vaccine would be aided if HTLV-III had the ability to infect and cause disease in a laboratory animal. However, attempts to infect nonhuman primates other than chimpanzees with HTLV-III have generally been unsuccessful (*25*). Because of the endangered status of chimpanzees, their use for this purpose will probably be limited. If STLV-III is indeed pathogenic in macaques, useful approaches to the development and testing of a vaccine for AIDS may emerge.

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A Novel Mechanism of Somatic Rearrangement Predicted by a Human T-Cell Antigen Receptor β-Chain Complementary DNA

Abstract. *The T-cell antigen receptor is a cell surface molecule vital in mediating the cellular immune response. The arrangement and rearrangement of the gene segments encoding the β-chain polypeptide of the receptor are similar to those of immunoglobulin gene segments. The two constant region genes of the human T-cell antigen receptor are 8 kilobases apart with a cluster of joining segments located 5′ of each constant region gene. Although most β-chain gene rearrangements involve the variable, diversity, and joining segments, analysis of a β-chain complementary DNA clone suggests the occasional occurrence of another type of rearrangement.*

The antigen receptor expressed on the surface of T cells allows the specific recognition of a wide variety of antigens. This receptor contains two disulfide-linked polypeptide chains designated α and β (*1–4*). The β-chain genes of both murine and human T-cell receptors are encoded in variable (V), diversity (D), joining (J), and constant (C) region segments in germline DNA (*5–14*). These segments rearrange during somatic development to produce an active gene in a fashion precisely analogous to immunoglobulin heavy-chain gene segments.

Murine D, J, and C gene segments are arranged D-J-C_1-D-J-C_2 in a 15-kilobase

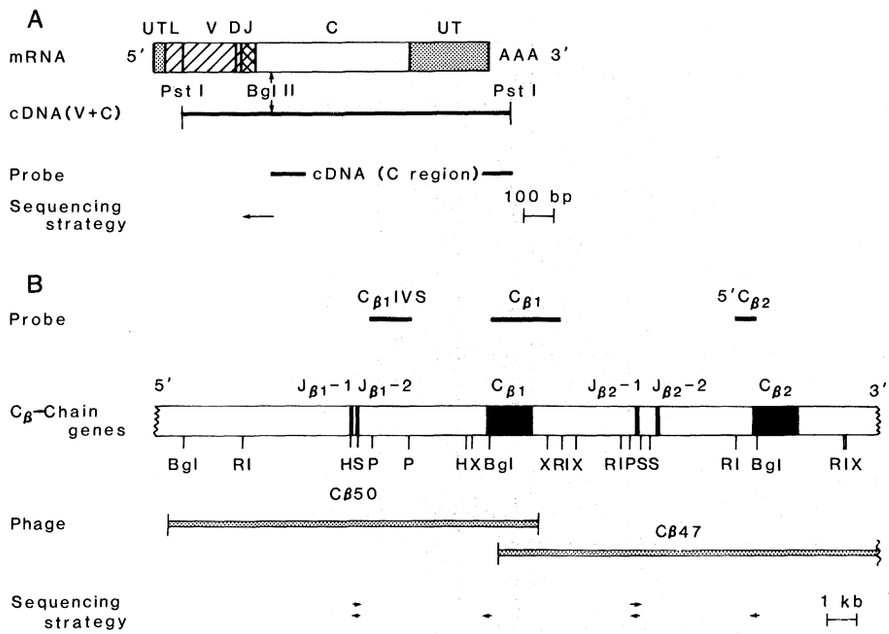

Fig. 1. Organization of the β chain mRNA (A) and constant region genes (B) of the human T-cell antigen receptor. A cDNA library was screened with two 20-base oligonucleotide probes (*21*) constructed to hybridize to the C region of a human β chain cDNA (*14*). The nucleotide sequence pTβ1 [cDNA (V + C)], containing a 1.1-kb nearly full-length β-chain cDNA insert, was isolated. Four J segments have been mapped near the C-region locus; other J segments probably exist close to the $J_{\beta1}$ and $J_{\beta2}$ clusters. The location of Eco RI (RI), Bgl II (Bgl), Hind III (H), Sma I (S), Pvu II (P), and Xba I (X) restriction enzyme sites are indicated. Probes used for analysis of C-region genes are restriction enzyme fragments. Other restriction enzyme fragments were cloned into the single-stranded bacteriophage M13 and their nucleotide sequence determined with the dideoxy chain termination method as described (*22*). The direction and extent of sequencing are indicated by individual arrows.

(kb) region (*10, 11, 13*). As a first approach to the question of how and when human β-chain genes rearrange, we and others (*15*) have cloned the genes encoding the C region of the β chain for the human T-cell antigen receptor and have established their physical map. A near full-length β-chain complementary DNA (cDNA), pTβ1 (Fig. 1A), was isolated from a cDNA library (*16*) that had been made with polyadenylated RNA isolated from REX, a human T-cell tumor line. The nucleotide sequence of pTβ1 is identical to the sequence of the β-chain cDNA from MOLT-3 cells (*14, 17*). A bacteriophage library constructed by cloning human placental DNA into Charon 28 (*18*) was screened with pTβ1 as described (*16*). Two bacteriophage clones, λCβ50 and λCβ47 (Fig. 1B), that hybridized to the cDNA–C-region probe (Fig. 1A), were isolated and used for further analysis.

The physical relationships between the genes for the C regions of the β chains is diagramed in Fig. 1B. Because the inserts in phages λCβ50 and λCβ47 share only about 1.5 kb of human DNA, the overlap between the two pieces of DNA was difficult to assess by comparison of restriction fragment maps. However, subcloned fragments (designated $C_{\beta 1}$ and 5′ $C_{\beta 2}$ in Fig. 1) derived from λCβ47 and λCβ50 hybridized to the same 11-kb Eco RI fragment of human DNA as well as to a 3.7-kb fragment (Fig. 2) (*17*). Furthermore, analyses of restriction enzyme sites and nucleotide sequences of the subcloned fragments is consistent with the conclusion that both bacteriophages contain part or all of the $C_{\beta 1}$ gene and that λCβ47 also contains the $C_{\beta 2}$ segment. The restriction map of the locus for the C region of the β chain thus determined is similar to that described in (*15*).

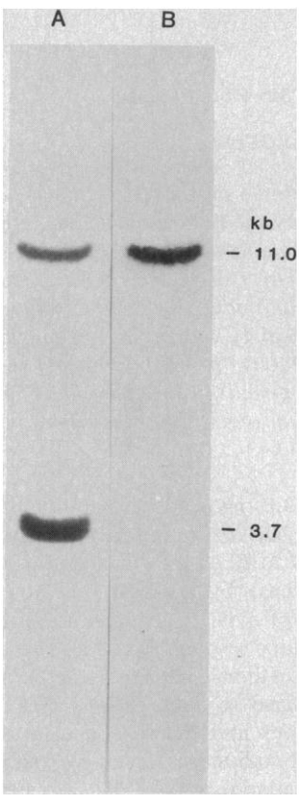

Fig. 2. Identification of C-region gene containing restriction fragments by Southern blot analysis. Human B-cell DNA was digested with Eco RI, fractionated on a 0.9 percent agarose gel, transferred to a nitrocellulose filter (*16*), and hybridized to the 5′ $C_{\beta 2}$ (lane A) and $C_{\beta 1}$ IVS (lane B) probes diagramed in Fig. 1. The sizes of the hybridizing fragments were determined by comigration with DNA fragments of defined length.

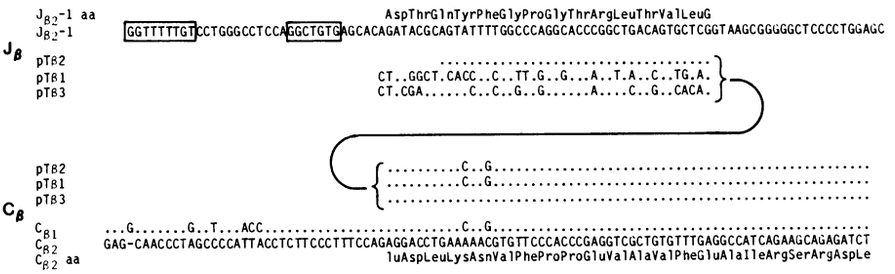

Fig. 3. Nucleotide sequences of a J segment ($J_{\beta 2}$-1) found 5′ of the $C_{\beta 2}$-region gene, three segments found on three β-chain cDNA's (pTβ1, pTβ2, and pTβ3), associated C-region segments, and the corresponding portions of the 5′ ends of the $C_{\beta 1}$ and $C_{\beta 2}$ gene segments. Two plasmids, pTβ2 and pTβ3, were isolated from a human thymus cDNA library; they were constructed by the G-C (G, guanine; C, cytosine) tailing method (*16*). Restriction fragments from pTβ2 and pTβ3 were used to identify the germline J segments, $J_{\beta 2}$-1 and $J_{\beta 2}$-2, respectively (Fig. 1). The 9- and 7-bp sequences that are found 5′ of all J segments are boxed. The deduced amino acid (aa) sequences of $J_{\beta 2}$-1 and $C_{\beta 2}$ are indicated.

There is a cluster of J segments 5′ of each murine C-region gene (*5, 7, 11*). There are at least two J segments 5′ of the human $C_{\beta 1}$ gene (*12*) and the J region found on pTβ1 is derived from this cluster (*12, 17*). The precise location of the J-region cluster between $C_{\beta 1}$ and $C_{\beta 2}$ could be identified by using probes that would specifically hybridize to this cluster.

A cDNA library was constructed with polyadenylated RNA from human thymus. This cDNA library was screened with the C-region cDNA probe (Fig. 1A) and several different cDNA clones were obtained. J-region probes were obtained from Pst I–Bgl II digests of these cDNA clones (Fig. 1A) and were used to probe Southern blots of restriction enzyme digests of λCβ50 and λCβ47 DNA. Plasmids pTβ2 and pTβ3 were selected for further analysis because the J probes derived from these plasmids hybridized to the region between $C_{\beta 1}$ and $C_{\beta 2}$ ($J_{\beta 2}$-1 and $J_{\beta 2}$-2 in Fig. 1B).

The nucleotide sequences of the J segments from pTβ1, pTβ2, and pTβ3 were determined (Fig. 3) so that they could be compared to the nucleotide sequences of the J segments encoded on the bacteriophage λCβ47. The germline sequence

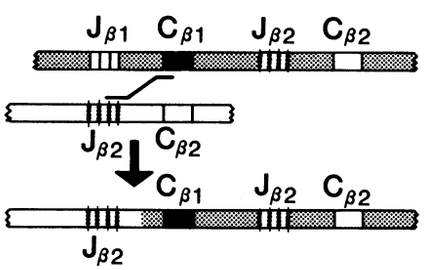

Fig. 4. A model involving sister chromatid exchange to explain the formation of an active gene containing a $J_{\beta 2}$ cluster-derived J segment 5′ of the $C_{\beta 1}$ gene segment.

corresponding to $J_{\beta 2}$-1 and the expressed J segment in pTβ2 are identical, suggesting that there are no polymorphic differences in $J_{\beta 2}$-1 segments of the individual whose DNA was used to make the genomic library and the individual whose thymus was used to make the cDNA library.

The nucleotide sequence of the 5′ C-region segment derived from pTβ2 and that derived from pTβ3 differed by two nucleotides (Fig. 3) as did the corresponding portions of the germline $C_{\beta 1}$ and $C_{\beta 2}$ genes. The nucleotide difference in the coding portions of $C_{\beta 1}$ and $C_{\beta 2}$ resulted in a change from Asn-Lys to Lys-Asn. This observation was unexpected because both plasmids contain J segments derived from the cluster of J segments encoded between $C_{\beta 1}$ and $C_{\beta 2}$. Subsequent nucleotide sequence analysis and restriction enzyme mapping demonstrated that pTβ2 contains the $C_{\beta 1}$ gene segment, whereas pTβ3 contains the $C_{\beta 2}$ segment (*17*).

Comparison of the pTβ2 cDNA with the structure of the locus for the C region of the β chain revealed an unexpected finding; the $J_{\beta 2}$-1 segment is joined to the $C_{\beta 1}$ segment. One explanation for this observation might have been the presence of a segment resembling $J_{\beta 2}$-1 in the $J_{\beta 1}$ cluster; however, the $J_{\beta 2}$-1 probe does not hybridize to the 9 kb of DNA 5′ of $C_{\beta 1}$ (*17*). We demonstrated (Fig. 2) that sequences 5′ of the $C_{\beta 1}$ segment are significantly different from sequences 5′ of $C_{\beta 2}$. Thus, there is only a single sequence like $J_{\beta 2}$-1 in the C-region locus and this segment is encoded 3′ of $C_{\beta 1}$.

Presumably, pTβ2 is encoded by an active gene in which the $J_{\beta 2}$-1 segment is 5′ of the $C_{\beta 1}$ segment. Since this gene structure is not encoded in the germline DNA, we suggest that it is formed during the somatic development of T cells. Perhaps a somatic recombination event involving an unequal crossover occurred during maturation of the cell expressing the pTβ2 messenger RNA (mRNA) and this recombination event placed $J_{\beta 2}$-1 5′ of $C_{\beta 1}$ (Fig. 4). Consistent with this hypothesis is the evidence that T-cell extracts contain an enzymatic activity that is able to promote homologous recombination in vitro (*19*).

The isolation of a β-chain cDNA derived from the $C_{\beta 1}$ segment and the $J_{\beta 2}$ cluster suggests an unexpected mechanism by which any J segment can be associated with either C_β segment. Since the $C_{\beta 1}$- and $C_{\beta 2}$-region genes are highly homologous, differing by six amino acid residues, the biological significance of these new forms of mRNA remains to be determined. Whether β-chain mRNA's utilizing one C-region segment are functional in one type of T cell while the other C_β segment is utilized in another T-cell subset remains an unanswered question. We do not yet know if this hypothesized somatic recombination occurs by a special mechanism or if this type of recombination occurs between all homologous genes during somatic development. We also do not know the frequency at which these unusual mRNA's are made in the thymus. The β-chain cDNA's derived from murine thymus do not have this structure (*7*). Eleven human β-chain cDNA's have been examined (*20*) (Fig. 3) and only one of them has the unexpected structure found in pTβ2. Analysis of active T-cell genes that have been formed by these unusual recombination events should provide some answers to these questions.

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Syrian Hamster Female Protein: Analysis of Female Protein Primary Structure and Gene Expression

Abstract. *The concentration in plasma of the female protein (FP) of the golden Syrian hamster is regulated by sex steroids and by mediators of the acute-phase response to tissue injury or inflammation. A complementary DNA (cDNA) clone corresponding to FP was isolated from a hamster liver cDNA library and used to determine the nucleotide sequence and derived amino acid sequence of native FP. The primary sequence of FP is 69 percent identical to human serum amyloid P component and 50 percent identical to human C-reactive protein. Evidence showed that sex-limited and acute-phase control of the FP gene is pretranslational. The FP protein is thus a useful model for investigating dual regulation of expression of a single gene.*

The plasma of golden Syrian hamsters contains a protein, designated female protein (FP), that is a member of the pentraxin family (*1*). Pentraxins, such as human C-reactive protein (CRP), serum amyloid P component (SAP), and other related proteins, have a characteristic subunit structure and are present in many species, including primitive invertebrates (*2*). Many pentraxins (for example, human CRP and murine SAP) are acute-phase proteins, which means that their concentrations in plasma increase after tissue injury or acute inflammation. The plasma concentration of FP in the Syrian hamster is under dual control; that is, concentrations are altered by sex steroids and by stimuli that elicit an acute-phase response. The concentration in plasma of female Syrian hamsters (0.5 to 3.0 mg/ml) is 100 to 1000 times greater than that in the plasma of normal males (4 to 20 μg/ml). Concentrations of FP increase by a factor of 5 to 10 in plasma of male hamsters during an inflammatory response but decrease by 50 percent in female hamsters after a similar acute-phase stimulus. Resting FP concentrations and the response to acute-phase stimuli are reversed by castration or by administration of sex steroids (*3*).

Complementary DNA (cDNA) clones corresponding to human CRP and SAP have been isolated (*4, 5*). Comparison of the structure and regulation of expression of the FP gene with those of other pentraxin genes would provide an opportunity to study the molecular basis of hormonal and acute-phase regulation of