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Immunology

Correction of accelerated autoimmune disease by early replacement of the mutated *lpr* gene with the normal *Fas* apoptosis gene in the T cells of transgenic MRL-*lpr/lpr* mice

(systemic lupus erythematosus/autoimmunity)

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ABSTRACT **MRL-*lpr/lpr* mice develop a generalized autoimmune disease which includes increased autoantibody production, glomerulonephritis, and development of lymphadenopathy. The *lpr* genetic defect has been identified as a mutation in the *Fas* apoptosis gene that results in low expression of *Fas* mRNA. To determine the significance of the *lpr* mutation and T cells in the development of the autoimmune disease, we constructed transgenic MRL-*lpr/lpr* mice using a full-length murine *Fas* cDNA under the regulation of the T-cell-specific CD2 promoter and enhancer. Here we show that the early correction of the *lpr* gene defect in T cells eliminates glomerulonephritis and development of lymphadenopathy and decreases the levels of autoantibodies. In this model, early correction of the *lpr* defect in T cells is sufficient to eliminate the acceleration of autoimmune disease even in the presence of B cells and other cells that express the mutant *lpr* gene.**

MRL-*lpr/lpr* mice develop lymphadenopathy, hypergammaglobulinemia, serum autoantibodies, and a generalized autoimmune disease including glomerulonephritis and arthritis and have been used as a model for the study of systemic lupus erythematosus (1). T cells and serum autoantibodies contribute to the development of autoimmune disease, but it has been difficult to determine which of these abnormalities are primary defects and which are secondary. Neonatal thymectomy was shown to reduce autoimmunity and lymphadenopathy in MRL-*lpr/lpr* mice (2, 3). $CD4^+$ peripheral T-cells have been indirectly implicated by the observation that autoimmune disease can be blocked by treatment with anti-CD4 (4). The *lpr* gene also confers an intrinsic B-cell defect that is independent of the T-cell defects, as increased serum immunoglobulin levels and autoantibody production are produced preferentially after bone marrow transfer by *lpr/lpr* B cells, but not +/+ B cells (5, 6).

In CBA/J-lpr^{cg} mice the *lpr* gene has been identified as a mutation of the intracellular signaling domain of the *Fas* apoptosis gene (7, 8). In MRL-*lpr/lpr* mice, the *lpr* gene is a mutation of the extracellular domain of the Fas gene and is due to the insertion of a retroviral transposon of the ETn type (9–11). Despite this evidence that the Fas gene is mutated in MRL-*lpr/lpr* mice, it is not clear how this gene mutation could lead to the profound lymphadenopathy and autoimmunity that is observed in these mice, since only minor defects in apoptosis have been detected during the development of T cells (12–16).

During neonatal tolerance to staphylococcal enterotoxin B (SEB) in $V_{\beta 8}$ T-cell receptor transgenic MRL-*lpr/lpr* and MRL-+/+ mice, clonal deletion of self-reactive T cells occurred normally in the thymus of MRL-*lpr/lpr* mice (17). However, we observed a dramatic loss of tolerance to SEB in the thymus and periphery of MRL-*lpr/lpr* mice but not MRL-+/+ mice, after ceasing SEB treatment. These results suggested that, in *lpr/lpr* mice tolerance loss might be related to factors other than defective apoptosis.

To directly determine whether defective *Fas* expression is responsible for the lymphoproliferative autoimmune disease in *lpr/lpr* mice, we have restored normal Fas gene expression by injection of a *CD2–Fas* transgene into single-cell embryos of MRL-*lpr/lpr* mice. This approach has allowed production of Fas transgenic MRL-*lpr/lpr* mice without the necessity of backcrossing. By placing the Fas gene under the regulation of a *CD2* promoter/enhancer, we ensured that high Fas expression was present in T cells, but not in B cells (18, 19). Analysis of these *CD2–Fas* transgenic mice indicated that restoration of *Fas* expression in the T cells of MRL-*lpr/lpr* mice led to correction of the lymphoproliferative disease, elimination of the $CD4^-CD8^-B220^+$ T cells, and abrogation of autoimmune disease. Furthermore, there was normalization of serum immunoglobulin levels and elimination of autoantibody production of the IgG2a isotype, indicating that the intrinsic defect of *Fas* expression in B cells is not sufficient for production of these features of autoimmunity.

MATERIALS AND METHODS

Preparation of cDNA. The 1.1-kb full-length *Fas* cDNA was obtained by polymerase chain reaction (PCR) amplification of cDNA made from thymus mRNA from MRL-+/+ mice. The full-length cDNA PCR primers were P1 (5′-GGC-CGC-CCG-CTG-CCG-CTG-TTT-TCC-CTT-GCT-GCA-GAG-3′, position +20) and P4 (5′-ATT-GAC-ATT-GGC-AAC-TCC-TGG-TGT-3′, position 1110). Sequence positions are referenced to the published murine *Fas* sequence (20). The full-length *Fas* cDNA was cloned into an *Eco*RI site in front of exon 1 of a human *CD2* minigene consisting of 5.5 kb of 5′ flanking sequence, exon 1, the first intron, fused exons 2–5, and 2.1 kb of the 3′ flanking sequence (19). The 3′ sequence has been shown to be sufficient to allow T-cell-specific, copy-dependent, integration-independent expression in transgenic mice (18, 19).

Production of Transgenic Mice. MRL-*lpr/lpr* male and female mice were obtained from The Jackson Laboratory. Single-cell MRL-*lpr/lpr* embryos were produced by mating of male and pseudopregnant female MRL-*lpr/lpr* mice. These embryos were injected with ≈100 copies of the *CD2–Fas* transgene and then placed into the distal oviduct of CD-1

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Abbreviations: dsDNA, double-stranded DNA; HPRT, hypoxanthine phosphoribosyltransferase; PE, phycoerythrin; FITC, fluorescein isothiocyanate.

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pseudopregnant female mice as described (21). Tail DNA was prepared from offspring (22), digested with *Eco*RI, and probed with a ^{32}P-labeled 345-bp *Fas* cDNA corresponding to a portion of the extracellular domain (8).

Northern Blot Analysis. RNA was prepared from thymus and lymph node of the *CD2–Fas* transgenic and nontransgenic MRL-*lpr/lpr* and MRL-+/+ mice, electrophoresed, blotted, and probed with the *Fas* cDNA as described (12). An identical blot was probed with a ^{32}P-labeled actin cDNA probe. The relative expression of *Fas* RNA relative to β-actin RNA was determined by quantifying the amount of hybridized probe on a PhosphorImager system (Molecular Dynamics).

Immunofluorescence Analysis and Cell Sorting. Single-cell suspensions were prepared and samples of 10^6 cells were surface-labeled for two-color immunofluorescence analysis and sorting (16). Thymocytes were labeled with phycoerythrin (PE)-conjugated anti-CD8 and fluorescein isothiocyanate (FITC)-conjugated anti-CD4. Lymph node cells were labeled with PE-conjugated anti-Thy-1.2 and FITC-conjugated anti-B220. Cells (10,000 per sample) were analyzed by flow cytometry on a FACScan (Becton Dickinson) equipped with logarithmic scales, and the data were processed in a Hewlett–Packard computer.

Cells were sorted and total RNA was then extracted from the homogenates of equal numbers (10^5) of cells by the guanidinium/cesium chloride method. Total RNA (2–4 μg) was used for cDNA synthesis followed by PCR amplification using the Perkin–Elmer RNA-PCR kit. Various numbers of PCR cycles were performed followed by extension for 10 min. Gels were blotted and hybridized to a labeled internal *Fas* probe to verify that the bands were *Fas*-specific. A unique larger PCR product was observed with thymic RNA from different MRL-*lpr/lpr* mice, as previously described (8). For each sample, PCR was also simultaneously carried out using hypoxanthine phosphoribosyltransferase (HPRT) primers (Perkin–Elmer), and the products were electrophoresed, blotted, and probed with a labeled internal HPRT oligonucleotide probe. The amount of hybridized probe was quantitated on a PhosphorImager and the intensity [log(cpm)] of each specific PCR product was plotted against the number of PCR cycles. The ratio of *Fas* expression was determined relative to HPRT gene expression for different PCR cycles.

Histologic Evaluation. Kidneys were removed and fixed in neutral buffered 10% formalin and prepared for routine light microscopy according to standard techniques. Histologic evaluation was carried out on sections stained with hematoxylin and eosin. At least 25 glomeruli of each specimen were graded semiquantitatively by an observer who was unaware of the source of tissue. Kidney sections were graded for number of cells (0, normal; 4, maximum proliferation) or sclerosis (0, normal; 4, maximum sclerosis). The grades were averaged to provide a glomerular disease severity score for each group as previously described (22).

Blood Urea Nitrogen. Blood urea nitrogen was assayed on whole blood samples by the Azostix method (Ames, Elkhart, IN).

Serum Immunoglobulin and Levels of Antibodies to Double-Stranded DNA (dsDNA). Serum immunoglobulin levels and antibodies to dsDNA were measured by an isotype-specific ELISA (16).

RESULTS

The *CD2–Fas* transgene was injected directly into single-cell embryos of MRL-*lpr/lpr* mice. Southern blot analysis indicated the presence of the *Fas* transgene in founder mouse 140 and its offspring as indicated by the presence of the 1.1-kb transgenic *Fas* band in addition to a single 8-kb endogenous *Fas* band found in MRL-*lpr/lpr* mice (Fig. 1*A*, lanes 4 and 5). MRL-+/+ mice had a single germ-line band at ≈10 kb (lane 1). Parental MRL-*lpr/lpr* mice had a single germ-line band at ≈8 kb (lane 3), whereas (MRL-*lpr/lpr* × MRL-+/+)F_1 mice had germ-line bands at both 8 and 10 kb (lane 2).

To confirm that the *Fas* transgene was expressed in lymphoid cells, we first analyzed the expression of the *Fas* transgene in offspring from founder mouse 140. RNA was prepared from thymus and lymph nodes of MRL-+/+ mice as well as nontransgenic and *CD2–Fas* transgenic MRL-*lpr/lpr* littermate mice. The RNA was electrophoresed and blotted, and identical blots were probed with either *Fas* cDNA or β-actin cDNA (Fig. 1*B*). The blots were washed, and the amount of hybridized probe was quantitated by PhosphorImager. The expression ratio of *Fas* relative to β-actin is shown below the *Fas* expression blot (Fig. 1*B*). There were nearly equivalent levels of *Fas* expression in the thymus and lymph nodes of MRL-+/+ mice compared with the thymus and lymph nodes of *CD2–Fas* transgenic MRL-*lpr/lpr* mice (Fig. 1*B*, lanes 1 and 3 and lanes 4 and 6). By Northern blot analysis, the level of *Fas* expression relative to

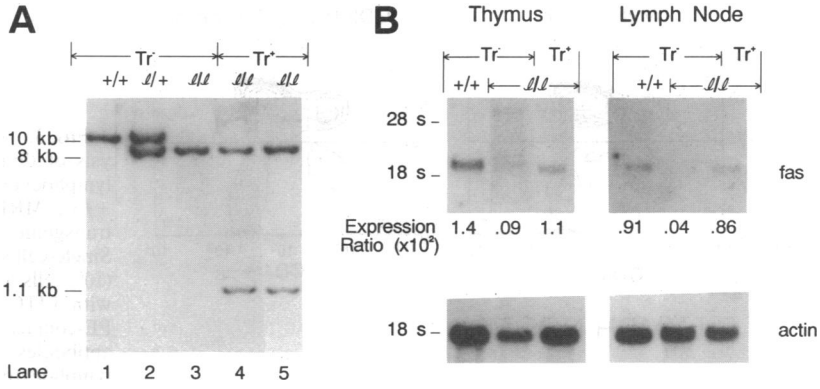

FIG. 1. (*A*) Southern blot analysis of DNA from *CD2–Fas* transgenic (Tr⁺) MRL-*lpr/lpr* mice. Tail DNA samples from MRL-+/+ (lane 1), (MRL-*lpr/lpr* × MRL-+/+)F_1 (lane 2), MRL-*lpr/lpr* (lane 3), and two CD2-*Fas* transgenic MRL-*lpr/lpr* mice (lanes 4 and 5) were digested with the *Eco*RI restriction enzyme, which releases the 1.1-kb *Fas* cDNA insert from the *CD2–Fas* transgenic mice. After blotting and hybridization with a *Fas* extracellular-domain cDNA probe (9), the washed blot was exposed for 72 hr. (*B*) RNA prepared from thymus and lymph node of 10-week-old MRL-+/+, MRL-*lpr/lpr*, and *CD2–Fas* transgenic MRL-*lpr/lpr* mice. RNA (≈20 μg) was electrophoresed, blotted, and hybridized with a probe for either *Fas* or β-actin on identical Northern blots. The autoradiographs were exposed for 48 hr (*Fas*) or 2 hr (actin) to Kodak XAR film, and also the signal was quantitated after exposure for equal times to a PhosphorImager (Molecular Dynamics), to allow an accurate determination of the ratio of Fas expression relative to actin expression as indicated below the *Fas* Northern blot. Positions of 28S and 18S rRNA are indicated.

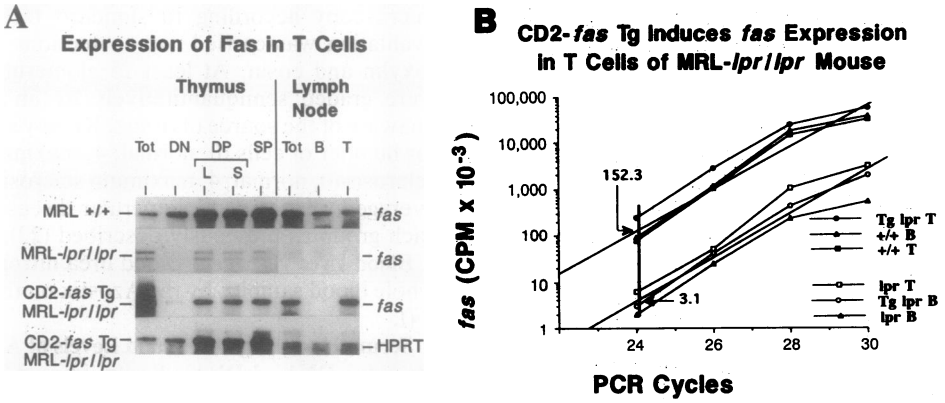

FIG. 2. (*A*) Thymocytes from MRL-+/+, MRL-*lpr/lpr*, and *CD2–Fas* transgenic MRL-*lpr/lpr* mice were either unsorted (Tot) or fluorescence-sorted into $CD4^-CD8^-$ double-negative (DN), $CD4^+CD8^+$ double-positive large (DP L), $CD4^+CD8^+$ small (DP S), or $CD4^+$ or $CD8^+$ single-positive (SP) populations and *Fas* expression was determined by PCR analysis. Lymph node populations were either unsorted (Tot) or sorted into B cells and T cells and *Fas* expression was determined by PCR analysis. (*B*) RNA, cDNA, and PCR analyses for *Fas* expression were carried out using different numbers of PCR cycles to ensure that relative *Fas* expression was determined using the linear portion of PCR production curve. The PCR products were electrophoresed, blotted, and hybridized with an internal *Fas*-specific cDNA probe that had been labeled with ^{32}P. After washing and autoradiography, the *Fas*-specific PCR products were quantitated with a PhosphorImager to accurately determine the intensity of the hybridizing probe in terms of cpm. There was a log-linear relationship of cpm to PCR cycles. The line indicates the least-squares fit to *Fas* expression in B cells from MRL-+/+ mice (▲, $r = 0.96$) and *Fas* expression in B cells from *CD2–Fas* transgenic (Tg) MRL-*lpr/lpr* mice (○, $r = 0.95$). B-cell *Fas* expression, in terms of cpm, derived from these curve fits at 24 PCR cycles is 152.3×10^3 and 3.1×10^3 for MRL-+/+ and *CD2–Fas* transgenic MRL-*lpr/lpr* mice, respectively. There was no significant change in expression of a control HPRT gene for different populations of thymocytes and lymph node cells in the *CD2–Fas* transgenic MRL-*lpr/lpr* mice.

β-actin expression in the thymus of nontransgenic MRL-*lpr/lpr* mice was 0.09×10^{-2} (≈8%) and 0.04×10^{-2} (≈5%) of that observed in MRL-+/+ mice and in the *CD2–Fas* transgenic MRL-*lpr/lpr* mice (Fig. 1*B*, lanes 2 and 5).

We next examined the expression of the *Fas* transgene in subpopulations of thymocytes and lymphocytes by PCR analysis on fluorescence-sorted cell populations. Since abnormal T-cell development in MRL-*lpr/lpr* mice has been proposed to occur during early T-cell development in the thymus, we first wanted to determine whether *Fas* expression was restored in both early and late stages of T-cell development in the thymus. In MRL-+/+ mice there was high RNA expression of *Fas* in total thymocytes (Fig. 2). High *Fas* expression was present both in early-stage $CD4^-CD8^-$ double-negative and $CD4^+CD8^+$ double-positive large thymocytes, and in later-stage $CD4^+CD8^+$ small and $CD4^+$ or $CD8^+$ single-positive thymocytes as well as in fluorescence-sorted T cells and B cells from the lymph nodes. In MRL-*lpr/lpr* mice, *Fas* expression was decreased in all thymocyte populations as well as lymph node B cells and T cells (Fig. 2*A*). In contrast, in *CD2–Fas* transgenic MRL-*lpr/lpr* mice, *Fas* expression was increased relative to nontransgenic MRL-*lpr/lpr* mice in both early- and late-stage thymocytes and in lymph node T cells, but not in lymph node B cells (Fig. 2). The lack of *Fas* expression in B cells of *CD2–Fas* transgenic MRL-*lpr/lpr* mice was not due to the absence of B-cell RNA, since there was no significant change in expression of the HPRT gene in different populations of thymocytes and lymph node cells (Fig. 2*A*). The expression of nearly equivalent levels of RNA encoding the HPRT gene was verified for different cell populations of nontransgenic MRL-+/+ and MRL-*lpr/lpr* mice (data not shown).

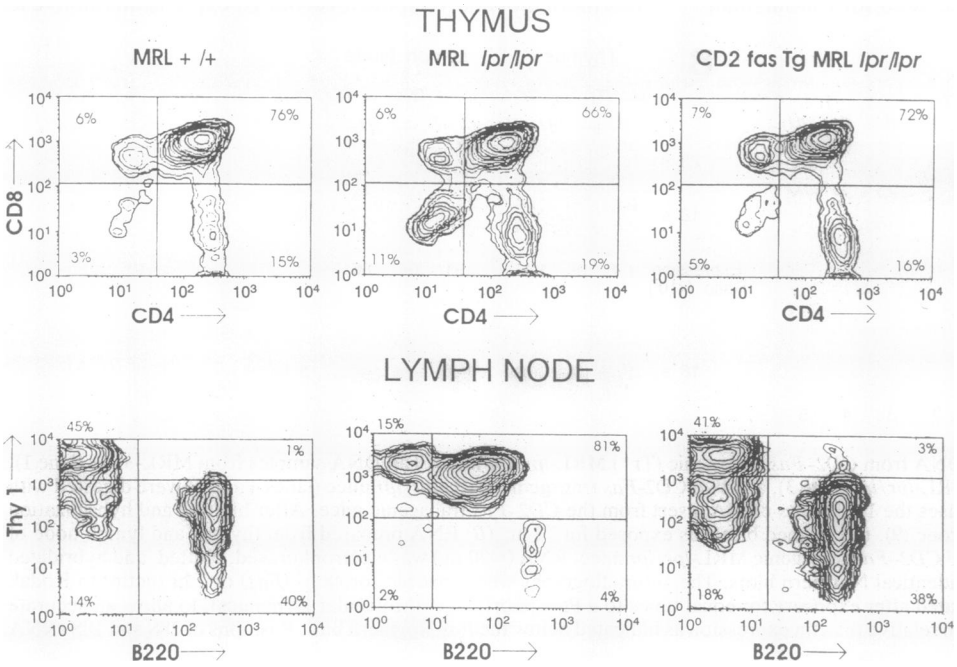

FIG. 3. Immunofluorescence analysis of thymocytes and lymph node lymphocytes from 18-week-old MRL-+/+, MRL-*lpr/lpr*, and *CD2–Fas* transgenic (Tg) MRL-*lpr/lpr* mice. Single-cell suspensions of thymocytes (10^6 cells per sample) were labeled with FITC-conjugated anti-CD4 and PE-conjugated anti-CD8 monoclonal antibodies. Single-cell suspensions of lymphocytes (10^6 per sample) were labeled with FITC-conjugated anti-B220 and PE-conjugated anti-Thy-1. Viable cells (10,000 per sample) were analyzed by flow cytometry on a FACScan with logarithmic scales. The gates used to define the populations are indicated by solid lines and the calculated percentage of cells in each quadrant is displayed. Each graph is representative of six mice.

Table 1. Effect of the *CD2–Fas* transgene on lymphadenopathy, blood urea nitrogen, and autoantibody production in MRL-*lpr/lpr* mice

Strain	Transgene	*n*	B220+ T cells,* %	LN cells,† no. × 10^{-6}	BUN,‡ mg/dl	GN§	Ig,¶ mg/ml IgG1	Ig,¶ mg/ml IgG2a	Anti-dsDNA‖ IgG1	Anti-dsDNA‖ IgG2a
MRL-*lpr/lpr*	−	30	81 ± 10	56.5 ± 8.5	64.2 ± 9.1	3.6 ± 0.8	2.4 ± 0.5	1.7 ± 0.26	0.21 ± 0.02	0.77 ± 0.12
MRL-*lpr/lpr*	+	13	3 ± 1	0.59 ± 0.07	29.6 ± 6.3	0.8 ± 0.4	0.97 ± 0.08	0.67 ± 0.13	0.14 ± 0.01	0.09 ± 0.02
MRL-+/+	−	35	1 ± 1	0.45 ± 0.06	21.1 ± 6.1	0.6 ± 0.3	0.82 ± 0.08	0.51 ± 0.16	0.04 ± 0.01	0.05 ± 0.01

CD2-fas transgenic (+) and nontransgenic (−) mice were evaluated at 18 weeks of age. *n*, No. of mice.
*Thy-1+B220+ lymph node T-cells were determined by flow cytofluorimetry, and the results are presented as the mean ± SEM.
†No. of cells per lymph node (mean ± SEM).
‡Blood urea nitrogen on whole blood assayed by Azostix (Ames) (0, not present; 4+, 80–100 mg/dl).
§Glomerulonephritis assayed by histologic analysis (0, not present; 4+, maximum severity).
¶Serum levels (mean ± SEM) measured by an isotype-specific ELISA including isotype standards of known concentrations.
‖Serum anti-dsDNA (mean ± SEM) determined by ELISA and expressed as OD at 405 nm.

Fas expression in lymph node T and B cells from MRL-+/+ mice, nontransgenic, and *CD2–Fas* transgenic mice was quantitated by analysis of the PCR results using a PhosphorImager as described in Fig. 1*B*. There was a log-linear increase in *Fas* expression as a function of PCR cycle number for each sample (Fig. 2*B*). There was a 25- to 50-fold increase in expression of *Fas* in T cells and B cells from the lymph nodes of MRL-+/+ mice relative to T cells and B cells from MRL-*lpr/lpr* mice, as indicated by the expression level at 24 PCR cycles (152.3 compared with 3.1). Expression of *Fas* in T cells from *CD2–Fas* transgenic MRL-*lpr/lpr* mice (●) was comparable to *Fas* expression in T cells from MRL-+/+ mice (■) (Fig. 2*B*). The curves are a least-square log-linear fit of *Fas* expression in B cells of MRL-+/+ mice (▲) relative to *Fas* expression in B cells of *CD2–Fas* transgenic MRL-*lpr/lpr* mice (○). There was a marked decrease in *Fas* expression in B cells from *CD2–Fas* transgenic MRL-*lpr/lpr* mice compared with B cells from MRL-+/+ mice. There was no significant difference in *Fas* expression in B cells from *CD2–Fas* transgenic MRL-*lpr/lpr* mice compared with B cells from nontransgenic MRL-*lpr/lpr* mice. These results are consistent with previous findings that the *CD2* promoter/enhancer used in these transgenic mice results in preferential transgene expression in T cells (18, 19).

Having established that approximately normal levels of *Fas* expression were present in thymocytes and lymph node T cells in *CD2–Fas* transgenic MRL-*lpr/lpr* mice, we analyzed the mice to determine whether expression of the *Fas* transgene could eliminate the development of the Thy-1.2+B220+ T cells. Peripheral blood T cells were analyzed in *CD2–Fas* transgenic mice and in control littermates at 4.5 months of age. There was a decrease of CD4−CD8− thymocytes in 18-week-old *CD2–Fas* transgenic MRL-*lpr/lpr* mice compared with nontransgenic MRL-*lpr/lpr* mice (Fig. 3 *Upper*). The predominant effect of the *CD2–Fas* transgene was to nearly eliminate the Thy-1.2+B220+ subset of T cells in the lymph node of *CD2–Fas* transgenic MRL-*lpr/lpr* mice compared with nontransgenic MRL-*lpr/lpr* mice (Fig. 3; Table 1). Further, although massive lymphoproliferative disease occurred in all nontransgenic siblings, none of the *CD2–Fas* transgenic mice had developed lymphadenopathy by 4.5 months of age.

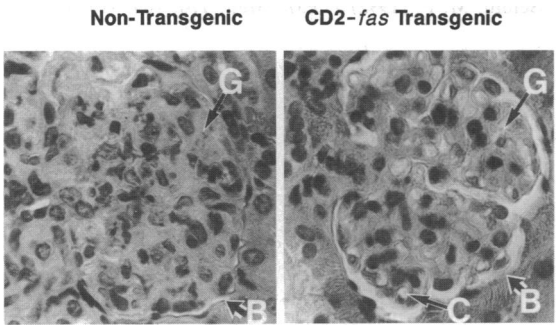

FIG. 4. Histological analysis of glomerulonephritis in nontransgenic (*Left*) and *CD2–Fas* transgenic (*Right*) 4.5-month-old female MRL-*lpr/lpr* mice. Tissue sections were stained with hematoxylin and eosin and the glomerulus (G) was photographed. In nontransgenic mice, there was thickening of the capillary walls and mesangium and loss of Bowman's space (B). In CD2-*Fas* transgenic mice, capillary tufts (C) and Bowman's space were present.

MRL-*lpr/lpr* mice developed elevated blood urea nitrogen and glomerulonephritis by 4.5 months of age (Table 1; Fig. 4 *Left*). Blood urea nitrogen was normalized in the *CD2–Fas* transgenic MRL-*lpr/lpr* mice (Table 1) and the glomerulonephritis was eliminated (Table 1; Fig. 4 *Right*). These results indicate that normalization of *Fas* expression in T cells of MRL-*lpr/lpr* mice is sufficient to eliminate autoimmune renal disease.

To determine whether selective expression of normal levels of *Fas* in T cells, but not B cells, of MRL-*lpr/lpr* mice, could affect the development of autoantibody-secreting B cells, we compared the levels of serum immunoglobulin and anti-dsDNA autoantibodies in 10-week-old transgenic and nontransgenic MRL-*lpr/lpr* mice (Table 1). The *Fas*-transgenic MRL-*lpr/lpr* mice expressed normal levels of IgG2a immunoglobulin and IgG2a anti-dsDNA. Interestingly, although the levels of IgG1 immunoglobulin and IgG1 anti-dsDNA were reduced, IgG1 anti-dsDNA was significantly elevated compared with MRL-+/+ mice.

DISCUSSION

The relative contributions of T-cell anergy loss and B-cell autoantibody production to the autoimmune disease process in the *lpr* mouse model of systemic lupus erythematosus have not been established (1). B cells of MRL-*lpr/lpr* mice have an intrinsic defect in autoantibody production (5, 6), and it has been suggested that in *lpr/lpr* mice, autoantibody production and possibly autoimmune disease are independent of the T-cell environment. We therefore placed the normal murine *Fas* gene under control of the *CD2* promoter and enhancer to target *Fas* expression to T cells, but not B cells. Despite the fact that these *CD2–Fas* transgenic MRL-*lpr/lpr* mice expressed no detectable *Fas* RNA in B cells, hypergammaglobulinemia and anti-dsDNA production were eliminated. This genetic correction of defective expression of *Fas* in the T cell of MRL-*lpr/lpr* mice is consistent with previous experiments suggesting that T cells are required for production of antibody production in MRL-*lpr/lpr* mice (1–4, 23, 24). The observation that increased production of anti-dsDNA of the IgG2a isotype was completely normalized, whereas IgG1 anti-dsDNA remained elevated, suggests that correction of the T-cell defect results in a shift from a T_{h1}-type helper T-cell response, which is relatively more dependent on interleukin 2 and interferon γ and favors production of IgG2a antibodies, to a T_{h2}-type response, which is relatively more dependent on interleukin 4 and tends to result in production of IgG1 antibodies (25). This is supported by the observation of

decreased interferon γ levels in the sera of aged *CD2–Fas* transgenic MRL-*lpr/lpr* mice compared with nontransgenic MRL-*lpr/lpr* mice (T.Z., unpublished data). It is also important to recognize that the semiquantative PCR technique does not exclude the possibility that the *CD2–Fas* transgene could have restored *Fas* expression in a subpopulation of B cells as has been observed by other investigators (26, 27), thereby directly correcting the *Fas* defect in B cells as well as T cells. Additionally, we cannot exclude the possibility that expression of *Fas* in the nonphysiologic *CD2* expression system might interfere directly with either T-cell development or development of the lymphoproliferative autoimmune disease in *CD2–Fas* transgenic MRL-*lpr/lpr* mice. These questions can be answered, in part, once the murine anti-Fas antibody becomes available and is used to detect surface Fas on lymphocytes (28).

Consistent with the observation that the *Fas* apoptosis gene is mutated in MRL-*lpr/lpr* mice, it has recently been possible to detect an apoptosis defect in these mice (29, 30). However, it remained unclear as to whether a mutation in an apoptosis gene could lead to the profound lymphadenopathy and autoimmunity that are observed in these mice. In addition, MRL-+/+ mice develop a milder form of autoimmune disease later in life, and it was not clear whether the *Fas* defect was a critical autoimmune accelerator gene in the presence of other autoimmune MRL background genes (31, 32). The data presented here demonstrate that replacement of the mutated *Fas* gene with a *Fas* transgene results in elimination of the abnormal $CD4^-CD8^-B220^+$ T cells and directly demonstrate that normal *Fas* expression is sufficient to prevent both the lymphoproliferative disease and generalized autoimmune disease in *lpr/lpr* mice.

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