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Research Article

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The *Yaa* Gene Abrogates the Major Histocompatibility Complex Association of Murine Lupus in (NZB × BXSB)F_1 Hybrid Mice

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Abstract

To investigate the specific contribution of select MHC class II genes on the development of murine lupus, H-2 congenic (NZB × BXSB)F_1 hybrid mice bearing either H-$2^{b/b}$, H-$2^{d/b}$, or H-$2^{d/d}$ haplotypes were generated. We compared the clinical development (autoantibody production and glomerulonephritis) of systemic lupus erythematosus (SLE) in these three F_1 hybrids in the presence or absence of the mutant gene, *Yaa* (Y chromosome-linked autoimmune acceleration), which normally accelerates the progression of murine SLE. (NZB × BXSB)F_1 hybrid female mice bearing either the H-$2^{b/b}$ or H-$2^{d/b}$ haplotype developed a rapid course of severe SLE, while the appearance of disease was markedly delayed in H-$2^{d/d}$ hybrid females. However, in the presence of the *Yaa* gene, H-$2^{d/d}$ F_1 males developed SLE as severe as H-$2^{b/b}$ and H-$2^{d/b}$ F_1 males. These data indicate that (*a*) the conventional H-2^b is a haplotype leading to susceptibility for murine SLE, while H-2^d is a relatively resistant haplotype; (*b*) the H-2^b haplotype exhibits a dominant effect on autoimmune responses, similar to the classical MHC-linked *Ir* gene effect; and (*c*) most strikingly, the *Yaa* gene totally abrogates the MHC effect on murine lupus in (NZB × BXSB)F_1 hybrid mice. (*J. Clin. Invest.* 1994. 94:521–525.) Key words: systemic lupus erythematosus • immune response genes • autoantibody • autoimmunity • mutant mice

Introduction

There exists ample evidence suggesting that many genetic factors play an essential role in the pathogenesis of systemic lupus erythematosus (SLE).[1] Early genetic studies in New Zealand mice have demonstrated that multiple, unlinked genes are responsible for the production of a variety of autoantibodies and the expression of various disease manifestations (1–5). However, the nature of the genes implicated in SLE is still unclear.

Since the genes encoding for MHC molecules participate in both the regulation of the immune response and the selection of T cell specificities from the repertoire, it has been postulated that MHC class II genes are primary candidates to determine susceptibility for the development of SLE. In fact, an association of particular MHC class II haplotypes with SLE has been previously reported in patients with SLE (6). Studies on H-2 congenic (NZB × NZW)F_1 and (NZW × BXSB)F_1 hybrid mice and on F_2 offspring of NZB × SWR crosses have suggested that unique hybrid MHC class II molecules produced in these F_1 hybrid mice may act as restriction elements for their autoimmune disease (7–9). More recently, it has been proposed that a mixed haplotype molecule, I-Eα^dEβ^z, may be responsible for the predisposition to the SLE in (NZB × NZW)F_1 hybrid mice (10). Other evidence that supports the role of MHC class II molecules in the regulation of murine SLE is that the introduction of the *bm12* mutation in the I-Aβ chain in NZB mice results in a dramatic increase of autoantibody production and the rapid development of severe lupus nephritis (11, 12). Although C57BL/6 (B6) and C3H/HeJ mice bearing the H-2^b and H-2^k haplotype, respectively, are able to develop significant autoimmune responses in the presence of the *lpr* or *gld* gene (13, 14), these mice fail to develop the typical glomerulonephritis characteristic in SLE. Thus, it has not yet been clear whether a unique mixed class II haplotype molecule and/or a mutant class II molecule is critical for the development of classic murine lupus or whether conventional MHC class II molecules are sufficient in mice with the appropriate autoimmune genetic background.

Recently, we have generated BXSB mice bearing the H-2^d haplotype, which develop less severe SLE, as compared with mice bearing the wild-type H-2^b haplotype (15). However, since only male BXSB mice develop severe SLE, which results in part from the action of a mutant gene, *Yaa* (Y chromosome-linked autoimmune acceleration), the study of BXSB mice alone cannot elucidate whether the protective effect of the H-2^d haplotype is mediated by preventing the accelerating effect of the *Yaa* gene or alternatively by inhibiting the development of autoimmune responses, independently of the *Yaa* gene. Since (NZB × BXSB)F_1 H-$2^{d/b}$ heterozygous female mice develop typical SLE in the absence of the *Yaa* gene (16), it is possible to study the contribution of the H-2 haplotype to the development of SLE and to critically test whether the *Yaa* gene may exhibit any effect on the possible MHC association of SLE. In the present study, using NZB (H-2^d) or NZB.H-2^b females and BXSB (H-2^b) or BXSB.H-2^d males, we have generated (NZB × BXSB)F_1 hybrids bearing the H-$2^{b/b}$, H-$2^{d/b}$, or H-$2^{d/d}$ haplotype and compared their development of autoimmune manifestations in the presence or absence of the *Yaa* gene. We report herein that the conventional H-2^b promotes the development of SLE occurring in (NZB × BXSB)F_1 hybrid female mice in a dominant fashion, as compared with H-2^d; however, this MHC effect is completely abrogated by the presence of the *Yaa* gene.

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1. *Abbreviations used in this paper:* B6, C57BL/6; gp70 IC, gp70-anti-gp70 immune complexes; SLE, systemic lupus erythematosus; *Yaa*, Y chromosome-linked autoimmune acceleration.

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Methods

Mice. BXSB (H-2^b) mice were purchased from The Jackson Laboratories, Bar Harbor, ME. NZB (H-2^d) mice were obtained from Bomholtgard, Ltd. (Ry, Denmark). BXSB.H-2^d and NZB.H-2^b congenic mice, created by backcross procedures at the 12th and 10th generation, respectively, were recently described (11, 15). The F_1 hybrid mice used in this study were obtained by local breeding. Mice were bled from retroorbital sinus puncture, and resulting sera were stored at −20°C until use.

Serological assays. Serum levels of IgG anti-DNA autoantibodies were determined by ELISA, and results are expressed in titration units, as described previously (17). Serum levels of gp70–anti-gp70 immune complexes (gp70 IC) were quantified by an ELISA combined with the precipitation of the serum with polyethylene glycol (average molecular weight 6,000), and results are expressed as μg/ml of gp70 complexed with anti-gp70 antibodies, as described previously (15).

Histopathology. Samples of all major organs were obtained at autopsy, and histological sections stained either with the periodic acid-Schiff reagent or with hematoxylin eosin. Glomerulonephritis was scored on 0 to 4 scale, in blind, based on the intensity and extent of histopathological changes, as described previously (2), according to Pirani and Salinas-Madrigal (18). Grades 3 and 4 glomerulonephritis were considered significant contributors to clinical disease and/or death.

cDNA cloning and sequencing. Total cellular RNA from spleens of BXSB (H-2^b) mice were extracted using the guanidine isothiocynate-CsCl method (19). First strand of cDNA was synthesized with a mixture of an oligo(dT) and 3′ primers, and 5 μg of total RNA (20). For amplification with *Taq* DNA polymerase (Perkin-Elmer Corp., Norwalk, CT), the following primers (5′-3′) were used: 5′ primer (5′-CAGCATGCTCAGCCTCTGTGGA-3′) and 3′ primer (5′-TCGTCGACTGACTTGCTATTTCTGAG-3′) for the I-Aα chain, and 5′ primer (5′-GGCTGCAGTGGTGGTGCTGATGGTG-3′) and 3′ primer (5′-TGTCTAGAAATCTGTCACTGAGCAGA-3′) for the I-Aβ chain. Polymerase chain reaction (PCR) was performed following the recommendations of the manufacturer (Perkin-Elmer Corp.). The amplified fragments were purified on 5% polyacrylamide gel and inserted into pBluescript II SK or pUC118 vector. The nucleotide sequences corresponding to the $\alpha 1$ and $\beta 1$ domains of the I-Aα and I-Aβ chains were determined by dideoxynucleotide chain termination method (21) using M13 primers. Predicted amino acid sequences were derived from nucleotide consensus sequences of independent clones for each $\alpha 1$ and $\beta 1$ domain.

Statistical analysis. Survival curves were estimated with BMDP Statistical Software (22) and compared using the Breslow statistic (23). Statistical analysis for serological parameters was performed with the Wilcoxon two-sample test. Probability values > 5% were considered insignificant.

Results

MHC association of SLE in (NZB × BXSB)F_1 females. To study the contribution of MHC class II genes on the development of SLE, (NZB × BXSB)F_1 hybrids bearing different H-2 haplotypes (H-$2^{b/b}$, H-$2^{d/b}$, or H-$2^{d/d}$) were generated, and the development of murine lupus was analyzed in female mice lacking the *Yaa* gene. (NZB × BXSB)F_1 females of both H-$2^{b/b}$ and H-$2^{d/b}$ haplotypes developed typical lupus in the first year of life; 50% of them died of glomerulonephritis by 7 and 9 mos, and only 10% were alive at 10 and 11 mos, respectively (Fig. 1 *A*). In contrast, the development of a lupus-like syndrome in H-$2^{d/d}$ female hybrid mice was markedly delayed; their 50% cumulative mortality rate was at 15 mo of age.

The spontaneous production of IgG anti-DNA autoantibodies and gp70 IC was examined in these three F_1 hybrid females. Serum levels of IgG anti-DNA autoantibodies increased with age; by 6 mo of age, the majority of H-$2^{b/b}$ and H-$2^{d/b}$ (NZB × BXSB)F_1 female hybrid mice demonstrated significant anti-DNA antibody activities at comparable titers (Fig. 1 *B* and Table I). In addition, both groups of female mice had essentially identical amounts of gp70 IC at 4 mo of age (Fig. 1 *C* and Table I). In contrast, the production of IgG anti-DNA antibodies and gp70 IC in H-$2^{d/d}$ female hybrids was limited until 6 months of age, as compared with H-$2^{b/b}$ and H-$2^{d/b}$ females. However, H-$2^{d/d}$ females produced progressively increasing amounts of anti-DNA and gp70 IC with age (data not shown).

Since the *H-2^b* gene in H-$2^{b/b}$ and H-$2^{d/b}$ (NZB × BXSB)F_1 mice is derived from BXSB mice (the *H-2^b* gene of the NZB.H-2^b strain is derived from non-autoimmune B6 mice) (11), it may be that a possible mutation in the BXSB *H-2^b* gene is involved in the development of lupus-like autoimmune disease in these two F_1 hybrid female mice. To exclude this possibility, we sequenced the DNA of the $\alpha 1$ and $\beta 1$ domains of the BXSB I-Aα and I-Aβ chains. The consensus nucleotide sequence reveals that both $\alpha 1$ and $\beta 1$ domains of the BXSB I-A molecules are identical to those of the previously sequenced *b* haplotype (24, 25) (data not shown).

Abrogation of MHC association of SLE by the Yaa gene in (NZB × BXSB)F_1 males. Since the *Yaa* gene is able to accelerate lupus-like autoimmune disease, the effect of the *Yaa* gene on the development of SLE in (NZB × BXSB)F_1 hybrid mice was examined in the context of the MHC class II haplotype. As shown in Fig. 2, the accelerating effect of the *Yaa* gene was most dramatic in H-$2^{d/d}$ hybrid male mice ($P < 0.0001$). This contrasted with its slight, but still significant accelerating effect in H-$2^{b/b}$ ($P < 0.05$) and H-$2^{d/b}$ hybrid males ($P < 0.005$). As a consequence, all three F_1 hybrid males bearing the *Yaa* gene, independently of their H-2 haplotypes, developed uniformly a very rapid course of SLE (50% mortality rate due to glomerulonephritis: 5.5 mo in H-$2^{b/b}$ and H-$2^{d/b}$ male hybrids; 6.5 mo in H-$2^{d/d}$ male hybrids; $P > 0.3$) (Fig. 2 *A*).

In correlation with their mortality rates, these three hybrid males bearing the *Yaa* gene had comparable levels of IgG anti-DNA autoantibodies and gp70 IC in sera at 4 mo of age (Fig. 2, *B* and *C*; Table I). The presence of the *Yaa* gene most remarkably potentiated the production of these autoantibodies in H-$2^{d/d}$ hybrid male mice: approximately a 10-fold increase in IgG anti-DNA autoantibodies and an 80-fold increase in gp70 IC, as compared with corresponding females. Although the *Yaa* gene mediated significant acceleration of both autoimmune responses in H-$2^{b/b}$ and H-$2^{d/b}$ mice, increases in IgG anti-DNA autoantibodies and gp70 IC in their males are relatively limited: 2–3-fold for IgG anti-DNA autoantibodies and 5-fold for gp70 IC (Table I).

Discussion

In the present study, we have investigated the specific contribution of select MHC class II molecules and the *Yaa* gene on the development of murine lupus in (NZB × BXSB)F_1 hybrid mice. We demonstrate herein that the conventional H-2^b promotes the development of SLE occurring in (NZB × BXSB)F_1 hybrid female mice in a dominant fashion, while H-2^d is a relatively resistant haplotype, and that most strikingly, this MHC effect is completely abrogated by the presence of the *Yaa* gene.

Our data indicate that the conventional H-2^b class II molecules exert an effect sufficient to initiate and promote autoimmune responses responsible for the development of SLE in

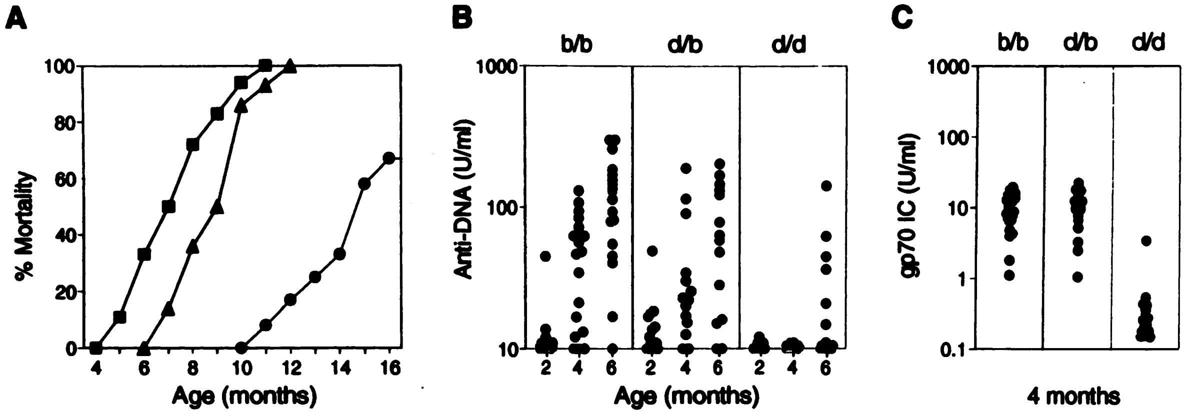

Figure 1. (*A*) Cumulative rates of mortality with glomerulonephritis in H-$2^{b/b}$ (■), H-$2^{d/b}$ (▲), and H-$2^{d/d}$ (●) (NZB × BXSB)F_1 hybrid female mice. 18 H-$2^{b/b}$, 15 H-$2^{d/b}$, and 15 H-$2^{d/d}$ female mice were followed for establishing the mortality rate. Statistical analysis of mortality curves for H-$2^{d/d}$ females and two other females were highly significant ($P < 0.0001$), while no significant difference was observed between H-$2^{b/b}$ and H-$2^{d/b}$ females ($P > 0.05$). (*B*) Serum levels of IgG anti-DNA autoantibodies in H-$2^{b/b}$, H-$2^{d/b}$, or H-$2^{d/d}$ (NZB × BXSB)F_1 hybrid female mice at 2, 4, and 6 mo of age. Results are expressed as U/ml. Differences of IgG anti-DNA autoantibodies for H-$2^{d/d}$ females and two other females (H-$2^{b/b}$ and H-$2^{d/b}$) at 4 and 6 mo of age were highly significant ($P < 0.001$), while differences between H-$2^{b/b}$ and H-$2^{d/b}$ females was not significant (4 mo, $P > 0.1$; 6 mo, $P > 0.05$). (*C*) Serum levels of gp70 IC in H-$2^{b/b}$, H-$2^{d/b}$, or H-$2^{d/d}$ (NZB × BXSB)F_1 hybrid female mice at 4 mo of age. Results are expressed as μg/ml of gp70 complexed with anti-gp70 antibodies. Differences of gp70 IC for H-$2^{d/d}$ females and two other females were highly significant ($P < 0.001$), while differences between H-$2^{b/b}$ and H-$2^{d/b}$ females was not significant ($P > 0.1$).

(NZB × BXSB)F_1 hybrid female mice in the absence of the *Yaa* gene, while the H-2^d is rather a resistant haplotype for SLE. This notion is supported by a recent observation made in H-2 congenic B6-*lpr/lpr* mice, in which the H-2^d, but not H-2^b, inhibits the *lpr* gene-induced autoantibody production (26). It should be stressed that H-$2^{d/b}$ heterozygous F_1 hybrid female mice develop SLE at an extent almost comparable to that seen in H-$2^{b/b}$ homozygous hybrid females, as observed in BXSB mice (15). This observation excludes the implication of hybrid MHC class II molecules in the development of SLE in (NZB × BXSB)F_1 hybrids, as opposed to (NZB × NZW)F_1, (NZW × BXSB)F_1 and (NZB × SWR)F_1 hybrid mice (7–9). Furthermore, the dominant effect of the H-2^b haplotype on autoimmune responses argues against the concept that the H-2^d haplotype may lead to the generation of CD8$^+$ regulatory T cells vetoing the activation of autoreactive cells. However, in studies on B6-*lpr/lpr* mice, the dominant effect of the H-2^b haplotype was less evident: the autoantibody production in H-$2^{d/b}$ B6-*lpr/lpr* mice was substantially lower than that of H-$2^{b/b}$ B6-*lpr/lpr* mice (26). It is possible that there may exist some gene-dosage effect of the MHC class II molecules on the development of autoimmune responses, as described for immune responses to hepatitis B core antigens (27). The MHC class II gene-dosage effect on autoimmune responses can be dependent on other factors in the genetic background of various mouse strains; it may be more apparent in mice whose genetic background is not predisposed to autoimmune diseases, such as B6 mice, than in lupus-prone (NZB × BXSB)F_1 mice. Alternatively, the expression of the I-E molecules in H-$2^{d/b}$ heterozygotes (absent in H-2^b mice) may exert some inhibitory effect on autoimmune responses, as recently observed in I-Eα^d BXSB transgenic mice (28). Although the protective effect conferred by the $E\alpha^d$ transgene has not been totally elucidated, our recent studies on different lupus-prone mice have shown that the protective effect of an $E\alpha^d$ transgene varies in lupus-prone mice, depending on the severity of the disease developing in mice studied (Iwamoto, M., N. Ibnou-Zekri, R. Merino, M. E. Gershwin, and S. Izui, manuscript in preparation). Thus, it may not be surprising to see that the inhibitory effect by the I-E molecules on autoimmune responses can be much greater in B6-*lpr/lpr* mice than in lupus-prone (NZB × BXSB)F_1 mice, which develop more severe SLE than B6-*lpr/lpr* mice (13, 16).

It is striking that the *Yaa* gene completely abolishes the MHC effect on the development of SLE occurring in (NZB × BXSB)F_1 female mice; this resulted from a remarkable accelerating effect mediated by the *Yaa* gene in H-$2^{d/d}$ males, as compared with its limited effect on H-$2^{b/b}$ and H-$2^{d/b}$ males. These data are consistent with the thesis that the capacity of the *Yaa* gene to promote autoimmune responses depends on the levels of autoantibodies spontaneously produced in different lupus-prone mice (15, 29–34). Marked enhancement of autoantibody production by the presence of the *Yaa* gene was observed

Table I. Anti-DNA and gp70 IC in H-$2^{b/b}$, H-$2^{d/b}$, and H-$2^{d/d}$ (NZB × BXSB)F_1 Hybrid Female and Male Mice at Four Months of Age

H-2	Sex	Anti-DNA‡	gp70IC‡
b/b	Female (21)*	45±38	9.8±5.1
	Male (11)	123±93	50.0±29.5
d/b	Female (15)	41±51	10.3±6.1
	Male (11)	89±81	47.0±33.0
d/d	Female (17)	7±2	0.4±0.8
	Male (13)	78±63	31.1±14.1

* Numbers of mice studied are indicated in parenthesis. ‡ Serum levels (means±1 SD) of IgG anti-DNA (U/ml) and gp70 IC (μg/ml). Although differences of IgG anti-DNA and gp70 IC between females and males in H-$2^{d/d}$ mice are most significant ($P < 0.001$), differences between females and males in H-$2^{b/b}$ and H-$2^{d/b}$ mice were still very significant (anti-DNA, $P < 0.005$; gp70 IC, $P < 0.001$).

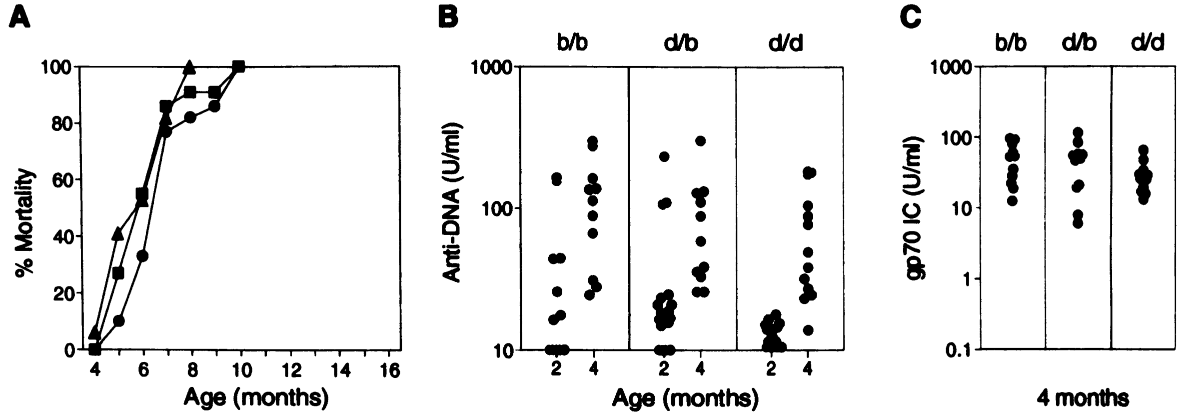

Figure 2. (*A*) Cumulative rates of mortality with glomerulonephritis in H-$2^{b/b}$ (■), H-$2^{d/b}$ (▲), and H-$2^{d/d}$ (●) (NZB × BXSB)F_1 hybrid male mice bearing the *Yaa* gene. 15 H-$2^{b/b}$, 17 H-$2^{d/b}$, and 22 H-$2^{d/d}$ male mice were followed for establishing the mortality rate. There were no statistical differences in moratlity curves of these three different hybrid males ($P > 0.3$). (*B*) Serum levels of IgG anti-DNA autoantibodies in H-$2^{b/b}$, H-$2^{d/b}$, or H-$2^{d/d}$ (NZB × BXSB)F_1 hybrid male mice bearing the *Yaa* gene at 2 and 4 mo of age. Results are expressed as U/ml. Differences of IgG anti-DNA autoantibodies among these three different hybrid males at 4 mo of age were not statistically significant ($P > 0.1$). (*C*) Serum levels of gp70 IC in H-$2^{b/b}$, H-$2^{d/b}$, or H-$2^{d/d}$ (NZB × BXSB)F_1 hybrid male mice bearing the *Yaa* gene at 4 mo of age. Results of gp70 IC are as μg/ml of gp70 complexed with anti-gp70 antibodies. Differences of gp70 IC among these three different hybrid males were not statistically significant ($P > 0.05$).

only in lupus-prone mice which spontaneously produce relatively low amounts of autoantibodies, but not in mice which already produce substantially high titers. This is in agreement with our recent observation that the *Yaa* gene is able to potentiate immune responses against foreign antigens only in mice who are genetically (MHC-linked) low-responding, but not high-responding (Fossati, L., M. Iwamoto, R. Merino, and S. Izui, manuscript in preparation). Thus, the selective autoimmune enhancing activity of the *Yaa* gene may be related to the capacity of T helper cells specific for autoimmune responses in different lupus-prone mice, which is likely to be in part regulated by the MHC class II genes. Accordingly, the *Yaa* gene only slightly potentiates anti-DNA and anti-gp70 autoimmune responses in the F_1 hybrids bearing the H-$2^{b/b}$ or H-$2^{d/b}$ haplotype, which apparently provides sufficient T cell help for both autoantibody responses, as documented by high titers of both antibodies in the absence of the *Yaa* gene; consequently, the progression of SLE is only slightly accelerated in the presence of the *Yaa* gene. In contrast, the *Yaa* gene markedly enhances autoimmune responses, resulting in a dramatic acceleration of SLE in the F_1 hybrids bearing the H-$2^{d/d}$ haplotype, which provides a limited T cell help for autoimmune responses.

It should be emphasized that (NZB × BXSB)F_1 H-$2^{d/d}$ males bearing the *Yaa* gene rapidly develop severe SLE, while the *Yaa* gene fails to provoke an accelerated form of SLE in BXSB.H-2^d mice (15). This can be explained by the fact that the *Yaa* gene effect becomes apparent only in lupus-prone mice, but not in mice which do not spontaneously develop significant autoimmune responses (32, 33). Accordingly, in the context of a BXSB background, the H-2^d haplotype almost completely prevents the development of autoimmune responses; therefore, the *Yaa* gene is unable to promote autoimmune responses, as is the case of non-autoimmune B6 and CBA/J mice bearing the *Yaa* gene (32, 33). In contrast, in (NZB × BXSB)F_1 hybrid mice, the presence of NZB and BXSB genomes and their genetic complementation apparently allow the development of a limited, but significant autoimmune responses, even in the context of the H-2^d; thus, the *Yaa* gene is able to accelerate the progression of their SLE.

Our results indicate that the conventional H-2^b is a more susceptible haplotype for SLE than the H-2^d, while such a MHC effect can be completely masked by the presence of accelerating factors, such as the *Yaa* gene. The precise role of the MHC class II molecules and the *Yaa* gene in the development of SLE remains to be determined. Considering the central role of the MHC molecules in the generation of the T cell repertoire, we favor the hypothesis that the MHC control is likely to be a consequence of thymic selection by the predisposing MHC haplotype of a harmful autoreactive T cell repertoire. It is possible that an autoimmune genetic background or the *Yaa* gene may be implicated in this selection process. As shown in recent studies on two different models of transgenic mice (35, 36), autoreactive T cells specific to minor determinants of autoantigens can be positively selected and evade tolerance induction even in non-autoimmune mice. However, they cannot be optimally stimulated in non-autoimmune mice, because the avidity of these T cell receptors to self peptide–MHC complexes may not be high enough to be activated. An attractive hypothesis is that the action of the *Yaa* gene may be to help promote the low-avidity interaction and subsequent activation of autoreactive T and B cells, possibly through T cell recognition of a *Yaa*-related molecule expressed on B cells, as we have recently proposed (37, 38). Further studies on these H-2 congenic mice in the context of the *Yaa* gene and the molecular identification of the *Yaa* gene product would help elucidate this important question.

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