LINKAGE ANALYSIS OF THE MURINE INTERFERON-α LOCUS ON CHROMOSOME 4

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The human interferon-α and -β (IFN-α and -β)[1] gene family has been mapped to chromosome 9 and several studies have shown these genes to be closely linked (1–6). In view of the relevance and potential of the mouse model for the genetics of IFN production and action, the mapping of murine IFN-α (MuIFN-α) genes is important; using Southern blot analysis of hamster-mouse somatic cell hybrid DNA, we recently located the MuIFN-α gene family on chromosome 4 (7). In the present paper, we confirm by a different method the location of the MuIFN-α genes on this chromosome and define the region involved.

Since restriction fragment analysis using a MuIFN-α cDNA probe revealed polymorphism between BALB/c and C57BL/6 DNA, we took advantage of the seven recombinant inbred (RI) lines derived from these two strains (8, 9) and determined the strain distribution pattern (SDP) of the polymorphism. This SDP suggested linkage to several histocompatibility loci situated on chromosome 4. Restriction fragment analysis of DNA from several bilineal congenic (BLC) lines (8, 9) showed linkage of the MuIFN-α genes to the histocompatibility locus *H-15*.

Materials and Methods

Mice. Parental strains (BALB/c and C57BL/6) originally purchased from The Jackson Laboratory, Bar Harbor, ME have been maintained by brother-sister mating either at the Laboratory of Infectious Diseases at NIH or at Orsay for several years. The seven RI strains used, derived by D. Bailey from a BALB/c × C57BL/6 (CXB) cross (8), were either obtained from The Jackson Laboratory directly or maintained at Orsay by brother-sister mating.

BLC strains carrying different fragments of chromosome 4 of BALB/c origin on a C57BL/6 background (9, 10) were obtained from The Jackson Laboratory. These were HW 13 (B6.C-H-15^c-H-16^c-H-20^c-H-21^c/By), HW 13J (B6.C-H-15^c/By), HW 13K (B6.C-H-16^c/By), HW 17 (B6.C-H-18^c/By), HW 21 (B6.C-H-20^c-H-21^c/By), HW 35 (B6.C-H-21^c/By).

Three other BLC strains, HW 81, HW 94, HW 97 (all B6.H-28^c-If-1^1), which carry

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[1] *Abbreviations used in this paper*: BLC, bilineal congenic; IFN, interferon; MuIFN, murine interferon; NDV, Newcastle disease virus; RI, recombinant inbred; SDP, strain distribution pattern; SSC, 0.15 M sodium chloride, 0.015 M sodium citrate.

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a fragment of chromosome 3 of BALB/c origin on a C57BL/6 background, have been originally received from D. Bailey and were maintained at Orsay (9, 11, 12).

DNA. DNA isolated from the liver of the parental strains and F_1 hybrids was prepared by D. Safars, Institut Curie, Orsay, France. Spleen and liver DNA from the seven RI strains and from the various BLC strains was either purchased from The Jackson Laboratory or provided by J. Silver from the Laboratory of Infectious Diseases, NIH.

Southern Blot Analysis. Southern blot analysis was performed according to either of the following two procedures: (*a*) High molecular weight spleen or liver DNA (10 μg per lane) was digested with a fivefold excess of restriction endonuclease purchased from Bethesda Research Laboratories (BRL), Gaithersburg, MD, electrophoresed through 0.5% agarose gels and transferred to nitrocellulose membranes as described by Southern (13). A 292 base pair (bp) *Bq*lII–*Hin*cII fragment from the plasmid pMF1204, which represents a MuIFN-α_2 cDNA, was used as a probe (7). This fragment was nick-translated according to Rigby et al. (14) and hybridizations were done at 37°C in 50% formamide for 48 h. Membranes were washed two times in 0.15 M sodium chloride, 0.015 M sodium citrate (SSC) (2×), 0.1% sodium dodecyl sulfate (SDS) at 55°C, followed by two 15-min washes in 0.5× SSC, 0.1% SDS at 55°C, and were exposed to Kodak XAR-5 films for 1–3 d (see Figs. 1, 2, and 4). (*b*) High molecular weight DNA (20 μg per lane), digested for 4 h with a 10-fold excess of restriction endonuclease (BRL), was electrophoresed through 1% agarose gels and transferred to Genescreen (New England Nuclear, Boston, MA). The probe consisted of the 820-bp cDNA insert from pMF1204 (7). After nick-translation (14), hybridizations were done at 30°C in 50% formamide for 24 h. Membranes were washed according to the procedure recommended for Genescreen and exposed for 4–7 d to Dupont Cronex 4 films (see Figs. 3, 5 and 6).

Results

Detection of Polymorphism Between BALB/c and C57BL/6. Southern blot analysis of high molecular weight mouse DNA with a human IFN-α (15) or a MuIFN-α_2 cDNA probe has previously (7) revealed the presence of a multiple IFN-α gene family. As shown in Fig. 1, most of the fragments observed for various restriction endonucleases are common to both BALB/c and C57BL/6 mice. There are, however, several polymorphic fragments observable in some of the digests. There is a 6.6-kb *Eco*RI fragment (Fig. 1, lanes 1 and 2) and a 23-kb *Bam*HI fragment (Fig. 1, lanes 3 and 4) present in BALB/c but absent in C57BL/6. The *Hin*dIII digests (Fig. 1, lanes 5 and 6) reveal the presence of 11.0 and 2.2 kb fragments observable only in C57BL/6, whereas 9.4 and 2.1 kb fragments are common to BALB/c. No polymorphic restriction endonuclease fragments are detectable in the *Bg*lII digest (Fig. 1, lanes 7 and 8).

The existence of a polymorphism between C57BL/6 and BALB/c was confirmed by the analysis of the restriction pattern of the (C57BL/6 × BALB/c)F_1 hybrid DNA. Digestion with *Hin*dIII revealed the presence of both the 9.4 kb (BALB/c) and the 11.0 kb (C57BL/6) fragments as well of both the 2.1 kb (BALB/c) and the 2.2 kb (C57BL/6) fragments (data not shown), whereas upon digestion with *Eco*RI, the restriction pattern of the F_1 DNA contained the 6.6-kb fragment characteristic of the BALB/c DNA (see Fig. 3). Since both *Hin*dIII and *Eco*RI enabled the detection of polymorphism between BALB/c and C57BL/6, these two enzymes were selected for further analysis of RI and BLC strains.

Southern Analysis of DNA from CXB RI Strains. DNA from the seven CXB RI strains were restricted with *Hin*dIII or *Eco*RI and examined for the inheritance of the parental polymorphic restriction fragments (Figs. 2 and 3). Digestion with *Hin*dIII revealed that RI strains E and K contain both the 11.0 and 2.2 kb

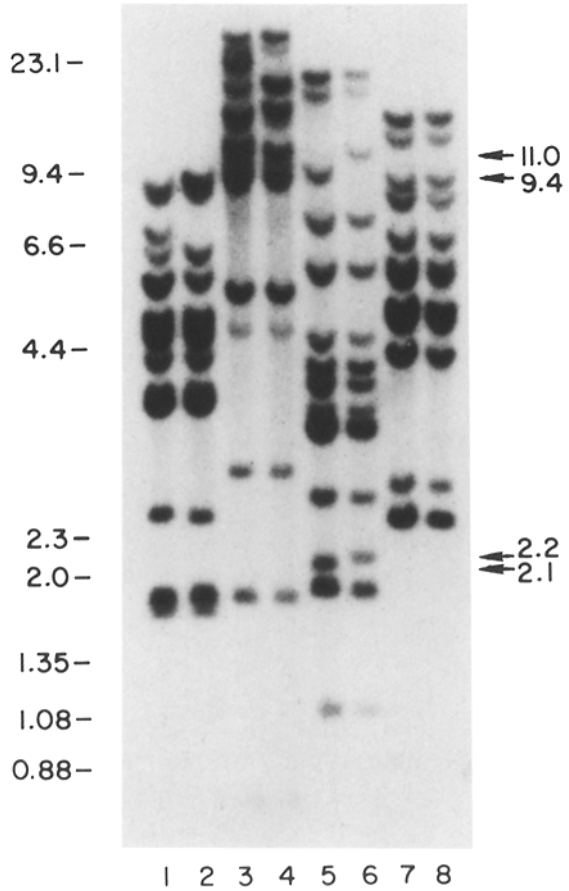

FIGURE 1. Southern blot analysis of BALB/c and C57BL/6 DNA using a *Bgl*II-*Hin*cII fragment of a MuIFN-α_2 cDNA probe. (Lanes *1*, *3*, *5*, and *7*) BALB/c DNA restricted with *Eco*RI, *Bam*HI, *Hin*dIII, and *Bq1*II, respectively; (lanes *2*, *4*, *6*, and *8*) C57BL/6 DNA restricted with *Eco*RI, *Bam*HI, *Hin*dIII, and *Bql*II, respectively. The 11.0 and 2.2 kb *Hin*dIII fragments of C57BL/6 and the 9.4 and 2.1 kb *Hin*dIII fragments of BALB/c are indicated by the arrows on the right. Sizes of *Hin*dIII-restricted λ-DNA and *Hae*III-digested φX174 DNA markers are indicated in kilobases on the left.

fragments characteristic of C57BL/6, while strains D, G, H, I, and J have both the 9.4 and 2.1 kb fragments of BALB/c (Fig. 2). Similarly, digestion with *Eco*RI showed that RI strains E and K lack the 6.6-kb fragment and, therefore, have a C57BL/6 pattern, whereas strains D, G, H, I, and J have the 6.6-kb fragment of BALB/c (Fig. 3).

Thus, the SDP of genomic DNA restricted with both *Eco*RI and *Hin*dIII indicates that, for IFN-α, E and K strains display the C57BL/6 restriction pattern whereas the other RI has the BALB/c pattern. This SDP was then compared with the SDP of other loci mapped on chromosome 4 (Table I). None of these SDP was completely identical to that of IFN-α, but four (*b*, *H-15*, *H-16*, *H-21*) differed only by one strain, suggesting that the IFN-α gene complex is located either near *H-15*, *H-16*, *H-21*, or *b* loci. The DNA from several BLC strains corresponding to these loci was further analyzed.

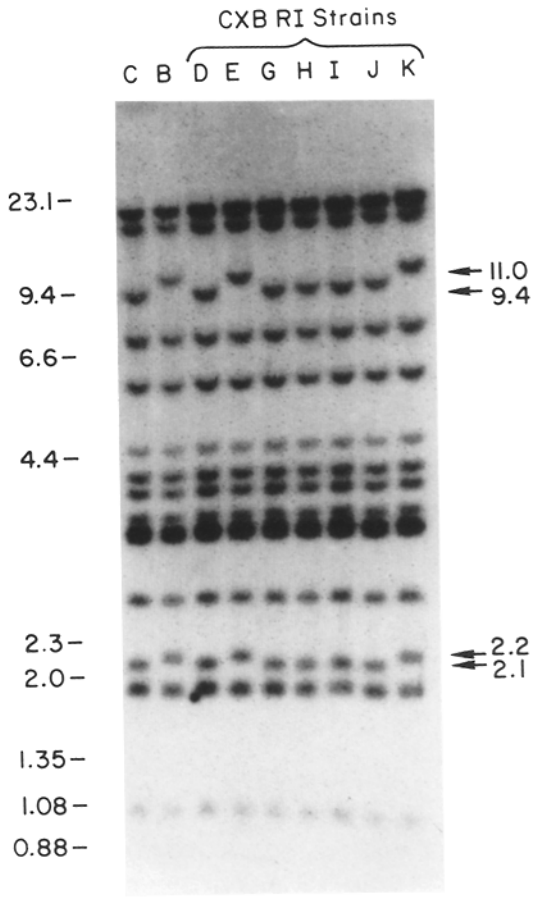

FIGURE 2. Hybridization of MuIFN-α_2 *Bq*1II-*Hinc*II fragment to BALB/c (*C*), C57BL/6 (*B*), and CXB RI DNA restricted with *Hin*dIII. The polymorphic *Hin*dIII fragments characteristic of BALB/c and C57BL/6 are indicated by arrows on the right and sizes are in kilobases. Sizes of *Hin*dIII-restricted λ-DNA and *Hae*III-digested φX174 DNA markers are indicated in kilobases on the left.

Southern Analysis of DNA from BLC Strains. DNA from lines HW 13J (*H-15*^{c}), HW 13K (*H-16*^{c}), HW 17 (*H-18*^{c}), HW 21 (*H-20*^{c} and *H-21*^{c}), and HW 35 (*H-21*^{c}) was examined for the polymorphic *Hin*dIII fragments. Only one BLC line, HW 13J (*H-15*^{c}) (Fig. 4, lane 3) had the polymorphic fragments characteristic of BALB/c, while the other BLC lines contained the C57BL/6 polymorphic fragments.

Further evidence was obtained by analyzing the *Eco*RI restriction pattern (Fig. 5). DNA from lines HW 13K (*H-16*^{c}), HW 17 (*H-18*^{c}), HW 21 (*H-20*^{c} and *H-21*^{c}), and HW 35 (*H-21*^{c}) showed the C57BL/6 restriction pattern, whereas DNA from line HW 13 (*H-15*^{c}, *H-16*^{c}, *H-20*^{c}, and *H-21*^{c}) showed the BALB/c pattern. This, by exclusion, again indicated linkage of IFN-α to *H-15*. Thus, apparently, during the construction of the BLC lines, the BALB/c IFN-α locus was introduced along with the BALB/c *H-15* locus into the C57BL/6 background.

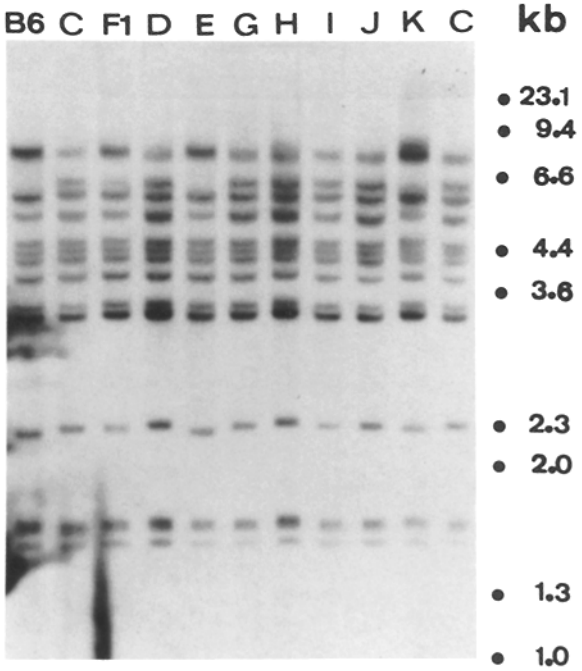

FIGURE 3. Southern blot analysis with the MuIFN-α_2 cDNA probe of DNA from C57BL/6 (*B6*), BALB/c (*C*), (B6XC)F_1, and CXB RI restricted with *Eco*RI. Sizes of *Hin*dIII-restricted λ-DNA and *Hae*III-digested φX174 DNA markers are indicated in kilobases on the right.

TABLE I

Strain Distribution Patterns in CXB RI strains of Polymorphic Loci on Chromosome 4

Locus	CXB RI Strain						
	D	E	G	H	I	J	K
b	B	B	C	C	C	C	B
Bfo	C	C	C	C	C	B	C
Exa	C	B	B	B	C	C	B
Gpd-1	C	C	C	B	C	B	B
Mup-1	B	C	C	C	B	C	B
H-15	C	B	C	C	B	C	B
H-16	C	B	C	C	C	B	B
H-18	B	B	C	B	C	B	B
H-20	B	B	C	B	C	B	B
H-21	B	B	C	C	C	C	B
IFN-α	C	B	C	C	C	C	B

C and B represent alleles characteristic of BALB/c and C57BL/6, respectively.

The *Hin*dIII and *Eco*RI restriction pattern of DNA derived from congenic strains carrying the BALB/c allele of the *If-1* locus on a C57BL/6 background (HW 81, HW 94, and HW 97) is of C57BL/6 type (Figs. 4 and 6).

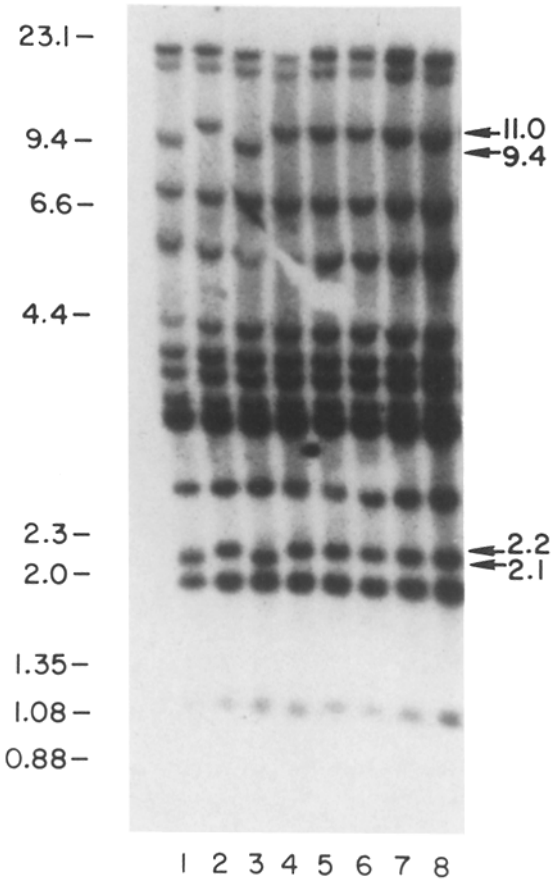

FIGURE 4. Hybridization of MuIFN-α_2 *Bq*II-*Hin*cII fragment to BLC DNA restricted with *Hin*dIII. (Lane *1*) BALB/c, (lane *2*) C57BL/6, (lane *3*) HW 13J, (lane *4*) HW 13K, (lane *5*) HW 17, (lane *6*) HW 21, (lane *7*) HW 35, and (lane *8*) HW 81 (B6.C-*H-28*^{c} *If-1*^{1}/By). The polymorphic *Hin*dIII fragments characteristic of BALB/c and C57BL/6 are indicated by arrows on the right and sizes are in kilobases. Sizes of *Hin*dIII-restricted λ-DNA and *Hae*III-digested φX174 DNA markers are indicated in kilobases on the left.

Discussion

Our previous analysis of a series of hamster-mouse somatic cell hybrids indicated that the MuIFN-α genes are located on chromosome 4 (7). The present results confirm this assignment to chromosome 4 using a different approach and taking advantage of restriction pattern polymorphisms of BALB/c and C57BL/6 DNA. The fact that in the seven RI and in the BLC strains, the different restriction patterns segregated out either as BALB/c or C57BL/6 type (Figs. 2–5) favors the existence of an IFN-α gene cluster; this situation would be comparable to the cluster of IFN-α genes on chromosome 9 in man (5, 6). Moreover, the nearly identical SDP of the IFN-α restriction pattern and of several loci already mapped on chromosome 4 in the seven RI lines (Table I) strongly suggested the presence of the IFN-α gene cluster on this chromosome. Firm evidence for this was provided by the observation that only the two BLC lines

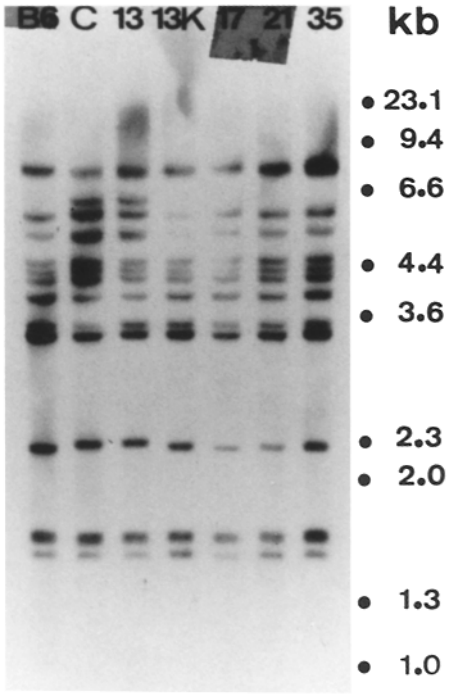

FIGURE 5. Southern blot analysis with MuIFN-α_2 cDNA probe of *Eco*RI-restricted DNA from C57BL/6 (*B6*), BALB/c (*C*), HW 13 (*13*), HW 13K (*13K*), HW 17 (*17*), HW 21 (*21*), and HW 35 (*35*). Sizes of *Hin*dIII-restricted λ-DNA and *Hae*III-digested φX174 DNA markers are indicated in kilobases on the right.

carrying the BALB/c fragment of chromosome 4 coding for *H-15* (lines HW 13 and HW 13J) displayed the BALB/c type restriction pattern (Figs. 4 and 5). On the contrary, DNA from BLC lines congenic at other histocompatibility loci on chromosome 4, but lacking the *H-15* region, have the C57BL/6 type restriction pattern for IFN-α (Figs. 4 and 5).

This indicates that the IFN-α gene cluster is situated near the *H-15* region, between the loci *Mup*-1 (major urinary protein) and *b* (brown). The distance between *Mup*-1 and *b* is ~4 centimorgans (cM), but the distance between *H-15* and either *Mup-1* or *b* is not known (D. Bailey, personal communication). It remains to be determined whether the linkage of the IFN-α gene cluster to *H-15* loci has a functional role such as abnormal immunological response.

The levels of circulating interferon after challenge with virus in vivo is affected by both the mouse genotype and the type of inducing virus, and several loci designated as *If* are involved (11, 12). BALB/c mice produce low amounts of IFN compared with C57BL/6 after challenge with Newcastle disease virus (NDV), and the *If-1* locus responsible for the difference has been linked to the *H-28* locus. It has been shown, however, that *H-28* is localized on chromosome 3 (DeMaeyer, unpublished results) and *If-1* has been recently mapped on chromosome 3 (17). It is, therefore, not surprising that the DNA from the B6.C-*H-28*^{c} *If-1*^{1} congenic strains showed the C57BL/6 restriction pattern. Since the structural genes for IFN-α are on chromosome 4, the mechanism by which *If-1*

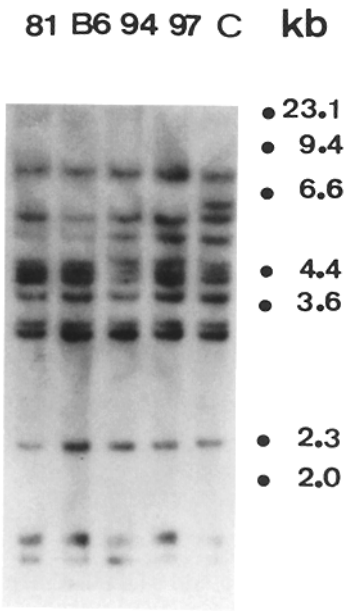

FIGURE 6. Southern blot analysis with the MuIFN-α_2 cDNA probe of *Eco*RI-restricted DNA from HW 81 (*81*), C57BL/6 (*B6*), HW 94 (*94*), HW 97 (*97*), and BALB/c (*C*). Sizes of *Hin*dIII-restricted λ-DNA and *Hae*III-digested φX174 DNA markers are indicated in kilobases on the right.

influences NDV-induced IFN levels is probably independent from differences in structural genes. It is, however, of interest that the *Lps* locus, influencing IFN production when bacterial lipopolysaccharide is used as an inducer, is situated on chromosome 4, also near the *b* locus.

In man, the IFN-α genes and the IFN-β_1 gene are clustered on the same chromosome (1–5), and preliminary evidence (Kelley and Pitha, manuscript in preparation, and 7 and 16) indicates that this linkage exists also in mouse.

Summary

Southern blot analysis with a murine interferon-α_2 (MuIFN-α_2) cDNA probe revealed restriction fragment polymorphism of *Eco*RI- and *Hin*dIII-digested C57BL/6 and BALB/c DNA. The inheritance pattern of this polymorphism was examined using DNA from each of the seven recombinant inbred strains derived from C57BL/6 and BALB/c; the strain distribution pattern suggests linkage of INF-α genes to two histocompatibility loci on chromosome 4. Southern blot analysis of DNA from six bilinear congenic strains carrying different fragments of the BALB/c chromosome 4 on a C57BL/6 background showed linkage of IFN-α genes to the histocompatibility locus *H-15*. It can therefore be concluded that the IFN-α gene cluster is situated on chromosome 4 near the *H-15* locus, between loci *Mup-1* and *b*.

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