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Protection of Murine Systemic Lupus by the Ea Transgene without Expression of I-E Heterodimers

Eduardo Martınez-Soria, Marie-Laure Santiago-Raber, Liza Ho, Thomas Moll, and Shozo Izui

A high-level expression of the Ea transgene encoding the MHC class II I-E α-chain is very effective in the protection from systemic lupus erythematosus (SLE) in mice. However, it has not been elucidated whether this protection results from the induction or increased expression of I-E heterodimers or from the generation of I-E α-chain-derived peptides displaying high affinity for I-A molecules, because previous studies were conducted in lupus-prone mice expressing I-E β-chains. To address this question, we assessed the protective effect of the Ea transgene in lupus-prone BXSB mice bearing the H2b haplotype (i.e., unable to express I-E heterodimers because of a deficiency in I-E β-chains). We observed that the Ea transgene expression resulted in a marked suppression of the development of SLE in H2b BXSB mice despite the absence of I-E expression. The observed protection was not associated with any detectable levels of T cell depletion and regulatory T cell expansion. Significantly, transgenic I-E α-chains were substantially expressed on the surface of B lymphocytes and dendritic cells, but not of macrophages, without apparent formation of mixed-isotype heterodimers with I-A β-chains. Our results demonstrate for the first time that the Ea transgene is able to prevent the development of SLE without induction of I-E heterodimer expression, indicating a critical role of I-E α-chains, but not I-E heterodimers, in the Ea transgene-mediated protection from SLE. Taken together, our data favor a model of autoimmunity prevention based on competition for Ag presentation between I-E α-chain-derived peptides and autoantigens. The Journal of Immunology, 2008, 181: 3651–3657.

The BXSB strain of mice spontaneously develops an autoimmune syndrome with features of systemic lupus erythematosus (SLE) that affects males much earlier than females (1), which results in part from the genetic abnormality, designated Y-linked autoimmune acceleration (Yaa), present on BXSB Y chromosome (2). Recently, the Yaa mutation was shown to be a consequence of a translocation from the telomeric end of the X chromosome onto the Y chromosome (3, 4). Based on the presence of the gene encoding TLR7 in this translocated segment of the X chromosome, the Tlr7 gene duplication has been proposed to be the etiologic basis for the Yaa-mediated enhancement of disease (3–5).

In addition to Yaa, it has been demonstrated that MHC class II molecules also play a critical role in development of SLE in BXSB mice and their F1 hybrids with New Zealand Black (NZB) mice, with lupus susceptibility more closely linked to the H2b haplotype than the H2d and H2k haplotypes (6–8). Because BXSB and (NZB × BXSB)F1 mice bearing the H2b haplotype do not express I-E MHC class II molecules, due to deletion of the promoter region of the Ea gene (9), the inhibitory effect of the H2b haplotype on lupus development may in part be related to the expression of I-E molecules. Indeed, the protective role of I-E was documented by the demonstration that the introduction of two copies of the Ea transgene encoding the I-E α-chain (Ea-chain) in H2b BXSB mice was sufficient to prevent the development of SLE (8) and that (NZB × New Zealand White (NZW))F1 mice lacking I-E molecules developed more severe disease than those expressing I-E (10). Furthermore, overexpression of the Ea transgene led to a marked suppression of the development of SLE not only in I-E−/−BXSB mice, but also in their I-E+/− F1 hybrids with three other lupus-prone strains, NZB, NZW, and MRL (11–13).

The mechanism responsible for the Ea transgene-mediated protection from SLE has not yet been fully understood. Studies on transgenic and nontransgenic mixed bone marrow chimeras revealed that anti-DNA autoantibodies and T cell-dependent Abs against foreign Ags were preferentially produced by nontransgenic B cells, indicating that B cells are the major target of transgene-mediated suppression of autoimmune responses (14, 15). In addition, we have recently observed in vitro that transgene expression in B cells can inhibit the activation of Ag-specific CD4+ T cells in an epitope-dependent manner (16). These data suggest that Ea transgene expression in B cells may interfere with the interaction and subsequent activation of autoreactive T and B cells. Because B cells from transgenic mice express not only I-E heterodimers, but also an Ea-derived peptide, Eα(52–68), displaying a high affinity to I-A molecules (17, 18), it has been speculated that the protective effect of the Ea transgene could be a consequence of thymic negative selection of a harmful autoreactive T cell repertoire and/or of competition for self-peptide presentation by the Eα(52–68) peptide (11, 14).

To address more specifically these questions, we generated BXSB Ea transgenic mice bearing the H2b haplotype, in which no I-E molecules are expressed, because of the lack of the expression of Ea chains due to the creation of a premature translation stop codon as a result of the single base insertion (19) and Eb chains due to aberrant Eb RNA splicing (20). Then, we determined...
whether the Ea transgene is able to efficiently prevent the development of lupus-like autoimmune manifestations even in the absence of I-E heterodimer expression. We report in this study that, in H2b BXSB mice, the Ea transgene was still very effective in protecting against SLE despite the lack of I-E expression, thus indicating a critical role of Ea chains, but not I-E heterodimers, in the Ea transgene-mediated protection from SLE.

Materials and Methods

Mice
BXSB-E3 (H2b) mice expressing the transgenic Ea chains were established, as previously described (11, 14). B10.G (H2b) mice were purchased from The Jackson Laboratory. The BXSB.H2b congenic strain was generated by selective backcrossing of (B10.G × BXSB-E3)F1, to BXSB mice for eight generations. The presence of the H2b allele was controlled by surface staining of peripheral blood B cells with anti-I-Aq (MKD6) and anti-I-Aq (Y-3P) mAb. The presence of the Ea transgene was screened by Southern blot analysis, as described previously (11, 14). Marker-assisted selection was used to obtain mice homozygous for all known BXSB lupus-predisposing Bxs loci on chromosomes 1, 3, and 13 (21). All experiments described in this study were approved by the Cantonal Veterinary Office in Geneva.

Flow cytometric analysis
Flow cytometry was performed using two- or three-color staining of PBMC, spleen cells, peritoneal cavity cells, and bone marrow-derived macrophages, and analyzed with a FACSCalibur (BD Biosciences). Dendritic cells (DC) from spleen were prepared according to the method described by Kamath et al. (22). Briefly, spleen fragments were digested with a mixture of 1 mg/ml Liberase (Roche Diagnostics) and 1 mg/ml DNase I (Sigma-Aldrich) for 20 min at room temperature and treated with 10 mM EDTA to disrupt T cell-DC complexes. Cells were then filtered with a cell strainer (70 μm), and stained in PBS containing 1% BSA and 2 mM EDTA. Bone marrow-derived macrophages were obtained by culturing bone marrow cells with 30% L cell conditioned medium for 7 days, according to the procedure by Vairo and Hamilton (23), and stimulated with murine IFN-γ (100 U/ml; PepToTech) for 48 h. The following mAb were used: anti-Eα (14,4,4S), anti-Eβ (H81.98.21), anti-I-Aq (MK66) and anti-I-Aq (Y-3P) mAb. The presence of the Ea transgene was screened by Southern blot analysis, as described previously (11, 14). Marker-assisted selection was used to obtain mice homozygous for all known BXSB lupus-predisposing Bxs loci on chromosomes 1, 3, and 13 (21). All experiments described in this study were approved by the Cantonal Veterinary Office in Geneva.

Immunoprecipitation and Western blot analysis
Total lysates of spleen cells from different BXSB mice were cleared of nonspecific binding to Sepharose beads by incubation with GammaBind Plus Sepharose (Amersham Biosciences) for 15 min at 4°C. Cleared lysates were incubated with GammaBind Plus Sepharose coupled with polyclonal rabbit anti-Eβ and anti-Alb antisera specific against the cytoplasmic tail of Eβ and Alb chains, respectively (provided by R. Germain, National Institutes of Health, Bethesda, MD), rat 14.4.4S anti-Eα mAb, rat anti-li (In-1), or rat anti-Ig-β (MCA2209) for 2 h at 4°C. Immunoprecipitates were washed, boiled in Laemmli’s loading buffer, loaded onto 12% SDS-PAGE gel, and transferred to Immobilon-P transfer membrane (Millipore) with a Bio-Rad transcell apparatus. After 2 h of blocking at room temperature in 10 mM Tris-HCl (pH 7.4), 100 mM NaCl, and 0.05% Tween 20 containing 5% low-fat, dry milk powder, membranes were incubated with rabbit anti-Alb, rabbit anti-Eβ, rabbit anti-li, or rat anti-Ig-β (MCA2209) Abs overnight at 4°C. Thoroughly washed membranes were incubated with HRP-conjugated goat anti-rabbit IgG (Bio-Rad) or goat anti-rat IgG (Jackson ImmunoResearch Laboratories) for 1 h at room temperature. Chemiluminescence development was conducted with the ECL reagents (Amersham Biosciences), and the membranes were exposed to HyperFilm ECL (Amersham Biosciences).

Serological assays
Serum levels of IgG autoantibodies against chromatin and dsDNA were determined by ELISA. Chick erythrocytes prepared from chick erythrocytes was directly coated on ELISA plates, whereas dsDNA was coated on ELISA plates precoated with poly(l-lysine) (Sigma-Aldrich). Then, the plates were incubated with 1/100 diluted serum samples, and the assay was developed with alkaline phosphatase-labeled goat anti-mouse IgG. Results are expressed in U/ml in reference to a standard curve derived from a serum pool of MRL-Fas−/− mice. Serum levels of gp70-anti-gp70 immune complexes (IC) were quantified by ELISA, combined with the treatment of sera with 10% polyethylene glycol (average m.w. 6000), which precipitates only Ab-bound gp70, but not free gp70, as described previously (6). Results are expressed as μg/ml gp70 in precipitates by referring to a standard curve obtained with a serum pool of NZB mice with known amounts of gp70.

Histopathology
Histological sections of kidney samples were stained with periodic acid/Schiff reagent. The extent of glomerulonephritis was graded on a 0–4 scale based on the intensity and extent of histopathological changes, as described previously (24). Glomerulonephritis with grade 3 or 4 was considered a significant contributor to clinical disease and/or death.

Mixed leucocyte reaction
A total of 2.5 × 10^6 responder lymph node cells from H2b or H2b BXSB-E3 nontransgenic mice was stimulated with 10^5 irradiated spleen cells from Ea transgenic or nontransgenic BXSB-E3 mice of either H2b or H2q haplotype in a total volume of 200 μl of DMEM containing 1% normal mouse serum. Proliferative responses were assessed by the measurement of [3H]thymidine incorporation for the final 6 h of 3 days’ culture.

Statistical analysis
Statistical analysis was performed with the Mann-Whitney U test. Values of p less than 0.05 were considered significant.

Results
Protection of SLE in BXSB.H2q mice bearing the Ea transgene
We have previously shown that the development of lupus-like autoimmune diseases occurring in I-E BXSB male mice (H2q) was markedly prevented in BXSB-E3 transgenic mice, which contain ∼100 copies of the Ea transgene (11). The transgenic mice not only express Ea/Eβ heterodimers, but also carry Ea/I-α complexes on the surface of B cells (11). To define the role of I-E molecules in the Ea transgene-mediated protection from the disease, we determined the development of SLE in H2q BXSB-E3 transgenic mice deficient in both endogenous Ea and Eβ chains, and, hence, unable to express I-E heterodimers even in the presence of the Ea transgene. BXSB.H2q nontransgenic male mice developed typical SLE: 55% of them died of glomerulonephritis by 8 mo, and only 10% were still alive at 12 mo (Fig. 1). In contrast, the development of lethal glomerulonephritis in BXSB-E3.H2q transgenic male mice was markedly delayed: 80% of them were still alive at 12 mo of age. Kidney histologies showed that all nontransgenic mice dead by 8 mo of age had developed severe glomerulonephritis (histological grade ≥3) with increased glomerular cellularity, obliteration of glomerular architecture, and tubular cast formation in nontransgenic mice. In contrast, at 8 mo of age, transgenic mice exhibited only mild glomerular changes, characterized by modest increases in the mesangium (mean histological grade of 7 mice analyzed: 1.1 ± 0.5). The transgene-mediated prolongation of life span correlated well with reduction of serum levels of IgG anti-nuclear autoantibodies (anti-chromatin and anti-DNA) and gp70 IC, all of which are known to be implicated in the development of glomerulonephritis in murine SLE (25–27). When tested at 5 mo of age (Fig. 2), the strongest suppression was observed with anti-gp70 autoimmune responses (transgenic, 1.5 ± 1.9 μg/ml; nontransgenic, 7.9 ± 8.6 μg/ml; p < 0.0001). A lesser, but still significant suppression was also obtained for anti-chromatin
It has been shown that CD4+ T cells from aged BXSB male mice developing SLE were predominantly CD45RBlow, CD44high, and CD62Llow (phenotype associated with activated/memory T cells), and nontransgenic littermates (63.0 ± 7.1%) was ~3 times lower than in nontransgenic littermates (63.0 ± 13.8%, p < 0.0001; Fig. 3B). CD62L staining confirmed that CD45RB low and CD45RB high T cells in the circulating blood between BXSB-E3.H2b transgensics and nontransgenic littermates at 5 mo of age. As shown in the representative results of CD45RB expression on CD4+ T cells in transgenic and nontransgenic mouse (Fig. 3A), the proportion of CD45RBlow cells among CD4+ T cells from transgenic mice (20.1 ± 7.1%) was ~3 times lower than in nontransgenic littermates (63.0 ± 13.8%, p < 0.0001; Fig. 3B). CD62L staining confirmed that CD45RBlow and CD45RBhigh CD4+ T cells expressed low and high levels of CD62L, respectively, in BXSB-E3.H2b transgenic mice (Fig. 3A). This result indicated a remarkable reduction of T cell activation in BXSB-E3 transgenic mice, consistent with markedly delayed development of SLE. Furthermore, a possible expansion of Treg cells in the transgenic BXSB mice was assessed by enumerating Treg cells among all CD4+ T cells in the peripheral blood from 5-mo-old BXSB-E3.H2b transgenic and nontransgenic male mice. A representative results of anti-CD45RB staining among CD4+ T cells from transgenic (Tg) and nontransgenic (Non-Tg) male mice, and anti-CD62L staining on CD45RBlow vs CD45RBhigh CD4+ T cells from transgenic male mice are shown. PBMC were stained with PE-labeled anti-CD4, FITC-labeled anti-CD45RB, and biotinylated anti-CD62L in the presence of 2.4G2 anti-FcRII/III mAb, followed by CyChrome-conjugated streptavidin. B. Percentages of CD45RBlow activated/memory CD4 T cells among all CD4+ T cells from transgenic (Tg) and nontransgenic (Non-Tg) male mice are shown. For intranuclear staining for Foxp3, PBMC were membrane stained with PE-labeled anti-CD4 and allophycocyanin-labeled anti-CD25, and then intranuclear stained with FITC-labeled anti-Foxp3, in the presence of 2.4G2 anti-FcγRII/III mAb. Percentages of CD25+ Foxp3+ CD4 Treg cells among all CD4+ T cells from transgenic (Tg) and nontransgenic (Non-Tg) male mice are shown. Each symbol represents an individual animal. Mean values are indicated by horizontal lines.

Expression of Eα chains without association of Ab chains on B cells from BXSB-E3.H2b transgenic mice

Using 14.4.4S anti-Eα mAb, the expression of the transgenic Eα chains was examined by surface staining of spleen cells from BXSB-E3.H2b transgensics and their nontransgenic littermates, in comparison with H2b BXSB-E3 transgenic mice. B220+ B cells from H2b transgenic mice displayed a high density of Eα molecules on their surface, as shown previously (11, 16), whereas H2b

FIGURE 1. Cumulative rates of mortality due to glomerulonephritis in BXSB-E3.H2b Eα transgenic and nontransgenic male mice. Groups of 16 transgenic (Tg) and 22 nontransgenic (Non-Tg) male mice were followed for 12 mo to establish mortality rates. The development of glomerulonephritis was evaluated by histological analysis of kidneys collected when mice were moribund or at the end of the experiment (12 mo of age). Glomerulonephritis with grade 3 or 4 was considered a significant contributor to clinical disease and/or death. Serum levels of gp70 IC and IgG anti-DNA and anti-chromatin autoantibodies in 5-mo-old BXSB-E3.H2b Eα transgenic and nontransgenic male mice. Results are expressed as U/ml for anti-DNA and anti-chromatin autoantibodies, and as μg/ml for gp70 IC. Each symbol represents an individual transgenic (Tg) and nontransgenic (Non-Tg) male mouse. Mean values are indicated by horizontal lines.
nontransgenic B cells failed to exhibit reactivity to 14.4.4S mAb (Fig. 4A). However, despite the absence of expression of Eβ chains, B cells from H2q transgenic mice displayed Eα molecules recognized by 14.4.4S mAb, although the mean fluorescence intensity with these transgenic B cells (means ± SD of three mice: 42.8 ± 4.4) was ~4 times less than that of B cells from H2β transgenic mice (178.1 ± 16.5). This was also the case when the surface expression level of Eα proteins was assessed with another anti-Eα mAb, H81.98.21 (Fig. 4A).

We next explored the possibility that the transgenic Eα chains formed heterodimers with Aβ chains in B cells, as the formation of mixed-isotype heterodimers between Eα and Aβ chains in H2q mice has been reported (29, 30). For this purpose, Eα or Aβ proteins in spleen cell extracts were immunoprecipitated with polyclonal Abs specific for the cytoplasmic tail of Eα or Aβ chains, and the obtained immunoprecipitates were separated on SDS-PAGE and blotted with these polyclonal Abs. Eα and Aβ chains were clearly visible in the respective Eα or Aβ precipitates from both H2q and H2β transgenic spleen cells, whereas neither Eα nor Aβ chains were detectable after immunoprecipitation with the reciprocal polyclonal Abs (Fig. 5A). Furthermore, in contrast to H2q Eα transgenic mice, Eα proteins in spleen extracts from H2q transgenic mice were not immunoprecipitated with 14.4.4S mAb (Fig. 5B), which is specific for a conformational epitope of the Eα protein resulting from association with Eβ or Aβ chains, but not for the free protein (31). These results ruled against the possible association of the transgenic Eα chains with the endogenous Aβ chains in B cells from BXSB-E3.H2q transgenic mice.

The possibility that Eα chains were expressed in association with Aβ chains on the cell surface of H2q transgenic B cells was unlikely, because immunoprecipitation with anti-Eα or anti-li Abs failed to coimmunoprecipitate li and Eα chains, respectively.
H2\textsuperscript{\alpha} transgenic B cells (Fig. 5C). Notably, no surface staining with anti-H2\textsuperscript{\alpha} mAb was observed (data not shown). It has been reported that Ag stimulation of B cells induced MHC class II molecules to associate with Ig-\alpha-Ig-\beta heterodimers, as demonstrated by reciprocal immunoprecipitation (32). Therefore, the possible association of the transgenic Ea chains with Ig-\alpha-Ig-\beta dimers was assessed by immunoprecipitation with either anti-Ea or anti-Ig-\beta Abs. However, our analysis showed no noticeable association of Ea chains and Ig-\beta in spleen cells from BXSB-E3.H2\textsuperscript{\alpha} transgenic mice (data not shown).

Expression of Ea chains on DC, but not on macrophages, in BXSB-E3.H2\textsuperscript{\alpha} transgenic mice

To better define the molecular basis of Ea expression on H2\textsuperscript{\alpha} Ea transgenic B cells, we determined whether DC and macrophages from BXSB-E3.H2\textsuperscript{\alpha} transgenic mice also expressed Ea chains on their surface. The staining with 14.4.4S mAb showed that Ea chains were expressed on splenic DC in H2\textsuperscript{\alpha} transgenic mice, although their levels were lower than those on DC from H2\textsuperscript{\beta} transgenic mice, as in the case of B cells (Fig. 4B). In contrast, 14.4.4S mAb failed to bind peritoneal macrophages from H2\textsuperscript{\alpha} transgenic mice, whereas macrophages from H2\textsuperscript{\beta} transgenic mice displayed an appreciable density of Ea proteins on their surface. The absence of expression of Ea proteins on H2\textsuperscript{\alpha} transgenic macrophages was further confirmed by the failure to induce the surface expression of Ea proteins on IFN-\gamma-stimulated bone marrow-derived macrophages from H2\textsuperscript{\alpha} transgenic mice, which contrasted with a substantial induction of Ea proteins on bone marrow-derived macrophages from H2\textsuperscript{\beta} transgenic mice (data not shown). Moreover, flow cytometry analysis confirmed the absence of expression of Ig-\beta chains in DC of H2\textsuperscript{\alpha} transgenic BXSB mice (Fig. 4C), further excluding a possible implication of Ig-\alpha-Ig-\beta heterodimers in the surface expression of Ea chains, at least for DC from BXSB-E3.H2\textsuperscript{\alpha} transgenic mice.

To address further the question about the possible expression of the transgenic Ea chains on the surface of B cells and DC without association of A\beta chains, we tested whether spleen cells from BXSB-E3.H2\textsuperscript{\alpha} transgenic mice were able to induce a proliferative response in nontransgenic T cells. In vitro MLR revealed that H2\textsuperscript{\alpha} nontransgenic lymph node cells failed to exhibit measurable proliferative responses in the presence of irradiated H2\textsuperscript{\alpha} transgenic splenocytes, whereas H2\textsuperscript{\alpha} nontransgenic lymph node cells robustly proliferated in the presence of H2\textsuperscript{\alpha} transgenic splenocytes (Fig. 6). Moreover, the analysis of the T cell repertoire in lymph nodes showed no significant decreases in V\beta\textsuperscript{6} and V\beta11 T cells in BXSB-E3.H2\textsuperscript{\alpha} transgenic mice, as compared with their nontransgenic littermates (data not shown). This contrasted with the finding that lymph nodes from H2\textsuperscript{\alpha} BXSB-E3 transgenic mice contained less V\beta\textsuperscript{6} and V\beta11 T cells than those from their nontransgenic counterparts, as described previously (14).

Discussion

We and others have previously shown that the development of SLE in lupus-prone mice is strongly suppressed by overexpression of the Ea transgene, which led not only to induction or increased expression of I-E heterodimers, but also to the generation of the Ea(52–68) peptide displaying a high affinity to I-A molecules (11–15). However, because previous studies were conducted in lupus-prone mice expressing E\beta chains, it has not yet been possible to define the relative contribution of I-E heterodimers and Ea peptides to the protective effect of the Ea transgene. In the present study, by introducing the Ea transgene and the H2\textsuperscript{\alpha} haplotype (lacking both endogenous Ea and E\beta chains) into lupus-prone BXSB mice, we have demonstrated for the first time that the Ea transgene is able to prevent the development of SLE without the induction of I-E heterodimer expression.

Based on the fact that a substantial fraction of T cells is eliminated in I-E-positive strains of mice during the thymic maturation of T cells (33), the clonal deletion of autoreactive T cells in the thymus has been proposed as one of the mechanisms explaining the I-E-mediated protection from several autoimmune diseases, such as autoimmune diabetes in NOD mice (34), collagen-induced arthritis in B10.RQBB3 mice (35), and SLE in (NZB × NZW)F1 mice (10). Indeed, we have previously observed a significant deletion of V\beta\textsuperscript{6} and V\beta11 T cells in H2\textsuperscript{\alpha} BXSB-E3 transgenic mice, as compared with their nontransgenic littermates (14). However, our present results demonstrate that the development of SLE in BXSB-E3.H2\textsuperscript{\alpha} transgenic mice was markedly suppressed without the expression of I-E heterodimers and also of EaA\beta mixed-isotype heterodimers (as discussed below). This indicates that thymic negative selection of a potentially harmful autoreactive T cell repertoire is not the major, if any, mechanism for the protective effect of the Ea transgene in the development of SLE.

It is significant that B cells and DC from BXSB-E3.H2\textsuperscript{\alpha} transgenic mice express substantial levels of Ea proteins on their surface, despite the absence of E\beta chains. This expression could be the result of the formation of a mixed-isotype heterodimer with the endogenous A\beta chains. The assembly of Ea and A\beta chains apparently occurs under physiological condition in mice, although very limited because of serious pairing restrictions between Ea and A\beta chains (29, 30). However, our reciprocal immunoprecipitation analysis was unable to reveal association of Ea and A\beta chains in spleen cells from BXSB-E3.H2\textsuperscript{\alpha} transgenic mice. Moreover, the absence of Ea expression on the surface of macrophages and the lack of MLR between H2\textsuperscript{\alpha} BXSB transgenic and nontransgenic lymphocytes further argue against the formation of EaA\beta heterodimers as an explanation for the cell surface expression of Ea in B cells and DC. Finally, the lack of immunoprecipitation, but positive surface staining, of Ea proteins by 14.4.4S mAb is an additional support for the absence of EaA\beta heterodimers, because this mAb immunoprecipitates only Ea chains when associated with E\beta or A\beta chains due to detection of an association-dependent conformational epitope (31). Collectively, our results strongly suggest that in BXSB-E3.H2\textsuperscript{\alpha} transgenic mice the Ea chains form a
complex with a protein other than Aβ chains, but this association is not stable enough to resist the treatment with detergent for the preparation of spleen cell lysates for immunoprecipitation with 14.4.4S mAb.

It has previously been reported that Ea proteins were also expressed on the surface of B cells from an Eaαβ recombinant strain, A.TFR5 (36, 37). In agreement with our results, no Eaαβ heterodimers were detectable by immunoprecipitation analysis in A.TFR5 mice expressing the I-Aβ molecule. Significantly, the expression level of Ea proteins on their B cells, as determined by staining with 14.4.4S mAb, was ~4 times less than that of the I-Eβ parental A.TL strain, and thus comparable to BXSB-E3.H2q transgenic mice despite their excessive expression of Ea chains. This also supports the idea that the Ea proteins present on the surface of B cells and DC of BXSB-E3.H2q transgenic mice are not free proteins, but associated with another protein, and that the amount of the latter available in B cells and DC is a limiting factor for surface expression of Ea proteins. Notably, transfection experiments have shown that free Ea chains cannot reach the cell surface of murine L cells (31). It was therefore speculated that Ea chains associated with li chains may reach the cell surface in A.TFR5 mice. However, this is apparently not the case in BXSB-E3.H2q transgenic mice, because anti-li mAb failed to show any significant surface staining of B cells from BXSB-E3.H2q transgenic mice, and because the association of Eaαβ and li chains was not detectable by reciprocal immunoprecipitation analysis.

Another candidate molecule may be Ig-α/Ig-β dimers, because it has been reported that stimulation of B cells through BCR induced association of MHC class II molecules with Ig-α/Ig-β heterodimers, which then act as signal transducers through the engagement with TCR, thereby promoting the activation of B cells (32). Thus, an attractive hypothesis is that overexpressed Ea chains may compete with MHC class II molecules for the Ig-α/Ig-β binding, thereby down-regulating T cell-dependent activation of autoreactive B cells. However, the failure of reciprocal immunoprecipitation of Eaαβ and Ig-β proteins and the absence of expression of Ig-β in DC do not support this possibility. Accordingly, our present data strongly suggest that Ea chains in BXSB-E3.H2q transgenic mice are likely to be associated with MHC-unrelated molecules and then transported to the cell surface of B cells and DC.

The remarkable suppression of the development of SLE in BXSB-E3.H2q transgenic mice in association with the expression of Ea proteins on the surface of B cells and DC favors a model of competitive autoimmunity suppression: Ea-chain-derived peptides with a high affinity to I-A molecules decrease the use of the latter for presentation of pathogenic self-peptides by B cells and DC, thereby limiting the excessive activation of autoreactive B and T cells (11, 14). It is known that the majority of the peptides associated with MHC class II molecules are derived from endogenous transmembrane proteins (17, 18). Thus, the Ea peptide could be generated from these surface Ea chains in the H2q transgenic mice. A competitive mechanism of this sort is consistent with the following findings. First, the transgene-mediated protection paralleled the expression levels of the Ea peptide presented by I-A, but not those of I-E heterodimers (11, 12). Second, B cells expressing the Ea transgene produced far less anti-DNA autoantibodies than I-Eαβ transgenic B cells in transgenic and nontransgenic double bone marrow chimeras (14, 15). Third, the expression of the Ea transgene in B cells can inhibit the activation of Ag-specific CD4+ T cells in vitro in an epitope-dependent manner without the modulation of I-E expression (16). Thus, the Ea transgene-mediated suppression of T cell responses can be related to the relative affinity of the Ea peptide vs antigenic peptides for individual MHC class II molecules. Finally, the protection from SLE in BXSB-E3.H2q transgenic mice was associated with a marked reduction of CD45RBlow, CD62Llow T cells, a phenotype associated with activated/memory T cells. The generation of the Ea peptide and its subsequent presentation by I-A molecules might result in the induction of Treg cells, thereby suppressing autoimmune responses in BXSB-E3.H2q transgenic mice. However, the lack of any measurable expansion of Treg cells in the periphery strongly argues against this possibility.

Our present results indicate a critical role of Ea chains, but not I-E heterodimers, in the Ea transgene-mediated protection from SLE, further supporting a model of autoimmunity prevention based on competition for autoantigen presentation. Moreover, given the critical role of DC and B cells in the T cell activation, it is of interest to identify and define the function of the putative molecule that pairs with Ea chains in these cells, because we cannot exclude the possibility that this interaction may additionally contribute to the Ea transgene-mediated prevention of SLE. As far as we know, there have been no reports describing the protection from human SLE conferred by certain HLA molecules. However, it has been shown that a DQ65–79 peptide, derived from the α-chain of HLA DQA03011, is able to bind proliferating cell nuclear Ag, thereby inducing T cell apoptosis (38). Such an immunosuppressive mechanism could be additionally implicated in the protective effect conferred by the Ea transgene.

The Ea transgene selectively suppresses autoimmune responses, but not immune responses to foreign Ags (11, 14). Clearly, further understanding of the protective mechanism(s) conferred by the Ea transgene would help to elucidate the molecular and cellular basis central to the development of SLE. This could help to establish a new strategy for the development of more specific therapeutic approaches in SLE.

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Disclosures

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