Dissection of the role of MHC class II A and E genes in autoimmune susceptibility in murine lupus models with intragenic recombination

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Systemic lupus erythematosus (SLE) is a multigenic autoimmune disease, and the major histocompatibility complex (MHC) class II polymorphism serves as a key genetic element. In SLE-prone (NZB × NZW)_{F1} mice, the MHC H-2^{d/z} heterozygosity (H-2^d of NZB and H-2^z of NZW) has a strong impact on disease; thus, congenic H-2^{d/d} homozygous _{F1} mice do not develop severe disease. In this study, we used *Ea*-deficient intra-H-2 recombination to establish A^{d/d}-congenic (NZB × NZW)_{F1} mice, with or without E molecule expression, and dissected the role of class II A and E molecules. Here we found that A^{d/d} homozygous _{F1} mice lacking E molecules developed severe SLE similar to that seen in wild-type _{F1} mice, including lupus nephritis, autoantibody production, and spontaneously occurring T cell activation. Additional evidence revealed that E molecules prevent the disease in a dose-dependent manner; however, the effect is greatly influenced by the haplotype of A molecules, because wild-type H-2^{d/z} _{F1} mice develop SLE, despite E molecule expression. Studies on the potential of dendritic cells to present a self-antigen chromatin indicated that dendritic cells from wild-type _{F1} mice induced a greater response of chromatin-specific T cells than did those from A^{d/d} _{F1} mice, irrespective of the presence or absence of E molecules, suggesting that the self-antigen presentation is mediated by A, but not by E, molecules. Our mouse models are useful for analyzing the molecular mechanisms by which MHC class II regions regulate the process of autoimmune responses.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the appearance of autoantibodies to several nuclear components. The deposition of formed immune complexes mediates the disease in a wide variety of tissues and organs, including the kidney and the vascular system. There is evidence that the development of SLE is under the control of multiple susceptibility genes (1). Among these, genes in the major histocompatibility complex (MHC) have been implicated as a key genetic element. Because SLE is an antibody-mediated disease, MHC class II polymorphisms are probably involved in the pathogenesis. However, because of the complex multifactorial inheritance and heterogeneity of SLE, and because of the linkage disequilibrium that exists among the class I, II, and III genes within the MHC complex, the absolute contribution of individual MHC class II loci has been difficult to dissect. Thus, our knowledge of the molecular mechanism of MHC class II contribution to SLE remains incomplete.

Substantial progress in research in this area has been achieved through studies using SLE-prone mice with genetic recombination and manipulation of MHC (H-2) genes. In (NZB × NZW)_{F1} mice that spontaneously develop disease closely resembling human SLE, the disease is strongly associated with H-2 haplotypes from both parents (H-2^d from NZB and H-2^z from NZW) (2–6). Genetic dissection by producing H-2-congenic mice revealed that an early onset of severe SLE occurs in only

heterozygous H-2^{d/z} mice and not in homozygous H-2^{d/d} and H-2^{z/z} mice (2, 3, 5). Although both A and E class II molecules may be involved, evidence has suggested that mixed haplotype class II Aαβ^z molecules are responsible for the pathogenesis by promoting the production of pathogenic high-affinity IgG autoantibodies to nuclear components (7, 8).

In contrast to class II A molecules, E molecules are suggested to be a suppressive genetic element for SLE. This notion was based mainly on the results obtained by using a transgene technique. A BXS^B strain of mouse, another spontaneous SLE model, carries H-2^b haplotype and expresses A^b, but not E, molecules, because of a defect in the *Ea* gene (9). The development of BXS^B disease is closely associated with the H-2^b haplotype (10) and is almost completely prevented by a transgene encoding *Ea*^d chains (11). Similar findings were noted in the nonobese diabetic (NOD) mouse, a model of spontaneous autoimmune diabetes. NOD mice express class II A^{g7}, but not E, molecules, because of a defect in the *Ea* gene (12). Evidence indicated that whereas the class II A^{g7} gene is critical for the disease susceptibility (13), the transgenic introduction of *Ea*^d or *Ea*^k does prevent the disease (14–16). Thus, A and E molecules seem to provide the susceptible and protective genetic elements for autoimmune diseases, respectively, at least in these mouse models.

Nevertheless, the conclusion awaits further studies, because the transgene possibly induces unexpected improper effects on immune cells. For example, unpaired or mispaired transgene-derived class II molecules can be toxic to B cell maturation (17). Furthermore, there are reports suggesting that excessively generated transgenic *Ea*^d molecules bind to A molecules, thereby decreasing the availability of A molecules for antigen presentation (18), and that overexpression of E molecules suppresses expression levels of endogenously encoded A molecules (19). In the (NZB × NZW)_{F1} model, severe SLE occurs despite the presence of intact E molecules. To examine the role of A and E molecules in (NZB × NZW)_{F1} lupus, we generated several kinds of congenic (NZB × NZW)_{F1} mice with intra-MHC recombination at the *E* subregion, taking advantage of natural recombinants, including those we found among ≈3,000 meioses in crosses of NZB strains.

Materials and Methods

Mice. NZB (H-2^d) and NZW (H-2^z) mice were purchased from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) and were maintained in our animal facility. The H-2-congenic

Abbreviations: DC, dendritic cell; NOD, nonobese diabetic; SLE, systemic lupus erythematosus; TCR, T cell receptor.

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Table 1. H-2 haplotypes of established MHC-congenic and intra-MHC recombinant-congenic New Zealand strains of mice

Strains	H-2 haplotype	K	Ab	Aa	Eb	Ea	Tnfa	D
NZB	d	d	d	d	d	d	d	d
NZB.GD	g2	d	d	d	d	//	b	b
NZW.GD	g2	d	d	d	d	//	b	b
NZB.GDr	g2r	d	d	d	d	d	//	b
NZW.H-2 ^d	d	d	d	d	d	d	d	d
(NZB × NZW.H-2 ^d)F ₁	d/d	d	d	d	d	d	d	d
(NZB × NZW.GD)F ₁	d/g2	d	d	d	d	d/b	d/b	d/b
(NZB.GDr × NZW.GD)F ₁	g2r/g2	d	d	d	d	d/b	d	b
(NZB.GD × NZW.GD)F ₁	g2/g2	d	d	d	d	//	b	b

//, Intra-H-2 recombination site between d and b haplotype.

NZW.H-2^d (2, 3, 5) strain was established by selective backcrossing of (NZB × NZW)F₁ to NZW for 15 generations. NZB.GD (H-2^{g2}) (20) and NZW.GD strains were established by selective backcrossing of (B10.GD × NZB)F₁ and (B10.GD × NZW)F₁ with NZB and with NZW mice, respectively, for 15 generations. The NZB strain with an intragenic recombination between *Ea* of NZB and *Tnfa* of NZB.GD was obtained in crosses of NZB and NZB.GD strains and was tentatively designated NZB.GDr. Alleles at the H-2 loci in established H-2-congenic and recombinant H-2-congenic New Zealand mice are shown in Table 1. These mice were crossed to produce (NZB × NZW)F₁ hybrids with the same d haplotype of the upstream region of the *Eb* gene but with different haplotypes of downstream regions of the *Ea* gene (Table 1), and the disease severity was compared among these female F₁ mice.

Typing of H-2 Haplotype. Peripheral blood was obtained from the periorbital sinus, followed by lysis of red blood cells with ammonium chloride. Aliquots of 5×10^5 to 10×10^5 cells were incubated with anti-A^d (K24–199) (21); anti-E (ISCR, which reacts with a common determinant of the E molecule) (a kind gift from Dr. N. Shinohara, Kitasato University, Kanagawa, Japan); anti-D^d (T19–191); and anti-D^b (H141–30) mAbs, followed by FITC-labeled anti-mouse polyclonal IgG antibodies (ICN). Incubations were run for 30 min at 4°C, and the stained cells were analyzed by using FACSTAR and CELLQUEST software (Becton Dickinson).

Microsatellite DNA Polymorphism in the *Tnfa* Promoter. *Tnfa* promoter was shown to have microsatellite polymorphism, and different tumor necrosis factor alleles have different lengths of microsatellites (22, 23). To determine the tumor necrosis factor alleles of each mouse strain, PCRs were performed with genomic DNAs, using 5' primer (5'-GGACAGAGAAGAAATGGGTTC-3') and 3' primer (5'-TCGAATCTGGGGCCAATCAGGAGGG-3') (22), and differences in lengths of PCR products were determined by using electrophoresis of PCR products on 7% denaturing polyacrylamide gels, as described in ref. 24.

Measurement of Proteinuria. The onset of renal disease was monitored by biweekly testing for proteinuria, as described in ref. 25. Mice with a proteinuria of 111 mg/ml or more in repeated tests were regarded as being positive.

Measurements of Anti-DNA and Anti-Chromatin Antibodies. Serum levels of IgG autoantibodies to DNA and chromatin were determined by ELISA, using peroxidase-conjugated polyclonal anti-mouse IgG antibodies (ICN). The DNA- and chromatin-binding activities were expressed in units, referring to a standard curve obtained by serial dilutions of a standard serum pool from 7- to 9-month-old (NZB × NZW)F₁ mice, containing 1,000

units/ml (5). DNA was obtained from calf thymus (Sigma). Chromatin was prepared as described in ref. 26. Briefly, nucleosomes were isolated by solubilizing chromatin from purified chicken erythrocyte nuclei with micrococcal nuclease. The solubilized chromatin was fractionated into sucrose gradients that were analyzed for monomers by using electrophoresis, and the appropriate fractions were dialyzed and pooled.

Analysis of T Cell Activation and T Cell Receptor (TCR) V_β Repertoires. To examine the activation states of CD4⁺ T cells, aliquots of 10⁶ spleen cells were double-stained with FITC-conjugated anti-CD4 mAb and phycoerythrin-conjugated anti-CD69 mAb. To analyze TCR V_β repertoires, spleen cells were stained with biotin-labeled mAbs to each TCR V_β repertoire (Pharmingen), followed by incubation with avidin-phycoerythrin and FITC-conjugated anti-CD4 mAb. All incubations were run for 30 min at 4°C, and the frequency of CD4⁺ T cells with each TCR V_β repertoire per total CD4⁺ T cells was calculated by using FACSTAR and CELLQUEST software.

Preparation of Retroviral Construct with Chromatin-Specific TCR Genes and Transduction to Splenocytes. Chromatin-specific TCR V_α and V_β DNA fragments were synthesized, using PCR based on the published sequences of the nucleosome-specific T cell line derived from the lupus-prone (SWR × NZB)F₁ mouse (27, 28), as described in ref. 29. These fragments were cloned into a pMXW retroviral vector (30) and transfected into PLAT-E packaging cell lines (31) by using FuGENE6 transfection reagent (Roche Diagnostics). The viral supernatant of transfected cells were placed on fibronectin-coated 24-well plates, and total spleen cells from 2-month-old (NZB × NZW)F₁ mice prestimulated for 48 h with Con A (10 μg/ml) and interleukin 2 (50 ng/ml) were added to the wells (1 × 10⁶ cells per well). Cells were cultured further for 36 h to allow infection to occur.

Purification and Functional Analysis of Dendritic Cells (DCs). Spleen cells were treated with collagenase type IV (Sigma) and DNase I, and CD11c⁺ cells were positively collected by passing spleen cells twice through MACS CD11c microbeads and magnetic separation columns. The purity (85% in average) of DCs was determined by flow cytometry with anti-CD11c-biotin, followed by streptavidin-phycoerythrin.

To analyze the potential of chromatin presentation, CD4⁺ T cells were purified by negative selection, using MACS microbeads with anti-CD19, -CD11c, and -CD8 mAbs 24 h postinfection, and 2 × 10⁴ cells per well of T cells were cocultured with 1 × 10⁵ cells per well of irradiated CD11c⁺ DCs in 96-well flat-bottom plates with 1 μg/ml chromatin. After 24 h of culture, the cells were pulse-labeled with 1 μCi (1 Ci = 37 GBq) of [³H]thymidine per well (NEN) for 15 h, and the [³H]thymidine incorporation was determined.

Statistical Analysis. Statistical analysis was performed by using Student's *t* test and the χ^2 test. *P* < 5% was considered to have a statistical significance.

Results

Establishment of Intra-MHC Recombinant-Congenic New Zealand Mice. By selective backcrossing, we first introduced the H-2^{g2} haplotype derived from the B10.GD strain into NZB (H-2^d) and NZW (H-2^e) and established H-2-congenic NZB.GD (20) and NZW.GD strains. H-2^{g2} has an intragenic recombination between *d* and *b* haplotype in the *E* gene and the H-2 haplotype is *K^dAb^dAa^dEb^dEa^bTnfa^bD^b* (Table 1) (32). Because the *Ea^b* gene is defective, H-2^{g2} mice do not express E molecules (20). To establish the strain that carries the H-2 haplotype of *K^dAb^dAa^dEb^dEa^dTnfa^bD^b*, we conducted a search for the mouse with a spontaneously occurring intragenic recombination between *Ea* and *Tnfa* in the progeny of NZB and NZB.GD crosses. In $\approx$ 3,000 meioses, there was a single mouse carrying this recombination, and the recombination-congenic NZB line, provisionally designated NZB.GDr (H-2^{g2r}), was generated. This haplotype is valid to evaluate the effect of E molecule expression on the same *Tnfa^bD^b* background. Table 1 summarizes the haplotypes of the intra-MHC-congenic New Zealand mouse and the related strains.

E Molecule Expression Levels in (NZB $\times$ NZW)F₁ Mice with Different Intra-H-2 Haplotypes. Congenic and recombinant-congenic New Zealand mice were crossed to obtain (NZB $\times$ NZW)F₁ mice with four different combinations of the H-2 haplotype (Table 1). Fig. 1 shows flow cytometric analyses for expression profiles of A, E, and D molecules on peripheral blood lymphocytes. As expected, although levels of A^d expression were almost identical, levels of E molecules differed significantly among these F₁ mice, i.e., full expression levels in H-2^{d/d} homozygous, approximately one-half of expression levels in H-2^{d/g2} and H-2^{g2r/g2} heterozygous, and no expression in H-2^{g2/g2} homozygous F₁ mice. Profiles of the class I D molecule expression showed that lymphocytes from H-2^{d/g2} F₁ mice were positive for both D^d and D^b, whereas those from H-2^{g2r/g2} and H-2^{g2/g2} F₁ mice were positive for D^b and negative for D^d. As also shown in Fig. 1, E molecules in wild-type H-2^{d/z} heterozygous F₁ mice were fully expressed. Because these F₁ mice are heterozygous H-2^{d/z}, the level of A^d and D^d expression was approximately one-half of that seen in the H-2^{d/d} homozygote.

Comparisons of Disease Features. Fig. 2A compares cumulative incidences of proteinuria in wild-type H-2^{d/z} heterozygous (NZB $\times$ NZW)F₁ mice with intact E molecule expression and four kinds of H-2-congenic (NZB $\times$ NZW)F₁ (H-2^{d/d}, H-2^{d/g2}, H-2^{g2r/g2}, and H-2^{g2/g2}) carrying identical homozygous A^{d/d} molecules but different levels of E molecule expression. Compared with findings in wild-type F₁ mice, the incidence was markedly reduced in homozygous A^{d/d} F₁ mice with intact E molecule expression (H-2^{d/d}). In a striking contrast, homozygous A^{d/d} F₁ mice deficient in E expression (H-2^{g2/g2}) showed an early onset and a high incidence of proteinuria comparable to those found in the wild-type F₁ mice. Findings in H-2^{d/g2} and H-2^{g2r/g2} with one-half of E expression levels were in between. Together with the finding that heterozygous H-2^{g2r/g2} and homozygous H-2^{g2/g2} F₁ mice share the same H-2 haplotype except for the *Ea* subregion (Table 1), it is strongly suggested that the development of lupus nephritis in A^{d/d} F₁ mice is down-regulated by E molecules in a dose-dependent manner.

As shown in Fig. 2B, the decrease in survival rate was associated with an increase in the incidence of proteinuria in all groups of mice. Whereas all H-2^{g2/g2} F₁ mice and 90% of wild-type F₁ mice died of disease by 12 months of age, 80% of H-2^{d/d} F₁ mice and $\approx$ 50% of H-2^{d/g2} and H-2^{g2r/g2} F₁ mice

Fig. 1. Flow cytometry analysis for cell surface expression of A^d, E, D^d, and D^b molecules on peripheral lymphocytes in (NZB $\times$ NZW)F₁ mice with different H-2 haplotypes. The upper four groups of F₁ mice with homozygous A^{d/d} showed the same expression level of A^d molecules, and the level in wild-type H-2^{d/z} heterozygous F₁ mice was almost one-half of that seen in the former groups. When E molecule expression levels were examined with a mAb to a common determinant, H-2^{d/g2} and H-2^{g2r/g2} F₁ mice showed approximately one-half the level (E^{1/2}) of that seen in H-2^{d/d} and wild-type H-2^{d/z} F₁ mice (E¹). H-2^{g2/g2} F₁ mice did not express E molecules (E⁰). D^d expression levels in H-2^{d/g2} and H-2^{d/z} F₁ mice were approximately one-half of that seen in H-2^{d/d} F₁ mice. D^b expression level in H-2^{d/g2} F₁ mice was approximately one-half of that seen in H-2^{g2r/g2} and H-2^{g2/g2} F₁ mice.

remained alive. In Fig. 2C and D, we compare serum levels of IgG autoantibodies to DNA and to chromatin, respectively, in 6-month-old homozygous A^{d/d} F₁ mice with different levels of E expression. Whereas H-2^{g2/g2} F₁ mice lacking E molecules showed high levels of both autoantibodies, comparable to those found in wild-type F₁ mice, the levels were greatly reduced in mice expressing E molecules.

Increase in Activated T Cells in E-Deficient Mice. Frequencies of CD69⁺ activated CD4⁺ T cells increase with age in (NZB $\times$ NZW)F₁ mice, as animals develop SLE (33). Fig. 3A compares frequencies of CD69⁺ activated splenic CD4⁺ T cells in total CD4⁺ T cells among four groups of A^{d/d} F₁ mice at 6 months of age. The frequency (mean $\pm$ SE) in E-negative H-2^{g2/g2} F₁ mice (31.1 $\pm$ 8.6) was significantly higher than those found in three other groups of F₁ mice (11.0 $\pm$ 2.2 in H-2^{d/d} F₁ mice, 14.2 $\pm$ 4.6 in H-2^{d/g2} F₁ mice, and 17.2 $\pm$ 6.3 in H-2^{g2r/g2} F₁ mice) (*P* < 0.02). Frequencies in H-2^{d/g2} and H-2^{g2r/g2} F₁ mice showed a tendency to be higher than those found in H-2^{d/d} F₁ mice; however, there were no significant differences among the groups.

Fig. 2. Comparisons of the cumulative incidence of proteinuria (A), survival rate (B), and serum levels of IgG anti-DNA antibodies (C) and anti-chromatin antibodies (D) among F₁ mice with different H-2 haplotypes. The number of mice examined is shown in parentheses. H-2^{g2/g2} F₁ showed a significantly higher incidence of proteinuria and a lower survival rate, as compared with three other A^{d/d} F₁ strains ($P < 0.001$). Incidence of proteinuria and survival rate in H-2^{d/d} F₁ mice were significantly reduced, as compared with H-2^{d/g2} F₁ and H-2^{g2r/g2} F₁ mice ($P < 0.01$). Compared with H-2^{g2/g2} F₁ mice, wild-type H-2^{d/z} F₁ mice showed a subtle but significantly lower incidence of proteinuria after 7 months of age ($P < 0.05$) and an improved survival rate after 9 months of age ($P < 0.05$). Serum levels of autoantibodies are compared at 6 months of age. Column and bar represent mean and SE, respectively. Asterisks indicate a significant difference.

TCR V β Repertoire Skewing in E-Deficient Mice. E molecule expression levels on thymic epithelial cells affect TCR V β repertoire selection in the thymus (34–36). As shown in Fig. 3B, V β 11 and V β 12 repertoires in splenic CD4⁺ T cells were negatively selected in E molecule-positive H-2^{d/d}, H-2^{d/g2}, and H-2^{g2r/g2} F₁ mice, and there was no significant difference in these repertoire frequencies among the three groups. In contrast, significant proportions of V β 11 (mean and SE, $10.8 \pm 0.2\%$) and V β 12 ($6.0 \pm 0.3\%$) repertoires were observed in E-negative H-2^{g2/g2} F₁ mice.

Effects of E Molecule Expression on DC Function. Differences in haplotypes of class II molecules may affect autoimmune manifestations through the self-antigen presenting capacity of antigen-presenting cells. To determine whether the presence or absence of E molecules affects the potential for self-antigen presentation, we took advantage of a T cell proliferation assay against self-antigen. Splenic T cells from (NZB $\times$ NZW)F₁ mice were transfected *in vitro* with chromatin-specific TCR V α and V β , originally derived from a (SWR $\times$ NZB)F₁ mouse, which can recognize the immunodominant nucleosomal epitope (amino acids 71–94 in histone H4) in the context of A^{d/d} (27, 28) and other A haplotype molecules (37). These T cells can reconstitute the specificity to the nucleosome (38). Such T cells and the T cells transfected with vector alone (mock) were cocultured with CD11c-positive splenic DCs obtained from (NZB $\times$ NZW)F₁ with different levels of E expression, in the presence of chromatin. As shown in Fig. 4, although DCs from A^{d/d} F₁ mice with H-2^{d/d}, H-2^{d/g2}, and H-2^{g2/g2} haplotypes induced significant levels of chromatin-specific T cell responses, there were no significant

Fig. 3. Changes in splenic CD4⁺ T cells among 6-month-old A^{d/d} F₁ mice with different E molecule expression levels. (A) Representative flow cytometry profiles for the expression of activation marker CD69 on splenic CD4⁺ T cells. Frequency of CD69⁺CD4⁺ T cells per total CD4⁺ T cells is shown. (B) Comparisons of each TCR V β repertoire frequency in splenic CD4⁺ T cells. V β 11 and V β 12 repertoires were significantly higher in H-2^{g2/g2} F₁ mice than those in other three F₁ mice ($P < 0.001$).

differences among the three groups of F₁ mice. Compared with findings in these A^{d/d} F₁ mice, DCs from wild-type A^{d/z} heterozygous (NZB $\times$ NZW)F₁ mice induced a significantly greater response of chromatin-specific T cells. Thus, it is suggested that, as compared with A^d β ^d, A^{d/z}-unique A^d β ^z and/or A^z β ^d have an increased capacity of DCs to present chromatin and that the presence or absence of E molecules does not influence the potential of DCs for chromatin presentation.

Discussion

Our newly generated intra-MHC recombinant-congenic (NZB $\times$ NZW)F₁ mice make it feasible to examine the role of class II A and E molecules in the regulation of autoimmune disease, with disregard to the effect of other MHC genes such as *Tnfa* and class I D, which also have been implicated in autoimmune susceptibility (39, 40). The results clearly indicated that class II A and E serve as promoting and protective genetic elements, respectively.

H-2^{d/z} heterozygosity has a strong impact on SLE in (NZB $\times$ NZW)F₁ mice, because the disease is largely reduced in H-2^{d/d} and H-2^{z/z} homozygous mice (2, 3, 5). This observation means that a combination of H-2-linked genes from both parents play a role in an epistatic manner. In this context, we earlier speculated that the polymorphic class II α and β chain genes from both parents may form F₁-unique mixed haplotype α/β heterodimers, such as A^d β ^z and A^z β ^d, either of which may serve as a restriction element for self-reactive T cells (5). Consistent was the finding in the present studies that, compared with DCs from A^{d/d} homozygous F₁ mice, DCs from wild-type A^{d/z} (NZB $\times$ NZW)F₁ mice showed a greater likelihood of chromatin presentation to T cells. Because the antigen-presenting capacity was not influenced by the presence or absence of E molecules, class II A molecules can be attributed to this event. The importance of mixed haplotype α/β heterodimers also was supported by findings of Gotoh *et al.* (7), in

Fig. 4. The potential for chromatin presentation by CD11c-positive splenic cells from (NZB $\times$ NZW) F_1 mice with different H-2 haplotypes. Splenic T cells from (NZB $\times$ NZW) F_1 mice transfected with chromatin-specific TCR V_α and V_β or with vector alone (mock) were cocultured with CD11c-positive splenic DCs obtained from F_1 mice with different H-2 haplotypes, in the presence of chromatin, and T cell proliferative responses were compared. DCs from all these F_1 mice induced significant levels of chromatin-specific T cell responses; however, the potential of DCs for chromatin presentation was significantly higher in wild-type H-2^{d/z} F_1 mice than that in other F_1 groups. Means and SE of three experiments are shown.

which the A $\alpha^d\beta^z$ -restricted self-reactive T cell clone isolated from (NZB $\times$ NZW) F_1 mice had a potential to induce IgG anti-DNA antibodies *in vivo* (8). These F_1 -unique mixed haplotype class II α/β heterodimers also may be involved in the aberrant selection of self-reactive T cells in the thymus. Based on studies by Braunstein and Germain (41), the assembly of A α and A β chains of different haplotypes is under serious pairing restrictions. Thus, low expression levels of these heterodimers in the thymic epithelial cells may allow α/β heterodimer-restricted self-reactive T cells to escape from negative selection in the thymus.

In contrast to A molecules, the mixed-haplotype E molecules are not formed in most of the H-2 heterozygotes, because E α chains are usually nonpolymorphic (42). However, E α^z is unique among the E α chains of other haplotypes and has two amino acid substitutions in the $\alpha 1$ domain (43, 44). Thus, F_1 unique E molecules can be formed in H-2^{d/z} F_1 mice. In this context, Nygard *et al.* (45) proposed the possible preferential formation of E $\alpha^d\beta^z$ mixed haplotype molecules and their involvement in the promotion of F_1 disease. However, evidence indicated that E $\alpha^d\beta^z$ molecules are protective rather than promoting (20).

The protective mechanism by E molecules remains elusive; however, several possibilities are suggested. First is the E-mediated clonal deletion model of self-reactive T cells in the thymus. It has been shown that T cells bearing TCR $V_\beta 5$, $V_\beta 11$, $V_\beta 12$, and $V_\beta 17a$ are eliminated in E-positive strains of mice (34–36), although the extent of negative selection is influenced by background genes (46–48). In this context, there are reports indicating that no clear-cut difference in the expression of TCR V_β repertoire was observed between E⁺ and E[−] transgenic NOD (16) as well as collagen-induced arthritis-susceptible B10.RQB3 mice (49), although the E transgene protected against the disease. Hence, the clonal deletion hypothesis for E-mediated

protection of autoimmune disease has been dismissed in cases of these models. In the (NZB $\times$ NZW) F_1 genetic background, however, CD4 T cell repertoires bearing TCR $V_\beta 11$ and $V_\beta 12$ were significantly negatively selected in mice expressing E molecules. Thus, clonal deletion remains one possible mechanism at work in our mouse models.

Second is the determinant capture model. In E α^d -transgenic BXSB mice, transgene-derived E α^d peptides bind to A^b molecules, possibly decreasing the use of A molecules for presentation of pathogenic self-peptides (18). Rudensky *et al.* (50) showed evidence for the binding of E α^d peptides to A^b molecules in a sequence analysis of peptides derived from the cell surface. This mechanism may be involved in the E-transgene-mediated protection of the collagen-induced arthritis model of E β -deficient B10.RQB3 mice (51, 52). However, such a mechanism may not be operative in our model, because the potential for chromatin presentation by CD11c⁺ DCs was not affected by E molecules expressed in A^{d/d} F_1 mice. This notion is in agreement with the finding by Nakano *et al.* (53) that antigen-presenting cells from both E⁺ and E[−] NOD mice can similarly stimulate diabetogenic T cells.

Third is the cytokine balance model. Hanson *et al.* (19) proposed that E molecule-mediated Th1/Th2 cytokine imbalance is one possible mechanism for the disease protection in E α^d -transgenic NOD mice. Indeed, there is evidence that the differential MHC class II expression on antigen-presenting cells mediated by class II promoter polymorphism exerts a codominant effect on the Th1/Th2 cytokine balance (54, 55). In our preliminary studies, however, there was no clear difference in the potential of anti-CD3-stimulated T cells to produce IL-4 and IFN- γ between E⁺ and E[−] A^{d/d} F_1 mice (data not shown).

An alternative is the signal transducer competition model, in which B cells may be the major cellular sites of E molecule-mediated autoimmune protection. Lang *et al.* (56) reported that antigen stimulation by means of the B cell antigen receptor on resting B cells induces association of MHC class II molecules with B cell antigen receptor-derived Ig- α /Ig- β heterodimers, which function as signal transducers on class II aggregation by the TCR. Because both A and E molecules physiologically associate with Ig- α /Ig- β heterodimers (56), when self-reactive B cells were stimulated by A-restricted T helper cells plus self-peptides, E molecules possibly associate competitively with Ig- α /Ig- β heterodimers. Hence, activation signals should be lower in E⁺ than in E[−] B cells. This idea well explains the observed dose dependency of E-mediated protection of SLE seen in the present studies.

Wild-type H-2^{d/z} F_1 mice develop severe SLE, although they do express intact E molecules. This finding is in striking contrast to findings in H-2^{d/d} F_1 mice that develop the disease only when lacking E molecules. Two mechanisms are thought to be involved. First, compared with H-2^{d/d} F_1 , the self-antigen-presenting capacity of DCs in H-2^{d/z} F_1 is much higher, so that effects of E molecules may be insufficient for suppression. Alternatively, although not mutually exclusive, generation of H-2^{d/z} F_1 -unique self-reactive T cells restricted to haplotype-mismatched A α/β heterodimers in the thymus, as discussed above, may play a role in an E molecule-independent manner.

Taken collectively, our mouse models may provide means for further clarification of molecular mechanisms involved in the positive and negative regulation of autoimmune disease mediated by class II A and E molecules. Studies on the suppressive effect of E molecules are of particular importance, because one can apply this knowledge to future prophylactic and therapeutic approaches to autoimmune diseases.

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1. Tsao, B. P. (2002) in *Dubois' Lupus Erythematosus*, eds. Wallace, D. J. & Hahn, B. H. (Lippincott Williams & Wilkins, Philadelphia), pp. 97–119.
2. Hirose, S., Nagasawa, R., Sekikawa, I., Hamaoki, M., Ishida, Y., Sato, H. & Shirai, T. (1983) *J. Exp. Med.* **158**, 228–233.
3. Hirose, S., Ueda, G., Noguchi, K., Okada, T., Sekigawa, I., Sato, H. & Shirai, T. (1986) *Eur. J. Immunol.* **16**, 1631–1633.
4. Kotzin, B. L. & Palmer, E. (1987) *J. Exp. Med.* **165**, 1237–1251.
5. Hirose, S., Kinoshita, K., Nozawa, S., Nishimura, H. & Shirai, T. (1990) *Int. Immunol.* **2**, 1091–1095.
6. Kono, D. H., Burlingame, R. W., Owens, D. G., Kuramochi, A., Balderas, R. S., Balomenos, D. & Theofilopoulos, A. N. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 10168–10172.
7. Gotoh, Y., Takashima, T., Noguchi, K., Nishimura, H., Tokushima, M., Shirai, T. & Kimoto, M. (1993) *J. Immunol.* **150**, 4777–4787.
8. Tokushima, M., Koarada, S., Hirose, S., Gotoh, Y., Nishimura, H., Shirai, T., Miyake, K. & Kimoto, M. (1994) *Immunology* **83**, 221–226.
9. Murphy, E. D. & Roths, J. B. (1979) *Arthritis Rheum.* **22**, 1188–1194.
10. Merino, R., Fossati, L., Lacour, M., Lemoine, R., Higaki, M. & Izui, S. (1992) *Eur. J. Immunol.* **22**, 295–299.
11. Merino, R., Iwamoto, M., Fossati, L., Muniesa, P., Araki, K., Takahashi, S., Huarte, J., Yamamura, K. I., Vassalli, J. D. & Izui, S. (1993) *J. Exp. Med.* **178**, 1189–1197.
12. Hattori, M., Buse, J., Jackson, R., Glimcher, L., Dorf, M., Minami, M., Makino, S., Moriwaki, K., Kuzuya, H., Imura, H., et al. (1986) *Science* **231**, 733–735.
13. Acha-Orbea, H. & McDevitt, H. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2435–2439.
14. Nishimoto, H., Kikutani, H., Yamamura, K. & Kishimoto, T. (1989) *Nature* **328**, 432–434.
15. Lund, T., O'Reilly, L., Hutchings, P., Kanagawa, O., Simpson, E., Gravely, R., Chandler, P., Dyson, J., Picard, J., Edwards, A., et al. (1990) *Nature* **345**, 727–729.
16. Bohme, J., Schuhbauer, B., Kanagawa, O., Benoist, C. & Mathis, D. (1990) *Science* **249**, 293–295.
17. Labrecque, N., Madsen, L., Fugger, L., Benoist, C. & Mathis, D. (1999) *Immunity* **11**, 515–516.
18. Iwamoto, M., Ibnou-Zekri, N., Araki, K. & Izui, S. (1996) *Eur. J. Immunol.* **26**, 307–314.
19. Hanson, M. S., Cetkovic-Cvrlje, M., Ramiya, V. K., Atkinson, M. A., Maclaren, N. K., Singh, B., Elliott, J. F., Serreze, D. V. & Leiter, E. H. (1996) *J. Immunol.* **157**, 1279–1287.
20. Hirose, S., Zhang, D., Nozawa, S., Nishimura, H. & Shirai, T. (1994) *Immunogenetics* **40**, 150–153.
21. Figueroa, F., Tewarson, S., Neufeld, E. & Klein, J. (1982) *Immunogenetics* **15**, 431–436.
22. Jongeneel, C. V., Acha-Orbea, H. & Blankenstein, T. (1990) *J. Exp. Med.* **171**, 2141–2146.
23. Jacob, C. O., Hwang, F., Lewis, G. D. & Stall, A. M. (1991) *Cytokine* **3**, 551–561.
24. Fujimura, T., Hirose, S., Jiang, Y., Koder, S., Ohmuro, H., Zhang, D., Hamano, Y., Ishida, H., Fukukawa, S. & Shirai, T. (1998) *Int. Immunol.* **10**, 1467–1472.
25. Knight, J. G. & Adams, D. D. (1978) *J. Exp. Med.* **147**, 1653–1660.
26. Bates, D. L., Butler, P. J., Pearson, E. C. & Thomas, J. O. (1981) *Eur. J. Biochem.* **119**, 469–476.
27. Mohan, C., Adams, S., Stanik, V. & Datta, S. K. (1993) *J. Exp. Med.* **177**, 1367–1381.
28. Kaliyaperumal, A., Mohan, C., Wu, W. & Datta, S. K. (1996) *J. Exp. Med.* **183**, 2459–2469.
29. Fujio, K., Misaki, Y., Setoguchi, K., Morita, S., Kawahata, K., Kato, I., Nosaka, T., Yamamoto, K. & Kitamura, T. (2000) *J. Immunol.* **165**, 528–532.
30. Zufferey, R., Donello, J. E., Trono, D. & Hope, T. J. (1999) *J. Virol.* **73**, 2886–2892.
31. Morita, S., Kojima, T. & Kitamura, T. (2000) *Gene Ther.* **7**, 1063–1066.
32. Lilly, F. & Klein, Y. (1973) *Transplantation* **16**, 530–532.
33. Ishikawa, S., Akakura, S., Abe, M., Terashima, K., Chijiwa, K., Nishimura, H., Hirose, S. & Shirai, T. (1998) *J. Immunol.* **161**, 1267–1273.
34. Kappler, J. W., Roehm, N. & Marrack, P. (1987) *Cell* **49**, 273–280.
35. Bill, J., Appel, V. & Palmer, E. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9184–9188.
36. Bill, J., Kanagawa, O., Woodland, D. L. & Palmer, E. (1989) *J. Exp. Med.* **169**, 1405–1419.
37. Shi, Y., Kaliyaperumal, A., Lu, L., Southwood, S., Sette, A., Michaels, M. A. & Datta, S. K. (1998) *J. Exp. Med.* **187**, 367–378.
38. Fujio, K., Okamoto, A., Tahara, H., Abe, M., Jiang, Y., Kitamura, T., Hirose, S. & Yamamoto, K. (2004) *J. Immunol.* **173**, 2118–2125.
39. Jacob, C. O. & McDevitt, H. O. (1988) *Nature* **331**, 356–358.
40. La Cava, A., Balasa, B., Good, A., van Gunst, K., Jung, N. & Sarvetnick, N. (2001) *J. Immunol.* **167**, 1066–1071.
41. Braunstein, N. S. & Germain, R. N. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2921–2925.
42. Mathis, D. J., Benoist, C. O., Williams, V. E., II, Kanter, M. R. & McDevitt, H. O. (1983) *Cell* **32**, 745–754.
43. Sciffenbauer, J., McCarthy, D. M., Nygard, N. R., Woulfe, S. L., Didier, D. K. & Schwartz, B. D. (1989) *J. Exp. Med.* **170**, 971–984.
44. Ogawa, S., Nishimura, H., Awaji, M., Nozawa, S., Hirose, S. & Shirai, T. (1990) *Immunogenetics* **32**, 63–67.
45. Nygard, N. R., McCarthy, D. M., Schiffenbauer, J. & Schwartz, B. D. (1993) *Immunol. Today* **14**, 53–56.
46. Kappler, J. W., Staerz, U., White, J. & Marrack, P. (1987) *Nature* **332**, 35–40.
47. MacDonald, H. R., Schneider, R., Lees, R. K., Howe, R. C., Acha-Orbea, H., Festenstein, H., Zinkernagel, R. M. & Hengartner, H. (1988) *Nature* **332**, 40–45.
48. Pullen, A. M., Marrack, P. & Kappler, J. W. (1988) *Nature* **335**, 796–801.
49. Gonzalez-Gay, M. A., Nabozny, G. H., Bull, M. J., Zanelli, E., Douhan, J., III, Griffiths, M. M., Glimcher, L. H., Luthra, H. S. & David, C. S. (1994) *J. Exp. Med.* **180**, 1559–1564.
50. Rudensky, A. Y., Preston-Hurlburt, P., Hong, S. C., Barlow, A. & Janeway, C. A., Jr. (1991) *Nature* **353**, 622–627.
51. Gonzalez-Gay, M. A., Zanelli, E., Krco, C. J., Nabozny, G. H., Hanson, J., Griffiths, M. M., Luthra, H. S. & David, C. S. (1995) *Immunogenetics* **42**, 35–40.
52. Gonzalez-Gay, M. A., Zanelli, E., Khare, S. D., Krco, C. J., Griffiths, M. M., Luthra, H. S. & David, C. S. (1996) *Immunogenetics* **44**, 377–384.
53. Nakano, N., Kikutani, H., Nishimoto, H. & Kishimoto, H. (1991) *J. Exp. Med.* **173**, 1091–1097.
54. Guardiola, J., Maffei, A., Lauster, R., Mitchison, N. A., Accolla, R. S. & Sartoris, S. (1996) *Tissue Antigens* **48**, 615–625.
55. Baumgard, M., Moos, V., Schuhbauer, D. & Müller, B. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 6936–6940.
56. Lang, P., Stolpa, J. C., Freiberg, B. A., Crawford, F., Kappler, J., Kupfer, A. & Cambier, J. C. (2001) *Science* **291**, 1537–1540.