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Differences in Cytolytic T Cell Response of BALB/c Mice Infected with Myocarditic and Non-Myocarditic Strains of Coxsackievirus Group B, Type 3

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Two strains of coxsackie B-3 virus, indistinguishable by neutralization with acute mouse antiserum to coxsackie B-3 group virus, differ markedly in pathogenicity. One strain induces extensive mononuclear cell infiltration and necrosis in the heart, and 92% of the infected animals die by day 10 after infection. Inflammation, cardiac damage, and mortality are reduced in mice infected with the nonmyocarditic virus. Peak virus replication occurs on day 3 with both viruses. Virus concentrations decrease to undetectable levels after day 12 of infection. Cytolytic T cells could be isolated from the spleens of animals inoculated with either virus. The spleen cells preferentially lysed target cells infected with the immunizing virus strain. These results suggest that the myocarditic and nonmyocarditic viruses induce only minimally noncross-reacting virus-specific antigens on the target cell surface.

Coxsackie B group viruses are a common cause of viral myocarditis in humans (16–18). However, not all who become infected develop myopericarditis, and the severity of the disease and of the permanent sequela resulting from it differ markedly among individuals (8, 18). This variability in the pathogenicity of infection may result from either host factors, including genetic, physiological, and environmental differences (2, 5, 9, 13, 20), or from differences in the virus (3, 4, 7, 16).

Two strains of coxsackievirus group B type 3 (CVB3) have been described which induce significantly different amounts of myocarditis in CD-1 mice (7, 15). These variants are indistinguishable by infectious or replicative properties in HeLa cells in vitro or in the heart in vivo (7). Nor does the nonmyocarditic coxsackie B-3 virus (CVB3N) induce greater levels of interferon or interfering particles in infected cells (7). Therefore, both virus strains appear equally capable of reaching and infecting the heart.

Cellular immunity has been shown to play an important role in the pathogenesis of myocarditic CVB3 (CVB3M) infections. Cytolytic T cells, present in the spleens of infected mice, are capable of lysing both infected and uninfected myofibers in vitro at times correlating to inflammation in the heart (10, 11). Secondly, T-lymphocyte-deficient mice fail to develop significant myocarditis after infection with CVB3M. Adoptive transfer of T cells into these animals restores their susceptibility to the disease (22). It has been suggested that the failure of CVB3N to induce myocarditis results from the inability of the virus to induce readily recognizable virus-specific antigens on the myofiber cell surface (14). The absence of this antigen could mean either that a T-lymphocyte immune response would not be induced in CVB3N-infected mice, or that any T cells induced would not be able to lyse infected myofibers in vitro or in vivo. The purpose of this work was to investigate whether T cell immunity occurred in CVB3N-infected animals and to determine whether differences in T-cell responses to CVB3N and CVB3M could explain the variations in myocarditis produced by these viruses.

MATERIALS AND METHODS

Animals. BALB/c mice were purchased originally from Cumberland Farms, Clinton, Tenn. Neonates and 6- to 8-week-old adult males were obtained from colonies maintained at this institution.

Virus preparation and purification. A "myocarditic" coxsackievirus B-3 (Nancy strain) (CVB3M) (adapted to the heart by J. F. Woodruff, Cornell University Medical College) and a second strain of the same virus (kindly supplied by Richard Crowell, Hahnemann Medical College, Philadelphia, Pa.) which produces little or no myocarditis (designated CVB3N), were passaged three times in HeLa cell monolayers (Gey strain; Flow Laboratories, Inc., McLean, Va.) and thereafter were grown in HeLa cell suspensions (Mandel strain; courtesy of Richard Crowell) by a previously described technique (10). The maintenance medium was Joklik modified minimal essential medium (Flow) containing double-strength essential amino acids and vitamins in Hanks basic salt solution, 5% fetal calf

serum (FCS), and antibiotics (100 μg of streptomycin and 100 U of penicillin per ml). Virus purification was performed by using CsCl gradients according to the method of Oberg and Philipson (12). The titers of purified virus ranged from 9×10^{10} to 10×10^{10} PFU/ml; samples were stored at −70°C in phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+}. Purified virus was used in all experiments.

Infection of mice. Each animal was infected by intraperitoneal inoculation of 0.5 ml of PBS containing 10^4 PFU of purified coxsackievirus B-3 and was sacrificed 3 to 39 days after infection.

Titration of virus in organs and neutralizing antibody in the serum. Organ virus titers were obtained by homogenization of the organs in minimal essential medium, centrifugation of the cellular debris, and titration of the viruses by plaque formation on monolayers of HeLa cells as described previously (4). For serum neutralizing antibody titrations, blood was obtained from the heart, and the serum was harvested, heat inactivated at 56°C for 30 min, and stored at −20°C. Samples from individual animals were assayed by incubation of twofold dilutions of the serum with 100 PFU of coxsackie B-3 virus for 45 min before the addition to monolayers of HeLa cells in the PFU assay. A 50% reduction of PFU was taken as the endpoint (19).

Spleen cell suspensions. Spleens from three to five mice were pooled and teased in cold basal medium Eagle (BME) containing 5% FCS, 100 μg of streptomycin per ml, and 100 U of penicillin per ml. The cells were washed twice ($300 \times g$ for 10 min), suspended in medium, layered on a Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), and centrifuged at $178 \times g$ for 10 min; the cells at the interface were removed and washed with medium. Adherent cells were removed by incubating spleen cells in plastic petri dishes (60 by 15 mm; Falcon Plastics, Oxnard, Calif.) at a concentration of 20×10^6 cells per ml for 1 h at 37°C. The nonadherent cells were harvested, washed once, and suspended in BME containing FCS at a concentration of 10^7 viable nucleated cells per ml. These cells were kept on ice until they were used in cytotoxicity assays. Cell viability was at least 90%, as assayed by trypan blue dye exclusion.

Preparation, culture, and characterization of myofibers. Hearts were removed aseptically from BALB/c mice within 48 h of birth. Single-cell suspensions of myofibers were prepared by using a modification of the procedure of Bollon et al. (1). Briefly, hearts were minced and then were subjected to stepwise enzymatic digestion with 0.25% pancreatin (GIBCO Laboratories, Grand Island, N.Y.). The isolated cells were washed with BME containing penicillin, streptomycin, 5% FCS, 10% horse serum, 0.2 mg of crystalline insulin, and 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (complete BME) per 100 ml of medium and then were depleted of endothelial cells and fibroblasts by two sequential 1-h adsorptions in 25-cm^2 plastic flasks (Falcon) at 37°C. The nonadherent myofibers were recovered, washed once, resuspended in complete BME, and dispensed into 6-mm tissue culture wells (Linbro Chemical Co., Hamden, Conn.). After 48 h (the time needed for these cells to become firmly adherent to plastic), the myofibers were used in the cytotoxicity assay.

In a separate experiment, 2×10^6 myocardial cells in complete BME were seeded onto cover slips (9 by 22 mm) and maintained in Leighton tubes (16 by 100 mm; Wheaton Scientific, Millville, N.J.) for examination by light microscopy. At 48 h, the cultures contained individual cells and cell clusters which contracted rhythmically (50 to 80 beats per min). In addition, 90% or more of the cells were identified as muscle fibers after staining with phosphotungstic acid-hematoxylin; sarcomeres were observed under light microscopy.

Cytotoxicity assay. The technique for the cytotoxicity assay has been described in previous reports (11, 20). Briefly, 7×10^4 neonatal myofibers in 0.2 ml of complete BME were dispensed into 6-mm plastic tissue culture wells and incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 48 h, the cells were infected with 100 PFU of virus per cell, a dose known to result in infection of 75% of the myofibers by 16 h. After 1 h, unabsorbed virus was removed by washing the monolayers. Target cells not exposed to virus were processed in identical fashion. Myofibers were then labeled by adding 5 μCi of ^{51}Cr to each well ($Na_2{}^{51}CrO_4$; Amersham/Searle, Arlington Heights, Ill.). After 45 to 60 min at 37°C, the monolayers were washed three times and overlaid with 0.2 ml of immune or nonimmune spleen cells in BME-FCS or with medium alone. The cultures were incubated at 37°C for 18 h at a spleen cell-to-target cell ratio of 150:1. The ^{51}Cr levels in the supernatants and in the cells were determined as described previously (11, 22); radioactivity was measured by using an Intertechnique CG 4000 gamma counter. ^{51}Cr release was calculated by using the following expression: [(counts per minute in supernatant)/(counts per minute in supernatant + counts per minute in cells)] × 100.

Cytotoxicity was expressed as the percentage of lysis, as calculated by the following expression: [(average percentage of ^{51}Cr released from test group) − (average percentage of ^{51}Cr release from medium group)]/[(average percentage released after freeze-thaw) − (average percentage released from medium group)] × 100.

The percentage of specific lysis represented the percentage of lysis by sensitized lymphocytes minus the percentage of lysis by nonimmune lymphocytes.

Anti-Thy 1.2 and anti immunoglobulin serum treatment of spleen cells. Monoclonal anti-Thy 1.2 serum (OLAC Ltd., Blackthorn, England) was titrated as described previously (11). The antiserum used in these experiments had a cytotoxicity titer against 1.25×10^6 BALB/c thymocytes of $1:5 \times 10^5$. Lysis of Thy 1.2-positive cells was performed as described previously (10, 11), using a 1:1,000 dilution of anti-Thy 1.2 serum and a 1:5 dilution of guinea pig complement (GIBCO) which had been absorbed with BALB/c thymocytes. This treatment increased the percentage of immunoglobulin-containing spleen cells from 43 to 91%.

Affinity purified rabbit anti-mouse immunoglobulin serum (Bionetics Laboratory Products, Kensington, Md.) was used at a 1/10 dilution. This treatment increased the percentage of Thy 1.2-positive cells in the spleen from 23 to 57%.

Histology. Organs were fixed in 10% buffered Formalin fixative and stained with hematoxylin and eosin. The extent of myocardial necrosis and inflammation was graded on a scale of 0 to 4 as described in detail elsewhere (21). Briefly, for necrosis a score of 0 indicates no necrotic or hypereosinophilic myofibers

per section, whereas a score of 4 indictaes widespread frank necrosis of myofibers per section. Inflammation was scored on the basis of the number of foci of inflammatory cells per section, with 0 indicating no inflammation and 4 denoting more than 90 foci. The mean score for myocardial necrosis and inflammation per group of animals was calculated from the scores of the individual animals.

Statistical analysis. The Student *t* test was used to analyze the significance of differences among groups.

RESULTS

Comparison of virus concentration in the hearts of BALB/c mice infected with CVB3M and CVB3N to the severity of cardiac injury induced. Male BALB/c mice were infected with 10^4 PFU of CVB3M or CVB3N. At intervals from 3 to 39 days later, the hearts were removed and examined for virus concentrations, inflammation, and necrosis (Table 1). Sera were obtained from mice infected 6 and 12 days earlier and assayed for virus-neutralizing antibody. Virus concentrations were maximum on day 3, decreased rapidly thereafter, and were generally not detected in the hearts after day 12. No significant differences were observed in either cardiac virus or serum antibody titers between the CVB3M and CVB3N groups, yet by day 6 postinfection, mice injected with CVB3M developed significantly greater amounts of inflammation and necrosis. In addition to increased myocarditis lasting throughout the 39-day experimental period, the myocardia of CVB3M-infected animals suffered apparent permanent cardiac injury, characterized by extensive interstitial fibrosis and calcification. Similar damage was not observed in CVB3N-infected animals (Fig. 1). The relative pathogenicity of the two virus variants was also reflected by cumulative animal mortality after infection. Two groups of 24 animals each were infected with either CVB3M or CVB3N. Animals began to die in the CVB3M group 3 days postinfection, with 58% of the mice dying by day 9. No animals in the CVB3N group died before day 10, although 58% died during the 39-day observation period. The delayed mortality in the CVB3N group probably does not reflect cardiac damage since little inflammation or necrosis of the myocardium was evident. However, CVB3 viruses can infect a wide range of tissues, and death may occur from irreparable damage to some organ other than the heart.

Demonstration that CVB3N and CVB3M virions are antigenically similar. Despite the differnces in the pathogenicity of infection with CVB3N and CVB3M, the virus variants appeared very close antigenically. Sera from individual mice obtained 6 days after infection with either CVB3M or CVB3N neutralized both viruses equally (Table 2). A second indication of the antigenic similarity of the viruses is shown in double diffusion Ouchterlony between hyperimmune rabbit antiserum to CVB3 and the viruses which results in a single line of identity (data not shown).

Characterization of cellular immune responses

TABLE 1. Characteristics of infection of BALB/c with two strains of coxsackie B-3 virus[a]

Days after infection	CVB3M-infected mice				CVB3N-infected mice			
	Virus titer[b]	Lesions (N/I)[d]	Serum-neutralizing antibody titer[f]	% Cumulative mortality[g]	Virus titer[b]	Lesions (N/I)[d]	Serum-neutralizing antibody titer[f]	% Cumulative mortality[g]
3	8.4 ± 2.3 (NS)[c]	0.7 ± 0.4		4	4.0 ± 1.6	0.5 ± 0.4		0
		0.5 ± 0.4				0.5 ± 0.4		
6	2.2 ± 1.3 (NS)	0.8 ± 0.2[e]	320 ± 65 (NS)	33[e]	0.9 ± 0.6	0.0 ± 0.0	256 ± 39	0
		1.0 ± 0.2[e]				0.5 ± 0.1		
9		1.6 ± 0.7[e]		58[e]		0.2 ± 0.2		0
		1.6 ± 0.6[e]				0.5 ± 0.1		
12	0.003 ± 0.0 (NS)		544 ± 64 (NS)	92[e]	0.012 ± 0.004		628 ± 187	25
39		3.0 ± 0.6[e]		96[e]		0.3 ± 0.2		58
		0.7 ± 0.2				0.2 ± 0.1		

[a] Mice were inoculated with 10^4 PFU of virus intraperitoneally in 0.5 ml of PBS; two to eight animals were used per group.

[b] PFU × 10^5 per heart ± standard error of the mean of six to eight animals per group.

[c] NS, Not significantly different from the value obtained with nonmyocarditic virus.

[d] N, Necrosis; I, inflammation. Lesions were graded on a scale of 0 to 4. Each value is the mean score of 2 to 10 animals in each group ± standard error of the mean.

[e] Significantly different from the value obtained with nonmyocarditic virus at $P \leq 0.05$.

[f] Reciprocal of the arithmetic mean ± standard error of the mean.

[g] Twenty-four mice.

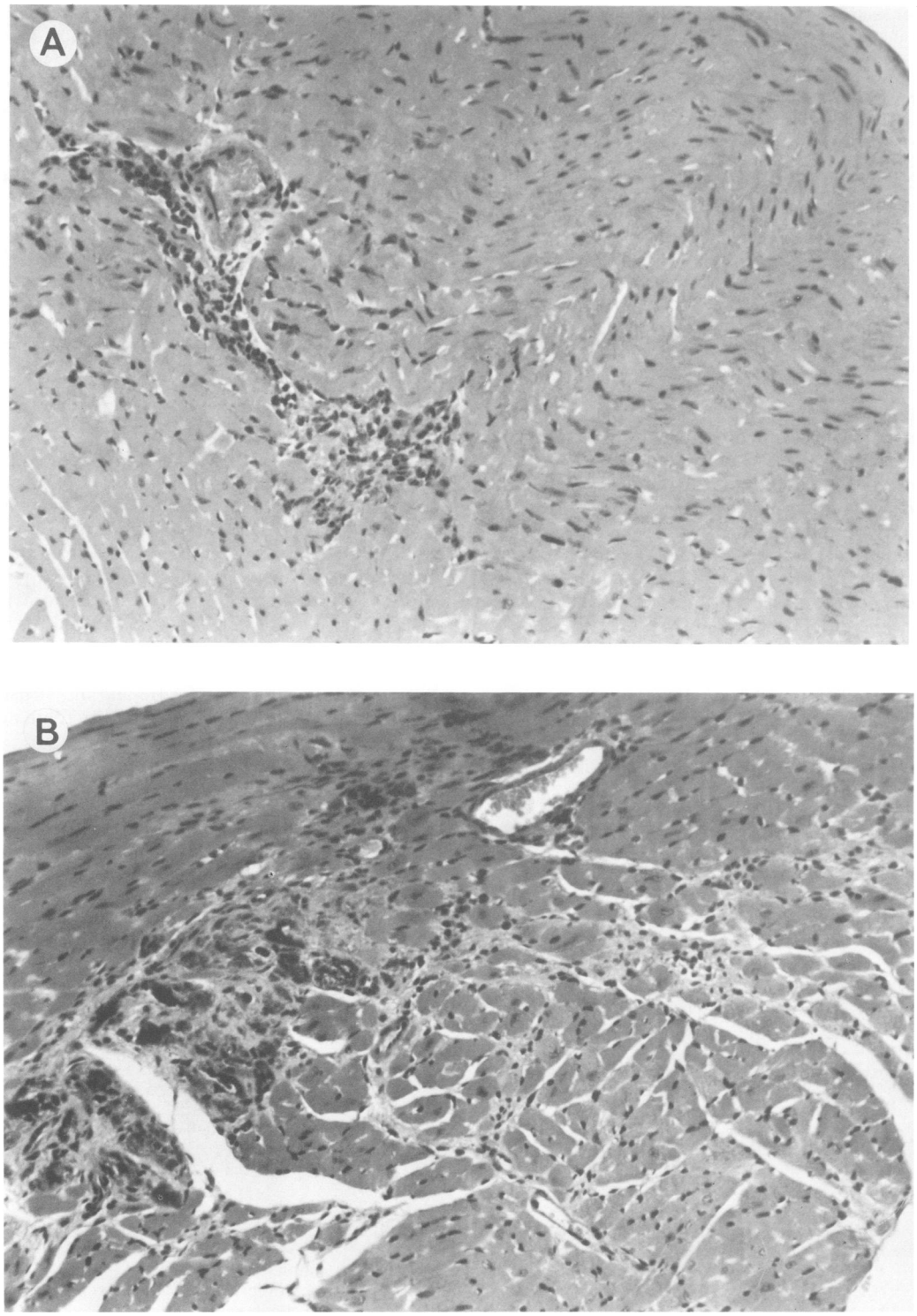

FIG. 1. Hearts from BALB/c male mice infected 6 and 39 days earlier with 10^4 PFU of CVB3M (A and B), respectively, and CVB3N (C and D, respectively). Tissues were stained with hematoxylin and eosin. Magnification, approximately 160×.

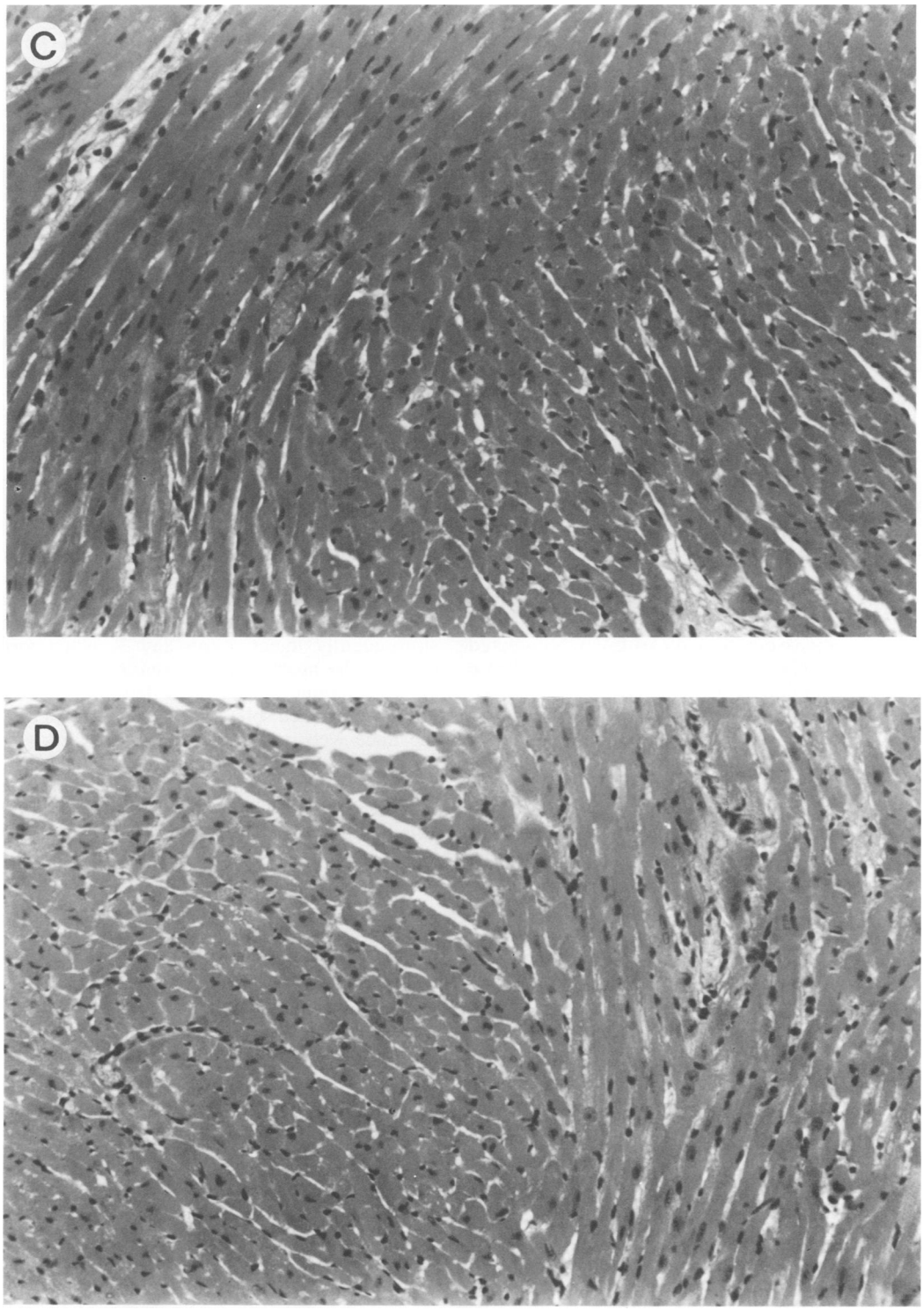

FIG. 1—*Continued*

TABLE 2. Neutralization of CVB3N and CVB3M, using mouse antisera to CVB3

Serum source[a]	Serum neutralization titer[b]	
	CVB3M	CVB3N
BALB/c anti CVB3M	640 ± 72	667 ± 105
BALB/c anti CVB3N	853 ± 67	747 ± 67

[a] Sera were obtained from BALB/c mice injected intraperitoneally 6 days earlier with 10^4 PFU of virus in 0.5 ml of PBS.

[b] Reciprocal of arithmetic mean of sera from five individual mice per group ± standard error of the mean.

to CVB3N and CVB3M. Spleens were removed from mice infected 3 to 39 days earlier with 10^4 PFU or CVB3M or CVB3N and were assayed on uninfected myofibers and cells infected with CVB3N or CVB3M (Table 3). CVB3M-immune spleen cells showed cytolytic activity to both uninfected and CVB3M-infected myofibers but had only minimal significant cytolytic activity to CVB3N-infected targets on day 12. Spleen cells from CVB3N-infected mice obtained before day 39 failed to lyse uninfected target cells (before day 39) and lysed only 14% of CVB3M-infected cells, whereas cytotoxicity to CVB3N-infected myofibers was 44.5%. These results indicate that CVB3N-infected myofibers do express virus-related antigens on the cell surface which can induce and react with immune lymphocytes. However, the virus-related antigens of CVB3N and CVB3M appear largely antigenically distinct and only minimally cross-reactive or not at all.

Cytolytic activity on or after day 6 was mediated by T lymphocytes. The spleen cells from CVB3N- and CVB3M-infected mice were treated with anti-Thy 1.2 or anti mouse immunoglobulin serum and complement before assay. Cytolysis of uninfected and CVB3M-infected myofibers by CVB3M-immune cells was significantly reduced by the anti-Thy 1.2 serum but was not affected by anti-immunoglobulin (Table 4). Similarly, treatment of CVB3N-immune cells with anti-Thy 1.2 reduced activity to CVB3N target cells. Interestingly, although untreated CVB3M- and CVB3N-immune cells had no activity to CVB3N-infected and uninfected myofibers, respectively, treatment with either antiserum and complement enhanced the reactivity of the remaining cells. This increased cytotoxicity probably represents natural killer cell activity since sequential treatment of the spleen cells with both anti-immunoglobulin and anti-Thy 1.2 serum and complement failed to remove the activity and the non-T, non-B cells were also lytic to HeLa cells.

Since both CVB3N and CVB3M infections result in the induction of significant virus-specific T cell immunity, it was considered possible that only the T cell activity to uninfected myofibers noted early during CVB3M infections was important in inducing myocarditis. Therefore, mice were infected with CVB3M or CVB3N and subsequently injected (on day 3, when virus titers in the heart would be maximal) with 3×10^7 nonimmune or day 6 CVB3M- or CVB3N-immune enriched spleen T cells. Four days later, the mice were sacrificed, and the hearts were examined for myocarditis (Table 5). Transfer of nonimmune or immune T cells into uninfected recipients failed to induce noticeable myocardial damage indicating (i) that any virus transferred in the spleen cells was insufficient by itself to induce myocarditis and (ii) that immune cells

TABLE 3. Cytolytic activity of spleen cells from mice infected with two strains of coxsackie B-3 virus to virus-infected and uninfected myofibers[a]

Spleen immune to:	Days after inoculation	% Specific lysis[b]		
		Uninfected myofibers	CVB3M-infected myofibers	CVB3N-infected myofibers
CVB3M	3	25.2 ± 3.5[c]	19.4 ± 1.0[c]	0.5 ± 0.5
	6	13.0 ± 2.9[c]	22.5 ± 3.2[c]	1.4 ± 2.7
	9	4.3 ± 0.6	25.2 ± 5.2[c]	8.3 ± 3.5
	12	−4.7 ± 1.4	46.0 ± 3.3[c]	9.2 ± 1.7[c]
CVB3N	3	−2.0 ± 1.8	1.9 ± 0.3	10.7 ± 5.7
	6	−6.1 ± 1.7	14.0 ± 1.7[c]	44.5 ± 3.9[c]
	9	−3.5 ± 1.0	4.1 ± 1.5	30.7 ± 3.5[c]
	12	3.6 ± 1.2	−8.5 ± 2.0	−2.6 ± 1.7

[a] Mice were inoculated with 10^4 PFU of virus intraperitoneally in 0.5 ml of PBS.

[b] Spleen cells were inoculated on uninfected or virus-infected myofibers at an effector/target cell ratio of 150:1 and an incubation time of 18 h. Spontaneous ^{51}Cr release was 33.0, 26.9, and 34.4% for the uninfected, CVB3M-infected, and CVB3N-infected targets, respectively. Percent lysis with nonimmune lymphocytes on the three targets was −10.0, 5.0, and −0.6%. Data are reported as mean percent specific lysis ± standard error of the mean of two experiments.

[c] Significantly greater than 0% at $P \leq 0.05$.

TABLE 4. Demonstration that cytolytic activity is mediated by T lymphocytes

Spleen cells immune to[a]:	Treatment[b]	% Specific lysis[c]			
		Uninfected myofibers	CVB3M-infected myofibers	CVB3N-infected myofibers	HeLa cells
CVB3N	Untreated	−12.3 ± 4.7	1.9 ± 5.7	54.8 ± 8.3	28.9 ± 2.5
	Anti Ig + C	−2.7 ± 6.2	4.5 ± 5.8	53.9 ± 12.4	27.7 ± 9.8
	Anti Thy 1.2 + C	−12.0 ± 5.5	11.9 ± 8.4	5.3 ± 5.0	22.1 ± 4.2
	Anti Ig + C + anti Thy 1.2 + C	1.2 ± 8.5	44.1 ± 13.4[d]	42.6 ± 10.3	31.8 ± 7.1
CVB3M	Untreated	12.8 ± 3.0	41.8 ± 11.9	19.5 ± 10.4	26.9 ± 2.4
	Anti Ig + C	8.8 ± 1.1	26.4 ± 7.3	19.8 ± 3.9	19.9 ± 6.1
	Anti Thy 1.2 + C	−17.3 ± 6.5[d]	4.7 ± 4.9[d]	−5.7 ± 8.9	21.8 ± 6.0
	Anti Ig + C + anti Thy 1.2 + C	5.6 ± 9.0	−1.1 ± 6.9[d]	19.3 ± 5.6	26.1 ± 5.7

[a] Mice were inoculated with 10^4 PFU virus intraperitoneally in 0.5 ml of PBS 6 days before assay.

[b] All spleen cells were depleted of adherent cells. Samples were then treated with antisera to either Thy 1.2 or mouse immunoglobulin (Ig) and complement (C).

[c] Mean percent specific lysis ± standard error of the mean of three experiments. The effector/target cell ratio used was 150:1, and the incubation period was 18 h. Mean spontaneous ^{51}Cr release ranged from 29.7 to 45.0%. Percent lysis with nonimmune lymphocytes ranged from −5.0 to 17.7%.

[d] Significantly enhanced or decreased lysis relative to untreated spleen cells at $P \leq 0.05$.

alone could not produce cardiac injury. Transfer of CVB3M T cell-enriched populations into CVB3N-infected recipients resulted in enhanced myocarditis in the recipients. CVB3N T cell-enriched populations were fully capable of inhibiting myocarditis in either CVB3N- or CVB3M-infected mice.

DISCUSSION

Different substrains of CVB3 vary markedly in the induction of myocarditis in inbred BALB/c mice. The decreased pathogenicity of CVB3N does not represent an inability to infect and replicate in myofibers in vitro or in vivo, and CVB3N and CVB3M virions appear antigenically identical by reaction with CVB3-specific antibody. However, myofibers infected with CVB3N and CVB3M express noncross-reactive antigens on the cell surface which can be recognized by T lymphocytes.

Cellular immunity in the CVB3M-infected host is important to the induction of myocarditis. T lymphocytes cytolytic to both infected and uninfected myofibers are readily demonstrated in the spleens of CVB3M-infected mice at times correlating to maximum inflammation and necrosis in the heart (10, 11). In addition, mice made deficient of T lymphocytes before infection do not develop significant myocarditis, although virus concentrations in the heart equal those in moribund T lymphocyte-sufficient littermates (22). The most likely hypothesis for T cell involvement in this disease is that the T lymphocytes directly mediate cardiac injury by lysis of the myofibers. However, virus-specific cytolytic T cells are also induced in CVB3N-infected mice which are as active in lysing CVB3N-infected myofibers in vitro as CVB3M-immune cells are in lysing CVB3M-infected targets. Neither CVB3N- nor CVB3M-immune cells appear capable of recognizing myofibers infected with the other CVB3 strain. Despite the differences in the antigenic specificity of the cytolytic T cells, it is surprising that CVB3N-immune T cells do not induce significant cardiac damage in vivo, since the virus is itself lytic to infected cells.

CVB3M-immune spleen cells are consistently lytic to uninfected myofibers, a phenomenon not found with CVB3N-immune cells. One explanation for these results is that there are "autoreactive" T cells induced in CVB3M infection which recognize myofiber-specific antigens. These myofiber-specific antigens must either be lacking in infected cells, or, more probably, are significantly altered by virus-related antigens expressed on these cells, preventing the recognition of the original myofiber-specific antigen by autoreactive T cells in CVB3M-immune spleen cells. The absence of this "autoimmune" reactivity and the minimal myocardial injury in CVB3N-infected mice are conducive to the theory that autoimmune T cells are more important than virus-specific immune T cells in this disease. There are two arguments to support this belief. First, virus concentrations in the heart rapidly diminish before the development of detectable T cell immunity in the host, and inflammation in the heart may persist for days or weeks after virus can no longer be detected. Therefore, major cardiac damage occurs when detectable virus infection of the cells is limited or not evident. Secondly, since the virus is itself lysogenic to infected cells, T cell-mediated lysis of infected target cells should not produce signif-

TABLE 5. Adoptive transfer of CVB3M-immune spleen T cells into recipient mice infected with CVB3N or CVB3M virus

Recipient infected with[a]:	Cardiac lesion score in mice adoptively transfused with[b]:			
	None	Nonimmune T lymphocytes	CVB3M-immune T lymphocytes	CVB3N-immune T lymphocytes
None	0 ± 0	0 ± 0	0 ± 0	0 ± 0
CVB3M	2.77 ± 0.32	2.31 ± 0.41	2.77 ± 0.25	0.95 ± 0.09[c]
CVB3N	1.09 ± 0.17	1.20 ± 0.34	2.10 ± 0.37[c]	0.75 ± 0.34

[a] Infected mice received 10^4 PFU of virus intraperitoneally in 0.5 ml of PBS.

[b] Recipient mice were injected intraperitoneally on day 3 of infection with 3.0×10^7 spleen T lymphocyte-enriched cells in 0.5 ml of PBS. The donor T cells were obtained by depletion of nonimmune or day 6 CVB3M-immune spleen cells of macrophage by adsorption to plastic, followed by treatment with antiserum to mouse immunoglobulin and complement. The recipient mice were sacrificed 7 days after infection. Values indicate the mean score ± standard error of the mean of 5 to 14 mice in each group.

[c] Significant decrease or increase in myocarditis compared with similarly infected animals not receiving spleen cells ($P \leq 0.05$).

icantly more myofiber destruction than virus infection alone. Furthermore, adoptive transfer of CVB3M spleen cells into CVB3N-infected mice results in levels of myocarditis similar to those obtained in CVB3M-infected animals. The increased myocarditis in the CVB3N recipients cannot be explained by inadvertent transfer of CVB3M virus since no myocarditis is induced in uninfected mice receiving CVB3M-immune spleen cells. The lack of myocardial injury in this group also suggests that immune cells in the absence of some insult to the heart are probably not able to migrate to and infiltrate the myocardium.

Although day 6 CVB3M-immune T cells can enhance myocarditis in CVB3N-infected animals when given early after infection, day 6 CVB3N-immune T cells inhibit the development of myocarditis in CVB3M-infected mice. We do not yet understand the mechanism by which the inhibition is occurring, and we are presently investigating this phenomenon. Several possibilities are under consideration. First, the day 6 CVB3N-immune spleen cell population may have greater numbers of suppressor cell precursors than CVB3M-immune spleen cells on the same day. When the CVB3N-immune cells are given on day 3, precursor suppressor T cells may be able to differentiate more quickly than the cytolytic cell precursors of the recipient, inhibiting cytolytic effector cell generation and subsequent cardiac damage. Alternate explanations include greater production-induction of interferon, natural killer cell, or virus neutralizing antibody by the CVB3N-immune cells. In each of these instances, virus may be more rapidly eliminated in the recipient, thus aborting myocarditis.

At present, we are not able to prove conclusively that the autoreactive rather than the virus-specific T cells in CVB3M-immune animals are more important to the induction of myocarditis since we have not tested the autoreactive and virus-immune cytolytic cells against one another. It is well known that virus-immune T cells recognize infected targets by both major histocompatibility and viral antigens (6, 11). It is therefore possible that CVB3M-immune cells lyse uninfected targets on the strength of the major histocompatibility antigens alone. Alternatively, autoreactivity in the CVB3M system may indicate secondary sensitization of the immune system to myofiber cell surface antigens. Further elucidation of the characteristics of the T cells lytic to both target cells is currently under investigation.

The nature of the virus-specific antigens induced by CVB3N and CVB3M on the myofiber are also not known. Since these viruses do not bud from the infected cell surface, it is not clear how virus-specific antigens would be expressed which could be recognized by the immune system. Recently, however, investigators using reoviruses, another nonbudding RNA virus, have shown that the viral hemagglutinating protein is the antigen recognized by cytolytic T cells in this system (6). Whether a similar antigen is important in coxsackieviral infections has not been thoroughly investigated. Whatever antigen is eventually determined to be recognized by the T cell, it appears likely that the B cells must respond to a different antigen in the virion. This conclusion is based on the observations that an acute mouse antiserum to CVB3N and CVB3M is unable to differentiate between the virus strains and that Ouchterlony between the viruses and a rabbit antiserum produces a single line of identity between the viruses (unpublished data). In addition, hyperimmune serum to coxsackie B-3 virus cannot lyse infected cells in the presence of complement and cannot inhibit T cell-mediated cytolysis (11), indicating that the antigen recognized by the immunoglobulin is not expressed on the target cell surface.

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