Passive immunotherapy for retroviral disease: Influence of major histocompatibility complex type and T-cell responsiveness

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ABSTRACT Administration of virus-specific antibodies is known to be an effective early treatment for some viral infections. Such immunotherapy probably acts by antibody-mediated neutralization of viral infectivity and is often thought to function independently of T-cell-mediated immune responses. In the present experiments, we studied passive antibody therapy using Friend murine leukemia virus complex as a model for an immunosuppressive retroviral disease in adult mice. The results showed that antibody therapy could induce recovery from a well-established retroviral infection. However, the success of therapy was dependent on the presence of both CD4$^+$ and CD8$^+$ T lymphocytes. Thus, cell-mediated responses were required for recovery from infection even in the presence of therapeutic levels of antibody. The major histocompatibility type of the mice was also an important factor determining the relative success of antibody therapy in this system, but it was less critical for low-dose than for high-dose infections. Our results imply that limited T-cell responsiveness as dictated by major histocompatibility genes and/or stage of disease may have contributed to previous immunotherapy failures in AIDS patients. Possible strategies to improve the efficacy of future therapies are discussed.

There is currently a great deal of interest in antibody therapy as a treatment for human immunodeficiency virus (HIV) infections. Such therapy has been successful in treating human exposure to a variety of viruses such as hepatitis A and B, poliovirus, rabies, cytomegalovirus, and others (1–3). Experimentally, antibody therapy has been demonstrated to be effective in preventing disease in rabies-infected nude mice when administered 72 hr postinfection (4) and in curing influenza pneumonia in *scid* mice when administered as late as 7 days postinfection (5). Passive antibody therapy has also induced cures of Sindbis virus and Theiler's virus infections in immunodeficient mice (6, 7). Such findings suggest that successful antibody therapies work independently of T-cell-mediated immune responses such as cytolytic T-lymphocyte (CTL) responses. However, this may not be generally true for all viruses, especially in cases where cell-mediated responses function in ways that cannot be compensated for by antibodies.

Currently, no small animal model exists for investigating passive antibody treatments as an immunotherapy for HIV infections. However, some insight may be gained from studying infections with other retroviruses. We have used Friend virus (FV) (8), an immunosuppressive retroviral complex that induces erythroleukemia in adult immunocompetent mice (9–13). Previously, passive transfers of specific antisera have been demonstrated to disrupt or delay the early pathogenic and oncogenic processes induced by murine leukemia viruses including FV (14–17). However, data from this lab demonstrated that spontaneous recovery from FV-induced disease in genetically resistant animals was dependent on cell-mediated

responses (18) as well as humoral responses (9). Therefore, we wished to determine whether antibody therapy was dependent on or independent of T-cell responses. The present experiments were conducted in strains of mice that fail to mount FV-specific antibody responses due to a homozygous genetic defect at the *Rfv3* locus (19, 20). We were able to demonstrate that passive antibody therapy could induce a cure of an established retroviral infection *in vivo*. However, in contrast to what was seen with rabies and influenza viruses, passive antibody therapy was effective only in the presence of CD4$^+$ and CD8$^+$ T-cell populations. These results suggest that antibody therapy may have a role in treatment of HIV infections in humans, particularly if it is instituted prior to virus-induced decline in functional T-cell populations.

MATERIALS AND METHODS

Mice. A/WySn (*H2^a*) and A.BY (*H2^b*) congenic mice obtained from Jackson Laboratories were used in these experiments. Both strains are Rfv3$^{s/s}$ and do not mount primary anti-FV humoral responses (19, 20). A sample of mice from each strain was serotyped for major histocompatibility complex (MHC) antigens to confirm their haplotype. All animal experiments were done in accordance with the guidelines of the Animal Care and Use Committee of Rocky Mountain Laboratories and the National Institutes of Health.

Virus. The B-tropic strain of FV was used in these experiments (21). This stock contains the polycythemia strain of defective spleen focus-forming virus. Mice were inoculated i.v. with either 250 or 1500 spleen focus-forming units (SFFU) as indicated. Erythroleukemia induction and progression were followed by spleen palpation under general anesthesia. We have previously demonstrated splenomegaly to be a reliable indicator of recovery and prognosticator of long-term survival (22).

Antibody Therapy. At 10 days postinfection, test groups were treated by i.p. injection with 0.2 ml of monoclonal antibody (mAb) 48 ascites fluid specific for envelope protein gp70 (23). This pooled ascites fluid contained 0.75 mg of total IgG per ml and had a FV neutralizing titer of 1:5000. mAb 48 has been demonstrated to reduce FV viremia levels *in vivo* while isotype-matched antibodies to other viral proteins were ineffective (23). Injections of mAb 48 were given three times per week from day 10 to day 40 postinfection. The dosage and schedule were empirically determined to neutralize FV viremia *in vivo*.

T-Cell Subset Depletions. Mice were depleted of T-cell subsets with mAbs specific for CD4 and CD8 antigens as described (18, 24). CD4$^+$ T cells were depleted by i.p. injection with 0.2 ml of mAb 191.1 ascites fluid on days −4, −2, 0, +3, and +5 relative to the day of infection. For CD8$^+$ T cells, 0.5 ml of mAb 169.4 supernatant was used. These doses were determined empirically to give reduction of T-cell subsets to

Abbreviations: CTL, cytolytic T lymphocyte; HIV, human immunodeficiency virus; FV, Friend virus; MHC, major histocompatibility complex; SFFU, spleen focus-forming unit(s); mAb, monoclonal antibody.

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<1% of the nucleated cells in peripheral blood. Such reductions were verified for these experiments 10 days after the first injections of depleting antibodies. The isotype-matched negative control ascites was mAb I9 (25).

CTL Assays. CTL analyses on individual mice were performed as described (18). Spleen cells from FV-infected mice were used in a direct assay at a 200:1 effector/target cell ratio against EL4 murine leukemia cells expressing the Friend murine leukemia virus envelope protein, a target previously demonstrated to possess the major epitopes recognized by primary FV-specific CTL (18). Control effector cells were obtained from uninfected mice. Blocking was done by pretreatment of effectors for 30 min with 1.0 μl of the same ascites used for the *in vivo* depletion studies.

RESULTS

Antibody Therapy. Previous experiments indicated that administration of FV envelope-specific mAb 48 reduced viremia levels *in vivo* (23). We wished to determine whether the antiviral effects of this antibody extended to full protection from an established infection. Ten days after infection of adult A.BY (Rfv3$^{s/s}$) mice with a high dose of FV, antibody therapy was initiated. This time point was chosen because it is when both spleen infectious centers and plasma viremia levels peak (9). Treatment was highly effective, decreasing 14-week mortality from 100% to 21% (Fig. 1).

T-Cell Requirements. We next examined whether T-cell responses were also required for recovery, even in the presence of therapeutic antibody. Immunotherapy experiments were repeated in mice that had been depleted of either CD4$^+$ or CD8$^+$ T cells by using mAbs. Depletion of either T-cell subset completely ablated the efficacy of the treatments (Fig. 2). These data indicated that FV-specific neutralizing antibody was essential but not sufficient for resolution of infection and that T-cell effectors were also required. T-cell help was not required for production of antibody since neutralizing antibody was provided experimentally. Thus, the dependence on CD4$^+$ T cells suggested a requirement for immunological help in the CD8$^+$ CTL response or possible involvement of CD4$^+$ CTL responses.

CTL assays were performed to establish which T-cell subsets were involved and revealed significant CTL activity that was blocked by the addition of anti-CD8 antibody but not anti-CD4 antibody (Fig. 3). Thus, the CTL were predominantly of the
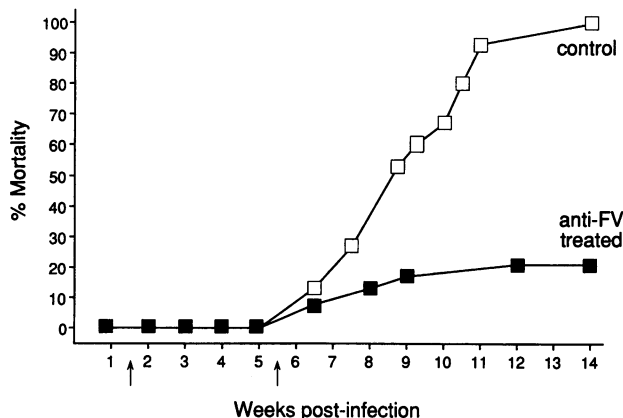
FIG. 1. Effect of anti-FV antibody treatment in A.BY mice. Adult age-matched female mice were inoculated i.v. with 1500 SFFU of FV. Intraperitoneal injections of FV-neutralizing antibody were administered three times per week from day 10 to day 40 (arrows). Erythroleukemia induction and progression were followed by spleen palpation under general anesthesia. For untreated A.BY (□), $n = 15$; for treated A.BY (■), $n = 24$. The difference between the two groups is statistically significant by Fisher's exact test ($P < 0.0001$).
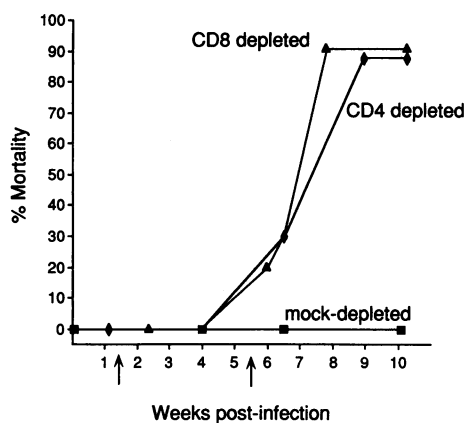
FIG. 2. Mice in the T-cell depletion studies were treated with mAb48 therapeutic antibody as in Fig. 1. The therapeutic mAb treatment period is indicated by arrows. The FV dose was 250 SFFU to show that T cells were necessary even at a low dose. For the CD8-depleted group (▲), $n = 13$; for the CD4-depleted group (◆), $n = 10$. Mock-depleted I9 control group (■) had 100% survival over 10 weeks ($n = 11$).

CD8$^+$ phenotype and CD4$^+$ T cells provided immunological help for generation of the CTL response *in vivo* (26, 27) and/or provided other antiviral mechanisms such as interferon production.

MHC Effects. Initially, it may appear that an antibody therapy that is effective in one mouse strain should also be effective in another. However, in an infection such as FV, where T lymphocytes are also required for protection, the situation may be more complex. For example, mice with certain MHC haplotypes might possess a genetic barrier to protection by passive immunization because they present FV peptides (28–30) poorly to CD4$^+$ or CD8$^+$ T cells. We investigated this possibility in A/Wy mice ($H2^a$), which are genetically identical to A.BY mice ($H2^b$) except for MHC genes. The $H2^a$ haplotype has previously been associated with high susceptibility to FV-induced immunosuppression and poor FV-specific T-cell responses (9, 31). A/Wy mice could not be successfully treated with antibody under identical conditions in which the majority of A.BY mice recovered (Fig. 4A). This potent MHC effect is consistent with the importance of virus-specific T-cell responses in recovery from disease.

Since virus dose is also an important factor in the ability of mice to recover from FV infection (9), we next examined whether antibody therapy might be successful in A/Wy mice
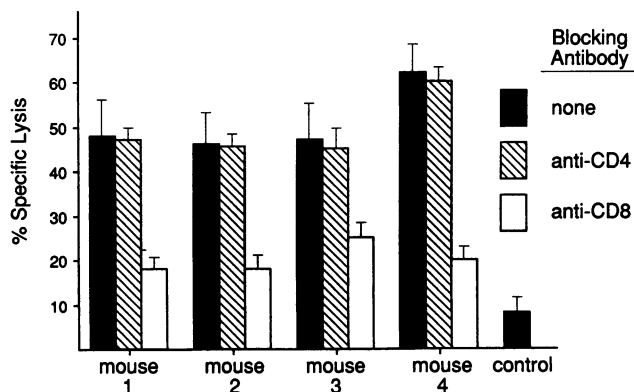
FIG. 3. CD8$^+$ CTLs in A.BY mice. Effector cells were derived from spleens 2 weeks postinfection with FV and were used without *in vitro* restimulation. Control effectors were obtained from an uninfected mouse. Blocking was done by pretreatment of effectors for 30 min with 1.0 μl of the same ascites used for *in vivo* depletion studies. Standard errors were calculated from triplicate sample raw data.
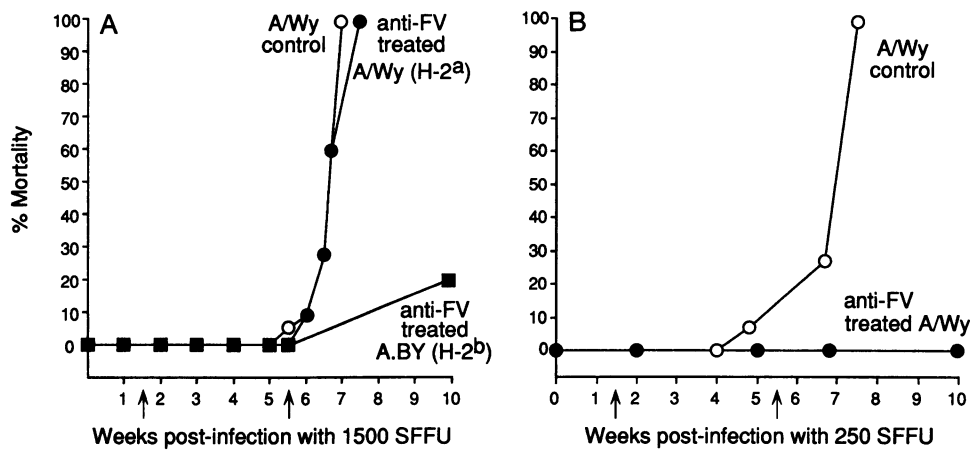
FIG. 4.   (*A*) Anti-FV antibody treatment in A/Wy (*H2^a*) mice after infection with high-dose FV (1500 SFFU). Experiments were performed as described in Fig. 1. Intraperitoneal injections of FV-neutralizing antibody were administered three times per week from day 10 to day 40 (arrows). For the A/Wy untreated group, $n = 11$; for the A/Wy group treated with mAb 48, $n = 22$; for the A.BY group treated with mAb 48, $n = 10$. Difference in mean survival times between A/Wy groups was not considered significant by Mann–Whitney test ($P = 0.0523$). (*B*) Anti-FV antibody treatment in A/Wy (*H2^a*) mice after infection with low-dose FV (250 SFFU). For the A/Wy untreated group, $n = 11$; for the A/Wy group treated with mAb 48, $n = 10$ ($P < 0.0001$ by Fisher's exact test). Recovered A/Wy mice were immune to subsequent challenge with a high dose (1500 SFFU) of FV (data not shown).

infected with a lower virus dose. Lowering the virus dose 6-fold decreased 10-week mortality from 100% to 0 (Fig. 4*B*). Thus, the MHC genetic barrier to antibody therapy was overcome under conditions of decreased viral load.

The MHC barrier to protection from high-dose FV in A/Wy mice appeared to be due to weak CTL responses. CTL assays with effector cells from A/Wy mice revealed a 2- to 3-fold lower response than that seen in A.BY mice (data not shown). Furthermore, *in vivo* depletion of CD8$^+$ T cells from FV-infected A/Wy mice produced only a minor decrease in mean survival time compared to a dramatic effect in A.BY mice (Table 1). The mean survival times of both CD8-depleted groups were virtually identical, indicating that the major reason for longer postinfection survival in untreated A.BY mice versus A/Wy mice was due to CTL activity. Importantly, immunotherapy appeared much less dependent on the relative strength of the CTL response at low than at high FV dose (Fig. 4).

DISCUSSION

The results from the present studies contrast with those obtained from experiments with influenza virus where transfer of either immune CTLs (32, 33) or neutralizing antibody alone (5) can prevent death, even in immune-deficient mice. For influenza, there appears to be enough overlap or redundancy in the functions of different immune responses to compensate for deficiencies in any one compartment. On the other hand, recovery from high-dose FV infections requires both CD4$^+$ and CD8$^+$ T cells as well as antibody, indicating that nonoverlapping immune functions are contributed by these immune components. The data from depletion experiments, which indicated necessary roles for CD4$^+$ and CD8$^+$ T cells, were

Table 1.   Comparison of FV survival in normal and CD8-depleted mice of different MHC types

| Mouse | MHC | Mean survival time, days post FV infection | | |
		Normal (*n*)	CD8 depleted (*n*)	Difference (*P*)
A/Wy	*H2^a*	47.2 (10)	40.8 (11)	6.4 (0.002)
A.BY	*H2^b*	66.5 (16)	40.0 (8)	26.5 (<0.001)

FV dose was 1500 SFFU. *n*, Number of mice per experiment. *P* values were determined by Mann–Whitney test. Mean survival times between the two CD8-depleted groups were not significantly different ($P = 0.825$).

supported by the finding of a MHC influence on immunotherapy success. This influence is consistent with previous studies which showed that both MHC class I (9, 34, 35) and class II genes (36, 37) can confer susceptibility or resistance to FV infections. Furthermore, the presence of high-recovery alleles at both MHC class I and class II is necessary for resistance (9), consistent with a requirement for antigen presentation to both CD8$^+$ and CD4$^+$ T cells.

Although antibody therapy was unsuccessful in A/Wy mice infected with high-dose FV, the success of therapy in curing low-dose infections is still extremely significant, since this mouse strain has a MHC type associated with low recovery and is highly susceptible to FV-induced erythroleukemia and immunosuppression. Under conditions of low-dose infection, the initiation of therapy 10 days postinfection preceded peak viremia titers and peak infectious center levels in the spleen. Such slowing of viral spread probably allowed critical CD4$^+$ and CD8$^+$ T-cell responses to develop in time to control the FV infection with the help of therapeutic antibody. This interpretation is consistent with the present data, demonstrating that CD4$^+$ and CD8$^+$ T cells are necessary even at low-dose FV infections, and with previous data, showing that MHC effects on FV recovery can act at the level of controlling the kinetics of T-cell responsiveness (9, 38).

In terms of high susceptibility to HIV-induced immunosuppression and high mortality rate, humans may be considered analogous to highly susceptible mice such as A/Wy. CD8$^+$ CTLs undoubtedly play an important role in anti-HIV immunity and are known to dissipate as CD4$^+$ T-cell levels become critically low (39–44). This may partially explain why antibody therapy was shown to have some beneficial effects but did not cure disease in AIDS patients (45–53). Although the reasons are undoubtedly complex, the study by Levy *et al.* (52) demonstrated greater benefit in patients with CD4$^+$ lymphocyte counts between 100 and 200 cells per ml than in patients with lower counts. Our data suggest that the benefit might be further increased in patients with higher CD4$^+$ lymphocyte counts.

Early intervention for HIV infection may be even more critical than for FV infection because of complications arising from virus variability and infection of lymphocytes. However, the early HIV isolates that are involved in sexual transmission appear to have limited antigen diversity in important antibody neutralizing domains (54–59) and thus may be much more sensitive to antibody therapy than later isolates, which are more likely to contain immune escape variants (60). The key

will be to find the right antibody or combination of antibodies that will most effectively cross-neutralize the greatest number of variants (58, 59, 61).

In other retrovirus models, antibody therapy has been experimentally used to prevent feline immunodeficiency virus (62), simian immunodeficiency virus, and HIV infections (63, 64) when administered prior to virus infection and in one case when administered 10 min postinfection (65). These experiments demonstrate *in vivo* neutralization of virus by passive antibody but do not address the critical issue of treating well-established infections in which significant spread has occurred and symptoms such as splenomegaly are present. The current success in treating established retroviral infections in mice suggests that similar immunotherapy could be beneficial for treating human retroviral diseases such as HIV even after significant virus spread has occurred. However, as illustrated with the FV model, success would likely be dependent on cell-mediated responses as well. Therefore, HIV immunotherapy might be most successful when used in combination with antiviral drugs such as reverse transcriptase inhibitors and protease inhibitors.

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