CLUSTERING OF CYTOKINE GENES ON MOUSE CHROMOSOME 11

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A large number of cytokines, growth factors, growth factor receptors, and oncogenes, including *Il-3, Il-4, Csfgm, Csfg, Ngfr, Pdgfr, Erba, Erbb-2*, and *Fms*, have been mapped to mouse chromosome 11 (1, 2). *Csfg* has also been reported to lie on chromosome 11 (3), although this claim has recently been challenged (4). Comparative mapping studies have identified regions of extensive homology between mouse chromosome 11 and human chromosomes 5 and 17 (4), allowing linkage data for mouse chromosome 11 to be applied to the human genome. One example involves the translocation breakpoint associated with acute promyelocytic leukemia (APL).[1] The APL breakpoint is located on the long arm of human chromosome 17 at band 17q12 (5), which corresponds to a region on the distal portion of mouse chromosome 11 (6). Because of the linkage conservation between the mouse and human chromosomes, genes may be identified as candidates for involvement in APL based on their chromosomal position on mouse chromosome 11. Similarly, a variety of human hematological disorders are associated with deletions of the long arm of chromosome 5. A number of genes with known hematopoietic functions, including *Il-3, Il-4*, and *Csfgm*, map to this region and have thus been implicated in the etiology of these diseases.

Recently, we (7) and others (8–10) have proposed that the genes encoding the mouse cytokines TCA3, P500, MIP1-α, MIP1-β, and JE belong to a new gene family. The criteria for inclusion as part of this gene family are first, the conservation of specific amino acid residues within the polypeptide sequence, and second, an overall conservation of the polypeptide hydropathy profile. Each member of the TCA3 family is

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[1] *Abbreviations used in this paper*: ANLL, acute nonlymphocytic leukemia; APL, acute promyelocytic leukemia; G-CSF, granulocyte CSF; GM-CSF, granulocyte/macrophage CSF; M-CSF, macrophage/monocyte CSF; MDS, myelodysplastic syndrome; RI, recombinant inbred; SDP, strain distribution pattern.

characterized by activation-specific expression in selected cell types including T cells (TCA3, P500, MIP1-α, MIP1-β), mast cells (TCA3, MIP1-α, MIP1-β, JE), macrophages (MIP1-α, MIP1-β, JE) (10–14) and nerve cells (JE) (Dorf, M. E., and M. Greenberg, unpublished data). Activation results from stimulation through the presumed physiologic pathways for each cell type: the T cell receptor pathway for T cells, the FcR$_\epsilon$ pathway for mast cells, LPS stimulation for macrophages, and NGF for nerve cells. The proteins encoded by the MIP1-α and MIP1-β genes induce chemotaxis and activation of neutrophils (15), while the apparent human homologue of JE, termed MCAF, has similar effects on monocytes (9). The functions of TCA3 and P500 are unknown, although preliminary data suggest that TCA3 is a proinflammatory protein (Wilson, S. D., unpublished observations).

As part of our investigation into the evolutionary relationship between members of the TCA3 family, and to identify candidates for etiologic involvement or linkage with clinically important loci, we set out to localize the genes encoding TCA3, MIP1-α, MIP1-β, and JE (provisionally termed *Tca-3*, *Mip-1a*, *Mip-1b*, and *Sigje*, respectively), as well as the unrelated lymphokine gene *Il-5*. Using a combination of mouse-hamster and mouse-rat somatic cell hybrids and recombinant inbred mouse strains, we demonstrate that *Tca-3*, *Mip-1a*, *Mip-1b*, *Sigje*, and *Il-5* all lie on mouse chromosome 11. Furthermore, we show that the members of the *Tca-3* gene family form a tightly linked cluster between the *Evi-2* and *Hox-2* loci, making these genes potentially important as disease-linked markers for the analogous portion of human chromosome 17, and as candidates for etiologic involvement in APL. Additionally, we show that *Il-5* maps to the *Il-3/Il-4/Csfgm* gene cluster, suggesting that *Il-5* could potentially be involved in the hematologic disorders involved in deletions of the long arm of human chromosome 5.

Materials and Methods

Somatic Hybrids. BALB/c × EBS6 mouse-hamster somatic hybrid cell lines (16) and mouse-rat microcell hybrid cell lines (17) were derived and maintained as previously described.

Genomic DNA. Genomic spleen DNA samples from BXD, AKXL, C57BL/6, DBA/2J, AKR/J, C57L/J, and BALB/cJ mice were purchased from The Jackson Laboratory, Bar Harbor, ME. DNA from STS/A, 020/A, OXA RI lines, and CXS RI lines were generously provided by Dr. J Hilgers, The Netherlands Cancer Institute, Amsterdam. All others were extracted using standard methods involving cell lysis in SDS, digestion with proteinase K and RNAse A, followed by repeated rounds of phenol/chloroform extraction and ethanol precipitation.

cDNA Probes. cDNA probes were obtained as follows: TCA3 was cloned in this laboratory (18); MIP1-α/Ty-5 and MIP1-β/H400 (10) (referred to as MIP1-α and MIP1-β in this study) were gifts of Dr. G. Zurawski (DNAX Research Institute, Palo Alto, CA); JE (14) was a gift of Dr. B. Rollins (Dana-Farber Cancer Institute, Boston, MA); IL-5 (19) and macrophage (M)-CSF (20) were gifts of Dr. S. Clark (Genetics Institute, Cambridge, MA); and IL-7 (21) was a gift of Dr. S. Gillis (Immunex Corp., Seattle, WA). Full-length cDNA inserts were excised by restriction digestion and twice gel purified by NaI/glass extraction according to the recommendations of the manufacturer (Bio 101, La Jolla, CA). Probes were labeled by random hexamer priming using protocols and reagents suggested by the manufacturer (Bethesda Research Laboratories, Gaithersburg, MD).

Southern Blotting. 10 μg genomic DNA were digested with appropriate endonucleases (5–10-fold enzyme excess, 4 h), electrophoresed in 0.8% agarose gels, and transferred to nylon reinforced nitrocellulose (MSI, Westboro, MA). Hybridizations were performed at 42°C in 50% formamide, 5× Denhardt's solution, 0.66 M NaCl, 0.09 M NaPO$_4$, 0.4 mM Na$_3$EDTA,

0.09 M sodium pyrophosphate, 0.3% SDS, and 130 μg/ml sonicated, denatured salmon sperm DNA. Hybridized blots were washed in 30 mM NaCl, 3 mM sodium citrate, and 0.1% SDS at 60°C. After washing, blots were exposed using a Betascope (Betagen, Waltham, MA).

Linkage Analyses. Linkage analyses were performed using RI SDP data maintained in the data banks of the NYU Biochemistry Department (New York, NY) and The Jackson Laboratory.

DNA Sequencing. The isolation of *Tca-3* genomic clones from a BALB/c spermatocyte library has been described (18). Genomic DNA was subcloned from Lambda Charon 4A into pUC 18 and 19, and DNA sequence determination was performed after further subcloning into M13 mp18 and mp19 vectors by the dideoxy chain termination method of Sanger et al. (22).

Results

Localization of Five Cytokine Genes to Chromosome 11. To determine the chromosomal location of *Tca-3*, a panel of 10 BALB/c × EBS6 mouse-Chinese hamster somatic hybrids, each retaining a defined complement of mouse chromosomes on a hamster background (16), was screened twice using Southern blotting. Each mouse chromosome with the exception of chromosome 11 was represented in this panel of hybrids, with most represented multiple times (16). When probed with the TCA3 cDNA probe, common hybridizing restriction fragments were detected in all the hybrid lanes corresponding to the hamster hybridization pattern. However, no mouse-specific hybridization could be detected in any of the hybrids (data not shown). Since chromosome 11 was the only mouse chromosome not represented in the panel of BALB/c × EBS6 hybrids, the absence of hybridizing mouse genomic fragments suggested that *Tca-3* is located on chromosome 11.

To test this interpretation, and to extend the findings, Southern blots containing Pvu II–digested genomic DNA from four rat-mouse microcell hybrid cell lines were screened with the probes for *Tca-3, Mip-1a, Mip-1b,* and *Sigje,* as well as *Il-5, Csfm,* and *Il-7.* Two of these cell lines (F(11)U and F(11)J) had received a single intact chromosome, chromosome 11, from the mouse parent cell line (17). The other two cell lines (FB(11)U and FB(11)J) represent chromosome 11 segregants (lacking mouse chromosome 11) derived from F(11)U and F(11)J, respectively (17). DNA from C57BL/6 and from the rat parent cell line FT0-2B was also analyzed. Results for one pair of hybrids, F(11)U and FB(11)U, are shown in Fig. 1. The results for the other pair of hybrids were identical (data not shown). TCA3, MIP1-α, MIP1-β, JE, and IL-5 cDNA probes all hybridized to mouse-specific restriction fragments in the chromosome 11–positive hybrids F(11)U and F(11)J. These mouse-specific fragments were absent from the chromosome 11 segregants FB(11)U and FB(11)J as well as from the rat parent line FT0-2B. The mouse *Il-7* and *Csfm* genes were also tested. No hybridization corresponding to the mouse patterns for *Il-7* or *Csfm* was detected in any of the rat-mouse hybrids. These results demonstrated that the genes *Tca-3, Mip-1a, Mip-1b, Sigje,* and *Il-5* are contained on mouse chromosome 11. The *Il-7* and *Csfm* genes apparently do not lie on this chromosome (although the possibility could not be formally excluded that a portion of chromosome 11 containing *Il-7* and/or *Csfm* was missing from the two mouse-hamster hybrids), thus neither was examined further in this study.

The Genes Encoding TCA3, MIP1-α, MIP1-β, and JE Are Clustered. To verify the chromosomal assignment of these genes and to determine their relative positions on chromosome 11, several panels of recombinant inbred (RI) mouse strains were

examined. Southern blot analysis of genomic DNA digested with a panel of restriction enzymes revealed restriction fragment length variants associated with each gene among the strains 020/A, STS/A, BALB/cJ, AKR/J, C57BL/6J, CS7L/J and DBA2/J (Table I). The strain distribution patterns (SDPs) of the variants were determined for all informative OXA, AKXL, CXS, and BXD RI strains of mice (Table II). *Mip-1a* and *Mip-1b* yielded identical SDPs among 44 informative RI strains, indicating at 95% confidence that the two genes are <2.3 cM apart. The one recombinant between these genes and *Tca-3* indicates a distance of 0.6 cM (95% confidence limits of 0.02–3.7 cM). In turn, the one recombinant among 18 AKXL strains informative for *Tca-3* and the viral integration site *Evi-2* (1), and the five recombinants among 32 AKXL and CXS strains informative for *Tca-3* and the homeobox *Hox-2* (23), suggest a location for the complex on chromosome 11 ~5 cM proximal to the *Hox-2* complex (24) (Table III). The viral integration sites *Xmv-42* (one recombinant with *Mip-1a/Mip-1b* out of 44 BXD and AKXL strains) (25) and *Xmmv-3* (one recombinant with *Mip-1a/Mip-1b* out of 18 AKXL strains) (26), along with several other loci, also showed tight linkage to the *Tca-3* cluster (Table III).

We were unable to identify a segregating polymorphism for *Sigje* in either BXD
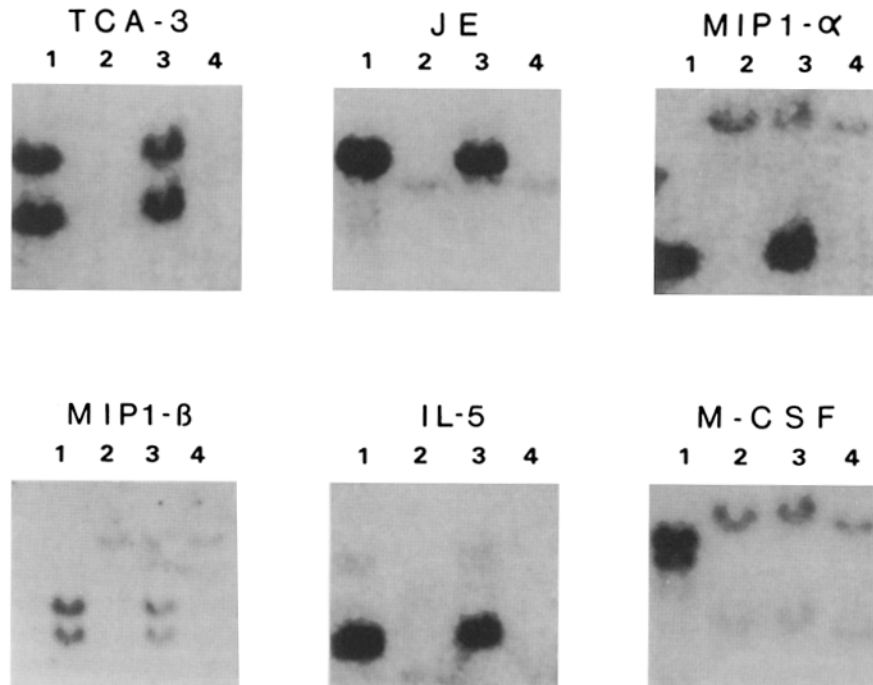
FIGURE 1.   Chromosomal location of six cytokine genes. 10 μg of genomic DNA per lane were digested with Pvu II and subjected to agarose gel electrophoresis and Southern blotting. Blots were probed with TCA3, MIP1-α, MIP1-β, JE, IL-5, and M-CSF cDNA probes and exposed using a Betascope. (Lane *1*) C57BL/6 mouse, (lane *2*) FT0-2B rat fusion partner, (lane *3*) F(11)U mouse chromosome 11–bearing rat–mouse microcell hybrid (see text), (lane *4*) FB(11)U chromosome 11 segregant (lacking chromosome 11) derived from F(11)U. Rat-specific hybridization was detected in all the hybrid lanes, confirming the presence of hybridizable DNA.

TABLE I

Restriction Fragment Length Polymorphisms Used to Assess
Strain Distribution Patterns in Recombinant Inbred Mice

Locus	Enzyme	Informative strain(s)	Fragment lengths
			kb
Tca-3	Taq I	A, D, S	4.0
		B, C, L, O	2.4, 1.7
Mip-1a	Taq I	B, L	9.4
		A, D	6.2, 1.9
Mip-1b	Taq I	A, D	5.9, 1.2
		B, L	1.8, 1.6
Sigje	Eco RI	S	10.2
		C	5.1
Il-5	Taq I	A, B, C	7.9
		D, O, S	3.8

B, C57BL/6J; D, DBA/2J; A, AKR/J; L, C57L/J; C, BALB/cJ; S, STS/A; O, O20/A.

or AKXL RI lines. The SDP data for *Sigje* in the CXS lines by themselves (two of 14 strains recombinant between *Sigje* and *Tca-3*) did not allow conclusions to be drawn as to linkage. However, when the BAYLOC algorithm (27) was applied to these data, using the assignment of *Sigje* to chromosome 11 from somatic cell hybrid data (Fig. 1) to reduce the effective genome size for the computation to 150 cM, *Sigje* showed linkage to *Tca-3* at 95% confidence.

P500 Represents an Alternative RNA Splice Product of the Tca-3 Gene. Brown et al. (10) described the cloning of a cDNA that they termed P500. P500 was identical in sequence to TCA3 through ~260 bp pairs (encoding the first 63 amino acids). However, the P500 sequence contains 99 additional base pairs in the middle of the cDNA as compared with the TCA3 sequence. This results in dramatically different amino acid sequences from amino acid position 64 to the end of each protein (10). To determine whether these cDNAs represent the products of different, closely related genes or are alternatively spliced transcripts from a single gene, we sequenced a genomic clone comprising the relevant region of the *Tca-3* gene. The results are shown in Fig. 2. The additional 99 bp present in the P500 cDNA are accounted for by an insert of the 99 bp immediately 5' to the start of exon 3 of the TCA3 transcript. Both of the alternative 5' boundaries for exon 3 are immediately preceded by the universally conserved splice donor signal sequence A G at positions −2, −1 (28). Thus, P500 is formed by an alternate choice of splice sites to exon 3, and represents yet another cytokine encoded on chromosome 11 within the cluster of genes that includes *Tca-3*.

TCA3 and JE Have Very Similar Intron/Exon Structures. The genes *Tca-3, Mip-1a, Mip-1b*, and *Sigje* have been grouped together into a gene family based largely on specific positional similarities in the amino acid sequences they encode. The overall protein identities range from 60% between MIP1-α and MIP1-β to only 27% between MIP1-α and TCA3 (29). Furthermore, the cDNA sequence similarities range from 51% be-

TABLE II

RI Strain Distribution Patterns for Five Cytokine Genes Located on Chromosome 11

Locus	RI Set	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	28	29	30	31	32	37	38
Tca-3	BXD	B	D			D	B		B	D	C	B	D	B	D	D	D		B	D	D	D	D	B	B	B	B	B	D	B	B	D		
	AKXL			S	S	A	A	A	A	L			L	L	A		L	A		A		L			A	L		L	L				A	A
	CXS	C	C	S	S	S	S	S	C	S	C	S	S	C	S																			
	OXA	A	A	O	A	A	A	O	O	A	O	A	A	A	A																			
Mip-1a	BXD	B	D			D	B		B	D		B	D	B	D	D	D		B	D	D	D	D	B	B	B	B	B	D	B	B	D		
	AKXL					A	A	A	A	L			L	L	A		A	A		A		L			A	L		L	L				A	A
Mip-1b	BXD	B	D			D	B		B	D		B	D	B	D	D	D		B	D	D	D	D	B	B	B	B	B	D	B	B	D		
	AKXL					A	A	A	A	L			L	L	A		A	A		A		L			A	L		L	L				A	A
Sigje	CXS	C	C	S	C	C	S	S	C	S	C	C	S	S	S																			
IL-5	BXD	D	D			B	D		B	B		B	D	D	D	B	D		B	B	B	D	B	B	B	B	B	D	D	B	D	D		
	CXS	C	C	C	C	C	C	S	C	S	S	C	C	C																				
	OXA	O	O	A	A	A	A	O	O	A	O	A	A	A	O																			

TABLE III

Comparison of Strain Distribution Patterns for Linked Loci Mapping to Chromosome 11

AKXL strain	Evi-2		Tca-3		Mip-1a/Mip-1b		Xmv-42/Xmmv-3		Hox-2
No. 16	L		L	X	A		A		A
19	A		A		A		A	X	L
24	A		A		A	X	L		L
29	A	X	L		L		L	X	A

CXS strain	Tcn2		Hba		Il-5/Il-4		Il-3		Sigje		Ula1/Tca-3		D1b1/Hox-2
No. 2	S		S	X	C		C		C		C		C
3	S	X	C		C		C	X	S		S		S
4	C		C		C		C		C	X	S		S
5	S	X	C		C		C	X	S		S		S
6	C	X	S		S		S		S		S		S
9	C		C	X	S		S		S		S		S
10	S		S		S		S	X	C		C	X	S
11	C		C		C		C	X	S		S		S
12	C		C		C	X	S		S		S		S
13	C		C		C		C	X	S	X	C		C

18 AKXL and 14 CXS strains were tested. Only strains in which crossovers occur between tabulated loci are shown. An X indicates a chromosome crossover point. 26 BXD strains were also tested for *Tca-3*, *Mip-1a*, and *Mip-1b*, revealing identical SDPs for each of these genes and for Xmv42. 14 OXA strains were tested for *Il-5* revealing the identical SDP as *Il-3* and *I-4*.

tween MIP1-α and MIP1-β to only 18% between MIP1-α and TCA3 (29). Thus, an alternative interpretation could not be excluded; that what appeared to be conservation of specific amino acid residues in evolutionarily related genes might instead represent a convergence of unrelated genes due to constraints dictated by folding patterns or receptor–ligand interactions. The localization of *Tca-3*, *Mip-1a*, *Mip-1b*, and *Sigje* to a cluster on chromosome 11 adds support to the contention that these genes are related at the genetic level. To further investigate this question, we looked at the fine structure of the intron/exon boundaries of two representative genes: *Tca-3* and *Sigje*. To determine the exact boundaries, we sequenced the genomic locus of the *Tca-3* gene (complete sequence submitted to Genbank) and compared it with the sequence data available for *Sigje* (14). The results are shown in Fig. 3. With the introduction of a single gap in the TCA3 amino acid sequence to maximize homology between the TCA3 and JE polypeptides, the intron/exon junctions of the two

P500 intron 2   P500 exon 3
.....AGATCTTGTAAAATTTCTTCATTTACAG ‖ AGTCAGGAGTTCAGGGGT

CCCTGGTCTCACAGAAGCAGAGAAGACTGTTACAGATTCCAGTGAGTGAC

TCA3 intron 2   TCA3 exon 3
CAAGGTCCTTGTCCGTGTCTTCTGTTCACAG ‖ ATTCAGGCTGAACAAAGG....

FIGURE 2. Sequence of the TCA3 genomic locus surrounding the boundary between intron 2 and exon 3. AG indicates the universally conserved nucleotide pair found at positions −1, −2 of 5' exon boundaries. This sequence reveals the basis for the P500 cDNA. Exons 1 and 2 are spliced identically for TCA3 and P500 (not shown). A different 5' boundary for exon 3 accounts for the 99-base insert found in P500 as compared with TCA3. In the TCA3 transcript, these 99 bp represent the 3' end of intron 2.
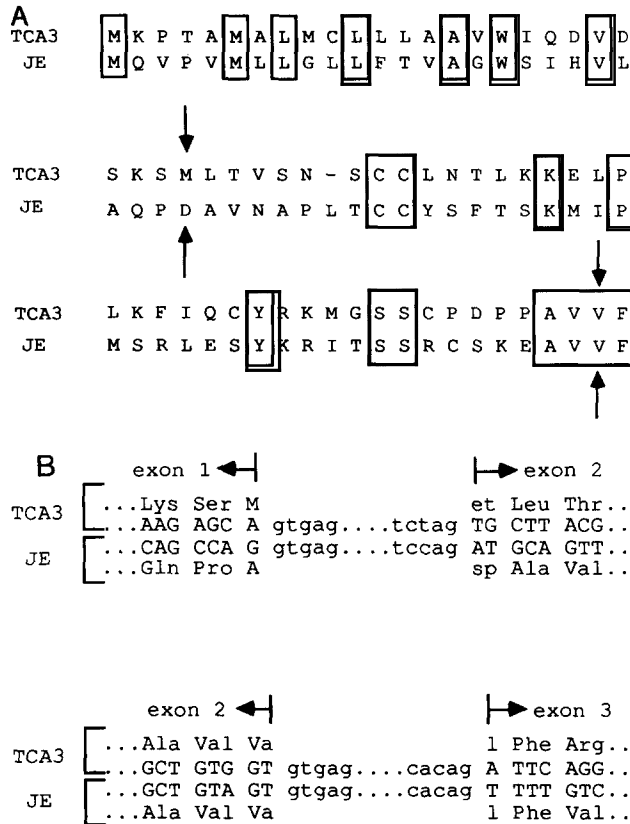
A

TCA3   M K P T A M A L M C L L L A A V W I Q D V D
JE   M Q V P V M L L G L L F T V A G W S I H V L

↓

TCA3   S K S M L T V S N - S C C L N T L K K E L P
JE   A Q P D A V N A P L T C C Y S F T S K M I P

↑   ↓

TCA3   L K F I Q C Y R K M G S S C P D P P A V V F
JE   M S R L E S Y K R I T S S R C S K E A V V F

↑

B

exon 1 ◄┤   ├► exon 2

TCA3   ...Lys Ser M   et Leu Thr...
        ...AAG AGC A gtgag....tctag TG CTT ACG...
JE   ...CAG CCA G gtgag....tccag AT GCA GTT...
        ...Gln Pro A   sp Ala Val...

exon 2 ◄┤   ├► exon 3

TCA3   ...Ala Val Va   l Phe Arg...
        ...GCT GTG GT gtgag....cacag A TTC AGG...
JE   ...GCT GTA GT gtgag....cacag T TTT GTC...
        ...Ala Val Va   l Phe Val...

FIGURE 3. Comparison of TCA3 and JE polypeptide and nucleotide sequences around intron/ exon boundaries. (A) Comparison of polypeptide sequences (using single-letter code). Identical amino acids are outlined. Arrows indicate the positions of exon junctions. A single gap (-) has been introduced into the TCA3 sequence. (B) Fine structure of intron/exon boundaries. Nucleotides contained within exons are indicated with capital letters; nucleotides contained within introns are in lower case letters. Amino acids are represented using three letter code.

genes line up exactly. The border of exon 1 and exon 2 occurs in codon 26 between the first and second nucleotides of the codon (codon 26 encodes a methionine in TCA3 and an aspartic acid in JE). In both *Tca-3* and *Sigje*, intron 2 falls between the second and third nucleotides of the codon encoding the second in a pair of valine residues that is highly conserved throughout the gene family (codons 64 and 65, respectively) (7–10). Thus, despite the relatively modest nucleotide and protein sequence identities between the two genes, their genomic structures show striking similarity.

Il-5 Is Part of the IL-3, IL-4, Csfgm Lymphokine Gene Cluster.   The SDP for the lymphokine gene *Il-5* was scored in the BXD, OXA, and CXS strains of mice (Table II). *Il-5* did not show close linkage to *Tca-3, Mip-1a, Mip-1b*, or *Sigje* (21/54, 11/26, and 6/14 discordant, respectively). However, *Il-5* showed tight linkage with *Il-4* and *Il-3* (0/28 and 1/28 discordancies, respectively), confirming that *Il-5* does map on mouse chromosome 11 and localizing this gene to a region between the genes *Hba* and *Il-3* (Table III). Analysis of the BXD RI lines indicated that *Il-5* is also linked to the gene *D4S10h* (0/26 discordancies). *Il-4* and *Il-3*, along with *Csfgm*, have already been shown to form a lymphokine gene cluster, with *Il-3* and *Csfgm* located no more than 230 kbp from each other (30). *Il-5* apparently falls within this lym-

phokine cluster and is located ~25 cM proximal to the *Tca-3* cluster on chromosome 11.

Discussion

We have used interspecific somatic cell hybrids and recombinant inbred mouse strains to demonstrate that *Tca-3*, *Mip-1a*, *Mip-1b*, and *Sigje* all map to the same region of mouse chromosome 11. This result is consistent with the hypothesis that these genes are evolutionarily related, a proposition originally provoked by similarities in their protein products. The striking similarities in organization between the *Sigje* and *Tca-3* genomic loci argue further that these genes have evolved from a common ancestral gene. In the human, a putative member of the Tca-3 gene family, RANTES (8) (for which no mouse homologue has yet been described), was recently assigned to human chromosome 17 (31). Given the extensive linkage conservation between human chromosome 17 and distal mouse chromosome 11 (4), it seems likely that once isolated, the mouse equivalent of RANTES will also fall into the same gene cluster as *Tca-3* and its related genes.

It is interesting to speculate on why such a large number of cytokine genes are located on one chromosome. Of the ~20 known cytokine genes involved in immune function (encoding IL-1-7, IFN-α, IFN-β, IFN-γ and G-, M-, and GM-CSF, TNF-α, TNF-β, TGF-β, TCA3, MIP1-α and -β, and JE), 10 map to mouse chromosome 11. Furthermore, these genes are organized into two main clusters on the chromosome, one corresponding to human chromosome 5 (*Il-3*, *Il-4*, *Il-5*, *Csfgm*), the other to human chromosome 17 (*Tca-3* [including *P500*], *Mip-1a*, *Mip-1b*, and *Sigje*). Other cytokine genes are similarly clustered, with the genes encoding TNF-α and TNF-β tightly linked on mouse chromosome 17, and those encoding IFN-α and IFN-β linked on mouse chromosome 4 (3). Certainly, the proximity of some of these genes is a vestige of their evolutionary relationship (i.e., arising via gene duplication and subsequent divergence). However, it may also reflect a functional relationship involving, for example, a mechanism of coordinate expression using common *cis*-acting regulatory elements. *Il-4* and *Il-5* provide one example of such coordinate regulation. Both genes are expressed in the Th2 but not the Th1 subset of T cells (32, 33); furthermore, they are coexpressed in the only other cell type (mast cells) known to release either IL-4 or IL-5 (12, 34). Similarly, MIP1-α and MIP1-β appear to be consistently coexpressed (29). The lack of detectable recombination between members of these gene pairs suggests that they are closely linked. A more precise estimation of the true physical distances involved using pulsed field gel electrophoresis would be useful in evaluating the viability of this hypothesis.

The malignant cells from every APL patient studied show a balanced translocation between chromosomes 15 and 17 (5). The translocation breakpoint on chromosome 17 lies between two regions: one containing the genes *Erba*, *Erbb-2*, and *Csfg*, the other containing the genes *Mpo*, *Cola-1*, and *Hox-2* (6). In the mouse genome, the second of these regions is inverted relative to human chromosome 17 (6), making precise localization of the mouse correlate of the APL breakpoint difficult. However, the human equivalents of *Mpo* and *Evi-2* have both been mapped near the APL breakpoint (6, 35). The localization of *Tca-3*, *Mip-1a*, *Mip-1b*, and *Sigje* near *Evi-2* and *Mpo* (which like the *Tca-3* cluster lies between *Evi-2* and *Hox-2* [4]) makes the human

equivalents of these genes potential candidates for association or linkage with the APL translocation breakpoint.

The disease resulting from the 15;17 APL translocation represents a malignant expansion of the myeloid precursor (promyelocyte) compartment. TCA3, MIP1-α, MIP1-β, and JE are all expressed by cell types of the myeloid lineage (macrophages and/or mast cells) (10, 12–14). Furthermore, MIP1-α/MIP1-β (15), and MCAF (the apparent human homologue of JE [9]) have chemotactic and activating activities on neutrophils and macrophages, respectively, suggesting the presence of specific receptors on these myeloid cell types. It is tempting to postulate that in APL, the altered expression of one or more of the members of this gene family may be responsible for the development of the malignant phenotype.

In addition to the APL breakpoint, the gene defect involved in von Recklinghausen neurofibromatosis (vRNF) has also been mapped to this region of human chromosome 17 (band 17q11-12) (36). The locations of *Tca-3*, *Mip-1a*, *Mip-1b*, and *Sigje* on distal mouse chromosome 11 makes it likely that their human homologues would be linked to the vRNF gene and might serve as useful disease-linked markers for this locus. The recent demonstration that JE is selectively induced following nerve growth factor (NGF) but not epidermal growth factor (EGF) or K$^+$ activation of PC12 nerve cells supports the potential involvement of products from this cluster or related genes in vRNF (Dorf, M. E., and M. Greenberg, unpublished data).

The tight linkage of *Il-5* to *Il-4* and *Il-3* demonstrates that *Il-5* is part of a second cluster of cytokine genes which also includes *Csfgm* (2). The human *Il-5* gene has been recently localized to chromosome 5, although its inclusion as part of the *Il-3*, *Il-4*, *Csfgm* gene cluster could not be established (37). In the human genome, the *Csfm* gene is located in the same region of chromosome 5 as *Il-3*, *Il-4*, and *Csfgm* (38). Our results (Fig. 1) agree with those of Buchberg et al. (5) and demonstrate that the *Csfm* gene is not a part of this lymphokine gene cluster in the mouse as had been previously suggested (3), and does not even lie on mouse chromosome 11. The absence of *Csfm* from mouse chromosome 11 is an interesting exception to the conservation of linkage groups normally seen between mouse and man (1, 4).

The loss of the long arm of human chromosome 5 [del (5q)] is associated with a host of myeloid disorders including therapy-related myeloid dysplastic syndrome (t-MDS) or acute nonlymphocytic leukemia (t-ANLL) (39, 40), as well as some cases of primary MDS/refractory anemia (RA) and de novo ANLL (41, 42). The variability of the breakpoint in these cases suggests that the loss of a gene or genes, rather than the juxtaposition of two genes, is the mechanism behind these diseases (38). The presence of *Il-3*, *Il-4*, *Csfgm*, and *Csfm*, along with *Fms* (which encodes the M-CSF receptor) in a cluster of 5q has implicated these genes in the etiology of disorders associated with del(5q) (38, 43). The tight linkage of the *Il-5* gene to this cluster makes it another candidate for involvement in these disorders.

Summary

The presence of positionally conserved amino acid residues suggests that the mouse proteins TCA3, P500, MIP1-α, MIP1-β, and JE are members of a single gene family. These proteins are activation specific and can be expressed by both myeloid and lymphoid cells. MIP1-α/MIP1-β and MCAF (the putative human homologue of JE)

act as chemotactic and activating agents for neutrophils and macrophages, respectively. The functions of TCA3 and P500 are unknown. We have used interspecies somatic cell hybrids and recombinant inbred mouse strains to show that the genes encoding TCA3, MIP1-α, MIP1-β, and JE (provisionally termed *Tca3, Mip-1a, Mip-1b,* and *Sigje,* respectively) map as a cluster on the distal portion of mouse chromosome 11 near the *Hox-2* gene complex. DNA sequence analysis indicates that the P500 and TCA3 proteins are encoded by alternative splicing products of one genomic gene. Additionally, the genes encoding TCA3 and JE are found to be strikingly similar with respect to the positions of intron–exon boundaries. Together, these data support the model that the cytokines TCA3, P500, MIP1-α, MIP1-β, and JE are encoded by a single cluster of related genes.

The gene encoding IL-5 (*Il-5*), which acts as a T cell–replacing factor, a B cell growth factor, and an eosinophil differentiation factor, is also mapped to mouse chromosome 11. *Il-5* maps ∼25 cM proximal to the *Tca-3* gene and appears tightly linked to a previously described gene cluster that includes *Il-3, Il-4,* and *Csfgm.*

We discuss the potential relevance of the two cytokine gene clusters described here with particular attention to specific human hematologic malignancies associated with chromosomal aberrations at corresponding locations on human chromosomes 5 and 17.

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