Imbalance Towards Th1 Predominance Is Associated with Acceleration of Lupus-like Autoimmune Syndrome in MRL Mice

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Abbreviations used in this paper: AHGG, aggregated HGG; DN, double negative; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HGG, human IgG; ll, long lived; lpr, lymphoproliferation; RDU, relative densitometric unit; RT, reverse transcriptase; Yaa, Y-linked autoimmune acceleration.

Abstract

To investigate the respective roles of Th1 and Th2 cells in the pathogenesis of lupus-like autoimmune disease, we have analyzed the spontaneous and antigen-induced productions of IgG1 vs IgG2a and IgG3 subclasses in relation to the mRNA expression of INF-γ (Th1 cytokine promoting IgG2a and IgG3 production), IL-4 (Th2 cytokine promoting IgG1 production), and IL-10 (Th2 cytokine) in CD4⁺ T cells from lupus-prone MRL mice. For this purpose, two paired sets of MRL mice were chosen for the comparison of these parameters: (a) MRL-lpr/lpr (lpr for lymphoproliferation) and its recently described substrain with a prolonged survival, termed MRL-lpr/lpr.ill (ll for long lived) and (b) MRL male mice bearing the Yaa (Y-linked autoimmune acceleration) gene (MRL.Yaa) with an accelerated disease and their male counterparts lacking the Yaa gene. We demonstrate herein that the accelerated development of lupus-like autoimmune disease in MRL-lpr/lpr and MRL.Yaa mice, as compared with MRL-lpr/lpr.ill and MRL-+/-/+ mice, respectively, was correlated with an enhanced expression of IFN-γ vs IL-4 and IL-10 mRNA in CD4⁺ T cells, which paralleled with an increase of spontaneous and foreign T cell–dependent antigen-induced productions of IgG2a and IgG3 vs IgG1 antibodies. These data suggest that an imbalance towards Th1 predominance may play a significant role in the acceleration of lupus-like autoimmune disease in MRL mice. (J. Clin. Invest. 1996. 97:1597–1604.) Key words: systemic lupus erythematosus • T helper subset • autoantibody • cytokine • mutant mice

Introduction

The MRL strain spontaneously develops an autoimmune syndrome resembling human SLE characterized by the production of various autoantibodies and the development of fatal glomerulonephritis (1). It has been shown that the progression of lupus-like autoimmune syndrome in MRL mice is markedly accelerated by the presence of the lpr (lymphoproliferation) gene and the Yaa (Y-linked autoimmune acceleration) gene (1, 2). Although the molecular nature of the Yaa gene abnormality has not yet been identified, the lpr gene was recently found to cause defects in the Fas antigen which mediates apoptosis (3). More recently, we have established a substrain of MRL-lpr/lpr mice which live almost twice as long with delayed development of glomerulonephritis, termed MRL-lpr/lpr.ill (ll for long lived), as compared with conventional MRL-lpr/lpr mice (4). Since MRL-lpr/lpr.ill mice still carry the lpr mutation, a new mutation is likely to be responsible for the retardation of the lupus-like autoimmune syndrome.

It is now well established that the lpr or Yaa gene–mediated acceleration of lupus-like autoimmune disease is dependent on the presence of CD4⁺ T cells (5–9). However, the respective roles of two different T helper cell subsets, Th1 and Th2, exhibiting different capacities of cytokine secretion, in the development and acceleration of SLE have not yet been well defined. Since several cytokines produced by Th2 cells, such as IL-4, IL-5, IL-6, and IL-10, are known to promote antibody production by B cells (reviewed in reference 10), it has been speculated that Th2 cells may play an active role in the development of autoantibody-mediated autoimmune diseases such as SLE (11). In fact, a lupus-like autoimmune syndrome occurring in mice during graft-versus-host and host-versus-graft reactions has been well documented to be a result of the selective activation of Th2 type cells (12–15). However, the cytokine generated by the Th1 cells such as IFN-γ is also known to promote the secretion of IgG2a and IgG3 antibodies (10, 16, 17). In addition, the acceleration of SLE by repeated injections of recombinant IFN-γ and inhibition by anti–IFN-γ antibodies in (NZB × NZW)F1 hybrid mice (18) suggest the possible involvement of Th1 type cells in the spontaneous development and progression of SLE.

To assess the respective roles of Th1 and Th2 cells in the pathogenesis of SLE, we have analyzed the expression of IFN-γ, IL-4, and IL-10 mRNA in CD4⁺ T cells from lupus-prone MRL mice in relation to the production of IgG subclasses. To better compare these parameters, we have chosen two paired sets of MRL mice, i.e. (a) MRL-lpr/lpr and its substrain MRL-lpr/lpr.ill with a prolonged survival and (b) MRL male mice bearing the Yaa gene (MRL.Yaa) with an accelerated disease and their male counterparts lacking the Yaa gene (MRL-+/-/+); this is because serum levels of total IgG are al-

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1. Abbreviations used in this paper: AHGG, aggregated HGG; DN, double negative; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HGG, human IgG; ll, long lived; lpr, lymphoproliferation; RDU, relative densitometric unit; RT, reverse transcriptase; Yaa, Y-linked autoimmune acceleration.

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most comparable between MRL-lpr/lpr and MRL-lpr/lpr II mice and between MRL-Yaa male and MRL-lpr/lpr male mice, yet the progression of lupus-like autoimmune disease markedly differs between these two pairs of MRL mice (2, 4). We report herein that the acceleration of autoimmune disease in MRL-lpr/lpr or MRL-Yaa mice is correlated with an increased production of IgG2a and IgG3 vs IgG1 autoantibodies in parallel to an enhanced expression of IFN-γ vs IL-4 and IL-10 mRNA in CD4+ T cells, suggesting an involvement of Th1 type cells in the acceleration of lupus-like autoimmune disease in MRL mice.

Methods

Mice. MRL-lpr/lpr mice were originally obtained from the Jackson Laboratory in 1978 and were maintained at Centre de Service des Animaux de Laboratoire (Orléans, France). In 1988, offspring of a single pair of long-lived male and female MRL-lpr/lpr founder mice were bred by brother-sister mating. At the sixth generation, a subline with a prolonged survival, referred to as MRL-lpr/lpr II, was obtained (4). MRL-lpr/lpr male mice were purchased from Olac Laboratory, Oxon, UK. MRL-Yaa mice bearing the Yaa gene were obtained by transferring the Yaa gene from BXSB mice into MRL-lpr/lpr mice by backcross procedure as described (2). All lines of MRL mice have been kept under the same condition and only male mice were used for the present study. Mice were bred from the retroorbital plexus, and resulting sera were stored at −20°C until use.

Immunization. Human IgG (HGG) was heat-aggregated at 65°C for 30 min. Mice were immunized with an intravenous injection of 400 µg of heat-aggregated HGG (AHGG) in PBS. 10 µg of pneumococcal capsular polysaccharide antigen (Pneumovax-23; Merck Sharp & Dohme, West Point, PA) in PBS were intraperitoneally injected.

Serological assays. Serum levels of IgG subclasses were determined by ELISA as described (19). Briefly, rabbit anti-IgG subclass-specific antibodies (Litton Bionetics Inc., Kensington, MD) were used for coating the plates, and all assays were developed with the specific antibodies (Litton Bionetics Inc., Kensington, MD). Results are expressed in mg/ml in reference to a standard used for coating the plates, and the assays were developed with the specific antibodies (Litton Bionetics Inc., Kensington, MD). Results are expressed in titration units (U/ml) in reference to standard curves obtained from anti-DNA mAb.

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

Results

Enhanced serum levels of IgG2a and IgG3 vs IgG1 in MRL mice with an accelerated lupus-like autoimmune disease. The presence of the lpr mutation markedly shortens the life span of MRL male mice as a result of the accelerated development of lethal lupus-like nephritis (1). This was accompanied by a remarkable increase in serum levels of all the IgG subclasses and by the enhanced production of a large spectrum of autoantibodies, as compared with MRL-lpr/lpr II mice (27, 28). However, MRL-lpr/lpr II mice, a recently described substrain of MRL-
showed 1.5-fold and threefold increases in IgG2a and IgG3, re-

Table I. 50% Mortality Rates and Serum Levels of IgG in Four Different Lines of MRL Male Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>50% Mortality*</th>
<th>Total IgG</th>
<th>IgG1</th>
<th>IgG2a</th>
<th>IgG2b</th>
<th>IgG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>lpr/lpr</td>
<td>6 mo</td>
<td>58.7±19.9</td>
<td>9.6±3.5</td>
<td>41.2±17.3</td>
<td>3.1±1.3</td>
<td>4.7±2.4</td>
</tr>
<tr>
<td>lpr/lpr.ll</td>
<td>12 mo</td>
<td>50.3±11.9</td>
<td>18.3±8.2</td>
<td>27.1±8.6</td>
<td>2.9±1.0</td>
<td>1.7±0.7</td>
</tr>
<tr>
<td>Yaa</td>
<td>12 mo</td>
<td>25.5±9.7</td>
<td>7.9±2.2</td>
<td>15.7±7.2</td>
<td>0.6±0.1</td>
<td>1.4±0.9</td>
</tr>
<tr>
<td>+/+</td>
<td>18 mo</td>
<td>16.6±5.3</td>
<td>7.2±2.0</td>
<td>8.2±3.2</td>
<td>0.5±0.1</td>
<td>0.7±0.3</td>
</tr>
</tbody>
</table>

* 50% mortality rate due to glomerulonephritis. Serum levels of total IgG (addition of all the IgG subclasses) and IgG subclasses in MRL-lpr/lpr and MRL-lpr/lpr.ll male mice at 4 mo of age (18 mice in each group) and in MRL-Yaa and MRL-+/+ male mice at 6 mo of age (16 mice in each group). Results are expressed in mg/ml (±1 SD). † P < 0.001.

lpr/lpr mice with a prolonged survival (4), exhibited hypergam-
maglobulinemia and autoantibody production at an extent comparable to that of conventional MRL-lpr/lpr mice, yet their development of lupus-like nephritis was markedly re-
tarded (Table I). Although serum levels of total IgG did not signifi-
cantly differ between MRL-lpr/lpr and MRL-lpr/lpr.ll mice at 4 mo of age (P > 0.1), the analysis of IgG subclasses showed 1.5-fold and threefold increases in IgG2a and IgG3, respectively, but a twofold decrease in IgG1 in MRL-lpr/lpr mice, as compared with MRL-lpr/lpr.ll mice (P < 0.001) (Ta-
ble 1). Similarly, serum levels of IgG2a and IgG3 anti-DNA autoantibodies in MRL-lpr/lpr mice were significantly higher than those of MRL-lpr/lpr.ll mice (P < 0.001), while IgG1 anti-
DNA antibody levels were diminished in MRL-lpr/lpr mice (P < 0.001) (Table II). Consequently, when relative concentra-
tions of IgG2a and IgG3 anti-DNA antibodies vs IgG1 anti-
dNA antibodies were analyzed in individual animals, the differ-
ces between MRL-lpr/lpr and MRL-lpr/lpr.ll mice were highly significant (P < 0.001) (Fig. 1). However, no differences in ratios of IgG2a/IgG3 anti-DNA antibodies were observed in both MRL-lpr/lpr mice (P > 0.1).

The Yaa gene accelerated the progression of lupus-like au-
toimmune syndrome in MRL mice, as documented by early development of lethal glomerulonephritis, although to a lesser extent than that induced by the lpr gene (2). MRL-Yaa male mice had moderately but significantly increased concentrations of total IgG in their sera at 6 mo of age, as compared with MRL-+/+ males lacking the Yaa gene (P < 0.001) (Table I). The analysis of IgG subclass concentrations revealed that sera from MRL-Yaa male mice had twofold higher levels of IgG2a and IgG3 than those from MRL-+/+ male mice (P < 0.001), while levels of IgG1 and IgG2b were comparable (P > 0.05) (Table I). Comparison of serum levels of anti-DNA IgG sub-
classes showed relatively limited differences in IgG1 and IgG3 anti-DNA (P < 0.05), but not in IgG2a anti-DNA autoanti-
bodies (P > 0.1) (Table II). However, the analysis of relative concentrations of anti-DNA IgG subclasses in individual ani-
mals disclosed that MRL-Yaa mice exhibited highly enhanced ratios of IgG2a/IgG1 and IgG3/IgG1 anti-DNA antibodies, as compared with those of MRL-+/+ males (Fig. 1; P < 0.001).

Ratios of IgG2a/IgG3 anti-DNA antibodies did not signifi-
cantly differ in both MRL male mice (P > 0.05).

Enhanced expression of IFN-γ and IL-4 and IL-10 mRNA by CD4+ T cells in MRL mice with an accelerated lupus-like au-
toimmune disease. The demonstration of an increased produc-
tion of IgG2a and IgG3 vs IgG1 in association with an accel-
erated lupus-like autoimmune disease in MRL mice bearing the lpr or Yaa gene raised a possibility that these differences in IgG subclass expression may be related to differential activation of Th1 vs Th2 type cells during the course of autoimmune

Table II. Serum Levels of IgG1, IgG2a and IgG3 Anti-DNA Autoantibodies in Four Different Lines of MRL Male Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IgG1</th>
<th>IgG2a</th>
<th>IgG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>lpr/lpr</td>
<td>70±88†</td>
<td>405±329†</td>
<td>169±113†</td>
</tr>
<tr>
<td>lpr/lpr.ll</td>
<td>263±199†</td>
<td>271±222†</td>
<td>78±60†</td>
</tr>
<tr>
<td>Yaa</td>
<td>39±36§</td>
<td>73±37</td>
<td>53±35§</td>
</tr>
<tr>
<td>+/+</td>
<td>62±16§</td>
<td>67±23</td>
<td>29±18§</td>
</tr>
</tbody>
</table>

Serum levels of IgG1, IgG2a, and IgG3 anti-DNA antibodies in MRL-
lpr/lpr and MRL-lpr/lpr.ll male mice at 4 mo of age (18 mice in each group) and in MRL-Yaa and MRL-+/+ male mice at 6 mo of age (16 mice in each group). Results are expressed in U/ml (±1 SD). † P < 0.001; ‡ P < 0.05.

Figure 1. Relative concentrations of IgG2a/IgG1, IgG3/IgG1, and IgG2a/IgG3 anti-DNA antibodies in individual sera from 4-mo-old MRL-lpr/lpr and MRL-lpr/lpr.ll male mice and in sera from 6-mo-old MRL-Yaa and MRL-+/+ male mice.

Th1-mediated Acceleration of Murine Lupus
disease in MRL mice. To address this question, we used the RT-PCR and dot blot analysis to compare the in vivo expression of Th1 cytokine (IFN-γ) and Th2 cytokine (IL-4 and IL-10) mRNA in lymph node cells from different lines of MRL mice.

Comparative measurements of IFN-γ and IL-4/IL-10 mRNA expression in 4-mo-old MRL mice bearing the lpr mutation revealed that lymph nodes from MRL-lpr/lpr mice had ~ threefold higher IFN-γ signals than those from MRL-lpr/lpr.II mice (Fig. 2). In contrast, levels of IL-4 transcripts were conversely diminished in MRL-lpr/lpr mice, which were about three to ninefold lower than those of MRL-lpr/lpr.II mice, although no significant differences in IL-10 mRNA abundance were observed between these two lpr mice. However, densitometric measurements of dot blots revealed that in all MRL-lpr/lpr mice tested, relative expression of IFN-γ vs IL-10 mRNA as well as IFN-γ vs IL-4 mRNA was significantly elevated, as compared with MRL-lpr/lpr.II mice ($P < 0.01$) (Fig. 3).

In MRL-Yaa and MRL-++/+ male mice at 6 mo of age, the expression of IFN-γ mRNA in total lymph node cells was almost comparable, while MRL-++/+ lymph nodes exhibited approximately threefold increased levels of IL-4 and IL-10 specific mRNA (Fig. 2). Semiquantitatively, relative expression of IFN-γ vs IL-4 and IL-10 mRNA was highly enhanced in 5 of 6 MRL-Yaa mice tested, as compared with that in MRL-++/+ mice ($P = 0.025$) (Fig. 3).

To confirm that the observed differences in IFN-γ, IL-4, and IL-10 mRNA expression was due to a differential activation of Th1 vs Th2 type cells in MRL-lpr/lpr and MRL-Yaa mice having an accelerated disease, a similar analysis was performed on CD4+ T cells purified from their lymph nodes. Both CD4+ T cells from MRL-lpr/lpr and MRL-Yaa mice again exhibited an upregulated expression of IFN-γ vs IL-4 and IL-10 mRNA, as compared with those from MRL-lpr/lpr.II and MRL-++/+ mice, respectively (Fig. 3 and 4).

In addition to CD4+ T cells, we assessed the contribution of the lpr DN T cells, a major subpopulation of T cells in lpr lymph nodes, to the IFN-γ, IL-4, and IL-10 mRNA expression. Both DN T cells from MRL-lpr/lpr and MRL-lpr/lpr.II mice expressed IFN-γ and IL-10 mRNA at comparable levels, but failed to express detectable amounts of IL-4 mRNA (Fig. 4).

Since it has been recently shown that IL-12 plays an important role in inducing the generation of Th1 type cells from the Th0 cells (29), the expression of IL-12 mRNA in lymph node
cells was determined by RT-PCR combined with dot blot analysis. No significant increased expression of IL-12 mRNA, in relation to the GAPDH mRNA expression, was observed in lymph nodes from MRL-lpr/lpr or MRL.Yaa mice, as compared with those from MRL-lpr/lpr or MRL-+/+ mice (Fig. 2). No IL-12 transcripts were detected in purified CD4+ T cells and DN T cells (Fig. 4). Since the lpr DN T cells did not express IL-12 mRNA, the absent differences in IL-12 mRNA expression between MRL-lpr/lpr and MRL-lpr/lpr ili may be due to a massive accumulation of the lpr DN T cells in their lymph nodes. However, this possibility was unlikely, because IL-12 mRNA levels in lymph node or spleen cells depleted of T cells were comparable in both lpr mice (data not shown). Notably, levels of IL-12 mRNA in T cell–depleted lymph node or spleen cells did not differ between MRL.Yaa and MRL-+/+ mice (data not shown).

Enhanced production of IgG2a vs IgG1 antibodies against T cell–dependent foreign antigens in MRL-lpr/lpr and MRL.Yaa mice. To further assess an enhanced activity of Th1 type cells in MRL-lpr/lpr ili vs MRL-lpr/lpr ili mice and in MRL.Yaa vs MRL-+/+ mice, we determined whether the immunization with T cell–dependent foreign antigens led to an increased production of IgG2a vs IgG1 antibodies. 2-mo-old MRL male mice were immunized with a T cell–independent pneumococcal capsular polysaccharide, both males developed comparable titers of IgM (MRL.Yaa, 98±21 U/ml; MRL-+/+, 111±31 U/ml) and IgG3 (MRL.Yaa, 31±14 U/ml; MRL-+/+, 57±43 U/ml) anti-polysaccharide antibodies 7 and 14 d after immunization, respectively.

Discussion

In the present study, to assess the respective roles of Th1 and Th2 type cells in the acceleration of lupus-like autoimmune disease occurring in MRL mice bearing the lpr or Yaa gene, we have analyzed the spontaneous and foreign antigen-induced production of IgG subclasses in relation to the expression of Th1 cytokine (IFN-γ) and Th2 cytokine (IL-4 and IL-10) mRNA by using RT-PCR. We demonstrate herein that the accelerated development of lupus-like autoimmune disease in MRL-lpr/lpr ili and MRL.Yaa mice, as compared with MRL-lpr/lpr ili and MRL-+/+ mice. In contrast, when MRL.Yaa and MRL-+/+ mice were immunized with a T cell–dependent pneumococcal capsular polysaccharide, both males developed comparable titers of IgM (MRL.Yaa, 98±21 U/ml; MRL-+/+, 111±31 U/ml) and IgG3 (MRL.Yaa, 31±14 U/ml; MRL-+/+, 57±43 U/ml) anti-polysaccharide antibodies 7 and 14 d after immunization, respectively.

This conclusion is based on the fact that relative expression of IFN-γ vs IL-4 and IL-10 mRNA, as determined by RT-PCR in combination with a semiquantitative dot blot analysis, is highly enhanced in whole lymph nodes as well as CD4+ T cells from MRL-lpr/lpr ili or MRL.Yaa mice, as compared to appro-
appropriate control MRL mice. Although our measurements are not quantitative but only relative between IFN-γ and IL-4/IL-10 mRNA expression, the present conclusion is further substantiated by the demonstration that spontaneous and foreign T cell–dependent antigen-induced productions of IgG2a and IgG3 antibodies, including anti-DNA autoantibodies, are highly significantly elevated, as compared with that of IgG1 antibodies. It should be emphasized that the comparison between MRL-lpr/lpr and MRL-lpr/lpr,ll mice, though differing only in the presence of the lpr mutation, is not appropriate for the present purpose, since the lpr mutation causes defects in the Fas antigen which mediates apoptosis (3), resulting in a marked increase in all the IgG subclasses; this is partly due to an extension of the functional life span of B cells bearing the lpr mutation, as is the case of mice overexpressing a bcl-2 transgene in B-lineage cells (30). Nevertheless, it is worth noting that the increase in IgG1 levels in MRL-lpr/lpr vs MRL-+/+ mice is relatively limited, as compared with those of IgG2a and IgG3.

It should be mentioned that the lpr DN T cells, a unique T cell subset accumulating in lymph nodes of mice bearing the lpr mutation (23), express substantial levels of IFN-γ and IL-10 mRNA, yet we observed remarkable differences in IFN-γ mRNA expression, but not in IL-10 mRNA abundance, when mRNA levels in whole lymph nodes were compared between MRL-lpr/lpr and MRL-lpr/lpr,ll mice. This is in fact compatible with the findings that the lpr DN T cell subset is the major population (~70%) in MRL-lpr/lpr lymph nodes containing the increased presence of the Th1 type cells, while the lpr DN T cells are substantially diminished (~20%) in MRL-lpr/lpr,ll lymph nodes, in which CD4+ T cells exhibiting the Th2 predominance are the major population (~40%) (4). It can be speculated that a prolonged survival in MRL-lpr/lpr,ll mice may be in part related to the decreased presence of the
lpr DN T cells, since IFN-γ and IL-10 may promote the IgG2a and IgG3 production in MRL-\(lpr/lpr\) male mice. In fact, IL-10 has been shown to be a potent growth and differentiation factor for activated B cells (31) and to play a significant role in the autoantibody production in murine and human SLE (32, 33). However, the contribution by the \(lpr\) DN T cells, if any, may not be essential, since our recent study has shown that the spontaneous production of IgG2a and IgG3 was not significantly reduced in anti-CD8 mAb-treated MRL-\(lpr/lpr\) mice developing an only limited number of the \(lpr\) DN T cells (8).

At present it is difficult to answer how the \(Yaa\) gene abnormality is associated with the preferential activation of Th1 cells for spontaneous autoimmune and foreign antigen-induced immune responses, and how a possible new mutation present in MRL-\(lpr/lpr\) mice leads to the downregulation of Th1 responses in MRL-\(lpr/lpr\) mice. An attractive hypothesis is that these mutations may modulate the expression of molecules such as cytokines or adhesion molecules involved in the differentiation of Th0 cells towards Th1 or Th2 cells. In this regard, lack of significant increases in IL-12 mRNA transcripts in lymph nodes and spleens from MRL mice developing an accelerated disease at least argues against the possible involvement of an IL-12-dependent pathway in the observed modulation of Th1/Th2 responses.

It is significant that a relatively enhanced activation of Th1 vs Th2 type cells, leading to an increased production of IgG2a and IgG3, but a diminished production of IgG1, is associated with the acceleration of lupus nephritis in MRL mice bearing the \(lpr\) or \(Yaa\) gene. This is highly relevant to the immunopathogenesis of lupus nephritis. Since murine IgG2a, but not IgG1, antibodies activate far better the complement system, it is conceivable that the complement activating IgG2a autoantibodies can be more nephritogenic than IgG1 autoantibodies. More significantly, murine IgG3 mAb have been shown to be extremely nephritogenic, generating “wire-loop”-like glomerular lesions (34–36), characteristic in human lupus nephritis, because of their cryoglobulin activity associated with a unique physicochemical property of γ3 heavy chain constant region (37). In addition, several studies have provided evidence that the IgG3 production correlates well with the development of murine lupus nephritis (4, 38–40). Thus, an enhanced production of IgG3 antibodies is likely to be an important factor for the accelerated development of murine lupus nephritis in MRL mice bearing the \(lpr\) or \(Yaa\) gene.

The present observation adds a further insight to understanding how the \(Yaa\) gene accelerates the development of lupus-like autoimmune disease. We and others have previously shown that the autoimmune enhancing activity of the \(Yaa\) gene markedly differ in different lupus-prone mice, depending on the levels of autoantibodies spontaneously produced in the absence of the \(Yaa\) gene (2, 41–44). The \(Yaa\) gene-mediated enhancement of autoantibody production is most dramatic in mice that spontaneously synthesize relatively low amounts of autoantibodies, but limited or absent in mice that already produce substantially high titers. In addition, the present study revealed that in the latter mice, the \(Yaa\) gene apparently modifies the quality of autoantibody responses—upregulation of IgG2a and IgG3 production and downregulation of IgG1 production—by promoting the Th1 responses. Thus, the role of the \(Yaa\) gene for the acceleration of lupus-like autoimmune disease is twofold. First, the \(Yaa\) gene enhances autoimmune responses against antigens to which mice respond poorly; and second, it promotes Th1 responses over Th2 responses against antigens to which mice respond relatively well, thereby potentiating the production of more nephritogenic autoantibodies.

Our result is consistent with the fact that repeated injections of recombinant IFN-γ can accelerate the development of SLE, but the treatment with anti–IFN-γ mAb inhibits the progression of SLE in (NZB × NZW)F1 hybrid mice (18). One of the accelerating effects ascribed to IFN-γ, in addition to its immune potentiating activity, may be related to the enhanced production of cryogenic IgG3 and complement-activating IgG2a autoantibodies with immunopathological consequences. Although available data have demonstrated a good correlation between the relative predominance of Th1 cells and the accelerated development of lupus-like autoimmune disease, it remains to be determined at what extent Th1 cells are involved in the pathogenesis of SLE. Clearly, studies in lupus-prone mice overexpressing or deficient in Th1 or Th2 cell activity should provide further insight towards our understanding on the respective roles of Th1 and Th2 cells in SLE.
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