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Autoimmune Myocarditis Does Not Require B Cells for Antigen Presentation

Susan Malkiel, Stephen Factor, and Betty Diamond

T cells constitute the pathogenic effector cell population in autoimmune myocarditis in BALB/c mice. Using mice rendered deficient for B cells by a targeted disruption to the IgM transmembrane domain or by treatment with anti-IgM Ab from birth, we asked whether B cells are a critical APC in the induction of autoimmune myocarditis. B cell-deficient mice immunized with cardiac myosin develop myocarditis comparable in incidence and severity to that in wild-type mice, suggesting that autoreactive T cells that cause myocarditis in BALB/c mice are activated by macrophages or dendritic cells. Since it does not appear that presentation of cryptic epitopes is critical for the breakdown of self tolerance, potentially pathogenic T cells recognizing dominant myosin epitopes must have escaped tolerization. Either anatomic sequestration of cardiac myosin peptide-MHC complexes or subthreshold presentation of cardiac myosin peptides by conventional APC can explain the survival of these autoreactive T cells.

Materials and Methods

Mice

BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in the Albert Einstein College of Medicine barrier animal facility. µMT mice on a C57BL/6 × 129/Sv background were obtained from K. Rajewsky (Cologne, Germany) (8) and backcrossed to the BALB/c strain for seven to nine generations. At each generation, the heterozygous breeders were identified by Southern blot analysis of tail DNA. They were then intercrossed to generate homozygous B cell-deficient mice.

BALB/c anti-µ mice were injected i.p. with 50 μg of goat anti-mouse IgM Ab (Southern Biotechnology Associates, Birmingham, AL), administered five times a week for the first week of life and on alternate days thereafter. Following cardiac myosin immunization, the dosage was increased to 100 μg of Ab every other day. Normal goat IgG (NIGlG)3 (Cappel Research Products, Durham, NC) was used as a negative control.

Cardiac myosin

Cardiac myosin was purified from BALB/c hearts according to the method of Pollack et al. (10). The concentration was determined by spectrophotometry, using an extinction coefficient of 5.4. Purity was determined by SDS-PAGE.

Induction of myocarditis

γ-Irradiated cardiac myosin (10, 30, and 100 μg) was emulsified in sterile CFA (Difco, Detroit, MI) and injected into 5- to 12-wk-old mice at four sites s.c. Mice were boosted s.c. with the same dose of cardiac myosin in CFA 1 and 3 wk after the initial immunization. They were sacrificed 1 wk after the last boost.

Histology

Mouse hearts were fixed in 10% buffered formalin and embedded in paraffin. Sections of each heart were obtained at five different levels and stained with hematoxylin and eosin. These were evaluated by a cardiac pathologist (S.F.), who was blinded to the status of each mouse. The diagnosis was according to the Dallas criteria, which established myocarditis as the presence of an inflammatory infiltrate and accompanying myocyte necrosis (1).

FACS analysis

The presence of B220-, CD4-, and CD8-positive cells in the spleen was determined by staining with anti-B220-PE (PharMingen), anti-CD4-PE (Becton Dickinson Immunocytometry Systems, San Jose, CA), and anti-CD8-FITC (PharMingen) and analysis with a FACScan.

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2 This study was supported by a grant from the National Institute of Arthritis and Musculoskeletal and Skin Diseases and by Grant T32HL07675 (to S.M.).

3 Abbreviations used in this paper: NIGlG, normal goat IgG; GCytc, cytochrome c; snRNPs, small ribonucleoprotein particles.
ELISAs

Mouse serum was tested for IgM and IgG anti-cardiac myosin Abs. Falcon microtiter plates (Becton Dickinson Labware, Lincoln Park, NJ) were coated with either cardiac myosin (0.5 µg/well) or anti-IgM Ab at a 1/1000 dilution (Southern Biotechnology Associates). Anti-IgM-coated plates were blocked with 1% BSA/PBS, and cardiac myosin-coated plates were blocked with 2% BSA/PBS. Serum samples were serially diluted, and titers were detected by an alkaline phosphatase conjugated anti-mouse IgG or IgM Ab (Southern Biotechnology Associates) diluted 1/1000 and the substrate, p-nitrophenyl phosphate (Sigma, St. Louis, MO). OD was measured at 405 nm.

Results

Induction of myocarditis in µMT mice

The µMT gene disruption that deletes the transmembrane domain of IgM was backcrossed onto the susceptible BALB/c strain for more than seven generations. The backcrossed homozygous mice had no detectable mature B cells expressing B220 in the spleen, but still possessed CD4+ and CD8+ T cells (Fig. 1). Additionally, they had little or no detectable IgM (<0.2 µg/ml) in their sera (Table I). Although homozygous immunized with cardiac myosin had some IgG in their sera (data not shown), they demonstrated no Ag-specific IgG response (Fig. 2). Eight of 10 homozygotes immunized with cardiac myosin developed myocarditis (data not shown). However, immunizing with 10 µg of cardiac myosin did not induce disease in either homozygous or wild-type mice (data not shown).

Induction of myocarditis in anti-µ mice

Because it is possible that a lack of B cells during fetal development can alter the T cell repertoire in mice, we decided to analyze BALB/c mice that were depleted of B cells after birth. Chronic injection of animals with anti-IgM Ab from birth is a technique previously demonstrated to deplete mice of B cells. Anti-µ-treated mice had no detectable B220+ splenocytes, but still possessed CD4+ and CD8+ T cells (Fig. 4). As observed with the µMT homozygous mice, anti-µ mice produced little or no detectable IgM (Table I). Although they did produce some IgG (data not shown), they also failed to demonstrate cardiac myosin-specific IgG following immunization (Fig. 2). All eight anti-µ mice immunized with cardiac myosin developed myocarditis, and there was no difference in the incidence or the severity of disease compared with that in the wild-type mice that were treated with normal goat IgG (Table II and Fig. 3).

Discussion

We found that mice genetically deficient in B cells and mice rendered B cell deficient after birth develop myocarditis following immunization with cardiac myosin. Although these B cell-deficient mice had little or no detectable IgM (Table I), they also failed to demonstrate cardiac myosin-specific IgG titers, in contrast to myosin-immunized µMT heterozygous mice (n = 10) and NGIgG-treated mice (n = 6).

Table I. Serum levels of IgM in immunized B cell-deficient BALB/c mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>IgM (µg/ml)</th>
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<tbody>
<tr>
<td>µMT+/−/−</td>
<td>&lt;0.2 µg/ml (n = 10)</td>
</tr>
<tr>
<td>µMT+/−/+</td>
<td>627.7 ± 154.6 (n = 9)</td>
</tr>
<tr>
<td>µMT−/−/+</td>
<td>439.2 ± 51.7 (n = 2)</td>
</tr>
<tr>
<td>Anti-µ</td>
<td>&lt;0.2 µg/ml (n = 8)</td>
</tr>
<tr>
<td>NGIgG</td>
<td>1279.9 ± 512.2 (n = 5)</td>
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</tbody>
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Table II. Induction of autoimmune myocarditis in B cell-deficient BALB/c mice

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Incidence of Myocarditis</th>
<th>Disease Severity</th>
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<tbody>
<tr>
<td>µMT+/−/−</td>
<td>8/10</td>
<td>1.45 ± 1.01</td>
</tr>
<tr>
<td>µMT+/−/+</td>
<td>10/11</td>
<td>1.68 ± 0.64</td>
</tr>
<tr>
<td>µMT+/−/+ (unimmunized)</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Anti-µ</td>
<td>8/8</td>
<td>2.25 ± 0.67</td>
</tr>
<tr>
<td>NGIgG</td>
<td>7/7</td>
<td>2.57 ± 0.49</td>
</tr>
</tbody>
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* Mice were immunized with cardiac myosin using the immunization protocol described in Materials and Methods.

The severity of myocarditis was graded on a scale of 0–3: 0, no disease; 1, one small focus involving <5 cells; 2, one large focus involving >10–20 cells or multiple small foci (<5); 3, multiple small or large foci (>5 foci).
mice are not completely depleted of serum IgG, there are essentially no B220+ cells in the spleen as determined by FACS analysis, and the mice clearly do not make myosin-specific B cells. This suggests that myosin-specific autoreactive T cells in BALB/c mice can be activated in the absence of myosin-specific B cells, and dendritic cells or macrophages are sufficient for the priming of these autoreactive T cells. The induction of myocarditis in the μMT homozygotes immunized with a lower dose of myosin is further evidence that myosin-specific B cells are not required to break T cell tolerance to myosin in BALB/c mice. We were unable to find a dose of Ag that led to disease in B cell-competent mice and no disease in B cell-deficient mice.

The role of B cells in priming naive T cells specific for foreign Ag has been controversial, and over recent years the role of B cells in activating autoreactive T cells has proven equally complex (11–20). In an initial study to determine whether B cells can break T cell tolerance, Mamula et al. found that adoptive transfer of B cells cross-reactive to foreign and self cytochrome c (Cyt c) into naive recipients resulted in the activation of an autoreactive T cell response (21). Parallel transfer of macrophages from mice immunized with foreign Cyt c did not break T cell tolerance (21). These results were confirmed using small ribonucleoprotein particles (snRNPs) as an Ag (22) and using genetically B cell-deficient mice (23). Presumably due to altered processing of Ag, the activated cross-reactive B cells present a novel self peptide or cryptic epitope to which T cells have not been tolerized. In other studies, nonobese diabetic (NOD) mice genetically deficient in B cells failed to develop diabetes, insulitis, or autoreactive T cells (24, 25), again demonstrating a critical role for B cells as APCs in autoimmunity. However, in a number of other models of autoimmune disease, B cells were shown to be unnecessary for the activation of autoreactive T cells. In collagen-induced arthritis and autoimmune myasthenia gravis, the T cell responses to collagen and to the acetylcholine receptor, respectively, are normal in the absence of B cells (26, 27). In experimental autoimmune encephalomyelitis, a T cell-mediated disease, mice genetically deficient in B cells developed disease in a manner comparable to that of wild-type mice (28).

The commonality of the cardiac myosin-induced model of myocarditis and the other models of autoimmune disease in which B cells are not needed to prime autoreactive T cells is that immunizing with native self Ag in adjuvant is sufficient to break T cell tolerance. In these models it can be assumed autoreactive T cells have not been tolerized by macrophages and dendritic cells and that T cells recognizing immunodominant epitopes of self Ag are still present in the T cell repertoire. This most likely reflects a lack of T cell exposure to dendritic cells and macrophages presenting self-peptides or a subthreshold density of peptide-MHC complexes presented by dendritic cells and macrophages. Immunization with self Ag in these models may either increase self-peptide presentation to the threshold required for T cell signaling and activation or expose normally anatomically sequestered MHC-self peptide complexes for the first time. Autoreactive T cell responses, such as the response to Cyt c or snRNPs, cannot be induced by immunization with native self-Ag in adjuvant. Because autoreactive T cells may have already been tolerized to immunodominant peptides presented by macrophages and dendritic cells, B cells presenting cryptic epitopes of self Ag are required for activation of an autoreactive T cell response.
Consistent with this hypothesis, Smith and Allen have shown that cardiac APCs in naive mice constitutively process and present cardiac myosin and can activate a T cell hybridoma derived from a mouse with myocarditis (29). These data demonstrate that epitopes of myosin that activate pathogenic T cells are constitutively presented in the heart. This may explain how cardiac damage in the absence of foreign Ag can give rise to autoimmune myocarditis. In the ischemic heart, the release of intracellular myosin exposes autoreactive T cells to APCs presenting myosin peptides and increases the level of that presentation.

In general, it may be that B cells are needed as APCs in diseases where the autoantigen is normally presented in a tolerogenic fashion, to expose cryptic epitopes of self Ag. For autoantigens to which T cells have not been tolerized, exposure to immunodominant peptides of self Ag by macrophages and dendritic cells may be sufficient to initiate autoreactivity and, perhaps, autoimmune disease.

References