It is useful to compare our observed distribution with the distribution of other features that provide evidence for former ground ice and ground water on Mars. The most definitive such evidence comes from valley systems, chaotic terrain, outflow channels, and fretted terrain. Valley systems are believed to have formed by sapping and are found only in the ancient cratered terrain (*2*). They are present over a wide range of latitudes and may be weakly concentrated toward equatorial regions. Chaotic terrain and outflow channels are thought to result from melting and rapid release of large amounts of ground ice (*3*), while fretted terrain may result from more gradual release of ground ice by either melting or sublimation (*1*). All are most common at low to middle latitudes. Chaotic and fretted terrain dissect the highland materials; outflow channels, commonly arising from chaotic terrain, debouch onto the lowland plains. Crater age dating shows that outflow channel formation took place after the formation of the ancient highlands but still fairly early in martian history (*18*), while valley systems formed during the very earliest part of the planet's history (*2*). Their distributions are therefore consistent with the concept that the equatorial regions were once rich in water and ice and have subsequently been desiccated.

The origin of the ice in the martian regolith is unclear. The many lines of evidence implying that ice was common in the cratered uplands early in martian history suggest that the ice was emplaced during an early period of intense outgassing. The alternative extreme is continuous outgassing throughout the planet's history at a rate substantially lower than the low-latitude depletion rates in order to keep the low latitudes ice-free. In either case, intense early meteoritic brecciation was probably largely responsible for the apparent capability of the deep regolith to hold large amounts of water. We cannot determine what fraction of ice must be present in the near-surface materials to produce the features we have mapped. Even if only modest fractions (5 to 10 percent by volume) are present, then the data presented here suggest that the total inventory of water at the surface is close to the upper limit of the post-Viking estimates of 10 to 100 m spread evenly over the surface of the planet (*19*).

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Human T-Cell γ Chain Genes: Organization, Diversity, and Rearrangement

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The human T-cell γ chain genes have been characterized in an attempt to better understand their role in immune response. These immunoglobulin-like genes are encoded in the genome in variable, joining, and constant segments. The human γ genes include at least six variable region genes, two joining segments, and two constant-region genes in germline DNA. Variable and joining segments recombine during the development of T cells to form rearranged genes. The diversity of human γ genes produced by this recombinational mechanism is greater than that produced by the murine genome but is more limited than that of other immunoglobulin-like genes.

AN ACTIVE γ GENE IS FORMED BY immunoglobulin-like rearrangement of variable (V) and joining (J) segments that occurs during somatic differentiation of T cells (*1–3*). It has been suggested that the γ chain plays a role as a cell surface receptor because of its similarities to immunoglobulin and T-cell antigen receptor α and β chains (*2–9*). However, the polypeptide chain has not yet been identified and its precise function remains uncertain. One approach to understanding the function of this molecule is to characterize the diversity of expressed γ chains. The mouse expresses a very limited number of γ chains because its genome encodes only three variable region genes. Only one of these participates in the formation of a functional gene (*3*).

There are two γ chain constant regions in the human genome that reside on the short arm of chromosome 7 (*10*). One of these constant (C) region gene segments is deleted from the genome of most human T cells. Here we report that the two C region segments are encoded about 12 kilobases (kb) apart, with at least one J region segment 5′ of each constant region. These J region segments recombine with multiple (at least three) variable region segments, suggesting that the human genome encodes more γ chain diversity than the murine genome.

To determine the structure of the human γ chain polypeptide and to obtain probes to the human V and J region segments we isolated a γ chain complementary DNA (cDNA) clone (*11*). The cDNA clone, pTγ-1, was isolated by screening a cDNA library made from the cell HPB-MLT with a DNA probe specific for the human γ chain C region (*10*). The nucleotide sequence of pTγ-1 was determined (*12*) and V, J, and C regions were identified by homology to the mouse γ gene (Fig. 1B).

The two γ chain C region genes were

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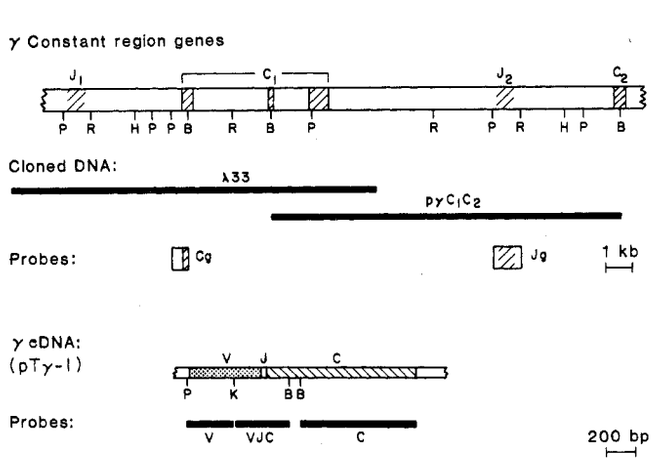

Fig. 1. Organization of the C regions of (A) the human γ chain gene and (B) the cDNA clone pTγ-1. A C region probe derived from genomic clones described previously (*10*) was used to screen a cDNA library constructed from T-cell polyadenylated messenger RNA cloned into the Eco RI site of pUC9 (*12*) by standard techniques (*11*). A human placenta bacteriophage Charon 4A library (*13*) was screened with a C region probe and a single clone, 33, was isolated. The clone $p\gamma C_1C_2$ containing a 12-kb Bam HI restriction fragment was isolated from a subgenomic library cloned into pBR322 by means of the C region probe with methods previously described (*10*). Location of the C region exons was determined by hybridization and nucleotide sequence analysis, and is indicated by closed-end, crosshatched areas. Open crosshatch symbols indicate the closest localization of J regions as detected by hybridization. The locations of the relevant Eco RI (R), Bam HI (B), Hind III (H), Kpn I (K), and Pst I (P) restriction enzyme sites are indicated; not all restriction enzyme sites are shown.

cloned on overlapping segments of DNA by standard techniques. A human placenta bacteriophage Charon 4A library (*13*) was screened with a probe to the γ constant region. A single clone, 33, was isolated and the location of the three C_1 exons and the associated J region were defined by hybridization of restriction fragments to the cDNA probes. The third coding block of C_1 and first coding block of C_2 were shown to reside on the same 12-kb Bam HI fragment by Southern blot analysis of genomic DNA (Fig. 1A). This fragment was cloned into pBR322 by screening a subgenomic library constructed from placental DNA fragments partially purified on a preparative agarose gel. This fragment encodes the 3′ end of the C_1 constant region, J_2, and the C_2 constant region (Fig. 1A).

J regions 4 kb 5′ of each C region were defined by hybridization to the J segment of pTγ-1 (designated VJC in Fig. 1B). The number and relation of J segments associated with each C region were not determined by these experiments. A striking feature of the human γ genes is the marked degree of homology between C_1 and C_2 which extends outside of the J and C coding regions. This is evident from the similarity of restriction maps in the region of C_1 and C_2. Cross-hybridization between restriction fragments from corresponding flanking segments and intervening sequences as well as analysis of short stretches of nucleotide sequences suggest that there is extensive homology in these regions. Such similarity provides evidence for recent duplication of this region of the genome. The extent of the duplication is not yet defined.

The V region probe (Fig. 1B) was used to obtain an estimate of the size of the family of V region genes homologous to the V region used in HPB-MLT. As shown in Fig. 2, when analyzed by the Southern blot method, the V probe produces seven bands of varying intensity. The greater intensity of some of the bands could be due to greater homology with the V region genes of the cDNA, or to multiple comigrating fragments that contain V regions or to multiple V region copies per fragment. Since Eco RI restriction sites within a V region would inflate the estimate of the number of V region encoding bands visualized with this type of hybridization experiment, we also hybridized the VJC probe to germline DNA (Fig. 2, lanes 3 and 4). Because this probe also contains J and C sequences, Eco RI fragments encoding portions of J_1, J_2, C_1, and C_2 also hybridized. These J- and C-containing fragments were identified with genomic probes Jg and Cg (Fig. 2, lanes 1 and 2). Five of the seven restriction fragments noted with the V probe produced isolated bands with the VJC probe. The 2.5-kb band noted with the V probe did not

Table 1. Classification of rearranged γ genes in various T-cell tumor DNA's. The DNA's were obtained from T-cell tumor lines or peripheral blood of patients with T-cell malignancy. The size of the Eco RI fragment and its association with the HPB-MLT V region is indicated for each type of rearrangement. Clinical and pathological diagnosis is indicated where known. Abbreviation: T-ALL, T-cell acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; ATL, adult T-cell leukemia; B-ALL, B lineage acute lymphoblastic leukemia; CL, cell line; PB, peripheral blood lymphocytes; ND, not determined.

Rearrangement	Eco RI (kb)	HPB-MLT V family	Cell	Diagnosis	CD4	CD8
I	9.5	No	REX (CL)		+	+
II	5.3	Yes	CEM (CL)	T-ALL	+	+
			HPB-MLT (CL)	ATL	+	+
III	4.2	Yes	SKW3 (CL)	CLL	+	+
			PELC (CL)		+	+
			SA (PB)	T-ALL	ND	ND
			REX (CL)		+	+
			ND (PB)	Sezary cell	+	−
			WH (PB)	T-ALL	ND	ND
			WA (PB)	ATL	+	−
			FALC (CL)	ATL	−	−
IV	2.5	No	SKW3 (CL)	CLL	+	+
			PELC (CL)		+	+
			DN (PB)	B-ALL	ND	ND
			HSB (CL)	T-ALL	−	−
			ROB (CL)	ATL	ND	ND
			ND (PB)	Sezary cell	+	−
			LS (PB)	T-ALL	ND	ND
			FALC (CL)	ATL	−	−
V	0.94	Yes	CEM (CL)	T-ALL	+	+
			SA (PB)	T-ALL	ND	ND
			8402T (CL)	T-ALL	−	−
			ROB (PB)	ATL	+	−
			TB (PB)	T-ALL	ND	ND
			RC (PB)	Sezary cell	+	−
VI	0.58	No	8402T (CL)	T-ALL	−	−
			HSB (CL)	T-ALL	−	−

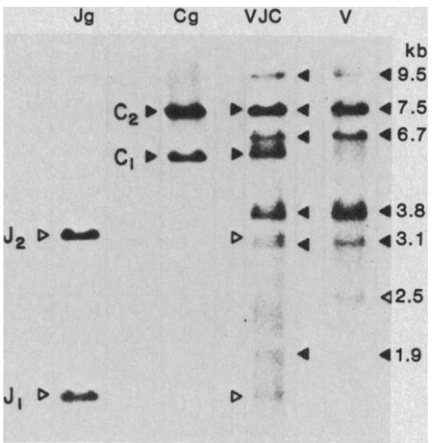

Fig. 2. Southern blot analysis of γ-chain V region number. Germline (B cell) DNA was digested with Eco RI and size-fractionated on a 0.9 percent agarose gel, transferred to nitrocellulose paper, and hybridized to nick-translated ^{32}P-labeled DNA fragments (*11*). The J probe was a 1-kb genomic fragment containing J_2 (designated Jg in Fig. 1), the C probe (Cg in Fig. 1) was a 0.6-kb genomic fragment derived from C_1, and probes VJC and V were derived from the cDNA (Fig. 1). The molecular weights (in kilobases) of bands hybridizing to the V probe are indicated on the right. Arrows to the right of the VJC lane indicate bands in common with the V probe, arrows to the left of the VJC lane indicate bands due to C or J segments. The open arrow (lane V) indicates a fragment that hybridized to the V probe but not to the VJC probe.

hybridize to the VJC probe, and the structure of the V region encoded by this fragment is unclear. The 7.5-kb V region encoding fragment comigrates with the C_2 encoding fragment and hence its hybridization to the VJC probe cannot be evaluated with certainty. However, the absence of new bands or change in intensity of common bands when the VJC probe is used indicates that the 7.5-kb band contains a complete V region not cleaved by Eco RI. Thus there are approximately six V region genes in this family.

The J segment probe derived from genomic DNA (designated Jg in Fig. 1A) was used to define the rearrangement of γ genes in various T-cell DNA's. Because the restriction enzyme sites around J_1 and J_2 are conserved we could not determine whether the altered restriction fragments detected in these experiments derived from J_1 or J_2. However, hybridization of the Eco RI digested DNA's to the C region (Cg) probe showed that C_1 was deleted from each T-cell DNA except REX. In this T-cell, one J_1 is in germline configuration, while J_1 on the other chromosome could be rearranged or deleted. T-cell DNA's were prepared from 17 T-cell tissue culture lines, isolated leukemic peripheral T cells, and the peripheral blood of one patient with a B-cell leukemia. Southern blot analyses of representative T-cell DNA's are displayed in Fig. 3. Most of the T-cell DNA digests have two nongermline restriction fragments (Fig. 3A). HSB DNA appears to have only a single nongermline fragment; however, other experiments demonstrated a second rearranged Eco RI fragment, 0.58 kb in size, which had migrated off this particular gel. DN DNA was prepared from peripheral lymphocytes of a patient with B-cell lineage acute lymphoblastic leukemia (ALL), and although the germline pattern is prominent there is a γ chain rearrangement. Whether this rearrangement has occurred in the leukemic B cells of this patient or in a monoclonal T-cell population in this patient's blood is uncertain. From 27 different rearranged bands only six unusual rearrangements were noted (Fig. 3A and Table 1).

Each rearrangement type appears to be the product of recombination between a particular V region gene and a J region gene. That is, we have digested these T-cell DNA's with other restriction enzymes and identified comigrating fragments that correspond to the unique types. The possibility that new bands in the T-cell DNA's represent restriction length polymorphisms is unlikely because of the absence of these bands in peripheral blood T-cell DNA of 11 normal volunteers and the consistency of rearrangements noted with other restriction enzymes. Since only one T cell was found with the type I rearrangement, we suggest that other rearrangements may be identified in other T cells.

To determine if the V regions in these rearrangements belong to the HPB-MLT V region family, we used the V probe hybridized to Eco RI–digested T-cell DNA's (Fig. 3, lanes V). The 5.3-, 4.2-, and 0.94-kb rearranged bands were visualized with the V probe; however, the 9.5- and 2.5-kb rearranged bands comigrated with germline V region fragments and the 0.58-kb rearrangement was not detected in this experiment. To determine whether these rearranged genes use a member of the HPB-MLT variable region family we digested the DNA's with other enzymes and repeated the hybridization to the J and V probes (Fig. 3B). Those T cells containing the type I, IV, and VI rearranged fragments do not use the HPB-MLT V region family because the rearranged fragments derived from these

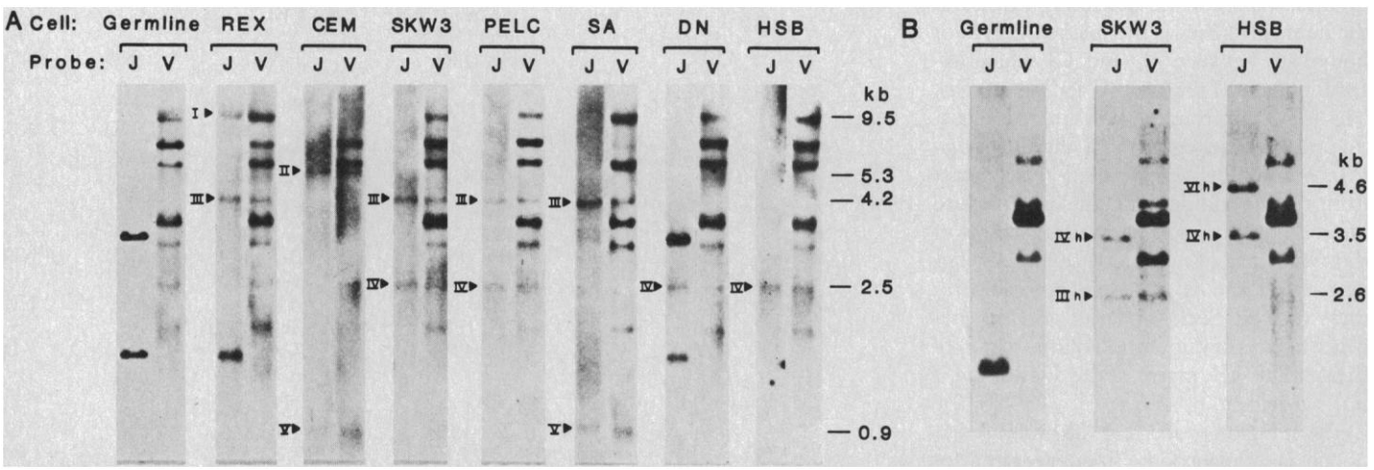

Fig. 3. Southern blot analysis of cells containing rearranged γ genes. Genomic DNA was prepared from human T-cell tumors (CEM, HSB, PELC, SKW3, REX), peripheral lymphocytes of patients with T-cell leukemia (SA) or B lineage leukemia (DN), or B cells (LAZ 509; nonrearranged γ genes). DNA's were digested with (A) Eco RI or (B) Hind III, fractionated on a 0.9 percent agarose gel, blotted onto nitrocellulose, and hybridized to ^{32}P-labeled nick-translated DNA probes. The J and V probes are designated Jg and V in Fig. 1. Arrows on the left of each pair of lanes indicate rearranged bands labeled with the J probe. The type of rearrangement is indicated to the left of each lane (see Table 1). In (B), IIIh, IVh, and VIh indicate the location of the rearranged fragments produced by Hind III digestion of T-cell DNA's.

cells do not hybridize to the HPB-MLT V region (Fig. 3B and Table 1). Because the type IV rearrangement is very common (Table 1) we suggest that this rearrangement involves V-J recombination with the V region of another non–cross-hybridizing family of V region genes. Thus, the human γ chain genes can rearrange with at least four different V region genes. The maximum number of V region genes is not defined by these experiments; however, we suggest that there will be approximately 15 based on the number of V regions in the HPB-MLT family and the number of different rearrangements observed. This appears to be significantly more recombinational diversity than is available to the murine γ genes (*2, 3*).

The role of the γ chain gene remains uncertain. However, we note that the gene is rearranged in human T cells with diverse cell surface phenotype (CD4, CD8; Table 1). Further studies to determine whether these rearrangements lead to expression of functional γ chains in these T cells may help to define the role of this polypeptide.

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T-Cell Recognition of Ia Molecules Selectively Altered by a Single Amino Acid Substitution

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T lymphocytes recognize foreign antigen together with allele-specific determinants on membrane-bound class I and class II (Ia) gene products of the major histocompatibility complex. To identify amino acids of class II molecules critical to this recognition process, the genes encoding the β chains of the I-A^k molecule were cloned from a wild-type B-cell hybridoma and from an immunoselected variant subline showing distinct serological and T-cell stimulatory properties. Nucleotide sequencing and DNA-mediated gene transfer established that a single base transition (G → A) encoding a change from glutamic acid to lysine at position 67 in the I-A^k_β molecule accounted for all the observed phenotypic changes of the variant cells. These results confirm the importance of residues 62 to 78 in the amino terminal domain of I-A_β for class II–restricted T-cell recognition of antigen and demonstrate the ability of a single substitution in this region to alter this recognition event.

CLASS II (OR IA) MAJOR HISTOCOMPATIBILITY molecules are integral membrane glycoproteins that exist as heterodimers of two noncovalently associated chains, α (33 kD) and β (29 kD). In the mouse, two isotypic forms of Ia have been demonstrated: I-A ($A_\beta A_\alpha$) and I-E ($E_\beta E_\alpha$) (*1, 2*). These molecules are predominantly expressed on B lymphocytes, some macrophages and T lymphocytes, dendritic cells, and thymic epithelial cells (*3, 4*). There is evidence that the primary role of Ia molecules is to act as corecognition elements during antigen-specific interactions of helper-inducer T lymphocytes with accessory cells (macrophages or dendritic cells) or with B lymphocytes [reviewed by Schwartz (*5*)]. A striking feature of class II molecules is their extensive intraspecies polymorphism, and it is this allelic variation that is directly involved in the Ia-restricted recognition of antigen by T cells. Nucleotide sequence analysis of cloned class II genes has revealed that the principal amino acid polymorphisms among alleles are in the NH_2-terminal domains of the I-A_β, I-A_α, and I-E_β chains (*6–12*). [The I-E_α chain shows relatively little variation among haplotypes (*13, 14*).] The amino acid substitutions appear clustered in three or four hypervariable regions spread across the entire domain.

Identification of the location of allelic variation in these molecules is the first step toward understanding the relation between the fine structure of Ia and its immunological function. Further analysis is needed to map more precisely the regions and residues participating in haplotype-restricted T-cell antigen recognition and to assign functions to the mapped regions with respect to such features as interaction with antigen or direct binding to the T-cell receptor. One of the two major approaches to exploring these issues is the use of DNA-mediated gene transfer technology to obtain expression of class II genes recombined in vitro ("exon shuffled") by cells capable of antigen presentation. The alterations in structure are then systematically correlated with those seen in function. This method has proved successful in confirming the predicted unique role of the NH_2-terminal I-$A_{\beta 1}$ or I-$E_{\beta 1}$ domains in Ia-restricted T-cell stimulation (*15, 16*).

The other approach is to identify mutations in class II genes that lead to altered immunologic function and then to determine the precise nature of the structural change in the mutated gene. However, in contrast to the many spontaneous mutations that have been described in genes encoding class I (H-2K) major histocompatibility complex (MHC) molecules, only one has been found affecting a class II gene (*17*). The B6.CH2^{bm12} mouse has an altered I-A^b_β gene, with three nucleotide changes compared to the wild type giving rise to three amino acid substitutions—at positions 67, 70, and 71 (*18*). The changes have resulted in an altered Ia serologic reactivity, a new immune response phenotype, mixed lymphocyte reactivity against cells of the parent strain, and a distinct pattern of MHC-restricted antigen presentation by cells bearing the mutant I-A molecule (*19*). It is not yet known, however, whether all the changes in the bm12 I-A_β molecule are required for each altered trait. Thus, it would be useful to have a panel of cells bearing mutant class II

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