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A Spontaneous Model for Autoimmune Myocarditis Using the Human MHC Molecule HLA-DQ8[1]

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Genome-wide analyses have shown that the MHC class II region is the principal locus that confers susceptibility to a number of human autoimmune diseases. Due to the high degree of linkage disequilibrium across the MHC, it has been difficult to dissect the contribution of individual genes to disease susceptibility. As a result, intensive efforts have been made to generate mice transgenic for human class II molecules as models of autoimmune disease. However, in every case, additional manipulations—such as immunization with Ag in adjuvant, expression of immunostimulants on target tissues, or coexpression of TCR transgenes—have been required to induce disease. In this study, we show that expression of the human HLA-DQ8 (DQA1*0301/DQB1*0302) molecule alone in three lines of transgenic nonobese diabetic murine class II-deficient (mII$^{-/-}$) mice results in the spontaneous development of autoimmune myocarditis. The disease shares key features of human myocarditis and was characterized by lymphocytic infiltrates in the myocardium and cardiac myocyte destruction, circulating IgG autoantibodies against cardiac myosin heavy chain, and premature death due to heart failure. We demonstrate that myocarditis could be transferred into healthy HLA-DQ8^{+}RAG-1$^{-/-}$mII$^{-/-}$ nonobese diabetic recipients with lymphocytes, but not sera. It has been widely thought that autoimmune myocarditis is of infectious etiology, with the immune responses arising secondary to cardiac damage from pathogens. These studies provide direct experimental evidence that spontaneous autoimmune myocarditis can occur in the absence of infection and that expression of HLA-DQ8 confers susceptibility to this organ-specific autoimmune disease. ***The Journal of Immunology,*** **2004, 172: 2651–2658.**

Myocarditis is a major cause of sudden death in children and young adults (1–3) and is a frequent precursor of dilated cardiomyopathy, a common indication for cardiac transplantation (4). Myocarditis presents a challenging clinical problem because the etiology is heterogeneous, the diagnosis is difficult to establish, and the treatment options are limited (4, 5). Although cardiotropic viruses are considered to be the most common causative agent, in the majority of cases it has not been possible to isolate viral genome from the myocardium, and the etiology of the disease is therefore unknown (4).

There is increasing evidence suggesting that subgroups of patients with myocarditis may have disease of autoimmune origin. Support for this theory has come from several observations. Patients with myocarditis have an increased prevalence of other autoimmune disorders such as celiac disease (6), an inflammatory disease of the small bowel that is associated with the structurally similar HLA class II molecules DQ8 and DQ2 (7–9). Circulating autoantibodies against cardiac Ags have been found in patients with dilated cardiomyopathy and their first-degree relatives, suggesting familial clustering characteristic of autoimmune diseases (9). Familial dilated cardiomyopathy is associated with the HLA-DR4/DQ8 haplotype, which is prevalent in other autoimmune diseases (10). In addition, recent studies have shown that patients with myocarditis who have circulating cardiac autoantibodies, but no evidence of viral genome in the myocardium, may benefit from immunosuppression (11). Because myocarditis is difficult to study in humans and the diagnosis is most often made postmortem (4), much of our understanding of the disease pathogenesis has come from animal models. However, to date, all models of autoimmune myocarditis have involved experimental induction of disease, either by infection with cardiotropic viruses or by immunization with adjuvants containing cardiac myosin (12).

In this study, we describe a spontaneous animal model of autoimmune myocarditis. This discovery arose unexpectedly during the course of other studies to make a mouse model to investigate the role of HLA-DQ8 in the development of type 1 diabetes. Genome-wide analyses in humans and mice have shown that susceptibility to organ-specific autoimmune diseases is strongly linked to the MHC class II region. This is exemplified by type 1 diabetes, in which the MHC class II region represents the most important genetic susceptibility locus with a logarithm of odds score of 65.8 (13). However, because of the high degree of linkage disequilibrium within the MHC complex, it has been difficult to analyze the contribution of individual loci to disease susceptibility. For this reason, several groups have generated mice that are transgenic for various HLA class II molecules, including HLA-DQ8, to model human autoimmune diseases. However, in no instance did a spontaneous autoimmune disease phenotype arise that could reproduce a human disease. For example, in an HLA-DQ8 type 1 diabetes model, both the initiation and adoptive transfer of disease were

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dependent upon the expression of the costimulatory molecule, B7.1, on the target tissues that do not physiologically express costimulatory molecules (14). In HLA-DR and -DQ transgenic models of arthritis, immunization with cartilage Ag in adjuvant was required to induce disease (15–18). More recently, HLA-DQ transgenic mice have also been evaluated as a potential model for polychondritis (19). In an HLA-DR2 model of multiple sclerosis, coexpression of a myelin basic protein-specific, human TCR transgene was required for spontaneous disease (20). In this study, we show that the expression of a human class II molecule alone—in the absence of immunization, adjuvants, or other transgenes—can serve as a restriction element for murine $CD4^+$ T cells in generating a full-blown spontaneous autoimmune disease syndrome that closely resembles the corresponding human disease.

Materials and Methods

Generation of the HLA-DQ8 transgenic mice

The *HLA-DQ8* (*DQA1*0301*/*DQB1*0302*) gene was excised from the cosmid clone (pDCβ2 (21); a gift from J. Strominger (Harvard Medical School, Boston, MA)) by digestion with *Eco*RV/*Kpn*I. The 2.6- and 10.8-kb fragments of DQA1*0301 and the 13-kb *Cla*I/*Kpn*I and 6.9-kb *Kpn*I fragments of DQB1*0302 were subcloned into separate pCR-Script (Stratagene, La Jolla, CA) vectors. The 13.4-kb *DQA1*0301* and the 19.9-kb *DQB1*0302* genes were then excised with *Srf*I/*Sac*II, and *Sac*II, respectively, and then subcloned back together into the pWE15 cosmid vector (BD Biosciences, Palo Alto, CA) that was first modified by adaptors in the *Bam*HI site to create the sites *Srf*I, *Kpn*I, *Sac*II, and *Bam*HI. The 33.3-kb *HLA-DQ8* transgene fragment was excised free of vector sequences by digestion with *Not*I. The transgene DNA was purified and injected into fertilized eggs of MHC class II-deficient, I-$A^b{}_\beta{}^{-/-}$ (hereafter, $mII^{-/-}$) C57BL/6 (B6) mice (Ref. 22; Diabetes Endocrinology Research Center Transgenics Mouse Core Facility, Joslin Diabetes Center).

Three HLA-DQ8 founders (70, 275, and 73) were identified by PCR and Southern blot analysis. Cell surface expression of HLA-DQ8 was analyzed by staining PBLs with a pan anti-HLA-DQ mAb (Leu 10; BD PharMingen, San Diego, CA) and FACS analysis. Genotyping for HLA-DQ8 transmission was performed by PCR with a DQA1 exon 1 primer set: 5′-AACTCT TCAGCTAGTAACTGAG-3′ and 5′-GCTCTGGTTTCCTGAAGGAG-3′. The $mII^{-/-}$ knockout mutation was identified by PCR analysis as described (22). The three HLA-DQ8$mII^{-/-}$ lines (70, 73, and 275) were initially established on the B6 background and were then backcrossed onto the nonobese diabetic (NOD)[4] background. Heterozygous carriers of the HLA-DQ8 and *neo* genes were used as breeders for each backcross generation. At the N_2 generation, mice were analyzed with the microsatellite markers 5′-AGCCCCTTCCAAGTGTCTCT-3′ and 5′-GGTTTCGGAAT GAGATGAGC-3′ and 5′-TGCTCCTCACCAGTCATGC-3′ and 5′-TAGTGTTTGCCATGGCTCTC-3′, which are linked to insulin-dependent diabetes (*idd*)*3* and *idd10*, respectively. Segregants homozygous for allelic variants characteristic of NOD served as the progenitors for all mice in subsequent backcross generations. The animals used in the experiments described in this study are the intercrossed HLA-$DQ8^{+/-}$ (hereafter, HLA-$DQ8^+$)$mII^{-/-}$ and HLA-$DQ8^-mII^{-/-}$ littermates from the N_5 to N_8 backcross generations. The possibility that these transgenic mice expressed I-A^{g7} was also excluded by negative staining of splenocytes with the I-A^{g7}-reactive mAb 10-3.6 (BD PharMingen). Because the severity of myocarditis did not increase in any of the three HLA-DQ8$mII^{-/-}$ lines from the N_5 to N_8 generations, the HLA-$DQ8^+mII^{-/-}$ mice from each of the lines were pooled and are referred to as 70, 275, or 73. The nontransgenic (HLA-$DQ8^-$) $mII^{-/-}$ littermates of these mice have been pooled and are referred to as $mII^{-/-}$.

Recombination-activating gene $(RAG)^{-/-}$ NOD mice were generated by backcrossing the RAG-$1^{-/-}$ mutation, originally on a 129/SvJ background (Ref. 23; gift of S. Tonegawa (Massachusetts Institute of Technology, Cambridge, MA)), onto the NOD strain for nine generations after selecting for homozygosity at the *idd1*, *idd3*, and *idd10* NOD loci. Line 73 HLA-$DQ8^+RAG^{-/-}mII^{-/-}$ NOD mice were generated by crossing line 73 HLA-$DQ8^+mII^{-/-}$ N_8F_3 NOD mice with $RAG^{-/-}$ $N_{10}F_7$ NOD mice. Offspring heterozygous at both *mII* and *RAG* loci and transgenic for HLA-DQ8 were intercrossed to create HLA-$DQ8^+RAG^{-/-}mII^{-/-}$ NOD pups. All mice were maintained in a specific pathogen-free barrier facility in accordance with the guidelines established by the Institutional Animal Care and Use Committee of Harvard Medical School.

Histology and immunohistochemistry

Hearts from mice were fixed overnight in 10% formalin, embedded in paraffin, sectioned, and stained for H&E by conventional methods. Immunohistochemistry was performed on frozen sections as described (24) with biotinylated anti-CD4 (clone H129.19), anti-CD8 (clone 53-6.7), or IVD12 (anti-HLA DQ (7–9)) followed by peroxidase-conjugated streptavidin (all from BD PharMingen). Stains were developed with either 3-amino-9-ethylcarbazole (Sigma-Aldrich, St. Louis, MO) or 3,3′-diaminobenzidine substrates (BD PharMingen). Double staining was performed with biotinylated IVD12 and either B220 (clone RA3-6B2; BD PharMingen) or F4/80 (clone A3-1; Serotec, Oxford, U.K.), followed by alkaline phosphatase-conjugated streptavidin and peroxidase-conjugated goat anti-rat (both from Jackson ImmunoResearch, West Grove, PA). Stains were developed first with alkaline phosphatase substrate (Vector Laboratories, Burlingame, CA) and then with 3-amino-9-ethylcarbazole substrate (Sigma-Aldrich).

Indirect immunofluorescence

$RAG^{-/-}$ NOD hearts were fixed in 2% paraformaldehyde and embedded in OCT. Frozen sections (4-μm thick) were incubated with 1/50 dilutions of sera from HLA-$DQ8^+$ (70, 275, or 73) or HLA-$DQ8^-mII^{-/-}$ NOD mice, followed by detection with FITC-conjugated $F(ab')_2$ of goat anti-mouse IgG (Jackson ImmunoResearch). Slides were mounted using Vectashield and stained with 4′,6′-diamidino-2-phenylindole (Vector Laboratories) for identification of nuclei. Images were taken on a Zeiss LSM-410 confocal microscope (Zeiss, Oberkochen, Germany).

Immunoblotting

To avoid interference from endogenous Igs, all tissues were obtained from $RAG^{-/-}$ NOD mice. Myofibrillar extracts were prepared from hearts as previously described (25). Kidney and liver were homogenized in Laemmli sample buffer. Proteins, including porcine cardiac myosin (Sigma-Aldrich), were separated by SDS-PAGE (8%) and transferred onto polyvinylidene fluoride membranes (Millipore, Bedford, MA) overnight. Blots were incubated with sera from line 275, 73 (both at 1:10,000), or 70 (at 1:1,000) mice. This was followed by incubation with peroxidase-labeled $F(ab')_2$ of goat anti-mouse IgG and development with ECL (Amersham Biosciences, Arlington Heights, IL).

ELISA

Plates were coated overnight with porcine cardiac myosin at 5 μg/ml. Plates were then washed and incubated with mouse sera starting at a 1/100 dilution with serial four-fold dilutions to 1/6400. Abs were detected with a peroxidase-conjugated $F(ab')_2$ of goat anti-mouse IgG, with ABTS (Sigma-Aldrich) as substrate. Absorbance was read at 405 nm.

Adoptive transfer

For both cell and serum transfers, donors consisted of line 73 or 275 HLA-$DQ8^+mII^{-/-}$ NOD mice that showed symptoms of advanced heart failure (labored breathing, lethargy, and cyanosis) and exhibited cardiomegaly at autopsy. For the cell transfers, splenocytes were depleted of RBC by ACK lysis and were injected i.v. into 6- to 8-wk-old line 73 HLA-$DQ8^+RAG^{-/-}mII^{-/-}$ NOD recipients ($1.5–2 \times 10^6$ cells/recipient). For serum transfers, 300 μl of sera, pooled from the diseased mice that exhibited the highest titers of cardiac myosin Abs (>1:6400), was injected i.v. into 6- to 8-wk-old NOD or $RAG^{-/-}$ NOD recipients. All recipients were checked daily for manifestations of heart failure. At the onset of symptoms or at the termination of the experiments 8 wk after adoptive transfer, mice were euthanized and the hearts were removed for histology. Serum was collected from the recipients before and at weekly intervals after the cell/serum transfers, and at the time of sacrifice.

Statistical analysis

Comparisons between the Kaplan-Meier survival curves of the different transgenic lines were performed using the log-rank test. All other results are presented as means $\pm$ SEM using the unpaired Student's *t* test.

Results

Transgenic mice were generated by the microinjection of a 33-kb genomic fragment containing the genes encoding HLA-DQ8 (DQA1*0301/DQB1*0302), along with their endogenous promoters and flanking sequences (21), into embryos of MHC class II-deficient (I-$A^b{}_\beta{}^{-/-}$; hereafter, $mII^{-/-}$) C57BL/6 (B6) mice (22).

[4] Abbreviations used in this paper: NOD, nonobese diabetic; RAG, recombination-activating gene; *idd*, insulin-dependent diabetes.

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Three HLA-DQ8 transgenic lines (70, 275, and 73) were established and maintained on the $mII^{-/-}$ B6 background. In parallel, the HLA-DQ8 transgenes and the $mII^{-/-}$ knockout mutation were backcrossed onto the NOD mouse strain. The transgenic mice were then intercrossed to create mice expressing human HLA-DQ8 as the only class II molecule. The animals used in the experiments described in this study were intercrossed mice from the N_5–N_8 backcross generations. Because B6 and NOD mice carry the same promoter deletion in the *I-Eα* gene (26, 27), expression of HLA-DQ8 could be examined in the absence of both I-A and I-E. The possibility that the HLA-DQ8$mII^{-/-}$ transgenic mice expressed hybrid I-Aα-DQB1*0302 molecules was excluded by the lack of staining of splenocytes by FACS with an I-$A\alpha^b$ mAb (AF6-120.1); expression of endogenous I-A^b proteins was also biochemically excluded by the lack of signals after immunoprecipitation of [^{35}S]methionine-labeled splenocytes with the mAb Y-3P (data not shown).

Immunohistochemical analysis of frozen thymic sections revealed that lines 73 and 275 displayed similar amounts of HLA-DQ8 staining in the thymic cortex, which was significantly greater than that seen in line 70 (Fig. 1*A*). Consistent with these findings, line 275 and 73 mice showed near or complete restoration of the peripheral $CD4^+$ T cell compartment (Fig. 1*B*) with mean CD4:CD8 ratios of 2.5 ± 0.15 ($n = 17$) and 3.0 ± 0.13 ($n = 14$), respectively, similar to levels seen in control NOD mice (3.0 ± 0.08; $n = 17$). In contrast, CD4:CD8 ratios in line 70 (Fig. 1*B*) were ~4- to 5-fold lower at 0.61 ± 0.04 ($n = 10$; $p < 0.0001$, compared with line 73 or 275). As expected, $mII^{-/-}$ mice that lacked HLA-DQ8 (Fig. 1*B*) had minimal $CD4^+$ T cells with a mean CD4:CD8 ratio of 0.09 ± 0.2 ($n = 10$; $p < 0.0001$, compared with line 70). Cytofluorimetric analysis of splenic $B220^+$ cell populations showed less striking differences between the lines with HLA-DQ8 expressed on similar percentages of B cells from lines 70 and 275, with mean levels of 40 ± 3.3% ($n = 8$) and 50 ± 4.2% ($n = 9$), respectively, compared with line 73, which showed mean levels of 76 ± 4.2% ($n = 5$). Immunization of HLA-$DQ8^+$(70, 275, and 73)$mII^{-/-}$ mice with the known HLA-DQ8-restricted peptides HSV-2 VP16 (28) and insulin B_{9-23}, revealed that line 70 and 275 mice made vigorous proliferative responses against both peptides with stimulation indices 7- to 20-fold above baseline, but weaker responses were noted in line 73, due to lower levels of HLA-DQ8 expression on dendritic cells (data not shown).

Unexpectedly, as lines 73 and 275 were bred onto the NOD background, the HLA-$DQ8^+mII^{-/-}$ mice developed the acute onset of labored breathing, lethargy, edema, and cyanosis, symptoms consistent with congestive heart failure (Fig. 2*A*). This was associated with a high incidence of premature sudden death, starting at ~12 wk of age. By 40 wk of age, >50% of line 73 and 275 HLA-$DQ8^+mII^{-/-}$ NOD mice died prematurely, compared with <5% of nontransgenic HLA-$DQ8^-mII^{-/-}$ NOD control littermates (Fig. 2*B*). Autopsy examination of the diseased mice revealed massively enlarged hearts with ischemic discoloration, varying degrees of pleural effusions, and enlargement and darkening of the liver. Surprisingly, marked cardiac enlargement was also seen in the majority of healthy appearing line 73 and 275 mice that survived to 40 wk (Fig. 2*C*). Histological analysis of the hearts (Fig. 3*A*) revealed extensive mononuclear infiltrates in the atria (RA), accompanied by cardiac myocyte degeneration and vacuolization, histopathological findings consistent with the diagnosis of active myocarditis in humans (29). Adjacent to the damaged tissues, massive mural thrombi were often observed (Fig. 3*A*, t). The ventricles (Fig. 3*A*, RV) were focally infiltrated, with line 73 exhibiting more severe pathology than line 275. Histological examination of the enlarged livers showed pathological changes char-

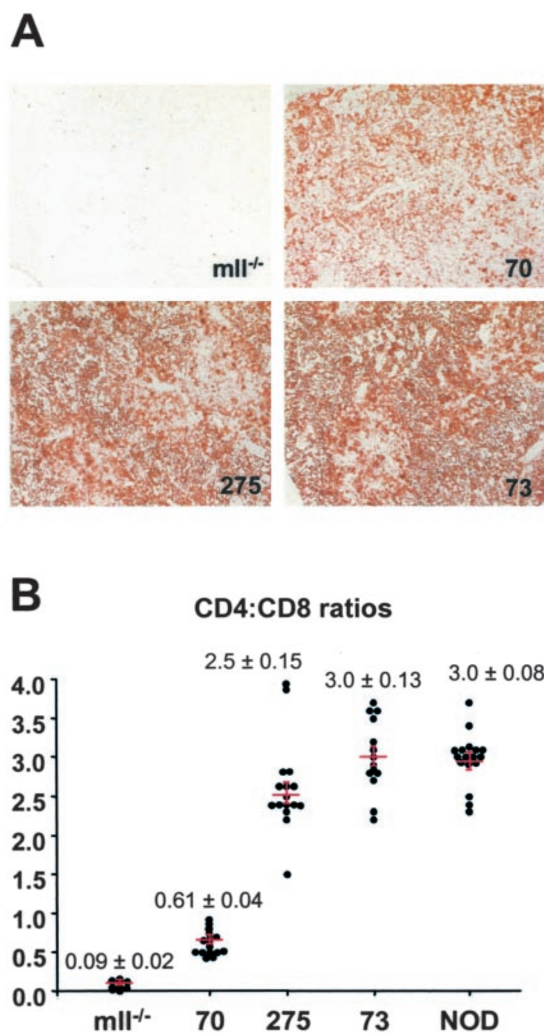

FIGURE 1. Comparison of HLA-DQ8 expression in the thymus and the levels of peripheral $CD4^+$ T cells in line 70, 275, and 73 HLA-$DQ8^+mII^{-/-}$ (70, 275, and 73) NOD mice and HLA-$DQ8^-mII^{-/-}$ ($mII^{-/-}$) NOD mice. *A*, Frozen thymus sections stained with the anti-HLA-DQ (7–9) Ab IVD12. *B*, CD4:CD8 ratios in PBMCs. NOD (HLA-$DQ8^-mII^{+/+}$) mice are included as controls.

acteristic of severe cardiac insufficiency with centrilobular hepatic necrosis and hemorrhage. Immunohistochemical analyses of the cellular composition of the ventricular lesions (Fig. 3*B*) revealed large numbers of $CD4^+$ and $CD8^+$ T cells along with abundant $F4/80^+$ macrophages, with only a few B cells present which often clustered in areas containing $CD4^+$ T cells (*B*, arrow). A striking feature of the inflammatory heart lesions was the marked induction of MHC class II Ag expression (Fig. 3*B*), potentially serving to amplify responses of primed $CD4^+$ T cells in situ. Based on colocalization studies with F4/80, it also appeared that some of the recruited macrophages expressed HLA-DQ8 (Fig. 3*B*). The de novo expression of MHC class II Ags on inflamed target cells is also a characteristic feature of human myocarditis lesions (4, 30).

In contrast to lines 73 and 275, line 70 HLA-$DQ8^+mII^{-/-}$ NOD mice, even when followed up to the N_8F_1 generation, did not develop clinical symptoms of congestive heart failure or show significant premature mortality (Fig. 2*B*). However, the majority of mice showed histological evidence of myocarditis with lymphocytic infiltrates and cardiac myocyte necrosis, especially in the atria (Fig. 3*A*), indicating that the same basic disease process was occurring in all three lines. The development of myocarditis was

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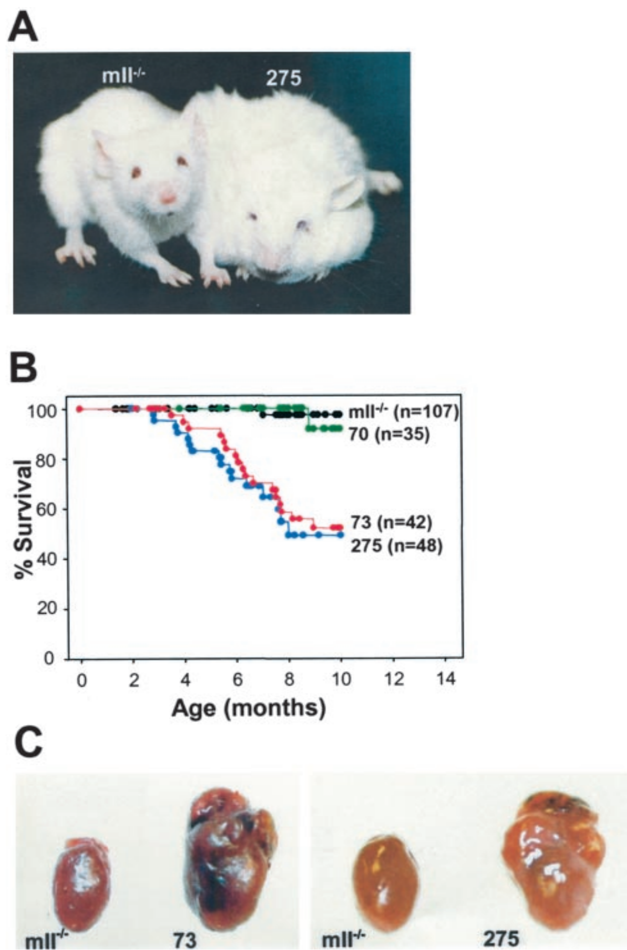

FIGURE 2. HLA-DQ8$^+$mII$^{-/-}$ NOD mice develop sudden death due to heart failure. *A,* A 16-wk-old line 275 HLA-DQ8$^+$mII$^{-/-}$ NOD female mouse (275) in severe congestive heart failure and a healthy HLA-DQ8$^-$mII$^{-/-}$ female littermate (mII$^{-/-}$). Note extensive edema and evidence of impaired oxygenation in the diseased 275 mouse, with the normal red eye color appearing cyanotic and the pronounced pallor of the ears and nose. At autopsy, this mouse exhibited massive cardiac and hepatic enlargement, and severe pulmonary edema. *B,* Kaplan-Meier survival curves for line 70, 275, and 73 HLA-DQ8$^+$mII$^{-/-}$ NOD mice (70, 275, and 73) and their HLA-DQ8$^-$mII$^{-/-}$ (mII$^{-/-}$) littermates. No significant difference in survival was seen between line 73 and 275 mice, or between line 70 and mII$^{-/-}$ mice; all other comparisons were highly significant ($p <$ 0.001). *C,* Representative enlarged hearts from line 73 and 275 HLA-DQ8$^+$ mice that survived to 40 wk compared with hearts of their HLA-DQ8$^-$mII$^{-/-}$ littermates.

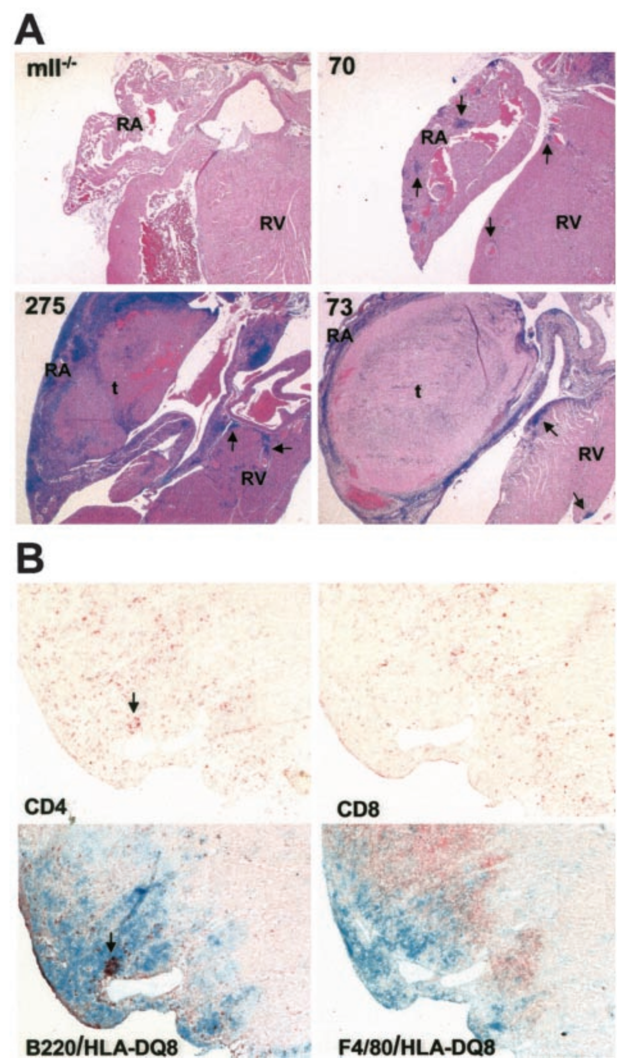

FIGURE 3. All three HLA-DQ8$^+$mII$^{-/-}$ transgenic NOD mouse lines develop myocarditis. *A*, Photomicrograph of formalin-fixed heart sections from a HLA-DQ8$^-$mII$^{-/-}$ mouse and HLA-DQ8$^+$ (70, 275, and 73) mice stained with H&E, shown at the same magnification. RA, Right atrium; RV, right ventricle; t, thrombus. Arrows indicate mononuclear cell infiltration. *B*, Immunohistochemical analysis of the inflammatory lesions from the left ventricle of a diseased line 73 mouse. Serial cryosections were stained with mAbs to CD4 (red) alone; CD8 (red) alone; or double stained with the anti-HLA DQ (7–9) mAb, IVD 12 (blue), and either B220 (red) or F4/80 (red). Note the marked induction of HLA-DQ8 expression in these lesions. Arrows point to an area containing a cluster of CD4$^+$ T cells and B cells.

HLA-DQ8- and NOD strain-specific, because it did not develop in nontransgenic mII$^{-/-}$ NOD mice, heterozygous HLA-DQ8$^+$mII$^{+/-}$ NOD mice, or HLA-DQ8$^+$mII$^{-/-}$ mice on the B6 background. We have recently generated fully congenic line 73 N_{10} HLA-DQ8$^+$mII$^{-/-}$ NOD mice (n = 56) and line 275 N_{10} HLA-DQ8$^+$mII$^{-/-}$ NOD mice (n = 37), and by Kaplan-Meier survival analysis and log-rank sum tests, there was no statistical difference in the time of onset or frequency of fatal myocarditis in either of these congenic HLA-DQ8 NODmII$^{-/-}$ lines compared with that of the N_{5-8} generation 275 and 73 mice shown in Fig. 2*B*. Thus, the myocarditis phenotype we observed cannot be attributed to background genes other than those from the NOD strain. The immune-mediated damage was cardiac muscle specific, because infiltrates were not seen in skeletal (Fig. 4*A*) or smooth muscle except in the pulmonary vein, which, in rodents, contains cardiac muscle. All three lines showed insulitis (represented in Fig. 4*B*), but the extent of pancreatic β cell damage was not sufficient to result in clinical diabetes or the production of autoantibodies (data not shown). Infiltration of the salivary gland (sialitis) was also present but occurred with similar frequency and severity in nontransgenic HLA-DQ8$^-$mII$^{-/-}$ NOD mice, consistent with its known control by non-MHC susceptibility genes (31).

Myocarditis was associated with the production of circulating IgG autoantibodies to cardiac tissues in all three lines of HLA-DQ8$^+$mII$^{-/-}$ NOD mice. By indirect immunofluorescence and confocal microscopy on RAG$^{-/-}$ NOD heart sections, the staining pattern localized to the striations within myocytes, producing a distinctive myofibrillar pattern (Fig. 5*A*). Although the prevalence and intensity of staining varied in mice from the different lines, the staining pattern was identical, suggesting that the autoantibodies were directed against similar antigenic determinants (Fig. 5*A*).

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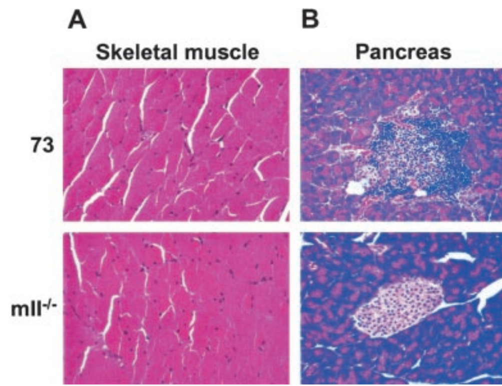

FIGURE 4. *A*, HLA-DQ8$^+$mII$^{-/-}$ NOD mice develop myocarditis, but not skeletal muscle myositis. Representative H&E staining of the latissimus dorsi muscle (skeletal muscle) of a line 73 mouse that had fulminant myocarditis showing normal skeletal muscle structure and absence of lymphocytic infiltrates (*upper panel*), with a similar appearance to skeletal muscle from a HLA-DQ8$^-$mII$^{-/-}$ NOD mouse (*bottom panel*). *B*, Histology of the pancreas of a 73 HLA-DQ8$^+$mII$^{-/-}$ NOD mouse showing insulitis (*upper panel*); islets from HLA-DQ8$^-$mII$^{-/-}$ NOD mice were intact and devoid of infiltration (*bottom panel*). Both HLA-DQ8$^+$- and HLA-DQ8$^-$mII$^{-/-}$ NOD mice showed inflammation in the exocrine pancreas, which is a feature of mII$^{-/-}$ mice (55).

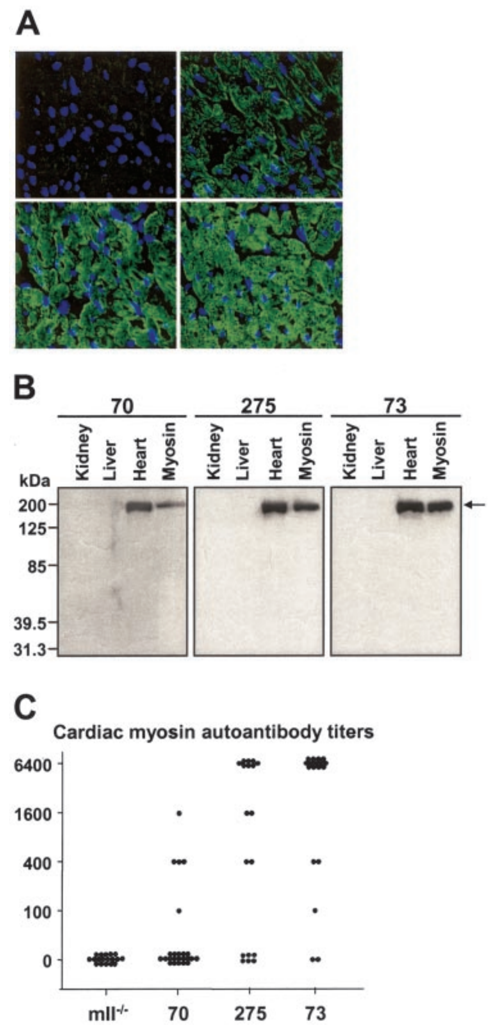

FIGURE 5. HLA-DQ8$^+$ (70, 275, and 73) mice develop circulating cardiac autoantibodies against cardiac myosin heavy chain. *A*, Indirect immunofluorescence and confocal microscopy of cryosections from a RAG$^{-/-}$ NOD mouse heart, stained with sera from mII$^{-/-}$, 70, 275, and 73 mice, and visualized using an FITC-conjugated anti-mouse IgG secondary Ab. Green color indicates serum staining, and blue color indicates nuclear staining with the marker 4′,6′-diamidino-2-phenylindole. *B*, Protein immunoblot analysis with sera from line 70, 275, and 73 mice using porcine cardiac myosin (myosin; 1 μg) and extracts (20 μg) from kidney, liver, heart of RAG$^{-/-}$ NOD mice. Samples were subjected to SDS-PAGE (8%), blotted onto a polyvinylidene fluoride membrane, and probed with serum (1:1,000 for the line 70 mouse; 1:10,000 for lines 275 and 73) followed by incubation with anti-mouse IgG. Arrowhead, ~200-kDa myosin heavy chain. *C,* ELISA of anti-cardiac myosin IgG-specific autoantibodies using serum samples from mII$^{-/-}$ (n = 16), 70 (n = 24), 275 (n = 18), and 73 (n = 18) mice. Each dot represents an individual mouse.

This was confirmed by immunoblot analysis (Fig. 5*B*), which revealed that sera from HLA-DQ8$^+$(70, 275, and 73)mII$^{-/-}$ mice, but not from NOD or control HLA-DQ8$^-$mII$^{-/-}$ mice (data not shown), reacted predominantly against a ~200-kDa protein in heart extracts that corresponded with the heavy chain of cardiac myosin, a major autoantigen in human myocarditis (25). Reactivity was not detectable in other tissues such as liver or kidney, but a ~200-kDa protein was seen in skeletal muscle that corresponded to skeletal muscle myosin (data not shown). This cross-reactivity was not unexpected, because the α-cardiac and β-skeletal muscle myosin H chain isoforms in rodents are highly (>97%) homologous (32); similar cardiac and skeletal muscle Ab cross-reactivity is also seen in human myocarditis (33). These findings sharply contrasted with the cardiac specificity of this autoimmune disease process, because autoimmune infiltrates developed in the heart (Fig. 3), but not in skeletal muscle (Fig. 4*A*). Comparison of the titers of cardiac myosin IgG autoantibodies by ELISA (Fig. 5*C*), revealed that line 73 mice exhibited the highest prevalence and titers of autoantibodies (16 of 18 positive), followed by line 275 (12 of 18 positive), and finally, line 70 (5 of 24 positive), with no Abs detectable in control DQ8$^-$mII$^-$ NOD mice (n = 16). In separate studies, we found that mice that exhibited macroscopic evidence of cardiomegaly (mean heart weight, 337 ± 16 mg (n = 9); compared with a mean heart weight, 154 ± 5.9 mg (n = 18) in HLA-DQ8$^-$mII$^{-/-}$ littermates) exhibited titers >1:1600. Thus, the titers of the myosin autoantibodies appeared to serve as a good serological marker of disease severity in the different HLA-DQ8 transgenic lines and in individual mice. High-titer autoantibodies (>1:6400) were detectable in mice as young as 6 wk of age, and were highly predictive of early progression to fatal myocarditis.

Myocarditis did not develop in lymphocyte-deficient line 73 HLA-DQ8$^+$mII$^{-/-}$RAG$^{-/-}$ NOD mice (n = 6), confirming that the development of disease was dependent on the function of mature T and/or B lymphocyte subsets. Importantly, we found that myocarditis could be successfully transferred into healthy line 73 HLA-DQ8$^+$mII$^{-/-}$RAG$^{-/-}$ NOD mice by the i.v. injection of splenocytes (1.5–2.0 × 10^7) from affected donors. Starting at ~3 wk after transfer, the recipients (n = 4) developed the acute onset of symptoms of severe heart failure accompanied by production of high-titer circulating autoantibodies against cardiac myosin. Histological analysis of the hearts revealed severe myocarditis (Fig. 6*A*) with extensive infiltration of lymphocytes into the myocardium and cardiac myocyte destruction (Fig. 6*A*, *insets*), reproducing the disease of the original donor mice. In contrast, the i.v. injection of serum (300 μl, i.v.) from severely diseased donors with the highest titers of anti-cardiac myosin Abs (>1:6400) into either immunocompetent NOD mice (n = 4) or RAG$^{-/-}$ NOD (n = 4) mice, did not cause myocarditis as assessed by the lack of clinical symptoms and histological changes in the heart (Fig. 6*B*), and the steady decline in Ab titers to undetectable levels (data not

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shown). This cell transfer model contrasts with virus- and cardiac myosin-induced immunization models of myocarditis, in which the transfer of disease usually requires either in vitro activation of donor splenocytes or pretreatment of the recipients with immunoadjuvants (12, 34, 35). Our HLA-DQ8$^+$RAG$^{-/-}$mII$^{-/-}$ NOD transfer system should thus provide a useful in vivo assay for further identifying the relevant cell type(s) responsible for this spontaneous autoimmune disease process.

Discussion

We describe a spontaneous animal model of autoimmune myocarditis that shares many clinical, histological, and immunological characteristics with human myocarditis (4, 12). Like humans, HLA-DQ8$^+$mII$^{-/-}$ mice develop the sudden onset of heart failure, leading to their early demise. This was associated with the presence of inflammatory cellular infiltrates in the myocardium and adjacent myocyte damage, histological features reminiscent of active myocarditis in humans (29). The inflamed lesions also contained abundant macrophages and CD4$^+$ and CD8$^+$ T cells, and exhibited marked induction of MHC class II Ag expression, which are also characteristic of human myocarditis lesions (4, 12). In addition, the diseased mice developed high-titer IgG autoantibodies that were specifically targeted against cardiac myosin heavy chain, a major autoantigen in human myocarditis (12). Despite their differing levels of HLA-DQ8 expression, all three transgenic lines developed the same basic disease process, indicating that HLA-DQ8 plays a fundamental role in the observed spontaneous autoimmune disease. The autoimmune phenotype of this model sharply contrasts with that described in the dilated cardiomyopathy syndrome of mice lacking the negative immunoregulatory receptor PD-1, which was characterized by a noninflammatory, fibrotic reaction in the heart and the production of pathogenic autoantibodies against a 33-kDa protein that was recently identified to be cardiac troponin I (36, 37).

This study shows that a human MHC class II gene can mediate a spontaneous disease process in the absence of other transgenes or immunization. While this manuscript was under consideration, an article describing the development of autoimmune myocarditis in double-transgenic human CD4/HLA-DQ8mII$^{-/-}$ NOD mice appeared (38). However, only one transgenic NOD line was described, raising the possibility that this phenotype may have been caused by founder effects, e.g., due to the transgene insertion site (38). In addition, the expression of the HLA-DQ8 transgene was examined on only a single genetic background. We show that the expression of HLA-DQ8 alone in three independently derived transgenic lines results in the spontaneous development of autoimmune myocarditis, with the severity of disease correlating with levels of expression of the HLA-DQ8 transgene. In addition, we show that autoimmune myocarditis develops on the NOD—but not on the B6—genetic background, suggesting that both MHC and non-MHC background genes are necessary for disease expression (39, 40). These findings are consistent with studies on human type 1 diabetes that have shown that, although HLA-DQ8 is relatively common in the general population, only a small proportion of carriers, presumably those individuals with additional non-MHC susceptibility genes, develop disease (41, 42). Interestingly, despite the autoimmune propensity of the NOD strain and the close structural and functional similarities of HLA-DQ8 and I-A^{g7} molecules (43, 44), NOD mice do not normally develop myocarditis. However, peptide binding studies have shown that, although the binding specificities of I-A^{g7} and HLA-DQ8 are similar, they are not identical, because there are naturally processed peptides from NOD mice that do not bind to HLA-DQ8, and vice versa (43).

Similar to previous studies (45, 46), none of our three lines of HLA-DQ8mII$^{-/-}$ NOD mice developed spontaneous diabetes, and NOD mice heterozygous for I-A^{g7} were also profoundly resistant to disease (47), likely a result of the inhibitory effects of the H-2^b haplotype-linked genes that were introgressed into the NOD background from the original mII$^{-/-}$ mutation made in 129/Sv-derived ES cells (48). Interestingly, although line 70 HLA-DQ8$^+$I-A$^{g7+/-}$ NOD mice were also protected from diabetes, both line 275 and 73 HLA-DQ8$^+$I-A$^{g7+/-}$ NOD mice showed a markedly enhanced incidence of spontaneous diabetes (data not shown). Thus, line 73 and 275 NOD mice displayed enhanced susceptibility to two different organ-specific autoimmune disease processes. In this context, it is interesting to note that autoimmune myocarditis and diabetes in NOD mice share a number of features. Both conditions are MHC class II dependent but require additional non-MHC background genes for disease expression. Both diseases are associated with circulating organ-specific Abs that precede the onset of disease and serve as markers of the ongoing disease

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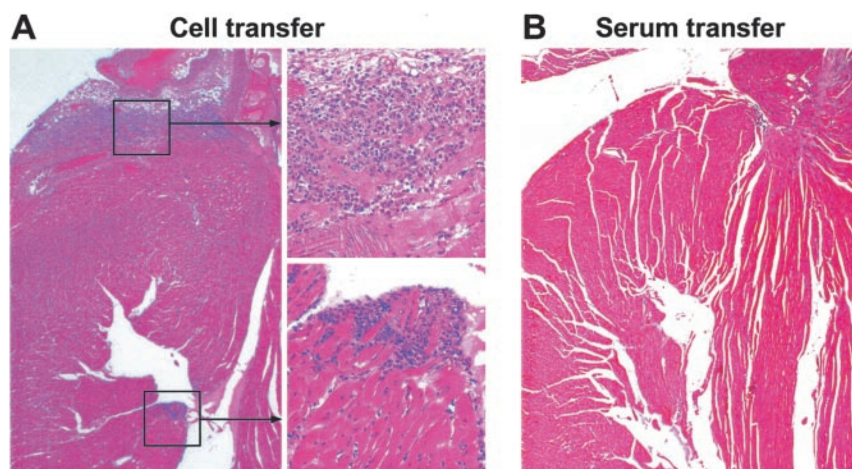

FIGURE 6. Myocarditis can be adoptively transferred with lymphocytes, but not serum. *A*, H&E staining of the left ventricle of a HLA-DQ8$^+$mII$^{-/-}$RAG$^{-/-}$ NOD recipient 21 days after receiving 20×10^6 splenocytes from an affected donor. The recipient showed symptoms of fulminant heart failure with pallor, lethargy, and labored breathing, and produced autoantibodies against cardiac myosin (1:400). *Insets* provide a higher magnification of the inflammatory lesions, showing extensive lymphocytic infiltration and destruction of cardiac myocytes. *B*, H&E staining of the heart of a RAG$^{-/-}$ NOD mouse 8 wk after receiving 300 μl of high-titer (>1:6400) sera. The myocardium is intact and free of mononuclear cell infiltration.

process, but are primarily T cell mediated. It will be of considerable interest to determine whether any of the key non-MHC *idd* loci that have been linked to susceptibility to type 1 diabetes (49) will also be associated with susceptibility to autoimmune myocarditis. Bottazzo and colleagues (9) have previously drawn parallels between the autoimmune features of human type 1 diabetes and those of familial dilated cardiomyopathy.

Autoimmunity has long been linked to myocarditis, but it has been postulated that the autoimmune responses are initiated by cardiac damage from infectious pathogens (12, 50). The results presented in this study suggest that some forms of myocarditis may be of primary autoimmune origin and mediated by HLA-DQ8 molecules. Interestingly, it has been shown that cardiac myosin-MHC class II complexes are normally constitutively expressed on residential cardiac APCs of healthy mice, suggesting that cell injury is not required for the exposure of cardiac Ags to the immune system (51). In addition, recent studies have suggested that cardiac myocytes undergo a remodeling process involving apoptosis and regeneration that plays an important role in normal cardiac development and tissue homeostasis (52). This raises the intriguing possibility that the physiological turnover of apoptotic cardiac myocytes may serve as an additional source of Ag to activate autoreactive $CD4^+$ T cells as has been hypothesized to occur for pancreatic β cells in the initiation of autoimmune diabetes (53, 54). Our hypothesis is that HLA-DQ8 shapes the T cell repertoire in the thymus such that significant numbers of $CD4^+$ T cells specific for cardiac Ags are selected that initiate this severe organ-specific inflammatory disease in the absence of infection with cardiotropic viruses. In keeping with this, the HLA-DQ8 transgenic lines with the highest levels of HLA-DQ8 expression in the thymic cortical epithelial cells and the largest numbers of peripheral $CD4^+$ T cells develop the most aggressive form of the disease.

Despite extensive investigation, it has not yet been possible to identify how HLA-DQ8 molecules function in conferring susceptibility to human autoimmune diseases. However, given the close resemblance of spontaneous myocarditis in HLA-DQ8 transgenic NOD mice to human myocarditis, a detailed cellular and molecular analysis of this model should enhance our understanding of the role of HLA-DQ8 molecules in pathogenesis of this disease. This knowledge could lead to improved detection and treatment of this serious human condition.

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References

1. Liberthson, R. R. 1996. Sudden death from cardiac causes in children and young adults. *N. Engl. J. Med. 334:1039*.
2. Nugent, A. W., P. E. Daubeney, P. Chondros, J. B. Carlin, M. Cheung, L. C. Wilkinson, A. M. Davis, S. G. Kahler, C. W. Chow, J. L. Wilkinson, and R. G. Weintraub. 2003. The epidemiology of childhood cardiomyopathy in Australia. *N. Engl. J. Med. 348:1639*.
3. Lipshultz, S. E., L. A. Sleeper, J. A. Towbin, A. M. Lowe, E. J. Orav, G. F. Cox, P. R. Lurie, K. L. McCoy, M. A. McDonald, J. E. Messere, and S. D. Colan. 2003. The incidence of pediatric cardiomyopathy in two regions of the United States. *N. Engl. J. Med. 348:1647*.
4. Feldman, A. M., and D. McNamara. 2000. Myocarditis. *N. Engl. J. Med. 343:1388*.
5. Mason, J. W., J. B. O'Connell, A. Herskowitz, N. R. Rose, B. M. McManus, M. E. Billingham, and T. E. Moon. 1995. A clinical trial of immunosuppressive therapy for myocarditis. *N. Engl. J. Med. 333:269*.
6. Frustaci, A., L. Cuoco, C. Chimenti, M. Pieroni, G. Fioravanti, N. Gentiloni, A. Maseri, and G. Gasbarrini. 2002. Celiac disease associated with autoimmune myocarditis. *Circulation 105:2611*.
7. Sollid, L. M., G. Markussen, J. Ek, H. Gjerde, F. Vartdal, and E. Thorsby. 1989. Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. *J. Exp. Med. 169:345*.
8. Maki, M., K. Mustalahti, J. Kokkonen, P. Kulmala, M. Haapalahti, T. Karttunen, J. Ilonen, K. Laurila, I. Dahlbom, T. Hansson, et al. 2003. Prevalence of celiac disease among children in Finland. *N. Engl. J. Med. 348:2517*.
9. Caforio, A. L., P. J. Keeling, E. Zachara, L. Mestroni, F. Camerini, J. M. Mann, G. F. Bottazzo, and W. J. McKenna. 1994. Evidence from family studies for autoimmunity in dilated cardiomyopathy. *Lancet 344:773*.
10. McKenna, C. J., M. B. Codd, H. A. McCann, and D. D. Sugrue. 1997. Idiopathic dilated cardiomyopathy: familial prevalence and HLA distribution. *Heart 77:549*.
11. Frustaci, A., C. Chimenti, F. Calabrese, M. Pieroni, G. Thiene, and A. Maseri. 2003. Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. *Circulation 107:857*.
12. Rose, N. R., and K. L. Baughman. 1998. Immune-mediated cardiovascular disease. In *The Autoimmune Diseases*, 3rd Ed. N. R. Rose and I. R. Mackay, eds. Academic Press, San Diego, p. 623.
13. Cox, N. J., B. Wapelhorst, V. A. Morrison, L. Johnson, L. Pinchuk, R. S. Spielman, J. A. Todd, and P. Concannon. 2001. Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am. J. Hum. Genet. 69:820*.
14. Wen, L., F. S. Wong, J. Tang, N. Y. Chen, M. Altieri, C. David, R. Flavell, and R. Sherwin. 2000. In vivo evidence for the contribution of human histocompatibility leukocyte antigen (HLA)-DQ molecules to the development of diabetes. *J. Exp. Med. 191:97*.
15. Nabozny, G. H., J. M. Baisch, S. Cheng, D. Cosgrove, M. M. Griffiths, H. S. Luthra, and C. S. David. 1996. HLA-DQ8 transgenic mice are highly susceptible to collagen-induced arthritis: a novel model for human polyarthritis. *J. Exp. Med. 183:27*.
16. Andersson, E. C., B. E. Hansen, H. Jacobsen, L. S. Madsen, C. B. Andersen, J. Engberg, J. B. Rothbard, G. S. McDevitt, V. Malmstrom, R. Holmdahl, et al. 1998. Definition of MHC and T cell receptor contacts in the HLA-DR4 restricted immunodominant epitope in type II collagen and characterization of collagen-induced arthritis in HLA-DR4 and human CD4 transgenic mice. *Proc. Natl. Acad. Sci. USA 95:7574*.
17. Rosloniec, E. F., D. D. Brand, L. K. Myers, Y. Esaki, K. B. Whittington, D. M. Zaller, A. Woods, J. M. Stuart, and A. H. Kang. 1998. Induction of autoimmune arthritis in HLA-DR4 (DRB1*0401) transgenic mice by immunization with human and bovine type II collagen. *J. Immunol. 160:2573*.
18. Bradley, D. S., P. Das, M. M. Griffiths, H. S. Luthra, and C. S. David. 1998. HLA-DQ6/8 double transgenic mice develop auricular chondritis following type II collagen immunization: a model for human relapsing polychondritis. *J. Immunol. 161:5046*.
19. Lamoureux, J., K. Durick, D. O'Bryant, C. S. David, M. M. Griffiths, and D. S. Bradley. 2003. Spontaneous polychondritis occurs in middle-aged mice containing the HLA-DQ6α8β transgene. In *American College of Rheumatology 67th Annual Scientific Meeting, October 23–28*, Vol. 48. Orlando, FL, p. S451 (Abstr.).
20. Madsen, L. S., E. C. Andersson, L. Jansson, M. Krogsgaard, C. B. Andersen, J. Engberg, J. L. Strominger, A. Svejgaard, J. P. Hjorth, R. Holmdahl, et al. 1999. A humanized model for multiple sclerosis using HLA-DR2 and a human T-cell receptor. *Nat. Genet. 23:343*.
21. Okada, K., J. M. Boss, H. Prentice, T. Spies, R. Mengler, C. Auffray, J. Lillie, D. Grossberger, and J. L. Strominger. 1985. Gene organization of DC and DX subregions of the human major histocompatibility complex. *Proc. Natl. Acad. Sci. USA 82:3410*.
22. Grusby, M. J., R. S. Johnson, V. E. Papaioannou, and L. H. Glimcher. 1991. Depletion of $CD4^+$ T cells in major histocompatibility complex class II-deficient mice. *Science 253:1417*.
23. Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and V. E. Papaioannou. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell 68:869*.
24. Lipes, M. A., E. M. Cooper, R. Skelly, C. J. Rhodes, E. Boschetti, G. C. Weir, and A. Davalli. 1996. Insulin secreting non-islet cells are resistant to autoimmune destruction. *Proc. Natl. Acad. Sci. USA 93:8595*.
25. Caforio, A. L., M. Grazzini, J. M. Mann, P. J. Keeling, G. F. Bottazzo, W. J. McKenna, and S. Schiaffino. 1992. Identification of α- and β-cardiac myosin heavy chain isoforms as major autoantigens in dilated cardiomyopathy. *Circulation 85:1734*.
26. Mathis, D. J., C. Benoist, V. E. Williams II, M. Kanter, and H. O. McDevitt. 1983. Several mechanisms can account for defective $E\alpha$ gene expression in different mouse haplotypes. *Proc. Natl. Acad. Sci. USA 80:273*.
27. Lund, T., E. Simpson, and A. Cooke. 1990. Restriction fragment length polymorphisms in the major histocompatibility complex of the non-obese diabetic mouse. *J. Autoimmun. 3:289*.
28. Kwok, W. W., M. E. Domeier, M. L. Johnson, G. T. Nepom, and D. M. Koelle. 1996. HLA-DQB1 codon 57 is critical for peptide binding and recognition. *J. Exp. Med. 183:1253*.
29. Aretz, H. T., M. E. Billingham, W. D. Edwards, S. M. Factor, J. T. Fallon, J. J. Fenoglio, Jr., E. G. Olsen, and F. J. Schoen. 1987. Myocarditis: a histopathologic definition and classification. *Am. J. Cardiovasc. Pathol. 1:3*.
30. Herskowitz, A., A. Ahmed-Ansari, D. A. Neumann, W. E. Beschorner, N. R. Rose, L. M. Soule, C. L. Burek, K. W. Sell, and K. L. Baughman. 1990. Induction of major histocompatibility complex antigens within the myocardium of patients with active myocarditis: a nonhistologic marker of myocarditis. *J. Am. Coll. Cardiol. 15:624*.
31. Garchon, H. J., P. Bedossa, L. Eloy, and J. F. Bach. 1991. Identification and mapping to chromosome 1 of a susceptibility locus for periinsulitis in non-obese diabetic mice. *Nature 353:260*.

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32. McNally, E. M., R. Kraft, M. Bravo-Zehnder, D. A. Taylor, and L. A. Leinwand. 1989. Full-length rat α and β cardiac myosin heavy chain sequences: comparisons suggest a molecular basis for functional differences. *J. Mol. Biol. 210:665*.
33. Lauer, B., K. Padberg, H. P. Schultheiss, and B. E. Strauer. 1994. Autoantibodies against human ventricular myosin in sera of patients with acute and chronic myocarditis. *J. Am. Coll. Cardiol. 23:146*.
34. Smith, S. C., and P. M. Allen. 1991. Myosin-induced acute myocarditis is a T cell-mediated disease. *J. Immunol. 147:2141*.
35. Bachmaier, K., N. Neu, L. M. de la Maza, S. Pal, A. Hessel, and J. M. Penninger. 1999. *Chlamydia* infections and heart disease linked through antigenic mimicry. *Science 283:1335*.
36. Nishimura, H., T. Okazaki, Y. Tanaka, K. Nakatani, M. Hara, A. Matsumori, S. Sasayama, A. Mizoguchi, H. Hiai, N. Minato, and T. Honjo. 2001. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science 291:319*.
37. Okazaki, T., Y. Tanaka, R. Nishio, T. Mitsuiye, A. Mizoguchi, J. Wang, M. Ishida, H. Hiai, A. Matsumori, N. Minato, and T. Honjo. 2003. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat. Med. 9:1477*.
38. Elliott, J. F., J. Liu, Z. N. Yuan, N. Bautista-Lopez, S. L. Wallbank, K. Suzuki, D. Rayner, P. Nation, M. A. Robertson, G. Liu, and K. M. Kavanagh. 2003. Autoimmune cardiomyopathy and heart block develop spontaneously in HLA-DQ8 transgenic IAβ knockout NOD mice. *Proc. Natl. Acad. Sci. USA 100:13447*.
39. Vyse, T. J., and J. A. Todd. 1996. Genetic analysis of autoimmune disease. *Cell 85:311*.
40. Wakeland, E. K., K. Liu, R. R. Graham, and T. W. Behrens. 2001. Delineating the genetic basis of systemic lupus erythematosus. *Immunity 15:397*.
41. Todd, J. A., J. I. Bell, and H. O. McDevitt. 1987. HLA-DQ β gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature 329:599*.
42. Ueda, H., J. M. Howson, L. Esposito, J. Heward, H. Snook, G. Chamberlain, D. B. Rainbow, K. M. Hunter, A. N. Smith, G. Di Genova, et al. 2003. Association of the T-cell regulatory gene *CTLA4* with susceptibility to autoimmune disease. *Nature 423:506*.
43. Yu, B., L. Gauthier, D. H. Hausmann, and K. W. Wucherpfennig. 2000. Binding of conserved islet peptides by human and murine MHC class II molecules associated with susceptibility to type I diabetes. *Eur. J. Immunol. 30:2497*.
44. Lee, K., K. Wucherpfennig, and D. Wiley. 2001. Structure of a human insulin peptide-HLA-DQ8 complex and susceptibility to type 1 diabetes. *Nat. Immunol. 2:501*.
45. Wen, L., F. S. Wong, L. Burkly, M. Altieri, C. Mamalaki, D. Kioussis, R. A. Flavell, and R. S. Sherwin. 1998. Induction of insulitis by glutamic acid decarboxylase peptide-specific and HLA-DQ8-restricted $CD4^+$ T cells from human DQ transgenic mice. *J. Clin. Invest. 102:947*.
46. Liu, J., L. E. Purdy, S. Rabinovitch, A. M. Jevnikar, and J. F. Elliott. 1999. Major DQ8-restricted T-cell epitopes for human GAD65 mapped using human CD4, DQA1*0301, DQB1*0302 transgenic IA^{null} NOD mice. *Diabetes 48:469*.
47. Katz, J., C. Benoist, and D. Mathis. 1993. Major histocompatibility complex class I molecules are required for the development of insulitis in non-obese diabetic mice. *Eur. J. Immunol. 23:3358*.
48. Hattori, M., E. Yamato, N. Itoh, H. Senpuku, T. Fujisawa, M. Yoshino, M. Fukuda, E. Matsumoto, T. Toyonaga, I. Nakagawa, et al. 1999. Cutting edge: homologous recombination of the MHC class I K region defines new MHC-linked diabetogenic susceptibility gene(s) in nonobese diabetic mice. *J. Immunol. 163:1721*.
49. Todd, J. A., and L. S. Wicker. 2001. Genetic protection from the inflammatory disease type 1 diabetes in humans and animal models. *Immunity 15:387*.
50. Rose, N. R. 2000. Viral damage or "molecular mimicry"—placing the blame in myocarditis. *Nat. Med. 6:631*.
51. Smith, S. C., and P. M. Allen. 1992. Expression of myosin-class II major histocompatibility complexes in the normal myocardium occurs before induction of autoimmune myocarditis. *Proc. Natl. Acad. Sci. USA 89:9131*.
52. Nadal-Ginard, B., J. Kajstura, P. Anversa, and A. Leri. 2003. A matter of life and death: cardiac myocyte apoptosis and regeneration. *J. Clin. Invest. 111:1457*.
53. Finegood, D. T., L. Scaglia, and S. Bonner-Weir. 1995. Dynamics of β-cell mass in the growing rat pancreas: estimation with a simple mathematical model. *Diabetes 44:249*.
54. Mathis, D., L. Vence, and C. Benoist. 2001. β-Cell death during progression to diabetes. *Nature 414:792*.
55. Vallance, B. A., B. R. Hewlett, D. P. Snider, and S. M. Collins. 1998. T cell-mediated exocrine pancreatic damage in major histocompatibility complex class II-deficient mice. *Gastroenterology 115:978*.

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