Theiler’s Murine Encephalomyelitis Virus-Induced Cardiac and Skeletal Muscle Disease

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The DA strain of Theiler’s murine encephalomyelitis virus, a member of the cardiovirus genus of picornaviruses, induces a restricted and persistent infection associated with a demyelinating process following intracerebral inoculation of mice; both virus infection and the immune response are believed to contribute to the late white matter disease. We now report that intraperitoneal inoculation with DA produces an acute myositis that progresses to a chronic inflammatory muscle disease in CD-1 mice as well as several inbred mouse strains. Some mouse strains also develop central nervous system white matter disease and a focal myocardiitis. Infectious virus in skeletal muscle falls to undetectable levels 3 weeks postinoculation (p.i.), although viral genome persists for at least 12 weeks p.i., the longest period of observation. Severe combined immunodeficient animals have evidence of muscle pathology as long as 5 weeks p.i., suggesting that DA virus is capable of inducing chronic muscle disease in the absence of an immune response. The presence in immunocompetent mice, however, of prominent muscle inflammation in the absence of infectious virus suggests that the immune system also contributes to the pathology. T lymphocytes are the predominant cell type infiltrating the skeletal muscle during the chronic disease. This murine model may further our understanding of virus-induced chronic myositis and help to clarify the pathogenesis of human inflammatory myopathies.

The human idiopathic inflammatory myopathies include polymyositis (PM), dermatomyositis (DM), and inclusion body myositis. All three are characterized by a chronic mononuclear cell infiltration of skeletal muscle and have been hypothesized to have a dysimmune pathogenesis. The factors responsible for the initiation and maintenance of the immune responses remain unclear, but roles for an infectious agent and host genetic susceptibility have been proposed (3).

Picornaviruses are attractive candidate etiologic agents for these chronic myositides (reviewed in reference 6). Of special interest is the observation that a substantial number of patients with agammaglobulinemia develop a persistent echovirus infection of muscle which is associated with a DM-like disease (reviewed in reference 18). In addition, several picornaviruses are known to cause inflammatory muscle disease following experimental inoculation of mice (reviewed in reference 31).

Theiler’s murine encephalomyelitis virus (TMEV), a member of the cardiovirus genus of picornaviruses, produces enteric and neurologic disease in mice (reviewed in reference 23). Strains of TMEV can be divided into two subgroups on the basis of their differing biologic properties and antigenicity when tested with neutralizing monoclonal antibodies (MAbs). The first subgroup, typified by GDVII strain, is highly virulent and produces a fatal, acute polioencephalomyelitis with no virus persistence following intracerebral inoculation of mice. In contrast, inoculation of DA strain and other members of the less virulent TO subgroup induces a transient mild, subclinical neuronal pathology followed by a chronic progressive inflammatory demyelinating disease with persistence of the virus. There are only small amounts of infectious virus, with little evidence of viral antigen and genome during the late demyelinating disease, suggesting that there is a restricted expression of the virus (e.g., reference 4). The immune response, age, and genetic background of the host as well as the route of inoculation are critical determinants of disease.

In 1949, Rustigian and Pappenheimer reported that TO and GDVII subgroup strains of TMEV replicate in muscle and induce a severe acute myositis following intramuscular inoculation of mice (26). In the present study, we describe an acute as well as chronic myositis following intraperitoneal (i.p.) inoculation of several strains of mice. No infectious virus was detectable at the time of the chronic myositis. This model may increase our understanding of virus-induced chronic myositis and provide insights into the pathogenesis of human inflammatory myopathies.

MATERIALS AND METHODS

Cells and virus. The DA strain of TMEV was obtained following transfection of in vitro-derived transcripts generated from an infectious full-length DA cDNA clone, pDAFL3 (24). BHK-21 cells were used for the growth of virus and infectivity assays.

Animal inoculation and tissue collection. CD-1 and BALB/c (H-2q) mice were obtained from Charles River Breeding Laboratories (Wilmington, Mass.), and ABY/Sal (H-2r), C57BL/6J (H-2b), DBA/1J (H-29), SJL/J (H-2r), SWR/J (H-2q), and BALB/c BySmn-scid/J (SCID) mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). Ten- to fourteen-day-old mice were generally inoculated i.p. with 50 μl of Hanks’ balanced salt solution containing 10^5 PFU of DA strain and were sacrificed at 1, 2, 3, 6, and 10 to 12 weeks postinfection (p.i.), except SCID mice, which were inoculated at 4 weeks of age and were sacrificed at 2, 3, 4, and 5 weeks p.i.

For infectivity studies, tissue samples were aseptically removed, weighed, homogenized in 2 ml of Hanks’ balanced salt solution, and clarified by centrifugation. Tissue homogenates were titrated by measurement of a 50% tissue culture infective dose (TCID50) on BHK-21 cells. If no virus was detected, some homogenates were blindly passed one to three times on BHK-21 cells.

For routine histology, specimens of heart, brain, muscles (hind limb), and spinal cord were fixed in 10% neutral buffered formalin. Samples were embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin for histopathological examination.

Two samples of muscle from the hind limbs of each animal were quick-frozen in isopentane that had been chilled in liquid nitrogen and were then stored at

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Immunohistochemistry. Immunohistochemical evaluation for TMEV antigen was performed on paraffin-embedded tissue sections according to a modification of a previously published method (2). Muscle sections were rehydrated in graded alcohols, incubated with 3% hydrogen peroxide-methanol for 20 min for blocking of endogenous peroxidase, and processed with a Histomouse SP kit (ZYmed, San Francisco, Calif.) according to the manufacturer's protocol. After washing with 1 M phosphate-buffered saline (PBS), sections were overlaid overnight at 4°C, or for 1 h at 37°C, with 1:100 dilution of two biotinylated anti-DA strain MAbs. After several washes with a solution containing PBS with 0.5% casein and 0.01% Tween 20, the section was incubated with streptavidin-peroxidase conjugate (Zymed) and then developed with 3-amin-9-ethylcarbazole (AEC) (Zymed). Slides were lightly counterstained with hematoxylin. In some cases, an antigen unmasking solution (Vector, Burlingame, Calif.) was used after rehydration according to the manufacturer's protocol. Positive controls consisted of brain sections from suckling mice 7 days following intracerebral inoculation with DA strain. Negative controls included a nonspecific biotinylated mouse MAb and muscle sections from uninoculated mice.

For characterization of the phenotype of inflammatory cells infiltrating muscle, 8-μm cryostat sections were prepared, air dried for 10 min, and stored at −70°C until use. Prior to staining, sections were fixed for 10 min at −20°C in acetone-methanol (1:1) and air dried. To reduce background, slides were incubated for 20 min with a blocking solution (1 M PBS with 10% goat serum and 0.5% casein). The following rat anti-mouse MAbs were obtained from Frank Firth (University of California): anti-CD4 (clone GK1.5), anti-CD8 (clone 53-6.7), antimacrophage (MAC-1 and F4/80), and an anti-T-cell receptor (clone HST-597). In addition, we used a rat anti-mouse CD45R/B220 MAb (clone RA3-6B2; Pharmingen, San Diego, Calif.) which is directed against B cells, subsets of natural killer cells, and non-major histocompatibility complex-restricted cytolytic T cells. MAbs were applied to sections overnight at 4°C, washed for 15 min, and then incubated for 20 min with biotinylated rabbit anti-rat immunoglobulin G (Zymed). The slides were then incubated overnight at −20°C in precoated streptavidin–peroxidase (Zymed) followed by incubation for 10 min with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, Mo.) 0.015% nickel ammonium sulfate, and 0.05% hydrogen peroxide in PBS. After washing in PBS, sections were dehydrated in ethanol and cleared in xylene, and coverslips were mounted in Permount (Fisher Scientific, Pittsburgh, Penn.).

Reverse transcriptase (RT)-PCR and Southern blot analysis. Selected tissues were quick-frozen in vials containing 2 ml of Ultraspec RNA (Biotecx, Houston, Tex.) and 5 to 10 mm glass balls (PGC Scientific Corp., Gaithersburg, Md.) and were stored at −70°C until use. At the time of RNA extraction, tissues were thawed and then shaken for 5 min in a SpeedVac (Eppendorf, Norwalk, Conn.). RNA was extracted according to the manufacturer's protocol (Biotecx).

The RT reaction mixture was prepared in a final volume of 20 μl containing 200 U of Moloney murine leukemia virus reverse transcriptase (Gibco BRL, Grand Island, N.Y.), 10 μM of each primer, 5 μg of RNA, first-strand buffer (50 mM Tris-HCl [pH 8.3], 75 mM KCl, 3 mM MgCl2), 10 mM dithiothreitol, 10 U of RNAsin (Promega, Madison, Wis.), 0.5 M deoxynucleoside triphosphates, and 20 pmol of a negative-sense primer which binds to DA RNA nucleotides 1409 to 1432. Following incubation at 37°C for 30 min, 5 U of reverse transcriptase (Perkin-Elmer Cetus, Norwalk, Conn.), 20 pmol of a positive-sense primer with sequence identical to DA RNA nucleotides 1044 to 1064, 1 μl of 10× PCR buffer (Perkin-Elmer Cetus), and water were added to make a final volume of 100 μl. Forty cycles of PCR were performed in a 2400 Perkin-Elmer thermal cycler as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s.

The amplified products were analyzed on a 2% agarose gel and ethidium bromide stained. The gel was then blotted onto a MaxiFilter (Schleicher & Schuell, Keene, N.H.) by capillary transfer; the membrane was then air dried, exposed to UV in a Stratalinker 1800 (Stratagene, La Jolla, Calif.) in “auto” mode (1,200 J), then washed in saline, exposed to UV in a Stratalinker 1800 (Stratagene, La Jolla, Calif.), and baked at 80°C for 1 h. Membranes were rehydrated in 1× PBS containing 200 μg/ml of sheep anti-rat immunoglobulin G (Bethyl, Montgomery, Tex.) and 80 μg/ml of goat anti-rabbit immunoglobulin G (Bethyl) and then incubated with alkaline phosphatase-conjugated rabbit anti-goat immunoglobulin G (Bethyl). The membranes were washed and exposed to UV as above. The presence of β-actin mRNA was determined by amplification in parallel with each RT-PCR reaction using a multiple tissue primer set (11) (5′-TGAAGCATGCTCTCTCGGACC-3′ and 5′-TGACTACCTGGCGCTACACGACTCGTGA-3′), which amplify a 192-nucleotide product of β-actin mRNA.

RESULTS

Clinical-pathological aspects of disease. Our initial experiments were directed at determining the effect of DA strain infection on CD-1 mice because of the known susceptibility of this mouse strain to chronic myositis following inoculation with the Tucson strain of coxsackievirus B1 (CVB1T) (20). Following i.p. inoculation of newborn CD-1 mice, DA strain had a 50% lethal dose of less than 10 PFU, with mice dying within 2 weeks of inoculation of a severe myositis. In contrast, inoculation of immunocompetent mice from 3 weeks of age induced only an occasional mild myositis. We selected 10- to 14-day-old animals for our studies since inoculation of 104 PFU of DA strain consistently induced a severe acute myositis that progressed to a chronic inflammatory muscle disease, with only a moderate mortality (7 of 42, or 17%).

The most remarkable pathology seen in the inoculated mice was present in muscle. One week p.i., small areas of necrosis within the muscle appeared, with relatively little inflammation. By the second week, a severe necrotizing myositis with diffuse polymorphonuclear and mononuclear cell infiltrates was apparent (Fig. 1A). There was also evidence of calcification and muscle regeneration beginning at this time. By 3 weeks p.i., there was a significant decrease in the necrosis, although mononuclear cell infiltrates persisted (Fig. 1B). After 6 to 12 weeks, most of the muscle had regenerated, and some regions of the destroyed muscle had been replaced with connective tissue. Atrophy of some myofibers occurred with no clear restriction to a perifascicular distribution, as described with DM. Central nuclei were not infrequently present within the remaining fibers. Foci of inflammation with evidence of muscle degeneration and regeneration continued in 70% (five of seven) of the animals for at least 12 weeks p.i., the longest time that was studied. The chronic infiltrates were usually located endomysially, perivascularly, or in association with calcification.

i.p. inoculation of the DA strain of TMEV was also performed in a number of inbred strains of mice. To determine the relationship of myositis to DA strain-induced demyelinating disease, we inoculated SJL/J mice (the strain most susceptible to the latter disease) as well as C57BL/6J and BALB/c mice (strains that are known to be resistant to the late white matter disease). We also inoculated other strains that vary in susceptibility to CVB3-induced chronic myocarditis (13): ABY/SnJ and SWR/J mice (susceptible) and DBA/1J mice (resistant).

Mortality varied among the different inbred strains: DBA/1J (18 of 22 [82%]), SJL/J (14 of 29 [48%]), SWR/J (13 of 31 [42%]), C57BL/6J (7 of 19 [37%]), BALB/c (5 of 27 [18%]), and ABY/SnJ (5 of 42 [12%]). Most of animals died between 7 to 14 days p.i. with evidence of tremulousness, inactivity, weakness, or paralysis. All mice developed acute myositis, and about 70 to 80% had evidence of progression to a chronic myositis. The chronic muscle disease, however, seemed most widespread and with larger foci of infiltrated cells in ABY/SnJ and C57BL/6J mice (Fig. 1C), which are both H-2b. In contrast, SWR/J and DBA/1J mice, which are both H-2d, had evidence of more muscle regeneration with less inflammation 6 weeks p.i. These results suggest that the H-2 background influences the disease; however, studies involving congenic inbred mouse strains are necessary to substantiate these findings. The muscle pathology in inoculated SJL/J mice was difficult to interpret because un inoculated adult controls occasionally manifest in-
FIG. 1. Histopathology of tissues from mice following i.p. inoculation of DA strain. Shown are paraffin sections of skeletal muscle from a CD-1 mouse 2 (A) and 3 (B) weeks p.i. and from a C57BL/6J mouse 10 weeks p.i. (C), spinal cord from an SJL/J mouse 6 weeks p.i. (D), skeletal muscle from a SCID mouse 3 (E) and 5 (F) weeks p.i., and heart from a SCID mouse 3 (G) and 5 (H) weeks p.i. Sections were stained with hematoxylin-eosin. (Magnifications: ×60 [A to C], ×45 [D], ×230 [E], ×115 [F], ×75 [G], and ×10 [H].)
Inflammatory infiltrates, as has been previously reported (12). In contrast, there was no evidence of muscle pathology in the other uninoculated adult mouse strains (data not shown).

There was evidence of myocarditis in some of the inoculated mouse strains. A prominent myocarditis was seen in 11 of 13 (85%) inoculated ABY/SnJ mice. Necrotic foci were primarily located in the myocardial ventricular wall by 1 week p.i. By 3 to 6 weeks p.i., these foci became fibrotic without associated inflammatory cells. About 60% (four of seven) of inoculated SWR/J mice had a myocarditis with scattered necrotic foci that seemed to leave few if any residual abnormalities; these mice generally had inflammatory cell infiltrates larger than those seen with ABY/SnJ mice. C57BL/6J and DBA/1J mice developed only rare necrotic cardiac myocytes by 1 to 2 weeks p.i., with no evidence of late pathology. No acute or chronic lesions were observed in heart tissue from CD-1, BALB/c, or SJL/J mice.

Although there was no evidence of brain pathology, myelitis was present in approximately 80% of infected SJL/J (10 of 12) (Fig. 1D) and SWR/J (8 of 10) mice and was found to a lesser extent in CD-1 (9 of 18 [50%]) and BALB/c (3 of 10 [30%]) mice. The spinal cord showed evidence of progressive inflammatory white matter involvement by 3 weeks p.i. The presence of early spinal cord white matter disease in the absence of brain pathology suggests that virus enters the spinal cord via retrograde axonal flow from the affected muscle, a route that has been demonstrated to occur in the case of poliovirus, another picornavirus (21). Central nervous system pathology was not apparent in the other inoculated mouse strains. Histopathological results are summarized in Table 1.

![FIG. 2. Viral titers in tissues from CD-1 mice following inoculation with DA strain. Tissue homogenates that demonstrated no cytopathic effect are indicated below the dashed line, which represents the limit of sensitivity of the assay.](http://jvi.asm.org/)

### TABLE 1. Effects of DA strain infection of varied mouse strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mortality</th>
<th>Myositis</th>
<th>Myocarditis</th>
<th>Myelitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-1</td>
<td>17 (7/42)</td>
<td>70 (5/7)</td>
<td>0 (0/19)</td>
<td>50 (9/18)</td>
</tr>
<tr>
<td>SJL/J</td>
<td>48 (14/29)</td>
<td>77 (10/13)</td>
<td>0 (0/12)</td>
<td>80 (10/12)</td>
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<tr>
<td>BALB/c</td>
<td>18 (5/27)</td>
<td>78 (11/14)</td>
<td>0 (0/17)</td>
<td>30 (3/10)</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>37 (7/19)</td>
<td>78 (7/9)</td>
<td>30 (3/10)</td>
<td>0 (0/9)</td>
</tr>
<tr>
<td>ABY/SnJ</td>
<td>12 (5/42)</td>
<td>78 (7/9)</td>
<td>85 (11/13)</td>
<td>0 (0/13)</td>
</tr>
<tr>
<td>DBA/1J</td>
<td>82 (18/22)</td>
<td>67 (2/3)</td>
<td>25 (1/4)</td>
<td>0 (0/3)</td>
</tr>
<tr>
<td>SWR/J</td>
<td>42 (13/31)</td>
<td>67 (8/12)</td>
<td>60 (4/7)</td>
<td>80 (8/10)</td>
</tr>
</tbody>
</table>

*Percentage of inoculated animals that died or had particular pathological features. All animals had evidence of acute myositis (data not shown); animals that are listed as having myositis had inflammatory muscle pathology ≥3 weeks p.i.*
**Virus studies.** At 1 and 2 weeks p.i., CD-1 mice had evidence of infectious virus in homogenates of multiple tissues, with the highest titer in skeletal muscle (Fig. 2). Without blind passage, all tissue homogenates from CD-1 mice at 3 and 6 weeks p.i. were negative for virus (Fig. 2); however, samples from the spinal cord (but not other tissues) were positive when blindly passed. In addition, skeletal muscle from several SWR/J mice at 3 weeks p.i. had evidence of infectious virus when blindly passed.

DA viral antigen was detected in the cytoplasm of cells with the morphology of myocytes at 1 week p.i. (Fig. 3A). This antigen was usually present in areas of focal muscle damage that had a relatively modest inflammatory cell infiltration. No viral antigen was detectable in muscle later than 2 weeks p.i.

To detect whether viral genome was present despite an absence of infectious virus or viral antigen, RNA was extracted from varied tissues and analyzed by RT-PCR followed by Southern blotting. DA viral genome was detected in approximately 50 to 75% of the skeletal muscle samples from CD-1, ABY/SnJ, C57BL/6J, BALB/c, and SJL/J mice from 6 to 12 weeks p.i. and in a lower percentage of samples from SWR/J and DBA/1J mice. A representative autoradiograph is shown in Fig. 4. Viral genome was detected in about 50% of cardiac tissue samples across all mouse strains at 6 weeks p.i., the only time that was studied. As expected, spinal cord samples were consistently positive for DA viral genome. The detection of DA viral genome in the absence of infectious virus or viral antigen indicates that the virus is restricted in its expression.

**Skeletal muscle inflammatory infiltrates.** The number of inflammatory cells within the muscle increased, peaking at 3 weeks p.i., and then decreased to levels seen during the first 2 weeks p.i. (Fig. 5). To characterize the inflammatory cell infiltrates, immunohistochemical studies using cell-specific MAbS were performed on muscle samples from C57BL/6J mice (Fig. 5); similar results were found in muscle samples from ABY/SnJ mice (data not shown). At 1 week p.i., 43% of the positively identified infiltrating cells were composed of T lymphocytes (28% CD8\(^{+}\) cells and 15% CD4\(^{+}\)) and 38% were macrophages (MAC-1\(^{+}\)). At 3 weeks p.i., there was a relative increase in the percentage of T lymphocytes (55%), especially CD4\(^{+}\) lymphocytes (representing 31% of the total), and a relative decrease in the percentage of CD45R\(^{+}\) (Fig. 3B to D) compared with 1 week p.i. (5% at 3 weeks, compared with 19% at 1 week). Macrophages were frequently associated with calcified fibers. The cellular infiltrates remained similar in composition at 6 weeks p.i. (Fig. 3E to G). At 12 weeks p.i., T lymphocytes still represented more than half of the infiltrating cells (51%, with 33% CD4\(^{+}\) and 18% CD8\(^{+}\), a CD4\(^{+}\)/CD8\(^{+}\) ratio which was almost the reverse of that seen at 1 week p.i.), but macrophages were substantially reduced (31%) and CD45R\(^{+}\) cells significantly increased (18%) (Fig. 3H).

**SCID mouse studies.** To further clarify the pathogenesis of DA strain-induced disease, 4-week-old SCID mice were inoculated i.p. with the DA strain. Occasional scattered muscle fibers with degeneration, regeneration, and calcification were observed in animals from 2 to 5 weeks p.i. (Fig. 1E and F), with titers of virus ranging from 10\(^{6}\) to 10\(^{7}\) TCID\(_{50}\)/g; some fibers had evidence of viral antigen by immunohistochemical staining. In contrast to skeletal muscle, cardiac pathology was progressive and extensive, beginning with scattered foci of necrotic muscle fibers with calcification at 3 weeks p.i. (Fig. 1G) and culminating at 5 weeks p.i. with a devastating heart disease affecting more than 80% of the myocardium (Fig. 1H). Histopathological examination at 5 weeks p.i. demonstrated foci of necrotic and calcified myocytes as well as patches of fibrosis, suggesting the coexistence of new and old lesions. Infectious virus in the heart was approximately 10\(^{5}\) TCID\(_{50}\)/g at 2 weeks p.i. and 10\(^{5}\) TCID\(_{50}\)/g at 5 weeks p.i. Viral antigen was detected in a few muscle fibers. No apparent pathology was detected in the brain or spinal cord, although infectious virus was present in both, with the highest titer being approximately 10\(^{4}\) TCID\(_{50}\)/g in the spinal cord at 5 weeks p.i. The presence of chronic pathology in the skeletal and cardiac muscle of SCID mice indicates that the DA strain of TMEV has the ability to cause chronic muscle disease in the absence of a specific immune response.

**DISCUSSION**

There are relatively few experimental models of virus-induced chronic myositis (10). One well-studied system involves infection of neonatal CD-1 mice with CVB1/T. That virus causes an acute and chronic myositis in which virus genome is present in the muscle for months (29), although no infectious virus (30) or viral antigen (28) can be detected later than 2 weeks p.i. T cells appear to be important in the mediation of this disease since myositis is not present 4 weeks after inoculation of nude mice (32). We found the CVB1/T model a difficult one to work with because there was a significant mortality (>50%) following inoculation with even small amounts of the virus into neonates, the age required to induce a substantial incidence of chronic myositis in survivors (unpublished data). For this reason, we attempted to develop a more easily studied model. TMEV DA strain-induced myositis is an attractive model of virus-induced chronic myositis for a number of reasons. Mortality is relatively low, with a significant incidence of a late inflammatory disease of muscle. In addition, the powerful molecular tools that are available to investigate DA strain-induced central nervous system disease, such as the infectious full-length DA cDNA clone, can be used to study DA strain-induced myositis.

Although the human idiopathic inflammatory myopathies have cellular infiltrates, their pathogenesis is thought to involve different arms of the immune system. The earliest lesion in DM is a membrane attack complex located in the capillary wall, emphasizing the role of a complement-mediated vasculopathy (7). PM is considered to be mediated by cytotoxic T cells; CD8\(^{+}\) cells and macrophages surround normal muscle fibers and appear to trigger their destruction (11). A possible role for Th2 cells and their cytokines in PM has also been proposed (17). The pathogenesis of inclusion body myositis and its relationship to autoimmunity remain unclear (8). Studies of human myositis are limited because one or relatively few samples from one or two points in time are generally available for study, and only after the disease is established. DA strain-induced myositis provides the opportunity to characterize inflammatory muscle disease from its inception as well as during progression in order to more finely characterize the role of the immune system in a virus-induced chronic myositic disease.

DA strain-induced myositis resembles DM because of the development of calcification in the damaged muscle; however, in contrast to DM, the atrophy in DA strain-induced myositis does not have a perifascicular distribution and there is an absence of microvascular injury. Early after infection, the inflammatory infiltrate primarily consists of T lymphocytes. An initial CD8\(^{+}\)/CD4\(^{+}\) T-cell ratio of 2:1 reverses over time. The CD8\(^{+}\) T cells may have a protective role early, perhaps mediating cytotoxicity of virus-infected cells, as has been proposed for DA strain-induced central nervous system disease (16). CD4\(^{+}\) T cells may also be important early to help in the generation of the cytotoxic CD8\(^{+}\) T cells and a protective B-cell response. Later, CD4\(^{+}\) cells and CD8\(^{+}\) cells may con-
FIG. 3. Immunohistochemical studies of paraffin-embedded sections (A) or frozen sections (B to H) of skeletal muscle from mice inoculated with DA strain. Shown are skeletal muscle from ABY/SnJ mice 1 week p.i. stained with DA strain-specific MAb (A); C57BL/6J mice at 3 weeks p.i. stained for CD4 (B), CD8 (C), and CD45R (D) antigens; ABY/SnJ mice at 6 weeks p.i. stained for CD4 (E) and CD8 (F); and C57BL/6J mice at 6 weeks p.i. stained for MAC-1 (G) and at 12 weeks p.i. stained for CD45R (H). See Materials and Methods for details regarding immunohistochemical staining. Sections were counterstained with hematoxylin. (Magnifications: ×115 [A] and ×230 [B to H].)
infiltrating cells per grid is shown. Bars represent the standard errors of the average number of mice inoculated with DA strain at various times p.i. Quantitation of the cells was performed as described in Materials and Methods. The average number of molecular weight markers (not visualized in the autoradiogram). Lane 21 contains transcripts derived from pDAFL3 (see Materials and Methods). Lanes 3, 4, 7, 14, 16, 17, 18, and 21 demonstrate evidence of DA viral genome.

FIG. 4. Representative autoradiogram of Southern blot hybridization of RT-PCR products from RNA of skeletal muscle of several strains of mice (listed above the lanes) 6 weeks p.i. Lane 1 contains RT-PCR products with no template, while lane 2 contains RT-PCR products derived from RNA extracted from the muscle of an uninoculated BALB/c mouse. Lane 10 contained stained molecular weight markers (not visualized in the autoradiogram). Lane 21 contains transcripts derived from pDAFL3 (see Materials and Methods). Lanes 3, 4, 7, 14, 16, 17, 18, and 21 demonstrate evidence of DA viral genome.

however, the pathology was relatively slight, perhaps because of the age of the SCID mice at the time of inoculation (4 weeks old), regeneration of the damaged muscle, or significant contribution of the immune system to the myositis. A role for a pathogenic immune response is supported by finding severe muscle inflammation and degeneration at 3 weeks p.i., which continued at later times—although in a less severe form, in the absence of infectious virus and viral antigen; our data also suggest that at least one locus in the H-2 region may influence the muscle disease, although more definitive tests are needed. The contribution of the immune system to the muscle-induced disease, however, remains unclear. Roles for a restricted virus infection as well as a pathogenic immune response have also been proposed in the pathogenesis of DA strain-induced demyelinating disease.

The abilities of TMEV to infect the heart (25) and induce cardiomyopathy (19) have been noted in the past. The immune system may not play as important a role in DA strain-induced myocarditis as in myositis, since i.p. inoculation of SCID mice with DA strain produced a remarkably severe and progressive heart pathology. Our findings related to DA strain-induced heart pathology in SCID mice are similar to those seen following CVB3 infection of SCID mice, providing further evidence for the importance of a direct picornavirus infection in the pathogenesis of chronic myocardial injury (5). The presence of myocardial fibrosis with little or no cellular infiltrates in DA strain-infected immunocompetent and SCID mice makes this experimental disease a potentially valuable model of human dilated cardiomyopathy (9).

A virus infection, perhaps by inducing a pathogenic immune response, has been implicated in the pathogenesis of the human idiopathic inflammatory myopathies. There is evidence that viruses, especially picornaviruses, can cause acute myositis in humans (reviewed in reference 10). In addition, echoviruses, which are members of the picornavirus family, are known to induce a chronic DM-like disease in gammadoglobulinemic patients (reviewed in reference 18). Human T-cell leukemia virus type 1 (HTLV-1) has been localized in chronic myositis lesions of HTLV-1-infected individuals (27). In addition, recent studies have demonstrated the presence of enterovirus genome in human chronic myocardial diseases (e.g., reference 1). Studies have failed, however, to demonstrate evidence of infectious enterovirus or the genome of enteroviruses or HTLV-1 in muscle biopsies from patients with idiopathic inflammatory myopathies (14, 15). Although the results of these investigations have been negative, they do not rule out the possibility that a picornavirus can trigger an autoimmune inflammatory muscle disease and then disappear or, considering our study’s results, have a restricted expression in focal areas of the muscle. It may also be that unusual picornaviruses are involved in these muscle syndromes or that mutations occur in the viral genome over the course of the disease, causing a failure in binding of the PCR primers and a subsequent lack of detection of the genome by RT-PCR.

FIG. 5. Composition of cellular infiltrates in skeletal muscle from C57BL/6J mice inoculated with DA strain at various times p.i. Quantitation of the cells was performed as described in Materials and Methods. The average number of infiltrating cells per grid is shown. Bars represent the standard errors of the means.

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