Idiopathic Dilated Cardiomyopathy
A Superantigen-Driven Autoimmune Disease

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Background—Many cases of idiopathic dilated cardiomyopathy (IDC) result from an inflammatory myocarditis. The specific immunological mechanisms are not yet defined. Various autoimmune diseases are associated with superantigen-triggered immune responses, resulting in massive T-cell activation and tissue damage. We studied 3 cases in a search for evidence that such a phenomenon is also implicated in IDC.

Methods and Results—Myocardial, lymph node, and thymic tissue samples were obtained from IDC patients who were undergoing heart transplantation. Infiltrating immune-cell phenotypes and gene expression of T-cell receptor (TCR) α- and β-chain variable (Vα and Vβ) regions were analyzed by immunostaining and polymerase chain reaction. Similar technical approaches were used to assay the tissues for the presence of coxsackievirus B (CVB). In all the specimens analyzed, an overexpression of the TCR Vβ3, Vβ7, and Vβ13.1 gene families was detected among the infiltrating T cells. These tissues were also found to be CVB3-positive. In vitro exposure of peripheral blood mononuclear cells to lysates of cells infected with CVB3 was capable of stimulating expansion of the same TCR Vβ families. The TCR Vα repertoire was never found to be skewed.

Conclusions—A superantigen-mediated immune response is involved in human heart disease. CVB3 may directly or indirectly trigger this response, suggesting a possible mechanistic link between CVB infection and myocarditis development progressing to IDC. (Circulation. 1998;98:777-785.)

Key Words: cardiomyopathy ■ immunology ■ myocarditis ■ viruses

Myocarditis exhibits a wide spectrum of clinical manifestations, ranging from essentially asymptomatic, transient inflammation to severe congestive heart failure, dysrhythmias, and death. The overall prognosis of myocarditis in children remains poor. In a comprehensive study, only 7 of 34 patients (21%) had resolution of their illness. Despite intensive support, the overall mortality was 62%.1 Furthermore, myocarditis is a major cause of sudden, unexpected death in adults <40 years old, with ~20% of such individuals dying of this disease.2 There is substantial evidence that viral and/or inflammatory myocarditis can progress to IDC,3 which, second to ischemic heart disease, is the most common indication for heart transplantation.4

Enteroviruses, and especially CVB, are thought to be responsible for >50% of myocarditis in North America and have been also implicated in the pathogenesis of IDC.5 To date, the association between enterovirus infection and heart disease has been based primarily on serological studies demonstrating the presence of CVB-specific neutralizing IgM and IgG antibodies in the sera of patients suffering from acute myocarditis6 and their persistence in the chronic phase.7 Attempts to detect CVB in patients’ cardiac tissue itself by use of strain-specific monoclonal antibodies and molecular techniques has varied considerably between different reports. Culturing CVB from patients’ myocardial tissue generally fails.3,8–14 Autoantibodies against cardiac antigens have been found in the mouse CVB3 model of the disease,15 as well as in patients with IDC and their relatives.16 However, a cell-mediated immune response seems to play the most central role in further expanding the virus-induced myocardial damage.3,6,16

Although it is not yet known whether autoimmune mechanisms are the cause of tissue injury or merely a reaction to autoantigens released after the tissue damage has already occurred, they are considered the most important mediators of the pathogenesis of myocardial inflammation progressing to IDC.3,5,16,17 Although this is the general consensus regarding IDC pathogenesis, a more specific process explaining the immune reaction that leads to these clinical consequences has remained elusive.
Superantigens are a class of bacterial and viral proteins that elicit a powerful immune response by their ability to activate T cells polyclonally. In their native conformation, they preferentially bind outside of the MHC class II binding groove on antigen-presenting cells, cross-linking the lateral side of the class II molecule and the Vβ portion of the TCR. Thus, superantigen specificity is mainly and almost entirely determined by the Vβ element of the TCR, bypassing the normal constraints of TCR specificity and MHC restriction. Because of its ability to target one or a limited number of Vβ families, in vivo exposure to a superantigen results in “skewing” of the T-cell repertoire, manifested either by expansion of T cells expressing particular Vβ families or by deletion and/or anergy of specific T-cell subsets. Because the T-cell repertoire encompasses a limited number of families with Vβ elements very similar in sequence, any superantigen is able to activate a large fraction of the pool of circulating T cells (5% to 30%), in contrast to conventional antigens, which normally activate ~1 in 10^3 T cells. Various reports have demonstrated the association of superantigens with the development of several human pathological conditions, some autoimmune diseases included.

We have had the opportunity to further investigate the immunological mechanisms involved in the pathogenesis of acute myocarditis that progressed to IDC in 3 children who subsequently received heart transplants. To this aim, the presence of CVB and the phenotypes of the immunocompetent tissue-infiltrating cells in the diseased hearts and, when available, mediastinal lymph nodes and thymus were analyzed. The pathogenic scenario resulting from these analyses was consistent with a CVB3-triggered, superantigen-mediated immune reaction as a possible mechanism leading to heart failure.

Methods

Subjects

Patient 1 was a 5½-year-old girl; patient 2, a 6-month-old boy; and patient 3, a 14-year-old boy. The symptomatic course of the disease was similar in all of them: healthy until the onset, these children began experiencing several days of fever, vomiting, diarrhea, and dehydration, for which they received antipyretics and oral and/or intravenous fluid rehydration. Rapid progression to the signs and symptoms of congestive heart failure led to hospitalization and cardiac evaluation. Findings included radiographic features of cardiomegaly and pulmonary edema, together with echocardiographic demonstration of globally depressed ventricular function without evidence of structural abnormalities or pericardial effusion. Cardiac catheterization confirmed a poor global ventricular function. Preliminary histological analyses of a few endomyocardial biopsies were not sufficiently informative. All patients were diagnosed as having IDC.

Because of the rapid and progressive deterioration of cardiomyopathy (patients 2 and 3) and cardiopulmonary arrest without response to cardiopulmonary resuscitation (patient 1), the children were placed on mechanical circulatory assist devices. They were listed for heart transplantation after serial echocardiographic examinations documented unrelenting severe myocardial dysfunction despite maximal circulatory support.

All 3 patients underwent orthotopic heart transplantation within a few days of decompensation. Intraoperative courses were uncomplicated and early graft function was good.

The patients and the allograft hearts continue to function well ≈2.5, 2, and 1.5 years after transplantation, respectively. Antirejection therapy with azathioprine, prednisone, and either tacrolimus or cyclosporine was used. The few episodes of clinically silent rejection have responded to methylprednisolone.

Biopptic specimens from the hearts of 3 additional patients (3 boys, 3, 8, and 9 years old) who clinically recovered from an episode of acute IDC without evidence of CVB3 infection were used as controls.

All the specimens were obtained only if not used by the pathologist. Their use for research was approved by the Institutional Review Boards at the Children’s Hospital of Boston or at Children’s Hospital of Pittsburgh. The latter Institutional Review Board also approved the use of both heart tissue obtained at the autopsy of an infant who died of renal dysplasia without histological and histochemical evidence of other abnormalities and blood samples from subjects ranging from 3 to 16 years of age who had been admitted to Children’s Hospital of Pittsburgh for orthopedic intervention. These samples were used as “normal” controls for immunostaining and TCR analyses, respectively.

PBMCs for in vitro stimulation studies were obtained from 9 adult blood donors.

All the subjects were HLA class II molecularly typed.

Immunostaining Procedure

Heart, lymph node, and thymus fragments obtained at the time of transplantation were snap-frozen in isopentane chilled to −150°C and stored at −80°C. Standard histological and histochemical techniques were used to prepare 5-μm-thick frozen sections. Before staining, the sections were exposed to absolute acetone for 10 minutes.

Mouse anti-human MAbs (MAbs to leukocyte common antigen, CD3 (pan–T cells), CD4 (helper/inducer T cells), CD8 (cytotoxic/suppressor T cells), and activated monocytes/macrophages (Ber-MAC3)) were obtained from Dako Corp. The presence of different strains of CVB was determined histologically by use of MAbs (B1, IgG2a, IgG2b, IgG2a, and B6; IgG2a ascites, 1/1000 dilution) from Accurate Chemical & Scientific Corp.

Control sections were set up with irrelevant isotype-matched MAbs (IgG1 and IgG2a). Bound MAbs were detected with alkaline phosphatase-anti-alkaline phosphatase followed by fast red TR alkaline phosphatase substrate (Dako Corp).

Detection of CVB Genome

For the detection of CVB genome, RT-PCR using a 5’-sense primer (5’-CGTACCTTTGGGCGCTGT-3’) and 3’-antisense primer (5’-GAAACACGGACACCCAAAAGTA-3’) was performed. The amplified DNA was isolated and tested for the presence of CVB sequences with nested primers P6 and P9. Twenty clones were isolated and automatically sequenced with an ABI 377 DNA Sequencer (Applied Biosystems Inc.).

Evaluation of TCR Vα and Vβ Repertoires

The study of the TCR Vα and Vβ gene segment usage of 29 TCR Vα and 26 TCR Vβ families and subfamilies was performed by RT-PCR as previously described.
Two microliters of the fluorescently labeled PCR products corresponding to the TCR Vα and Vβ gene families were resolved by electrophoresis on an ABI 377 DNA Sequencer and automatically analyzed by Genescan software (Applied Biosystems Inc). For each tissue specimen, the analysis was performed at least 3 times from RT-PCR to gel reading. Genescan also allows the analysis of the different lengths of the CDR3, inclusive of Vβ, (N) Dβ (N), and Jβ segments, the corresponding peaks, and their areas (spectratypes). In the case of the most relevant specimens, DNA sequencing of the cloned PCR products was also performed.

In Vitro PBMC Stimulation With CVB-Infected Vero Cell Lysates

PBMCs from healthy adults were isolated from heparinized peripheral venous blood with a Ficoll-Hypaque gradient (1-Step Lymphoprep, Accurate Chemical & Scientific Corp). For each donor, RNA was extracted from 3×10^6 cells. Also, 1×10^6 cells/mL were seeded into flat-bottom 24-well culture plates (Corning) and exposed to sonicated Vero cell lysates in complete RPMI-1640 medium supplemented with 10% human AB serum (Normolcera-Plus). Noninfectious lysates from Vero cells exposed to CVB1, CVB3, CVB4, or CVB6 (Virion Inc) were first tested in dose-response experiments to select the concentration of 2 μL/mL used in the study. Cultures with noninfected Vero cell lysates were used as negative controls, and phytohemagglutinin at 5 mg/L was used for positive controls.

PBMCs were cultured at 37°C in 5% CO_2 for 15 days and independently stimulated with each of the different lysates on day 1 and every 3 days thereafter. Purified human interleukin-2 (Boehringer Mannheim) was added (5 IU/mL) on days 4, 8, and 12. Twelve hours before harvesting, human interleukin-2 (5 IU/mL) was also added to allow regeneration of potentially modulated TCR. At days 4, 9, and 15 of in vitro culture, portions of the cells were harvested, washed, and counted, and the total RNA was extracted. Activated (CD71+) cells were isolated from the remaining portion by immunomagnetic separation with DynaBeads M-450 CD71 (Dynal, Inc). RNA extraction was performed directly on cells bound to the beads, and the TCR repertoire was analyzed.

Results

Immunohistochemical Analysis

Heart

The cardiac tissue from each of the patients studied showed a considerable infiltration of leukocyte common antigen–positive white blood cells that were mostly CD4+ and CD8+ T lymphocytes (Figure 1). CD4+ cells were disseminated within the affected tissue, whereas CD8+ elements were mostly clustered in multifocal perivascular sites in which areas of cell damage were also evident. Activated monocytes/macrophages were seen in histological sections, as indicated by their morphology and by their reactivity with Ber-MAC3 MAb (Figure 1). No signs of fibrosis or calcification were found in the sections analyzed. However, the histological scenario was notably heterogeneous, with areas of significant T-cell infiltration and parenchymal damage interspersed with areas of apparently normal myocardium.

When an MAb specific for the CVB3 serotype was used, the myocardium of these patients showed strong reactivity compared with normal control heart (eg, Figure 2). Within the tissue, multifocal areas were, in fact, characterized by the presence of myocytes and infiltrating cells containing cytoplasmic granules that were positively stained. The immunostaining for CVB1, CVB4, and CVB6 serotypes was negative (eg, Figure 2).

Mediastinal Lymph Nodes

In patient 1, the architecture of the lymph nodes appeared to be largely altered, such that we were unable to clearly distinguish the primary and secondary germinal centers. Large nucleated cells reacted with MAb specifically directed against CVB3, whereas MAbs against CVB1, CVB4, and CVB6 were negative (eg, Figure 2). CVB3-positive elements, organized in cellular cords within the
lymphocytic component of the tissue, were also positive for Ber-MAC3 (data not shown). These results suggest that these cells, belonging to the monocyte lineage, may have phagocytosed viral material. Lymph nodes from patients 2 and 3 were not available.

**Thymus**

The thymic tissue from patients 1 and 2 (the third patient’s thymus was not available) was clearly positive for CVB3 (Figure 2). No positivity for CVB3 was demonstrated in the thymic cortex. With MAbs directed against CVB1, CVB4, and CVB6, no reactivity was found either in the medulla or in the thymic cortex (eg, Figure 2). CVB3+ staining was seen mostly on hyalinized epithelial reticular elements surrounding Hassall’s corpuscles (Figure 2). A large population of monocytes/macrophages was present within the epithelial constituents. The monocytes appeared to be activated, as demonstrated by their reactivity with Ber-MAC3 MAb (data not shown).

**Determination of the Presence of CVB Genome in Tissue Samples**

Among heart specimens, we were able to amplify the CVB genome in patient 1 only (not shown). The RT-PCR–amplified DNA segment (present also in the thymus) was cloned and sequenced. The segment matches 460 out of 464 base pairs from the 5′-nontranslated region of a CVB4 strain (GeneBank S76772, base pairs 84 to 547). This strain was previously found to be the active cause of myocarditis and type I diabetes in a patient who died as a consequence of this infection. Compared with this published sequence, the 4 differing nucleotides were located at positions 260 (adenine not cytosine), 276 (guanine not adenine), and 341 to 342 (cytosine-uridine not uridine-cytosine). It is perhaps noteworthy to point out that the distinction between different strains of CVB (eg, CVB3 versus CVB4), defined on the basis of antibody neutralization assays, does not consistently reflect corresponding nucleotide variations of the 5′-nontranslated region. Differences of 30% to 50% in nucleotide sequences are frequently present in variants of the same immu-
nologically defined strain. This may explain why the specimens analyzed were immunologically CVB3-positive, even if the isolated CVB 5'-nontranslated region was more similar to a CVB4 published sequence.30

**TCR Vα and Vβ Repertoires in the Heart, Lymph Nodes, and Thymus**

Semiquantitative analysis of the TCR Vβ repertoire of the cells infiltrating the heart tissues of our patients revealed an exceptionally high level of expression of the Vβ3, Vβ7, and Vβ13.1 gene families compared with the expression pattern observed in both the biopsy specimens from the hearts of control subjects and the PBMCs of age-matched children (Table 1 and Figure 3). In each case, specimens from different areas of the same organ showed different percentages of TCR Vβ skewing (eg, 56% Vβ7 in the right ventricle versus 18% in the left ventricle of patient 2; see Table 1 and Figure 3). In patient 3, the TCR repertoire analysis was possible only in the

**TABLE 1. TCR Vβ Expression in Heart Specimens From Patients and Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Vβ3</th>
<th>Vβ7</th>
<th>Vβ13.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>21.2</td>
<td>4.4</td>
<td>14.0</td>
</tr>
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<td>DRB1*07, <em>13; DQA1</em>01, <em>0201; DQB1</em>0201, *0603</td>
<td></td>
<td></td>
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<tr>
<td>Patient 2</td>
<td>2.6</td>
<td>55.6</td>
<td>5.0</td>
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<tr>
<td>DRB1<em>13, <em>15; DQA1</em>01; DQB1</em>0602, *0603</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 3</td>
<td>0.8</td>
<td>13.7</td>
<td>9.5</td>
</tr>
<tr>
<td>DRB1*11, <em>14; DQA1</em>01, <em>05; DQB1</em>0502, *0601</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart biopsies (n=3)</td>
<td>8.4±4.99</td>
<td>4.2±0.41</td>
<td>7.6±2.11</td>
</tr>
<tr>
<td>PBMC (n=16)</td>
<td>5.4±3.55</td>
<td>5.1±1.64</td>
<td>6.4±1.06</td>
</tr>
</tbody>
</table>

The value of each Vβ is expressed as a percentage of corrected ratios. Median values are shown for patients, mean values (±SD) for control subjects.

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**Figure 3.** TCR Vα and Vβ repertoires of T cells infiltrating heart, lymph node, and thymus. Analysis of Vβ repertoire of T cells infiltrating right (red) and left (orange) ventricle and thymus (yellow) of IDC patient 2. Mean values (±SD) of TCR Vβ families of peripheral blood mononuclear cells from 16 normal control subjects are shown in green, and control hearts in blue. Exceptionally high expression of Vβ7 family is apparent in right ventricle, where TCR Vα repertoire was found unskewed (inset upper right).
right ventricle. In the left ventricle, the repertoire was not interpretable, possibly because of the paucity of T-cell infiltrates in the tissue available from this section of the heart. When additional tissues were available, a similar, though less prominent, skewing of the TCR Vβ repertoire was also seen in the thymus (eg, patient 1, 23.6% Vβ3, 10.8% Vβ7, and 10.2% Vβ13.1; patient 2, 4.6% Vβ3, 12.7% Vβ7, and 7.2% Vβ13.1) and in lymph nodes (eg, patient 1, 17.0% Vβ3, 7.4% Vβ7, and 12.4% Vβ13.1). The TCR Vα repertoire analysis did not reveal overexpansion of any Vα gene family in any of the specimens from patients or control subjects (eg, Figure 3). The coexpression of all the Vα gene families argues against a possible “founder” effect for the Vβ skewing due to a potentially limiting number of T cells in the successfully analyzed tissue samples.

In addition, the polyclonality of the infiltrating T cells belonging to the most highly expressed Vβ families was demonstrated by the results of Genescan spectratype analysis and by DNA sequencing of cloned CDR3 regions (data not shown).

TCR repertoires of the patients’ PBMCs were found not to be skewed. These data on PBMCs, however, are probably not conclusive because the repertoires may have been affected by blood transfusions that the patients had received before transplantation.

Specific TCR Vβ Skewing of PBMCs Stimulated With CVB3-Infected Vero Cell Lysates
The TCR repertoire analysis of PBMCs from 9 donors expressing different HLA class II alleles, cultured with CVB3-infected Vero cell lysates, showed increases of different combinations of the Vβ3, Vβ7, and Vβ13.1 families in the activated (ie, CD71+) PBMCs compared with the values found in the respective PBMCs analyzed before culture. Although expression of these 3 Vβ families increased up to 4 times in different donors’ PBMCs, the other Vβ families did not significantly change in percentage of expression. The values for each skewed Vβ family are listed in Table 2. A time-course experiment with the PBMCs from donor 1 is shown as an example of a progressive increase of the Vβ7 family peaking at 15 days (from 9.1% of the uncultured cells to 18.7%) (Figure 4). Cultures with CVB1-, CVB4-, and CVB6-infected Vero cell lysates did not significantly increase any Vβ gene family values in the CD71+ PBMCs (eg, Figure 5). In all donors, incubation with lysates from noninfected Vero cells did not result in a predominant increase of any Vβ family (eg, Figure 5). The polyclonality of the CD71+ cells, belonging to the most highly expressed Vβ gene families after CVB3-infected Vero cell lysate incubation, was demonstrated by the results of Genescan spectratype analysis (eg, Figure 5).

Discussion
In humans, IDC is generally considered to be part of the sequelae of a viral myocarditis. Myocarditis is a disease in which a link between viral infections and autoimmunity has long been suspected but, so far, not confirmed. T cells and the development of a cell-mediated immune response are considered to be the primary factors responsible for long-term myocardial damage after the early process of myocarditis.

Evidence of a viral link in cases of IDC has been provided, although the mechanism(s) of virally stimulated injury still remain elusive.

In our study of the myocardial inflammatory infiltrates, the immunohistochemical analysis showed the presence of a population of activated helper (CD4+) and cytotoxic (CD8+) T lymphocytes. In addition, strong and selective immunostaining for the CVB3 serotype was observed not only in the heart but also in the mediastinal lymph nodes and in the thymus of the patients. More noteworthy, the analysis of the TCR Vβ repertoire of the tissue infiltrate showed a remarkable skewing of 3 Vβ gene families, 3, 7, and 13.1. Specimens from different sections of the same organ (eg, left versus right ventricle in patients 2 and 3) showed various degrees of inflammation. The heterogeneous infiltration

### Table 2. Specific TCR Vβ3, 7, and 13.1 Overexpression of PBMCs Exposed to CVB3-Infected Vero Cell Lysates

<table>
<thead>
<tr>
<th>Donors</th>
<th>Vβ3</th>
<th>Vβ7</th>
<th>Vβ13.1</th>
<th>Vβ15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1<em>4, <em>7; DQA1</em>01, 03; DQB1</em>0302, *0602</td>
<td>2.8</td>
<td>9.1</td>
<td>7.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Uncultured PBMCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD71+PBMCs</td>
<td>6.9</td>
<td>18.7</td>
<td>10.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Donor 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*4, <em>13; DQA1</em>01, <em>03; DQB1</em>0302, *0603</td>
<td>0.9</td>
<td>7.7</td>
<td>7.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Uncultured PBMCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD71+PBMCs</td>
<td>2.9</td>
<td>12.1</td>
<td>18.4</td>
<td>0.8</td>
</tr>
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<td>Donor 6</td>
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<tr>
<td>DRB1*07, <em>15; DQA1</em>01, <em>02; DQB1</em>0201, *0602</td>
<td>2.2</td>
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<td>5.8</td>
<td>9.3</td>
<td>11.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Highest percentages of corrected ratios are given as examples of TCR Vβ3, 7, and 13.1 families in CD71+PBMCs after 15-day incubation with cell lysates. Uncultured cells were used as baseline controls. The other unskewed Vβ families are represented by Vβ15.
might explain why in some cases, the magnitude of the specific skewing of these Vb families was lower than in the other cases studied. Specimens from other sites of the same organ might have been highly infiltrated and could show a more impressive TCR Vb skewing. In addition, and perhaps for similar reasons, CVB3 infection was ascertained immunologically in all the specimens, but the presence of the CVB genome was detected by RT-PCR in only 1 case. These aspects can be taken into consideration to explain why a correct diagnosis of myocarditis may be made without the support of histopathological and molecular evidence.

In certain autoimmune diseases,\textsuperscript{22,36,37} and malignancy,\textsuperscript{38,39} it has been shown that in situ activated T cells carry a restricted set of rearranged TCR genes, as we have found in the hearts of our patients. Although a preferential usage of certain Vb families could be explained by the presence of an oligoclonal T-cell response to a conventional antigen, the polyclonality of the T-cell populations we have found in the specimens studied, together with the presence of a nonrestricted Va repertoire, is most consistent with an immune response initiated by a superantigen.\textsuperscript{18,28}

The ability of lysates from CVB3-infected Vero cells to stimulate in vitro T cells from healthy donors carrying the same 3 TCR Vb families we have found skewed in vivo (ie, Vb3, 7, and 13.1) further supports the hypothesis of a superantigen-driven immune response. Although superantigen-mediated immune responses are notoriously not classically MHC-restricted, the binding efficiency and presentation of superantigens vary in the presence of different MHC alleles,\textsuperscript{40} as well as in the presence of different peptides lodged in the MHC molecule-binding groove.\textsuperscript{41} To this point, however, due to the limited number of donors we were able to test, we are not in the position of associating positive or negative reactions against CVB3 to any specific HLA allele. Nonetheless, it is certainly worthwhile to note that the donors most reactive to CVB3 had DQA1*01 and DQB1*06 alleles in common with the myocarditis patients.

We do not yet know whether a protein of the CVB3 itself has superantigenic properties or whether CVB3 infection transcriptionally activates human endogenous retroviruses encoding molecules with superantigen-like activity.\textsuperscript{42} This has been proposed in cytomegalovirus infection.\textsuperscript{43} However, we were able to provide evidence that in vitro, a CVB3-triggered, superantigen-driven response gave results very similar to those observed in the pathogenic scenario of the cases of IDC studied.

Some clear evidence exists in humans for virus-derived superantigens.\textsuperscript{44,45} Evidence for a superantigen-mediated preferential expansion of TCR Vb7-positive T cells in patients suffering from type I diabetes was described a few years ago,\textsuperscript{22} and more recently, a human endogenous retrovirus distantly related to mouse mammary tumor virus has been isolated from the same specimens\textsuperscript{46} and was found to be able to trigger in vitro a similar immune reaction.\textsuperscript{47}
Our observations of a limited TCR Vβ gene family usage in the presence of variable CDR3 regions allow us to hypothesize that CVB3 might encode a superantigen (or upregulate an endogenous superantigen-like molecule) whose relevance in myocarditis/IDC pathogenesis and perhaps in other autoimmune diseases seems to be clinically relevant and should be further evaluated.

Acknowledgments

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References


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