Protection of Murine Lupus by the $Ea^d$ Transgene Is MHC Haplotype-Dependent

Nabila Ibnou-Zekri, Masahiro Iwamoto, M. Eric Gershwin and Shozo Izui

J Immunol 2000; 164:505-511; doi: 10.4049/jimmunol.164.1.505
http://www.jimmunol.org/content/164/1/505

References
This article cites 33 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/164/1/505.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Protection of Murine Lupus by the \( E_a^d \) Transgene Is MHC Haplotype-Dependent

Nabila Ibnou-Zekri, Masahiro Iwamoto, M. Eric Gershwin, and Shozo Izui

A high-level expression of a transgene, \( E_a^d \), encoding the I-E\(^d \) \( \alpha \)-chain is very effective in protection against murine lupus. To investigate the specific contribution of select H-2 haplotypes on the \( E_a^d \) transgene-mediated disease-suppressing effect, we generated H-2 congenic (NZB \( \times \) BXSB)\( F_1 \) hybrid mice bearing either H-2\(^{b0} \), H-2\(^{d1} \), or H-2\(^{d0} \) haplotype, and compared the transgene-mediated protective effect on the clinical development (autoantibody production and glomerulonephritis) of lupus in these \( F_1 \) hybrids. The level of protection was most remarkable in mice bearing the I-E\(^- \) H-2\(^{b0} \) haplotype but was only minimal in I-E\(^+ \) H-2\(^{d0} \) \( F_1 \) hybrids. Additional analysis demonstrated a marked suppression of lupus in I-E\(^+ \) H-2\(^{d0} \) (MRL \( \times \) BXSB)\( F_1 \), hybrid mice, indicating that the transgene is able to suppress autoimmune responses even in mice already expressing I-E molecules at a homozogous level. Our results indicate that the level of the transgene-mediated protection is dependent on the host H-2 haplotype.

This suggests that the autoimmune suppressive activity of the \( E_a^d \) transgene is likely to be determined through the interaction of the transgene product with the host MHC class II molecules, providing new insight into the role of MHC in lupus-like autoimmunity.

\( \text{The Journal of Immunology, 2000, 164: 505–511.} \)

---

Studies on lupus-prone mice expressing different levels of the \( E_a^d \) transgene revealed that the protection conferred by the transgene is dependent on its level of expression, but not on that of whole I-E molecules on the surface of B cells (12, 13). In addition, it has been observed that the level of protection markedly differs among lupus-prone mice studied: the highest in BXB6, intermediate in (MRL \( \times \) BXSB)\( F_1 \), and lowest in (NZB \( \times \) BXSB)\( F_1 \) mice (10, 12, 13). This indicates that the disease-suppressing effect of the \( E_a^d \) transgene is likely influenced by the presence or absence of specific disease-associated alleles present in different lupus-prone strains. Although the precise mechanisms responsible for the \( E_a^d \) transgene-mediated protection of SLE have not yet been defined, the demonstration that autoantibodies were selectively produced by nontransgenic B cells in transgenic and nontransgenic double bone marrow chimeras indicated that B cells, and not T cells, are the major site of the transgene effect on the suppression of autoimmune responses (10, 14).

Thus, we postulated that \( E_a^d \) transgene expression may lead to interference with an efficient interaction between autoreactive T and B cells by modulating the presentation of pathogenic self-peptides by MHC class II molecules; this can result from increased formation of I-E \( \alpha \)-chain-derived peptides (Ea peptides) displaying a high affinity to the I-A molecules or from the induction or enhanced expression of mixed-haplotype I-E molecules (10, 12, 13). If this is indeed the case, the protective ability of the \( E_a^d \) transgene can be markedly influenced by the host H-2 haplotype because of variabilities in the binding affinity of individual MHC class II molecules to Ea peptides and in the expression level of potential autoimmunity-inhibitory mixed-haplotype I-E molecules in the transgenic mice bearing different H-2 haplotypes.

Due to the potential importance of this thesis, we generated (NZB \( \times \) BXSB)\( F_1 \) hybrids bearing the H-2\(^{b0} \), H-2\(^{d1} \), or H-2\(^{d0} \) haplotype and (MRL \( \times \) BXSB)\( F_1 \) hybrids bearing the H-2\(^{b0} \) or H-2\(^{d1} \) haplotype, and compared the level of the \( E_a^d \) transgene-mediated protection from autoimmune manifestations (autoantibody production and glomerulonephritis) in relation to the host H-2 haplotype. We report in this paper that the autoimmune inhibitory effect of the \( E_a^d \) transgene is dependent on the H-2 haplotype.
haplotype, suggesting that the action of the Ea
transgene is mediated through interaction with the host MHC class II molecules.

Materials and Methods

Mice

A BXSB Ea transgenic line, BXSB-E-1, which expresses the transgene at a high level, was established as previously described (10). BXSB.H-2d, BXSB.H-2b, and NZB.H-2b congenic mice, created by backcross procedures at the 12th, 12th, and 10th generations, respectively, were described (6, 8, 15). NZB and MRL mice were purchased from Bomholtgard (Ry, Denmark). BXSB mice were purchased from Janvier (Le Genest-Saint-Isle, France). 

Lastly, they were analyzed with FACScan (Becton Dickinson, Mountain View, CA).

Serological assays

Serum levels of IgG anti-DNA autoantibodies were determined by ELISA, and results are expressed in titration units, as described previously (16). Serum levels of gp70-anti-gp70 immune complexes (gp70 IC) were quantified by an ELISA combined with the precipitation of sera with polyethylene glycol (average m.w. 6000), and results are expressed as μg/ml of gp70 complexed with anti-gp70 Abs, as described previously (6).

Histopathology

Samples of all major organs were obtained at autopsy, and histological sections were stained with either the periodic acid-Schiff reagent or with hematoxylin and eosin. Glomerulonephritis was scored on a 0-to-4 scale, in grades 3 and 4 glomerulonephritis were considered significant contributors to clinical disease and/or death.

Cytocfluorometric analysis

The expression of IgE molecules in peripheral blood B cells was analyzed by first staining them with FITC-conjugated anti-mouse μ chain (LO-MM-9) mAb (19), and then by incubation with biotinylated anti-Ea (H81.98.21.1) (20) or anti-Eα (Y-17) (21) mAb, and then with PE-conjugated streptavidin (Caltag Laboratories, San Francisco, CA). Lastly, they were analyzed with FACSscan (Becton Dickinson, Mountain View, CA).

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

H-2 haplotype-dependent protection of SLE by the Ea
transgene in (NZB × BXSB)F1 mice

To assess the role of the H-2 haplotype on the Ea
transgene-mediated protection of SLE, (NZB × BXSB)F1 hybrids bearing different H-2 haplotypes (H-2b/b, H-2d/b, or H-2d/d) with or without the transgene were generated, and the effect of the transgene on the clinical development of SLE was assessed in these F1 female mice.

Table I. Effect of the Ea
transgene on serum levels of IgG anti-DNA and gp70 IC in (NZB × BXSB)F1 hybrid female mice

<table>
<thead>
<tr>
<th>H-2 Age (mo)</th>
<th>Anti-DNA*</th>
<th>gp70 IC*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tg</td>
<td>Non-Tg</td>
</tr>
<tr>
<td>b/b 4</td>
<td>5 ± 3</td>
<td>31 ± 16</td>
</tr>
<tr>
<td>6</td>
<td>8 ± 8</td>
<td>93 ± 76</td>
</tr>
<tr>
<td>8</td>
<td>29 ± 38</td>
<td>104 ± 53</td>
</tr>
<tr>
<td>d/b 4</td>
<td>26 ± 17</td>
<td>55 ± 41</td>
</tr>
<tr>
<td>6</td>
<td>31 ± 34</td>
<td>85 ± 64</td>
</tr>
<tr>
<td>8</td>
<td>103 ± 113</td>
<td>116 ± 103</td>
</tr>
<tr>
<td>d/d 4</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>6</td>
<td>16 ± 13</td>
<td>29 ± 28</td>
</tr>
<tr>
<td>8</td>
<td>38 ± 38</td>
<td>86 ± 81</td>
</tr>
<tr>
<td>10</td>
<td>52 ± 44</td>
<td>100 ± 86</td>
</tr>
</tbody>
</table>

* Serum levels (means ± 1 SD) of IgG anti-DNA (U/ml) and gp70 IC (μg/ml) in different ages of (NZB × BXSB)F1 transgenic (Tg) and nontransgenic (Non-Tg) female mice (15–30 mice in each group, except 10 mice for 8-mo-old H-2b/b and H-2d/d F1 nontransgenic groups).
(NZB × BXSB)F1 nontransgenic females of H-2\textsuperscript{b/b}, H-2\textsuperscript{d/b}, and H-2\textsuperscript{d/d} haplotypes developed typical SLE; 50% of them died of glomerulonephritis by 7, 9, and 15 mo, respectively (Fig. 1). Although the high-level expression of the transgene was very effective in the protection from SLE occurring in both H-2\textsuperscript{b/b} and H-2\textsuperscript{d/b} F1 females, the protective effect of the Ea\textsuperscript{d} transgene was far stronger in the H-2\textsuperscript{d/d} F1 females (Fig. 1). In fact, two-thirds of the H-2\textsuperscript{d/b} F1 females bearing the transgene were still alive at 18 mo of age, whereas 50% of the H-2\textsuperscript{b/b} F1 female transgenics died of glomerulonephritis by 17 mo of age (p < 0.001). Most strikingly, the expression of the transgene barely altered the survival rate of the H-2\textsuperscript{d/d} F1 females (p > 0.1; Fig. 1).

The extent of the transgene-mediated prolongation of the life-span observed among these three F1 females bearing different H-2 haplotypes correlated well with the level of suppression of spontaneous autoantibody production, as determined by serum levels of IgG anti-DNA autoantibodies and gp70 IC (Table I). Both autoantibody responses were nearly completely suppressed in the H-2\textsuperscript{b/b} F1 female transgenics at any age tested (4, 6, and 8 mo of age; p < 0.001), but the level of the suppression was significantly less in the H-2\textsuperscript{d/b} female transgenics, despite comparable development of these autoantibody responses in both H-2\textsuperscript{b/b} and H-2\textsuperscript{d/b} nontransgenics. Consistent with a marginal effect of the transgene on the survival rate in the H-2\textsuperscript{d/d} F1 females, these mice exhibited small decreases in serum levels of gp70 IC at 6 and 8 mo of age (p < 0.001) and of IgG anti-DNA autoantibodies at 8 mo of age (p < 0.05).

Because the onset, progression, and level of autoimmune responses were not comparable among the three different (NZB × BXSB)F1 female hybrids, the comparative analysis of the transgene-mediated protective effect on their development of SLE may not be pertinent. Therefore, we conducted a similar analysis in their male counterparts, because all three F1 hybrid males, independently of their H-2 haplotypes, uniformly developed a very rapid course of the disease as a result of the autoimmune accelerating effect of the Yaa (Y-linked autoimmune acceleration) mutation (7). The transgene-induced disease-suppressing effect was relatively limited in the H-2\textsuperscript{b/b} hybrid males compared with their female counterparts. Nevertheless, the transgene expression in F1 hybrid males bearing the H-2\textsuperscript{b/b} haplotype led to the most remarkable reduction in serum levels of IgG anti-DNA autoantibodies and gp70 IC (Table II), and significantly prolonged their survival rate (50% mortality rate due to glomerulonephritis: 8 mo in transgenics and 5 mo in nontransgenics) (Fig. 2). In contrast, significant but relatively small decreases in serum levels of gp70 IC, but not of IgG anti-DNA autoantibodies, were noted in F1 males bearing either the H-2\textsuperscript{d/b} or H-2\textsuperscript{d/d} haplotype, whose life-spans were barely extended by the presence of the transgene (50% mortality rate in H-2\textsuperscript{d/d} mice: 7 mo in transgenics and 6 mo in nontransgenics; 50% mortality rate in H-2\textsuperscript{d/d} mice).

It should be mentioned that circulating B cells from all three different transgenic mice expressed I-E molecules, as examined by surface staining using anti-E\textsubscript{a} (H81.98.21.1) mAb, at levels comparable to those found in nontransgenic H-2\textsuperscript{d/d} F1 hybrid mice (Fig. 3A). However, as expected, the surface density of a mixed-haplotypic I-E molecule, E\textsubscript{a}E\textsubscript{b}, as recognized by the Y-17 mAb, was higher in the H-2\textsuperscript{b/b} transgenic F1 hybrids than that of the H-2\textsuperscript{d/b} F1 transgenics, although the latter mice expressed a higher level of the E\textsubscript{a}E\textsubscript{b} heterodimer than their nontransgenic H-2\textsuperscript{b/b} littermates did (Fig. 3B).

![FIGURE 2. Cumulative rates of mortality due to glomerulonephritis in (NZB × BXSB)F1 transgenic (Tg) and nontransgenic (N-Tg) male mice bearing the H-2\textsuperscript{b/b} (A), H-2\textsuperscript{d/b} (B), or H-2\textsuperscript{d/d} (C) haplotype. A group of 20–30 transgenic or nontransgenic mice were followed to calculate the mortality rate.](http://www.jimmunol.org/Downloadedfrom/507)
Comparable protection of SLE by the \textit{Ea}^d transgene in (MRL × BXSB)\textit{F}_1 mice bearing the H-2\textit{b}/b and H-2\textit{b}/k haplotypes

The data obtained with (NZB × BXSB)\textit{F}_1 hybrid mice bearing the three different H-2 haplotypes have shown that the protective ability of the \textit{Ea}^d transgene is strongly linked to the host H-2 haplotype. Because the disease-suppressing activity of the transgene was the lowest in the H-2\textit{d}/d (NZB × BXSB)\textit{F}_1 hybrids already expressing I-E molecules at a homozygous level, we studied the protective ability of the transgene in lupus-prone mice bearing another I-E\textsuperscript{1} haplotype, H-2\textit{k}. Because NZB mice bearing the H-2\textit{k} haplotype are not available, we generated (MRL × BXSB)\textit{F}_1 hybrids bearing either H-2\textit{k}/b or H-2\textit{k}/k haplotype, and the effect of the transgene on the clinical development of SLE was assessed in both H-2\textit{k}/b and H-2\textit{k}/k \textit{F}_1 male mice (only male hybrids carrying the \textit{Yaa} gene are able to develop a lethal form of SLE) (24).

The transgene-induced suppression of a lupus-like syndrome was highly significant in both H-2\textit{k}/b and H-2\textit{k}/k (MRL × BXSB)\textit{F}_1 transgenic males (Fig. 4). The 50% cumulative mortality rates of both transgenic \textit{F}_1 males were 14 and 15.5 mo, respectively, compared with their nontransgenic \textit{F}_1 male littermates (H-2\textit{b}/b, 5.5 mo; H-2\textit{k}/k, 8 mo). The greatly prolonged survival in the transgenic \textit{F}_1 males was reflected most in serum levels of gp70 IC (Table III). At 4 mo of age, serum gp70 IC concentrations in both H-2\textit{k}/b and H-2\textit{k}/k \textit{F}_1 transgenic males were markedly reduced compared with those of nontransgenic \textit{F}_1 male mice (\(p < 0.001\)). A partial reduction in IgG anti-DNA levels was seen only in the H-2\textit{k}/b \textit{F}_1 males (\(p < 0.01\)) and not in the H-2\textit{k}/k \textit{F}_1 males (\(p > 0.1\)). These results suggest that the protective capacity of the \textit{Ea}^d transgene may be more effective in the H-2\textit{k}/b \textit{F}_1 males than in the H-2\textit{k}/k \textit{F}_1 males. This was further supported by the analysis of their \textit{F}_1 female hybrids, which develop a mild, but not lethal, form of lupus. The expression of the transgene reduced the production of IgG anti-DNA autoantibodies only in H-2\textit{k}/b (MRL × BXSB)\textit{F}_1 female hybrids (\(p < 0.001\)), and not in H-2\textit{k}/k \textit{F}_1 females (\(p > 0.1\)) (Table III). Although the H-2\textit{k}/k \textit{F}_1 females developed low levels of gp70 IC detectable at 8 mo of age, its production was completely inhibited by the presence of the transgene (\(p < 0.01\)), as is the case for the H-2\textit{k}/b \textit{F}_1 females (\(p < 0.001\)). Notably, the surface density of I-E molecules, as determined by staining with
anti-Eα mAb, on circulating B cells from these two different transgenic mice was comparable to that found in nontransgenic H-2^k/k F_1 hybrid mice (data not shown).

**Discussion**

In this study, we have investigated the specific contribution of select H-2 haplotypes on the Ea\textsuperscript{d} transgene-mediated protection from SLE in (NZB × BXSB)F_1 and (MRL × BXSB)F_1 hybrid mice. We demonstrate that the level of the protection conferred by the transgene is highly dependent on the host H-2 haplotype. Although the molecular mechanism responsible for this H-2 association with the transgene-mediated protection remains to be defined, these results suggest that the autoimmune suppressive activity of the transgene is likely to be determined through the interaction of the transgene product with the host MHC class II molecules, thereby possibly modulating the presentation of pathogenic self-peptides by MHC class II molecules implicated in murine SLE.

The comparative analysis of (NZB × BXSB)F_1 hybrid mice bearing three different H-2 haplotypes has demonstrated that the inhibitory effect of the Ea\textsuperscript{d} transgene on clinical development of SLE (autoantibody production and glomerulonephritis) is the strongest with the H-2^k/b haplotype, intermediate with the H-2^k/d haplotype, and the weakest with the H-2^d/d haplotype (Table IV). This strongly suggests that the disease-suppressing effect conferred by the transgene is determined by specific mechanism(s) linked to the host H-2 haplotype. This is consistent with the observation that the autoimmune-inhibitory effect mediated by the transgene is somehow more selective for anti-gp70 autoantibodies than for anti-DNA Ab, as best shown in H-2^k/d (MRL × BXSB)F_1 hybrid mice. All these data strongly argue against the idea that the prevention of SLE results from a nonspecific functional defect in B cells (and/or other APCs) secondary to overexpression of the transgene, as was the case in mice bearing a high-copy number of the Ab^b transgene (25). Instead, as discussed below, the action of the Ea\textsuperscript{d} transgene may be more specifically involved in the process of autoimmune responses.

Additional analysis of (MRL × BXSB)F_1 mice bearing either the H-2^k/b or H-2^k/k haplotype revealed that the H-2^k/k is also a sensitive haplotype for the disease-suppressing effect of the Ea\textsuperscript{d} transgene. However, the (MRL × BXSB)F_1 mice bearing the H-2^k/b haplotype were more susceptible to the transgene effect than those bearing the H-2^k/k haplotype because spontaneous production of IgG anti-DNA autoantibodies was suppressed only in the H-2^k/b hybrids but not at all in the H-2^k/k hybrids. Because the experiments were not conducted in mice bearing the same genetic background, direct comparison for the relative role the H-2^b and H-2^k haplotypes in the transgene-mediated protection was not possible. However, we and others observed that expression of the transgene consistently inhibited the production of IgG anti-DNA autoantibodies in BXSB, (NZB.H-2^b × BXSB)F_1, and C57BL/6-lpr/lpr mice bearing the H-2^b haplotype (10, 14). Therefore, the protective effect conferred by the Ea\textsuperscript{d} transgene is likely to be more effective in mice with the H-2^a haplotype than in those with the H-2^k haplotype (Table IV).

Our demonstration, a remarkable association of the Ea\textsuperscript{d} transgene-mediated protective effect with the host H-2 haplotype, favors a model that the transgene expression in B cells may modulate the presentation of pathogenic self-peptides by MHC class II molecules (I-A and/or I-E), thereby interfering with an excessive activation of potential autoreactive T and B cells. We propose two possible mechanisms of modulation for autoantigen presentation as a result of either excessive generation of Eα peptides with a high affinity to the I-A molecules, thereby decreasing the use of I-A molecules for presentation of pathogenic self-peptides, or the induction or increased production of unique mixed-haplotype I-E molecules, such as Eα^bEβ^d, which interfere with the presentation of pathogenic determinants by other MHC class II molecules as a consequence of capturing self-peptides (10, 12, 13, 26). In this regard, it is important to note that the transgenic H-2^a mice express only a single type of I-E\textsuperscript{d} molecule and no additional novel I-E molecules with different peptide-binding specificities (Table IV). This likely is also the case in mice bearing the H-2^b haplotype. The transgenic H-2^b mice can produce a novel mixed-haplotype Eα^bEβ^d heterodimer (Table IV); however, its peptide-binding specificity should be identical with that of the conventional I-E\textsuperscript{b} (Eα^bEβ^d) molecule because a single amino acid difference localized at the junctional region between the α2 domain and the transmembrane domain between the extracellular region of the Eα\textsuperscript{d} and Eα\textsuperscript{b} chains cannot affect the peptide-binding specificity of these two I-E heterodimers (27). Thus, a marked suppression observed

---

**Table III. Effect of the Ea\textsuperscript{d} transgene on serum levels of IgG anti-DNA and gp70 IC in (MRL × BXSB)F_1 hybrid mice**

<table>
<thead>
<tr>
<th>H-2</th>
<th>Sex</th>
<th>Age (mo)</th>
<th>Tg</th>
<th>Non-Tg</th>
<th>p</th>
<th>Tg</th>
<th>Non-Tg</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>k/b</td>
<td>Male</td>
<td>4</td>
<td>55 ± 59</td>
<td>133 ± 118</td>
<td>0.01</td>
<td>2.3 ± 3.7</td>
<td>29.6 ± 25.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>k/k</td>
<td>Male</td>
<td>4</td>
<td>72 ± 62</td>
<td>80 ± 59</td>
<td>&gt;0.1</td>
<td>1.1 ± 1.4</td>
<td>6.3 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>k/b</td>
<td>Female</td>
<td>6</td>
<td>15 ± 10</td>
<td>72 ± 59</td>
<td>&lt;0.001</td>
<td>&lt;0.1</td>
<td>3.5 ± 2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>k/k</td>
<td>Female</td>
<td>6</td>
<td>51 ± 69</td>
<td>42 ± 30</td>
<td>&gt;0.1</td>
<td>&lt;0.1</td>
<td>0.4 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Serum means (means ± 1 SD) of IgG anti-DNA (U/ml) and gp70 IC (μg/ml) in (MRL × BXSB)F_1 male and female transgenic (Tg) and nontransgenic (Non-Tg) mice (15 mice in each group).

**Table IV. I-E molecules expressed in H-2^a, H-2^b, and H-2^d mice with or without the Ea\textsuperscript{d} transgene and the level of the transgene-mediated protection from SLE**

<table>
<thead>
<tr>
<th>H-2</th>
<th>Non-Tg</th>
<th>Tg</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>Eα^aEβ^b + Ea\textsuperscript{d}</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>Eα^dEβ^d + Eα\textsuperscript{d}</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>Eα\textsuperscript{b}Eβ\textsuperscript{d} (Eα\textsuperscript{b}Eβ\textsuperscript{d}) + Eα\textsuperscript{d}</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* H-2^a mice bearing the Ea\textsuperscript{d} transgene can express a novel mixed-haplotype Eα\textsuperscript{d}Eβ\textsuperscript{b} heterodimer. However, its peptide-binding specificity should be identical to that of the I-E\textsuperscript{a} (Eα\textsuperscript{a}Eβ\textsuperscript{b}) molecule (see text).
in the H-2^d/k (MRL × BXSB)F_1 transgenic mice supports the importance of the Ea peptide-dependent mechanism for the inhibition of autoantibody production. In addition, the protection of the disease in the H-2^d/k transgenic mice, whose expression of I-E molecules does not quantitatively and qualitatively differ from nontransgenic littermates, further argues against the proposal that the transgene effect is a consequence of thymic selection of I-A^* molecules because the introduction of two copies of the Ea transgene is able to almost completely prevent the development of SLE in BXSB (I-E^d, H-2^b) mice (8), whose I-A^* molecule also has a high affinity to the Eo peptide, like the I-A^* molecule (29). If this is so, it can be speculated that I-E^* molecules may be responsible for the development of relatively weak autoimmune responses occurring in the H-2^d/k hybrid mice and that these I-E^* dependent autoimmune responses can only be slightly suppressed, if at all, by the Ea peptide-dependent mechanism, because of a possible limited affinity of the I-E^* molecules to Eo peptides. In contrast to the H-2^d and H-2^k mice, the H-2^b mice express only the mixed-haplotype Ea^Eg^b heterodimer in the presence of the transgene (Table IV), and its expression level is much higher than that seen in H-2^d/k and H-2^k/k heterozygous mice. If the Ea^Eg^b heterodimer is indeed implicated in the autoimmune suppressive activity, the best protection observed in H-2^b mice can be explained by the additive effect of the Ea peptide-mediated and Ea^Eg^b mixed haplotype-mediated inhibition of the presentation of pathogenic self-peptides by MHC class II molecules.

The present results indicate that the protective effect conferred by the Ea^a transgene is likely to be much more complex than we thought initially, but support that the overall disease-suppressing effect of the Ea^a transgene can be determined by several different factors. They include: 1) the affinity of individual MHC class II molecules to peptides derived from the transgene product, I-E^a-chains (28–30); 2) the expression level of potential autoimmune inhibitory mixed-haplotype I-E molecules generated in the transgenic mice; and 3) the relative contribution of individual MHC class II molecules to the development of autoimmune response characteristics of SLE. Accordingly, the interpretation for a better protection observed in H-2^d/k heterozygous (NZB × BXSB)F_1 mice than H-2^d/k F_1 mice and in H-2^b^ (MRL × BXSB)F_1 mice than H-2^b/k F_1 mice is complex because these H-2 heterozygous mice express multiple I-A and I-E molecules in which the precise contributions of each class II molecule to the development of SLE and whose affinities to Ea peptides have been poorly defined. This would also explain why the introduction of the Ea^a transgene in (C57BL × NZB) × NZB backcross mice has no effect on the development of autoantibody production (31).

In conclusion, our data indicate that the H-2 haplotype is an important genetic factor which controls the protective effect of the Ea^a transgene. Because the expression of two copies of the Ea gene is capable of providing protection from SLE (8, 9, 32), the possible role of the MHC class II Ea gene as a lupus-protective gene in mice is likely to be influenced by the host MHC haplotype. However, it should be stressed that the effect of the Ea^a transgene can be modulated by genetic factors other than the MHC class II genes present in the genetic background of individual lupus-prone mice. This was best exemplified by the demonstration that the level of the protection conferred by the transgene is complete in H-2^b BXSB males (10) but only partial in H-2^b/k (NZB × BXSB)F_1 male mice, as shown in this study. Furthermore, our present and previous studies also revealed that the protective effect of the transgene is counteracted by the presence of the Yaa gene in mice highly predisposed to SLE such as (NZB × BXSB)F_1 and (NZW × BXSB)F_1 mice. This observation is consistent with the thesis that the action of the Yaa gene may be implicated in the process of efficient interaction of autoreactive T and B cells (24, 33, 34). Clearly, further understanding of the Ea transgene-mediated protective mechanism and the Yaa gene-mediated accelerating mechanism would help elucidate the molecular and cellular basis central to the development of murine SLE.

Acknowledgments

We thank Liliane Fossati Jimack and Luc Reininger for critically reading the manuscript, and Geneviève Leyvraz, Agnès Bapst, and Christine Mono-Hinard for their excellent technical help.

References

by immunization with activated T cells: possible role of T cellbound Ia antigens as targets of immunoregulatory T cells. J. Immunol. 112:662.