Genetic Mapping of Lymphocytic Choriomeningitis Virus Pathogenicity: Virulence in Guinea Pigs Is Associated with the L RNA Segment†

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The Armstrong CA 1371 (ARM) and WE strains of lymphocytic choriomeningitis virus (LCMV) differ in the ability to produce disease in adult guinea pigs. Infection with the ARM strain is not lethal, even at high virus doses ($>10,000$ PFU), whereas the WE strain causes 100% mortality even at low doses (<10 PFU). To determine the genetic basis of this virulence, intertypic reassortants were made between the ARM and WE strains of LCMV. The two reassortants with the genotypes WE/ARM (L segment of WE and S segment of ARM) and ARM/WE (L segment of ARM and S segment of WE) were tested for their pathogenicity in guinea pigs. The ARM/WE reassortant was avirulent like the ARM/ARM parental strain. Minimal viral replication was observed in organs of guinea pigs inoculated with 10^2 or 10^5 PFU of ARM/ARM or ARM/WE, and all animals survived. In contrast, the WE/ARM reassortant was highly virulent like the WE/WE parental strain and killed all of the infected animals. High levels of viral replication were observed in guinea pigs infected with the latter two strains. In contrast to these *in vivo* observations, both the parental strains and the ARM/WE or WE/ARM reassortants had similar growth potential in cultured guinea pig fibroblasts. Thus, the L RNA segment of LCMV WE is important for viral replication *in vivo* and is associated with fatal acute disease after infection of adult guinea pigs.

Lymphocytic choriomeningitis virus (LCMV) is the prototypic arenavirus. The genome consists of two single-stranded RNA species (15), L and S, with approximate molecular weights of 2.85×10^6 and 1.35×10^6 , respectively (5). The following four major viral structural proteins have been identified: a 63,000-molecular-weight (63K) internal nucleoprotein, NP, associated with the genomic RNA; two surface glycoproteins, GP1 (44K) and GP2 (35K) (3) and a large protein, L (180K) (7; M. J. Buchmeier et al., Abstr. Negative Strand Virus Meeting 1985). Several individual LCMV strains have been distinguished and segregated on the basis of hybridization with cDNA probes, T1 oligonucleotide mapping, tryptic peptide mapping, and monoclonal antibody analysis (2, 3, 5, 19). In addition, variations in the pathogenicity of the LCMV strains have been observed. For example, C3H newborn mice inoculated with the Armstrong CA1371 strain of LCMV (LCMV ARM) develop growth retardation and low blood sugar due to viral curtailment of growth hormone synthesis, but infection with the WE strain has no effect on growth, development, or blood sugar levels (17). Adult guinea pigs resist infection by the ARM strain but succumb to infection by the WE strain (5, 10).

To define the precise role of the L and the S RNA segments and their respective gene products in disease, we selected reassortants formed by double infection of BHK₂₁ cells with the ARM and WE strains. When these reassortants were tested for their pathogenicity in guinea pigs, the ARM/WE reassortant was avirulent like the ARM parental strain, and the guinea pigs survived the infection. In contrast, the WE/ARM reassortant was virulent like the WE parental strain and caused a lethal infection with 100%

mortality. Thus, the gene products encoded by the L RNA segment of WE are required for the virulence of LCMV in adult guinea pigs. The novelty of our findings incriminating the L RNA segment in disease of guinea pigs is underscored by recent observations that the other RNA segment, the S RNA, and the products it encodes are responsible for the virus-induced growth hormone deficiency (17).

MATERIALS AND METHODS

Animals. Male adult Hartley guinea pigs weighing 300 to 400 g were obtained from Sprague-Dawley, Indianapolis, Ind. They were inoculated with either 10^2 or 10^5 PFU of virus by intraperitoneal (IP) injection (0.5 ml).

Virus. The origins of the two LCMV parental strains used in this study, ARM and WE, have been described (5). Reassortants between LCMV/ARM and LCMV/WE were generated and characterized as reported (16). Virus stocks of the two parental genotypes (L RNA/S RNA: ARM/ARM, WE/WE) and reassortant genotypes (ARM/WE, WE/ARM) were cloned and plaque purified three times on Vero cells. Thereafter, a stock was prepared through one passage in BHK₂₁ cells. Infectious LCMV was quantitated by plaque assay on Vero cell monolayers (5).

Immunosuppression. A single dose of cyclophosphamide (Neosar; Adria Laboratories, Inc., Columbus, Ohio) was administered to guinea pigs IP 1 day before or 3 days after inoculation with 10^5 PFU of virus.

Interferon titration. Guinea pig interferon activity was measured by the inhibition of the cytopathic effect of vesicular stomatitis virus on guinea pig fibroblasts (ATCC CCL 158, JH4 clone 1) with a microassay technique as described previously (8).

Immunofluorescence and histopathological studies. Tissues were removed from animals sacrificed after ether anesthesia at 4, 8, 10, and 11 days after infection. Samples for immu-

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FIG. 1. Survival of guinea pigs infected IP with the parental strains (ARM/ARM or WE/WE) or reassortants (ARM/WE or WE/ARM) of LCMV. Groups of eight adult male Hartley guinea pigs were injected (0.5 ml) with 10^2 (solid line) or 10^5 (dotted line) PFU of each virus, and mortality was scored for 30 days.

no fluorescence were snap frozen in liquid nitrogen, and frozen sections (4 μ m) were cut on a cryostat, fixed with acetone, and stained with fluorescein isothiocyanate-conjugated antibody to LCMV. The sections were then mounted under buffered glycerol and examined with a Zeiss fluorescence microscope. Samples for histology were fixed in Bouin solution, stained with hematoxylin-eosin, and examined by light microscopy.

RESULTS

Mortality after infection with the four different viruses. Groups of eight guinea pigs were injected IP with 10^2 or 10^5 PFU of each LCMV genotype (ARM/ARM, ARM/WE, WE/ARM, or WE/WE). The infected guinea pigs were checked daily over a 1-month period (results summarized in Fig. 1). No fatalities were observed in guinea pigs infected with LCMV containing the L RNA segment of ARM, genotypes ARM/ARM and ARM/WE. These guinea pigs were resistant to a lethal challenge with 10^5 PFU of WE/WE 1 month after the first injection (data not shown), indicating that they developed immunity after infection with ARM/WE and ARM/ARM viruses. In contrast, all guinea pigs receiving reassortants containing the L RNA segment of WE died between days 11 and 22 after infection was initiated. A similar result was obtained with another independently derived reassortant with the WE/ARM genotype. All of the guinea pigs infected with the parental WE/WE virus died between days 9 and 12 after infection. About 2 to 3 days before death, animals infected with WE/ARM or WE/WE virus lost weight, became moribund, and breathed with difficulty. Results were similar with viral doses of 10^2 and 10^5 PFU.

Mortality of guinea pigs infected with the WE/ARM or

WE/WE genotype is associated with high viremia. No infectious virus was detected in the sera of animals 1, 2, 3, 4, 8, and 11 days after they received 10^5 PFU of LCMV containing the L RNA segment of ARM (ARM/ARM, ARM/WE) (Fig. 2). In contrast, infectious virus was recovered from sera of guinea pigs receiving the WE/WE or the WE/ARM LCMV. Guinea pigs receiving WE/ARM LCMV had a 3-day delay in viremia and a 3- to 8-day delay in death, when compared with guinea pigs inoculated with WE/WE LCMV. Nevertheless, guinea pigs inoculated with either WE/WE or WE/ARM LCMV possessed infectious titers close to 10^5 PFU/ml of serum by 8 and 11 days after infection, the time when the animals started to die.

Next, we determined whether the absence of infectious virus in the sera of guinea pigs infected with the ARM/ARM and ARM/WE LCMV was associated with interferon induction. The level of serum interferon was below the limit of detection (37.5 U/ml) from days 1 to 4 after infection with

FIG. 2. Virus titers in the sera and tissues of guinea pigs. Adult male guinea pigs were injected IP at day zero with 10^5 PFU of parental strains (ARM/ARM [○] or WE/WE [□]) or reassortants (ARM/WE [●] or WE/ARM [■]). Guinea pigs were bled at the indicated intervals after infection, and their sera were collected for virus titration. Each symbol represents the mean virus titer in the serum of an individual guinea pig at days 1, 2, 3, and 11 and the mean of two titers from two different guinea pigs at days 4, 8, and 10. Two or three guinea pigs in each group were sacrificed at days 4, 8, and 10 to 11 after infection, and organs were collected and stored at -70°C until virus titration. Vertical bars indicate the range of values.

ARM/ARM or ARM/WE. Interferon was present at days 2 and 3 (375 and 187 U/ml) in WE/WE infection and at day 2 (125 U/ml) in WE/ARM infection. Therefore, the absence of infectious virus in the sera of ARM/ARM- and ARM/WE-infected guinea pigs did not correlate with heightened interferon production.

Level of viral replication in tissues in vivo correlates with the L RNA of WE LCMV. Virus titers were determined in the adrenal glands, kidneys, liver, lungs, and spleen of two or three guinea pigs infected 4, 8, 10, and 11 days earlier with 10^5 PFU of LCMV. Results are summarized in Fig. 2. In guinea pigs infected with LCMV containing the L RNA segment of WE (WE/WE and WE/ARM), there were high levels of infectious virus as early as 4 days after virus inoculation. The highest value was in the spleen (approximately 10^6 PFU/g). By day 8, virus titers had increased in all organs. From days 8 to 11, virus titers in most organs remained constant or increased, except in the spleen, where a 100-fold reduction in the virus titer occurred. In the guinea pigs infected with the ARM/WE genotype, infectious virus was detected from days 4 to 11 in the spleen and was prominent from days 8 to 10 in the liver. These titers peaked at day 10 and then decreased. Virus was detected only at day 10 in the kidneys and adrenals and at days 10 and 11 in the lungs. In all organs, the titers were 10- to 1,000-fold lower in guinea pigs infected with ARM/ARM or ARM/WE than in those infected with LCMV containing the L RNA segment of WE (WE/WE, WE/ARM). In guinea pigs infected with the parental ARM strain, virus was detected only in the spleen 4 and 8 days after infection; titers were about 10-fold lower in comparison to the ARM/WE-infected guinea pigs and about 1,000-fold lower in comparison to the WE/ARM- or WE/WE-infected guinea pigs. Thus, there was a correlation between the viral replication in organs and the L RNA segment of WE in vivo. When tested for growth potential, LCMV ARM/ARM, ARM/WE, WE/ARM, and WE/WE replicated equally well in guinea pig fibroblast cells (line JH4, CCL 158, ATCC) (Fig. 3).

Expression of viral antigens and histopathology correlates with the L segment of WE LCMV. Guinea pigs infected with 10^5 PFU of each LCMV genotype were sacrificed 4, 8, and 10 or 11 days after infection, and tissues were obtained for histological and immunofluorescent analysis. Histopathological changes were marked in the spleen, liver, and adrenal tissues of guinea pigs infected with LCMV carrying the L RNA segment of WE. The liver showed fatty change with no or minimal necrosis (not shown); the adrenal tissue had focal cortical necrosis, and the spleen developed red-pulp and white-pulp necrosis and marked cell loss (Fig. 4). These changes were maximal 8 days after infection. Guinea pigs infected with LCMV ARM/WE showed a similar splenic lesion at day 4, but it was less intense than that observed with WE/ARM or WE/WE infection. Lesions were not observed in ARM/ARM-infected guinea pigs. Despite high virus titers in the brain (approximately 10^5 PFU/g) and kidneys (Fig. 2) 10 days after infecting guinea pigs with LCMV WE/ARM and WE/WE, neither tissue injury nor cellular necrosis followed.

When these tissues were studied by direct immunofluorescence with fluorescein-isothiocyanate-labeled guinea pig antibody to LCMV, high concentrations of LCMV antigen were detected in livers and spleens of guinea pigs infected 8 days previously with the WE/ARM or WE/WE genotype. Study of the liver revealed viral antigens in about 50% of hepatocytes and a few Kupffer cells; viral antigens were present in the red pulp and in the marginal zone of the

FIG. 3. Virus titers in fibroblasts. Guinea pig fibroblast cells (line JH4) were infected at a multiplicity of infection of 0.2 with the parental strains (ARM/ARM [○] or WE/WE [□]) or reassortants (ARM/WE [●] or WE/ARM [■]). Culture fluids were harvested 6, 24, 48, 72, and 96 h after infection, and virus titer was determined by plaque assay on Vero cells. Each symbol represents the mean of titers from two different samples of culture fluid. Vertical bars indicate the range of values.

follicles of the spleen. In adrenal tissue, foci of infected cells were detected in the cortex (Fig. 4). Viral antigens were localized in the peritubular region in the kidneys and in the endothelium of blood vessels in the brain. In contrast to the abundant display of LCMV antigens in guinea pigs infected with WE/ARM or WE/WE, animals infected with ARM/WE or ARM/ARM genotypes had only a few cells containing LCMV antigens in the livers and spleens. Comparatively, there were significantly fewer viral antigen-containing cells in animals infected by ARM/ARM than in those receiving ARM/WE LCMV.

Effect of cyclophosphamide. Cyclophosphamide treatment suppresses the host immune response. For example, this therapy protects against the ordinarily lethal disease of adult mice after intracerebral LCMV infection presumably mediated by cytotoxic T lymphocytes and not by direct virus cytolytic action (4). The effect of this drug on the course of both lethal infection by WE and subclinical infection by ARM was investigated. There was no sign of illness in guinea pigs treated with a single dose of cyclophosphamide (200 mg/kg, IP) 1 day before infection with LCMV ARM (10^5 PFU). Similarly, cyclophosphamide treatment administered 1 day before or 3 days after infection with LCMV WE (10^5 PFU) did not change the course of the disease (Fig. 5).

FIG. 4. Immunofluorescence and histopathology of spleen and adrenal tissues from guinea pigs infected with LCMV. (A, C) Spleen of a guinea pig at 10 days postinfection with 10^5 PFU of WE/ARM. (B, D) Adrenal tissue of a guinea pig at 10 days postinfection with 10^5 PFU of WE/WE. (A) Hematoxylin-eosin staining. Red-pulp necrosis ($\times 150$). (B) Hematoxylin-eosin staining. Focus of necrosis in the cortical zone with karyorrhexic debris ($\times 150$). (C) Immunofluorescent stain showing viral antigens localized predominantly in cells of the red pulp ($\times 150$). (D) Immunofluorescent stain showing a focus of cells infected in the cortical zone ($\times 250$).

DISCUSSION

Here we record the first evidence that the L RNA segment of LCMV and the proteins which it encodes are associated with disease. To develop this evidence, we made intertypic reassortants, one that contained the L RNA from the virulent WE parental strain and the other containing the S RNA of that viral strain. Disease was defined as the lethal infection of guinea pigs with as little as 10^2 PFU, evidence of significant viral replication and antigen expression in several tissues of an infected animal. By contrast, the characteristics of the nonpathogenic parental strain and reassortant were the inability to cause death even after inoculations of 10^5 PFU, minimal viral replication, and little or no viral antigen expression in tissues from inoculated animals.

The genome of LCMV consists of two single-stranded RNA species designated L and S according to their size (15). Utilizing intertypic reassortants, monoclonal antibodies (16), and antibodies to selected linear amino acid sequences in the open reading frames (Buchmeier et al., Abstr. Negative Strand Virus Meeting), we have determined that the S RNA segment encodes the nucleoprotein and the two viral glycoproteins. The cloning and sequencing of the L segment are not yet complete. However, analysis of open reading frames coded for by the L RNA indicates that, at the very least, an

approximately 180K protein is encoded on that segment (Buchmeier et al., Abstr. Negative Strand Virus Meeting). Although the activity of the protein is not yet known, based on structural similarities with other known polymerases, it has tentatively been identified as the putative LCMV polymerase. This is in agreement with other studies (7, 11) with Pichinde virus which indicate that the L RNA segment encodes the viral polymerase. In our studies, by identifying the L RNA as responsible for the acute disease of LCMV-infected guinea pigs, we have ruled out any major participation in this disease of either of the major LCMV glycoproteins. This is in contrast to reassortants used to map reovirus (6), bunyavirus (1), and influenza virus diseases (18); in those experiments, the viral surface glycoproteins or outer-capsid proteins were implicated in causing disease. Interestingly, the S RNA of LCMV which encodes its two major structural glycoproteins has been correlated with a different LCMV disease. The growth hormone deficiency disorder leading to death from hypoglycemia has been clearly mapped to the S RNA segment with the same intertypic reassortants as those used in this study (17). Additionally, recent studies show that the generation and recognition of virus-specific cytotoxic T lymphocytes restricted to interactions with class I major histocompatibility complex antigens in *in vitro* murine assays are encoded by genes located on the S RNA

FIG. 5. Effect of cyclophosphamide treatment on the course of lethal LCMV WE infection of guinea pigs. Groups of six guinea pigs were either treated with saline 1 day before infection (□...□) or treated with cyclophosphamide (200 mg/kg, IP) 1 day before (□---□) or 3 days after infection (□- - -□). Mortality in the infected (10^5 PFU of LCMV WE, IP, on day zero) and mock-infected groups treated with cyclophosphamide (200 mg/kg, IP) (Δ) was scored over 3 weeks.

of LCMV (Y. Riviere et al., submitted for publication). Thus, the fact that the S RNA is not associated with the acute disease of guinea pigs described here complements our observation that cyclophosphamide treatment has no effect on this disease. Other studies show that cyclophosphamide treatment prevents the immune response disease caused by intracerebral inoculation of LCMV into adult mice, the mechanism of injury most likely being the destruction of virus-infected cells caused by virus-specific H-2-restricted cytotoxic T lymphocytes (20).

Acute lethal disease of adult guinea pigs is primarily associated with the L RNA segment of LCMV. Infection of adult guinea pigs with WE/WE or WE/ARM was lethal, since all guinea pigs inoculated IP with doses of 10^2 PFU died. Infectious virus was found in their sera and was present in all organs studied 4, 8, 10, and 11 days after infection. Immunofluorescence studies indicated the presence of viral polypeptides in heavy concentrations in all of the organs studied. Light microscopy showed frequent evidence of necrosis in the spleen and adrenal glands with spotty necrosis in the liver, where fatty changes were also observed. In contrast, infection of adult guinea pigs with ARM/ARM led to a subclinical infection with viral replication largely restricted to the spleen and with ARM/WE to a subclinical infection with viral replication in several organs. In all instances, the amount of infectious virus found in several organs of ARM/ARM or ARM/WE-infected guinea pigs was 10- to 1,000-fold lower than those of WE/WE- or WE/ARM-infected animals. Thus, in addition to its lethal properties, the L RNA segment of WE is important for viral replication in vivo. If the L segment of LCMV codes for the viral polymerase, as suspected, the difference observed for the virulence in guinea pigs may be explained by a difference in the polymerase activities of the parental strains. However, this interpretation must be constrained until the entire L RNA segment has been cloned and sequenced and the

coding capacity for any unknown protein is uncovered. The fact that the ARM/WE genotype of virus replicated to higher levels in vivo in comparison to parental ARM/ARM suggests a possible interaction between the S RNA of WE and the L RNA of ARM that may augment viral replication in vivo. It was of interest that all the virus forms used—ARM/ARM, ARM/WE, WE/ARM, and WE/WE—replicated with equal efficiency in vitro in guinea pig fibroblasts. These results make two points. First, the difference in susceptibility noted in vivo may reflect selection of and replication in a primary target cell such as the macrophage or in other cells of the reticuloendothelial system. The reticuloendothelial system has been shown to play an essential role after viral infection, particularly that by several arenaviruses (12–14). Second, caution should be used in extending in vitro observations to in vivo situations and vice versa.

Previously, Kirk et al. (9) generated and characterized intertypic recombinant viruses between the ARM and WE strains of LCMV. Their experience was limited to the WE/ARM genotype only, which they found to be as avirulent in guinea pigs as the parental strain, ARM. Our results contrast with theirs, since we find that the ARM/WE genotype is similar to the parental ARM in avirulence, but that the WE/ARM genotype is extremely virulent, like the parental WE. Our results are strengthened by the use of both intertypic reassortants (WE/ARM, ARM/WE) and by similar data with another independently generated WE/ARM reassortant. Currently we can offer no explanation for the discrepancy with the findings of Kirk et al. The ARM strain used in our lab was derived from ARM 1371, whereas that utilized by Kirk came from E-350. ARM 1371 and E-350 are both closely homologous by oligonucleotide mapping (5).

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LITERATURE CITED

1. Bishop, D. H. L., and R. E. Shope. 1980. Bunyaviridae, p. 1–21. In H. Fraenkel-Conrat and R. R. Wagner (ed.), *Comprehensive virology*, vol. 14. Newly characterized vertebrate viruses. Plenum Publishing Corp., New York.
2. Buchmeier, M. J., H. A. Lewicki, O. Tomori, and M. B. A. Oldstone. 1981. Monoclonal antibodies to lymphocytic choriomeningitis and Pichinde viruses: generation, characterization and cross-reactivity with other arenaviruses. *Virology* **113**: 73–85.
3. Buchmeier, M. J., and M. B. A. Oldstone. 1981. Molecular studies of LCM virus induced immunopathology: development and characterization of monoclonal antibodies to LCM virus, p. 71–77. In D. H. L. Bishop and R. W. Compans (ed.), *The replication of negative strand viruses*. Elsevier/North Holland Publishing Co., New York.
4. Buchmeier, M. J., R. M. Welsh, F. J. Dutko, and M. B. A. Oldstone. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Adv. Immunol.* **30**: 275–331.
5. Dutko, F. J., and M. B. A. Oldstone. 1983. Genomic and biological variation among commonly used lymphocytic choriomeningitis virus strains. *J. Gen. Virol.* **64**: 1689–1698.

6. **Fields, B. N., and M. I. Greene.** 1982. Genetic and molecular mechanisms of viral pathogenesis: implications for prevention and treatment. *Nature (London)* **300**:19-23.
7. **Harnish, D. G., K. Dimock, D. H. L. Bishop, and W. E. Rawls.** 1983. Gene mapping in Pichinde virus: assignment of viral polypeptides to genomic L and S RNAs. *J. Virol.* **46**:638-641.
8. **Hovanessian, A. G., and Y. Riviere.** 1980. Interferon-mediated induction of 2-5A synthetase and protein kinase in the liver and spleen of mice infected with Newcastle disease virus or injected with poly(I) poly(C). *Ann. Virol. Inst. Pasteur* **131E**: 180-196.
9. **Kirk, W. E., P. Cash, C. J. Peters, and D. H. L. Bishop.** 1980. Formation and characterization of an intertypic lymphocytic choriomeningitis recombinant virus. *J. Gen. Virol.* **51**:213-218.
10. **Lehmann-Grube, F.** 1971. Lymphocytic choriomeningitis virus. *Virol. Monogr.* **10**:78-81.
11. **Leung, W.-C., M. F. K. L. Leung, and W. E. Rawls.** 1979. Distinctive RNA transcriptase, polyadenylic acid polymerase and polyuridylic acid polymerase activities associated with Pichinde virus. *J. Virol.* **30**:98-107.
12. **Mims, C. A., and F. A. Tosolini.** 1969. Pathogenesis of lesions in lymphoid tissue of mice infected with lymphocytic choriomeningitis virus (LCM) virus. *Br. J. Exp. Pathol.* **50**:584-592.
13. **Mogensen, S. C.** 1979. Role of macrophages in natural resistance to virus infections. *Microbiol. Rev.* **43**:1-26.
14. **Murphy, F. A., M. J. Buchmeier, and W. E. Rawls.** 1977. The reticuloendothelium as the target in a virus infection. Pichinde virus pathogenesis in two strains of hamsters. *Lab. Invest.* **37**:502-515.
15. **Pedersen, I. R.** 1979. Structural components and replication of arenavirus. *Adv. Virus Res.* **24**:277-330.
16. **Riviere, Y., R. Ahmed, P. J. Southern, M. J. Buchmeier, F. J. Dutko, and M. B. A. Oldstone.** 1985. The S RNA segment of lymphocytic choriomeningitis virus codes for the nucleoprotein and glycoproteins 1 and 2. *J. Virol.* **53**:966-968.
17. **Riviere, Y., R. Ahmed, P. J. Southern, and M. B. A. Oldstone.** 1985. Perturbation of differentiated functions during viral infection *in vivo*. II. Viral reassortants map growth hormone defect to the S RNA of the LCMV genome. *Virology* **142**:175-182.
18. **Scholtissek, C.** 1979. Influenza virus genetics. *Adv. Genet.* **20**:1-36.
19. **Southern, P. J., and M. B. A. Oldstone.** 1984. Studies on the molecular biology of lymphocytic choriomeningitis virus, p. 59-64. *In* R. W. Compans and D. H. L. Bishop (ed.), *Segmented negative strand viruses (arenaviruses, bunyaviruses, and orthomyxoviruses.)* Academic Press, Inc., New York.
20. **Zinkernagel, R. M., and P. C. Doherty.** 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T cell restriction specificity, function and responsiveness. *Adv. Immunol.* **27**:51-177.