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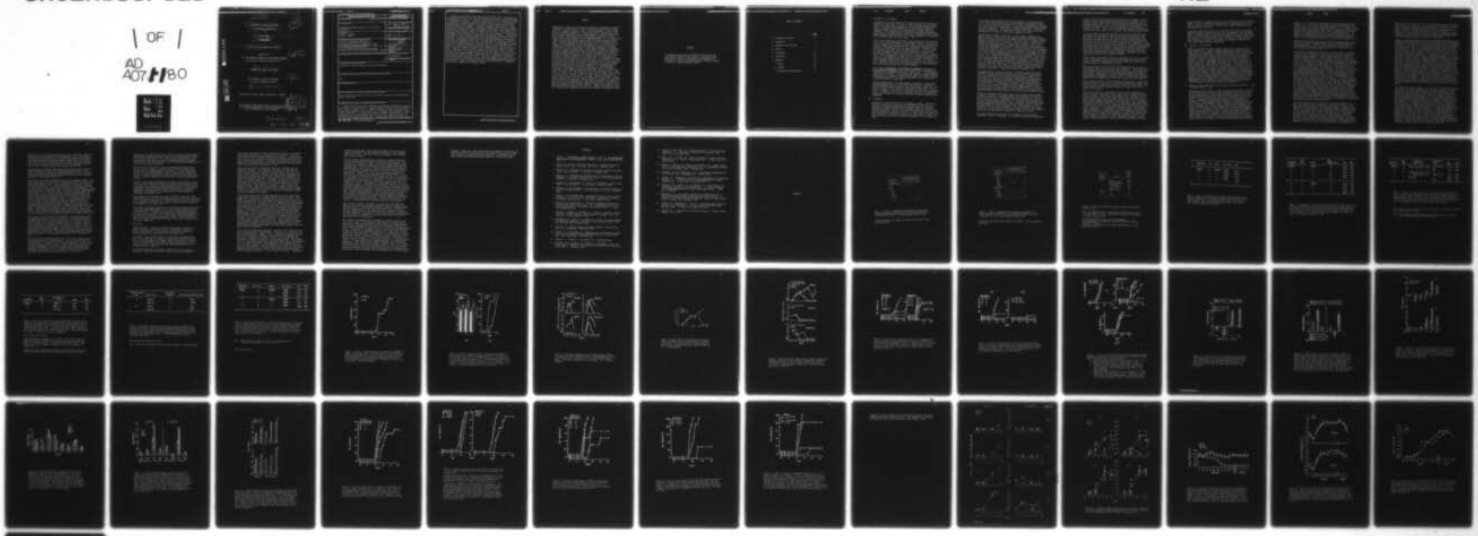

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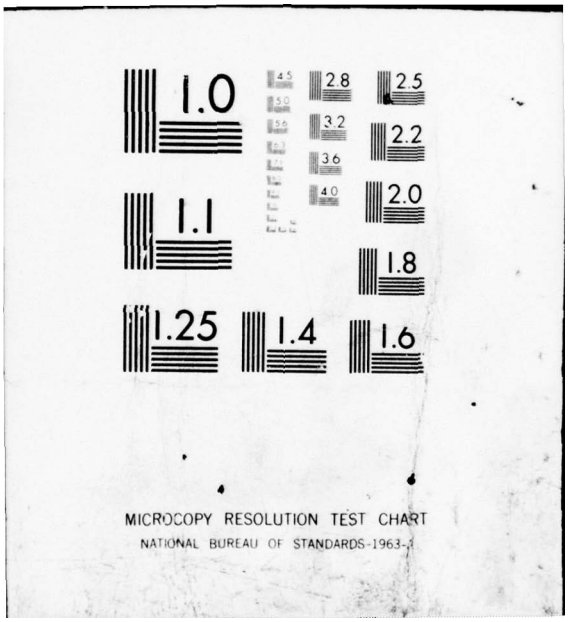
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Immunological factors that influence the phenotypic expression of genetic resistance or susceptibility to lethal flavivirus infection were investigated in 2 congenic strains of mice: C3H/RV (resistant to lethal infection) and C3H/He (susceptible to lethal infection). Resistance was abrogated by immunosuppression with cyclophosphamide or rabbit antimouse thymocyte serum. Immunosuppressed mice had higher yields of virus from target tissues such as brain than non-immunosuppressed mice. Paradoxically, C3H/He mice given anti-

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thymocyte serum had an increased average survival time. Following immunization with inactivated virus and adjuvant both strains of mice withstood ip challenge with virus. In contrast only resistant mice (C3H/RV) were unable to withstand ic challenge. Similarly C3H/RV mice were able to withstand ic challenge after passive immunization with anti-Banzi immune ascitic fluid, whereas C3H/He mice succumbed. Attempts to protect susceptible mice from lethal infection by transfer of non-immune C3H/RV splenic cells were generally unrewarding, although in several experiments recipients of non-immune resistant type cells had an increase in average survival time of several days. An in vitro microcytotoxicity test for Banzi virus was developed. It was shown that splenic cells from both resistant and susceptible mice were specifically cytotoxic for virus-infected L-929 target cells. Cytotoxicity was T cell-dependent. Methods were established for adoptive immunization of susceptible mice with virus-immune splenic cells from resistant or susceptible donors. Protection of recipients from lethal challenge was T cell dependent. Furthermore, dose response experiments indicated, that on a cell-for-cell basis, RV splenic cells were more protective than He splenic cells. Replication of Banzi virus was compared in brains and in "brain" cell cultures of RV and He mice. Yields of virus from cell cultures were identical. Yields from RV brains were about 1 log higher than for He brains. Results overall indicate that an intact immune response is required for full phenotypic expression of resistance and that both cell-mediated and humoral immunity are activated during Banzi virus-infection of mice. They also suggest that immune RV cells are more capable of defending an adoptively immunized susceptible mouse than are He cells. Genetic resistance is viewed as a multifactorial phenomenon involving both immune and non-immune events.

SUMMARY

Immunological factors that influence the phenotypic expression of genetic resistance or susceptibility to lethal flavivirus infection were investigated in 2 congenic strains of mice: C3H/RV (resistant to lethal infection) and C3H/He (susceptible to lethal infection). Resistance was abrogated by immunosuppression with cyclophosphamide or rabbit antimouse thymocyte serum. Immunosuppressed mice had higher yields of virus from target tissues such as brain than non-immunosuppressed mice. Paradoxically, C3H/He mice given antithymocyte serum had an increased average survival time. Following immunization with inactivated virus and adjuvant both strains of mice withstood ip challenge with virus. In contrast only resistant mice (C3H/RV) were able to withstand ic challenge. Similarly C3H/RV mice were able to withstand ic challenge after passive immunization with anti-Banzi immune ascitic fluid, whereas C3H/He mice succumbed. Attempts to protect susceptible mice from lethal infection by transfer of non-immune C3H/RV splenic cells were generally unrewarding, although in several experiments recipients of non-immune resistant type cells had an increase in average survival time of several days. An in vitro microcytotoxicity test for Banzi virus was developed. It was shown that splenic cells from both resistant and susceptible mice were specifically cytotoxic for virus-infected L-929 target cells. Cytotoxicity was T cell-dependent. Methods were established for adoptive immunization of susceptible mice with virus-immune splenic cells from resistant or susceptible donors. Protection of recipients from lethal challenge was T cell dependent. Furthermore, dose response experiments indicated, that on a cell-for-cell basis, RV splenic cells were more protective than He splenic cells. Replication of Banzi virus was compared in brains and in "brain" cell cultures of RV and He mice. Yields of virus from cell cultures were identical. Yields from RV brains were about 1 log higher than for He brains. Results overall indicate that an intact immune response is required for full phenotypic expression of resistance and that both cell-mediated and humoral immunity are activated during Banzi virus-infection of mice. They also suggest that immune RV cells are more capable of defending an adoptively immunized susceptible mouse than are He cells. Genetic resistance is viewed as a multifactorial phenomenon involving both immune and non-immune events.

FOREWARD

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 Resources, National Academy of Sciences - National Research Council.

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I. Statement of the Problem

Arthropod-borne (toga) viruses are a hazard to United States military personnel in many areas of the world. More specifically, neurotropic flaviviruses constitute a significant class of human pathogens and include: 1) Japanese B Encephalitis virus (Japan, Korea, Thailand, Vietnam and other regions of Southeast Asia); 2) Murray Valley Encephalitis virus (Australasia); 3) Russian Spring-Summer Encephalitis virus (central and eastern Europe and Asia); 4) West Nile virus (Africa, Middle East and Western Europe); 5) St. Louis Encephalitis virus (U.S.) and, 6) Powasan virus (U.S. and Canada). (1)

All of these viruses can cause severe, and occasionally, fatal encephalitis. Yet, overt encephalitis is an uncommon sequella of flavivirus infection among people living in endemic areas. Studies with mumps and measles viruses have shown that mild or asymptomatic encephalitis accompanies a significant portion of human infections. Similar studies for flavivirus infection are not readily available. It is not clear why some individuals are able to deal effectively with neurotropic flaviviruses whereas others develop severe or fatal illness. Nevertheless, it seems reasonable to speculate that U.S. military personnel introduced to an endemic area would constitute a highly susceptible population and would experience a high rate of infection. In contrast, local inhabitants would likely be immune to infection from previous contact. Moreover, effective vaccination procedures for most neurotropic flavivirus infections have not yet been developed.

In our view, clarification of host defense mechanisms, including the role of genetic influences, could play a vital role in delineating the pathogenesis of arthropod-borne viral encephalitides. Such information could be applied toward: 1) predicting the clinical and epidemiological consequences of introducing susceptible personnel into endemic regions; 2) determining prognoses in infected individuals and, 3) developing a rational procedure for vaccination.

Prevailing explanations for innate genetic resistance to lethal flaviviral encephalitis hold that resistance gains phenotypic expression through nonimmunological virus-host cell interactions. Thus cells from resistant hosts, be they neurons, macrophages or fibroblasts, are believed to support virus replication less well than cells from susceptible hosts. In contrast, data from our laboratory indicate that expression of genetic resistance to flaviviral encephalitis involves immunological mechanisms.

II. Background

Resistance to virus infection can be acquired or innate. Acquired resistance is generally a reflection of immunological reactivity to a previously encountered pathogen whereas innate resistance indicates defiance to initial infection with a pathogen and may not require immunological defenses. Innate resistance to viruses can occur in species totally refractory to infection (2), but, as used in this discussion, innate resistance is defined as the ability of individuals to survive initial encounters with viruses ordinarily pathogenic for the species as a whole.

It has been well established that innate resistance or susceptibility to flaviviruses (group B togaviruses) is hereditable in Mendelian patterns (2-7). Thus, resistance of inbred mice to flaviviruses apparently depends on a single autosomal dominant gene. Resistance is probably group specific since mice resistant to flavivirus are highly susceptible to alphaviruses (group A togaviruses). The adjectives "innate" and "genetic" are used synonymously with respect to resistance throughout the text.

The mechanism of genetic resistance to flaviviruses is unknown, but 2 general theories have evolved. The first has gained considerable support among virologists and proposes that genetic resistance is expressed, phenotypically, in many types of cells in a resistant animal. Indeed several studies, particularly those utilizing primary cultures of cells from resistant and susceptible donors, indicate that resistant cells (brain, fibroblasts, macrophages) do not support replication of some flaviviruses as well as susceptible cells (6-8). It has been suggested that macrophages in particular play a key role in genetic resistance, since they are a major first line of defense against many viruses and mirror, in vitro, the resistance to virus replication displayed by the host from which they are collected (6,7). Recent evidence indicates that even embryonic cells (fibroblasts) from resistant mice produce smaller quantities of infectious flavivirus (West Nile (WN) virus) than embryonic fibroblasts from susceptible mice. Further, there are preliminary data suggesting that this effect may be due to enhanced production of defective interfering (DI) particles in resistant cells (8). This theory, by implication, reduces immunological defenses to a secondary role in genetic resistance and does not require phenotypic expression of "resistance" genes by lymphoid cells.

The second theory proposes that genetic resistance is expressed phenotypically through the lymphoreticular system and implies that lymphoid cells from resistant mice are functionally superior to lymphoid cells from susceptible mice in defending host tissues against flaviviruses. This theory was supported by experiments showing that immunosuppression abrogated genetic resistance to WN virus (5) and to mouse hepatitis virus (MHV) (10-11). Furthermore, resistance to MHV failed to develop in mice or their cultured macrophages after neonatal thymectomy (11). The studies with WN virus, however, were not complemented by virological and histological characterizations of infected mice, so the effects of immunosuppression on viral replication and brain lesions remained unknown.

Goodman and Koprowski tried a more direct test of this theory by making reciprocal transfers of nonimmune lymphoid cells between resistant and susceptible mice before challenging them with flavivirus (5). They were able to partially protect susceptible mice from lethal WN encephalitis by giving them resistant lymphoid cells. Conversely, they presented some evidence suggesting resistant mice given susceptible lymphoid cells were more susceptible to lethal viral infection. Unfortunately, they used allogeneic mice for their experiments. Thus, graft-versus-host disease occurred in recipient mice and only small numbers of mice, whose chimeric status was not characterized, remained for infectivity studies.

The second theory forms the basis of our approach to understanding mechanisms of genetic resistance. We have been studying the pathogenesis

of Banzi virus (flavivirus) infection in genetically resistant and susceptible congenic mice originally obtained from Dr. Koprowski. The resistant strain is designated C3H/RV (RV) and the susceptible strain is designated C3H/He (He). They are histocompatible as determined by tumor grafting, serological assay (12), skin grafting and mixed leukocyte culture assay (13). Banzi virus was originally isolated from an African child (14) and was a gift of Dr. J. Casals, Yale Arbovirus Research Unit.

Our early studies, indicated that Banzi virus was about 100,000 times more lethal for adult He mice than for adult RV mice following intraperitoneal (IP) inoculation. Banzi virus caused encephalitis in both strains, but RV mice were able to limit infection, remain asymptomatic, and recover, whereas He mice developed an encephalitic syndrome in 7 to 9 days and died. Virus replicated in tissues of both strains, but yields were greater from He brains than from RV brains. Similarly, meningoencephalitis occurred in both strains, but neuronal necrosis was observed only in brains of He mice. Finally, immunofluorescent studies showed that viral antigen was detectable in the cytoplasm of many neurons and possibly glial cells of He mice during the height of infection, but viral antigen was not detected in brains of RV mice.

Further studies confirmed that genetic resistance of RV mice to togavirus infection was group specific since He and RV adults were essentially equally susceptible to alphavirus infection (Semiliki forest virus and Venezuelan equine encephalomyelitis virus).

Resistance to Banzi virus in RV mice developed postnatally and did not reach significant levels until mice were 4 weeks old. Intracerebral (IC) inoculation of Banzi virus produced high mortality in both strains and virus titers, distribution of viral antigen and lesions were similar in both strains. Resistance of RV mice was also abrogated by several immunosuppressive treatments: sublethal (400 R) x-irradiation; cyclophosphamide (CY) (150 mg/kg) given 1 day after virus; and T cell depletion.

These findings indicated that full phenotypic expression of genetic resistance to Banzi virus requires an intact lymphoreticular system. They also indicated that genetic resistance of RV mice to lethal Banzi viral infection does not result from the inability of their brain cells to support virus growth.

Banzi virus infection of congenic mice offers several advantages as a model for genetic resistance. First, Banzi virus is highly infectious for both strains of mice following parenteral (IP) inoculation, but is highly lethal only for He mice. This finding is in contrast to previously reported models utilizing WN and yellow fever (YF) virus where best results followed intracerebral (IC) inoculation (5,6). Parenteral inoculation mimics the natural route of transmission, ostensibly allows a more natural interplay of host defenses with virus and avoids traumatic injury to brain (i.e. - needle tracts resulting from IC inoculation). Second, responses to virus within each strain of mouse are uniform and highly reproducible, so experiments with small numbers of mice produce definitive data on morbidity and mortality. Third, Banzi virus is not, in our experience, virulent for laboratory personnel. Since it does, however, belong to a group of viruses containing a number of agents that are highly pathogenic for man, it offers an opportunity to under-

stand mechanisms of togaviral infection for extrapolation to more hazardous agents. Fourth, utilization of congenic hosts allows transfers of cells from one strain of mouse to the other without encountering significant histocompatibility barriers.

It should be emphasized that we view the Banzi viral model as an experimental prototype for neurotropic flavivirus encephalitis. The low pathogenicity of Banzi virus for laboratory personnel make it much easier and safer to work with than some of the more pathogenic viruses in this group. Nevertheless, once the mechanism of Banzi virus-host interactions have been clarified, we expect to extrapolate our findings to some of the more common human pathogens listed above.

III. Approach to the Problem

Phenotypic expression of innate genetic resistance to flaviviruses has been thought to be a largely nonimmunological event mediated by host target cells and macrophages. We have hypothesized, rather, that genetic resistance gains phenotypic expression through one or more classes of lymphoreticular cells. More specifically, we propose that thymus-derived (T) lymphocytes assume a major role in initiating protective responses to virus. Macrophages are viewed as effector cells that require activation by genetically-capable T cells to become defenders against infection. Furthermore, we suggest that T cells serve not only as macrophage activators, but also as initiators of responses to virus during early stages of infection. We also imply that T cells from susceptible animals may be either functionally defective or may actively suppress the response of other lymphoid cell populations to flaviviruses or flaviviral antigens. These concepts are consistent with prevailing explanations for acquired resistances to viral infection and for genetic control of immune responsiveness to chemically-defined antigens. The overall goal of the research is to elucidate the role of host immune responses in genetic resistance and to determine how lymphoreticular cells influence the phenotypic expression of resistance.

IV. Results of Experiments Performed During Contracting Period March 1, 1975 through December 31, 1976.

Resistance of adult RV mice to lethal infection was previously shown to be compromised by intracerebral (IC) inoculation of virus (15). Virus titers, distribution of viral antigen and brain lesions (necrotizing meningoencephalitis) were also similar in both strains after IC inoculation. We had also shown that resistance of infected RV mice was severely compromised by immunological crippling with sublethal x-irradiation; cyclophosphamide (CY) treatment or T lymphocyte depletion (adult thymectomy followed by lethal x-irradiation and syngeneic bone marrow grafting) (16). Recent results indicate that treatment with rabbit antimouse thymocyte serum (RAMTS) also compromises resistance (Figure 1). Paradoxically, T-cell depletion of He mice with RAMTS prolonged average survival time (AST) of Banzi virus-infected mice by several days compared to infected He mice given normal rabbit serum (NRS) (Figure 2). CY treatment did not prolong survival of He mice. Virus yields, distribution of viral antigen and brain lesions among CY treated RV mice were similar to those in non-immunosuppressed He mice

(Figure 3,4). Virus yields and lesions in T-cell-depleted mice were similar to, but less severe than, those of infected CY-treated mice and the course of infection was longer (Figure 5). For example, virus titers peaked earlier and at higher levels, the spread of viral antigen was faster and more diffuse and the extent of neuronal necrosis was greater in CY treated RV mice than in T cell-depleted RV mice. In addition, CY-treated mice had no detectable HAI antibody titers comparable to nonimmunosuppressed control RV mice infected with Banzi virus. HAI titers were not reduced by T-cell depletion (Tables 1 & 2).

When AST among infected RV mice given various immunosuppressive treatments were compared CY-treated mice always had a shorter AST than T-cell-depleted mice. Similarly ASTs among RV mice compromised by several methods were always one to several days longer than for He mice infected the same way (Table 3).

We have begun to examine the responses of He and RV mice to immunization with formalinized Banzi virus vaccine (BV) or with vaccine incorporated into incomplete Freund's adjuvant (BV+IFA) or complete Freund's adjuvant (BV+CFA). RV mice primed IP with one dose of BV+CFA were protected from lethal IC challenge with virus by postvaccination day 6 whereas He mice primed with BV+CVA succumbed to IC challenge given up to 15 days post priming (Figure 6). BV+IFA rendered RV mice only partially immune to IC challenge and BV alone did not protect. Viral replication in brains of vaccinated IC-challenged RV mice was low and encephalitis was mild and transient. In contrast, viral titers in vaccinated IC-challenged He mice were high and severe necrotizing choriomeningoencephalitis developed. Viral antigen appeared in only small quantities in RV brain whereas antigen was widespread in He brains. He mice were able to survive IP or IV challenge when primed with any of the 3 vaccine preparations (Figure 7). He mice were also protected from IP challenge but not IC challenge, by passive immunization with anti-Banzi immune ascitic fluid. Passively immunized RV mice were, however, protected from IC challenge with live virus (Figure 8). These preliminary studies together with our previous serological data indicate that both He and RV mice can respond to Banzi antigen, but more detailed kinetic studies of the primary response to virus are required to determine if differences critical to the outcome of infection exist. It should also be determined if the massive inflammation in vaccinated-challenged He mice has immunopathological components and if the differences in response to IC challenge can be explained by the slightly lower level of viral replication previously observed in unprimed RV compared to unprimed He mice.

Since the immunosuppression experiments cited above suggested that T cells are required for full phenotypic expression of resistance we began additional examination of T-cell participation. First, we have adapted an in vitro assay for cell mediated immunity based on release of ^{51}Cr from target cells infected with Banzi virus after exposure to virus-primed spleen cells (17,18). The targets are L cells (mouse fibroblasts) which were derived from C3H mice and are, therefore, syngeneic with RV and He donor cells. Results show that: 1) RV and He spleen cells from Banzi virus-primed donors are specifically cytotoxic for infected L cells by 6 days post-priming; 2) pretreatment of immune RV or He spleen cells with anti-thymocyte serum abolishes cyto-

toxic activity of spleen cells and 3) nylon passaged (T-cell enriched) spleen cells are as cytotoxic for target cells as unfractionated spleen cells. Representative data are shown in Figures 9 through 14. The cytotoxic activity of immune antiBanzi serum is being examined, but to date, no cytotoxic activity has been detected. This assay is, to our knowledge, the first in vitro system for studying cell-mediated immune responses to flaviviruses. It has provided additional evidence for T cell involvement in primary responses to Banzi virus.

Second, we began testing various adoptive immunization procedures prior to more detailed in vivo dissection of immunological defenses in genetic resistance. We first tried to confirm the previous work of Goodman and Koprowski with WV virus (5) by attempting to protect He mice from lethal IP challenge with Banzi virus using non-immune donor spleen or lymph node (LN) cells. We also tried compromising resistance of RV mice by adoptive transfer of non-immune He spleen or lymph node cells. Various protocols were examined including: 1) transfer of up to 10^9 spleen or LN cells to unaltered recipients; 2) challenging recipients 1, 7 or 14 days after adoptive transfer 3) more extensive repopulation of recipients with donor cells by single or repeated transfers of $2x10^8$ cells to thymectomized, lethally-irradiated (950R) recipients reconstituted with donor type bone marrow cells; 4) parabiosing RV and He mice where partners were unaltered or where 1 partner had been thymectomized, irradiated and reconstituted with congenic bone marrow prior to parabiosis. (Mice were surgically separated about 10 days before challenge). The upshot of these experiments was that He mice given non-immune RV donor spleen or LN cells frequently but not invariably, had ASTs several days longer than He mice given syngeneic He cells, dead RV cells or no cells. Best results were obtained in unaltered or sublethally irradiated recipient mice challenged 1 or 7 days after transfer. All attempts to render RV mice susceptible to lethal Banzi viral infection by injecting He lymphoid cells before challenge were unsuccessful. Results of some typical experiments with non-immune donor cells are shown in Figures 15 and 16.

More recently we have used donor cells from live virus-primed mice and results have been more consistent. The original protocol, which was a modification of adoptive immunization procedures reported for other viruses (19, 20), consisted of infecting donor mice with Banzi virus and then transferring primed spleen cells to unaltered recipients who had been challenged with 100 LD_{50} one day before adoptive transfer. With this procedure, primed RV donor cells conferred partial protection (increased AST) to complete protection from lethal IP challenge on He mice (Figure 17). Significant prolongation of AST in He recipients occurred after IV injection of RV spleen cells harvested as early as 6 days post-priming. Dose response trials of 6 day post-priming donor cells indicated that $2x10^8$ cells resulted in longer AST among He recipients than smaller cell doses (Table 4). We have occasionally used suppression of viral replication in adoptively immunized mice as a marker, but we feel that AST or average day to death provide more rigorous tests of donor cell activity. We have also used donor cells primed with BV+CFA, but results, thus far, indicate significant protection of He recipients occurs only if cells are injected before virus challenge. Further, protection is enhanced by sublethal x-irradiation (250R) of recipients prior to cell transfer (Figure 18).

Rabinowitz (21) has used SQ challenge with virus for adoptive immunization protocols in his studies of host responses to VEE. Preliminary trials with this modification in the Banzi-C3H system were encouraging. For example, He mice given $2x10^8$ primed RV spleen cells, half IV and half IP, one day after receiving $100LD_{50}$ of Banzi virus subcutaneously (SQ) had low mortality whereas control mice given nonimmune cells and virus or virus alone had 100 percent mortality (Figure 19).

More recently the adoptive immunization protocols have been refined to establish optimum conditions for demonstrating the transfer of resistance. Experiments confirm that susceptible (He) mice can be protected from mortality after SQ challenge with virulent Banzi virus by adoptive transfer of virus-primed spleen cells.

Adult C3H/He mice were inoculated SQ with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus and 24 hours later were adoptively immunized with $2x10^8$ live Banzi virus-primed C3H/RV spleen cells. Cells transferred by 5 days post-priming prevented mortality in 7% of infected recipients while cells transferred at 7 days post-priming or later usually protected 67-100 percent of infected recipients. Recipients were not protected by $2x10^8$ nonimmune C3H/RV spleen cells or by heat-killed spleen cells from optimally immune C3H/RV mice (Tables 5 & 6). Protection after transfer was abrogated by pretreating immune spleen cells with rabbit antimouse brain (RAMB) serum (anti-T cell serum) and complement but not by pretreating cells with normal rabbit serum and complement (Table 7). By postinfection day 8, titers of Banzi virus in brains of adoptively immunized C3H/He mice were less than 3.0 $\log_{10}$ $TCID_{50}$/0.1gm whereas titers in mice given nonimmune donor spleen cells exceeded 7.0 $\log_{10}$ $TCID_{50}$/0.1gm (Table 8). Live virus-primed C3H/He spleen cells ($2x10^8$) protected C3H/He recipients against lethal Banzi virus infection in some experiments, but not in others. Dose-response experiments indicate that immune C3H/RV spleen cells were more effective than equivalent numbers of immune C3H/He spleen cells in protecting infected C3H/He recipients from mortality (Table 9).

Virus yields from mice given various numbers of virus-immune SC during a pathogenesis study are shown in figure (20). Mice given $2x10^8$ RV or He immune SC had little or no virus in spleen or brain with the exception of one He cell recipient harvested on day 12. However, titers of virus in brain of He mice given $1x10^8$ He cells and collected on days 9 and 12 were generally higher than in recipients of RV cells. This differential effect also was apparent, among mice given $5x10^7$ cells in that virus titers in spleens and brains of mice given He cells were higher than in mice given RV cells. Furthermore, remaining recipients of $5x10^7$ RV SC survived up to 14 days whereas recipients of He cells died by 9 days.

Viral antigen was not detected before day 9 in brain or spinal cord of recipient mice from any treatment group. Two recipients of $5x10^7$ RV cells examined on or after day 9 had small amounts of fluorescing intracytoplasmic viral antigen in frontal cortex, hippocampus, corpus striatum and mesencephalon. Banzi viral antigen was not detected in recipients of $2x10^8$ He cells, whereas massive cortical and hippocampal fluorescence developed in 2 of 4 mice given $1x10^8$ cells and 1 of 4 mice given $5x10^7$ cells that were examined on or after day 9.

Mice given 2 or $1x10^8$ RV SC did not have central nervous system lesions. Nonsuppurative myelitis was detected on day 12 in one mouse that died from the group given $5x10^7$ RV SC. This mouse had high titers of virus in brain and large amounts of viral antigen were detected by immunofluorescence. Most mice examined at day 5 and beyond had lymphoid hyperplasia of spleen and lymph nodes.

Mice given He SC did not have central nervous system lesions through day 5. By day 9, however, all mice given $5x10^7$ SC had necrotizing encephalitis or encephalomyelitis. By day 12 1 of 4 mice given $2x10^8$ SC and 3 of 4 mice given $1x10^8$ SC also had encephalitis or myelitis. In each case where encephalitis occurred, titers of virus in brain were elevated. Lymphoid hyperplasia was apparent in spleen and lymph nodes by day 2.

Groups of control mice given diluent alone also were evaluated. Virus titers in their spleens and brains rose sharply as previously reported (3) (Figure 20). Massive fluorescence was detected in cerebral cortex, hippocampus, corpus striatum and mesencephalon by day 9. Histological examination of brains and spinal cords revealed diffuse severe nonsuppurative encephalomyelitis. Neuronal necrosis was prominent in hippocampus and frontal cortex.

HAI antibody titers during early stages of infection increased faster among recipients of RV cells than among recipients of He cells (Figure 21). By day 9, however, mean titers for recipients of RV cells were less than for recipients of He cells. This biphasic response was more pronounced in recipients of 1 or $2x10^8$ cells.

In contrast to in vitro work with other flaviviruses discussed previously Banzi virus replicates equally well in target cells from resistant and susceptible mice. For example, virus yields from macrophage cultures or from cultures of infant or adult brain were essentially identical for He and RV mice (Figures 22 & 23). Nevertheless, closer examination of virus replication in brains during early stages of infection revealed that virus titers were persistently about one log lower in RV mice than in He mice (Figure 24).

V. Discussion

Taken collectively, these data indicate that phenotypic expression of genetic resistance requires host factors independent of innate resistance of tissues to viral replication and that among these host factors immunological competence plays an important role.

In addition, these studies support the notion that cell-mediated immunity is an important host defense in experimental flavivirus infection. They also suggest that immune T cells are required for adoptive transfer of protection and that immune C3H/RV spleen cells are, on a cell for cell basis, more efficient than immune C3H/He spleen cells at conferring adoptive immunity.

The adoptive immunization experiments indicate that by 5 days postinoculation acute infection with live Banzi virus elicits immune SC

which when transferred to lethally-challenged recipients, can suppress viral growth, development of encephalitis and mortality. Viable SC were required since SC killed by heat did not protect. The requirement for live cells also indicates that possible preformed antibody in the donor cell preparation was not responsible for protection. Protection appeared to be virus-specific in that mice adoptively immunized against Banzi virus succumbed to challenge with SFV.

Immune SC protected mice challenged 1 day before transfer, but not 3 days before transfer. This indicates that host virus interactions critical to the outcome of infection occurred early. The fact that doses of immune donor SC required to protect infected recipients suppressed viral replication in spleen and brain whereas suboptimal cell doses associated with mortality did not prevent viral replication in either site supports this idea. Furthermore, the failure of mice protected with optimum numbers of SC to develop encephalitis or to have detectable virus titers in brain indicates that virus may have never infected brain, but was eliminated in spleen by immune donor cells homing to spleen. Under these circumstances a delay of several days between viral challenge and transfer of even optimal numbers of immune SC may have allowed virus to seed and replicate in brain and overwhelm developing immunological defenses.

T cells were required for adoptive immunization to Banzi virus since immune donor cells exposed to RAMB+C did not confer protection. This finding is in contrast to a previous study with West Nile virus in which adoptive immunization was not abrogated by exposing donor cells to anti-T cell antibody (22). Although it has been suggested that host-dependent variations may occur in the relative importance of CMI and humoral immune responses to different flaviviruses (22), contrasting results with Banzi and West Nile virus may also reflect differences in experimental design. For example, in the WN study donor mice were given multiple immunizations of virus over several weeks before SC were harvested. The prolonged period of immunization would favor proliferation of antibody producing cells (B cells) refractory to treatment by anti-T cell antibody. In our system SC were harvested 5 to 10 days after donor mice were given a single dose of live virus. These conditions favor T cell-dependent adoptive immunization (21). Nevertheless, our passive immunization experiments using anti-Banzi hyperimmune ascitic fluid clearly indicate that humoral immunity is effective in preventing lethal Banzi-induced encephalitis.

T cells are functionally heterogeneous. They may act as killer cells in CMI, as helper cells in humoral antibody formation or as cells that suppress the development of immunity (23). Although serum antibody was not detected in recipients of immune SC unless they were challenged with virus, it is reasonable to assume that donor cells contributed to the development of humoral responses in virus-challenged mice. HAI antibody titers in adoptively immunized mice appeared earlier than in non-immunized mice, but titers remain low. Thus challenge of SC recipients did not elicit a prompt secondary response despite the fact that such mice were protected from mortality. This may indicate that CMI is the primary host defense during early phases of Banzi virus infection and that humoral immunity enhances protection at a later stage of infection. Experiments

utilizing purified donor cell populations should help delineate the relative contributions of CMI and humoral immunity to host defenses against Banzi virus. The Banzi virus system appears to be a viable model for this purpose.

The cell transfer experiments were carried out within the framework of exploring mechanisms of genetic resistance to flaviviruses. Comparisons of the ability of immune SC from genetically resistant (RV) and genetically susceptible (He) donor mice to combat Banzi infection suggested that, cell for cell, RV SC were more effective than He SC. The differential effect was not apparent when large numbers ($2x10^8$) of RV or He cells were transferred since, by clinical, virological and morphological criteria, SC of both types protected recipient mice equally well. However, when suboptimal doses ($1x10^8$, $5x10^7$) of cells were transferred, mortality and viral replication were usually greater in recipients of He cells than in recipients of RV cells. Furthermore, encephalomyelitis developed in recipients of He cells, but not in recipients of RV cells. Retrospective studies indicate that total nucleated cell counts of SC harvested on postinoculation day 7 were also consistently higher for RV donors than He donors. Time course data (not included) showed that SC yields from He mice dropped after day 5 which is just after peak viral replication in spleen and just before the development of necrotizing encephalitis. Lower SC yields from He mice may reflect a greater susceptibility of He SC to Banzi-induced cytolysis. Alternatively, stress-associated adrenocorticoid-mediated cytolysis may also contribute to SC loss in He mice. Whether the SC losses reflect selective or non-selective depletions of SC sub-populations that contribute to significantly host resistance remains to be determined.

Early studies of genetic resistance to flaviviruses using BRVR and BSVS mice indicated that non-immune lymphoid cells from resistant mice reduced mortality in susceptible, infected recipients. Similar effects have not been detected in C3H congenics despite recourse to heroic transfer technics such as total bone marrow and lymphoid cell replacement and parabiosis. Selection of a less severe criterion than mortality may be necessary to demonstrate differential protective capacities of non-immune cells to flaviviruses.

With respect to nonimmunological mechanisms in genetic resistance, we feel that the small, but potentially significant, differences in AST, LD_{50}, brain titers and lesions between IC-inoculated or immunosuppressed RV adults and untreated He adults observed in our earlier work may be due to innate resistance of target cells or to some other non-immunological events. In contrast to *in vitro* work with other flaviviruses discussed previously Banzi virus replicated equally well in target cells from resistant and susceptible mice. Nevertheless, virus replication in brains during early stages of infection revealed that virus titers were presistently about one log lower in RV mice than in He mice. Therefore, on balance, genetic resistance to flaviviruses appears to be a multifactorial process. Our model system offers several advantages for studying this phenomenon. First, Banzi virus is highly infectious and, for He mice, highly lethal after parenteral challenge of adults. This is in contrast to several other prototype flaviviruses used for this type of research since their virulence for adult mice after parenteral inoculation is low. Second, Banzi virus is adaptable for use in *in vitro* assays of immunological

phenomena. Third, mice used in this study are congenic so reciprocal cell transfer studies can be performed without danger of immunological rejection. Fourth, the system offers the possibility of exploring a non H-2 linked genetically-determined immune response to an infectious agent.

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APPENDIX

Treatment, titer of antibody	No. of mice on indicated day after inoculation of virus							
	2	4	6	8	10	12	15	18
Cyclophosphamide*								
<1:20	3	3	3	1	3	1		
Saline								
<1:20	1	2						
1:40			1					
1:80				2				
1:160			1				1	
1:320				1		2		2
1:640					2		1	1
1:1,280					1			

Table 1. Titers of hemagglutination-inhibiting antibody in cyclophosphamide-treated and saline-inoculated C3H/RV mice given ip inoculations of 3.0 $\log_{10}$ $TCID_{50}$ of Banzi virus.

*Cyclophosphamide (150 mg/kg) was given one day after inoculation of virus.

Treatment, titer of antibody	No. of mice on indicated day after inoculation of virus						
	3	6	9	12	15	19	21
T-cell depletion							
<1:20	3				1		
1:20		2					
1:40					2		
1:80		1	1	3			1
1:160			1			1	1
1:320			1				
1:1,280						1	
Sham operation							
<1:20	3	1			1	1*	
1:40		2					
1:160				1			
1:320			3	1	2		1
1:640				1			
1:1,280						1	1

Table 2. Titers of hemagglutination-inhibiting antibody in T-(thymus-derived) cell-depleted and shamoperated C3H/RV mice given ip inoculations of 2.5 $\log_{10}$ $TCID_{50}$ of Banzi virus.

*This mouse had neither HAI antibody nor lesions. It was considered uninfected.

Strain of mice, route of inoculation	Treatment	Average survival time (days)
C3H/RV		
ip	None	21.0*
	Cyclophosphamide†	10.0
	X irradiation (400 rad)‡	10.0
	Thymus-derived (T-) cell depletion	17.0
	None	8.5
ic	Cyclophosphamide	8.0
C3H/He§		
ip	None	8.0
ic	None	6.3

Table 3. Survival of C3H/RV and C3H/He mice given inoculations of Banzi virus.

Note: All C3H/He mice and ic-inoculated C3H/RV mice were given 2.0 $\log_{10}$ $TCID_{50}$ of virus. C3H/RV mice inoculated ip were given 3.0 $\log_{10}$ $TCID_{50}$ of virus.

*The 21st day was the last day of the experiment.
†Cyclophosphamide (150 mg/kg) was given one day after administration of virus.
‡Mice were x-irradiated one day before administration of virus.
§Data are from [3].

Recipient	No.	Donor	No. cells	AST
C3H/He	6	C3H/RV	$2x10^8$	11.5
"	"	"	$1x10^8$	10.5
"	"	"	$5x10^7$	10.0
"	"	"	$1x10^7$	9.2
"	"	"	-	8.0

Table 4. Effect of various doses of C3H/RV primed spleen cells on adoptive immunization of C3H/He recipients as measured by AST after lethal challenge (2.0 $\log_{10}$ $TCID_{50}$) of recipients 1 day prior to cell transfer. Donor cells were harvested 6 days after priming with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus.

Recipient	No. Mice	Donor	Days Postpriming	AST	MR
C3H/He	6	C3H/RV	3	11.5	6/6
"	"	"	5	16.5	2/6
"	"	"	7	17.8	2/6
"	"	"	10	21.0	0/6
"	"	"	14	18.7	1/6
"	"	C3H/He	3	13.7	5/6
"	"	"	5	12.8	4/6
"	"	"	7	12.7	5/6
"	"	-	-	12.0	6/6

Table 5. Comparison of the ability of $2x10^8$ C3H/RV or C3H/He spleen cells, harvested at different days after donor priming with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus, to protect C3H/He recipients from lethal SQ challenge with 2.0 $\log_{10}$ $TCID_{50}$ of virus. Recipients were challenged with virus 1 day before transfer. AST = average survival time in days. MR = mortality ratio. Mice were observed for 21 days.

Recipient	No. Mice	Treatment of Donor Cells	Challenged with	AST	MR
C3H/He	6	Primed with Banzi virus	Banzi virus	17.3	1/6
"	"	" " " "	SFV	7.7	5/6
"	"	Primed with Banzi virus and heat-killed*	Banzi virus	8.8	6/6
"	"	No cells	" "	10.5	4/4
"	"	" "	SFV	10.8	4/4

Table 6. Effects of various treatments on the ability of C3H/RV spleen cells to protect C3H/He recipients from lethal SQ challenge with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus or Semlike Forest virus (SFV). Recipients were challenged with virus 1 day before adoptive immunization with $2x10^8$ spleen cells. Primed cells were harvested at 7 days. AST = average survival time. MR = mortality ratio. Mice were observed for 21 days.

*Cells were killed by heating to 56C for 30 minutes. Viability was checked by trypan blue dye exclusion.

Note: Mice adoptively immunized against Banzi virus were not protected from lethal infection with SFV (alphavirus).

Recipient	No. Mice	Treatment of cells	AST	MR
C3H/He	6	RAMB + C'	11.8	5/6
"	"	*NRS + C'	18.2	1/6
"	"	no cells	8.7	6/6

Table 7. Effect of pretreatment in vitro with rabbit antimouse brain (RAMB) serum on the ability of virus-primed RV spleen cells to protect adoptively immunized He recipients from a lethal SQ challenge with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus. Recipients were challenged 1 day before receiving $2x10^8$ donor cells. Cells were harvested 7 days postpriming. AST = average survival time. MR = mortality ratio. Mice were observed for 21 days. Results of a typical experiment.

NRS = Normal Rabbit Serum

RAMB was prepared by immunization of rabbits with C3H/RV thymocytes. Serum was absorbed with mouse liver and heat-inactivated before use. RAMB was cytotoxic for 100% of thymocytes and 20-40% of spleen cells from He or RV mice. RAMB was cytotoxic only in the presence of C' (guinea pig).

Spleen cells were counted after treatment and concentrations were adjusted to provide $2x10^8$ viable nucleated spleen cells per recipient.

Post Challenge day	Donor cells	No. Brains tested	$\bar{x}$ titer $\log_{10}$ $TICD_{50}$/0.1gm
5	Primed*	4	<3.0
	Unprimed	3	Trace
8	Primed	4	<3.0
	Unprimed	3	≥8.6

Table 8. Preliminary evaluation of the effect of adoptive immunization of C3H/He mice with Banzi virus-primed or unprimed spleen cells on virus replication in brain. Donors were primed with 2.0 $\log_{10}$ $TCID_{50}$ of virus. Recipients were challenged SQ with 2.0 $\log_{10}$ $TCID_{50}$ of virus 1 day before receiving 2.10^8 donor spleen cells. Brains were harvested on post-challenge days 5 and 8.

*Primed cells were harvested on day 7.

Note: Primed cells suppressed virus replication compared to unprimed cells.

Recipient	No. Mice	Donor	No. Cells	AST	MR *
C3H/He	6	C3H/RV	$2x10^8$	21.0	0/6
"	"	"	$1x10^8$	19.3	1/6
"	"	"	$5x10^7$	15.0	4/6
"	"	C3H/He	$2x10^8$	12.7	5/6
"	"	"	$1x10^8$	14.3	4/6
"	"	"	$5x10^7$	8.5	6/6
"	"	-	-	9.2	6/6

Table 9. Effect of various doses of C3H/RV and C3H/He spleen cells primed with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus on adoptive immunization of C3H/He recipients against lethal SQ challenge (2.0 $\log_{10}$ $TCID_{50}$) of Banzi virus. Donor cells were harvested 7 days after priming and injected IP and IV (1:1). Recipients were challenged with virus 1 day before cell transfer. AST = average survival time in days. Mice were observed for 21.0 days. Results of a typical experiment.

Note: Based on AST, RV spleen cells protect recipients better than equivalent numbers of He spleen cells.

*MR = mortality rate

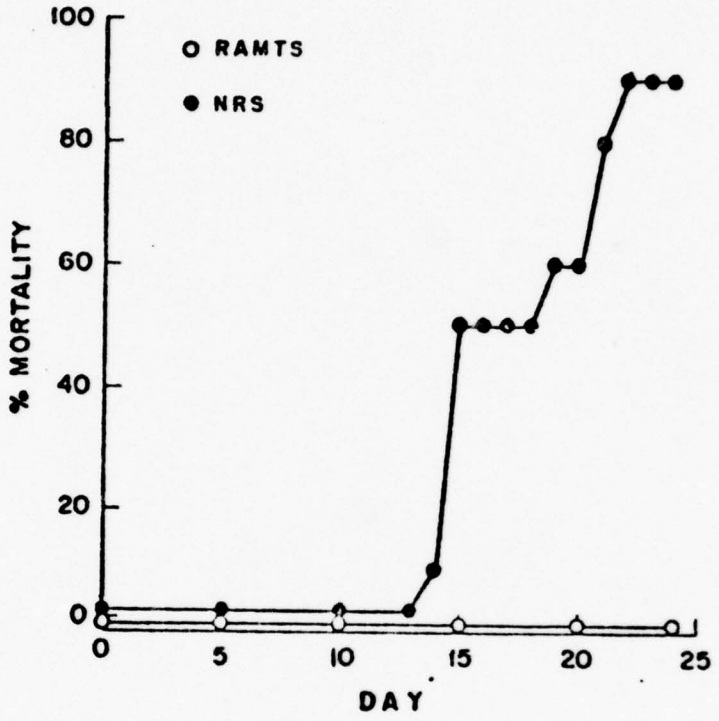

Figure 1. Effect of rabbit antimouse thymocyte serum (RAMTS) or normal rabbit serum (NRS) on mortality rate of adult C3H/RV mice given ip inoculations of 3.0 $\log_{10}$ $TCID_{50}$ of Banzi virus serum was inoculated in 0.2 ml aliquots ip beginning 2 days before virus challenge and inoculations were continued every other day for 16 days. Ten mice per group.

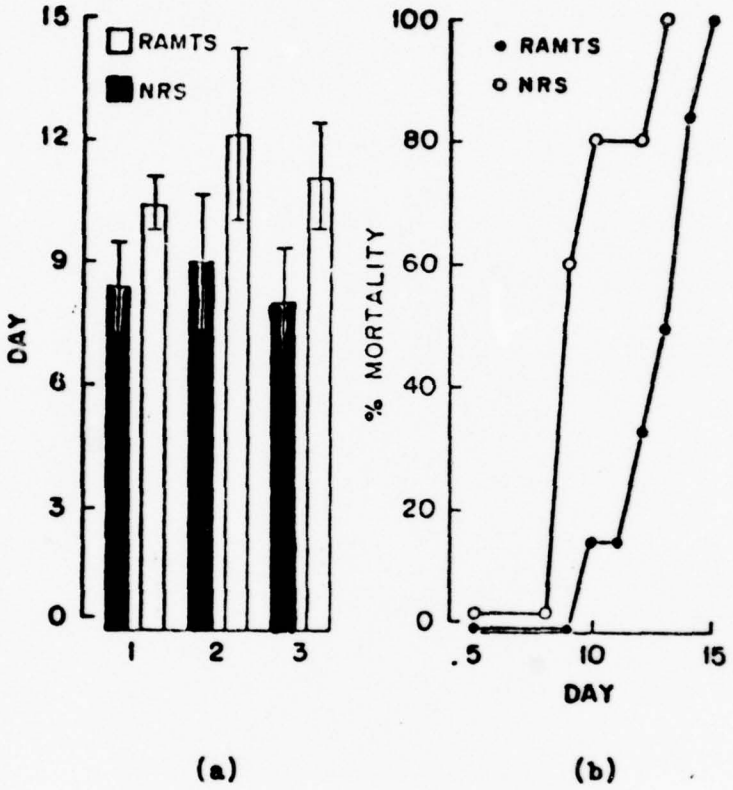

Figure 2. Effect of rabbit antimouse thymocyte serum (RAMTS) or normal rabbit serum (NRS) on average survival time (AST±SD) (a) and mortality rate (b) of adult C3H/He mice given ip inoculations of 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus. 0.2 ml of serum was inoculated ip every other day beginning 2 days before virus challenge. (a) results of 3 typical experiments with 6 to 10 mice per group. (b) results of typical mortality with 10 mice per group.

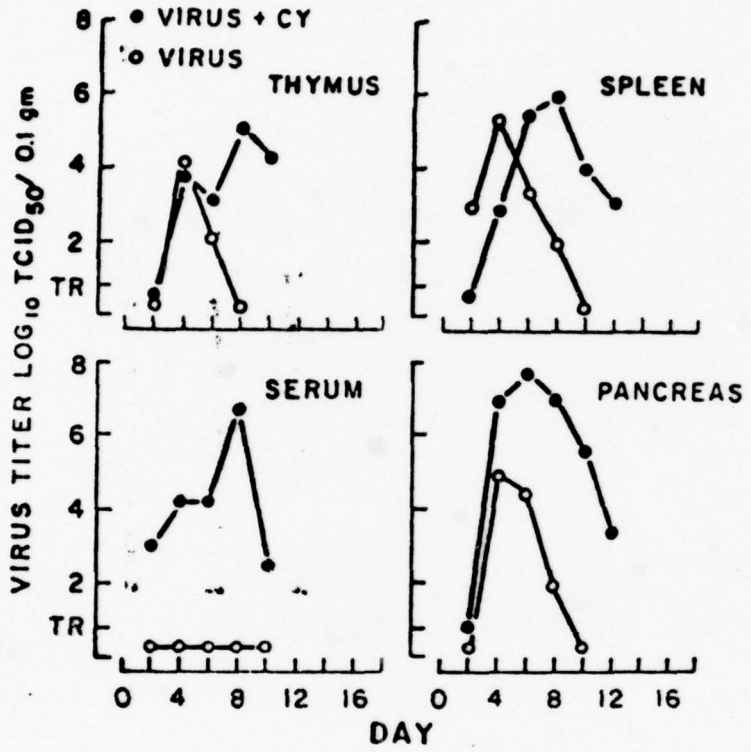

Figure 3. Mean titers of Banzi virus in thymus, spleen, serum, and pancreas of adult C3H/RV mice given cyclophosphamide (150 mg/kg) one day after ip inoculation of 3.0 $\log_{10}$ $TCID_{50}$ of Banzi virus. Each point represents two or three mice. Cy = cyclophosphamide.

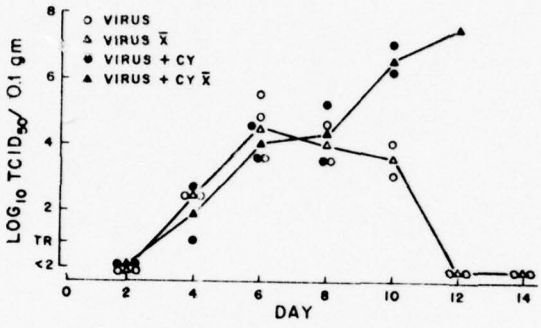

Figure 4. Mean ($\bar{X}$) titers of Banzi virus in brain of adult C3H/RV mice given cyclophosphamide (150 mg/kg) one day after ip inoculation of 3.3 $\log_{10}$ $TCID_{50}$ of Banzi virus. Each point represents two or three mice. Cy = cyclophosphamide.

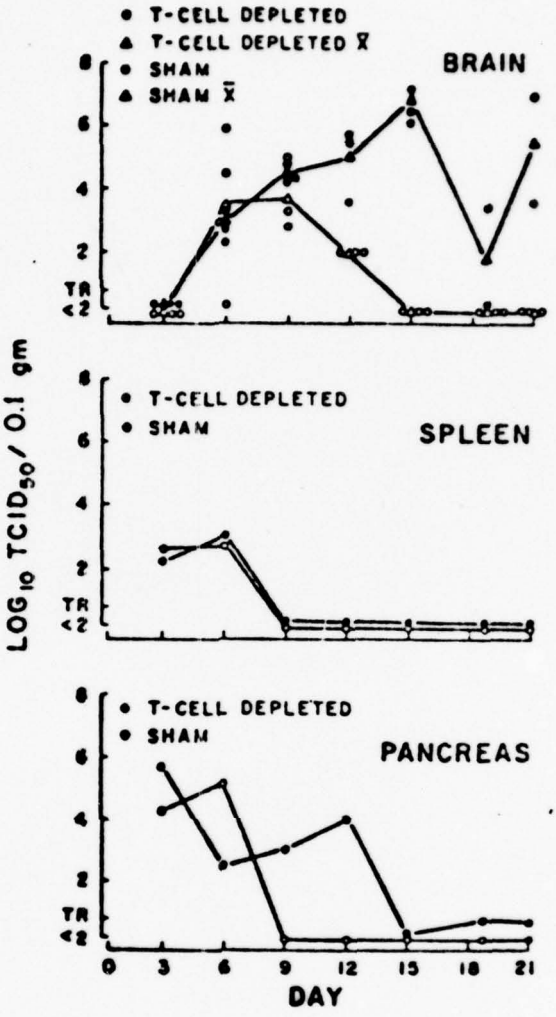

Figure 5. Mean ($\bar{X}$) titers of Banzi virus in brain, spleen, and pancreas of thymus (T-) cell-depleted (thymectomy, 850 rad of X-irradiation, and bone marrow reconstitution) adult C3H/RV mice given 2.5 $\log_{10}$ $TCID_{50}$ of Banzi virus. Each point represents two or three mice.

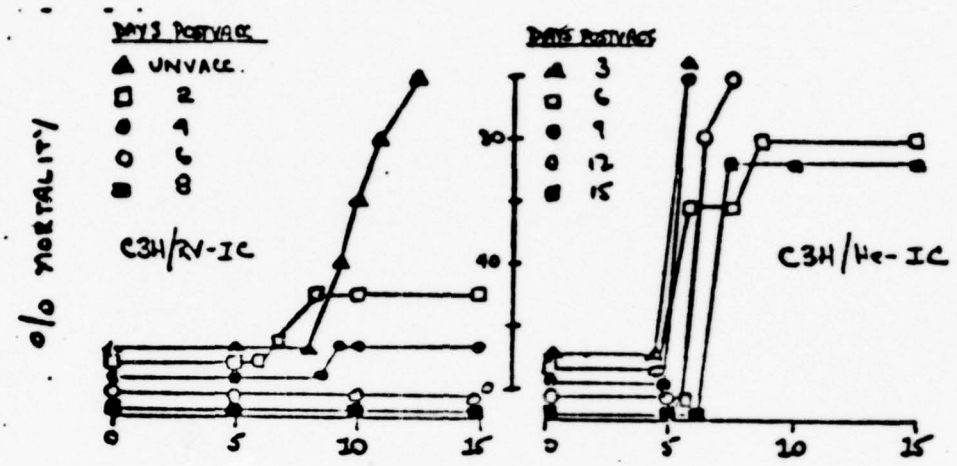

Figure 6. Effect of ip vaccination with 0.2 ml formalinized Banzi virus in complete Freund's adjuvant on mortality of C3H/RV and C3H/He adult mice challenged IC with 2.0 $\log_{10}$ $TCID_{50}$ of live Banzi virus at various days after vaccination. Note RV mice have complete protection by day 6 whereas He mice have little to no protection up to day 15.

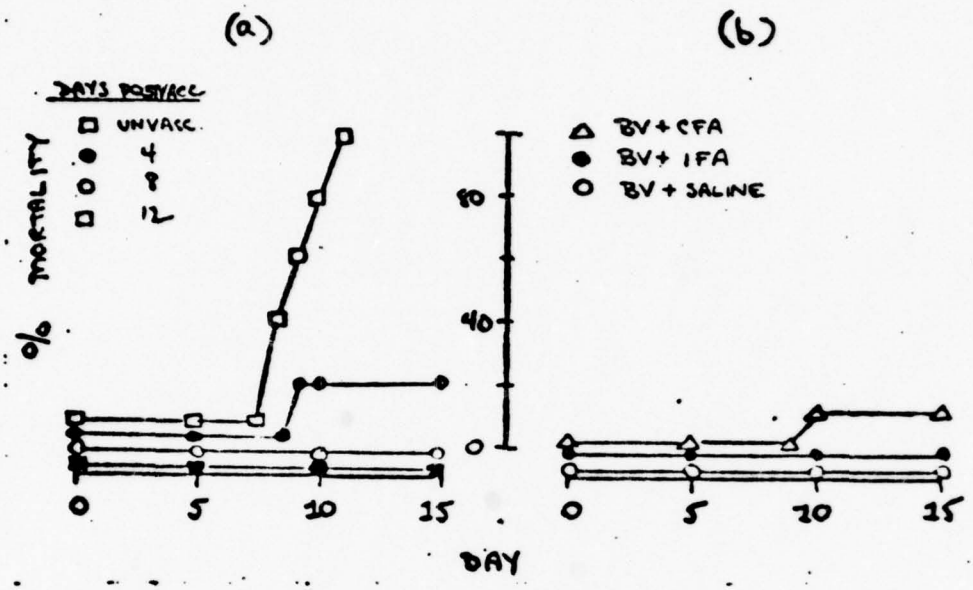

Figure 7. Effect of ip vaccination with 0.2 ml formalinized Banzi virus on mortality of C3H/He adult mice challenged ip with 2.0 $\log_{10}$ $TCID_{50}$ of live Banzi virus (a) Mice were primed with BV+CFA and were challenged at various days. (b) Mice were primed with 1 of 3 vaccines and were challenged 6 days after priming.

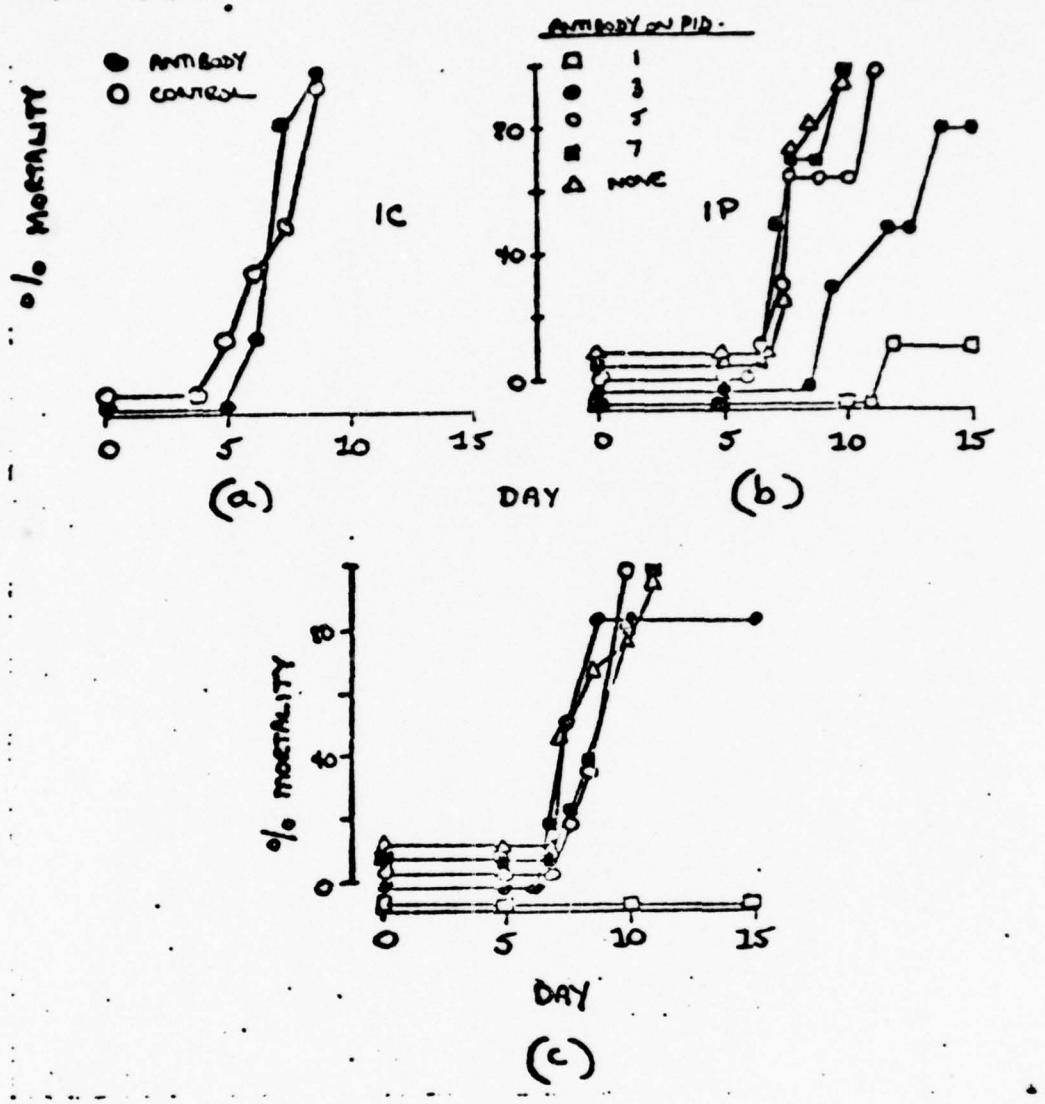

Figure 8. Effect of passive immunization with anti-Banzi antibody (immune ascitic fluid); 0.5ml-ip on mortality of C3H/He and C3H/RV adult mice challenged by various methods.

a) C3H/He mice given antibody or normal ascitic fluid 1 day after ic challenge with 2.0 $\log_{10}$ $TCID_{50}$ of virus.

b) C3H/He mice given antibody on days k,3,5 or 7 after ip challenge with 2.0 $\log_{10}$ $TCID_{50}$ of virus. Note protection is adequate only in group given antibody by 1 day after challenge.

c) C3H/RV mice given antibody 1,3,5 or 7 days after ic challenge with 2.0 $\log_{10}$ $TCID_{50}$ of virus. Legend is the same as for (b). Note protection against ic challenge occurs but only when antibody is given by 1 day after challenge.

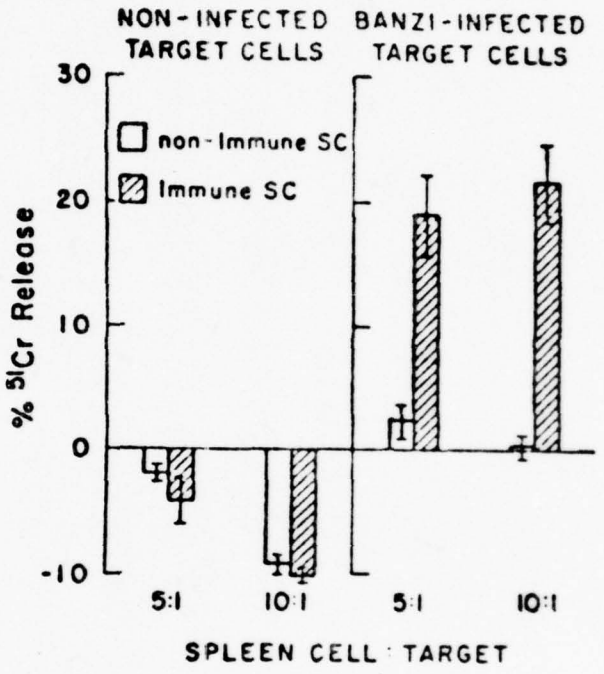

Figure 9. Cytotoxic activity of nonimmune and Banzi-immune C3H/RV spleen cells (SC) for Banzi infected or uninfected L cell monolayers. Mice were immunized with $10^{2.0}$ of live Banzi virus and SC were harvested 7 days postinfection. The data represents the mean and standard error for four replicates.

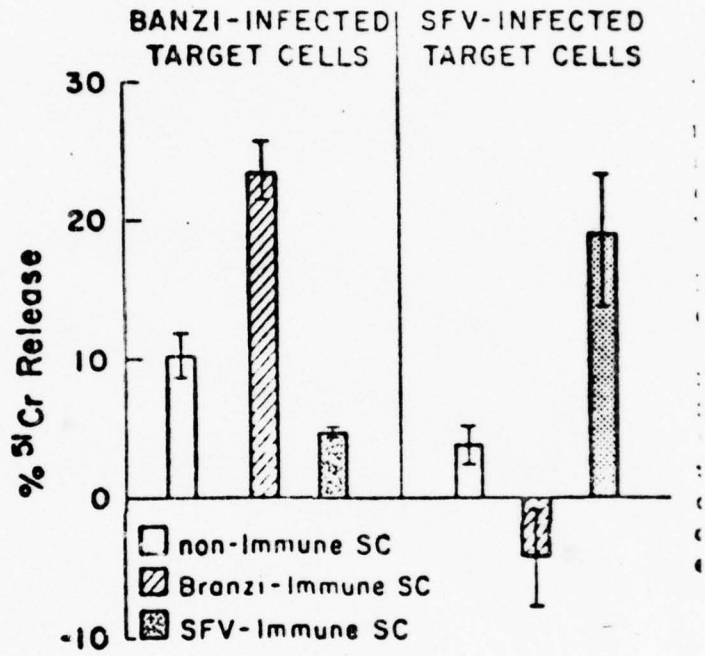

Figure 10. Specificity of the cytotoxic response. C3H/RV mice (three mice per group) were injected with supernatant from uninfected VERO cell cultures (open bars), $10^{2.0}$ LD_{50} Banzi virus-infected VERO cell cultures (hatched bars) or $10^{2.0}$ LD_{50} Semliki Forest virus (SFV) infected VERO cell cultures (shaded bars). Spleen cells (SC) were harvested 7 days later and incubated with L cells which had been inoculated with either Banzi or SFV ($10^{3.0}$ to $10^{4.0}$ $TCID_{50}$). The medium used in this experiment was RPMI 1640 containing 0.5% normal mouse serum. The data represents the mean and standard error for three to four wells per group.

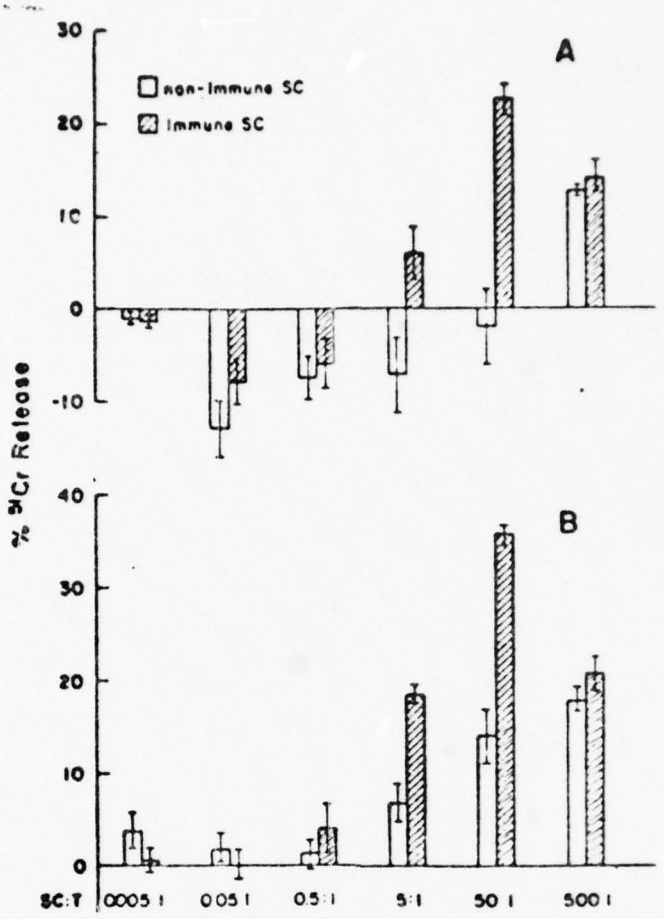

Figure 11. Effect of various spleen cell: target cell ratios on cytotoxic response. SC were from non-immune mice (three mice per group) or from mice injected 6 days previously with $10^{2.0}$ LD_{50} of Banzi virus. A) SC from C3H/RV mice. B) SC from C3H/He mice. SC:T = spleen cell to target ratio. Data represent the mean and standard error of four replicates.

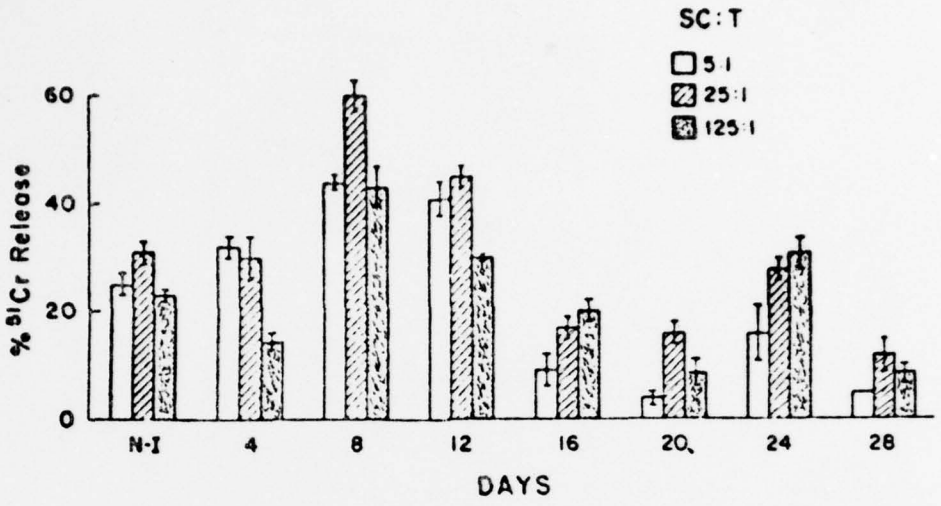

Figure 12. Cytotoxic responses of C3H/RV spleen cells (SC) harvested at various days after immunization. C3H/RV mice (three per group) received a single inoculation of a mouse brain suspension containing $10^{2.0}$ LD_{50} of Banzi virus at 4 day intervals for 28 days. One group of C3H/RV mice received a single inoculation of normal mouse brain (N-I = non-immune) 6 days prior to harvest. SC from these mice were harvested and incubated with Banzi virus infected ($10^{3.0}$ to $10^{4.0}$ $TCID_{50}$) L cells. SC:T = spleen cell to target ratio. Data represent the mean and standard error or four replicates.

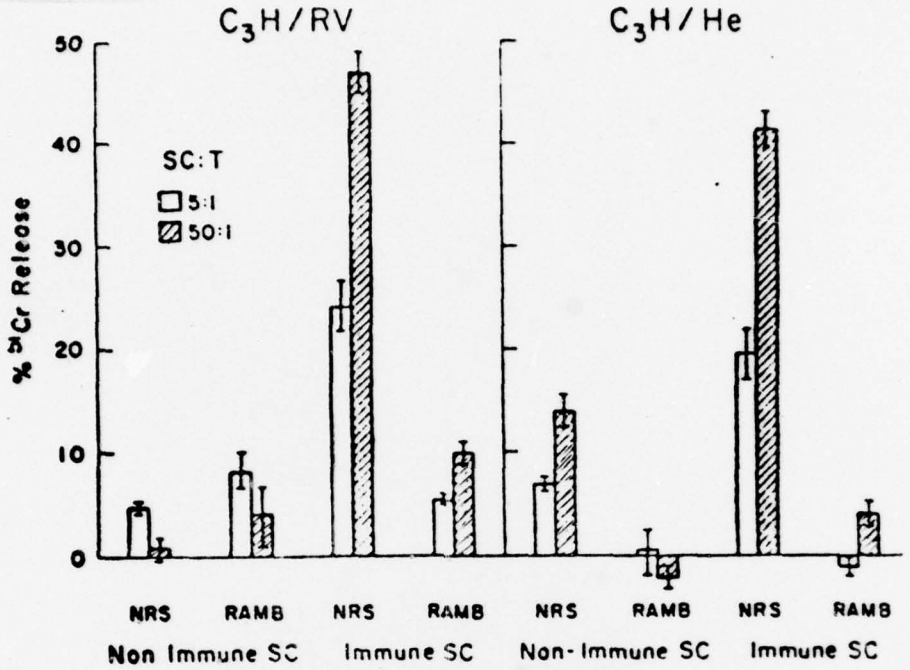

Figure 13. Effect of pretreatment of spleen cells (SC) with either rabbit antimouse brain (RAMB) serum and complement or normal rabbit serum (NRS) and complement of cytotoxic activity. C3H/RV and C3H/He mice (three mice per group) received either an injection of normal mouse brain (open bars or $10^{2.0}$ LD_{50} of Banzi virus (hatched bars). Spleen cells were harvested 6 days later, incubated for 30 minutes with either RAMB serum or NRS, washed and then incubated with Banzi virus infected L cells. SC:T = spleen cell to target ratio. A) SC from C3H/RV mice. B) SC from C3H/He mice. Data represent mean and standard error of four replicates.

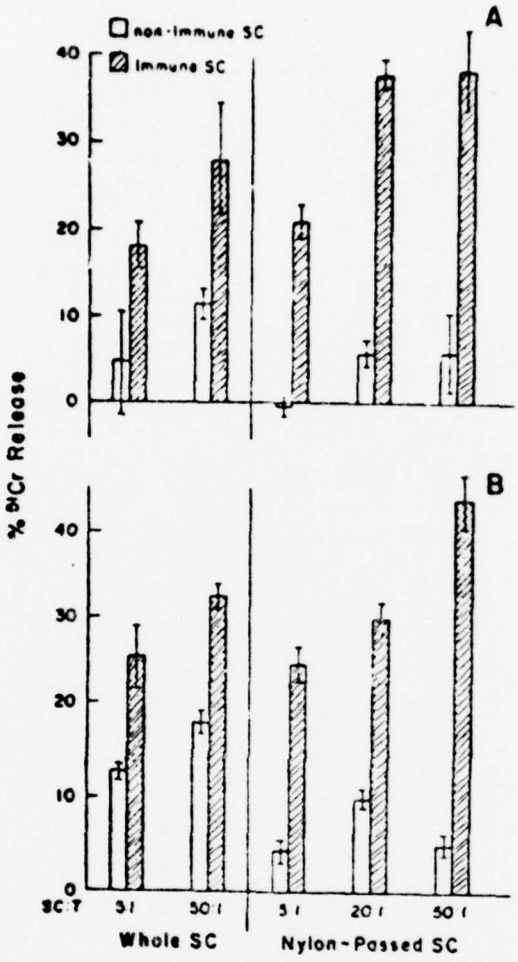

Figure 14. Cytotoxic activity of nylon wool filtered and unfiltered spleen cells (SC). C3H/RV and C3H/He mice (three mice per group) received either normal mouse brain suspension or $10^{2.0}$ LD_{50} Banzi virus and their spleens were harvested 6 days later. 2 x 10^7 SC from each group were filtered through nylon wool columns. Whole spleen and nylon filtered SC were incubated with Banzi virus infected ($10^{3.0}$ to $10^{4.0}$ $TCID_{50}$) L cells. A) SC from C3H/RV mice. B) SC from C3H/He mice. SC:T = spleen cell to target ratio. Data represent the mean and standard error of four replicates. These are the results of two separate experiments.

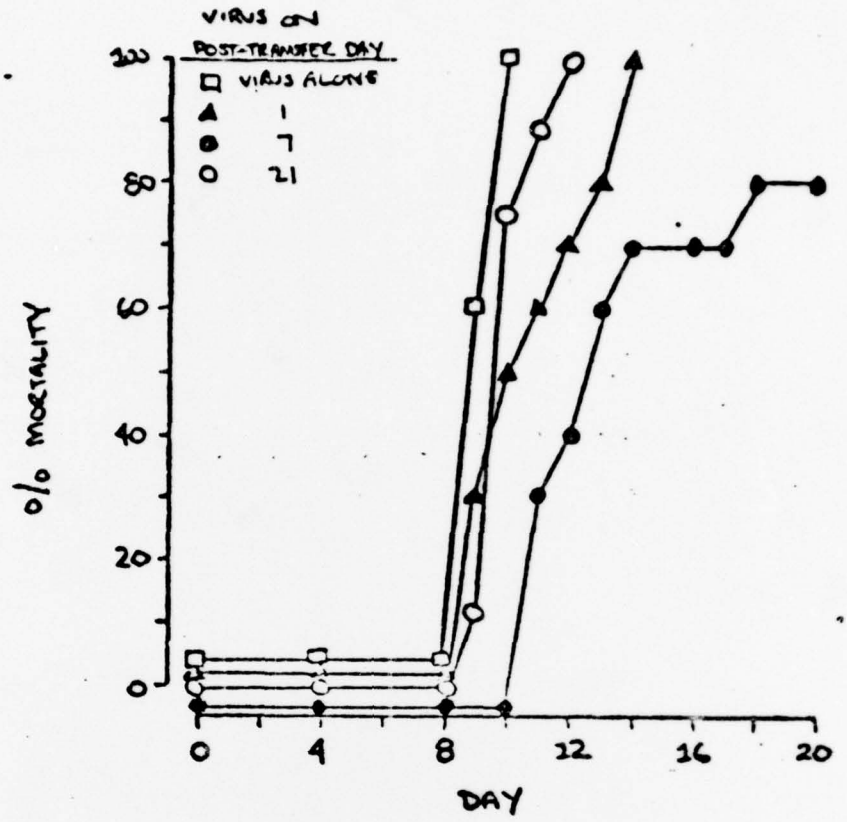

Figure 15. Mortality among groups of C3H/He mice challenged with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus ip at various intervals after receiving $7x10^7$ pooled spleen cells and lymph node cells from unprimed C3H/RV mice. Recipients were given 400R of x-irradiation on the day of cell transfer. Recipients were challenged 1 day, 1 week or 3 weeks after receiving cells. Six to 10 mice per group.

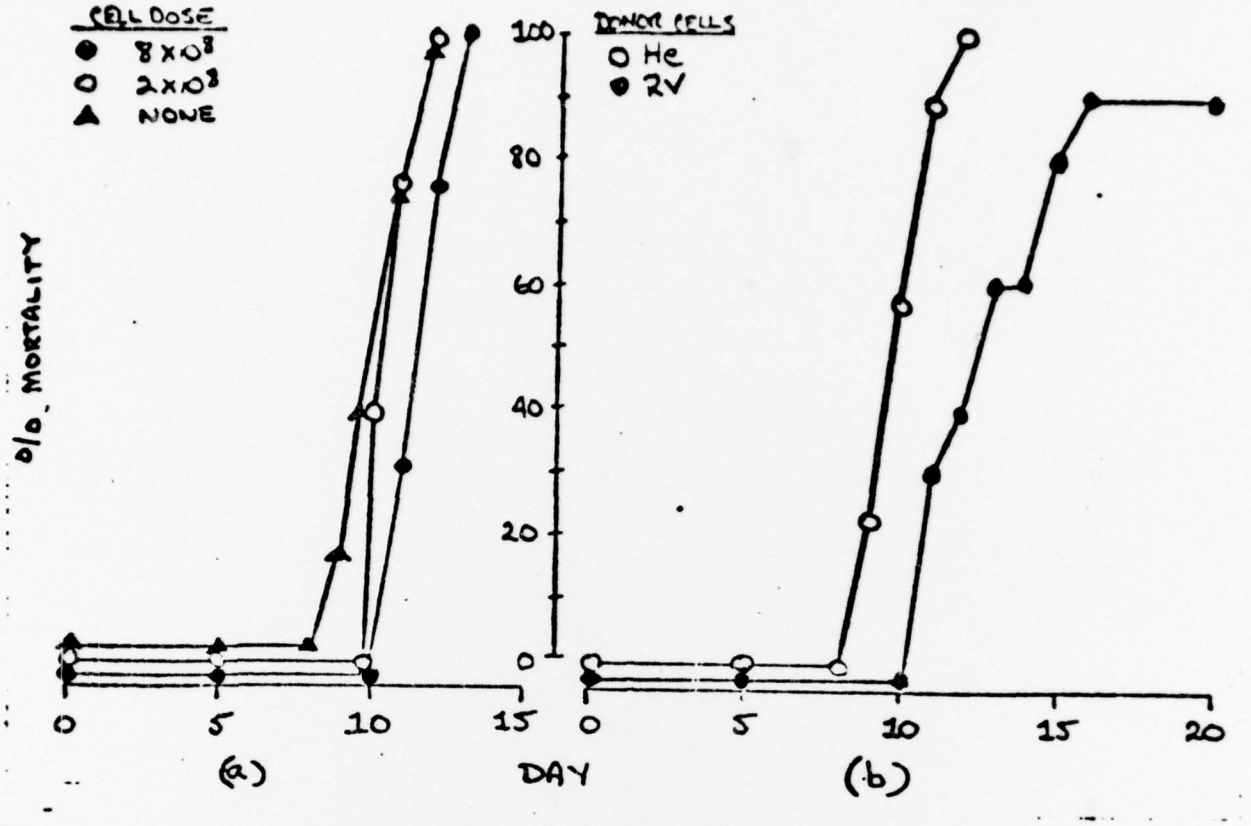

Figure 16. Mortality among groups of C3H/He mice challenged with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus up after receiving unprimed donor lymphoid cells.

a) Mortality among groups of C3H/He mice challenged with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus ip one day after receiving $8x10^8$, $2x10^8$ or no unprimed RV spleen cells. Recipients also received 250R x-irradiation on the day of transfer. Six mice per group.

b) Mortality of C3H/He recipients given 10^8 non-immune RV or He lymph node and spleen cells one week before challenged mice were given 400R x-irradiation prior to transfer. Other experiments using non-irradiated recipients gave similar results. That is He recipients of RV cells had an AST several days longer than He recipients of He cells and there was an occasional permanent survivor. Also, spleen cells or lymph node cells given separately produced the same effect as pooled cells. Experiments utilized 6 to 10 mice per group.

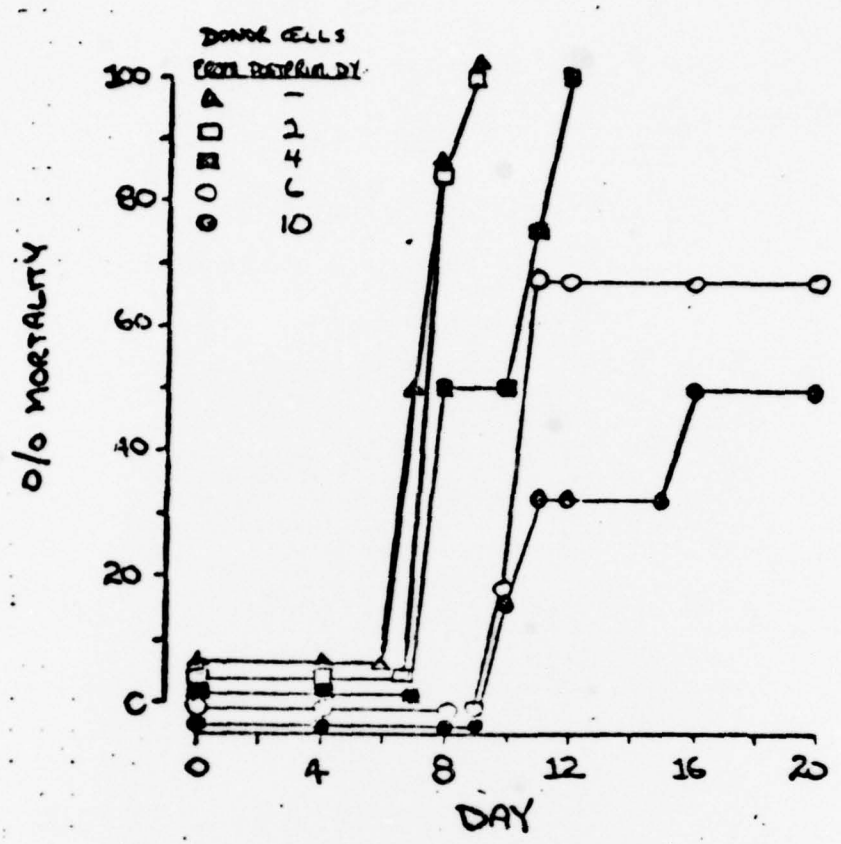

Figure 17. Mortality among groups of C3H/He mice adoptively immunized with $2x10^8$ primed C3H/RV spleen cells one day after lethal challenge with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus ip. Donor cells were harvested at 2, 4, 6 or 10 days after priming with live Banzi virus and were inoculated IV.

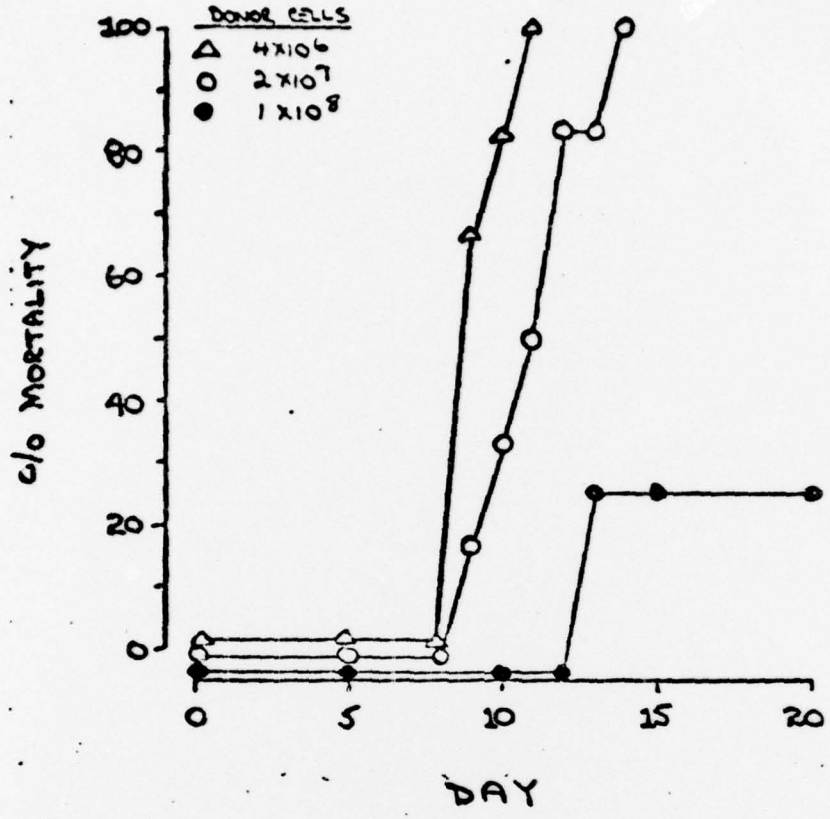

Figure 18. Effect of donor spleen cells primed with BV+CFA on adoptive transfer of resistance to lethal challenge with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus in C3H/He mice. Spleen cells were harvested 6 days after priming. Recipients were sublethally x-irradiated (250R) on the day of transfer (day 6) and were challenged with virus 1 day later. Six mice per group.

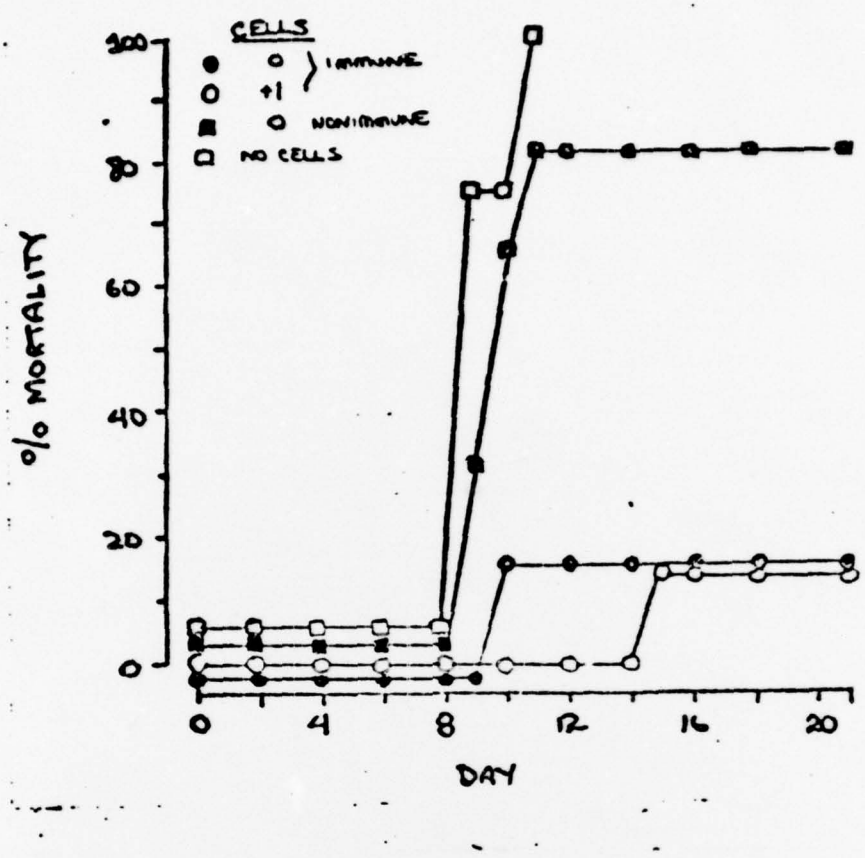

Figure 19. Effect of immune or nonimmune C3H/RV spleen cells on adoptive transfer of resistance to lethal challenge with 2.0 $\log_{10}$ $TCID_{50}$ of Banzi virus in C3H/He mice. Donor cells were harvested 7 days after priming with 2.0 $\log_{10}$ $TCID_{50}$ of live Banzi virus. Recipients received $2x10^8$ cells (half ip and half IV). Groups of 6 recipient mice included: 1) mice given no cells (open boxes); 2) mice given nonimmune cells and virus simultaneously (closed boxes); 3) mice given immune cells and virus similtaneously (closed circles); and 4) mice given immune cells 1 day after virus (open circles).

Figure 20. Yields of Banzi virus from tissues of He mice adoptively immunized with various numbers of Banzi virus-immune RV or He splenic cells one day after lethal challenge with 100 $TCID_{50}$ of virus.

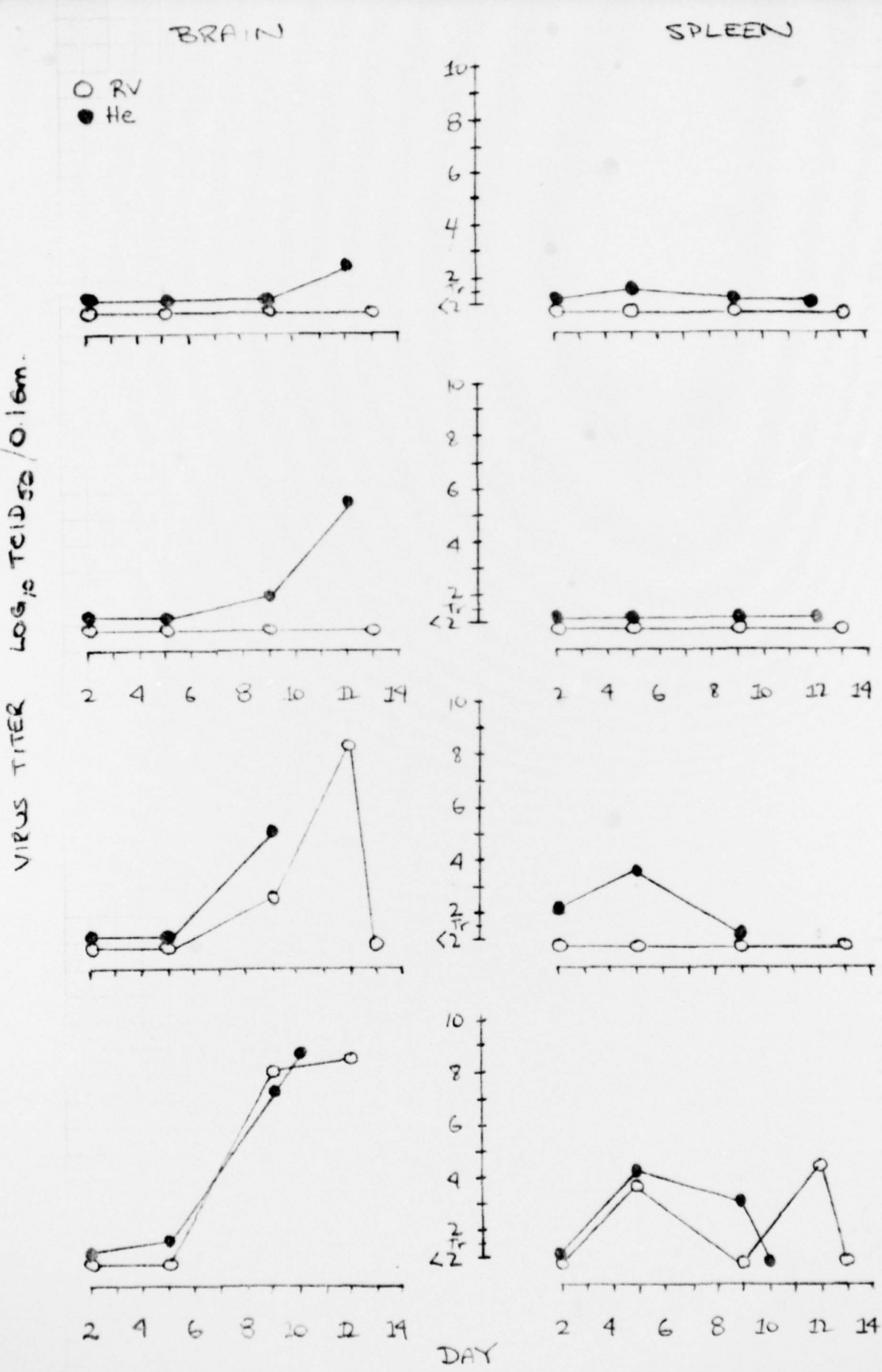
BRAIN
SPLEEN
SCXIC 8
○ RV
● He
VIRUS TITER LOG10 TCID50/0.16m.
2.0
1.0
0.5
0
DAY
2 4 6 8 10 12 14

Figure 20.

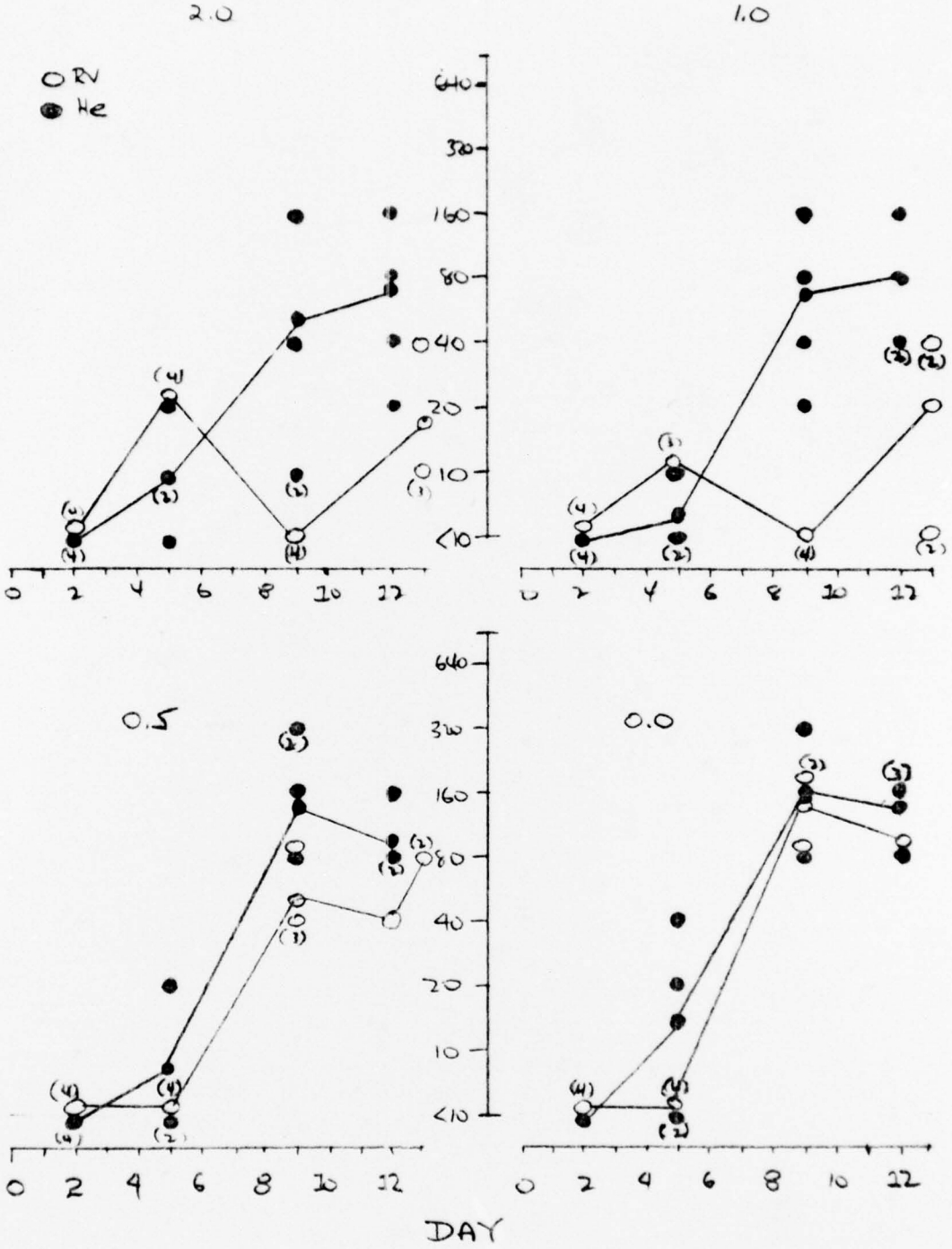

Figure 21. Serum HAI antibody titers in He mice adoptively immunized with various numbers of Banzi virus-immune RV or He splenic cells one day following challenge with 100 $TCID_{50}$ of virulent virus.

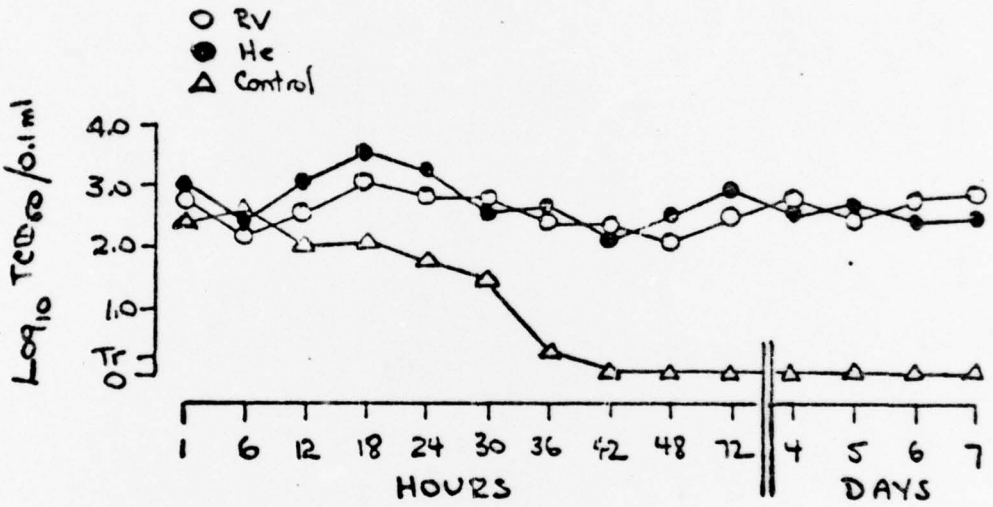

Figure 22. Replication of Banzi virus in cultures of unstimulated peritoneal macrophages. Macrophage monolayers were established in triplicate in 25 cm^2 plastic bottles by seeding $1.5x10^6$ cells/ml of medium. Cultures were inoculated with $10^{7.6}TCID_{50}$ of virus and washed 5 X after 1 hour incubation. Medium was collected and completely replaced at each of the sampling times indicated. Titers represent an average of 3 samples (1 per culture) for each sampling time. Controls consisted of bottles containing medium, but no cells.

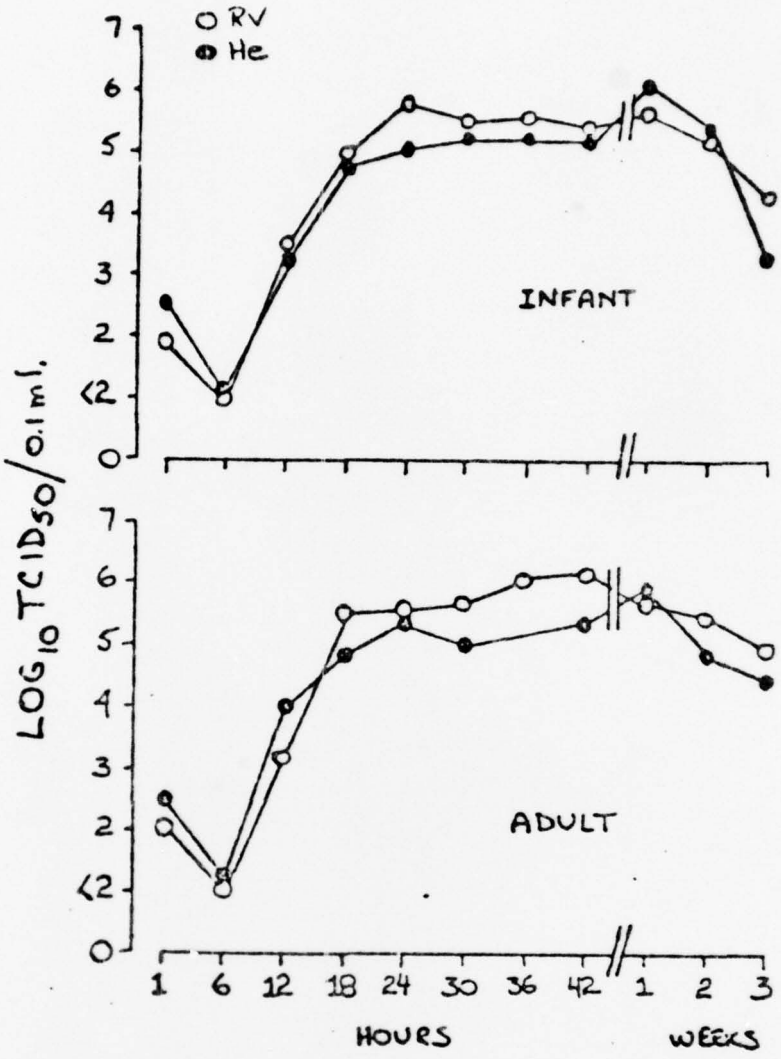

Figure 23. Mean titers of Banzi virus in monolayer cultures of brain cells from infant and adult C3H/RV and C3H/He mice. Cells were obtained by growing trypsinized brain fragments in plastic bottles using 80% minimum essential medium (Eagle's base) and 20% fetal bovine serum with 3.3% glucose. Cultures were at 3rd and 4th passage. They were inoculated with 6.6 $\log_{10}$ $TCID_{50}$ of virus and incubated for 1 hr. on a rotating platform. Medium was completely replaced after each collection.

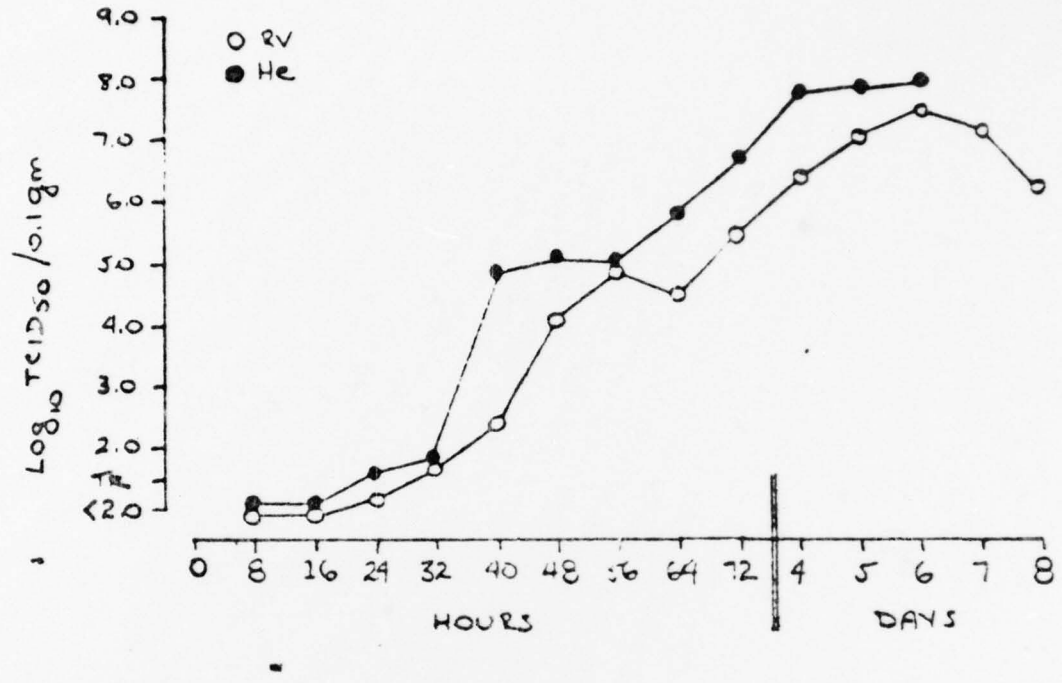

Figure 24. Average titers of Banzi virus in brains of adult RV and He mice inoculated intracerebrally with $10^{1.0}$ $TCID_{50}$ of virus. Whole brains were collected from 3 mice at each of the times indicated. Note that there is a small (± 1.0 $10g_{10}$) but persistent difference in the virus titer: RV brain titers are lower than He brain titers. Note also that He mice were all dead by day 6 whereas enough RV mice remained alive to sample through day 8.

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