Autoimmune Disease in Mice Due to Integration of an Endogenous Retrovirus in an Apoptosis Gene

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Summary

The MRL-lpr/lpr mouse strain is a model of systemic autoimmune disease. In this model, intrinsic defects of intrathymic T cell development include defective deletion of self-reactive T cells and expression of endogenous retroviruses. Defective deletion of self-reactive T cells in the thymus has been proposed to be due to a germline mutation in the Fas apoptosis gene. Using different fragments of a Fas cDNA probe, we determined that the Ipr/lpr mutation is a 5.3-kb insertion of DNA within the second intron of the Fas gene. cDNA corresponding to this region was then derived from thymic RNA from MRL-lpr/lpr and MRL-+/+ mice using the polymerase chain reaction. All thymic RNA samples from MRL-lpr/lpr mice yielded a unique product that was 168 bp larger than that of MRL-+/+ mice. Complete sequence analysis indicated that this inserted sequence had 98% homology with a sequence from the 3' long terminal repeat of the early transposon (ETn). RNA analysis indicated higher expression of ETn RNA in the thymus of MRL-lpr/lpr than MRL-+/+ mice. The interdependence of ETn expression and abnormal Fas expression was then analyzed in a CD2-Fas transgenic mouse model in which a full-length murine Fas cDNA under the regulation of the CD2 promoter and enhancer was used to correct defective Fas expression in T cells of MtLL-lp$/lpr$ mice. In these mice, reduced thymic ETn expression was observed, confirming that high ETn expression is related to abnormal Fas expression. These results establish a link between endogenous retrovirus expression, abnormal Fas expression, and autoimmune disease.

The homozygous expression of the lpr/lpr gene leads to autoimmunity and lymphadenopathy in different strains of mice, including MRL, C57BL/6, C3H, AKR, and BALB/c (1). The lpr gene has been identified as a point mutation in the intracellular region of the Fas gene in CBA/J-lpr$^g$ mice (2). The functional significance of this point mutation has been demonstrated in transfection studies using a normal intracellular region of the Fas gene, or an intracellular domain containing this point mutation, ligated to the extracellular domain of the human Fas gene. After transfection into L929 cells and Fas crosslinking of the expressed Fas molecules with anti-human Fas antibody, apoptosis occurred in cells transfected with the normal chimeric Fas gene, but not in cells expressing the chimeric Fas gene using the mutated intracellular domain (2). The Fas gene also has been found to be abnormal in MRL-lpr/lpr mice in which Southern blot analysis indicated altered restriction enzyme digestion (2, 3) and Fas RNA expression was not detectable in the thymus (2). These results led to the conclusion that the Fas mutation in MRL-lpr/lpr mice was different from the mutation in CBA/J-lpr$^g$ mice, and that in MRL-lpr/lpr mice the mutation leads to disruption of normal transcription of the Fas gene.

Different strains of lpr/lpr mice develop different types of lymphoproliferative autoimmune disease (1). Genetic differences between the different strains of lpr/lpr mice play a role in determining the levels in autoantibody production, the type and severity of autoimmune disease, and extent of lymphoproliferation (1, 4, 5). Genes determining the severity of renal disease in mice expressing the lpr/lpr gene have been mapped to chromosomes 7 and 12, whereas genes associated with arthritis, although known to exist, have not yet been mapped (3-5). The heterozygous expression of the lpr gene also leads to a less severe form of lymphoproliferative autoimmune disease (6). It is not yet known if these disease differences are related to differences in expression of the Fas gene, or to the influence of other genes in the immune response.

We have previously reported that the Fas mutation was due to the insertion of a retrotransposon in the Fas gene (7). This early transposon (ETn) was found to be inserted within transcripts of the otherwise normal Fas gene. At least two abnormal sized transcripts of the Fas gene contained tran-

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1 Abbreviation used in this paper: ETn, early transposon.
scripts derived from the E\(Tn\). One transcript was a short 168-bp sequence inserted between the second and third exon. In addition, there was a larger E\(Tn\)-Fas transcript of \(\sim\)10.5 kb.

In this paper we have confirmed our previous findings and have further characterized the E\(Tn\) sequence within the second intron of the Fas gene in MRL-lpr/lpr mice. This mutation leads to the abnormal transcription and splicing of the Fas gene in MRL-lpr/lpr mice, and results in reduced amounts of normally spliced Fas mRNA (2%). The expression of E\(Tn\) is increased in the thymus of younger mice, but decreases with age. In addition, in CD2-Fas transgenic MRL-lpr/lpr mice, increased expression of Fas mRNA results in decreased expression of E\(Tn\) in the thymus. These results indicate that the extent of interruption of the Fas transcription by E\(Tn\) is not constant, and that Fas transcription is less disrupted under conditions that suppress expression of the E\(Tn\) retrotransposon.

Materials and Methods

Normal Mice. The original breeding pairs of MRL-lpr/lpr and MRL+/- mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Production of CD2-Fas Transgenic MRL-lpr/lpr Mice. The 1.1-kb full-length Fas cDNA was obtained by PCR amplification of cDNA made from thymus mRNA from MRL+/+ mice as described below. This was cloned into an EcoRI 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. The resultant plasmid was shown to be sufficient to allow T cell-specific, copy-dependent, integration-independent expression in transgenic mice (8, 9). MRL-lpr/lpr male and female mice were obtained from The Jackson Laboratory. Single-cell MRL-lpr/lpr embryos were produced, injected with \(\sim\)100 copies of the CD2-Fas transgene, and then place into the distal oviduct of CD1 pseudopregnant female mice. Tail DNA prepared from offspring was digested with EcoRI and probed with a 32p-labeled full-length Fas cDNA to identify CD2-Fas transgenic mice (Wu, J., T. Zhou, J. Zhang, J. He, and J. D. Mountz, manuscript in preparation).

Southern Blot Analysis. MRL-lpr/lpr and MRL+/- mice were obtained from The Jackson Laboratory. DNA was prepared from the thymus and digested with the indicated restriction enzymes. Approximately 10 \(\mu\)g of the digested DNA was separated on a 0.7% agarose gel, blotted to a nylon membrane, and hybridized with 32p-labeled cDNA probes.

Northern Blot Analysis. RNA was prepared as described previously (10). 5 \(\mu\)g of poly(A)+ RNA was denatured at 65°C for 5 min in electrophoresis buffer (0.4 M 3-morpholino propanesulfonic acid, 0.1 M sodium acetate, 2 mM EDTA pH = 7.0) containing 6% formaldehyde and 50% formamide, and size fractionated by electrophoresis through 1% agarose gels containing 0.6% formaldehyde. Gels were stained with ethidium bromide to assure integrity of the loaded RNA. RNA was transferred to nylon membranes (Nitroplus 2000; Micron Separations Inc., Eastboro, MA) and baked for 2 h at 80°C in a vacuum oven. Membranes were prehybridized and then hybridized with 1-3 \(\times\) \(10^6\) cpm/ml of different DNA probes that had been labeled with 32P by random priming to a specific activity of 109 cpm/\(\mu\)g. Filters were then washed with 2X SSC + 0.1% SDS at 42°C for 30 min and then with 0.1X SSC + 0.1% SDS at 60°C for 30 min; they were then exposed to XAR-2 film (Eastman Kodak, Rochester, NY) at \(-70^\circ\text{C}\) with intensifying screens.

PCR Analysis. Thymuses of mice were homogenized and total RNA was extracted from the homogenates by the guanidinium-Cl method. Total RNA (2-4 \(\mu\)g) from each tissue was used for cDNA synthesis followed by PCR amplification using an RNA-PCR kit (Perkin-Elmer Corp., Norwalk, CT). Reaction conditions were as specified by the manufacturer. An oligo(dT) primer was used to initiate cDNA synthesis. 30 PCR cycles (1 min at 95°C; 1.5 min at 55°C; 2.5 min at 72°C) were run followed by extension for 10 min, and the amplification products were visualized after electrophoresis on agarose gels (1.0%) under ultraviolet illumination in the presence of ethidium bromide. Gels were blotted and hybridized to a labeled internal Fas probe to verify that the bands were Fas specific. The full-length Fas cDNA was obtained by PCR amplification of cDNA made from thymus mRNA and MRL-lpr/lpr or MRL+/- mice. The full-length cDNA PCR primers were (P1, 5'-GCC-GGC-CGG-CTG-TTTC-CCT-GCT-GCA-GAG-3'; position +20) and (P4, 5'-ATT-GAC-ATT-GGC-AAC-CTCC-TGG-TGT-3'; position 1110). Internal primers were used to obtain the extracellular domain cDNA that was sequenced. These primers were (P2, 5'-CA-CAG-TTA-AGA-GTGT-CAC-CAA-GGT-247); position +93) and (P3, 5'-AA-AGT-CCC-AGG-GGC-CTA-TGG-TTG-3'; position 540). 5' primers used in sequencing were the universal vector 5' primer: (5'-CTG-TTG-GATC-TGG-GCT-3'; position 53), (5'-TGT-CAA-CCA-GTCG-CAA-CCT-3'; position 215), (5'-CGA-AAG-TAC-CGG-AAA-AGA-3'; position 608), and (5'-CGA-GAA-AAT-AAC-ATC-AAG-3'; position 773). 3' primers used in sequencing were the 3' universal vector primer: (5'-GAA-CTC-AGA-ACC-TCC-GAT-3'; position 656), (5'-TGTT-GGC-TTC-GGC-TCT-5'; position 464), (5'-ACA-GAA-GGG-AAG-GAG-TAC-3'; position 293), and (5'-TGTT-GAG-GAC-TGCCC-AA-AAT-3'; position 245). Sequence positions are referenced to the published murine Fas sequence (11).

Genomic Cloning. High molecular weight DNA from the thymus of MRL-lpr/lpr and MRL+/- mice was digested with PstI and HindII. The digested sample was electrophoresed at 65°C in 0.6% agarose gel and probed with the 345-bp HindII fragment of Fas and the 168-bp E\(Tn\) fragment, and another portion of the gel was sliced into thin sections representing different molecular weights. The gel slices corresponding in size to bands hybridizing with the E\(Tn\) or Fas probes were extracted from the gel using GeneClean II (Bio 101, La Jolla, CA). DNA fragments ranging from 3.8 to 5.6 kb were amplified using the appropriate 3' or 5' Fas cDNA primers in combination with E\(Tn\) primers from the 168-bp sequence (see Fig. 2) and published E\(Tn\) sequences (12). PCR products were cloned into the PCR 2000 vector (Invitrogen, San Diego, CA) for sequence analysis.

Sequence Analysis. Sequence analysis was carried out on double-stranded DNA derived from PCR, amplification and cloning into the PCR 2000 (Invitrogen). Sequence analysis was carried out in both directions using 5' and 3' universal primers and Fas-specific primers. For genomic sequencing, universal primers were used to determine the sequence of both the 3' end of the second exon and the 5' end of the third exon and flanking intronic sequences.

DNA Probes. The full-length murine Fas cDNA probe (49-1033 bp) was obtained by PCR amplification of cDNA from MRL+/- thymus mRNA as described above and using previously described Fas primers (11). A probe corresponding to the first and second exons of Fas, which are 5' of the normal E\(Tn\) insertion, was the 170-bp PstI-HindII fragment (49-219 bp) derived from the full-length Fas cDNA clone.

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the remainder of the extracellular domain was the 345-bp HindIII fragment (219-569 bp) derived from the full-length Fas cDNA clone. The ETn probe was the 168-bp ETn sequence that was isolated by PCR amplification of cDNA prepared from MRL-Ipr/Ipr thymus mRNA and reamplified using primers specific for the 5' and 3' sequences of ETn. The β-actin probe was a gift from Dr. K. Gordon (Genzyme Corp., Framingham, MA) (13).

Results

The Ipr Mutation Results from a 5.3-kb Insertion of DNA.

High molecular weight DNA from the kidney and thymus of MRL-Ipr/Ipr and MRL-+/+ mice was digested with various restriction enzymes and probed with a 345-bp sequence corresponding to the extracellular domain of the Fas gene (Fig. 1 a). There was no difference in restriction fragment lengths between high molecular weight DNA from the kidney and the thymus (data not shown). There was an additional 5.3 kb of DNA within the extracellular domain of the genomic Fas gene from MRL-Ipr/Ipr mice as determined by restriction fragment length analysis of Southern blots prepared using multiple single and double enzyme digestions and hybridization with a 170-bp cDNA probe corresponding to the first and second exons of Fas cDNA (49-219 bp), an extracellular domain probe (219-569 bp), and a full-length Fas cDNA probe (49-1033 bp). The inserted DNA contained additional digestion sites for EcoRI, HindIII, and PvuII but not for BamHI (Fig. 1 b). Using probes corresponding to exons 1 and 2, or a HindIII cDNA fragment corresponding to the extracellular domain, the insert was localized to the region of the Fas gene corresponding to the second intron (Fig. 1 b).

Insertion of ETn in the Extracellular Domain of Fas cDNA from Ipr/Ipr Mice. To determine if the Ipr/Ipr mutation in the extracellular domain of the Fas gene results in abnormal Fas RNA, cDNA corresponding to the extracellular domain was derived from thymus RNA from several MRL-Ipr/Ipr and MRL-+/+ mice using the PCR and primers P2 and P3 (Fig. 2 a). All RNA samples from the thymus of different MRL-Ipr/Ipr mice yielded a unique PCR product that was 168 bp larger than that of wild-type MRL-+/+ mice (Fig. 2 b). The mutation of the Fas gene in MRL-Ipr/Ipr mice was confirmed to be in the extracellular domain by sequencing, using full-length primers P1 and P4 (Fig. 2 a) and using the extracellular Fas cDNA clones from MRL-Ipr/Ipr and MRL-+/+ mice. The sequence of the transmembrane and cytoplasmic domains were identical in MRL-Ipr/Ipr and MRL-+/+ mice. Complete sequence analysis of cDNA corresponding to the extracellular domain of the Fas gene was carried out using two different MRL-Ipr/Ipr mice and indicated that there was a 168-bp insert into the Fas cDNA sequence at position 232 of an otherwise normally encoded extracellular domain (11) (Fig. 2 c). The 268-bp ETn sequence found within the Fas gene was 99% homologous to sequence Mus ETn Xi (12, 14) (base pairs 1120-1285) and Mus ETn IgM (15) (base pairs 270-435) as determined by searching GenBank.

Fas Gene Expression in Ipr and +/+ Mice. A full-length Fas cDNA was used to probe Northern blots of poly(A) RNA prepared from the thymus of MRL-+/+, MRL-Ipr/Ipr, MRL-Ipr/+, BXSB male, and NZB mice and from the BW5147 cell line. In MRL-+/+ mice there was a 2.2-kb normal-sized Fas cDNA (Fig. 3 a, lane 1-3). In contrast, in 1-mo-old MRL-Ipr/Ipr mice there were multiple bands ranging from 2 to 10.5 kb (Fig. 3 a, lanes 4 and 6). Fas expression was highest in the thymus of 1-mo-old MRL-Ipr/Ipr mice, and decreased in 3-mo-old mice (Fig. 3 a, lane 5). When identical blots were hybridized with a 170-bp PstI/HindIII Fas cDNA fragment corresponding to the first and second exons, Fas expression in MRL-Ipr/Ipr mice was very low compared with Fas expression in MRL-+/+ mice (Fig. 3 b, lanes 4-6). A faint abnormal high molecular weight species of 10.5 kb was present using this 5' probe (Fig. 3 b, lane 4, arrow). When blots were probed with a 345-bp HindIII Fas cDNA fragment corresponding to the extracellular domain of Fas, the primary species of RNA expressed in the thymus of young MRL-Ipr/Ipr mice was a high molecular weight 10.5- and 9.5-kb...
Figure 2. MRL-Ipr/Ipr mice express an abnormal Fas RNA containing Etn. (a) The position of the Etn sequence inserted within the Fas gene was determined by sequence analysis of cDNA prepared from the thymus of MRL-Ipr/Ipr and MRL+/- mice. The PCR primers used and their relative locations within the Fas gene are indicated as P1, P2, P3, and P4. The PCR products using primers P2 and P3 were subjected to agarose gel electrophoresis and visualized by ultraviolet illumination in the presence of ethidium bromide. A unique larger PCR product was observed using thymic RNA from six different MRL-Ipr/Ipr mice. (b) The wild-type sequence of the Fas gene is numbered as previously described (11). The Etn sequence found within the otherwise normal extracellular coding region of Fas cDNA from MRL-Ipr/Ipr mice is 98% homologous to a portion of an Etn previously found to be integrated into the Ig locus of mice (12, 14, 15). The 168-bp Etn insert in the Fas gene of MRL-Ipr/Ipr mice results in an in-frame amino acid sequence shown below the cDNA sequence.

Figure 3. Northern blot analysis of Fas RNAs from the thymus of wild-type MRL+/+ and MRL-Ipr/Ipr mice. Thymus poly(A)+ RNA from the indicated mouse strains was analyzed by probing four identical blots with: (a) a full-length Fas cDNA probe, (b) a 5′ Pst-I/HinClI Fas cDNA probe corresponding to position 49-219, (c) a Fas cDNA probe corresponding to the 345-bp HindIII fragment of extracellular domain, and (d) a 168-bp Etn probe derived from Etn sequences within the abnormal sized Fas transcript obtained by PCR amplification of the extracellular domain Fas cDNA from lpr/lpr mice. MRL+/+, BXSB male, and NZB female mice were 2 mo of age. MRL-Ipr/Ipr mice were 1 mo old (lanes 4 and 6) and 3 mo old (lane 5). (a–c) Arrows indicate the abnormal Fas transcripts in MRL-Ipr/Ipr mice that correspond in size to a unique transcript that also hybridizes to the Etn probe used in d.

These results indicate that the Fas mutation leads to production of abnormal high molecular weight Fas transcripts in the thymus. There was high expression of the 2.2-kb Fas transcript in (MRL-Ipr/lpr × MRL+/-)F1 mice, and also in BXSB male and NZB autoimmune mice (Fig. 3, a–c; lanes 7–9). Expression of normal levels of Fas RNA in BXSB and NZB mice indicates that autoimmune disease in these mice is not related to defective expression of Fas RNA.

The 168-bp Etn probe, derived from within the Fas cDNA prepared from thymus RNA of MRL-Ipr/lpr mice, strongly hybridized to a 5.7-kb full-length Etn transcript that was expressed in the thymus of younger MRL-Ipr/lpr mice (Fig. 3, d, lanes 4 and 6), but not strongly expressed in the thymus of older MRL-Ipr/lpr mice (Fig. 3, d, lane 5) or in the thymus of MRL+/+ mice (Fig. 3, d, lanes 1–3). RNA corresponding to the full-length 5.7-kb Etn transcripts was also abundant in the thymus of MRL-Ipr/+ and BXSB mice, and also in

transcript (Fig. 3 c). These results indicate that the Fas mutation leads to production of abnormal high molecular weight Fas transcripts in the thymus. There was high expression of the 2.2-kb Fas transcript in (MRL-Ipr/lpr × MRL+/-)F1 mice, and also in BXSB male and NZB autoimmune mice (Fig. 3, a–c; lanes 7–9). Expression of normal levels of Fas RNA in BXSB and NZB mice indicates that autoimmune disease in these mice is not related to defective expression of Fas RNA.

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Figure 4. Sequence analysis of the ETn in the germline Fas gene. (Top) The 168-bp ETn insert, which is aberrantly spliced between exons 2 and 3 in some of the Fas transcripts in MRL-Ipr/Ipr mice. (Middle) The short 14-bp germline sequence between exon 2 and the 168-bp ETn transcript within Fas, followed by an addition of ~5.3 kb of ETn sequence and then by 3.5 kb of intron sequence. (Bottom) The 5.3 kb of ETn sequence that is integrated into the second intron of the Fas gene of MRL-Ipr/lpr mice.

Discussion

Watson et al. (3) previously reported that there was a 1.4-kb deletion near the BamH1 site of the Fas gene. Restriction map analysis and intron sequencing described in the present paper indicate that there is a 5.3-kb insert containing the ETn retrotransposon. The discrepancy between our results and the previous results may be related to our use of a greater number...
of restriction enzymes, including HindIII and BanII, which has a restriction site in ETn (Fig. 1), and HincII and PstI (data not shown). It is unlikely that there is a difference in the mice, since both analyses were carried out on MRL-lpr/lpr mice obtained from The Jackson Laboratory.

ETn retrotransposon transcription occurs during early embryonic development in mice (16, 17). Increased expression of endogenous retroviruses in the thymus of autoimmune strains has been proposed to be related to development of autoimmune disease (18-20). In this paper, we report that there is also increased expression of full-length ETn endogenous retrovirus in the thymus of MRL-lpr/lpr mice and BXSB mice (Fig. 3). Inhibition of translation of retroviral transcripts by antisense RNA has been reported to result in increased proliferation of lymphocytes leading to the speculation that full-length retroviral transcripts and protein products are a compensatory mechanism for increased lymphocyte proliferation in autoimmune mice (18). A second mechanism of association of retroviruses with autoimmunity is suggested by the present data, which suggest that increased retroviral expression may be related to defective Fas expression in MRL-lpr/lpr mice.

The ETn retrotransposon in the second intron of the Fas gene might interfere with Fas expression by promoting abnormal transcription initiation and interfering with abnormal splicing. High expression of ETn correlates with high expression of an abnormal large-sized Fas transcript with a molecular weight of ~10.5 kb (Fig. 3, lanes 4 and 6). The largest Fas transcript corresponds in size with an abnormal ETn transcript of the same size. This transcript contains both 5’ and 3’ Fas cDNA sequences because it is detected by both the 5’ 170-bp Pst-1/HincII Fas probe and the 3’ 345-bp HincII Fas probe. A lower unusual Fas transcript with an approximate molecular weight of 7.5 kb, which retains 5’ and 3’ Fas sequences (Fig. 3, lane 4), does not hybridize with the 168-bp ETn probe, indicating that the 168-bp portion of ETn is spliced out of this transcript. Other aberrant splicing events can lead to Fas transcripts that contain only 168 bp of ETn sequences (Fig. 2). These results suggest that thymic developmental factors that lead to high ETn expression also promote production of an abnormally large Fas transcript in MRL-lpr/lpr mice due to the integration of ETn within the second intron of the Fas gene.

In MRL-lpr/+ heterozygous mice, there was increased expression of ETn and abnormal Fas, despite the presence of apparently normal levels of Fas transcription from the unmутated allele. It is possible that abnormal Fas and high ETn are expressed in a subpopulation of thymocytes that express low levels of normal Fas and exhibit abnormal thymic development. This was investigated in CD2-Fas transgenic MRL-lpr/lpr mice. In these mice, Fas expression is regulated by the CD2 promoter/enhancer, which results in high expression of Fas in all thymocytes, and elimination of ETn expression (Fig. 5). These results suggest that Fas expression and ETn expression are functionally related.

We have also observed that ETn expression is decreased and Fas expression is partially normalized in TCR-β transgenic mice (Wu, J., and J. D. Mountz, manuscript in preparation). We have previously demonstrated that in TCR-β transgenic mice, there is nearly total elimination of the CD4+ CD8+ B220+ subpopulation of T cells and lymphoproliferation, but not elimination of autoimmunity (21, 22). We have recently demonstrated that there is decreased apoptosis of thymocytes of MRL-lpr/lpr mice and an increase of a large, proliferating CD4+ CD8+ thymocyte subset in thymocytes (22). The TCR-β transgene was found to reduce these large, proliferating CD4+ CD8+ thymocytes, and there was no difference between this population in TCR-β transgenic MRL-lpr/lpr mice and the same population in MRL-+/+ mice. These results suggested that the presence of the TCR-β transgene corrected the defect in early T cell development related to lymphoproliferation despite the presence of a germ-line mutation of the Fas apoptosis gene. Rearrangement of the TCR-β chain gene has been proposed to play a critical role in early T cell development in the thymus (23, 24). The TCR-β transgene suppresses rearrangement of the endogenous TCR-β gene (25). Suppression of rearrangement of the endogenous TCR-β gene might accelerate T cell maturation resulting in decreased levels of retroviral LTR and eukaryotic gene enhancer binding proteins associated with T cell development (26). Prevention of aberrant transcription initiation at the site of the ETn integration within the second intron of the murine Fas gene could result in normal transcription initiation from the 5’ end of the Fas gene. This would lead to the observed increased levels of Fas expression in the thymus of the TCR-β transgenic MRL-lpr/lpr mice. This interpretation is consistent with the concept that ETn expression and abnormal Fas expression are functionally related in lpr mice.

Abnormal Fas expression and T cell development in the thymus of autoimmune mice might lead to continued high expression of retroviruses. Abnormal populations of T cells, or B cells found in the periphery of autoimmune mice, exhibit common features of developmental defects and retrovirus expression. In the case of the lpr/lpr gene the present data suggest that retrovirus expression is intimately related to abnormal Fas gene transcription and abnormal lymphocyte development. For other autoimmune strains of mice, there is no evidence for such a direct association of retrovirus expression and altered expression of genes related to autoimmunity. For the CBA/J-lpr/g mice, the genetic defect of the Fas gene is a single point mutation in the cytoplasmic signaling region of the gene, and currently there is no evidence to suggest that this is related to the presence of the ETn retrotransposon within this gene. It will be necessary to characterize the genomic organization of the Fas gene in CBA/J-lpr/g mice to determine if a retrovirus might result in abnormal splicing and this point mutation. Likewise, cloning and characterization of defective autoimmune genes in other strains of mice, including gld/gld, NZB, and BXSB, will be necessary to determine if retroviruses play a critical role in abnormal expression of autoimmune genes other than the Fas gene.
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Note added in proof: Integration of ETn in an intron of the Fas gene in lpr mice has recently been published (27).

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