Induction of Experimental Autoimmune Myocarditis in Mice Lacking CD3 or CD8 Molecules

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Summary

Experimental induction of most autoimmune diseases appears to depend on the activation of CD4 + T helper cells, while CD8 + lymphocytes may have a role in disease progression. To study the role of CD4 + and CD8 + T cell subsets in T cell-dependent autoimmunity, mice lacking CD4 or CD8 molecules after gene targeting were injected with cardiac myosin to induce organ specific autoimmune myocarditis. Mice homozygous for the CD8 mutation (CD8 -/-) developed significantly more severe disease as compared to CD4 +/-CD8 +/- controls. Surprisingly, CD4 -/- mice developed autoimmune myocarditis with infiltration of TCRèB+CD4-CD8- T cells in the heart tissue and appearance of autoantibodies. These data demonstrate that the lack of CD4 + or CD8 + T cells has no significant influence on the initiation of autoimmune myocarditis. CD4 + and CD8 + cells regulate disease severity and these results may explain the occurrence of autoimmunity in CD4 immunodeficiencies.

In mice, autoimmune diseases arise either spontaneously or can be experimentally induced by injection of the specific autoantigen. Experimental induction of most autoimmune disease requires CD4 + T helper lymphocytes responsive to the self-antigen presented on major histocompatibility complex (MHC) class II molecules (reviewed in reference 1). In virtually all experimental and genetic animal models of autoimmunity, administration of monoclonal antibodies (mAb) against CD4 molecules blocks disease (reviewed in references 2-5). Thus, CD4 + T helper cells are postulated to be crucial for the initiation and development of T cell dependent autoimmunity and clinical trials targeting the CD4 + cell lineage to treat various human autoimmune diseases are currently underway (2-5). Besides CD4 + cells, MHC class I restricted CD8 + lymphocytes can be found in autoimmune lesions (1-5). The significance and role of CD8 + lymphocytes in the development of autoimmunity, especially in experimentally induced autoimmune diseases, is controversial.

Coxsackie virus (CB3)-induced myocarditis and dilated cardiomyopathy are the most frequent cardiac complications in young patients, and several clinical and experimental studies suggest that later stages of this disease are mediated by an autoimmune response (6-8). The human disease can be mimicked in mice by inoculation of CB3-virus, which in the mouse model probably leads to an autoimmune response to cardiac myosin exposed after virus-mediated myocyte damage (9-11). This hypothesis is supported by the fact that myocarditis can be induced with purified cardiac myosin, i.e., in the absence of virus (12). Cardiac myosin-induced autoimmune myocarditis is strictly organ-specific and cannot be induced with other isoforms of muscle myosin (12). Experimental induction of autoimmune myocarditis is MHC class II haplotype dependent and can be inhibited by treatment with mAbs against MHC class II or CD4 molecules (13). Adoptive transfer studies in SCID mice have also demonstrated that autoantigen-induced myocarditis is mediated by CD4 + cells (14). By contrast, immunodepletion studies suggested that CD8 + cells have a pathogenic role in later phases of the disease (13).

Mice rendered CD4 or CD8 deficient as a result of gene targeting (15, 16) are ideal models to study the role of CD4 + and CD8 + T cell subsets in the development of autoimmune diseases. To investigate whether CD4 -/-, CD8 -/- or control CD4 +/-CD8 +/- mice are susceptible to antigen-induced myocarditis, these mice were injected with purified cardiac myosin (12). We show that both CD4 -/- and CD8 -/- mice develop autoimmune myocarditis suggesting that the initiation of antigen-induced autoimmunity does not depend on CD4 + or CD8 + T cells.

Materials and Methods

Mice. To generate mice in an MHC haplotype susceptible to cardiac myosin-induced autoimmune disease, CD4 or CD8 gene deficient mice (15, 16) were backcrossed into a B10.Br strain (H-2b) (CD4 -/- = 4th backcross; CD8 -/- = 5th backcross). As
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a control, male CD8⁻⁻ (B10.Br; 5th backcross) were bred with female CD4⁺⁻ (B10.Br; 4th backcross) mice of the same breeding nucleus to generate mice heterozygous at both loci (CD4⁺⁻/⁻ CD8⁻⁻/⁻). It should be noted that CD4⁻⁻ or CD8⁻⁻ throughout the text refers to CD4⁻⁻/⁻ CD8⁻⁻/⁻ or CD4⁻−/CD8⁻⁻/⁻ genotypes. Care of animals was in accordance with guidelines of the Canadian Research Council.

Immunization Procedure. Cardiac myosin was purified from mice as described (17). 7-wk-old mice were immunized twice at a 7-d interval (subcutaneously) with 150 µg of cardiac myosin emulsified in Freund’s complete adjuvant (FCA) or with FCA alone (12). 21 d after the first immunization, mice were sacrificed and analyzed histologically for infiltration of the heart muscle and serologically for the presence of IgG auto-Abs against cardiac myosin (10, 12). For histological analysis, hearts were fixed in formaldehyde and processed for hematoxylin and eosin staining. IgG auto-Ab titers were determined by ELISA-technique using the cardiac myosin isoform as test antigen (12, 17). Specificity of myosin IgG auto-Abs against the cardiac isoform has been demonstrated previously (17).

Immunocytochemistry. For immunoperoxidase staining (13), cryostat sections were fixed in acetone (10 min) and endogenous peroxidase reactivity blocked with NaBi and H2O2. After incubation with mAbs (30 min), sections were washed in tri-buffered saline (TBS, pH 7.4) and further incubated with peroxidase-conjugated rabbit anti-rat Ig (0.25 mg/ml; DAKOPATTS, Copenhagen, Denmark) or unconjugated rabbit anti-hamster IgG (0.1 mg/ml; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) followed by peroxidase conjugated swine anti-rabbit Ig (0.25 mg/ml; DAKOPATTS). All sections were developed for 10 min with 0.06% diaminobenzidine and 0.01% H2O2 and counter-stained with hematoxylin.

The following mAbs were used: Mac-1 (rat IgG2b; clone M1/70); GK1.5 (anti-CD4; rat IgG2b); 53-6.72 (anti-CD8α/ε/Ly2; rat IgG2a); KT3 (anti-CD3; rat IgG); GL 3 (anti-pan TCR-γδ; hamster IgG); H57-597.2.1 (anti-pan TCRαβ; hamster IgG). All mAbs were derived from American Type Culture Center, Rockville, MD and used as supernatants.

Results and Discussion

CD4⁻⁻, CD8⁻⁻, or control CD4⁺⁺ CD8⁺⁺ mice were injected with purified cardiac myosin in FCA (12). In comparison to CD4⁺⁺ CD8⁻⁻ mice, CD8⁻⁻ animals developed a significantly more severe disease as assessed by the histological extent of heart infiltration (Table 1 and Fig. 1, a and b) suggesting that CD8⁺ lymphocytes do not only act as cytotoxic effector cells in autoimmune disease but may also regulate the severity of disease (18). A similar regulatory role of CD8⁺ T cells has been previously described in experimental autoimmune encephalitis (EAE), i.e., increased frequency of relapses in CD8⁻⁻ mice and resistance to a second induction of EAE in mice depleted of CD8⁺ cell using mAbs (19, 20). More surprisingly, CD4⁻⁻ animals injected with the autoantigen also developed autoimmune myocarditis (Fig. 1 c). Incidence and severity of autoimmune myocarditis in CD4⁻⁻ mice were comparable to CD4⁺⁺/CD8⁺⁺ control mice (Table 1).

Table 2 summarizes immunoperoxidase staining of cells infiltrating the heart tissue of animals that had developed autoimmune disease. In all mice analyzed, the cellular infiltrate was composed of macrophages (Mac-1⁺, 70–80%) and CD3⁺ T lymphocytes (16–24%). In CD4⁺⁺/CD8⁺⁺ control mice almost all (>95%) infiltrating T cells were TCRαβ⁺ and expressed either CD4 or CD8 accessory molecules. The same results have been previously obtained in normal B10.Br and other mouse strains (data not shown and reference 13). In CD8⁻⁻ mice, virtually all infiltrating TCRαβ⁺ cells coexpressed CD4 molecules. Strikingly, in CD4⁻⁻ mice <30% of TCRαβ⁺ lymphocytes infiltrating the heart expressed CD8 molecules. These data show that most TCRαβ⁺ lymphocytes present in autoimmune lesions of CD4⁻⁻ mice have a CD4⁺ CD8⁻ phenotype. TCRγδ⁺ cells could be found in hearts of CD4⁺⁺/CD8⁺⁺, CD4⁻⁻, and CD4⁻⁻ mice in a low frequency (Table 2).

The antibody response to a T cell-dependent antigen, Incidence and disease severity were scored histologically on hematoxin and eosin stained heart sections in a double blind test by two independent investigators. Histological grading of severity is (12, 17): 0 = no infiltration in heart muscle; 1 = up to 5% of histological section is infiltrated; 2 = 5–10%; 3 = 10–20%; 4 >20%. Disease severity was compared between different groups injected with cardiac myosin using a Student’s t test: CD4⁺⁺/CD8⁺⁺ vs. CD4⁻⁻, no significant difference (p >0.05); CD4⁺⁺/CD8⁺⁺ vs. CD8⁻⁻ and CD4⁻⁻/CD8⁻⁻, significantly different (p <0.05). The incidence of disease in both CD4⁻⁻ and CD8⁻⁻ animals did not differ significantly from CD4⁺⁺/CD8⁺⁺ controls (χ²-test, p >0.05). One representative experiment is shown. The results were confirmed in two additional experiments.

Table 1. Incidence and Histological Severity of Cardiac Myosin-induced Myocarditis in CD4⁻⁻, CD8⁻⁻, and CD4⁺⁺/CD8⁺⁺ Mice

| Genotype      | Immunization | Incidence (positive animals/total) | Severity in positive animals |
|---------------|--------------|------------------------------------|------------------------------|
| CD4⁺⁺/8⁺⁺     | Cardiac myosin | 5/12 | 1.8 ± 0.8 |
| CD4⁺⁺/8⁺⁺     | FAC only     | 0/6 | - |
| CD4⁻⁻         | Cardiac myosin | 3/6 | 1.0 ± 0.0 |
| CD4⁻⁻         | FAC          | 0/7 | - |
| CD8⁻⁻         | Cardiac myosin | 4/5 | 3.3 ± 1.0 |
| CD8⁻⁻         | FAC          | 0.8 | - |

Figure 1. Cellular infiltrates in hearts of CD4⁺⁺/CD8⁻⁻ (a), CD8⁻⁻ (b), and CD4⁺⁺/CD8⁺⁺ mice (c) injected with cardiac myosin. In all cases, the infiltrates of mononuclear cells are diffuse and interstitial. (d) shows the normal morphology of heart muscle from a CD4⁻⁻ mouse injected with FCA alone. It should be noted that heart areas are shown to illustrate infiltration and that the infiltrations shown to not correspond to histological grading of the whole heart as described in Table 1. Hearts were taken from mice 21 d after first injection of cardiac myosin (12), fixed in formaldehyde, and processed for conventional histology (hematoxin and eosin staining). x80.
Table 2. Phenotype of Cells Infiltrating the Hearts of CD4*/-CD8*-, CD4 +/-, and CD8 +/− Mice

| Phenotype | CD4*+/−CD8*+/− | CD4*+/− | CD8*+/− |
|-----------|----------------|--------|--------|
| Mac-1     | 78.4           | 74.3   | 69.6   |
| CD3       | 16.1           | 17.7   | 23.7   |
| TCRαβ     | 16.2           | 14.1   | 20.1   |
| TCRγδ     | 1.3            | 3.0    | 3.4    |
| CD4       | 8.2            | 0      | 20.9   |
| CD8       | 7.1            | 3.9    | 0      |

Heart sections were stained with indicated mAbs followed by peroxidase labeled second stage Abs (13). In each instance, at least 1000-2500 infiltrating cells were counted. Numbers indicate percentages of positive cells among total infiltrating cells. Percentages are shown for one animal representative of each haplotype. Phenotypes and percentages of infiltrating cells, especially the prevalence of CD4-CDS-TCRαβ* lymphocytes in the heart infiltrate of CD4 −/− mice, were confirmed in more animals (not shown). Unspecific background staining was in all instances <1% of infiltrating cells.

SRBC, is markedly reduced in CD4 −/− mice (15). Since mice with myocarditis develop high titers of IgG autoantibodies (auto-Ab) against cardiac myosin (10, 21, 22), we analyzed whether auto-Ab were present in CD4 −/− or CD8 −/− mice. Fig. 2 shows titers of anti-myosin IgG auto-Ab in mice injected with cardiac myosin or FCA alone. All CD4 +/−CD8 +/− and CD8 −/− mice with cellular heart infiltrate developed high titers of IgG auto-Ab, and high auto-Ab titers correlated with the severity of cellular infiltration. Surprisingly, experimental autoimmune myocarditis in CD4 −/− animals was accompanied by the appearance of IgG auto-Ab (Fig. 2). Mice injected with FCA alone did not show any heart infiltration (Table 1 and Fig. 1 d) or high titers of auto-Ab (Fig. 2).

Development of specific IgG auto-Ab against cardiac myosin in the absence of help by CD4 + T cells suggests that cells other than CD4 + lymphocytes are functional that can induce immunoglobulin-class switching and myosin specific IgG auto-Ab production. Alternatively, IgG auto-Ab production in myocarditis may not require T cell help since, however, experimental autoimmune myocarditis is T cell dependent (13, 14) and auto-Ab titers are abrogated after treatment with anti-CD4 and anti-MHC class II mAbs (14), the production of IgG auto-Ab appears to depend on T helper cells. Few examples of autoimmunity have been described that may be abrogated by CD4 −CD8 −TCRαβ* (23) or CD4 −CD8 −TCRγδ* cells (24) and vast numbers CD4 −CD8 −TCRαβ* lymphocytes arise in autoimmune gld/gld or lpr/lpr mice (reviewed in 25). Approximately 10% of peripheral T cells in CD4 −/− mice have a CD4 −CD8 −TCRαβ* phenotype (15) and it has been demonstrated that these cells can provide MHC class II restricted help for T cell dependent antibody production (Rahemtulla, A., T. M. Kuendig, A. Narendran, M. F. Bachmann, M. Julius, P. S. Ohashi, C. J. Paige, R. M. Zinkernagel, and T. W. Mak; manuscript submitted for publication). It should be noted that CD4 −CD8 −TCRαβ* T cells in CD4 −/− mice display a phenotype previously described for various normal mouse strains (26-29) suggesting that these cells constitute a T cell population that is also present in normal mice but that is expanded in CD4 −/− mice.

Our data demonstrate that T cell-dependent and organ-specific autoimmune diseases, i.e., autoimmune myocarditis, can develop in mice lacking CD4 + or CD8 + T cells. Both T cell subsets, however, participate in disease propagation in which CD4 −/− mice develop a mild, but CD8 −/− mice a more severe form of disease. In CD4 −/− mice, most T cells infiltrating the heart muscle have a TCRαβ*CD4 −CD8 −phenotype, a cell population is probably also responsible for initiation of autoimmunity in these mice. The same population is probably also responsible for the development of EAE in CD4 −/− mice (Koh, D. R., A. Ho, A. Rahemtulla, and T. K. Mak, manuscript submitted for publication). These data caution against attempted treatments of autoimmune diseases with mAbs against CD4 or CD8 molecules (2-5) since other than CD4 + or CD8 +, cell populations have the functional capability to induce organ-specific autoimmunity and might explain the occurrence of autoimmunity in patients with genetic or acquired immunodeficiency syndromes, e.g., the high incidence of non-viral myocarditis in AIDS patients (30).
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