicine; 1503 Defense

13. START DATE: 80 10
14. ESTIMATED COMPLETION DATE: CONT
15. FUNDING ORGANIZATION: DA
16. PERFORMANCE METHOD: C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE — EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	9.0	3326
87	11.0	3039

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: USA Medical Research Institute of Infectious Diseases
b. ADDRESS (include zip code): Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL: Huxsoll, D L
d. TELEPHONE NUMBER (include area code): 301-663-2833

20. PERFORMING ORGANIZATION
a. NAME: Disease Assessment Division, USAMRIID
b. ADDRESS: Fort Detrick, MD 21701-5011
c. NAME OF PRINCIPAL INVESTIGATOR: Bailey, C L
d. TELEPHONE NUMBER (include area code): 301-663-7244
f. NAME OF ASSOCIATE INVESTIGATOR (if available): Johnson, E D
g. NAME OF ASSOCIATE INVESTIGATOR (if available): Turell, M J

21. GENERAL USE: FIC
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense; (U) Rapid Detection; (U) Vaccines; (U) Lab Animals; (U) Mice (U) Rats; (U) Guinea Pigs; (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To define the disease spectrum of arboviruses to include vector and reservoir competence, pathogenesis of viral strains, and therapy and immunoprophylaxis.

24. (U) Longitudinal epidemiological studies correlate clinical manifestations with seroconversions and identify sites for in-depth ecological studies to recover viral strains from reservoirs. Ecological and genetic factors relating to vector and reservoir competence are studied under controlled conditions.

25. (U) 8510 - 8609 Seroepidemiological surveys in the Central African Republic indicate that filovirus-reactive, antibody-positives are family-clustered and limited to select areas within each village. Antibody results suggest that Ebola and Marburg viruses are more active than originally expected and are more likely to cause disease during the drier seasons. Hemostatic impairment in Ebola- and Marburg-infected rhesus monkeys suggest significant differences between these viruses with regard to pathogenesis and hemorrhage. The aerosol infectivity of Hantaan and related viruses was demonstrated in outbred Wistar rats. Vector competence studies revealed a newly discovered route of dissemination for arboviruses in mosquito vectors. The escape of Rift Valley fever virus from the alimentary canal of mosquitoes into the hemocoel and salivary glands is at the foregut-midgut junction.

PROJECT NO. 3M162770A871: Medical Defense Against Biological Warfare

WORK UNIT NO. 871-AB-150: Exploratory Development Studies of Conventional Agents of Biological Origin for Development of Medical Defensive Countermeasures

PRINCIPAL INVESTIGATOR: C. L. Bailey, LTC, Ph.D.

ASSOCIATE INVESTIGATORS: E. D. Johnson, MPH, Ph.D.
J. W. LeDuc, LTC, Ph.D.
K. J. Linthicum, CPT, Ph.D.
J. C. Morrill, MAJ, D.V.M.
M. J. Turell, Ph.D.
C. T. Liu, Ph.D.
T. M. Cosgriff, LTC, M.D.
J. White, Ph.D.

BACKGROUND

Highly pathogenic viruses, with invertebrate and vertebrate vectors, are known to be potential threats to U.S. military personnel in endemic areas. Many of these viruses cause severe human disease, mainly hemorrhagic fevers, and include Rift Valley fever, Marburg, Ebola, Pichinde, and Hantaan viruses. In most cases, little is known about the epidemiology and life cycles of the viruses. In many cases, we have virtually no information on pathogenesis, therapy, or immunoprophylaxis of the diseases caused by these viruses. The main emphasis of this work unit is to (i) evaluate the natural threat and impact of the group of highly pathogenic viruses by accurately establishing the incidence and distribution of apparent and inapparent infections and determining epidemiological, ecological, and agent-related factors influencing endemic viral activity; and (ii) to alter the highly pathogenic nature of these agents by defining the pathogenic mechanisms involved in the disease processes and developing therapeutic measures to intervene in the hemorrhagic fever.

SUMMARY

Seroepidemiological surveys in the Central African Republic indicate that filovirus-reactive, antibody-positive members of the indigenous population are family-clustered and limited to select areas within each village. Antibody data suggest that Ebola and Marburg viruses are more active than originally expected and are more likely to cause disease during the drier seasons. Hemostatic impairment in Ebola- and Marburg-infected rhesus monkeys suggests significant differences between these viruses with regard to pathogenesis and hemorrhage. The aerosol infectivity of Hantaan and related viruses was demonstrated in outbred Wistar rats.

In vector competence studies with *Culex pipiens* and Rift Valley fever virus, the virus was detected outside the alimentary canal in 10 to 20% of the specimens as early as 4 h after ingestion of a viremic blood meal. Several of these mosquitoes were capable of transmitting the virus as early as 48 h after the bloodmeal.

We have established that guinea pigs infected with Pichinde virus show symptoms of dehydration and hyponatremia. Significant amounts of water and sodium ions were transported from the plasma into the red blood cells without changing the cellular potassium ion content 11 days postinoculation with the virus.

MGLB Biologic Principles and Epidemiology of Filoviridae Infections

PRINCIPAL INVESTIGATOR: E. D. Johnson, Ph.D.

Cross-sectional seroepidemiological surveys were conducted in five previously studied and six new villages located along the Central African Republic's (CAR) northern frontier bordering Chad and Sudan. The surveys were conducted to identify risk factors influencing the high Ebola virus- (EBOV) and Marburg virus (MBGV)-reactive antibody prevalence rates previously demonstrated among select populations living in this arid region. With the findings from our 1985 survey and maps and census data produced while in the field, follow-up studies were completed successfully in the seropositive population, yielding a resurvey rate of 65 to 85%. The findings indicate that filovirus-reactive, antibody-positives are family-clustered and limited to select areas in each village. In two representative villages with filovirus antibody prevalence rates of 22% (39/179) and 29% (43/149), 56% of the seropositives were found among 7.9% (15/189) of the total number of families living in these villages. In several families approximately 60% (9/15) of the total members were filovirus-reactive, antibody-positives. Surprisingly, three types of seropositives were identified: those positive for EBOV-reactive antibody; those positive for MBGV-specific antibody; and those positive for antibody reactive to both viruses. To date, we thought that the filovirus group was comprised of only two distinct, non-cross-reacting serotypes, Ebola or Marburg virus-reactive. The high frequency of seropositives to both viruses (7.6%) is unique to central Africa and suggests that either both of these viruses may be circulating independently of one another or that a third cross-reacting filovirus is active in the CAR. Previously, the lack of overt disease associated with the high antibody frequency strongly supported the presence of a third, less-virulent filovirus in the CAR. However, detailed questioning of seropositive individuals with elevated antibody titers ($\geq$1024), revealed that many of the Marburg virus antibody-reactives had experienced an acute illness comprised of headache, fever, pharyngitis, general aches and pains, coughing, and nose bleeds; deaths were not uncommon among the young. The illnesses occurred each year during the dry season. In at least one village, 80% (5/6) of the MBGV-seropositives were among the 9 to 11-year-old-male population and at least one fatal illness accompanied by uncontrollable hemorrhage occurred in this age group. Though the

clinical picture is sketchy and will require detailed documentation and etiological agent identification, the findings strongly suggest that filovirus antibody development in northern CAR is associated with an overt illness. The observation that the CAR illness takes place during the dry season is consistent with previous east African findings that peak filoviral activity occurs during the drier months. The means by which the filoviruses are maintained and transmitted are unknown. Four hundred rodent specimens were collected from antibody-positive and antibody negative-households in an attempt to incriminate select rodent species as the reservoir host. The processing of these specimens has not been completed. In light of the behavior of other African hemorrhagic fever viruses, rodents are probably part of the natural cycle. Person-to-person spread cannot be dismissed. Aerosol transmission studies have been completed, indicating that the Mayinga prototype EBOV strain is highly infectious by the aerosol route. As few as 300 infectious units per liter of inhaled air produces a uniformly fatal disease in rhesus monkeys. All animals develop a typical filoviral hemorrhagic diathesis with significantly prolonged prothrombin and partial thromboplastin times.

Collectively, these findings suggest that the filoviruses are more active than expected and are capable of inflicting severe disease during the drier seasons of each year. The high frequency of seropositives reacting to both non-cross-reacting serotypes suggest that both highly pathogenic viruses, EBOV and MBGV, circulate in nature independently and pose significant health threats in central Africa; recent preliminary surveys suggest that this activity extends to western Africa. The clustering of filovirus-reactive, antibody-seropositives indicates the importance of environmental factors in the spread of these agents. Aerosol spread from an infected human contact or reservoir host seems likely in light of recent laboratory experiments.

MGLJ Studies of Arbovirus Infection, Dissemination, and Transmission in Vectors

PRINCIPAL INVESTIGATOR: K. Linthicum, CPT, Ph.D.

ASSOCIATE INVESTIGATOR: M. E. Faran, MAJ, Ph.D.

A manuscript has been published describing the avidin-biotin-peroxidase (ABC) immunocytochemical procedure used to locate Rift Valley fever (RVF) viral antigen in infected mosquito cells and in serial sections of infected *Culex pipiens* mosquitoes. This procedure has facilitated the study of intrinsic factors affecting vector competence and led to the discovery of a newly recognized route of arboviral dissemination from the mosquito midgut. Studies were completed demonstrating the dissemination pattern of RVF virus in *Cx. pipiens*.

Investigation of the effect RVF virus has on adult survival of *Cx. pipiens* has been completed. The above studies determined that: (a) virus disseminates from the alimentary canal very rapidly in 10 to 20% of the population. In some cases virus was detected outside the

alimentary canal as early as 4 h after ingestion of a viremic blood meal. A proportion of mosquitoes were biologically competent (i.e., virus isolated from salivary glands) to transmit virus as early as 48-h postexposure; (b) dissemination occurred continuously or sporadically after some event, such as blood feeding, until at least day 18, resulting in recruitment of new potential transmitting mosquitoes from the nontransmitting subpopulation. This contradicts the traditional concept of an "extrinsic incubation period"; (c) *Cx. pipiens* mosquitoes, infected either orally or by inoculation with RVF virus, survive a significantly shorter time than do uninfected individuals. The decreased survival associated with RVF Viral infection should be considered in mathematical models of this diseases's epidemiology; (d) trauma, simulated by vigorous shaking after a viremic bloodmeal, does not affect either infection or dissemination rates in *Cx. pipiens*. This indicates that early dissemination is not an artifact of trauma that might result from artificial laboratory conditions; and (e) the salivary glands and thoracic ganglion are the last organs that become infected of the six tissues examined. Infection of these organs require from 24 to 48 h after virus has escaped from the alimentary canal.

A long-term project was initiated to investigate the temporal and spatial morphological distribution of RVF virus and viral antigen in African mosquitoes from endemic regions of disease.

An electron microscopic study of the morphogenesis and pathogenesis of RVF virus in *Cx. pipiens* will be completed this year.

MGLK Factors Affecting Vector Competence of Mosquitoes for Rift Valley Fever Virus

PRINCIPAL INVESTIGATOR: M. J. Turell, Ph.D.

Further characterization of the substance responsible for inhibition of the recovery of Rift Valley fever (RVF) virus from pools of mosquito larvae indicates that it may be an enzyme, as heating to 56°C for 45 min reduced the effect. Also, the virus inactivation curve was temperature- as well as dose-dependent. Preliminary studies indicate that mosquitoes are highly sensitive to several snake toxins and that mosquito inoculation may serve as a rapid, sensitive, and inexpensive assay system for these toxins. Temperature of extrinsic incubation was not found to affect infection of *Aedes fowleri* with RVF virus. However, both the time interval until virus disseminated to the hemocoel and the dissemination rate once virus had begun to disseminate were directly correlated with extrinsic incubation temperature.

MGBE Microbes and Other Biological Agents. Pathogenetic and immunogenetic mechanisms after Aerosol Exposure

PRINCIPAL INVESTIGATOR: E. H. Stephenson, COL, D.V.M.

AND

MGLI Epidemiology of Hantaan-Like Agents

PRINCIPAL INVESTIGATOR: J. W. LeDuc, LTC, Ph.D.

ASSOCIATE INVESTIGATORS: E. O. Nuzum, Ph.D.
C. A. Rossi
E. H. Stephenson, COL, D.V.M.

Hantaan, Seoul (Urban Rat), and Puumala viruses are antigenically related members of the *Hantavirus* genus, family Bunyaviridae, and are responsible for human disease, ranging in severity from subclinical to severe hemorrhagic fever with renal syndrome (HFRS). Previous studies have documented infectious virus in feces and body secretions of naturally infected wild rodents and experimentally infected laboratory rats. Inhalation of aerosolized virus has been suggested as an important route of transmission among the hantaviruses. Experiments were designed to demonstrate aerosol transmission of virus to laboratory rats and to contrast the relative sensitivity of aerosol and intramuscular (i.m.) exposures. Groups of five to eight rats were exposed nose-only to varying concentrations of aerosolized Hantaan, Seoul, or Puumala virus for 10 min, or were inoculated i.m. with 0.5 ml of serially diluted virus. Infection was detected by demonstrating anti-hantavirus antibody in 29-day postinfection sera by immunofluorescence assay (IFA), with Hantaan-or Puumala virus-infected, Vero E-6-cell, spot slides. We showed rats to be extremely sensitive to both routes of infection, although the ID_{50} for i.m. exposure was less than for aerosol exposure. Furthermore, *in vivo* infectivity determinations consistently were more sensitive than conventional cell culture assays. Future efforts will attempt to demonstrate shedding of infectious virus in rats after aerosol infection.

MGLC Pathophysiology of Arenavirus Disease

PRINCIPAL INVESTIGATOR: C. T. Liu, Ph.D.

Pathogenesis of Pichinde virus infection and treatment Previously, we demonstrated that dehydration and hyponatremia were associated with Pichinde viral infection in strain 13 guinea pigs. Decreases in water and Na+ intake plus renal tubular dysfunction were considered partially as the mechanism. Further investigation revealed that significant amounts of water and NA+ were transported from the plasma into the red blood cells without changing the cellular K+ content 11 days postinoculation.

When a leukotriene antagonist, FPL-55712, was infused i.v. (420 $\mu g^{-1} kg^{-1} h^{-1}$) for 2 h following a priming dose (300 µg/kg) to virus-

infected guinea pigs on day 10, the infection-induced, low cardiac output showed significant improvement. These results provide direct evidence that leukotrienes play a role in the pathogenesis of cardiac dysfunction. The depressed cardiac function was demonstrated to be reversed by a leukotriene antagonist through competitive mechanisms.

The disease course of Pichinde virus infection The entire disease course of Pichinde viral infection in strain 13 guinea pigs was video recorded. Major clinical signs and behavior changes were clearly shown with verbal explanations. This tape establishes a vivid document, showing a serious and lethal arenaviral infection in an animal model. The video taping technique provides a better approach in presenting scientific evidence than a written description.

Establishment of a computer network In order to modernize the laboratory and facilitate physiological data collections, analyses, or graph-plotting and creating documents, a computer network has been established, including connections between several microcomputers (IBM PC/AT) and the Wylbur computer system. A data acquisition system for weighing animals, water, and food to meet with criteria of the "Good Laboratory Practices Regulations" was also developed. More data acquisition software for various physiological measurements are being requested, and a future commercial contract is pending. Furthermore, by using telemetric devices, an automatic system for simultaneous recording of body temperatures from several guinea pigs was completed.

Development of techniques Techniques for studying total-body capillary permeability were initiated with the collaboration of Dr. Gabriel G. Pinter. The approaches involve an i.v. injection of ^{125}I-albumin (25 μCi) and subsequent collections of arterial blood and lymph samples from the right lymphatic vessel and the left thoracic duct at different time intervals for 3 to 4 h. Preliminary results confirm the feasibility, despite a high failing rate. Once these difficult techniques are developed, they will be used for measuring changes of capillary permeability in Pichinde virus-infected strain 13 guinea pigs.

Significant progress was made in establishing techniques for measuring plasma and tissue levels of epinephrine, norepinephrine, dopamine, NA^+, K^+-ATPase, and MG^{++}-ATPase. Using the laser doppler capillary perfusion monitor, relative capillary flow values through the skin and internal organ (liver) surfaces were obtained. However, it was not possible to measure the capillary flow in a moving organ (contracting heart or ventilating lungs). Surface temperatures of different regions of control and Pichinde virus-infected strain 13 guinea pigs were also measured with the infrared thermometer.

MGFE Genesis of Hemorrhage Manifestations of Viral Hemorrhagic Fevers

PRINCIPAL INVESTIGATOR: T. M. Cosgriff, LTC, M.D.

ASSOCIATE INVESTIGATOR: R. M. Lewis, Ph.D.

In the last year, data from multiple studies on hemostatic impairment in Pichinde viral infection of strain 13 guinea pigs were reviewed and submitted in manuscript form for publication. The data are compatible with the occurrence of disseminated intravascular coagulation as an important pathogenic mechanism for coagulation abnormalities. Included in the data are results of an assay for guinea pig fibrinogne degradation products developed by Dr. James Chen, a visiting Professor in our laboratory. This assay is of great value for investigations of mechanisms of coagulation abnormalities in guinea pigs and is the only assay available which is specific for guinea pig fibrinogen degradation products.

Initial studies on hemostatic impairment in Ebola and Marburg viral infections of rhesus monkeys have also been completed and manuscripts for publication are in preparation. Data from these studies suggest significant differences between the two viral infections with regard to the pathogenesis of hemorrhage.

Multiple studies of Rift Valley fever viral infection in rhesus monkeys point to the importance of liver injury, as well as disseminated intravascular coagulation mechanisms of hemorrhage in this model. They also demonstrate the therapeutic efficacy of interferon in infected animals. Computer analysis of the extensive amount of data generated in these studies is nearly completed.

New research studies initiated in the last year include a protocol designed to evaluate the mechanism of thrombocytosis produced by the antiviral drug, ribavirin, as well as coagulation studies in patients with epidemic hemorrhagic fever (EHF), as part of a clinical trial of ribavirin for treatment of EHF in China. In collaboration with Dr. Chen, an electrophoresis assay for detection of factor VIIIR ag multimers in supernates and lysates of cell cultures is under development.

MGGA Analysis of Subcellular Structures in Microbial Infections of Potential BW Importance

PRINCIPAL INVESTIGATOR: J. White, Ph.D.

Cell cultures infected with the prototype strain of Hantaan virus (HTNV), examined by conventional procedures for electron microscopy (IEM), had consistently failed to reveal the presence of cell-associated viral particles. More recently, we examined plaque foci from HTNV-infected cultures of the E-6 Vero line. Monolayer sections containing plaque foci were examined in a plane perpendicular to that of the original growth surface. Cellular morphology was normal, and extracellular viral particles were seen only between the plasma membrane and growth substrate. Viral morphology was compatible with HTNV particles. This polarity in E-6 Vero cells suggested that our previous IEM studies were unsuccessful, because reagents were always applied *in situ* to the upper surface of intact monolayers. Monolayer fragments of plaque foci were used to examine by IEM the prototype strain on HTNV, other murine isolates, and several isolates associated with human disease. These viral isolates, identical in morphology,

were specifically labeled with either human convalescent sera, a specific mouse polyclonal ascites fluid, or a pool of monoclonal antibodies reactive with the viral envelope as the detecting antibody, and protein A or the appropriate antiglobulin serum bound to 10-nm gold particles as the immune probe. All infected cultures contained hollow membranous structures, 30 nm in diameter, that reacted specifically with the envelope monoclone pool.

Consistent with the responsibility, various services and professional assistance were provided to other investigators of the Institute. Collaborative studies on the pathogenesis of Marburg virus and Ebola virus infections showed that viral replication occurs in the hepatic parenchyma of a simian host.

PUBLICATIONS

1. **Faran, M. E., W. S. Romoser, R. G. Routier, and C. L. Bailey.** 1986. Use of the avidin-biotin-peroxidase complex immunocytochemical procedure for detection of Rift Valley fever virus in paraffin sections of mosquitoes. *Am. J. Trop. Med. Hyg.* 35: (In Press).

2. **Gargan, II, T. P., and K. J. Linthicum.** 1986. Variation in the length of the median pale band on the proboscis of *Aedes taeniorhynchus*. *J. Am. Mosq. Contr. Assoc.* 2:222-224.

3. **Linthicum, K. J., F. G. Davies, A. Kairo, C. L. Bailey, H. F. Kaburia, and K. J. Lindquist.** 1986. Field ecological studies on Rift Valley fever virus. To be published in the *Proc. Ann. Mtg., Kenya Med. Res. Inst. and Kenya Trypanosomiasis Inst.*

4. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and A. Kairo.** 1986. Observations on the dispersal and survival of a population of *Aedes lineatopennis* (Ludlow) (Diptera:Culicidae) in Kenya. *Bull. Entomol. Res.* 75:661-670.

5. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker.** 1986. Prediction of Rift Valley fever virus activity in Kenya by satellite remote sensing imagery. Submitted to *Science*.

6. **Liu, C. T.** 1986. Techniques for isolation and performance of the perfused guinea pig working heart. *Am. J. Vet. Res.* 47:1032-1043.

7. **Liu, C. T., P. B. Jahrling, and C. J. Peters.** Evidence for the involvement of sulfidopeptide leukotrienes in the pathogenesis of Pichinde virus infection of strain 13 guinea pigs. *Prostaglandins Leukotrienes Med.* (In Press).

8. **Liu, C. T., P. B. Jahrling, and C. J. Peters.** 1986. Evidence for the involvement of leukotrienes in the pathogenesis of Pichinde virus infection. *Fed. Proc.* 45:457.

9. **Liu, C. T.** 1986. Changes of ECG in guinea pigs infected with Pichinde virus. *Proc. Int. Union Physiol. Sci. (30th Congress)*. 16:410.

10. **Peters, C. J., P. B. Jahrling, C. T. Liu, et al.** Experimental studies of arenaviral hemorrhagic fevers. *In* Current Topics in Microbiology and Immunology (In Press).

11. **Romoser, W. S., M. E. Faran, and C. L. Bailey.** 1986. A newly recognized route of arbovirus dissemination from the mosquito midgut. Submitted to *Science*.

12. **Turell, M. J., C. A. Rossi, and C. L. Bailey.** 1985. Effect of extrinsic incubation temperature on the ability of *Aedes taeniorhynchus* and *Culex pipiens* to transmit Rift Valley fever virus. *Am. J. Trop. Med. Hyg.* 34:1211-1218.

13. **Rossignol, P. A., J. M. C. Ribeiro, M. Jungery, M. J. Turell, and C. L. Bailey.** 1985. Enhanced mosquito blood-finding success on parasitemic hosts: evidence for vector-parasite mutualism. *Proc. Nat. Acad. Sci.* 82:7725-7727.

14. **Turell, M. J., C. A. Rossi, R. F. Tammariello, Jr., and C. L. Bailey.** 1986. Reduced recovery of Rift Valley fever virus associated with assay of mosquito (Diptera: Culicidae) larval pools. *J. Med. Entomol.* 23:426-422.

15. **Turell, M. J., and C. L. Bailey.** Transmission studies in mosquitoes (Diptera: Culicidae) with disseminated Rift Valley fever virus infection. *J. Med. Entomol.* (In Press).

16. **Turell, M. J.** Horizontal and vertical transmission of viruses in insect and tick vectors. *In* T. Monath, (Ed.), Epidemiology of arthropod-borne viral diseases, CRC Press, Inc. (In Press).

PRESENTATIONS

1. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker.** 1986. Use of satellite remote sensing imagery to predict Rift Valley fever virus activity in Kenya. Presented at the Organization of African Unity Symposium on Viral Diseases in Africa, Nairobi, Kenya, May.

2. **Turell, M. J., and C. L. Bailey.** 1985. Reduced infection rates in mosquitoes after ingestion of artificial viremias of Rift Valley fever virus. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Miami, FL, November.

3. **Turell, M. J.** 1985. Competence of potential vectors of Rift Valley fever virus. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Miami, FL, November.

4. **Knauert, F. K., J. C. Morrill, and M. J. Turell.** 1985. A nucleic acid hybridization assay for detecting Rift Valley fever virus. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Miami, FL, November.

5. **Turell, M. J., and C. L. Bailey.** 1986. Effect of environmental temperature on the replication, dissemination, and transmission of Rift Valley fever virus by *Aedes fowleri*. To be presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene to be held in Denver, CL, December.

6. **Turell, M. J., R. A. Tammariello, and C. L. Bailey.** 1986. Reduced recovery of Rift Valley fever virus associated with assay of larval pools. To be presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene to be held in Denver, CO, December.

7. **Nuzum, E. O., C. A. Rossi, E. H. Stephenson, and J. W. LeDuc.** 1986. Aerosol infectivity of Hantaan and related viruses in outbred Wistar rats. To be presented at the 35th annual meeting of the American Society for Tropical Medicine and Hygiene, Denver, CO, December.

8. **Romoser, W. S., M. E. Faran, and C. L. Bailey.** 1986. Is the basal lamina the mesenteronal escape barrier to Rift Valley fever virus in mosquitoes? Presented at the Annual meeting of the American Mosquito Control Association, New Orleans, LA, April.

9. **Liu, C. T.** 1986. Pathogenetic mechanisms of Pichinde virus infection in strain 13 guinea pigs: the possible involvement of mediators. Presented at the USAMRIID Professional Staff Conference, April.

10. **Johnson, E. D., K. T. McKee, Jr., N. Jaax, S. Dixon, R. Lewis, and T. Cosgriff.** 1985. Filovirus infections of rhesus macaques. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Miami, FL, November.

11. **Huggins, J. W., C. Hsiang, T. M. Cosgriff, Z. Wu, M. J. Meegan, J. W. LeDuc, Z. Zhen, J. I. Smith, S. Ge, M. Guan, C. Wang, T. Zhang, G. Yuan, and X. Gui.** 1986. Double-blind, placebo-controlled clinical trial of ribavirin therapeutic efficacy in the treatment of epidemic hemorrhagic fever: open phase for dose setting. Presented at the IXth International Congress of Infectious and Parasitic Diseases, Munich, July.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA307135	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	131
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Isolation and Characterization of Immunogenic Components of Anthrax Toxin

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 04	CONT	DA	C. In-House

17. CONTRACT/GRANT
- a. DATE EFFECTIVE — EXPIRATION
- b. CONTRACT/GRANT NUMBER
- c. TYPE — d. AMOUNT
- e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	1.0	110
87	3.0	120

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Bacteriology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Welkos, S L
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7341
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Ivins, B
MILITARY/CIVILIAN APPLICATION H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Greene, R.

22. KEYWORDS (Precede EACH with Security Classification Code) (U) BW Defense; (U) Lab Animals; (U) Mice; (U) RAI (U) Guinea Pigs; (U) Rabbits; (U) Anthrax Toxin; (U) Lac Fusion Proteins; (U) Cloning

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To isolate polypeptide fragments of anthrax toxin components by using plasmid cloning vectors that allow construction of hybrid genes. To characterize the structure, immunogenicity, and biological activity of the hybrid toxin proteins. This work may ultimately lead to an improved anthrax vaccine to protect military personnel.

24. (U) Isolate protective antigen (PA)-lac Z gene fusions and characterize these fusions by using restriction endonuclease digestion and agarose gel electrophoresis. Once purified, the different PA-lac hybrids will be assayed by in vitro and in vivo techniques. Purified, fully characterized hybrid proteins might be conjugated with other anthrax antigenic material to form a new vaccine.

25. (U) 8510 - 8609 The protective antigen (PA) gene of *Bacillus anthracis* was inserted into the fusion vector, and 6 - 7 classes of mutants with different lengths of insert fused to the β-galactosidase gene were isolated. Isolation of PA-containing fusion proteins was attempted and proteins characterized electrophoretically, immunologically, and enzymatically. Candidate proteins consisting of PA fused to β-galactosidase could not be clearly demonstrated. However the nucleotide sequence of the PA gene was recently determined, and we are exploiting additional technologies for cloning anthrax toxin genes (ie. a *Bacillus* cloning system, transposon mutagenesis, and plasmid expression vectors). We developed an inbred mouse model for anthrax and are using it to assay the safety and efficacy of the prototype recombinant vaccines being developed.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-00-131: Isolation and Characterization of Immunogenic Components of Anthrax Toxin

PRINCIPAL INVESTIGATOR: S. L. Welkos, Ph.D.

ASSOCIATE INVESTIGATORS: B. E. Ivins, Ph.D.
R. S. Greene, Ph.D.

BACKGROUND

Bacillus anthracis is an important pathogen of animals and of people exposed to infected animals or their products. The organism is a significant BW threat due to the stability of its spores, the nonspecific symptoms of the infection, and the severity of systemic anthrax. Our major goals are to understand the pathogenesis of disease and basis of host susceptibility, and to develop an improved vaccine for military personnel.

B. anthracis produces a three-part exotoxin which appears to have an essential role in the disease manifestations of anthrax. This toxin is composed of the protein components: protective antigen (PA), edema factor (EF), and lethal factor (LF). A major goal of research in anthrax is to clone and express the genes encoding the individual components. The initial recombinant work on anthrax toxin has focused on cloning of the gene for PA. This protein is required to mediate the toxic effects of EF and LF and it induces immunity to infection. A fragment of DNA from *B. anthracis* was cloned into an *Escherichia coli* plasmid vector, but expression of the PA gene was low (1). The goal of current research is to determine the sequence within the cloned DNA that encodes the PA protein, identify the structural domains of PA associated with biological and immunogenical activity, and improve the yield of PA by subcloning the gene into expression vectors. Recently *E. coli* fusion vectors have been developed to simplify cloning and analysis of gene products (2). These new plasmid vectors allow the construction of hybrid genes, composed of cloned DNA fused with the *lac* Z gene of *E. coli*. A hybrid protein is produced, consisting of the product of the cloned DNA fused to β-galactosidase, the product of *lac* Z. By using this technique, a series of gene fusions can be isolated, each having differing lengths of the cloned gene fused to *lac* Z. Analyses of the protein fusions that result would help to define the domains of the PA molecule responsible for induction of protective immunity, recognition of target cell receptors, and binding to LF and EF.

Additional collaborative approaches to the cloning and optimal expression of virulence factors and vaccine antigens are being pursued. These studies include the cloning of the PA gene into the gram-positive host, *Bacillus subtilis* (to improve production of recombinant PA); transposon mutagenesis of the *B. anthracis* genome;

and subcloning of toxin component genes into *E. coli* expression plasmids. Recently, the nucleotide sequence of the protective antigen gene was determined and this information is facilitating our research. Also, we developed a murine model of anthrax which is being used to assay the safety and efficacy of new vaccine candidates and to identify host defense mechanisms.

SUMMARY

The PA gene of *B. anthracis* was inserted into the fusion vector, and six to seven classes of mutants were isolated which have different lengths of the insert fused to the β-galactosidase gene. Isolation of PA-containing, fusion proteins was attempted, and proteins were characterized electrophoretically, immunologically, and enzymatically. Candidate proteins consisting of PA fused to β-galactosidase could not be clearly demonstrated. However, the nucleotide sequence of the PA gene, which was recently determined, is allowing us to better exploit new techniques of cloning and vaccine production. In collaboration with Dr. B. Ivins, the PA gene was subcloned into a *B. subtilis* host; production of the PA protein was characterized; and protective immunization studies was begun (3). Additional collaborative studies were initiated with Drs. Bruce Ivins and Robert Greene to investigate systems that could be used to identify and express vaccine antigens and virulence factors. These projects include transposon mutagenesis of the *B. anthracis* genome and cloning of toxin component genes into an *E. coli* plasmid-expression vector. Finally, we developed an inbred mouse model of anthrax and are using it to identify host mechanisms critical for defense against *B. anthracis* and to assay safety and efficacy of prototype anthrax vaccines, ie. the *B. subtilis*, PA-producing strains (4).

PUBLICATIONS

1. Welkos, S. L., T. J. Keener, and P. H. Gibbs. 1986. Differences in susceptibility of inbred mice to *Bacillus anthracis*. *Infect. Immun.* 51:795-800.

2. Ivins, B. E., and S. L. Welkos. 1986. Cloning and expression of the *Bacillus anthracis* protective antigen gene in *Bacillus subtilis*. *Infect. Immun.* 54:537-542.

PRESENTATIONS

1. Welkos, S. L., and T. Keener. 1986. Susceptibility of mice to anthrax and genetics of resistance to the Sterne vaccine strain. Presented at the Annual Meeting of the American Society for Microbiology, Washington, DC, March.

LITERATURE CITED

1. Vodkin, M. H. and S. H. Leppla. 1983. Cloning of the protective antigen gene of *Bacillus anthracis*. *Cell* 34:693-697.

2. **Berman, M. L., and D. E. Jackson.** 1984. Selection of *lac* gene fusions in vivo: *ompR-lacZ* fusions that define a functional domain of the *ompR* gene product. *J. Bacteriol.* 159:750-756.

3. **Ivins, B. E., and S. L. Welkos.** Cloning and expression of the *Bacillus anthracis* protective antigen gene in *Bacillus subtilis*. *Infect. Immun.* 54:537-542.

4. **Welkos, S. L., T. J. Keener, and P. H. Gibbs.** 1986. Differences in susceptibility of inbred mice to *Bacillus anthracis*. *Infect. Immun.* 51:795-800.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA309249	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 85 12 16	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	132
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code)
Medical Defensive Studies on Crimean-Congo Hemorrhagic Fever Virus

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 12	86 12	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	1.0	230
87	1.0	175

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Disease Assessment Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD. 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Watts, D M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7244
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION L	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) RAI; (U) Crimean-Congo Hemorrhagic Fever; (U) Arbovirus; (U) Lab Animals; (U) Mice; (U) Guinea Pigs; (U)Nairovirus; (U) Bunyaviridae

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) To evaluate and develop virological and immunological techniques, and an animal model system representing human Crimean Congo hemorrhagic fever (CCHF) virus. The knowledge gained will strengthen the U.S. military's capabilities to combat natural or BW CCHF human viral infections.

24. (U) Evaluate techniques for propagating and detecting CCHF virus; the most sensitive, specific, and practical methods will be made standard. By a standard approach, an animal species will be selected for CCHF modeling. Candidate techniques and the animal model will be perfected for use in implementing comprehensive, multidisciplinary, biomedical investigations.

25. (U) 8512 - 8609 A reliable in-vitro quantitative plaque assay employing a human adenocarcinoma cell line has been developed and shown to be effective for propagating and detecting CCHF viral strains from Asia, the Middle East, and Africa. Preliminary results indicate that this technique can also be employed successfully as a virus-specific antibody assay. Other CCHF viral and antibody techniques have been developed, including the direct and indirect fluorescent antibody assay and the agar-gel diffusion assay, but these techniques remain to be evaluated. In-vitro replication of CCHF virus was readily inhibited by ribavirin, thus demonstrating its potential as a therapeutic drug. In-house collaborative research was initiated to define the molecular and biochemical properties of CCHF viruses, and to assess subhuman primates as a model for describing the histopathology and immunopathogenesises of CCHF viral infection.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-LA-132: Medical Defensive Studies on Crimean-Congo Hemorrhagic Fever Virus

PRINCIPAL INVESTIGATOR: D. M. Watts, Ph.D.

BACKGROUND

Crimean-Congo hemorrhagic fever (CCHF) is a severe and often fatal, tick-borne, viral disease of man. The geographical distribution, which includes several countries in African, Asia, Middle East, and Europe, is among the largest of all areas of recognized, arthropod-borne, viral diseases affecting human populations. Morbidity rates appear to be higher than for other arboviral diseases, and mortality rates during outbreaks have ranged from 15 to more than 50%. Despite the recognized human health significance, effective prevention and treatment strategies are lacking. This can be attributed largely to the lack of and/or unreliable biotechnology needed to generate knowledge required to understand the ecology, epidemiology, pathogenesis, and molecular biology of CCHF virus. Thus, the objective of this research is to establish the technological capability for developing effective strategies to combat the natural or biological warfare threat posed by this virus to U.S. military forces.

SUMMARY

We have employed a pioneering approach in an attempt to develop basic quantitative serological and virological techniques for CCHF virus. More than 10 continuous vertebrate cell lines and one continuous tick cell line were evaluated for propagating the 10200 viral strain, and for use as a plaque assay. Although all cell lines were susceptible to infection, VERO, SW-13 (human adenocarcinoma), and *Dermacentor variabilis* tick cultures yielded the highest viral titers, ranging from 10^6 to $10^{7.0}$ PFU per ml. Among the different cell lines, VERO and CW-13 proved to be superior for plaquing the 10200 viral strain; however, the more pathogenic strains from Africa, the Middle East, and the Soviet Union, consistently produced plaques only in the SW-13 cell line. Furthermore, plaques were clearly visible by days 3 and 4 post-infection, in contrast to 7 or more days for VERO cells. Preliminary observations indicated that the plaque assay employing SW-13 cells may also be suitable for performing plaque-reduction neutralization tests. Emphasis will continue to focus on the perfection of the latter technique and studies are in progress to develop other indirect and direct techniques for detecting CCHF viral antigen and antibody.

While definite studies have not been conducted, observations revealed that the replication of the 10200 CCHF viral strain was readily inhibited by ribavirin. Studies are in progress to assess the

potential of this drug, as well as interferon, for suppressing the in vitro replication of the more virulent CCHF viral strains.

PUBLICATIONS

1. **Watts, D. M., G. G. Clark, R. R. Pinger, and C. L. Crabbs.** 1986. Experimental assessment of the susceptibility of *Aedes* mosquitoes to infection with Jamestown Canyon virus. *J. Med. Entomol.* 23:454-456.

2. **Watts, D. M., G. G. Clark, C. L. Crabbs, C. Rossi, and T. Olin.** 1986. Ecological evidence against transovarial transmission of Eastern equine encephalitis virus on the Delmarva Peninsula. *J. Med. Entomol.* (In Press).

3. **Watts, D. M., D. S. Burke, B. A. Harrison, A. Nisalak, and R. W. Whitmire.** 1986. Effects of temperature on the transmission of dengue virus type 2 by *Aedes aegypti*. *Am. J. Trop. Med. Hyg.* (In Press).

4. **Clark, G. G., C. L. Crabbs, D. M. Watts, and C. L. Bailey.** 1986. An ecological study of Jamestown Canyon virus on the Delmarva Peninsula with emphasis on its possible vector. *J. Med. Ent.* 23:588-599.

5. **Clark, G. G., F. J. Dein, C. L. Crabbs, J. W. Carpenter, and D. M. Watts.** 1986. Antibody response for sandhill and whooping cranes to an Eastern equine encephalitis (EEE) virus vaccine. *J. Wildl. Dis.* (In Press).

6. **Carpenter, J. W., G. G. Clark, and D. M. Watts.** 1986. The impact of Eastern equine encephalitis virus on the recovery of the endangered whooping crane. *Ann. Proc. Internat. Council for Bird Preservation* (In Press).

REVIEW ARTICLES

1. **DeFoliart, G. R., P. R. Grimstad, and D. M. Watts.** 1986. Advances in mosquito-borne arboviruses/vector research. *Ann. Rev. Entomol.* (In Press).

2. **DeFoliart, G. R., D. M. Watts, and P. R. Grimstad.** 1986. Changing patterns in mosquito-borne arboviruses. *Am. J. Mosq. Control Assoc.* (accepted for publication).

BOOK CHAPTERS

1. **Watts, D. M., T. G. Ksiazek, K. J. Linthicum, and H. Hoogstraal.** 1986. Crimean-Congo hemorrhagic fever. *In* Epidemiology of arthropod-borne viral diseases, T.G. Monath (ed.), (submitted for publication).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA303476	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 85 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	138
b. CONTRIBUTING				
c. CONTRIBUTING	None			

11. TITLE (Precede with Security Classification Code) (U) Application of Recombinant DNA Technology to Develop New Generation of Q Vaccines

12. SUBJECT AREAS 0613 Microbiology; 1503 Defense; 0601 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 02	87 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE | d. AMOUNT
e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	1.0	150
87	1.0	78

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Airborne Diseases Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Vodkin, M H
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7453
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Stephenson, E H
MILITARY/CIVILIAN APPLICATION: H	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Williams, J C

22. KEYWORDS (Precede EACH with Security Classification Code) (U) DNA Recombinant Technology; (U) Vaccine; (U) Q Fever; (U) Coxiella burnetii (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) *Coxiella burnetii* is perceived to be a prime candidate for biological warfare (BW). Current vaccines are reasonably effective against this rickettsial disease, but cause many side-effects. This is a pioneering study to determine the feasibility of using DNA recombinant technology to develop an entire new generation of safe and highly effective vaccines against Q fever. If successful, U.S. troops can be immunized without lost time due to sterile abscesses and sore arms.

24. (U) Standard techniques of gene cloning will be used to transform a host bacterial cell with *Coxiella burnetii* DNA. Screening for proteins in recombinant clones will be pursued by a number of techniques. Any clone producing an antigen that is recognized by specific antisera becomes a candidate for a vaccine seed. This study was approved by Institutional Biosafety Committee of WRAIR representing DoD and the Office of Recombinant DNA Activities, National Institutes of Health (NIH).

25. (U) 8410 - 8509 A number of cosmid clones screened positive with different immune sera. One of them, p JB 196, was examined more closely. After subcloning, a 5 kilobase fragment has been shown to encode a 62 kilodalton (kD) polypeptide and the amino terminal fragment of a 29.5 kD polypeptide. Both of these proteins are immunodominant antigens and the vaccine potential of them is being assessed.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-00-138: Application of Recombinant DNA Technology to Develop New Generation of Q Vaccines

PRINCIPAL INVESTIGATOR: M. H. Vodkin, Ph.D.

ASSOCIATE INVESTIGATORS: J. C. Williams, Ph.D.
E. H. Stephenson, COL, DVM, Ph.D.

BACKGROUND

Q fever, the disease caused by *Coxiella burnetii*, is distributed worldwide. In humans, it most frequently leads to an acute, debilitating, pulmonary disease. In about 5% of these cases, a chronic form of the disease involving liver (granuloma) or cardiac tissue (endocarditis) ensues. The acute disease could be disruptive to military operations and at-risk civilians in endemic areas. The chronic disease would have long-term implications for both military and civilian populations in the absence of immunization. The currently available phase I, whole-cell vaccine has led to undesirable reactions in a proportion of the vaccinees. There is evidence that reactogenic and immunogenic properties reside in different compounds. The main purpose of this work unit is to clone the DNA of the pathogen and screen the clones for the production of immunogenic compounds. This approach will complement biochemical attempts to separate immunogens from reactogens and has the advantage of not requiring large amounts of biological material, which must be produced in P-4 containment facilities.

At the DNA level, heterogeneity has been demonstrated with restriction fragment-length polymorphism (RFLP). A deletion of chromosomal DNA has been implicated in one of the RFLPs to be associated with alterations in lipopolysaccharide structure and virulence components. The portion of DNA which covers the deletion has been cloned successfully. In addition to chromosomal DNA, all strains examined so far contain a 37 to 45-kilobase plasmid. Plasmids also show variability, and the variations are associated with organisms that can lead to altered pathology.

SUMMARY

Our work concerning a deletion of chromosomal DNA involved in the phase transition of *Coxiella burnetii* has been expanded. We have cloned another strain carrying a similar, but larger, deletion and it appears to control functions involved in lipopolysaccharide functions.

We have cloned the plasmid characteristic of laboratory strains of *C. burnetii*. Killed *Escherichia coli* bearing this plasmid specifically induced an antibody that strongly cross-reacted with a 24-kd polypeptide of *C. burnetii*. Thus, the plasmid appears to encode

for the polypeptide. This is the first function ascribed to a rickettsial plasmid.

One of the cosmid clones of chromosomal DNA screened positive with both a hyperimmune and a monoclonal antibody. The hyperimmune serum recognized a 62-kd polypeptide in lysates from both *C. burnetii* and the recombinant *E. coli*. The monoclonal antibody reacted with a 24-kd polypeptide of *C. burnetii*. However, the recombinant *E. coli* reacted with monoclonal antibody only in a colony blot assay. Since the monoclonal antibody did not react with a specific polypeptide, our data are compatible with the successful cloning and expression of a small peptide of the 24-kd protein. Our hypothesis is that a 5-kd truncated polypeptide, which is the amino terminal portion of an immunodominant 29.5-kd polypeptide, is also encoded by that clone.

PUBLICATIONS

1. **Vodkin, M. H., J. C. Williams, and E. H. Stephenson.** 1986. Genetic heterogeneity among isolates of *Coxiella burnetii*. *J. Gen. Microbiol.* 132:455-463.

2. **Vodkin, M. H., and J. C. Williams.** 1986. Overlapping deletion in two spontaneous phase variants of *Coxiella burnetii*. *J. Gen. Microbiol.* 132:2587-2594.

3. **Stover, C. K., M. H. Vodkin, and E. V. Oaks.** 1986. Use of conversion adaptors to clone antigen genes in *ggtll*. Submitted to Nucl. Acid Res.

PRESENTATIONS

1. **Vodkin, M. H., and J. C. Williams.** 1986. Cloning and subcloning plasmids of *Coxiella burnetii* for diagnosis of altered pathogenicity. Presented at the First Annual American Society for Microbiology Conference on Biotechnology, Washington, DC, March.

2. **Vodkin, M. H.** 1986. Cloning of antigens from *Coxiella burnetii*. Presented at the Sixth American Society for Rickettsiology Meeting, Williamsburg, September.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA305755	86 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISS'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	139
b. CONTRIBUTING			LA	
c. CONTRIBUTING	None			

11. TITLE (Precede with Security Classification Code) (U) In Vitro Effect of Hemorrhagic Fever Viruses on Endothelial Cells

12. SUBJECT AREAS 0613 Microbiology; 0605 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 12	87 06	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE	EXPIRATION
b. CONTRACT/GRANT NUMBER	
c. TYPE	d. AMOUNT
e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	1.0	220
87	1.0	158

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Medical Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Lewis R M
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7655
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available)
MILITARY/CIVILIAN APPLICATION: L	g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Endothelial Cells; (U) Coagulation Factors; (U) Hemostasis; (U) Hemorrhagic Fever Viruses (U) RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) Devise an in vitro model to determine the pathogenesis of the hemostatic derangement produced by hemorrhagic fever viruses. This work will ultimately benefit military personnel exposed to these viruses either in endemic areas or because of their use in biological warfare.

24. (U) Endothelial cell cultures utilizing human, bovine, and hybrid cells will be infected with hemorrhagic fever viruses and the effects on these cells, particularly with regard to hemostatic functions, will be determined.

25. (U) 8510 - 8609 Cultures of endothelial cells have been routinely grown for several passages. These cultures have been shown to be susceptible to productive infection by Ebola, Marburg and Lassa viruses. Other cell lines which have been established in the laboratory because of their importance for coagulation. These cultures, hepatoma cells and monocyte-like cells, have also been shown to allow virus infection. In addition, assays for factor VIII related antigen, a number of prostaglandins and leukotrienes and tissue plasminogen activator are being used to evaluate the effects of these viruses on the respective cell types.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-LA-139: In Vitro Effect of Hemorrhagic Fever Viruses on Endothelial Cells

PRINCIPAL INVESTIGATOR: R. M. Lewis, Ph.D.

BACKGROUND

Hemorrhagic fever is a clinical syndrome which is often associated with a number of viral diseases. These infections are a world health problem. Though not endemic to the United States, they do present health risks to American travelers, especially military personnel who might be stationed in endemic areas. In addition, many hemorrhagic fever viruses can be transmitted by aerosol and are thus potential biological weapons against non-immune American troops.

These diseases share the ability to cause drastic changes in the hemostatic mechanism. It is not clear whether these changes are associated with altered coagulation protein activity, impaired platelet function, or altered capillary reactivity. Pathologically, the changes in hemostasis appear widespread and not confined to specific organs.

The endothelium lines vessels throughout the entire vascular system. Endothelial cells produce important molecules which function in coagulation, platelet aggregation, and capillary integrity. In addition, these cells respond to immune effector molecules and produce immunologically important products.

Alterations in the important control functions exerted by endothelial cells could result in drastic changes in the ability to control bleeding. If we find that viral infection can elicit such changes, we might be able to explain the hemorrhagic syndrome.

SUMMARY

Endothelial cells are known to support viral growth of Ebola, Marburg, and Lassa hemorrhagic fever viruses. We have continued these infectivity studies and have found positive growth of Hantaan and Junin viruses in cells of EA926, an endothelial-like cell line. We have adapted the assay for factor VIII-related antigen to the EA926 culture supernatant; no effect on Ebola-infected cell cultures could be measured. Additional cell lines important for coagulation have been established in our laboratory: the HEPG2 line, hepatocyte-like cells which synthesize vitamin K-dependent coagulation factors; and the U937 line, monocyte-like cells which produce a number of coagulation initiators.

We have found that the U937 cells are permissive for Rift Valley fever virus replication. Furthermore, this replication was enhanced

in cultures that were treated by phorbol ester. Phorbol ester has been demonstrated to induce changes in the U939 cells. These changes are consistent with those seen with monocyte maturation.

We have found that HEPG2 cells support the replication of Pichinde, Crimean-Congo, Marburg, and Hantaan viruses. In addition, our studies have shown that, when these cells are treated with T-2 mycotoxin, there is altered production of prothrombin.

PUBLICATIONS

1. **Cavagnaro, J., and R. M. Lewis.** 1986. Toxicological evaluation of drugs, pp.---. *In* M. Williams and J. B. Melnick (ed.), Drug discovery and development, Chapter - . Humana Press, Clifton, NJ. (In Press).

2. **Cosgriff, T. M., P. B. Jahrling, J. P. Chen, L. A. Hodgson, R. M. Lewis, and D. E. Green.** 1986. Studies of the coagulation system in arenaviral hemorrhagic fever: experimental infection of strain 13 guinea pigs with Pichinde virus. Submitted to *Am. J. Trop. Med. Hyg.*

3. **Lewis, R. M., T. M. Cosgriff, C. J. Peters, and J. C. Morrill.** 1986. Differentiation of a human monocytic cell line is associated with increased production of Rift Valley fever virus by infected cells. Submitted to *J. Infect. Dis.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA306147	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	LA	140
b. CONTRIBUTING				
c. CONTRIBUTING	None			

11. TITLE (Precede with Security Classification Code) (U) Cloning of Military Relevant Toxin Genes for Novel Vaccine Development

12. SUBJECT AREAS 0605 Clinical Medicine; 1503 Defense

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 12	88 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE | EXPIRATION

b. CONTRACT/GRANT NUMBER

c. TYPE | d. AMOUNT

e. KIND OF AWARD | f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	1.0	315
87	1.0	158

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases	a. NAME Pathology Division, USAMRIID
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011	b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L	c. NAME OF PRINCIPAL INVESTIGATOR Smith, L A
d. TELEPHONE NUMBER (include area code) 301-663-2833	d. TELEPHONE NUMBER (include area code) 301-663-7211
21. GENERAL USE FIC	f. NAME OF ASSOCIATE INVESTIGATOR (if available) Baksi, K
MILITARY/CIVILIAN APPLICATION: L	g. NAME OF ASSOCIATE INVESTIGATOR (if available) Alcaide, C

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) BW Defense;(U) Lab Animals; (U) Cloning; (U) Vaccines; (U) Snakes; (U) Toxin; (U) Genes; (U) Arachnids;(U)RAI

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The objectives of this research are to clone, sequence, and express the genes coding for snail conotoxins; scorpion toxins; and the snake toxins, myotoxin, cobrotoxin, cardiotoxin, crotoxin, taipoxin, and bungarotoxins. The knowledge gained is expected to lead directly to the development of novel vaccines for these toxins.

24. (U) Three approaches have been or will be utilized in the cloning of the above toxin genes: (1) synthetic gene cloning, (2) cDNA cloning, and (3) genomic cloning. The genes will be inserted into a suitable vector (plasmid) and transformed into *E. coli*. Cloned DNA will be analyzed with oligonucleotide probes homologous to toxin gene sequences and the DNA, subsequently, sequenced. The products of the clones will be examined immunologically and the genes will be manipulated into producing maximum quantities of products for vaccine development. Finally, site-specific mutagenesis will be utilized at the molecular level to produce non-toxic antigenic proteins.

25. (U) 8510 - 8609 In 1986, we report (1) the synthesis of conotoxin M1, myotoxin, and cobrotoxin on a Biosearch 8600 DNA synthesizer. Conotoxin has been cloned into *E. coli*. (2) The generation of two cDNA libraries, one for *Naja naja atra* sequences (cobrotoxin and cardiotoxin) and one for *Crotalus virdis virdis* sequences (myotoxin). cDNA libraries are being screened for the toxin genes. One clone from the *Naja naja atra* library that hybridized to cobrotoxin probes and one from the *Crotalus virdis virdis* library that hybridized to the myotoxin probes are presently being sequenced. Presumably, these clones contain the toxin genes, and (3) production of a cosmid library containing *Naja naja atra* DNA. This library is presently being screened using a labelled cDNA probe.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-LA-140: Cloning of Military Relevant Toxin Genes for Novel Vaccine Development

PRINCIPAL INVESTIGATOR: L. A. Smith, Ph.D.

ASSOCIATE INVESTIGATORS: K. Baksi, Ph.D.
C. Alcaide, Ph.D.

BACKGROUND

There exists in nature a wide variety of poisonous animals which produce many different types of toxins. Animals, such as spiders, scorpions, beetles, insects, snails, etc., have venom glands which produce a mixture of different compounds, some of which are acutely deleterious to man. In recent years, there has been much research done on the purification, characterization, and pharmacology of toxic factors from various venoms. Although venom toxicity is based on an amalgamation of constituents, one protein family stands out in having very pronounced pharmacological activities. Some members of this family are potent neurotoxins, while others produce cardiotoxic and cytolytic effects. For example, conotoxin omega (snail) and snake toxins, such as notexin, crotoxin, taipoxin, β-bungarotoxin, and textilotoxin, affect presynaptic nerve endings, causing an inhibition in the release of the neurotransmitter, acetylcholine. Other snail conotoxins and snake toxins, such as cobrotoxin and α-bungarotoxin, affect the postsynaptic nerve endings by binding to the acetylcholine receptor, inhibiting the transmission of nerve impulses. There are yet more toxins, such as alpha scorpion toxins, which affect postsynaptic sodium channels, and bee apamin, which affects CNS and spinal cord potassium channels. The commonality in all of the above toxins is the fact that they are low molecular weight protein neurotoxins. The other family of toxins are referred to as membrane-damaging, cardiotoxic, or cytolytic toxins. Representatives of this family are snake cardiotoxins and myotoxins. Although there has been extensive pharmacological and protein chemical studies on the above toxins, genetic investigations on the toxin genes has not been an active research area. Since our goa'・ focus on the development of vaccines against many of these toxins, it is our intent to utilize recombinant DNA technology to clone and express the genes coding for snail contotoxins, scorpion toxins, and snake myotoxin, cobrotoxin, cardiotoxin, crotoxin, bungarotoxin, and taipoxin. The toxoided cloned gene products or non-toxic CRMs, generated from site-directed mutagenesis of the cloned genes, will be tested for their efficacy as vaccines.

SUMMARY

In 1985, we reported (i) the purification, to apparent homogeneity, of cobrotoxin, cardiotoxin, and phospholipase A_2 from the

venom of *Naja naja atra*; (ii) the production of affinity-purified, rabbit antibodies against these three toxins; and (iii) the insertion of liver DNA fragments from *N. n. atra* and *Crotalus vegrandis* into the plasmid vector, pUC8, and the subsequent cloning into *Escherichia coli*. One *C. vegrandis* clone strongly hybridized with an oligonucleotide probe prepared to the acidic subunit of crotoxin, but not to the two probes made to the basic subunit. The cloned DNA is being sequenced. The neurotoxin from *C. vegrandis*, crotoxin, is composed of two, non-identical subunits. Although the basic subunit contains the phospholipase A_2 activity, both components are required for their synergistic neurotoxic action.

In 1986, we report (i) the synthesis of genes for the toxins, conotoxin M1 from the marine snail, *Conus magus*; myotoxin from *C. v. virdis*; and cobrotoxin from *N. n. atra* on a Biosearch 8600 DNA Synthesizer. The cobrotoxin gene was synthesized with the start/stop codons, two restriction enzyme sites, and the bacterial alkaline phosphatase leader sequence. Short, synthetic, oligodeoxyribonucleotide leader sequences described by Narang et al. (1) will be used with the conotoxin and myotoxin genes. Conotoxin M1 has been cloned into *E. coli*. The synthetic genes for the myotoxin and cobrotoxin are presently being cloned into *E. Coli*. (ii) We also report the production of two cDNA libraries, one for *N. n. atra* (venom contains cobrotoxin and cardiotoxin) and one for *C. v. virdis* (venom contains myotoxin). cDNA libraries were screened for the toxin genes by using oligonucleotide probes homologous to the toxin sequences. The DNA from two positive clones, one from *N. n. atra*, which hybridized to the crotoxin probe, and one from *C. v. virdis*, which hybridized to the myotoxin probe, is presently being sequenced. The cDNA from *N. n. atra* is 500 base pairs, and the cDNA from *C. v. virdis* is 450 base pairs. Products from the expression of the two clones have not been detected by polyclonal antibodies prepared against the native toxins. Menez et al. (2) have shown recently that antibodies prepared against reduced and carboxymethylated erabutoxin (a neurotoxin from *Laticauda semifasciata*) bind to the product of the expressed gene in *E. coli*, while antibodies made to the native toxin do not. It has been shown that the unprocessed leader sequence from the expressed erabutoxin inhibits the proper folding of the protein. Presumably, most of the snail, snake, and scorpion toxins will have signal sequences which will not be processed in *E. coli*. The leader sequences will have to be removed by using either site-directed mutagenesis (to create a specific sensitive site for CNBr or protease cleavage) or by cloning into another host that will process the leader sequences. Our future research will pursue both avenues, with the erabutoxin and cobrotoxin as models.

PRESENTATIONS

1. Smith, L. A. 1986. Effect of presynaptic neurotoxins on the uptake of [^{3}H]-myoinositol in guinea pig synaptosomes. Presented at the Annual Meeting of the European International Society of Toxicology, Prague, Czechoslovakia, August.

LITERATURE CITED

1. Sung, L. W., P. L. Yao, D. M. Zahab, and S. A. Narang. 1986. *Proc. Natl. Acad. Sci. U.S.A.* 83:561-565.

2. Tamiya, T., G. G. Prelat, D. Mariat, and A. Menez. (Manuscript in preparation).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA307174	86 10 01	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D. CHANGE	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	141
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) Molecular Approaches to Alphavirus Vaccines

12. SUBJECT AREAS
0605 Clinical Medicine; 1503 Defense; 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 04	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATE EFFECTIVE — EXPIRATION
b. CONTRACT/GRANT NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	0.3	250
87	1.0	122

19. RESPONSIBLE DOD ORGANIZATION
a. NAME USA Medical Research Institute of Infectious Diseases
b. ADDRESS (include zip code) Fort Detrick, MD 21701-5011
c. NAME OF RESPONSIBLE INDIVIDUAL Huxsoll, D L
d. TELEPHONE NUMBER (include area code) 301-663-2833

20. PERFORMING ORGANIZATION
a. NAME Virology Division, USAMRIID
b. ADDRESS Fort Detrick, MD 21701-5011
c. NAME OF PRINCIPAL INVESTIGATOR Dalrymple, J M
d. TELEPHONE NUMBER (include area code) 301-663-2665
f. NAME OF ASSOCIATE INVESTIGATOR (if available)
g. NAME OF ASSOCIATE INVESTIGATOR (if available)

21. GENERAL USE FIC
MILITARY/CIVILIAN APPLICATION L

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Medicine; (U) Vaccines; (U) Alphavirus; (U) RAI; (U) Recombinant DNA; (U) Monoclonal Antibodies; (U) Cross-protection

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The objectives of this research are to: a) develop the ability to rapidly identify DNA sequences in structural protein coding genes of alphaviruses and related virus variants, b) define the major antigenic determinants of alphavirus pathogens of military medical importance and prepare diagnostic reagents, c) evaluate the feasibility of incorporating different viral genes or gene segments into a vaccine virus vector, and d) evaluate the potential prophylactic value of broadly reactive alphavirus immunogens derived from recombinant DNA technology.

24. (U) Investigate alphaviruses that are antigenically closely related. Lymphocyte hybridomas will be prepared, and specific antibodies to determinants associated with neutralization and/or animal protection will be emphasized. Genes coding for the structural proteins of selected alphaviruses will be cloned and sequenced. Sequences conserved among alphaviruses will be used for rapid sequence analysis of different isolates and virus mutations. Sequences will be inserted into vaccinia virus as a vector for the expression of potentially protective immunogens.

25. (U) 8504-8509-A series of 11 different alphavirus isolates have been propagated to high titers, plaque-purified to provide genetically homogeneous populations, and polyclonal hyperimmune antisera prepared to each. Antigenic cross-reactivity has been evaluated by radioimmune assay and plaque reduction neutralization testing. Viral purification, nucleic acid extraction, and RNA analysis are being performed preparatory to cloning.

PROJECT NO. 3A161101A91C: Independent Laboratory In-House Research

WORK UNIT NO. 91C-00-141: Molecular Approaches to Alphavirus Vaccines

PRINCIPAL INVESTIGATOR: J. M. Dalrymple, Ph.D.

BACKGROUND

Conventional methodology that leads to protection of humans against viral infections requires the development of a live, attenuated or inactivated vaccine for each individual virus posing a threat to U.S. military personnel. However, the number of different viral pathogens, strains, and variants clearly demonstrates the complexity of such an undertaking. The development of recombinant DNA technology has provided a mechanism for dissecting viral gene products and examining their individual pathogenic effects and their potential for use in immunization and protection against virus-induced disease. If the individual genes coding for protective immunogens could be identified, it should be possible theoretically to construct a vaccine, capable of broad-spectrum protection, containing multiple genes from a variety of human pathogens.

SUMMARY

The propagation, purification, and characterization of 11 different alphaviruses belonging to the Semliki Forest virus subgroup, including five geographically separate strains of Chikungunya virus, have been accomplished. I have extracted RNA from each of these purified virion preparations by techniques developed to minimize degradation resulting, presumably, from contaminating ribonuclease. Preliminary molecular cloning and sequencing experiments have resulted in small clones of cDNA from two strains of chikungunya virus and a strain of Mayaro virus. Contract efforts have resulted in the cloning of significant portions of the O'nyong nyong viral genome. Continued cloning of the complete structural protein-coding regions of each of the alphaviruses under consideration will be required for the successful completion of the project.

Polyclonal antisera have been prepared to each of the viruses and cross-neutralization and cross-protection tests performed. In the absence of an optimal animal protection model for each virus, preliminary data suggest that protection of mice from lethal chikungunya viral infection may be more cross-reactive than the plaque-reduction neutralization test suggests. Lymphocyte hybridomas secreting monoclonal antibodies have been prepared to three of the alphavirus strains and high-titer mouse ascitic fluids obtained for detailed analysis. Neutralizing monoclonal antibodies were not abundant in these original hybridoma libraries, which may necessitate additional fusions to obtain antibodies to these important epitopes. Epitope mapping of the monoclonal antibodies is in progress by using competitive radioimmune assays and "western blotting" procedures.

A collection of Sindbis viral strains representing isolates from throughout the world has been added to this study to investigate the extent of genome variation occurring in natural habitats. The genome of Sindbis virus has been completely cloned and sequenced, providing a prototype nucleotide sequence against which the variants will be compared. Preliminary testing in animals suggests that this virus collection contains isolates which vary in their mouse neurovirulence and reactivity with a previously characterized battery of Sindbis monoclonal antibodies.

APPENDIX A

PUBLICATIONS BY

UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE FOR INFECTIOUS DISEASES

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1. **Allam, I. H., P. M. Feinsod, R. McN. Scott. C. J. Peters, A. J. Saah, S. A. Ghaffar, S. El Said, and M. A. Darwish.** 1986. Rift Valley fever surveillance in mobile sheep flocks in the Nile delta. *Am. J. Trop. Med. Hyg.* 35:1055-1060.

2. **Amano, K-I., J. C. Williams, S. R. Missler, and V. N. Reinhold.** 1986. Structural and biological relationships of *Coxiella burnetii* lipopolysaccharides: chemical composition and microheterogeneity of smooth, semi-rough, and rough LPS. Submitted to *J. Biol. Chem.*

3. **Anderson, A. O., O. L. Wood, A. D. King, and E. H. Stephenson.** 1986. Studies of antiviral mucosal immunity using the lipoidal amine adjuvant Avridine. Submitted to *Adv. Exp. Med. Biol.*

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14. **Clark, G. G., F. J. Dein, C. L. Crabbs, J. W. Carpenter, and D. M. Watts. 1986.** Antibody response of sandhill and whooping cranes to an eastern equine encephalitis (EEE) virus vaccine. Submitted to *Am. J. Vet. Res.*

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118. **Wilson, K. E., and D. M. Driscoll.** 1986. High containment isolation: a unique patient care modality. Submitted to *R N.*

119. **Wood, O. L., J. M. Meegan, J. Morrill, and E. Stephenson.** 1986. Rift Valley fever, p. . *In* Z. Dinter and B. Morein (ed.), Viral infections of ruminants. Elsevier, Amsterdam. To be published.

APPENDIX B

CONTRACTS, GRANTS, MIPRS, and PURCHASE ORDERS IN EFFECT

UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

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CONTRACT NUMBER	TITLE, INVESTIGATOR, INSTITUTION
DAMD-17-86-C-6063	Structure-Function Relationship of Hydrophiidae Postsynaptic Neurotoxins. A. Tu, Colorado State University, Fort Collins, CO.
DAMD-17-86-G-6016	Epidemiology and Epizootiological Investigations of Haemorrhagic Fever Viruses in Kenya. P.M. Tukei, Virus Research Center (KMRI), Nairobi, Kenya.
DAMD-17-85-C-5002	Analysis of Trichothecene Mycotoxins by Combined HPLC-MS. P. Vouros, Northeastern University, Boston, MA.
DAMD-17-86-C-6041	Synthesis of Nucleoside Analogues with Potential Antiviral Activity against Negative Strand RNA Virus Targets. R.D. Walker, Birmingham University, Birmingham, England
PO-84-PP-4828	Kinetics of Percutaneous Absorption of Mycotoxins In Vitro. B.M. Wallner, U.S. Department of Agriculture, Athens, GA.
MIPR-85-MM-5511	Preparation and Structural Analysis of Toxins, and Modeling of Toxins, Antibody Binding Sites and Antiviral Drugs. K.B. Ward, Naval Research Laboratory, Washington, DC.
DAMD-17-85-G-5030	Engineered Organisms in the Environment: Scientific Issues. R.D. Watkins, American Society for Microbiology, Washington, DC.
DAMD-17-85-C-5192	Toxin Photoaffinity Probes. D.S. Watt, University of Kentucky, Lexington, KY
DAMD-17-86-C-6075	Mechanism of Action of Low Molecular Weight Toxins in the Cardiovascular System. W.T. Woods, Alabama University, Birmingham, AL.
DAMD-17-83-G-9565	Studies of Biological and Molecular Basis of the Inhibition of Activity of Phagocytic Cells by Anthrax Toxin. G.G. Wright, University of Virginia School of Medicine, Charlottesville, VA.

DAMD-17-86-C-6134	Biosystematics of *Aedes* (Neomelaniconion). T.J. Zavortink, University of San Francisco, San Francisco, CA.
PO-85-PP-5825	Pathophysiology of Peptide Toxins of *M. Aeruginosa* and *A. Phylloides*. W.H. Adams, Department of Energy, Upton, NY.
DAMD-17-86-C-6107	Research in Drug Development for Therapeutic Treatment of Neurotoxin Poisoning: Studies of Conotoxins. A. Almquist, SRI International, Menlo Park, CA.
DAMD-17-86-C-6101	Use of Nucleic Acid-Protein Cytophotometry and Cytointerferometry in Characterizing Cellular Mode of Trichothecene Toxicant Action. A. Anthony, Pennsylvania State University, University Park, PA.
DAMD-17-85-G-5006	Seroepidemiological Survey for Congo-Crimean Hemmorrhagic Fever and Hantaan Virus. A. Antoniadis, University of Thessaloniki, Aristolean Thessaloniki, Greece.
DAMD-17-85-C-5171	Characterization of the *P. Brevis* Polyether Neurotoxin Binding Component in Excitable Membranes. D. Baden. University of Miami, Coral Gables, FL.
DAMD-17-85-C-5236	A Multi Disciplinary Study of Ciguatoxin and Related Low Molecular Weight Toxins from Marine Sources. L.E. Bailey, University of Hawaii, Honolulu, HI.
DAMD-17-86-C-6119	Combination Chemotherapy using Immune Modulators and Antiviral Drugs Against Togaviruses and Bunyaviruses. S. Baron, University of Texas, Galveston, TX.
PO-86-PP-6814	Regulatory Peptides: Behavioral and Neurochemical Effects. J.E. Barrett, Uniformed Services University, Bethesda, MD.
DAMD-17-85-C-5241	Pathophysiology and Toxicokinetic Studies of Blue-Green Algae Toxication in the Swine Model. V. Beasley, University of Illinois, Urbana, IL.
DAMD-17-86-C-6056	Mechanisms of Action of Clostridial Neurotoxins on Dissociated Mouse Spinal Cord Neurons in Cell Culture. G. Bergey, University of Maryland, Baltimore, MD.

DAMD-17-86-G-6012	Second American Symposium on Animal, Plant and Microbial Toxins. A.L. Bieber, Arizona State University, Tempe, AZ.
DAMD-17-84-G-4035	Genetic and Molecular Studies of the Phlebotomus Fever Group of Viruses. D.H.L. Bishop, Natural Environment Research, Swindon, UK.
DAMD-17-86-C-6057	Development and Testing of an In Vitro Assay for Screening of Potential Therapeutic Agents Active Against Na Channel Neurotoxins. G.B. Brown, University of Alabama, Birmingham, AL.
DAMD-17-86-C-6234	Synthetic Vaccines for the Control of Arenavirus Infection. M.J. Buchmeier, Scripps Clinic and Research Foundation, La Jolla, CA.
DAMD-17-83-C-3013	Synthetic Peptide Vaccines for the Control of Arenavirus Infection. M.J. Buchmeier, Scripps Clinic and Research Foundation, La Jolla, CA.
DAMD-17-85-C-5224	Diagnosis and Management of Trichothecene Toxicosis in the Swine Model. W.B. Buck, University of Illinois, Urbana, IL.
DAMD-17-84-C-4146	The Use of a Resin Bound Monoclonal Antibody in the Purification of the Various Components in Anthrax Vaccines. J.W. Burnett, University of Maryland, Baltimore, MD.
DAMD-17-86-C-6080	Studies on the Binding Site for Staphylococcal Enterotoxins on Murine Lymphoid Cells. S.B. Buxser, The Upjohn Company, Kalamazoo, MI.
DAMD-17-84-C-4156	Freshwater Cyanobacteria (Blue-Green Algae) Toxins: Isolation and Characterization. W.W. Carmichael, Wright State University, Dayton, OH.
DAMD-17-84-C-4130	Molecular Basis of Paralytic Neurotoxin Action on Voltage-Sensitive Sodium Channels. W.A. Catterall, University of Washington, Seattle, WA.
DAMD-17-85-C-5249	Immunologic Approach to the Identification and Development of Vaccines to Various Toxins. T. Chanh, Georgetown University Medical, Washington, DC.
DAMD-17-86-C-6173	Immunological Techniques for Detection of Fungal and Dinoflagellate Toxins. F.S. Chu, University of Wisconsin, Madison, WI.

DAMD-17-86-C-6154	Evaluation of Immune Response Modifying Compounds Utilizing Virus-Specific Human T Lymphocyte Clones. M. Cohn, Georgetown University, Washington, DC.
DAMD-17-85-C-5266	Human Hybridomas for Exotic Antigens. M. Cohn, The Salk Institute for Biology, La Jolla, CA.
DAMD-17-85-C-5226	Rift Valley Fever Virus: Molecular Biologic Studies of the M Segment RNA for Application in Disease Prevention. M. Collett, Molecular Genetics, Inc., Minnetonka, MN.
DAMD-17-85-C-5009	Development of General Antisera for Trichothecenes. C.E. Cook, Research Triangle Institute, Chemistry and Life Sciences Group, Research Triangle Park, NC.
DAMD-17-86-C-6062	Receptor Binding and Membrane Transport of Botulinum Toxins. J.R. Dankert, University of Florida, Gainesville, FL.
DAMD-17-84-C-4245	Study of Antigenic Structures of Botulinum Neurotoxin. B. Das Gupta, University of Wisconsin, Madison, WI.
DAMD-17-86-C-6120	Development of Methods for Carrier-Mediated Targeted Delivery of Antiviral Compounds Using Monoclonal Antibodies. M. Dawson, SRI International, Menlo Park, CA.
PO-86-PP-6815	The Regulation of a Post-Translational Peptide Acetyltransferase: Strategies for Selectively Modifying the Biological Activity of Neural and Endocrine Peptides. W.R. Millington, Uniformed Services University, Bethesda, MD.
PO-86-PP-6813	Cardiovascular Physiology and Pathophysiology of Substance P. D.E. Dobbins, Uniformed Services University Bethesda, MD.
DAMD-17-84-C-4139	Potential Vaccine for Anthrax. R.J. Doyle, University of Louisville, Louisville, KY.
DAMD-17-85-C-5202	Preparation and Characterization of Antiparalytic Shellfish Poison Poly and Monoclonal Antibodies for Development of Identification and Prophylaxis/Therapy Techniques. P. Duquette. Bio-Metric Systems, Inc., Eden Prairie, MN.
DAMD-17-86-C-6019	Enzyme Immunoassay for T-2 Tetraol (SBIR 85.1). P.H. Duquette, Bio-Metric Systems, Inc., Eden Prairie, MN.

PO-84-PP-4861	The Role of Plasmids and Bacteriophages in Toxigenicity of *Clostridium Botulinum* and Characterization of Converting Bacteriophages. M.W. Eklund, U.S. Department of Commerce, Seattle, WA.
PO-85-PP-5804	Investigate Immunoassay Development for Field Detection of Saxitoxin and Gonyautoxins. R.A. Elston, U.S. Department of Energy, Richland, WA.
PO-86-PP-6800	Fieldable Diagnostic Monoclonal Immunoassay and Immunoprophylactic Reagent for Tetrodotoxin. R. Elston, U.S. Department of Energy, Richland, WA.
PO-86-PP-6807	Acute T-2 Intoxication: Physiologic Consequences and New Therapeutic Approaches. G. Feuerstein, The Uniformed Services University, Bethesda, MD.
PO-86-PP-6811	Dermorphin as a Behavioral and Autonomic Modulator. G. Feuerstein, The Uniformed Services University, Bethesda, MD.
DAMD-17-86-C-6156	Structure and Expression of Genes for Flavivirus Immunogens. M.J. Fournier, University of Massachusetts, Amherst, MA.
DAMD-17-86-G-6004	UCLA Symposia Conference: Positive Strand RNA Viruses. C.F. Fox, The University of California, Los Angeles, CA.
DAMD-17-84-C-4144	Selective Targeting of Antiviral Immunomodulating Agents in the Treatment of Arenavirus Infections. D.J. Gangemi, The University of South Carolina, Columbia, SC.
DAMD-17-86-C-6118	Biology of Immunomodulators. D.J. Gangemi, The University of South Carolina, Columbia, SC.
DAMD-17-86-C-6239	Research, Development and Delivery of Second Generation Fiber Fluorescent Immunoassay Instrument. T.R. Glass, ORD, Cambridge, MA.
DAMD-17-86-C-6071	Development of Monoclonal Antibodies Against Viral Agents. M. Haspel, Litton Bionetics, Inc., Kensington, MD.
DAMD-17-85-C-5047	Analysis of a Pilot Study on Acute and Subchronic Toxicology of T-2 Toxin in Cynomologus Monkeys. C. Hassler, Battelle Columbus Laboratories, Columbus, OH.

PO-84-PP-4854	Expression of Immunogenic Virus Proteins in Eukaryotic Vector Systems. J. Hay, The Uniformed Services University, Bethesda, MD.
DAMD-17-85-C-5054	*Bordetella* Extracytoplasmic Adenylate Cyclase: Structural and Functional Analogies with *Bacillus Anthracis* Edema Factor Adenylate Cyclase. E.L. Hewlett, The University of Virginia, Charlottesville, VA.
DAMD-17-86-C-6059	An In Vitro System for Studying Presynaptically Acting Neurotoxins. B. Howard, The University of California, Los Angeles, CA.
DAMD-17-86-C-6055	Cloning, Sequencing and Structural Manipulation of The Enterotoxin D and E Genes from *Staphylococcus aureus*. J.J. Iandola, Kansas State University, Manhatten, KS.
PO-86-PP-6816	Use of Radar for Malaria and Rift Valley Fever Vector Breeding Habitat Delineation. M. Imhoff, NASA Goddard Space Flight Center, Greenbelt, MD.
DAMD-17-85-C-5129	Preparation of Radio Labelled Macrocyclic Trichothecenes, Simple Trichothecenes for Generation of Generic Antibodies. B.B. Jarvis, The University of Maryland, College Park, MD.
DAMD-17-86-C-6017	Enterotoxins: Synthetic Peptide Approach to Study of Structure/Function and Immune Properties. H.M. Johnson, The University of Florida, Gainesville, FL.
DAMD-17-86-C-6110	Development of Synthetic Immunizing Agents Against Staphylococcal Enterotoxins. A.K. Judd, SRI International, Menlo Park, CA.
DAMD 17-86-C-6061	Crotoxin: Structural Studies, Mechanism of Action and Cloning of its Gene. I.I. Kaiser, The University of Wyoming, Laramie, WY.
DAMD-17-85-G-5020	Tetrodotoxin, Saxitoxin, and The Molecular Biology of The Sodium Channel. C.Y. Kao, The New York Academy of Science, New York, NY.
DAMD-17-85-C-5280	Mechanism of Action of Tetanus Toxin. M. Klempner, New England Medical Center Hospital, Boston, MA.
DAMD-17-85-C-5008	The Synthesis of Radiolabelled Mycotoxins. G.A. Kraus, Iowa State University, Ames, IA.

DAMD-17-86-G-6006	Small Molecular Weight Mycotoxins: The Trichothecenes. R. Krauss, Federation of American Societies for Experimental Biology. Bethesda, MD.
PO-85-PP-5872	Structure Elucidation of Algal Toxins. T. Krishnamurthy, Chemical Research and Development Laboratory, Aberdeen Proving Ground, Aberdeen, MD.
DAMD-17-85-C-5283	Functional Consequences of Chemical Modification of The Saxitoxin Binding Site on Neuronal Sodium Channels. B.K. Krueger, The University of Maryland, Baltimore, MD.
DAMD-17-86-G-6024	Seminar on Carrier-Mediated Antiviral Therapy. E. Kurstak, The University of Montreal, PQ, Canda.
DAMD-17-86-C-6121	Screening of Immunoenhancing Drugs with Antiviral Activity Against Members of The Arena-, Alpha-, and Adenoviridae. P.A. Leblanc, The University of Alabama, University, AL.
DAMD-17-86-G-6011	Hemorrhagic Fever with Renal Syndrome (HFRS) (Korean Hemorrhagic Fever). H-W. Lee, Korean University Medical College, Seoul, Korea.
DAMD-17-84-G-4016	Hemorrhagic Fever with Renal Syndrome (Korean Hemorrhagic Fever). H-W. Lee, Korean University Medical College, Seoul, Korea.
DAMD-17-86-C-6043	Neurotoxin Binding Site on The Acetylcholine Receptor. T.L. Lentz, Yale University, New Haven, CT.
PO-85-PP-5844	Immunologic Studies on The Effect of Trichothecenes. D. Lubaroff, Veterans Administration, Iowa City, IA.
MIPR-85-MM-5512	Development of Vaccine to Mycotoxin T-2. V. Manohar, Immuquest Labs, Rockville, MD.
DAMD-17-83-C-3020	Approaches to The Detoxication of Mycotoxins Using Glutathione Precursors and Analogs. A. Meister, Cornell University Medical College, New York, NY.
DAMD-17-86-C-6047	Detoxication of Mycotoxins and Other Toxins and Compounds of Military Interest. A. Meister, Cornell University Medical College, New York, NY.

DAMD-17-85-C-5204	Metabolism, Mass Spectral Analysis and Mode of Action of Trichothecene Mycotoxins. C. Mirocha, University of Minnesota, St. Paul, MN.
DAMD-17-86-C-6117	Efficacy and Mode of Action of Immune Response Modifying Compounds Against Alphaviruses and Flaviviruses. P.S. Morahan, Medical College of Pennsylvania, Philadelphia, Pa.
DAMD-17-86-G-6028	Epidemiology, Haemorrhagic Fever In Kenya. R.P. Mouton, University Hospital, Leiden, The Netherlands.
PO-85-PP-5833	Synthesis of Prodrugs of L-Cysteine as Prophylactic Agents Against Low Molecular Weight Toxins. H.T. Nagasawa, VA Medical Center, Minneapolis, MN.
DAMD-17-86-C-6001	Rare 2-Substituted Purine Nucleosides. V. Nair, University of Iowa, Iowa City, IA.
DAMD-17-82-C-2004	Mechanisms of Bunyavirus Virulence; A Genetic Approach. N. Nathanson, University of Pennsylvania, Philadelphia, PA.
DAMD-17-86-C-6002	Synthesis of Nucleoside Mono- and Dialdehydes as Antiviral Agents. J.P. Neenan, Rochester Institute of Technology, Rochester, NY.
DAMD-17-85-C-5208	Trichothecenes Mycotoxin Studies. P.M. Newberne, Massachusetts Institute of Technology, Cambridge, MA.
DAMD-17-86-C-6073	Study of Nephropathia Epidemica in Sweden. B. Niklasson, National Bacteriological Laboratory, Stockholm, Sweden.
PO-86-PP-6803	Collaborative Research Program on Seafood Toxins. S.W. Page, Department of Health and Human Services, Washington, DC.
DAMD-17-85-C-5232	Genetically-Engineered Poxviruses and The Construction of Live Recombinant Vaccines. E. Paoletti, Laboratory of Immunology, Albany, NY.
DAMD-17-85-C-5274	*Coxiella burnetii* Lipopolysaccharides: Structural Characterization, Chemical Synthesis and Immunogen Development. V. Reinhold, Harvard School of Public Health, Boston, MA.

DAMD-17-85-C-5167	Use of Recombinant DNA Techniques for The Production of a More Effective Anthrax Vaccine. D. Robertson, Brigham Young University, Provo, UT.
DAMD-17-86-C-6160	Mechanism of The Presynaptic Neurotoxin Tetanus Toxin. T.B. Rogers, The University of Maryland, Baltimore, MD.
DAMD-17-86-C-6133	Studies of Infection and Dissemination of Rift Valley Fever Virus In Mosquitoes. W.S. Romoser, Ohio University, Athens, OH.
MIPR-86-MM-6502	Rapid Screening and Structural Characterization of Biological Toxins. M.M. Ross, Naval Research Laboratory, Washington, DC.
DAMD-17-85-C-5282	Alphavirus Epitopes of Vaccine Relevance. A.L. Schmaljohn, The University of Maryland, Baltimore, MD.
DAMD-17-86-C-6058	Mass-Screening of Curarimimetic Neurotoxin Antagonists. J. Schmidt, State University of New York, Albany, NY.
DAMD-17-85-C-5071	Chemical Preparation Laboratory for IND Candidate Compounds. E. Schubert, Pharm-Eco Laboratories, Inc., Simi Valley, CA.
DAMD-17-86-C-6011	Synthesis Laboratory for USAMRIID Selection Panel. J.A. Secrist, Southern Research Institute, Birmingham, AL.
DAMD-17-84-C-4135	The Synthesis of Certain Carbocyclic Nucleoside Analogs as Antiviral Agents. J.A. Secrist, Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6003	Drug Development Against Viral Diseases of Military Importance. J.A. Secrist, Southern Research Institute, Birmingham, AL.
DAMD-17-84-C-4015	Epizootiology of Hantaan and Related Viruses in Baltimore. J. Childs, John Hopkins University, Baltimore, MD.
DAMD-17-85-C-5276	Development of Systems for Delivery of Antiviral Drugs. W. Shannon, Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6013	Research in Drug Development Against Viral Diseases of Military Importance (Biological Testing). W. Shannon, Southern Research Institute, Birmingham, AL.

DAMD-17-86-C-6028	Determination of The In Vitro and In Vivo Activity of Compounds Tested Against Punto Toro Virus. R. Sidwell, Utah State University, Logan, UT.
DAMD-17-86-C-6064	Structure/Function Studies of Presynaptic Neurotoxins. P.B. Sigler, University of Chicago, Chicago, IL.
DAMD-17-86-C-6161	A Core Facility for The Study of Neurotoxins of Biological Origin. L.L. Simpson, Jefferson Medical College, Philadelphia, PA.
DAMD-17-85-C-5285	Therapeutic Approaches to The Treatment of Botulism. L. Simpson, Jefferson Medical College, Philadelphia, PA.
PO-85-PP-5829	Development of Capillary Zone Electrophoresis/Mass Spectrometry for Analysis of Marine Toxins. R.D. Smith, Battelle Memorial Institute, Richland, WA.
PO-85-PP-5830	Development and Evaluation of Supercritical Fluid Chromatography (SFC) and SFC-Mass Spectrometry For Analysis of Trichothecenes, Marine Toxins and Neurotoxins. R.D. Smith, Battelle Memorial Institute, Richland, WA.
DAMD-17-86-C-6169	Colonization and Containment of *Hyalomma marginatum* Rufipes for Studies on Transmission of Crimean-Congo Hemorrhagic Fever. D.E. Sonenshine, Old Dominion University Research, Norfolk, VA.
DAMD-17-84-C-4003	Function of Mosquito Saliva in Delivery of Pathogens. A. Spielman, Harvard University, Cambridge, MA.
DAMD-17-85-C-5199	Identification and Selective Acquisition of Chemicals and Drugs for Antiviral Chemotherapy. E.L. Stephen, Technassociates, Inc., Rockville, MD.
DAMD-17-85-C-5023	Studies of The Biology of Phleboviruses in Sandflies. R. Tesh, Yale University, New Haven, CT.
DAMD-17-85-C-5212	Genetic and Physiological Studies of *Bacillus anthracis* Related to Development of an Improved Vaccine. C. Thorne, University of Massachusetts, Amherst, MA.

DAMD-17-86-C-6162	Development of New Immunogens and A Controlled Release Delivery System for Oral Immunization Against Staphylococcal Enterotoxin B. T.R. Tice, Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6044	Enchancement of Antiviral Agents Through The Use of Controlled Release Technology. T. Tice, Southern Research Institute, Birmingham, AL.
DAMD-17-86-C-6042	Drug Development Against Viral Diseases (Biological Testing). G. Tignor, Yale University, New Haven, CT.
DAMD-17-86-C-6060	Synthesis and Testing of Tetrodotoxin and Batrachotoxin Antagonists. L. Toll, SRI International, Menlo Park, CA.
DAMD-17-86-C-6166	Effects of Immunomodulatory Drugs on T Lymphocyte Activation and Function. C. Tsoukas, Scripps Clinic and Research Foundation, La Jolla, CA.

APPENDIX C

PRESENTATIONS
(Abstracts)

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1. **Driscoll, D. M., and J. D. Mims.** High hazard disease containment and evaluation. Presented at the 8th Medical Symposium of the 102nd ARDOM, Kansas City, MO, October 1985.

2. **Driscoll, D. M., and J. D. Mims.** Containment and transportation of patients with highly contagious infectious disease. Presented at the Annual Health Professionals Conference, Uniformed Services University of the Health Sciences, Bethesda, MD, October 1985.

3. **Liu, C. T., R. P. Sanders, R. S. Dixon, and C. J. Peters.** Treatment with IV infusion of lactated Ringer's or human albumin solution in Pichinde virus-infected guinea pigs. Presented at the Annual Meeting of the American Physiology Society, Niagara Falls, NY, October 1985.

4. **Welkos, S. L., and T. Keener.** Susceptibility of mice to anthrax and genetics of resistance to the Sterne vaccine strain. Presented at the Annual Meeting of the American Society for Microbiology, Washington DC, March 1986.

5. **Snyder, C. E., Jr., and J. C. Williams.** Purification and chemical characterization of a major membrane protein from *Coxiella burnetii*. Presented at the Annual Meeting of the American Society for Microbiology, Washington, DC, March 1986.

6. **Snyder, C. E., Jr., and J. C. Williams.** Production of monoclonal antibodies reactive with envelope components of *Coxiella burnetii*. Presented at the Annual Meeting of the American Society for Microbiology, Washington DC, March 1986.

7. **Wannemacher, R. W., Jr., D. L. Bunner, J. G. Pace, and R. E. Dinterman.** Efficacy of a non-occlusive barrier (B) model to study toxin pharmacokinetics after dermal (D) exposure (E). Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March 1986.

8. **Martin, D. G., G. W. Parker, and S. R. Davio.** Medical defense against saxitoxin. Presented at the Army Science Conference, West Point, NY, June 1986.

9. **Ezzell, J. W., T. M. Abshire, and R. F. Berendt.** Identification of two *de novo* extractable antigens from *Bacillus anthracis* and their potential use as vaccines. Presented at the Army Science Conference, West Point, NY, June 1986.

10. Fricke, R. F. Effect of T-2 toxin on activities of trans-stilbene oxide-induced hepatic microsomal enzymes. Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March 1986.

11. Fricke, R. F., and J. Jorge. Effect of monoethyl glutathione ester (GEE) on total intracellular GSH levels of acetaminophen (ApAp) and buthione sulfoxime (BSO)-treated rat hepatocytes. Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March 1986.

12. Pace, J. G., W. J. Canterbury, and C. Matson. Fate and distribution [^{3}H]T-2 toxin in topically exposed guinea pigs. Presented at the Annual Meeting of the Society for Toxicology, Atlanta GA, March 1986.

13. Meegan, J., F. Knauert, and J. W. LeDuc. Utilization of novel techniques for the rapid diagnosis of viral diseases of military importance. Presented at the Army Science Conference, West Point, NY, June 1986.

14. Thompson, W. L., and R. F. Fricke. The effect of cellular glutathione (GSH) levels on cytotoxicity of T-2 mycotoxin (T-2) in rat hepatocytes. Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March 1986.

15. Creasia, D. A., R. W. Wannemacher, Jr., and D. L. Bunner. Acute inhalation toxicity of aerosols of T-2 toxin in solution and as a suspension. Presented at the Annual Meeting of the Society for Toxicology, Atlanta, GA, March 1986.

16. Williams, J. C., M. H. Vodkin, D. H. Waag, K. -I. Amano, and E. H. Stephenson. Strategies for vaccine against Q fever: genetic and molecular basis of virulence and attenuation in *Coxiella burnetii*. Presented at the Army Science Conference, West Point, NY, June 1986.

17. King, A. D., A. O. Anderson, and E. H. Stephenson. Early pathogenesis of Venezuelan equine encephalitis virus: detection of specific adsorption of virus in brain tissue. Presented at the Army Science Conference, West Point, NY, June 1986.

18. Middlebrook, J. L. Cellular mechanism of action of botulinum neurotoxin. Presented at the Annual Meeting of the American Chemical Society, Symposium on Natural toxins, Denver, CO, June 1986.

19. Liu, C. T., P. B. Jahrling, and C. J. Peters. Evidence for the involvement of leukotriene in the pathogenesis of Pichinde virus infection. Presented at the Annual Meeting of the Federation of the American Society for Experimental Biology, St. Louis, MO, April 1986.

20. Liu, C. T. Alterations of ECG in guinea pigs infected with Pichinde virus. Presented at the 30th International Congress of Physiological Sciences, Vancouver, Canada, November 1985.

21. Fricke, R. F., and J. Jorge. Protective effect of ascorbic acid in decreasing T-2 toxin-induced lethality in mice. Presented at the Annual Meeting of the Federation of the American Society for Experimental Biology, St. Louis, MO, April 1986.

22. Wannemacher, R. W., Jr., D. L. Bunner, and R. E. Dinterman. The relationship between concentration and exposed area on absorption and excretion of T-2 mycotoxin through rabbit skin. Presented at the Annual Meeting of the Federation of the American Society for Experimental Biology, St. Louis, MO, April 1986.

23. Creasia, D. A., J. D. Thurman, R. W. Wannemacher, Jr., and D. L. Bunner. Acute inhalation toxicity of T-2 toxin in the rat and mouse. Presented at the Annual Meeting of the Federation of the American Society for Experimental Biology, St. Louis, MO, April 1986.

24. Bunner, D. L. Pathophysiology and treatment of low molecular weight toxin exposure. Presented at a joint US/Israel Bilateral Symposium on R & D in Military Medicine. Tel Aviv, Israel, December 1985.

25. Kaiser, I. I., J. L. Middlebrook, and M. H. Crumrine. Cross-reactivity and neutralization of crotalid toxins by antisera raised against crotoxin, its subunits, and two related toxins. Presented at the Symposium of Natural Toxins, part of the Rocky Mountain Regional American Chemical Society meeting, Denver, CO, June 1986.

26. Lowe, J. R. Transportable microcomputer programs for DNA and protein analysis. Presented at the 1986 ASBC/ACS-DBC Joint National Meeting; special poster session on the Use of Microcomputers in Biochemical Research and Teaching, Washington, DC, January 1986.

27. Trusal, L. R., and S. R. Watiwat. Ultrastructural effects of T-2 mycotoxin on chick heart cells in vitro. Presented at the Annual Meeting of the American Branch of the International Society for Toxinology, Tempe, AZ, May 1986.

28. Huggins, J. W., C. Hsiang. T. M. Cosgriff, Z. Wu, J. M. Meegan, J. W. LeDuc, Z. Zhen, J. I. Smith, S. Ge, M. Guan, C. Wang, T. Zhang, G. Yuan, and X. Gui. Double-blind, placebo-controlled clinical trial of ribavirin therapeutic efficacy in the treatment of epidemic hemorrhagic fever: open phase for dose setting. Presented at the IXth International Congree of Infections and Parasitic Diseases, Munich, Germany, July 1986.

29. **Kende, M., J. Brown, J. Smith, A. Johnson, M. Ussery, and P. Canonico.** Treatment of yellow fever virus (YFV) infection in African green (AG) monkeys with human recombinant alpha-2 interferon (HuRaI). Presented at the IXth International Congress of Infections and Parasitic Diseases, Munich, Germany, July 1986.

30. **Vodkin, M. H., and J. C. Williams.** Cloning and subcloning plasmids of *Coxiella burnetii* for diagnosis of altered pathogenicity. Presented at the First Annual Meeting of the American Society for Microbiology Conference on Biotechnology, Washington, DC, March 1986.

31. **Knauert, F. K.** Use of a DNA probe to detect Rift Valley fever virus RNA in epidemiologically relevant specimens. Presented at the Annual Meeting of the American Society for Virology, University of California at Santa Barbara, CA, June 1986.

32. **Clark, C. G., C. L. Crabbs, C. L. Bailey, and C. H. Calisher.** Isolation of western equine encephalitis virus for mosquitoes in south-central New Mexico. Presented at the Annual Meeting of the American Mosquito Control Association, New Orleans, LA, April 1986.

33. **Bunner, D. L., E. R. Morris, J. G. Pace, and C. F. Matson.** Effect of T-2 mycotoxin on amino acid uptake in L-6 myoblasts. Presented at the Second American Symposium on Animal, Plant and Microbial Toxins, Tempe, AZ, May 1986.

34. **Hewetson, J. F., and J. E. Beheler.** Monoclonal antibodies against saxitoxin binding of ^{3}H-saxitoxin to the sodium channel. Presented at the Second American Symposium on Animal, Plant and Microbial Toxins, Tempe, AZ, May 1986.

35. **Ussery, M. A., R. M. Bunte, and P. G. Canonico.** Kinetics of peripheral Japanese encephalitis virus (JEV) infection in C57 black mice. Presented at the XIth International Congress of Infections and Parasitic Diseases, Munich, Germany, July 1986.

36. **Kenyon, R. H., D. E. Green, and C. J. Peters.** Differences in Junin virus (JV) strains. Presented at the Annual Meeting of the American Society for Virology, Santa Barbara, CA, June 1986.

37. **Ussery, M. A., Y. Mine, T. Takaya, and P. G. Canonico.** Broad spectrum antiviral activity of kanamycin A derivatives with lipophilic acyl groups at the N-1 position. Presented at the Annual Meeting of the American Society for Virology, Santa Barbara, CA, June 1986.

38. **Kende, M., and P. G Canonico.** Design of carrier-mediated drug delivery for antiviral therapy. Presented at the 5th International Conference on Comparative Virology, Alberta, Canada, May 1986.

39. **Smith, J. F., and M. C. Osterling.** Characterization of monoclonal antibodies specific for a Rift Valley fever virus nonstructural protein. Presented at the Annual Meeting of the American Society for Virology, Santa Barbara, CA, June 1986.

40. **Dalrymple, J. M., J. C. Morrill, P. Sridhar, S. L. Hu, and M. S. Collett.** Evaluation of s recombinant vaccinia virus vaccine candidate of Rift Valley fever. Presented at the Annual Meeting of the American Society for Virology, Santa Barbara, CA, June 1986.

41. **Kende, M., W. Rill, H. Levy, R. Williams, and P. Canonico.** Nonspecific and specific immunomodulation with poly(ICLC) for antiviral therapy. Presented at the International Symposium on Immunological Adjuvants and Modulators of Nonspecific Resistance to Microbial Infections, Columbia, MD, June 1986. (sponsored by the Office of Naval Research, USAMRDC, and the Air Force Office of Scientific Research.)

42. **Schmaljohn, C. S.** The coding strategies of the M and S genome segments of Hantaan virus. Presented at the Annual Meeting of the American Society for Virology, University of California, Santa Barbara, CA, June 1986.

43. **Linthicum, K. J., C. L. Bailey, F. G. Davies, and C. J. Tucker.** Use of satellite remote sensing imagery to predict Rift Valley fever virus activity in Kenya. Presented at the Organization of African Unity Symposium on Viral Diseases in Africa, Nairobi, Kenya, May 1986.

44. **Ivins, B., G. Knudson, and D. LeBlanc.** Transposon Tn*916* mutagenesis of *Bacillus anthracis*. Presented at the 86th Annual Meeting of the American Society for Microbiology, Washington, DC, March 1986.

45. **Burnett, A. M., R. J. Doyle, and J. Ezzell.** Release of *Bacillus* spores from sporangia by sodium sulfate. Presented at the 86th Annual Meeting of the American Society for Microbiology, Washington, DC, March 1986.

46. **Doyle, R. J., T. Beveridge, M. Stewart, and J. Ezzell.** Evidence for a surface array on *Bacillus anthracis*. Presented at the 86th Annual Meeting of the American Society for Microbiology, Washington, DC, March 1986.

47. **Hewlett, E. L., H. J. Anderson, G. A. Meyers, M. R. Conboy, A. A. Weiss, V. G. Reddy, R. D. Pearson, G. G. Wright, and S. H. Leppla.** Differential activities of adenylate cyclase toxins from *Bordetella pertussis* and *Bacillus anthracis* in target cells. Presented at the 86th Annual meeting of the American Society for Microbiology, Washington, DC, March 1986.

48. **Levitt, N. H., H. H. Ramsburg, S. E. Hasty, P. M. Repik, F. E. Cole, Jr., and H. W. Lupton.** Development, production, and preclinical testing of a live, attenuated Chikungunya virus vaccine. Presented at the 86th Annual Meeting of the American Society for Microbiology, Washington, DC, March 1986.

49. **Thompson, W. L., M .B. Allen, and R. W. Wannemacher, Jr.** Comparison of in vivo and in vitro protein and DNA synthesis inhibition in response to T-2 mycotoxin. Presented at the FASEB Summer Conference, Copper Mountain, CO, July 1986.

50. **Thompson, W. L., and J. G. Pace.** Studies of in vitro protein synthesis inhibition and metabolism of the 12,13-epoxytrichothecenes by tissue culture and primary cell lines. Presented at the Federation for the Society for Experimental Biology and Medicine Summer Conference, Copper Mountain, CO, July 1986.

51. **Smith, L. A.** Effect of presynaptic neurotoxins on the uptake of [^{3}H]-myoinositol in guinea pig synaptosomes. Presented at the meeting of the European International Society of Toxicology, Prague, Czechslovakia, August 1986.

52. **Romoser, W. S., M. E. Faran, and C. L. Bailey.** Is the basal lamina the mesenteronal escape barrier to Rift Valley fever virus in mosquitoes? Presented at the Annual meeting of the American Mosquito Control Association, New Orleans, LA, April 1986.

53. **Middlebrook, J. L., and D. L. Leatherman.** Cell surface interactions of T-2 mycotoxin: evidence for a receptor. Presented at the meeting of the European International Society of Toxicology, Prague, Czechoslovakia, August 1986.

54. **Kende, M., W. Rill, H. Levy, and P. Canonico.** Antiviral effects of polyICLC in immunocompromised hosts. Presented at the 26th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, July 1986.

55. **Ussery, M. A., M. A. Balady, P. G. Canonico, and C. W. Czarniecki.** Prophylactic and therapeutic efficacy of recombinant interferons alpha and gamma in Japanese encephalitis virus-infected C57 black mice. Presented at 26th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, September 1986.

56. **Hewetson, J. F., and J. E. Beheler.** Monoclonal antibodies against saxitoxin inhibiting binding of ^{3}H saxitoxin to the sodium channel. Presented at the first annual meeting of the International Society of Toxinology, American Branch, Tempe, AR, May 1986.

57. **Bunner, D. L., E. R. Morris, J. G. Pace, and C. F. Matson.** Effect of T-2 mycotoxin on amino acid uptake in L-6 myoblasts. Presented at the first annual meeting of the International Society of Toxinology, American Branch, Tempe, AR, May 1986.

58. **Liu, C. T.** Changes of ECG in guinea pigs infected with Pichinde virus. Presented at the International Congress of Physiological Sciences, Vancouver, BC, July 1986.

59. **Trusal, L. R., and S. R. Watiwat.** Ultrastructural effects of T-2 mycotoxin on chick heart cells in vitro. Presented at the Summer Conference of the Federation of American Societies for Experimental Biology, Copper Mountain, CO, July 1986.

60. **Trusal, L. R., S. R. Watiwat, and J. C. O'Brien.** Biochemical and ultrastructural effects of T-2 mycotoxin on rat hepatocytes in vitro. Presented at the Summer Conference of the Federation of American Societies for Experimental Biology, Copper Mountain, CO, July 1986.

61. **Templeton, C. B., and D. A. Creasia.** Blood gas changes following aerosol T-2 exposure in rats. Presented at the Summer Conference of the Federation of American Societies for Experimental Biology, Copper Mountain, CO, July 1986.

62. **Ruwe, W., and G. A. Saviolakis.** Identification and characterization of sites in the brain at which interleukin-1 initiates fever. Presented at the Congress on Research in Lymphokines and Other Cytokines, Boston, MA, August 1986.

63. **Waag, D., J. Williams, R-I. Amano, M. England, and J. Beveridge.** Relationship between the ability to induce in vitro splenocyte hyporesponsiveness and the LPS phenotype of *Coxiella burnetii* strains. Presented at the 6th Annual Conference of the American Society for Rickettsiology, Williamsburg, VA. September 1986.

GLOSSARY

3,4-DAP	3,4 Diaminopyridine
ABC	Avidin-biotin-peroxidase
ADCC	Antibody-dependent, cell-mediated cytolysis
AG	African green
A/J	Mouse strain
BALB/c	Mouse strain
BSA	Bovine serum albumin
BSO	Buthione sulfoxime
BW	Biological warfare
CAR	Central African Republic
CCHF	Crimean-Congo hemorrhagic fever
CDC	Centers for Disease Control
CFA	Complement fixation assay
cGMP	Cyclic guanidine monophosphate
CHIK	Chikungunya virus
CM	Chloroform-methanol
CMI	Cell-mediated immunity
CMR	Chloroform-methanol-extracted residue
CNS	Central nervous system
CRM	Cross-reacting material
DNA	Deoxyribonucleic acid
DOD	Department of Defense
EA1	Extractable protein
EBOV	Ebola virus
EEE	Eastern equine excephalitis

EF	Edema factor
EHF	Epidemic hemorrhagic fever
EP	Embryonated egg passage
FDA	Food and Drug Administration
GEE	Glutathione ester
GMP	Guanidine monophosphate
GTP	Guanidine triphosphate
HFRS	Hemorrhagic fever with renal syndrome
HPLC	High-pressure liquid chromatography
HTLV-III	Human T-lymphotropic virus (Aids)
HTN	Hantaan virus
HTNV	Hantaan virus
IEM	Electron microscopy
IFA	Immunofluorescence assay
IgG	Immunoglobulin G
IND	Investigational new drug
ISC	Immune suppressive complex
i.m.	Intramuscular
JEV	Japanese encephalitis virus
JV	Junin Virus
KHF	Korean hemorrhagic fever
KLH	Keyhole limpet hemocyanin
LCMV	Lymphocytic choriomeningitis virus
LDH	Lactose dehydrogenase
LF	Lethal factor
LIB	Liberia
LNI	Neutralizing antibody
LPS	Lipopolysaccharides

LT	Lymphocyte transformation
LV	Lassa virus
M	Medium (RNA)
MAA	Microagglutination assay
MBGV	Marburg Virus
MHS	Major histocompatibility complex
mPA	Meta-periodate
NIH	National Institutes of Health
NS	Nonstructural
NSP	Nonstructural protein
NaOH	Sodium hydroxide
PA	Protective antigen
PAGE	Polyacrylamide gel electrophoresis
PFU	Plaque-forming units
Ph1	Phase 1
QAE	QAE chomatography
RFLP	Restriction fragment-length polymorphism
RIA	Radioimmunossay
RNA	Ribonucleic acid
RVF	Rift Valley Fever
RVFV	Rift Valley fever virus
S	Small (RNA)
SGOT(AST)	Serum glutamic-oxaloacetic transaminase
SL	Sierra Leone
TCA	Trichloroacetic acid
VEE	Venezuelan equine encephalitis
YFV	Yellow fever virus