A MURINE MODEL OF POLYMYOSITIS INDUCED BY COXSACKIEVIRUS B1 (TUCSON STRAIN)

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A murine model of polymyositis induced by coxsackievirus B1, Tucson strain (CVB_T) is described. Intraperitoneal CVB_T inoculation of CD 1 Swiss mice less than 48 hours old resulted in proximal hindquarter weakness that was first apparent 7 days after viral challenge and persisted for more than 10 weeks. Electromyographic and histologic evidence of a continuing myositis was present during this entire period of time. However, virus was not detectable later than 2 weeks post infection, despite clinical progression of disease. The finding of electromyographic and histologic abnormalities in CVB_T-infected mice, long after virus had cleared and neutralizing antibody production evoked, suggests that persistent myositis may be immunologically mediated, triggered by the initial acute viral infection.

Polymyositis (PM) is a treatable myopathic disease whose etiology is unknown. Previous investigations of this disorder have centered on examining the role of viral infection and autoimmune dysfunction in the pathogenesis of PM, but to date these studies have failed to provide an understanding of the pathogenetic mechanisms involved (1). Further progress in unraveling the etiology of this disorder has been hampered by lack of a good experimental model.

Investigation of the role of virus in the pathogenesis of PM dates back to 1959 when Sussman et al isolated coxsackievirus B (CVB) from the muscle of a 1-year-old child who died of a diffuse myositis (2). Numerous subsequent investigators have identified picornavirus-like particles by electron microscopic (EM) examination of clinically involved muscle from patients with PM (3–5). The importance of these reports is further supported by the demonstration of a serial rise in serum neutralizing antibody titers to CVB in several patients who developed PM (6) and isolation of CVB from the stool and muscle of a patient with fatal PM (5). As well, CVB has been implicated in other human muscle disorders (i.e., Bornholm disease or epidemic myalgia) in which viral replication in skeletal muscle is thought to be responsible for clinical manifestations (7).

Recently, a number of disorders in mice have been shown to arise as a consequence of CVB infection (8,9). Studying the pathogenesis of diabetes mellitus (DM), Yoon et al were able to develop strains of CVB that, upon infection, resulted in the selective destruction of pancreatic beta cells and the subsequent development of clinical DM (8). Virologic studies and serial determination of anti-CVB titers in the mice as well as in humans with juvenile onset DM have confirmed the importance of these findings (10). The etiologic role of CVB in chronic myocarditis has similarly been examined in a murine system. Data from the latter studies suggest that CVB infection may stimulate autoimmune destruction of myocardial tissue. These observations have recently been extended to primates (11,12).

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Such investigations thus underscore the potential role of CVB in triggering autoimmune disorders as well as the importance of murine models in elucidating the pathophysiology of human disease. Therefore, based on reports confirming the myotropism of CVB, the documented role of CVB in stimulating the development of other postulated autoimmune disorders, and strong support in the literature implicating CVB pathogenetically in the development of PM, we undertook to develop the murine CVB-induced model of PM described below.

MATERIALS AND METHODS

Virus and cells. CVB isolates with known myotropism were obtained from Dr. L. Minnich, University of Arizona (CVB 1, Tucson, CVB_T) and Dr. C. Gauntt, University of Texas (CVB 3, Woodruff strain, myocarditic). CVB with undefined myotropisms were also examined; these included clinical isolates of CVB 1, 4, 5, and 6 (Clin 1, 4, 5, and 6, respectively) obtained from the virology laboratory of The University of Michigan Medical Center, and an amyocarditic strain of CVB 3, supplied by Dr. C. Gauntt. All viruses were maintained and plaque-purified 3 times in either Vero or Buffalo green monkey kidney (BMK) cells. Virus was stored at −70°C. All dilutions of virus used in vivo were made in phosphate buffered saline (PBS). The working inoculum of virus selected for all studies was that dose which resulted in the greatest morbidity (proximal limb girdle weakness) and overall lowest mortality.

Tissue culture. Virus was propagated in BMK cell monolayers by standard techniques (7). Muscle tissues examined for the presence of infectious virus were obtained from the proximal extensor muscles of the right hindlimb, diaphragm, and heart by blunt dissection and ground using a mortar and pestle in 2 ml Dulbecco's modified essential medium (DMEM) and sand. The slurry was then spun at 2,000 revolutions per minute for 10 minutes and the supernatant used to infect BMK cell monolayers (7). Plaques were counted after 48 hours of incubation at 37°C in a 5% CO_2 atmosphere, 12 hours following neutral red overlay. Results are expressed as the $\log_{10}$ of the number of plaque-forming units (PFU) per ml.

Animals used and experimental design. CD 1 Swiss mice, a murine line previously reported to develop focal myositis, were used in all experiments. Pregnant breeder females were obtained from the Charles River Breeding Laboratories (Portage, MI) and allowed to deliver. Offspring were then randomly selected, inoculated, and caged separately in groups of 7–10 animals with surrogate mothers. Mice were challenged intraperitoneally (IP) at less than 48 hours of age with serial tenfold dilutions of 0.1 cc virus or PBS (control), using a Hamilton syringe to ensure accurate delivery of the inoculum. Animals were observed daily for clinical evidence of muscle weakness. Groups of 5–10 were killed every other day during the first 7 days of the study and thereafter weekly by cervical dislocation for histologic and serologic examinations. Serum samples were obtained by subclavian cutdown and processed individually or pooled when only small quantities were obtained. All serum samples were stored at −20°C.

Histopathologic studies. All tissue samples were obtained immediately after killing and fixed in either 10% paraformaldehyde or 2% glutaraldehyde or snap-frozen in isopentane cooled in liquid nitrogen for light microscopy, electron microscopy or frozen section studies, respectively (13). Several tissues were examined for evidence of viral infection; these included liver, heart, peripheral nerves, and central nervous system tissue, as well as skeletal muscle and the diaphragm.

Immunofluorescence studies. Transverse and longitudinal sections (8μ) of right hindquarter muscle tissue, mounted on cover slips and fixed for 20 minutes in cold acetone (−20°C), were examined by indirect immunofluorescence for CVB 1 antigens. A 1:32 dilution of monkey anti–CVB 1 serum (10μ, NIH reference research sera, Bethesda, MD) was applied to fixed tissue which was incubated in a moist chamber at room temperature for 1 hour. The cover slips were then washed in PBS for 20 minutes, followed by application of FITC-conjugated goat anti-monkey gamma globulin (Antibodies Inc., La Jolla, CA) and incubated in a moist chamber for 1 hour at 37°C. Just prior to mounting of these tissues in Elvanol, the cover slips were again washed in PBS (10 minutes) then distilled water (30 seconds), and counterstained for 5 seconds in Eriochrome black.

Antibody determinations. The functional ability of serum to neutralize CVB_T was studied by a microneutralization assay. Briefly, serial fourfold dilutions of heat-inactivated (30 minutes, 56°C) serum from CVB_T-infected and control mice, diluted in 10% heat-inactivated newborn calf serum and DMEM, were incubated with 10^6 PFU/ml CVB_T at 37°C for 1 hour. Two hundred microliters of the incubated sample was then added to microtiter wells containing BMK monolayers and allowed to incubate at 37°C in a 5% CO_2 atmosphere overnight. The cells were then fixed with 20% formaldehyde and stained with Giemsa stain. Fifty percent neutralization of CVB_T was recorded and the neutralization titer expressed as $\log_2$ of the reciprocal.

Electromyography (EMG). Serial EMGs were performed on both CVB-challenged and control mice. All animals were anesthetized with sodium pentothal, 5 mg/ml, 0.3–0.8 ml/animal. Dosages were titrated to keep the animals as lightly anesthetized as possible. Mice were restrained on their backs and the skin and superficial tissues of the abdomen and right limb incised and reflected to expose the musculature of the limbs. In mice less than 18 days old, both hindlimbs were examined. In older mice, only the right hindlimb was subjected to investigation. The muscles of the leg proper were considered "distal," whereas those of the thigh were considered "proximal." No attempts were made to evaluate individual muscles within these groups due to the relative size of the concentric needle as compared with the muscle fibers. Concentric needle electrodes (DISA 13L50, 30 mm long, 0.45 mm diameter) were used throughout the study. All examinations were performed using a TECA TD-20 EMG machine. Attempts to evaluate motor unit action potentials (MUAP) were not made since they were rarely seen and, when visualized, usually accompanied tremor or myoclonic jerking, thus making them unsuitable for analysis. Since it was impossible to produce voluntary movements,

electromyographic evaluation of interference patterns was also not performed.

RESULTS

Identification of myotropic virus. Of the 7 CVB strains initially screened in the mice (CVB_T, CVB 3 myocarditic [Woodruff], CVB 3 amyocarditic, and Clin 1, 4, 5, and 6), 3 produced clinical muscle weakness: in Clin 1– and Clin 6–infected animals these symptoms resulted from primary neurologic damage, while disease induced by CVB_T was due to histologically demonstrated myositis. Subsequent studies were hence performed using plaque-purified CVB_T. An infectious IP dose of 3×10^2 PFU was used in all subsequent experiments after preliminary studies revealed that this inoculum was optimal, producing clinical limb girdle proximal muscle weakness in 70–80% of challenged animals with an accompanying mortality of less than 30%. Studies using large numbers of mice have repeatedly substantiated these findings (Figure 1).

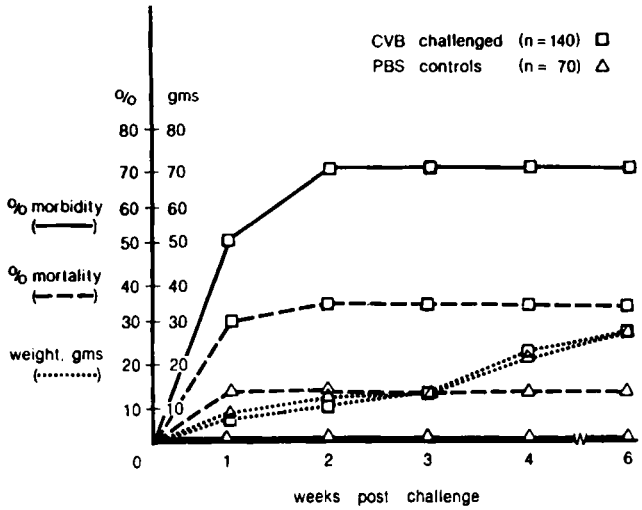


Figure 1. Coxsackievirus B1, Tucson strain (CVB_T)-induced murine polymyositis. One hundred forty mice less than 48 hours old were challenged intraperitoneally with CVB_T; 70 animals treated with phosphate buffered saline (PBS) were used as controls. Clinical symptoms were manifested within 7–10 days.

Clinical symptoms. Clinical evidence of muscle disease was occasionally observed as early as 4–5 days after infection, but more commonly was apparent 7–10 days following viral challenge. Characteristically, muscle weakness was evident in the proximal extensor muscle groups of both hindquarters with one limb often being preferentially affected. This resulted in a waddling gait, thereby simplifying clinical recognition of disease. Weakness increased in severity throughout the first 3 weeks following inoculation, after which it stabilized but persisted for the length of the experiment. As many as 70–80% of surviving animals developed clinical evidence of myositis. No weakness was ever observed in the control animals. There was, additionally, no significant difference in the growth and development of virus-infected mice as compared with controls (Figure 1).

Histopathology. In the inoculated animals the pathologic changes varied in severity among animals, and even in the same animal among different muscle groups. For each reported observation, 2–5 animals were examined. In all CVB-infected mice, the hip extensors showed the most extensive and severe changes. Furthermore, even within the same muscle the distribution, extent, and intensity of the lesions were different among various fascicles. The earliest changes were observed on day 4 and consisted of interstitial edema and acute necrosis of isolated muscle fibers which were surrounded by acute and chronic inflammatory cells. On day 6 there was widespread necrosis of muscle cells, severe interstitial edema, and diffuse infiltration with polymorphonuclear leukocytes, lymphocytes, and plasma cells. Phagocytosis of necrotic fiber segments was evident.

These changes were also prominent on day 8 (Figure 2). By then, active regeneration of muscle cells was apparent, characterized by numerous slender, basophilic myotubes with central vesicular nuclei and prominent nucleoli. Occasional cells were undergoing mitosis (see insert Figure 2), but their definite identification as either myoblasts or other interstitial cells was not possible.

Fairly extensive dystrophic myofiber calcifications were also present from days 8–14 and to a lesser extent throughout the study. By day 14 marked variation in fiber size, centralization of myocyte nuclei, rounding of cell contours, and focal round cell infiltrates with occasional eosinophils, commonly surrounding blood vessels or dystrophic calcifications, were prominent (Figure 3). On day 21 adipose tissue began to replace lost myofibers (Figure 4). Myocyte regeneration remained active and could be seen in some animals as late as 72 days.

Overall there appeared to be an initial acute generalized inflammatory reaction which progressed to a stage of focal, chronic inflammation and then gradually began to resolve (Figure 5). At no stage were

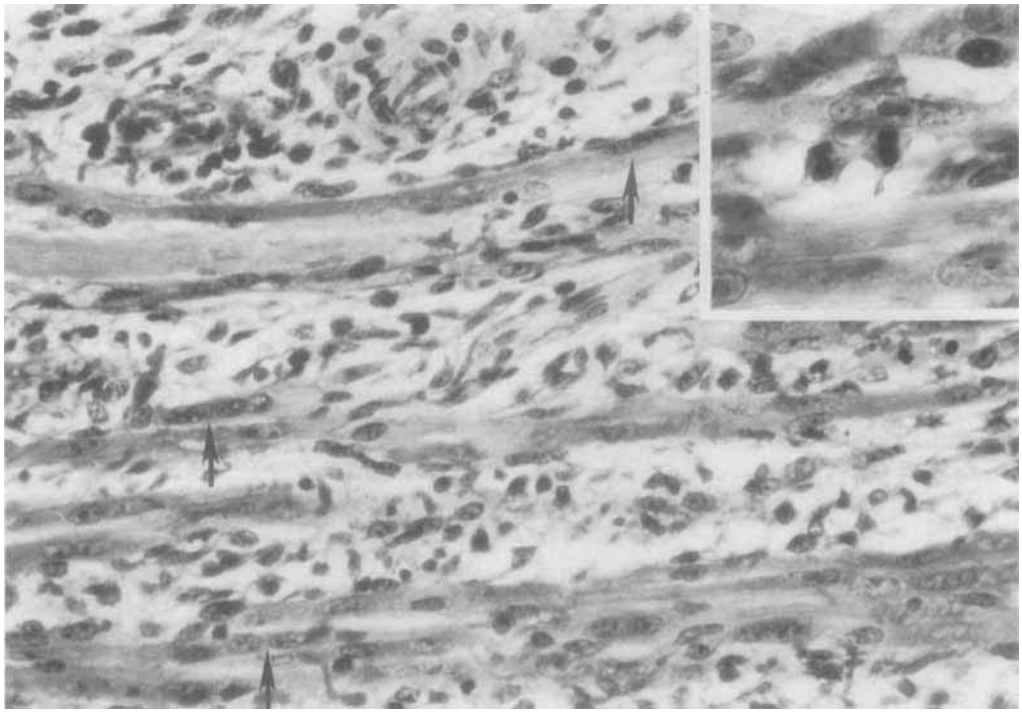


Figure 2. Eight days after inoculation with coxsackievirus B1, Tucson strain. Phagocytosis of necrotic muscle fibers, diffuse and perivascular acute and chronic inflammatory reaction, regeneration (**arrows**), and occasional mitotic figures (**insert**) (hematoxylin and eosin, original magnification × 470).

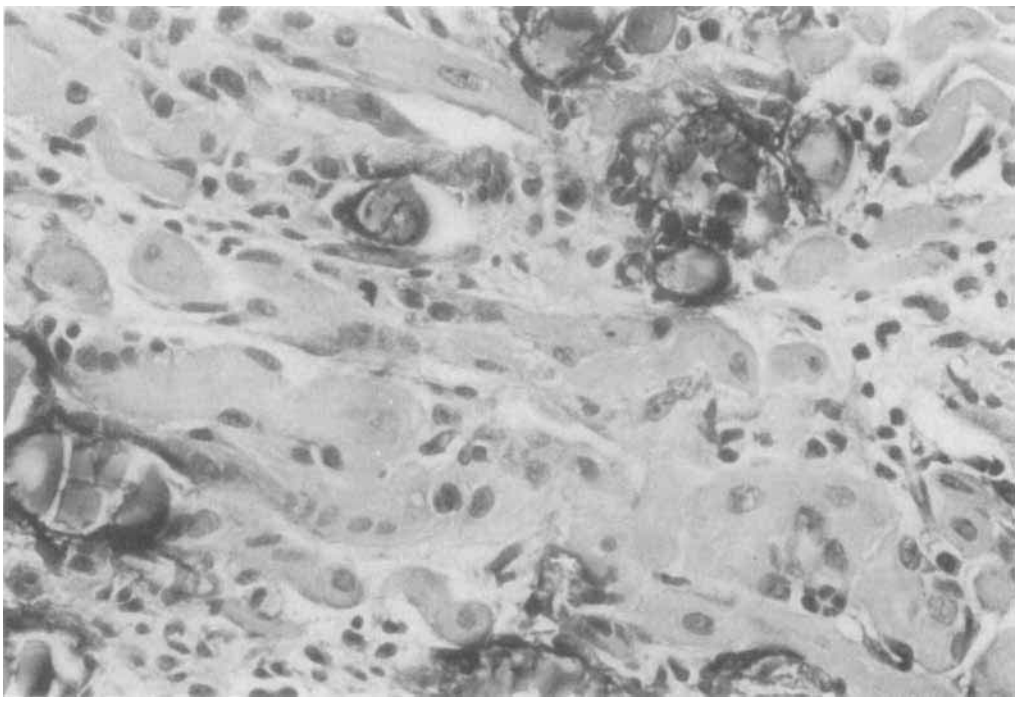


Figure 3. Fourteen days after inoculation with coxsackievirus B1, Tucson strain. Dystrophic calcification of muscle cells. Interstitial inflammation is less intense than in Figure 2. Many muscle fibers have central nuclei (hematoxylin and eosin, original magnification × 470).

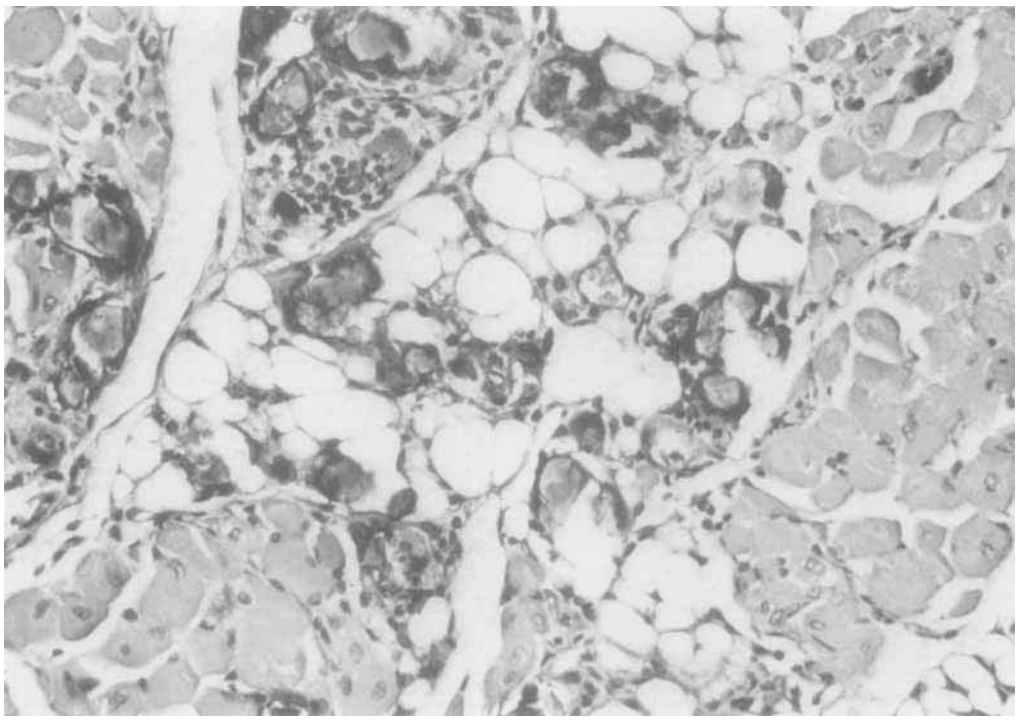


Figure 4. Twenty-one days after inoculation with coxsackievirus B1, Tucson strain. Fat replacement, prominent in the involved fascicles. Inflammatory cells mainly gathered about areas of calcification. Surviving muscle fibers have rounded contours and central nuclei (hematoxylin and eosin, original magnification × 296).

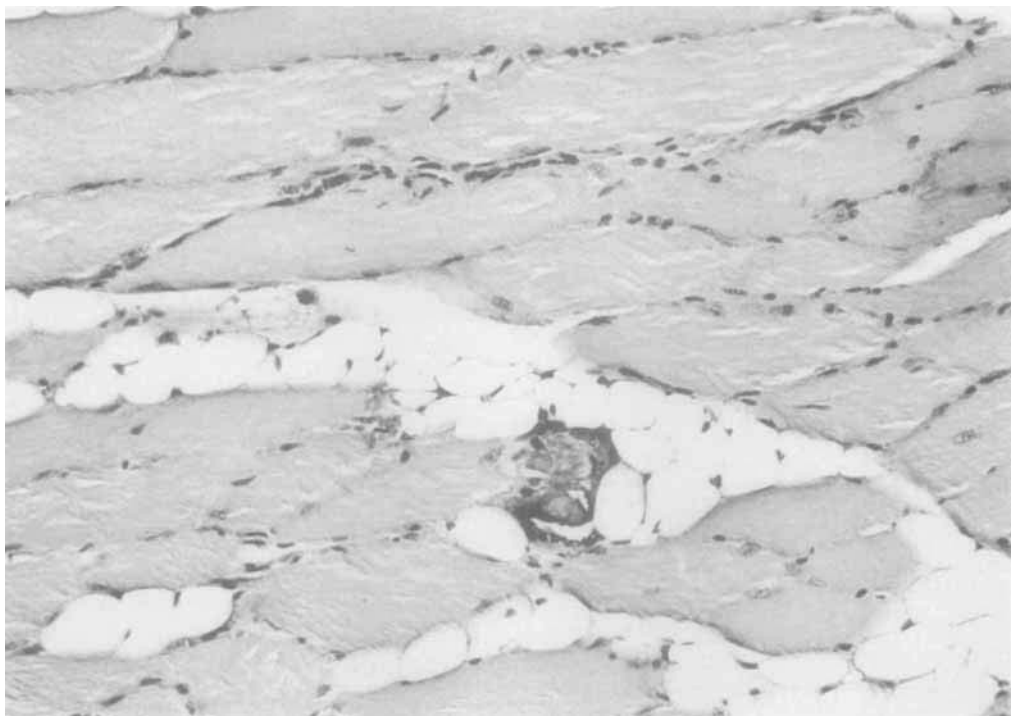


Figure 5. Fifty-four days after inoculation with coxsackievirus B1, Tucson strain. Fat replacement. Occasional calcified muscle cells still present (hematoxylin and eosin, original magnification × 296).

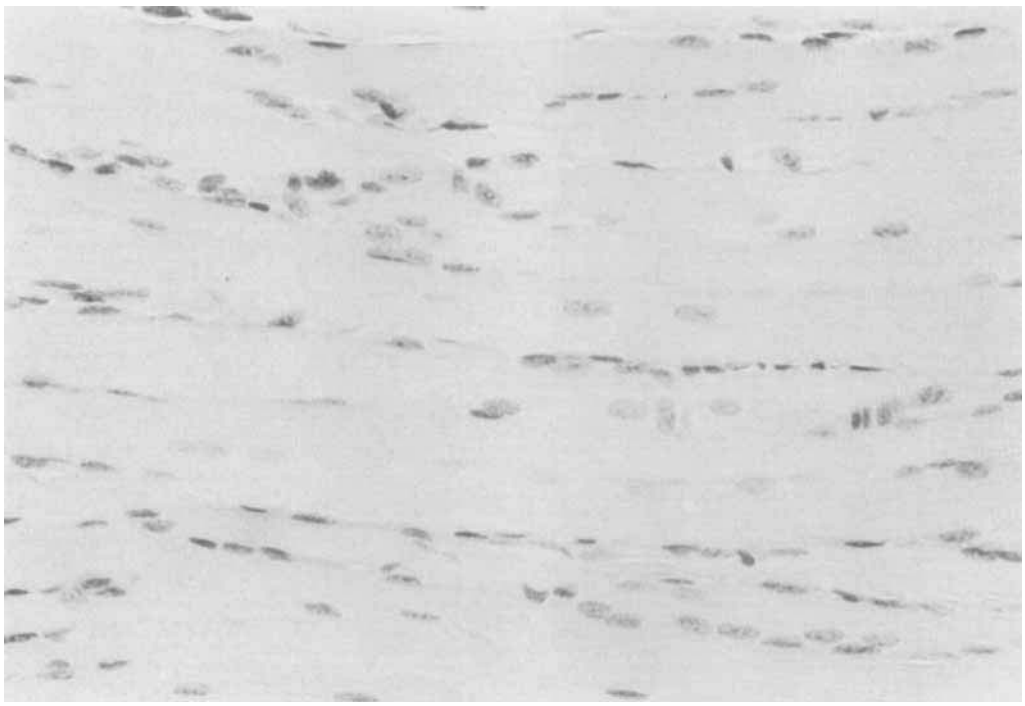


Figure 6. Fourteen days after control inoculation with phosphate buffered saline. Muscle is normal (hematoxylin and eosin, original magnification × 470).

similar changes seen in the control mice (Figure 6). No other muscle tissue involvement was present as demonstrated by serial examination of myocardial and diaphragmatic tissues, nor was there hepatic or nervous system disease. Electron microscopic examination of 2 mice less than 2 weeks old failed to reveal evidence of picornavirus infection.

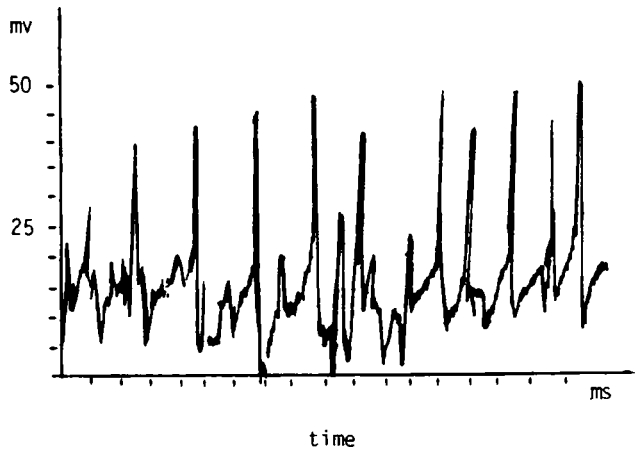


Figure 7. Complex repetitive discharge. Experimental animals studied 18 days following challenge with coxsackievirus B1, Tucson strain manifest positive sharp waves and fibrillations, as well as the complex repetitive discharges seen here (see Table 1, experiment 2).

Electromyography. The electrical activity of muscle tissue involved in an ongoing inflammatory process is usually abnormal. Specific electromyographic evidence of active myositis includes the presence of: 1) increased myofiber irritability as manifested by positive sharp waves, fibrillations, and complex repetitive discharges (CRDs); 2) short-duration, low amplitude MUAPs; and 3) a full interference pattern with mild voluntary contraction. Due to technical limitations no attempts were made to examine for the latter 2 manifestations (see Materials and Methods).

EMG results were abnormal in CVB_T-challenged mice through 53 days, after which these studies were not performed (Figure 7, Table 1). The abnormalities seen included fibrillations, positive sharp waves, and CRDs, findings consistent with an inflammatory myopathy. These EMG findings were most marked in the proximal hindquarter muscle groups early in the course of these experiments (experiments 1–3) and later became virtually confined to the proximal muscles (experiments 4, 5, and 7).

Overall, there was continued EMG evidence of inflammation for at least 53 days reflected by the presence of CRDs (experiment 7 and Figure 7), though

Table 1. Electromyography findings after challenge with coxsackievirus B1, Tucson strain*

Experiment	Days from challenge	Fibrillations	Positive sharp waves	Complex repetitive discharges
1	11	++	+	Profuse
2	18	+	+	Moderate
3	24	+	0	Minimal
4	32	0	0	Minimal
5	39	0	0	Minimal
6	46	0	0	0
7	53	0	0	Minimal

* Age-matched controls demonstrated no significant electromyographic abnormalities.

there was a decrease in the extent of these abnormalities with time. A neurogenic etiology for these abnormalities could not be ruled out by EMG alone due to the inability to accurately evaluate MUAPs. However, histologic studies (above) suggested relative sparing of neurologic tissue by CVB_T infection. Control animals did display a significant amount of "spontaneous activity" consisting of short-duration (1–2 ms) spike potentials with an initial negative deflection consistent with endplate phenomenon. These were, however, easily distinguishable from the pathologic abnormalities observed in the infected mice.

Virus recovery. CVB has recognized myotropism and is thought to induce its pathology by direct tissue lysis. In self-limited viral illnesses, the normal host is usually capable of clearing virus within 2 weeks (7). The continued presence of infectious viral particles in muscle tissue for longer periods would directly implicate CVB_T in the myopathic process which, by clinical and electromyographic evidence, appeared to persist for at least 7 weeks. Attempts were therefore made to examine for persistence of infectious virus in 3 separate organs: diaphragm, cardiac, and skeletal muscle (Table 2, Figure 8).

Virus was first recovered 2 days following IP challenge, with peak titers of infectious virus cultured from both diaphragm and skeletal muscle on the fifth and sixth days after viral challenge. CVB titers were highest in skeletal muscle, followed by diaphragmatic tissues. Virus was entirely cleared first from cardiac tissue, followed by the diaphragm and skeletal muscle on days 8, 10, and 14, respectively. The short-lived presence and low concentration of virus ($<10^2$ PFU/ml) in cardiac tissues probably reflect transient, early viremia: the histologic appearance of the heart did not reflect CVB-induced lytic injury, as opposed to

Table 2. Persistence of virus after challenge with coxsackievirus B1, Tucson strain (CBV_T)

Tissue	Peak CVB_T titer (log_{10})*	Culture negative (days from challenge)
Skeletal muscle†	6.0	14
Diaphragm	4.5	10
Myocardium	2.0	8

* Plaque-forming units/ml.
† Right hindquarter extensor muscle tissue.

the generalized inflammation seen in skeletal muscle. The inability to recover virus from any muscle tissues after 2 weeks argues against a primary lytic process mediating the observed myopathy which persists for months.

Examination for viral antigens. To examine the possibility that noninfectious forms of virus persisted in CVB_T-challenged animals, indirect immunofluorescence examination of hindquarter proximal muscle tissue for the presence of CVB or CVB-associated antigens was performed in control and infected animals. CVB antigens were first detected 6 days post infection, and antigen expression was greatest on days 9–12. Figures 9A and 9B illustrate the appearance of positively and negatively immunofluorescent muscle tissue from an uninfected and an infected animal, respectively. In pathologically involved muscle, immunofluorescence was confined to the cytoplasm of

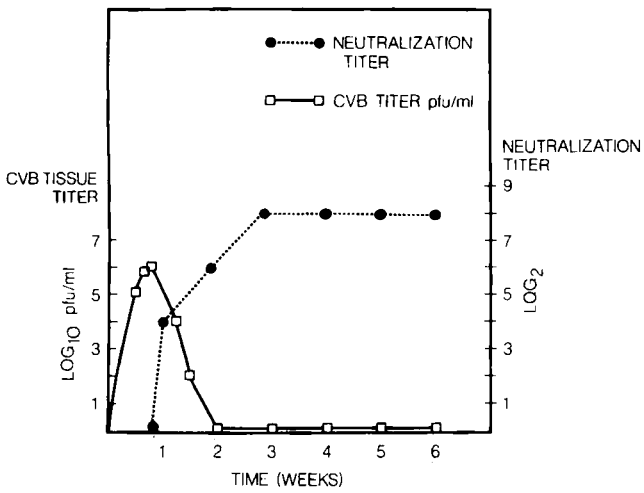
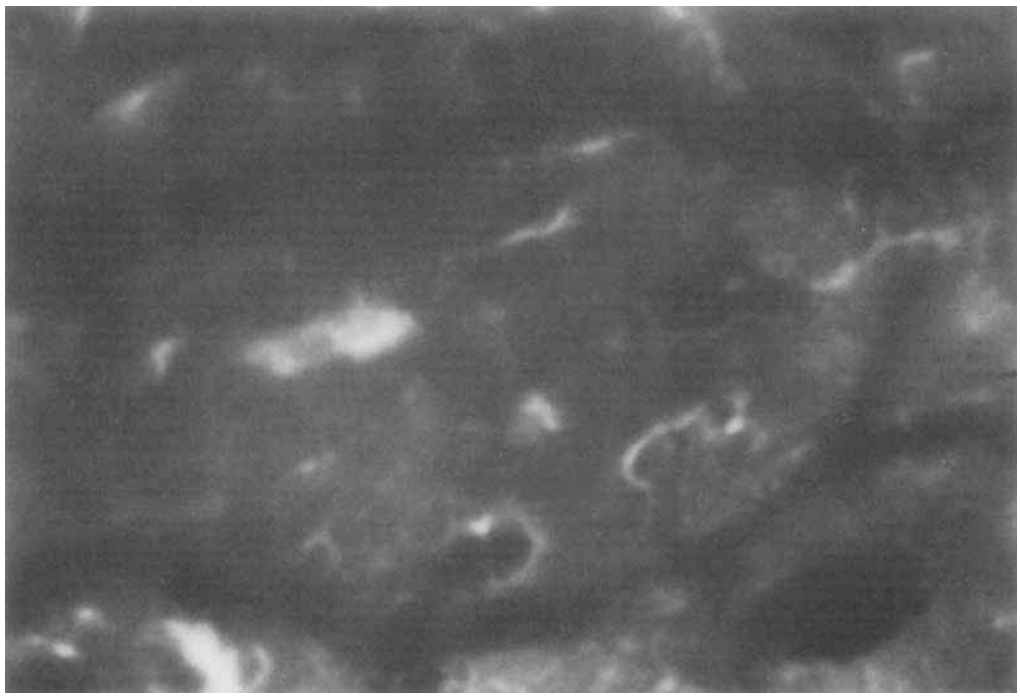


Figure 8. Coxsackievirus B1, Tucson strain (CVB_T) recovery from right hindquarter extensor muscle and antibody response to CVB_T challenge, as assessed by microneutralization assay. Significant antibody titers began to rise after 7 days and reached peak concentrations by 3 weeks. Virus was entirely cleared after 2 weeks. PFU = plaque-forming units.



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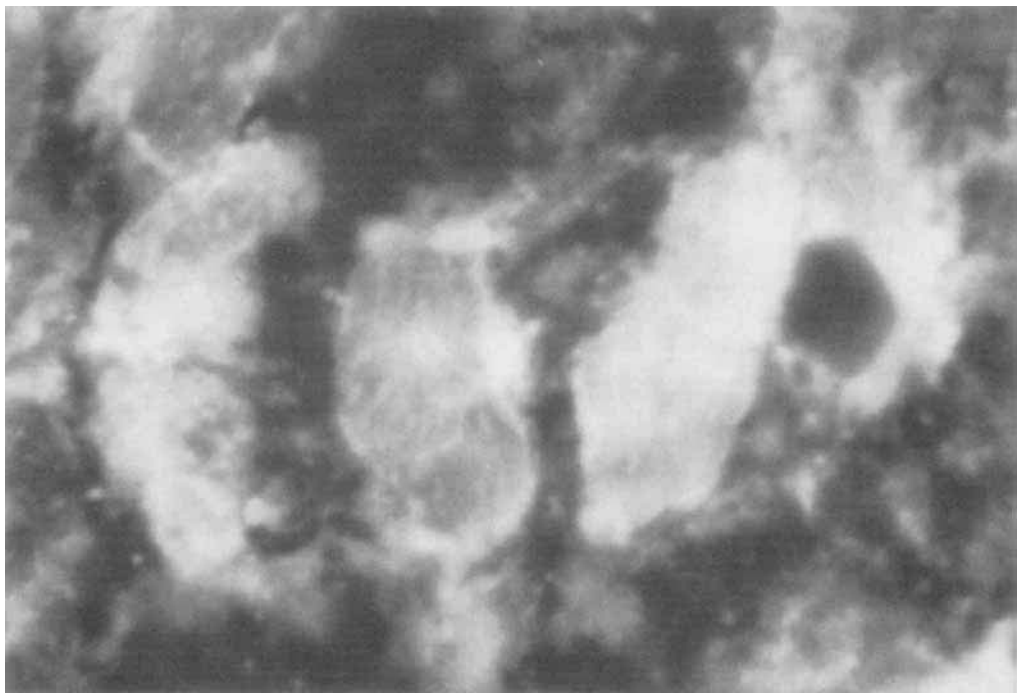

B

Figure 9. Indirect immunofluorescence of control (**A**) and coxsackievirus B1, Tucson strain (CVB_T)–challenged (**B**) mice 9 days following treatment. Positive immunofluorescence is bright white (**B**) and reflects the presence of CVB_T or the group B specific antigen (original magnification × 400).

individual myofibers, consistent with previously demonstrated intracytoplasmic picornavirus replication. By 2 weeks post infection, all tissues studied had no demonstrable evidence of CVB antigens. Parallel histologic studies on frozen section materials examined at 14 days or later confirmed the presence of ongoing myositis despite the negative immunofluorescence findings.

Antibody determinations. To assess the humoral immune response of mice to CVB_T challenge, specific serum antibody titers directed against CVB were measured by a microneutralization assay. A rise in anti-CVB immunoglobulin titer was first detected by day 6 in infected animals. By day 12 there was an eightfold increase in serum neutralizing capacity. Neutralizing titers thereafter remained elevated throughout the 6-week experimental period (Figure 8).

DISCUSSION

Significant progress in understanding the pathogenesis of polymyositis has been hampered by the lack of an appropriate experimental model. Earlier attempts to develop an animal system in which to study this disease have been patterned after other successful models of autoimmune disorders, such as experimental allergic encephalitis and autoimmune myasthenia gravis and have resulted in the description of a myopathic model, experimental allergic myositis (EAM), which develops as a consequence of repetitive challenge with homologous or heterologous muscle tissue in complete Freund's adjuvant (1,14).

This model suffers from several major shortcomings when compared with human PM: 1) Muscle weakness is often subclinical and thus difficult to detect; 2) An accompanying adjuvant-induced arthritis hampers clinical evaluation of muscle disease; 3) There is frequently a 6-week delay until the myositis begins; 4) The disease is monophasic and short-lived; 5) To foster continued myositis, "booster" injections are necessary; and finally 6) The nature of the antigen responsible for the muscle disease has not been clearly defined. Thus, there is a clear need for an experimental model which more closely resembles the clinical and laboratory features of PM and which can be easily manipulated in subsequent laboratory investigations.

The CVB-induced murine model of PM described in this paper differs significantly from others studied and closely approximates the human disorder. The cardinal clinical feature of PM is proximal muscle weakness. In the model described herein, mice infected as neonates with sublethal doses of CVB_T clearly developed weakness which preferentially affected the proximal muscle groups. The onset of these symptoms occurred shortly after viral challenge, often as soon as 4 days following infection. Muscle weakness was progressive, much as in humans, ultimately affecting the ability of these animals to ambulate normally. Daily inspection confirmed the progressive nature of this syndrome, which persisted well beyond what would be expected from a self-limited viral illness. Muscle weakness increased in severity for 3 weeks and was easily recognizable for as long as 10 weeks. Despite the severity of the developing myositis, there was no significant difference in the growth pattern of infected animals as compared with controls, emphasizing the selective nature of this disease (Figure 1).

The pathologic features of murine CVB_T-induced murine myositis paralleled those seen in human PM. There was evidence of selective muscle tissue involvement, characterized by mononuclear cell infiltrates accompanied by degenerating and regenerating myofibers. Histologic abnormalities, first evident on day 4, consisted of intense and generalized muscle tissue inflammation (Figures 1–5). By 2 weeks after infection, when muscle weakness was clinically progressive, the myositis had become more focal and was almost indistinguishable from that seen in human PM. Ongoing muscle tissue inflammation persisted with easily recognizable pathologic abnormalities for at least 6 weeks, long after infectious virus had cleared (Figure 8). Transient myocyte calcifications were present for 3 weeks, and may be related to the calcinosis which accompanies childhood PM, but the explanation for this finding is not clear.

Despite the fact that infectious virus could be recovered in tissue culture for at least 2 weeks post infection and viral antigen could be detected by immunofluorescence for an equivalent period of time, electron microscopic examination of this tissue failed to reveal the icosahedral particles characteristic of picornavirus known to be present by culture techniques. These findings emphasize that EM examination is not a sensitive means of screening for viral involvement in disease and may help explain why viral particles are not detected more frequently in cases of human PM.

Although there is little question that CVB_T infection played a primary role in the development of early myositis, the exact mechanisms responsible for prolonged muscle tissue injury are unclear. At least two hypotheses can be advanced to explain the CVB-induced disease: 1) Either primary CVB infection of skeletal muscle myofibers resulted in myolysis, attendant release of inflammatory mediators, and the observed pathology and clinical symptoms which only very gradually resolved, and/or 2) the virus stimulated

a host response capable of perpetuating a myositis. Three lines of experimental evidence presented above tend to support the latter hypothesis. First, virus could not be detected by either direct culture or EM studies beyond 2 weeks after infection, yet muscle weakness remained progressive and electromyographic and histologic evidence of ongoing myositis persisted for long periods of time thereafter. Second, indirect immunofluorescence failed to reveal the presence of any CVB antigens, making it unlikely that a chronic viral infection had occurred to explain the inflammatory process. Finally, antibody to CVB, which has previously been demonstrated to prevent the spread and replication of virus, was present in the serum in amounts sufficient to neutralize virus throughout the period of progressive clinical and histologic muscle disease.

The fact that the host immune response is capable of inducing an inflammatory myositis has been known for some time from investigations of EAM (14). In addition, Woodruff and Woodruff have obtained significant data implicating the immune response in the development and perpetuation of muscle tissue injury in a similar CVB-induced murine disorder, chronic myocarditis (9). In that model, CVB infection results in the development of cytotoxic T lymphocytes capable of specifically lysing CVB-infected myofibers by an H-2 restricted, antibody-independent mechanism (9,15). In vivo studies using antithymocyte serum confirmed the T cell–dependent nature of this process by demonstrating a significant reduction in CVB-induced myocarditis with little effect on the ability of host defenses to clear virus.

Further investigations in both murine and primate systems have suggested that these specifically activated T cells may be reacting to the presence of a novel cell surface antigen which is found after CVB infection (11,12). It is also possible that T cell–dependent antiviral antibodies react with these antigens via complement or nonimmune cell cytotoxic mechanisms, resulting in myocyte injury. In humans, recent studies by Rowe et al also have implicated T cells in the pathogenesis of PM by demonstrating large numbers of T cells, probably bearing the HLA–DR antigen, in the inflammatory infiltrates of muscle (16).

Thus the clinical, laboratory, and immunologic characteristics of murine CVB_T-induced myositis bear a strong resemblance to human PM and provide an ideal model to explore the pathogenesis and treatment of this disorder. Furthermore, study of CVB_T-induced murine myositis can be expected to contribute to an overall understanding of the role of virus in the initiation and maintenance of PM as well as other collagen vascular disorders, and to the mechanisms by which the elicited immune responses give rise to the observed inflammatory reactions.

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