Selective Autoantibody Production by *Yaa*$^+$ B Cells in Autoimmune *Yaa*$^+$-*Yaa*$^-$ Bone Marrow Chimeric Mice

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Summary

The accelerated autoimmune syndrome observed in BXSB/MpJ male mice is associated with the presence on the Y chromosome of an as yet unidentified mutant gene, designated Y chromosome–linked autoimmune acceleration (*Yaa*). To study the mechanisms by which the *Yaa* gene accelerates and/or induces the production of autoantibodies, we have developed double-congenic bone marrow chimeras containing B cells from autoimmune males carrying the *Yaa* gene, and from nonautoimmune male or female mice lacking it and differing by the Igh allotype. The analysis of the allotype of total immunoglobulins and anti-DNA antibodies in *Yaa*$^+$ male-normal female (*Yaa*$^-$) chimeric mice revealed that the selective activation of B cells from autoimmune *Yaa*$^+$ male mice was responsible for the hypergammaglobulinemia and autoantibody production. This phenomenon was not due to an anti-HY interaction between female T helper cells and male B cells, because first, *Yaa*$^+$ B cells were selectively stimulated to produce autoantibodies in *Yaa*$^+$ male–*Yaa*$^-$ male chimeric mice; and second, normal male and female chimeras failed to develop an autoimmune syndrome. In addition, the fact that both B cell populations in *Yaa*$^+$-*Yaa*$^-$ chimeras similarly responded to a foreign antigen, human IgG, argues against the possibility that the selective activation of *Yaa*$^+$ B cells may be due to their hyper-responsiveness to T helper signals. We propose that a cognate interaction of T helper cells with *Yaa*$^+$ B cells, because of possible T cell recognition of a *Yaa*-related molecule expressed on *Yaa*$^+$ B cells, may be responsible for the acceleration and/or induction of autoantibodies in BXSB/MpJ mice.

The BXSB/MpJ (BXSB) strain spontaneously develops a progressive and lethal autoimmune disease, similar to human systemic lupus erythematosus, 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*)[1] gene present in the Y chromosome of the BXSB strain is responsible for the accelerated autoimmune abnormalities and immunopathological lesions in mice predisposed to autoimmune diseases (3–6). However, the lack of induction of the autoimmune syndrome in *Yaa* gene–congenic normal mice (4, 5) indicates that the effect of this gene is dependent on the presence of the abnormal autosomal genome in autoimmune-prone mice. Although it has been demonstrated that CD4$^+$ T helper cells play an essential role in the autoantibody production and in development of fatal glomerulonephritis in male BXSB mice (7), the cellular and molecular mechanisms by which the *Yaa* gene abnormality promotes autoimmune responses are poorly understood. In the present study, we have analyzed whether a unique interaction between T and B cells could result in autoantibody production in autoimmune mice bearing the *Yaa* gene. For this purpose, we have constructed radiation bone marrow chimeric mice consisting of autoimmune male cells carrying the *Yaa* gene, and of nonautoimmune male or female cells lacking the *Yaa* gene and differing by the Igh allotype, and determined the origin of B cells producing autoantibodies. Our results indicate that B cells from male mice bearing the *Yaa* gene are selectively activated to produce anti-DNA autoantibodies and hypergammaglobulinemia.

Materials and Methods

Mice. The C57BL/6 (B6, Igh^b) strain bearing the *Yaa* gene (B6.*Yaa*) was developed in our laboratory by backcross procedures

[1] *Abbreviations used in this paper:* AHGG, aggregated human IgG; BMC, bone marrow cells; *Yaa*, Y chromosome–linked autoimmune acceleration.

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as described (5). NZW (Igh^n) mice were purchased from Harlan Olac Ltd. (Oxon, UK). The B.C20/Icr (BC20) strain carrying the Igh^a allotype on the B6 background was kindly provided by Dr. M. Bosma (Fox Chase Cancer Center, Philadelphia, PA). The hybrid mice used in this study were obtained as follows: B6.*Yaa* male mice carrying the *Yaa* gene and $Igh^{a/b}$ allotype, obtained by mating B6.*Yaa* males with BC20 females, were crossed with NZW females. The expression of the Igh allotype in these F_1 hybrid mice was documented by an ELISA using polyclonal anti-Igh allotype antibodies as described below. It should be noted that like BXSB males, (NZW × B6.*Yaa*)F_1 male, but not female mice, spontaneously developed a severe lupus-like syndrome (5).

Preparation of Bone Marrow Chimeras. 2-mo-old recipients were irradiated at 800 rad and reconstituted with bone marrow cells (BMC) from 6–7-mo-old mice. A mixture of 10^7 viable BMC were intravenously injected into irradiated recipients as detailed in Table 1. Chimeric mice were bled every 2 mo by retro-orbital sinus puncture, and resulting sera were stored at −20°C until use.

Antigens and Antibodies. Calf thymus DNA (Sigma Chemical Co., St. Louis, MO) was heat-denatured and used as single-stranded DNA. Aggregated human IgG (AHGG) was prepared by heating HGG at 63°C for 30 min. IgG fractions of goat anti-mouse γ chain–specific antibodies were purchased from Cappel Laboratories (Cochranville, PA). Polyclonal anti-Igh^a and anti-Igh^b allotype antibodies were obtained in SJL mice immunized with BALB/c IgG and in BALB/c mice immunized against B6 IgG, respectively, as described previously (8). Anti-IgG2a^a (Ig[1a]8.3) mAb reacting also with IgG2a of the Igh^n allotype (9) and anti-IgG2a^b (C506FS) mAb were generously provided by Dr. L.A. Herzenberg (Stanford, CA) and Dr. J. Van Snick (Brussels, Belgium), respectively. Polyclonal antibodies and mAbs were purified with a protein A column and conjugated with alkaline phosphatase according to the procedure of Engvall and Perlmann (10).

Immunization Protocol. *Yaa*$^+$ male–*Yaa*$^-$ female chimeric mice (group I: Table 1) and (NZW × B6)F_1 males were intraperitoneally injected and boosted 60 d later with 500 μg of AHGG. Mice were bled 10 d after challenge.

Serological Assays. Serum levels of IgG were determined by ELISA as described (8). Results are expressed in mg/ml in reference to a standard curve obtained with mouse Ig reference serum (Miles Scientific, Naperville, IL).

To determine serum levels of Igh^a and Igh^b allotypes in sera, a previously described ELISA was used with alkaline phosphatase-conjugated anti-Igh^a or anti-Igh^b allotype antibodies (8). Results are expressed in titration units in reference to a serum pool from 6-mo-old (B6 × BC20)F_1 ($Igh^{a/b}$) mice. Note that the anti-Igh^a polyclonal antibodies used in the present study crossreact with the Igh^n allotype, while anti-Igh^b antibodies do not react with the Igh^a and Igh^n (Fig. 1).

Serum IgG2a^a and IgG2a^b levels were determined by ELISA. Microtiter plates were coated with 5 μg/ml of Ig(1a)8.3 (anti-IgG2a^a) or C506FS (anti-IgG2a^b) mAb. After incubation with 0.5% BSA for 1 h at room temperature, serum samples diluted at 1:10^4 with 2% BSA in PBS containing 0.05% Tween 20 were added to the wells for an overnight incubation at 4°C. Wells were then incubated with alkaline phosphatase-conjugated polyclonal anti-Igh^a antibody for the detection of IgG2a^a or with C506FS (anti-IgG2a^b) mAb conjugates for the detection of IgG2a^b. Although both the Ig(1a)8.3 mAb and anti-Igh^a allotype polyclonal antibody are able to bind to IgG2a of the Igh^n allotype (IgG2a^c), this ELISA method fails to detect IgG2a^c, most probably because the binding of IgG2a^c to solid-phase anti-IgG2a^a mAb interferes with the recognition of the IgG2a^c epitope by anti-Igh^a antibodies (Fig. 1). Results are expressed in mg/ml in reference to a standard curve obtained with purified IgG2a^a and IgG2a^b mAb.

The activity of IgG anti-DNA antibodies in sera was measured by an ELISA as described previously (8). The results are expressed in titration units referring to a standard curve obtained by serial dilutions of a serum pool from 4-mo-old MRL/MpJ-*lpr*/*lpr* mice. To determine the allotype of anti-DNA antibodies, an ELISA was performed using alkaline phosphatase-conjugated antiallotype antibodies. The results are expressed in titration units in reference to a standard curve derived from a serum pool of (NZW × BXSB)F_1 ($Igh^{n/b}$) male mice.

The Ig allotype of anti-HGG antibodies raised during the course of the secondary immune response was tested by an ELISA (11) using alkaline phosphatase-conjugated antiallotype antibodies. The results are expressed in titration units in reference to a standard curve obtained from immunized (B6 × BALB/c)F_1 ($Igh^{a/b}$) mice.

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

Results

Generation of Yaa$^+$-Yaa$^-$ BMC Chimeras. To investigate whether a possible unique interaction between B cells from mice bearing the *Yaa* gene and T helper cells is involved in the spontaneous production of autoantibodies, irradiated recipient (NZW × B6)F_1 mice were reconstituted with a mixture of BMC from autoimmune male mice bearing the *Yaa* gene ($Igh^{n/a}$ allotype) and female or male mice lacking the *Yaa* gene ($Igh^{n/b}$ allotype), as described in Table 1 (groups I and II). As control, irradiated B6 female mice were reconstituted with BMC from both BC20 (Igh^a) male and B6 (Igh^b) female mice (group III). 2 mo after reconstitution, the chimerism was checked by measuring serum levels of Ig

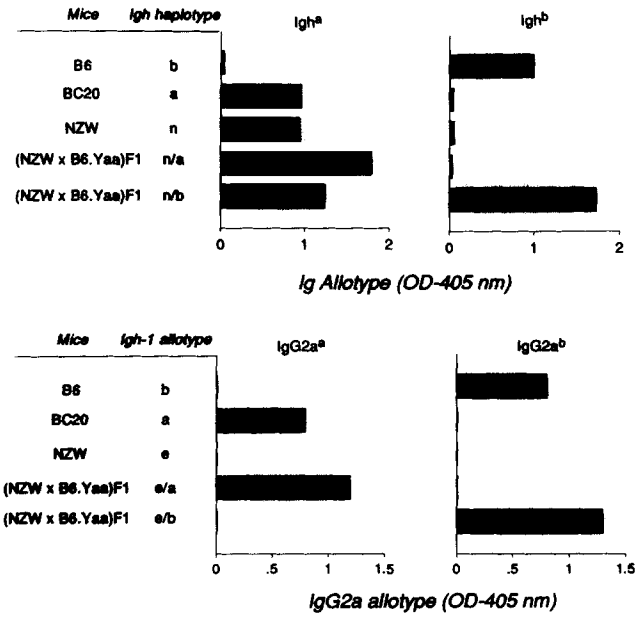

Figure 1. Specificity of Igh and IgG2a allotype-specific ELISA. Results (mean of 7–10) from 6-mo-old mice are expressed as OD at 405 nm.

Table 1. *BMC Chimeric Mice*

Group	BMC donor	Recipient
Male.*Yaa* + female → female (group I)	(NZW × B6.*Yaa*)F_1 male ($Igh^{n/a}$) + (NZW × B6.*Yaa*)F_1 female ($Igh^{n/b}$)	(NZW × B6.*Yaa*)F_1 female ($Igh^{n/b}$)
Male.*Yaa* + male → male (group II)	(NZW × B6.*Yaa*)F_1 male ($Igh^{n/a}$) + (NZW × B6)F_1 male ($Igh^{n/b}$)	(NZW × B6)F_1 male ($Igh^{n/b}$)
Male + female → female (group III)	BC20 male + B6 female	B6 female

Table 2. *Ig Allotype Levels in BMC Chimeric Mice*

Mice	Age	Igh allotype	$Igh^{a,n}$	Igh^{b}
			U × 10^{-3}/ml	
Group I	2	a, n, b	3.6 ± 1.9	1.0 ± 0.4
Group II	2	a, n, b	2.0 ± 1.4	0.7 ± 0.2
Group III	2	a, b	2.2 ± 1.1	1.3 ± 0.6
(NZW × B6)F_1 male	6	n, b	2.1 ± 1.0	1.2 ± 0.3
(NZW × B6.*Yaa*)F_1 male	6	n, b	10.0 ± 4.8	7.3 ± 3.1
(NZW × B6.*Yaa*)F_1 male	6	n, a	25.2 ± 18.7	<0.01

Serum levels of $Igh^{a,n}$ and Igh^{b} allotypes were measured in BMC chimeras (2 mo after reconstitution) and in 6-mo-old unmanipulated control mice by ELISA. Results are the mean of 10–20 mice ± 1 SD.

allotypes. Concentrations of $Igh^{a,n}$ allotypes detectable by the anti-Igh^{a} allotype assay were approximately three times higher than those of Igh^{b} allotype in groups I and II, as expected (Table 2). When IgG2a^{a} and IgG2a^{b} allotypes were specifically detected, their levels were comparable in group I (IgG2a^{a}: 4.1 ± 2.1 mg/ml; IgG2a^{b}: 3.2 ± 1.9 mg/ml; $p > 0.05$) and in group II (IgG2a^{a}: 2.4 ± 0.7 mg/ml; IgG2a^{b}: 2.7 ± 1.6 mg/ml; $p > 0.1$). In mice of group III

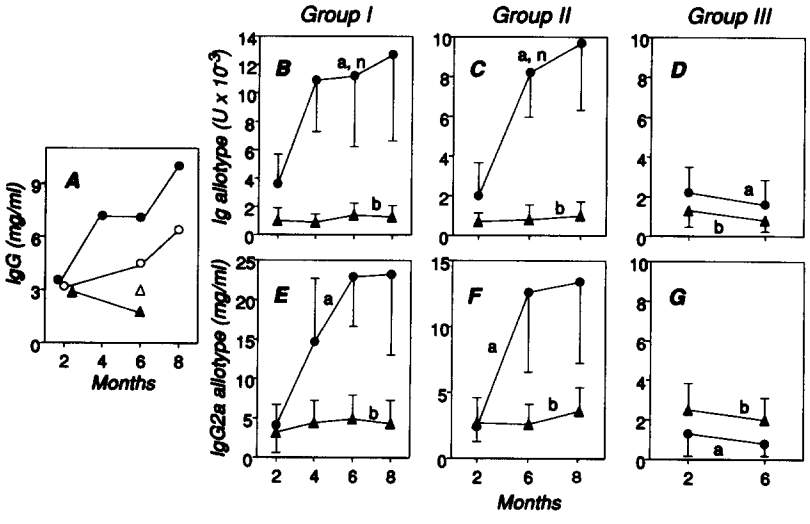

Figure 2. Kinetics of serum levels of IgG (*A*), Igh allotypes (*B–D*), and IgG2a allotypes (*E–G*) in BMC chimeras. Mean levels (10–20 mice in each group) of IgG in chimeric mice (group I, ●; group II, ○; group III, ▲) and control B6 mice (△) are expressed in mg/ml (*A*). Mean levels (± 1 SD) of Igh and IgG2a allotypes (group I, *B*, E; group II, *C* and *F*; group III, *D* and *G*) are expressed in U/ml × 10^{-3} and mg/ml, respectively. Statistical analysis has shown that increases in $Igh^{a,n}$ and IgG2a^{a} allotypes (*Yaa*$^{+}$ origin) at 4, 6, and 8 mo vs. at 2 mo in both groups I and II were significant ($p < 0.001$), while levels of the b allotype (*Yaa*$^{-}$ origin) did not significantly differ during the period of observation ($p > 0.1$).

expressing both Igh^a and Igh^b allotypes, their Ig allotype levels were essentially identical to those found in unmanipulated 6-mo-old (NZW × B6)F$_1$ Igh$^{a/b}$ male mice.

Kinetics of IgG Levels and Allotype Expression in Yaa$^+$-Yaa$^-$ BMC Chimeras. To study if chimeric mice developed hypergammaglobulinemia, they were bled every 2 mo and serum levels of IgG were measured by ELISA. An age-dependent increase of IgG levels was observed in mice (groups I and II) receiving a mixture of BMC from mice with and without the *Yaa* gene (Fig. 2 *A*). In contrast, mice reconstituted with normal male and female BMC of B6 origin (group III) failed to develop hypergammaglobulinemia.

In parallel to the elevation of total IgG levels, a selective age-related increase of the Igh^{a,n} allotype was observed in groups I and II, while the Igh^b allotype levels remained constant during the period of observation (up to 8 mo after the reconstitution) (Fig. 2, *B* and *C*). The specific detection of IgG2a allotypes in these chimeras again revealed an age-dependent, selective increase of the IgG2a^a allotype in both group I (8 mo post-reconstitution, IgG2a^a: 23.2 ± 9.9 mg/ml) and group II (8 mo post-reconstitution, IgG2a^a: 13.4 ± 8.5 mg/ml) (Fig. 2, *E* and *F*). In contrast, IgG2a^b allotype levels remained detectable and unchanged during the whole study (8 mo post-reconstitution, group I: 4.3 ± 2.3 mg/ml, and group II: 3.6 ± 1.4 mg/ml). Notably, no increase in levels of both Ig allotypes was observed in mice of group III (Fig. 2, *D* and *G*).

Selective Production of Anti-DNA Antibodies by Yaa$^+$ Male B Cells. The spontaneous production of IgG anti-DNA antibodies was examined in chimeric mice in comparison with unmanipulated (NZW × B6.*Yaa*)F$_1$ mice and normal control mice. Serum levels of IgG anti-DNA progressively increased in both groups of mice reconstituted with a mixture of BMC from mice with and without the *Yaa* gene. 6 mo after cell transfer, their titers were almost comparable to those found in 6-mo-old unmanipulated (NZW × B6.*Yaa*)F$_1$ male mice (Fig. 3). In contrast, no significant titers of IgG anti-DNA antibodies were detected in mice from group III, as well as in (NZW × B6)F$_1$ males, (NZW × B6.*Yaa*)F$_1$ females, and control B6 females.

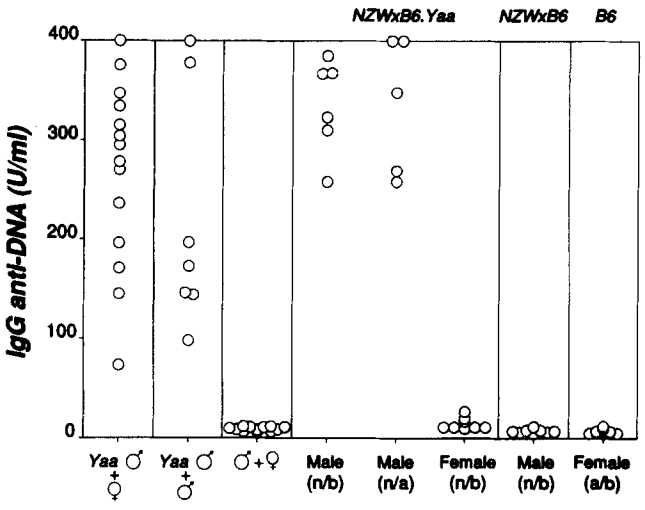

Figure 3. Serum levels of IgG anti-DNA antibodies in BMC chimeras (6 mo after reconstitution) and 6-mo-old unmanipulated control mice. Results are expressed in U/ml. Group I, *Yaa* ♂ + ♀; group II, *Yaa* ♂ + ♂; group III, ♂ + ♀.

The analysis of the Ig allotype of anti-DNA antibodies has shown that, as in the case of the hypergammaglobulinemia, anti-DNA antibodies produced in mice of groups I and II were exclusively of the Igh^{a,n} allotype (Fig. 4), indicating that anti-DNA antibodies in *Yaa*$^+$-*Yaa*$^-$ BMC radiation chimeras were selectively produced by B cells from mice expressing the *Yaa* gene. Notably, anti-DNA antibodies of both

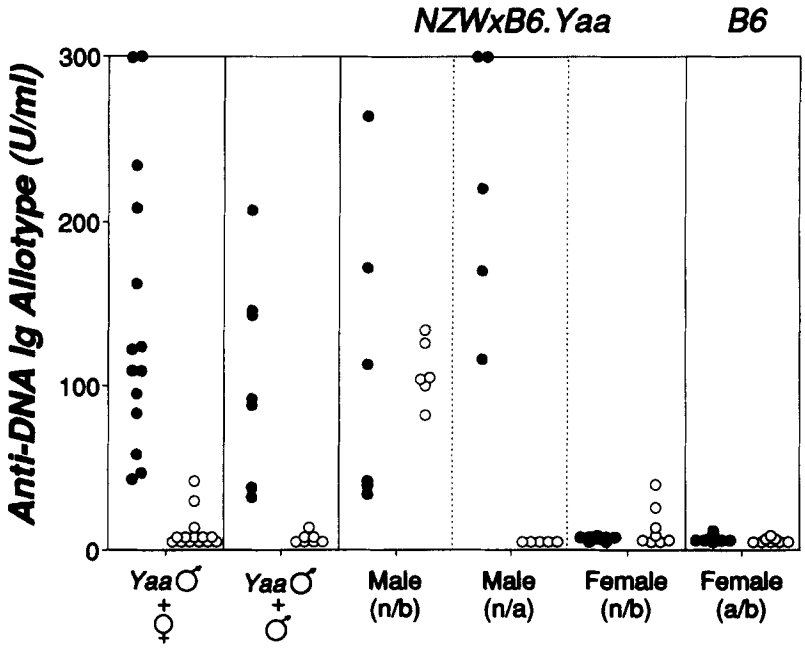

Figure 4. Serum levels of Igh^{a,n} (●) and Igh^b (○) anti-DNA antibodies in BMC chimeras (6 mo after reconstitution) and 6-mo-old unmanipulated control mice. Results are expressed in U/ml. Group I, *Yaa* ♂ + ♀; group II, *Yaa* ♂ + ♂.

Table 3. *Ig Allotype Levels of Total Ig, Anti-HGG, and Anti-DNA Antibodies in BMC Chimeric Mice*

Group	Age*	Igh allotype*		Anti-HGG*		Anti-DNA*	
		$Igh^{a/n}$	Igh^{b}	$Igh^{a/n}$	Igh^{b}	$Igh^{a/n}$	Igh^{b}
		U × 10⁻³/ml		*U/ml*		*U/ml*	
Male.*Yaa* + female → female	2	9.1 ± 3.4	1.4 ± 0.3	1,579 ± 702	618 ± 174	35 ± 14	2 ± 1
	6	12.7 ± 5.7	1.5 ± 0.3	979 ± 575	394 ± 166	72 ± 41	3 ± 2
(NZW × B6)F_1 male	6	2.3 ± 1.2	1.8 ± 0.3	1,227 ± 374	1,038 ± 394	3 ± 1	3 ± 1

* 500 μg of AHGG was intraperitoneally injected into *Yaa*$^+$ male–*Yaa*$^-$ female chimeric mice at 2 or 6 mo after reconstitution and 6-mo-old (NZW × B6)F_1 male mice. 2 mo later, mice were boosted with 500 μg of AHGG. The Ig allotype of anti-HGG antibodies was determined 10 d after challenge. Results are the mean of 5–10 mice ± 1 SD. The titers of anti-HGG-specific allotype antibodies in nonimmunized mice were <1 U/ml. Serum levels of $Igh^{a,n}$ and Igh^{b} allotypes and of anti-DNA allotypes were measured by ELISA.

allotypes were similarly detectable in unmanipulated (NZW × B6.*Yaa*)F_1 male ($Igh^{n/b}$) mice.

Lack of Selective Production of Anti-HGG Antibodies by Yaa$^+$ *B Cells.* It has been previously suggested that B cells from BXSB male mice may be hyper-responsive to appropriate T helper cell–mediated signals (12). To investigate if such a hyperreactivity is responsible for the selective production of autoantibodies by the *Yaa*$^+$ B cells in our double bone marrow chimeric mice, two groups of *Yaa*$^+$ male–*Yaa*$^-$ female BMC chimeric mice were immunized with AHGG either at 2 or 6 mo after the reconstitution and boosted 2 mo later. If B cells of the *Yaa*$^+$ origin are indeed hypersensitive to T helper signals, we could expect in the anti-HGG immune response a distribution of the $Ig^{a,n}$ and Ig^{b} allotypes similar to that found for the total Ig allotype (6.5 times increase at 4 mo and 8.5 times at 8 mo) or for the anti-DNA antibodies (17.5 times increase at 4 mo and 24 times at 8 mo). However, anti-HGG levels of the $Igh^{a,n}$ allotype in both groups of chimeric mice were only 2.5 times higher than those of the Ig^{b} allotype (Table 3). Notably, (NZW × B6)F_1 male mice produced both allotypes of anti-HGG antibodies at a comparable level. This indicates that *Yaa*$^+$ B cells and *Yaa*$^-$ B cells were activated in a similar fashion to produce anti-HGG antibodies in the double-chimeric mice.

Discussion

The BXSB Y chromosome–linked mutant gene, *Yaa*, accelerates the progression of a lupus-like autoimmune syndrome only in mice that are predisposed to autoimmune diseases. To better define the cellular mechanisms responsible for the *Yaa* gene effect, we have made BMC radiation chimeras containing two sets of B cells from mice with or without the *Yaa* gene, and the origin of anti-DNA autoantibodies was identified by using the Igh allotype marker. Our results have demonstrated that B cells from mice bearing the *Yaa* gene were selectively activated in these chimeric mice to produce anti-DNA autoantibodies and hypergammaglobulinemia.

The analysis of the Igh allotype in *Yaa*$^+$-*Yaa*$^-$ BMC chimeras has clearly shown that hypergammaglobulinemia and anti-DNA autoantibodies arose from the selective activation of B cells from mice bearing the *Yaa* gene, but not from those lacking it. To explain this phenomenon, one may postulate a prevalent expansion of B cells from the *Yaa*$^+$ mice, which results from the preferential repopulation of *Yaa*$^+$ lymphoid cell lineages over the others in irradiated hosts. In fact, in the case of radiation chimeras constructed with a mixture of BMC from mice with or without the *lpr* gene, the Igh allotype of the donor lacking the *lpr* gene progressively decreased from 2 mo post-transplantation and was barely detectable by 4–8 mo post-transplantation due to the selective repopulation by *lpr* donor cells (13). However, the persistence of Igh allotype of the *Yaa*$^-$ donor at a constant level and the production of anti-HGG antibodies by the *Yaa*$^-$ B cells at a level comparable to that by *Yaa*$^+$ B cells rule out the selective elimination of the normal *Yaa*$^-$ B cell population from our *Yaa*$^+$-*Yaa*$^-$ chimeras. In addition, the implication of HY antigen–specific female T cells in the observed selective activation of the *Yaa* gene–bearing male B cells is unlikely because of two reasons: first, the *Yaa*$^+$ B cells still produced autoantibodies in chimeras constructed with a mixture of male B cells with or without the *Yaa* gene, and second, normal male and female chimeras of B6 origin failed to develop an autoimmune syndrome.

The fact that autoreactive B cells of the *Yaa*$^+$ origin are selectively activated in *Yaa*$^+$-*Yaa*$^-$ bone marrow chimeric mice indicates that the *Yaa* gene abnormality is likely to be expressed at least at the level of B cells. At present, the mechanism leading to the selective activation of *Yaa*$^+$ autoreactive B cells is unknown, but the following possible mechanism can be considered.

First, the *Yaa* gene may cause an abnormality in B cells, which renders them hyper-responsive to appropriate T cell–mediated helper signals, due to abnormalities either in receptors for T helper factors or in regulatory molecules controlling cellular responsiveness to appropriate signals. The finding of Prud'homme et al. (12) may be relevant, showing the in vitro hyper-responsiveness of male BXSB B cells to T helper signals, although this issue has never been addressed

in the context of the *Yaa* gene expression. However, this first possibility appears to be less likely because of the absence of preferential production of anti-HGG antibodies by B cells of the Yaa^+ origin in Yaa^+-Yaa^- chimeric mice after immunization with AHGG.

Second, the *Yaa* gene may be involved in the expression of intercellular adhesion molecules on B cells, thereby facilitating their interaction with T helper cells. Again, results of anti-HGG responses argue against this possibility.

Third, an attractive hypothesis is that the *Yaa* gene abnormality may be associated with the expression of an antigen on the surface of B cells, which can be directly or indirectly related to the *Yaa* gene, and its recognition by T helper cells could induce the activation of autoreactive Yaa^+ B cells. In fact, the recognition by T helper cells of a self or modified Ia molecule expressed on B cells has been shown to be responsible for the development of a lupus-like autoimmune syndrome observed during the course of chronic graft-vs.-host disease (14, 15), after induction of neonatal tolerance to alloantigens (8, 16) or after injection of drugs or chemical agents (17, 18). In addition, such a possibility has been suggested to be involved in the initiation and/or progression of a lupus-like autoimmune disease (19). The selective production of anti-DNA autoantibodies, but not antibodies to foreign antigens by Yaa^+ B cells in Yaa^+-Yaa^- chimeric mice, may well be explained by differences in the capacity of T helper cells specific for DNA autoantigens and for HGG antigens to respond against their respective antigens. Because of the presence of sufficient anti-HGG-specific T cell help in Yaa^+-Yaa^- chimeric mice, as documented by the development of high and comparable titers of anti-HGG antibodies in both (NZW × B6)F_1 and (NZW × B6. *Yaa*)F_1 male mice (5), the hypothetical *Yaa*-specific T helper cells may not provide any additional help for anti-HGG responses. Consequently, there was no preferential production of anti-HGG antibodies by Yaa^+ B cells. In contrast, such *Yaa*-specific T helper cells may be essential for the promotion of anti-DNA autoimmune responses in (NZW × B6)F_1 mice, which have a markedly limited activity of T helper cells specific for anti-DNA autoimmune responses. This hypothesis is consistent with our recent observations that BXSB male mice bearing the *Yaa* gene developed enhanced T cell–dependent antibody production to certain exogenous antigens, to which female BXSB mice mounted only limited immune responses (Fossati et al., manuscript in preparation). In addition, our previous finding that male BXSB mice are able to develop T-dependent IgG anti-HGG responses in the absence of HGG-specific T helper cells, but the presence of non-HGG specific T helper cells (11), further supports the possible existence of *Yaa*-specific T helper cells in BXSB male mice. Clearly, the present demonstration that the *Yaa* gene abnormality is expressed at the level of B cells would help to identify the molecular nature of the *Yaa* gene and to elucidate the molecular and cellular basis of the *Yaa* gene–induced acceleration of the autoimmune disease in BXSB mice.

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