Greatly Reduced Lymphoproliferation in \textit{lpr} Mice Lacking Major Histocompatibility Complex Class I

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Summary

Mice homozygous for the \textit{lpr} gene have a defect in \textit{fas} (CD95), a cell surface receptor that belongs to the tumor necrosis factor receptor family and that mediates apoptosis. This genetic abnormality results in lymphoproliferation characterized by the accumulation of CD4−CD8− (double negative [DN]) T cells, autoantibody production, and background strain-dependent, end-organ disease. Our previous results suggested that major histocompatibility complex (MHC) class I may be involved in the development of DN cells. To test this hypothesis, we derived C57BL/6-\textit{lpr}/\textit{lpr} (B6/\textit{lpr}) mice that were deficient for the \textit{B2m-\textit{lpr}} (B6/lpr) mice that were deficient for the \textit{B}2\textit{m-\textit{lpr}} and had no detectable class I expression. At 6 mo of age, compared with B6/\textit{lpr} littermates with normal class I genes, these mice showed greatly reduced lymphadenopathy, mostly due to a dramatic decrease in the number of DN cells. Significant changes in the percentage of other T cell subsets were noted, but only γ/δ T cells showed a marked increase in both percentage and absolute numbers. Analysis of T cell receptor Vβ expression of the remaining DN T cells in \textit{B2m-\textit{lpr}} mice showed a shift to a CD4-like repertoire from a CD8-like repertoire in control B6/\textit{lpr} mice, indicating that a small MHC class II selected DN population was unmasked in \textit{lpr} mice lacking class I. We also found that the production of immunoglobulin G (IgG) autoantibodies (antichromatin and anti-single stranded DNA), total IgG and IgG2a, but not total IgM or IgM rheumatoid factor, was significantly reduced in the \textit{B2m-\textit{lpr}} mice. This work suggests that \textgreater{}90\% of DN T cells in \textit{lpr} mice are derived from the CD8 lineage and are selected on class I. However, a T cell subset selected on class II and T cells expressing γ/δ are also affected by the \textit{lpr} defect and become minor components of the aberrant DN population.

The \textit{lpr} murine model is characterized by autoantibody production, strain-dependent, end-organ autoimmune disease, and severe lymphadenopathy (1). The bulk of the lymphadenopathy is accounted for by the accumulation of TCR α/β T cells that are CD4− and CD8− (double negative [DN]) and aberrantly express other markers such as B220. Recent work has demonstrated that the \textit{lpr} defect is due to a transposon disrupting the gene coding for \textit{fas} (CD95), a cell surface receptor that belongs to the TNF-receptor family and that is found on activated lymphocytes (2) and other tissues (3). Cross-linking by \textit{fas} ligand or by antibodies to \textit{fas} induces apoptosis in cells expressing this receptor (4–9).

Abbreviations used in this paper: \textit{B2m}, \textit{B}2\textit{m}-microglobulin; DN, double negative; ss, single stranded.

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The derivation of the DN T cells in \textit{lpr} mice and the reason they develop in the setting of defective expression of \textit{fas} is mostly unknown. Previous studies demonstrated that these cells undergo negative selection during intrathymic development, despite the markedly decreased expression of \textit{fas} in the \textit{lpr} thymus (10–12). These studies also suggested that the DN T cells are derived from thymic precursors that express CD4 and CD8, a conclusion supported by the the methyl-ation pattern of the CD8 gene in these cells (13). Other studies have indicated that the DN T cells proliferate predominantly in the periphery (11, 14–16). It has been postulated that DN T cells may represent autoactive cells that cannot be deleted secondary to their \textit{fas} defect; this may explain their possible role in the autoimmune process (17). Mature CD4+ T cells from MRL-\textit{lpr}/\textit{lpr} mice have been shown to be resistant to anti-CD3 or antigen-mediated apoptosis (18, 19) and peripheral CD8+ cells appear to be resistant to superantigen-mediated deletion (20). Whether the cells that fail to undergo apoptosis subsequently downregulate CD4 or CD8 and become DN is unknown.
Previous work in our laboratories analyzing the TCR V~ repertoire of DN T cells demonstrated a pattern similar to that of CD8+ T cells (1). When comparisons were made to determine the influence of different class I and II MHC molecules on T cell repertoire formation, the results suggested that DN T cells in lpr mice may be selected on class I MHC molecules. Selection on MHC class II could not be demonstrated. In the present studies, we have extended these observations by studying lpr mice lacking β2-microglobulin (β2m-). These mice have deficient expression of class I MHC molecules, which mostly prevents the development of the CD8+ T cell subset (21). Compared with lpr β2m+ littermates, β2m- lpr mice showed a dramatic decrease in absolute numbers and percentages of CD8+ and DN T cells, and consequently greatly reduced lymphadenopathy. Analysis of the remaining DN T cells showed that their TCR V~ repertoire resembled that of CD4+ cells, suggesting selection on MHC class II. We also noted a significant decrease in IgG, but not IgM, total immunoglobulin and autoantibody levels in these knockout mice.

Materials and Methods

Mice. Mice with the β2m gene disrupted (β2m-) and backcrossed six generations to the C57BL/6 (B6) strain were kindly provided by Beverly Keller (University of North Carolina at Chapel Hill (22, 23)). We backcrossed these mice twice to B6-lpr/lpr mice originally obtained from The Jackson Laboratory (Bar Harbor, ME). Individual mice genotyped as homozygous for lpr and heterozygous for the β2m knockout were interbred to generate lpr/β2m- mice deficient for the β2m gene (β2m-lpr/lpr) and β2m- lpr littermate controls homozygous for the β2m wild-type gene (β2m+/lpr). β2m and lpr phenotypes were confirmed by lack of expression of MHC class I antigens, as determined by cell surface staining and cytotoxicity analysis, and the accumulation of Thy1.2+B220+ T cells, respectively. Mice were maintained at the University of North Carolina School of Medicine animal facility.

Genotyping. Genomic DNA was prepared from tails. Oligonucleotide primers for the β2m- gene were located at the 5’ and 3’ ends of the disrupted β2m exon II and the 3’ end of the inserted Neo gene (β2m3': GTG GCC CCT AGA AAC CCC TC; β2m5': AGA CGG TCT TGG CTT CGG CC; and Neo3': ACG GTA TCG CCG CTC CGG AT, respectively; 22). Oligonucleotide primers for lpr locus were designed based on the published nucleotide sequence for the insertion of the early transposable element (ETn) into intron II of the fas gene in lpr mice (2). Primers were located 5’ to the ETn insertion site, 3’ to the ETn insertion site, and in the 3’ end of the ETn (FAS5': CAA GCC GTG CCC TAG GAA ACA CAG; FAS3': GCA GAG ATG CTA AGC AGC AGC CGG; and ETN5': GTG GAG CTC CAA TGC AGT CCT, respectively). Amplifications were performed in separate three-primer 50-μl PCR reactions under standard conditions with 1.0 mM MgCl2 using AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). Amplification was performed for 30 cycles with temperatures of 94°C melting, 55°C annealing, and 72°C extension. PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining. Using the three primers mentioned above, the β2m+ three-primer PCR generated products of 284 and 200 bp for the wild-type and knockout genes, respectively. Similarly, the lpr three-primer PCR generated products of 240 and 445 bp for the wild-type fas and mutant lpr genes, respectively. This permitted the detection of the heterozygous and both homozygous states utilizing a single PCR reaction for each gene.

Preparation of Cells. At 6 mo of age, mice were killed, and cervical, axillary, scapular, femoral, mesenteric, periaortic, and subrenal LN and spleens were removed and weighed. Single-cell suspensions of all LN and spleen cells were made by passing them through a mesh screen in cold medium (RPMI 1640 with 15 mM Heps, 5% FCS [HyClone Laboratories, Logan, UT], 100 U/ml penicillin, and 100 μg/ml streptomycin from the University of North Carolina Cancer Center, and 5 x 10^-4 M 2-ME [Sigma Chemical Co., St. Louis, MO]). RBC were lysed with NH4Cl, and cells were washed twice before counting by an automated cell counter. For experiments analyzing expression of Vβ chains, lymph node T cells were first prepared by nylon wool purification (10).

Antibodies. Antibodies used for immunofluorescence staining included: anti-IgM (AF6-78-FITC, mouse IgG1; PharMingen, Inc., San Diego, CA), anti-FcRIII (CD16) (2.4G2, rat IgG1) (24), anti-CD45R/B220 (RA3-6B2-BNHS, rat IgG2a; PharMingen), anti-H-2Dk (28-14-8S, mouse IgG2a; courtesy of Jeffrey Frelinger, University of North Carolina at Chapel Hill, Chapel Hill, NC) (25), and anti-mouse IgG2a (goat polyclonal-FITC; Boehringer Mannheim, Indianapolis, IN). Anti-CD4, anti-CD8, anti-Thyl.2, and mouse anti-rat κ light chain were previously described (26, 27). The mAb to CD4, CD8, and TCR Vβ chains used in the Vβ repertoire studies were previously described (11).

Immunofluorescence Staining and Flow Cytometry. In 96-well microtiter plates, 1.5 x 10^4 cells were stained in cold media containing 3% FCS and 0.1% NaN3. In general, antibodies were incubated with cells on ice for 30 min. Streptavidin–R–phycoerythrin (Southern Biotechnology Associates, Birmingham, AL) was used as a second reagent. Cell surface immunofluorescence analysis of LN and spleen cells was performed by single- and two-color flow cytometry analysis of at least 10^4 events for each sample on a FACScan® (Becton Dickinson & Co., Mountain View, CA) with Cytometry data acquisition and software (Fort Collins, CO) with size gating on the lymphocyte population.

Two-color immunofluorescence was used to analyze TCR Vβ expression separately in the CD4+, CD8+, CD4−CD8− (analyzed by costaining with both fluoresceinated anti-CD4 and CD8 antibodies), and B220+ T cell populations as described (11). In initial experiments, results for DN cells and B220+ T cell were virtually identical, and therefore data are only presented for DN cells. Cell stainings with anti-Vβ mAb and their analysis were performed as described (11).

Quantification of Immunoglobulin and Autoantibodies Levels by ELISA. Mice were bled monthly from 3 to 6 mo of age. Serum samples were assayed in duplicate for total IgG, IgG2a, and IgM levels, as well as IgG antichromatin, IgG anti-single stranded (ss) DNA, and IgM RF anti-IgG2b activity, as previously described (28, 29). Results for antichromatin and anti-ssDNA antibodies are reported in equivalent dilution factors (EDF) of standardized reference MRL/lpr sera as previously defined by the formula: EDF = (dilution of standard reference sera which gives the equivalent OD of the test serum) x 10^6 (30).

Statistics. Student’s t-test was used to determine the statistical significance of differences between groups.

Results

Decreased Lymphadenopathy in β2m−/lpr Mice. Lpr (i.e., lpr/lpr) mice genotyped as homozygous for the β2m-knockout locus (β2m") and lpr littermates carrying wild-type
Figure 1. Weights and total cell numbers for LNs and spleens in 
\( \beta_{2m}^{-}\) Ipr (■) (n = 10), \( \beta_{2m}^{+}\) Ipr (◼) (n = 12), and C57BL/6 (□) (n = 6). Data (mean ± SE) are shown for mice killed at 6 mo of age. Statistical comparisons are to the \( \beta_{2m}^{-}\) Ipr group: (ns) not significant using \( p = 0.05 \) as a cutoff, (*) \( p < 0.05 \) and (**) \( p < 0.005 \).

\( \beta_{2m} \) genes (\( \beta_{2m}^{+}\) Ipr) were followed for the development of lymphoproliferation and autoimmunity. None of the mice showed evidence of obvious clinical disease or infection during the first 6 mo of life. Both groups of mice were killed at 6 mo of age, and LNs and spleens were removed and weighed. The lack of expression of class I MHC in \( \beta_{2m}^{-}\) Ipr mice was confirmed by cell surface staining of LN and spleen cells with an anti-H-2D b reagent. Cytofluorographic patterns for \( \beta_{2m}^{-}\) Ipr cells were indistinguishable from the background staining of control cells from MP, L(H-2 k) mice (data not shown). As shown in Fig. 1, the \( \beta_{2m}^{-}\) Ipr mice showed a marked decrease in lymphadenopathy compared with the control group. This difference reflected a fourfold decrease in total LN cell numbers in the \( \beta_{2m}^{-}\) Ipr group compared with controls. Compared with non-Ipr B6 mice, however, the LN cell numbers in the \( \beta_{2m}^{-}\) Ipr group were still increased sevenfold. Fig. 1 also shows that spleen weights and cell numbers in \( \beta_{2m}^{-}\) Ipr mice were only slightly reduced, and that these differences were not significant.

**Reduced Accumulation of DN T Cells in \( \beta_{2m}^{-}\) Ipr Mice.** Similar to \( \beta_{2m} \)-knockout mice that do not carry the Ipr mutation, \( \beta_{2m}^{-}\) Ipr mice showed the expected decrease in the percentage and number of CD8 + cells (Fig. 2, A and B). Importantly, \( \beta_{2m}^{-}\) Ipr mice also demonstrated a marked reduction in the percentage and number of aberrant DN T cells, best identified as the Thy1.2 + CD4 - CD8 - subset. The difference between the Thy1.2 + CD4 + CD8 - and the Thy1.2 + B220 + subset was due to the inclusion of the CD4 + B220 + subsets in the latter population (data not shown) (31). Whereas DN T cells accounted for 50–70% of total LN cells in \( \beta_{2m}^{-}\) Ipr mice, this percentage decreased to <20% in \( \beta_{2m}^{-}\) Ipr. Coupled with the decrease in total cell numbers, this resulted in a 90% decrease in the number of DN cells in the \( \beta_{2m}^{-}\) Ipr group (Fig. 2 B).

Nearly all DN T cells in the \( \beta_{2m}^{-}\) Ipr mice expressed the \( \alpha/\beta \) TCR (Fig. 3). However, TCR \( \alpha/\beta^{+} \) cells accounted for only 55% of the DN T cells in \( \beta_{2m}^{-}\) Ipr mice (Fig. 3). The remaining DN T cells expressed TCR \( \gamma/\delta \) receptors, which meant a 30-fold increase in the percentage of TCR \( \gamma/\delta \) in \( \beta_{2m}^{-}\) Ipr DN cells compared with controls. Despite the decrease in total T cells in \( \beta_{2m}^{-}\) Ipr mice, the absolute number of \( \gamma/\delta^{+} \) cells was actually increased in these mice (22 ± 1.4 × 10^6 vs. 11 ± 3.3 × 10^6, \( p < 0.005 \)).

The reduction in DN cells and loss of CD8 + cells accounted for a significant increase in the percentage of CD4 + cells in the \( \beta_{2m}^{-}\) Ipr group when compared with controls (\( p = 0.001 \)). However, because of the reduced total T cell numbers, this difference in percentage did not result in a significant increase in total CD4 + cells numbers (Fig. 2 B). The CD4 + analysis included both the CD4 + B220 - and the abnormal CD4 + B220 + subsets found in Ipr mice. Separate experiments staining for this latter population showed no difference between the \( \beta_{2m}^{-}\) Ipr and \( \beta_{2m}^{+}\) Ipr mice (data not shown).

**Changes in TCR Vß Repertoire of Residual DN Cells in \( \beta_{2m}^{-}\) Ipr Mice.** We compared the TCR Vß repertoire of DN T cells to that expressed by CD4 + and CD8 + single positive cells in \( \beta_{2m}^{-}\) Ipr and \( \beta_{2m}^{+}\) Ipr mice. The repertoire
of the CD4+ cells was remarkably similar in the two groups of mice (Fig. 4 A). Because of the very low percentage of CD8+ cells in β2m−/− mice, the CD8+ repertoire was analyzed only in β2m−/−lpr mice. The results of the CD4+ and CD8+ repertoires in these experiments were similar to our previous reports (11). Particular differences in Vβ expression between the CD4+ and CD8+ populations should be emphasized. For example, the percentage of Vβ5+ cells was significantly greater in the CD8+ population, whereas the opposite was true for Vβ14 expression. These differences reflect the relative selection of CD8+ cells on MHC class I in B6 (i.e., H-2b) mice (32, 33).

The DN cells from the β2m−/−lpr group showed a TCR Vβ usage pattern distinct from that of the β2m−/−lpr group (Fig. 4 B). As previously observed in B6-lpr/lpr mice, the repertoire of the β2m−/−lpr DN cells was similar to that of the CD8+ subset. Remarkably, TCR Vβ expression of DN cells in β2m−/−lpr mice shifted to a pattern that resembled more the CD4+ subset (compare Fig. 4, A and B). In particular, the significant decrease in Vβ5 and increase in Vβ14 usage by β2m−/−lpr DN cells distinguished them from DN T cells in β2m−/−lpr and paralleled the CD4+ cells from both groups.

Previous studies from our laboratories have also demonstrated an increased percentage of DN Vβ8.3+ cells, out of proportion to levels in the CD4+ or CD8+ subsets (11). This selective expansion of DN Vβ8.3+ T cells was also apparent in β2m−/−lpr mice (Fig. 4 B). Thus, the percentage expressing Vβ8.3 in the DN T cell population was 20 vs. 8.7% of CD4+ cells and 8.2% of CD8+ cells in the control group (p <0.001 for both groups). However this expansion of Vβ8.3+ DN cells was no longer apparent in β2m−/−lpr mice (11% in DN cells).

Decreased Total IgG and IgG Autoantibodies Levels in β2m−/−lpr Mice. We also determined the effect of class I deficiency on autoantibody production and total Ig levels. Fig. 5 A shows the increased production of IgG antichromatin in β2m−/−lpr mice, which is markedly reduced in β2m−/−lpr mice. Similarly, β2m−/−lpr mice produced much less IgG anti-ssDNA with age than controls (Fig. 5 B). In contrast, IgM RF anti-IgG2b activity levels were equally elevated at 3 mo of age and increased at the same rate in both groups (Fig. 5 C). β2m−/−lpr mice showed the expected age-dependent increase in total IgG2a (Fig. 5 D), total IgG (Fig. 5 E), and total IgM levels (Fig. 5 F). Interestingly, the levels in the

**Figure 3.** Percentage of DN cells expressing CD3, TCR α/β, or TCR γ/δ in β2m−/−lpr (■) (n = 7) and β2m−/−lpr (■) (n = 7) mice (mean ± SE). LN cells from 6-mo-old mice were purified over nylon wool columns before staining for CD4, CD8, and the respective TCR marker. (*) p <0.015 and (**) p <0.005.

**Figure 4.** TCR Vβ repertoire in β2m−/−lpr compared with β2m−/−lpr. LN cells were purified over nylon wool columns before staining for different Vβs and CD4, CD8, or CD4 and CD8. Results are shown for the mean percentage (+_ SE) of CD4 or CD8 cells (A) and of DN cells in (B) (4–11 different mice in each group were analyzed for each Vβ). Because of the low percentage of CD8+ cells in β2m−/−lpr mice (see Fig. 2), the repertoire of this population could not be reliably determined. Values for the CD8 population for Vβ3 and Vβ6 were determined in a separate group of age-matched B6-lpr/lpr mice. Statistical comparisons shown are between CD4-β2m+ or CD4-β2m+ and CD8-β2m+ subsets in (A) and between DN subsets for Vβ5, 8.3, and 14 in (B): (*) p <0.005. Statistical analysis was not performed for the other Vβ subsets.
\( \beta_{2m}^{-lpr} \) mice paralleled the pattern seen in the autoantibodies with significantly lower total IgG and total IgG2a levels but no difference in the total IgM levels.

In contrast to MRL/lpr mice, B6/lpr mice demonstrate minimal glomerular pathology (34 and our unpublished data). Kidneys from two \( \beta_{2m}^{-lpr} \) mice were examined by light microscopy for pathologic abnormalities. Not surprisingly, especially considering the decreased IgG autoantibody levels in these mice, no abnormalities were noted.

**Discussion**

The current studies were undertaken to understand better the derivation of DN T cells in \( lpr \) mice. The results demonstrate that the accumulation of aberrant DN cells is mostly abrogated in \( lpr \) mice deficient in \( \beta_{2m} \) expression. However, the reduction in lymphoproliferation in these mice also unmasked a minor TCR \( \alpha/\beta^{+} \) DN subset with a repertoire similar to that of the CD4^{+} population as well as an expanded DN population expressing \( \gamma/\delta \) TCR. We also noted that \( lpr \) mice carrying the \( \beta_{2m} \) knockout gene had decreased levels of total IgG and IgG autoantibodies, although the explanation for this effect may be complex.

We found that >90% of the DN cells were eliminated in \( lpr \) mice deficient in \( \beta_{2m} \) expression. In a previous TCR repertoire analysis of \( lpr \) mice, we noted that DN cells expressed a V\( \beta \) pattern resembling that of the CD8^{+} population (11). Furthermore, particular class I molecules influenced both DN and CD8^{+} V\( \beta \) repertoires in a similar fashion. The present results extend these earlier studies and strongly support the hypothesis that most of the DN population is selected and/or expanded on class I MHC. The close connection of DN cells with the CD8 lineage is further supported by recent studies (35) showing that anti-CD8 mAb treatment prevents the development of most DN cells. The findings are also consistent with the methylation pattern of the CD8 gene in these cells, reflecting previous expression (13).

It seems likely that both intrathymic selection and peripheral proliferation of most DN cells involve recognition of class I. Lymphoproliferation in \( lpr \) mice requires peripheral expansion of lymphocyte subsets. Thus, thymectomy performed more than a few weeks after birth, and before the appearance of B220^{+} T cells, has little effect on the subsequent accumulation of DN T cells (11, 16). We previously showed that V\( \beta \)8.3^{+} T cells are overrepresented in the DN population compared with the CD4^{+} or CD8^{+} subsets. Longitudinal studies and studies of thymectomized animals indicated that this subset of DN cells is selectively expanded in the periphery.

In the present studies, the accumulation of DN V\( \beta \)8.3^{+} T cells was also apparent in \( \beta_{2m}^{-lpr} \) mice but was absent in \( \beta_{2m}^{-lpr} \) mice, indicating that this V\( \beta \)-selective increase is a class I-dependent phenomenon. The nature of the class I MHC-antigen complex which interacts with V\( \beta \)8.3 is unclear. In preliminary work (Kotzin, B. L., V. Kakkanaiah, E. Roper, P. Cohen, and R. Eisenberg, unpublished observations), the DN V\( \beta \)8.3^{+} cells in \( lpr \) mice have not been found to be oligoclonal and there have not been any repeated CDR3 motifs. The only similar V region element among these cells therefore appears to be the V\( \beta \). We do not yet understand this observation, because neither a class I–presented superantigen nor a V\( \beta \)8.3-selective superantigen has yet been described.

The presence of a residual DN population in \( \beta_{2m}^{-lpr} \)
mice prompted us to determine whether these cells were CD8- or CD4-like in their TCR repertoire. β2m-knockout mice may have detectable levels of class I, most likely related to surface expression of small amounts of class I α chain alone, and the few CD8+ T cells they produce can be shown to proliferate in the periphery (36, 37). Therefore, it was possible that the remaining DN cells in β2m- lpr mice were selected on this unusual type of class I. However, our analysis of Vβ expression in DN cells indicated a shift from a CD8-like pattern in control β2m+ lpr mice to a CD4-like Vβ pattern in β2m- lpr mice. These results suggest that a relatively small component of DN cells in lpr mice has been selected on class II rather than class I MHC. The fact that this subset is <10% of the total DN population in lpr mice may explain apparent discrepancies with previous studies. For example, our previous attempt to demonstrate selection of DN Vβ17+ cells on class II could have been obscured by opposite trends in the subset of Vβ17+ cells selected on class I (11). Similarly, other studies showed little change in lymphoproliferation in class II knockout lpr mice, but it seems unlikely that a <10% decrease of DN cells would have been detected by the methods used in these experiments (38).

It is of interest that Bix et al. (39) also used β2m- mice to show that the normal DN TCR α/β+ thymocytes in B6 mice are mostly selected on MHC class I. Although some characteristics of this thymic population suggest a possible relationship to peripheral lpr DN T cells, major differences between these two DN T cell populations are apparent. Importantly, the thymic DN cells are strongly skewed towards Vβ8.2 expression, whereas the lpr DN show a more diverse Vβ repertoire with a preferential use of Vβ8.3. The small peripheral DN T cell population present in non-lpr mice has also been proposed as a normal counterpart of lpr DN T cells, but TCR Vβ analysis has demonstrated a lack of evidence for intrathymic positive and negative selection that is characteristic of lpr DN T cells (11, 40).

Nearly all DN cells in lpr mice express the α/β TCR (1). It is unclear whether a very small component of the aberrant T cell population may express γδ TCR. Interestingly, in β2m- lpr mice, we noted a striking increase in the percentage and number of γδ+ T cells. Compared with normal animals (41), the increase in γδ+ T cells in β2m- lpr mice was >100-fold, reflecting a 10–15-fold increase in percentage and a ninefold increase in LN cell number. Thus, the lpr mutation may cause an expansion of the γδ+ T cell population, most likely because peripheral elimination of a subset of these cells is prevented by decreased expression of fas. Why the number of γδ+ T cells was increased twofold in β2m- lpr compared with β2m+ lpr mice remains unclear, especially since CD4+ cells did not increase in a similar fashion.

IgG autoantibodies and total IgG levels, but not IgM autoantibodies or total IgM levels were reduced in β2m- lpr mice. The patterns suggest that there was not a selective effect on autoantibody versus normal antibody population. These findings may also relate to previous studies of nonautoimmune β2m- mice in which antibody production to exogenous antigens and total IgG, but not total IgM, levels were similarly reduced (42). These earlier studies have led to the hypothesis that there is a role for class I in B cell development and/or in the B-T cell interactions necessary for efficient IgG antibody production (21). Previous studies of lpr mice have shown the expected dependence of autoantibody production on CD4+ T cells and MHC class II expression (27, 38, 43, 44). In the present studies, it is possible but less likely that the absence of class I-dependent DN cells is responsible for the reduced autoantibody production in these mice.

Previous studies from our laboratories and others indicate that positive and negative selection within the thymus is mostly normal in lpr mice (10–12). Thus, intrathymic events appear to be independent of Fas, implying that another system mediating apoptosis must be involved. In contrast, other studies (17–20) suggest that peripheral tolerance mechanisms may be severely affected by the lpr mutation and decreased expression of Fas. The present work supports the concept that all T cell subsets, including CD4+, CD8+, and γδ+ cells, are influenced by the impairment of Fas-mediated apoptosis, although the effect on CD8+ cells appears to be the major factor in generating the DN population. Downregulation of accessory CD4 or CD8 molecules also appears to be a consequence of a lesion in the Fas pathway (11). Despite evidence for some selectivity for particular TCR in the peripheral expansion process, the heterogeneous repertoire of the DN T cells indicates that many clones are expanded. Whether these clones have an autoreactive potential remains a major question.

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