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The human T cell antigen Leu-2 (T8) is encoded on chromosome 2

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Summary. The locus encoding the human T lymphocyte cell surface antigen Leu-2 has been assigned to chromosome 2 with a DNA mapping panel derived from somatic cell hybrids. The two genomic components identified by a cDNA clone for Leu-2 segregated with human chromosome 2 in all 24 independent hybrid clones examined. The cosegregation of the Leu-2 and immunoglobulin kappa (IgK) loci in hybrids with spontaneous rearrangements of chromosome 2 is consistent with the possibility that the Leu-2 locus is on proximal human 2p near IgK. In the mouse, a locus for a T lymphocyte cell surface antigen with properties similar to Leu-2 is closely linked to the IgK locus on mouse chromosome 6. Hence the syntenic relationship of a gene implicated in T cell killing with the immunoglobulin kappa locus would then be conserved in the mouse and human genomes.

Introduction

Leu-2 (synonym T8, CD 8 [Bernard et al. 1984]) is a human T lymphocyte cell surface antigen expressed on most T cells having either cytotoxic or suppressor function, but not expressed on helper T cells. A possible role for the protein bearing the Leu-2 antigenic site in T cell killing has been proposed, based on the observation that monoclonal antibodies against Leu-2 block killing by many cytotoxic T cells which recognize foreign antigen in association with a Class I major histocompatibility complex molecule (Evans et al. 1981; Landgren et al. 1982; Malisson et al. 1982; Reinherz et al. 1982). The mouse antigen Lyt-2 is thought to be the homologue of Leu-2 because of similarities in (i) expression and density distribution on lymphocyte subpopulations, (ii) trypsin sensitivity, and (iii) biochemical characteristics (Ledbetter et al. 1981). In addition, in the mouse, antibodies against Lyt-2 also show the blocking effect on cytotoxic T cells (MacDonald et al. 1982; Nakayama et al. 1980; Shinohara and Sachs 1979).

To investigate further the homology between Leu-2 and Lyt-2, we cloned the gene for Leu-2 (Kavathas et al. 1984). In this report, we assign the gene encoding the Leu-2 human T cell surface antigen to human chromosome 2, most probably proximal 2p, by Southern blotting analysis of DNA from human-mouse and human-hamster somatic cell hybrids using a Leu-2 cDNA probe.

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Methods

1. Somatic cell hybrids

The somatic cell hybrids used for DNA mapping panels were derived from fusions of the hypoxanthine phosphoribosyl transferase deficient mouse cell RAG or Chinese hamster cell E36 with white blood cells (WBC) or fibroblasts from four unrelated individuals. Two of the WBC donors were female carriers of different, reciprocal X/19 translocation chromosomes. These included: the X/19W translocation t(X;19)(q23−25::q13) (Latt et al. 1976) and the X/19B translocation t(X;19)(q1::?p13) (Brook et al. 1984). One of the fibroblast donors was a karyotypically normal male and the second, a female carrier of a reciprocal X/13 translocation (X;13)(q21−23::q21−31) (Latt et al. 1976). The hybrid clones have been extensively characterized for human chromosome complements by analysis of human isozyme markers characteristic of each chromosome and by cytogenetic techniques (Bruns et al. 1978, 1979). Cloned DNA probes have also been used to monitor 22 of the human autosomes and the X chromosome in the DNAs used in the present study (Bruns et al. 1984; Kurnit et al. 1984; Whitehead et al. 1983).

2. Southern blot hybridization

Parental and hybrid DNAs were digested to completion with the restriction endonuclease EcoRI, separated by electrophoresis on 0.8% agarose gels, and transferred to nitrocellulose (Southern 1975). Prehybridization and hybridization were performed in 4X SSC, 5% dextran sulfate at 68°C (Kunkel et al. 1982; Wahl et al. 1979). Filters were washed in 0.1X SSC, 0.1% sodium dodecyl sulfate (SDS) at 60°C. Autoradiograms were exposed with an intensifying screen at −80°C for 2–5 days.

3. Hybridization probes

a) Leu-2 cDNA and genomic probes. The Leu-2 cDNA probe, a 1.7 kb cDNA fragment, is about ⅔ of the full length cDNA (2.5 kb) and corresponds to the 3′ end of the clone. Although the cDNA clone does not contain an EcoRI site, the portion of the genomic sequence which it spans contains a single EcoRI site, presumably in an intron. The genomic probe for Leu-2 used in this study was a HindIII-SphI 0.9 kb fragment that maps immediately 5′ to the cDNA clone.

b) Immunoglobulin kappa probe. The human immunoglobulin kappa constant region probe was a 2.5 kb EcoRI fragment from

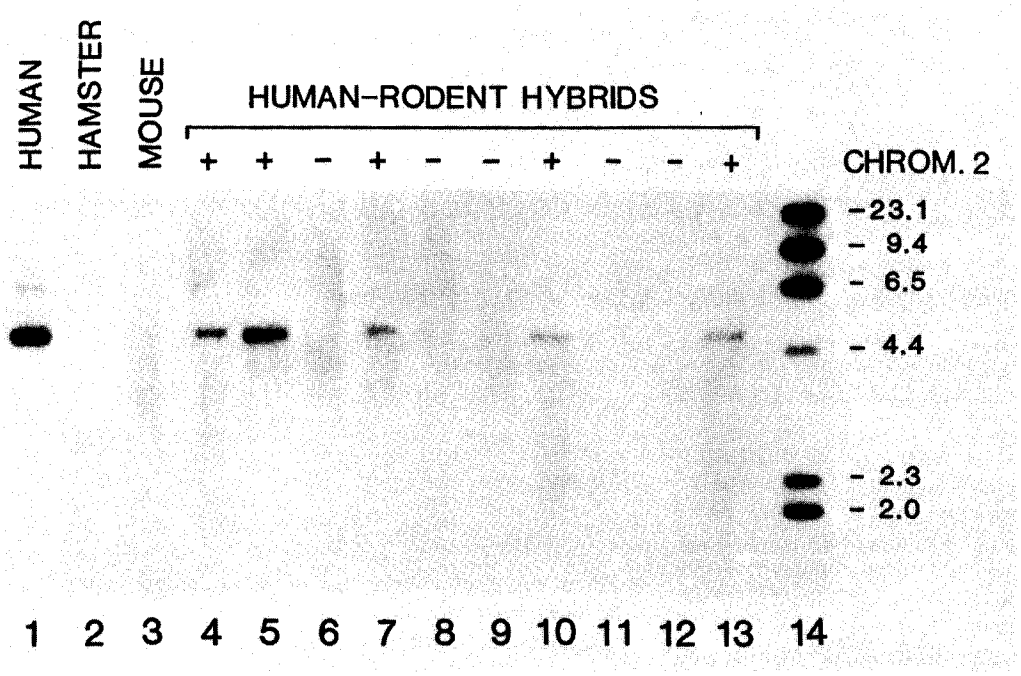

Fig. 1. Hybridization of the Leu-2 cDNA probe with EcoRI digested DNAs from human-rodent hybrids. The DNAs are from: *(1)* HeLa cells; *(2)* Chinese hamster E36 cells; *(3)* mouse RAG cells; *(4–13)* human-hamster and human-mouse hybrids; and *(14)* a HindIII digest of lambda DNA labeled by means of T4 DNA polymerase and dCTP. The chromosome 2 complement of the hybrids is indicated above each lane. The additional ~ 6 kb component present in HeLa DNA was also shown to segregate with chromosome 2 in these hybrids with a genomic probe specific for this sequence (not shown)

Table 1. Segregation of human chromosomes with Leu-2 in human-rodent hybrids[a]

Chromosome	Enzyme markers	Probe/chromosome[b]				
		Concordant		Discordant		Discordancy (percent)
		+/+	−/−	+/−	−/+	
1	PGM1, PEPC	3	12	2	5	32
2	MDH1, IDH1	5	19	0	0	0
3	GLB1, GPX1	4	11	1	7	35
4	PGM2, ALB	3	9	2	9	48
5	HEXB	2	11	3	8	46
6	ME1, SOD2	4	10	1	7	36
7	GUS, MDH2	4	13	1	6	29
8	GSR	3	14	2	5	29
9	AK1, ACO1	1	12	4	5	41
10	GOT1	5	9	0	10	42
11	LDHA, APOAI/CIII	3	12	2	6	35
12	LDHB, GAPD, PEPB	3	9	2	8	45
13	ESD	1	9	3	8	52
14	NP	4	8	1	11	50
15	HEXA, MPI	3	14	2	5	29
16	APRT	4	12	1	7	33
17	GALK	2	12	1	4	26
18	PEPA	4	11	0	6	29
19,19q+[c]	PHI, MANB	3	3	1	16	74
20	ADA	3	8	2	10	52
21	SOD1	3	8	2	11	54
22	ACO2	2	9	3	10	54
X,Xq−[c]	G6PD, PGK	2	8	3	9	55
Y		1	18	4	1	21

[a] The segregation of the 4.5 kb component recognized by the Leu-2 cDNA probe is tabulated

[b] Clones in which a chromosome was present in less than 15% of metaphases, the characteristic isozyme was weakly expressed, or a chromosome-specific DNA probe exhibited a weak hybridization signal were excluded from analysis for that chromosome. Clones with a rearranged chromosome were similarly excluded from the analysis

[c] The chromosome 19 column represents the intact 19 and the two different 19q+ translocation chromosomes. Likewise, the X column represents the intact X and the two Xq−-derivative chromosomes

Table 2. Leu-2 and IGK segregation in hybrids with rearrangements of chromosome 2

Hybrid cell line	Chromosome 2 loci[a]						
	IDH1	MDH1	Probe 10	Probe 6	N-myc	IGKC	Leu-2
G35A5	−	+	+	+	+	+	+
G35D3	−	+	+	−	−	+	+
G24A4	−	+	+	+	+	−	−
R5-3	+	−	−	−	−	−	−

[a] MDH1 and IDH1 were analyzed by isozyme electrophoresis whereas probe 10, probe 6, N-myc, IGKC and Leu-2 were determined by nucleic acid hybridization techniques. None of the four cell lines had an intact chromosome 2 by cytogenetic analysis (Bruns et al. 1979). G35D3 appears to have a complex rearrangement of chromosome 2 with both a terminal and interstitial deletion of 2p

the 12.5 kb BamHI genomic fragment described by Hieter et al. (1980) that had been subcloned into pBR322. The 12.5 kb genomic fragment was generously provided by Dr. P. Leder.

c) Cloned DNA sequences, all localized to chromosome 2p, both by hybrid cell analysis and by in situ hybridization (Kanda et al. 1983; Kohl et al. 1983; Shiloh et al. 1985). Probes 6 and 10 were isolated from a library enriched for sequences from the homogeneously staining region (HSR) of IMR-32 neuroblastoma cells (Kanda et al. 1983). Both sequences, 1.85 and 1.65 kb respectively, had been subcloned in the HindIII site of pBR322. The N-myc probe, a 2.1 kb fragment inserted into the EcoRI site of pBR325, was originally identified and cloned in lambda phage Charon 16A by virtue of its homology with the v-myc gene (Kohl et al. 1983).

Results

The Leu-2 cDNA clone hybridized with two DNA fragments in EcoRI digested human DNAs: a major 4.5 kb component and a less intense 6 kb component (Fig. 1). No cross-hybridization between the Leu-2 cDNA and mouse or hamster DNA was observed. Only the 4.5 kb human component was easily identified in DNAs from a series of human-rodent hybrids when the Leu-2 cDNA clone was nick translated and used as a probe (Fig. 1). This component segregated solely with chromosome 2 in 24 primary human-hamster and human-mouse hybrids that had no rearrangement of this chromosome (Table 1). The pattern of hybridization of the 4.5 kb component in these hybrid DNAs was discordant with the segregation of the other autosomes and of the sex chromosomes (discordancy fractions 0.26–0.74) (Table 1). Chromosome 2 segregation in the hybrids was determined by cytogenetic techniques and by analysis of malate dehydrogenase-1 (MDH-1) and isocitrate dehydrogenase-1 (IDH-1) isozymes (Bootsma and Kidd 1984; Bruns et al. 1979). In addition, the DNAs from the hybrid cell lines had been used to assign a number of DNA sequences amplified in the neuroblastoma cell line, IMR-32, to chromosome 2 (Kanda et al. 1983; Kohl et al. 1983); assignments which were confirmed by in situ hybridization (Kanda et al. 1983; Shiloh et al. 1985).

The less intense 6 kb component identified by the Leu-2 cDNA clone was analyzed in a subset of the hybrids of Table 1 and in four hybrid cell lines with rearrangements of chromosome 2 using a specific genomic probe for this component. The probe was a 0.9 kb HindIII-SphI fragment of the genomic Leu-2 clone from a region 5′ to the cDNA clone. Hybridization of the 6 kb component was concordant with that of the 4.5 kb major human component in all 14 hybrids analyzed (seven clones positive for both components; seven clones negative for both components, data not shown). Segregation of the 6 kb component was also independent of that of the other human autosomes and of the sex chromosomes in the hybrids analyzed (discordancy fractions 0.21–0.80). These observations suggest that the Leu-2 locus is on human chromosome 2.

To assess the relationship of the Leu-2 locus to other regionalized chromosome 2 loci, four hybrid clones with spontaneous rearrangements of chromosome 2 were analyzed for hybridization with the Leu-2 cDNA clone; with three chromosome 2p probes for sequences amplified in a neuroblastoma cell line and with a genomic probe for the constant region of the human immunoglobulin kappa locus (IgGKC) (Table 2). The IGK locus has been regionalized to the proximal short arm of chromosome 2 at band p12 (Rabbitts 1983) whereas the MDH-1 locus is more distal at band p23 (Bootsma and Kidd 1984). N-myc has been localized to 2p23–24 (Schwab et al. 1984); probe 6 to 2p15–16 (Kanda et al. 1983; Shiloh et al. 1985); and probe 10 to 2pcen–p13 (Kanda et al. 1983; Shiloh et al. 1985). The IDH-1 gene is on the distal long arm of the chromosome (q23–qter) (Bootsma and Kidd 1984). In all hybrids, hybridization of Leu-2 was concordant with that of IgKC and discordant with each of the other chromosome 2 loci in at least one cell line (Table 2).

Discussion

The data derived from the human-hamster and human-mouse somatic cell hybrids analyzed in this study indicate that the locus specifying the T lymphocyte cell surface antigen Leu-2 is on human chromosome 2. The locus of the mouse T cell surface antigen, Lyt-2, previously postulated to be the homologue of human Leu-2 (Ledbetter et al. 1981), is closely linked to the IgK locus and to the locus for a related T cell surface antigen, Lyt-3 (Gottlieb 1974), on mouse chromosome 6. The frequency of recombination between the Lyt-2,3 locus and IgK is estimated at 0.3% (Gibson et al. 1983). The segregation of Leu-2 with the IgK locus in four independent hybrid clones with spontaneous rearrangements of chromosome 2 in this study suggests that the Leu-2,IgK linkage group has likely been conserved in the human genome.

To establish structural homology between two genes, either amino acid or nucleotide sequence comparisons must be made. The gene coding for Lyt-2 has recently been cloned by screening a mouse genomic library with the Leu-2 cDNA

clone used in the present study. The genomic clone has been shown to code for Lyt-2 by transfection. Nucleotide sequence and predicted amino acid comparison confirms that Leu-2 and Lyt-2 are homologous (Nakauchi et al. 1985).

Note: A similar assignment of Leu-2 was published after this paper was submitted (Sukhatme et al. 1985).

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References

Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF (1984) Leucocyte typing: Human leucocyte differentiation antigens detected by monoclonal antibodies. Springer-Verlag, Berlin

Bootsma D, Kidd KK (1984) Report of the committee on the genetic constitution of chromosomes 2, 3, 4 and 5. Cytogenet Cell Genet 37:22–46

Brook JD, Shaw DJ, Meredith L, Bruns GAP, Harper PS (1984) Localization of genetic markers and orientation of the linkage group on chromosome 19. Hum Genet 68:282–285

Bruns GAP, Leary AC, Eisenman RE, Bazinet CW, Regina VM, Gerald PS (1978) Expression of ACONs and GALT in man-rodent somatic cell hybrids. Cytogenet Cell Genet 22:172–176

Bruns GAP, Mintz BJ, Leary AC, Regina VM, Gerald PS (1979) Human lysosomal genes: Arylsulfatase A and beta-galactosidase. Biochem Genet 17:1031–1059

Bruns GAP, Karathanasis SK, Breslow JL (1984) The human apolipoprotein AI-CIII complex is located on chromosome 11. Atherosclerosis 4:97–102

Evans RL, Wall DW, Patsoucas CD, Siegal FP, Fikrig SM, Testa CM, Good RA (1981) Thymus-dependent membrane antigens in man: Inhibition of cell-mediated lympholysis by monoclonal antibodies to T_{H2} antigen. Proc Natl Acad Sci USA 78:544–588

Gibson DM, Maclean SJ, Cherry M (1983) Recombination between kappa chain genetic markers and the Lyt-3 locus. Immunogenetics 18:11–116

Gottlieb PD (1974) Genetic correlation of a mouse light chain variable region marker with a thymocyte surface antigen. J Exp Med 140:1432–1437

Hieter PA, Max EE, Seidman JG, Maizel JV, Leder P (1980) Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments. Cell 22:197–207

Kanda N, Schreck R, Alt F, Bruns G, Baltimore D, Latt SA (1983) Isolation of amplified DNA sequences from IMR-32 human neuroblastoma cells: Facilitation by fluorescence-activated flow sorting of metaphase chromosomes. Proc Natl Acad Sci USA 80:4069–4073

Kavathas P, Sukhatme VP, Herzenberg LA, Parnes JR (1984) Molecular cloning of the gene coding for the human T lymphocyte differentiation antigen Leu-2. Proc Natl Acad Sci USA 81:7688–7692

Kohl NE, Kanda N, Schreck RR, Bruns G, Latt SA, Gilbert F, Alt FW (1983) Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell 35:359–367

Kunkel LM, Tantravahi U, Eisenhard M, Latt SA (1982) Regional localization on the human X of DNA segments cloned from flow sorted chromosomes. Nucleic Acids Res 10:1557–1578

Kurnit DM, Neve RL, Morton CC, Bruns GAP, Ma NSF, Cox D, Klinger HP (1984) Recent evolution of DNA sequence homology in the pericentromeric regions of human acrocentric chromosomes. Cytogenet Cell Genet 38:99–105

Landgren U, Ramstedt U, Axberg I, Ullberg M, Kondal M, Wigzell H (1982) Selective inhibition of human T cell cytotoxicity at levels of target recognition or initiation of lysis by monoclonal OKT3 and Leu-2a antibodies. J Exp Med 155:1579–1584

Latt SA, Willard HF, Gerald PS (1976) BrdU-33258 Hoechst analysis of DNA replication in human lymphocytes with supernumerary or structurally abnormal X chromosomes. Chromosoma 57:135–153

Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA (1981) Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. J Exp Med 153:310–323

MacDonald RH, Glasebrook AL, Bron C, Kelso A, Cerottini J-C (1982) Clonal heterogeneity in the functional requirement for Lyt-2/3 molecules on cytolytic T lymphocytes (CTL): Possible implications for the affinity of CTL antigen receptors. Immunol Rev 6:89–115

Malisson B, Rebai N, Mawas C (1982) Inhibition of human T cell mediated cytolysis by monoclonal antibodies to effector cell surface structures. Adv Exp Med Biol 146:563–573

Nakauchi H, Nolan G, Hsu C, Huang H, Kavathas P, Herzenberg LA (1985) Molecular cloning of Lyt-2, a membrane glycoprotein marking a subset of mouse T lymphocytes: Molecular homology to its human counterpart. Leu-2/T8, and to immunoglobulin variable regions. Proc Natl Acad Sci USA (in press)

Nakayama E, Dippol W, Shiku H, Oettgen HF, Old LJ (1980) Alloantigen-induced T-cell proliferation: Lyt phenotype of responding cells and blocking of proliferation by Lyt antisera. Proc Natl Acad Sci USA 77:2890–2894

Rabbitts TH (1983) The human immunoglobulin genes. Biochem Soc Trans 11:119–126

Reinherz EL, Meuer S, Fitzgerald KA, Hussey RE, Levine H, Schlossman SF (1982) Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. Cell 30:735–743

Schwab M, Varmus HE, Bishop JM, Grzeschik K-H, Naylor SL, Sakaguchi AY, Brodeur G, Trent J (1984) Chromosome localization in normal human cells and neuroblastomas of a gene related to c-myc. Nature 306:245–248

Shiloh Y, Brodeur G, Schreck R, Donlon T, Sakai K, Korf B, Shipley J, Bruns G, Seeger R, Latt (1985) Differential amplification of a specific domain of chromosome 2 in untreated neuroblastoma tissues. Proc Natl Acad Sci USA 82:3761–3765

Shinohara N, Sachs DH (1979) Mouse alloantibodies capable of blocking cytotoxic T-cell function. J Exp Med 140:1432–1437

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Sukhatme VP, Vollmer AC, Erickson J, Isobe M, Croce C, Parnes JR (1985) Gene for the human T cell differentiation antigen Leu-2/T8 is closely linked to the light chain locus on chromosome 2. J Exp Med 161:429–434

Wahl GM, Stern M, Stark GR (1979) Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc Natl Acad Sci USA 76:3683–3687

Whitehead AS, Bruns GAP, Markham AP, Colten HR, Woods DR (1983) Isolation of human C reactive protein cDNA and localization of the gene to chromosome 1. Science 221:69–71

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