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Human T-Cell Receptor α-Chain Genes: Location on Chromosome 14

Abstract. *The genes encoding the α chain of the human T-cell receptor have been mapped to chromosome 14, the chromosome on which the human immunoglobulin heavy chain locus resides. Thus, genes encoding two different classes of antigen receptor are present on the same chromosome. Furthermore, breaks involving chromosome 14 are frequently seen in tumors of T-cell origin. The potential relation of these chromosome abnormalities to α-chain genes is discussed.*

The major histocompatibility complex (MHC) restricted antigen receptor on T lymphocytes is a disulfide-linked heterodimer and is composed of an α and a β chain (*1*). The complementary DNA (cDNA) clones encoding the β chain have been isolated and described in mice and man (*2*). The β-chain locus consists of several gene segments that encode variable (V), diversity (D), joining (J), and constant (C) regions of the β-chain protein, which is itself related to immunoglobulins. The β chain is encoded on chromosome 7 in man (*3*) and on chromosome 6 in mouse (*4*). The locus for murine immunoglobulin (Ig) kappa light chains is encoded on chromosome 6 as well (*5*), although the evolutionary significance of this linkage is not understood. The α-chain cDNA clones have been isolated and characterized in humans (*6*) and in mice (*7*). The sequence of these α-chain cDNA's suggest that, like the β chain and the Ig's, the α chain is encoded in separate, noncontiguous gene segments (V, J, and C). We have now mapped the genes encoding the α chain and have assigned these genes to human chromosome 14.

We used a series of human-rodent somatic cell hybrids, containing characterizing combinations of human chromosomes (see legend to Fig. 1 for description of hybrids); the isolation and characterization of most of these hybrids have been described (*8*), and those not previously described are discussed in the legend to Fig. 1. Hybrid cells were grown in culture, and DNA was prepared from each hybrid (*9*). The presence or absence of human α-chain genes in each hybrid was determined by probing Southern blots of genomic DNA with a labeled α-chain cDNA probe isolated from the human T-cell tumor, HPB-MLT. This human α-chain cDNA has been characterized by one of us (E.P.) and is described in (*6*).

Analysis of hybrid DNA's with an α-chain cDNA probe by the Southern blot method (Fig. 1) shows three Hind III fragments (2.6, 4.4, and 9.6 kb) in human DNA. A cDNA fragment specific for the V region of this particular α chain hybridizes to the 9.6-kb Hind III fragment, whereas a cDNA fragment specific for the α-chain C region hybridizes to the 2.6- and 4.4-kb Hind III fragments (*10*).

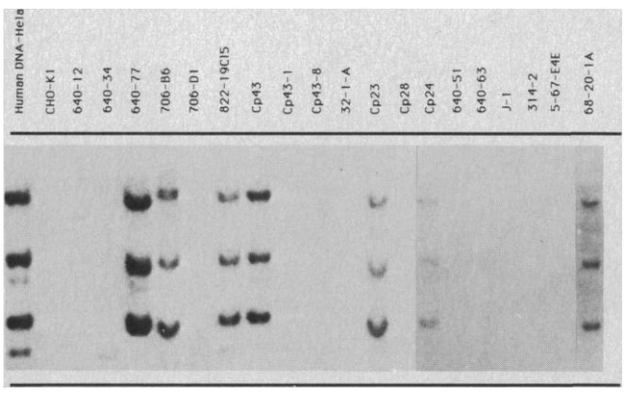

Fig. 1. Southern analysis of human-rodent cell hybrids for human α-chain genes. Hybrid or tumor cell DNA was digested with Hind III and subjected to electrophoresis through 0.7 percent agarose gels. Samples of DNA were blotted and hybridized by the method of Wahl *et al.* (*14*), except that the acid depurination step was omitted and the gel was irradiated with shortwave ultraviolet for 8 minutes prior to denaturation. The blots were hybridized with the 1.3-kb insert of pGA5, a cDNA clone that encodes the α chain from the human T-cell tumor HPB-MLT. This insert contains variable joining and constant region sequences and has been described (*6*). The upper, middle, and lower bands are 9.6, 4.4, and 2.6 kb, respectively. The faint bands seen in human DNA are apparently unrelated to the α-chain locus since they do not segregate with the major bands. They may represent α-chain pseudogenes, which are unlinked to the α-chain locus. The hybrid cell lines in (*8*) were used except for 32-1-A, a human lymphoblast-mouse L-cell hybrid; Cp23, a human fibroblast–Chinese hamster ade$^-$B hybrid; Cp24, a human fibrosarcoma–Chinese hamster ade$^-$B hybrid; 314-2, a human lymphocyte–Chinese hamster uri$^-$C, ade$^-$G hybrid; 706-D1, a human lymphocyte–Chinese hamster ade$^-$F hybrid.

Under the conditions of hybridization and washing used in this study, no detectable hybridization to Chinese hamster DNA (or in the case of hybrid 32-1-A, mouse DNA) is seen. This has simplified the analysis of human α-chain genes in this collection of human-rodent hybrids.

The human chromosomes present in each hybrid as well as the presence or absence of human α-chain DNA are shown in Fig. 2. The presence of human α-chain genes and chromosome 14 are concordant at a frequency of 100 percent. The concordance of α-chain genes with all other human chromosomes is ≤72 percent. Furthermore, three hybrids, each with only two human chromosomes [706-B6 (8, 14), Cp23 (14, 17), and Cp24 (9, 14)] were found to contain α-chain DNA. Chromosome 14 is the only common chromosome. Finally, α-chain DNA is present in the subclone Cp43, which was derived from 706-B6 and contains only chromosome 14, while one of its segregants, Cp43-1, which has lost chromosome 14 lacks α-chain genes. Taken together, these data allow us to assign the α locus to chromosome 14 in man.

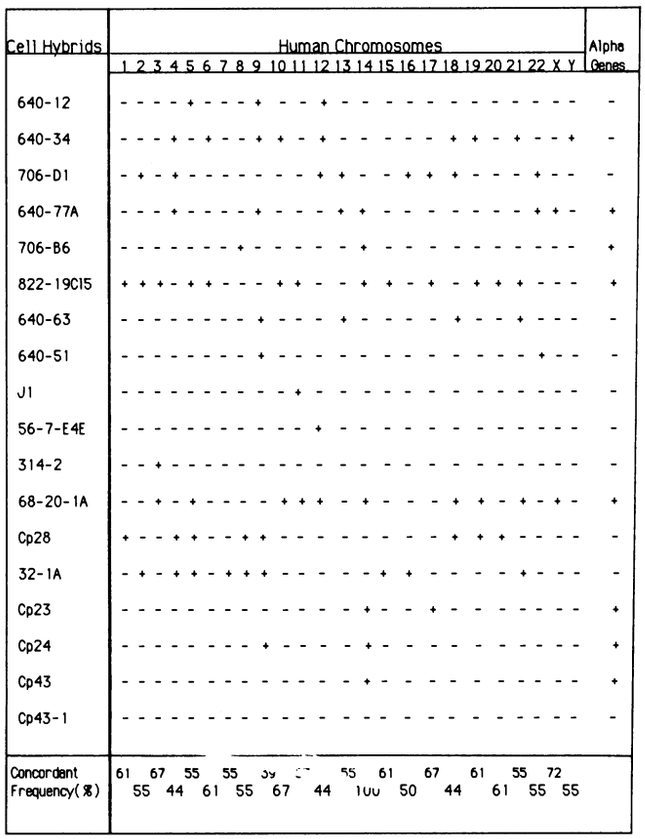

Fig. 2. Human–CHO-K1 cell hybrids for human chromosomal assignment of the T-cell receptor α-chain genes.

For regional mapping of the α-chain locus, we have used a deletion segregant of Cp43, Cp43-8, which contains only the distal half of chromosome 14 including 14q22-qter, translocated to a Chinese hamster chromosome. The data in Fig. 3 show metaphase spreads of hybrids Cp43 and Cp43-8. Since hybrid Cp43-8 lacks both the proximal half of chromosome 14 and α-chain genes, we can provisionally locate the α locus on chromosome 14, proximal to 14q22.

The assignment of the human α-chain locus to chromosome 14 is of interest for two reasons. First, the human Ig heavy (Ig_H) chain genes are also encoded on chromosome 14 (*5*). While the α chain is clearly an Ig-like protein, it is difficult to determine whether it is more homologous to Ig_H or Ig_L (light) chains. The Cys^{206} in the α-chain sequence is postulated to be the site of disulfide linkage to the β chain, and there is some homology in this region to the hinge region of Ig_H chains (*6*). However, other regions of the

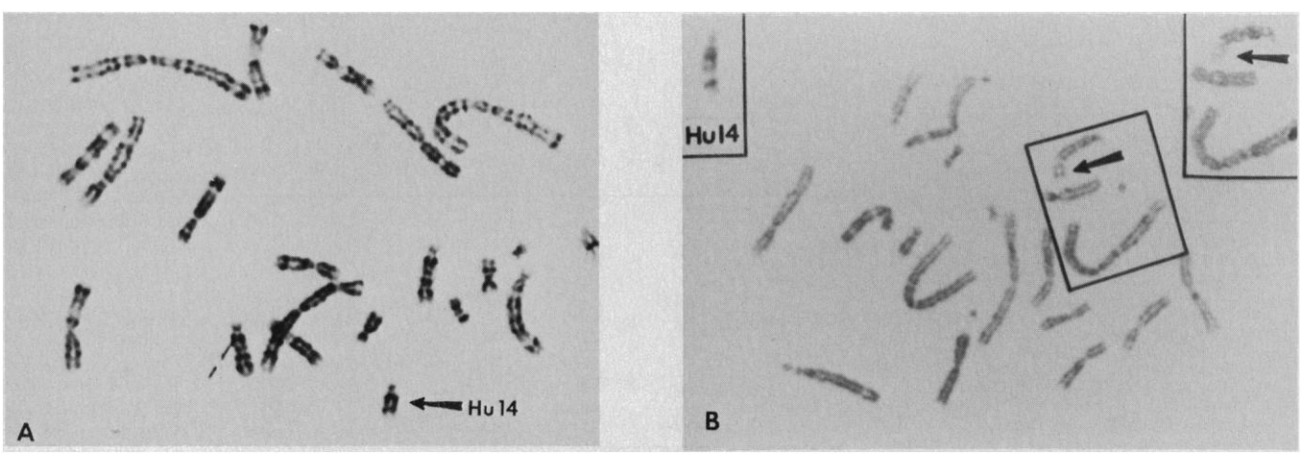

Fig. 3. Metaphase chromosomes of hybrid Cp43 and a deletion segregant, Cp43-8. (A) This metaphase cell of Cp43 shows a single human chromosome 14 (arrow) identified by trypsin banding and by Giemsa-11 differential staining techniques. This hybrid is also positive for human nucleoside phosphorylase (NP), an isozyme marker for human chromosome 14. The human α-chain genes are present in this hybrid. (B) Trypsin-banded metaphase chromosomes of Cp43-8 showing that the distal portion of human chromosome 14 including 14q22-qter was translocated to a hamster chromosome (arrow). Left upper inset shows the single intact human chromosome 14 originally present in the parental line, Cp43. The human material in the translocation chromosome was further confirmed by a sequential staining technique (*15*), as shown in the right upper inset. The translocation chromosome contains a hamster arm and a human arm. In the sequential staining procedure, the metaphase spread was trypsin banded, photographed, destained with a mixture of methanol and acetic acid (3:1), soaked for 1 hour at 60°C, and restained with Giemsa-11. Hamster chromosomes were stained heavily (magenta) and human chromosomes were stained lightly (blue) (see arrow). This deletion hybrid lacks α-chain genes and nucleoside phosphorylase, a marker regionally localized to 14q13.1(5).

α-chain sequence are not more homologous to Ig_H chains than they are to Ig_L chains. Furthermore, our data suggest that α-chain genes are located on chromosome 14 proximal to 14q22. Since Ig_H chain genes have been mapped to 14q32(5), this would place these two loci ≥45 centimorgans (cM) apart. It would be difficult to detect such a relatively weak linkage by classical genetic analysis in that genes 50 cM apart behave as independently assorting elements. Whereas the Ig_H chain and T-cell receptor α-chain loci may be the products of an ancient gene duplication, the actual evolutionary relation between these two loci still needs to be determined. Perhaps examination of these genes in several other species will clarify this point.

The second point of interest regarding chromosome 14 concerns tumors of T-cell origin. Cytogenetic studies have been performed on cells from patients with T-cell malignancies, such as chronic lymphocytic leukemia. T-cell lymphoma, and lymphosarcoma (*11*). Although several chromosomal abnormalities can be seen in the malignant cells derived from these patients, a common feature in many of these T-cell tumors is a break involving chromosome 14. The breakpoint has been located in bands 14q11 → 13 and is associated with chromosome inversions and translocations (*12*). The involvement of bands 14q11 → 13 in these tumors, had led Hecht *et al.* (*12*) to propose that genes relating to T-cell function are encoded at this site. Our data are consistent with the possibility that the human α-chain locus is located in bands 14q11 → 13. Given the precedent of chromosome abnormalities involving Ig genes in B-cell tumors (*13*), there remains the possibility that the chromosome 14 rearrangements seen in some T-cell tumors involve α-chain genes.

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Human T-Cell Clones from Autoimmune Thyroid Glands: Specific Recognition of Autologous Thyroid Cells

Abstract. *The thyroid glands of patients with autoimmune diseases such as Graves' disease and certain forms of goiter contain infiltrating activated T lymphocytes and, unlike cells of normal glands, the epithelial follicular cells strongly express histocompatability antigens of the HLA-DR type. In a study of such autoimmune disorders, the infiltrating T cells from the thyroid glands of two patients with Graves' disease were cloned in mitogen-free interleukin-2 (T-cell growth factor). The clones were expanded and their specificity was tested. Three types of clones were found. One group, of T4 phenotype, specifically recognized autologous thyroid cells. Another, also of T4 phenotype, recognized autologous thyroid or blood cells and thus responded positively in the autologous mixed lymphocyte reaction. Other clones derived from cells that were activated in vivo were of no known specificity. These clones provide a model of a human autoimmune disease and their analysis should clarify mechanisms of pathogenesis and provide clues to abrogating these undesirable immune responses.*

Autoimmune diseases involving the thyroid gland are characterized by a variety of humoral and cell-mediated immunological phenomena (*1*). These lead to either tissue destruction, with consequent inhibition of function as in Hashimoto's thyroiditis (*2*) or primary myxedema (*3*), or to hyperstimulation of hormone secretion and hypertrophy of the gland as in Graves' disease (*4*) and in

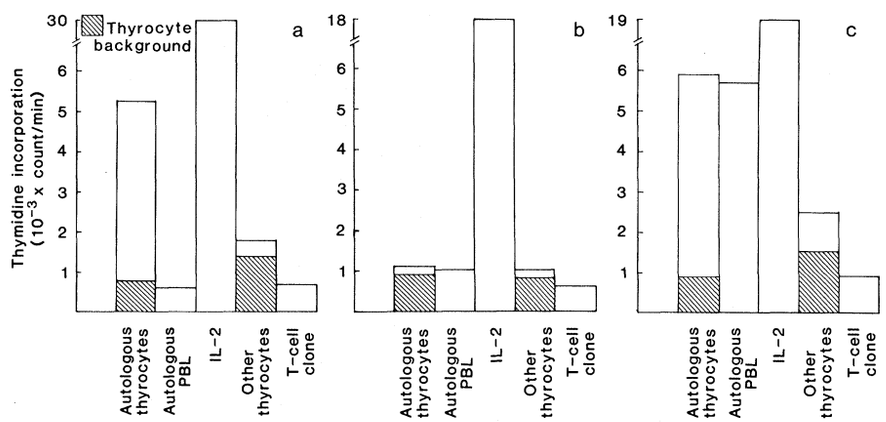

Fig. 1. Proliferative response to one clone from each of the groups. (a) Clone 17 is thyrocyte-specific. Response of T cells alone and to autologous thyrocytes is clearly seen with the thyrocyte background (no T cells, shaded); 10^4 T cells and 10^4 thyrocytes or peripheral blood cells per well, response assayed as in Table 1. (b) Clone 15 does not respond to thyroid or blood cells, only to IL-2. (c) Clone 51 is an autologous MLR clone, responding equally to autologous thyroid (autol thyr) and peripheral blood mononuclear cells (pbmnc).