Prevention of Systemic Lupus Erythematosus in Autoimmune BXSB Mice by a Transgene Encoding I-E α Chain

By Ramón Merino,* Masahiro Iwamoto,* Liliane Fossati,* Pedro Muniesa,‡ Kimi Araki,* Satoru Takahashi,* Joaquín Huarte,‡ Ken-Ichi Yamamura,§ Jean-Dominique Vassalli,‡ and Shozo Izui*

*From the Departments of *Pathology and ‡Morphology, Centre Médical Universitaire, University of Geneva, 1211 Geneva 4, Switzerland; and the §Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 862, Japan*

Summary

Males from the BXSB murine strain (H-2^b) spontaneously develop an autoimmune syndrome with features of systemic lupus erythematosus (SLE), which results in part from the action of a mutant gene (*Yaa*) located on the Y chromosome. Like other H-2^b mice, the BXSB strain does not express the class II major histocompatibility complex antigen, I-E. Here we report that the expression of I-E ($E\alpha^d E\beta^b$) in BXSB males bearing an $E\alpha^d$ transgene prevents hypergammaglobulinemia, autoantibody production, and subsequent autoimmune glomerulonephritis. These transgenic mice bear on the majority of their B cells not only I-E molecules, but also an I-E α chain–derived peptide presented by a higher number of I-A^b molecules, as recognized by the Y-A*e* monoclonal antibody. The I-E^+ B cells appear less activated in vivo than the I-E^- B cells, a minor population. This limited activation of the I-E^+ B cells does not reflect a functional deficiency of this cell population, since it can be stimulated to IgM production in vitro by lipopolysaccharides at an even higher level than the I-E^- B cell population. The development of the autoimmune syndrome in the transgenic and nontransgenic bone marrow chimeric mice argues against the possibility that the induction of regulatory T cells or clonal deletion of potential autoreactive T cells as a result of I-E expression is a mechanism of the protection conferred by the $E\alpha^d$ transgene. We propose a novel mechanism by which the $E\alpha^d$ transgene protects BXSB mice against SLE: overexpression of I-E α chains results in the generation of excessive amounts of a peptide displaying a high affinity to the I-A^b molecule, thereby competing with pathogenic autoantigen–derived peptides for presentation by B lymphocytes and preventing their excessive stimulation.

The BXSB mouse strain spontaneously develops a progressive and lethal autoimmune disease, similar to human SLE, which affects male animals much earlier than females (1, 2). Cell transfer and Y chromosome transfer studies have clearly demonstrated that the Y chromosome-linked autoimmune acceleration (*Yaa*) gene present in the Y chromosome of the BXSB strain is responsible, in mice predisposed to autoimmune diseases, for the accelerated autoimmune abnormalities and immunopathological lesions (3–6). In addition, it has been demonstrated that the MHC genes play a critical role in the development of SLE-like autoimmune syndrome in BXSB mice (7) and in their F_1 hybrids with NZB mice (Merino, R., M. Iwamoto, and S. Izui, manuscript in preparation): the H-2^b haplotype appears to be associated with the development of the autoimmune disease, whereas the H-2^d haplotype protects against this disease. Since BXSB and (NZB × BXSB)F_1 mice bearing the H-2^b haplotype do not express one of the class II MHC antigens, I-E (because of the deletion of the promoter region of the $E\alpha$ gene [8]), the inhibitory effect of the H-2^d haplotype may in part be related to the expression of the I-E molecule. In fact, it has been shown that I-E molecules could exhibit a suppressive activity on immune responses (9), and more recently the expression of I-E molecules through an I-E α chain transgene was found to result in the prevention of autoimmune diabetes in NOD (I-E^-) mice (10–13). In the present study, we found that the expression of a transgene encoding the I-E α chain, $E\alpha^d$, is highly protective against the development of the lupuslike autoimmune disease in BXSB mice, and defined the possible protective mechanism(s) conferred by the expression of this transgene.

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Materials and Methods

Generation of BXSB Eα^d Transgenic and BXSB.Igh^a Congenic Mice. BXSB mice were purchased from The Jackson Laboratories (Bar Harbor, ME). A 14-kb SacII/XhoI fragment containing the entire Eα^d gene sequence (14) was microinjected into fertilized eggs of BXSB mice, as described (15). Mice were screened for the transgene by the surface staining of PBMC using an anti-I-E mAb, Y-17 (16), as described below.

BXSB.Igh^a congenic mice were established by transfer of the Igh^a gene complex of the B.C20/Icr strain as follows: BXSB females were mated with B.C20/Icr males, male offsprings which carry the Igh^a allotype, as determined by ELISA (described below), were then backcrossed with BXSB females for 11 generations. Then, females carrying the Igh^a allotype were mated with BXSB males to introduce the *Yaa* gene. The BXSB.Igh^a novel strain was obtained by mating female and male BXSB Igh$^{a/b}$ heterozygotes at the 12th backcross generation.

Southern Blot Analysis. 5 μg of high molecular weight genomic DNA prepared from tails of BXSB mice were digested with SacI, electrophoresed on a 1.0% agarose gel, and transferred to a nylon membrane (GeneScreen Plus; Du Pont Co., Boston, MA) as described (17). Hybridization was carried out under high stringency with a ^{32}P-labeled 2.6-kb SalI fragment containing the first exon of the Eα^d gene (18), which recognizes two 1.9 and 2.9-kb fragments of the injected Eα^d gene and a 4.2-kb fragment of the endogenous defective Eα^b gene in SacI-digested DNA (see Fig. 1 *A*).

Northern Blot Analysis. Total RNA was extracted, using the guanidine isothiocyanate/CsCl method (19), from spleen, thymus, lymph nodes, liver, kidneys, lungs, and brain of BXSB mice. RNA (30 μg) were electrophoresed on a 1% formaldehyde-containing agarose gel, transferred to nylon membrane, and hybridized with a ^{32}P-labeled 3.5-kb SalI fragment containing exons 2, 3, and 4 of the Eα^d gene (18) or ^{32}P-labeled cDNA corresponding to β-actin.

Cytofluorometric Analysis. The expression of I-E and/or I-A molecules in peripheral blood and spleen cells was analyzed by staining first with FITC-conjugated anti-mouse μ chain mAb (LO-MM-9) (20), and then incubating with biotinylated anti-I-E (Y-17) and/or anti-I-A^b (Y-3P) mAb (21), followed by PE-conjugated avidin (Caltag Laboratories, San Francisco, CA) and analyzed with a FACScan® (Becton Dickinson & Co., Mountain View, CA). The expression of the I-A^b-Eα peptide complexes, recognized by the Y-A*e* mAb (22, 23), was determined by two different staining procedures. First, spleen cells were first stained with FITC-conjugated anti-mouse μ chain mAb (LO-MM-9), and then incubated with biotinylated Y-A*e* mAb, followed by PE-conjugated avidin. Second, spleen cells were first stained with FITC-labeled anti-I-E (Y-17) mAb, then with PE-conjugated goat anti-mouse μ chain Abs (Caltag Laboratories), and incubated with biotinylated Y-A*e* mAb, followed by Streptavidin-RED670™ (Gibco BRL, Gaithersburg, MD). The expression of Vβ segments of TCR in Thy-1$^+$ lymph node cells was analyzed as described previously (7). Monocytes in peripheral blood were enumerated using anti-Mac-1 mAb (M1/70) (24) followed by FITC-conjugated goat anti-rat IgG Abs. Surface IgM^a and IgM^b positive cells in peripheral blood from bone marrow chimeric mice were stained with biotinylated mAb, RS-3.1 (anti-IgM^a) (25), and MB86 (anti-IgM^b) (26), followed by PE-conjugated avidin.

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 eosin. Glomerulonephritis was scored on a 0–4 scale based on the intensity and extent of histopathological changes, as described previously (5).

Serological Assays. Serum levels of total IgG, IgG Abs against single-stranded DNA or human IgG (HGG)[1] and the allotype of anti-DNA Abs were determined by ELISA as described (6, 27, 28). Anti-DNA or anti-HGG activities in sera are expressed in titration units as described previously (6, 27). 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), as described (6, 7). Results are expressed as μg/ml of gp70 complexed with anti-gp70 Abs by referring to a standard curve obtained from NZB sera with known amounts of gp70. Serum levels of Igh^a and Igh^b allotypes were determined by a previously described ELISA (28).

Isolation of I-E$^+$IgM$^+$ and I-E$^-$IgM$^+$ B Cells, and Cell Culture. Spleen cells, depleted of Thy-1$^+$ cells by treatment with anti-Thy-1.2 (AT83) mAb (29) and rabbit complement from transgenic BXSB mice were stained for surface IgM and I-E molecules as described above. Then, I-E$^+$IgM$^+$ or I-E$^-$IgM$^+$ B cells were purified by a FACStar® (Becton Dickinson & Co.) For the spontaneous IgM and IgG secretion, 10^5 I-E$^+$IgM$^+$ or I-E$^-$IgM$^+$ B cells were incubated in 200 μl DME supplemented with additional amino acids, 10 mM Hepes, 5×10^{-5} M 2-ME, and 10% FCS for 24 h at 37°C in a humidified incubator containing 5% CO_2 in air. For the LPS-induced IgM production, 3×10^4 I-E$^+$IgM$^+$ or I-E$^-$IgM$^+$ B cells in 200 μl culture were stimulated with 50 μg/ml of *Escherichia coli* LPS (Difco Laboratories, Inc., Detroit, Michigan) for 5 d. IgM and IgG concentrations in supernatants were determined by ELISA (6, 7).

Preparation of Bone Marrow Chimeras. 2-mo-old BXSB female recipients were irradiated at 850 rad and reconstituted with bone marrow cells (BMC) from 3–4-mo-old BXSB mice. A mixture of 10^7 viable BMC were intravenously injected into irradiated recipients as detailed in Table 1.

Statistical Analysis. Statistical analysis was performed with the Wilcoxon two-samples test. Probability values >5% were considered insignificant.

Results and Discussion

To explore a possible protective effect of the I-E molecule on the development of SLE in autoimmune BXSB male mice, we introduced the Eα^d gene into this strain, and nine founder transgenic BXSB mice were generated. A line from one of these founder mice, BXSB-E-1, which contains approximately 50 copies of the Eα^d transgene (Fig. 1 *A*), was established and analyzed in more detail. The Eα transcript was strongly expressed in thymus, spleen, and lymph nodes, and at a low level in lungs, liver, and kidneys (Fig. 1 *B*), in agreement with previous results in I-Eα^d transgenic C57BL/6 (B6.Eα^d) mice (14). Comparative measurements

[1] *Abbreviations used in this paper:* BMC, bone marrow cells; B6.Eα^d, I-Eα^d transgenic C57BL/6; gp70 IC, gp70-anti-gp70 immune complexes; HGG, human IgG.

Table 1. *BMC Chimeric Mice*

Group	BMC donor	Recipient
I*	BXSB.Igh^a male (Igh^a) + BXSB-E-1 male (Igh^b)	BXSB female
II	BXSB.Igh^a male (Igh^a) + BXSB male (Igh^b)	BXSB-E-1 female
III	BXSB.Igh^a male (Igh^a) + BXSB male (Igh^b)	BXSB female
IV	BXSB.Igh^a female (Igh^a) + BXSB-E-1 female (Igh^b)	BXSB female

* 2 mo after the reconstitution, the chimerism was checked by measuring serum levels of Ig allotype and by enumeration of IgM^a and IgM^b allotype-positive circulating B cells. These analyses showed that both populations of donor B cells were equally repopulated in recipient mice (data not shown).

of $E\alpha$ mRNA and β-actin mRNA levels, as determined by densitometric analysis on Northern blots, showed an ~20-fold-greater expression of $E\alpha$ mRNA in the spleen from the BXSB-E-1 mice than in BXSB.H-2$^{b/d}$ heterozygous mice.

Expression of the $E\alpha$ gene product was examined by the surface staining of spleen cells using an anti-IE mAb, Y-17, which recognizes combinations of Eα and Eβ molecules of various haplotypes, including Eα^dEβ^b (16). Spleen cells from the BXSB-E-1 mice expressed the I-E molecule exclusively on surface IgM$^+$ B cells at levels similar to those found on the spleen B cells of CBA/J (H-2^k, I-E$^+$) mice (Fig. 1 *C*). The transgenic mice contained, however, a significant percentage of I-E$^-$IgM$^+$ B cells (about 15–20% of splenic B cells), which expressed the I-A^b molecule at a density as high as that found on I-E$^+$IgM$^+$ B cells (data not shown). Such a disparate expression of I-E antigens among B cells has been previously noted in other $E\alpha^d$ transgenic mice generated using transgenes containing only a short 5′ flanking sequence of the $E\alpha^d$ gene (30). However, our $E\alpha^d$ transgene construct contains about 4 kb of 5′ flanking sequence, which is enough to allow an appropriate expression of $E\alpha^d$ mRNA and I-E α chain. In the spleens of B6.Eα^d transgenic mice generated using the same construct (14), no significant numbers of I-E$^-$IgM$^+$ B cells were found (Fig. 1 *C*). The presence of I-E$^-$IgM$^+$ B cells may thus be a peculiarity of the BXSB-E-1 mice or of mice with a lupus background. It is also possible that the I-E$^-$ and I-E$^+$ populations may represent two different subsets of B cells at different stages of activation or differentiation. In the transgenic mice, the I-E molecule was also present on a very small fraction of unstimulated monocytes, but on most IFN-γ–stimulated monocytes, as is also the case for normal I-E$^+$ mice (data not shown). Immunofluorescence staining with the Y-17 anti-I-E mAb of thymus cryosections of these transgenic mice showed typical confluent staining of the medulla and reticular staining of the cortex (data not shown).

Expression of the I-E antigen in these transgenic mice was accompanied by a decrease in the Vβ5$^+$ and Vβ11$^+$ T cells, which potentially contain anti-I-E autoreactive T cells. Vβ5$^+$ and Vβ11$^+$ represented 1.48 and 2.91% of Thy-1$^+$ cells from the BXSB-E-1 lymph nodes, as compared with 7.48 and 5.14%, respectively, in the BXSB nontransgenic I-E$^-$ littermates. In contrast, no differences were found in the percentages of Vβ6$^+$ and Vβ8.2$^+$ cells between the transgenic mice (10.55 and 12.45%, respectively) and their nontransgenic littermates (8.04 and 12.12%). The extent of this depletion of Vβ5$^+$ and Vβ11$^+$ T cells, presumably due to I-E expression in the transgenic mice, was comparable with that observed in I-E$^+$ BXSB H-2$^{b/d}$ heterozygous mice (7). The spleen cells of the BXSB-E-1 mice induced a significant proliferation of I-E$^-$ BXSB lymphocytes in MLC (data not shown).

The lupuslike autoimmune syndrome developing in the male nontransgenic I-E$^-$ littermates was dramatically prevented in the male BXSB-E-1 transgenic mice. Whereas 50% of the nontransgenic I-E male littermates had died of glomerulonephritis within the first 8 mo, with no survivors after 1 yr, none of the transgenic mice died within the first year (Fig. 2 *A*). Kidney histology at 8 mo showed in I-E$^-$ male mice a severe glomerulonephritis with increased mesangial and glomerular cellularity, obliteration of glomerular architecture, and tubular cast formation. In contrast, transgenic males exhibited minimal glomerular changes (Fig. 3). In correlation with the renal lesions, serum levels of total IgG, nephritogenic gp70 IC (6, 7, 31, 32) and IgG anti-DNA autoantibodies in the male transgenic mice at 6 mo were markedly lower than those of their male nontransgenic littermates ($p < 0.001$), and almost comparable with those of female BXSB mice (Fig. 2, *B–D*). Blood monocytosis, a unique abnormality characteristic of conventional BXSB male mice (33) was, however, unaltered in transgenic mice (percent Mac-1$^+$ PBMC at 8 mo of age [mean of 5 mice ± 1 SD]: transgenic males, 20.6 ± 2.6%; transgenic females, 6.6 ± 0.9%; nontransgenic males, 19.5 ± 5.8%; nontransgenic females, 6.2 ± 1.2%). This indicates that monocytosis in BXSB males is neither a cause nor a consequence of the autoimmune syndrome, but rather is somehow related to the nature of the *Yaa* mutation. This also suggests that the expression of the I-E α chain transgene prevents the development of autoimmune responses, rather than the acceleration effect mediated by the *Yaa* gene in BXSB mice. This is further supported by our recent observation that the lupuslike autoimmune syndrome developing in (NZB × BXSB-E-1)F$_1$ female mice in the absence of the *Yaa* gene is similarly inhibited by the presence of the $E\alpha^d$ transgene (Iwamoto, M., R. Merino, and S. Izui, unpublished observation).

The present results demonstrate that the expression of the I-E α chain transgene prevents the autoimmune syndrome

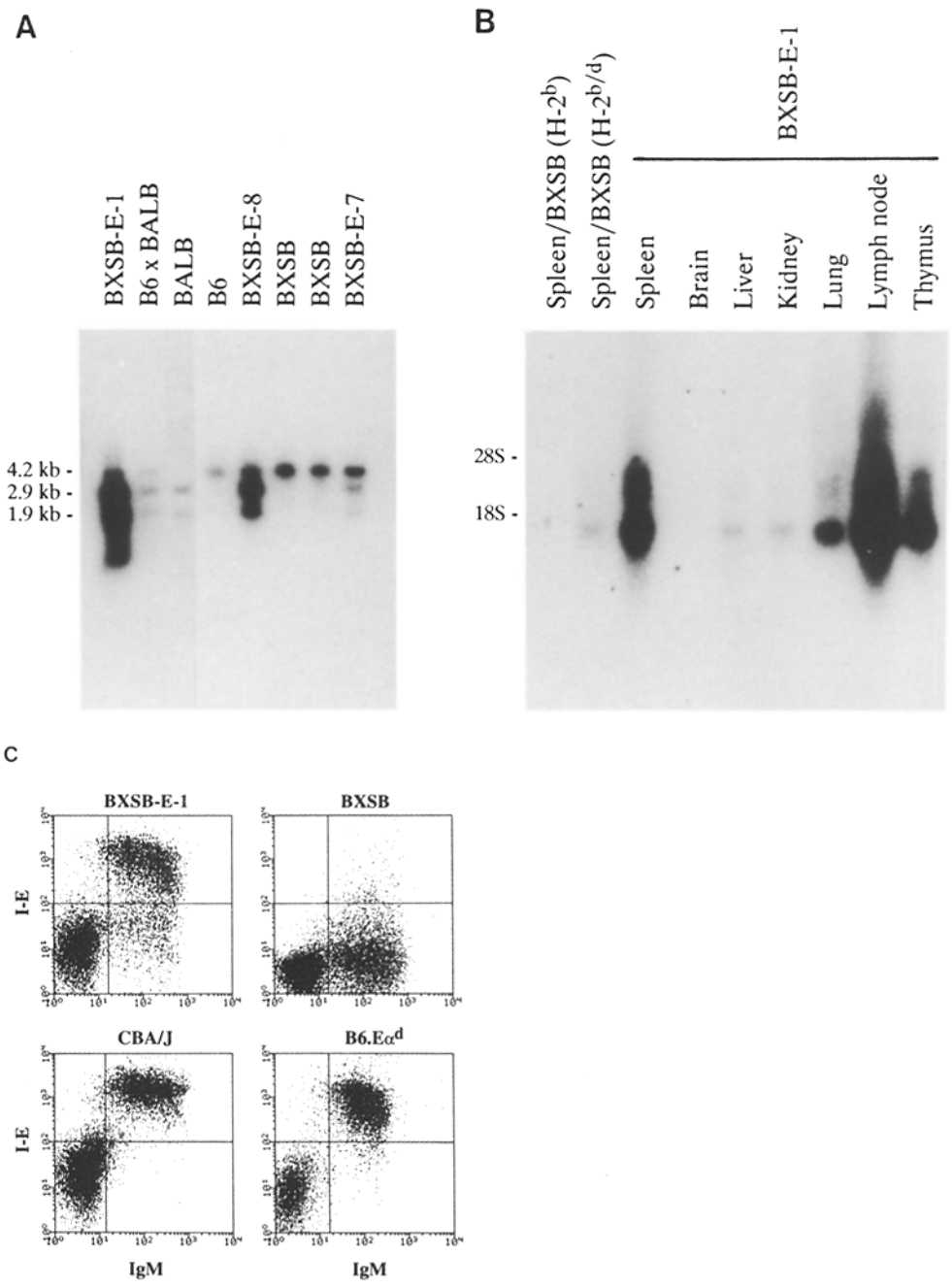

Figure 1. (*A*) Southern blot analysis of the *Eα* gene in transgenic (*BXSB-E-1*, *BXSB-E-7*, *BXSB-E-8*) or nontransgenic BXSB, C57BL/6 (*B6*) (*H-2b*), BALB/c (*H-2d*) and their F_1 hybrids (*B6 × BALB*) mice. The host BXSB mice carry a deletion in this region, as other H-2^b mice (8), and their SacI-digested DNA yielded a 4.2-kb band, larger than those (1.9 and 2.9 kb) obtained from BALB/c DNA. DNA from transgenic mice (*BXSB-E-1*, *BXSB-E-7*, *BXSB-E-8*) gave, in addition, the two 1.9- and 2.9-kb bands characteristic of the *Eαd* gene, indicating that these mice contain the injected BALB/c *Eαd* gene. (*B*) Northern blot analysis of *Eα* mRNA from various tissues of the BXSB-E-1 mice. As controls, RNA from I-E^+ BXSB.H-$2^{b/d}$ (7) and I-E^- BXSB (*H-2b*) spleens were also analyzed. Positions of 18S and 28S ribosomal RNA are indicated. (*C*) Expression of the I-E ($E\alpha^dE\beta^b$) antigen on spleen cells of the BXSB-E-1 mice. Spleen cells from 2-mo-old male transgenic and nontransgenic BXSB, CBA/J (*I-E+*) and B6.Eα^d transgenic (14) mice was first stained with FITC-conjugated anti-mouse μ chain mAb (LO-MM-9), and then incubated with biotinylated anti-I-E (Y-17) mAb, followed by PE-conjugated avidin.

in BXSB mice. This is reminiscent of the prevention of spontaneous autoimmune diabetes in NOD (I-E^-) mice by the expression of the I-E antigen through an I-E α chain transgene (10–13). However, NOD mice (H-2^{g7}) have a unique I-A^{NOD}, made of an I-Aα^d chain and of a distinct type of I-A β chain (34). The mechanisms by which I-E expression protects NOD mice are still unclear, and protection is also afforded by the expression of I-A^k (35, 36). It has been speculated that expression of another class II MHC molecule in the cells of NOD mice prevents the peculiar self-antigen–presenting property of I-A^{NOD}. In contrast, the I-A α and β chains of BXSB mice are apparently conventional H-2^b class II MHC molecules. Expression of I-E molecules at the level provided by the H-$2^{b/d}$ heterozygous state does not protect BXSB mice from their autoimmune syndrome (7), despite the fact that it is accompanied by a decrease in V$\beta5^+$ and V$\beta11^+$ T cells comparable with that observed in the BXSB-E-1 mice. This suggests that lack of the autoimmune syndrome in the BXSB-E-1 mice may be related to an unusually high level of I-E α chains, as suggested by the very high levels of corresponding mRNA detected (probably related to the large copy number of *Eαd* transgenes carried by these

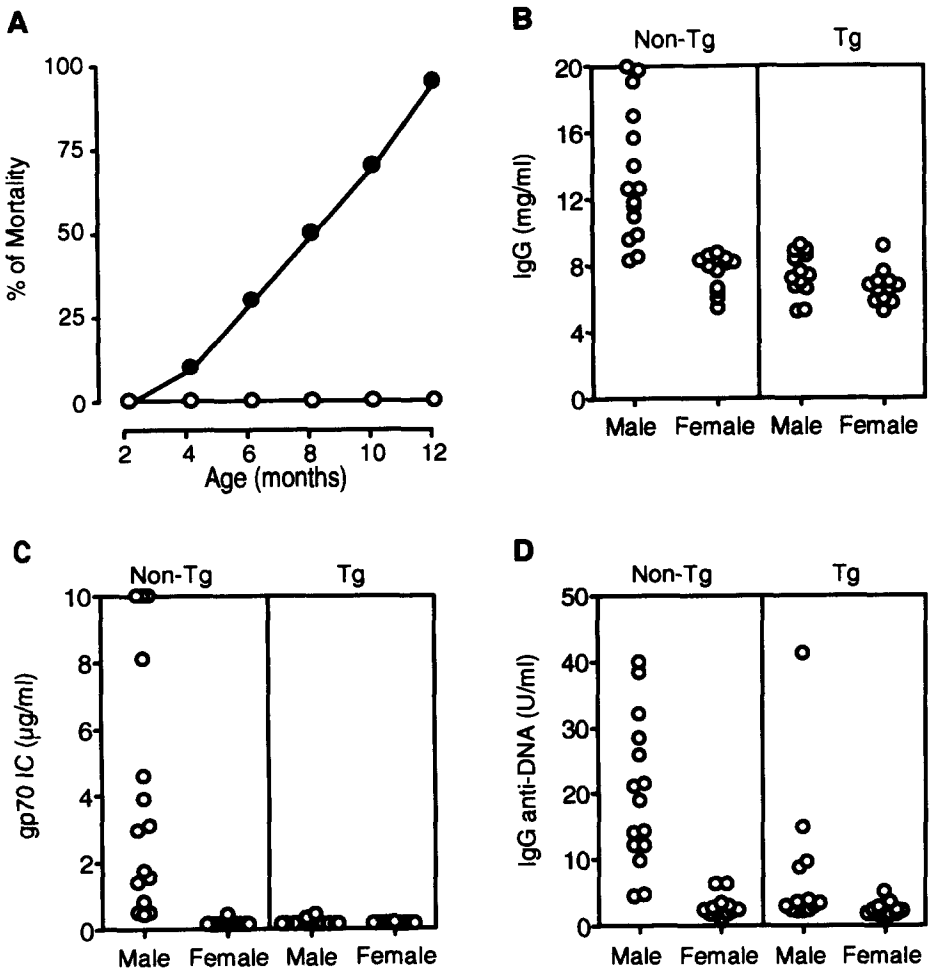

Figure 2. (*A*) Cumulative mortality with glomerulonephritis in male BXSB transgenic (O) and nontransgenic (●) littermates (15 animals from each group). (*B–D*) Serum levels of total IgG, gp70 IC, and IgG anti-DNA autoantibodies in 6-mo-old BXSB transgenic and nontransgenic male and female mice.

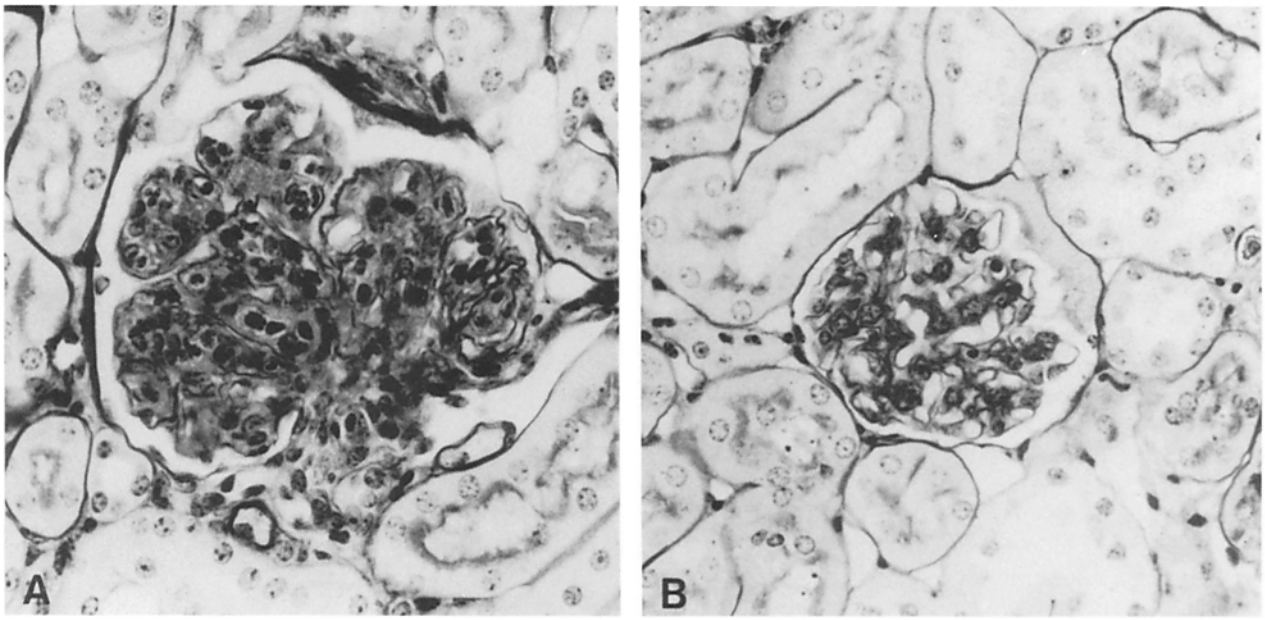

Figure 3. (*A*) Representative histological appearance of glomerular lesions of kidneys from 8-mo-old BXSB nontransgenic male littermates showing increased glomerular cellularities and obliteration of the glomerular architecture. (*B*) Representative histological appearance of glomeruli from 8-mo-old BXSB transgenic males exhibiting minimal glomerular changes. Note a marked difference in the size of glomeruli between BXSB transgenic and nontransgenic littermates. The tissues were stained with periodic acid-Schiff reagent (×200).

mice). Preliminary findings indicate that the second transgenic line, BXSB-E-2, which also carries a high copy number of $E\alpha^d$ transgenes and expresses $E\alpha$ mRNA at a level similar to the BXSB-E-1 mice, fails to develop the autoimmune syndrome. An immune deficiency syndrome has been observed in mice bearing a high copy number (>50) of the $A\beta^k$ transgene (37). It should be emphasized here that the BXSB-E-1 mice did not show any feature of immune deficiency, had an IgG level in male transgenic mice comparable with that found in nontransgenic BXSB female mice (Fig. 2 *B*), and displayed a normal immune response against the T cell–dependent antigen HGG (serum IgG anti-HGG levels 14 d after aggregated HGG injection: BXSB-E-1, 528 ± 154 U/ml; BXSB, 555 ± 215 U/ml; IgG anti-HGG levels before immunization were <5 U/ml).

Overexpression of I-E α chain in these transgenic mice appears to lead to an increased formation of peptides derived from this chain which are presented in the groove of I-A^b molecules. In mice bearing I-A^b and I-E molecules, it has been shown that one of the major self-peptides presented by the I-A^b molecules is derived from the I-E α chain. It appears that the I-A^b–I-Eα peptide complexes are recognized by the Y-*Ae* mAb (22, 23). When this mAb was used to stain spleen cells of male mice derived from a cross between the BXSB-E-1 and BXSB.H-2^d mice, surface IgM^+ B cells of mice bearing the $E\alpha^d$ transgene expressed a higher density of the Y-A*e* epitope than similar cells from nontransgenic littermates. This was not due to a difference in density of the I-A^b molecule on these cells, since B cells from both types of mice expressed identical I-A^b density (Fig. 4). The likeliest interpretation of this observation is that on splenic B cells of mice bearing the transgene, an increased fraction of the I-A^b molecules contain in their groove the I-E α chain–derived peptide.

To explore whether an increased presentation of I-E α chain peptides by the I-A^b molecule might prevent excessive activation of the B cells bearing these peptides, and thus explain the lack of autoimmune disease in the BXSB-E-1 mice, splenic $I\text{-}E^+IgM^+$ and $I\text{-}E^-IgM^+$ B cells isolated from 1 yr-old BXSB-E-1 male mice were fractionated and compared for their spontaneous Ig production. As shown in Fig. 5 *A*, $I\text{-}E^-$ B cells secreted four to eight times more IgM and IgG than $I\text{-}E^+$ B cells during a 24-h culture, suggesting that $I\text{-}E^-$ B cells were selectively activated in vivo in the BXSB-E-1 mice. The smaller spontaneous Ig production of $I\text{-}E^+$ B cells was not due to a general functional deficiency of these cells, since after stimulation with bacterial LPS, they were capable of producing IgM Abs at an even higher level than $I\text{-}E^-$ B cells (Fig. 5 *B*). The relatively lower stimulation of $I\text{-}E^-$ B cells in vitro by LPS is consistent with the notion that these latter cells are selectively activated in vivo, as is the case for B cells derived from diseased lupus-prone mice (38).

It may be that a mechanism operates in transgenic mice overexpressing I-E α chains which inhibits the activation of B cells by some other indirect way. To explore this possibility, two types of chimeras were constructed, using nontransgenic or transgenic BXSB female mice lethally irradiated and reconstituted with BMC from transgenic and/or

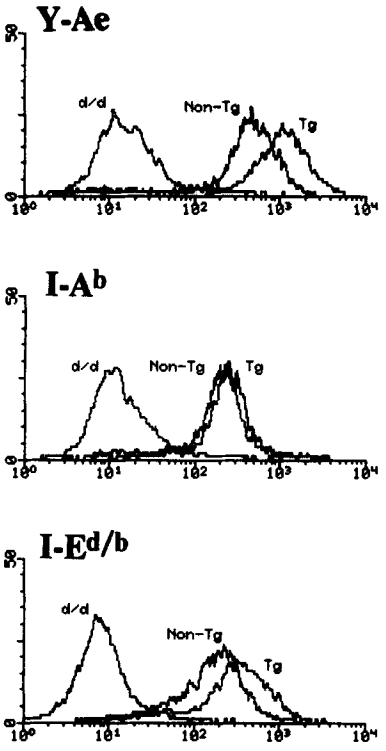

Figure 4. Increased surface expression of the Y-A*e* epitope on surface IgM^+ B cells expressing the $E\alpha^d$ transgene in BXSB mice. Spleen cells from 2-mo-old transgenic and nontransgenic BXSB male mice of H-$2^{b/d}$ haplotype, obtained from a cross between the BXSB-E-1 and BXSB.H-2^d (7) mice, were first stained with FITC-conjugated anti-mouse μ chain mAb (LO-MM-9), and then incubated with biotinylated Y-A*e*, Y-3P (anti-I-A^b), or Y-17 (anti-I-$E^{d/b}$) mAb, followed by PE-conjugated avidin. As controls, spleen cells from BXSB.H-2^d male mice were stained with Y-A*e*, Y-3P, or Y-17 mAb.

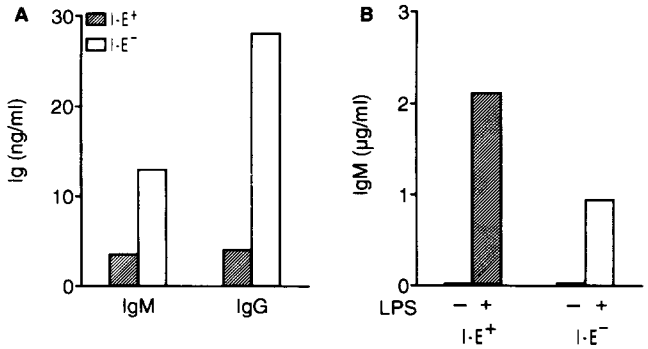

Figure 5. In vitro spontaneous IgM and IgG secretion (*A*), and LPS-stimulated IgM production (*B*) by $I\text{-}E^+IgM^+$ and $I\text{-}E^-IgM^+$ B cells isolated from the BXSB-E-1 mice. For the spontaneous IgM and IgG secretion, 10^5 $I\text{-}E^+IgM^+$ or $I\text{-}E^-IgM^+$ B cells sorted from spleen cells by a FACStar® were cultured for 24 h at 37°C in a humidified incubator containing 5% CO_2 in air. For the LPS-induced IgM production, 3×10^4 $I\text{-}E^+IgM^+$ or $I\text{-}E^-IgM^+$ B cells were stimulated with LPS for 5 d. IgM and IgG concentrations in supernatants were determined by ELISA. Representative results of three separate experiments are shown.

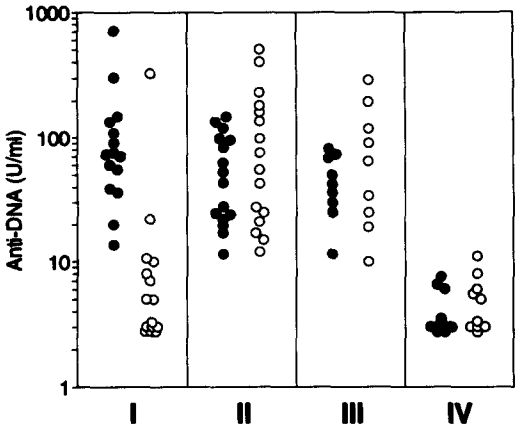

Figure 6. Serum levels of Igh^a (●) and Igh^b (○) anti-DNA Abs in four different groups of BMC chimeras (4-mo after reconstitution). Results are expressed in U/ml. The chimeras are those listed in Table 1.

nontransgenic BXSB male mice (Table 1). In these chimeras, the origin of B cells could be recognized because they bear different Igh allotypes. First, irradiated nontransgenic BXSB female mice were reconstituted with a mixture of BMC from nontransgenic (Igh^a) and transgenic (Igh^b) male BXSB mice (group I). These mice developed lethal glomerulonephritis by 8 mo after the reconstitution as did BMC chimeras not involving transgenic bone marrow, i.e., female BXSB mice reconstituted with nontransgenic male BMC (group III). Significantly, Igh allotype analysis of the anti-DNA Abs revealed that they all originated from the nontransgenic male bone marrow population (Fig. 6). Second, irradiated BXSB transgenic female mice were reconstituted with a mixture of Igh^a and Igh^b BMC of nontransgenic males (group II). These chimeras had a glomerular disease of the same severity, and comparable levels of anti-DNA Abs bearing in this condition the two Ig allotypes. It should be noted that chimeras reconstituted with a mixture of BMC from transgenic and nontransgenic female mice (group IV) failed to develop a comparable autoimmune syndrome.

All this shows that two explanations for the prevention of the autoimmune syndrome in the transgenic mice can be ruled out: I-E expression by cells of the transgenic animal or resulting from the graft of transgenic BMC did not lead to the generation of CD8$^+$ regulatory T cells "vetoing" the activation of autoreactive cells or to any form of clonal deletion of potential autoreactive T cells. It should be empha-

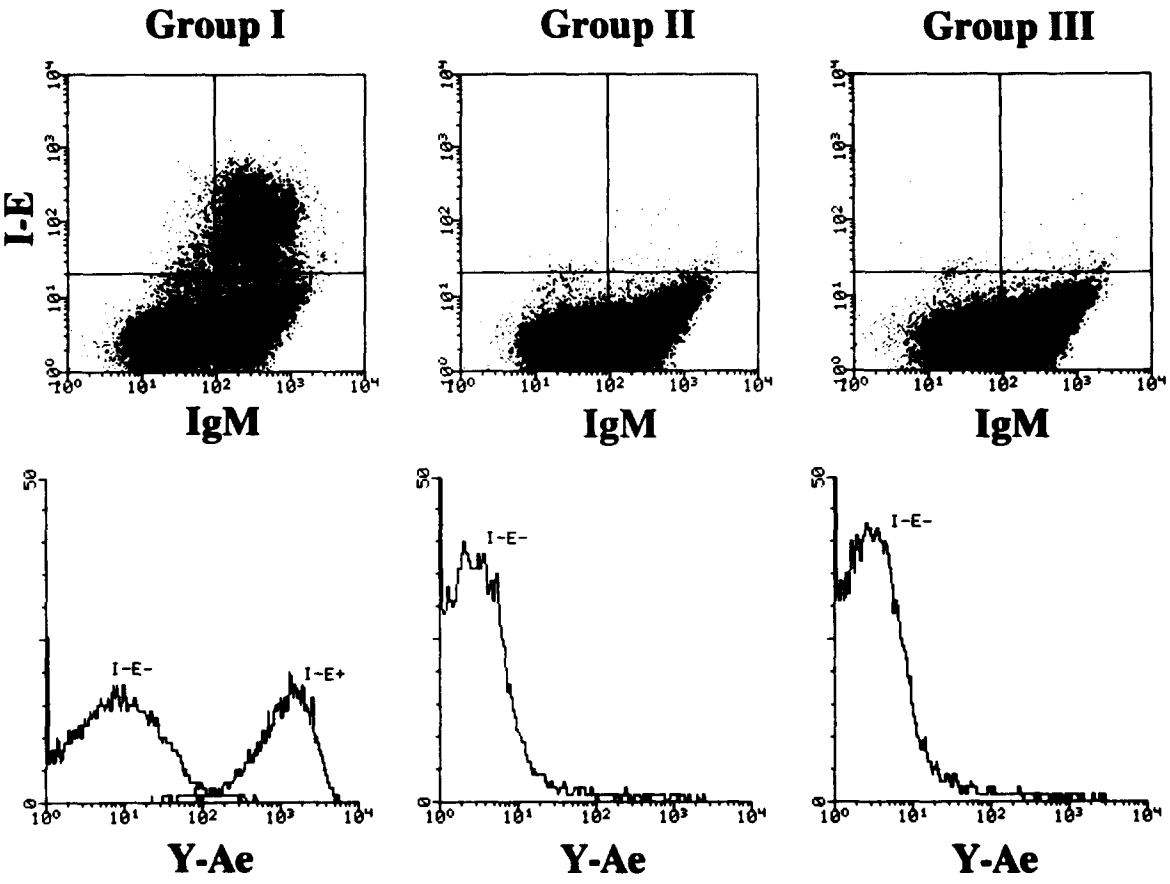

Figure 7. Surface expression of the Y-A*e* epitope on I-E$^+$IgM$^+$ B cells from BMC chimeric mice. 2 mo after the reconstitution, spleen cells from three groups of BMC chimeras were first stained with FITC-labeled Y-17 (anti-I-E) mAb, then with PE-conjugated goat anti-mouse μ chain Abs, and finally with biotinylated Y-A*e* mAb, followed by Streptavidin-RED670™. (*Top*) Surface I-E and IgM stainings in spleen cells. (*Bottom*) Expression of the Y-A*e* epitope in the I-E$^+$IgM$^+$ and I-E$^-$IgM$^+$ B cells. Note the selective expression of the Y-A*e* epitope in the I-E$^+$IgM$^+$ B cell population in mice of group I.

sized that the presentation of I-E α chain peptides by the I-A^b molecule, as determined by the Y-A*e* staining, was limited only to I-E^+ B cells derived from the transgenic bone marrow population in transgenic and nontransgenic double BMC chimeras (group I; Fig. 7), and that these B cells produced far less anti-DNA autoantibodies than I-E^- B cells in these chimeras (group I; Fig. 6). These results are entirely consistent with the hypothesis that a lower activation of B cells bearing an I-E α chain peptide in their I-A^b molecule is the mechanism preventing autoimmunity in the transgenic mice.

Our results suggest a novel mechanism explaining how the expression of a transgene encoding the I-E α chain prevents autoimmune diseases. Since I-E α chain–derived peptides apparently have a high affinity for I-A molecules other than the I-A^b (39), this mechanism might also be responsible for the protection against the development of autoimmune diabetes by the expression of an $E\alpha^d$ transgene in NOD mice (10–13). Further understanding of the protective mechanism(s) conferred by the $E\alpha^d$ transgene may elucidate the molecular and cellular bases central to the development of murine SLE and possibly of autoimmune diabetes. These mechanisms could have clinical implications in the design of future therapeutic strategies with self-peptides in several autoimmune disorders.

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Address correspondence to Dr. Shozo Izui, Department of Pathology, Centre Médical Universitaire, University of Geneva, 1, rue Michel Servet, 1211 Geneva 4, Switzerland.

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