Structural Organization of the Cytokine Gene Cluster on Human Chromosome 5

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Introduction

Cytokines make up a family of glycoprotein growth factors that have been shown to support clonal proliferation of hematopoietic progenitor cells [1]. Interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (CSF-2) stimulate differentiation and proliferation of cell progenitors along multiple (myeloid and erythroid) pathways [2–4]. Interleukin-4 (IL-4) can affect B cell, T cell, mast cell and macrophage functions [5]. Interleukin-5 (IL-5) is a more specific cytokine, acting on later stages of eosinophil differentiation [6].

The human genes for CSF-2, IL-3, IL-4, and IL-5 have been cloned, sequenced [3, 7–9], and mapped by in situ hybridization to human chromosome 5 at bands q23–31 [10–15], a region that is frequently deleted in patients with myeloid disorders [del(5q)] and acute myeloid leukemia (AML) [16, 17]. A close genomic linkage of human IL-3 and CSF-2 genes was reported [18, 19]. The distance between the genes was found to be only 10.5 kilobases (kb). The physical linkage of the IL-4 and IL-5 genes to within 240–310 kb was also demonstrated by long-range mapping, using pulse-field gel electrophoresis (PFGE) [15, 20].

Close linkage between the IL-3 and CSF-2 genes and between IL-4 and IL-5 genes, together with the similar gene structure, regulation, and biological activities of the four genes [21], suggests that they may have been derived from a common ancestral gene(s) and might be a part of a gene cluster related to the putative antioncogene that is involved in the development of AML and therapy-related acute nonlymphocytic leukemia.

We report the molecular cloning and characterization of three regions of the long arm of human chromosome 5 that contain the CSF-2, IL-3, IL-4, and IL-5 genes; we also studied the physical organization of these genes using PFGE and hybridization probes derived from chromosome walking.

Results and Discussion

To isolate genomic DNA clones containing the genes for human CSF-2, IL-3, IL-4, and IL-5, phage and cosmid genomic libraries of 1.5×10^6 and 2×10^6 clones, respectively, were prepared from human leukocyte DNA. The libraries were probed with synthetic oligonucleotides from the published sequences.

Three cosmids and eight phage clones identified with the IL-3 and CSF-2 probes at the first step of the genomic walk cover 70 kb (Fig. 1) [22]. To continue the cosmid walk in both directions, we set out to isolate single copy fragments devoid of repetitive sequences from both extremities of the 70-kb region. We subcloned the end fragments of cosmids cos-2 and cos-C by digestion with restriction enzymes that have a unique site in the polylinker region of the cosmid vector, followed by circularization [23]. On the basis of primary structure of the insert DNA of these clones, oligonucleotide

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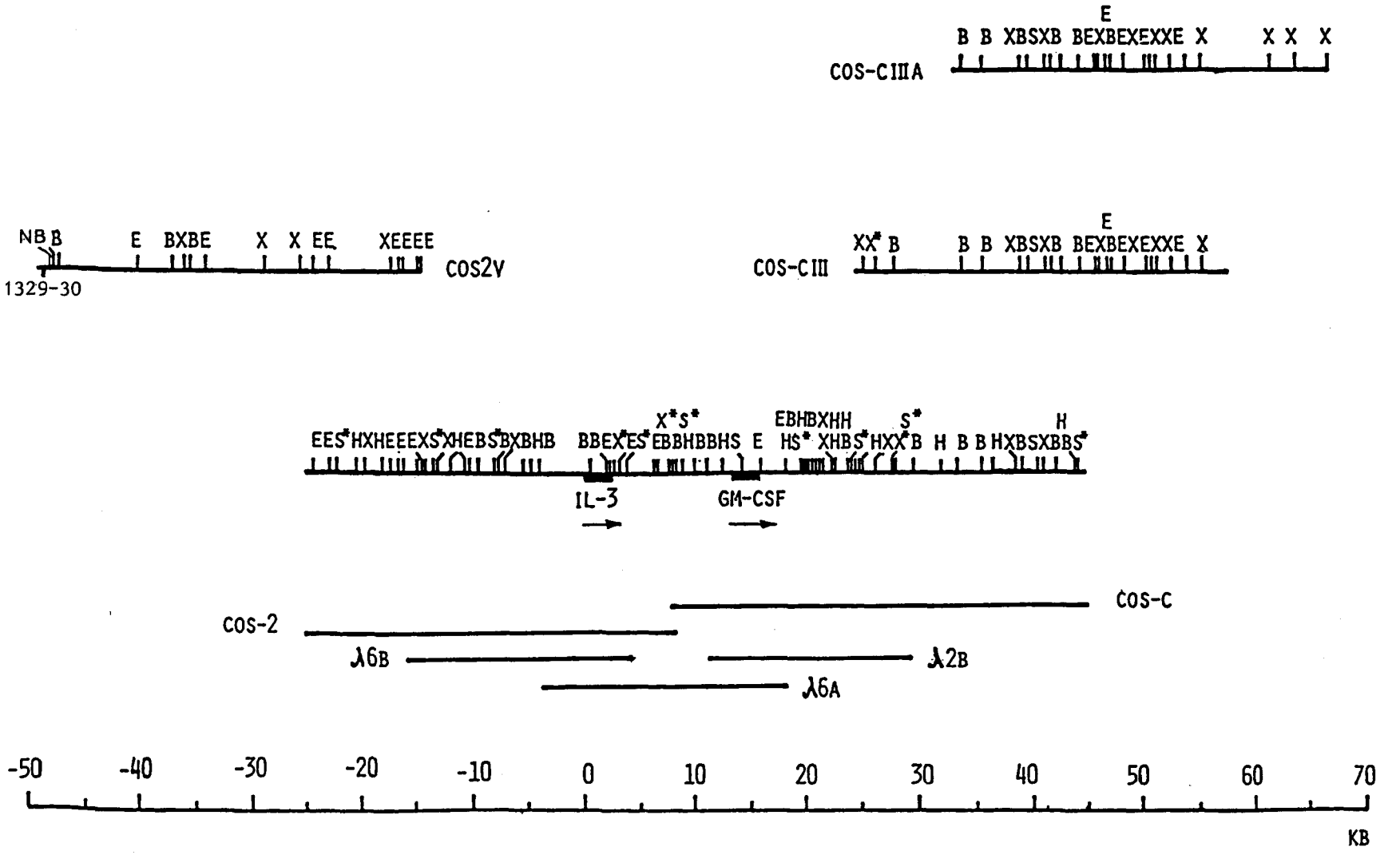

Fig. 1. Maps of the cloned 70-kb region that includes the genes for IL-3 and CSF-2 and cosmid clones that represent a 125-kb region: *E*, *Eco* RI; *X*, *Xba* I; *B*, *Bam* HI; *H*, *Hin* dIII; *S*, *Sal* I; *S**, *X**, *Xho* I; *N*, *Not* I; *Sf*, *Sfi* I; *B**, *Bss* HII. Cosmid and phage overlap. Localization of oligonucleotide probe 1329-30 is shown

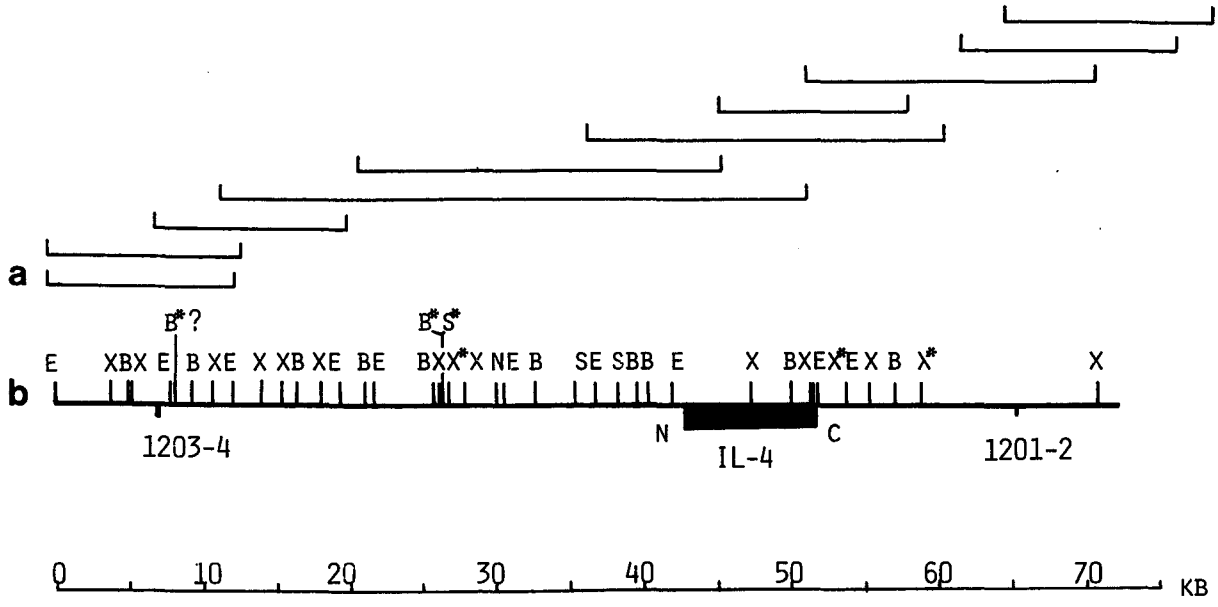

Fig. 2a,b. Map of the cloned 75-kb region that includes the IL-4 gene: *E*, *Eco*I; *X*, *Xba*I; *B*, *Bam*HI; *X**, *Xho*I; *S*, *Sfi*I; *B**, *Bss*HII; *S**, *Sac*II. Cosmid and phage overlap. Localization of oligonucleotide probes 1201-2 and 1203-4 is shown

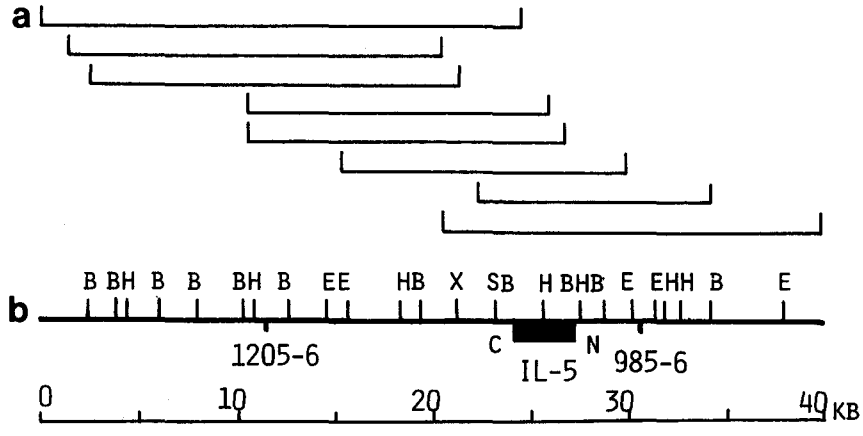

Fig. 3a,b. Map of the cloned 40-kb region that includes the IL-5 gene: *E*, *Eco*I; *X*, *Xho*I; *B*, *Bam*HI; *H*, *Hin*dIII; *S*, *Sal*I. Cosmid and phage overlap. Localization of oligonucleotide probes 1205-6 and 985-6 is shown

probes were synthesized. They were used to hybridize the cosmid library, yielding cosmids cos-2V, cos-CIII, and cos-CIIIA (Fig. 1). In total, the cosmid and phage walk comprised at least eight overlapping clones covering a 125-kb region around the IL-3 and CSF-2 genes.

Analogous genomic walks were performed around the IL-4 and IL-5 genes. More then six overlapping phage and cosmid clones represented the 75-kb IL-4 gene region (Fig. 2). A 40-kb region of the IL-5 gene was covered by two cosmid and at least three phage clones (Fig. 3). Further walking in both directions from the IL-5 gene was blocked by the presence of repetitive sequences.

We chose to analyze the sites for the rare-cutting restriction enzymes in three cloned regions. These enzymes usually have one or two CpGs in their recognition sequences and are methylation sensitive. The dinucleotide CpG, which is frequently methylated in a tissue-specific fashion, is underrepresent in bulk mammalian DNA by a factor of 5. Unmethylated restriction sites for the rare-cutting enzymes are often found clustered in

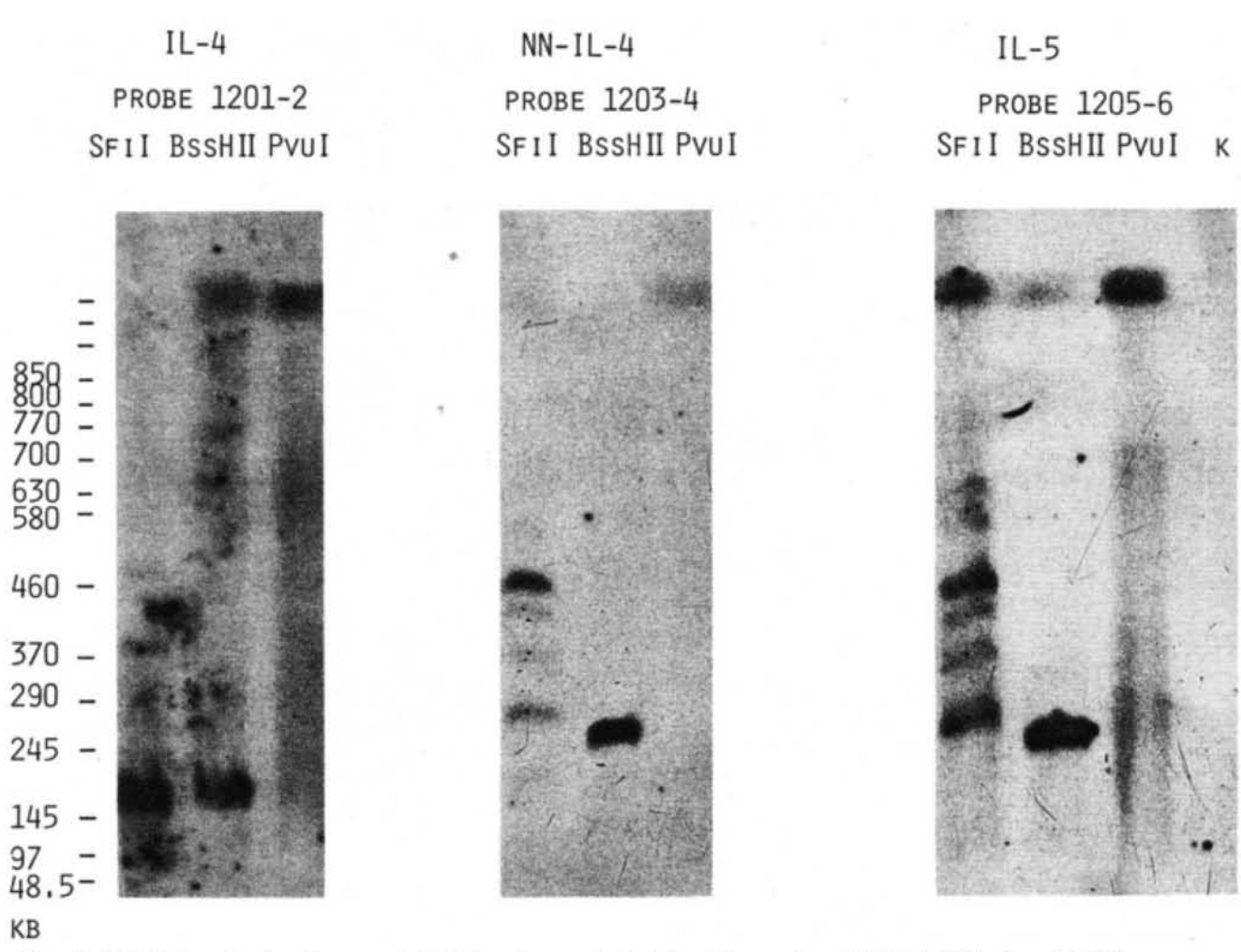

Fig. 4. PFGE analysis of genomic DNA using *Sac*II, *Mlu*I, and *Nae*I. Filter was sequentially hybridized to probes 1201-2, 1203-4, and 1205-6. The sizes of the markers are indicated

"CpG-rich islands," GC-rich regions of 1–2 kb where CpG is found at close to the frequency expected from local base composition and where methylation is suppressed. These islands are frequently found at, and mark, the 5′ ends of genes [24–26]. We mapped *Bss*HII, *Not*I, *Sfi*I, and *Sac* II restriction sites to trace possible CpG-rich islands.

As shown in Figs. 1 and 2, within the IL-3–CSF-2 region and the IL-4 region we found single restriction sites for *Bss*HII and *Sac*II. Only one *Not*I restriction site was present around 50 kb upstream from the IL-3–CSF-2 gene cluster (Fig. 1); this CpG-rich island may correspond to an unidentified gene because it lies adjacent to unique sequences conserved in evolution (data not shown).

To generate a large-scale map of the regions around the IL-3–CSF-2 and IL-4–IL-5 gene clusters we used rare-cutting restriction enzymes and PFGE. Several probes from each region derived from walking and partial sequencing were used for hybridization (Figs. 1–3). The results of several hybridization experiments are shown in Figs. 4–6; the sizes of the restriction fragments that hybridized to different probes are given in Tables 1 and 2. Our data confirmed physical linkage of the IL-4 and IL-5 genes shown earlier by hybridization with other probes [15, 20]: *Bss*HII, *Sfi*I, and *Nae*I digests revealed the same bands with probes 1203-4 and 1205-6 (Figs. 4, 5). Based on the length of the *Bss*HII and *Sac*II fragments (Table 2), we estimated the distance between the two genes as 240 kb. Genomic walks performed in the regions of the IL-4 and IL-5 genes allowed us to determine the orientation of both genes by using for hybridization the probes specific for the 5′ and 3′ ends of the genes separately (Figs. 4–6; Tables 1, 2). The results of these experiments allowed us to place the

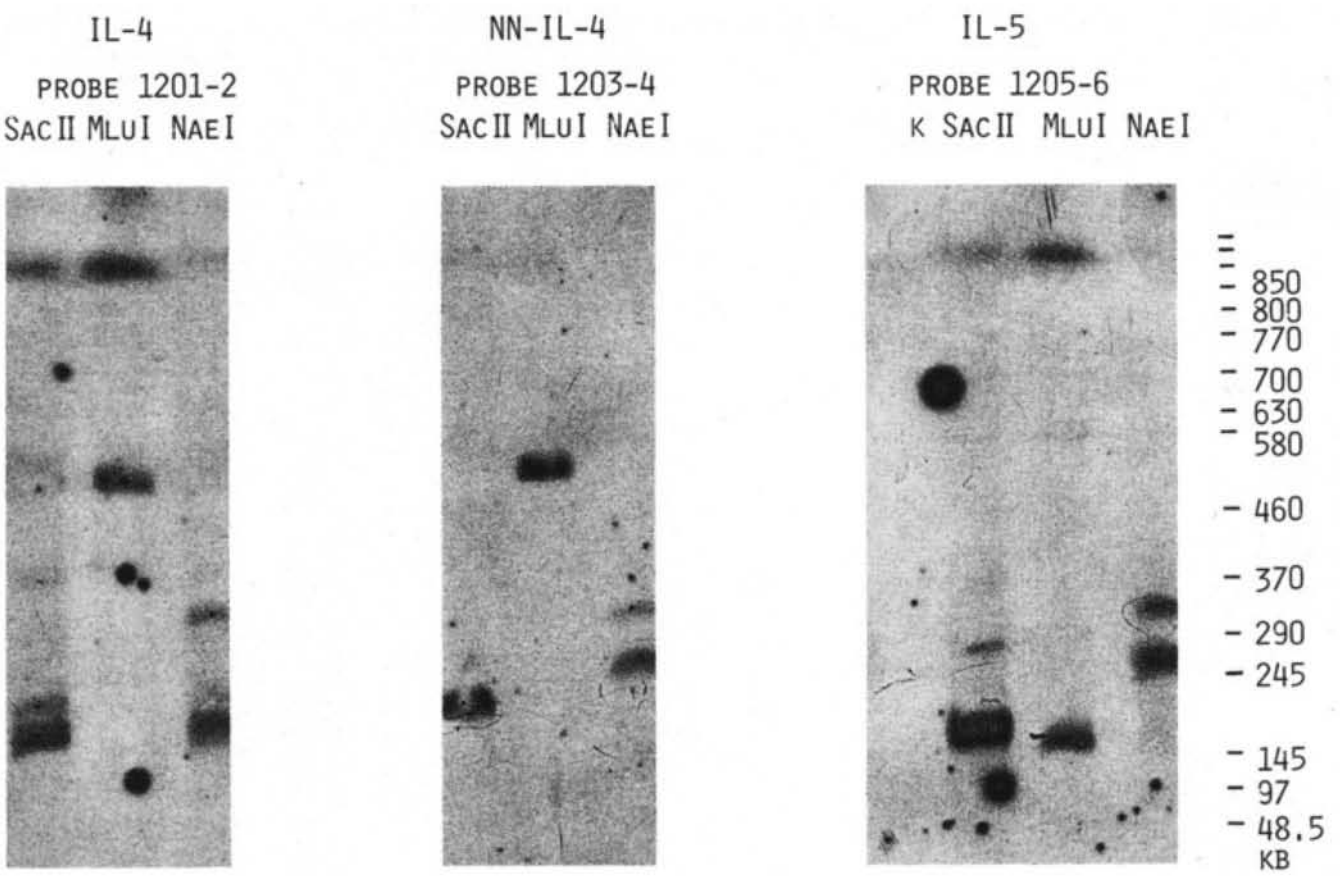

Fig. 5. PFGE analysis of genomic DNA using *Sfi*I, *Bss*HII, and *Pvu*I. Filter was sequentially hybridized to probes 1201-2, 1203-4, and 1205-6. The sizes of the markers are indicated

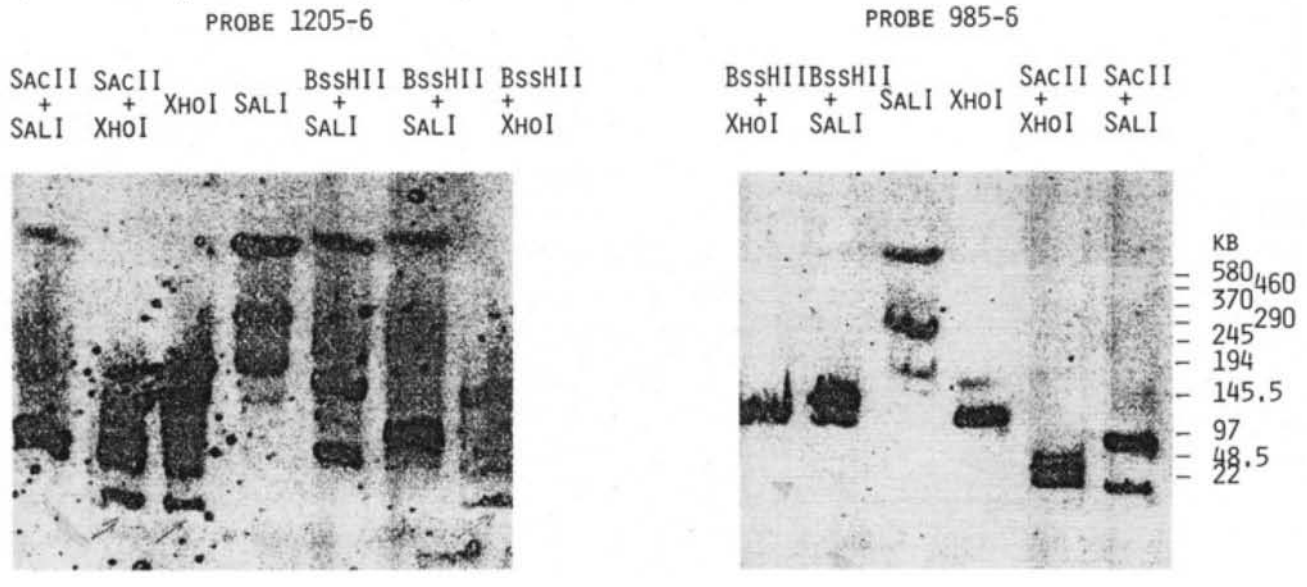

Fig. 6. PFGE analysis of genomic DNA using *Sal*I, *Xho*I, *Bss*HII + *Xho*I, *Bss*HII + *Sal*I, *Sac*II + *Xho*I, *Sac*II + *Sal*I. Filter was hybridized to probes 1205-6 and 985-6. The sizes of the markers are indicated

genes on a long-range restriction map of this cluster in head to head orientation (Fig. 8).

To test a possible linkage between the IL-4–IL-5 and IL-3–CSF-2 gene clusters we used for hybridization the probe 1329-30 mapped 50 kb upstreme from the 5′-end of the IL-3 gene (Fig. 1). No common bands were detected in *Not*I digests with this probe and IL-4-specific probe 1201-2 (Fig. 7) and we were unable to demonstrate physical linkage of the two gene

Table 1. Sizes in kilobase of restriction fragments obtained from complete and partial digestions

Fragment	Probe			
	1201–2	1203–4	1205–6	1329–30
Bss HII				
complete	180	245	245	275
Mlu I				
complete	550	550	155	>900
Nae I				
partial	200	260	260	
	340	340	340	300
Not I				
complete	345	345	345	950
Sac II				
complete	185	200	175	310
partial	225		265	
	415			
Sfi I				
complete	160	260	260	145
partial		350	350	
		410	410	
		450	450	

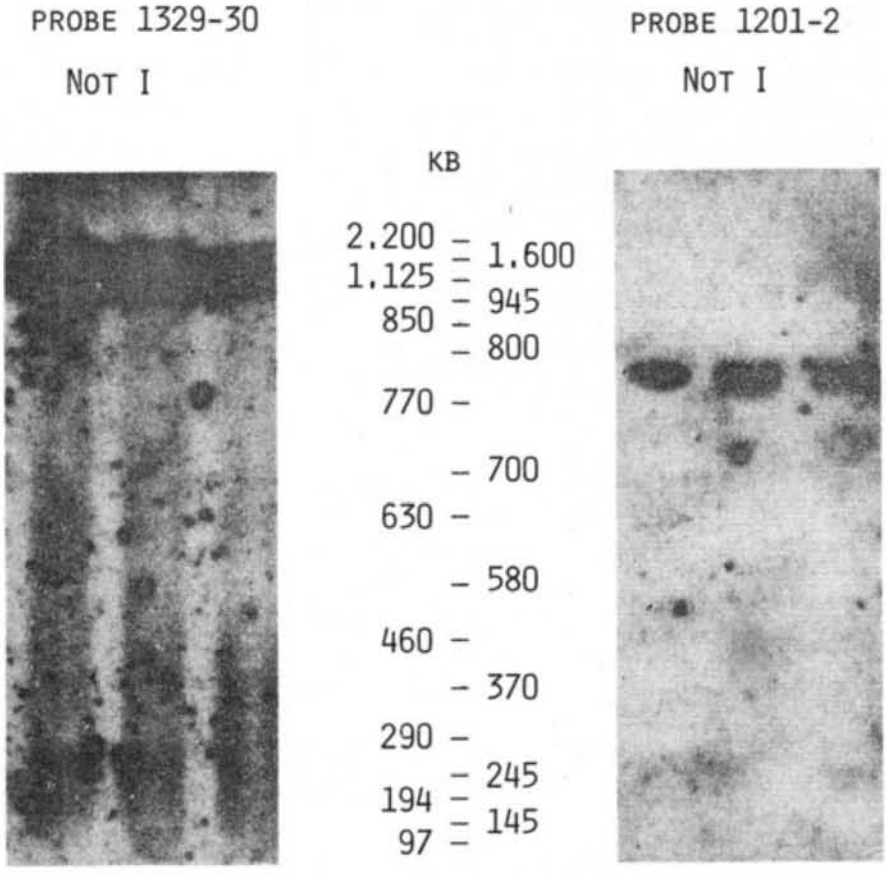

Fig. 7. PFGE analysis of genomic DNA using *Not*I. Filter was hybridized to probes 1329-30 and 1201-2. The sizes of the markers are indicated

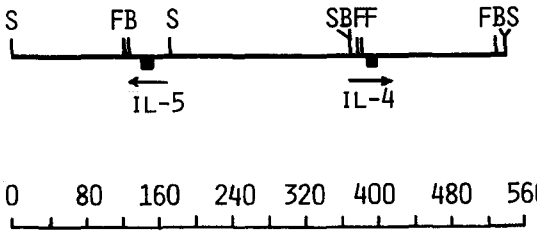

Fig. 8. Long-range physical map around IL-4 and IL-5 genes: *S*, *Sac*II; *F*, *Sfi*I; *B*, *Bss*HII. *Arrows* show the orientation of the genes from the 5′ end to the 3′ end

clusters. The data obtained by Huebner et al. [27] on somatic cell hybrids and clinical samples from patients with acquired deletions suggested the following order of these four genes on the long arm of chromosome 5: cen–(IL-4–IL-5)–IL-3–CSF-2–qter. Based on this order of the genes and on the length of *Not*I fragments (Table 1), we propose that the distance separating the IL-3 and IL-4–IL-5 genes was not less than 1050 kb.

Because of the close linkage of the four related genes, particularly the very close linkage of the IL-3 and CSF-2 genes, it might be suggested that they may have coordinate regulation during T-lymphocyte gene expression, and (or) that they diverged from a common ancestral gene producing a cluster of hematopoietic genes on chromosome 5. It

Table 2. Sizes in kilobases of restriction fragments obtained from complete and double digestions

Fragment	Probe	
	985–6	1205–6
*Sal*I		
partial	160	130
	195	195
	290	290
	> 600	> 600
Bss HII + *Sal*I		
complete	–	40
partial	130	–
	150	150
Sac II + *Sal*I		
complete	19	45
partial	65	65
*Xho*I		
complete	–	10
partial	130	22
	170	130
Bss HII + *Xho*I		
complete	–	10
partial	–	22
	130	140
Sac II + *Xho*I		
complete	21	10
partial	44	44
	55	55
Sac II + *Bss* HII		
complete	60	

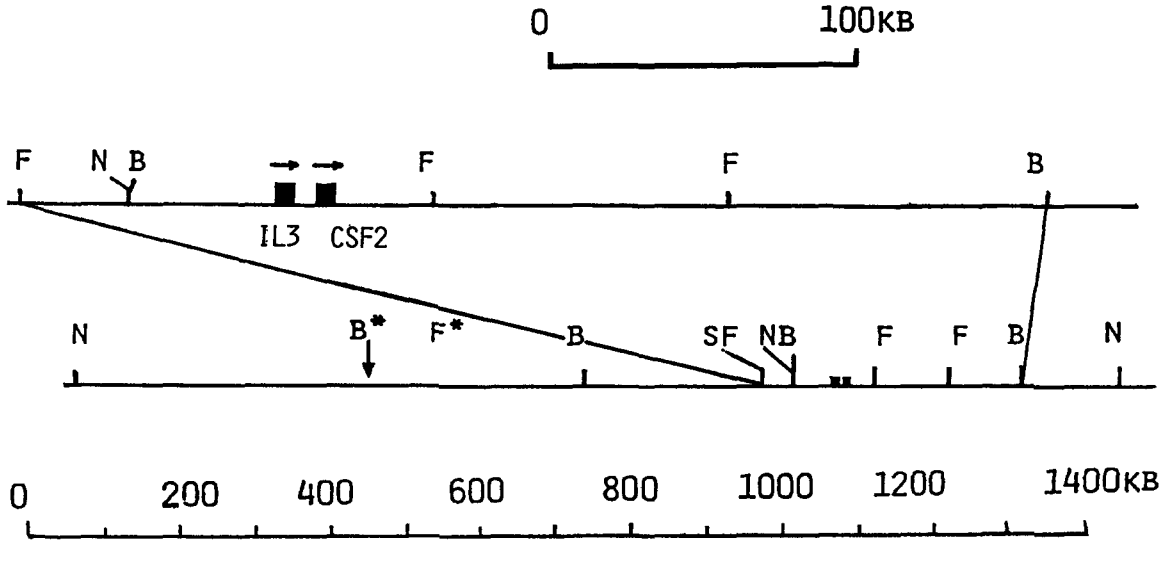

Fig. 9. Long-range physical map around IL-3 and CSF-2 genes: *F*, *Sfi*I; *B*, *Bss*HII; *N*, *Not*I. *Arrows* show the orientation of the genes from the 5′ end to the 3′ end

was shown that in the mouse genome the IL-3 and CSF-2 genes are also closely physically linked – they are only 14 kb apart and have the same orientation as in the human genome [15]. The conservation of linkage relationships supports both suggestions, and further study of the DNA between and surrounding the four genes is important in order to determine whether any other genes related to regulation of hematopoiesis are localized in the same cluster of genes.

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