IMMUNOGLOBULIN $V_H$ GENES ARE TRANSCRIBED BY T CELLS IN ASSOCIATION WITH A NEW 5' EXON

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For a number of years it seemed likely that Ig $V_H$ region genes were expressed in T cells. The presence of $V_H$ determinants on the surface of T lymphocytes was reported (1) and clonotypic transcription of a germline $V_H$ gene segment was observed in a cytotoxic T cell clone (2). Nevertheless, other examples of $V_H$ transcription in T cells have failed to emerge (3, 4). However, recent studies of inversion of chromosome 14 long arm in a T cell lymphoma showed that recombination had occurred between the T cell receptor $J\alpha$ locus at 14q11 and an Ig $V_H$ gene at 14q32 (5, 6). In addition, 14q32 sequences adjacent to the inversion breakpoint hybridize to a 5.2-kb mRNA in a panel of T cells (7), whether or not they have a chromosome 14 inversion. We have now characterized cDNA clones from a human T cell line and show that the 14q32 T cell mRNA corresponds to a transcript of an unrearranged $V_H$ gene in which a novel, 5' exon replaces the hydrophobic leader sequence normally associated with $V_H$ transcripts in B cells. In genomic DNA, the new exon is adjacent to a pseudo-$V_H$ gene, not observed in mature mRNA, implying that RNA splicing controls association of the new exon with the expressed $V_H$ segments.

Materials and Methods

Analysis of Recombinant Clones

cDNA and Genomic Clones. Poly(A)$^+$ mRNA from the RPMI 8402 cell line was cloned into the Eco RI site of Xgt10 (8) (Clonetech Laboratories Inc., Palo Alto, CA). Restriction fragments of isolated recombinant $\lambda$ DNA were subcloned into pUC9 and M13 (9) and sequenced as M13 single-stranded DNA (10, 11). A $\lambda$ phage library made from RPMI 8402 DNA (12) was screened with cR18ES (Fig. 1 D). Positive clones were analyzed as above.

Analysis of Genomic DNA

Southern filters were prepared (13) and hybridized as described (12). DNA probes were prepared by random oligolabeling (14).

Results and Discussion

A 5.2-kb RNA was originally detected in 11 different human T cell lines with probes containing 14q32 sequences, adjacent to the centromeric breakpoint of the SUP-T1 chromosome 14 inversion (7). This RNA was derived from a 4.3-kb region separating...
the Ig V\textsubscript{\textalpha} and V\textsubscript{\textbeta} genes (Fig. 1 A). The structure of the 5.2-kb transcript was analyzed in cDNA clones from the T cell line RPMI 8402. 10\textsuperscript{5} recombinant cDNA clones were screened with the probe XS9X (Fig. 1 B). 35 phages were isolated, representing a frequency of \textasciitilde1 in 2,500 recombinants.

Nine clones formed a set of overlapping cDNA fragments, whose total length is 4.7 kb (Fig. 1 C). The cDNA restriction pattern is identical for 4.1 kb, at the 3' end, to that of the spacer region between V\textsubscript{\textalpha} and V\textsubscript{\textbeta}. The structure of the 5' region of the 5.2-kb transcript was analyzed by DNA sequencing. 700 bp of this sequence, beginning at the 5' end of clone cR18, is shown in Fig. 2 A. Amino acid translation of this sequence shows that the 5.2-kb RNA includes the V\textsubscript{\textalpha} gene segment (V\textsubscript{\textalpha}II subgroup), represented by nucleotides 302–605. The transcribed V\textsubscript{\textalpha} segment is not rearranged, since it still has the conserved heptamer (nucleotides 606–612) and nonamer (nucleotides 636–644) recombination signal sequences (15). Thus, most of the 5.2-kb RNA in RPMI 8402 cells is derived from transcription beginning at the 5' end of the V\textsubscript{\textalpha} (nucleotide 302) and extending through most of the downstream spacer region up to, but not including, the V\textsubscript{\textbeta} gene segment.

In B lymphocytes, the transcription of V\textsubscript{\textgamma} gene segments involves splicing of a 5' leader exon to the V\textsubscript{\textgamma} segment, with consequent removal of a small intron. However, the sequence immediately upstream of V\textsubscript{\textgamma} in the 5.2-kb RNA (residues 1–301, Fig. 2 D) does not correspond to the 5' leader exon, but instead to new exonic sequences (designated as E\textsubscript{\textgamma}). Normally the AUG codon used for the initiation of protein translation is present in the leader exon of the V\textsubscript{\textgamma} gene. Therefore this function must be fulfilled by the E\textsubscript{\textgamma} sequence in this mRNA. Analysis of the E\textsubscript{\textgamma} sequence revealed an initiation codon in phase with the V\textsubscript{\textgamma} segment. The available E\textsubscript{\textgamma} sequence actually includes another ATG triplet (nucleotides 255–257), which is out of phase with V\textsubscript{\textgamma} and ends at a stop codon just 10 residues downstream (nucleotides 285–287).
Translation of the 5.2-kb RNA from the putative ATG initiation codon would yield a polypeptide of 122 amino acids, of which the four NH₂-terminal residues are encoded by E₉. Furthermore, the E₉ sequence does not show significant homology to any other known sequence. Since it seems that the E₉ exon can only encode for four amino acids, and does not seem hydrophobic, a V₉ protein domain with this sequence attached would most likely remain inside the cell. At present, we do not know whether the mRNAs described in this paper are translated into V₉ polypeptides. However, the potentially intracellular protein products of these T cell V₉ transcripts may, like other Ig-like molecules, have the capacity to recognize and bind particular antigens. This raises some interesting possibilities for control of Ig rearrangement in T cells.

To further investigate the occurrence of E₉ sequences in T cell mRNA, other cDNA clones were isolated using cR8EP (Fig. 1 D). Two new clones (cR15 and cR19) were analyzed and found together to encompass 1.8 kb of mRNA sequence. Sequence analysis (Fig. 2 B) again revealed the presence of a V₉ segment unrelated to V₉. This V₉ segment (V₉, sub-I subgroup) is also not rearranged, since it still retains 3' recombination signals (Fig. 2 B). Furthermore, the sequences upstream of V₉ (nucleotides 1-66, Fig. 2 B) again consist of the E₉ sequences (nucleotides 235-301, Fig. 2 B). Thus, the same E₉ exon(s) appear to be spliced with two distinct Ig V₉ gene transcripts in RPMI 8402 cells.

Since the normal leader exon is replaced in both of the T cell V₉ mRNAs by a new exon, we investigated the genomic organization by isolating clones from a λ phage library of RPMI 8402 DNA (12). The restriction map of a representative clone (λRES4) appears in Fig. 3 A. The E₉ sequence was located by hybridization with
cR18ES (Fig. 1 D) and was found to occur as a single exon (Fig. 3 B). An RNA splice site occurs at the end of the exon where it is spliced to the V\textsubscript{H} segments. Therefore, the sequence is a single exon used in the production of the mRNAs represented by clones cR18 and cR19.

In addition to the E\textsubscript{T} exon, RES4 contains a V\textsubscript{H} gene, located 1.4 kb downstream of E\textsubscript{T}. The sequence of this gene is given in Fig. 3 C. The sequence shows a V\textsubscript{H} gene with a normal leader exon. However, the sequence differs from the V\textsubscript{H} segments found in the cDNA clones. Furthermore, the gene is a pseudo-V\textsubscript{H} (related to the V\textsubscript{H}II subgroup) with reading frame mutations. Therefore, the E\textsubscript{T} exon is not adjacent in the genome to the expressed V\textsubscript{H} segments.

The hybridization complexity of the E\textsubscript{T} sequences was investigated (Fig. 4) in human and mouse DNA with either cR8ES (E\textsubscript{T}) or cR8EP (V\textsubscript{H}II). The V\textsubscript{H} probe detects multiple hybridizing bands in human and mouse DNA. The hybridization characteristics of the cR8ES probe appear quite different. Only two hybridizing bands appear in human DNA, demonstrating that the E\textsubscript{T} sequences are less complex than the V\textsubscript{H} genes with which they are associated in RNA. Furthermore, the E\textsubscript{T} sequences appear conserved in mouse DNA.

Thus, we have found that V\textsubscript{H} gene segments are indeed transcribed in T cells, and also that the pattern of V\textsubscript{H} transcription differs from that observed in B cells. The primary E\textsubscript{T}-V\textsubscript{H} T cell transcript must presumably carry at least one V\textsubscript{H} segment (i.e., ΨV\textsubscript{H} segment), which is removed during splicing to generate the mature mRNA. Since the genomic and cDNA E\textsubscript{T} exons are identical, it seems prob-
able that E<sub>r</sub> associated with V<sub>n</sub><sup>α</sup> and V<sub>n</sub><sup>θ</sup> are derived by relatively long-range RNA splicing from the same genomic E<sub>r</sub> sequence. Clearly, the genomic E<sub>r</sub> arrangement provides a simple explanation for the replacement of the leader exon in T cell mRNA, because the normal V<sub>n</sub> leader exon does not have a 5' acceptor splice site that could join with the donor site of the E<sub>r</sub> exon. This latter donor site can however splice directly onto the acceptor site of the V<sub>n</sub> segment.

Summary

We previously detected mRNAs in a number of human T cell lines with a probe from within the Ig V<sub>n</sub> gene locus. We now show these mRNAs consist of Ig V<sub>n</sub> genes expressed in T cells. In one human T cell line, two RNA species have been studied and found to come from transcripts of unrearranged V<sub>n</sub> segments in which the leader exon, normally associated with V<sub>n</sub> transcripts in B cells, is replaced by a novel 5' exon (E<sub>r</sub>) not encoding a hydrophobic leader peptide. In genomic DNA, this new E<sub>r</sub> exon is adjacent to a pseudo-V<sub>n</sub> gene that has not been observed in mature mRNA. This implies that RNA splicing controls association of the new exon with the expressed V<sub>n</sub> segments. Hence, V<sub>n</sub> transcription does indeed occur in T cells, but is qualitatively different from that in B cells.

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