Localization of the Gene Encoding the Human Interleukin-2 Receptor on Chromosome 10

Abstract. *The human interleukin-2 receptor is an inducible growth factor receptor present on the surface of activated T lymphocytes. The receptor is required for a normal T-cell immune response. High-resolution fluorescence-activated chromosome sorting and DNA spot-blot analysis with complementary DNA's for the interleukin-2 receptor indicated that the receptor gene was located on chromosome 9, 10, 11, or 12. In situ hybridization studies showed that the interleukin-2 receptor gene is on the short arm of chromosome 10, p14 → 15.*

Interleukin-2 (IL-2, also referred to as T-cell growth factor) is a lymphokine synthesized and secreted by T cells after they are activated with an antigen or mitogen that permits long-term growth of human lymphoid cells in vitro (*1*). Interleukin-2 has been biochemically characterized (*2*), molecularly cloned (*3*), and identified as being encoded by a single gene on human chromosome 4 (*4*). The entire DNA sequence of the IL-2 gene has been determined (*5*).

Interleukin-2 acts by binding to specific high-affinity membrane receptors present on the surface of activated T cells (*6*) and may also in some circumstances have biological effects on B cells (*7*). Receptors for IL-2, which are absent on resting T cells, are expressed after cellular stimulation with antigen or lectin. Under appropriate conditions, IL-2 itself can upregulate the expression of IL-2 receptors (*8*). These receptors have been biochemically characterized (*9*) and are present in large numbers on leukemic cells infected with type I human T-cell lymphotrophic virus (HTLV-I) (*10*). Complementary DNA's (cDNA's) for this receptor have been isolated and sequenced (*11, 12*), and both alternate polyadenylation and messenger RNA splicing events have been described (*11*). The finding of relatively simple hybridization patterns on genomic Southern blots suggested that the human IL-2 receptor was encoded by a single structural gene (*11*). We have now localized this gene to chromosome 10p14 → 15.

The 24 human chromosome types were sorted into 21 fractions according to the complementary Hoechst and chromomycin fluorescent emission (*13*). Filter-bound chromosomal DNA was denatured and hybridized to ^{32}P-labeled IL-2 receptor cDNA's prepared by the random priming method (*14*). We used IL-2 receptor cDNA's pIL2R2 and pIL2R4 in these studies. The pIL2R2 cDNA contains an insert corresponding to bases 1 to 937 of the published sequence for pIL2R3 (*11*); pIL2R4 is a full-length cDNA except that it lacks a 216–base pair segment in the middle of the protein-coding region (*11*). A gene-specific hybridization signal was obtained with the filter-bound DNA from chromosomes 9, 10, 11, and 12, indicating that the IL-2 receptor gene was on one of these chromosomes (Fig. 1). Fluorescent sorting technology does not separate these four Hoechst-chromomycin–stained chromosomes.

In situ hybridization studies (conducted by T.A.D.) with ^{3}H-labeled IL-2 receptor cDNA's were used to determine the specific location of the receptor gene. When these studies were performed, the results of the DNA spot-blot analysis had not yet been obtained. The pIL2R2 and pIL2R4 cDNA's were labeled by nick translation to specific activities of 1.9×10^7 and 2.0×10^7 cpm/μg, respectively, with the ^{3}H-labeled nucleotides deoxyadenosine triphosphate (51.9 Ci/mmol), deoxycytidine triphosphate (61.1 Ci/mmol), and thymidine triphosphate (103.3 Ci/mmol) (all from New England Nuclear). Peripheral blood lymphocytes from normal males were cultured in RPMI 1640 medium (Gibco) supplemented with 10 percent fetal bovine serum and synchronized in the cell cycle by the method of Yunis and Chandler (*15*). The hybridization technique was as described by Harper and Saunders (*16*), except that a fluorescent R-band procedure was used to stain the hybridized chromosome preparations after autoradiography (*17*). This modification permitted consistent, unequivocal chromosome identification through the use of simultaneous fluorescent and transmitted illumination. The hybridizations were performed at 42°C for 14 hours, with nick-translated IL-2 receptor DNA at a final concentration of 0.02 ng/μl. The autoradiographs were exposed at 4°C for 9 days and developed in Kodak Dektol. The preparations were analyzed, then destained in methanol and G-band–stained with Wright's stain (*15*) to show the location of the silver grains.

The IL-2 receptor cDNA's pIL2R2 and pIL2R4 contain overlapping sequences and gave virtually identical results when used as probes. The major site of hybridization was to chromosome 10 at bands p14 and p15. In 56 of 300 cells, the label was associated with 10p14 → 15, and 57 of the 520 grains were found at 10p14 → 15. In Fig. 2, both chromosomes 10 were labeled over this region, which was the strongest site of hybridization.

Figure 3 shows the intensity of hybridization to 10p14 → 15 relative to the rest of the karyotype. These data permit assignment of the IL-2 receptor to this locus. Five other loci (3p24 → pter, 12q24 → qter, 7p15 → p21, 7q32 → q34, 11p15 → pter) showed lower but statistically significant degrees of hybridization to the IL-2 receptor cDNA. These secondary sites of hybridization include three regions to which oncogenes have been mapped (*RAF1* at 3p25, Harvey *ras* at 11p15, and Kirsten *ras* at 12q24 → ter) (*18*). The enhanced autoradiographic signal in these chromosomal regions may be due at least in part to (i) possible active transcription of these regions in stimulated lymphocytes, which would result in greater accessibility of the probe and

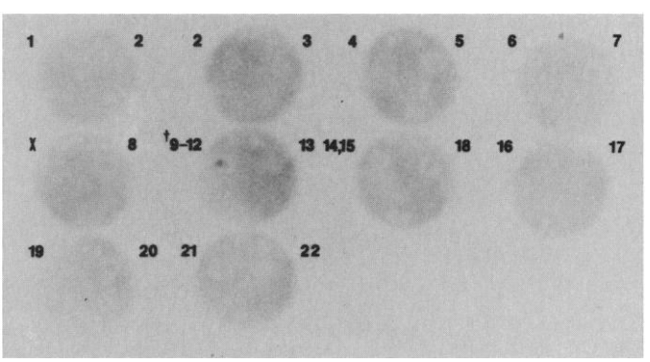

Fig. 1. Chromosome suspensions were prepared from a lymphocyte cell line in tris-spermine buffer and stained with the Hoechst - chromomycin A3 stain pair (*13*). A dual-laser custom FACS IV chromosome sorter (*24*) was used to sort 30,000 chromosomes of each type directly onto a single spot of a nitrocellulose filter paper. The filter-bound chromosomal DNA was denatured, neutralized, prehybridized, and hybridized in 10 percent dextran sulfate (*13*) for 18 hours to IL-2 receptor cDNA's labeled with ^{32}P by the "random priming" method (*14*). After excess probe was removed by washing, a specific hybridization signal was detected autoradiographically over the area of the nitrocellulose filter onto which DNA from chromosomes 9 to 12 was spotted (denoted by dagger).

therefore greater nonspecific in situ hybridization or (ii) the possible occurrence of DNA sequences homologous to the IL-2 receptor cDNA in these regions in addition to the oncogene sequences, which are not homologous to the IL-2 receptor.

No oncogenes, growth factors, or growth factor receptors have previously been localized to chromosome 10. Other key T-lymphocyte membrane proteins such as the β chain of the antigen receptor and proteins of the major histocompatibility complex are located on different chromosomes [chromosomes 7 and 6, respectively (*19, 20*)] and thus are not linked to the IL-2 receptor gene. Chromosome 10 contains the hexokinase locus, which when absent leads to a hemolytic anemia (*21*). Thus, a polymorphism for the IL-2 receptor locus, when identified, might be useful to test for segregation frequency in families with hexokinase deficiency. A polymorphic IL-2 receptor can also serve as a known mapped marker to test for segregation with other unmapped disease genes in studies analogous to those of the Huntington's disease locus, which was subsequently mapped to chromosome 4 (*22*). Polymorphic sites of the IL-2 receptor gene that exhibit tight linkage with genetic disease loci could be useful in prenatal diagnosis.

The short arm of chromosome 10, although apparently not involved in translocations in T-cell lymphomas, was identified in a translocation t(10:11) (p15;q23.3) in an acute nonlymphocytic lymphoma (*23*). Whether these neoplastic cells displayed IL-2 receptors is unknown. However, augmented IL-2 receptor expression is a hallmark of HTLV-I–induced adult T-cell leukemia (*10*). Since no uniform karyotypic abnormality of chromosome 10 has been found in this leukemia, the deregulated IL-2 receptor expression does not appear to be due to a chromosomal translocation involving the IL-2 receptor gene.

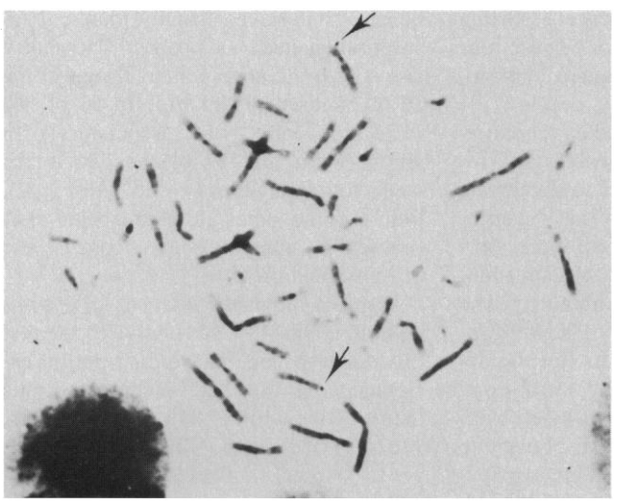

Fig. 2. Peripheral blood lymphocytes from normal males were activated with phytohemagglutinin. The chromosomes were prepared, hybridized to nick-translated pIL2R4, and autoradiographed for 9 days. Both chromosome 10's are labeled at band p15 (indicated by arrows).

Fig. 3. Histogram indicating the location and number of grains from experiments with either pIL2R2 or pIL2R4. The distributions observed with the two probes were identical and therefore the data have been combined. Of 300 cells, 18.7 percent had label over 10p14 → 15. Of 520 total grains, 11 percent were found at this site.

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Malignant Transformation of Erythroid Cells in Vivo by Introduction of a Nonreplicating Retrovirus Vector

Abstract. *DNA from a replication-defective spleen focus-forming virus (SFFV) was reconstructed and transfected into ψ-2 cells containing a packaging-defective mutant of Moloney murine leukemia virus. Replication-incompetent retrovirus particles (helper virus–free) containing genomes that express the transforming envelope gene of SFFV (gp52) transformed bone marrow cells in vitro and, after direct intravenous introduction of the vector, induced malignant erythroid disease in vivo. Disease induction was dependent on prior treatment of mice with phenylhydrazine, which probably increased the availability of erythroid target cells. Since there was no evidence of virus particle expression in mice with malignant disease, this study demonstrates the acute oncogenic potential of a limited number of erythroid cells expressing SFFV gp52. Direct inoculation of animals with nonreplicating retroviral vectors containing transforming genes may be useful in studying the oncogenic effects of such genes.*

Retroviruses can transfer their own genes into mammalian cells and can also be used to transfer genes that have been artificially inserted into them by genetic engineering. The successful use of retroviruses as vectors for the introduction of genes into hematopoietic cells has been demonstrated (*1–3*). With the development of helper cell lines that provide viral particles containing only the minimum genetic information required for integration and expression, genes can be directly introduced into recipient cells without the presence of replication-competent helper virus (*4, 5*). We used a helper cell line to produce viral particle preparations containing only the genome of the spleen focus-forming virus (SFFV), a replication-defective retrovirus, which—in the presence of replication-competent virus—causes an acute erythroleukemia (*6*). Our results show that helper cell lines can facilitate studies of transforming genes, as in the case of SFFV, by delivering them directly to cells in vitro or in vivo in the absence of a spreading infection.

The SFFV expresses a pathogenic 52-kilodalton (kD) glycoprotein encoded by a retroviral envelope gene (*env*) that is highly altered from its putative parent gene in Friend murine leukemia virus (F-MuLV) as a result of deletion, insertion, and recombination (*7*). This gene is essential for the acute erythroleukemia induced by this virus (*6, 8*), but it is unknown whether the replicating helper virus used to rescue the defective SFFV from cells plays a role in the disease process—either directly through expression of its own genes or indirectly through its ability to cause a systemic infection and, therefore, continual infection of new erythroid progenitor cells. To answer this question, we reconstructed SFFV DNA so that it could be efficiently expressed in helper cell lines and be incorporated into particles. Preparations derived this way were tested for their ability to induce transformation of bone marrow cells in vitro and to cause an erythroleukemia by direct inoculation in vivo.

DNA fragments derived from permuted clones of SFFV were used to prepare structures analogous to integrated proviruses that have a long terminal repeat (LTR) at each end (see Fig. 1). We prepared two constructs ($SFFV_A$-L and $SFFV_{AP}$-L) that would have different phenotypic characteristics in vitro—one that would induce abnormal erythroid proliferation and differentiation in the absence of exogenous erythropoietin (Epo) (analogous to the $SFFV_P$ strain), and one that would require Epo (analogous to the $SFFV_A$ strain) (*6*). The $SFFV_A$-L construct is entirely composed of DNA fragments derived from $SFFV_A$ (*9*). The 5′ end consists of a Cla I–Eco RI fragment from the full-size genomic clone (Cla I–Cla I), which has two internal LTR's (Fig. 1A); the 3′ end of the envelope gene and the 3′ LTR consist of Eco RI–Kpn I [1200 base pairs (bp)] and Kpn I–Bam HI (200 bp) fragments derived from a subclone of the same virus. The extra 5′ LTR was not removed during construction since it should be eliminated on formation of viral RNA; the message presumably initiates at the second 5′ LTR and terminates at a polyadenylation site in the 3′ LTR. The recombinant $SFFV_{AP}$-L is identical except that the Eco RI–Kpn I fragment is derived from $SFFV_P$. This Eco RI–Kpn I fragment was chosen for the recombinant virus because it has been suggested that sequences in the 3′ end of the envelope gene may be responsible for the phenotypic differences between $SFFV_P$ and $SFFV_A$ (*10*). When the structures were checked by restriction analysis and Southern blotting (see Fig. 1B), both were found to produce three similar LTR-hybridizing, Kpn I fragments, confirming the presence of three LTR's. The derivation of the Eco RI–Kpn I fragments in each of the constructions could be shown by the presence or absence of a unique Cla I site in the 3′ region of the virus; while the $SFFV_A$-L DNA was seen as a single band at 8.7 kilobases (kb) after Cla I digestion, the $SFFV_{AP}$-L remained supercoiled (Fig. 1B). Further double digestions and hybridization with a 5′ envelope probe (Fig. 1B) confirmed the structures depicted in Fig. 1A.

The phenotypic characterization in vitro and in vivo of helper virus–containing stocks of the constructed viruses has been carried out and will be the subject of another report. Briefly, $SFFV_A$-L (with F-MuLV) behaves in vitro like the original $SFFV_A$ (with F-MuLV) (*8*), inducing transformation of erythroid cells in the presence, but not in the absence, of Epo-containing preparations. The

Table 1. Induction of erythroid bursts by helper-free virus. Bone marrow cells from phenylhydrazine-treated mice were incubated for 2 hours at 4°C with the test preparation and then plated in methylcellulose by previously described procedures (*15*). Hemoglobinized (benzidine-positive) erythroid bursts were counted on day 5. Media from fibroblast cultures were tested undiluted.

Test virus	Erythroid bursts per 10^6 cells	
	No Epo added	Epo added (0.2 U/ml)
None	0	0
F-MuLV/$SFFV_P$*	89	177
$SFFV_{AP}$-L (ψ-2)	23	55
$SFFV_A$-L (ψ-2)	0	>200
ψ-2	0	0

*Positive control virus produced by NIH 3T3 cells.