## *Brief Definitive Report*

# ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN LYMPHOKINE GENES OF NORMAL AND AUTOIMMUNE MICE

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The genetic origin for the lupus-like disease in (NZB × NZW)$F_1$, MRL/*lpr* or BxSB mice is largely unknown (reviewed in reference 1). The mice develop symptoms such as generalized B cell hyperactivation, early IgM to IgG class switch, or functional abnormalities in the $CD4^+$ T cell subpopulation indicating the potential involvement of lymphokines in the pathogenesis of the disease. This assumption is supported by findings such as decreased IL-2 and IL-2 receptor expression in (NZB × NZW)$F_1$, MRL/*lpr* and BxSB mice (2), abnormal IL-1 transcripts (3), increased IL-4 expression (4), spontaneous transcription of IFN-$\gamma$ (5), and increased TNF-$\alpha$ activity in MRL/*lpr* mice (6). In contrast, TNF-$\alpha$ production was found to be decreased in NZW and (NZB × NZW)$F_1$ mice (7). The authors proposed that decreased TNF-$\alpha$ production might be related to a mutation in the TNF-$\alpha$ gene as demonstrated by RFLP analysis. Moreover, administration of TNF-$\alpha$ partially abolished the development of the disease. Altogether, the participation of lymphokines in autoimmunity suggested to analyze lymphokine genes at the genetic level. First, we sought to reevaluate the relevance of the TNF-$\alpha$ polymorphism in NZB by determining the frequency of this allele in a large number of laboratory and wild mice and found that the TNF-$\alpha$ allele of NZW is inherited in most normal inbred strains and wild mice arguing against an involvement of the NZW TNF-$\alpha$ gene as a causal agent in the development of lupus in (NZB × NZW)$F_1$ hybrids. Second, we searched for polymorphisms in other lymphokine or lymphokine receptor genes whose expression seems to be abnormal in autoimmune mice. As judged from the RFLP analysis, the genes encoding IL-1$\alpha$, IL-2, IL-2 receptor $\alpha$ chain, IL-4, IL-5, and IFN-$\gamma$ seem to be normal in autoimmune strains.

## Materials and Methods

The sources of (C57BL/6 × DBA/2)$F_1$, BALB/c, C3H, DBA/2, BxSB, MRL/*lpr*, NZW, NZB, MOLF/EI (*Mus musculus molossinus*), CAST/EI (*Mus musculus castaneus*), *Mus musculus musculus*, *Mus spretoides* (4A), *Mus spicilegus* (4B), *Mus spretus*, *Mus cervicolor*, *Mus cookii*, *Mus caroli*, *Mus pyromys saxicola* (plathythrix), and *Mus coelomys pahari*, and of Lewis rat have been described (8, 9). Preparation of liver DNA and Southern blot analysis have been described

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(9). As nick-translated probes, purified fragments carrying the following lymphokine cDNA regions were used: TNF-α, 1.0-kb Sal I fragment of pSVd2-3 mTNF (10); IL-1α, 1.0-kb Bam HI fragment of pIL-1-1 1301 (11); IL-2, 0.8-kb Sal I full-length cDNA of psupDol-IL-2 (Blankenstein, T., unpublished); IL-4, 0.5-kb Bgl II fragment of pXEP.IL-4 (Blankenstein, T., unpublished); IL-2 receptor α chain: 0.95-kb Hind III-Rsa I fragment of pMIL2R-2 (12); IL-5: 0.5-kb Bam HI-Acc I fragment of pSP6K-mTRF23 (13); IFN-γ, 0.6-kb Pst I fragment of pAT153 MIFN G (Fiers, W., unpublished).

## Results

Bam HI-digested liver DNA of a large number of autoimmune, normal laboratory mice and murine rodents representing the phylogenetical distance between house mice and the rat were analyzed by Southern blots using the TNF-α probe (Fig. 1, *a* and *b*). A 10.5-kb Bam HI fragment lights up in NZB, DBA/2, BALB/c, and the heterozygous (C57BL/6 × DBA/2)$F_1$. After longer exposure, an additional 1.5-kb fragment appears in these mice. The 12-kb Bam HI allele present in NZW is also inherited in MRL/*lpr*, BxSB, C3H, (C57BL/6 × DBA/2)$F_1$, MOLF/EI (inbred *M. m. molossinus*), CAST/EI (inbred *M. m. castaneus*), and in outbred *M. m. musculus*, *M. spretoides*, *M. spicilegus*, *M. spretus* and *M. caroli*. The phylogenetically more distantly related *M. cervicolor*, *M. cookii*, *M. c. pahari*, *M. p. saxicola*, and the rat exhibit TNF-α-specific bands of different size. The complete lack of allelic forms within the wild mice species and the frequent occurrence of the 12-kb Bam HI band indicate the strong evolutionary constraint of the TNF-α gene (9). The allele represented by the 12-kb band in NZW is inherited in most laboratory and wild mice, arguing against a defect TNF-α gene in NZW mice based on this polymorphism (7). Additionally, no polymorphism could be detected when NZW and other autoimmune mice were compared with normal inbred strains in Eco RI digests and hybridized

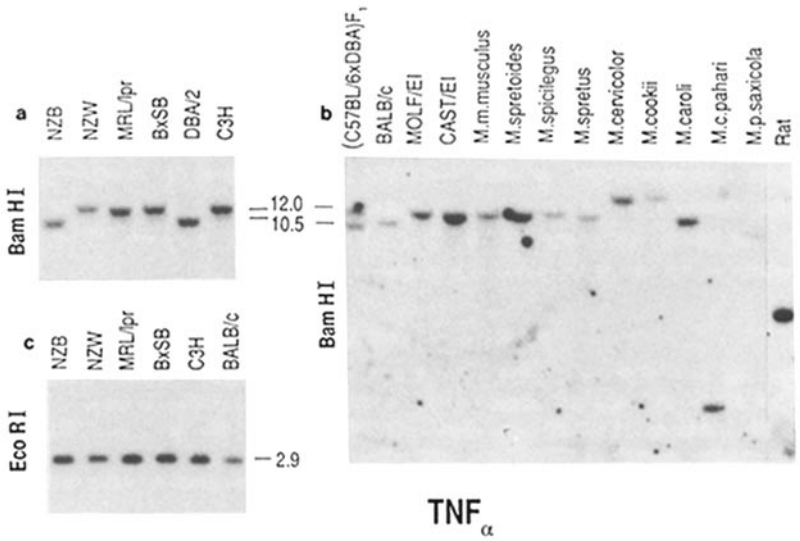


FIGURE 1. Southern blot analysis of indicated animals with the TNF-α probe. (*a*) Normal and autoimmune laboratory strain and (*b*) wild mice including rat DNA digested with Bam HI; (*c*) DNA of laboratory strains digested with Eco RI.

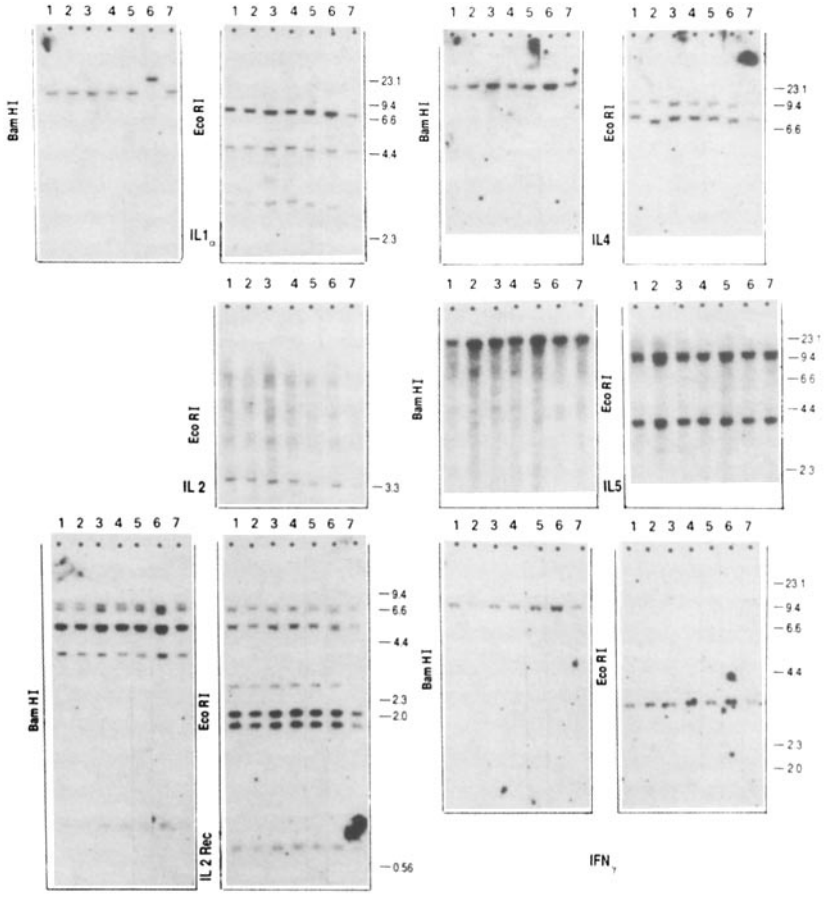


FIGURE 2. DNA of autoimmune and normal laboratory mice were hybridized in Southern blots to indicated lymphokine or lymphokine receptor probes. Probes and restriction enzyme used are indicated below and to the left, respectively. Size marker in all experiments was λ Hind III-digested DNA. Fragment size is given in kilobases. (*1*) NZB, (*2*) NZW, (*3*) MRL/*lpr*, (*4*) BxSB, (*5*) C3H, (*6*) BALB/c, (*7*) (C57BL/6 × DBA/2)$F_1$.

to the TNF-α probe (Fig. 1 *c*). Next, we searched for RFLPs in other lymphokine or lymphokine receptor genes of autoimmune mice in comparison to normal mice. Bam HI- and Eco RI-digested DNA were hybridized in Southern blots to the IL-1α, IL-2, IL-4, IL-5, IFN-γ, and IL-2 receptor probes (Fig. 2). For IL-2 only the Eco RI Southern blot is shown. No polymorphism could be observed in either of IL-2, IL-2 receptor, IL-5, or IFN-γ loci. BALB/c shows a Bam HI polymorphism in the IL-1α gene, NZW exhibits an IL-4 polymorphism for Eco RI.

## Discussion

The purpose of this investigation was to assess the potential genetically determined involvement of lymphokine genes in the pathogenesis of lupus-like disease on the basis of RFLP analysis. Similar studies have been performed concerning the Ig heavy chain V-, TCR $V_\alpha$-, and $V_\beta$-genes and the IL-6 locus without obvious success to demonstrate a correlation between disease and certain alleles or haplotypes (9, 14–16).

Based on the finding that a dominant gene closely linked to the MHC complex of NZW mice is one of the factors responsible for autoimmunity in (NZB × NZW)$F_1$ hybrids (16), it was proposed that a mutated TNF-$\alpha$ gene represented by a Bam HI polymorphism and therefrom resulting low TNF-$\alpha$ expression may be involved in the pathogenesis in (NZB × NZW)$F_1$ mice (7). The TNF-$\alpha$ gene has been located within the MHC complex (17). In contrast, the lupus gene or closely linked genes in NZW have been mapped by genetic analysis ~10 cMorgan apart from the MHC locus (16). Therefore, it seemed necessary to analyze whether this TNF-$\alpha$ polymorphism is found exclusively in NZW mice. RFLP analysis in a large number of inbred strains and wild mice showed that only BALB/c, DBA/2, and NZB possess the apparent normal TNF-$\alpha$ gene, whereas 11 other mice have inherited the same allele as NZW mice. Furthermore, both allelic forms do not seem to result from a larger mutation since no polymorphism could be detected upon Eco RI digests. These results argue against an involvement of the NZW TNF-$\alpha$ gene in the pathogenesis of (NZB × NZW)$F_1$ hybrids. This is supported by the recent finding of Boswell et al. who found increased TNF-$\alpha$ expression in MRL/*lpr* mice (6). MRL/*lpr* exhibit the same restriction site pattern for the TNF-$\alpha$ gene as NZW mice (Fig. 1).

The general B cell hyperactivation, increased numbers of lymphokine-secreting T cell populations, and a number of studies describing deregulated lymphokine expression in autoimmune mice indicate that lymphokines may be involved in the pathogenesis of autoimmune mice. We therefore extended the RFLP analysis to other lymphokine genes of autoimmune mice in comparison to normal mice.

As previously shown for IL-6 (9), no polymorphism could be detected in the loci containing IL-2, the IL-2 receptor, IL-5, and IFN-$\gamma$ genes. Allelic forms were identified in the IL-1$\alpha$ gene of BALB/c and in the IL-4 gene of NZW. The IL-4 allele in NZW is also inherited in MOLF/EI, the inbred *M. m. molossinus* (unpublished observation). Together, our results do not support the involvement of lymphokine genes as primary agent in the development of the lupus-like disease. The possible significance of an Eco RI RFLP in the IL-3 locus unique to NZB and NZW mice (unpublished observation) remains to be elucidated.

## Summary

Analysis of RFLP has been employed in lymphokine genes of autoimmune and normal mice. No polymorphism could be detected in the loci containing IL-2, IL-2 receptor, IL-5, and IFN-$\gamma$ in NZB, NZW, BxSB, and MRL/*lpr* mice when compared with normal mice. Allelic forms were identified in the IL-1$\alpha$ gene of BALB/c and in the IL-4 gene of NZW. The frequency of the Bam HI RFLP in the TNF-$\alpha$ gene of NZW which has been proposed to be associated with the development of autoimmune disease in (NZB × NZW)$F_1$ mice has been analyzed in a number of different inbred strains and in wild mice. Since the same allele is inherited in most autoimmune, healthy laboratory and wild mice the TNF-$\alpha$ gene does not seem to be one of the causal agents that contributes to the development of autoimmunity in (NZB × NZW)$F_1$ mice.

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