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Development of Lupus in BXSB Mice Is Independent of IL-4

Dwight H. Kono, Dimitrios Balomenos, Miyo S. Park, and Argyrios N. Theofilopoulos

Although systemic lupus erythematosus appears to be a humorally mediated disease, both Th1 and Th2 type responses have been implicated in its pathogenesis. The Th1 response, as exemplified by IFN-γ production, has been uniformly shown in mouse lupus models to be critical for disease induction. The role of Th2 type responses, however, is more complicated, with some studies showing detrimental and others beneficial effects of IL-4 in these models. To further address this issue, we generated and analyzed IL-4 gene-deficient BXSB mice. Mice homozygous for this deletion had significantly lower serum levels of total IgG1 compared with wild-type BXSB, consistent with the lack of IL-4. However, no significant differences were observed in mortality, spleen weight, severity of glomerulonephritis, levels of anti-chromatin and anti-ssDNA Abs, or frequency of activated (CD44high) CD4+ T cells. The anti-chromatin Ab isotype response was virtually all Th1 type in both the knockout and wild-type BXSB. These findings directly demonstrate that IL-4 and, by inference, Th2 cells are not obligatory participants in the induction and maintenance of lupus in this strain. The Journal of Immunology, 2000, 164: 38–42.

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Yaa chlorophyll protein; EAMG, experimental autoimmune myasthenia gravis; GN, glomerulonephritis; D4+ T cells following activation become polarized into two main subsets, designated Th1 and Th2, that manifest distinct cytokine profiles and effector functions (1, 2). The Th1 subset, which secretes IL-2, IFN-γ, and TNF-β, promotes mainly cellular-mediated responses that provide protection from intracellular pathogens, activation of phagocytes, and delayed-type hypersensitivity (DTH), but also, through IFN-γ, class switching to the IgG2a isotype. In contrast, Th2 cells that secrete IL-4, IL-5, IL-6, IL-10, and IL-13 protect against extracellular pathogens, induce IgE-mediated allergic responses, activate eosinophils, and promote humoral responses, particularly of the IgG1 and IgE subclasses. The development of CD4+ cells into Th1 or Th2 cells can be influenced by a number of factors, including cytokine milieu, type of APC, Ag affinity and concentration, costimulatory molecules, and duration of exposure. The molecular basis for this functional dichotomy has not been fully defined; however, a number of key cytokine and chemokine receptors expressed on either Th1 or Th2 cells have been identified that are important for differentiation and effector functions (3–6). Several protooncogenes, kinases, and transcription factors have also been implicated in the development of the Th subsets (7–12).

Systemic lupus erythematosus (SLE) is a humoral-mediated disease that is nonetheless dependent on CD4+ T cells for disease induction (13), presumably because pathogenic autoantibodies require T-dependent affinity maturation (14–16). The functional dichotomy of Th subsets suggests that T cell-dependent autoantibody-mediated diseases like SLE might involve Th2-type responses, whereas cell-mediated responses in organ-specific autoimmune diseases might be mediated by Th1-type responses. This attractive but simplistic model, however, has not been supported by studies of humoral autoimmune diseases, such as lupus (17–22) and myasthenia gravis (23), nor by studies of certain organ-specific diseases such as experimental autoimmune encephalomyelitis (24) and experimental autoimmune uveitis (25, 26). In the case of lupus, considerable evidence points to the importance of the Th1 response for disease induction and acceleration. Cytokine profiles of spleen cells in BXSB and MRL-Fas+ mice showed mainly increases in the Th1 cytokine, IFN-γ (27). Accelerated disease in closely related lupus susceptible and non-susceptible mouse strains was associated with increases in both IFN-γ and the Th1-mediated IgG isotypes, IgG2a and IgG3 (28). IFN-γ accelerated, while anti-IFN-γ Ab or soluble IFN-γR, prevented disease in spontaneous murine lupus (17, 18). Moreover, gene knockout of IFN-γ or IFN-γR eliminated disease in spontaneous and xenobiotic-induces models of lupus (19–22, 29).

Th2-type responses have also been associated with the development of SLE. Increases in number of IL-4-producing cells have been found in some lupus-susceptible strains (30, 31); treatment with blocking anti-IL-4 Ab or soluble IL-4R reduced autoantibody production and nephritis in (NZB × NZW)F1 and MRL-Fas+ mice (32, 33), and IL-4 knockout (MRL-Fas+ × B6)F2 mice had less lymphadenopathy and end-organ disease compared with IL-4 wild-type littermate controls. Overall these studies suggest both Th1 and Th2 responses are important for the development of lupus.

Other findings, however, suggest that the Th2 response may be less important than Th1 response in lupus or even protective. In BXSB and MRL-Fas+ mice, the increases in IL-4 levels and IL-4 producing cells although present are much less than the increases observed in IFN-γ levels or IFN-γ-producing cells (27, 30, 34). Knockout of the IL-4 gene had no effect on disease induction in the mercury-induced model of systemic autoimmunity, which was previously considered a prototypic Th2-mediated humoral disease (29, 35). Finally, expression of an IL-4 transgene by B cells in (NZW × C57Bl/6.Yaa)F1 mice protected rather than enhanced the development of lupus nephritis, and this protection appeared to be due to deviation of the autoantibody response away from especially pathogenic Th1-mediated IgG subclasses such as IgG3 (36).

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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; PerCP, peridinin chlorophyll protein; EAMG, experimental autoimmune myasthenia gravis; GN, glomerulonephritis; Yaa, Y chromosome accelerated autoimmunity and lymphoproliferation.

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The Journal of Immunology, 2000, 164: 38–42.
To further define the role of the Th2-type response in SLE we backcrossed the IL-4 gene knockout mutation onto the BXSB lupus-prone background and assessed its effect on autoimmunity. Strikingly, homozygous deletion of the IL-4 gene did not affect autoantibody production, glomerulonephritis (GN) or mortality in this strain.

Materials and Methods

Mice

Wild-type and IL-4 knockout (IL-4−/−) BXSB mice were bred and maintained under specific pathogen-free conditions at The Scripps Research Institute. IL-4 knockout mice of mixed 129 × C57BL/6 background were kindly provided by Dr. W. Müller (University of Cologne, Germany) (37). BXSB IL-4-deficient mice were generated by seven backcrosses to the BXSB strain, followed by intercrossing of the heterozygous IL-4−/+ offspring. Wild-type or heterozygous IL-4 knockout littermates from this intercross at N7 were used as controls. The BXSB Y chromosome containing the Yaa (Y chromosome-accelerated autoimmunity and lymphoproliferation) gene was bred into the backcross mice, and only male mice with the Yaa gene were analyzed in this study. Genotyping of the IL-4 wild-type and knockout mutation by PCR was performed as described (38).

Pathology

Autopsy and histologic examination of mice were performed as previously described (38) at 5 mo or if mice developed severe disease. Tissues were fixed in Bouin’s solution, and sections were stained with periodic-acid Schiff reagent. GN was graded on a 0–4+ scale (38).

Serology

Serum IgG1 and IgG2α subclass levels were determined by ELISA as previously described (29), with standard curves generated using calibrated mouse serum (Binding Site, Birmingham, England). Levels of Ig were calculated by regression plots of standard values (Statview, SAS Institute, Cary, NC). Abs against chromatin or ssDNA were assayed as previously described (14). HRP-conjugated anti-mouse IgG1- (Caltag, Burlingame, CA) or IgG2α-specific (PharMingen, La Jolla, CA) detecting Abs were used for identifying IgG1 or IgG2α anti-chromatin Abs. Positive and negative control sera were included on each plate for normalization. The amount of chromatin-bound IgG1 or IgG2α Ab was calculated from an IgG1 or IgG2α subclass standard curve that was included on each plate. This standard curve was generated as above for detecting serum IgG1 and IgG2α subclass levels (29).

Flow cytometry

Spleen cell suspensions were stained with anti-CD8-FITC, anti-CD4-peridinin chlorophyll protein (PerCP), anti-CD44-PE, and anti-CD62L-biotin/streptavidin-APC (PharMingen). Cell data were acquired on a FACSORT and analyzed with the Cell Quest program (Becton Dickinson, Mountain View, CA). Live cells were gated based on their forward and side scatter characteristics.

Results

Survival of IL-4−/− and wild-type BXSB mice

BXSB background mice deficient in IL-4 expression and IL-4−/− littermate controls were produced by backcrossing IL-4−/+ mice to the BXSB strain and then intercrossing the N7 generation. When the resulting wild-type and IL-4−/− mice were followed for disease, both developed early mortality with no significant difference in survival at 5 mo (43% and 44%, respectively, Fig. 1). The IL-4−/− mice had greater survival (90%) compared with both wild-type and homozygous knockout BXSB mice, but this did not reach statistical significance (p > 0.05). The overall 62% (16/26) combined survival at 5 mo of these mice was comparable to our colony of BXSB mice, which have a 50% mortality around 5[1–2] mo of age. This suggests that the backcrossing has been sufficient to fix most if not all of the BXSB lupus-predisposing alleles.

IL-4 knockout mice develop lymphoaccumulation and GN

Wild-type and both partial and complete IL-4 knockout mice all developed lymphoaccumulation. The average spleen weight of the IL-4−/− mice, however, was slightly lower than the IL-4−/+ mice (p > 0.05), and significantly smaller than the IL-4−/− mice (p < 0.01, Fig. 1). Histologic examination of the kidneys revealed that IL-4 deficient BXSB mice, with either homozygous or heterozygous deletions, developed the typical features of GN observed in wild-type BXSB mice. Glomeruli had periodic-acid Schiff-staining deposits, cellular proliferation primarily mesangial, occasional polymorphonuclear cells, and rare crescents (not shown). There were no significant differences in the type and severity of GN among the three groups (Fig. 1).

IgG1 levels are decreased in IL4−/− mice

IL-4-deficient BXSB had significantly lower serum levels of total IgG1 compared with wild-type or heterozygous IL-4−/− mice (+/+; 171 ± 85 μg/ml; +/−; 182 ± 95 μg/ml; −/−; 78 ± 21 μg/ml; p < 0.05), but similar levels of IgG2a, a subclass that is dependent on IFN-γ (Th2 cytokine) and not IL-4 (Fig. 2). This is consistent with the deficiency of IL-4.
Levels of autoantibodies in IL-4-deficient and wild-type BXSB mice

The effect of IL-4 deficiency on autoantibody production was also examined. Levels of both IgM and IgG Abs to chromatin and ssDNA were measured at 4–5 mo of age (Fig. 3). Although there was some variability in mean values among the IL-4+/+/, IL-4−/−, and IL-4+/− littermates, none of the differences reached statistical significance ($p > 0.05$).

Similar to what was observed with total IgG1 and IgG2a levels, IL-4 deficiency should skew the IgG1 and IgG2a anti-chromatin Ab response away from IgG1 production toward the IgG2a response. When this was examined, however, no difference in the IgG1 anti-chromatin levels could be detected because the IgG1 anti-chromatin levels were exceedingly low, below the lower limit of our standard curve at a serum dilution of 1:100, indicating a concentration <0.31 μg/ml (Fig. 4). In contrast, levels of IgG2a were similar for all IL-4 groups, with levels in the 300–400 μg/ml range. Thus, even in the IL-4+/− BXSB mice, the relative amounts of anti-chromatin IgG2a to IgG1 is much greater than would be expected from the ratio of total IgG2a to IgG1. This would strongly suggest a greater role for the Th1-type responses in the production of anti-chromatin Ab.

IL-4-deficient BXSB exhibit similar levels of memory/effector subset CD4+ T cells

A characteristic feature of autoimmune BXSB mice is the accelerated accumulation of effector/memory CD4+ T cells compared with nonautoimmune strains of mice (30, 39). As shown in Table I, splenocytes from 2-mo-old wild-type and IL-4-deficient BXSB mice exhibited similar percentages of CD4+ and CD8+ T lymphocytes in the spleen ($p > 0.05$). Importantly, there were no significant differences ($p > 0.05$) in the percentage of effector/memory phenotype CD4+ T cells in wild-type and IL-4−/− BXSB mice, either by the presence of CD44+ (22.0 ± 4.4% and 26.8 ± 7.0%, respectively) or CD62Llow (13.8 ± 3.2% and 16.0 ± 5.7%) cell surface expression. In contrast, non-autoimmune C57BL/6 mice had lower levels of CD4+ and CD4+CD44low T cells (p < 0.028) compared with both wild-type and IL-4+/− mutant BXSB mice.

Discussion

In this study, no significant difference in a broad range of autoimmune manifestations, including early mortality, GN, hyperIgG2a, anti-chromatin autoantibody, and increases in memory/effector phenotype Th cells were observed between homozygous IL-4−/− gene knockout BXSB mice and their IL-4 wild-type littermates. Interestingly, slight differences between these two groups and the IL-4−/− heterozygous group were noted for spleen weight and mortality, although the latter was not statistically significant. Overall, however, any differences between the IL-4−/− mice and the other groups were small as there was significant GN at 5 mo for most of the IL-4−/− heterozygous mice and autoantibody levels between the groups were similar. Because of the number of mice examined, small effects of IL-4 deficiency on GN and spleen size cannot be excluded. Nevertheless, the findings clearly demonstrate that IL-4 is not required for lupus-like disease in BXSB mice and contradict the notion that the Th2 response is important for the development of lupus.
The findings herein differ from studies of IL-4-deficient (MRL-Fas<sup>−/−</sup> × B6)F<sub>1</sub> mice, wherein IL-4-deficient mice generated similar levels of IgG2a and autoantibodies, but had significantly less lymphadenopathy and GN compared with IL-4 wild-type controls (3). This may reflect different immunopathologic mechanisms because of background gene differences between the (MRL-Fas<sup>−/−</sup> × B6)F<sub>1</sub> mice and the BXSB strain, or may possibly be due to a MRL susceptibility gene in linkage disequilibrium with the IL-4 gene on chromosome 11. In fact, a suggestive MRL locus on chromosome 11 linked to autoantibody production and vasculitis has been recently reported (40). On the other hand, studies showing reduced autoantibodies and nephritis in (NZB × NZWF<sub>1</sub>) and MRL-Fas<sup>−/−</sup> mice after treatment with blocking anti-IL-4 Ab or soluble IL-4R (32, 33) favors the argument that background genes are the determining factor. This implies the existence of IL-4-dependent and -independent mechanisms for developing lupus.

The current finding that lupus-like disease in BXSB mice does not require IL-4 is similar to what has been observed for several other humoral-mediated autoimmune diseases, including mercury-induced systemic autoimmunity (29, 35) and experimental autoimmune myasthenia gravis (EAMG) (41). In the case of EAMG, IL-4 deficiency may in fact facilitate development of disease (42). Thus, in most cases of Ab-mediated autoimmune diseases, knock-out of the IL-4 gene has resulted in no reduction or even worsening of disease.

The failure of the IL-4 deletion to alter disease severity in BXSB mice could be due to compensatory mechanisms that are sometimes observed in gene knockout mice. This seems unlikely, however, since IL-4 has been shown to be required for the generation of Th2 T cells in vitro (43, 44) and since the IL-4 knockout blocks Th2 type cytokines and IgG isotype responses (37, 45). Furthermore, the virtual absence of IgG1 anti-chromatin autoantibody relative to IgG2a and the lack of differences in IgG1 anti-chromatin autoantibody between the wild-type and IL-4 knockout BXSB mice support the contention that Th2 type responses are not important for disease in this model. Recent evidence, however, indicates that IL-13 is also important for development of Th2 T cells (46) and double knockout of IL-4 and IL-13 had more profound effect on Th2 development than knockout of either gene alone (47). Some of this overlap in function is due to shared IL-4 and IL13 receptor components and signal transduction molecules (48, 49). Nevertheless, IL-4 and IL-13 have nonoverlapping functions (48, 50), with IL-4 important for class switching to IgG1 (47) and Th2 cell development and IL-13 important for expulsion of parasites from the gut and in experimental models of allergic asthma (51, 52). Although it seems unlikely that IL-13 could completely compensate for the lack of IL-4 in the BXSB model, the availability of a double IL-4/IL-13 knockout created by simultaneously mutating these adjacent genes with a single construct (47) will make it possible to directly address this issue.

Santiago et al. (36) reported that overexpression of an IL-4 transgene in B cells reduced disease severity in (NZB × C57BL/6.YaaF<sub>1</sub>, lupus mice. Deletion of the IL-4 gene in BXSB mice, however, did not result in greater disease severity, which would be expected if normal levels of IL-4 were acting to down-regulate the autoimmune response. This suggests that disease suppression by transgenic expression of IL-4 may not represent the normal function of IL-4, but a consequence of pharmacologic doses of this cytokine. In this case, the reduction in GN may also be due to the antiinflammatory effects of IL-4 (53) in addition to the postulated deviation of pharmacologic doses of this cytokine.

Table I. Splenic T cell populations in IL-4<sup>−/−</sup> BXSB mice*  

<table>
<thead>
<tr>
<th>Strain</th>
<th>% T cells</th>
<th>% CD4&lt;sup&gt;+&lt;/sup&gt;</th>
<th>% CD4&lt;sup&gt;+&lt;/sup&gt; CD44&lt;sup&gt;dim&lt;/sup&gt;</th>
<th>% CD4&lt;sup&gt;+&lt;/sup&gt; CD62L&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>% CD8&lt;sup&gt;+&lt;/sup&gt;</th>
<th>% CD8&lt;sup&gt;+&lt;/sup&gt; CD44&lt;sup&gt;dim&lt;/sup&gt; CD62L&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>40.1 ± 2.4</td>
<td>63.8 ± 0.7</td>
<td>10.2 ± 2.1</td>
<td>9.4 ± 1.2</td>
<td>14.0 ± 1.8</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>BXSB</td>
<td>40.3 ± 1.8</td>
<td>67.9 ± 0.6*</td>
<td>22.0 ± 2.6***</td>
<td>13.8 ± 1.9</td>
<td>11.3 ± 0.4</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>IL-4&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>39.7 ± 1.5</td>
<td>71.0 ± 1.2**</td>
<td>26.8 ± 4.1*****</td>
<td>16.0 ± 3.3</td>
<td>11.6 ± 1.9</td>
<td>3.5 ± 0.4***</td>
</tr>
</tbody>
</table>

* Mean and SE are shown. Percentages are of all live-gated cells (column 2), T cells (column 3), CD4<sup>+</sup> cells (columns 4 and 5), or CD8<sup>+</sup> cells (columns 6 and 7). T cells included all CD4- or CD8-staining cells. Spleen cell suspensions from three to mo-old male mice were stained with anti-CD4-PerCP, anti-CD8-FITC, anti-CD44-PE, and anti-CD62L-biotin/streptavidin-APC. BXSB mice in this experiment are from the main colony.

* p = 0.011; ** p = 0.007; *** p < 0.028 (compared with B6 mice).

Acknowledgments

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References

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