Lack of Involvement of the c-fms and N-myc Genes by Chromosomal Translocation t(2;5)(p23;q35) Common to Malignancies With Features of So-Called Malignant Histiocytosis

By Rodman Morgan, Stephen D. Smith, Barbara K. Hecht, Valerie Christy, Julia D. Mellentin, Roger Warnke, and Michael L. Cleary

We report the molecular, cytogentic, and immunologic characterization of three hematologic malignancies that contained characteristic t(2;5) chromosomal translocations. The clinicopathologic features in all three cases fit the disease spectrum of so-called malignant histiocytosis (MH). All cases expressed activation antigens including Ki-1 (CD 30), but no lineage-restricted pattern of cellular antigen expression was observed. Cell lines SUP-M2 and SU-DHL-1 established from two of the cases showed rearranged β T-cell receptor (β TCR) genes nonproductive of full-length β TCR mRNA and therefore not helpful in unequivocal establishment of lineage derivation. The common cytogenetic feature was a reciprocal translocation between chromosomes 2 and 5, involving bands p23 and q35 near the reported chromosomal locations of the N-myc and c-fms genes, respectively. Normal-sized and truncated c-fms RNAs were observed in both cell lines, whereas no N-myc transcripts were detected. Sequence analysis of the truncated fms mRNA showed that it consisted of the 3' half of the c-fms mRNA, but its derivation was not the result of a structural alteration of the c-fms gene. Our studies show that the t(2;5) does not involve the N-myc and c-fms protooncogenes and that this cytogenetic abnormality may be characteristic of a subset of primitive malignancies with an indeterminate lineage but with clinicopathologic features of so-called MH.

MALIGNANT histiocytosis (MH) is a clinicopathologic syndrome that may affect the entire reticuloendothelial system at presentation. The biology of this aggressive malignancy is not yet fully understood, but morphologic and histochemical studies suggest that it involves proliferation of mononuclear phagocytes and their precursors. Pathologically, MH represents a subset of the presumed "true histiocytic malignancies," which recent immunologic studies have shown to be a rare yet diverse group with no common diagnostic features, since they variably express a number of hematolymphoid antigens. Immunogenotype studies of these malignancies have shown frequent β T-cell receptor (β TCR) gene rearrangements, suggesting that many lymphomas with histiocytic morphologic features may be derived from or related to the T-lymphoid lineage.

We describe our studies using immunologic, molecular, and cytogenetic methods on the neoplastic cells from three primitive hematologic malignancies with the clinicopathologic features of so-called MH. Two cultured cell lines, one of them newly established, appear to be representative of the patients' malignancies. Lineage derivation could not be precisely established based on immunologic studies or rearrangement and transcription of antigen receptor genes. All three cases had a consistent translocation between chromosomes 2 and 5 in bands p23 and q35, respectively. Although the breakpoints occur near the sites of known protooncogenes, our molecular studies provide no evidence for their involvement by t(2;5). Review of the literature showed that five additional cases of MH contain a t(2;5)(p23;q35) among other chromosome changes. This translocation may be specific to a subset of tumors that show the clinicopathologic features of MH, although the precise derivation of these tumors does not fit clearly into the T-lymphoid or myeloid/histiocytic lineages.

MATERIALS AND METHODS

Patients

Case 1. A 10-year-old boy (M.S.) was originally diagnosed by Rappaport classification as having diffuse histiocytic lymphoma, with fever, painful lymphadenopathy, and skin and lung involvement. The cell line SU-DHL-1 was established from the malignant cells; its detailed characterization has been published elsewhere. Review of the pathologic and clinical features indicated that this patient's disease was most consistent with a diagnosis of MH.

Case 2. A 5-year-old girl (T.S.) had an 8-week history of weight loss, painful lymphadenopathy, fever, leukemoid reaction, bilateral pulmonary infiltrates, pleural effusion, and ascites. After treatment with chemotheraphy (CHOP), she developed CNS involvement. A cell line (SUP-M2) was established from cells in cerebrospinal fluid (CSF). In the initial pathologic evaluation process, the lymph node was suggestive of malignant hematolymphoid neoplasia, not otherwise specified; however, subsequent review indicated that the pathologic features of this patient's tumor were most consistent with a diagnosis of MH.

Case 3. A 19-year-old man (M.L.) had splenomegaly, skin infiltrates, and a high WBC count with 80% atypical cells. There were no distinguishing morphologic or ultrastructural features to the neoplastic cells, although special histochemical stains revealed evidence of predominantly monocytoic differentiation due to the prominent nonspecific esterase positivity. A diagnosis of M4 acute nonlymphocytic leukemia (ANLL) was made. After treatment with...
chemotherapy, he developed CNS involvement. Reevaluation of the pathologic material led to the diagnosis of MH.

Cell Lines. The cell lines SUP-M2 and SU-DHL-1 were established from CSF and lymph node biopsy tissue, respectively, by procedures described in detail elsewhere.

Chromosome preparation and analysis. Chromosomes were prepared for analysis from the SUP-M2 and SU-DHL-1 cell lines and from peripheral blood (PB) and bone marrow (BM) cells. For cell synchronization and high-resolution chromosome banding, cells were exposed to methotrexate (10^{-7} mol/L) for 18 hours and released with thymidine (10^{-5} mol/L) for six hours. After incubation at 37°C in 5% CO₂, air for 24 hours, cells were arrested by addition of colcemid for 30 minutes. The arrested cells were pelleted, suspended in hypotonic KCl solution, and fixed as described previously. Chromosome spreads were trypsin-Giemsa banded, and 20 cells were analyzed for karyotype preparation.

DNA probes. The βTCR probe consisted of a 3-kilobase (kb) HindIII-EcoRI fragment containing the β constant region gene. This probe also crosshybridizes with the CD2 gene. Probes for the c-fms gene consisted of the pSM3 and pSM7C v-fms fragments and a 3.5-kb EcoRI genomic DNA fragment from near the 5' end of the c-fms gene containing the c-fms exon that codes for amino acids 244 through 296. The N-myc probe consisted of a 1.7-kb EcoRI-BglII fragment containing the third exon and 3' flanking sequences of the N-myc gene.

Isolation and blotting of nucleic acids. DNA was isolated from cultured cell lines and lymph node biopsy tissues by previously described procedures. Total cellular RNA was isolated as previously described, and poly (A⁺) RNA was enriched using oligo-dT cellulose. Conditions for genomic Southern blot and Northern blot analyses have been described previously. DNA probes were labeled with α[3²P]dCTP by random hexanucleotide priming.

Recombinant DNA. A cDNA library was constructed with 3 µg poly (A⁺) RNA from the cell line SUP-M2, according to procedures described elsewhere. Approximately 100,000 recombinant phages were screened with the v-fms pSM3 probe. Hybridizing phages were plaque purified. The cDNA inserts from three independently isolated phages containing cDNA inserts >2 kb were subcloned into M13 vectors for sequence analysis.

Nucleotide sequence analysis. Nucleotide sequences were determined by dideoxy chain termination on DNA fragments subcloned into either M13mp18 or M13mp19 single-strand phage vectors.

Characterization of cellular antigens. All fresh tumor specimens were snap-frozen, processed, and stained as previously described. For patients M.S. and M.L., fresh-frozen biopsy material was unavailable and limited immunophenotype studies were performed on paraffin-embedded formalin-fixed sections. The staining consisted of a first-stage incubation with one of the antibodies shown in Table 1. After washing, horse anti-mouse immunoglobulin (Vector Laboratories, Burlingame, CA) was applied before a third stage of horseradish peroxidase-conjugated avidin. Sections were then incubated in diaminobenzidine followed by copper sulfate, fixed in absolute methanol, and counterstained with methylene blue. Cytospin preparations from cell lines SUP-M2 and SU-DHL-1 were stained in an identical manner.

RESULTS

Cyto genetic Studies

Cyto genetic analysis of the cell lines SUP-M2 (patient T.S.) and SU-DHL-1 (patient M.S.) and patient M.L. showed multiple chromosomal aberrations in each of the three cases. The only common cytogenetic feature was a reciprocal translocation between chromosomes 2 and 5 involving bands 2p23 and 5q35 (Figs 1 and 2). Cytogenetic examination of the cell line SU-DHL-1 revealed the following representative karyotype: 65,X,—Y, +1, +2, +2, +3, +3, +5, +5, +6, +6, —7, —9, +10, +12, +14, +15, +18, —20, +21, +21, +22, del(1)(p21?),(t;2;5),(p23;q35), t(2;5)(p23;q35), del(6)(q23), del(6)(q23), +der(9)(t;9;9) (p21;q13), del(10)(p12), (t;14)?(p11.2;?)t(19;?)(q13;?), +5 mar (Fig 1).

When this representative karyotype was compared with that of the same cell line reported by Hecht et al, the only obvious similarity was the del(6q), although the markers observed previously were not described in written or photographic form. However, review of the original unpublished quinacrine-banded karyotype showed 2p— and 5q+ chromosomes which may have been interpreted as a t(2;5)(p23;q35) had the banding been of superior quality (R. Morgan, unpublished observations). The SU-DHL-1 cell line has maintained its Ig-phenotype since its original description.

Cytogenetic examination of the cell line SUP-M2 revealed the following karyotype: 47,XX, +X,—9, +der(1)(t;1;?)(q44;?),del(1)(p34),t(2;5)(p23;q35) (Fig 2). Cytogenetic examination of the unstimulated (no phytohemagglutinin, PHA) blood cells in a sample from patient M.L. revealed an abnormal karyotype consisting of a translocation between chromosomes 2 and 5, i.e, t(2;5)(p23;q35) as the only abnormality. Blood cells stimulated with PHA had a normal male karyotype. Analysis of a sample obtained 8 months later showed that the karyotypic findings in patient M.L. progressed and underwent a radical change, but with persistence of the t(2;5). The evolving karyotypic analyses showed:

<table>
<thead>
<tr>
<th>MoAb</th>
<th>SUP-M2</th>
<th>MS</th>
<th>DHL-1</th>
<th>ML</th>
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<tbody>
<tr>
<td>B-cell</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Leu-17</td>
<td>CD19</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
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<td>B1</td>
<td>CD20</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>CD22</td>
<td>—</td>
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<td>L26</td>
<td></td>
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<td>—</td>
</tr>
<tr>
<td>Macrophage/myeloid</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Leu-M1</td>
<td>CD15</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
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<td>CD14</td>
<td>+</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Leu-M5</td>
<td>CD11C</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Y182</td>
<td>—</td>
<td>+ (±50%)</td>
<td>+ (&gt;50%)</td>
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<tr>
<td>Activation</td>
<td></td>
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<td>TAC</td>
<td>CD25</td>
<td>+</td>
<td>—</td>
<td>+</td>
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<tr>
<td>Ki-1/BerH2</td>
<td>CD30</td>
<td>+</td>
<td>—</td>
<td>+</td>
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<td>OKT9</td>
<td>—</td>
<td>+</td>
<td>—</td>
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Table 1. Summary of Immunophenotypic Findings

<table>
<thead>
<tr>
<th>T-cell</th>
<th>Leu-1</th>
<th>CD5</th>
<th>—</th>
<th>(±5%)</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-cell</td>
<td></td>
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Fig 1. Karyotype of SU-DHL-1.

Fig 2. Karyotype of SUP-M2.
46,XY,t(2;5)(p23;q35) → 46,XY,t(2;5)(p23;q35)/47,XY,+7,t(2;5)(p23;q35),t(15;16)(q22;p13.3)/78 count with the t(2;5)(p23;q35).

**Immunophenotype Studies**

The results of analyses for cellular antigens were notable for a marked absence of expression of most lymphoid and macrophage/myeloid antigens (Table 1). In general, the few antigens expressed on the cell lines or patient's tumor cells are known not to be completely restricted to the T lymphocyte lineage or to the macrophage/myeloid lineage (eg, Leu-1 on SU-DHL-1). Phenotype studies on tissues from patients M.S. and M.L. were confined to antigens preserved in formalin-fixed, paraffin-embedded sections due to the unavailability of frozen tissues from these cases. The only antigens consistently expressed in all cases were those associated with cellular activation, and these were observed in both cell lines, frozen tissues of patient T.S., and formalin-fixed tissues of patients M.S. and M.L. None of the cases expressed any of the B-cell antigens tested. Although the phenotype results do not unequivocally establish lineage of these cells, they are consistent with either a T-cell origin associated with loss of most T-cell surface marker expression or a histiocytic origin for each of the three cases.

**Configuration and Expression of \( \beta \)TCR Genes**

To help establish the lineage derivation of these tumors and cell lines, the configurations of the \( \beta \)TCR genes were examined by Southern blot analysis (Fig 3). In the cell line SUP-M2, two rearranged \( \beta \)TCR bands were observed with a \( \beta \) constant-region DNA probe on EcoRI-digested DNA, but no rearranged bands were observed after HindIII digestion. These results indicated that both \( \beta \)1 alleles were rearranged in this cell line, whereas the \( \beta \)2 alleles remained in the germline configuration. The pattern of rearrangements noted in the SUP-M2 cell line was identical to that observed in DNA obtained from a lymph node biopsy from the same patient (Fig 3, lanes 2 and 3), thus confirming that the cell line was representative of the tumor. A similar analysis of the SU-DHL-1 cell line showed a single rearranged band and a germline band in HindIII-digested DNA, whereas only a germline pattern was observed after EcoRI digestion of the cell line DNA. These results indicated that a single \( \beta \)2 allele had undergone rearrangement in the SU-DHL-1 cell line, either with deletion of the upstream \( \beta \)1 gene or simply as a result of a D\( \beta \)2-J\( \beta \)2 rearrangement without deletion of the upstream \( \beta \)1 gene. The \( \beta \)1 and \( \beta \)2 genes on the allelic chromosome remained in the germline configuration. We did not observe immunoglobulin gene rearrangements in either cell line with a heavy chain joining segment probe (data not shown).

To determine whether the rearranged \( \beta \)TCR genes were transcriptionally active, Northern blot analyses were performed on poly(A)\(^+\) mRNAs purified from the two cell lines (Fig 4). A very low abundance 1.0-kb \( \beta \)TCR transcript similar in size to the \( \beta \)TCR transcript observed in two lymphoblastoid cell lines (Fig 4) was observed in SUP-M2; no \( \beta \)TCR transcripts were observed in SU-DHL-1. The size of the \( \beta \) mRNA in SUP-M2 corresponds to a truncated D-J transcript and is shorter than the 1.3-kb \( \beta \)TCR mRNA that codes for a full-length \( \beta \) polypeptide observed in SUP-T7 (Fig 4). The results of Northern blot analyses indicated that the rearranged \( \beta \)TCR genes in these two cell lines were not productive of full-length \( \beta \) mRNA, and (at least in SUP-M2) were indicative of only partial (ie, D-J) rearrangement of the \( \beta \)TCR genes.

**Molecular Studies of the c-fms Gene and Its Transcripts**

Molecular studies were performed to determine the relationship of the t(2;5) breakpoints with respect to potential involvement of protooncogenes N-myc and c-fms, which have been mapped to regions 2p24 and 5q33.2-34, respectively. Messenger RNAs purified from SU-DHL-1 and SUP-M2 were analyzed on Northern blots by a radiolabeled C\( \beta \)1 TCR DNA probe. Positions of expected germline \( \beta \) constant region fragments (-); rearranged \( \beta \)TCR bands (arrows). Numbers refer to sizes in kilobases and positions of DNA standards.

**Fig 3.** Southern blot analysis of the \( \beta \)TCR genes in patient TS and cell lines SUP-M2 and SU-DHL-1. After digestion with either EcoRI (A) or HindIII (B), DNA was subjected to Southern blot analysis with a radiolabeled C\( \beta \)1 TCR DNA probe. Positions of expected germline \( \beta \) constant region fragments (-); rearranged \( \beta \)TCR bands (arrows). Numbers refer to sizes in kilobases and positions of DNA standards.

The results of analyses for cellular antigens were notable for a marked absence of expression of most lymphoid and macrophage/myeloid antigens (Table 1). In general, the few antigens expressed on the cell lines or patient’s tumor cells are known not to be completely restricted to the T lymphocyte lineage or to the macrophage/myeloid lineage (eg, Leu-1 on SU-DHL-1). Phenotype studies on tissues from patients M.S. and M.L. were confined to antigens preserved in formalin-fixed, paraffin-embedded sections due to the unavailability of frozen tissues from these cases. The only antigens consistently expressed in all cases were those associated with cellular activation, and these were observed in both cell lines, frozen tissues of patient T.S., and formalin-fixed tissues of patients M.S. and M.L. None of the cases expressed any of the B-cell antigens tested. Although the phenotype results do not unequivocally establish lineage of these cells, they are consistent with either a T-cell origin associated with loss of most T-cell surface marker expression or a histiocytic origin for each of the three cases.
Fig 4. Northern blot analysis of βTCR mRNAs in SUP-M2, SU-DHL-1, lymphoblastoid cell lines (IM-9, SUP-B6), and T-cell leukemia cell line SUP-T7. Polyadenylated RNA from cultured cell lines was size-fractionated in a formaldehyde agarose gel. After transfer to nylon membrane, hybridization was performed with a βTCR constant region DNA probe. Positions of 1.3-kb and 1.0-kb C-β-containing mRNAs (arrows).

Fig 5. Northern blot analysis of c-fms mRNAs in monocytic/histiocytic lineage cell lines. Polyadenylated RNA from cultured cell lines was size-fractionated in a formaldehyde agarose gel. After it was transferred to nylon membrane, hybridization was performed with v-fms probes SM3 and SM7C. Numbers and arrows (right) refer to the 4.0-kb full-length c-fms mRNA and the 2.2-kb truncated c-fms mRNA, respectively. Size markers consisted of 28S and 18S ribosomal RNAs.

band corresponds to the previously described full-length c-fms mRNA, which was present at moderate levels in both SUP-M2 and SU-DHL-1. This c-fms mRNA is present at about tenfold less abundant levels than in U937 after exposure to phorbol esters, indicating that there was probably not a notable overexpression of the mRNA in the two t(2;5)-carrying cell lines. The presence of the 2.2-kb v-fms RNA appeared to correlate with expression of the c-fms gene since uninduced U937 cells lacked both the 4.0-kb and 2.2-kb transcripts and the latter was not observed in other cell lines that did not express the 4.0-kb c-fms mRNA (data not shown). The 2.2-kb RNA was difficult to observe in Northern blots of total cellular RNA preparations (data not shown), which may explain its lack of detection by other investigators, although at least one other published Northern blot analyses using several v-fms and c-fms DNA probes showed no structural alterations of the c-fms gene (Figure 6) although this analysis did not include the extreme 5' end of the gene.22

To analyze the structure of the 2.2-kb truncated c-fms RNA further, we prepared a cDNA library from mRNA purified from the SUP-M2 cell line. Recombinant phages that hybridized to the v-fms SM3 probe were purified, and cDNA inserts >2 kb from three independently isolated phages were analyzed by nucleotide sequencing (Fig 7). The sequence data from these three clones were essentially identical (except for small differences in the extent of 5' extension) and showed that most of the truncated c-fms RNA was identical to the 3' half of the previously described c-fms mRNA with the homology starting at nucleotide number 1,923 of the published sequence23 and extending 3' to the polyadenylation site. The homology with c-fms mRNA did not extend 5' of nucleotide 1,923; instead, 113 to 141 nucleotides of unrelated sequence were present at the 5' ends
of each of several cDNAs analyzed. Computer analysis showed that the additional 5' nucleotides were not homologous to any portion of the c-fms mRNA. This analysis also showed that just 5' to the point of divergence with c-fms mRNA there was a short stretch of nucleotides that perfectly matched the consensus 3' intron splice site sequence (Y)_nNYAGG as shown in Fig 7. The sequence data suggested that the 141 nucleotides at the 5' end of the truncated c-fms RNA originated from the 3' portion of a c-fms intron, most likely the intron just downstream of the exon coding for the transmembrane segment of the c-fms protein. Translating the sequence data showed that although there were four ATG codons in the 5' 141-nucleotide segment, they could not initiate a continuous open reading frame with the c-fms protein coding sequence since an in-frame TGA termination codon occurred before the start of homology with c-fms at nucleotides 77 through 79 of the truncated RNA.

We were unable to detect N-myc mRNA in either of the cell lines SUP-M2 or SU-DHL-1 on Northern blot hybridization analyses (data not shown). Therefore, the t(2;5) breakpoint on chromosome 2 probably does not involve the N-myc gene.

**DISCUSSION**

We used immunologic, molecular, and cytogenetic techniques to study three hematologic malignancies that had characteristic t(2;5) chromosomal translocations in common. We were specifically interested in assessing the lineage derivation of these malignancies and determining whether the c-fms and N-myc genes were involved by the t(2;5) translocation. All three cases were retrospectively judged to possess the clinicopathologic characteristics of MH. The cell of origin for MH has traditionally been considered to be a mononuclear phagocyte intermediate in its differentiation between the BM-derived monoblast/monocyte of acute monocytic leukemia and the fixed tissue histiocyte of true histiocytic lymphomas. This intermediate stage of differentiation creates difficulties in distinguishing MH from other histiocytic malignancies (ie, histiocytic lymphoma used for lymphoid tumors in the Rappaport scheme). In addition, the distinction between MH and acute monocytic leukemia may be arbitrary, and these two entities may merge, as occurred in patient M.L. MH and histiocytic malignancies in general may morphologically resemble peripheral T-cell lymphomas, and it has been proposed that many of these malignancies may indeed be of T-cell origin. Unfortunately, monoclonal antibodies (MoAbs) are not always helpful in distinguishing tumors of histiocytic lineage from those of lymphoid lineage, since some antigens expressed by mononuclear phagocytes are not specific for these cells and can also be found on other cell types. For the three cases in this report, no consistent or lineage-restricted pattern of cellular antigen expression was observed, although activation antigens were present in all cases and none expressed B-cell antigens. Recently, immunogenotype analyses were applied to so-called histiocytic malignancies, and many of these tumors showed bTCR gene rearrangements, supporting the proposal that these malignancies may be related to or derived from the T-lymphocytic lineage. 

The availability of two cell lines representative of tumors from patients with histiocytic lineage malignancies permit-
ned an extensive molecular analysis of the βTCR genes and their expression. Our results showed that although βTCR gene rearrangements were present in both cell lines, the extent of rearrangement appeared incomplete. The most compelling evidence for this conclusion was obtained from Northern blotting experiments that showed no detectable full-length βTCR mRNA in either cell line. These findings indicated that a v13 gene had not been rearranged by a V13D3 joining event which is required for production of full-length J mRNA. Thus, at most, only D-J joining had occurred in the βTCR genes, and such rearrangements are not lineage-specific. For instance, D-J transcripts are apparent in the lymphoblastoid cell lines in Fig 4. Therefore, the βTCR gene rearrangement data cannot be interpreted as unequivocal evidence for a T-cell origin in two of the three cases described here as MH. The data do not rule out a monocytic-histiocytic derivation and suggest that D8β rearrangements may occur in monocytic/histiocytic cells as well as B-lineage lymphocytes. Alternatively, the incomplete nature of the βTCR gene rearrangements in our cases may indicate that the cells are arrested at an early stage of T-cell differentiation, although this seems less likely based on the extremely low levels of transcription for these genes and the lack of early T-cell antigen expression by the cells. Our results suggest that use of βTCR gene rearrangements to assign so-called histiocytic malignancies to the T-cell lineage may not be reliable in all cases.

Our studies extend our previous observations regarding the association of chromosome 5q35 abnormalities with a specific morphologic subtype of hematolymphoid malignancy. Among other chromosome changes, the translocation t(2;5) (p23;q35) was common to all three of the cases we report, and in one case it was the sole detectable deviation from the normal karyotype. Review of the literature showed that eight cases of MH of 22 studied cytogenetically (Table 2) contain the same translocation t(2;5) (p23;q35).
The cytologic location of the t(2;5) breakpoints was initially provocative in that band q35 of chromosome 5 was just distal to the reported location for the c-fms protooncogene,\textsuperscript{45} although more recent studies have mapped c-fms toSq32.2-33.3.\textsuperscript{25} Since the product of the c-fms gene is related to the receptor for mononuclear-phagocyte growth factor (CSF-1),\textsuperscript{46} we proposed that it may be involved in the t(2;5) translocation, accounting for the restricted presence of this translocation to malignancies with the features of MH.

An observation that initially appeared to support the above proposal was the presence of an abundant, abnormal-sized c-fms RNA in two MH cell lines. Because of the cytologic localization of the chromosome 5 breakpoints distal to the site of the c-fms protooncogene, we explored the possibility that the abnormal c-fms RNA may have resulted from a structural alteration of the c-fms gene. However, extensive molecular studies demonstrated no apparent structural abnormalities of the c-fms gene in the MH cell lines that would account for production of an abnormal-sized fms RNA. The truncated c-fms RNA was also observed in two other monocytic cell lines that lacked the t(2;5) translocation but expressed the c-fms gene, it was not present in U937 before phorbol ester exposure. These results suggest that the presence of the truncated RNA correlates with transcriptional activity of the c-fms gene in the cell lines examined in this study. Sequence analysis of the truncated RNA showed an RNA processing splice site in the 5' portion, indicating that the small c-fms-homologous RNA was not a product of the processed c-fms messenger RNA since it retained intron sequences at its 5' end. The abnormal-sized c-fms RNA may have resulted from aberrant transcription that initiated within the middle of the gene. This would require the presence of a cryptic promoter in the intron that lies just downstream of the exon that codes for the transmembrane segment of the c-fms protein. More likely, the truncated RNA resulted from abnormal cleavage or degradation of the c-fms precursor RNA. Although our results cannot discriminate between these alternative origins for the smaller c-fms RNA present in all of the MH and monocytic/histiocytic lineage cell lines we examined, the apparent enrichment of this RNA after poly(A) selection procedures is most consistent with it being a specific degradation product of the c-fms precursor RNA. Our results thus indicate that the RNA does not derive from a t(2;5) translocation-mediated interruption of the c-fms gene.

In summary, our results indicate that, although the N-myc and c-fms genes map to the cytologic regions of the t(2;5) breakpoints, they are not involved by this translocation. Apparently, other undescribed loci participate in this characteristic cytogenetic abnormality which may be a common feature of a small clinicopathologically distinct subset of hematolymphoid malignancies.

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Lack of involvement of the c-fms and N-myc genes by chromosomal translocation t(2;5)(p23;q35) common to malignancies with features of so-called malignant histiocytosis

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