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Coxsackievirus B-3 Myocarditis

Acute and Chronic Forms of the Disease Caused by Different Immunopathogenic Mechanisms

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Male BALB/c, DBA/2 and A mice inoculated with a myocarditic variant of coxsackievirus group B, Type 3 (CVB3) developed three distinct patterns of myocarditis. Most BALB/c and all DBA/2 mice developed acute cardiac inflammation lasting only 2 weeks, while A animals consistently showed twice as much myocardial damage as the other two strains, with active myocarditis continuing for 56 days after virus inoculation. Although virus elimination from the heart was also delayed in A mice, immunopathogenic, not viral, mechanisms caused cardiac injury in all strains. *In vivo* depletion of L3T4$^+$ (T helper) and Lyt 2$^+$ (T cytolytic/suppressor) cells using monoclonal antibodies showed that myocarditis in BALB/c mice depended predominantly on Lyt 2$^+$ cells, in DBA/2 mice on L3T4$^+$ cells and in A mice on both Lyt 2$^+$ and L3T4$^+$ cells. IgM heart reactive antibodies (HRAs) occurred in all three strains, while IgG HRAs were detected only in DBA/2 and A mice. Presumably, only the IgG antibody was pathogenic in this system. These data suggest that the host's genetic makeup determines the type of immune response to CVB3 infection of the heart and therefore the pattern of myocarditis which develops. (Am J Pathol 1987, 128:455–463)

COXSACKIEVIRUS group B, type 3 infection of mice provides a useful model to study pathogenic mechanisms of myocarditis. As in humans, the murine disease appears heterogeneous because genetically distinct inbred strains vary markedly, not only in the extent of heart damage following infection and the form of the disease (acute or chronic),[1–5] but also in predominant pathogenic mechanisms of cardiac injury.[6] Thus, work by Wolfgram et al[4] associates myocarditis induction in some susceptible strains with generation of specific heart reactive antibodies, while other investigators[7–9] implicate cellular autoimmune mechanisms in different animals. Which pathogenic mechanism develops in a particular individual may be of considerable importance for two reasons. First, immunosuppressive therapy using cyclosporin A fails to prevent cardiac damage mediated by cellular autoimmunity presumably because the pathogenic cytolytic T-lymphocyte response is both T-helper cell and interleukin-2 independent.[10–13] This therapy, however, is highly effective against T-helper cell dependent (humoral?) autoimmunity found in other mouse strains.[14] Therefore, success in treating myocarditis might depend upon the pathogenic mechanisms involved. Secondly, as suggested by other investigators,[4] the type of autoimmunity arising in an individual could determine whether the acute or chronic form of myocarditis develops.

Materials and Methods

Animals

BALB/c mice were originally purchased from Cumberland Farms (Clinton, Tenn). DBA/2J and A/J mice were purchased from Jackson Laboratories, Bar Harbor, ME. Neonates and 6–10-week-old adult

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males of these strains were obtained from colonies of these mice maintained at the University of Vermont.

Virus

A myocarditic variant of coxsackievirus B-3 (Nancy strain) (CVB3M) was obtained from J. F. Woodruff (Cornell University Medical College, New York, NY). Propagation of this virus has been described in detail elsewhere.[15] Virus preparations were titered by the plaque-forming assay, aliquoted, and stored at −70 C in phosphate-buffered saline (PBS) until use.

Infection and Sacrifice of Animals

Animals were inoculated intraperitoneally with 10^5 plaque-forming units (PFU) of CVB3M in 0.5 ml PBS. The animals were anesthetized by sodium pentobarbital and sacrificed by exsanguination.

Organ Virus Titer

Hearts were removed aseptically, and the upper half of the heart was homogenized in minimal essential medium. Cellular debris was removed by centrifugation at 300*g* for 10 minutes. The supernatant was serially diluted and titered on HeLa cell monolayers in the plaque-forming assay.

Antibody Treatment of Mice

Monoclonal antibodies directed against Lyt 2.2 (2.43) and L3T4 (GK 1.5) were generously supplied by Dr. S. Sriram, University of Vermont. Approximately 2 mg GK 1.5 and 0.5 mg 2.43 were injected intraperitoneally into mice 2 and 1 day before, and 1 day after inoculation with the virus. Relative depletion of Lyt 2^+ and L3T4$^+$ cells was determined by flow cytometry on mesenteric lymph node cells obtained from animals at the time of sacrifice.

Analysis of Lymphocyte Populations by Fluorescence Activated Cell Sorter (FACS)

Mesenteric lymph nodes were removed and pressed between two glass slides. The single-cell suspension obtained in this manner was washed in PBS and resuspended in PBS containing 1% bovine albumin and 0.1% sodium azide (blocking buffer) at a concentration of 5×10^6 cells/ml. Fifty microliters of the cell suspension was mixed with the appropriate monoclonal antibody and incubated on ice for 30 minutes. The cells were then washed and mixed with 50 μl of fluorescein-conjugated monoclonal antibody to rat kappa chain (Becton Dickinson, Mountain View, Calif) and incubated on ice for 30 minutes. The cells were washed again and suspended in 0.5 ml of blocking buffer containing 1% paraformaldehyde. The percentage of stained cells was determined by flow cytometry using a System 50-H/H Cytofluorograf and 2150 Data Handling System (Ortho Diagnostics Systems Inc., Westwood, Mass) which contains an argon laser and uses a light control mode set at 488 nm and 200 mw. Green fluorescence histograms on 10,000 single cells (FITC fluorescence defined by 530/30 band pass filter) were analyzed by setting the threshold with negative control cells (lymphocytes incubated with FITC-labeled antibody but not with either anti-L3T4 or anti-Lyt 2) such that only 1–2% of negative cells appeared in the positive region.

Preparation and Culture of Myocytes

The procedure for the preparation of myocyte cultures has been extensively reported.[9] Briefly, hearts were aseptically removed from neonatal mice less than 72 hours old, minced, and subjected to stepwise digestion with 0.4% collagenase Type II (Cooper Biomedical, Freehold, NJ). The dissociated cells were washed twice and suspended in Dulbecco's modification of Eagle's medium (DMEM) containing 5% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured in 96-well tissue culture plates (Nunc) at a concentration of 7×10^4 cells per well.

Enzyme-Linked Immunosorbent Assay (ELISA)

Preparation of Plates With Antigen

We used a modification of the ELISA procedure described by Cobbold and Waldemann.[16] Tissue reactive antibodies were assayed on cell monolayers which were prepared as described above and fixed with 0.25% glutaraldehyde for 1 minute. The glutaraldehyde was removed, and the cells were washed twice with a blocking buffer consisting of PBS with 0.1% sodium azide and 1% bovine serum albumin. Virus-specific antibodies were assayed in wells of Dynatech Immulon I plates containing 4×10^7 PFU sucrose gradient-purified CVB3 per well.

ELISA

Fifty microliters of the test serum, diluted in PBS–Tween (PBS containing 0.05% Tween 20 and 0.02% sodium azide), was added to wells containing antigen. The plates were incubated at room temperature for 30 minutes. After removing excess serum, the wells were

washed six times with PBS–Tween. Fifty microliters of either goat anti-mouse IgG (γ-chain-specific) or goat anti-mouse IgM (μ-chain-specific) conjugated to β-galactosidase (Zymed laboratories, South San Francisco, Calif) was added and the plates were incubated for 30 minutes at room temperature. The antibody conjugate was removed by washing the wells six times with PBS–Tween. The substrate, o-nitrophenyl-beta-galactopyranoside, was added at a concentration of 5 mM and allowed to react for 45 minutes at room temperature before the reaction was stopped by the addition of 1 M Na_2CO_3. Changes in optical density (OD) of the supernatant were determined using a micro-ELISA Minireader (Dynatech MR 590). Positive test sera gave 0.1 units of more greater absorbance than an equivalent dilution of normal mouse serum. The antibody titer was defined as the reciprocal of the last positive serum dilution.

Histology

The apical portion of the heart was fixed in 10% formalin, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin (H&E) and projected onto paper, where the total area of the myocardium and the areas of inflammation were outlined. The percent area of myocardium undergoing inflammation and necrosis was determined by image analysis with an Apple computer and the formula (total area of inflammation)/ (total area of myocardium) × 100.

Statistical Analysis

The Wilcoxon Ranked Score test was used for statistical evaluations.

Results

Kinetics of Myocarditis

Six-to-eight-week-old male BALB/c, DBA/2, and A mice were inoculated with 10^5 PFU CVB3 and sacrificed at various times thereafter. Hearts were evaluated for myocardial inflammation (Figure 1). Both BALB/c and DBA/2 mice developed acute myocarditis with maximal cardiac injury occurring between 7 and 10 days after infection and inflammation decreasing to background levels within approximately 2 weeks. No active myocarditis was observed in DBA/2 mice after this time, but a small proportion of BALB/c mice (10%) developed chronic disease lasting through Day 56. The disease pattern differed strikingly in A mice with maximum inflammation of 7% occurring on Day 14. Although the extent of cardiac involvement decreased substantially in A animals by Day 27, considerable inflammation persisted throughout the observation period. Figure 2 shows representative lesions in the three strains. Lesions in BALB/c mice 7 days after virus inoculation (Figure 2A) contained densely packed mononuclear cells, while lesions in DBA/2 mice appeared to be more necrotic (Figure 2C). Later in infection, areas of inflammation resolved completely in BALB/c mice

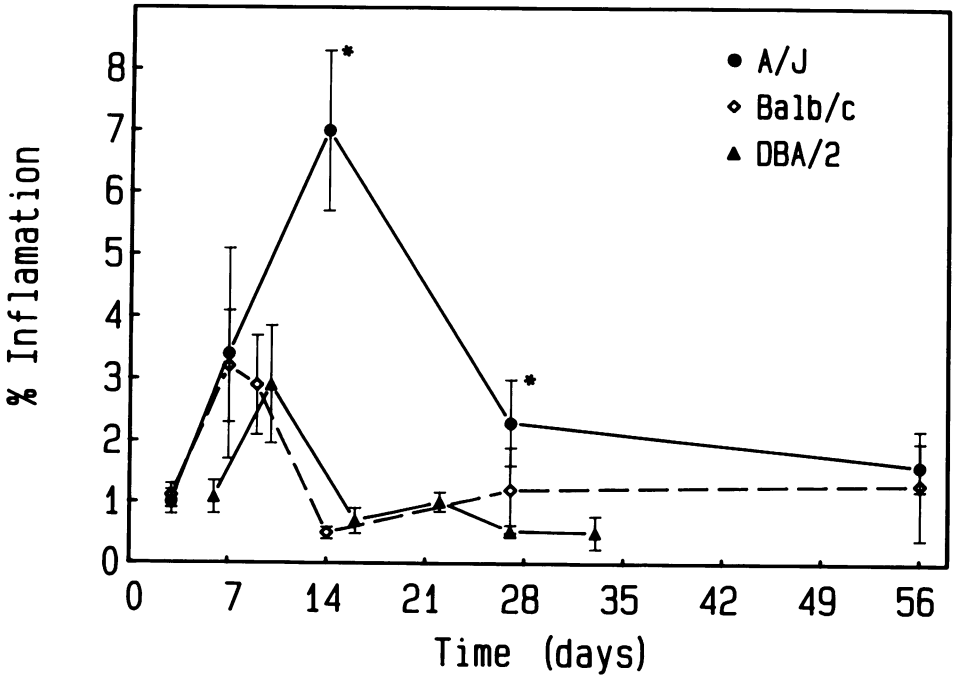

Figure 1—Kinetics of myocardial inflammation. Mice were inoculated intraperitoneally with CVB3 and sacrificed at various times thereafter. Hearts were divided in two with the apical half undergoing histologic analysis for myocarditis. Results represent the mean percent of the myocardium inflamed + SEM of 8 or more animals for each time point. *Significantly greater than either BALB/c or DBA mice at $P \leq 0.01$.

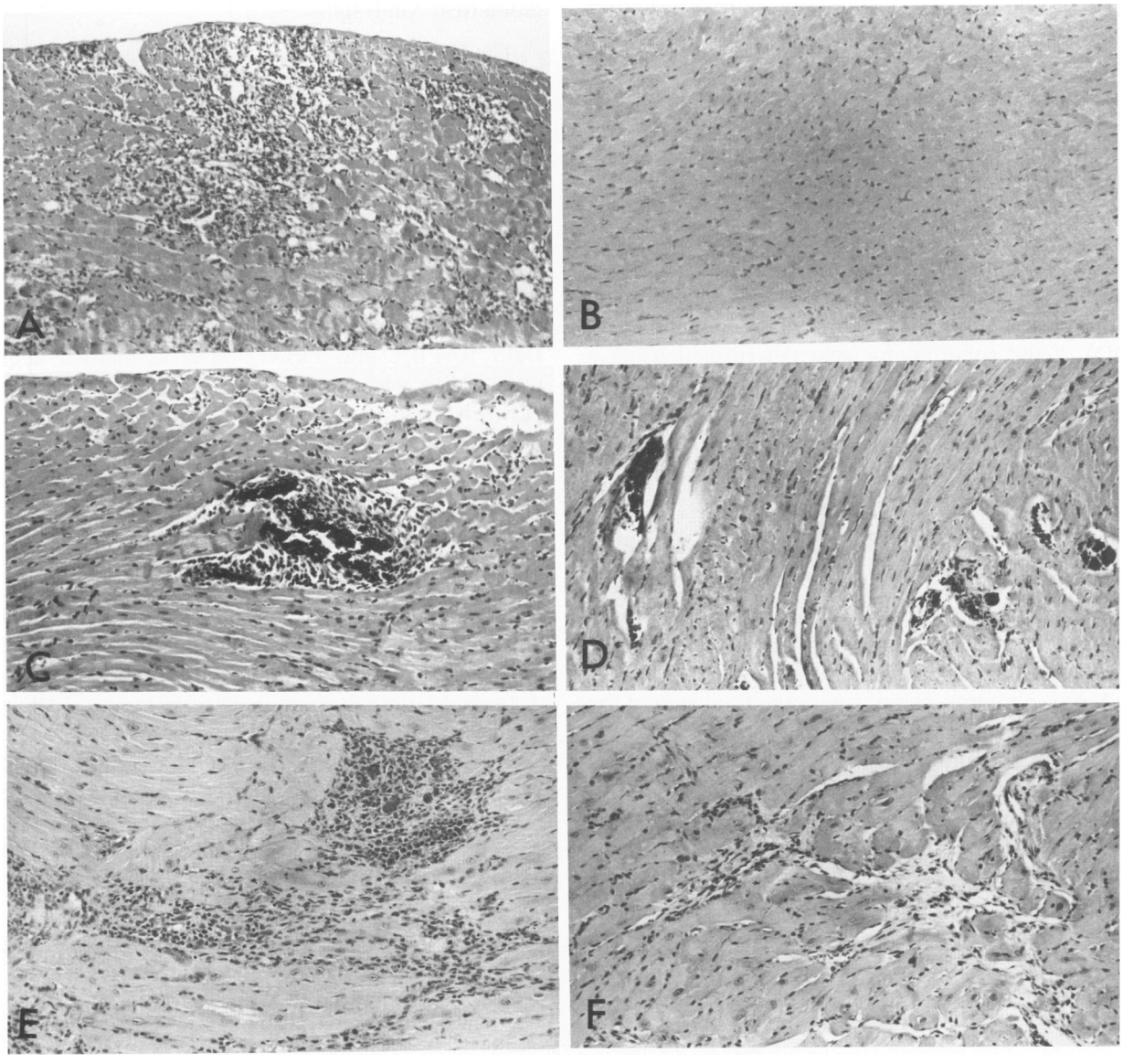

Figure 2—Representative photomicrographs of myocardial lesions from animals given in Figure 1. Tissue sections were stained with H&E. Examples shown are from BALB/c mice 7 (**A**) and 14 (**B**) days after virus inoculation, from DBA/2 mice 10 (**C**) and 33 (**D**) days after virus, and A mice 14 (**E**) and 56 (**F**) days after virus.

(Figure 2B) or condensed to calcified scars in DBA/2 mice (Figure 2D). A mice developed early cellular infiltrates similar to those seen in BALB/c mice while later lesions (Day 56) showed interspersed areas of calcification and fibrosis with areas of active inflammation (Figure 2E and F).

Virus concentrations in all three strains reached similar maximal levels between 3 and 7 days after infection and decreased to undetectable levels by Days 16 (DBA/2), 27 (BALB/c) and 56 (A) (Figure 3). The rapidity of virus elimination roughly correlated to the speed and size of the CVB3-antibody response (Figure 4), with DBA/2 and BALB/c mice mounting vigorous humoral responses to the virus between Days 7 and 10, while maximal antibody responses in A animals were delayed until Day 27. Although this suggests that clearance of the virus largely depends upon the virus-specific antibody, other antiviral defense mechanisms (interferon, natural killer cells) were not investigated and may also differ between the strains.

All three strains developed heart reactive antibodies (HRAs) but the predominant class arising in the various strains differed (Figure 5). By days 7 and 14 after infection 75% (6/8) of the BALB/c mice had developed IgM HRAs, while at the same time, only

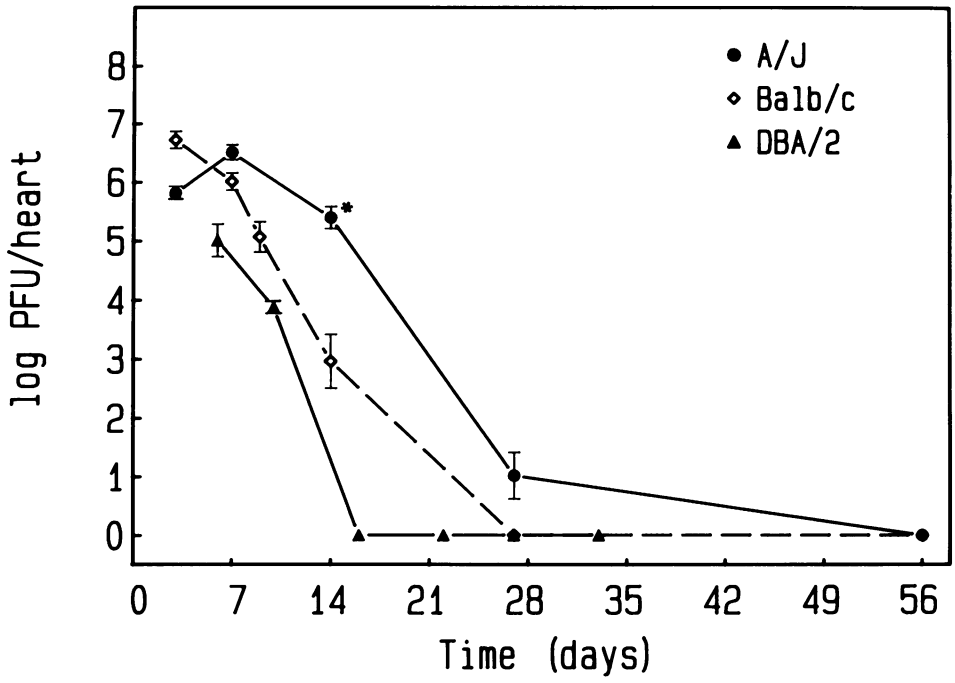

Figure 3—Kinetics of virus elimination. The atrial halves of the hearts from animals presented in Figure 1 were analyzed for infectious virus concentrations by means of the plaque-forming assay. Results represent mean $\log_{10}$ PFU virus/heart + SEM of 6 or more animals per time point. *Significantly greater than either BALB/c or DBA/2 mice at $P \leq 0.01$.

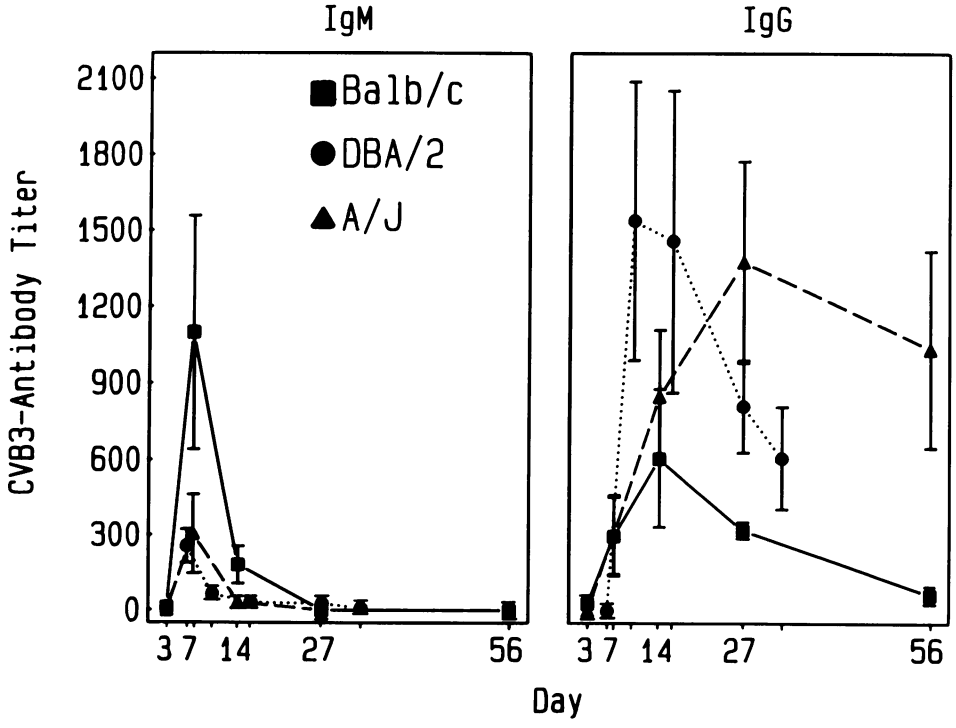

Figure 4—Kinetics of anti-CVB3 antibody response. Sera from animals given in Figure 1 were analyzed for reactivity to the virus using the ELISA. Results represent mean antibody titers of serum from 6 or more individual animals + SEM.

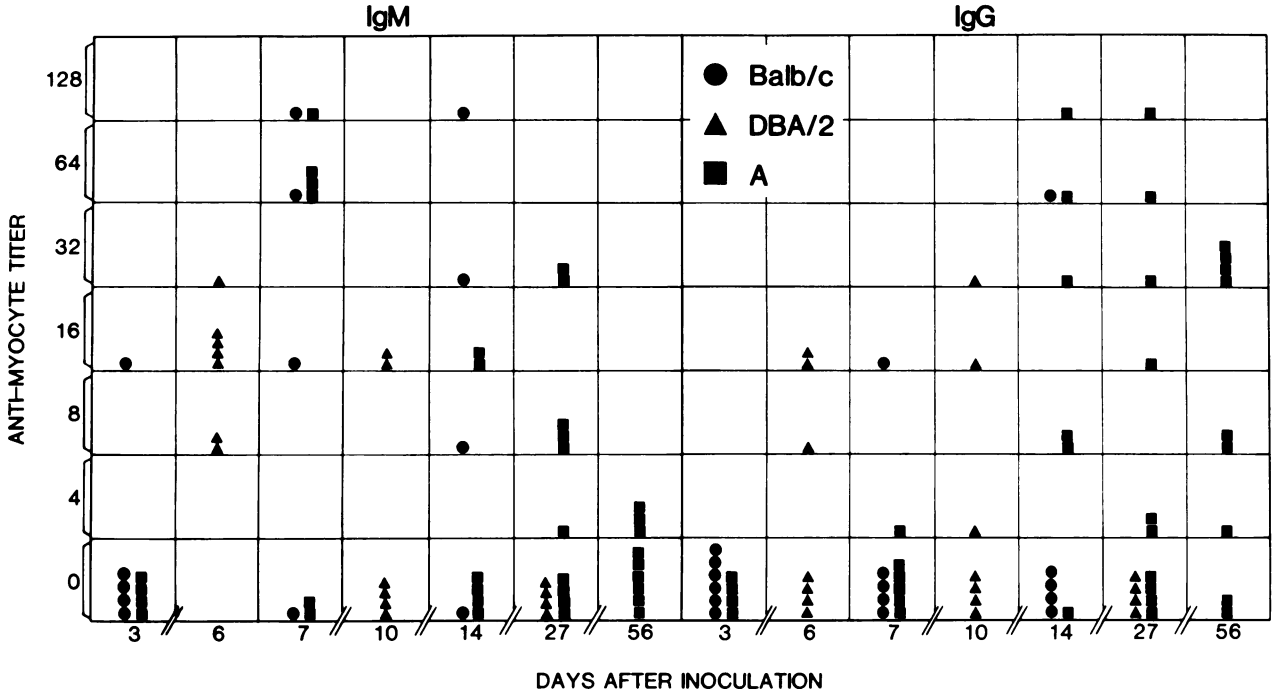

Figure 5—Kinetics of anti-myocyte antibody response. Sera collected at sacrifice from animals given in Figure 1 were analyzed by ELISA for antibodies to cultured myocytes. Each point represents an individual animal, and each individual was tested for both IgG and IgM antibodies.

25% (2/8) showed IgG HRA. In A and DBA/2 mice, IgM antibodies usually peaked early (7/7 DBA/2 and 4/6 A mice were positive at 6 and 7 days, respectively) and decreased as the disease progressed (2/4 and 0/4 DBA/2 mice were seropositive at 10 and 27 days; 6/10 and 3/6 A mice had these antibodies at 27 and 56 days). In contrast to BALB/c mice, 43% of the DBA/2 mice (3/7 on Days 6 and 10) and 83% of the A mice (5/6 at Day 14) also produced IgG HRA. The IgG response was transient in DBA/2 animals, because no activity was observed in serum collected after day 10 but remained elevated in A animals through Day 56 (7/9 mice seropositive).

Depletion of Lymphocyte Subpopulations

Mice were depleted separately of either L3T4$^+$ or Lyt 2.2$^+$ cells, and some mice were simultaneously depleted of both subpopulations, as described in the Materials and Methods section. Animals were infected, sacrificed 7 (DBA/2 and BALB/c) and 14 (A) days after virus inoculation and evaluated for myocardial lesions. Mesenteric lymph node cells were also stained for numbers of L3T4$^+$ and Lyt 2$^+$ cells by flow cytometry for confirmation of the efficiency of antibody depletion (Table 1). Removing either T-cell population separately afforded BALB/c mice some protection, but only depletion of Lyt 2$^+$ cells gave statistically significant reduction of cardiac lesions (Figure 6). Importantly, removal of both L3T4$^+$ and Lyt 2$^+$ cells gave no added protection from heart disease, indeed, cardiac injury in animals simultaneously depleted of both T-cell subpopulations was slightly enhanced, compared with mice depleted of Lyt 2$^+$ cells alone. While these results might indicate that both L3T4$^+$ and Lyt 2$^+$ cells participate in myocarditis of BALB/c mice, clearly, the Lyt 2$^+$ cell is predominantly pathogenic. However, the pattern is different in DBA/2 animals, where depletion of L3T4$^+$ cells gives complete elimination of myocarditis. DBA/2 mice were not treated with anti-Lyt 2.2 because T-cytolytic/suppressor cells in this strain express the Lyt 2.1 antigen. Finally, removing L3T4$^+$ cells alone in A mice had little effect on subsequent myocarditis, but elimination of Lyt 2$^+$ cells dramatically aggravated the disease, which suggests that T-suppressor cells might be present which incompletely regulate T-cell autoimmunity. However, when both Lyt 2$^+$ and L3T4$^+$ cells were eliminated, myocarditis was completely prevented. Thus, both T-cell populations must be involved in myocarditis in A mice.

Table 1—Proportion of Mesenteric Lymph Node Cells Belonging to L3T4+ (T-Helper) and Lyt2+ (T-Cytolytic/Suppressor) Cell Populations After Treatment of Mice with Monoclonal Antibodies to These T Cells *in Vivo*

Strain	Treatment	% Total cells FITC*	
		L3T4+	Lyt2+
BALB/c	None	47.1	21.0
	Anti-L3T4	3.4	39.2
	Anti-Lyt2	51.3	3.7
	Anti-L3T4 + anti-Lyt2	7.4	5.5
DBA/2	None	54.7	ND
	Anti-L3T4	3.9	ND
A	None	20.6	13.5
	Anti-L3T4	7.1	24.0
	Anti-Lyt2	17.6	5.4
	Anti-L3T4 + anti-Lyt2	8.7	7.9

Discussion

Animal models provide circumstantial evidence linking viral infections to chronic myocarditis and possibly cardiomyopathy.[1,2,4] Various investigators over the years have described persistent cardiac injury lasting up to 18 months in mice infected with either coxsackie B or encephalomyocarditis viruses.[1,2] Most recently, independent investigations by Wolfgram et al[4] and Kishimoto et al[17] indirectly implicate humoral immunity in the chronic stages of the disease. Myocarditis susceptible mice developing HRA showed nearly twice as much late-stage (Day 45 after infection) myocarditis as animals lacking this antibody.[4] Furthermore, while B lymphocytes comprise only 6% of the early (Day 7) inflammatory cell infiltrate (T lymphocytes represent 80%), by Day 75, the proportions of T and B cells change to 25% and 33%, respectively. This suggests that B lymphocytes and presumably humoral immunity become increasingly important in persistent disease.[17]

Our results indicate that while there may be a connection between HRA induction and chronic myocarditis, the association is complex. Evidence presented here and in a previous report[18] indicates that the IgM HRA of BALB/c mice is not pathogenic. Equivalent titers of this antibody arise in T-lymphocyte-deficient mice infected with the myocarditic virus and in animals given a nonmyocarditic CVB3 variant despite nearly complete absence of cardiac lesions in either group of animals. Therefore, not all HRA can be associated with cardiac injury. The IgG HRA of DBA/2 mice probably causes tissue injury, because complement depletion using cobra venom anticomplementary protein protects this strain, but not BALB/c animals, from myocarditis. CVB3-infected DBA/2 hearts show significant IgG deposits, while equally damaged BALB/c myocardium has none.[6] However, this HRA does not lead to chronic myocarditis, because disease in DBA/2 mice is mild and transient. Nonetheless, this result does not elimi-

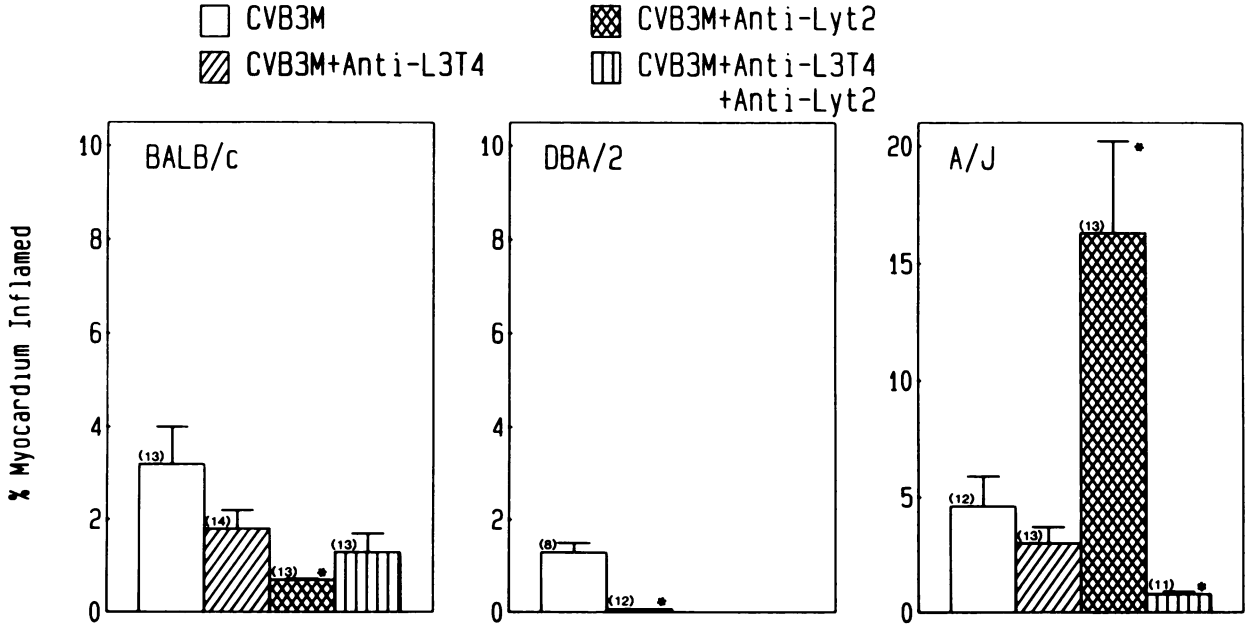

Figure 6—*In vivo* depletion of T-cell subpopulations. Mice were given injections of monoclonal antibody to T-helper (anti-L3T4) and T-cytolytic/suppressor (anti-Lyt 2) cells 2 and 1 day before and 1 day after inoculation with virus. BALB/c and DBA/2 mice were sacrificed 7 days after infection. A mice were sacrificed 14 days after infection. Hearts were evaluated for cardiac inflammation. Results represent the mean percent myocardium inflamed + SEM. Number of animals/group in parenthesis. *Results statistically different from those of mice given virus only at $P < 0.01$.

nate IgG HRA as a factor in late myocarditis. Humoral autoimmune-induced tissue injury presumably lasts as long as antibody persists. Thus, in DBA/2 mice where HRA antibodies disappear after Day 10, cardiac damage rapidly resolves, while in A animals which have autoantibodies through Day 56, myocarditis progresses. The reasons humoral responses differ between these two strains are not known but may reflect 1) the lower incidence and mean titer of IgG HRA in DBA/2 than in A mice, 2) the antigenic specificities recognized by the HRA in the two strains, or 3) the longer persistence of virus in A than in DBA/2 mice. The last possibility suggests that HRA responses are stimulated by antigens released from infected or damaged myocytes. Once virus is eliminated from the organ, less stimulation of the immune system occurs, and consequently antibody titers decrease. While this explanation is speculative, virus is eliminated quickly in BALB/c and DBA/2 mice, and both strains have only acute myocarditis, while infection is protracted in A animals and cardiac damage persists.

The difference in viral clearance may be due to several factors. Virus-neutralizing antibody and nonspecific cellular immunity (natural killer cells and macrophage) are predominantly involved in eliminating virus from the host.[19,20] Both BALB/c and DBA/2 mice generate maximal CVB3 antibody responses between 7 and 10 days after infection, but responses in A animals are considerably delayed until Day 27. In addition, A strain mice have decreased natural killer cell activity and poor macrophage function.[21,22] These factors combined may explain the prolonged infection of A animals.

Although chronic myocarditis correlates best with virus persistence in these three strains, clearly virus is not directly responsible for cardiac injury. Fifty-eight percent of A mice at Day 27 and 100% of these mice at Day 56 had no detectable virus in their hearts; yet all animals showed significant cardiac inflammation. Therefore, myocarditis can continue for many weeks after infectious virus elimination. One may wonder whether undetected virus remains and contributes to cardiac injury. Bowles et al[23] report 53% of myocardial biopsies removed from myocarditis and cardiomyopathy patients have evidence of persistent coxsackieviral genomic material, while only rarely is infectious virus present in the heart. Chronically infected myocytes might show decreased or aberrant function, compared with healthy cells, and ultimately lead to cardiomyopathy. Secondly, such cardiocytes could express neoantigens recognized by infiltrating inflammatory cells. Because cardiac injury in this murine model is mediated exclusively by immunopathogenic mechanisms (removal of one or both T-cell subpopulations prevent myocarditis), virus contribution to chronic disease should depend predominantly on neoantigen expression.

While duration of infection might influence disease chronicity, the amount of viral replication probably does not affect myocarditis severity, because maximal virus concentrations in the three strains are equivalent. Interestingly, mild disease occurs in DBA/2 and BALB/c mice where the immunopathogenic mechanism depends predominantly on only one T-cell subset ($L3T4^+$ cell-dependent humoral immunity in DBA/2 and Lyt 2^+ cell-dependent cell-mediated immunity in BALB/c). Cardiac damage is twice as severe in A mice where both $L3T4^+$ and Lyt 2^+ cell-dependent responses develop. The simplest explanation could be that cardiac injury mediated by these two immunopathogenic mechanisms is additive. Alternatively, either a more vigorous immune response in A strain mice or greater susceptibility of A strain cardiocytes to immune-mediated destruction could produce the aggravated disease observed in these animals. $L3T4^+$ cell-dependent mechanisms alone can be extraordinarily pathogenic, as demonstrated in A animals depleted of Lyt 2.2^+ cells. The increased inflammation could not be due to more extensive infection, because viral titers in the hearts of these mice were not increased. Possibly suppressor cells are important in limiting inflammation in A mice, and the loss of Lyt 2.2^+ suppressor cells results in a runaway immune response. More effective suppressor cell generation in BALB/c and DBA/2 mice might explain the less severe disease noted in these animals.

References

1. Reyes MP, Lerner AM: Coxsackievirus myocarditis with special reference to acute and chronic effects. Prog Cardiovasc Dis 1985, 27:373–394
2. Matsumori A, Kawai C, Sawada S: Encephalomyocarditis virus myocarditis in inbred strains of mice: Chronic stage. Jpn Circ J 1982, 46:1192–1196
3. Matsumori A, Kawai C: Coxsackievirus B-3 perimyocarditis in Balb/c mice: Experimental model of chronic perimyocarditis in the right ventricle. J Pathol 1980, 131:97–106
4. Wolfgram LJ, Beisel KW, Hershkowitz A, Rose NR: Variations in the susceptibility to coxsackievirus B-3 induced myocarditis among different strains of mice. J Immunol 1986 136:1846–1852
5. Gauntt CJ, Gomez PT, Duffey PS, Grant JA, Trent DW, Witherspoon SM, Paque RE: Characterization and myocarditic capacities of coxsackievirus B3 variants in selected mouse strains. J Virol 1984, 52:598–605
6. Huber SA, Lodge PA: Coxsackievirus B-3 myocarditis: Identification of different pathogenic Mechanisms in DBA/2 and Balb/c mice. Am J Pathol 1986, 122:284–291
7. Woodruff JF: Viral myocarditis: A review. Am J Pathol 1980, 101:427–483

8. Guthrie M, Lodge PA, Huber SA: Cardiac injury in coxsackievirus group B, type 3 induced myocarditis in Balb/c mice is mediated by Lyt 2^+ cytolytic lymphocytes. Cell Immunol 1984, 88:558–566
9. Huber SA, Lodge PA: Coxsackievirus B-3 myocarditis in Balb/c mice: Evidence for autoimmunity to myocyte antigens. Am J Pathol 1984, 116:21–29
10. O'Connell JB, Reap EA, Robinson JA: The effects of cyclosporin on acute murine coxsackie B3 myocarditis. Circulation 1986, 73:353–359
11. Monrad ES, Matsumori A, Murphy JC, Fox JG, Crumpacker CS, Abelmann WH: Therapy with cyclosporin in experimental murine myocarditis with encephalomyocarditis virus. Circulation 1986, 73:1058–1064
12. Estrin E, Smith C, Huber SA: Coxsackievirus B3 myocarditis: T-cell autoimmunity to heart antigens is resistant to cyclosporin A treatment. Am J Pathol 1986, 125:244–251
13. Estrin M, Huber SA: Coxsackievirus B3 induced myocarditis: Autoimmunity is $L3T4^+$ T helper cells and IL-2 independent in BALB/c mice. Am J Pathol 1987, 127:335–341
14. Estrin M, Herzum M, Lodge PA, Huber SA: Immunosuppressives in myocarditis. Eur Heart J (In press)
15. Wong CY, Woodruff JJ, Woodruff JF: Generation of cytotoxic lymphocytes during coxsackievirus B-3 infection: I Model and virus specificity. J Immunol 1977, 118:1159–1164
16. Cobbold SP, Waldmann H: A rapid solid-phase enzyme-linked binding assay for screening monoclonal antibodies to cell surface antigens. J Immunol Methods 1981, 44:125–130
17. Kishimoto C, Kuribayashi K, Masuda T, Tomioka N, Kawai C: Experimental and immunological studies in viral myocarditis and dilated cardiomyopathy: Significance of T lymphocytes in the severity of myocarditis, Myocarditis and Related Disorders. Edited by M Sekiguchi, EGJ Olsen, JF Goodwin. Tokyo, Springer-Verlag, 1985, pp 218–220
18. Huber SA, Lyden DC, Lodge PA: Immunopathogenesis of experimental coxsackievirus induced myocarditis: Role of autoimmunity. Herz 1985, 10:1–10
19. Woodruff JF: Lack of correlation between neutralizing antibody production and suppression of coxsackievirus B3 replication in target organs: Evidence for involvement of mononuclear inflammatory cells in host defense. J Immunol 1979, 123:31–36
20. Godeny EK, Gauntt CJ: Involvement of natural killer cells in coxsackievirus B3 induced murine myocarditis. J Immunol 1986, 137:1695–1702
21. Kiessling R, Wigzell H: An analysis of the murine NK cell as to structure, function and biological relevance. Immunol Rev 1979, 44:165–208
22. Skamene E, James SL, Meltzer MS, Nesbitt MN: Genetic control of macrophage activation for killing of extracellular targets. J Leukocyte Biol 1984, 35:65–69
23. Bowles NE, Richardson PJ, Olsen EGJ, Archard LC: Detection of coxsackie-B-virus-specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. Lancet 1986, 2:1120–1122