AD _____

(2)

SYNTHETIC PEPTIDE VACCINES FOR THE CONTROL OF ARENAVIRUS INFECTONS

ANNUAL REPORT

M. J. BUCHMEIER and PETER SOUTHERN

August 25, 1988

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

Contract No. DAMD17-86-C-6234

Scripps Clinic and Research Foundation
La Jolla, California 92037

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Scripps Clinic and Research Foundation 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) La Jolla, California 92037	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. DAMD17-86-C-6234
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21702-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62787A; PROJECT NO. 3M16 62787A871; TASK NO. AB; WORK UNIT ACCESSION NO. WUDA310904

11. TITLE (Include Security Classification)
Synthetic Vaccines for the Control of Arenavirus Infection

12. PERSONAL AUTHOR(S)
Buchmeier, M.J.; Southern, Peter

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Report	FROM 8/1/87 TO 7/31/88	1988 August 25	11

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	Arenavirus	BD	vaccine
06	13		Glycoprotein	mice	LCMV
06	01		Peptide	lab animals	RA I

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Arenaviruses are endemic on both the African and South American continents and represent significant public health hazards. Prophylactic immunization, precise diagnostic methods, and effective treatment protocols are not currently available. We are using genetic cloning methods to develop an effective vaccine against arenaviruses. Developmental studies have been carried out and techniques established with the prototype arenavirus, lymphocytic choriomeningitis virus (LCMV), and these methods can now be applied for rapid development and evaluation of vaccines against the pathogenic arenaviruses Lassa, Junin, and Machupo. Using techniques of peptide and immunochemistry we have identified and mapped the gene products of the L and S-RNA (OVER)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller	22b TELEPHONE (Include Area Code) 301/663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

Abstract

Arenaviruses are endemic on both the African and South American continents and represent significant public health hazards. Prophylactic immunization, precise diagnostic methods, and effective treatment protocols are not currently available. We are using genetic cloning methods to develop an effective vaccine against arenaviruses. Developmental studies have been carried out and techniques established with the prototype arenavirus, lymphocytic choriomeningitis virus (LCMV), and these methods can now be applied for rapid development and evaluation of vaccines against the pathogenic arenaviruses Lassa, Junin, and Machupo. Using techniques of peptide and immunochemistry we have identified and mapped the gene products of the L and S-RNA segments of LCMV and mapped the important immunogenic regions of the viral glycoproteins. The LCMV genomic RNAs have been cloned and primary sequences of the RNAs and their gene products are being completed. Synthetic peptides corresponding to immunogenic regions of the viral structural proteins are being synthesized and will be evaluated for the ability to induce immune responses in experimental animals. Experimental approaches to immunization based upon synthetic peptides and polypeptides, vaccinia virus vectors containing LCMV genes, and anti-idiotypic antibodies will be explored. Experimental approaches to immunotherapy for acute arenavirus infections will also be investigated using cloned cytotoxic T-lymphocytes and neutralizing monoclonal antibodies in attempts to modify the course of acute disease. Finally, monoclonal antibodies and cDNA probes against defined type specific and common determinants and sequences will be made in order to facilitate precise diagnosis of arenaviral diseases.

Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

The investigators have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.

Table of Contents

A. Introduction and Overview

During the contract year 1 Aug 87 - 31 Jul 88 we have focused our efforts on two major issues related to the work described in contract proposal C6234. These issues are:

1) Precise description of the major neutralizing antigenic site on the GP-1 glycoprotein of the Old World arenavirus, lymphocytic choriomeningitis virus (LCMV). In previous topographical mapping studies we have defined two major neutralizing epitopes on GP-1 by competitive inhibition antibody binding assays. These epitopes, termed GP-1A and GP-1D, mapped to a topographically restricted region (site) as evidenced by mutual partial inhibition of binding. We have mapped one of these epitopes using viral escape mutants, and have attempted chemical synthesis of the epitope by synthetic peptide chemistry.

2) The requirements for folding transport and intracellular processing of GP-C precursor to form the GP-1 and GP-2 structural proteins have been examined in detail. These facts are important if we are to knowledgeably interpret and compare the biosynthesis and structure of arenavirus glycoproteins in viral and molecularly cloned expression systems. It is critical to ensure accurate expression of the native structure of viral proteins before their consideration for vaccine production regardless of whether a biological vector such as vaccinia is used, or a protein subunit approach is taken.

B. Identification of the neutralizing antigenic site on LCMV

During this contract year we focused our attention on efforts to map the major neutralizing domain of LCMV-GP-1 glycoprotein using neutralization escape mutants. Two selections for escape mutants were done using the monoclonal antibody 2-11.10 (epitope GP-1D) (Parekh and Buchmeier, 1986). The results of those selections are summarized in Table 1 below.

Table 1

Selection of Antigenic Variants with Monoclonal 2-11.10: Reactivity of Selected Strains with LCMV Monoclonals

Monoclonal (epitope)	Reactivity[a] Expt. 1	Positive/Total Expt. 2
2-11.10 (GP-1D)	7/14	4/9
WE 6.4 (GP-1A)	14/14	9/9
9-7.5 (GP-2B)	14/14	9/9
1-1.3 (NP-A)	14/14	9/9

[a]Reactivity with GP-1 specific antibodies was judged by ELISA, indirect immunofluorescence and by virus neutralization. Antibodies to GP-2 and NP were evaluated by ELISA and by indirect immunofluorescence.

It is evident from this selection that approximately 40-50% of the plaques selected were true escape mutants with regard to MAb 2-11.10, but showed no difference in binding of MAb WE 6.2. Other antibodies to the GP-1A site, including WE197.1, WE258.4 and WE36.1, behaved like WE 6.2 in their recognition of these strains. Four strains, two escape mutants (2-11.10 negative) and two representing the wild type phenotype (2-11.10 positive) were selected for detailed mapping studies. These strains and their 2-11.10 binding characteristics are summarized in Table 2 below.

Table 2

Antibody Binding Profiles of Four Strains of LCMV-Armstrong

Strains Isolate	Reactivity[a] with MAb 1-1.3 (NP)	33.6 (GP-2)	WE258.4 (GP-1A)	2-11.10 (GP-1D)
ARM-3	+	+	+	-
ARM-4	+	+	+	+
ARM-5	+	+	+	-
ARM-10	+	+	+	+

[a]Reactivity in ELISA and indirect immunofluorescence assays.

These strains were subjected to direct RNA sequencing by the dideoxy chain termination method of Sanger, and mutation was observed at a single codon (nucleotides 594-596) of the GP-C precursor corresponding to amino acid 173 of the GP-1 posttranslational product. This mutation is detailed below in Table 3.

Table 3

Sequence Comparison and Antibody Reactivity of LCMV Arm Isolates

LCMV Isolate	GP-C Sequence[a] nucleotides 582-620 amino acids 169-181	Epitope[b] A	Epitope[b] D
ARM-5	CAATACAACTTGACATTCTCAGATCGACAAAGTGCTCAG GlnTyrAsnLeuThrPheSerAspArgGlnSerAlaGln	+	-
	GCA		
ARM-4	----------Ala----------	+	+
	ACA		
ARM-3	----------Thr----------	+	-
	AAA		
ARM-10	----------Lys----------	+	+

[a]Sequences were determined by primer extension.
[b]Reactivity with MAbs to epitope A (MAbs 197-1 and 6.2) and epitope D (2-11.10) is scored. + indicates a positive reaction.

These results established that mutants which contained the sequence Asn-Leu-Thr at amino acids 171-173 of GP-1 failed to be recognized by MAb 2-11.10, suggesting that the mechanism of escape was insertion of a glycosylation site of the form (Asn x Ser/Thr) at this position. We therefore compared glycosylation of these strains and found that as predicted, the MAb 2-11.10 resistant strains carried an additional sixth oligosaccharide chain on GP-1 relative to those strains which bound 2-11.10. Moreover, we observed heterogeniety at position 173 between the antibody binding strains ARM-4 and ARM-10. The former had the sequence Asn-Leu-Ala at this position, while the latter was Asn-Leu-Lys, suggesting that limited heterogeniety was tolerable. Neither of these isolates showed any increased or decreased avidity of binding of MAb 2-11.10.

Further experiments were done to define the role of glycosylation and disulfide bond formation in the structure of this important epitope, and these results are described in detail in a manuscript submitted for publication (Wright, Salvato and Buchmeier, J. Virol., submitted 1988). Briefly, we found that the major neutralizing epitope was conformational in nature, that

it required native protein folding for expression, and that this folding required prior N-linked glycosylation of the GP-C polypeptide chain and intrachain disulfide bond formation. The epitope was expressed on monomeric (n = 1) and homopolymeric (n = 1-4) forms of GP-1 but was destroyed by heating and by reducing agents.

Based on this data we attempted to synthesize the GP-1D epitope chemically with limited success. It was reasoned that a peptide containing the 171-173 sequence and spanning the adjacent flanking cysteine residues might form a hairpin or loop structure containing the GP-1D epitope. To this end three peptides were synthesized as shown below:

GP-C peptide 160-185 CDFNNGITIQYNLAFSDEQSAQSQC
170-185 YNLAFSDEQSAQSQC
176-185 DEQSAQSQC

These peptides were used to coat ELISA plates and tested for reactivity with a large panel of 22 mouse and rat monoclonals to LCMV as well as normal and immune guinea pig antisera to LCMV. Binding was observed as described in Table 4 below.

Table 4

Antibody	Binding to Substrate Containing Native Virus	160-185	170-185	176-185
2-11.10	>1:10,000	1:160	<1:10	<1:10
WE 258.4	1:5,600	1:80	<1:10	<1:10
eE 197.4	ND	1:160	<1:10	<1:10
WE 36.1	ND	<1:10	<1:10	<1:10
WE 6.2	ND	1:40	<1:10	<1:10
WE 327.3	ND	1:160	<1:10	<1:10
WE 18.7	ND	<1:10	<1:10	<1:10
WE 67.8	ND	<1:10	<1:10	<1:10
Rat MAb #8-12	ND	<1:10	<1:10	<1:10
#8-13	ND	<1:10	<1:10	<1:10
#8-14	ND	<1:10	<1:10	<1:10
#8-21	ND	<1:10	<1:10	<1:10
#8-22	ND	<1:10	<1:10	<1:10
#8-24	ND	<1:10	<1:10	<1:10
#8-25	ND	1:20	<1:10	<1:10
#8-26	ND	<1:10	<1:10	<1:10
#8-29	ND	<1:10	<1:10	<1:10
#8-32	ND	1:20	<1:10	<1:10
#8-35	ND	<1:10	<1:10	<1:10
#8-40	ND	<1:10	<1:10	<1:10
#8-50	ND	<1:10	<1:10	<1:10
#8-55	ND	<1:10	<1:10	<1:10
Normal guinea pig	<1:20	1:20	<1:10	<1:10
Guinea pig immune serum	ND	1:20	<1:10	<1:10

ND = not determined. Qualitative determinations by indirect immunofluorescence established titers of >1:100.

The results suggested very low avidity specific binding of the GP-1 antibodies to peptide 160-185, however the titer was extremely low in proportion to the titer of the same monoclonals against native virus, hence this approach was not aggressively pursued pending availability of pertinent structural details of the GP-1 molecule to allow a more accurate prediction to be made.

C. Detailed studies of the biosynthesis folding, transport and intracellular processing of GP-C to GP-1 and GP-2.

Two lines of evidence suggested to us that a full understanding of the intracellular events in biosynthesis and processing of GP-C to form GP-1 and GP-2 was an important short-term goal of this project. First, as described above, native folding of the GP-C polypeptide chain as indicated by acquisition of reactivity with conformation dependent neutralizing monoclonal antibodies requires both prior glycosylation and intrachain disulfide bond formation. This observation must be taken into account in the design of any vaccine which is intended to mimic the relevant native epitope. Second, in an attempt to use the baculovirus expression system to produce glycoprotein for study, we obtained two vectors containing the full length LCMV NP and GP-C genes from Dr. David Bishop, NERC Insect Virus Unit, Oxford, U.K. These viruses, termed YON (NP) and YOG (GP-C), were grown in Spodoptera frugiperda cells and lysates prepared at the time of maximum CPE. These lysates were coated on microtiter wells in a standard ELISA format and several LCMV monoclonals were assayed for reactivity. The results of this assay are shown in Table 5 below:

Table 5

Reactivity of LCMV Monoclonal Antibodies with NP and GP-C Expressed by Baculovirus

Antibody	Specificity	Titer vs. Substrate[a] YON (NP)	YOG (GP-C)
Guinea pig anti LCMV	GP+NP	>1:10,000	1:1000
1-1.3	NP	>1:10,000	<1:10
WE258.4	GP-1	<1:10	<1:10
WE6.2	GP-1	<1:10	<1:10
WE33.6	GP-2	<1:10	1:100
9-7.9	GP-2	<1:10	1:100

[a]Substrate was 2 ug protein/well of Spodoptera lysate from cells infected with the indicated vector. Titer expressed as the highest dilution of antiserum scoring 2 times the background optical density.

These vectors clearly directed the synthesis of GP-C and NP, however neither of the conformation dependent, neutralizing MAb to site GP-1A recognized those proteins produced in insect cells. Moreover, Bishop has observed that the YOG-infected cells accumulate the GP-C polypeptide in the endoplasmic reticulum with little or no transport to the plasma membrane, again suggesting a defect in folding. Antisera produced in Bishop's laboratory by immunization of rabbits with YOG or YON lysates failed to recognize native GP-1 and did not neutralize viral infectivity. These results taken together suggest a defect in folding or processing of GP-C in the insect cell. We therefore sought to define the normal sequence of events in biosynthesis and processing of GP-C.

We first used a series of glycosylation inhibitors to define the minimal glycosyl chain structure required for proper folding of the chain to express the 2-11.10 epitope. We found that fully deglycosylated GP-C and GP-1 produced either by digestion of virions with N-glycanase (which removes complex sugar side chains) or by growth in the presence of tunicamycin (which blocks the en-bloc addition of the initial high mannose sugar chain to the growing polypeptide chain) were non-reactive with neutralizing antibodies. In contrast, addition of the most elementary high mannose oligosaccharide chain as expressed in the presence of the inhibitors castantospermine or 1-NM-deoxynojirimycin was sufficient to allow appropriate folding and acquisition of conformational epitopes. Significantly, intracellular transport via the normal secretory pathways was also dependent upon glycosylation.

By pulse chase and temperature blocking studies we showed that terminal glycosylation occurs in the medial to trans-golgi and precedes cleavage of GP-C at the GP-1/GP-2 junction.

D. Plans for the Coming Year

In the next contract year we intend to complete our studies of the biosynthesis and transport of GP-C and extend these to the structure of the glycoprotein spike, and we intend to explore the requirements for protection from lethal disease by antibody, and to seek evidence for a specific LCM viral receptor on susceptible cells which is recognized by GP-1 as the initial event in infection. These studies will yield a rational knowledge of the role of antibody in protection from infection and recovery from disease, and understanding of the virus-receptor interaction will provide information relevant to interpretation of the earliest stage of infection by arenaviruses. As Lassa is a close relative of LCM virus, it is anticipated that these observations will be directly applicable to that important human pathogen.

Appendix 1: Publications supported by this document, 1987-88

Published Papers

*Buchmeier, M. J., K. E. Wright, E. L. Weber, and B. S. Parekh. Structure and expression of arenavirus proteins. In Proceedings of the First International Symposium on Immunobiology and Pathogenesis of Persistent Virus Infections, Atlanta (C. Lopez, ed.), April 26-29, 1987, pp. 91-104.

Francis, S. J. and P. J. Southern. 1988. Deleted viral RNAs and lymphocytic choriomeningitis virus persistence in vitro. J. gen. Virol. 69:1893-1902.

*Fuller-Pace, F.V. and P. J. Southern. 1988. Temporal analysis of transcription and replication during acute infection with lymphocytic choriomeningitis virus. Virology 162:260-2[illegible].

Southern, P.J. and M.B.A. Oldstone. Biological and genomic variability among arenaviruses. In RNA Genetics, Vol. III, Domingo, Holland and Ahlquist, eds. Boca Raton: CRC Press, 1988, pp. 159-170.

*Weber, E.L. and M. J. Buchmeier. 1988. Fine mapping of a heptapeptide containing a group specific antigen conserved among arenavirus glycoproteins. Virology 164:30-38.

*previously listed as papers in press.

Papers in Press

Wright, K. E. and M. J. Buchmeier. Characterization of conformational neutralizing epitopes of lymphocytic choriomeningitis virus. Virology, in press, 1988.