Expression of Costimulatory Molecules B7–1, B7–2, and CD40 in the Heart of Patients With Acute Myocarditis and Dilated Cardiomyopathy

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Background—In patients with acute myocarditis and dilated cardiomyopathy (DCM), we previously reported that antigen-specific T cells infiltrate the heart and play an important role in the myocardial damage involved. For antigen-specific T-cell activation to occur, it is necessary for T cells to receive a costimulatory signal provided by costimulatory molecules expressed on antigen-presenting cells (APCs) as well as the main signal provided by binding of T-cell receptors to the antigen.

Methods and Results—To investigate the roles of the costimulatory molecules B7–1, B7–2, and CD40 in the development of acute myocarditis and DCM, we analyzed the expression of these antigens in the myocardial tissues of patients with acute myocarditis and DCM. We also examined the expression of a cytolytic factor, perforin, in the infiltrating cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, because both killer lymphocytes are thought to damage B7–1–expressing APCs. We found that B7–1, B7–2, and CD40 were moderately to strongly expressed in the cardiac myocytes of patients with acute myocarditis. Weak to moderate expression of these antigens was also found in the cardiac myocytes of patients with DCM. There was infiltration of perforin-expressing CTLs and NK cells in the myocardial tissues of patients with acute myocarditis and DCM.

Conclusions—Our findings strongly suggest that expression of B7–1, B7–2, and CD40 antigens on cardiac myocytes may make them APCs for CTLs and NK cells and that they may play an important role in the direct myocardial damage by these killer cells in acute myocarditis and DCM. (Circulation. 1998;97:637-639.)

Key Words: myocarditis | cardiomyopathy | immunology | immunohistochemistry

We previously reported that activated and antigen-specific T cells infiltrate the hearts of patients with acute myocarditis and DCM. For antigen-specific T-cell activation to occur, it is necessary for the T cell to receive two signals from the APC. The first signal is provided by T-cell receptor engagement with a combination of antigen and major histocompatibility complex, and the second signal, called the costimulatory signal, is provided by costimulatory molecules on the APC. Among these, the B7 family molecules B7–1 (B7, CD80) and B7–2 (B70, CD86), which are the ligands for CD28 and CTLA-4 on the T cell, are the most extensively characterized and appear to be the most critical. Another costimulatory molecule, CD40, expressed on various APCs, is known to induce expression of B7 antigens as well as to initiate T-cell–dependent antibody responses.

To investigate whether costimulatory molecules B7 and CD40 antigens really play an important role in myocardial damage in humans, we analyzed the expression of these antigens in the myocardial tissue of patients with acute myocarditis and DCM. We also examined the expression of the cytolytic factor perforin in the infiltrating CTLs and NK cells, because both killer lymphocytes are thought to directly damage B7–1–expressing APCs.

Methods

Patients

Myocardial tissue samples were obtained at biopsy or autopsy from 7 patients with acute myocarditis and 10 patients with DCM (11 men and 6 women; age, 45.7±13.6 years [mean±SD]) in whom clinical diagnoses of acute myocarditis and DCM had been previously determined by history, physical examination, blood analyses, and echocardiography. Myocardial tissue samples from 4 patients with other causes of congestive heart failure without inflammation, such as doxorubicin-induced myocardial injury, hypertrophic obstructive car-
diomyopathy, valvular heart disease, and old myocardial infarction, were used as controls.

**Monoclonal Antibodies**

Mouse anti-human B7–1 (L307) and B7–2 (IT2.2) mAbs were described previously. Mouse anti-human CD40 (5C3), CD8 (Leu-2a), and CD16 (3G8) mAbs were purchased from PharMingen, Becton Dickinson Immunocytometry Systems, and Immunotech, respectively. The procedures for preparing a rat anti-mouse perforin (P1–8) mAb, which was also shown to react with human perforin, were described previously.9

**Immunofluorescence**

In this study, to amplify the specific signals of antigen–antibody reaction, we used tyramide signal amplification technology for fluorescence (TSA-Direct [Green], NEN Life Science Products) according to the manufacturer’s instructions. Cryostat sections of freshly dissected myocardial tissue samples (6 mm thick) were fixed in acetone for 5 minutes at 4°C and incubated with mouse anti-human B7–1, B7–2, or CD40 mAb, respectively, for 1 hour at 37°C. The sections were reacted with fluorescein–tyramide for the appropriate time (3 to 10 minutes), then incubated with streptavidin–horseradish peroxidase for 30 minutes. The sections were then reacted with fluorescein–tyramide for the appropriate time (3 to 10 minutes), then examined and photographed under a fluorescence microscope.

**Immunohistochemistry**

Double staining was performed for surface markers (CD 8 or CD16) and perforin by an enzyme antibody method as described previously.10 Surface markers stained brown by the horseradish peroxidase reaction, and perforin stained blue by the alkaline phosphatase reaction.

**Results**

**Expression of B7–1, B7–2, and CD40 in Ventricular Tissue**

In normal ventricular tissue, several cells weakly to moderately expressed B7–1, B7–2, and CD40 antigens (Fig 1A, 1E, and 1I, respectively). These cells positive for B7–1, B7–2, and CD40 are thought to be dendritic cells.11 There was no expression of these antigens in cardiac myocytes, whereas in ventricular tissue of patients with acute myocarditis, most of the infiltrating cells strongly expressed B7–1, B7–2, and CD40 and some of the cardiac myocytes moderately to strongly expressed these antigens (Fig 1B, 1F, and 1J, respectively). In ventricular tissue of patients with DCM, most of the infiltrating cells strongly expressed B7–1, B7–2, and CD40, and a few cardiac myocytes near or in contact with the infiltrating cells moderately to strongly expressed these antigens (Fig 1C, 1G, and 1K, respectively; arrows). The expression of these antigens was found in all patients with acute myocarditis and DCM that we studied, with some variations. In contrast, there was no expression of these antigens in ventricular tissue of control patients with other causes of congestive heart failure without inflammation (Fig 1D, 1H, and 1L, respectively).

**Expression of Perforin in Infiltrating Killer Lymphocytes**

To analyze the pathogenic role these infiltrating cells might play, we examined the expression of perforin in CTLs and NK cells in ventricular tissues of patients with acute myocarditis and DCM, because both types of lymphocytes are thought to directly damage B7–1–expressing APCs. Fig 2 shows representative sections with double staining of the infiltrating cells for perforin as blue and cell surface markers (CD8 and CD16) as brown. There was clear expression of perforin in the peripheral cytoplasmic granules of CTLs (Fig 2A and 2B) and NK cells (Fig 2C and 2D) in patients with acute myocarditis (Fig 2A and 2C) as well as those with DCM (Fig 2B and 2D). This indicated that both infiltrating CTLs and NK cells were activated killer cells. There were almost no infiltrating CTLs or NK cells in ventricular tissue of control patients with other causes of congestive heart failure without inflammation. The
absence of the expression of B7–1, B7–2, and CD40 in ventricular tissue of these control patients supported the absence of infiltrating killer cells, because the infiltrating killer cells strongly expressed these antigens (Fig 1D, 1H, and 1L).

Discussion
In this study, we clearly demonstrated that expression of the costimulatory molecules B7–1, B7–2, and CD40 was induced on cardiac myocytes in acute myocarditis and DCM. Furthermore, infiltration of perforin–expressing activated CTLs and NK cells was found in myocardial tissues in acute myocarditis and DCM. Because CTLs and NK cells are known to directly damage B7–1–expressing APCs, these findings strongly suggested that the expression of costimulatory molecules, especially B7–1, may make cardiac myocytes the target cells for these infiltrating killer lymphocytes and play a critical role in the development of the myocardial damage involved. The expression of CD40 on cardiac myocytes was thought to play a role in inducing B7 antigens on cardiac myocytes on binding to CD40 ligand (gp39) expressed on the infiltrating T cells.5

Recently, we found that the expression of B7–1 and B7–2 was strongly induced on cardiac myocytes in a murine model of acute myocarditis caused by Coxsackievirus B3. We also found that in vivo, anti–B7–1 mAb treatment markedly reduced the myocardial inflammation, indicating the pivotal role of B7–1 in the development of the myocardial damage involved. Furthermore, the expression of B7–1 and B7–2 was clearly induced on cultured murine cardiac myocytes by treatment with interferon-γ in vitro (manuscript submitted). Therefore, the expression of B7–1, B7–2, and CD40 on cardiac myocytes revealed in the present study seems to have been induced by cytokines such as interferon-γ released from the infiltrating cells.

In conclusion, the expression of costimulatory molecules (especially B7–1) on cardiac myocytes may play a critical role in myocardial damage in patients with acute myocarditis and DCM; this raises the possibility of immunotherapy with anti–B7–1 mAb to prevent T-cell–mediated as well as NK cell–mediated myocardial damage in acute myocarditis and DCM.

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