TMEV, demyelination is initiated by CD4+ T-cells, which resulted in 42% mortality in SJL/J syngeneic transplants. 47% mortality in diseased DBA/2 recipients restored with marrow from naive B6D2 donors, and 12% in diseased DBA2 recipients receiving marrow from B6D2 donors previously infected with TMEV. Delayed type hypersensitivity (DTH) to both virion and myelin proteins was decreased in surviving mice that underwent transplantation; however, CNS viral titers were significantly elevated compared with nontransplanted controls. We conclude that a functional immune system with appropriate T-cell responses is important in prevention of lethal cytopathic CNS effects from TMEV. Relevant to the clinical use of bone marrow transplantation, attempts to ablate the immune system in viral-mediated immune diseases or virus-initiated autoimmune disease may have acute and lethal consequences. Our results raise concern about the attempted use of autologous hematopoietic transplantation in patients with MS, an autoimmune disease with a suspected virus etiology, particularly if the graft is aggressively depleted of lymphocytes.© 1999 by The American Society of Hematology.

Theiler’s murine encephalomyelitis virus (TMEV) establishes a persistent infection in the central nervous system (CNS) leading to an inflammatory demyelinating disease of the CNS in which the histology and clinical course is similar to multiple sclerosis (MS). Disease pathogenesis is primarily due to T-cell-mediated destruction of myelin, which has been attributed to cytotoxic effects of the virus, but immune-mediated destruction of myelin mediated via both virus-specific and myelin-specific T cells appear to play the major role. To determine if bone marrow transplantation would be an effective therapy for a virus-initiated autoimmune disease and to better separate viral cytotoxic effects from immune-mediated demyelination, we ablated the immune system of TMEV-infected animals with 1,100 cGy total body irradiation, and then the animal’s immunity was reconstituted by transplantation of disease-susceptible SJL/J mice with syngeneic and then the animal’s immunity was reconstituted by transplantation of disease-resistant (C57Bl/6 × DBA/2)F1 (B6D2) donors. Hematopoietic transplant performed after onset of disease resulted in 42% mortality in SJL/J syngeneic transplants, 47% mortality in diseased DBA2 recipients restored with marrow from naive B6D2 donors, and 12% in diseased DBA2 recipients receiving marrow from B6D2 donors previously infected with TMEV. Delayed type hypersensitivity (DTH) to both virion and myelin proteins was decreased in surviving mice that underwent transplantation; however, CNS viral titers were significantly elevated compared with nontransplanted controls. We conclude that a functional immune system with appropriate T-cell responses is important in prevention of lethal cytopathic CNS effects from TMEV. Relevant to the clinical use of bone marrow transplantation, attempts to ablate the immune system in viral-mediated immune diseases or virus-initiated autoimmune disease may have acute and lethal consequences. Our results raise concern about the attempted use of autologous hematopoietic transplantation in patients with MS, an autoimmune disease with a suspected virus etiology, particularly if the graft is aggressively depleted of lymphocytes.© 1999 by The American Society of Hematology.

From the Department of Medicine, the Department of Microbiology-Immunology and Interdepartmental Immunobiology Center, the Department of Pathology, and Robert H. Lurie Cancer Center, Northwestern University Medical School, Chicago, IL.

Submitted November 4, 1998; accepted June 6, 1999.

Address reprint requests to Richard K. Burt, MD, Department of Medicine, Northwestern University Medical School, 250 E Superior St, Room 1456, Wesley Pavilion, Chicago, IL 60611.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1999 by The American Society of Hematology.

0006-4971/99/9408-0042$3.00/0
autoimmune animal model of MS. Recent short-term outcome studies on limited numbers of patients with MS suggest that immune ablation and hematopoietic stem cell reconstitution with autologous hematopoietic stem cells may prevent progression of and in some cases improve the pathogenesis of MS.

Because the histology and clinical course of TMEV are similar to human MS and because epidemiological studies suggest MS may be initiated and/or exacerbated by virus infections, we evaluated the outcome of hematopoietic stem cell transplantation in TMEV-induced demyelinating disease. The results demonstrate that stem cell transplantation of mice with ongoing TMEV-induced demyelinating disease results in a high incidence of mortality concomitant with a significant elevation of CNS virus titers. Thus, attempts to ablate the immune system in viral-mediated immune diseases or virus-initiated autoimmune diseases associated with persistent infection may have acute and lethal consequences.

MATERIALS AND METHODS

Animals. Six-week-old female SJL mice were obtained from Harlan Laboratories (Madison, WI). B6D2 F1 mice were obtained from Jackson Laboratories (Bar Harbor, ME). Animals were maintained on standard mouse chow and water ad libitum in a containment animal facility. Neomycin sulfate (0.7 mmol/L), tetracycline (0.1 mmol/L), and trimethoprim/sulfamethoxazole (0.4 mmol/L) were added in the drinking water for 2 weeks after bone marrow transplantation (BMT) to prevent infections.

Induction of TMEV-induced demyelinating disease. BeAn 8386 virus was plaque purified and amplified in BHK-21 cells. A working stock was prepared by passage in BHK-21 cells. Female mice were anesthetized with methoxyflurane and intracerebrally inoculated in the right cerebral hemisphere with approximately 2.9 × 10^4 PFU of BeAn virus in 30 mL. All animals were examined several times per week for the first 4 weeks and at least once weekly thereafter. Sham-infected control animals received 30 µL perfused spinal cord from 3 mice in each group in guanidium isothiocyanate and isolating total RNA by CsCl gradient. First-strand cDNA synthesis was performed using 2 µL (0.5 µg/µL) of RNA in 10.5 µL of diethyl pyrocarbonate (DEPC)-treated water, 1 µL of oligo-(dT) primer, and 6.5 µL of a master mix (4 µL of 5 × reaction buffer, 1 µL of dNTP mix [10 mmol/L each], 0.5 µL RNase inhibitor, and 1.0 µL of Moloney murine leukemia virus [M-MLV] reverse transcriptase). Polymerase chain reaction (PCR) primers for interferon-γ (IFN-γ), interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α) encompass 234, 324, and 240 bp of wild-type cDNA, respectively. First-strand cDNA was amplified 35 cycles (Perkin Elmer Thermocycler; Perkin Elmer, Norwalk, CT).

Virus plaque assays. Standard plaque assays (previously described) were performed for quantification of virus titers in the spinal cord, brain, and spleen. Organs from 3 mice per group were homogenized with a Virtis tissue homogenizer (Virtisher-Gardiner, New York, NY) into a 10% solution and the homogenate was layered over BHK-21 cells. After 96 hours of incubation, live cells were stained using a 0.015% neutral red. Plates were incubated for 4 hours and plaques were enumerated.

Statistical analyses. Student’s t-tests were used to determine the statistical significance of clinical scores, DTH, and virus titers between experimental groups.

RESULTS

Syngeneic transplantation of TMEV-susceptible SJL mice. Syngeneic hematopoietic stem cell transplantation was performed on 2 separate groups of SJL mice with established TMEV-induced demyelinating disease at days 70 and 92 postinfection. Thirteen of 31 (42%) of these mice transplanted 70 days postinfection died within 2 weeks posttransplantation (Fig 1A). Interestingly, the mortality rate for SJL mice with TMEV-induced demyelinating disease was significantly greater than we have noted in similar transplants of SJL mice with established relapsing EAE, in which a mortality rate of less than 6% was observed in well over 100 mice transplanted at varying times during ongoing disease. There was no significant difference in median neurologic deficit in surviving transplanted animals compared with nontransplanted animals (P = .3858) as neurologic deterioration continued in both the transplanted and control groups (Fig 1B). Despite the progression of clinical disease, the myeloblation was apparently successful, because DTH responses to both virus epitopes (VP2 70-86 and VP3 23-47) and to the immunodominant epitope on proteolipid protein (PLP 139-151) were abrogated in transplanted mice assayed approximately 30 to 40 days postreconstitution (Fig 2).

Evaluation of the CNS for Th1-associated (IFN-γ) and TNF-α-
or Th2-associated (IL-10) cytokine mRNA levels using a semiquantitative PCR analysis showed no significant differences between transplanted and nontransplanted mice assayed approximately 40 days postreconstitution (Fig 3).

Interestingly, transplanted animals exhibited significantly higher titers of infectious TMEV within the spinal cord than did untreated controls (Fig 4). In transplanted mice, approximately 4-fold more infectious virus was present in the spinal cords than in the nontransplanted mice (12.6 × 10^3 PFU/mg v 3.1 × 10^3 PFU/mg, P = .004). No viral plaques were found in the brain or spleen of either TMEV-infected group or in any tissues in the uninfected controls. Histologic examination of the spinal cords showed an acute inflammatory infiltrate with less glial scarring in transplanted mice versus a prominent chronic demyelination and gliosis with minimal residual infiltration in nontransplanted mice (Fig 5).

Allogeneic transplantation using TMEV-resistant donors. To determine if the efficacy of the transplantation may be increased by using marrow from disease-resistant donors, disease-susceptible DBA/2J mice were treated with TBI and infused with marrow from either naive or TMEV-infected disease-resistant B6D2 mice. At day 20 postinfection, before the onset of clinical disease, susceptible DBA/2J mice were treated with TBI and infused with marrow from either naive or TMEV infected disease-resistant B6D2 mice (Fig 6). Control mice developed chronic progressive disease with no mortality (0/9).

**DISCUSSION**

Resistance and susceptibility to demyelination after TMEV infection are both immune-mediated processes. Susceptibility/resistance to TMEV-induced demyelinating disease is controlled by multiple loci, including Tmevd-1 on chromosome 6 near the genes encoding the β chain of the T-cell receptor; Tmevd-2 on chromosome 3 near the Car-2 locus; and the MHC class I H-2D region on chromosome 17. Disease susceptibility correlates with development of strong virus-specific Th1 responses, as exemplified by expression of DTH reactivity and predominant production of Th1-derived cytokines and IgG2a-predominant antivirus antibody responses. Recent evidence indicates that virus...
clearance is largely mediated by an abundant MHC class I-restricted CD8\(^+\) CTL response arising within the first 10 days postinfection.\(^{40}\) Studies have shown that depletion of CD8\(^+\) T cells can confer susceptibility to some otherwise resistant inbred strains.\(^{41}\)

In disease-susceptible strains, TMEV-induced demyelinating disease is a 2-stage process. Infection with the DA strain of TMEV leads to early inflammatory infiltration within the CNS gray matter that leads to a limited amount of neuronal necrosis that is consistent with lytic infection of neurons in cell culture.\(^{42}\) The gray matter inflammation is cleared within 2 weeks postinfection and is replaced by mononuclear inflammation and demyelination of white matter. Use of the BeAn strain of TMEV that was employed in these studies obviates much of the gray matter pathology. Relatively weak CTL responses in susceptible strains\(^{40}\) result in the inability to clear virus from the CNS and lead to establishment of persistent infection.\(^3\) White matter damage is initiated by virus-specific CD4\(^+\) T cells that lead to macrophage-mediated bystander destruction of myelin.\(^7,15,16\) Myelin-specific T-cell responses do not play a role in disease initiation as responses to immunodominant epitopes on MBP and PLP are not detected before disease onset.\(^{17,18}\) and peripheral tolerance to myelin components before virus infection fails to affect the course of demyelination.\(^{16}\) However, chronic disease is associated with the development of T-cell responses to multiple myelin epitopes that arise via epitope spreading.\(^{17}\) Taken together with the fact that TMEV-specific T-cell responses persist throughout the disease course in susceptible SJL mice,\(^3\) the data are consistent with a role for both anti–virus-specific and anti–myelin-specific T-cell responses in chronic disease.

Because MS\(^{27}\) and perhaps other T-cell–mediated autoimmune diseases may be initiated as a secondary consequence to a virus infection, we have attempted to use hematopoietic stem cell transplantation as a tool to separate the overlapping roles of direct virus cytopathology, virus-specific immunity, and myelin epitope-specific autoimmunity in TMEV-induced demyelinating disease. The results clearly show that transplantation of syngeneic marrow from naive TMEV-susceptible donors to
diseased SJL/J recipients resulted in 40% mortality within 10 to 15 days after transplantation (Fig 1). Although the deaths may have been due to radiation induced CNS injury, the mortality rate was significantly higher than that of SJL mice undergoing BMT for the treatment of relapsing EAE, a purely autoimmune inflammatory demyelinating disease, in which we and others have historically observed an approximate 6.0% mortality rate.22-26

Surviving mice displayed significantly diminished immune DTH responses to both virus and myelin epitopes, indicating the effectiveness of the myeloablative therapy (Fig 2). The acute neurologic deterioration correlated with an increased CNS viral load (Fig 4). Histologic evaluation (Fig 5) showed a predominance of acute gray matter inflammation in transplanted mice compared with predominant white matter demyelination in untreated animals. The slightly exacerbated clinical disease course, increased CNS virus levels, and pattern of histology are consistent with an exacerbation of direct viral cytopathology, not immune-mediated CNS damage. This finding is similar to that previously reported by Lipton and Dal Canto,11 who showed that high-dose cyclophosphamide or antithymocyte serum administered shortly after virus infection prevented TMEV-induced immune-mediated demyelination, but resulted in a mortality rate of 77% to 88%. Thus, severe immunosuppression of TMEV-infected animals is capable of causing fatal neurologic consequences that were also likely due to uncontrolled virus growth.

To reconstitute viral immunity more rapidly, we attempted allogeneic transplantation of marrow from naive disease-resistant B6D2 mice into diseased DBA/2J mice. This resulted in an equally high early mortality of 50%, although death was delayed to 60 to 70 days posttransplant. Therefore, we at-
Neurologic score of surviving mice inoculated with TMEV. (A) Percentage of animals surviving. (B) Resistant donor marrow for DBA/2J disease-susceptible recipients cytotoxic lymphocytes infused with the donor marrow.43 May have been due to adoptive transfer of viral-specific peptides from naive allogeneic or syngeneic donors. Lower mortality substantially lower than that of mice receiving bone marrow donors. This resulted in a mortality rate (20%) that was previously inoculated B6D2 donors rather than naive B6D2 tempted allogeneic transplantation using disease-resistant but previously inoculated B6D2 donors rather than naive B6D2 donors. This resulted in a mortality rate (20%) that was substantially lower than that of mice receiving bone marrow from naive allogeneic or syngeneic donors. Lower mortality may have been due to adoptive transfer of viral-specific cytotoxic lymphocytes infused with the donor marrow.43 Because mouse bone marrow contains relatively few lymphocytes, adoptive transfer of both splenocytes and marrow from previously infected disease-resistant mice may prevent or further decrease exacerbation of viral cytopathic effects after transplantation.

BMT is currently being investigated in clinical trials as therapy for human autoimmune diseases. Whereas the experimental results in autoimmune disorders such as EAE indicate amelioration or cure after transplantation, the current results in TMEV-induced demyelinating disease suggest that this therapy may be dangerous in virus-associated autoimmune diseases. Transplantation did improve the autoimmune component of TMEV by decreasing immune responses to viral and PLP peptides. However, nonspecific attempts to suppress this virus-initiated autoimmune process also suppress viral immunity and can apparently result in lethal consequences.

Further advances in transplant of virus-associated autoimmune diseases should recognize the importance of controlling viral cytotoxicity after transplantation with either peritransplant antiviral drugs and/or virus-specific adoptive immunotherapy at the time of graft infusion. This is particularly true in MS, in which an infectious etiology is strongly suspected based on a variety of observations, including epidemiological data,44-49 abnormal humoral and/or cellular responses to viruses,50-55 isolation of virus or presence of viral proteins within CNS plaques,56-58 and animal models of virus-initiated, inflammatory CNS demyelinating diseases that mimic MS clinically and historically.1,2 The rationale for treating MS by hematopoietic stem cell transplantation is based on the unproven assumption that MS is an autoimmune disease targeting myelin proteins and that the procedure will ablate activated autoreactive T cells and also lead to the re-establishment of self-tolerance to myelin epitopes. This theory is supported by cure or amelioration of disease after transplantation of autoimmune diseases such as EAE,22-26 collagen-induced arthritis,59 and adjuvant arthritis,60 which are induced by active immunization with self-proteins/peptides in adjuvant. However, the current data clearly show that stem cell transplantation of an autoimmune-like disease initiated with infection with TMEV, a natural mouse pathogen, although dampening autoimmune responses, also inhibits antiviral responses, resulting in virus reactivation and significant mortality.

It should be pointed out that TMEV-induced demyelinating disease may be an exception, because the virus establishes a lifelong persistent CNS infection. Hematopoietic stem cell transplantation may be perfectly appropriate for treating virus-induced autoimmune diseases in which the initial virus infection has been cleared, thus alleviating the danger of virus reactivation. Current results from transplantation of MS patients have not shown undue mortality,1,2,6 perhaps suggesting that the disease etiology is diverse, with only a subset being virally mediated, or that a potential virus infection may have already been cleared or is relatively noncytopathic. However, given the limited number of patients transplanted to date, the potential for virus reactivation is a real concern and may depend on the level of immunosuppression of the recipient and/or the degree of lymphocyte depletion of the graft.

REFERENCES

2. Lipton HL: Theiler’s virus infection in mice: An unusual biphasic disease process leading to demyelination. Infect Immun 11:1147, 1975
HEMATOPOIETIC STEM TRANSPLANTATION OF TMEV DISEASE


23. van Gelder M, Kinwel-Bohe EP, van Bekkum DW: Treatment of experimental allergic encephalomyelitis in rats with total body irradiation and syngeneic BMT. Bone Marrow Transplant 11:233, 1993

24. van Gelder M, van Bekkum DW: Treatment of relapsing experimental autoimmune encephalomyelitis in rats with allogeneic bone marrow transplantation from a resistant strain. Bone Marrow Transplant 16:343, 1995


43. Lin X, Thiemann NR, Pease LR, Rodriguez M: VP1 and VP2 capsid proteins of Theiler’s virus are targets of H-2D-restricted
cytotoxic lymphocytes in the central nervous system of B10 mice. Virology 214:91, 1995


Viral Hyperinfection of the Central Nervous System and High Mortality After Hematopoietic Stem Cell Transplantation for Treatment of Theiler’s Murine Encephalomyelitis Virus-Induced Demyelinating Disease

Richard K. Burt, Josette Padilla, Mauro C. Dal Canto and Stephen D. Miller