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 *lpr/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 MRL-*lpr/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*^{cg} 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*^{cg} 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*)[1] 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-

[1] *Abbreviation used in this paper: ETn*, early transposon.

scripts derived from the *ETn*. One transcript was a short 168-bp sequence inserted between the second and third exon. In addition, there was a larger *ETn-Fas* transcript of ~10.5 kb.

In this paper we have confirmed our previous findings and have further characterized the *ETn* 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 *ETn* 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 *ETn* in the thymus. These results indicate that the extent of interruption of the *Fas* transcription by *ETn* is not constant, and that *Fas* transcription is less disrupted under conditions that suppress expression of the *ETn* 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 3′ sequence has been 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 ~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 ^{32}P-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 μg of the digested DNA was separated on a 0.7% agarose gel, blotted to a nylon membrane, and hybridized with ^{32}P-labeled cDNA probes.

Northern Blot Analysis. RNA was prepared as described previously (10). 5 μg of poly(A)$^+$ RNA was denatured at 65°C for 5 min in electrophoresis buffer (0.4 M 3-morpholinopropanesulfonic 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 × 10^6 cpm/ml of different DNA probes that had been labeled with ^{32}P by random priming to a specific activity of 10^9 cpm/μg. Filters were then washed with 2× SSC + 0.1% SDS at 42°C for 30 min and then with 0.1× SSC + 0.1% SDS at 60°C for 30 min; they were then exposed to XAR-2 film (Eastman Kodak, Rochester, NY) at −70°C with intensifying screens.

PCR Analysis. Thymuses of mice were homogenized and total RNA was extracted from the homogenates by the guanidinium-CsCl method. Total RNA (2–4 μg) 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′-GGC-CGC-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). Internal primers were used to obtain the extracellular domain cDNA that was sequenced. These primers were (P2, 5′-CA-CAG-TTA-AGA-GTT-CAT-ACT-CAA-GGT-ACT-AAT-3′; position +93) and (P3, 5′-AA-AGT-CCC-AGA-AAT-CGC-CTA-TGG-TTG-TTG-3′; position 540). 5′ primers used in sequencing were the universal vector 5′ primer: (5′-CTG-TGG-ATC-TGG-GCT-3′; position 53), (5′-TGT-CAA-CCA-TGC-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-TCT-AGA-ACC-TCC-AGT-3′; position 656), (5′-TGT-GTT-CGC-TGC-GCC-TCG-3′; position 464), (5′-ACA-GAA-GGG-AAG-GAG-TAC-3′; position 293), and (5′-GTT-GAG-GAC-TGC-AAA-ATG-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 HincII. The digested sample was divided into several wells and subjected to electrophoresis through 0.8% agarose. After electrophoresis, a portion of the gel was blotted and probed with the 345-bp HincII fragment of *Fas* and the 168-bp *ETn* 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 *Fas* or *ETn* 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 *ETn* primers from the 168-bp sequence (see Fig. 2) and published *ETn* 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 derived by PCR amplification of cDNA prepared 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 *ETn* insertion, was the 170-bp PstI-HincII fragment (49–219 bp) derived from the full-length *Fas* cDNA clone (Fig. 2 *a*). A probe corresponding to

the remainder of the extracellular domain was the 345-bp HincII 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-*lpr/lpr* 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 lpr Mutation Results from a 5.3-kb Insertion of DNA. High molecular weight DNA from the kidney and thymus of MRL-*lpr/lpr* and MRL-+/+ mice was digested with various restriction enzymes and probed with a 345-bp sequence corresponding to the extracellular domain of the *Fas* cDNA (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-*lpr/lpr* 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 HincII 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 lpr/lpr Mice. To determine if the *lpr/lpr* 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-*lpr/lpr* and MRL-+/+ mice using the PCR and primers P2 and P3 (Fig. 2 *a*). All RNA samples from the thymus of different MRL-*lpr/lpr* 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-*lpr/lpr* 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-*lpr/lpr* and MRL-+/+ mice. The sequence of the transmembrane and cytoplasmic domains were identical in MRL-*lpr/lpr* and MRL-+/+ mice. Complete sequence analysis of cDNA corresponding to the extracellular domain of the *Fas* gene was carried out using two different MRL-*lpr/lpr* 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 lpr and +/+ Mice. A full-length *Fas* cDNA was used to probe Northern blots of poly(A) RNA prepared from the thymus of MRL-+/+, MRL-*lpr/lpr*, MRL-*lpr/+*, 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-*lpr/lpr* 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-*lpr/lpr* mice, and decreased in 3-mo-old mice (Fig. 3 *a*, lane *5*). When identical blots were hybridized with a 170-bp PstI/HincII *Fas* cDNA fragment corresponding to the first and second exons, *Fas* expression in MRL-*lpr/lpr* 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 HincII *Fas* cDNA fragment corresponding to the extracellular domain of *Fas*, the primary species of RNA expressed in the thymus of young MRL-*lpr/lpr* mice was a high molecular weight 10.5- and 9.5-kb

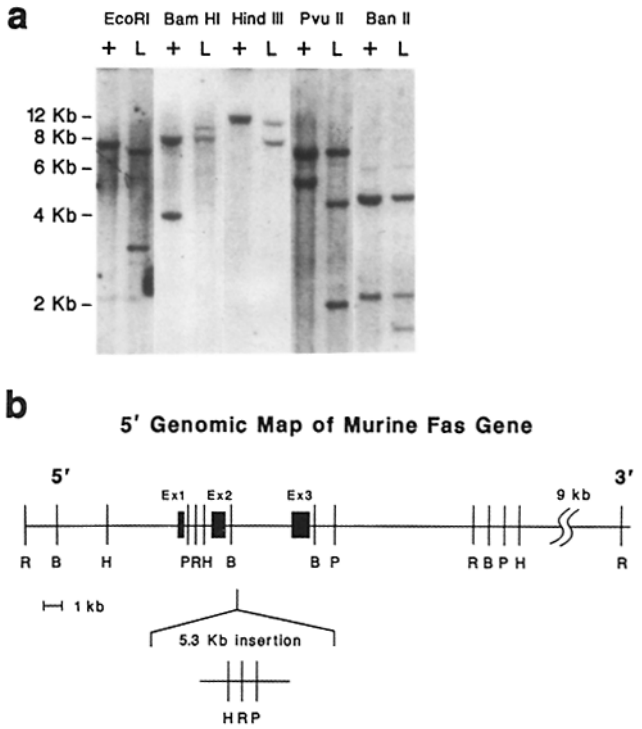

Figure 1. Southern blot analysis of the *Fas* gene in MRL-*lpr/lpr* and wild-type MRL+/+ mice. DNA was purified from thymuses of wild-type MRL+/+ (+) and MRL-*lpr/lpr* (*L*) mice. (*a*) High molecular weight DNA was digested with the indicated restriction enzyme and probed with a HincII cDNA fragment corresponding to the extracellular domain fragment extending from 219 to 569 bp of the murine sequence. (*b*) Restriction map derived by single and multiple double enzyme digestions, and probing with a cDNA fragment corresponding to either a 170-bp PstI-HincII cDNA fragment (+49 to +219 bp), which is entirely 5′ of the *ETn* insertion, a probe corresponding to the remainder of the extracellular domain, which was a 345-bp HincII cDNA fragment (+219 to +569 bp), or the full-length *Fas* cDNA probe (+49 to +1033 bp). The location of the 5.3-kb inserted DNA and the approximate location of Ex1, Ex2, and Ex3 were derived using the different probes on multiple identical blots. Additional enzyme sites present in the 5.3-kb insert are indicated. *R*, EcoRI; *B*, BamHI; *H*, HindIII; *P*, PvuII.

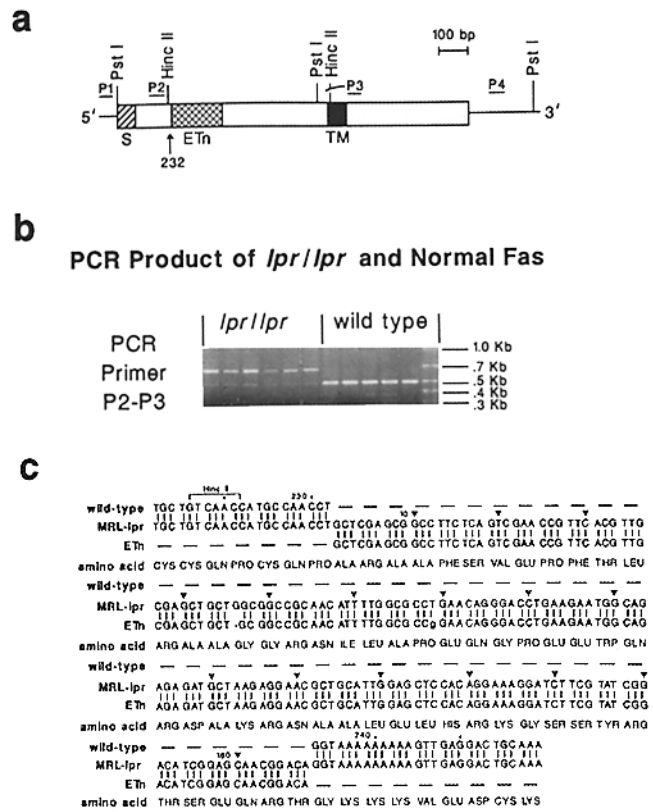

Figure 2. MRL-*lpr*/*lpr* mice express an abnormal *Fas* RNA containing *ETn*. (*a*) The position of the *ETn* sequence inserted within the *Fas* gene was determined by sequence analysis of cDNA prepared from the thymus of MRL-*lpr*/*lpr* and MRL-+/+ mice. The PCR primers used and their relative locations within the *Fas* gene are indicated as P1, P2, P3, and P4. Also shown is the location of the 170-bp 5′ probe, which is the PstI-HincII fragment, and the 345-bp extracellular domain probe, which is the HincII fragment derived from the normal *Fas* cDNA clone. (*b*) 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-*lpr*/*lpr* mice. (*c*) 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-*lpr*/*lpr* 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-*lpr*/*lpr* mice results in an in-frame amino acid sequence shown below the cDNA sequence.

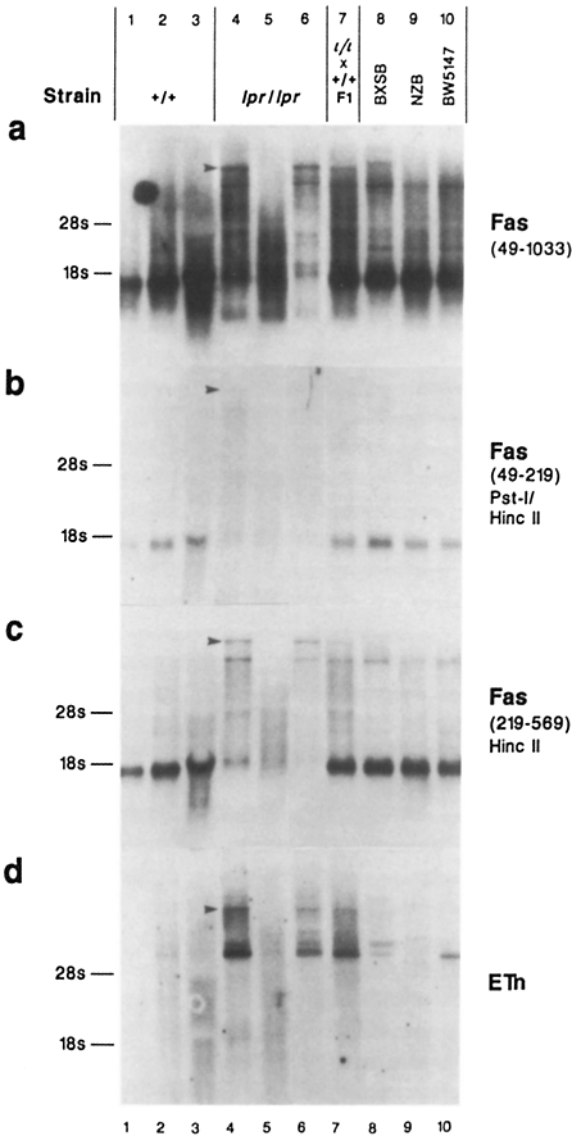

Figure 3. Northern blot analysis of *Fas* RNAs from the thymus of wild-type MRL-+/+ and MRL-*lpr*/*lpr* mice. Thymus poly(A)$^+$ RNA from the indicated mouse strains was analyzed by probing four identical blots with: (*a*) a full-length *Fas* cDNA probe, (*b*) a 5′ Pst-I/HincII *Fas* cDNA probe corresponding to position 49–219, (*c*) a *Fas* cDNA probe corresponding to the 345-bp HincII 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-*lpr*/*lpr* 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-*lpr*/*lpr* mice that correspond in size to a unique transcript that also hybridizes to the *ETn* probe used in *d*.

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-*lpr*/*lpr* × MRL-+/+)F_1 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-*lpr*/*lpr* mice, strongly hybridized to a 5.7-kb full-length *ETn* transcript that was expressed in the thymus of younger MRL-*lpr*/*lpr* mice (Fig. 3 *d*, lanes *4* and *6*), but not strongly expressed in the thymus of older MRL-*lpr*/*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-*lpr*/+ and BXSB mice, and also in

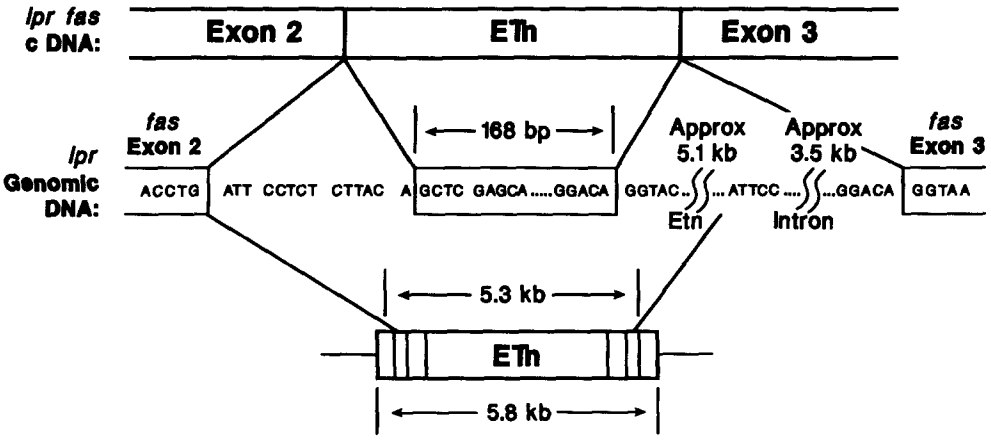

Figure 4. Sequence analysis of the *ETn* in the germline *Fas* gene. (*Top*) The 168-bp *ETn* insert, which is aberrantly spliced between exons 2 and 3 in some of the *Fas* transcripts in MRL-*lpr/lpr* mice. (*Middle*) The short 14-bp germline sequence between exon 2 and the 168-bp *ETn* transcript within *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-*lpr/lpr* mice.

the BW5147 cell line. The largest *Fas* transcript corresponds in size to an abnormal 10.5-kb *ETn* transcript in MRL-*lpr/lpr* mice (Fig. 3, *a–d*, lanes *4*, *arrows*), suggesting the presence of the *ETn* sequence within one of the abnormal-sized high molecular weight *Fas* transcripts of MRL-*lpr/lpr* mice. A higher ~10.5-kb molecular weight *Fas* transcript was also present in the thymus of MRL-*lpr/+* and BXSB mice, but not NZB mice or the BW5147 cell line (Fig. 3 *a*, lanes *7–10*).

Germline Organization of the Mutated Fas Gene in lpr Mice. Using PCR primer pairs to the 3′ end of *Fas* exon 2 and the 5′ end of the 168-bp *ETn* found in the *Fas* transcript or the 5′ end of *Fas* exon 3 and the 3′ end of *ETn*, the sequences of cloned genomic fragments isolated using the 345-bp HincII fragment of *Fas* and the 168-bp *ETn* fragment were determined (Fig. 4). In MRL-*lpr/lpr* mice the *ETn* sequence began at the 5′ terminal of exon 2 and continued for an additional 5.3 kb. There was a conserved splice consensus nucleotide sequence on the 3′ end of exon 2–intron 2 (G/A) and on the 5′ end of the 168-bp *ETn* (A/G), which was found to be spliced into the *Fas* transcript. Also, there was a conserved splice consensus at the 3′ end of the 168-bp *ETn* (A/G) and on the 5′ end of the *Fas* exon 3 (A/G). These splice consensus sequences allow for splicing of the 168-bp *ETn* into the *Fas* cDNA. Additional *ETn* sequence was present in the MRL-*lpr/lpr* mice directly adjacent to the 3′ terminus of the second exon (Fig. 4). In MRL-+/+ and MRL-*lpr/lpr* mice there was an additional 3.5 kb of intron sequence consistent with the restriction map shown in Fig. 1.

Downregulation of ETn in a CD2-Fas Transgenic MRL-lpr/lpr Mice. To determine if high *ETn* expression was dependent on abnormal *Fas* expression, CD2-*Fas* transgenic mice were produced that used a full-length murine *Fas* cDNA under the regulation of the CD2 promoter and enhancer (8, 9) to correct defective *Fas* expression in T cells of MRL-*lpr/lpr* mice. The presence of the *Fas* transgene resulted in reduction of expression of *ETn* in the thymus, suggesting that high *ETn* expression is related to abnormal *Fas* expression (Fig. 5 *A*). Northern blot analysis indicated that there was high expression of the *Fas* transgene in the thymus and lymph node, but not in the brain of 4-wk-old CD2-*Fas* transgenic MRL-*lpr/lpr* mice (Fig. 5 *B*).

Discussion

Watson et al. (3) previously reported that there was a 1.4-kb deletion near the BamHI 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

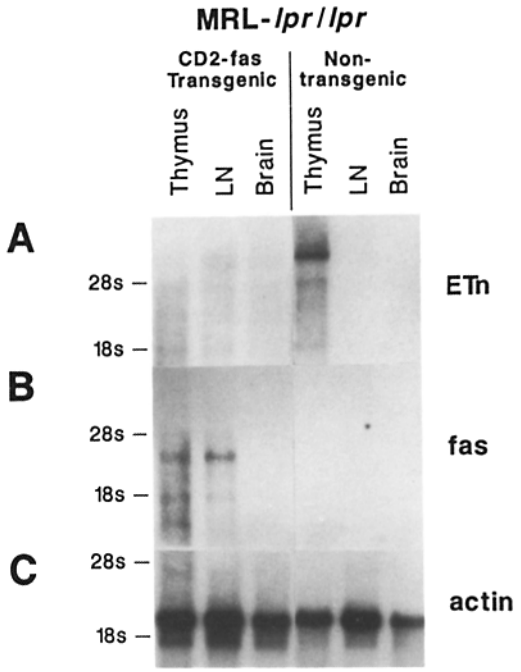

Figure 5. Decreased *ETn* expression in CD2-*Fas* transgenic MRL-*lpr/lpr* mice. Poly(A) RNA from thymus, lymph node (*LN*), and brain of 4-wk-old CD2-*Fas* transgenic and nontransgenic MRL-*lpr/lpr* mice was blotted as described in Fig. 3. (*A*) *ETn* expression is decreased in the thymus of 4-wk-old CD2-*Fas Transgenic MRL-lpr/lpr* mice but not in age-matched nontransgenic littermate control mice. (*B*) CD2-*Fas* transgenic MRL-*lpr/lpr* mice have high levels of *Fas* RNA in the thymus and LN but not in non-T cell sites, including the brain. Nontransgenic litter mate control mice do not express *Fas*. (*C*) The blot was stripped and hybridized with a β-actin probe to ensure that nearly equal amounts of RNA were present in all samples.

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 unmutated 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 $CD4^+CD8^+$ subpopulation of 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 germline 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^{cg} 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^{cg} 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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