Immunoglobulin treatment suppressed adoptively transferred autoimmune myocarditis in severe combined immunodeficient mice

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Shioji, Keisuke, Zuyi Yuan, Toru Kita, and Chiharu Kishimoto. Immunoglobulin treatment suppressed adoptively transferred autoimmune myocarditis in severe combined immunodeficient mice. Am J Physiol Heart Circ Physiol 287: H2619–H2625, 2004. First published August 12, 2004; doi:10.1152/ajpheart.01130.2003.—We investigated the suppressive effects of immunoglobulin (Ig) on effector T cells in autoimmune myocarditis. Treatment with Ig reduced production of the so-called T-helper type 1 (Th1) cytokines stimulated by concanavalin A or cardiac myosin in cultured lymph node (LN) cells from rats with myocarditis. The cytotoxic activities of LN cells from rats immunized with myosin and treated with Ig were reduced against cardiomyocytes and F-2 cells compared with those treated without Ig. The adoptive transfer of myocarditis from LN cells of Lewis rats with myocarditis to severe combined immunodeficient (SCID) recipients was successfully achieved. Treatment with Ig, but not with F(ab′)2 fragments of Ig, reduced the mortality and severity of myocarditis in SCID recipient mice. Decreased ability of LN cells of Ig-treated rats, but not rats treated with F(ab′)2 fragments, to transfer autoimmune myocarditis was also demonstrated. The findings of the present study suggest that autoimmune myocarditis was successfully transferred to SCID mice and that treatment with Ig ameliorated autoimmune myocarditis produced by the adoptive transfer method.

The therapeutic efficacy of high-dose immunoglobulin (Ig) has been reported in inflammatory and autoimmune diseases (7), e.g., idiopathic thrombocytopenic purpura (21), dermatomyositis (1), Kawasaki disease (16), and peripartum cardiomyopathy (5). We documented a case report of successful Ig treatment for fulminant myocarditis (24). We reported that treatment with Ig suppressed the severity of the disease in acute murine myocarditis (8, 27). Moreover, treatment with Ig suppressed the severity of the disease by suppressing the initial antigen-priming process in rats with experimental autoimmune myocarditis (23). Ig therapy with the Fc portion resulted in an anti-inflammatory action by the inhibitory Fc receptor (Fc receptor IIB) associated with suppression of dendritic cells in an autoimmune myocarditis model (23).

In the present study, we focused on the role of Ig in T cell responsiveness to cardiac myosin in SCID mice with autoimmune myocarditis produced by the adoptive transfer method. To investigate whether treatment with Ig could modify the functions of myosin-specific and -nonspecific T cells, we studied cytokine production in cultured lymph node (LN) cells and the cytotoxic activity of LN cells from rats immunized with cardiac myosin. In addition, we investigated the suppressive effects of Ig on autoimmune myocarditis in SCID to which cells from Lewis rats with myocarditis were adoptively transferred and the cytotoxic activity of LN cells of Ig-treated rats with autoimmune myocarditis.

MATERIALS AND METHODS

Animals. Six-week-old male Lewis rats and 5-week-old BALB/c mice (Shimizu Laboratory Supplies) and 5-week-old male CB-17scid/ scid and CB-17 mice (Crea) were maintained in our animal facilities and cared for in accordance with the institutional policies and guidelines of Kyoto University.

Cells. F-2 cells are murine endothelial cells established from an ultraviolet light-induced tumor; they were maintained in 10% FCS-supplemented DMEM at 37°C in 5% CO2. For culture of neonatal rat cardiomyocytes, cardiac ventricles from 1- to 4-day-old Lewis rats were minced and dissociated with 0.125% trypsin. Cardiomyocytes (2 × 106/well) were incubated in 96-microwell plates in 10% FCS-supplemented DMEM at 37°C in 5% CO2. Bromodeoxyuridine (100 μmol/l) was added during the first 48 h to prevent proliferation of nonmyocytes.

LN cells were isolated as described previously (9). They were removed from Lewis rats 12 days after immunization with myosin, during the phase of the autologous regulatory process in LN cells. LN cells were used for adoptive transfer to recipient rodents or suspended in RPMI 1640 with 10% FCS, 1% sodium pyruvate, 1% nonessential amino acids, 5 × 10−5 M 2-mercaptoethanol, and penicillin-streptomycin.

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Ig preparations. Intact human Ig (Venoglobulin-IH, Welfide), a polyethylene glycol-treated human Ig, or F(ab')2 fragments of human Ig (Gamma-Venin, Aventis Corp) were used. The doses of Ig used in the in vitro study were similar to those reported previously (23). The heterogeneous source of Ig did not appear to be a problem (8, 18, 23, 27). To exclude the possibility that human Ig preparations used in this study might cause cellular damage to rat lymphocytes by xenonantibodies, the trypan blue dye exclusion test was performed in lymphocytes cocultured for 30 min with various doses (0.1, 0.6, and 6 mg/ml) of both Ig preparations.

Measurement of cytokines. Viable LN cells (2 × 10⁵ per well) were cultured in the presence of concanavalin A (ConA), porcine cardiac myosin, or the untreated protein mouse myelin basic protein for 72 h. Human Ig was added to the medium 30 min before ConA or protein (porcine cardiac myosin and mouse myelin basic protein) stimulation.

For analysis of proinflammatory cytokine [tumor necrosis factor-α (TNF-α), Th1 cytokine [interferon-γ (IFN-γ)] and interleukin (IL)-β] and Th2 cytokine (IL-10), supernatants were assayed using antibody-sandwich enzyme-linked immunosorbent assay (ANALYZA, TECHNE) at the end of culture.

Cytotoxicity assay. Lewis rats were immunized with cardiac myo- sin, treated with Ig (1 g·kg⁻¹·day⁻¹, n = 3) or PBS (n = 3) every other day, and killed on day 12. The actual dose of Ig was calculated from the weight at the beginning of the experiment. LN cells from rats treated with Ig or PBS were used as effector cells. F-2 cells and cardiomycocytes (2 × 10⁵/well each) plated in 96-microwell plates were labeled with sodium chromate (⁵¹Cr, 37 kBq/well; Amersham International) for 1 h. After labeled target cells were washed three times with PBS, LN cells were incubated at effector-to-target ratios of 100:1 and 200:1 for 4 h. The supernatant was collected, and the radioactivity of ⁵¹Cr release into the supernatant was measured using a gamma counter (LKB Wallac Clinigamma 1272 Pharmacia). The percentage of cytotoxicity was calculated as follows

\[
\% \text{ cytotoxicity} = \frac{(E - S)}{(M - S)} \times 100
\]

where E represents counts per minute released in the presence of effector cells, S represents spontaneous counts per minute released from target cells incubated in the medium, and M represents maximal counts per minute released from target cells incubated with 2% Nonidet P-40.

Antigen. Cardiac myosin was prepared from the ventricular muscle of porcine hearts, as described previously (9). The cardiac myosin fraction was dissolved at 10 mg/ml in PBS containing 0.3 M KCl.

Active induction of experimental myocarditis in rats. Porcine car- diac myosin (1 mg/ml sc) mixed with Freund’s complete adjuvant supplemented with Mycobacterium tuberculosis H37Ra (Difco) was injected into the footpads of the rats on day 1.

Adoptive transfer and Ig treatment. In the first set of experiments, LN cells of Lewis rats with myocarditis were prepared as described above and injected intraperitoneally into SCID mice (n = 24), CB-17 mice (n = 2), BALB/c mice (n = 3), or Lewis rats (n = 3) at 2 × 10⁷ viable cells per animal. Ig (1 g·kg⁻¹·day⁻¹, n = 15), F(ab')² fragment (n = 5), or PBS (n = 15) was intraperitoneally administered to these SCID mice on days 2, 3, 4, 7, and 9. The actual dose of Ig was calculated from the weight at the beginning of the experiment. The animals were observed daily, and necropsy was performed immediately on mice that were found dead. Mice and rats surviving until day 12 were killed under ether anesthesia for pathological examination.

Control SCID mice without adoptive transfer were injected with PBS (n = 8) or Ig (n = 8) intraperitoneally and killed on day 12. LN cells of Lewis rats without myocarditis were also prepared and injected intraperitoneally into SCID and CB-17 mice (n = 5 each).

In the second set of experiments, 13 days after primary immunization, LN cells were collected from PBS- and from Ig-treated rats or rats treated with F(ab')² fragments and transferred to SCID mice (n = 5 each) as described above. The ability to transfer the disease to naive SCID recipients was assayed.

In the third set of experiments, viable LN cells of Lewis rats with myocarditis were injected intraperitoneally into SCID mice at 3 × 10⁷ viable cells per mouse. The mice were divided and injected with PBS (n = 5) or treated with Ig (1 g·kg⁻¹·day⁻¹, n = 4) from day 1 to day 3. The mice were killed on day 4 for quantitation of spleen cells and flow cytometric analyses. For analysis of surface markers, the collected cells were incubated with the appropriate dilution of R-phycocerythrin-conjugated antibodies: CD4, CD8, and IgGl isotypic control antibody (Immunotech). Cells (1 × 10⁵ per trial) were analyzed with a FACScan cytometer (Becton Dickinson) using CELLQuest. Control SCID mice without adoptive transfer were injected with PBS and killed on day 3 (n = 3).

Histopathology. At euthanasia, macroscopic findings were graded on a scale of 0–3: normal appearance (0), a spotty hemorrhagic or discolorated area (1), a few spotty hemorrhagic or discolorated areas (2), and multiple or diffuse hemorrhagic or discolorated areas (3). After macroscopic examination, the hearts, lungs, livers, intestine, and kidneys were fixed in 10% formalin, transversely sliced, embedded in paraffin, and stained with hematoxylin and eosin. The area of the total myocardium and that affected by myocarditis (i.e., regions showing myocardial hemorrhage, infiltration by inflammatory cells, and myocardial necrosis) was determined using a square lattice scale in an ocular lens of a microscope. The percentages of damaged area [(affected area/total myocardial area) × 100] were calculated.

Immunohistochemistry. Immunohistochemistry for surface markers was performed as described previously (23). The primary antibodies (Serotec) were as follows: anti-I-A antibody to recognize major histocompatibility complex class II-expressing cells, including mono- cytes and B lymphocytes; anti-rat CD4 antibody to detect helper T lymphocytes and macrophages; and anti-rat CD8 antibody to detect cytotoxic/suppressor T lymphocytes. (For negative controls, sections were processed through all steps of the immunohistochemical staining without primary antibodies.)

Immunohistochemistry for IL-1β was performed using mouse anti-mouse IL-1β antibody (Serotec) with a Mouse-on-Mouse Immunodetection Kit, Peroxidase (Vector), as described previously (28, 29). Cells were counterstained with hematoxylin. Positive-stained cells were counted in myocardial inflammatory 0.25 × 0.25-mm² fields, and the averaged number from five different fields was calculated.

Statistical analysis. Values are means ± SD. Statistical analysis of the data was performed by Student’s t-test for differences between two unpaired groups, χ²-test with the Yates’ correction for survival rate, or one-way ANOVA for differences among three or more groups and reanalysis with Fisher’s protected least-significant difference test to characterize significant differences between groups. Survival curves were analyzed by the method of Kaplan and Meier. P < 0.05 was considered statistically significant.

RESULTS

Neither intact Ig nor F(ab')² caused a significant loss of cell viability at ordinary in vitro doses by trypan blue test (viability >93%). Accordingly, the possibility of xenonantibodies of human Ig against rats as an in vitro mechanism may be low.

Ig suppressed production of TH1, but not TH2, cytokine in cultured LN cells from rats with myocarditis. IFN-γ production per cell in LN cells was increased by ConA stimulation (Table 1). TNF-α, IFN-γ, and IL-1β production was also increased by myosin stimulation (Table 1). IL-10 production was not changed by ConA or myosin stimulation. Among the four cytokines, only IFN-γ production stimulated by ConA was reduced by treatment with Ig in cultured LN cells from rats with myocarditis (Table 1). IFN-γ, IL-1β, and TNF-α production stimulated by myosin was also markedly reduced by treatment with Ig (Table 1). There was no significant change in
Table 1. Effect of Ig on production of cytokines in vitro

<table>
<thead>
<tr>
<th>Ig, mg/ml:</th>
<th>TNF-α, pg/ml</th>
<th>IFN-γ, pg/ml</th>
<th>IL-1β, pg/ml</th>
<th>IL-10, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.6</td>
<td>6.0</td>
<td>0</td>
</tr>
<tr>
<td>ConA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 µg/ml</td>
<td>65.3±17.2</td>
<td>50.1±15.6</td>
<td>49.8±15.7</td>
<td>12.2±3.5</td>
</tr>
<tr>
<td>1.0 µg/ml</td>
<td>78.2±16.3</td>
<td>49.5±10.7</td>
<td>46.4±19.0</td>
<td>12.6±5.0</td>
</tr>
<tr>
<td>Cardiac</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myosin</td>
<td>2.0 µg/ml</td>
<td>212.3±27.6</td>
<td>57.5±15.5†</td>
<td>34.4±12.6†</td>
</tr>
<tr>
<td>5.0 µg/ml</td>
<td>412.6±55.6</td>
<td>151.1±68.7†</td>
<td>40.9±29.7†</td>
<td>28.2±11.0</td>
</tr>
<tr>
<td>Myelin basic protein</td>
<td>5.0 µg/ml</td>
<td>39.5±13.4</td>
<td>42.6±10.5</td>
<td>25.7±15.2</td>
</tr>
</tbody>
</table>

Values are means ± SD expressed as cytokine concentration of lymph node (LN) cells of 4–6 wells each experiment. Cytokine production per cell in LN cells stimulated by concanavalin A (ConA), cardiac myosin, or myelin basic protein were reduced by treatment with Ig. *P < 0.05; †P < 0.01 vs. LN cells treated without Ig at the same dose of ConA, cardiac myosin, or myelin basic protein (1-way ANOVA with Fisher’s protected least-significant difference test).

production of the four cytokines stimulated by the unrelated protein, myelin basic protein. The results suggested that Ig suppresses myosin-specific and -nonspecific Th1 responses.

Ig suppressed cytotoxic activity of LN cells from Lewis rats with myocarditis. At the effector-to-target ratio of 100:1, the cytotoxic activities against cardiomyocytes, target cells with self-antigen, and F-2 cells, target cells with non-self-antigen, did not differ between LN cells from Lewis rats immunized with myosin and treated with Ig and those treated with PBS (Fig. 1). At the effector-to-target ratio of 200:1 but not with 100:1. Values are means ± SD of 4 individual wells. Findings were constant and are representative of 3 separate experiments. *P < 0.01 vs. PBS (Student’s t-test).

Adoptive transfer of myocarditis was successful into SCID mice but not into wild-type mice. Twelve of 15 SCID recipients (80.0%) showed pathological changes in the heart, and 10 of 15 PBS-injected SCID mice (66.7%) to which LN cells from Lewis rats with myocarditis were adoptively transferred died before the euthanasia (Table 2). Hemorrhagic perimyocarditis was induced macroscopically (Fig. 2A) and microscopically (Fig. 2, B–D). No pathological changes were observed in the other organs of mice treated by adoptive transfer. Accordingly, mice that died before euthanasia might have died by diastolic dysfunction due to hemorrhagic perimyocarditis.

No evidence of myocarditis was observed in SCID mice without adoptive transfer and treated with Ig or PBS (Table 2). In none of two CB-17 or three BALB/c mice or two of three Lewis rats was myocarditis induced by adoptive transfer from Lewis rats with myocarditis. In addition, in none of the SCID or CB-17 mice (n = 5 each) was myocarditis induced by adoptive transfer of LN cells from Lewis rats without myocarditis.

Immunohistochemistry for rat surface markers showed CD4-positive cells (Fig. 3A) and I-A-positive cells (Fig. 3B) in SCID mice treated by adoptive transfer from Lewis rats with myocarditis. Only a few cells in these SCID mice showed very weak immunoreactivity for anti-CD8 antibody (Fig. 3C). Accordingly, the adoptive transfer of self-reactive rat T cells to SCID mice was successfully achieved. Moreover, CD4-positive cells, but not CD8-positive cells, may play a pivotal role in autoimmune myocarditis induced by adoptive transfer.

Ig, but not the F(ab′)2 fragment, suppressed autoimmune myocarditis in adoptively transferred SCID mice. Only 3 of 15 adoptively transferred SCID mice treated with Ig (20%) died before euthanasia. Three of five adoptively transferred SCID...
mice treated with F(ab')2 fragments (60%) died (Table 2). Comparison of the survival curves by the method of Kaplan and Meier showed a significant difference between Ig-treated and PBS-injected mice (66.7%, 10 of 15, log rank 9.52, \( P < 0.01 \)). Treatment with Ig reduced the severity of the disease, as assessed by the ratio of heart weight to body weight and histological scores (Table 2, Fig. 2E). In addition, immunohistochemistry for IL-1β showed that treatment with Ig reduced the number of IL-1β-positive cells (\( P < 0.01 \); Fig. 4). In contrast, treatment with the F(ab')2 fragment did not suppress development of myocarditis (Table 2).

**DISCUSSION**

The findings of the present study suggested that autoimmune myocarditis was successfully transferred to SCID mice and that treatment with Ig suppressed Th1 cytokine production and cytotoxic activities of LN cells from rats with myocarditis, which leads to suppression of the severity of disease in SCID recipient mice with autoimmune myocarditis. In contrast, treat-

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**Table 2. Effect of Ig and F(ab')2 fragments on SCID mice with autoimmune myocarditis by adoptive transfer from Lewis rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Diseased/Total</th>
<th>Hemorrhage/Total</th>
<th>HW/BW, mg/g</th>
<th>Macroscopic Score</th>
<th>Damaged Area, %</th>
<th>Deaths/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adoptive transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>15</td>
<td>12/15 (80.0%)</td>
<td>10/15 (66.7%)</td>
<td>7.1±0.4</td>
<td>2.2±0.9</td>
<td>5.2±3.3</td>
<td>10/15 (66.7%)</td>
</tr>
<tr>
<td>Ig</td>
<td>15</td>
<td>9/15 (60.0%)</td>
<td>3/15† (20.0%)</td>
<td>5.6±0.7*</td>
<td>1.5±0.9*</td>
<td>1.9±1.3*</td>
<td>3/15† (20.0%)</td>
</tr>
<tr>
<td>F(ab')2 fragments</td>
<td>5</td>
<td>4/5 (80.0%)</td>
<td>4/5 (80.0%)</td>
<td>6.6±0.6</td>
<td>2.0±1.1</td>
<td>4.6±2.2</td>
<td>3/5 (60.0%)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>8</td>
<td>0/8 (0%)</td>
<td>0/8 (0%)</td>
<td>5.1±0.3</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>Ig</td>
<td>8</td>
<td>0/8 (0%)</td>
<td>0/8 (0%)</td>
<td>5.6±0.4</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0/8 (0%)</td>
</tr>
</tbody>
</table>

Heart weight-to-body weight ratio (HW/BW), macroscopic score, and damaged area values are means ± SD. SCID, severe combined immunodeficient. Significantly different from adoptively transferred mice injected with PBS: *\( P < 0.01 \); †\( P < 0.05 \).

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The severity of passively transferred myocarditis was significantly lower in SCID recipients of LN cells from Ig-treated rats (n = 5) than in SCID recipients of LN cells from PBS-treated rats (n = 5; Table 3).

In the analysis of splenocyte surface markers, no differences were observed in the ratio of CD4- and CD8-positive cells to total spleen cells between adoptively transferred SCID mice treated with Ig and those injected with PBS (data not shown).

**Fig. 2. Representative histopathology in adoptively transferred severe combined immunodeficient (SCID) mice.** Heart of an adoptively transferred SCID mouse injected with PBS, which died on *day 8*, shows multiple hemorrhagic lesions (arrows) macroscopically (A) and microscopically (B). Extensive injury of cardiomyocytes with inflammatory changes, including pericarditis, was observed in an adoptively transferred SCID mouse injected with PBS on *day 12* (C and D). Severity of the disease was reduced in an adoptively transferred SCID mouse treated with Ig on *day 12* (E). Cells were stained with hematoxylin and eosin. Original magnification: ×40 (B, C, and E) and ×200 (D).
ment with the F(ab')2 fragment did not suppress development of autoimmune myocarditis in SCID recipient mice. In addition, decreased ability of LN cells of Ig-treated rats, but not rats treated with the F(ab')2 fragment, to transfer autoimmune myocarditis was also demonstrated. Taken together with the previous report (23), Ig suppressed the function of effector T cells as well as the expression of dendritic cells, inducing selective myosin unresponsiveness in autoimmune myocarditis via the Fc portion.

We previously reported that treatment with Ig suppressed experimental autoimmune myocarditis in rats as a result of suppression of the expression of dendritic cells (23). In the present study, we investigated the effect of Ig on T cells, because T cells, especially CD4 T cells, were reported to play a pivotal role in myocardial injury in experimental autoimmune myocarditis (9, 17). Several effects of Ig on T cells might be postulated. Antibodies against the variable region of the T cell receptor, which are present in Ig, may regulate T cell function in autoimmune disorders (11). Anticytokine antibodies in Ig may modulate T cell function (26). Ig also contains agonistic and blocking antibodies against Fas (CD95), the receptor for the Fas ligand, which transduces apoptotic signals into cells. Thus apoptosis of self-reactive T cells may be induced by such antibodies (19). Indeed, refractoriness to autoimmune encephalomyelitis was transferred to Lewis rats by CD4 T cells from rats treated with Ig in an experimental autoimmune encephalomyelitis model (18). In addition to the above-mentioned effects of Ig on T cells, in the present study, measurement of Th1 cytokine production showed that Ig affects autoimmune myocarditis by suppressing T cell responsiveness to cardiac myosin, a specific stimulus, and to ConA, a nonspecific stimulus.

Extensive injury of cardiomyocytes with CD4-positive cells was shown in SCID rats treated by adoptive transfer from rats with myocarditis in the present study in addition to rats with experimental autoimmune giant cell myocarditis, as previously shown (9, 23). The cytotoxicity assay suggested that the cytotoxic activities of autoreactive LN cells, in addition to xenoreactive LN cells, were increased in rats with experimental giant cell myocarditis (Fig. 1) and that these cells were responsible for cardiac injury in SCID recipients of adoptive transfer from Lewis rats with myocarditis. Accordingly, treatment with Ig suppressed the experimental autoimmune myocarditis model, in part as a result of suppression of cytotoxic activities of LN cells.

SCID mice were used for transfer experiments in several autoimmune diseases, such as autoimmune myocarditis (25), systemic lupus erythematosus (6), and rheumatoid arthritis (14). The results of the present study showed that adoptive transfer of xenogenic LN cells was successful in SCID mice because of deficiency of B and T lymphocyte functions, but not in CB-17 or BALB/c mice, although these strains share the same genetic background, including the same H-2 complex (4, 25). The direct evidence of rat CD4-positive cells in the myocardium of SCID recipient mice also confirmed the successful transfer of the xenogenic autoimmune system (Fig. 3). CD4-positive T cells, but not CD8-positive T cells sorted from LN cells, induced myocarditis in naïve rats (17). Accordingly, transferred antigen-specific CD4-positive T cells might operate in an autoimmune manner in vivo in the adoptively transferred SCID mice, and treatment with Ig ameliorates immune-mediated myocarditis in SCID recipient mice, mainly as a result of suppressive effects on antigen-specific T cells.

Hemorrhagic perimyocarditis was characteristic not only in the present transfer model (Fig. 2) but also in the development of giant cell myocarditis (23). It is suggested that the cause of death in the present study may be acute constrictive heart failure. Accordingly, marked improvement in survival by treat-
ment with Ig might be reasonable, regardless of the rather small, but significant, improvement in cardiac pathology.

IL-1β is a principal proinflammatory cytokine produced mainly by macrophages. IL-1β is induced in autoimmune myocarditis, and IL-1β activates cytotoxic/helper T cells, which might cause direct cardiomyocyte injury and the induction of cell adhesion molecules (10). The present study showed that treatment with Ig leads to a significant reduction of IL-1β-positive cells (Fig. 4), which implies that treatment with Ig reduced the severity of myocarditis in adoptively transferred SCID mice with myocarditis, at least in part as a result of the anti-inflammatory action, as previously described (8, 23).

Human myocarditis includes various clinical etiologies: idiopathic, viral/immune, and giant cell origins. Diagnosis of myocarditis based on etiology before the beginning of the treatment has not been established in most clinical settings (20). McNamara et al. (13) reported that treatment with Ig did not augment the improvement of left ventricular function in patients with recent-onset cardiomyopathy. One of reasons may be that patients with dilated cardiomyopathy with noninflammatory causes occupied a large part of that trial. Ig therapy has been proven to be effective for autoimmune and inflammatory diseases (7). Because Ig suppressed not only autoimmune myocarditis in SCID mice by the transfer method but also experimental autoimmune giant cell myocarditis in rats (23), Ig therapy might be specifically effective for patients with autoimmune or immune-mediated myocarditis but not with noninflammatory myocarditis.

Some of the beneficial effects of Ig therapy in autoimmune and immune-mediated disorders have been attributed to Fc fragments of Ig preparations in vivo (21, 23). Indeed, in the present study, treatment with F(ab′)2 fragments did not suppress the development of autoimmune myocarditis in SCID recipient mice. However, recent reports revealed the inhibitory effects of F(ab′)2 fragments on dendritic cell maturation in vitro (2, 3). Recent studies in an animal model of idiopathic thrombocytopenic purpura suggest that Ig increases expression of Fcγ receptor IIB, an inhibitory receptor (21). Accordingly, precise analysis of the interaction between Fc or F(ab′)2 fragments and Fc receptors for the mechanisms of Ig appears to be warranted.

In conclusion, the findings of the present study provide evidence that treatment with Ig ameliorates immune-mediated myocarditis by suppressing Th1 cytokine production and cytotoxic activities of LN cells in SCID recipients treated by adoptive transfer from rats with myocarditis via the Fc portion. Namely, Ig treatment preferentially induces myosin unresponsiveness of effector T cells in autoimmune myocarditis. There is no general agreement on an effective treatment for immune or autoimmune myocarditis. Thus exploration of the clinical usefulness of Ig for autoimmune or immune-mediated myocarditis appears warranted.

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