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The interleukin 3 gene is located on human chromosome 5 and is deleted in myeloid leukemias with a deletion of 5q

(gene mapping/hematopoietic growth factors/chromosomal deletions/malignant transformation)

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ABSTRACT **The gene *IL-3* encodes interleukin 3, a hematopoietic colony-stimulating factor (CSF) that is capable of supporting the proliferation of a broad range of hematopoietic cell types. By using somatic cell hybrids and *in situ* chromosomal hybridization, we localized this gene to human chromosome 5 at bands q23–31, a chromosomal region that is frequently deleted [del(5q)] in patients with myeloid disorders. By *in situ* hybridization, *IL-3* was found to be deleted in the 5q− chromosome of one patient with refractory anemia who had a del(5)(q15q33.3), of three patients with refractory anemia (two patients) or acute nonlymphocytic leukemia (ANLL) *de novo* who had a similar distal breakpoint [del(5)(q13q33.3)], and of a fifth patient, with therapy-related ANLL, who had a similar distal breakpoint in band q33 [del(5)(q14q33.3)]. Southern blot analysis of somatic cell hybrids retaining the normal or the deleted chromosome 5 from two patients with the refractory anemia 5q− syndrome indicated that *IL-3* sequences were absent from the hybrids retaining the deleted chromosome 5 but not from hybrids that had a cytologically normal chromosome 5. Thus, a small segment of chromosome 5 contains *IL-3*, *GM-CSF* (the gene encoding granulocyte–macrophage-CSF), *CSF-1* (the gene encoding macrophage-CSF), and *FMS* (the human c-*fms* protooncogene, which encodes the CSF-1 receptor). Our findings and earlier results indicating that *GM-CSF*, *CSF-1*, and *FMS* were deleted in the 5q− chromosome, suggest that loss of *IL-3* or of other CSF genes may play an important role in the pathogenesis of hematologic disorders associated with a del(5q).**

Colony-stimulating factors (CSFs) are a family of glycoproteins that are believed to be required for growth and maturation of myeloid progenitor cells *in vivo* and *in vitro* (1, 2). In cell culture systems, macrophage-CSF (M-CSF or CSF-1) (3) and granulocyte-CSF (4, 5) primarily stimulate cells committed to the macrophage and granulocyte lineages, respectively, whereas GM-CSF stimulates the proliferation of cells from the granulocyte, granulocyte–macrophage, and macrophage lineages (6, 7). In addition to their capacity to stimulate the proliferation of progenitor cells, the CSFs can induce commitment to differentiate in these precursors, and can stimulate the functional activity of mature granulocytes and macrophages (1).

Human GM-CSF, CSF-1, and G-CSF have been purified and complementary DNA (cDNA) clones have been isolated (3–5, 8). Recently, another hematopoietic growth factor [interleukin 3 (IL-3), or multi-CSF], capable of supporting the proliferation of a broader range of hematopoietic cell types than G-CSF, CSF-1, or GM-CSF, has been identified (refs. 9 and 11; Y.-C.Y., R. Kriz, S. Kovacic, A. C. Leary, A. B. Ciarletta, J. S. Witek-Giannotti, R. E. Donahue, and S.C.C., unpublished results). The GM-CSF and CSF-1 genes (*GM-CSF* and *CSF-1*) have been mapped to human chromosome 5, at bands q23–31 and q33.1, respectively (12–14), a region that is frequently deleted in patients with myeloid disorders [del(5q)] (15). The protooncogene *FMS*, also located in this region of chromosome 5 (13), encodes a protein with an associated tyrosine kinase activity that is closely related, and possibly identical, to the receptor for CSF-1 (16). Thus, *FMS* also plays a role in the differentiation and proliferation of mononuclear phagocytic cells (16, 17).

Loss of a whole chromosome 5 or loss of a part (deletion) of the long arm of this chromosome [del(5q)] is frequently observed in the malignant cells of patients with a myelodysplastic syndrome (MDS) or those with acute nonlymphocytic leukemia (ANLL) secondary to cytotoxic therapy for a previous malignant disease [therapy-related MDS or ANLL (t-MDS, t-ANLL)] (18–20). It is also observed less frequently in patients with ANLL arising *de novo* (15, 21, 22). The relatively high frequency of loss of chromosome 5 or del(5q) in patients with t-ANLL, and the relative absence of these abnormalities in the leukemia cells of patients with ANLL *de novo* who are less than 30 years old, has led to the suggestion that these abnormalities may be a marker of mutagen-induced leukemia (23). A del(5q) also occurs in patients with a primary MDS characterized by refractory anemia (RA) with abnormal megakaryocytes (24); the disorder in this latter group of patients has been termed the "5q− syndrome."

The deletions of chromosome 5 observed in the RA 5q− syndrome and in ANLL are interstitial rather than terminal and are characterized by variability in the proximal and distal breakpoints. However, in a recent clinical and cytogenetic evaluation of t-ANLL and t-MDS patients, a region was identified, consisting of bands 5q23 and q31, that was deleted in all patients. This segment has been termed the "critical region" (20). The identification of a critical region suggests that the loss of genes located within this region plays an important role in the pathogenesis of these hematologic disorders (20, 23). Until recently, the identity of the genes located within the critical region of chromosome 5 was unknown; however, a number of genes encoding cellular growth factors or growth factor receptors have recently been mapped to this region of chromosome 5 (reviewed in ref. 25).

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Abbreviations: ANLL, acute nonlymphocytic leukemia; t-ANLL, therapy-related ANLL; RA, refractory anemia; del(5q), deletion of the long arm of chromosome 5; CSF, colony-stimulating factor; CSF-1, macrophage-CSF; GM-CSF, granulocyte–macrophage-CSF; IL-3, interleukin 3, or multi-CSF; DHFR, dihydrofolate reductase; *CSF-1*, *GM-CSF*, *IL-3*, and *DHFR*, genes encoding CSF-1, GM-CSF, IL-3, and DHFR, respectively; *FMS*, human c-*fms* protooncogene, the cellular homologue of the transforming gene (v-*fms*) of McDonough feline sarcoma virus.

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Table 1. Clinical and cytogenetic features of patients with a del(5q)

Patient	Age/sex	Hematologic disease	Karyotype
1	61/M	RA	46,XY(20%)/46,XY,del(5)(q15q33.3)(80%)
2	40/M	RA	46,XY(30%)/46,XY,del(5)(q13q33.3)(70%)
3	68/F	RA	46,XX(25%)/46,XX,del(5)(q13q33.3)(75%)
4	63/M	AML	46,XY,del(5)(q13q33.3),t(19;?)(p13;?)(95%)/46,XY,del(5)(5%)
5	76/F	t-ANLL	46,XX(3%)/46,XX,−7,del(5)(q14q33.3),+r(6%)/47,XX,−7,+8,del(5),+r(6%)/44,XX, −7,−8,−9,del(5),+der(9)t(8;9;?)(q1?1;p24;?),dic(12;15)(p11;p13),+mar1(42%)/45,XX, −7,−8,−9,del(5),+der(9),dic(12;15),+mar1,+r(19%)/45,XX,−7,−8,−9,del(5), +der(9),dic(12;15),+2mar1(16%)/NCA:46,XX,−7,−8,−9,+10,del(5),+der(9), dic(12;15),+mar1,+r(3%)/45,XX,−7,del(3)(p21),del(5)(3%)

AML, acute myeloblastic leukemia. NCA, nonclonal abnormality.

With respect to hematologic malignant disorders, the most notable of these genes are the *GM-CSF*, *CSF-1*, and *FMS* loci (12–14).

Analysis of somatic cell hybrids and *in situ* hybridization studies previously suggested that the deletion of *FMS*, *GM-CSF*, and *CSF-1* in the 5q− syndrome or in ANLL may result in hemizygosity for critical loci, thereby leading to abnormal hematopoiesis (13, 14, 17). Using a probe derived from the recently cloned IL-3 gene (*IL-3*) (9) for *in situ* chromosomal hybridization and somatic cell hybrid analysis, we have demonstrated that *IL-3* is closely linked to *GM-CSF* on human chromosome 5 and that it is located within the critical region of this chromosome.

MATERIALS AND METHODS

Patients. The clinical and cytogenetic features of the five patients with hematologic diseases characterized by a deletion of chromosome 5 and whose bone marrow cells were examined by *in situ* hybridization are listed in Table 1. Patients 1–3 had the RA 5q− syndrome, patient 4 had ANLL (specifically, acute myeloblastic leukemia, AML) arising *de novo*, and patient 5 had t-ANLL following chemotherapy and radiotherapy for breast carcinoma. Each patient had a del(5q) with a distal breakpoint in 5q33.3; patient 1 had a deletion extending from band q15 to band q33.3, patients 2 to 4 had a larger deletion extending from band q13 to band q33.3, and patient 5 had a deletion extending from band q14 to band q33.3.

DNA Probes. The *IL-3* probe is a 1-kilobase-pair (kb) *Pst* I fragment encompassing 680 base pairs (bp) of 5′ flanking sequence, the 213-bp first exon, and 30 bp of the first intron of the human genomic *IL-3* sequences, cloned in the *Pst* I site of plasmid pUC8. The dihydrofolate reductase (DHFR) probe is a 1.8-kb *Eco*RI genomic fragment from the 5′ end of the normal *DHFR* locus.

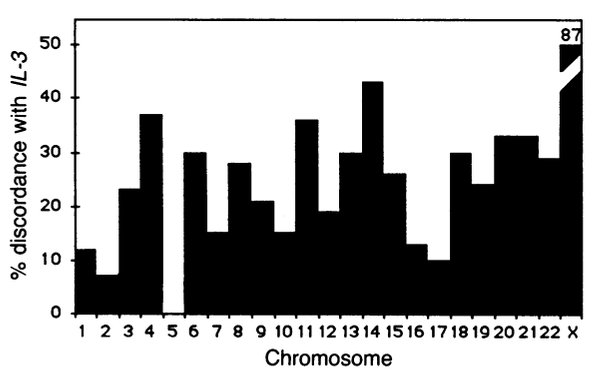

FIG. 1. Percent discordance of *IL-3* sequences and specific human chromosomes in a panel of rodent–human somatic cell hybrid clones. These results showed concordance between the presence of human *IL-3* sequences and human chromosome 5.

***In Situ* Chromosomal Hybridization.** A radiolabeled *IL-3* probe was prepared by nick-translation of the entire plasmid with all four ^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.3×10^8 dpm/μg. *In situ* hybridization was performed as described (26). Metaphase cells were hybridized at 4.0 and 8.0 ng of probe per ml of hybridization mixture. Autoradiographs were exposed for 11 days.

Somatic Cell Hybrids. Somatic cell hybrids were formed by polyethylene glycol-mediated fusion of human lymphocytes from a healthy adult woman and mouse RAG (70 series) or Chinese hamster E36 (80/81 series) cells. The rodent cells were mutant in their hypoxanthine phosphoribosyltransferase, permitting hybrid selection with hypoxanthine/aminopterin/thymidine (HAT) medium (27, 28, 36). A panel for mapping studies was established from a series of 46

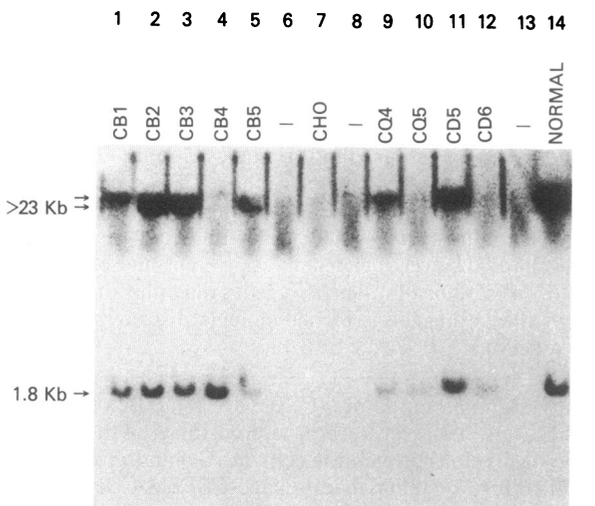

FIG. 2. Southern blot analysis of *Eco*RI-digested DNA from somatic cell hybrid clones containing the normal human chromosome 5 or the del(5q) chromosome, hybridized simultaneously with the *IL-3* and *DHFR* probes. The *DHFR* probe yields a 1.8-kb band, whereas the *IL-3* probe yielded bands at approximately 23-kb. CB1, CB2, CB3, and CB5 (lanes 1, 2, 3, and 5) are hybrid clones containing an intact human chromosome 5. The *DHFR* and *IL-3* sequences were detected in each of these clones. The presence of a *DHFR*-specific band in the absence of an *IL-3*-specific band in clone CB4 (lane 4) is consistent with retention of the proximal portion of the long arm of human chromosome 5 and loss of the distal portion. CQ4 (lane 9) and CD5 (lane 11) are hybrid clones containing the normal chromosome 5, whereas CQ5 (lane 10) and CD6 (lane 12) are clones retaining the del(5q) chromosome from the same patients, respectively. Lane 7 contained DNA from the CHO parental cells, and lane 14 contained DNA from normal human bone marrow cells used as a control. The *DHFR* sequences were detected in all CQ and CD hybrid clones (lanes 9–12); *IL-3* sequences were detected in hybrid clones retaining the normal human chromosome 5 (CQ4 and CD5, lanes 9 and 11) but not in hybrid clones retaining the del(5q) chromosome (CQ5 and CD6, lanes 10 and 12).

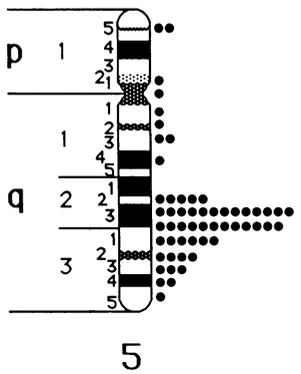

FIG. 3. Distribution of labeled sites on chromosome 5 in 100 normal metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes that were hybridized with a ^{3}H-labeled, *IL-3*-specific probe. Each dot indicates one labeled site observed in the corrresponding band. Sixty-five percent (36/55) of the labeled sites on chromosome 5 were located at bands q22–31; this cluster represented 19.9% of all labeled sites (36/181).

hybrids that contain the entire rodent genome but have selectively lost different combinations of human chromosomes. Extracts of each of the hybrids were typed for up to 36 diagnostic isozyme markers previously assigned to individual human chromosomes (27, 28, 36). Cytogenetic analyses by trypsin–Giemsa banding were used to determine the human chromosomal complement. High molecular weight DNA for Southern blot analysis and cell homogenates for isozyme studies were prepared from the same passages of cells. The *IL-3* probe was ^{32}P-labeled and was hybridized to Southern blots of *Eco*RI-digested DNAs from the hybrid cell panels as previously described (27, 28, 36).

Somatic cell hybrid clones containing the normal human chromosome 5 or the del(5q) chromosome were prepared by fusing human bone marrow cells with DHFR-deficient Chinese hamster ovary (CHO) cells. The CB series (CB1–CB5) was formed by the fusion of normal human bone marrow from a healthy individual with CHO cells, whereas CQ and CD clones were prepared by fusion of DHFR-deficient CHO cells with bone marrow cells of two patients with the RA 5q− syndrome (17). These hybrids retain human chromosome 5 when grown in culture medium deficient in hypoxanthine, thymidine, and glycine. Cytogenetic analysis using a trypsin–Giemsa banding technique demontrated that all CB clones except CB4 contained an intact human chromosome 5. G-11 staining revealed that variable numbers of other human chromosomes were present (17). Southern blots of *Eco*RI-digested high molecular weight DNA from these hybrid clones were hybridized simultaneously with the ^{32}P-labeled *DHFR* and *IL-3* probes. The *DHFR* and *IL-3* probes detect single 1.8-kb and 23-kb restriction fragments in *Eco*RI-digested DNA, respectively.

RESULTS

Analysis of Somatic Cell Hybrids. To determine the chromosomal location of *IL-3*, we established a panel from a series of somatic cell hybrids that contain the entire rodent genome but have selectively lost different combinations of human chromosomes. Southern blot analysis of this hybrid panel showed concordance between the presence of human *IL-3* sequences and human chromosome 5 (Fig. 1). In contrast, a highly significant frequency of discordancies was found with each of the other human chromosomes (7–87%).

The localization of the *IL-3* gene to human chromosome 5 was corroborated by the analysis of a series of somatic cell hybrids that were prepared by fusing normal human bone marrow cells with CHO cells. Southern blot analysis of *Eco*RI-digested DNA from this hybrid panel, hybridized simultaneously with the *IL-3* probe and a *DHFR*-specific probe (*DHFR* is located at 5q11–22) demonstrated the presence of *IL-3* and *DHFR* sequences in four of the five hybrid lines tested (CB1, CB2, CB3, and CB5; Fig. 2, lanes 1–3 and 5). Cytogenetic analysis of these lines revealed that an intact human chromosome 5 was present. Apparently the individual whose cells we used to obtain the CB hybrid series has an *Eco*RI site polymorphism. A mixed clonal population for both normal chromosomes 5 is seen in CB2 and CB3 (Fig. 2, lanes 2 and 3). In the remaining hybrid line (CB4), *DHFR* sequences were detected; however, CB4 lacked *IL-3* sequences and had no cytogenetically identifiable human chromosome 5 (Fig. 2, lane 4). These results are consistent with the retention of the proximal portion of the long arm of human chromosome 5 and loss of the distal portion of 5q in this clone.

***In Situ* Chromosomal Hybridization.** To determine the chromosomal sublocalization of the *IL-3* gene, we hybridized the *IL-3* probe to normal metaphase chromosomes. This resulted in specific labeling only of chromosome 5. Of 100 metaphase cells examined, 37 were labeled on region q2 or q3 of one or both chromosome 5 homologues. The distribution of labeled sites on this chromosome is illustrated in Fig. 3; of 181 labeled sites observed, 55 (30.4%) were located on this chromosome. These sites were clustered at band q22–31, and this cluster represented 19.9% (36/181) of all labeled sites ($P < 0.0005$). The largest cluster of grains was observed at 5q23–31. Similar results were obtained in a second hybridization experiment.

Analysis of Bone Marrow Cells with a del(5q). To determine the relationship of *IL-3* to the critical region of chromosome

Table 2. *In situ* hybridization of the human *IL-3* probe to bone marrow metaphase cells from five patients with a del(5q)

Patient	No. of cells analyzed	Total no. of labeled sites	No. of labeled sites (%)			
			Normal chromosome 5		5q− chromosome	
			Total	Bands q22–32	Total	Bands q13–35
1	91	116	23 (19.8%)*	16 (13.8%)	2 (1.7%)	1 (0.9%)†
2	100	117	25 (21.4%)*	17 (14.5%)	1 (0.9%)	1 (0.9%)‡
3	75	92	17 (18.5%)*	11 (12.0%)	2 (2.2%)	0‡
4	100	133	23 (17.3%)*	18 (13.5%)	4 (3.0%)	3 (2.3%)‡
5	100	125	22 (17.6%)*	15 (12.0%)	4 (3.2%)	1 (0.8%)§

*Cumulative probability for the Poisson distribution is <0.0005. The mean was estimated from the number of labeled sites on all chromosomes except the normal 5 and del(5q) chromosomes.
†Bands q15–33.3 are deleted.
‡Bands q13–33.3 are deleted.
§Bands q14–33.3 are deleted.

5, we examined somatic cell hybrids retaining the deleted chromosome 5 from two patients with the RA 5q− syndrome, and we hybridized the *IL-3* probe to metaphase cells obtained from bone marrow aspirates of five other patients with the RA 5q− syndrome, ANLL *de novo*, or t-ANLL characterized by a del(5q). Southern blot analysis of genomic DNA from the somatic cell hybrids in which we used the human *IL-3* and *DHFR* probes indicated that all clones contained a human *DHFR* fragment (Fig. 2, lanes 9–12). The human *IL-3* was detected in hybrids that had a cytologically normal chromosome 5 (CQ4 and CD5, lanes 9 and 11); however, *IL-3* sequences were absent in the hybrids retaining the deleted chromosome 5 (CQ5 and CD6, lanes 10 and 12) from the same patients.

The results of *in situ* hybridizations of the *IL-3* probe to metaphase cells from five patients with a del(5q) (Table 1) are listed in Table 2 and illustrated in Fig. 4. In all five cases, specific labeling was observed on the normal chromosome 5 homologues (Fig. 4 *Left* and Table 2), but not on the rearranged homologues (Fig. 4 *Right* and Table 2). Thus, the results of somatic cell hybrid analysis and *in situ* chromosomal hybridizations indicate that the *IL-3* locus was deleted as a result of an interstitial deletion of 5q.

DISCUSSION

By using *in situ* hybridization and somatic cell hybrid analysis, we have localized *IL-3* to the long arm of chromosome 5, at bands q23–31. Earlier studies localized *GM-CSF*, *CSF-1*, and *FMS* to this region of chromosome 5, specifically to 5q23–31, 5q33.1, and 5q33.2–33.3, respectively (13, 14, 25). The three CSF genes and *FMS* are located in a region of chromosome 5 that is frequently deleted in patients with neoplastic myeloid disorders (18–24). Our results, together with previous studies of *FMS* (17), showing that these genes are within the deleted segment of chromosome 5 in the RA 5q− syndrome and in ANLL, suggest that they play some role in the pathogenesis of these disorders.

The relative variability of the breakpoints noted in the deletion of chromosome 5 suggests that the event, which may be essential for malignant transformation, is the loss of a critical DNA sequence, rather than the consistent juxtaposition of two genes as in the case of *ABL* and breakpoint-cluster-region (bcr) sequences in chronic myelogenous leukemia (29). The identification of a critical region—i.e., a chromosomal segment that is deleted in all patients with this rearrangement—further supports this hypothesis. The genetic consequence of a deletion of a CSF allele or an *FMS* allele may be a reduction in the level of the gene products, or the loss of a wild-type gene, thereby allowing the expression of a recessive allele on the homologous chromosome. Alternatively, loss of function of both alleles may have occurred, in one case through a detectable chromosomal deletion and in the other, as a result of a mutation. Similar mechanisms have been proposed for the pathogenesis of retinoblastoma and Wilms tumor (30); the latter mechanism has recently been confirmed for retinoblastoma (31).

It is not known whether the deletion of one gene (or of several genes) encoding CSFs or the loss of the receptor gene(s) is the critical genetic alteration in malignant myeloid cells with a del(5q). With respect to the CSF genes, recent studies (32) show that, in some cases of ANLL, constitutive expression of *GM-CSF* by leukemia cells results in autonomous proliferation *in vitro* of leukemia colony-forming cells. Furthermore, other experimental data suggest that expression of CSF genes is important in malignant transformation. Specifically, GM-CSF or IL-3 production induced by retroviral expression vectors resulted in CSF-independence in factor-dependent murine hematopoietic cell lines (10, 33). Moreover, these cells were tumorigenic when inoculated into

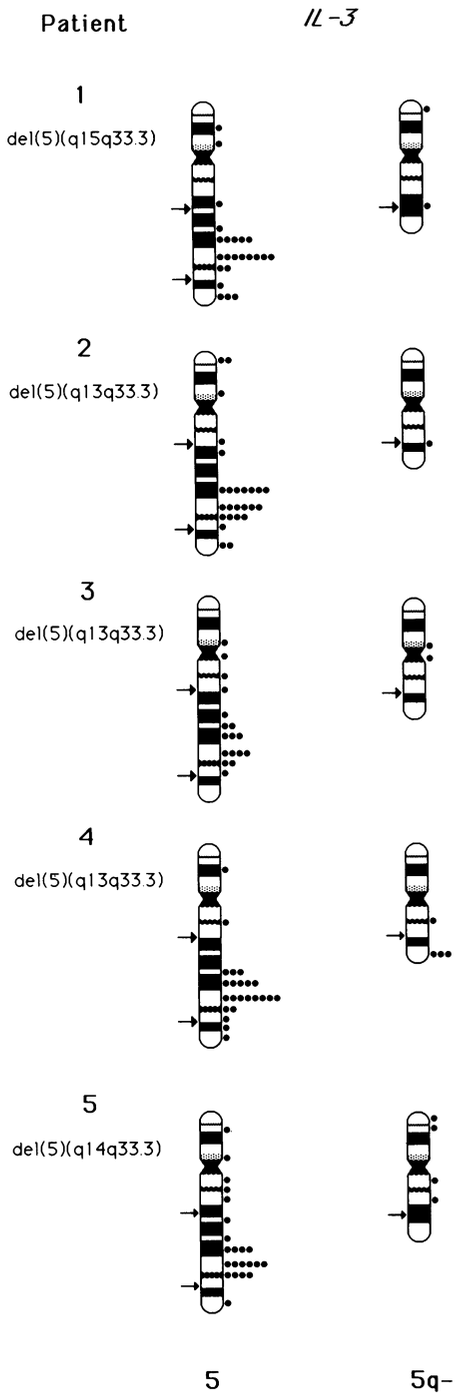

FIG. 4. Distribution of labeled sites on the normal chromosome 5 and on the deleted homologues in metaphase cells, from bone marrow aspirates of five patients, that were hybridized with the *IL-3* probe. The arrows to the left of each normal chromosome 5 (*Left*) illustrate the breakpoints and the segment that is deleted in each patient. The arrow on each 5q− chromosome (*Right*) identifies the breakpoint junction. The results of these hybridizations indicate that *IL-3* is located within the segment that is deleted in each patient.

mice, whereas the parental cell line was not. With respect to myeloid disorders associated with a del(5q), there is considerable evidence suggesting that the chromosomal abnormality occurs in a myeloid stem cell, and possibly in a common progenitor cell. If so, this would suggest that IL-3, which is capable of supporting the proliferation of a broad range of hematopoietic cells, may be the critical growth factor in these disorders.

On the other hand, loss of CSF-receptor genes may be the

relevant genetic alteration in these leukemia cells. The lack of capacity for autonomous growth of most leukemia cells *in vitro* has led to the hypothesis that leukemia stem cells have an intrinsic abnormality as a consequence of which CSF-stimulated proliferation results in an abnormally high ratio of self-regenerative divisions compared to divisions leading to the production of differentiated cells (1). Such a defect could result from an altered level of expression of CSF receptor(s) or from the production of an altered receptor. In this regard, recent data suggest that the v-*fms* product, which differs from the cellular *FMS*-encoded glycoprotein at the extreme carboxyl terminus, and by its constitutive autophosphorylation, is an unregulated kinase that provides growth-stimulatory signals in the absence of ligand (34). Moreover, introduction of v-*fms* into simian virus 40-immortalized, CSF-1-dependent macrophages resulted in factor-independence and tumorigenicity in nude mice (34). These results raise the possibility that critical alterations in the kinase domain of *FMS* might contribute to the pathogenesis of hematologic malignancies. Finally, with respect to growth factor receptor genes, it is notable that the gene encoding the receptor for platelet-derived growth factor, which shares structural features with the *FMS* protein, is also located on chromosome 5, at bands q31–32 (35). Moreover, this gene is deleted in patients with a del(5q) (M.M.L., J. A. Escobedo, and L. T. Williams, unpublished results).

Finally, our data provide evidence that a functional family of genes that regulate the growth of hematopoietic cells maps to a limited segment of chromosome 5. Thus, it may be possible to relate some of the clinical features of the somewhat heterogeneous group of patients with a del(5q) to the specific chromosomal deletions, or to the possible alterations of the CSF or CSF-receptor genes on the homologous chromosome 5.

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