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The Role of $\alpha\beta^+$ T Cells and Homeostatic T Cell Proliferation in Y-Chromosome-Associated Murine Lupus[1]

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Male BXSB mice develop an early life, severe lupus-like disease largely attributed to an undefined Y-chromosome-associated autoimmunity accelerator, termed *Yaa*. Although the exact disease pathogenesis is uncertain, indirect evidence suggests that T cells play an important role in the male BXSB disease. We have developed TCR α-chain gene-deleted BXSB mice to directly examine the role of $\alpha\beta^+$ T cells and the mode by which *Yaa* promotes disease in this strain. All disease parameters, including hypergammaglobulinemia, autoantibody production, glomerulonephritis, and the unique monocytosis of BXSB males, were severely reduced or absent in the $\alpha\beta^+$ T cell-deficient mice. Adoptively transferred CD4$^+$ T cells of either male or female BXSB origin showed equal homeostatic proliferation in $\alpha\beta^+$ T cell-deficient male recipients. Moreover, deficient male mice eventually developed equally severe lupus-like disease after adoptive transfer and homeostatic expansion of T cells from wild-type BXSB males or females. The results directly demonstrate that the *Yaa*-mediated disease requires $\alpha\beta^+$ T cells that are not, in themselves, abnormal in either composition or properties, but are engaged by a *Yaa*-encoded abnormality in a non-T cell component. In addition, homeostatic anti-self proliferation of mature T cells derived from a small number of precursors can induce systemic autoimmunity in an appropriate background. ***The Journal of Immunology,*** **2001, 167: 2354–2360.**

The primary role of T cells in several spontaneous and induced models of autoimmune syndromes, including lupus, has been clearly demonstrated (reviewed in Ref. 1). With regard to spontaneous models of lupus, the role of T cells in disease pathogenesis has been directly documented in the MRL-*Fas*^{lpr} strain. In initial studies, deletion of the TCRα gene, and thus of $\alpha\beta^+$-expressing T cells, led to a significant, but incomplete, disease amelioration (2). However, disease was almost completely eliminated upon deletion of both TCRα and -δ genes, and thus of $\alpha\beta^+$ and $\gamma\delta^+$ T cells (3). For two other spontaneous mouse models of lupus, (NZB $\times$ NZW)F$_1$ and BXSB, studies have only indirectly implicated a role for T cells in disease pathogenesis. In (NZB $\times$ NZW)F$_1$ mice, treatment with Abs to Thy1.2 (4), CD4 (5), MHC class II (6), and B7 (7) or treatment with CTLA4Ig alone (8) or in combination with anti-CD40 ligand (9) resulted in significant disease reduction. Initial efforts with anti-Thy1.2 Ab treatment of BXSB mice were confounded by an unexplained anaphylactic reaction (4), but subsequent experiments with anti-CD4 Ab (10) or CTLA4Ig (11) also reduced disease incidence and severity.

Despite many common characteristics, the three major mouse models of lupus exhibit unique histologic and serologic manifestations as well as unique disease accelerators. These accelerators include female hormones in New Zealand mice, the *Fas*^{lpr} mutation in MRL-*Fas*^{lpr} mice, and a Y-chromosome-associated accelerator of autoimmunity, termed *Yaa*,[3] in BXSB mice that remains to be identified (reviewed in Ref. 1). Radiation bone marrow chimera experiments with T and B cells of *Yaa* and non-*Yaa* origins showed that the determining factor for autoantibody production in BXSB mice was the presence of the *Yaa* gene in B, but not T cells (12, 13). Other studies have also demonstrated that *Yaa* primarily increases immune responses to weakly immunogenic Ags (14). Therefore, it was hypothesized that *Yaa* promotes autoimmunity by enhancing Ag presentation (for example, through increased peptide presentation or increased costimulation), which then facilitates the engagement of otherwise quiescent, low-affinity, self-reactive T cells (12, 13). Further evidence for MHC-dependent T cell engagement in the BXSB disease has been suggested by the inhibitory effects of high I-Eα transgenic expression in this H-2^b mouse (15). This effect appears to be due to competitive inhibition of autoantigen presentation by peptide fragments from processed I-Eα bound effectively to H-2^b class II MHC molecules.

We initially generated congenic TCR$\alpha^{-/-}$ BXSB mice to directly determine whether $\alpha\beta^+$ T cells are required in the male BXSB disease and found that such mice were indeed free of disease. We then performed adoptive transfers of small numbers of wild-type (WT) *Yaa*$^+$ or *Yaa*$^-$ mature CD3$^+$ T cells to determine whether proliferation of these cells in the lymphopenic *Yaa*$^+$ hosts were equally capable of inducing the full disease spectrum and found that this was the case. Hence, the *Yaa* gene defect does not modify thymic selection or properties of T cells, but, rather, induces their excessive activation through a non-T cell component.

Materials and Methods

Mice

129/Sv $\times$ C57BL/6 TCR$\alpha^{-/-}$ mice were obtained from M. J. Owen (Imperial Cancer Research Fund, London, U.K.). BXSB TCR$\alpha^{-/-}$ mice were generated by six backcrosses of the 129 $\times$ C57BL/6 TCR$\alpha^{-/-}$ mice

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[3] Abbreviations used in this paper: *Yaa*, Y chromosome-associated accelerator; GN, glomerulonephritis; LN, lymph node; WT, wild type.

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to the BXSB strain, followed by final intercrossing of heterozygous BXSB TCR$\alpha^{+/-}$ offspring. Control TCR$\alpha^{+/+}$ and homozygously deleted TCR$\alpha^{-/-}$ male BXSB littermates were analyzed in this study. Genotyping for the TCRα deletion was performed by PCR detection of the neo gene, followed by confirmation of the homozygous deletion with anti-CD3 staining and FACS analysis (16). Mice were maintained under specific pathogen-free conditions, and procedures were performed according to guidelines of the institutional animal research committee.

Pathology

Mutant and control littermates were bled bimonthly and followed for survival until the termination of the experiment. Blood urea nitrogen was measured using AZOSTIX strips (Bayer, Elkhart, IN) according to the manufacturer and was graded on a 1–4 scale (5–90 mg/dl). Histologic examination of periodic-acid Schiff-stained kidneys was performed in a blind manner at 5 mo of age, and the severity of glomerulonephritis (GN) was defined on a scale of 0–4$^+$ (16). OCT (Miles, Elkhart, IN)-embedded snap-frozen kidneys were thin sectioned, air dried, fixed in ice-cold acetone, and blocked with 10% horse serum in PBS. Sections were then incubated with anti-IgG-FITC (Vector Laboratories), and deposit intensity was scored as previously described (17).

Serologic analysis

IgG and autoantibody levels were detected by ELISA as previously described (17). Briefly, IgG in serial dilutions of sera was captured on 96-well plates coated with either the Fc-specific F(ab′)$_2$ of goat anti-mouse IgG (5 μg/ml; Jackson ImmunoResearch, West Grove, PA) or mouse chromatin (3.5 μg/ml). Bound IgG subclasses were measured using alkaline phosphatase-conjugated goat anti-mouse IgG subclass-specific Abs (Caltag, Burlingame, CA). Standard curves for each subclass were generated using calibrated mouse serum (Binding Site, Birmingham, U.K.).

FACS analysis

Splenocytes and PBMC were stained with various combinations of Abs to TCR β-chain, CD3, CD4, CD8, CD19, CD44, and/or CD11b (Mac-1$^+$; BD PharMingen, La Jolla, CA). FACS data were acquired (>10,000 events) on a FACSort and were analyzed with CellQuest analysis software (BD Biosciences, Mountain View, CA).

Adoptive transfers

To assess in vivo proliferative responses, two groups (nine mice per group) of $\alpha\beta^+$ T cell-deficient young male BXSB mice were injected i.v. with 2×10^6 highly purified (95.5% purity) CD4$^+$ lymph node (LN) cells from either male or female young BXSB mice (18) that were stained with the intracellular fluorescent dye CFSE (Molecular Probes, Eugene, OR), as previously described (19). Unlabeled donor T cells were analyzed by flow cytometry before injection. Both male and female T cells were <15% CD44^{high}, <15% CD69$^+$, <10% CD25$^+$, and >85% CD45RB^{high}, a phenotype typical of T cells from young unmanipulated BXSB mice. Three mice from each group were sacrificed at 2, 5, and 21 days after transfer, and patterns of cell division as well as total donor T cells (Thy 1.2$^+$, CD4$^+$) in LN (axillary, inguinal, cervical, mesenteric) and spleen were determined by FACS.

To assess disease induction, young (2-mo-old) $\alpha\beta^+$ T cell-deficient male BXSB mice were transfused with $4–5 \times 10^6$ FACS-sorted CD3$^+$ LN cells from 2-mo-old BXSB WT male or female donors (10–12 mice/group) and followed for up to 9 mo of age, and survivors were sacrificed for serologic, cellular, and histologic assessments. A cohort of 20 control WT male BXSB mice was followed in parallel for survival, and an additional 6 were sacrificed at 4 mo for serologic, cellular, and histologic comparisons.

Statistics

Student's *t* test was used for group mean comparisons, and survival was analyzed by the Kaplan-Meier method with comparisons by a log rank test. A value of $p < 0.05$ was considered to be significant.

Results

Survival characteristics

To determine to what extent $\alpha\beta^+$ T cells are required for the development of lupus-like disease in the BXSB model, BXSB mice deficient in the TCR α-chain were generated, and the severity of autoimmune manifestations in male (*Yaa*$^+$) TCR$\alpha^{-/-}$ and littermate TCR$\alpha^{+/+}$ controls was compared. The absence of detectable

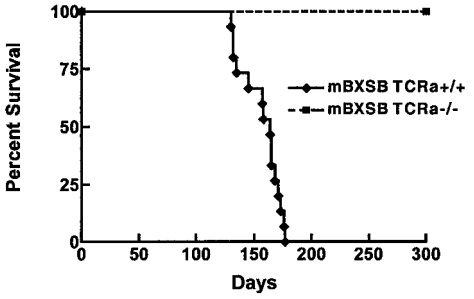

FIGURE 1. Cumulative survival rates for littermate TCR$\alpha^{-/-}$ and TCR$\alpha^{+/+}$ male BXSB mice (20 mice/group) followed for 9 mo.

$\alpha\beta^+$ T cells in the TCR$\alpha^{-/-}$ mice was confirmed by flow cytometry using Abs to the TCR β-chain. Mice were followed for survival for 9 mo, well beyond the life expectancy of BXSB males (1). Strikingly, the lack of TCR$\alpha\beta^+$ cells resulted in a dramatic increase in survival (Fig. 1). Control BXSB males exhibited 50% mortality at 5.3 mo and 100% mortality by 6.5 mo, while BXSB TCR$\alpha^{-/-}$ male mice were all alive at 9 mo ($p < 0.0001$). Notably, the mortality rate of the littermate controls was similar to that of our WT BXSB colony, indicating that the congenic line contained the major BXSB lupus susceptibility genes.

Serologic characteristics

Serum polyclonal IgG levels were determined in TCR$\alpha^{-/-}$ and TCR$\alpha^{+/+}$ littermates at 5 mo of age. Although the controls exhibited typical hypergammaglobulinemia, all IgG subclasses were significantly lower in TCR$\alpha^{-/-}$ mice ($p < 0.001$), resulting in a >9-fold reduction of total serum IgG (Fig. 2*A*). Nonetheless, the IgG levels and subclass distributions in these mutant mice were

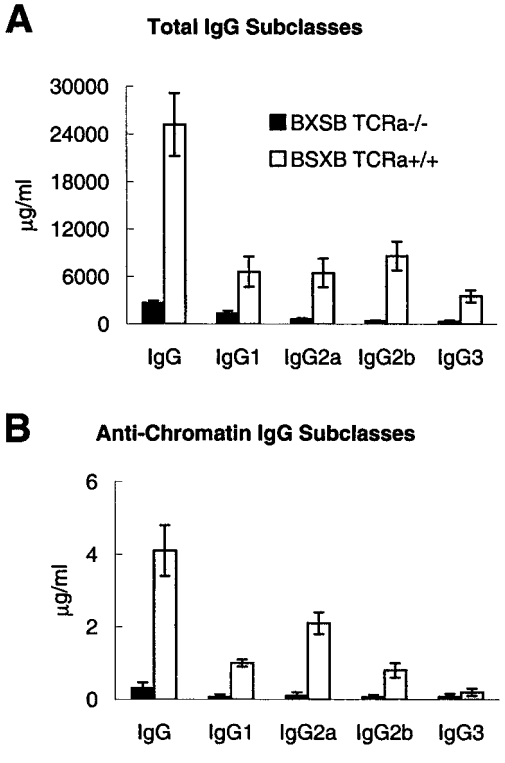

FIGURE 2. Polyclonal (*A*) and antichromatin (*B*) IgG subclass levels in 5-mo-old TCR$\alpha^{-/-}$ and TCR$\alpha^{+/+}$ littermate male BXSB mice. Data are the mean ± SEM of six to eight mice per group. Total polyclonal and antichromatin IgG are the sum of the four subclasses.

Table I. *Lymphoid organ weights and cellular subsets of BXSB TCR$\alpha^{-/-}$ mice*[a]

TCRα	Spleen Weight	LN Weight	CD4$^+$ Subset		CD8$^+$ Subset		CD19$^+$	PBMC Mac-1$^+$
			Total	CD44^{high}	Total	CD44^{high}		
−/−	0.2 ± 0.5*	0.04 ± 0.01*	1.2 ± 0.05		0.8 ± 0.01		85.5 ± 0.9*	8.9 ± 0.1*
+/+	0.4 ± 0.05	0.4 ± 0.02	17.7 ± 1.5	84.9 ± 2.7	2.3 ± 0.3	81.2 ± 2.7	74.9 ± 1.4	21.2 ± 3.2

[a] CD4$^+$, CD8$^+$, and CD19$^+$ are expressed as percentage of total splenocytes, CD44^{high} as percentage of splenic CD4$^+$ or CD8$^+$ cells, and Mac-1$^+$ as percentage of PBMC. LN (axillary, inguinal, cervical, and mesenteric) and spleen weights are in grams. Values are means ± SEM of six to eight mice per group at 5 mo.
*, $p < 0.05$ between knockout and WT mice.

similar to those commonly found in normal genetic background mice, an observation consistent with reports indicating considerable class switching in the absence of $\alpha\beta^+$ T cells (20). Our previous studies have shown that the autoantibody response to native chromatin in lupus-predisposed strains, including BXSB, generally preceded the appearance of autoantibodies against chromatin subcomponents, such as dsDNA and histones (21). To evaluate disease progression in the groups studied, we determined antichromatin titers at 5 mo of age and detected high levels, predominantly of the IgG2a subclass, in TCR$\alpha^{+/+}$ littermates, while such autoantibodies were virtually absent in the deficient mice (Fig. 2*B*).

Lymphoid cell assessment

Weights and cellular composition of spleen and lymph nodes were also analyzed (Table I). As expected, there was a significant reduction in the weights of both organs in the TCR$\alpha^{-/-}$ mice compared with controls ($p < 0.05$) with, on the average, a 10-fold decrease in LN and a 2-fold decrease in spleens. Similarly, deficient mice were devoid of T cells, while, as previously reported (22, 23), the controls showed a large proportion of CD44^{high} activated/memory phenotype T cells (Table I). In TCR$\alpha^{-/-}$ mice, however, there was an incremental increase in the frequency of CD19$^+$ B cells, probably due to the absence of $\alpha\beta^+$ T cells.

Peripheral blood monocytosis

Previous studies have shown the accumulation of a peculiar Mac-1$^+$ MHC class II$^-$ monocyte population in the peripheral blood of male, but not female, BXSB mice (24). To determine whether this monocytosis is T dependent, we analyzed Mac-1$^+$ expression in PBMC of 5-mo-old mice (Table I). Although control TCR$\alpha^{+/+}$ mice exhibited the typical expansions of Mac-1$^+$-expressing cells (21.2%), this population was significantly reduced (8.9%) in TCR$\alpha^{-/-}$ mice ($p < 0.01$). The reduction in absolute numbers of these cells is probably more severe than the indicated drop in percentage suggests, since $\alpha\beta^+$ T cells are not present in the deficient mice.

Renal histology

Kidney weights, GN scores, and blood urea nitrogen levels were reduced ($p < 0.05$) in 5-mo-old BXSB TCR$\alpha^{-/-}$ mice compared with TCR$\alpha^{+/+}$ controls (Table II). Control BXSB male mice exhibited typical glomerular pathology, including extended glomeruli, sclerosis of glomerular capillary walls, and heavy periodic acid-Schiff-positive material in the mesangial matrix (Fig. 3). Hypercellularity of the mesangium and the presence of both mononuclear and polymorphonuclear cells were also noted in the control mice, as were heavy glomerular IgG immune deposits in the mesangium and capillary walls. All these parameters were considerably reduced in TCR$\alpha^{-/-}$ mice (Fig. 3).

Homeostatic T cell proliferation

Several recent studies have shown that small numbers of T cells transferred into lymphopenic hosts proliferate extensively to reconstitute the original lymphocyte pool (reviewed in Refs. 25–27). This proliferation appears to be mediated by recognition of self-MHC/peptide ligands. Therefore, male mice deficient in $\alpha\beta^+$ T cells, and thus lymphopenic, were adoptively transferred with small numbers of CFSE-stained CD4$^+$ LN cells from WT male and female donors to determine the degree of homeostatic proliferation. In agreement with previous studies (18), no proliferation was detected on day 2 after transfer, since CFSE-stained cells were confined to a single peak on the far right of the histogram (data not shown). Thereafter, both WT male and female CD4$^+$ T cells transferred into $\alpha\beta^+$ T cell-deficient male mice proliferated extensively and equally, thereby leading to reduced CFSE intensity in 87–89% of donor cells on day 5 and 96% on day 21 after transfer (Fig. 4*A*). Consequently, enumeration of donor T cells (Thy 1.2$^+$, CD4$^+$) at these two time points showed a nearly identical recovery regardless of donor gender (Fig. 4*B*).

Adoptive transfers of mature T cells

Since $\alpha\beta^+$ T cell-deficient mice were free of disease and mature T cells transferred into lymphopenic hosts homeostatically expanded, we performed adoptive transfers of small numbers ($4–5 \times 10^6$) of WT male or female CD3$^+$ LN cells into male $\alpha\beta^+$ T cell-deficient mice to determine whether T cell origin affects disease reconstitution in the lymphopenic male BXSB background.

Male recipients of either male or female T cells exhibited equal mortality rates, with ~50% of the animals dead at 9 mo of age, or 7 mo after transfer, the latest point of observation (Fig. 5). This mortality rate is delayed ~2 mo compared with an unmanipulated cohort of control WT male BXSB mice, which may be explained by the time required for the small number of transfused cells to expand sufficiently. Serologic analyses showed that increases in

Table II. *Kidney disease in BXSB TCR$\alpha^{-/-}$ mice*[a]

TCRα	Kidney Weight	GN Score	Blood Urea Nitrogen	Immune Complex Deposit Score
−/−	0.2 ± 0.1*	2.0 ± 0.1*	1.5 ± 0.3*	1.3 ± 0.3*
+/+	0.35 ± 0.05	3.5 ± 0.3	3.2 ± 0.3	3.5 ± 0.4

[a] Kidney weights (grams) and severity of GN, blood urea nitrogen, and immune complex IgG deposits graded on a 0–4 scale. Mean ± SEM of six to eight mice per group at 5 mo.
*, $p < 0.05$ between deleted and WT mice.

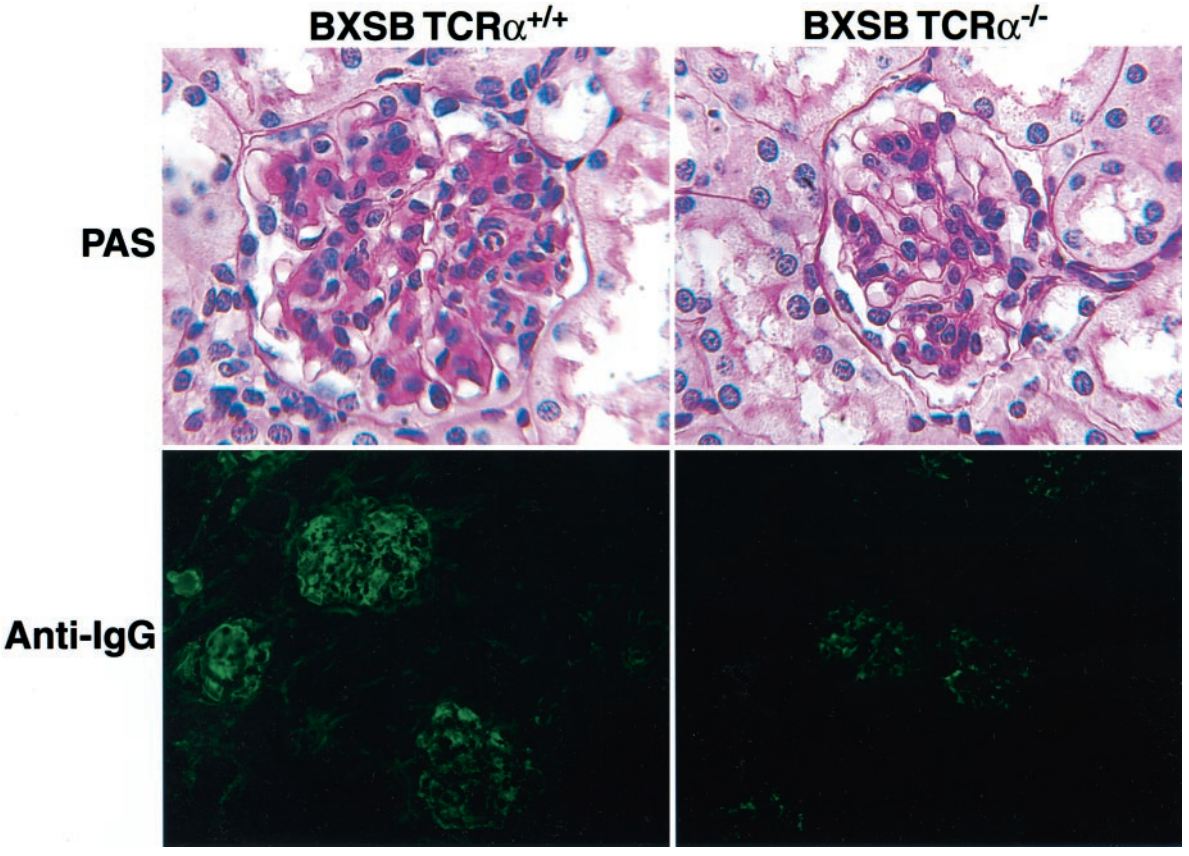

FIGURE 3. Glomerular pathology and immune complex IgG deposits in representative littermate 5-mo-old TCR$\alpha^{-/-}$ and TCR$\alpha^{+/+}$ male BXSB mice. *Upper panels*, Periodic acid-Schiff-stained sections of paraffin-embedded tissues (original magnification, ×64). There is substantially less cellular proliferation and periodic acid-Schiff-staining deposits in the TCR$\alpha^{-/-}$ glomeruli. Polymorphonuclear cells, which are unique to the glomerular lesions of the male BXSB mouse, can be readily seen. *Lower panels*, Direct IgG immunofluorescence of kidney sections (original magnification, ×32 at equal exposure).

polyclonal IgG (Fig. 6*A*) and antichromatin (Fig. 6*B*) subclasses of male $\alpha\beta^+$ T cell-deficient recipients of either male or female WT T cells were equal and approximated those of the unmanipulated WT mice. Similarly, spleen and LN weights and cellular compositions, including high percentages of activated/memory phenotype CD4$^+$CD44^{high} and CD8$^+$CD44^{high} cells were equal in the three groups, as was the frequency of Mac-1$^+$ monocytes in peripheral blood (Table III). Finally, blood urea nitrogen levels, GN severity, and intensity of IgG kidney deposits were also very similar between the T cell-reconstituted and control WT mice regardless of the gender origin of T cells (Table IV). Overall, the results indicate that homeostatically proliferating mature T cells of male or female BXSB origin are equally capable of inducing an early life, lupus disease in the $\alpha\beta^+$ T cell-deficient male BXSB background.

Discussion

We have conclusively demonstrated, through analysis of congenic TCRα-deleted BXSB mice, that $\alpha\beta^+$ T cells are necessary for lupus expression in this strain. The $\alpha\beta^+$ T cell-deficient male mice showed no mortality after 9 mo and had severely reduced serum IgG and antichromatin autoantibody levels, decreased peripheral blood monocytosis, and minor grades of GN. The full spectrum of

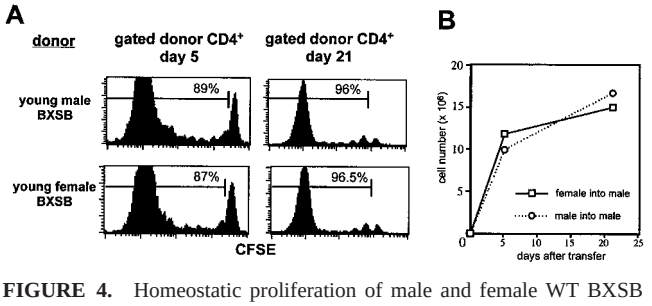

FIGURE 4. Homeostatic proliferation of male and female WT BXSB CD4$^+$ T cells transferred into male BXSB TCR$\alpha^{-/-}$ recipients. *A*, Cell division analyzed by FACS on gated CFSE-stained CD4$^+$Thy1.2$^+$ T cells on days 5 and 21. The large population on the *left* represents donor cells that have undergone seven, eight, or more divisions and are, therefore, CFSE negative. *B*, Numbers of CD4$^+$ donor T cells recovered from recipient's LN and spleens. Data are from three mice per group at each time point and are representative of two separate experiments.

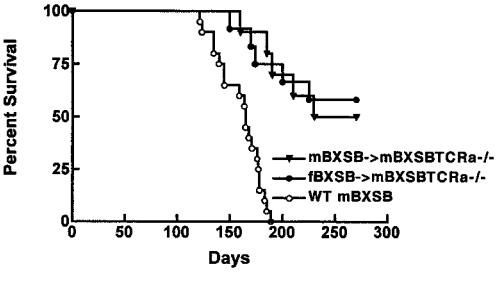

FIGURE 5. Cumulative survival rates for male BXSB TCR$\alpha^{-/-}$ mice adoptively transferred with 4–5 × 10^6 CD3$^+$ LN T cells from either male or female WT BXSB mice. Recipient mice (10–12 mice/group) were followed for up to 7 mo after transfer. The survival rate for a cohort of 20 unmanipulated WT male BXSB mice is depicted for comparison.

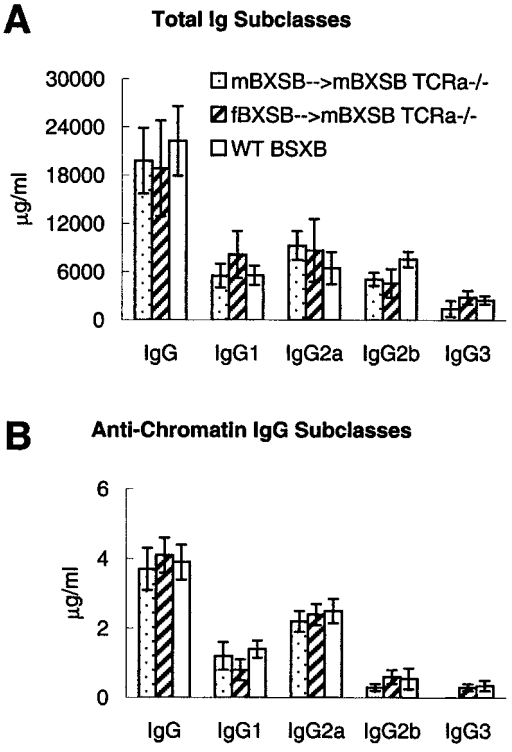

FIGURE 6. Polyclonal (*A*) and antichromatin (*B*) IgG subclass levels in male BXSB TCR$\alpha^{-/-}$ mice at 7 mo after transfer of WT male or female BXSB CD3$^+$ LN T cells. Comparisons are made with 4-mo-old unmanipulated male BXSB mice. Data are the mean ± SEM of six to eight mice per group. Total polyclonal and antichromatin IgG are the sum of the four subclasses.

disease could, however, be reconstituted in these male T cell-deficient mice by both *Yaa*$^+$ (male BXSB) and *Yaa*$^-$ (female BXSB) T cells, thereby documenting that homeostatic anti-self T cell proliferation can induce systemic autoimmunity in an appropriate background, and that the *Yaa* gene functions by impacting a non-T cell component.

It is of considerable interest that the absence of $\alpha\beta^+$ T cells led to an almost complete elimination of disease in the BXSB males. This effect was more pronounced than that observed by Craft and associates (2) in similarly TCRα-deleted MRL-*Fas*^{lpr} mice in which only partial protection from disease was afforded. In this strain concurrent deletion of both the α and δ genes, and thus of both $\alpha\beta^+$ and $\gamma\delta^+$ T cells, was required for nearly complete elimination of serologic and histologic disease parameters (3). The present findings in the BXSB mouse are also at some variance with the initial studies by Wen et al. (28), who reported that $\alpha\beta^+$ T cell-deficient normal background mice showed expansion of B cells and secretion of T-dependent isotype autoantibodies (IgG1 and IgE) with a spectrum of specificities similar to those in lupus, suggesting that autoantibody induction can be mediated by $\gamma\delta^+$ T cells. Adoptive transfer experiments into SCID mice by these investigators indeed showed that $\gamma\delta^+$ T cells are capable of providing help to B cells in the absence of $\alpha\beta^+$ T cells (20). It appears, therefore, that the ability of $\gamma\delta^+$ T cells to promote systemic autoimmunity is dependent upon additional genetic and/or environmental factors. Recent studies have shown that CD4$^+$ $\alpha\beta^+$ T cells of MRL-*Fas*^{lpr} mice are hyper-responsive to antigenic stimuli (29). Such enhanced responses may also be applicable to MRL-*Fas*^{lpr}, but not to BXSB, $\gamma\delta^+$ T cells.

A unique characteristic of the BXSB male disease, originally identified by Wofsy et al. (24), is the appearance of a late-onset monocytosis, almost exclusively detected in the peripheral blood. These cells appear atypical in that despite expressing Mac-1 and having morphological features of macrophages, they are devoid of MHC class II molecules. The origin and potential contribution of these cells to the disease process remain unclear. The present study as well as a previous study in which monocytosis was reduced in BXSB male mice treated with anti-CD4 Ab (10) document that this peculiar manifestation is highly T cell dependent. The means by which T cells induce mobilization of these monocyte-like cells remains unknown, but several products of activated T cells, including IFN-γ (30), M-CSF (30), and GM-CSF (31), might be the mediators. It is apparent, however, that simple activation of T cells and secretion of monocytosis-promoting products cannot fully account for this manifestation, since large numbers of activated T cells are also present in other lupus strains of mice that do not exhibit this characteristic. It is possible, therefore, that induction of the putative T cell-derived factor(s) is directly or indirectly connected to the presence of the *Yaa* gene. The picture becomes more complicated, however, if one considers that this monocytosis does not occur in a long-lived subline of BXSB male mice previously established in this laboratory (32). Because appropriate breeding experiments showed that longevity in this BXSB subline was not due to a modification of the *Yaa* gene, it should be concluded that the monocytosis-promoting factor(s) is an intermediary between T cells and the *Yaa* gene product. Further studies on the means of induction, homing patterns, marker acquisition, and functional characteristics of these monocyte-like cells are, therefore, highly warranted.

The mode by which the *Yaa* gene defect accelerates a lupus-like disease in appropriate backgrounds has not been fully elucidated, and the actual gene remains unknown. Nevertheless, Izui and associates (12–14) showed that in bone marrow chimeras containing two sets of T and B cells from mice with or without the *Yaa* gene,

Table III. *Lymphoid organ weights and cellular subsets in TCR$\alpha^{-/-}$ male BXSB recipients of WT male or female CD3$^+$ cells*

			CD4$^+$ Subsets		CD8$^+$ Subsets			
	Spleen	LN	CD4$^+$	CD4$^+$CD44^{high}	CD8$^+$	CD8$^+$CD44^{high}	CD19$^+$	Mac-1$^+$ Peripheral Blood
Male BXSB→male BXSB TCR$\alpha^{-/-}$	0.4 ± 0.05	0.4 ± 0.1	13.7 ± 0.5*	83.0 ± 1.8	3.4 ± 0.3	71.4 ± 1.4	58.9 ± 1.2	25.1 ± 2.8
Female BXSB→male BXSB TCR$\alpha^{-/-}$	0.4 ± 0.1	0.3 ± 0.1	16.6 ± 0.7	84.6 ± 1.7	2.4 ± 0.4	69.3 ± 3.4	54.6 ± 2.2	18.5 ± 2.9
WT male BXSB	0.38 ± 0.1	0.45 ± 0.05	16.8 ± 1.9	88.4 ± 4.0	2.7 ± 0.22	84.2 ± 2.1	79.2 ± 2.4**	23.7 ± 4.8

[a] Spleen and LN (axillary, inguinal, cervical, and mesenteric) weights (in grams) of 4-mo-old WT male BXSB and of 9-mo-old $\alpha\beta^+$ T cell-deficient male BXSB recipients of WT male or female CD3$^+$ LN T cells. Lymphoid subsets are in percentages of total splenocytes or PBMC (mean ± SEM of five to eight mice per group) and, in the case of CD44^{high} cells, as percentages of CD4$^+$ or CD8$^+$ cells.

*, $p < 0.05$ between male BXSB→male BXSB TCR$\alpha^{-/-}$ mice and either of the other groups.

**, $p < 0.05$ between the WT male BXSB mice and either male TCR$\alpha^{-/-}$ recipient groups.

Table IV. *Kidney disease in $\alpha\beta^+$ T cell-deficient male BXSB recipients of $CD3^+$ T cells from WT BXSB male or female mice*[a]

	GN	Blood Urea Nitrogen	Immune Complex Deposit Score
Male BXSB→male BXSB TCR$\alpha^{-/-}$	2.9 ± 0.2	3.0 ± 0.3	3.0 ± 0.2
Female BXSB→male BXSB TCR$\alpha^{-/-}$	3.0 ± 0.2	2.8 ± 0.2	2.8 ± 0.2
WT male BXSB	3.4 ± 0.2	3.0 ± 0.2	3.1 ± 0.3

[a] GN, blood urea nitrogen, and immune complex deposits of 4-mo-old WT male BXSB and of 9-mo-old $\alpha\beta^+$ T cell-deficient male BXSB recipients of WT male or female $CD3^+$ LN T cells (mean ± SEM of five to eight mice per group).

the T cells from either *Yaa*$^-$ nonautoimmune mice or *Yaa*$^+$ autoimmune mice were equally efficient in promoting anti-DNA and anti-gp70 autoantibody production by *Yaa*$^+$ B cells. Therefore, they concluded that the *Yaa* gene defect is not functionally expressed in T cells, but only in B cells (and/or other APCs), and suggested that this defect leads to the engagement of otherwise quiescent, low avidity, self-reactive T cells. Indeed, recent studies have shown that normal mice harbor a large cohort of these normally harmless cells, which, under certain circumstances, such as high peptide presentation and costimulation, may acquire effector function and become harmful (33).

Our findings with adoptive transfers of *Yaa*$^+$ and *Yaa*$^-$ BXSB mature T cells into the $\alpha\beta^+$ T cell-deficient BXSB male mice are in full congruence with the above conclusions and further extend the results of Izui and associates (12–14) in demonstrating full recapitulation of the male-like BXSB disease with either type of T cell. Adoptive transfer experiments with mature T cells became feasible through the availability of the lymphopenic (i.e., TCR$\alpha^{-/-}$) BXSB mice, wherein transferred T cells survived and homeostatically expanded to reconstitute the normal lymphocyte pool. Numerous recent studies have documented that homeostatic T cell proliferation in the periphery is based on recognition of self-MHC/peptide ligands similar to those used in intrathymic positive selection, and the expanded cells acquire activation markers as well as effector function (25–27). Of interest, however, only a small fraction (~15%) of mature T cells are expected to engraft and proliferate in a lymphopenic host (34). Why only a small fraction exhibits this capacity is unclear, but likely possibilities include the absence or low expression levels in the periphery of some of the positive selection-mediating thymic peptides, restriction of proliferative capacity to T cells with higher anti-self avidity, and/or cell death. Accordingly, skewing of the Vβ TCR repertoire and a reduction in humoral responses to diverse Ags following homeostatic expansion of polyclonal T cell populations have been observed (35). In our transfers of $4–5 \times 10^6$ mature T cells, it can therefore be calculated that reconstitution of the T cell pool occurred via the expansion of as few as $6–7 \times 10^5$ (~15%) T cells. These few cells, likely to encompass a small repertoire of TCRs, were, nevertheless, sufficient to induce the full serologic and histologic spectrum of the male BXSB disease. In future studies, we will determine the minimum number of T cells required to induce disease via homeostatic expansion in this setting. These issues aside, it can be hypothesized that anti-self-homeostatic expansion of T cells in appropriate backgrounds may play a primary or secondary role in the initiation and perpetuation of systemic autoimmunity, and the findings presented herein provide initial evidence that this might be the case.

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