Brain Pathology 9: 21-31(1999)

RESEARCH ARTICLE

CNS Cell Populations are Protected from Virus-Induced Pathology by Distinct Arms of the Immune System

Kristen M. Drescher[1,2], Paul D. Murray[1], Chella S. David[1], Larry R. Pease[1], Moses Rodriguez[1,2]

Departments of [1]Immunology and [2]Neurology, Mayo Medical School, Rochester, MN 55901

The basis for the distinct patterns of brain pathology in individuals experiencing virus-induced encephalitis may be related to either the tropism of the virus or the host's response to virus infection of the central nervous system (CNS). In these studies we used Theiler's murine encephalomyelitis virus (TMEV) and a series of mice deficient in various immune system components (α/β T cells, antibody, Class I MHC, and Class II MHC) to examine the hypothesis that discrete populations of CNS cells are protected differentially from virus infection by distinct arms of the immune response. Here we demonstrate that the Class I-mediated immune response provided more protection from areas of the brain (brainstem, corpus callosum and cerebellum) with abundant white matter as there was significantly more disease in these areas in β_2m -/- (Class I-deficient) mice as compared to Aβ^o (Class II-deficient) mice. In contrast, the striatum, with an abundance of neurons, was protected from virus-induced pathology primarily by antibody. In addition, we determined that antibody and α/β T cells provided protection from severe deficits and death during the acute phase of the disease. The data presented here support the hypothesis that distinct immune system components function to protect discrete areas of the CNS from virus-induced pathology.

Corresponding author:
Moses Rodriguez, M.D., Mayo Clinic/Foundation, 200 First St. S.W., Department of Immunology, Guggenheim (4), Rochester, MN 55905; Tel.: (507) 284-4663; Fax: (507) 284-1673

Introduction

Virus-induced encephalitis in both humans and animals is characterized by pathology in distinct areas of the brain, which varies depending on the particular pathogen and genetics of the host. Examples of this include herpes simplex type 1 which induces disease largely in the frontal and temporal lobes, HIV-1 which localizes to the cerebral white matter, brain stem, and gray matter of the thalamus and basal ganglia (5), and rabies which affects many areas, but predominantly localizes to the pons and medulla (22). In most instances it is not known whether the unique patterns of virus-induced pathology following infection is related not only to the tropism of the virus for a particular cell type but also to the host immune response.

Intracerebral infection with Theiler's murine encephalomyelitis virus (TMEV) produces an acute neuronal disease (resulting in encephalitis) in mice which are either susceptible or resistant to the demyelinating disease induced by this virus (13). The receptor for TMEV is not known although many CNS cell types can be infected *in vivo*. These include neurons (8), astrocytes (2), microglia (6, 8, 13, 20), and oligodendrocytes (2, 19). Despite the wide susceptibility of most CNS cell types by this virus, widespread brain pathology is not observed in infected mice suggesting that there is an intrinsic ability of the host to control disease. One possibility for the selective pathology observed is that the immune system is able to either clear or protect distinct regions of the brain from pathology. Alternatively, distinct arms of the immune response may contribute to neurologic injury in an attempt to clear the infection from specific regions of the brain. There are several lines of evidence to support, at least theoretically, the concept that distinct components of the immune system are involved in clearing virus from distinct cellular components of the CNS. For example, during TMEV infection neurons express relatively low levels of MHC Class I (12). As a result, one could speculate that the primary mechanism involved in clearing virus from neurons in

Strain	Mutation	Source	Phenotype
Scid	B, T cell deficient	Taconic	T, B cell, antibody deficient
Aβ°	β chain of MHC Class II disrupted	C. Benoist	CD4+ T cell, antibody deficient Class II deficient
β_2m	β_2 microglobulin deficient	R. Jaenisch	CD8+ T cell deficient; Class I deficient
μ MT	mutation of μ chain	J. Kenny	antibody deficient
α/β KO	β chain of TCR absent	Jackson Labs	T cell, antibody deficient

Table 1. Mice used in the study.

the gray matter would be class II or antibody-mediated. Other cell types such as glia, express high levels of MHC Class I relatively early in infection, supporting a hypothesis whereby a Class I-mediated response would be involved in virus clearance from these cells (12).

In this study, we hypothesized that protection from acute encephalitis following TMEV infection is mediated by specific components of the immune system and that distinct immune system components protect specific regions from virus-mediated damage. To dissect the relative contributions of various immune system constituents in protection from the early stages TMEV infection, we employed a strategy utilizing TMEV-infected knockout mice lacking either MHC Class I, MHC Class II, antibody or α/β T cells on an identical genetic background (H-2^b) resistant to demyelination and virus persistence. We assessed the relative contributions of these immune system components in protection from death and pathological changes in the brain following intracerebral infection with TMEV. The results presented here support a role for antibody and α/β T cells in protection from severe deficits and death in the acute phase of the disease. Of unique interest, each of the knockout mice of similar background and genotype had distinct areas of the brain with pathology, indicating a role for specific components of the immune response in clearing virus from selective areas of the brain.

Material and Methods

Virus. The Daniel's strain of TMEV was used in all experiments. The history of this virus has been published previously (13).

Mice. The knockout mice used in this study are described in Table 1. The mice have defects in the production of α/β T cells (α/β KO) (14), IgM (μ MT) (10), MHC Class I (β_2m -/-) (25) or Class II (Aβ° mice) (7). C.B 17-*Scid* mice have both B and T cell deficiencies (4). β_2m-/-Aβ° double knockout mice have both MHC Class I and Class II deficits. C57BL/6J (prototypic resistant strain) mice were purchased from the Jackson Laboratories (Bar Harbor, ME). C.B17-*Scid* mice were purchased from Taconic Farms (Germantown, NY).

Strain	Cbm	Bst	Ctx	Hip	Stm	CC
C57BL/6J	1/4 (4)	1/4 (2)	3/4 (1-6)	2/4 (6-50)	0/4 (0)	2/4 (1-10)
Scid	0/6 (0)	0/6 (0)	6/6* (1->50)	5/6 (3-18)	5/6* (0->50)	0/6 0
β_2m-/-Aβ°	0/7 (0)	4/7 (2-28)	5/7 (4-24)	2/7 (1-4)	5/7* (0->50)	0/7 0
β_2m-/-	7/9 (4-14)	6/9 (2-13)	5/9 (3-6)	3/9 (1-5)	5/9 (1-8)	1/9 5
Aβ°	2/6 (1)	1/6 (2)	5/6 (1-46)	4/6 (0->50)	3/6 (6-16)	0/6 0
α/β KO	2/7 (1-7)	3/7 (14-30)	6/7 (0->100)	5/7 (0->50)	5/7 (0->100)	1/7 1
μ MT	0/7 0	0/7 0	3/7 (2-86)	2/7 (1-20)	5/7 (1-46)	0/7 0

The first line indicates the number of mice with antigen-positive staining in the indicated region as a ratio of total number of animals examined. The second line in parentheses indicates the range of the number of positive staining cells in the sample. Abbreviations: Cbm – cerebellum; Bst – brainstem; Ctx – cortex; Hip – hippocampus; Stm – straitum; CC – corpus callosum. * indicates that the number of virus antigen positive cells is significantly higher ($p < 0.05$) in the indicated group when compared to the infected C57BL/6J mice of resistant genotype using the Mann-Whitney Rank Sum Test . No statistical differences in the number of virus antigen positive cells were found between either the β_2m-/-, Aβ° , α/β KO, or μ MT mice and the C57BL/6J mice.

Table 2. Virus antigen cells in the brains of mice following intracerebral infection with Theiler's virus.

Infection and sacrifice of mice, and brain pathology. At 4 to 6 weeks of age, mice were inoculated intracerebrally (i.c.) with 2 x 10^5 pfu of TMEV in a 10 μl volume. At day 16 post-infection (p.i.), mice were perfused with Trump's fixative (phosphate-buffered 4% formaldehyde with 1% glutaraldehyde) as previously described (18). Following perfusion with Trump's fixative, two coronal cuts were made in the intact brain at the time of removal from the skull (one section through the optic chiasm and a second section through the infundibulum). As a guide we used the *Atlas of the Mouse Brain and Spinal Cord* corresponding to sections #220 and 350, page 6 (21). This resulted in three blocks which were then embedded in paraffin. This allowed for systematic analysis of the pathology of the cortex, corpus callosum, hippocampus, brainstem, striatum, and cerebellum. The resulting slides were then stained with hematoxylin and eosin. Pathologic scores were assigned without knowledge of experimental group to the following areas of the brain: cortex, corpus callosum, hippocampus, brainstem, striatum, and cerebellum. Each area of the brain was graded on a scale of 0 to 4 as follows: 0=no pathology; 1=no tissue destruction but only minimal inflammation; 2=early tissue destruction (loss of architecture) and moderate inflammation; 3=definite tissue destruction (demyelination, parenchymal damage, cell death, neurophagia, neuronal vacuolation); 4=necrosis (complete loss of all tissue elements with associated cellular debris). Meningeal inflammation was assessed and graded as follows: 0=no inflammation; 1=one cell layer of inflammation; 2=two cell layers of inflammation; 3=three cell layers of inflammation; 4=four or more cell layers of inflammation. The area with maximal extent of tissue damage was used for assessment of each brain region. Care and handling of all mice conformed to the guidelines of the National Institutes of Health and the Mayo Clinic.

Immunohistochemical staining of virus antigen. Brains from mice perfused with Trump's fixative were embedded in paraffin for immunohistochemical staining. Slides were deparaffinized in xylene, then rehydrated through an ethanol series (absolute, 95%, 70%, 50%, PBS) prior to the addition of the primary antibody. A polyclonal antisera to purified Daniel's virus was used for the detection of TMEV virions (19). Incubation with a secondary biotinylated antibody followed, and detection was performed using the avidin-biotin complex technique (Vector Laboratories, Burlingame, CA). Staining was visualized using Hanker-Yates reagent (Polysciences, Warrington, PA) with hydrogen peroxide as the substrate as previously described (15). Slides were lightly counterstained with hematoxylin. The number of virus antigen positive cells was counted using a light microscope and expressed per region of brain examined. The relative areas of brain region examined were comparable between experimental groups due to the method used to process the tissue for embedding and sectioning as described above.

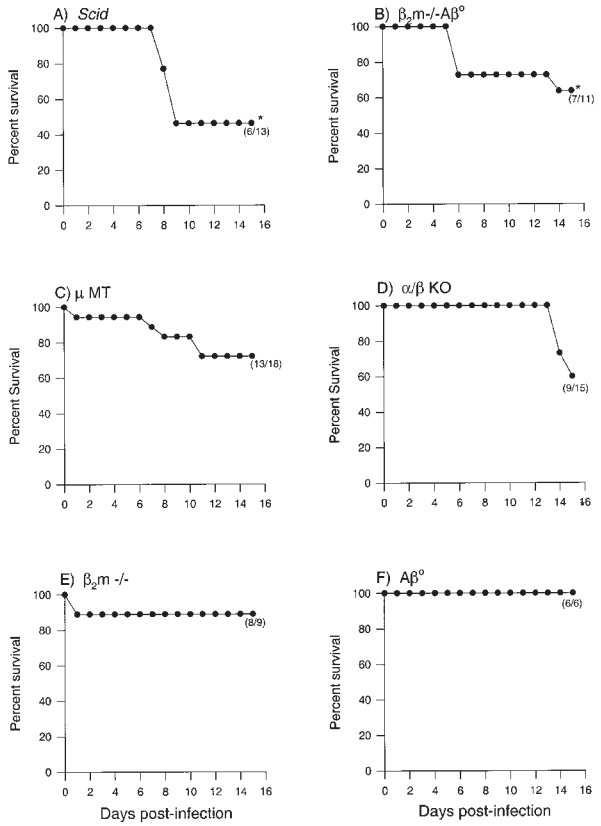


Figure 1. Survival of immune knockout mice following intracerebral (i.c.) infection with TMEV (Daniel's strain). Mice with profound antigen-specific immune defects such as *Scid* (**A**) and β_2mAβ° (**B**) mice experienced increased mortality compared with immunocompetent mice which are resistant (C57BL/6J) to chronic infection with Theiler's virus (100% survival to day 16 post-infection). Increased mortality was observed in the encephalitic phase of the disease in the m MT (antibody deficient) mice (**C**). Mice lacking α/β T cells (**D**) experienced increased death rates at a slightly later time point. The survival of β_2m(-/-) (**E**) and Aβ° (**F**) mice was not affected by Theiler's virus infection. All remaining animals were sacrificed at day 16 p.i. * indicates that all animals surviving to day 16 p.i. were either clinically ill or moribund at the time of sacrifice. The numbers on each graph indicate the number of mice surviving to day 16 over the total number of mice infected on day 0.

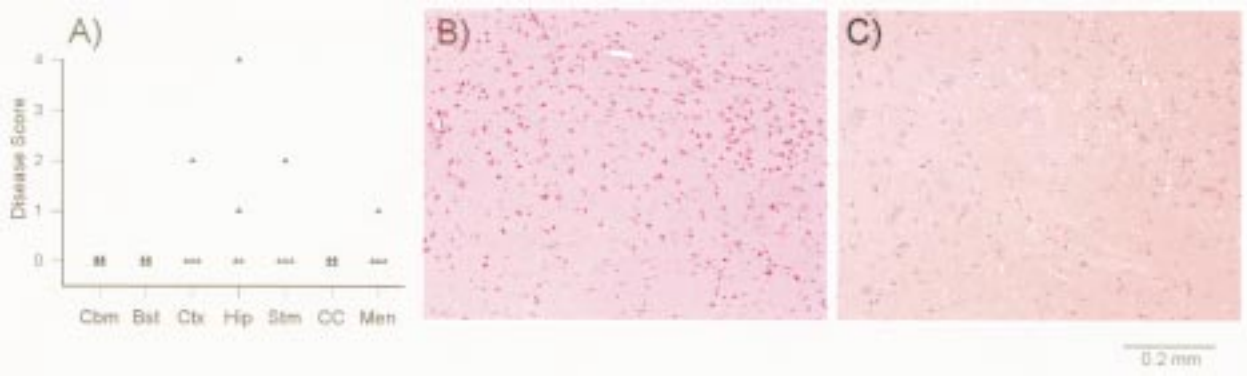


Figure 2. Immunocompetent resistant C57BL/6J mice experience minimal brain pathology at day 16 after infection with Theiler's virus. Disease scores are shown for the cerebellum (Cbm), brainstem (Bst), cortex (Ctx), hippocampus (Hip), striatum (Stm), corpus callosum (CC), and meninges (Men). Each symbol represents an individual mouse graded at each area of the brain according to the scale detailed in the **Material and Methods**. C57BL/6J mice which are resistant to chronic TMEV infection showed minimal or no brain disease at 16 days following infection with Theiler's virus (**A**). No inflammation or parenchyml damage is observed in the brainstem, and this section was graded as a "0" (**B**). Note the absence of TMEV antigen in the brainstem of the C57BL/6J mouse (**C**) as determined using the immunoperoxidase technique with a polyclonal rabbit antibody to TMEV.

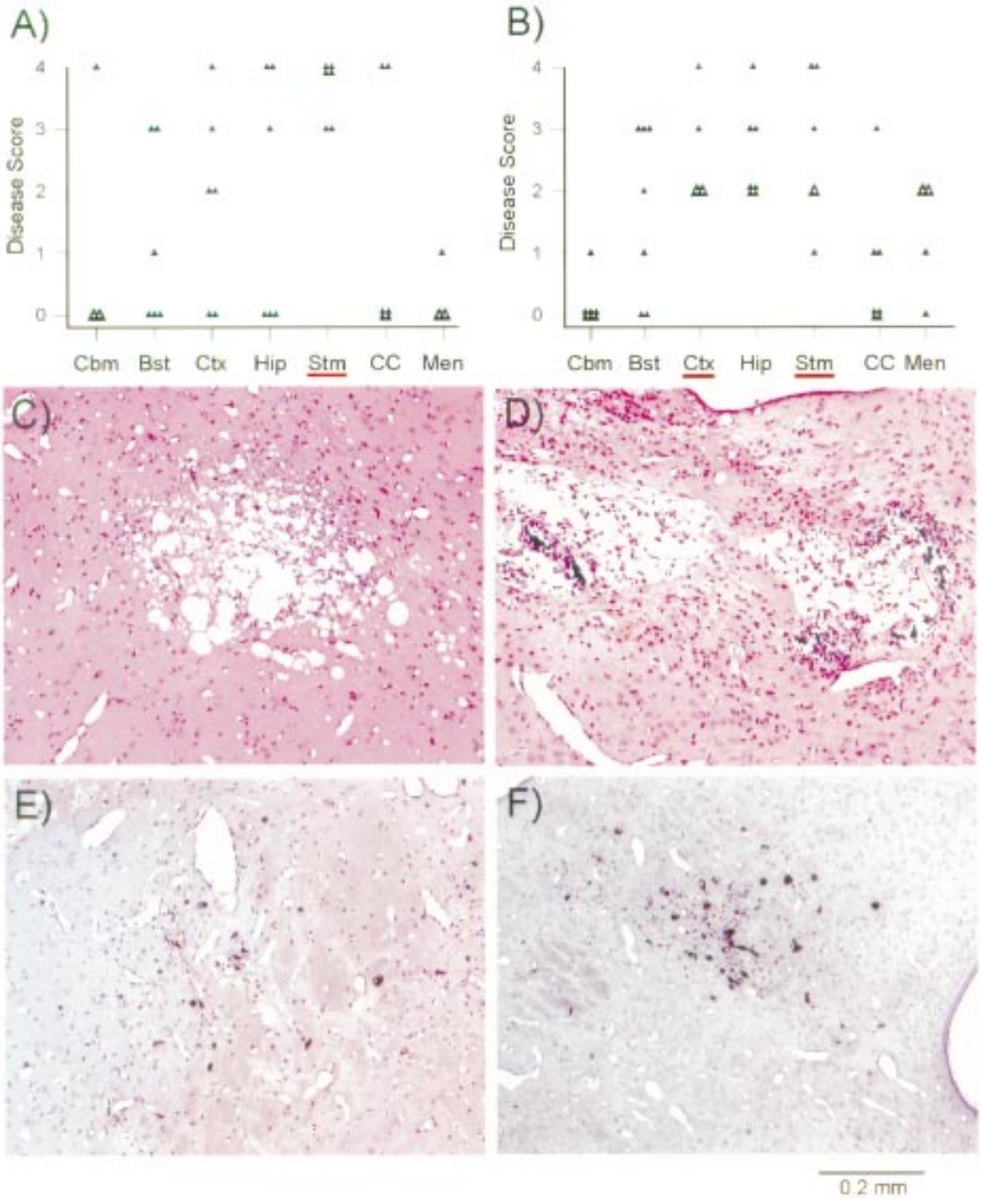
Statistics. Statistical analyses were performed using the Mann Whitney Rank Sum Test. p values ≤ 0.05 were considered significant.

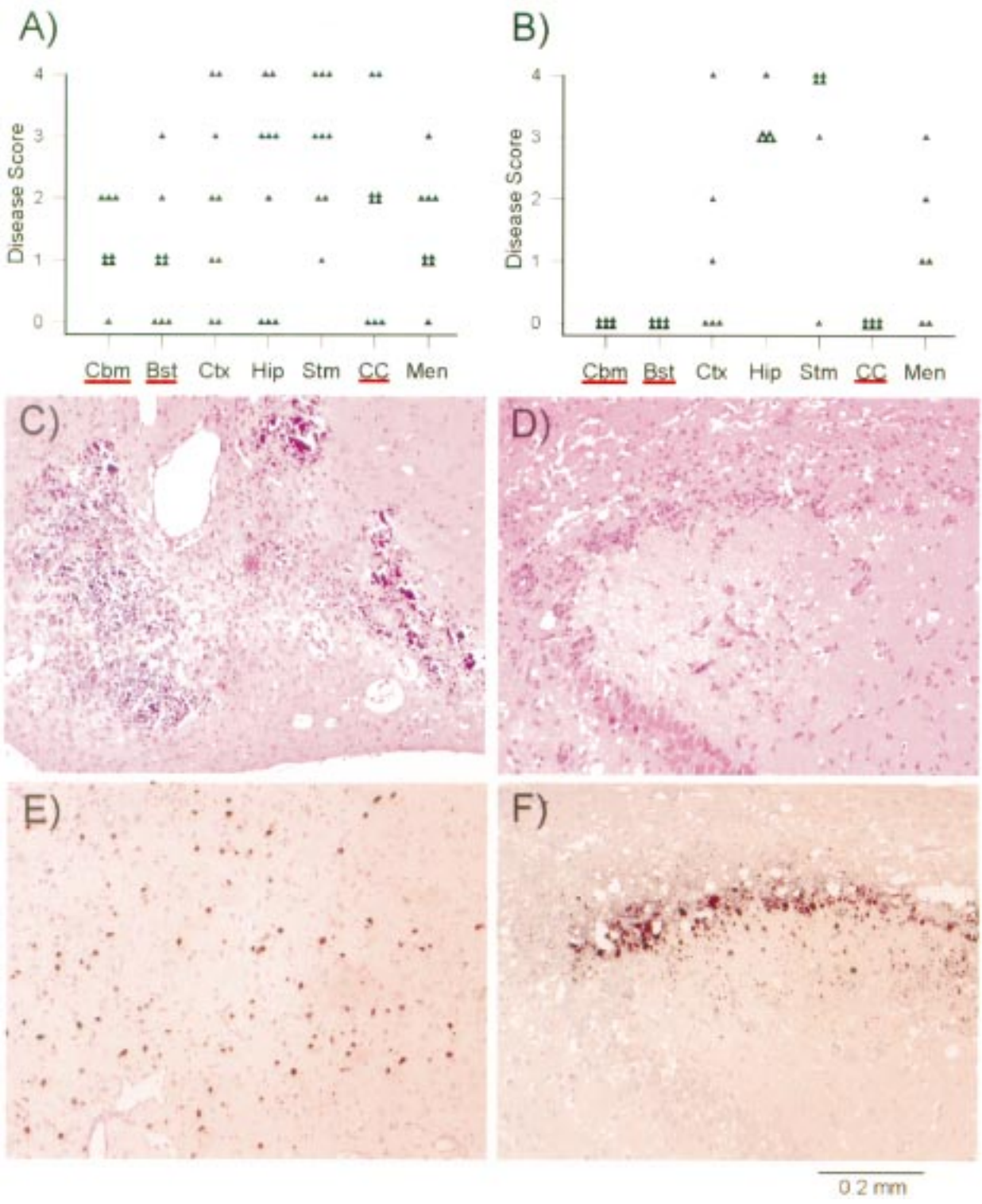
Results

α/β T cells and antibody are required for protection from TMEV-induced lethal encephalitis. Intracerebral (i.c.) infection of either severe combined immunodeficient (*Scid*) mice or MHC Class I/Class II double knockout (β_2m-/-Aβ^o) mice on a genetic background otherwise resistant to persistent infection with Theiler's virus resulted in death in the majority of mice between two and three weeks post-infection (p.i.) (Fig. 1A, 1B). The remaining mice were moribund and therefore were sacrificed. Both strains of mice have profound defects in their abilities to mount antigen-specific immune responses. In contrast, infection of immunocompetent mice (C57BL/6J) resistant to chronic virus persistence did not impair survival (100% survival at day 16 post-infection). To determine the components of the immune response required for protection from death, we infected μ MT (antibody-deficient), α/β KO (α/β T cell- deficient), β_2m-/- (Class I-deficient) and Aβ^o (Class II-deficient) with TMEV and determined the effects of these specific immune defects on mortality. As shown in Figures 1C and 1D, both the μ MT and α/β KO mice showed increased mortality compared to immunocompetent resistant mice. Five of the 18 μ MT mice died by day 16 p.i., while 6 of the 15 α/β KO mice died by the same timepoint. Antibody-deficient (μ MT) mice were more susceptible to death during the encephalitogenic phase of disease, as the mice became ill within 1 week of infection. Those μ MT that did not die recovered and were clinically normal at the time of sacrifice on day 16 p.i. α/β KO mice became ill at approximately day 10 p.i. and died usually on day 12 or 13 p.i. In contrast to the μ MT mice, one third of the α/β KO mice that survived to day 16 p.i. were moribund and had at least 2 limbs paralyzed. The survival of β_2m-/- mice and Aβ^o mice was unaffected by virus infection (Fig. 1E, 1F) indicating that the CD4-restricted and CD8-restricted immune response can independently protect mice from death.

The ability to mount a virus-specific immune response is vital for protection from direct TMEV-induced damage to the cortex and striatum. Little is known about the immune factors responsible for protection of specific brain regions from development of

Figure 3. (Opposing page) Severe brain pathology is observed at day 16 p.i. in mice lacking antigen-specific immune responses. Results are shown for *Scid* mice (**A**, **C**, **E**) and Class I/Class II double knockout (**B**, **D**, **F**) mice. Disease scores are shown for the cerebellum (Cbm), brainstem (Bst), cortex (Ctx), hippocampus (Hip), striatum (Stm), corpus callosum (CC), and meninges (Men). Areas underlined in red indicate that there was a statistically significant increase in pathology in this area when compared with the amount of disease in C57BL/6J mice as determined by the Mann Whitney Rank Sum test. Each symbol represents an individual mouse graded at each area of the brain according to the scale detailed in the **Material and Methods**. Both *Scid* (**C**, **E**) and Class I/Class II double knockout (**D**, **F**) mice had severe pathology (**C**, **D**) and high TMEV antigen levels (**E**, **F**) in the striatum. Due to the presence of necrosis, both (**C**) and (**D**) were graded as a "4".

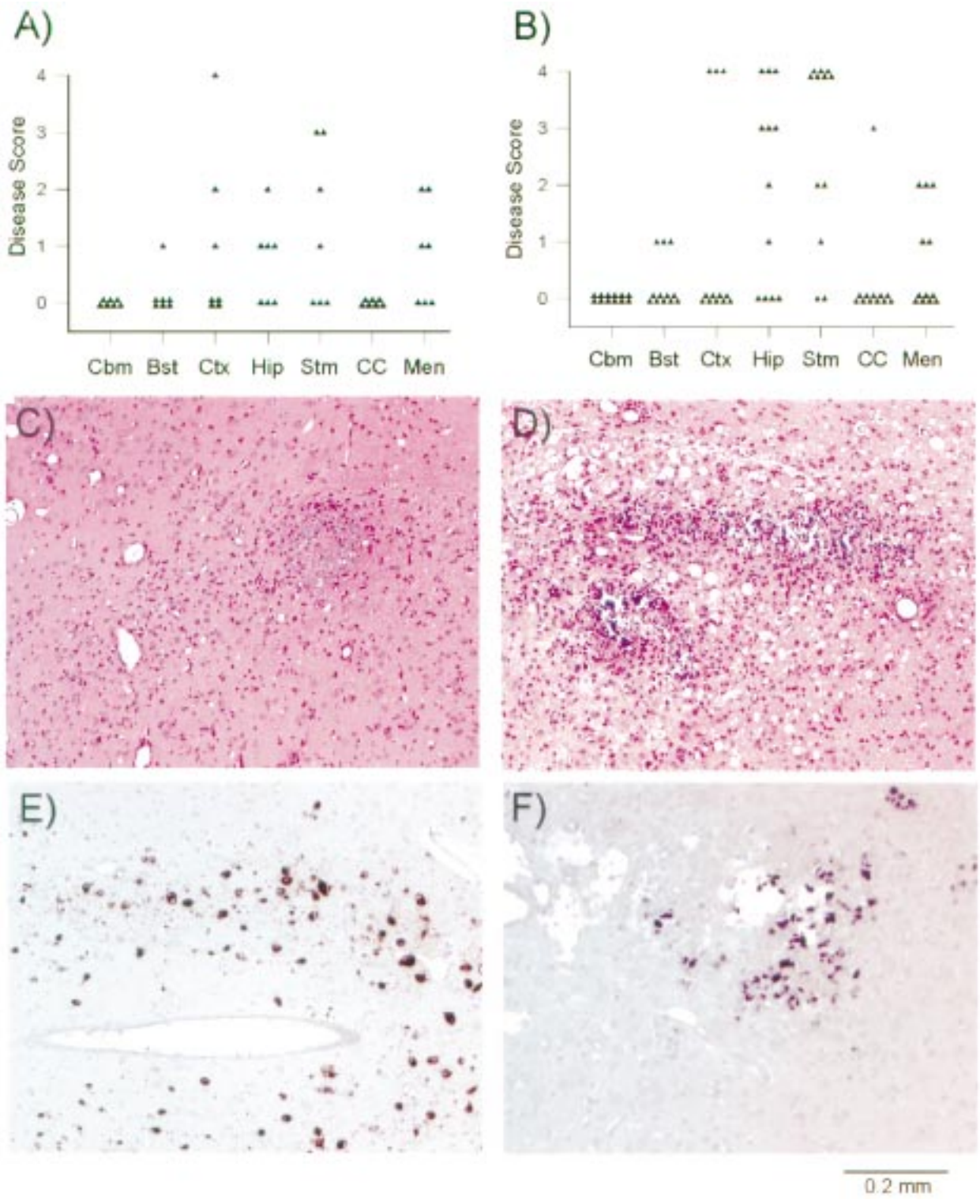

A)
B)
Disease Score
0
1
2
3
4
Cbm
Bst
Ctx
Hip
Stm
CC
Men
C)
D)
E)
F)
0.2 mm


A)
B)
Disease Score
0
1
2
3
4
Cbm
Bst
Ctx
Hip
Stm
CC
Men
C)
D)
E)
F)
0.2 mm

pathology following TMEV infection. We therefore examined the brains of mice with severe combined immune deficiency (*Scid*), significant, but incomplete T cell deficiency (Class I/Class II double knockout), and compared these to mice with intact immune systems (C57BL/6J). By analyzing these particular groups, we were able to address the relative contribution of the immune system in both protection from TMEV-induced disease and immune-mediated pathology. The mice were analyzed 16 days post-infection (p.i.) because this was the latest timepoint that could be realistically studied given the survival times observed in Fig.1. Pathological scores were assigned without knowledge of genotype using the scale described in the **Material and Methods**. Immunocompetent resistant mice (C57BL/6J) mice had minimal brain pathology (Fig. 2A, B). As shown in Figure 3A, B, C, and D, both *Scid* and Class I/Class II double knockout mice had severe brain pathology when compared to C57BL/6J mice (Fig. 2A and 2B). Both the cortex and the striatum showed significantly more disease in the Class I/Class II double knockout mice compared to C57BL/6J mice ($p=0.02$ and $p=0.02$, respectively). In the *Scid* mice the striatum also had significantly more pathology than what was observed in the resistant mice ($p=0.01$). *Scid* mice had significantly less meningeal inflammation than did the Class I/Class II double knockout mice ($p < 0.0001$; Mann-Whitney Rank Sum Test). This observation was expected, as the *Scid* mice have severe impairments of their T and B cell compartments. Minimal amounts of virus antigen (Table 2) were detected in the brains of C57BL/6J mice (Fig. 2C). The amount of viral antigen in both the cortex and the striatum varied between individual mice, but significantly greater virus burdens were found in both the cortex and striatum of the *Scid* mice as compared to C57BL/6J mice ($p=0.04$; Table 2; Fig. 3E). The differences in virus burdens in the striatum of Class I/Class II double knockout mice and C57BL/6J mice approached statistical significance ($p=0.07$; Table 2, Fig. 3F, 2C). The amount of virus antigen found in a lesion did not always correlate with the level of pathology, particularly when there was extensive damage to the parenchyma. Together these results suggest that the immune system protects mice from TMEV-induced brain disease, and that the cortex and striatum are particularly vulnerable to direct virus-mediated damage in mice with combined immune defects.

The MHC Class I - mediated immune response protects mice from TMEV - induced disease in the cerebellum, brainstem and corpus callosum. As mice with severe immune deficits have increased levels of virus-mediated brain disease compared to their immunocompetent counterparts, it was of interest to dissect the relative contributions of various arms of the immune system to protection from disease. Based on the observation that neurons express low levels of MHC Class I and glia express high levels of MHC Class I after TMEV infection, we predicted that β_2m-/- mice would have more pathology in areas abundant in white matter compared to the Aβ^o mice. To test whether protection was primarily Class I- (CD8+ T cell-) or Class II- (CD4+T cell-) mediated, we infected mice deficient in either MHC Class I (β_2m-/- mice) or MHC Class II (Aβ^o mice) with TMEV and examined their brains for pathology at day 16 p.i. In addition to an absence in MHC antigens, these mice are also severely deficient in CD8+ and CD4+ T cells, respectively. The lack of the Class I-mediated arm of the immune system (β_2m-/- mice) resulted in an increased amount of cerebellar pathology particularly in the white matter tracts (Fig. 4A). In contrast, deficiency in MHC Class II (Fig. 4B) resulted in no pathology in the cerebellum (β_2m vs. Aβ^o, $p=0.001$). Interestingly, neither *Scid* (Fig. 3A) nor Class I/Class II double knockout (Fig. 3B) mice experienced significant cerebellar disease. Therefore in the presence of an intact MHC Class II response Class I protects against cerebellar pathology. Furthermore a functional Class II mediated immune response is required for the induction of cerebellar pathology.

Figure 4. (Opposing page) The MHC Class I mediated immune response protects mice from TMEV-induced disease in the cerebellum, brainstem and striatum (areas with abundant white matter) at day 16 p.i. Results are shown for β_2m -/- (**A**, **C**, **E**) and Aβ^o (**B**, **D**, **F**) mice. Disease scores are shown for the cerebellum (Cbm), brainstem (Bst), cortex (Ctx), hippocampus (Hip), striatum (Stm), corpus callosum (CC), and meninges (Men). Each symbol represents an individual mouse graded at each area of the brain according to the scale detailed in the **Material and Methods.** Areas of the brain underlined in red have significantly different ($p<0.05$) levels of brain disease when comparing β_2m -/- and Aβ^o mice as determined by the Mann Whitney Rank Sum test. Mice deficient in MHC Class I (β_2m -/-) have exacerbated disease in the cerebellum, brainstem, and corpus callosum (**A**) when compared to their MHC Class II-deficient (Aβ^o) counterparts (**B**). Examples of brain pathology typical of the striatum of Class I-deficient (**C**) and hippocampus of Class II- deficient (**D**) mice are shown. The striatum (C) received a grade of "4" due to the frank necrosis in the tissue with associated cellular debris. The hippocampus (**D**) was graded as a "3" because of the loss of neurons and loss of architecture without necrosis. Abundant immunoreactivity to TMEV antigen was found in the striatum of the Class I-deficient mice (**E**) and the hippocampus of Class II-deficient mice (**F**).


A)
B)
Disease Score
0
1
2
3
4
Cbm
Bst
Ctx
Hip
Stm
CC
Men
C)
D)
E)
F)
0.2 mm

The brainstem and the corpus callosum, two additional regions of the brain containing abundant white matter, also appeared to rely on Class I for protection from disease. β_2m-/- mice (Fig. 4A) had significantly more disease in both the brainstem and corpus callosum when compared to their Class II-deficient counterparts (Fig. 4B) (p=0.03 and 0.02, respectively). As was observed in the cerebellum, the extent of disease observed in the corpus callosum of the β_2m-/- mice (Fig. 4A) was significantly (p=0.03) more severe than in the Class I/Class II double knockout mice (Fig. 3B). This indicated that in the in the absence of Class I, Class II exacerbates disease in the corpus callosum. In contrast, the pathology observed in the brainstems of *Scid* and Class I/Class II double knockout mice was comparable to that observed in the β_2m-/- mice. This observation supports the hypothesis that a MHC Class I, but not a Class II-restricted response, protects the brainstem from TMEV-induced pathology.

Both the Class I- and Class II-mediated immune responses appear to participate in protecting the cortex from viral-induced damage. While the most severe disease was found in animals with the most serious immune defects, the extent of disease found in the Class I-deficient mice (Fig. 4A) was higher than the Class II-deficient mice (Fig. 4B) and approached statistical significance (p=0.059). These data suggest that MHC Class I as compared to MHC Class II-mediated immune response may play a larger role in protecting the cortex from TMEV-induced disease. Both Class I-deficient and Class II-deficient mice had severe striatal (Fig. 4C) and hippocampal disease (Fig. 4D) with accompanying virus replication (Fig. 4E, 4F). These results support a role for both Class I and Class II-mediated protection in these areas of the brain.

Antibody protects the striatum from TMEV-induced disease. While the role of MHC Class I in protecting specific regions of the brain has been shown, the data presented here also implicate the Class II-mediated immune response in protection from TMEV-induced disease. To assess the relative contribution of antibody in disease protection, we infected α/β KO mice (deficient in α/β T cells and antibody) and μ MT mice (severely deficient in immunoglobulins with an intact T cell compartment) with Theiler's virus and sacrificed the mice at day 16 to assess the relative contribution of antibody in disease protection. Both μ MT and α/β KO mice had increased disease in the striatum, hippocampus, and cortex compared to the C57BL/6J mice, suggesting the participation of antibody in protection from these areas of brain (Fig. 5A-D). Antibody-deficient mice had significantly more disease in the striatum than C57BL/6J mice (p=0.04). No statistically significant differences in pathology were observed in α/β KO mice compared with the μ MT mice, although the increased level of disease observed in the striatum of the μ MT mice approached statistical significance (p=0.06; Fig. 5A, 5B). No significant differences in the levels of virus antigen were found between α/β KO mice and μ MT mice (Table 2). These results support the hypothesis that antibody plays a critical role in protecting areas of the brain with abundant neurons.

Discussion

The present studies addressed if particular immune system components protect discrete areas of the brain from virus-induced disease. We examined the contribution of the host immune system in protecting distinct areas of the brain by studying mice with specific immune defects infected with the same strain of Theiler's virus (DA strain). As the receptor for TMEV has not been identified, it was not possible to examine the issue of virus-induced pathology from the viewpoint of receptor distribution. The use of knockout mice allowed us not only to determine the contributions of the immune system components to protection, but also provided insights into the role of those remaining immune system components in the development of tissue injury.

Previous studies demonstrated that glial cells as compared to neurons can upregulate MHC Class I on their surface within 12 to 48 hours following virus infection (12). These data support a hypothesis that areas of the brain with abundant glia (white matter tracts) would be protected by a CD8+ T cell / Class I mediated immune response, whereas areas of the brain with abundant neurons (gray matter) would be protected by either a CD4+

Figure 5. (Opposing page) Antibody protects mice from TMEV-induced disease in the striatum at day 16 p.i. Results are shown for mice deficient in α/β T cells(α/β KO) (**A**) and antibody (m MT) (**B**). Disease scores are shown for the cerebellum (Cbm), brainstem (Bst), cortex (Ctx), hippocampus (Hip), striatum (Stm), corpus callosum (CC), and meninges (Men). No significant differences in the level of pathology was observed between α/β KO (**A**) and m MT (**B**) mice using the Mann Whitney Rank Sum test. Each symbol represents an individual mouse graded at each area of the brain according to the scale detailed in the **Material and Methods**. Both α/β KO (**C**, **E**) and antibody-deficient (**D**, **F**) mice have severe pathology (**C**, **D**) and high TMEV antigen levels (**E**, **F**) in the striatum. The striatum illustrated in (**C**) shows parancheymal damage and was graded as a "3." The presence of necrosis and vacuolar changes in (**D**) resulted in the sample being graded as a "4."

T cell / Class II mediated response, or by antibody. Data from the present study support this hypothesis. Using β_2m-/- mice deficient in both MHC Class I and CD8 single positive T cells, we found that TMEV-infected mice had higher propensity for cerebellar pathology, particularly in the white matter, as compared with mice deficient in MHC Class II. The role of the Class I arm of the immune response in protection was further underscored by the observation that the brainstem and the corpus callosum (regions also with abundant white matter) showed more significant pathology in the Class I-deficient compared to Class II-deficient mice. These results particularly highlighted the balance between protection and immunopathology, as there was significantly more damage in the β_2m-/- mice (which have intact Class II-mediated responses) than the Class I/Class II double knockout mice. Therefore the presence of an intact Class II immune response appears to be necessary for immune-mediated pathology in the brainstem, cerebellum and corpus callosum to ensue.

Our studies suggest that the main mechanism of protection for most regions of the brain from virus-induced pathology involves the Class I-mediated immune response. The two areas of the brain that are an exception are the striatum and the hippocampus, regions with a predominance of neurons. These findings are consistent with several reports from our laboratory and other laboratories highlighting the importance of the CD8+-Class I mediated immune response in protecting mice from TMEV persistence. H-2D^{b,d} expressing mice are resistant to virus persistence, while mice expressing H-2D^{q,r,s} are susceptible (3, 17). In addition, mice resistant to TMEV-induced demyelinating disease and virus persistence generate strong TMEV-specific H-2D-restricted CTL responses by day 7 p.i. whereas susceptible mice do not generate a TMEV-specific Class I-restricted response (11). An earlier study by Altintas et al. (1) reported an upregulation of both H-2D and H-2K Class I MHC in the brains of mice resistant to TMEV-induced demyelination. One of the most intensely positive structures in this study was the corpus callosum (1), an area that experienced a high degree of pathology in Class I deficient mice but was largely spared in immunocompetent mice of a resistant haplotype.

While MHC Class I appears to contribute to protection from TMEV-induced brain pathology, the Class II-mediated arm of the immune system appear to be also critically involved. The data supports the role for antibody in preventing TMEV-induced damage to the striatum. In addition, an intact Class II response , in the presence of Class I, protects the cerebellum from TMEV-induced pathology. In the absence of Class II cerebellar pathology is minimal.

As evidenced from the fatal outcome observed following infection of the *Scid*, α/β TCR KO mice, and Class I/Class II double knockouts, the intact immune system is required to protect mice from death during the early phases of TMEV. In contrast either the Class I-restricted or Class II-restricted response alone is sufficient to protect from death as minimal or no deaths were observed following infection of either β_2m-/- or Aβ^o mice. The humoral immune response alone also is important in protection from death, as there was an increased frequency of death in the μ MT animals. As neither μ MT nor α/β KO mice produce TMEV-specific antibodies (data not shown), this may be the critical component of the immune system required for protection from fatal encephalitis. Because neurons as compared to glia have less capacity to upregulate MHC molecules following virus infection, this is consistent with our results that antibody rather than T cells protect from early virus infection. The role of antibody in protecting the brain during virus infection has been described in several systems (16, 23, 24), most notably with Sindbis virus (9), where antibody is essential for clearing virus from neurons, thus protecting mice from death and chronic injury.

Together, the results of this study illustrate the vital role of components of the Class I and Class II immune response in preventing injury to distinct regions of the brain in mice genetically resistant to TMEV persistence. The results demonstrate that those processes involved in protection from parenchymal brain injury are distinct from those that protect the host from death in the acute phase of disease.

References

1. Altintas A, Cai Z, Pease LR, Rodriguez M (1993) Differential expression of H-2K and H-2D in the central nervous system of mice infected with Theiler's virus. *J Immunol* 151: 2803-2812.
2. Aubert C, Chamorro M, Brahic M (1987) Identification of Theiler's virus infected cells in the central nervous system of the mouse during demyelinating disease. *Microb Pathog* 3: 319-326.
3. Azoulay A, Brahic M, Bureau JF (1994) FVB mice transgenic for the H-2Db gene become resistant to persistent infection by Theiler's virus. *J Virol* 68: 4049-4052.
4. Bosma GC, Custer RP, Bosma MJ (1983) A severe combined immunodeficiency mutation in the mouse. *Nature* 301: 527-530.
5. Budka H (1991) Neuropathology of human immunodeficiency virus infection. *Brain Pathol* 1: 163-175.

6. Clatch RJ, Miller SD, Metzner R, Dal Canto MC, Lipton HL (1990) Monocytes/macrophages isolated from the mouse central nervous system contain infectious Theiler's murine encephalomyelitis virus (TMEV). *Virol* 176: 244-254.
7. Cosgrove D, Gary D, Dierich A, Kaufman J, Lemeur M, Benoist C, et al (1991) Mice lacking MHC Class II molecules. *Cell* 66: 1051-1066.
8. Dal Canto MC, Lipton HL (1982) Ultrastructural immunohistochemical localization of virus in acute and chronic demyelinating Theiler's virus infection. *Am J Path* 106: 20-29.
9. Griffin DE, Levine B, Ubol S, Hardwick JM (1994) The effects of alphavirus infection on neurons. *Ann Neurol* 35: S23-S27
10. Kitamura D, Roes J, Kuhn R, Rajewsky K (1991) A B-cell deficient mouse by targeted disruption of the membrane exon of the immunoglobulin m gene. *Nature* 350: 423-426.
11. Lin X, Pease LR, Rodriguez M (1997) Differential generation of Class I H-2D- versus H-2K-restricted cytotoxicity against a demyelinating virus following central nervous system infection. *Eur J Immunol* 27: 963-970.
12. Lindsley MD, Patick AK, Prayoonwiwat N, Rodriguez M (1992) Coexpression of class I major histocompatibility antigen and viral RNA in central nervous system of mice infected with Theiler's virus: a model for multiple sclerosis. *Mayo Clin Proc* 67: 829-838.
13. Lipton HL (1975) Theiler's virus infection in mice: an unusual biphasic disease process leading to demyelination. *Infect.Immun* 11: 1147-1155.
14. Mombaerts P, Clarke AR, Rudnicki MA, Lacomini J, Hohara S, Lafaille JJ, et al (1992) Mutations in T-cell antigen genes alpha and beta block thymocyte development at different stages. *Nature* 360: 225-231.
15. Njenga MK, Pavelko KD, Baisch J, Lin X, David C, Leibowitz J, et al (1996) Theiler's virus persistence and demyelination in major histocompatibility complex class II-deficient mice. *J Virol* 70: 1729-1737.
16. Rima BK, Duffy N, Mitchell WJ, Summers BA, Appel MJ (1991) Correlation between humoral immune responses and presence of virus in the CNS in dogs experimentally infected with canine distemper virus. *Arch Virol* 121: 1-8.
17. Rodriguez M, David CS (1995) H-2 Dd transgene suppresses Theiler's virus-induced demyelination in susceptible strains of mice. *J Neurovirol* 1: 111-117.
18. Rodriguez M, Lafuse W, Leibowitz J, David CS (1986) Partial suppression of Theiler's virus-induced demyelination in vivo by administration of monoclonal antibodies to immune response gene products (Ia antigens). *Neurol* 36: 964-970.
19. Rodriguez M, Leibowitz JL, Lampert PW (1983) Persistent infection of oligodendrocytes in Theiler's virus-induced encephalomyelitis. *Ann Neurol* 13: 426-433.
20. Rossi CP, Delcroix M, Huitinga I, McAllister A, Van Rooijen N, Claassen E, et al (1997) Role of macrophages during Theiler's virus infection. *J Virol* 71: 3336-3340.
21. Sidman RL, Angevine JB, Pierce ET (1971) *Atlas of the mouse brain and spinal cord*, Harvard University Press: Cambridge MA.
22. Tangchai P, Yenbutr DVA (1970) Central nervous system lesions in human rabies. A study of twenty-four cases. *J Med Assoc Thailand* 53: 471-486.
23. Tyler KL, Virgin HW, Bassel-Duby R, Fields BN (1989) Antibody inhibits defined stages in the pathogenesis of reovirus serotype 3 infection of the central nervous system. *J Exp Med* 170: 887-900.
24. Wright KE, Buchmeier MJ (1991) Antiviral antibodies attenuate T-cell-mediated immunopathology following acute lymphocytic choriomeningitis virus infection. *J Virol* 65: 3001-3006.
25. Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R (1990) Beta 2-microglobulin deficient mice lack CD4-CD8+ cytolytic T cells. *Nature* 344: 742-746.