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

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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 mutant strain MRL-lpr/lpr.ll with an accelerated disease and their male counterparts lacking the Yaa 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.ll 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 mouse

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.ll (ll for long lived), as compared with conventional MRL-lpr/lpr mice (4). Since MRL-lpr/lpr.ll 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 sub-strain MRL-lpr/lpr.ll 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-
most comparable between MRL-lpr/lpr and MRL-lpr/lpr ll mice and between MRL-Yaa male and MRL-Yaa +/+ 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 ll, was obtained (4). MRL-Yaa +/+ mice were purchased from Olac Laboratory, Oxon, UK. MRL-Yaa mice bearing the Yaa gene were developed by transferring the Yaa gene from BXSB mice into MRL-Yaa +/+ 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 bled from the retroorbital plexus, and resulting sera were stored at −20°C until use.

Immunization. Human IgG (HGG) was heat-aggregated at 63°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 the assays were developed with the same corresponding antibodies conjugated with alkaline phosphatase. Results are expressed in mg/ml in reference to a standard curve obtained with mouse Ig reference serum (ICN ImmunoBiology, Costa Mesa, CA). IgM, IgG1, IgG2a, and IgG3 anti-DNA, anti-HGG, and anti-polysaccharide antibodies were measured by ELISA using anti-IgM (LO-MM-9) (20), anti-IgG1 (Ig[4a]10.9) (21), anti-IgG2a (Ig[1a]8.3) (21) and anti-IgG3 (H139.61.1) (22) mAbs conjugated with alkaline phosphatase. Monospecificities of the same corresponding antibodies conjugated with alkaline phosphatase were measured by ELISA as described (19). Briefly, rabbit anti-IgG subclass-specific antibodies (Litton Bionetics Inc., Kensington, CA) were used for coating the plates, and the assays were developed with the same corresponding antibodies conjugated with alkaline phosphatase. Results are expressed in mg/ml in reference to standard curves obtained with anti-DNA mAbs of different IgG subclasses and from CBA/J mice (Igh1) immunized with foreign antigens (HGG and polysaccharides).

Purification of CD4+ and CD4+ CD8+ double negative (DN) T cell and depletion of T cells. Single cell suspensions were prepared from a pool of axillary, mediastinal, retroperitoneal, and inguinal lymph nodes. To purify CD4+ T cells, lymph node cells were stained with a rat anti-CD4 (H129.19) mAb, followed by goat anti-rat IgG FITC conjugates, and then sorted with a FACStar (Becton Dickinson, Mountain View, CA). Thy-1-1 CD4+ CD8- DN T cells, a characteristic phenotype expanding in lymph nodes of MRL-lpr/lpr mice (23), were sorted after staining lymph node cells with a rat anti-Thy-1.2 (30-H12) mAb, followed by goat anti-rat IgG FITC conjugates, and then incubated with biotinylated anti-CD4 (H129.19) and anti-CD8 (H35-17.2) mAb, followed by phycoerythrin-conjugated avidin (Caltag Laboratories, San Francisco, CA). Cytodifferentiation analysis on FACScan revealed that purified CD4+ and DN cell populations contained <5% contaminating cells. To deplete T cells, lymph node or spleen cells were treated with a rat anti-Thy-1.2 (AT-83) mAb and rabbit complement. The efficiency of the treatment was documented by cytofluorometric analysis.

Reverse transcriptase (RT)-PCR. Total cellular RNA from lymph nodes and sorted lymph node cells were extracted by the guanidine isothiocyanate/CsCl method (24) and acid guanidinium thiocyanate-phenol-chloroform method (25), respectively. RNA was retrotranscribed after annealing with 1 μM oligo-dT primers (15 mer) in the presence of 100 U of RT (Life Technology Inc., Gaithersburg, MD), 20 U of RNAsin (Boehringer Mannheim, Mannheim, Germany), 10 mM DTT, and 500 nM each deoxynucleotide (Boehringer Mannheim) in a total volume of 20 μl at 1 h at 37°C. Aliquots of the mixture were then amplified in a thermocycler (Perkin-Elmer Corp., Norwalk, CT) in a volume of 50 μl with 20 U/ml of Taq polymerase (Boehringer Mannheim), 200 μM of each deoxynucleotide and 200 nM of each of the two primers. The cycling conditions were 0.5 min at 94°C for denaturation, 1.5 min at 55°C for annealing and 1.5 min at 72°C for elongation, and then 5 min at 72°C after the last cycle. Based on results obtained by titrations of cycle number which gave a logarithmic increase in the amount of specific amplified products, samples were amplified at 32 cycles for IFN-γ, IL-4, and IL-12 and at 25 cycles for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers had the following sequences, 5′-TGA-AACCTACACAGTCTTG-G-3′ and 3′-CGACCTCTTTTTCGC-TCTCTGAG-5′; IL-4, 5′-GTCCTGCTGTCAGACGGC-3′ and 3′-CATGTTGGCTGACTA-TA-5′; IL-10, 5′-TCAACAAAAGGACCA-GCGTGAAACCATCTG-3′ and 3′-CTGTCATGGCTTCCG-AGTCCAGAAGCTACA-A-5′; IL-12 (p40), 5′-CAACATCAAGAGCAGTAGAGGA-3′ and 3′-GCTAGAAGATTTATTA-ACA-GC-5′; GAPDH, 5′-TGAAGCTTGTAGTGAAGCAGTTGG-3′ and 3′-ACGACATCTCAAGACAGCATC-5′. The primer pairs used were designed to span over introns to avoid the amplification of potential contaminating genomic DNA. PCR products were visualized after electrophoresis through 2% agarose gels by staining with ethidium bromide. The product generated by IFN-γ primers is 460 bp, by IL-4 primers is 459 bp, by IL-10 primers is 420 bp, by IL-12 primers is 517 bp, and by GAPDH primers is 277 bp. To ensure the absence of sample contamination, a reaction mixture with no added cDNA was run in parallel with each PCR.

Dot blot analysis. Serially diluted amplified PCR products were blotted to nylon membranes (GeneScreen Plus; DuPont Co., Boston, MA). Hybridization was carried out under high stringency with a full-length mouse IFN-γ cDNA (a kind gift of Dr. W. Fiers, Ghent, Belgium), mouse IL-4 cDNA (pSP6KmIL-4374 [26]) and PCR amplified IL-10, IL-12, and GAPDH fragments, which were subcloned into pUC19 vector and verified by nucleotide sequencing, were used for preparing 32P-labeled probes. Quantification was performed by densitometric analysis on dot blots (Image Quant Software FAST SCAN; Molecular Dynamics Ltd., Kemsing, UK). The ratios of IFN-γ to IL-4 or IL-10 mRNA are referred to as relative densitometric units (RDU).

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-Yaa +/+ mice (27, 28). However, MRL-lpr/lpr.ll mice, a recently described strain of MRL-
lpr/lpr mice with a prolonged survival (4), exhibited hypergammaglobulinemia and autoantibody production at an extent comparable to that of conventional MRL-lpr/lpr mice, yet their development of lupus-like nephritis was markedly retarded (Table I). Although serum levels of total IgG did not significantly differ between MRL-lpr/lpr and MRL-lpr/lpr.II 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.II mice ($P < 0.01$) (Table I). Similarly, serum levels of IgG2a and IgG3 anti-DNA autoantibodies in MRL-lpr/lpr mice were significantly higher than those of MRL-lpr/lpr.II mice ($P < 0.001$), while IgG1 anti-DNA antibody levels were diminished in MRL-lpr/lpr.II mice ($P < 0.001$) (Table II). Consequently, when relative concentrations of IgG2a and IgG3 anti-DNA antibodies vs IgG1 anti-DNA antibodies were analyzed in individual animals, the differences between MRL-lpr/lpr and MRL-lpr/lpr.II 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 autoimmune 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 subclasses showed relatively limited differences in IgG1 and IgG3 anti-DNA ($P < 0.05$), but not in IgG2a anti-DNA autoantibodies ($P > 0.1$) (Table II). However, the analysis of relative concentrations of anti-DNA IgG subclasses in individual animals 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 significantly differ in both MRL male mice ($P > 0.05$).

Enhanced expression of IFN-γ vs IL-4 and IL-10 mRNA by CD4+ T cells in MRL mice with an accelerated lupus-like autoimmune disease. The demonstration of an increased production of IgG2a and IgG3 vs IgG1 in association with an accelerated 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 disease.

### 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$^\dagger$</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$^\ddagger$</td>
<td>41.2±17.3$^\ddagger$</td>
<td>3.1±1.3</td>
<td>4.7±2.4$^\ddagger$</td>
</tr>
<tr>
<td>lpr/lpr.II</td>
<td>12 mo</td>
<td>50.3±11.9</td>
<td>18.3±8.2$^\ddagger$</td>
<td>27.1±8.6$^\ddagger$</td>
<td>2.9±1.0</td>
<td>1.7±0.7$^\ddagger$</td>
</tr>
<tr>
<td>Yaa</td>
<td>12 mo</td>
<td>25.5±9.7$^b$</td>
<td>7.9±2.2</td>
<td>15.7±7.2$^b$</td>
<td>0.6±0.1</td>
<td>1.4±0.9$^b$</td>
</tr>
<tr>
<td>+/+</td>
<td>18 mo</td>
<td>16.6±5.3$^b$</td>
<td>7.2±2.0</td>
<td>8.2±3.2$^b$</td>
<td>0.5±0.1</td>
<td>0.7±0.3$^b$</td>
</tr>
</tbody>
</table>

*50% mortality rate due to glomerulonephritis. $^\ddagger$Serum levels of total IgG (addition of all the IgG subclasses) and IgG subclasses in MRL-lpr/lpr and MRL-lpr/lpr.II 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). $^\dagger$P < 0.001.; $^b$P < 0.05.

### 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$^\ddagger$</td>
<td>405±329$^\ddagger$</td>
<td>169±113$^\ddagger$</td>
</tr>
<tr>
<td>lpr/lpr.II</td>
<td>263±199$^\ddagger$</td>
<td>271±222$^\ddagger$</td>
<td>78±60$^\ddagger$</td>
</tr>
<tr>
<td>Yaa</td>
<td>39±36$^b$</td>
<td>73±37</td>
<td>53±35$^b$</td>
</tr>
<tr>
<td>+/+</td>
<td>62±16$^b$</td>
<td>67±23</td>
<td>29±18$^b$</td>
</tr>
</tbody>
</table>

Serum levels of IgG1, IgG2a, and IgG3 anti-DNA antibodies in MRL-lpr/lpr and MRL-lpr/lpr.II 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). $^\ddagger$P < 0.001.; $^b$P < 0.05.
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 ll 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 ll 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 ll mice tested, relative expression of IFN-γ vs IL-10 mRNA was significantly elevated, as compared with MRL-lpr/lpr ll 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 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 ll 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 ll 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.ill or MRL-+/+ mice (Fig. 2). No IL-12 transcripts were detected in purified CD4+ T 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.ill 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 vs MRL-lpr/lpr.ill 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 AHGG in PBS in order to avoid any effects of Freund’s adjuvant on the expression of Th1 and Th2 phenotypes. An intravenous injection of AHGG induced primary IgG anti-HGG antibody responses at comparable levels in all four lines of MRL mice (data not shown). However, the analysis of IgG1 and IgG2a anti-HGG antibodies revealed that MRL-lpr/lpr and MRL.Yaa mice produced higher titers of IgG2a anti-HGG antibodies than MRL-lpr/lpr.ill and MRL-+/+ mice (Fig. 5). This markedly contrasted with a limited production of IgG1 anti-HGG antibodies in MRL-lpr/lpr and MRL.Yaa mice, as compared with MRL-lpr/lpr.ill and MRL-+/+ mice. In contrast, when MRL.Yaa and MRL-+/+ 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 and MRL.Yaa mice, as compared with MRL-lpr/lpr.ill and MRL-+/+ mice, is well correlated with an enhanced expression of IFN-γ vs IL-4 and IL-10 mRNA in CD4+ T cells, which parallels to an increase of spontaneous and foreign T cell–dependent antigen-induced productions of IgG2a and IgG3 vs IgG1. Our results suggest that an imbalance towards Th1 predominance may play a role in the acceleration of lupus-like autoimmune disease in MRL mice bearing the lpr or Yaa mutation.

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 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-+/+ and MRL-lpr/lpr 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.11 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.11 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.11 mice may be in part related to the decreased presence of the

Figure 4. IFN-γ, IL-4, IL-10, IL-12, and GAPDH mRNA expression in CD4+ and lpr DN T cells from 4-mo-old MRL-lpr/lpr and MRL-lpr/lpr.11 male mice (A) and in CD4+ T cells from 6-mo-old MRL-Yaa and MRL-+/+ male mice (B). PCR was performed with cDNA samples from CD4+ and lpr DN T cells purified from lymph nodes pooled from three animals for each different MRL lines. PCR products (10 μl of the PCR mix) were visualized after electrophoresis through 2% agarose gels by staining with ethidium bromide (M, molecular weight marker [DNA molecular weight marker VI, Boehringer Mannheim]. –, PCR mix without cDNA). Then, reciprocally diluted samples (1.2, 0.4, and 0.13 μl of the PCR mix) from amplified PCR products were applied to nylon membrane and hybridized with corresponding probes. Representative results of three separate experiments are shown.
Serum levels of IgG1 and IgG2a anti-HGG antibodies in MRL mice were undetectable in sera from unimmunized MRL mice (pressed in U/ml). IgG1 and IgG2a anti-HGG activities were determined by ELISA, and results are expressed in U/ml. IgG1 and IgG2a anti-HGG activities were undetectable in sera from unimmunized MRL mice (<1 U/ml).

Figure 5. Serum levels of IgG1 and IgG2a anti-HGG antibodies in MRL-lpr/lpr and MRL-lpr/lpr.II male mice and in MRL-Yaa and MRL-+/+ male mice. 400 μg of AHGG were intravenously injected into 2-mo-old MRL mice. 10 d later, serum levels of IgG1 and IgG2a anti-HGG antibodies were determined by ELISA, and results are expressed in U/ml. IgG1 and IgG2a anti-HGG activities were undetectable in sera from unimmunized MRL mice (<1 U/ml).

lpr DN T cells, since IFN-γ and IL-10 may promote the IgG2a and IgG3 production in MRL-lpr/lpr 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.II 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 immunopotentiating 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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