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Murine Infection by Vesicular Stomatitis Virus: Initial Characterization of the *H-2*^{d} System

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BALB/c mice and congenic *H-2L*^{d}-deficient BALB/c-*H-2*^{dm2} (dm2) mice were experimentally infected intranasally with isolates of vesicular stomatitis virus (VSV). The survival of infected hosts, viral replication in lungs and brains, and histopathologic changes in the two mouse strains were compared. In both strains of mice, mortality occurred during the period 7 to 10 days postinfection. However, dm2 mice were relatively resistant to lethal infections. Viral replication occurred at low levels in the lungs of both strains and did not evoke significant pathologic changes. In contrast, viral replication in the brains was much greater; in the BALB/c strain, this was accompanied by more frequent and more severe pathologic changes. In general, mice surviving at day 10 had effectively cleared virus from central nervous system but not respiratory sites. Evidence is presented that viral replication occurs first in the nasal cavity and is transmitted both to the lungs and to the olfactory bulb where focal cytopathology occurs. Virus enters the ventricles, causing encephalitis; necrosis occurs around the ventricles and in the lumbosacral region of the spinal cord. Necrotic lesions were accompanied by mononuclear infiltration. Mice immunized with virus of the same serotype or with a vaccinia virus hybrid encoding the VSV glycoprotein were protected from lethal infection; in contrast, mice immunized with heterotypic virus were susceptible to challenge.

Invasion of a susceptible host by a pathogen precipitates a cascade of immunologic events. Specific cell-mediated reactions are stimulated; among them are the induction (or memory recall) of helper T-cell activity, development of cytolytic T lymphocytes, delayed hypersensitivity, and antibody production. In addition, many cell types produce cytokines, which can have profound systemic effects. The infection itself can cause tissue or organ damage as a result of cytopathic effects. In an attempt to understand some of the complexities of the immune response to viral infection, we have initiated studies of the infection of BALB/c mice and a congenic strain, BALB/c-*H-2*^{dm2} (dm2), with vesicular stomatitis virus (VSV).

VSV is a well-characterized rhabdovirus with a broad host range whose natural host is the cow and which is transmitted by arthropod vectors. Neutralization tests have identified two major serotypes, designated Indiana (I) and New Jersey (NJ) (30). Intranasal instillation of either serotype in mice can lead to lethal infections. This was first described as a model system by Sabin and Olitsky (24, 25) more than 50 years ago.

Previous immunologic studies have demonstrated the class I major histocompatibility complex (MHC)-restricted cytolytic T-lymphocyte response to VSV in BALB/c mice. The class I-restricted cytolytic T-lymphocyte response is primarily specific for the nucleoprotein and is restricted exclusively to the *H-2L*^{d} gene product (7, 17, 32). Because of a naturally occurring deletion, the region encoding the *H-2L*^{d} gene is absent in dm2 mice (27). Experimental immunization of dm2 mice has demonstrated that in the absence of L^d, a compensatory class II MHC-restricted response to VSV was elicited (3, 4); dm2 mice respond normally, however, to influenza virus and Sendai virus immunizations (17).

The studies described here detail the differential responses and outcomes of BALB/c and dm2 mice to intranasal infection with VSV. Parameters examined include survival, viral replication, and histopathology.

MATERIALS AND METHODS

Infection studies. Mouse and virus strains used were as previously described (3, 4). In brief, BALB/c mice were purchased from Taconic Farms, Inc., Germantown, N.Y., and the Jackson Laboratory, Bar Harbor, Maine. VSV (both San Juan and Hazelhurt strains; respectively, Indiana and New Jersey serotypes), influenza A/Japan/305/57 virus, and hybrid vaccinia virus carrying the glycoprotein (G protein) gene of Indiana serotype VSV (VSV-G) (13) were prepared as previously described (3, 4).

Methoxyflurane (Metofane)-anesthetized mice (Pitman-Moore, Washington Crossing, N.J.) were infected intranasally with VSV diluted in RPMI 1640 medium. Mice were observed daily for 15 days after infection. All infected mice showed signs of infection which included ruffled dull fur, group huddling, and reduced activity level. Those mice which succumbed showed evidence of weight loss, hind-limb paralysis, prostration, and extremely labored breathing. Preliminary studies were performed with serial inocula of virus (ranging from 2×10^3 to 2×10^7 PFU per mouse) into the nares of anesthetized mice to determine the appropriate dose for the balance of the work (data not shown). In repeated experiments, a challenge of 2×10^6 PFU was selected and reproducibly resulted in mortality ranging from 30 to 70%. Both male and female mice, of ages ranging from 6 to 18 weeks (all groups were age and sex matched) as

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described in the figure legends, were utilized. Generally, 5- to 7-week-old male mice were used to minimize variations.

Determination of viral titer. Mice were given a lethal overdose of sodium pentobarbital-induced anesthesia (0.5 mg per mouse; Abbott Laboratories, North Chicago, Ill.). The animals were then exsanguinated, and the brain and paired lungs were aseptically removed. Specimens were placed in 2 ml of chilled RPMI 1640 medium containing 0.1 mg of gentamicin sulfate (Whittaker M.A. Bioproducts) per ml. Individual organs were homogenized with Potter-Elvejam homogenizers under standardized conditions, and the homogenates were aliquoted and stored at −70°C. Viral titers were determined as previously described (6) and expressed as PFU per homogenized organ. Tissue homogenates were serially diluted, and virus was adsorbed on Chinese hamster ovary cell monolayers for approximately 30 min at 37°C and then incubated under agar for an additional 18 h. The plates were then fixed with 10% formaldehyde and stained with 0.5% methylene blue. Plaques were then visually enumerated. Two replicates were performed for each sample. Positive (virus of known titer) and negative (no virus) controls were included in each plaque assay; positive controls did not vary significantly from one assay to another (range, 10^9 to 10^{10} PFU/ml).

Histological preparation. Pentobarbital-anesthetized animals were fixed by intracardiac perfusion with Bouin's modified solution (8.5 parts saturated aqueous picric acid, 0.5 part glacial acetic acid, 1.0 part formalin). Specimens were tagged with individual identifiers and were placed in Bouin's reagent at least 2 weeks before histological analysis. This process both fixed tissues and demineralized bone, allowing sectioning of bones of the nasal cavity and spine with spinal cord in situ.

From each mouse, 6-μm paraffin cross sections were prepared and stained with hematoxylin and eosin. The sections were representative of the nasal cavity, olfactory bulbs, frontal, parietal, and occipital lobes, diencephalon, midbrain, hindbrain with cerebellum, six cross sections of the spine and spinal cord including cervical, thoracic, lumbar, and sacral levels, and lung. From some mice, longitudinal sections through the nasal cavity, cribriform plate, and olfactory lobes were prepared.

In one experiment, simultaneous measurements of both virus replication and histology were performed. To do this, in deeply anesthetized mice, the cranium was aseptically opened, and the brain was divided along the midline; one half was preserved and the other half was processed for plaque analysis. Each individual was handled separately. The results were compared at 4, 7, 10, and 12 days postinfection. Group size ranged from 8 to 15 at the later time points, to allow for approximately 8 for each determination.

RESULTS

To determine the normal course of VSV infection in BALB/c mice, we introduced virus intranasally as described above. Observations of morbidity and mortality were made for 15 days, after which the process was essentially complete. At various intervals, brain and lung specimens were removed for determination of viral titers, and whole mice were perfused for histological examination. It was expected that the survival rates would be inversely proportional to the infection dose and that a lethal dose for 50% of the mice (LD_{50}) could be determined. Whether both serotypes, VSV-I and VSV-NJ, would result in similar infection and survival patterns was impossible to predict. Therefore, dose curves of viral inocula were tested (data not shown), and both serotypes of VSV were used in initial experiments.

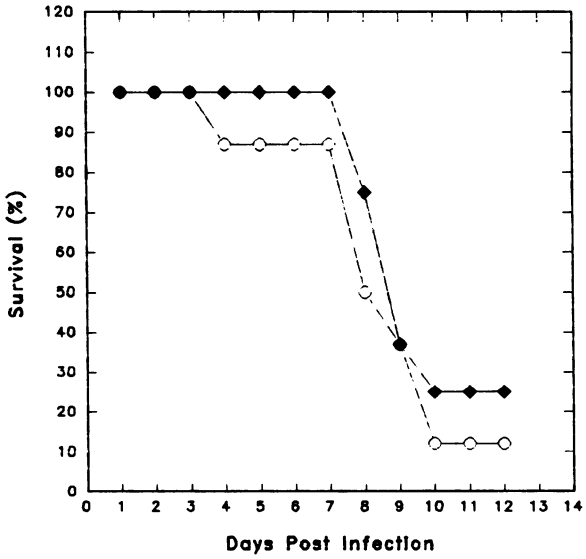

FIG. 1. Rates of survival are comparable after infection with 2 × 10^6 PFU of VSV-I or VSV-NJ. Each group of eight male BALB/c mice, 5 to 7 weeks old, was infected intranasally with the appropriate virus and scored daily after inoculation. ◆, VSV-I; ○, VSV-NJ.

In all eight experiments studied, the time course of the disease process was essentially the same: significant mortality began at day 7. All mice remaining alive at day 12 survived the infection; no subsequent deaths were observed in mice held for an additional 2 to 4 months nor were any significant sequelae noted. Occasionally, mice succumbed during the first 2 to 4 days after virus instillation. The symptoms were inconsistent with VSV infection. These early deaths may have been due to inadvertent bacterial pneumonia associated with the anesthesia. Figure 1 compares the effects of virus serotype on survival of infected mice; a single dose (2 × 10^6 PFU per mouse) of VSV-I or VSV-NJ was instilled in BALB/c mice. Survival frequencies at day 12 were similar in infections of both serotypes (Fig. 1).

We examined the recovery of virus from mice infected with VSV to correlate survival with evidence of differential host response to infection. Given the preliminary studies of Sabin and Olitsky (24, 25), we limited our virologic examination to lungs and brains. Figure 2a depicts the levels of virus recoverable from the homogenized brains of individual BALB/c mice at several times after intranasal instillation. These results are a composite of five experiments involving 101 individual mice. VSV recovery levels were low ($<10^4$ PFU per organ) on day 2 and gradually rose to approximately 10^7 PFU per organ on days 6 through 8. In all the surviving individual mice studied, virus levels were observed to be at low or undetectable values in the brain by day 10; in all cases examined, virus was below the level of detection in the brains of surviving mice on day 12.

The presence of VSV in the lung was in marked contrast to that in the brain (Fig. 2b). Data shown are from four separate experiments including 67 individual mice. On day 2, levels of up to 10^3 PFU per organ were noted. These levels changed comparatively little during the infection (rising to 10^4 PFU per organ) and persisted to at least day 10, when, in most cases, the amount of virus was below detectable levels in the

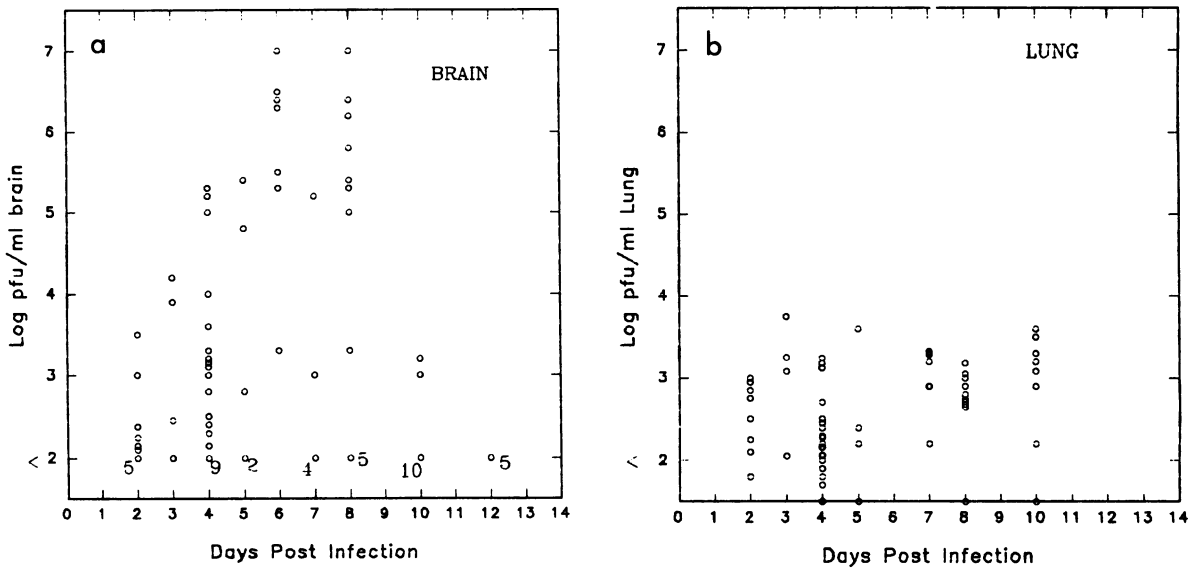

FIG. 2. Levels of virus recovered from the brains and lungs of animals infected with 2×10^6 PFU of VSV-I per mouse. Organs were aseptically removed on the days indicated and homogenized, and the virus titers were determined on Chinese hamster ovary cells. (a) Composite of data obtained from five experiments with the brain homogenates of 101 individual mice; (b) virus titrations from the lung homogenates obtained in four experiments with 67 individual mice. All mice were BALB/c males 5 to 7 weeks old.

brains of mice surviving the infection. Individuals showing little or no virus in brain homogenates invariably had evidence of virus in lung tissue and showed physical signs of infection. Thus, all mice were infected.

Comparison of VSV infection of BALB/c and BALB/c-*H-2*^{dm2} mice. To determine the effect of the absence of class I MHC-restricted cytotoxic T lymphocytes on the course of in vivo infection by VSV, we chose to study the congenic pair of BALB/c and dm2 mouse strains. They are identical genetically and immunologically except for the ability of BALB/c mice to produce *H-2L*^{d}-restricted VSV-specific effectors.

We could not predict a priori the outcome of infections of dm2 mice. It was possible that the class I-restricted lymphocytes would be protective, so that BALB/c mice would be more resistant than dm2 animals to immunopathology. On the other hand, class I effectors might be destructive, giving the opposite result. Alternatively, the susceptibility to infection might be distinct from any MHC effects but be associated with background genes.

These possibilities were explored by comparing the survival, virus recovery, and pathology of the two strains following infection with VSV. Figure 3 depicts the typical time course, from one of three replicate studies, of mortality in sex- and age-matched BALB/c and dm2 mice; two infection inocula are represented; 2×10^5 and 2×10^6 PFU per mouse. As noted earlier, significant mortality occurred on day 7 and beyond. The BALB/c mice alive on day 12 appeared healthy and survived the infection to day 18, when the experiment was terminated. In all cases studied (Fig. 3, and other data not shown), BALB/c mice were more susceptible to lethal infection than dm2 congenics.

The time course of virus recovery from the brain homogenates of both strains which received 2×10^6 PFU is depicted in Fig. 4a. This figure represents the data from a single experiment but is consistent with other studies. It indicates that all BALB/c mice had a measurable virus titer on day 4 postinfection; of those dm2 mice with a detectable virus titer in brain homogenates (one-half do not have a detectable titer), the highest level is 100 times less than the highest BALB/c titer. By day 7, in 70% of the dm2 mice, no virus was recoverable (sensitivity of assay, >10 PFU per organ), and the remainder had lower levels than the highest seen (days 4 and 7) in the BALB/c population. Two-thirds of the BALB/c mice cleared VSV from the brain by day 7. On day

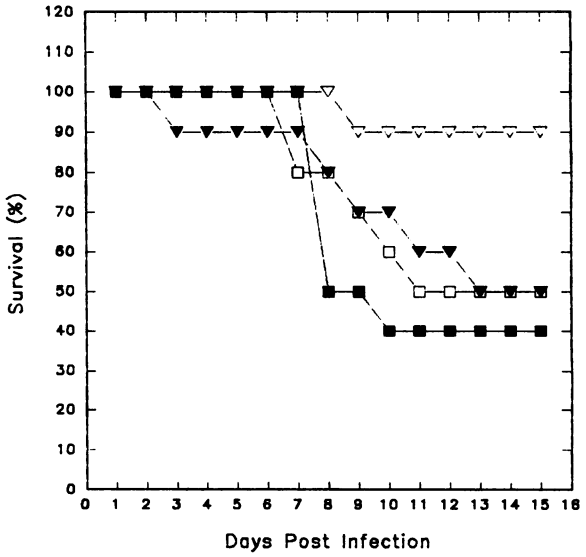

FIG. 3. BALB/c-*H-2*^{dm2} mice are more resistant than BALB/c mice to lethal intranasal infection by VSV-I. Groups of BALB/c (n = 11) or dm2 (n = 10) mice were infected intranasally with either 2×10^5 or 2×10^6 PFU of VSV-I and scored daily after inoculation. ▽, dm2, 2×10^5 PFU; □, dm2, 2×10^6 PFU; ▼, BALB/c, 2×10^5 PFU; ■, BALB/c, 2×10^6 PFU. Both male and female BALB/c and dm2 mice were used (see Table 2) in a single experiment. Ages ranged from 13 to 18 weeks. Groups were age and sex matched.

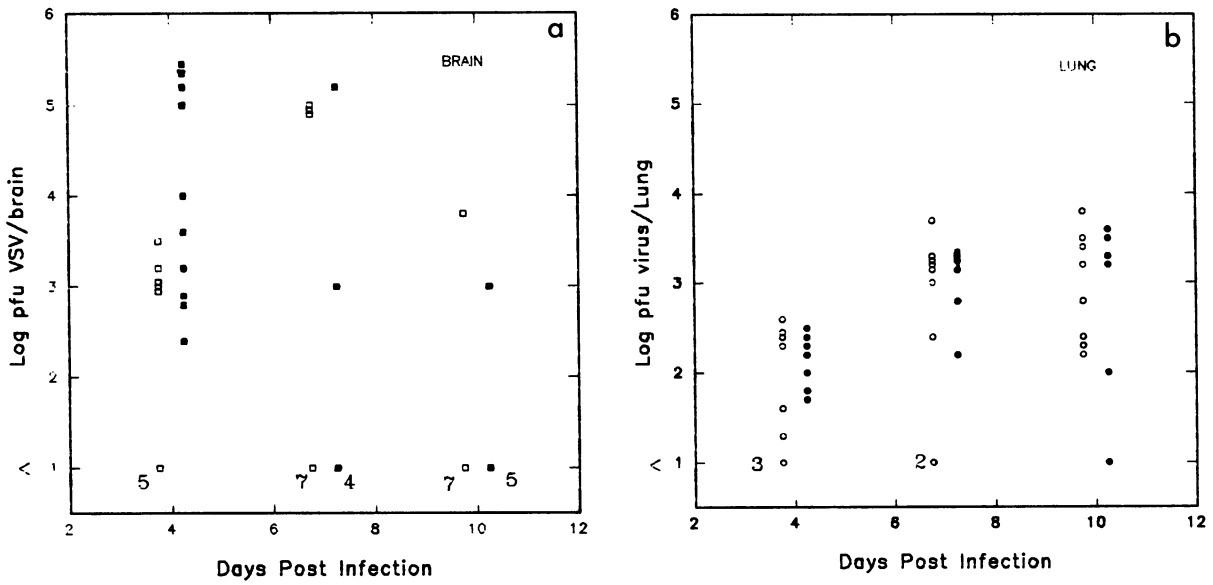

FIG. 4. Comparison of viral titers recovered from BALB/c and dm2 organs. Amounts of virus recovered from the brains (a) and lungs (b) of dm2 and BALB/c mice intranasally infected with 2×10^6 PFU of VSV-I per mouse. Organs were aseptically removed on the days indicated and homogenized, and the VSV titers were estimated on Chinese hamster ovary cells. Data are from a single experiment. ■, BALB/c brains; □, dm2 brains; ●, BALB/c lungs; ○, dm2 lungs. Female mice aged 6 to 16 weeks were used.

10, all surviving mice demonstrated significantly reduced or undetectable levels of VSV.

The lung virus assays (Fig. 4b) from this experiment were consistent with earlier observations (Fig. 2a). There was measurable virus at day 4, which increased until day 10. Thus, once again, the presence of detectable levels of VSV in the lung appeared unrelated to the course of the disease and was observed in both strains of mice.

It was possible that there was an effect on susceptibility or survival of VSV infections based on the gender of the host. There are both anecdotal and published accounts of increased frequencies of diseases in hosts of different sex (6, 14). To test this, we infected sex- and age-matched groups of dm2 and BALB/c mice with VSV-I. Female mice were more resistant to lethal viral infection than males; this dichotomy arose in both strains tested here and may be related to hormonal differences, although the mechanism(s) is not apparent (8a). dm2 mice were more resistant to lethal VSV infection than their congenic strain, BALB/c.

Histopathology. Lesions observed in histopathologic analyses of perfused samples were quite uniform and characteristic from one animal to another. The distribution and severity of lesions varied depending on the dose, the time after infection, and the mouse genotype. Most mice, regardless of experimental group, had various degrees of necrotizing fibrinopurulent rhinitis. The nasal epithelium was ulcerated, and the nasal cavity was filled with an exudate of neutrophils and fibrin (Fig. 5A and B). In some mice, focal or diffuse necrosis of the olfactory lobes was observed; this was characterized by liquefactive necrosis of neurons and neutropil with infiltration of lipid-laden macrophages (Fig. 5C). In no case did sections reveal continuity of necrosis from nasal cavity into olfactory lobes; random sections sometimes revealed chronic inflammation in the meninges surrounding the rootlets of the olfactory nerves.

In the brains of some mice, there were foci of mononuclear inflammatory cell infiltration in the meninges at the basilar aspects of the frontal lobes and around the medulla (Fig. 5D). Focal necrosis was rarely observed in white matter around the rostral aspects of the lateral ventricles (Fig. 5E) and rarely seen in the parenchyma adjacent to the third and fourth ventricles. In occasional mice, these foci were associated with perivascular cuffs of mononuclear inflammatory cells.

The spinal meninges were focally infiltrated by mononuclear cells at all levels in more severely affected mice. However, only in the lumbosacral levels was significant inflammation and necrosis of the parenchyma observed. This consisted of moderately dense infiltration of mixed inflammatory cells and liquefactive necrosis of parenchyma (Fig. 5F). The lumbosacral spinal cord lesions are consistent with the hind-limb paralysis seen in many mice before death from VSV infections.

Only 1 of 51 mice examined had any evidence of chronic interstitial pneumonia; all other lungs were normal. There was no sign of specific or nonspecific responses in the lungs despite the presence of infectious virus. There was no histopathologic evidence of cell death from virus-mediated cytopathology.

The variability of severity and distribution of lesions was consistent with the variability of viral titers. The data presented in Table 1 show that dm2 mice were affected to a moderately less severe degree than were BALB/c mice, particularly at 10 days postinfection.

To directly compare viral recovery from homogenates of brain with pathologic changes, we infected a group of 50 BALB/c mice with 2×10^6 PFU of Indiana virus per mouse. At intervals after infection, groups of four to seven mice were sacrificed and half-brains were processed for viral titer analysis, with the balance of the head fixed in Bouin's solution and analyzed for pathologic changes. The results are shown in Table 2. All mice showed evidence of necrotizing rhinitis, and this is not listed in Table 2. The most severe central nervous system lesions were present on days

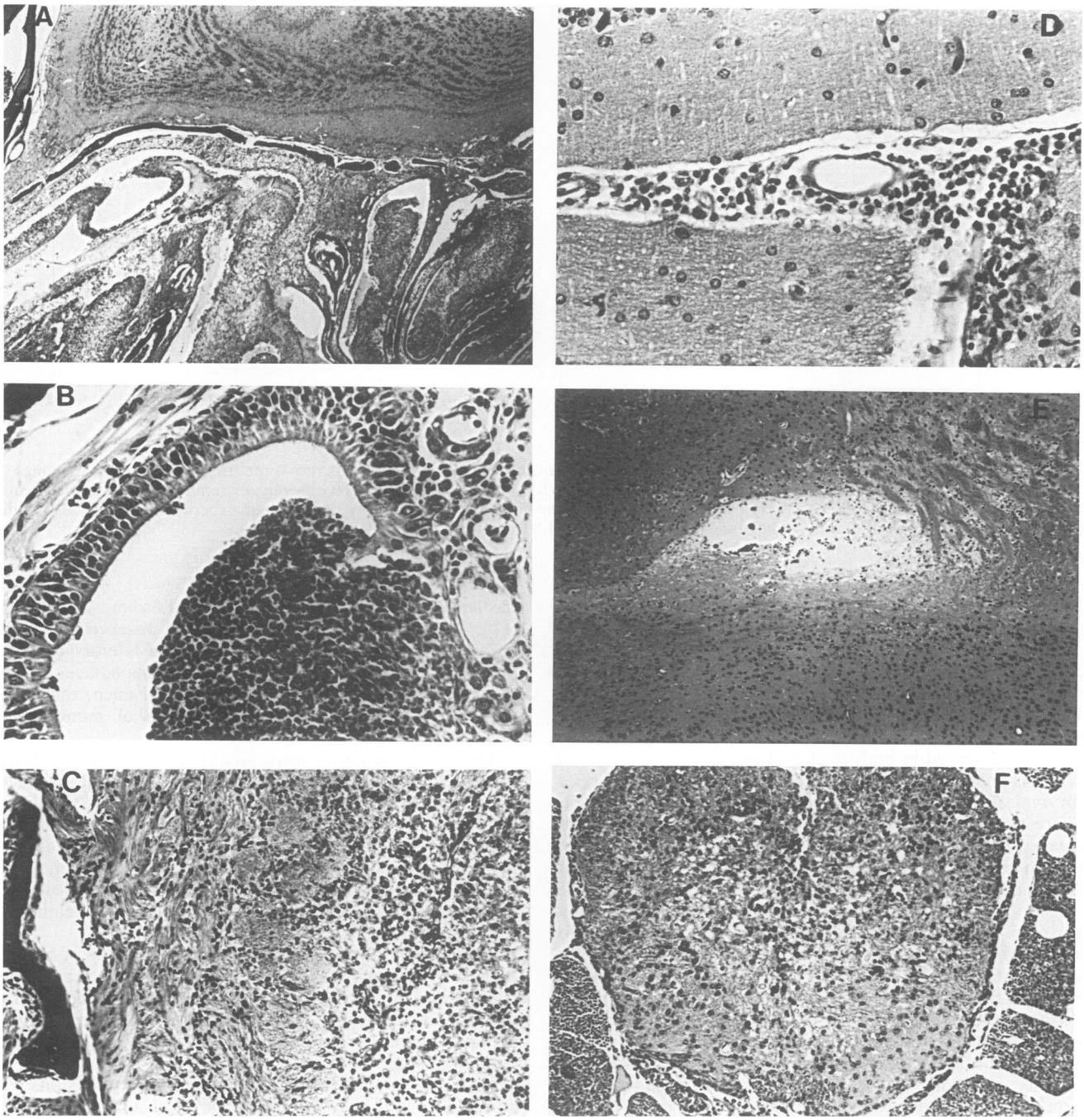

FIG. 5. Histopathology. This figure illustrates the aspects of histopathology seen repetitively in the mice examined. All sections are stained with hematoxylin and eosin. (A) Lumina of the nasal sinuses from a BALB/c mouse, 7 days postinfection, are filled with fibrinopurulent exudate. The olfactory lobe at the top of the figure, above the cribriform plate, is unaffected in this case. Original magnification, ×10. (B) One portion of the nasal epithelium from a BALB/c mouse, 9 days postinfection, is shown. It is ulcerated, and a fibrinopurulent exudate extends into the lumen of the nasal sinus. The rest of the nasal epithelium is relatively spared. Original magnification, ×100. (C) Olfactory lobe, 10 days postinfection, is diffusely infiltrated by lymphocytes. To the right, the parenchyma has undergone liquefactive necrosis. The necrotic area is infiltrated by lipid-laden macrophages. Original magnification, ×50. (D) Cerebral leptomeninges from a mouse, 9 days postinfection, are infiltrated by mononuclear inflammatory cells, predominantly lymphocytes. Original magnification, ×50. (E) Cerebrum at the level just rostral to the lateral ventrical of a mouse, 10 days postinfection. The cavity represents the effects of liquefactive necrosis. Original magnification, ×25. (F) Lumbar spinal cord from a mouse 7 days postinfection. There is diffuse infiltration of the parenchyma by lymphocytes and macrophages. The holes in the central portions of the tissue are consistent with necrosis. Original magnification, ×50.

TABLE 1. Histopathologic lesions observed in target organs[a]

Day postinfection (no. of mice)	Rhinitis	Necrosis in olfactory lobe	Meningitis, necrosis in brain	Necrosis in spinal cord
BALB/c				
4 (5)	5	0	0	0
7 (5)	5	2	4	3
10 (4)	4	1	1	1
dm2				
4 (5)	5	0	0	0
7 (3)	3	1	2	2
10 (4)	2	0	4	0

[a] Serial paraffin-embedded sections from perfused mice were stained with hematoxylin and eosin before examination. Data are scored for the presence or absence of lesions in groups.

6 and 8, at a time when mice were dying. By day 10, 5 of 6 surviving mice (10 were infected at that time point) had cleared virus from their brains, and 3 of those with no detectable virus had moderately severe lesions including meningitis and focal necrosis (olfactory lobe, inferior frontal lobe). Of 10 mice infected at that time point, 5 mice surviving to day 12 showed no virus and 60% had no lesions in the central nervous system. The remaining two mice had focal but not mortal areas of necrosis in their brains.

While there is a trend to have recovery of more virus associated with increased severity of histopathologic changes, this is not an absolute correlation, as the second mouse in the day 6 group had the lowest viral recovery but had lesions comparable with the mouse listed above it and also the penultimate mouse of that group (nonsuppurative meningitis and focal necrosis of the olfactory lobe). Thus, in general, the more viral replication in brains, the more severe the histopathologic lesions and the greatest likelihood of infection-related mortality.

TABLE 2. Split-brain analysis in VSV infection of BALB/c mice

Day postinfection	Viral titer (PFU)	Histopathology[a]
4	<20	NSL
	<20	NSL
	<20	NSL
	3×10^2	NSL
	<20	NSL
	<20	NSL
	<20	NSL
6	5×10^5	NSM, FNOL
	3×10^3	NSM, FNOL
	1×10^7	MM, OLN
	3×10^5	NSM, OLNS
	5×10^6	MM, OLN
	4×10^6	NSM, FNOL
	3×10^6	M, FNM, OLMI
8	$>1 \times 10^7$	M, NOL
	1×10^7	M, NOL
	4×10^5	M
	4×10^6	MM, OLN
10	2×10^3	NOL
	<20	NOL, MM
	<20	NSL
	<20	MM
	<20	MM, FNIFL
	<20	NSL
12	<20	NSL
	<20	FNOL, FNIFL
	<20	NSL
	<20	NOL, FNIFL, FNH
	<20	NSL

[a] NSL, no significant lesions; NSM, nonsuppurative meningitis; FNOL, focal necrosis, olfactory lobe; MM, mild meningitis; OLN, olfactory lobe normal; OLNS, olfactory lobe not sampled; FNM, focal necrosis, medulla; OLMI, mild inflammation, olfactory lobe; M, meningitis; NOL, necrosis, olfactory lobe; FNIFL, focal necrosis, inferior frontal lobe; FNH, focal necrosis, hippocampus.

Protective effect of priming. Having determined the normal course of VSV infection in the two strains of mice studied, we next studied the effect of priming. It was expected that presentation of the virus by the intraperitoneal route would activate the immune systems of these animals and provide protection from the virus (20, 21). In our laboratory and others, intraperitoneal priming with these agents has never resulted in morbidity or mortality in these mice and is therefore routinely employed to induce both cellular and humoral immunity (3, 4, 15–17).

The effects of parenteral injection of various viral or control preparations 13 days before intranasal challenge of 2×10^6 PFU of VSV-I to groups of mice were determined. The mortality time course after instillation of control (RPMI diluent or influenza A/Japan/305/57 virus) was similar to that in naive mice (Fig. 6). Those mice receiving intraperitoneal injections of VSV-I (10^5 to 10^7 PFU) or a recombinant vaccinia virus vector expressing the surface glycoprotein of VSV-I (13) were completely immune to subsequent intranasal challenge by VSV-I. As little as 10^5 PFU of VSV-I was found to protect BALB/c mice completely from an LD_{50} challenge of the same virus. Figure 6a shows no protective role of immunization with 10^7 PFU of VSV-NJ on VSV-I challenge. In another experiment, however (data not shown), partial protection associated with heterotypic immunity was observed.

In dm2 mice, a similar observation of protection by priming was seen (Fig. 6b). Control mice demonstrated mortality similar to that of those shown in Fig. 4 (2×10^6 PFU). Mice parenterally inoculated with VSV-I or the vaccinia vector encoding VSV-G were completely protected from the lethal consequences of intranasal VSV challenge.

It was possible that mice challenged after priming were not infected, possibly owing to the presence of neutralizing serum or secretory antibody. Table 3 presents the data of virus recoverable from mice primed intraperitoneally 10 days before infection and sacrificed 3 days postinfection. The effect of priming on the infection of the brain was unequivocal; no detectable virus was found in brain homogenates from either the BALB/c or dm2 mice. In all other cases (RPMI 1640 medium, VSV-NJ, or influenza virus), no discernible reduction in brain titer was seen. In the lungs, as expected from Fig. 3 and 6, low levels of virus were seen; these titers were independent of those in the brains of these animals and, although lower, were not significantly diminished in VSV-I-primed mice.

DISCUSSION

In contrast to intranasal influenza virus infection, which causes gross pulmonary pathology in mice (1, 20, 31), the effect of VSV infection of the lung is not associated with an intense infiltration of mononuclear cells and pathology. In contrast, the VSV disease is associated with infection of the olfactory bulb leading to direct retrograde central nervous system dissemination. This is evidenced by the correlation of mortality and brain virus levels as a function of time after infection. Mice surviving to day 12 have completely elimi-

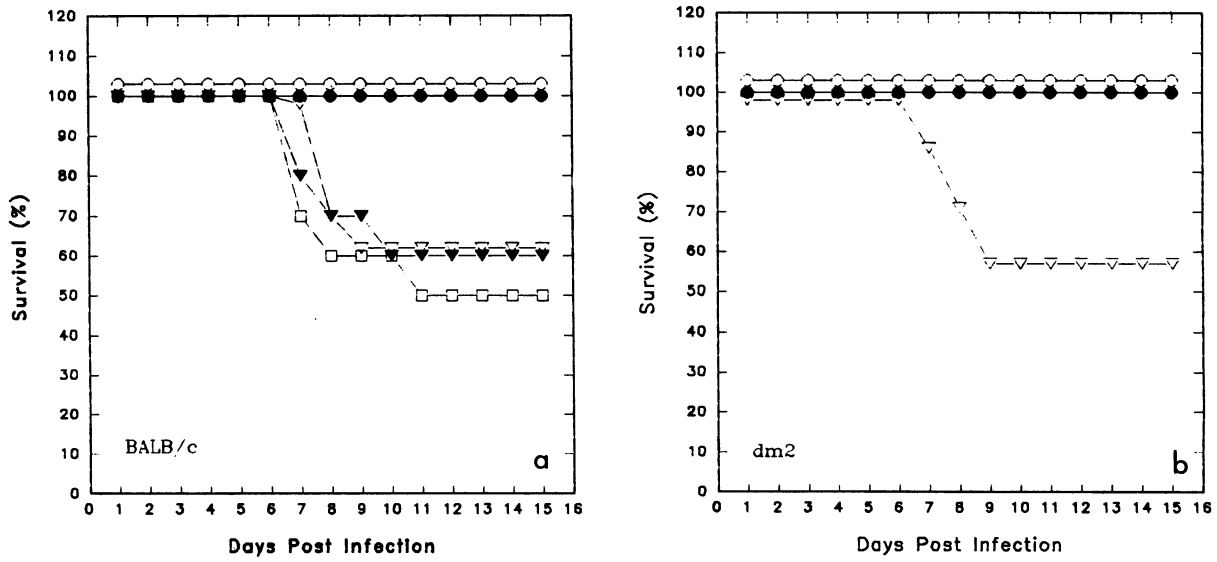

FIG. 6. (a) Protective effect of priming. In BALB/c mice, priming by VSV-I or vaccinia virus recombinant expressing VSV-G protein protected completely against later challenge with VSV-I; control preparations were ineffective. Groups of 10 male BALB/c mice, 5 to 7 weeks old, were primed by intraperitoneal injection of VSV-I (○) or vaccinia virus expressing VSV-G protein (●) (10^7 PFU), VSV-NJ. (10^7 PFU (▽), influenza A/Japan/305/57 virus (100 hemagglutinating units) (□), or RPMI 1640 medium (▼). After 10 days, all mice were challenged intranasally with 2×10^6 PFU of VSV-I and scored daily. (b) Priming of dm2 mice is protective. In dm2 mice, priming by VSV-I or vaccinia virus expressing VSV-G was completely protective against later challenge by VSV-I. Female mice aged 4 to 13 weeks were primed by intraperitoneal injection of VSV-I (○) or vaccinia virus expressing VSV-G (●) (10^7 PFU per mouse, eight mice) or RPMI 1640 medium (seven mice) (▽). Ten days later, all mice were challenged with 2×10^6 PFU of VSV-I intranasally and scored daily thereafter.

nated detectable levels of virus from the brain (Fig. 2 and 4). Presumably, those mice unable to marshall the necessary immunologic capacity to rapidly clear the virus from the brain and/or, alternatively, those mice in which the isolated sites of necrotizing lesions occurred in essential regions, succumbed (Fig. 5); work is in progress to determine whether viral damage or immune-mediated pathology of an essential central nervous system center(s) results in the lethal outcome. The data shown in Table 3 suggest that there can be a distinction between the severity of inflammation and the virus titer recovered from within the same brain (although there may be sampling errors in tissue sections viewed). There is a trend, however, between moderate lesions and clearance of virus, and lesions persist after viral clearance.

Pathology in the mouse VSV system differs from the exquisite neuron specificity of the related rabies virus infection (11). It appears that numerous cell types are decimated by the VSV infection. This correlates with its wide in vitro species and host-cell specificity. The retrograde passage of virus from nasal epithelium to olfactory bulb and then deeper into central nervous system sites and spinal cord (12) is not unlike the passage of pseudorabies virus (5); this is also consistent with the observed budding from the basolateral surface of apical cells (9, 22, 23), which would be associated with greater invasiveness. The pathology we observed was more widespread, involving more cell types, in VSV infection. In contrast to central nervous system transmission of reovirus infection, which is limited to neonatal mouse hosts (8), both Sabin and Olitsky (24, 25) and we found adult mice to be very susceptible to lethal infection (Fig. 1 and 3). We are in the process of determining which cell types infiltrate the nasal cavity, brain, and spinal cord, using immunohistopathology. In addition, we are also studying the course of

TABLE 3. Priming prevents central nervous system but not lung infection[a]

Strain	Priming	PFU/brain[b]	PFU/lung[b]
BALB/c	RPMI 1640 medium	<10	7×10^3
		2×10^4	1×10^2
		9×10^3	1×10^3
		5×10^2	2×10^3
BALB/c	VSV-I	<10	1×10^2
		<10	8×10^2
		<10	4×10^3
		<10	6×10^3
BALB/c	VSV-NJ	2×10^2	2×10^4
		2×10^4	7×10^2
		5×10^3	2×10^2
		<10	4×10^1
BALB/c	Influenza virus	7×10^3	<10
		3×10^4	1×10^3
		<10	ND[c]
		2×10^4	7×10^3
dm2	RPMI 1640 medium	<10	3×10^3
		2×10^1	3×10^3
		4×10^4	5×10^2
		2×10^4	1×10^3
dm2	VSV-I	<10	6×10^2
		<10	4×10^2
		<10	5×10^2
		<10	2×10^3

[a] Female mice were injected intraperitoneally with VSV-I (10^7 PFU), VSV-NJ (10^7 PFU), influenza A/Japan/305/57 virus (100 hemagglutinating units), or RPMI 1640 medium. All mice were challenged with 2×10^6 PFU of VSV-I intranasally; 3 days later, organs were removed and VSV-I titers were estimated on Chinese hamster ovary cells. Data from two separate experiments are shown; this experiment was performed at the same time as the experiment shown in Fig. 6.

[b] Virus titer recoverable 3 days postinfection in individual mice primed 10 days earlier.

[c] ND, not determined.

infection in immunodeficient mice and in mice selectively depleted of T-cell subsets.

By contrast, the peak pulmonary virus titers were found to be at levels 1,000 times lower than that in the brain and were not cleared in the same interval; animals surviving to day 10, and appearing normal on inspection, had lung titers comparable to those at earlier stages in the disease process (Fig. 2 and 4). Surprisingly, no overt inflammatory response was seen in the lungs (Fig. 5). This is puzzling, as the brain, not the lung, is considered a privileged site. There was no sign of cytopathic effect or of infiltration of mononuclear cells into the lungs which were infected. This suggests that the nonspecific cytokines such as interferons are containing the infection.

Plaque morphology in vitro was different in the two organs. Virus obtained from a low dilution of lung homogenates was observed repeatedly to form smaller, less diffuse plaques in Chinese hamster ovary cells (28). We attributed this to the ready induction of interferons in the lungs, which probably served to limit virus propagation. Work is in progress to determine whether this hypothesis is valid. As VSV is exquisitely sensitive to interferon and is used as the virus of choice for many bioassays for interferon (19), this is a reasonable interpretation. Indeed, the monolayer used for assays is of rodent origin and likely to be sensitive to a degree to murine cytokines.

The protection afforded by suitable priming arises from the activation of cellular and/or humoral effectors. Previous work (21) suggests that cytolytic T lymphocytes appear before antibody-forming cells after viral infection. Preliminary studies (data not shown) on the adoptive transfer of splenocytes from primed BALB/c hosts to naive animals, with simulatenous infection, showed a significant decrease in mortality. Since antibody-forming cells are not adoptively transferred in this fashion, these data suggest that clearance from the brains of these mice by day 3 (Table 3) resulted from the action of cytolytic effectors.

When the survival of female mice was compared with that of males in both strains of mice, females had a more likely chance of surviving infections at two different dose levels. Gender-associated morbidity has been previously described in several infectious systems (6, 14) and contrasts to the increased frequency of autoimmune phenomena in females of all species.

The partial protection offered by priming mice with VSV-NJ before infection with VSV-I (Table 3, Fig. 6a, and data not shown), considered with the vaccinia data (Fig. 6) showing that priming with endogenously produced G protein alone is effective, are consistent with the observation that the G proteins of the respective strains demonstrate a 50% homology (10). In the influenza virus system, there has been a slight protective role associated with heterotypic immunity, but complete protection is associated with glycoprotein (hemagglutinin) determinants (26). Indeed, the availability of vaccinia virus constructs encoding discrete viral genes has permitted further dissection of the responses and with both influenza virus and VSV has shown no protection afforded exclusively by nucleoprotein (1, 2). We are exploring whether cytotoxic T-lymphocyte epitopes derived from the G protein sequence (18) expressed by vaccinia virus constructs in the absence of serological epitopes (29) are protective for mice.

The increased incidence of morbidity and mortality in BALB/c mice contrasts with the dm2 strain, which differs by a small deletion encoding the *H-2L^d* gene. It is intriguing to speculate that there is more than one receptor for the virus and that the *H-2L^d* gene product is among them. We suggest this, as virus replicated to higher titers in the BALB/c mouse in the central nervous system. It is also possible that once virus has replicated in central nervous system, the availability of both inducible class II and constitutively expressed class I MHC restriction molecules puts intact BALB/c mice at a disadvantage because of increased immunopathology. We will test these hypotheses.

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