# Analysis of Thymic Endogenous Retroviral Expression in Murine Lupus

## Genetic and Immune Studies

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## Abstract

**Inbred mouse genomes contain two subclasses of proviruses related to mink cell focus-forming (MCF) retroviruses: polytropic (*Pmv*), and modified polytropic (*Mpmv*). To determine whether one of these subclasses is associated with murine lupus, oligonucleotide probes specific for *Pmv* or *Mpmv* sequences were used in Northern analyses. Thymus 8.4 kb *Mpmv* RNA was expressed in five of five lupus-prone strains and crosses and this expression was not affected by genes that retard or accelerate development of lupus. Two of four leukemia-prone strains expressed low levels of such thymic transcripts, but none of 11 control strains did. 8.4 kb *Mpmv* RNA expression was not induced in thymuses of control mice by the *lpr/lpr* or *gld/gld* genotypes (which cause polyclonal immune activation) nor by treatment with mitogens. In contrast to *Mpmv*, thymic 8.4 kb *Pmv* expression was poorly associated with autoimmunity: it was easily detected in nearly all strains, and was increased by polyclonal activation in control mice. These studies indicate that the organ-specific thymic 8.4 kb *Mpmv* expression (*a*) is characteristic of several genetic backgrounds which predispose to murine lupus, (*b*) precedes and does not correlate with disease development, (*c*) is not due to polyclonal activation, and (*d*) is regulated independently of 8.4 kb *Pmv* expression. (*J. Clin. Invest.* 1990. 86:809–816.) Key words: autoimmune diseases • endogenous retrovirus • mitogens • gene expression • thymus • provirus • leukemia**

## Introduction

Infectious retroviruses can induce features of several autoimmune diseases. For example, goats infected with the caprine arthritis-encephalitis virus develop a progressive proliferative synovitis with remarkable similarities to rheumatoid arthritis (1–3). Furthermore, patients infected with the human immunodeficiency virus can develop arthritis, autoimmune hemolytic anemia, thrombocytopenic purpura, vasculitis, glomerulonephritis, polymyositis, and Sjogren's syndrome (4–6).

Persistent retroviral infection may not be required for the development of autoimmunity. Indeed, a replication-defective retrovirus can cause lymphoproliferation and polyclonal B cell activation with autoantibody production (7–9). Furthermore, purified retroviral proteins can cause polyclonal immune activation, autoantibody production, and induction of abnormal gene expression (reviewed in 6).

The genomes of most or all vertebrates contain multiple copies of retroviral sequences, the great majority of which are defective for replication. Since many of these endogenous retroviral sequences encode proteins, they could potentially have immune effects contributing to the development of autoimmunity. Until recently, endogenous retroviral sequences had been thought to have no direct immune effects. However, using antisense oligonucleotides to murine endogenous retroviral sequences, we have provided evidence that endogenous retroviral envelope proteins related to those of mink cell focus-forming retroviruses (MCF)[1] have immunoregulatory properties (10).

We previously reported that lupus-prone mouse strains express endogenous full-length (8.4 kb) MCF-related transcripts in spleen and thymus RNA (11, 12). In contrast, only a few control strains expressed such transcripts. Although these transcripts were the same length as infectious type C retroviruses, they had a noninfectious structure (11). After our studies were completed, it was reported that endogenous MCF-related sequences could be subdivided into two subclasses: polytropic (*Pmv*) and modified polytropic (*Mpmv*) (13), which could be distinguished by oligonucleotide probes (14).

The probe used in our initial studies, $MCF_{env}$, was not subclass-specific: it was derived from an *env* sequence conserved in both *Pmv* and *Mpmv* proviruses. To determine whether the association of lupus with endogenous MCF-related expression included one or both of the *Pmv* and *Mpmv* subclasses, we have used sensitive oligonucleotide probes specific for these two subclasses in Northern blot analyses of spleen and thymus RNA from lupus-prone, control, and congenic mouse strains. We have also examined the induction of *Pmv* and *Mpmv* expression in control mice after in vivo mitogen stimulation.

## Methods

*Mice.* A/J, AKR/J, CBA/J, C3H/HeJ, C57Bl/6J, C58/J, DBA/1J, HRS/J, MRL-+/+, MRL-*lpr/lpr*, SB/Le, and SJL/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME. CBA/N mice were bred by NIH Animal Production; NZB/BlN, BXSB, and BXSB. Wild-Y (consomic BXSB mice carrying a Y chromosome bred from the inbred wild mouse strain *Cast*/Ei) were bred in our own facilities. Congenic NZB.*xid*, and BXSB.*xid* mice as well as (NZB × NZW) F1 mice were bred in the NIH SPF breeding facility; and NFS, DBA/2J, and BALB/c mice were obtained from the Frederick Cancer Research Facility, Frederick, MD. Lupus-prone mice are MRL-+/+, MRL-*lpr/lpr*, NZB, BXSB, and (NZB × NZW) F1. All mice were in good health

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1. *Abbreviations used in this paper:* LPS, lipopolysaccharide; LTR, long terminal repeat; MCF, mink cell focus-forming; *Mpmv*, modified polytropic; *Pmv*, polytropic.

when studied. For most strains, additional RNA samples were prepared from a different sex or age and gave results similar to those shown in the figures. In addition to our own NZB/BlN mice, we studied NZB/J mice immediately upon receipt from The Jackson Laboratory.

*Oligonucleotides and probes.* Oligodeoxynucleotides were synthesized on an Applied Biosystems, Inc. (Foster City, CA) model 380 B DNA synthesizer using standard phosphoramidite chemistry and were > 99% pure after purification by denaturing polyacrylamide gel electrophoresis (oligonucleotide sequences are given in Table I). Mouse beta 2 microglobulin (β2M) is a 0.6-kb cDNA provided by Dr. F. Mushinski (NIH). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was a rat 1.3-kb cDNA obtained from M. Piechaczyk.

*RNAs and Northern blots.* RNA was extracted using guanidinium isothiocyanate and centrifugation through cesium chloride. Cloned DNA from endogenous B14 (*Mpmv* subclass), B56 (*Pmv* subclass), and B77 (non-MCF-related) sequences (15) and RNA from ecotropic, xenotropic, and MCF MuLVs (16) were included with each blot to monitor hybridization specificity. RNA was size fractionated in 0.7% formaldehyde-agarose gels as described (12), transferred to Nytran membranes (Schleicher & Schuell, Keene, NH), and then UV cross-linked with a "Stratalinker" (Stratagene, La Jolla, CA) resulting in substantially improved hybridization efficiency as compared to our previous studies in which blots were vacuum baked.

Blots probed with $Pmv_{env21}$ and $Mpmv_{LTR21}$ oligonucleotide probes were prehybridized at 45°C in 50 mM Tris (pH 8), 1% SDS, 1 mg/ml yeast RNA (type X-S; Sigma Chemical Co., St. Louis, MO), and 1 M NaCl; hybridized at 45°C in the same mix containing $5 \times 10^6$ cpm/ml of oligonucleotide and labeled with [$^{32}$P]γATP and washed at 45°C for 15 min in 6× SSC, 0.1% SDS, and twice for 30 min in 1× SSC, 1% SDS. The $Mpmv_{env19}$ and $Pmv_{LTR21}$ probes were hybridized and washed at 48°C using the same conditions. Probes were stripped by washing in 0.1% SDS at 65°C for 10 min: stripping was confirmed by autoradiography before reprobing of the blots. Autoradiography was performed at −70°C with intensifying screens.

*Mitogens.* Concanavalin A (Con A) type IV-S and *Staphylococcus typhimurium* lipopolysaccharide (LPS) were purchased from Sigma Chemical Co. and were resuspended in sterile PBS before intraperitoneal injection in a final volume of 0.1 ml.

## Results

*Characterization of class-specific oligonucleotide probes.* Endogenous *Pmv* and *Mpmv* sequences are very similar, but may be distinguished by a characteristic 27-bp *env* deletion in *Mpmv* sequences (13). Conversely, there is an ~ 50-bp LTR deletion in cloned *Pmv* LTRs, but not *Mpmv* LTRs (17–19). The use of *Pmv* and *Mpmv env*-specific oligonucleotide probes on Southern blots has been described previously (14), but *Pmv* and *Mpmv* LTR-specific probes have not been reported previously. To distinguish *Pmv* and *Mpmv* RNA on Northern blots, we synthesized oligonucleotides specific for the *env* and LTR deletions characteristic of these two sequence subclasses (Table I) and determined the specific hybridization conditions given in Methods.

*Pmv and Mpmv expression in thymuses of inbred mouse strains.* Thymus RNA from all five lupus-prone mice (NZB/BlN, MRL-*lpr/lpr*, MRL-+/+, and BXSB, Fig. 1; (NZB × NZW) $F_1$, data not shown) contained 8.4-kb *Mpmv* transcripts that hybridized to the *env*-specific probe $Mpmv_{env19}$ (Fig. 1). Of the four high leukemia strains (AKR/J, C58/J, HRS/J, and SJL/J), thymuses of two, C58/J and HRS/J, expressed low levels of these transcripts. In contrast, none of 10 control strains, nor an additional control strain, Balb/c (not shown), expressed any detectable 8.4 kb *Mpmv* thymic transcripts. NZB/J mice had essentially identical expression to the NZB/BlN shown. 8.4 kb *Pmv* transcripts were not associated with autoimmunity; they were easily detected in all strains except C57Bl/6 (Fig. 1).

To confirm the association of thymus 8.4 kb *Mpmv* RNA and lupus observed with the *env* probes, the blots shown in Fig. 1 were probed with the LTR-specific probes, $Pmv_{LTR21}$ and $Mpmv_{LTR21}$ (Fig. 2). These studies confirmed that thymic 8.4-kb *Mpmv* transcripts are easily detected only in lupus-prone mouse strains, and that thymic 8.4-kb *Pmv* transcripts are in nearly all strains (Fig. 2).

*Table I. Subclass-specific Probes for Endogenous Pmv and Mpmv Sequences*

| Probe: | | |
|---|---|---|
| | 861 | 879 |
| $Pmv_{env21}$ | CCTCTATAGTCCCTGAGACTGCCCCACCTTCTCAACAACCTGGGAC | |
| $Mpmv_{env19}$ | CCTCTATA | CAACCTGGGAC |
| | 861 | 906 |
| | 305 | 372 |
| $Pmv_{LTR21}$ | AGAACAGATGG CTC | TCAGACG |
| $Mpmv_{LTR21}$ | AGAACAGATGGTTCTCAGATAAAGCGGAACCAGCAACAGACACAGAAGCCCGAT | AGACG |
| | 305 | 372 |

The sequences shown are from bases 861 to 879 of MX27 ( a *Pmv* provirus) (13); bases 861 to 906 of MX33 (an *Mpmv* provirus) (13); bases 305 to 372 of B-56 (a *Pmv* provirus) (15, Krieg, unpublished observation); and bases 305 to 372 of B-14 (an *Mpmv* provirus) (15, Krieg, unpublished observation). Deletions are indicated by gaps in the sequences. Oligonucleotide probes for Northern blots were synthesized complementary to the underlined sequences. $Pmv_{env21}$ and $Mpmv_{LTR21}$ probes, which are complementary to regions deleted from *Mpmv env* and *Pmv* LTR sequences, respectively, hybridized only to the corresponding cloned DNAs, even at low stringency (data not shown). In contrast, the $Mpmv_{env19}$ and $Pmv_{LTR21}$ probes potentially could hybridize to the conserved sequences on either side of the deletions. When hybridized at a moderate stringency as described in Methods, these probes bound only to the corresponding DNA hybridization standards (data not shown). Bands hybridizing to *env*-specific probes were always detected by the corresponding LTR-specific probes. Sequences detected with these probes are described in the text as *Pmv* or *Mpmv*, respectively.

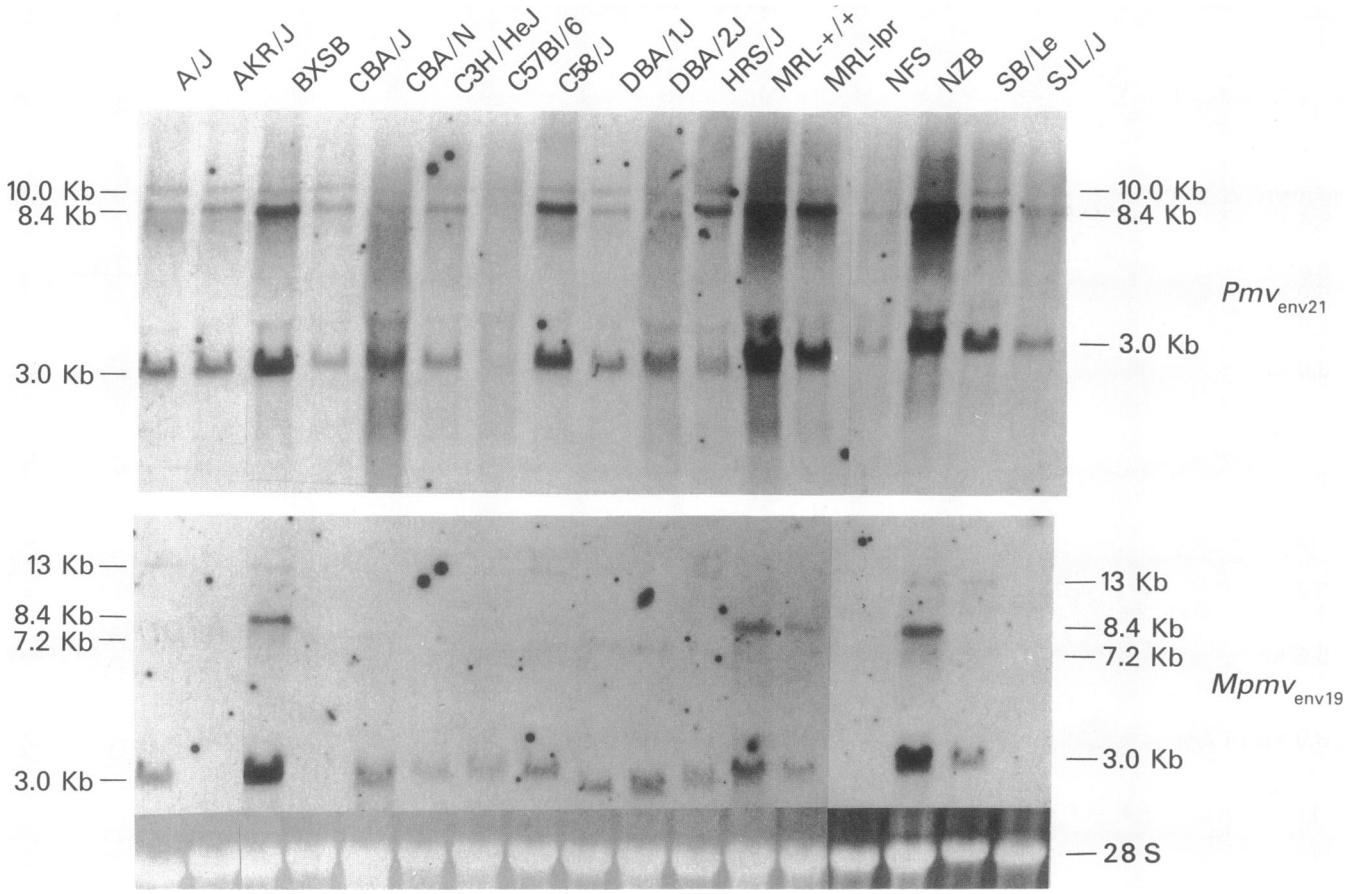


*Figure 1. Pmv* and *Mpmv env*-related expression in thymus RNA. Northern blot analyses with 10 μg of total thymus RNA were performed. Data represent a composite from two blots containing internal controls and probed with $Pmv_{env21}$ and then stripped and reprobed with $Mpmv_{env19}$. Similar amounts of RNA were present in each lane as determined by ethidium bromide staining (*bottom,* showing the 28 S rRNA band). Ages and sexes of the mice shown were: A/J, 2 mo, female; AKR/J, 7 wk, male; BXSB, 2 mo, male; CBA/J, 9 mo, male; CBA/N, 3 mo, male, C3H/HeJ, 5 mo, female; C57BL/6, 4 mo, male; C58/J, 2 mo, male; DBA/1J, 2 mo, female; DBA/2J, 6 mo, female; HRS/J, 2 mo, male; MRL-+/+, 5 mo, female; MRL-*lpr/lpr* (indicated on figure as MRL-*lpr*), 5 mo, female; NFS, 4 mo, male; NZB, 2 mo, male; SB/Le, 2 mo, male; SJL/J, 2 mo, female. Autoradiographic exposures were the same for all lanes.

*Lack of relationship between disease severity and thymus 8.4 kb Mpmv RNA in lupus-prone mice.* The *xid* mutation greatly retards development of autoimmune disease in lupus-prone strains, while the BXSB Y chromosome accelerates disease (20). Thymuses from the congenic lupus-prone mouse strains NZB/BlN and NZB.*xid* contained comparable amounts of 8.4-kb *Mpmv* RNA. Similarly, BXSB male, BXSB female, BXSB.Wild-Y, and BXSB.*xid* expressed comparable levels of 8.4-kb *Mpmv* RNA despite marked differences in disease severity (Fig. 3). MRL-+/+ and MRL-*lpr/lpr* mice also differ greatly from each other in disease severity, and also had very similar *Pmv* and *Mpmv* RNA levels (Fig. 1). Lupus-prone NZB/BlN, BXSB, and MRL-*lpr/lpr* mice expressed similar levels of thymus 8.4-kb *Mpmv* and *Pmv* RNA from birth through at least 6 mo of age, whereas control DBA/2 mice did not express thymus 8.4-kb *Mpmv* RNA at birth or later (data not shown).

*Effects of polyclonal immune activation on Pmv and Mpmv expression in control mice.* Thymus RNA of control mice injected with the T cell mitogen, Con A, showed no induction of 8.4-kb *Mpmv* RNA at the time points analyzed, despite induction of other *Mpmv* RNA and 8.4-kb *Pmv* RNA (Fig. 4 *A*, lanes *1–4*). The level of 8.4-kb *Pmv* RNA in lane *4* was similar to that present in a control NZB thymus RNA sample on the same blot. Both *Pmv* and *Mpmv* 8.4-kb transcripts were induced in the spleens of the same Con A-injected mice (Fig. 4 *B*). Thymus RNA from control mice injected with a B cell mitogen, LPS, also showed no induction of 8.4-kb *Mpmv* transcripts, despite induction of 5.6-kb *Mpmv* and 8.4-kb *Pmv* transcripts (Fig. 4 *A*, lanes *5* and *6*). 8.4-kb *Mpmv* and *Pmv* expression was highly induced in the spleens of mice injected with LPS (data not shown).

Control C3H/HeJ mice bearing the genotypes *lpr/lpr* or *gld/gld* develop polyclonal lymphoid activation with proportional increases in B cells producing antibodies to self and nonself antigens (21). These mice do not carry background genes for lupus and, in contrast to MRL-*lpr/lpr*, do not develop glomerulonephritis. Thus, the *lpr/lpr* and *gld/gld* genotypes exert immune effects similar to chronic polyclonal stimulation. C3H-*lpr/lpr* and *gld/gld* mice did not express thymus 8.4-kb *Mpmv* RNA despite increased expression of 8.4-kb *Pmv* and 13-kb *Mpmv* RNA (Fig. 5, and data not shown). Thus,

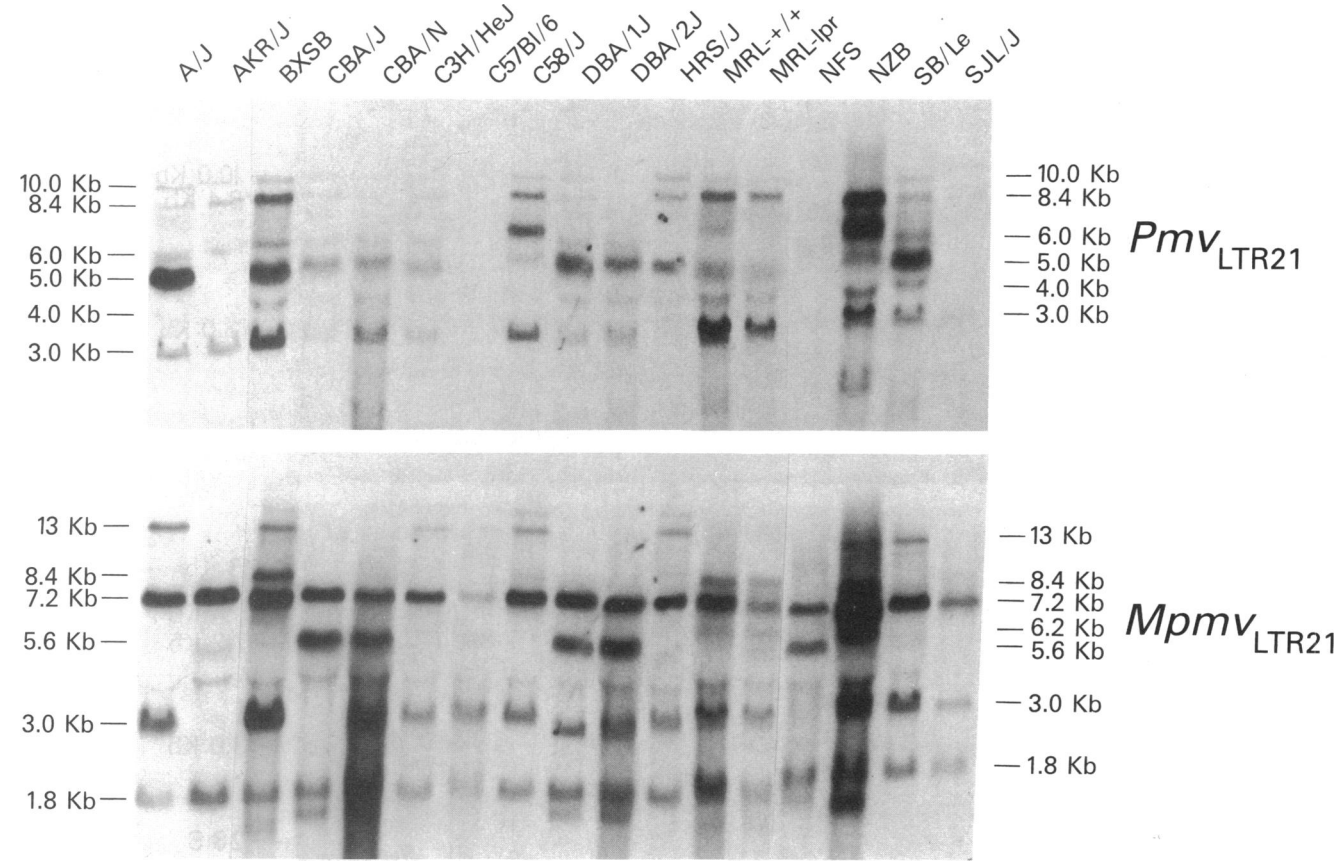


*Figure 2.* *Pmv* and *Mpmv* LTR-related expression in thymus RNA. This study represents a reprobing of the blots shown in Fig. 1 with $Pmv_{LTR21}$, and then with $Mpmv_{LTR21}$. Most of the subgenomic transcripts seen with LTR-specific probes (7.2-, 6.2-, 6.0-, 5.6-, 5.0-, 4.0-, and 1.8 kb) were not detected with *env*-specific probes (Fig. 1), probably because of *env* deletions present in many transcribed endogenous proviruses (17, 35–37). The 13- and 10.0-kb transcripts were greater than full-length, and may represent read-through transcripts (35).

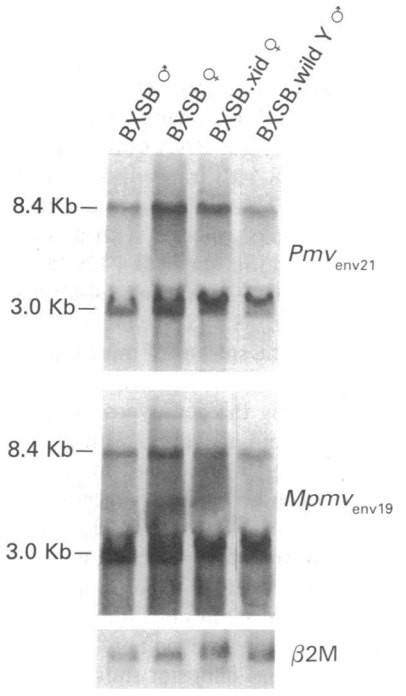


*Figure 3.* Studies of *Pmv* and *Mpmv* expression in BXSB congenic mice. Northern analyses were performed with 20 μg total thymic RNA with the indicated probes. RNA loading appeared comparable by reprobing the blot with β2M (*bottom*). All mice were 2 mo old.

polyclonal activation of control mice with either mitogens or the *lpr/lpr* or *gld/gld* mutations failed to induce thymus 8.4-kb *Mpmv* RNA.

*Pmv and Mpmv expression in spleens of inbred mouse strains.* More than half of the control mouse strains had detectable levels of 8.4-kb *Mpmv* RNA in their spleens with the sensitive techniques used herein (Fig. 6). Thus, the association of 8.4-kb *Mpmv* expression with autoimmunity is at least somewhat tissue specific. However, there was still a quantitative difference in expression between lupus-prone and control strains: the lupus-prone strains BXSB and NZB expressed substantially higher levels of 8.4-kb *Mpmv* RNA than did the control and high leukemia strains.

## Discussion

These studies demonstrate that five of five lupus-prone mouse strains and crosses express high levels of thymic 8.4 kb *Mpmv* transcripts; two of four high leukemia strains express low levels; and none of eleven control strains express detectable levels of these transcripts in the thymus. The association with lupus was tissue specific in that low levels of 8.4-kb *Mpmv* RNA were present in spleens of many control mice. Thymic 8.4-kb *Mpmv* RNA expression was present from birth in

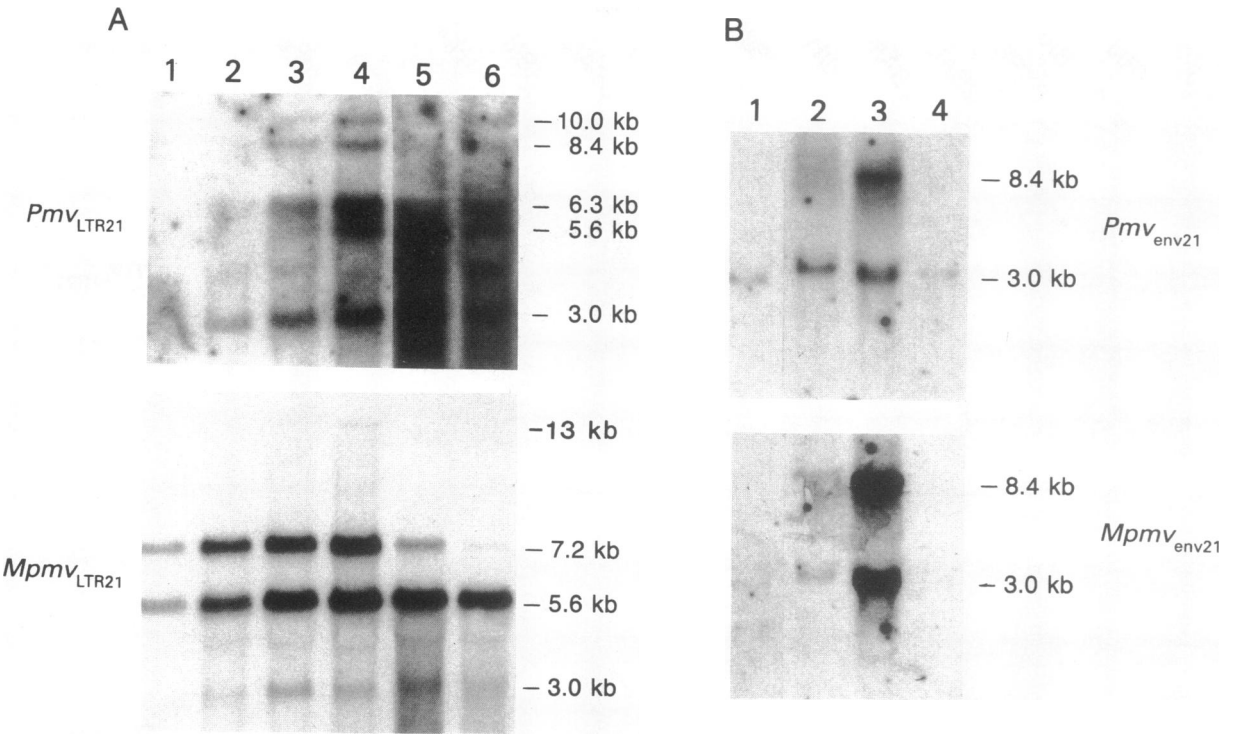


*Figure 4.* Effects of mitogens on *Pmv* and *Mpmv* expression in thymuses and spleens of NFS mice. (*A*) 4-mo-old male NFS mice were injected intraperitoneally with 100 μg Con A (lanes *2–4*) or 50 μg of LPS (lanes *5* and *6*), and thymus RNA prepared at 4 h (lanes *2* and *5*), 8 h (lane *3*), 24 h (lane *6*) and 26 h (lane *4*). Lane *1* is from a control uninjected mouse. Northern blot analyses were performed using 10 μg of total thymic RNA and probed with $Mpmv_{LTR21}$, stripped, and then probed with $Pmv_{LTR21}$. Equal amounts of RNA were present in all lanes as determined by hybridization to GAPDH (not shown). (*B*) Splenic RNA was prepared from the same Con A-injected mice whose thymus RNA is shown in *A*, lanes *1–4*. 20 μg of total spleen RNA was electrophoresed through a 1% agarose gel and hybridized to the *env*-specific probes.

lupus-prone mice and thus preceded pathologic changes. Studies of several congenic lupus-prone mouse strains demonstrated that genes that retard or accelerate disease do not substantially affect expression of thymic 8.4-kb *Mpmv* RNA. Thus, thymic 8.4-kb *Mpmv* expression is not directly associated with disease severity; instead, such constitutive expression may be determined by "background" genes in lupus-prone mice.

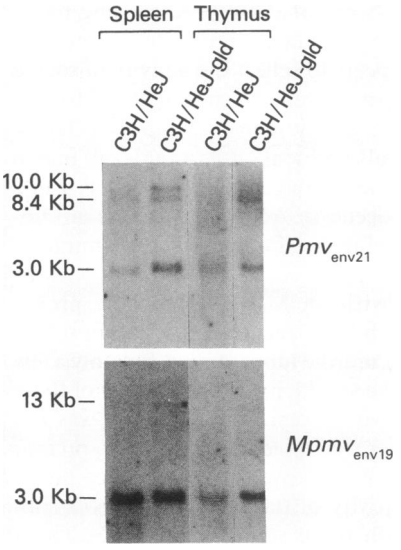


*Figure 5. Pmv* and *Mpmv* expression in C3H/HeJ and C3H-*gld/gld* mice. Northern analyses were performed with 5 μg of poly(A) + splenic RNA or 20 μg total thymic RNA with the indicated probes in a 1% agarose gel. Age and sex of the mice were: C3H/HeJ, 5 mo, male; C3H-*gld/gld*, 4 mo, male.

Polyclonal immune activation is an early feature of murine SLE and can be detected in the first week of life (21–23). Thus, it remained possible that thymic expression of 8.4-kb *Mpmv* RNA in autoimmune mice might be secondary to polyclonal immune activation rather than representing a novel genetic abnormality. However, injection of control mice with polyclonal immune activators failed to induce 8.4-kb *Mpmv* transcripts in the thymus. C3H-*lpr/lpr* and C3H-*gld/gld* mice with spontaneous polyclonal immune activation also failed to express detectable levels of thymus 8.4-kb *Mpmv* RNA. This did not represent a failure of the mitogens or genes to affect the thymus since they did induce thymic 8.4-kb *Pmv* transcripts up to the same levels expressed in lupus-prone mice. Thus, 8.4-kb *Mpmv* RNA expression does not appear to be induced by polyclonal immune activation, and can be regulated independently from 8.4-kb *Pmv* expression, which is induced by polyclonal activation. These data suggest that tissue-specific thymic 8.4-kb *Mpmv* expression in lupus-prone mice is not just secondary to polyclonal activation, which is characteristic of these mice, but rather requires background genes that are not readily induced in control mice. Alternatively, genes active in control mice may prevent thymus 8.4-kb *Mpmv* induction or expression.

The genetics of murine lupus are quite complex, with evidence for at least six genes contributing to the autoimmune

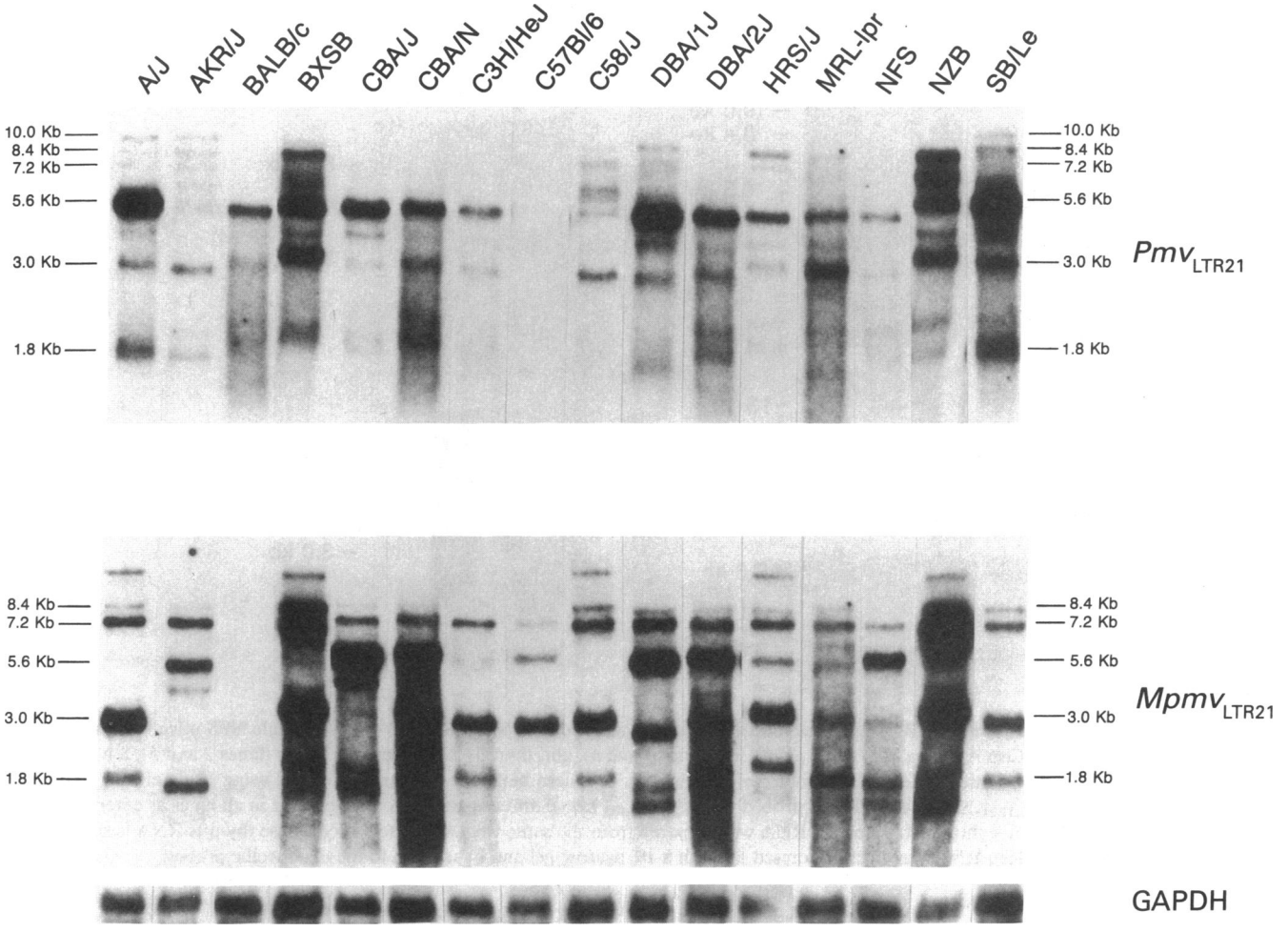


*Figure 6. Pmv* and *Mpmv* LTR-related expression in spleen RNA. Northern blot analyses with 5 μg of poly(A)+ RNA were performed in 1% agarose gels. Data are a composite from two blots containing internal controls and probed together with $Pmv_{LTR21}$, stripped, and then reprobed with $Mpmv_{LTR21}$. Similar amounts of RNA were present in each lane, as determined by reprobing with the housekeeping gene, GAPDH (*bottom*). Ages and sexes of the mice were the same as in Fig. 1 except for DBA/2, which was 2 mo old, and Balb/c, which was a 2-mo old female. *Pmv* and *Mpmv* expression was severalfold lower in spleen than thymus, an observation not readily apparent from the figures, since Figs. 1 and 2 used total RNA, while this figure uses poly (A)+ RNA.

phenotype of NZB mice (24). We distinguish background genes, which predispose to the development of autoimmunity, from other genes. For example, expression of the c-*myc* protooncogene is elevated in spleens of lupus-prone mice (25). This expression is closely related to disease severity: splenic c-*myc* expression is (*a*) greatly reduced in BXSB females as compared to BXSB males, (*b*) reduced in NZB.xid as compared to NZB, and (*c*) much lower in MRL-+/+ than in MRL-*lpr/lpr* (25). In normal mice, this expression can be induced by polyclonal activation (26). Thus, c-*myc* expression correlates well with disease severity in lupus-prone mice and with polyclonal activation. Therefore, elevated c-*myc* expression in lupus-prone mice is induced by other factors and does not itself represent a "background" gene for autoimmunity. We believe that many genes like c-*myc* are induced in lupus-prone mice and are likely important in mediating and regulating autoimmune organ pathology and other inflammatory responses. However, lupus-prone mouse strains must have different genes that predispose to the development of these autoimmune responses. None of these predisposing background genes have yet been identified. Constitutive expression of 8.4 kb *Mpmv* RNA appears to reflect the activity of such a background gene in lupus-prone mouse strains, and thus represents one of the earliest genetic defects in murine lupus. Since this expression was also present (at a lower level) in two of four high leukemia strains, it may also be associated with a predisposition to leukemogenesis. Additional factors are undoubtedly required for the full development of autoimmunity or leukemia.

In previous studies with an MCF *env*-specific probe, $MCF_{env}$, we observed 8.4 kb MCF-related transcripts in spleen and thymus RNA from all murine lupus strains, but only a few control strains (11, 12). The specificity and sensitivity of those studies were limited by two factors: firstly, $MCF_{env}$ was not subclass-specific, but rather hybridized to a sequence present in both the *Pmv* and *Mpmv* subclasses of MCF-related proviruses. Secondly, the sensitivity of the present studies is substantially greater than with those studies with $MCF_{env}$ as a

result of (*a*) technical refinements (see Methods) and (*b*) the more efficient hybridization of the subclass-specific probes (19 to 21 nucleotides long) compared to $MCF_{env}$ (16 nucleotides long). Previous studies of MCF expression with $MCF_{env}$ in young AKR mice did not detect 8.4 kb RNA (16; and unpublished observation), but with the improved techniques and probes of the current study, 8.4 kb *Pmv* RNA can now be readily detected in such mice (as shown in Figs. 1 and 2). Thus, studies performed using the $MCF_{env}$ probe gave a less complete impression of endogenous retroviral expression than that obtained with the new probes.

Southern analyses have demonstrated no unique *Mpmv* proviruses shared by lupus-prone strains that are not also present in some control strains (Stoye, J., W. Frankel, and J. Coffin, personal communication). Therefore, 8.4 kb *Mpmv* expression in lupus-prone mice most likely results from some difference in the regulation of gene expression between lupus-prone and control mice. This difference could be at the level of RNA transcription, processing, or degradation.

Thymic abnormalities in lupus-prone mice may contribute to the development of their autoimmunity. For example, in lupus-prone NZB mice tolerance defects develop in the thymus (27). Could abnormal thymic endogenous retroviral expression (such as 8.4 kb *Mpmv* RNA) directly or indirectly perturb thymic function in lupus-prone mice? Direct immune effects of endogenous MCF-related proteins have been suggested by our recent antisense studies (10). Indirect immune effects could result from autoimmune responses to endogenous retroviral envelope proteins such as SU (SU was formerly called gp70). SU is present on the membranes of murine lymphocytes, and is particularly highly expressed on thymocytes (28). Lupus-prone strains of mice (and many humans with lupus) produce antibodies reactive with such retrovirally encoded determinants, and thus potentially reactive with lymphocytes and thymocytes (6, 29). Indeed, antithymocyte antibodies, some of which may be antiretroviral, are present in sera from mice and humans with SLE and can interfere with normal T cell functions, especially those of immune regulation (30–34). Such antibodies might also interfere with normal intrathymic developmental processes. Thus, abnormal thymic endogenous retroviral expression could predispose to immune abnormalities found in murine and human lupus. Of note, many classes of endogenous retroviruses are expressed in peripheral blood mononuclear cells from lupus patients as well as controls (Krieg and Steinberg, unpublished data). Many new human endogenous retroviral sequences have been cloned in the past few years, and the possibility remains that an association of endogenous retroviral expression with human lupus exists. The special association of murine lupus with thymus 8.4 kb *Mpmv* expression could provide an important clue to the genetic and immune regulatory defects in murine and perhaps human SLE.

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