Brief Definitive Report

GENE FOR THE HUMAN T CELL DIFFERENTIATION ANTIGEN Leu-2/T8 IS CLOSELY LINKED TO THE κ LIGHT CHAIN LOCUS ON CHROMOSOME 2

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Leu-2/T8 is a human T cell differentiation antigen that is expressed by most T cells with cytotoxic or suppressor function (1–3). On peripheral blood lymphocytes, cytotoxic T lymphocyte clones, and various human leukemic lines, it is composed of dimers and higher multimers of a 32–34 kD glycopeptide (4–6). On thymocytes, an additional 43–46 kD glycoprotein is linked to the smaller subunit in different stoichiometric ratios via disulfide bridges (4–6). A possible role for the *Leu-2/T8* antigen in target cell recognition is suggested by observations that monoclonal antibodies against *Leu-2/T8* block killing activity of most cytotoxic T cells that recognize a class I major histocompatibility complex protein (either with or without foreign antigen) (7–9). This block is at the step of binding of the cytotoxic T cell to its target cell (10–12).

Lyt-2,3 is thought to be the functional murine homologue of *Leu-2/T8* (3, 4). Though protein sequences with which to make a direct structural comparison between these two molecules are not presently available, biochemical evidence, tissue distribution, correlation with effector function, and blocking studies using monoclonal antibodies all make this conclusion plausible. Using classical genetic methods, the gene(s) for *Lyt-2,3* have been mapped to chromosome 6, tightly linked to the mouse C_κ locus (13–16). We were, therefore, interested in determining the chromosomal location of *Leu-2/T8* and its possible linkage to the human κ locus on chromosome 2 (17, 18).

We have recently isolated *Leu-2/T8* complementary DNA (cDNA) clones (19). We have now used one such cDNA clone as a probe to determine the chromosomal localization of the *Leu-2/T8* gene by hybridization to DNA from mouse-human somatic cell hybrids, and by in situ hybridization to human chromosomes.

Materials and Methods

Somatic Cell Hybrids. The generation and characterization of the mouse-human somatic cell hybrids have been described previously (18, 20, 21). The human chromosomal contents of these cell lines are shown in Tables I and II. The JI cell line was the kind gift

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of Dr. G. Lenoir. Line GM1500 is from the Human Genetic Mutant Cell Repository, Camden, NJ.

Southern Blots. Cellular DNA from the hybrid cell lines or from controls was thoroughly digested with Eco RI (New England Biolabs, Beverly MA), fractionated by electrophoresis through a 0.9% agarose gel, and transferred by blotting to nitrocellulose filters according to the procedure of Southern (22). Blots were hybridized with the 2 kilobase (kb) insert of a *Leu-2/T8* cDNA clone labeled with [^{32}P]orthophosphate by nick-translation to a specific activity of 2–4 × 10^8 cpm/μg. Hybridizations were for 16 h at 42°C in 40% formamide, 4× saline sodium citrate (SSC), Denhardt's solution, 20 mM Tris, pH 7.6, 0.1% sodium dodecyl sulfate (SDS), 100 μg/ml denatured herring sperm DNA, and 10% dextran sulfate. The filters were each washed twice at room temperature for 30 min with 2× SSC, 0.1% SDS, and once at 52°C for 30 min with 0.1× SSC, 0.1% SDS. The filters were then exposed to XAR-5 film with an intensifying screen for 3 d at −70°C.

In Situ Hybridization. In situ hybridizations were performed as previously described (20). The probe was a *Leu-2/T8* cDNA clone containing a 2 kb insert, labeled with all four [^{3}H]nucleoside triphosphates by nick-translation.

Results

Hybridization to DNA From Mouse-Human Somatic Cell Hybrids. We used 10 mouse-human hybrid cell lines to map the *Leu-2/T8* gene within the human genome. DNA derived from these lines was digested with Eco RI, fractionated by agarose gel electrophoresis, blotted onto a nitrocellulose filter, and hybridized to a 2 kb *Leu-2/T8* cDNA clone insert labeled by nick-translation. Fig. 1 shows examples of hybridization to some of the somatic cell hybrid DNA and normal human DNA. The *Leu-2/T8* probe hybridized to two human-specific fragments: 7.5 kb and 4.8 kb (Fig. 1, lanes *1*, *2*, *8*, *10*, *11*). Comparison of the human chromosome composition of the hybrids with the presence of *Leu-2/T8*-specific DNA allows assignment of the *Leu-2/T8* gene to chromosome 2, since this is the only chromosome for which there is complete concordance (Table I). None of the hybrids used contains human chromosome 21. We feel we can exclude that chromosome because three independent hybrids without detectable chromosome 21 but containing chromosome 2 have hybridizing human sequences. This conclusion is confirmed by two additional types of evidence discussed below.

To further define the location of the *Leu-2/T8* gene on chromosome 2 we hybridized the *Leu-2/T8* probe to DNA derived from mouse-human fusions in which the human partner was a Burkitt lymphoma cell line (JI) carrying a t(2;8) translocation. In an earlier study (18) we had shown that the Ig κ locus on one chromosome 2 of this cell line is translocated to a region distal (3′) to an unrearranged *c-myc* oncogene on chromosome 8, thus enhancing *c-myc* transcription. The breakpoint on chromosome 2 is on band p11, between V_κ genes; regions distal to this (including the C_κ gene) are translocated (18). We digested DNA derived from several JI × NP3 hybrids with Eco RI and hybridized to the previously described *Leu-2/T8* cDNA probe (Fig. 1 and Table II). Human-specific bands appeared with JI 4-5B7 DNA (8q+), while no signal was present with JI 5-4 (8 and 2p−) DNA. The weak signal with JI 4-5B7 DNA can be explained by the low frequency (10–30%) of metaphases containing the 8q+ chromosome. These results show that the *Leu-2/T8* gene maps to the short arm of chromosome 2, either distal to the C_κ locus or, less likely, between some of the V_κ genes and C_κ.

In Situ Hybridization to Human Metaphase Chromosomes. We have confirmed

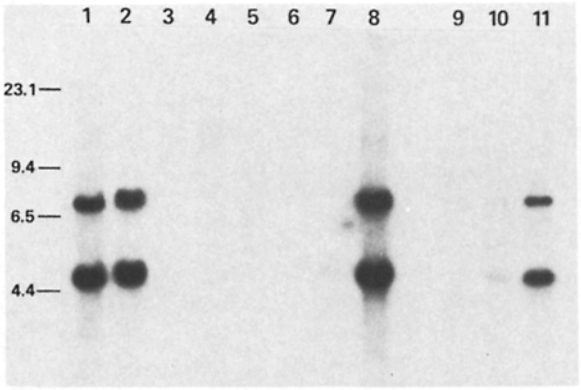

FIGURE 1. Hybridization of *Leu-2/T8* cDNA probe to mouse-human somatic cell hybrid lines. Southern blots of EcoR1 digested genomic DNA of hybrid and control cell lines were prepared and hybridized to a *Leu-2/T8* cDNA probe as described in Materials and Methods. Cell lines used were: (*1*) PAF × BALB IV-1; (*2*) PAF × BALB IV-5; (*3*) 77-B10 C1 31; (*4*) 106 2B44C4; (*5*) Nu 9; (*6*) GM54VA × BALB/c C1 31; (*7*) 53-87-1-F1-C1 21; (*8*) GM1500 (human control); (*9*) JI 5-4; (*10*) JI 4-5B7; (*11*) JI (human Burkitt lymphoma line). The human chromosomal contents of the hybrids are shown in Tables I and II.

TABLE 1

Presence of Leu-2/T8 Gene in Mouse-Human Somatic Cell Hybrids

Cell line	Human chromosome complement	Presence of human-specific sequences hybridizing to *Leu-2/T8* probe
NP3 (mouse)	None	−
GM1500 (human)	All	+
53-87-1-F1-C1 21	7	−
GM54VA × BALB/c C1 31	17	−
Nu 9	6, 7	−
106 2B44C4	14	−
77-B10 C1 31	1, 3, 5, 8, 9, 10, 13, 14, 18, 20, X	−
PAF × BALB IV-5	2, 5, 8, 12, 19	+
PAF × BALB IV-1	2, 8, 12, 19	+
GM × LM C1 6	4, 11, 12, 16, 20, 22	−
P × BALB IV-5 C1 5	2 (5?, 8?)*	+
CSKNS51C1 C1 11	1, 4, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, X	−

* This hybrid is a subclone of PAF × BALB IV-5, which has lost chromosomes 12 and 19 and retained chromosome 2. The presence or absence of chromosomes 5 and 8 has not been examined.

TABLE 2

Presence of Leu-2/T8 Gene in JI Burkitt's Lymphoma Cells and JI × NP3 Hybrids

Cell line	Human chromosome*				Presence of human sequence hybridizing to *Leu-2/T8* cDNA probe
	8	8q+	2	2p−	
JI	++	++	++	++	+
JI 4-5B7‡	−	+	−	−	+
JI 5-4‡	+	−	−	++	−
JI 6-3‡	−	−	++	−	+
PA682 (human control)	++	−	++	−	+
NP3 (mouse fusion partner)	−	−	−	−	−

* Frequency of metaphases with relevant chromosomes: −, none; +, 10–30; ++, >30% (18).
‡ JI × NP3 hybrids.

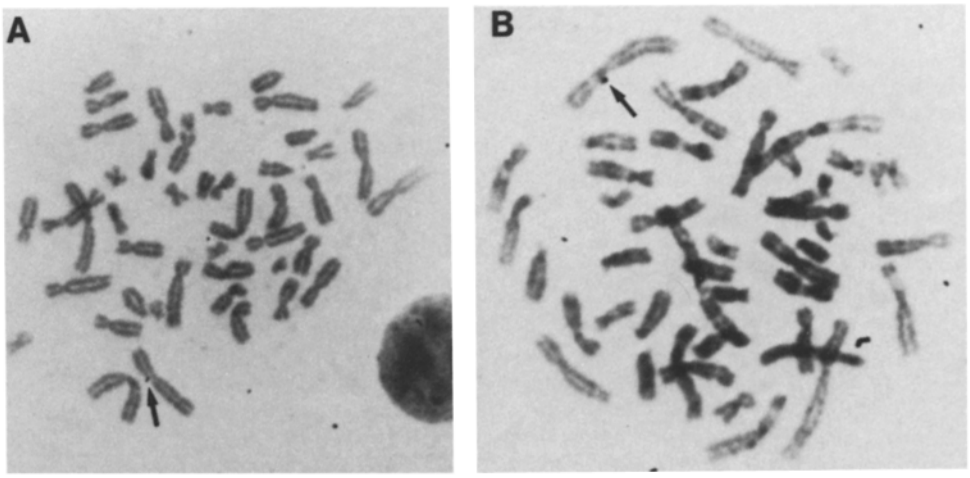

FIGURE 2. In situ hybridization of human chromosomes to a *Leu-2/T8* cDNA probe. Two representative metaphase spreads are shown (*A* and *B*). Arrows point to silver grains on the proximal portion of chromosome 2.

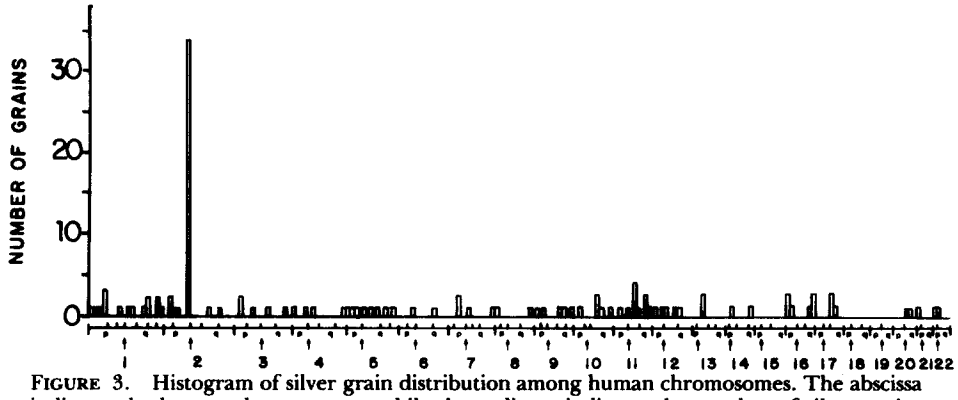

FIGURE 3. Histogram of silver grain distribution among human chromosomes. The abscissa indicates the human chromosomes, while the ordinate indicates the number of silver grains found on each chromosome in the 77 metaphases examined.

the results obtained with the somatic cell hybrids by in situ hybridization of a *Leu-2/T8* cDNA probe to human metaphase chromosome preparations from peripheral blood cells of a normal male. After autoradiography, we analyzed the metaphase spreads for grain localization. Examples of two such spreads are shown in Fig. 2. Over 27% of all grains were located on the short arm of chromosome 2. Over 91% of the 2p grains were between the centromere and band 2p13, with most grains at 2p12. A histogram depicting the silver grain distribution along the human chromosomes is shown in Fig. 3. The short arm of chromosome 2 represents ~3.3% of the haploid genome, and our observation that >27% of the *Leu-2/T8* probe hybridization is localized to the proximal half of this region is highly significant ($P \ll 0.01$). Thus, cytological hybridization localizes the *Leu-2/T8* gene to the p1 region of chromosome 2.

Discussion

The chromosomal location of the *Leu-2/T8* gene is significant because of the close linkage to the C_κ locus, which has previously (18) been mapped distally on

the p11 band of chromosome 2. This linkage to C_κ parallels that found for the mouse *Lyt-2,3* gene(s). The reasons for the maintenance of the linkage to C_κ in mouse and man are not known. Note that the genes encoding the β chain of the mouse T cell receptor are also linked to C_κ on chromosome 6 (23). However, that linkage is not maintained for the human T cell receptor β chain genes, which are located on chromosome 7, rather than 2 (23). The close linkage of both *Leu-2/T8* and *Lyt-2,3* to C_κ lends further support to the hypothesis that these two T cell differentiation antigens are homologous structures. We have now isolated mouse clones homologous to *Leu-2/T8* (Rose Zamoyska, V. P. Sukhatme, and J. R. Parnes, unpublished results) and can determine whether they in fact encode Lyt-2 and/or -3.

Summary

We have mapped the gene encoding the T cell differentiation antigen *Leu-2/T8* to human chromosome 2 by hybridization of a *Leu-2/T8* complementary DNA clone to DNA from a panel of mouse-human cell hybrids. In situ hybridization further localizes the gene to the 2p1 region in close proximity to the Ig κ light chain gene. The *Leu-2/T8* gene translocates with C_κ to chromosome 8 in a Burkitt lymphoma line carrying a t(2;8) translocation. These data support the hypothesis that *Leu-2/T8* is the human homologue of the mouse *Lyt-2,3* antigen.

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