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Coxsackievirus-Induced Disease

CD4+ Cells Initiate Both Myocarditis and Pancreatitis in DBA/2 Mice

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DBA/2 male mice inoculated intraperitoneally with 1.8 × 10^5 plaque-forming units (PFU) coxsackievirus B-3 (CVB3) showed extensive inflammatory cell infiltration of the myocardium and acinar tissue of the pancreas in 7 days. Selective depletion of T lymphocyte subpopulations indicated that CD4 cells were either completely or partially responsible for cell damage in both organs. Other organs such as the liver were infected and contained virus titers equivalent to those seen in the heart and pancreas but showed no apparent tissue injury. The role of the CD4 cell was confirmed by positive selection of either T cell subpopulation from CVB3-immune lymphocytes in vitro and adoptive transfer of these cells into T cell-deficient (thymectomized, irradiated, bone marrow reconstituted, TXBM) DBA/2 recipients. Lymphocytes from CVB3-infected donor mice were adsorbed to myocyte, skin fibroblast, or liver vascular endothelial cell (VEC) monolayers. The adherent population was retrieved and adoptively transferred into uninfected syngeneic recipients. When killed 7 days later, the animals receiving unfractionated immune lymphocytes or cells eluted from heart monolayers developed both myocarditis and pancreatitis. Anti-Thy 1.2 and C' treatment of the unfractionated cells completely abrogated transfer of disease. Cells eluted from either fibroblast or liver VEC monolayers showed no pathogenicity. Adsorption of immune cells to heart monolayers in the presence of anti-IA^d (class II major histocompatibility complex antigen, MHC) inhibited attachment of the pathogenic T cell, whereas anti K^dD^d (a class I MHC antigen) had no effect. (Am J Pathol 1989, 135:899–907)

Autoimmunity can occur in humans and experimental animals after infections with viruses.[1-7] Resulting diseases may be highly tissue specific, causing injury to selected organs, or multiple organs may become involved.[8,9] In the latter instances, immune effectors recognize broadly distributed target antigens such as RNA, DNA, and cytoskeletal materials, or more restricted elements found on cells with similar origins or function (polyendocrine autoimmunity and myositis). In some experimental virus infections, pathologic alterations were not restricted to single organs but occurred widely throughout the body.[9,10] Thus, encephalomyocarditis (EMC) virus reportedly caused simultaneously myocarditis, encephalitis, and insulin-dependent diabetes in mice,[11] whereas reoviruses induced autoantibodies reacting to diverse endocrine tissues.[9] Generally, affected tissues must be susceptible to viral infection, but virus replication with cytolysis does not appear necessary for pathogenesis. Rather, in each instance, the inflammatory or immunologic response has been injurious.[4,7]

In the present model, CVB3 infected many organs including the liver, heart, and pancreas but only caused myocarditis and pancreatitis in the latter two organs. Extensive investigations into the immune mechanisms of myocarditis have already been done. These showed that autoreactive cytolytic T lymphocytes (ACTL), obtained by selective adsorption of CVB3 immune lymphocytes to primary myocyte monolayers were capable of lysing uninfected myocytes. Furthermore, these cells were major histocompatibility complex (MHC) antigen restricted and were primarily responsible for tissue injury.[12-14] The present study found that pancreatitis is also immune mediated and depends on the same T cell population that causes myocarditis.

Materials and Methods

Mice

DBA/2 mice were originally purchased from Jackson Laboratories, Bar Harbor, ME. Adult male (7 to 9 weeks) and

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neonatal (0 to 3 days) animals obtained from breeding colonies maintained at the University of Vermont were used for all experiments.

Thymectomy, Irradiation, and Bone Marrow Reconstitution (TXBM) of Mice

Mice were thymectomized by opening the anterior mediastinum through an incision in the neck and sternum extending to the second rib. The thymus was removed by aspiration. One week later, the animals received 700 R whole body irradiation and 5×10^6 syngeneic bone marrow cells intravenously through the tail vein. Bone marrow cells were obtained by flushing the tibia and femoral shafts of normal donor animals with Hank's balanced salt solution (HBSS). The cells were washed, treated with a 100 μg/ml anti-Thy 1.2 MAb, and 20% rabbit complement at 37 C for 30 minutes, washed again, and counted by trypan blue exclusion. TXBM mice were maintained on drinking water containing 125 mg/l tetracycline and were given a solution of 100 μg testosterone propionate (Sigma Chemical Co., St. Louis, MO) subcutaneously in olive oil 2 weeks before use.

Virus

A myocarditic variant of Coxsackievirus B3 (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.[12] Virus preparations were titered using a plaque-forming assay.[13] Aliquots of the virus in phosphate-buffered saline (PBS) were stored at −70 C.

Infection and Killing of Mice

Mice were infected intraperitoneally with 1.8×10^5 plaque-forming units (PFU) of virus in 0.5 ml of PBS. Mice were killed by sodium pentobarbital overdose.

Organ Virus Titers

Organs were removed aseptically, weighed, and homogenized in Dulbecco's minimal essential medium (DMEM) containing 2% fetal bovine serum (FBS) and antibiotics (100 units penicillin and 100 μg streptomycin/ml). Cellular debris was removed by centrifugation at 300*g* for 10 minutes. Supernatants were serially diluted and titered on HeLa cell monolayers in the plaque-forming assay.[13] Virus titers were expressed as the number of PFU present per gram of tissue.

Virus Neutralizing Antibody Titers

Blood obtained by cardiac puncture at the time animals were killed was allowed to clot. Serum was heat inactivated at 56 C for 30 minutes, serially diluted in DMEM-2% FBS, and combined (0.1 ml) with an equal volume of DMEM-2% FCS containing 100 PFU virus. The mixture was incubated at 37 C for 45 minutes and added to 60 mm^2 plates containing confluent HeLa cell monolayers. After incubation for 45 minutes at 37 C, the monolayers were covered with DMEM-2% FBS containing 0.6% agar, and the plaques were determined 2 days later. The neutralizing antibody titer was the dilution of antibody required to reduce the PFU 50%.

Monoclonal Antibodies (MAb)

Hybridoma clones producing MAb to the Pan T cell (Thy 1.2, clone 30H12), CD4 (anti-L3T4, clone GK1.5), and CD8 (anti-Lyt 2.1, clone 1C6-13.1) T cell marker antigens and to class I (K^dD^d, clone 34-7-23S) and class II (IA^d, clone MK-D6) major histocompatibility complex (MHC) antigens were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The CVB3 neutralizing monoclonal antibody, 8A6, was developed in this laboratory and was described previously.[15] Hybridoma cells were grown in ascites form in Balb/c mice treated with 0.5 ml of 2,6,10,14-tetramethylpentadecane (pristane, Sigma) and 500 R irradiation. Immunoglobulin was purified from ascites fluid by precipitation with 40% ammonium sulfate and Sephadex G10 chromatography (Pharmacia Inc., Piscataway, NJ).

Histology

Tissue sections were fixed in 10% buffered formalin and stained with hematoxylin and eosin (H&E). Inflammation was scored on a 0 to 4 scale by either KL or SAH with 0 representing no inflammation and 4 representing confluent lesions throughout the tissue section.

Glucose Tolerance Test (GTT)

Mice were bled from the retroorbital plexus before and 1 hour after receiving 2 mg glucose/g body weight intraperitoneally. Serum glucose values were determined on a YSI glucose analyzer Model 23A (Yellow Springs Instruments, Yellow Springs, OH), and expressed as mg of glucose per deciliter of serum (mg%).

Preparation and Culture of Myocytes

The procedure for preparing myocyte cultures was detailed earlier.[16] Briefly, hearts were asceptically removed from neonatal mice younger than 72 hours, minced, and subjected to stepwise enzymatic dissociation using 0.4% collagenase II (Cooper Biomedical, Freehold NJ). The cells were washed twice and resuspended in DMEM containing 5% horse serum (Gibco, Grand Island, NY), 5% FBS, and antibiotics at a concentration of 3×10^5 cells/ml. For immunoadsorption, 5 ml of the cell suspension was dispensed into 75 cm^2 tissue culture flasks (Corning Scientific Products, Corning, NY) and incubated for 48 hours at 37 C in a humidified NAPCO model 5300 CO_2 incubator (NAPCO, Tualatin, OR) containing a 6% CO_2 atmosphere. The monolayers were fixed by incubating with 0.3% glutaraldehyde for 30 seconds followed by thorough washing of the cells and overnight incubation in blocking buffer consisting of phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0.1% sodium azide.

Preparation of Skin Fibroblasts

Skin from neonatal DBA/2 animals was asceptically removed, minced finely, suspended in a 0.25% solution of trypsin, and digested at 37 C for 20 minutes with constant stirring. The supernatant was removed. The cells were recovered by centrifugation at 300*g* for 10 minutes, resuspended in DMEM-5% FBS, and dispensed into 75 cm^2 tissue culture flasks (Corning). After overnight incubation, the monolayer was washed free of cellular debris and fresh DMEM-5% FBS was added. When the culture reached confluence, the monolayer was glutaraldehyde fixed as indicated earlier.

Preparation of Liver Vascular Endothelial Cells (VEC)

Cultures of liver VEC were a gift of Dr. Carl Haisch (Department of Surgery, University of Vermont). Briefly, livers were obtained from Balb/c ($H\text{-}2^d$) mice, minced, and rinsed with two to three changes of PBS. Minced tissue was digested with a mixture of 0.2% collagenase and 0.01% DNase in DMEM containing 5% FBS. The digestion was carried out at 37 C for approximately 45 minutes followed by disruption of the tissue by repeated pipetting. The resulting cell suspension was centrifuged to pellet the cells. The cell pellet was resuspended in 55% isotonic Percoll (Pharmacia) and a gradient was formed by layering 37% Percoll, 29% Percoll, and saline on top. The gradient was centrifuged at 400*g* for 15 minutes. The top band of the gradient contained mostly dead cells and debris, whereas endothelial cells were found at the interface between 29% Percoll and 37% Percoll.

Cultures were maintained in DME/F12 containing 5% FBS, 20 U/ml heparin, 10 μg/ml endothelial cell growth supplement (Sigma), 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium, and 200 μg/ml Endo-Gro (VEC TEC). Endothelial cells were subcultured by brief (2-minute) treatment with 0.01% trypsin at 37 C. This further enriched the culture with endothelial cells because they detached from the surface first and fibroblasts were left behind.

Lymphocyte Preparation

Either spleens or mesenteric lymph nodes were obtained, pressed through a fine mesh screen, washed twice in DMEM, and depleted of red blood cells by flotation on ficoll-hypaque (Pharmacia). B cells and macrophages were removed by incubating the lymphocyte suspension with nylon wool for 30 minutes at 37 C. The nonadherent cells were counted for viability by trypan blue dye exclusion.

Positive Selection of T Cell Subpopulations

The MAb to CD4 and CD8 antigens (GK 1.5 and HB129, respectively) were adjusted to 1 mg/ml in coating buffer (sodium carbonate buffer, pH 9.6), and 5 ml was added to 100 mm^2 tissue culture plates (Corning). After overnight incubation at 4 C, the plates were washed and incubated for 4 hours at 37 C with 5 ml blocking buffer (PBS containing 1% bovine serum albumin and 0.1% sodium azide) and washed again. Approximately 5×10^7 mesenteric lymph node cells from DBA/2 donor mice inoculated 7 days earlier with CVB3 were added to each plate. The plates were incubated for 90 minutes at 4 C, washed to remove nonadherent cells, and gently scraped with a rubber policeman to recover attached cells. The recovered cells were washed and resuspended in HBSS, counted by trypan blue exclusion, and resuspended to 5×10^6 viable cells/ml.

Antibody and Complement Treatment of Lymphocytes

Approximately 2×10^7 lymphocytes were resuspended in DMEM containing 100 μg/ml anti-Thy 1.2 MAb and 20% rabbit complement (Gibco) and incubated at 37 C for 45 minutes. The cells were washed, resuspended in HBSS, and counted by trypan blue exclusion.

Enumeration of T Lymphocyte Subpopulations

The cells were resuspended in blocking buffer, incubated with monoclonal antibody to T cell markers for 30 minutes at room temperature, washed, and incubated with a 1:100 dilution of FITC-conjugated anti-rat IgG (for L3T4) (Sigma) or FITC-conjugated anti-mouse IgG (for Lyt 2.1) for 30 minutes at room temperature. The cells were washed and fixed in blocking buffer containing 1% paraformaldehyde. Flow cytometry was performed using an Ortho Diagnostics Systems 50-H cytometer with an argon laser excitation source tuned to 488 nm and 250 mw and a 2150 Data Acquisition processor (Ortho Diagnostics Systems, Inc., Westwood, MA). FITC fluorescence was measured through a 525-nm bandpass filter producing cytograms of forward *versus* 90 degree scatter. Histograms were analyzed by setting the threshold on the basis of a negative control (cell preparations in which the primary antibody was excluded) so that 1% to 2% of negative cells fell into the positive range.

Adsorption of Lymphocytes to Cell Monolayers

Cells were obtained from mesenteric lymph nodes of DBA/2 mice 7 days after CVB3 infection, added to glutaraldehyde fixed monolayers, and incubated at 37 C for 90 minutes. The nonadherent cells were removed by gently washing the monolayers four to six times with PBS, and the adherent cell population was recovered by incubating the monolayer in medium containing 20 mM EDTA for 15 minutes at 37 C. In some cases, adsorption was done in the presence of 100 μg/ml of either MAb to IA^d or K^dD^d. Recovered cells were washed in DMEM, and both untreated and immunoadsorbed cell fractions were incubated with 100 μg neutralizing MAb to CVB3 (clone 8A6) for 30 minutes to eliminate any infecting virus present in the lymphocytes.

Lymphocyte Adoptive Transfer

TXBM mice

TXBM animals were injected intraperitoneally with 1.8×10^5 PFU CVB3 and, 2 days later, anesthetized and injected intravenously through the tail vein with 0.2 ml HBSS containing 1×10^6 positively selected CD4+ or CD8+ lymphocytes. Animals were killed 5 days after receiving lymphocytes.

Uninfected Mice

Approximately 1.2×10^6 cells were injected intravenously into uninfected DBA/2 recipients and the animals also received 1 mg virus-neutralizing MAb (8A6) 1 and 3 days after lymphocyte transfer to insure against virus infection. On day 7, the animals were killed. The heart, liver, and pancreas were removed for virologic and histologic examination. Serum was assayed for virus-neutralizing antibody.

Statistics

The Student's *t*-test was used for all statistical analyses and *P* values less than 0.05 were considered significant.

Results

Multi-organ Disease in CVB3 Infection

DBA/2 mice were inoculated with CVB3, treated with MAb to deplete specific T lymphocyte subsets, and killed 7 days later. The heart, pancreas, and liver were removed and evaluated histologically and virologically (Table 1). Virus concentrations generally were statistically equivalent in groups of immunologically intact and depleted mice. Virus titers were slightly higher in the pancreas and heart, ranging from 10^5 to 10^8 PFU/g tissue, and were slightly lower in the liver. In all cases, variations in virus titers with immunomodulation were moderate and averaged less than a $\log_{10}$. More significant effects were observed on inflammation and tissue pathology. Despite high virus content, the liver did not shown inflammation or necrosis except for occasional small foci of inflammatory cells, which could also be observed in uninfected mouse livers. In about half the infected mice, some nonspecific hepatocellular alterations were seen consisting primarily of cytoplasmic clearing, excess granularity, and changes in nuclear chromatin pattern. We considered these changes as metabolic in origin, related to pancreatitis or stress induced by the experimental manipulation. However, in both heart and pancreas, immunologically intact mice showed extensive lymphocytic infiltration of the myocardium and exocrine portion of the pancreas (Figure 1A to L). In the heart, tissue injury was exclusively mediated by CD4 cells because depletion of this population resulted in complete abrogation of disease in seven of 11 animals and significantly reduced myocarditis consisting of scattered small lymphocyte aggregates in the remaining mice. CD4 cells were also at least partly involved in pancreatic injury, although protection was not as effective as that observed in the heart because approximately one third of the CD4 depleted animals continued to develop severe

Table 1. *Comparison of Inflammation and Virus Titers in DBA/2 Mice Depleted of CD4+ or CD8+ T cells**

Treatment	Animals	Cells positive for T cell antigen (%)			Inflammation score (0 to 4 scale)			Oregan virus titer (log_{10} PFU/g tissue)		
		Thy 1.2+	CD4+	CD8+	Heart	Pancreas	Liver	Heart	Pancreas	Liver
None	9	83.5	57.2	15.8	1.6 ± 0.3† (8/9)‡	3.7 ± 0.2 (9/9)	0 ± 0 (0/9)	7.51 ± 0.38	6.99 ± 0.13	6.26 ± 0.16
CD4+ cell depleted	11	40.3	3.6	34.1	0.2 ± 0.1§ (3/11)	1.3 ± 0.5§ (4/11)	0 ± 0 (0/11)	6.57 ± 0.22	7.48 ± 0.28	5.47 ± 0.23§
CD8+ cell depleted	11	75.5	70.8	1.5	1.6 ± 0.3 (11/11)	3.8 ± 0.1 (11/11)	0 ± 0 (0/11)	6.97 ± 0.27	7.68 ± 0.26	5.95 ± 0.12
CD4+ and CD8+ cell depleted	11	18.2	10.7	2.9	0.5 ± 0.1§ (6/11)	1.7 ± 0.6§ (5/11)	0 ± 0 (0/11)	6.37 ± 0.18	7.53 ± 0.24	5.80 ± 0.49

* Male DBA/2 mice were injected intraperitoneally with 2 mg MAb to either CD4 or CD8 T cell antigens on two sequential days as indicated above and inoculated with 1.8×10^5 PFU CVB3 1 day after the last antibody injection. Control (infected but without MAb treatment) and experimental animals were killed 7 days after virus inoculation.

† Mean ± SEM.

‡ Number of animals positive/total number of animals per group.

§ Value significantly less than control group at $P \leq 0.05$.

pancreatitis. Possibly, either residual T cell activity in CD4-depleted animals remained capable of initiating pancreatic enzyme-mediated autolysis, or other non-T cell mechanisms also exist. Of great interest was the apparent specificity of the inflammatory disease for the acinar pancreas. Glucose tolerance tests further confirmed histologic evidence that islets were spared. Glucose values before and 1 hour after injection of 2 mg glucose/g body weight were 173 ± 11 and 226 ± 53 mg/dl, respectively, for uninfected DBA/2. Seven days after infection, pre-glucose and postglucose challenge values actually decreased slightly to 103 ± 13 and 151 ± 12 mg/dl, respectively.

Confirmation of the T cell role in myocarditis and pancreatitis was achieved by making DBA/2 mice T cell deficient by TXBM. Inoculation of TXBM mice with CVB3 resulted in no myocarditis and pancreatitis in half the animals, with minimal lesions evident in the remainder (Table 2). However, adoptive transfer of positively selected CD4 lymphocytes from virus infected donors significantly enhanced both incidence and severity of disease. No inflammation was seen in the liver, again evidence for the organ specificity of immune-mediated tissue injury. Adoptive transfer of immune CD8 cells also failed to increase significantly either myocarditis or pancreatitis. Thus, what immunologic damage occurred in these organs appeared to be exclusively CD4 cell mediated.

Effector T cells May React to Common Tissue Antigens in the Heart and Pancreas

Because immune effectors belonging to the same T cell subset initiated both myocarditis and pancreatitis, a single immune effector recognizing a common antigenic epitope on heart and pancreatic acinar cells may cause both diseases. Alternatively, two distinct immune responses may have occurred simultaneously, with each immune effector recognizing different tissue-specific antigens. To distinguish between these possibilities, immune T cells (IL) from DBA/2 donor animals infected with CVB3 7 days earlier were selectively adsorbed to cardiocyte, liver, or fibroblast monolayers. The adherent cell population was retrieved and adoptively transferred into uninfected syngeneic animals (Figure 2 shows experiment 1; Table 3 experiment 2). Controls consisted of animals given normal lymphocytes (NL) from uninfected DBA/2 donors. Both the cells and recipients were treated with CVB3-neutralizing MAb to prevent transfer of virus. The animals were killed 7 days after injection of immune cells, and the heart, liver, and pancreas were evaluated for virus and pathology. Serum was evaluated for neutralizing antibody titer. None of the recipients showed any detectable virus in the organs or virus-neutralizing antibody in the serum (data not shown). However, hearts and especially pancreases of recipient animals given unfractionated (line 2) or heart adsorbed and eluted (Ad/E heart) T cells (line 4) demonstrated significant inflammation and necrosis, whereas the liver showed no detectable inflammation. Livers did, however, demonstrate changes in hepatocyte cytology as discussed previously and as shown in Figure 1I. We believe these latter changes are a function of metabolic stress induced by pancreatitis and/or experimental manipulation. Adsorption of pathogenic T cells to cardiocyte monolayers could be inhibited by MAb to IA^d (line 7), as would be expected of CD4 T cells (which are usually class II MHC antigen restricted), but could not be inhibited by MAb to the class I MHC molecule (K^dD^d, line 8). The tissue injury caused by transfer was also clearly mediated by T cells, because treatment of unfractionated immune lymphocytes with anti-Thy 1.2 and complement before transfer completely abrogated disease induction (line 3). Furthermore, organ specificity of adsorption of pathogenic cells was demonstrated because lymphocytes re-

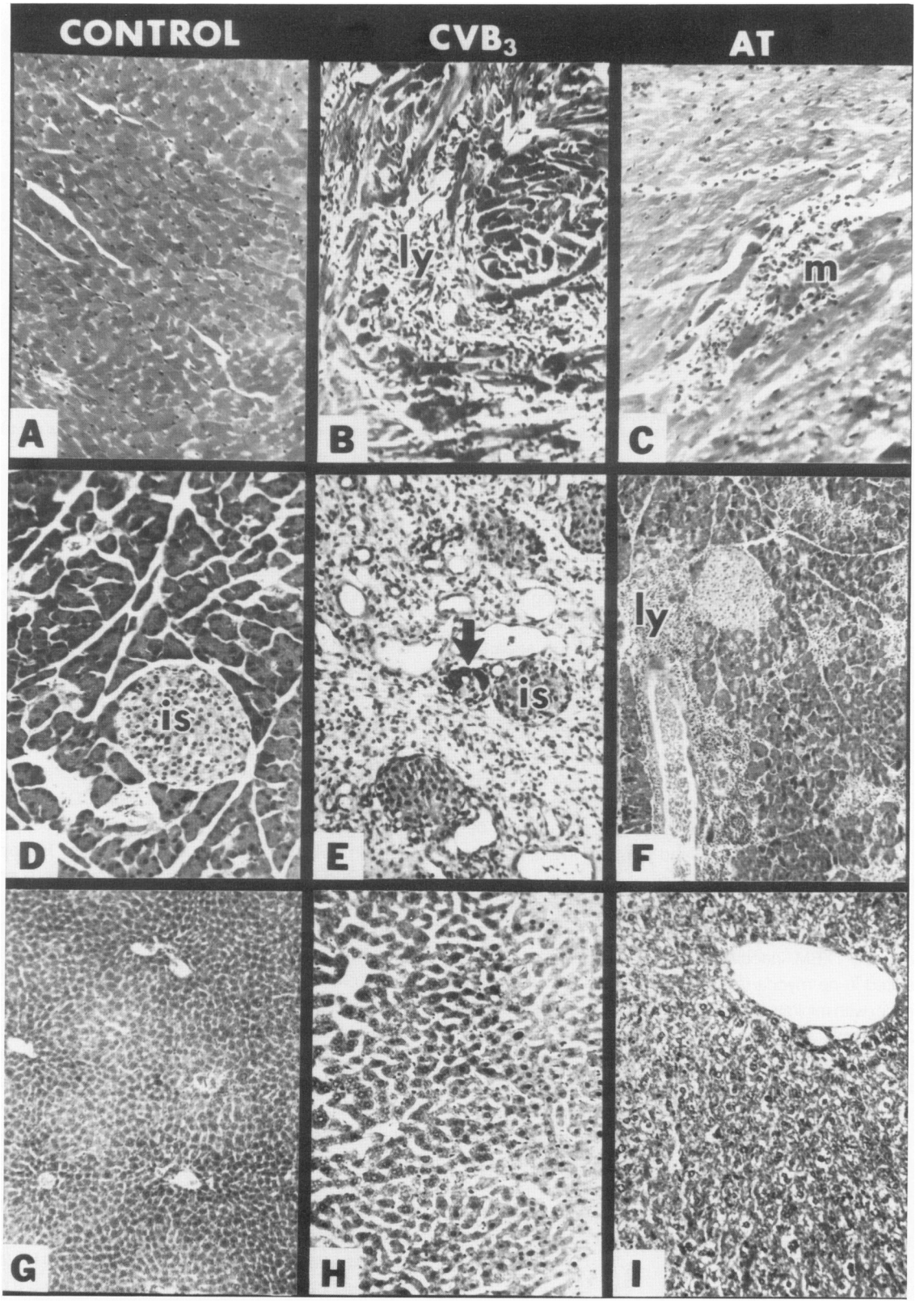

Figure 1. **A:** *A control animal heart. After CVB3 infection, the myocardium* **(B)** *developed large, frequently confluent, inflammatory lesions with myocyte necrosis and dense lymphocytic infiltration (ly). Adoptive transfer of lymphocytes from infected animals resulted in myocarditis* **(C)** *evidenced in this photomicrograph by the presence of hypereosinophilic (dark), partially degenerated myocytes (m) adjacent to an interstitial collection of mononuclear cells.* **D:** *A microscopic section of control animal pancreas showing a large islet of Langerhans (is) surrounded by typical rosettes of acinar exocrine pancreas.* **E:** *CVB3 infection resulted in dramatic obliteration of the acinar tissue. A single, partially spared acinus (arrow) can be seen adjacent to an intact islet (is).* **F:** *Partial destruction of acinar tissue was seen after adoptive transfer. A lymphocytic aggregate (ly) can be seen adjacent to a preserved islet. No inflammatory lesions were identified in control* **(G)**, *CVB3-infected* **(H)** *or adoptive transfer recipient* **(I)** *livers. Nonspecific reactive changes were noted in livers from both of the experimental groups. (H & E, ×25* **[F, G]**; *×50* **[A–E, H, I]**.*)*

Table 2. *Reconstitution of Disease Susceptibility with CD4 T Cells**

Treatment	Inflammation (score 0 to 4)		
	Heart	Pancreas	Liver
TXBM	0.3 ± 0.1 (3/6)	0.8 ± 0.4 (3/6)	0 ± 0 (0/6)
TXBM + CD4	1.0 ± 0.2* (6/6)	2.0 ± 0.5† (6/6)	0 ± 0 (0/6)
TXBM + CD8	0.3 ± 0.1 (5/8)	1.1 ± 0.5 (4/8)	0 ± 0 (0/8)

* DBA/2 mice were made T cell deficient by thymectomy, 700 R irradiation, and syngeneic bone marrow reconstitution (TXBM). TXBM mice were inoculated intraperitoneally with 1.8×10^5 PFU CVB3 and 2 days later received through the tail vein 1×10^6 positively selected CD4+ or CD8+ cells from DBA/2 donor mice infected 7 days earlier with CVB3. TXBM recipients were killed 7 days after receiving virus. Results represent men score ± SEM. Number in parentheses represents number of animals with inflammation/total animals in group.

† Results were significantly different in inflammation severity (mean score) from TXBM at $P \leq 0.05$.

trieved from either liver VEC (line 5) or skin fibroblast (line 6) monolayers showed no transfer of pancreatitis. Cells obtained from skin fibroblasts did induce minimal myocardial lesions in four of six animals, however, possibly suggesting some heterogeneity in the pathogenic response. These results strongly indicate that a common effector can cause both pancreatitis and myocarditis in CVB3-infected DBA/2 mice.

Discussion

How viral infections trigger autoimmunity is incompletely understood. Presumably, several mechanisms may be involved. First, infection and virus-mediated lysis of cells may release cellular components, and immune cells may

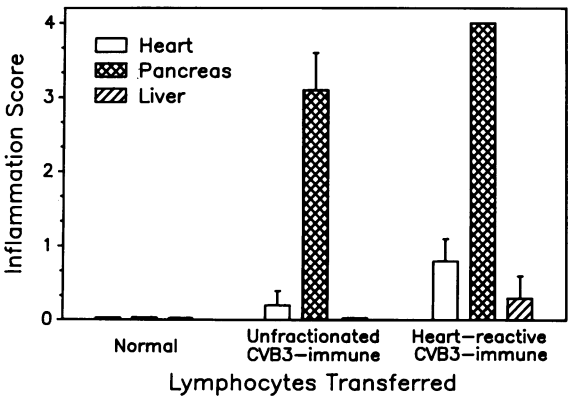

Figure 2. *Adaptive transfer of disease with immune lymphocytes. Lymphocytes were obtained from normal on day 7 CVBB immune donor mice. One half of the immune cells were adsorbed to myocyte monolayers and the adherent cells were retrieved. 1×10^6 of the normal, immune (unfractionated) and immune-myocyte adsorbed (heart-reactive) were adoptively transferred into uninfected DBA/$_2$ recipient animals. Seven days later the recipients were killed and various organs were evaluated for inflammation. Results represent mean score ± SEM of four animals per group.*

Table 3. *Pathogenicity of Tissue Adsorbed and Eluted Immune T Cells in Uninfected Recipients**

Lymphocytes	Treatment	Heart	Pancreas	Liver
NL	—	0 ± 0 (0/6)	0 ± 0 (0/6)	0 ± 0 (0/6)
IL	—	0.9 ± 0.2† (6/6)	2.0 ± 0.7† (6/6)	0 ± 0 (0/6)
IL	Anti-Thy 1.2+C	0 ± 0 (0/6)	0 ± 0 (0/6)	0 ± 0 (0/6)
IL	Ad/E heart	0.7 ± 0.1† (6/6)	1.8 ± 0.6† (5/6)	0 ± 0 (0/6)
IL	Ad/E liver VEC	0 ± 0 (0/6)	0 ± 0 (0/6)	0.5 ± 0 (6/6)
IL	Ad/E fibroblasts	0.2 ± 0.1 (4/6)	0 ± 0 (0/6)	0 ± 0 (0/6)
IL	Ad/E heart anti-IA^d	0.1 ± 0.1 (1/6)	0.3 ± 0.3 (1/6)	0 ± 0 (0/6)
IL	Ad/E heart anti-K^dD^d	0.8 ± 0.3† (6/6)	1.3 ± 0.4† (5/6)	0 ± 0 (0/6)

* Donor DBA/2 mice were inoculated with 1.8×10^5 PFU CVB3 7 days before they were killed. Mesenteric lymph node T cells were retrieved and divided. One portion (line 3) was treated with anti-Thy 1.2 and C'. Other portions (lines 4 to 6) were adsorbed to monolayers of the heart and liver, or fibroblast cells and adherent cells recovered. Cells were also adsorbed to heart cells in the presence of either 100 μg/ml mAb to IA^d (line 7) or K^dD^d (line 8) and adherent cells recovered. Control lymphocytes (line 1) were obtained from uninfected donor DBA/2 mice. All cells were treated with CVB3-neutralizing MAb adjusted to 6×10^6 cells/ml and 0.2 ml were injected into uninfected DBA/2 recipients through the tail vein. Recipients received 0.5 ml of a 1 mg/ml solution CVB3-neuralizing MAb intraperitoneally on days 1 and 3 after lymphocyte transfer and were killed on day 7. Results represent the mean inflammation score + SEM.

† Results were significantly different from the group receiving normal lymphocytes at $P \leq 0.05$. Numbers in parentheses represent number of animals showing inflammation lesions/number of total animals.

NL, nonimmune lymphocytes; IL, immune lymphocytes; VEC, vascular endothelial cell.

be presented with self antigens not normally present in high concentrations in uninfected individuals. Several proteins are well known for their antigenicity when inoculated into animals in complete Freund's adjuvant.[15–18] Examples include myelin basic protein and thyroglobulin, which initiate encephalitis and thyroiditis, respectively.[16–18] A second mechanism may be that virus attachment to viral receptors alters the cell surface molecules making these appear foreign to the immune system. Thus, injection of stabilized virus-membrane complexes readily stimulates autoantibody induction to the membrane components alone.[19,20] A third possibility involves antigenic mimicry by which the virus and cell share certain antigenic epitopes.[21] When infection stimulates anti-viral immunity, the effectors also react to the cellular entity. Finally, autoimmunity may arise through anti-idiotypic responses.[22–24] Here, the variable region of the virus neutralizing antibody initiates a complementary immunity that closely resembles the three-dimensional structure of the virus and therefore binds to the virus receptor.

The present study suggests that both myocarditis and pancreatitis after CVB3 infections result primarily from immunopathogenic mechanisms, because mice made T cell deficient by either depletion of cells with specific MAb, or thymectomy, irradiation, and bone marrow reconstitution, develop minimal disease. The same T cell population

was responsible for injury in both organs and the CD4 effector could restore disease susceptibility in TXBM animals. Whether or not the immune response is targeting virus epitopes (virus specific immunity) on infected tissues or represents autoimmunity is not completely certain. However, the immune T cells are capable of transferring disease into uninfected syngeneic mice that might indicate their autoreactive nature. The main question is whether virus could be transferred along with the immune T cells into the recipients. However, both the cells and the recipients were treated with CVB3-neutralizing MAb under a protocol that effectively abrogates infection of animals inoculated with 1.8×10^5 PFU (1988, unpublished observations). No virus was evident in the organs of the recipients at the time of killing, although animals inoculated with virus would have shown high titers if killed at the same time (Table 1). Finally, MAb and complement lysis of the T cells before transfer completely prevent both myocarditis and pancreatitis. If these diseases resulted from virus transfer into recipients, we would not expect lysis of the T cells to prevent disease induction because the T cell-specific antibody does not affect virus. We conclude, therefore, that CVB3 induces autoimmunity to both cardiocytes and pancreatic acinar cells, and the same effector may induce both diseases, presumably by recognizing shared antigenic epitopes. The evidence for the latter statement is primarily the demonstration that immune T cells adsorbed and eluted from the heart cell monolayers cause both myocarditis and pancreatitis. Although developing autoimmunity within 7 days of infection may seem overly rapid when compared with models such as experimental allergic encephalitis (EAE),[15,18] the conditions of immune sensitization are not necessarily equivalent in the two systems. This becomes especially clear should virus-induced autoimmunity reflect antigenic mimicry between the virus and tissue. Dr. Notkins and his colleagues showed that CVB3-neutralizing MAb can cross-react with mouse myocardium.[25] Thus, at the same time the virus stimulates a vigorous immune response to itself, it may simultaneously be stimulating the cross-reactive "autoimmune" effectors to heart and pancreatic tissue. Because the virus-specific response peaks between 7 and 14 days after infection in this model,[4,5,12] autoimmunity arising during the same period might not be unexpected. However, animal morbidity and mortality levels by day 10 in infected mice dictate that we kill mice on day 7 although peak organ pathology occurs later.

Multi-system autoimmunity may result from several mechanisms. Certainly, release of cellular components would sensitize lymphoid cells to any tissues expressing the same component. Presumably in the present system this would include pancreatic acinar cells and cardiocytes but exclude cells of the liver. Similarly, antigenic mimicry would also explain why only some tissues are pathologically involved because cells of the affected organs would be the only ones expressing the cross-reactive antigens. Whether or not the remaining mechanisms explain tissue selection in virus-induced autoimmunity is less certain. In the present system, the liver is heavily infected, yet no evidence of autoimmune hepatitis is evident. If virus-receptor complexes initiate autoimmunity, one might wonder why only virus interactions with myocytes and pancreatic acinar cells stimulate this response, whereas apparently similar virus-receptor interactions in other organs are ineffective. Similarly, if variable regions of anti-idiotypic antibodies resemble the virus, thus promoting antibody attachment to the virus receptor, one must question why only some receptor molecules are targeted. One explanation may be that the inoculating virus pool actually consists of multiple virus variants with distinctive organ and receptor tropisms. Such variants were described for coxsackieviruses[26,27] as well as other viruses.[28] In this case, characteristics of either the virus-receptor interaction in the heart and pancreas or of the receptor molecule itself are more likely to lead to their autosensitization as opposed to other organs. Thus, because T cell stimulation requires antigen presentation in conjunction with major histocompatibility complex (MHC) antigens,[29] infected organs stimulating autoimmunity may show greater inducibility of the appropriate MHC molecules[6] or virus receptor interactions (processing) with MHC antigens may be more effective in these, than in other, infected tissues.

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