Age Dependence of Viral Expression: Comparative Pathogenesis of Two Rodent-Adapted Strains of Measles Virus in Mice

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The pathogenesis of two rodent-adapted strains of measles virus was studied in 1- to 2-day-old suckling and 4-week-old weanling BALB/c mice. Both the mouse-adapted Edmonston (MAEd) strain and the hamster-neurotropic (HNT) strain caused necrotizing giant-cell encephalitis with a 90 to 100% mortality after intracerebral inoculation into suckling mice. After intracerebral inoculation into weanling mice, MAEd virus caused fatal disease in 20% of the mice; HNT virus caused fatal disease in 30%, but an additional 35% of these mice developed disease and then recovered. Even when mice were moribund there was little histological evidence of disease in weanling mice inoculated intracerebrally with either strain of virus. Fluorescent-antibody staining showed extensive measles virus antigen in the suckling mouse brain and focal areas of measles virus antigen in the weanling mouse brain. Infectious virus was recovered easily from the brains of suckling mice by plaquing on Vero cells, but no infectious virus could be recovered similarly from weanling mice. However, virus could be recovered by intracerebral inoculation of weanling mouse tissue homogenates into suckling animals. The immune response appeared to play no role in the recovery from infection or in these age-related differences in disease. It appears that maturation of the cells of the mouse central nervous system converted the production of measles virus from the infectious form in the suckling mouse to a primarily defective infection in the weanling mouse.

Measles virus can cause varied forms of human disease. Natural infection in an unimmunized individual causes an acute, febrile illness with an exanthem which appears at the time of the immune response. Complete recovery and life-long immunity usually follow, but occasionally giant-cell pneumonia or postinfectious encephalomyelitis complicate primary infection. The former usually occurs in immunodeficiency states (12, 16), and the latter, a perivenular inflammatory demyelinating disease, is thought to result from an abnormal immune response (10). In addition, a few children develop a chronic measles virus encephalitis, subacute sclerosing panencephalitis (SSPE), years after primary measles virus infection, and in this disease the measles virus appears to be defective in its replication (17). Finally, there is some evidence that measles virus may be a factor in the pathogenesis of multiple sclerosis. Patients with this chronic demyelinating disease have been shown to have higher levels of anti-measles antibody in their cerebrospinal fluid and serum than controls (19).

Hamsters and rats infected with measles virus also develop different diseases depending on the age of inoculation and on their immune status. An acute encephalomyelitis is produced in newborn hamsters (2) and rats (3) by a hamster-neurotropic (HNT) strain and in newborn hamsters by Edmonston (11) and hamster-adapted SSPE (9) strains of measles virus. A chronic infection resembling SSPE can be produced by inoculating hamster-adapted Schwarz virus into newborn progenies of immune hamsters (25) or by inoculating a hamster-adapted SSPE agent into weanling hamsters (4). Infection of adult hamsters with the hamster-adapted SSPE agent (4), or of weanling rats with HNT virus (21), produces a subclinical encephalitis.

These differences in disease processes associated with measles virus in both men and experimental animals may be related, in part, to strain variations of the virus, but also appear to be related to host factors. Age, secondary environmental factors, or the quality or quantity of the immune response may be important. For investigation of the host factors responsible

for the varied disease processes, the use of a mouse model would be preferable, since in this animal the genetics and immune responses are most clearly delineated. For this reason, the pathogenesis of two rodent-adapted strains of measles virus was studied in newborn and weanling BALB/c mice. Both virus strain differences and age of the host animals were found to markedly influence the disease process.

MATERIALS AND METHODS

Animals. Inbred BALB/c mice (Flow Research Animals, Dublin, Va.) were used. Suckling mice were 1 to 2 days old and weanlings were 4 weeks old. Pregnant hamsters were obtained (Lakeview Hamster Colony, Newfield, N.J.), and sucklings were used at 3 to 4 days of age.

Viruses. Two strains of measles virus were studied. A mouse-adapted measles virus (MAEd) was obtained from Fred Rapp (18). This virus was derived from the Edmonston strain of measles virus adapted to mice by David T. Imagawa (8) by repeated intracerebral passage in suckling mice. MAEd virus was maintained by intracerebral passage in suckling mice, and stock virus was prepared as an unclarified 10% suspension of brains from sick suckling mice. MAEd stock virus titered 10^6 plaque-forming units (PFU) per g of brain on Vero cells (Industrial Biological Laboratories, Rockville, Md.) and 10^3 50% intracerebral lethal doses (ICLD$_{50}$) per g of brain in suckling mice.

The hamster neurotropic (HNT) strain of measles virus was obtained from Theodore Burnstein. This virus had been adapted to hamsters by repeated intracerebral passage in suckling hamsters of the Philadelphia 26 strain of measles virus (2). HNT virus was maintained by intracerebral passage in suckling hamsters, but stock virus was prepared as an unclarified 10% suspension of brains of suckling mice 6 days after intracerebral inoculation. HNT stock virus titered $10^{4.8}$ PFU/g of brain on Vero cells and $10^{7.5}$ ICLD$_{50}$/g of brain in suckling hamsters. All stocks were stored in small volumes at -70 C until use.

Animal inoculations. Suckling animals were inoculated in the right cerebral hemisphere with 0.02 ml of undiluted stock virus suspension ($10^{3.3}$ PFU of MAEd virus or $10^{2.1}$ PFU of HNT virus). Weanling animals were inoculated in the right cerebral hemisphere, under ether anesthesia, with 0.03 ml of undiluted stock virus suspension ($10^{3.5}$ PFU of MAEd virus of $10^{2.3}$ PFU of HNT virus). Control animals were inoculated in like manner with an unclarified 10% suspension of normal suckling mouse brain.

Virus titrations. Tissues were individually assayed for measles virus by preparing a 10% fresh tissue homogenate with a chilled mortar and pestle by using Hanks basic salt solution with 5% fetal calf serum (diluent). This homogenate was frozen and thawed once, and 10-fold dilutions were prepared, and dilutions were assayed by plaquing in Vero cells by the methods of Schumacher et al. (20). Three 35-mm plastic dishes (Falcon) were inoculated with 0.2 ml of each dilution, incubated and rocked for 1 h at 37 C, washed once, and overlaid with 3 ml of minimal essential medium

(MEM) containing 0.5% agarose (Seakem Marine Colloids, Inc., Springfield, N.J.) and 5% fetal calf serum. MAEd plaques were read at 6 days after staining overnight with an overlay containing neutral red in 0.5% agarose and MEM (1:30,000). The tissues that were titrated were the brains of suckling animals and the brains, spleens, and blood of weanling animals.

On occasion, newborn mice or hamsters were used for viral titrations or recovery. Newborn animals were inoculated intracerebrally with 0.02 ml of 10-fold dilutions of tissue homogenates, using one litter per dilution.

Antibody determinations. Serum-neutralizing (SN) antibody was measured by mixing twofold dilutions of serum with an equal volume of diluent containing 50 PFU of MAEd measles virus per 0.1 ml. After incubation at room temperature for 60 min, Vero cell monolayers were inoculated with 0.2 ml of the virus serum mixture and then incubated, washed, overlaid, and stained as described above. Neutralizing antibody titers are reported as the highest dilution of serum causing 50% or greater plaque reduction. Complement-fixing (CF) antibody and hemagglutination-inhibiting (HI) antibody were determined by standard microtiter methods (11) with commercial antigen preparations (Microbiological Associates, Inc., Bethesda, Md.). Sera for HI tests were pretreated with manganese chloride and heparin to remove nonspecific inhibitors (14). Control positive mouse sera from animals inoculated with formalized, alum-adsorbed measles virus vaccine (Division of Biologic Standards, reference lot 2) were included with all tests. All sera were heated at 56 C for 30 min prior to testing.

Histology. Tissues for fluorescent-antibody staining were rapidly frozen in tubes immersed in dry ice and alcohol. Sections were cut in a cryostat at 7 μm, dried, fixed in acetone for 10 min, and stained by the indirect method (13). Optimal fluorescence was obtained with a 1:50 dilution of serum from a patient with SSPE and a 1:10 dilution of fluorescein-conjugated rabbit anti-human gamma globulin (Hyland Division Trevenol Laboratories, Costa Mesa, Calif.). The tissues examined for measles antigen by fluorescent-antibody staining were the brains from suckling animals and the brains and spleens from weanling animals. No nonspecific staining was obtained with the brains and spleens from control animals or with the fluorescein conjugate alone on infected tissue.

Tissues for routine histology were fixed in Formalin, embedded in paraffin, and sectioned horizontally at four levels. Hematoxylin and eosin, luxol fast blue, and Weil-Weigert stains were used.

RESULTS

Clinical studies. The results of intracerebral inoculation of the two strains of measles virus into suckling and weanling BALB/c mice are summarized in Fig. 1. MAEd virus killed 93% (26/28) of suckling mice with an incubation period of 6 to 12 days and 20% (6/30) of weanling mice with an incubation period of 14 to 21 days. HNT virus killed 100% (24/24) of suckling mice

with an incubation period of 5 to 8 days and 31% (13/42) of weanling mice with an incubation period of 8 to 13 days. However, 67% (28/42) of weanlings inoculated intracerebrally with HNT virus manifested disease with a 1- to 3-day period of hyperactivity followed by apparent full recovery in at least half of the mice. In contrast, those weanling mice showing signs of disease after inoculation with MAEd virus invariably died. Suckling or weanling mice inoculated intraperitoneally or subcutaneously with either MAEd or HNT viruses showed no signs of disease.

Virological studies—MAEd strain. After intracerebral inoculation of MAEd virus into suckling mice, measles virus antigen was first detectable in the cytoplasm of cells in the cerebral neocortex, basal ganglia, and hippocampus on days 6 to 8, and increased in these sites until death. The multiplication of MAEd virus in suckling mice is shown in Fig. 2. Maximal measles virus multiplication was reached on day 6, but at no time was virus recovered from all animals. These animals from which infectious virus was not recovered may represent the 10% of animals which in other studies did not die after MAEd virus inoculation and, therefore, may not have been infected. No infectious virus was recovered from the homogenized spleens of these same suckling animals (titer $<10^2$ PFU/g of spleen). Spleens were not examined for the presence of viral antigen.

After intracerebral inoculation of MAEd virus into weanling mice, measles virus antigen was found initially on day 6 in scattered cells in the spleen and persisted until day 18. Antigen was not found in the brain earlier than 13 days after inoculation, and antigen was found almost exclusively in clinically ill mice. In contrast to
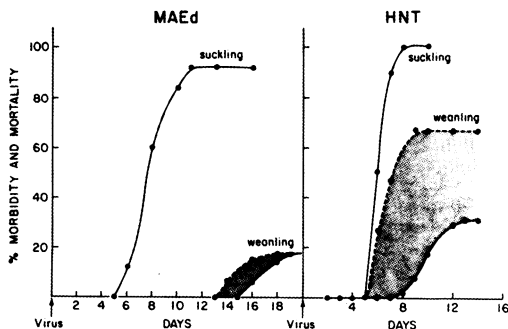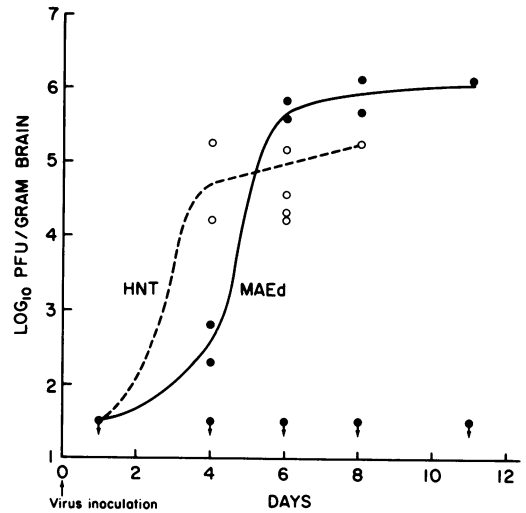
FIG. 2. *Growth of HNT (○) and MAEd (●) strains of measles virus in the brains of suckling BALB/c mice after intracerebral inoculation of $10^{2.1}$ PFU of HNT virus or $10^{3.3}$ PFU of MAEd virus.*

the suckling mouse, no infectious virus could be detected in homogenates of brain, spleen, or blood by inoculation on Vero cells. However, when a homogenate made from the brains of three saline-perfused weanling mice was inoculated intracerebrally into 20 suckling mice, two became ill, even though suckling mice had proven 1,000-times less sensitive than Vero cells in titrations of stock MAEd virus. Brains of these two suckling mice showed measles virus antigen by fluorescent-antibody staining, and MAEd virus was recovered on Vero cells.

Virological studies—HNT strain. After intracerebral inoculation of HNT virus into suckling mice, measles virus antigen was noted first in small foci in the cytoplasm of cells in the cortex and basal ganglia on day 3. These foci increased rapidly in size (Fig. 3a), with added involvement of the hippocampus and scattered cells in the cerebellum, until death on days 6 to 8. The multiplication of HNT virus in suckling mice is shown in Fig. 2. Virus was detectable first on day 4 and increased thereafter until death.

After intracerebral inoculation of HNT virus into weanling mice, measles virus antigen appeared first in scattered cells in the spleen on day 3, increased somewhat by day 9, and then decreased to undetectable levels by day 15. Measles virus antigen was first detectable in the brain on day 5, and increased in sick animals until death. In recovered animals, viral antigen was no longer detectable by day 17. Measles virus antigen was seen primarily in the cyto-
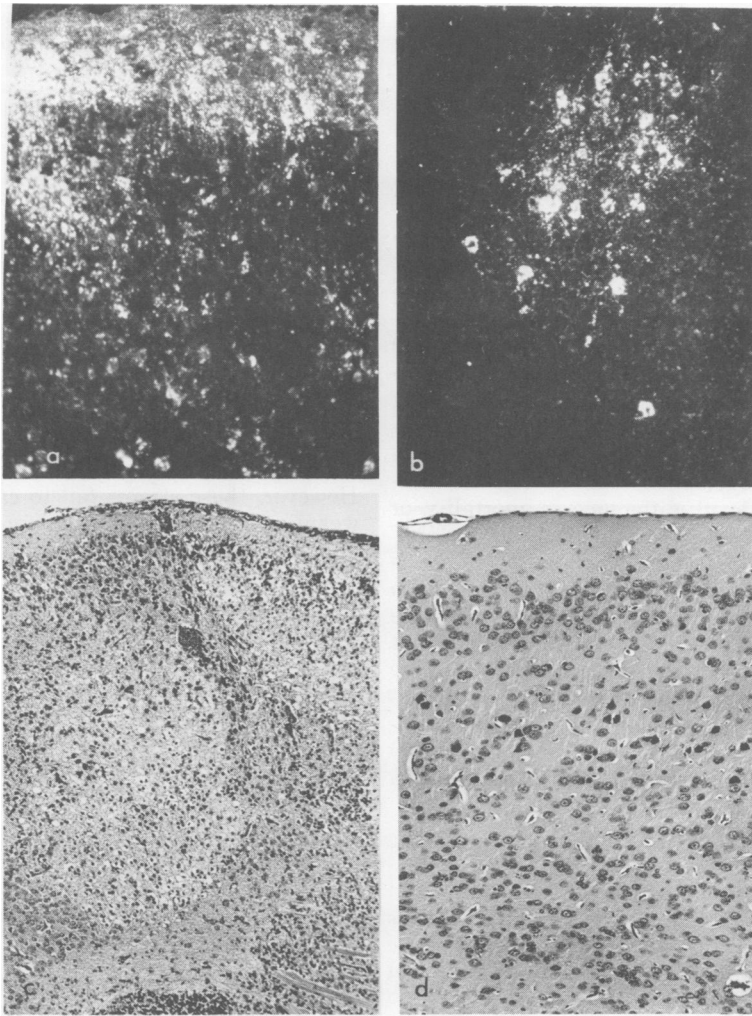
FIG. 1. *Cumulative morbidity (●-----●) and mortality (●———●) curves for suckling and weanling BALB/c mice inoculated intracerebrally with either MAEd or HNT strains of measles virus.*

FIG. 3. *Fluorescent antibody (FA) and hematoxylin-and-eosin (HE)-stained sections of cerebral cortex from HNT virus-infected mice (×69). FA, Suckling mouse d6 showing extensive viral antigen (a); FA, weanling mouse d8 showing focal viral antigen (b); HE, suckling mouse d6 showing extensive necrosis and inflammation (c); and HE, weanling mouse d8 showing minimal changes (d).*

plasm of cells in the cerebral cortex (Fig. 3b), basal ganglia, and hippocampus. No involvement of the cerebellum was noted. As with MAEd virus, no virus could be recovered on Vero cells from the homogenates of brains, spleens, or blood of weanling mice inoculated with HNT virus despite the presence of large amounts of viral antigen in brain. HNT virus could be recovered from brains and spleens of weanling mice when homogenates were inoculated into suckling hamsters. Even though Vero cells failed to detect virus, weanling mouse brain from day 9 titered $10^{5.4}$ $ICLD_{50}/g$, and spleen titered $10^{3.9}$ $ICLD_{50}/g$ in the suckling hamsters.

Pathological studies. Brains of suckling mice inoculated with either MAEd or HNT measles virus showed marked meningeal and perivascular inflammation, the formation of giant cells, and focal areas of necrosis in the cerebral neocortex and hippocampus that were infiltrated with polymorphonuclear leukocytes (Fig. 3c). In contrast, weanling mice had very little histological evidence of disease. There were occasional pyknotic cortical and hippocampal cells and small focal areas of vacuolization (Fig. 3d). However, perivascular or meningeal inflammation was remarkably absent even in hyperactive, moribund, or recovering mice. Neither suckling or weanling mice showed demyelination.

Immunological studies. Sera from two or

three weanling mice infected intracerebrally with MAEd or HNT viruses were pooled and assayed for the presence of HI, CF, and SN antibody on various days after infection. The humoral immune response appeared erratic and late in the course of infection, with no detectable antibody found until 10 to 15 days after infection (Table 1).

The role of the immune response in recovery from disease was studied further by immunosuppression of weanling mice. Weanling mice were given $10^{2.3}$ PFU of HNT virus intracerebrally on day 1. One group was treated with 250 mg of cyclophosphamide (cytoxan) per kg on day 1 and 125 mg of cyclophosphamide per kg on day 8 after infection. A second group received only HNT virus, and a third group received only cyclophosphamide. Results indicate no significant difference in mortality or morbidity between animals receiving HNT virus and cyclophosphamide and HNT virus alone (Table 2). Furthermore, the animals in each HNT virus group died during the same period of time (days 8 to 14), indicating that immunosuppression did not accelerate or delay the time of death. One death from cyclophosphamide alone occurred on day 13. The remaining animals from both groups were bled on day 18, and none demonstrated SN antibody at a 1:10 serum dilution.

DISCUSSION

For the study of the disease process and particularly the immune response to measles virus infection, it is advantageous to have models of disease in the mouse. Four strains of

TABLE 1. Antibody determinations on weanling mice infected intracerebrally with MAEd or HNT strains of measles virus

Day	MAEd		HNT		
	SN	CF	SN	HI	CF
4	<1:5	<1:8			
6	<1:10	<1:8	<1:20	<1:10	ND[a]
7			ND	<1:10	<1:8
8	<1:5	<1:8	ND	<1:10	ND
10	<1:5	<1:8	1:20	<1:10	<1:8
11			ND	<1:10	ND
13	ND	<1:8	ND	<1:10	ND
14			ND	<1:10	ND
15	1:50	ND	ND	<1:10	1:32
17	1:25	<1:8	<1:8	ND	1:8
20	1:5	ND	<1:8	<1:10	1:64
23	1:25	1:32			
24			ND	<1:10	1:8

[a] ND, Not done.

TABLE 2. Effect of cyclophosphamide immunosuppression on morbidity and mortality from HNT measles virus infection

Treatment	Morbidity		Mortality	
	Sick/total	%	Dead/total	%
HNT + cytoxan	12/18	67%	8/18	44%
HNT alone	11/17	65%	9/17	53%
Cytoxan alone	1/10	10%	1/10	10%

measles virus have been adapted to mice. In addition to the MAEd (18) and HNT (2) strains used in these studies, Carlstrom (5) and Matumoto (15) have adapted human isolates to suckling mice by serial intracerebral passage. However, these strains fail to cause disease in weanling animals and, therefore, only MAEd and HNT strains were used in the present study.

When these two strains of measles virus were compared, several differences are apparent. HNT virus replicated more rapidly than MAEd virus and resulted in earlier death in both suckling and weanling mice. In addition, HNT virus caused a more predictable cerebral infection than MAEd virus. However, although more predictable, death was not a certain consequence of cerebral infection with HNT virus in the weanling mouse, as it appeared to be with MAEd virus.

When the two age groups of mice were compared, the most striking difference was that infection with either strain of measles virus in the suckling mouse carried a high mortality and was productive of infectious virus which can readily be titered by plaque assay. In the weanling mouse, on the other hand, fewer animals died, and even when large amounts of viral antigen were evident in the brain, infectious virus could not be recovered in cell cultures. Similar difficulties with recovery of infectious measles virus from rodents have been reported by others. Carlstrom (5) could not recover virus in tissue culture from his infected suckling mice. Wear et al. (24) could not recover cell-free MAEd virus from brain homogenates of suckling Swiss mice, and Byington and Burnstein (3) could not recover infectious HNT virus in tissue culture from suckling rats. This suggests a defective infection which, in the weanling mouse, appears to be a host-mediated phenomenon dependent on age. This effect of age is unlikely to be dependent on a maturation of the immune response, since (i) only a late unpredictable antibody response was evoked,

(ii) no inflammatory response was present in the brains of infected animals as an indication of a cell-mediated immune response, and (iii) immunosuppression made no discernible difference in morbidity or mortality of HNT virus-infected weanling animals. Immune responses may be actually inhibited by measles virus infections. Anergy during measles is well documented in man (7, 22), and H. McFarland (unpublished data) has recently shown a specific inhibition of T-cell activity in mice infected with the MAEd strain of measles virus.

These data suggest first that some differences in disease such as predictability of infection and latent period of infection are properties of the virus strain causing that infection. However, the development of defective infection in mice appeared dependent on the maturation of the cells of the central nervous system itself. In the suckling mouse, appreciable quantities of infectious virus are produced in the brain, allowing rapid spread and cell death. In the weanling mouse, virus production is defective and, although cellular derangement is sufficient to cause death in some mice, this is not inevitable.

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