JOURNAL OF VIROLOGY, Oct. 1993, p. 6119–6124
0022-538X/93/106119-06$02.00/0

Vol. 67, No. 10

Lymphocytes Protect against and Are Not Required for Reovirus-Induced Myocarditis

BARBARA SHERRY,[1]* XI-YANG LI,[2] KENNETH L. TYLER,[3] JOHN M. CULLEN,[1] AND HERBERT W. VIRGIN IV[2]*

Department of Microbiology, Pathology, and Parasitology, North Carolina State University College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, North Carolina 27606[1]; Department of Neurology, Denver Veterans Affairs Medical Center and Departments of Neurology, Medicine, and Microbiology-Immunology, University of Colorado Health Sciences Center, Denver, Colorado 80262[3]; and Division of Infectious Diseases, Departments of Medicine, Pathology, and Molecular Microbiology, Washington University School of Medicine, Box 8051, 660 South Euclid Avenue, St. Louis, Missouri 63110[2]

Received 30 April 1993/Accepted 2 July 1993

Many studies suggest that host lymphocytes are damaging, rather than protective, in virally induced myocarditis. We have investigated the role of lymphocyte-based immunity in murine myocarditis by using a myocarditic reovirus (reovirus serotype 3 8B), nonmyocarditic reoviruses, adoptive transfer experiments, and mice with severe combined immunodeficiency (SCID mice). Prior to infection, passive transfer of monoclonal antibodies specific for 8B capsid proteins protected neonatal mice against 8B-induced myocarditis, indicating that humoral immunity can protect against myocarditis. Some monoclonal antibodies acted by blocking viral spread to and/or replication in the heart. Passive transfer of reovirus-immune, but not naive, spleen cells prior to infection protected neonatal mice from 8B-induced myocarditis. Depletion of either CD4 or CD8 T cells resulted in increased viral titer in the heart but did not abrogate immune cell-mediated protection against myocardial injury. This shows that both CD4 and CD8 T cells can act independently to protect myocardial tissue from reovirus infection. In addition, reovirus 8B caused extensive myocarditis in SCID mice. This confirms a prior report (B. Sherry, F. J. Schoen, E. Wenske, and B. N. Fields, J. Virol. 63:4840–4849, 1989) that T cells are not required for reovirus-induced myocarditis and demonstrates for the first time that B cells are not required for reovirus-induced myocarditis. We used SCID mice and a panel of reoviruses to assess (i) the relationship between growth in the heart and myocardial damage and (ii) the possibility that nonmyocarditic reoviruses exhibit a myocarditic phenotype in the absence of functional lymphocytes. Growth in the heart was not the sole determinant of myocarditic potential in SCID mice. Although 8B induced myocarditis in SCID mice, no or minimal myocarditis was found in SCID mice infected with four reovirus strains previously shown (B. Sherry and B. N. Fields, J. Virol. 63:4850–4856, 1989) to be nonmyocarditic or poorly myocarditic in normal neonatal mice. We conclude that (i) humoral immunity and cellular immunity are protective against, and not required for, reovirus-induced myocarditis and (ii) the potential to induce cardiac damage is a property of the virus independent of lymphocyte-based immunity.

Myocarditis is an important human disease, with a significant number of cases thought to occur as sequelae of viral infection (17, 18, 30). Injury to the heart may be due to (i) direct viral cytopathicity for myocardial cells (3, 9, 20, 21), (ii) inflammatory responses occurring in response to viral infection (14), or (iii) lymphocyte recognition of viral or self antigens on myocardial cells (10, 11, 15, 18, 31). Studies done with the coxsackievirus and murine cytomegalovirus systems have supported a role for lymphocytes as the predominant cause of virus-induced cardiac damage (4, 10, 11, 15, 18, 31). However, recent reports that coxsackievirus causes myocarditis in mice with severe combined immunodeficiency (SCID mice) (3) and that coxsackievirus and encephalomyocarditis virus RNA persist for prolonged periods in the heart (12, 13) raise questions about the need to invoke autoimmunity as well as the causative role of lymphocyte-based immunity in viral myocarditis.

To shed further light on these questions, we chose to investigate mechanisms of myocarditis by using a different viral model system. We selected murine infection with a myocarditic reovirus (reovirus serotype 3 8B) since reovirus infection has been extensively studied at both pathogenetic and genetic levels (19, 22). 8B is a reassortant virus derived from an in vivo cross between the prototypic serotype 1 Lang (T1L) and serotype 3 Dearing (T3D) reoviruses and contains at least one mutation (20, 21). Its ability to damage the heart is determined by the M1 gene, which encodes a viral core protein of unknown function (20, 27, 32). Two factors suggested to us that studies of 8B-induced myocarditis might provide new information. First, 8B-induced myocarditis is characterized by extensive myocardial necrosis and subsequent calcification with scant polymorphonuclear and monocytic infiltrate (20, 21, 26). This contrasts with the extensive cellular infiltrate seen in coxsackievirus myocarditis. Second, since 8B causes myocarditis in neonatal nude mice (21), T cells are not required for myocardial injury. This suggested that reovirus might provide a model for analysis of the protective effects of antibody and T cells in viral myocarditis.

In this paper we show that reovirus-specific antibody and both CD4 and CD8 T cells protected against, rather than contributed to, 8B-induced myocarditis. 8B was myocarditic in SCID mice. Moreover, reoviruses which are nonmyocarditic or poorly myocarditic in neonatal immunocompetent mice were similarly benign in adult SCID mice, indicating

* Corresponding authors.

TABLE 1. Characteristics of anti-reovirus MAbs used in this study[a]

MAb	Protein	Virus specificity		
		T1L	T3D	8B
G5	σ1	−	+	+
5C6	σ1	+	−	−
8H6	μ1	+	+	+
10F6	μ1	+	+	+
4F2	σ3	+	+	+
10C1	σ3	+	+	+

[a] All Mabs are IgG2a kappa, except 10F6, which is IgG2b kappa (24).

that they do not have a latent myocarditic potential masked by the normal host immune response. These findings show that, in the reovirus system, the genetic constitution of the virus rather than host lymphocyte function is the primary determinant of reovirus-induced myocarditis. Antibody and T cells play a predominantly protective role.

MATERIALS AND METHODS

Tissue culture media. L929 fibroblasts were maintained in spinner culture with minimal essential medium supplemented with 5% fetal calf serum (FCS; Hyclone, Ogden, Utah, or Biofluids, Rockville, Md.), 2 mM L-glutamine, 1 U of penicillin per ml, and 1 μg of streptomycin (Biofluids or Irvine Scientific, Santa Ana, Calif.) per ml. Spleen cells were isolated in Hanks balanced salt solution (Biofluids) containing 5% FCS, 1 U of penicillin per ml, 1 μg of streptomycin per ml, and 10 mM *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES; Biofluids), and RPMI 1640 (Biofluids) supplemented with 10% FCS, 2 mM L-glutamine, 1 U of penicillin per ml, and 1 μg of streptomycin per ml.

Viruses. Reoviruses 8B, T3D, and T1L were obtained from laboratory stocks. E3 and EB121 are T1L × T3D reassortant viruses obtained from Max Nibert (2). The viruses were plaque purified twice, passaged twice, and then stored at 4°C in supplemented minimal essential medium or frozen at −70°C before being used for inoculation into mice. Methods for virus growth, cesium chloride purification, quantitation, storage, and plaque assay were as published previously (20, 24). Virus titers were determined by plating serial 10-fold dilutions of sample on L929 fibroblast monolayers, overlaying them with agar, and staining them with neutral red.

Antibodies. Anti-reovirus monoclonal antibodies (MAbs) were prepared and stored as described previously (24). Briefly, MAbs were purified on protein A columns, concentrated, sterile filtered, and stored at −70°C in phosphate-buffered saline (PBS) at 1.0 mg/ml. The isotype, protein specificity, and viral specificity of anti-reovirus MAbs used here are shown in Table 1 (24). Cell lines producing the rat immunoglobulin G2b (IgG2b) anti-CD4 MAb GK1.5 (ATCC TIB 207 [5]) and the rat IgG2a anti-CD8 MAb 53-6.72 (ATCC TIB 105 [16]) were obtained from the American Type Culture Collection, Rockville, Md. Anti-T-cell MAbs were protein G purified and stored as described previously (25). Control rat IgG was purchased from Jackson Immunoresearch, West Grove, Pa., diluted to 1.0 mg/ml in PBS, and stored at −20°C. All antibodies were injected in 100-μl volumes (tuberculin syringe, 30-gauge needle) intraperitoneally (i.p.).

Mice. Mice were maintained at biosafety level 2 at Washington University School of Medicine in accordance with all Federal and University policies. Sentinel mice screened negative (every 2 to 4 months) for adventitious mouse pathogens. BALB/c AnNCr female and A/JCr male mice (National Cancer Institute, Frederick, Md.) were bred to produce CAF_1 neonatal mice. SCID mice on the CB17 background were maintained by serial brother-sister mating. SCID lines were tested for leaky phenotype by measuring IgG levels in serum by using the enzyme-linked immunoassay (results not shown), and some mice had detectable IgG levels. Experimental SCID mice were not individually assessed for leaky phenotype since infection with reovirus elicited no anti-reovirus antibody even in leaky SCID mice under conditions eliciting significant anti-reovirus antibody in congenic CB17 mice (9a).

Spleen cell preparation, adoptive transfer, and T-cell depletion. Adult CAF_1 mice were immunized i.p. with 100 μg of cesium chloride-purified T3D or T3D top component (empty capsids) in 200 μl of complete Freund's adjuvant (Colorado Serum Co., Denver, Colo.). T3D was used to generate immune spleen cells because of the ease of preparing large amounts of T3D antigen and the extensive antigenic relatedness of 8B and T3D. Spleens from normal mice or mice immunized no more than 6 weeks previously with adjuvant with or without T3D as antigen were removed and minced in supplemented HBSS. Erythrocytes were lysed with Tris-buffered ammonium chloride, and cells were resuspended in supplemented RPMI 1640. Cells were >95% viable by trypan blue exclusion. Immediately prior to adoptive transfer, cells were resuspended at 2×10^8 cells per ml in RPMI 1640 without FCS, in PBS, or in PBS containing 1.0 mg of anti-T-cell MAb or rat IgG per ml. After 30 min on ice, 10^7 cells in 50 μl were transferred i.p. to 1-day-old CAF_1 mice by using a 0.5-ml glass syringe with a 30-gauge needle.

T cells were depleted from adoptively transferred mice as follows. On the day of birth, neonatal CAF_1 mice were given 100 μl of 1.0-mg/ml anti-T-cell MAb or control rat IgG i.p. One day later, mice received spleen cells which had been incubated in the appropriate anti-T-cell subset-specific MAb or control rat IgG (1 mg/ml) for 30 min at 4°C. The efficacy of this procedure was assessed by FACScan analysis (Becton Dickinson, Braintree, Mass.) of spleen cells 3 and 7 days after adoptive transfer (data not shown). Anti-CD4 MAb and anti-CD8 MAb treatment resulted in greater than 98% depletion of the appropriate T-cell subset with no diminution in other cell populations. Rat IgG treatment did not alter T-cell subsets in the spleen at either time point.

Mouse inoculation and tissue harvest. All experiments were performed at least twice, and data from replicate experiments were pooled. Two-day-old CAF_1 mice were inoculated intramuscularly (i.m.) in the left hindlimb (via a 30-gauge needle) with 10 μl containing 1.6×10^3 PFU of 8B. Preliminary experiments showed that this dose was lethal by day 9 and caused extensive myocarditis 7 days after inoculation of 1- to 2-day-old CAF_1 mice. Seven days after infection, the apical one-third of the heart was removed to 1 to 2 ml of 10% buffered formalin prior to embedding and staining with hematoxylin and eosin. The rest of the heart, the entire brain, and the left hindlimb muscle were removed to 1.0 ml of gel saline each and kept at −70°C prior to freeze-thawing, sonication, and titer determination by plaque assay (reported as PFU per milliliter). Adult SCID mice were inoculated i.p. with 4×10^7 PFU of reovirus (in 0.2 to 1.0 ml of supplemented minimal essential medium via a 22-gauge needle). The mice were sacrificed 15 or 20 days postinoculation, and the heart was examined, divided, and

processed as above. No differences in viral titer or myocardial damage in SCID mice were seen between the two time points selected.

Evaluation of cardiac injury. Neonatal CAF_1 mice were sacrificed 7 days after infection, and the heart was examined by eye for gross myocarditis as evidenced by patches of white myocardium (this is equivalent to the gross myocarditis reported in the figures). Blinded sections from individual CAF_1 or SCID mice were scored for evidence of myocardial injury (equivalent to myocardial injury reported in the figures). Myocardial injury ranged from a single small focus of necrosis to multiple foci or large coalescing foci. A hematoxylin- and eosin-stained paraffin section was scored positive if there was evidence of any myocardial injury (cardiac injury was not further quantitated). Myocardial injury was characterized by necrosis of muscle fibers, pyknotic nuclear debris, mineralization of cell debris, and proliferation of sarcolemmal nuclei. Rare mononuclear inflammatory cells, primarily macrophages, and rare neutrophils were present in the necrotic foci.

Statistical analyses. Data were subjected to chi-square analysis.

RESULTS

Antibody-mediated protection against 8B-induced myocarditis. One-day-old CAF_1 mice were inoculated i.p. with control MAb or MAbs specific for the 8B σ1, σ3, or μ1 outer capsid proteins and then given a lethal dose of 8B i.m. 24 h later. MAbs were selected on the basis of their capacity to protect against T1L- and T3D-induced central nervous system disease (23). The mice were sacrificed 7 days later, and the effect of MAb treatment on myocarditis was assessed by determining the number of mice with gross myocarditis (Fig. 1), evaluating cardiac sections histologically (Fig. 1), and measuring the viral titer in the heart, muscle, and brain (Fig. 2). Control MAb 5C6, specific for the T1L but not the 8B σ1 protein, had no effect compared with PBS by any of these evaluations. In contrast, all other MAbs dramatically inhibited myocarditis as evidenced by the almost complete lack of gross myocarditis (Fig. 1), the sharply reduced incidence of myocardial injury (Fig. 1), and the significantly decreased viral titer in the heart (from 300- to 100,000-fold [Fig. 2]). Notably, some protective MAbs (10F6 and 4F2 [Fig. 2]) minimally affected viral titer in muscle, the site of primary replication, indicating that they protect by inhibiting viral spread, growth in the heart, or both. MAb-induced decreases in heart viral titer were comparable to those seen in the brain (Fig. 2), demonstrating that the heart is as sensitive to antibody-mediated protection as are other tissues and organs. MAb protective capacity was not determined by capsid protein specificity (although one MAb, 4F2, was less effective at inhibiting myocardial injury and decreasing viral replication in the heart).

Immune cell-mediated protection against 8B-induced myocarditis. One-day-old CAF1 mice were inoculated i.p. with reovirus-immune or control spleen cells and 1 day later were given a lethal dose of 8B i.m. Seven days later the mice were sacrificed and the extent of myocarditis was assessed as described above (Fig. 3 and 4). Control nonimmune spleen cells did not affect 8B-induced myocarditis by any of these evaluations. In contrast, reovirus T3D-immune spleen cells protected against gross myocarditis (Fig. 3) and microscopic myocardial damage (Fig. 3) and significantly decreased the viral titer in the heart (Fig. 4). Reovirus 8B-immune spleen cells also protected against gross myocarditis (data not shown). T3D immune cells decreased the viral titer in muscle (Fig. 4) to a greater extent than MAb did (Fig. 2), confirming that immune cells are more effective than MAbs in controlling viral replication at primary sites of infection (25). T3D-immune cells decreased the viral titer comparably in both brain and heart tissue (Fig. 4).

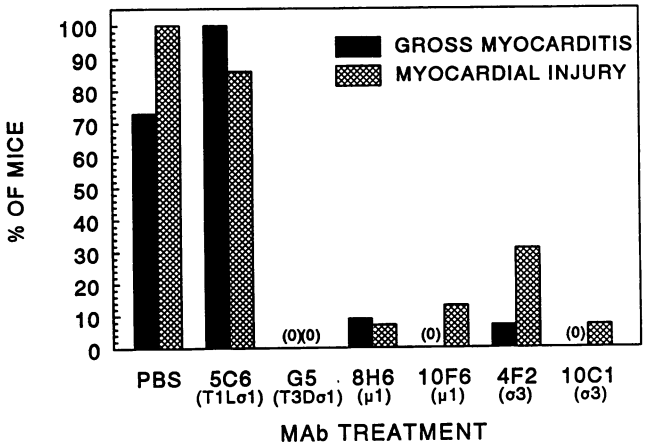

FIG. 1. Effect of MAbs on 8B-induced myocardial injury. One-day-old mice received PBS or MAb (Table 1) as indicated, and 1 day later they were challenged with 1.6×10^3 PFU of 8B i.m. Seven days later the mice were sacrificed and scored by eye for macroscopic external cardiac lesions (gross myocarditis) or microscopically for myocardial injury. Data were pooled from two experiments for each treatment (13 to 18 mice per treatment). For gross myocarditis, data from all MAbs recognizing 8B capsid proteins were significantly different from control MAb (5C6) data ($P < 0.0005$). 8H6, 10F6, 10C1, and 4F2 data were not significantly different from G5 data ($P > 0.1$). For myocardial injury, data from all MAbs recognizing 8B capsid proteins were significantly different from control MAb (5C6) data (8H6, 10F6, and 10C1, $P < 0.001$; 4F2, $P < 0.025$). 8H6, 10F6, and 10C1 data were not significantly different from G5 data ($P > 0.1$); 4F2 data were significantly different from G5 data ($P = 0.01$).

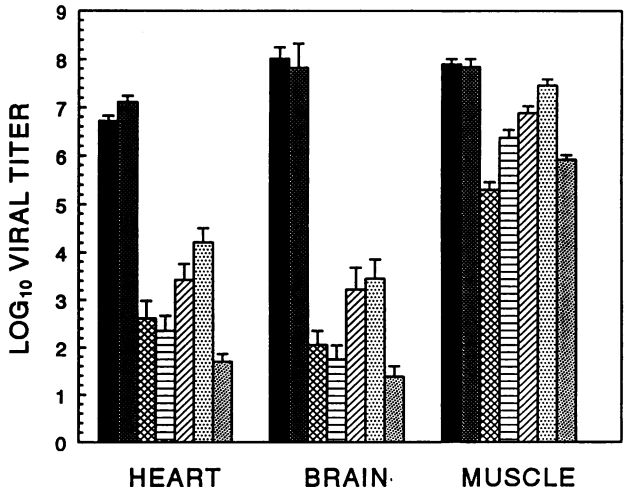

FIG. 2. Effect of MAbs on 8B replication. Viral titers in samples of heart, brain, and hindlimb muscle from individual mice described in the legend to Fig. 1 were determined. Data are presented as the mean log titer. Error bars denote the standard error of the mean. Symbols: ■, PBS; ▩, 5C6 (T1σ1); ▩, G5 (T3σ1); ▤, 8H6 (μ1); ▨, 10F6 (μ1); ▨, 4F2 (σ3); ▨, 10C1 (σ30).

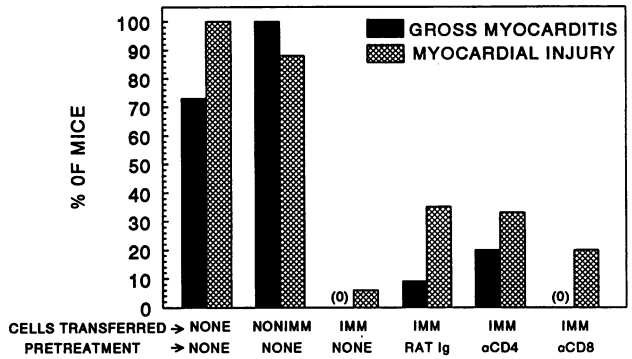

FIG. 3. Effect of cell transfer on 8B-induced myocardial injury. One-day-old mice received PBS, nonimmune spleen cells, immune spleen cells, or immune spleen cells depleted of either CD4 or CD8 T cells. Mice receiving depleted cells received control antibody (rat Ig) or anti-T-cell MAbs on the day of birth. All mice were challenged with 1.6×10^3 PFU of 8B i.m. when they were 2 days old. Seven days later the mice were sacrificed and scored by eye for macroscopic external cardiac lesions (gross myocarditis) or microscopically for myocardial injury. Data were pooled from two experiments for each treatment (15 to 18 mice per treatment). Data from all immune cell transfers were significantly different from data from control (PBS or nonimmune cell transfer) treatments ($P < 0.005$). Neither anti-CD4 nor anti-CD8 treatment had a significant effect compared with rat Ig treatment of immune cells ($P > 0.1$).

To assess the contribution of different T-cell subsets to protection against 8B-induced myocarditis, we depleted immune spleen cells of either CD4 or CD8 T cells and assessed their protective capacity (Fig. 3 and 4). Rat Ig was used as a control treatment of immune spleen cells since it did not protect against gross or microscopic myocarditis and did not alter the viral titer in the heart (data not shown). Immune cells depleted of either CD4 or CD8 T cells protected against gross and microscopic myocarditis (Fig. 3). It is likely that residual protection after single T-cell subset depletion is due

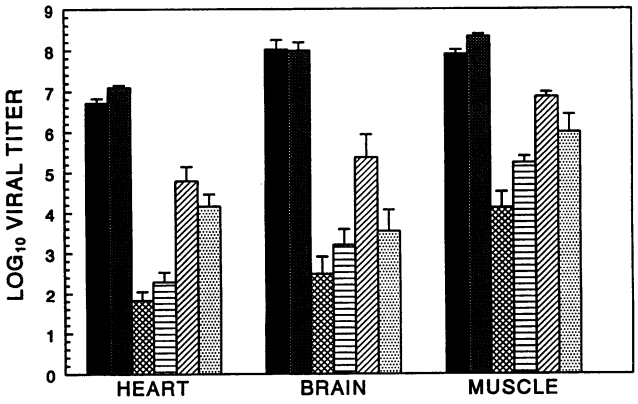

FIG. 4. Effect of cell transfer on 8B replication. Viral titers in samples of heart, brain, and hindlimb muscle from individual mice described in the legend to Fig. 3 were determined. Data are presented as the mean log titer. Error bars denote the standard error of the mean. Symbols: ■, no cells transferred, no pretreatment; ▩, nonimmune spleen cells transferred, no pretreatment; ▩, immune spleen cells transferred, no pretreatment; ▤, immune spleen cells transferred, pretreatment with rat Ig; ▨, immune spleen cells transferred, pretreatment with anti-CD4 MAb; ▩, immune spleen cells transferred, pretreatment with anti-CD8 MAb.

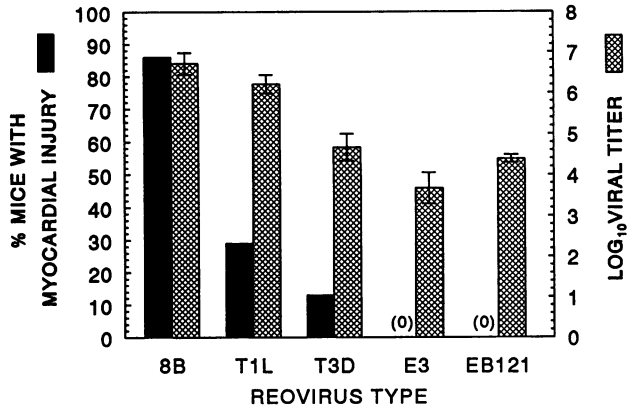

FIG. 5. Reovirus-induced myocardial injury in adult SCID mice. Adult SCID mice were infected with the indicated reovirus strain. Data from two experiments were pooled (seven or eight mice per group). Hearts were divided into two portions: one portion for histopathologic evaluation and one portion for virus titer determination. Blinded slides were scored for evidence of myocardial injury. For myocardial injury, T1L, T3D, E3, and EB121 data are not significantly different from each other ($P > 0.1$). T1L, T3D, E3, and EB121 data are all significantly different from 8B data (T3D, E3, and EB121 $P < 0.005$; T1L $P < 0.05$).

to T cells of the undepleted subset, with or without a contribution from immune B cells. Depletion of either T-cell subset increased the 8B titer in the heart significantly (Fig. 4), indicating that both CD4 and CD8 T cells independently contribute to control of viral replication in the heart. CD4 depletion increased viral titers in the brain, whereas CD8 depletion had little effect, confirming an important protective role for CD4 cells in the brain (25).

Reovirus myocarditis in SCID mice. The observation that lymphocytes protected against 8B-induced myocarditis suggested that reoviruses which are poorly myocarditic in immunocompetent mice might have a latent potential to induce myocarditis which could be unmasked in immunodeficient mice. We therefore evaluated the myocarditic potential of a series of reoviruses in adult SCID mice. Adult SCID mice developed myocarditis after i.p. injection with 8B (Fig. 5), confirming that T cells are not required for 8B-induced myocarditis (21). Moreover, since SCID mice lack functional B cells, this provides the first demonstration that humoral immunity is not required for 8B-induced myocarditis. SCID mice did not develop the macroscopic external cardiac lesions (gross myocarditis) which typified myocarditis in neonatal mice. However, histopathologic evaluation of cardiac sections from 8B-infected SCID mice revealed extensive myocardial damage with pathologic features similar to those seen in 8B-infected neonatal mice (Fig. 5; data not shown).

We then assessed the myocarditic potential in SCID mice of four viruses which were nonmyocarditic or poorly myocarditic in neonatal immunocompetent mice. T1L (poorly myocarditic) and T3D (nonmyocarditic) are the parental strains from which the reassortant 8B was derived (20, 21). E3 and EB121 are nonmyocarditic T1L-T3D reassortant viruses used for genetic analysis of the myocarditic capacity of 8B (20). Only T1L and T3D generated any detectable microscopic cardiac lesions in SCID mice (Fig. 5), and the damage was dramatically less severe than that caused by 8B (data not shown). Importantly, cardiac injury due to reoviruses E3 and EB121 did not appear in SCID mice. 8B and

T1L (Fig. 5) generated high viral titers in the heart (as they did in immunocompetent neonatal mice [21]), whereas T3D, E3, and EB121 viruses generated significantly lower titers (as T3D did in immunocompetent neonatal mice [21]). In SCID mice, as in immunocompetent mice (21), viral growth in the heart did not correlate with cardiac damage (compare titer versus myocardial injury for T1L and 8B [Fig. 5]). Thus viral genotype, rather than host lymphocytes, is the primary determinant of reovirus growth in the heart and reovirus-induced myocarditis.

DISCUSSION

We addressed the role of immunity in reovirus-induced myocarditis because of extensive data suggesting that lymphocytes are directly involved in damaging the heart during infection with other viruses. We used the reovirus model since a myocarditic reovirus has been well characterized and the molecular basis of reovirus myocarditic potential is under active investigation (20, 21). In addition, the fact that 8B causes myocarditis in nude mice suggested that reovirus myocarditis might differ significantly from other model systems and thus might provide new insights into relationships between lymphocyte function and cardiac injury (21). This paper shows for the first time that (i) CD4 T cells, CD8 T cells, and anti-reoviral antibody protect against both reoviral replication in the heart and myocarditis; (ii) B cells are not required for reovirus-induced myocarditis; and (iii) the ability to replicate in the heart and cause myocarditis is intrinsic to the reovirus strain independent of the immune system. The experiments confirm a previous report that T cells are not required for reovirus-induced myocarditis (21).

Role of lymphocyte-based immunity in virus-induced myocarditis. We investigated the role of lymphocyte-based immunity in reovirus-induced myocarditis in two ways. First, we showed that T3D-immune cells and 8B-specific MAbs protected against myocarditis (Fig. 1 to 4). Second, we showed that 8B caused extensive myocardial damage in SCID mice which lack both T and B cells (Fig. 5). Immune control of the viral titer in the heart paralleled that in the brain, showing that there was no organ selectivity for immune protective effects (Fig. 2 and 4). We ruled out the possibility that 8B has unique epitopes responsible for myocarditis induction by demonstrating that 8B-immune spleen cells protected against 8B-induced myocarditis. In our system, immune cells were transferred prior to infection, and they probably require reactivation in vivo for function. Therefore, these experiments do not address the possibility that preactivated T cells damage myocardial tissue expressing viral antigen prior to adoptive transfer. We conclude that lymphocyte-based immunity (both humoral and cellular) protects against and is not required for reovirus-induced myocardial damage in the mouse strains used in these studies.

These results contrast with some findings in other systems. Both humoral and cellular antiviral immunity and autoimmunity are thought to exacerbate coxsackievirus-induced (10, 11, 17, 18, 31) and murine cytomegalovirus-induced (4, 15) myocardial damage. However, other evidence argues that the reovirus and coxsackievirus system share significant similarities. Similar to findings presented here, coxsackievirus-induced myocarditis occurs in SCID mice (3), and immunization with avirulent coxsackievirus or transfer of immune antibody can protect against coxsackievirus-induced myocarditis (6, 28). In addition, coxsackievirus and encephalomyocarditis virus RNA persists in the mouse heart for prolonged periods (12, 13). The distribution of chronic myocardial lesions matches the distribution of coxsackievirus RNA (12). This suggests that the lymphocytic infiltrate characteristic of coxsackievirus-induced myocarditis may be targeting viral rather than self antigens.

We favor the hypothesis that, as in other organs, effective lymphocyte-based antiviral immunity protects against viral myocarditis. In the reovirus system, chronic myocarditis is not seen because the immune system is effective at clearing the virus. The chronic myocarditis seen in the coxsackievirus system might then be due to failure of the immune system to clear the virus, or viral nucleic acid, completely. According to this hypothesis, lymphocytes could exacerbate damage to the myocardium while clearing the virus but be critical for limiting viral replication and damage (3, 12).

The finding that both CD4 and CD8 T cells play a role in limiting reovirus replication in the heart is similar to our previous finding that both cell subsets are involved in protection against lethal disease mediated by a neurotropic reovirus (25). Transferred immune cells depleted of either CD4 or CD8 T cells protected against gross and microscopic myocarditis and controlled viral replication in the heart (Fig. 3 and 4). Therefore both CD4 and CD8 T cells are independently protective against reovirus infection of the heart. CD4 T cells may well act by helping B cells, resulting in antibody-mediated control of the myocardial virus titer. This is a likely mechanism since (i) adoptively transferred MAbs specific for the outer capsid proteins $\sigma 1$, $\sigma 3$, and $\mu 1$ are protective (Fig. 1 and 2) and (ii) depletion of CD4 T cells inhibits anti-reoviral antibody responses by adoptively transferred immune spleen cells (25).

Similar to our previous findings (25), MAb effects on primary replication were less impressive than their effects at secondary sites of infection (Fig. 2). This contrasts with finding with polyclonal immune spleen cells, which effectively decrease the virus titer at the primary site of infection (Fig. 4). This is consistent with a model in which MAbs control spread to and growth within secondarily infected organs while immune cells are more efficient at control of replication at the primary site.

Determinants of reovirus myocarditic capacity. We used several reovirus strains which are poorly myocarditic in normal neonatal mice (20, 21) to assess whether the protective capacity of the immune system plays a role in determining whether a reovirus is myocarditic. That is, do variations in myocarditic potential between different reovirus strains relate to differences in their relative sensitivities to immune control? If this hypothesis were correct, reoviruses which are poorly myocarditic in normal neonatal mice should induce myocarditis in SCID mice. However, our experiments show that the ability of several reovirus strains to cause myocarditis in SCID mice parallels their myocarditic potential in normal neonatal mice (Fig. 5) (20). We conclude that the myocarditic capacity of reoviruses is intrinsic to the virus and is independent of lymphocyte-based immunity.

Mechanism of reovirus-induced myocardial damage. We previously reported that 8B is efficiently myocarditic in neonatal nude mice, which lack functional T cells (21). The results presented here, obtained with SCID mice, confirm that T cells are not required, and provide the first evidence that B cells are not required, for reovirus-induced myocarditis. However, SCID mice have natural killer cell function in response to viral infection (29), leaving open the possibility that these cells participate in 8B-induced myocardial injury. We think that this is unlikely since natural killer cells protect against coxsackievirus-induced myocarditis (7, 8).

Another possibility is that 8B induces interleukin-1, tumor necrosis factor, or other cytokine expression in the heart with resultant myocardial damage. This mechanism has been proposed in the coxsackievirus model system (14). We cannot rule out a role for cytokines in our system since macrophages (which secrete both interleukin-1 and tumor necrosis factor) are present in SCID mice. However, 8B is directly cytopathogenic for cardiac myocytes in vitro (1). This, together with evidence that reovirus 8B directly infects cardiac myocytes (21), suggests that reovirus 8B is myocarditic because of direct interactions with cardiac myocytes. This direct cytopathogenicity could reflect inhibition of cell macromolecular synthesis, induction of cardiac myocyte cytokines, or other mechanisms.

ACKNOWLEDGMENTS

Research support came from Public Health Service Program project grant 2 P50 NS16998 from the National Institute of Neurological and Communicative Disorders and Stroke (to H.W.V.) and grant AI31250 from the NIH (to B.S.). H.W.V. performed this work while supported by a Burroughs Wellcome Young Virologist Award. K.L.T. is supported by a Merit Grant from the Veterans Administration.

B.S. thanks Mary Ann Blum for excellent technical assistance. We thank Stacy Smith for her careful review of the manuscript.

REFERENCES

1. **Baty, C., and B. Sherry.** 1993. Cytopathogenic effect in cardiac myocytes but not in cardiac fibroblasts is correlated with reovirus-induced myocarditis. J. Virol. **67**:6295–6298.
2. **Brown, E. G., M. L. Nibert, and B. N. Fields.** 1983. The L2 gene of reovirus serotype 3 controls the capacity to interfere, accumulate deletions and establish persistent infection, p. 275–287. *In* R. W. Compans and D. H. L. Bishop (ed.), Double-stranded RNA viruses. Elsevier Science Publishing, Inc., New York.
3. **Chow, L. H., K. W. Beisel, and B. M. McManus.** 1992. Enteroviral infection of mice with severe combined immunodeficiency. Evidence for direct viral pathogenesis of myocardial injury. Lab. Invest. **66**:24–31.
4. **Craighead, J. E., W. B. Martin, and S. A. Huber.** 1992. Role of CD4+ (helper) T cells in the pathogenesis of murine cytomegalovirus myocarditis. Lab. Invest. **66**:755–761.
5. **Dialynas, D., Z. Quan, K. Wall, A. Pierres, J. Quintans, M. Loken, and F. Fitch.** 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5. J. Immunol. **131**:2445–2451.
6. **Fohlman, J., N. G. Ilback, G. Friman, and B. Morein.** 1990. Vaccination of Balb/c mice against enteroviral mediated myocarditis. Vaccine **8**:381–384.
7. **Godeny, E. K., and C. J. Gauntt.** 1986. Involvement of natural killer cells in coxsackievirus B3-induced murine myocarditis. J. Immunol. **137**:1695–1702.
8. **Godeny, E. K., and C. J. Gauntt.** 1987. Murine natural killer cells limit coxsackievirus B3 replication. J. Immunol. **139**:913–918.
9. **Grun, J. B., M. Shultz, S. D. Finkelstein, R. L. Crowell, and B. J. Landau.** 1988. Pathogenesis of acute myocardial necrosis in inbred mice infected with coxsackievirus B3. Microb. Pathog. **4**:417–430.

9a. **Haller, B., and H. W. Virgin.** Unpublished data.

10. **Huber, S. A., N. Heintz, and R. Tracy.** 1988. Coxsackievirus B-3-induced myocarditis. Virus and actinomycin D treatment of myocytes induces novel antigens recognized by cytolytic T lymphocytes. J. Immunol. **141**:3214–3219.
11. **Kishimoto, C., and W. H. Abelmann.** 1990. In vivo significance of T cells in the development of Coxsackievirus B3 myocarditis in mice. Immature but antigen-specific T cells aggravate cardiac injury. Circ. Res. **67**:589–598.
12. **Klingel, K., C. Hohenadl, A. Canu, M. Albrecht, M. Seemann, G. Mall, and R. Kandolf.** 1992. Ongoing enterovirus-induced myocarditis is associated with persistent heart muscle infection: quantitative analysis of virus replication, tissue damage, and inflammation. Proc. Natl. Acad. Sci. USA **89**:314–318.
13. **Kyu, B., A. Matsumori, Y. Sato, I. Okada, N. M. Chapman, and S. Tracy.** 1992. Cardiac persistence of cardioviral RNA detected by polymerase chain reaction in a murine model of dilated cardiomyopathy. Circulation **86**:522–530.
14. **Lane, J. R., D. A. Neumann, A. Lafond-Walker, A. Herskowitz, and N. R. Rose.** 1992. Interleukin 1 or tumor necrosis factor can promote coxsackie B3-induced myocarditis in resistant B10.A mice. J. Exp. Med. **175**:1123–1129.
15. **Lawson, C. M., H. L. O'Donoghue, and W. D. Reed.** 1992. Mouse cytomegalovirus infection induces antibodies which cross-react with virus and cardiac myosin: a model for the study of molecular mimicry in the pathogenesis of viral myocarditis. Immunology **75**:513–519.
16. **Ledbetter, J., and L. Herzenberg.** 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. **47**:63–90.
17. **Leslie, K., R. Blay, C. Haisch, P. A. Lodge, A. Weller, and S. Huber.** 1989. Clinical and experimental aspects of viral myocarditis. Clin. Microbiol. Rev. **2**:191–203.
18. **Rose, N. R., A. Herskowitz, D. A. Neumann, and N. Neu.** 1988. Autoimmune myocarditis: a paradigm of post-infection autoimmune disease. Immunol. Today **9**:118–120.
19. **Schiff, L. A., and B. N. Fields.** 1990. Reoviruses and their replication, p. 1275–1306. *In* B. N. Fields and D. M. Knipe (ed.), Virology, 2nd ed. Raven Press, New York.
20. **Sherry, B., and B. N. Fields.** 1989. The reovirus M1 gene, encoding a viral core protein, is associated with the myocarditic phenotype of a reovirus variant. J. Virol. **63**:4850–4856.
21. **Sherry, B., F. J. Schoen, E. Wenske, and B. N. Fields.** 1989. Derivation and characterization of an efficiently myocarditic reovirus variant. J. Virol. **63**:4840–4849.
22. **Tyler, K. L., and B. N. Fields.** 1990. Reoviruses, p. 1307–1328. *In* B. N. Fields and D. M. Knipe (ed.), Virology, 2nd ed. Raven Press, New York.
23. **Tyler, K. L., M. A. Mann, B. N. Fields, and H. W. Virgin.** 1993. Protective anti-reovirus monoclonal antibodies and their effects on viral pathogenesis. J. Virol. **67**:3446–3453.
24. **Virgin, H. W., M. A. Mann, B. N. Fields, and K. L. Tyler.** 1991. Monoclonal antibodies to reovirus reveal structure/function relationships between capsid proteins and genetics of susceptibility to antibody action. J. Virol. **65**:6772–6781.
25. **Virgin, H. W., and K. L. Tyler.** 1991. The role of immune cells in protection against and control of reovirus infection in neonatal mice. J. Virol. **65**:5157–5164.
26. **Walters, M. N., P. J. Leak, R. Joske, N. F. Stanley, and D. Perret.** 1965. Murine infection with reovirus. III. Pathology of infection with types I and II. Br. J. Exp. Pathol. **46**:200–212.
27. **Weiner, J. R., J. A. Bartlett, and W. K. Joklik.** 1989. The sequences of reovirus serotype 3 genome segments M1 and M3 encoding the minor protein mu 2 and the major non-structural protein muNS, respectively. Virology **169**:293–304.
28. **Weller, A. H., M. Hall, and S. A. Huber.** 1992. Polyclonal immunoglobulin therapy protects against cardiac damage in experimental coxsackievirus-induced myocarditis. Eur. Heart J. **13**:115–119.
29. **Welsh, R. M., J. O. Brubaker, M. Vargas Cortes, and C. L. O'Donnell.** 1991. Natural killer (NK) cell response to virus infections in mice with severe combined immunodeficiency. The stimulation of NK cells and the NK cell-dependent control of virus infections occur independently of T and B cell function. J. Exp. Med. **173**:1053–1063.
30. **Wenger, N. K., W. H. Abelmann, and W. C. Roberts.** 1990. The heart, arteries, and veins, p. 1256–1277. McGraw-Hill Book Co., New York.
31. **Wolfgram, L. J., K. W. Beisel, and N. R. Rose.** 1985. Heart-specific autoantibodies following murine coxsackievirus B3 myocarditis. J. Exp. Med. **161**:1112–1121.
32. **Zou, S., and E. G. Brown.** 1992. Nucleotide sequence comparison of the M1 genome segment of reovirus type 1 Lang and type 3 Dearing. Virus Res. **22**:159–164.