EXPRESSION OF T CELL RECEPTOR GENES IN HUMAN B CELLS

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The human T lymphocyte antigen receptor (Ti)[1] is a disulphide-linked heterodimer composed of an α and a β chain (1–3). This molecule is responsible for the antigen specificity and MHC restriction of T cells, and participates in T lymphocyte activation (4, 5). Ti molecules have been found at the surface of T cells and some thymocytes (6). The genes that encode the human Ti_α and Ti_β molecules have been cloned and sequenced (7–11). A third homologous rearranging gene, Ti_γ, has also been cloned; its role in T lymphocyte recognition of antigen is unclear (12, 13).

Ti_α and Ti_β genes are remarkably similar to Ig genes. They consist of gene segments that rearrange to form unique variable-region genes. As with Ig genes in B cells, this rearrangement process occurs in an ordered fashion, and is probably subject to allelic exclusion (14, 15). V, D, and J segments participate in the formation of a complete Ti_β variable region gene (11, 16). In the human Ti_α gene, it is clear that V and J segments are involved in such rearrangements; whether D_α segments exist is still uncertain (9). The chief differences between Ig and Ti genes are that only Ti genes encode molecules that must recognize both antigen and MHC, and only Ig genes undergo somatic mutation.

Thymocytes and many T cell lines produce truncated Ti_α and Ti_β transcripts that lack V regions, as well as full-length mRNAs. In mature peripheral T cells, only full-length Ti_α and Ti_β transcripts are synthesized (17–19). A similar situation is seen in the B lymphocyte lineage, where pre–B cells and some B cell lines express truncated Ig H chain transcripts (20–24). Some, but not all, of these truncated Ig and Ti transcripts include D regions. In-frame translation initiation codons are found upstream of some D_H and D_β genes, and protein products arising from truncated IgH transcripts have been observed (24, 25) in some murine pre–B cell lines. The biological role of truncated Ig and Ti transcripts and their protein products is unclear. Others (26, 27) have proposed that transcription of unrearranged Ig gene segments may mediate their increased accessibility to "recombinases," or joining enzymes.

It is intriguing that many human T cell lines, some of which have undergone

[1] *Abbreviations used in this paper:* AET, 2-*S*-amino-ethylisothiouronium bromide; Ti, T cell antigen receptor.

D_H–J_H rearrangements, express low levels of truncated IgH transcripts (20, 28). We were interested in determining whether the converse situation exists, i.e., whether Ti genes are transcribed in B cells. In this study, we demonstrate that truncated Ti_α and Ti_β transcripts are indeed produced in small quantities in human B cell lines, and in the case of Ti_β, in freshly purified tonsillar B cells. However, rearrangements of Ti_β genes in B cells are infrequent. These observations yield new insights into the relationship between Ti gene transcription and rearrangement.

Materials and Methods

Cells. Cells were grown in suspension in RPMI 1640 medium supplemented with 10% FCS, 50 U/ml penicillin G, and 50 μg/ml streptomycin. RPMI 4265 is a B cell leukemia line obtained from Hugh McDevitt, Stanford University, Stanford, CA (29); Raji is a Burkitt lymphoma cell line obtained from the American Type Culture Collection, Rockville, MD (30); Josh-7 is a B cell precursor line from an X-linked agammaglobulinemia patient, obtained from Shu Man Fu, The Rockefeller University, New York (31); RPMI 8226 is a λ-secreting multiple myeloma cell line obtained from the American Type Culture Collection (32); Jurkat is a T cell leukemia line obtained from Art Weiss, UCSF (33); HL-60 and U-937 are promyelocytic leukemia cell lines obtained, respectively, from Robert Gallo (National Institutes of Health, Bethesda, MD) and Hillel Koren, Duke University, Durham, NC (34, 35); K562 is a leukemia cell line of the erythroid lineage obtained from Y. W. Kan, UCSF (36); HeLa is a cervical carcinoma cell line obtained from the American Type Culture Collection (37); and Colo-320 is a neuroendocrine tumor cell line obtained from Manfred Schwab, UCSF (38).

Northern Blots. Poly(A) RNA was prepared from cell lines by SDS lysis, proteinase K digestion, and oligo(dT) cellulose batch absorption (39). For cell lines HL-60 and U-937, due to high levels of endogenous ribonucleases, the guanidinium thiocyanate–cesium chloride procedure was used for RNA extraction, followed by two rounds of oligo(dT) cellulose chromatography (40, 41).

Poly(A) RNA was denatured in formamide and formaldehyde and electrophoresed through 1.2% formaldehyde-agarose gels, and blotted overnight to nitrocellulose paper as described (41). Blots were baked in vacuo for 2 h at 80°C, then prehybridized and hybridized to nick-translated probes under standard conditions (41). The Ti_α probe was the Eco RI insert of pY14, which contains the full-length Ti_α cDNA from Jurkat (19). The Ti_β probe was the Bam HI–Xba I insert of pTβF-neo, which contains the full-length Ti_β cDNA from Jurkat (5). Hybridized blots were washed stringently; the final two washes were 45 min each at 68°C in 0.2× SSPE, 0.1% SDS. Blots were autoradiographed at −70°C using preflashed Kodak XAR-5 film and Lightning-Plus intensifying screens (DuPont Co., Wilmington, DE)

cDNA Cloning and Sequencing. Poly(A) RNA from the cell line RPMI 4265 was prepared as described under Northern blotting, and used to construct a cDNA library in the bacteriophage vector λgt10 (42). The first-strand synthesis reaction contained 5 μg of RPMI 4265 poly(A) RNA, 4 μg of oligo(dT$_{12-18}$) (Collaborative Research, Lexington, MA), 0.06 U RNAsin (Promega Biotech, Madison, WI), 60 U avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim Diagnostics, Houston, TX), 35 mM 2-ME, 640 μM of each dNTP, 5 μCi of each [^{32}P]dNTP (800 Ci/mmol, Amersham Corp., Arlington Heights, IL), 100 mM Tris (pH 8.3), 60 mM KCl, and 7 mM MgCl$_2$. RNA-DNA duplexes were tailed with terminal transferase (P-L Biochemicals, Milwaukee, WI) and dGTP, and second-strand cDNA synthesis was primed with oligo(dC$_{12-18}$) (Collaborative Research) as described (42); since 3′ recessed ends are not tailed under these conditions, full-length first strands are preferentially primed for second-strand synthesis. After second-strand synthesis with DNA polymerase I, the pH was adjusted to 8.3, KCl and 2-ME were added to 60 mM and 30 mM, respectively, and 20 U of reverse transcriptase were added to finish elongation of second strands stalled at polymerase I

pause sites. After protection of cDNA Eco RI sites with Eco RI methylase (New England Biolabs, Beverly, MA) and S-adenosylmethionine, and purification of double-stranded cDNA on Sephadex G-50, a final elongation step was performed with DNA polymerase I to finish any incomplete syntheses and to generate blunt ends for ligation to Eco RI linkers. After linker addition and Eco RI digestion, double-stranded cDNA longer than 800 bp was isolated by preparative agarose gel electrophoresis and electroelution. The cDNA was ligated to λgt10 arms, packaged in vitro, and amplified on *Escherichia coli* strain C600 *hfl*, as described (42).

The library was screened with nick-translated Ti_α and Ti_β probes by standard techniques (41). Positive clones were plaque purified, and inserts were subcloned into Eco RI–digested M13 mp8 (43). ^{32}P and ^{35}S sequencing by the dideoxy method were performed essentially as described (44). Primers used were the M13 universal sequencing primer (Pharmacia Fine Chemicals, Piscataway, NJ), and internal 20-nucleotide primers complementary to the C_α and C_β genes near the J–C junctions (nucleotides 622–641 of pY14, and nucleotides 488–507 of YT35; references 7, 19).

Southern Blotting.   Genomic DNA samples from various cell lines were prepared by lysis of washed cells with sarcosyl and proteinase K as described under Northern Blots, followed by extraction with phenol and precipitation with ethanol. DNA samples were digested with Eco RI, electrophoresed on a 0.7% agarose gel, and transferred to a nylon membrane (41). After crosslinking of the DNA to the membrane by UV irradiation (45), the blot was probed with a ^{32}P-labeled, 4.0-kb genomic Eco RI fragment containing the human $C_{\beta 2}$ gene and a 4.1-kb genomic Eco RI fragment containing the human $J_{\beta 2}$ gene cluster (11). The blot was washed stringently and autoradiographed by standard procedures (41).

Preparation and Analysis of Tonsillar T and B Cells.   Lymphocytes were removed from a non-neoplastic adult human tonsil by perfusion and were purified by Ficoll-Hypaque centrifugation. Adherent cells were removed by incubation at 37°C for 45 min in plastic dishes. T and B cells were separated by nylon-wool chromatography (46). The B cell fraction was further purified by rosetting out contaminating T cells with 2-S-amino-ethylisothiouronium bromide (AET)-conjugated SRBC. Aliquots (2 × 10^6 cells) of T and B cell fractions were washed and stained with FITC-conjugated rabbit anti–human Ig or FITC-conjugated OKT11 (Ortho Diagnostic Systems Inc., Westwood, MA); fluorescence was determined by flow microfluorimetry on a FACS IV (Becton Dickinson Immunocytometry Systems, Mountain View, CA). From the remainder of the cells, poly(A) RNA was isolated as described under Northern Blotting. T and B cell RNA samples were electrophoresed on a 1.0% formaldehyde-agarose gel, blotted to nylon membrane, crosslinked to the membrane by UV irradiation (45), prehybridized, and hybridized to ^{32}P-labeled Ti_α and Ti_β probes, then were washed stringently and autoradiographed (41).

Results

Detection of Truncated Ti Transcripts in B Cell Lines.   Poly(A) RNA was prepared from four human B cell lines to examine their production of Ti_α and Ti_β mRNA. The cell lines were chosen to represent a wide variety of B cell phenotypes, including Burkitt's lymphoma (Raji), lymphocytic leukemia (RPMI 4265), Igλ-secreting myeloma (RPMI 8226), and a B cell line from an X-linked agammaglobulinemia patient (Josh-7) (29–32). By Southern and Northern blotting, we verified that all four cell lines were clonal, had rearranged their IgH and Igκ genes, and expressed Igκ mRNA (data not shown). Poly(A) mRNA was also prepared from the T cell line Jurkat, the myeloid lines HL-60 and U-937, and the neuroendocrine cell line Colo-320 (33–35, 38). RNA samples were electrophoresed through formaldehyde-agarose gels, blotted to nitrocellulose, hybridized to ^{32}P-labeled Ti_α and Ti_β cDNA probes, and washed at high stringency. With the Ti_α probe, Jurkat showed abundant levels of the expected 1.7-kb Ti_α
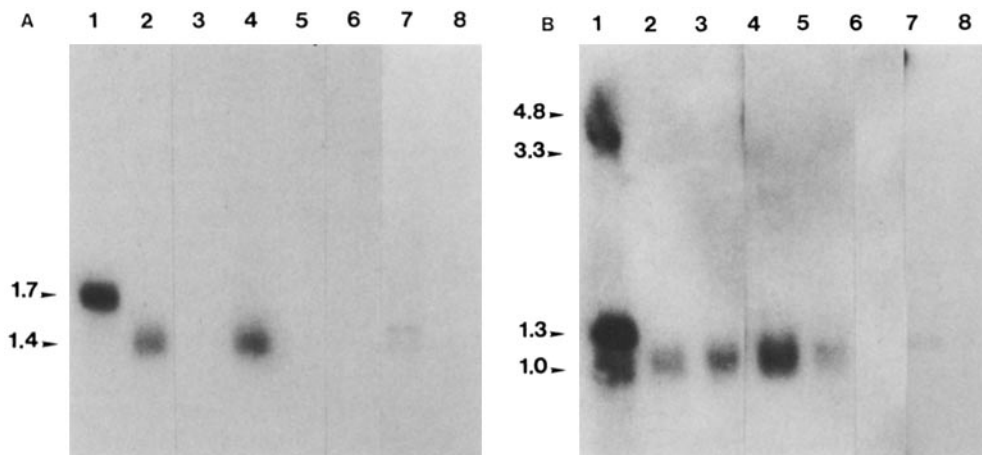
FIGURE 1. Northern blot analysis of Ti gene expression in human cell lines. Poly(A) RNAs from various cell lines were analyzed by Northern blotting. Lane *1*, Jurkat; lane *2*, RPMI 4265; lane *3*, Raji; lane *4*, Josh-7; lane *5*, RPMI 8226; lane *6*, Colo-320; lane *7*, HL-60; lane *8*, U-937. Lane *1* contains 1.4 μg of RNA; all other lanes contain 5 μg. (A) Ti_α probe, (B) Ti_β probe. Autoradiography exposure times: lanes *1–6*, 24 h; lanes *7–8*, 72 h.

mRNA, as shown in Fig. 1A. In contrast, the B cell lines Josh-7 and RPMI 4265 contained low levels of truncated 1.3-kb Ti_α transcripts. By densitometry, Josh-7 and RPMI 4265 contained 15% and 4%, respectively, of the amount of Ti_α mRNA seen in Jurkat. Raji and RPMI 8226 displayed no detectable Ti_α mRNA even after prolonged exposure. U-937 and Colo-320 were also totally negative for Ti_α transcripts, whereas the myeloid cell line HL-60 showed barely detectable levels of 1.3- and 1.4-kb Ti_α mRNA; this represented <1/200 of the levels seen in the T cell line. Failure to detect Ti_α mRNA in Colo-320 and some of the B cell lines was not due to RNA degradation or failure of the RNA to transfer to nitrocellulose, since actin mRNA was detected in all lanes when the blot was rehybridized with a γ-actin cDNA probe (data not shown).

When Northern blots were probed at high stringency with the full-length Ti_β cDNA probe, as seen in Fig. 1B, the T cell line Jurkat showed abundant quantities of the full-length 1.3-kb mRNA and smaller quantities of the truncated 1.0-kb mRNA, as well as small amounts of high molecular weight RNA which may represent unspliced precursors. The B cell lines RPMI 4265, Raji, Josh-7, and RPMI 8226 all expressed low levels of 1.0–1.1-kb truncated Ti_β transcripts. By densitometry, the truncated Ti_β transcripts in these B cell lines were 2–5% as abundant as the full-length transcripts in the T cell line Jurkat. Colo-320 and U-937 completely lacked Ti_β transcripts, while HL-60 expressed extremely low levels of 1.1-kb Ti_β transcripts.

When similar Northern blots were probed with the Ti_β probe at lower hybridization stringency, no additional RNA bands were seen in any of the cell lines (data not shown). In preliminary experiments using a human Ti_γ cDNA probe, we did not detect Ti_γ mRNA in any of the B cells used in this study (data not shown).

Sequence of Truncated Ti Transcripts in B Cells. We determined the structure of Ti_α and Ti_β transcripts in the B cell line RPMI 4265 by cDNA cloning and

nucleotide sequencing. cDNA was constructed from RPMI 4265 mRNA and inserted into the bacteriophage vector λgt10. Special efforts were made to ensure that the majority of clones were full length or nearly full length: after first-strand synthesis, the cDNA was tailed with dGTP using terminal transferase under conditions where only full-length first strands are tailed; the second strand was synthesized sequentially by DNA polymerase I and reverse transcriptase, using oligo(dC) as a primer; and cDNAs smaller than 800 bp were removed by preparative gel electrophoresis before insertion into the vector.

The RPMI 4265 cDNA library was screened with Ti_α and Ti_β probes. ~1 clone in 2,000 hybridized to the Ti_α probe, and 1 in 10,000 to the Ti_β probe. Individual clones were isolated and subcloned into M13 mp8. The 5' and 3' ends of these cDNA clones were sequenced by the dideoxy method, using the M13 universal primer and a synthetic oligonucleotide primer complementary to the 5' region of C_α or C_β. The lengths of these cDNA clones agreed with those of the corresponding mRNAs to within 100 nucleotides; thus, these are full-length or nearly full-length cDNA clones.

The DNA sequences of five independent Ti_α cDNA clones, all from the B cell line RPMI 4265, are shown in Fig. 2. All of them have a similar structure, consisting of 5' noncoding RNA, a heptamer-nanomer recombination sequence (47), a J segment, and a C segment. Each uses a different J_α segment, containing 19–21 codons. The putative amino acids that would be encoded by each of these J_α segments (if appropriate translation initiation codons existed [48]) are shown in Fig. 3. They show good homology to each other, and to a J_α gene previously identified in a T cell cDNA clone (8), particularly at the invariant residues F-G-X-G seen in all Ti and Ig J segments. However, none of these five Ti_α cDNA clones can be translated in the correct reading frame. Clones $R_\alpha2$, $R_\alpha3$, and $R_\alpha5$ have AUG initiation codons in the 5'-flanking DNA or J_α segments, but all are in incorrect reading frames, and all are followed by a translation stop codon within 3–28 amino acids. For clones $R_\alpha1$ and $R_\alpha4$, the first AUG codon occurs with the C_α region. This codon is also out of frame, and is followed by a translation termination codon 17 amino acids downstream (8). Thus, the only peptides that might be produced by translation of these truncated transcripts are out of frame and very short, and are not likely to have any function. While the genomic sequences surrounding these J_α genes are not yet known, the sizes and sequences of these five clones strongly suggest that they are transcribed from promoters upstream of unrearranged J_α genes.

Two Ti_β cDNA clones from RPMI 4265 were sequenced. As shown in Fig. 2, clone $R_\beta1$ contains the $J_{\beta2.6}$ and $C_{\beta2}$ segments (11). Clone $R_\beta2$, interestingly, contains two different J_β segments. Both $J_{\beta2.4}$ and $J_{\beta2.5}$ are present in this cDNA. The RNA splice donor site at the 3' end of $J_{\beta2.4}$ is not used; instead, the $J_{\beta2.5}$ splice donor site is spliced to $C_{\beta2}$ (11). Comparison with the known genomic sequence of this region demonstrates conclusively that these Ti_β cDNAs are the product of unrearranged genes (11). Neither of these clones contains a translation initiation codon in the 5'-flanking or J_β regions. The first AUG codon lies in the $C_{\beta2}$ gene; it is in an incorrect reading frame and is followed by a stop codon 28 amino acids downstream (11). Thus, these transcripts do not encode Ti proteins.

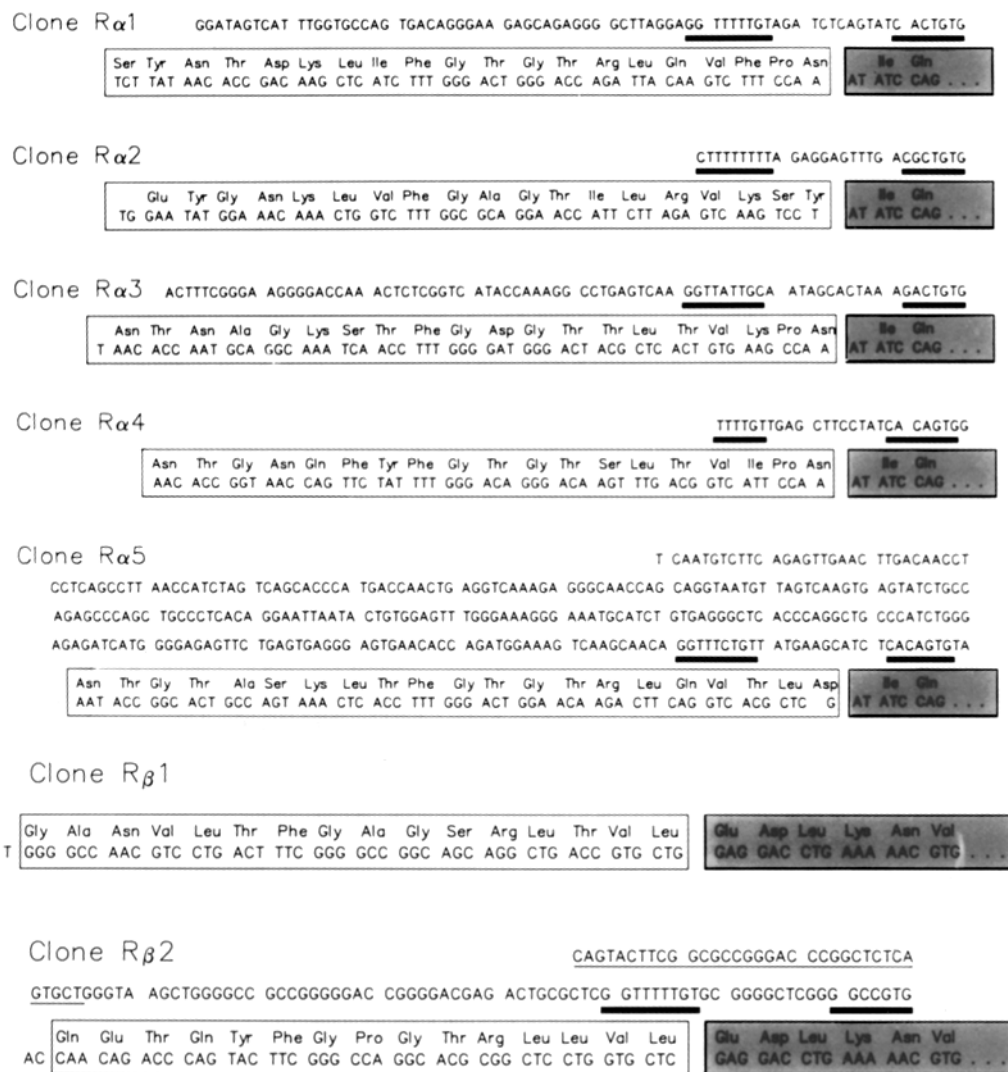*Comparison of Ti_α cDNA Sequences from T and B Cells: Evidence for D_α*

Clone Rα1   GGATAGTCAT TTGGTGCCAG TGACAGGGAA GAGCAGAGGG GCTTAGGAGG TTTTTGTAGA TCTCAGTATC ACTGTG

Ser	Tyr	Asn	Thr	Asp	Lys	Leu	Ile	Phe	Gly	Thr	Gly	Thr	Arg	Leu	Gln	Val	Phe	Pro	Asn	Ile	Gln
TCT	TAT	AAC	ACC	GAC	AAG	CTC	ATC	TTT	GGG	ACT	GGG	ACC	AGA	TTA	CAA	GTC	TTT	CCA	A	AT ATC CAG . . .	

Clone Rα2   CTTTTTTTTA GAGGAGTTTG ACGCTGTG

Glu	Tyr	Gly	Asn	Lys	Leu	Val	Phe	Gly	Ala	Gly	Thr	Ile	Leu	Arg	Val	Lys	Ser	Tyr	Ile	Gln
TG GAA	TAT	GGA	AAC	AAA	CTG	GTC	TTT	GGC	GCA	GGA	ACC	ATT	CTT	AGA	GTC	AAG	TCC	T	AT ATC CAG . . .	

Clone Rα3   ACTTTCGGGA AGGGGACCAA ACTCTCGGTC ATACCAAAGG CCTGAGTCAA GGTTATTGCA ATAGCACTAA AGACTGTG

Asn	Thr	Asn	Ala	Gly	Lys	Ser	Thr	Phe	Gly	Asp	Gly	Thr	Thr	Leu	Thr	Val	Lys	Pro	Asn	Ile	Gln
T AAC	ACC	AAT	GCA	GGC	AAA	TCA	ACC	TTT	GGG	GAT	GGG	ACT	ACG	CTC	ACT	GTG	AAG	CCA	A	AT ATC CAG . . .	

Clone Rα4   TTTTGTTGAG CTTCCTATCA CAGTGG

Asn	Thr	Gly	Asn	Gln	Phe	Tyr	Phe	Gly	Thr	Gly	Thr	Ser	Leu	Thr	Val	Ile	Pro	Asn	Ile	Gln
AAC	ACC	GGT	AAC	CAG	TTC	TAT	TTT	GGG	ACA	GGG	ACA	AGT	TTG	ACG	GTC	ATT	CCA	A	AT ATC CAG . . .	

Clone Rα5   T CAATGTCTTC AGAGTTGAAC TTGACAACCT

CCTCAGCCTT AACCATCTAG TCAGCACCCA TGACCAACTG AGGTCAAAGA GGGCAACCAG CAGGTAATGT TAGTCAAGTG AGTATCTGCC

AGAGCCCAGC TGCCCTCACA GGAATTAATA CTGTGGAGTT TGGGAAAGGG AAATGCATCT GTGAGGGCTC ACCCAGGCTG CCCATCTGGG

AGAGATCATG GGGAGAGTTC TGAGTGAGGG AGTGAACACC AGATGGAAAG TCAAGCAACA GGTTTCTGTT ATGAAGCATC TCACAGTGTA

Asn	Thr	Gly	Thr	Ala	Ser	Lys	Leu	Thr	Phe	Gly	Thr	Gly	Thr	Arg	Leu	Gln	Val	Thr	Leu	Asp	Ile	Gln
AAT	ACC	GGC	ACT	GCC	AGT	AAA	CTC	ACC	TTT	GGG	ACT	GGA	ACA	AGA	CTT	CAG	GTC	ACG	CTC	G	AT ATC CAG . . .	

Clone Rβ1

Gly	Ala	Asn	Val	Leu	Thr	Phe	Gly	Ala	Gly	Ser	Arg	Leu	Thr	Val	Leu	Glu	Asp	Leu	Lys	Asn	Val
T GGG	GCC	AAC	GTC	CTG	ACT	TTC	GGG	GCC	GGC	AGC	AGG	CTG	ACC	GTG	CTG	GAG	GAC	CTG	AAA	AAC	GTG . . .

Clone Rβ2   CAGTACTTCG GCGCCGGGAC CCGGCTCTCA

GTGCTGGGTA AGCTGGGGCC GCCGGGGGAC CGGGGACGAG ACTGCGCTCG GTTTTTGTGC GGGGCTCGGG GCCGTG

Gln	Glu	Thr	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	Arg	Leu	Leu	Val	Leu	Glu	Asp	Leu	Lys	Asn	Val
AC CAA	CAG	ACC	CAG	TAC	TTC	GGG	CCA	GGC	ACG	CGG	CTC	CTG	GTG	CTC	GAG	GAC	CTG	AAA	AAC	GTG . . .

FIGURE 2.   DNA sequences of Ti$_\alpha$ and Ti$_\beta$ cDNA clones from B cell line RPMI 4265. Nucleotides derived from J genes are in *unshaded boxes*. C genes are in *shaded* boxes. Heptamer-nanomer joining sequences are *heavily underlined*. Nucleotides from J$_{\beta2.4}$ in clone R$_\beta$2 are *lightly underlined*. The top five are Ti$_\alpha$ cDNA clones; the bottom two are Ti$_\beta$ cDNA clones.

Genes.   While it is clear that Ti$_\alpha$ transcripts in B cells contain only J and C segments, it is likely that D$_\alpha$ segments or non–germline-encoded N regions contribute to the formation of full-length Ti$_\alpha$ transcripts in T cells. This inference is based on the comparison of the DNA sequence of a single Ti$_\alpha$ cDNA clone with its corresponding genomic V$_\alpha$ and J$_\alpha$ sequences (9, 19). In this study we have been able to extend this conclusion. Since the B cell Ti$_\alpha$ transcripts have heptamer-nanomer recombination signals at the 5' ends of their J$_\alpha$ segments, they almost certainly arise from unrearranged genes. Thus, their sequences can be compared with the sequences of full-length transcripts in T cells to determine

	Vα	Jα	Cα
HPB-MLT	. . V Y F C	A L D S S A S K I I F G S G T R L S I R P N	I Q N . .
RPMI α1	. . . . . .	. ■ S T N T D K L I F G T G T R L Q V F P N	I . . . .
RPMI α2	. . . . . .	. ■ V G T G N K L V F G A G T I L R V K S T	I . . . .
RPMI α3	. . . . . .	. ■ N T N A G K S T F G D G T T L T V K P N	I Q N . .
RPMI α4	. . . . . .	. . ■ N T G N Q F Y F G T G T S L T V F Q	. . . . .
RPMI α5	. . . . . .	■ N T G T A S K L T F G T G T R L Q V T L D	I Q N . .

FIGURE 3. Comparison of hypothetical Tiα translated protein sequences. Sequences are shown for the five RPMI 4265 Tiα cDNA clones and for the T cell line HPB-MLT. Conserved amino acids are in *shaded boxes*. *Small shaded boxes* show the positions of heptamer-nanomer recombination signals.
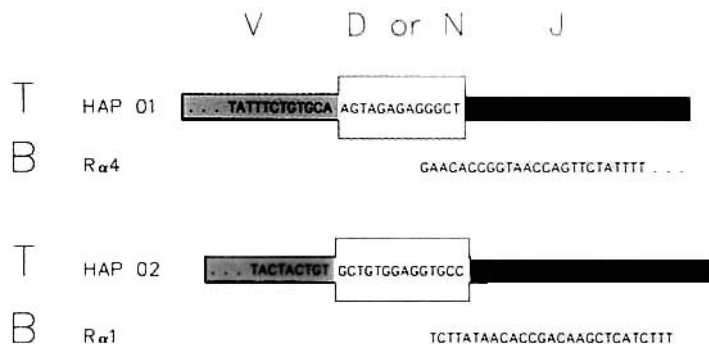
FIGURE 4. Comparison of Tiα cDNA sequences from T and B cells. The region near the V-J junction is shown for human peripheral T cell cDNA clones HAP 01 and HAP 02, and for RPMI 4265 cDNA clones Rα4 and Rα1. Nucleotides from Vα genes are *lightly shaded*; Cα nucleotides are *heavily shaded*. *Unshaded* nucleotides are derived from either Dα genes or N regions.

which nucleotides in the T cell cDNAs were derived from Jα segments. Similarly, comparison of full-length cDNAs from different T cell lines that use the same Vα gene but different Jα genes can reveal which nucleotides were contributed by Vα segments. Nucleotides that were not derived from Vα or Jα genes must have been contributed either by Dα genes or by N regions added by terminal transferase.

Yoshikai et al. (49) recently sequenced 24 different Tiα cDNA clones from human peripheral T lymphocytes. Two of these T cell cDNAs use the same Jα segments as two of our B cell cDNAs. The B cell clone Rα1 uses the same Jα segment as the T cell clone HAP02, as seen in Fig. 4. HAP02 and another T cell cDNA clone, HAP29, use the same Vα but different Jα segments; thus, the 3' boundary of the Vα segment can be precisely determined.

HAP02 contains 14 nucleotides that are not accounted for by Vα or Jα segments. These nucleotides must be derived from either a Dα gene or an N region added by terminal transferase (50). Similarly, the T cell cDNA clone HAP01 uses the same J segment as the B cell cDNA clone Rα4 (49), and the 3' boundary of its V region can be determined to within three nucleotides by analogy to other T cell Tiα cDNA clones. As seen in Fig. 4, HAP01 contains 13 ± 3 nucleotides derived from either a Dα gene or nongermline elements.

Lack of Rearrangement of Tiβ Genes in B Cell Lines. Because the Jα segments are spread out over >50 kb of genomic DNA, it is impossible to study Tiα gene rearrangements by ordinary Southern blotting using Cα probes (9). However,
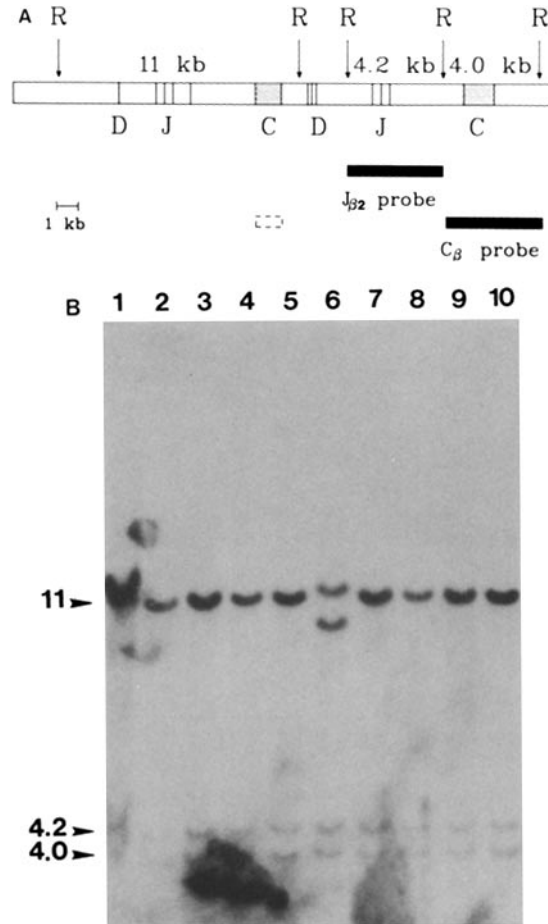
FIGURE 5. Southern blot analysis of Ti_β gene rearrangements. (A) restriction map of human Ti_β genes. Positions of Eco RI restriction sites are indicated by arrows labeled "R". Sizes of relevant Eco RI restriction fragments, in kilobase pairs, are shown. Shaded boxes indicate the locations of the $J_{\beta2}$ and $C_{\beta2}$ probes. The region of $C_{\beta1}$ that crosshybridizes with the $C_{\beta2}$ probe is indicated by a box outlined with dashed lines. (B) Southern blot. 10 µg of DNA from various cell lines were electrophoresed on a 0.8% agarose gel, blotted to nylon paper, hybridized with ^{32}P-labeled $J_{\beta2}$ and $C_{\beta2}$ probes, and autoradiographed. Lane 1, HeLa; lane 2, Colo-320; lane 3, HL-60; lane 4, U-937; lane 5, K562; lane 6, Jurkat; lane 7, Josh-7; lane 8, Raji; lane 9, RPMI 4265; lane 10, RPMI 8226. Autoradiography exposure was for 96 h.

the Ti_β genes can be studied by this technique. By digesting genomic DNA from various cell lines with the restriction enzyme Eco RI, and probing Southern blots of this DNA with a combination of C_β and J_β genomic probes, it is possible to detect all rearrangements involving either the $J_{\beta1}$ or $J_{\beta2}$ gene cluster (Figure 5A) (11). In Fig. 5B, such a Southern blot is shown, using DNA from the four B cell and plasma cell lines, the T cell line Jurkat, and five myeloid and nonhematopoietic cell lines. Only the T cell line displayed bands of altered mobility, indicating rearrangement of its Ti_β genes. All of the B cell and nonlymphoid cell lines displayed the germline pattern of DNA bands. These data confirmed the
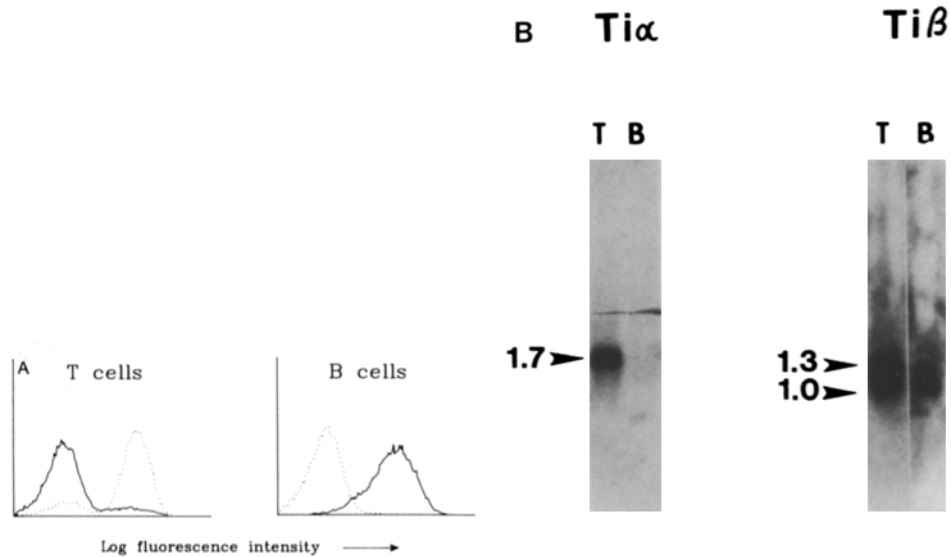
FIGURE 6.   Ti expression in human tonsilar lymphocytes. (A) FACS analysis of purified tonsilar T and B cells. *Solid lines,* cells stained with anti-Ig; *dotted line,* cells stained with OKT11. Fluorescence intensity is plotted on the abscissa on a logarithmic scale; relative cell number is plotted on the ordinate. (B) Northern blot analysis. mRNA was prepared from the same tonsilar T and B cell populations used in A. 500 ng samples of poly(A) RNA were analyzed by Northern blotting. *Left,* Ti$_\alpha$ probe. *Right,* Ti$_\beta$ probe. *T,* mRNA from purified T cells; *B,* mRNA from purified B cells. Autoradiography exposure times, 16 h for *T*; 48 h for B.

conclusion, from sequencing of cDNA clones, that the Ti$_\beta$ genes were not rearranged in these B cell lines.

Truncated Ti Transcripts in Tonsillar B Cells.   Finally, we sought to determine whether expression of low levels of truncated Ti$_\alpha$ and Ti$_\beta$ transcripts in human B cell lines represented a bona fide physiological phenomenon, or was merely an artifact of in vitro cell culture. We purified lymphocytes from a non-neoplastic adult tonsil, and fractionated T and B cells by nylon-wool chromatography. The B cell fraction was depleted of any residual T cells by rosetting with AET-treated SRBC. Aliquots of the T and B cell fractions were stained with FITC-conjugated rabbit anti–human Ig and OKT11, and examined by flow microfluorimetry; from the remainder of the cells, poly(A) RNA was purified. The right half of Fig. 6A shows the FACS analysis of the purified B cells; >99% of the cells were OKT11$^-$ and surface Ig$^+$. Thus, these tonsillar B cells were essentially free of contaminating T cells and nonlymphoid cells. In the left half of Fig. 6A, in contrast, ~85% of the purified T cells were OKT11$^+$ and 90% were surface Ig$^-$, suggesting that the T cells were slightly contaminated with B cells and nonlymphoid cells.

Poly(A) mRNA from the purified tonsillar T and B lymphocytes was analyzed by Northern blotting using Ti$_\alpha$ and Ti$_\beta$ probes. As seen in Fig. 6B, the T cells contained abundant full-length, 1.7-kb Ti$_\alpha$ transcripts; no Ti$_\alpha$ mRNA was seen in the B cells. In contrast, low levels of truncated Ti$_\beta$ transcripts of 1.0–1.1 kb were clearly seen in the purified B cells, while the T cells expressed abundant

1.3-kb transcripts and lower levels of truncated 1.0-kb transcripts. No clear 1.3-kb band was seen in the B cell lane; this is further evidence that the B cell preparation was not contaminated with T cells. Thus, truncated Ti$_\beta$ transcripts, but not Ti$_\alpha$ transcripts, are expressed in tonsillar B cells.

Discussion

We have examined the expression of truncated mRNA transcripts of Ti$_\alpha$ and Ti$_\beta$ genes in human B cells. Such transcripts are present at low levels in several B cell lines examined and, in the case of Ti$_\beta$, in tonsillar B cells. These transcripts arise from unrearranged genes, and do not encode Ti proteins. The fact that expression of these truncated transcripts is lymphocyte specific, and probably occurs from multiple, coordinately regulated promoters upstream of different J segments, suggests that the transcripts have some conserved function. Since this function is not mediated by a protein product, this transcription may itself play a role in lymphocyte function or development. Based on data from studies (26, 27) of Ig gene rearrangement, it is probable that transcription is involved in the process that renders Ti gene segments accessible to the enzymes that mediate rearrangement. This hypothesis is consistent with the fact that both the abundance of Ti$_\beta$ transcripts, and the frequency of Ti$_\beta$ gene rearrangements, are highest in T cells, intermediate in B cells (and some myeloid cells), and absent in nonhematopoietic cells (51, 52).

Ti$_\beta$ Transcripts are Found in Human B Cells. We used Northern blot analysis to test for the presence of Ti$_\alpha$ and Ti$_\beta$ poly(A) mRNA. We detected low-abundance, 1.0–1.1-kb Ti$_\beta$ truncated transcripts in all four human B cell lines examined, representing developmental stages from immature B cells to Ig-secreting plasma cells (26–29). Such transcripts are present at an abundance of roughly 1–5 copies per cell, based on the frequency of positive clones obtained in screening the RPMI 4265 cDNA library, and considering the cloning bias in favor of RNA species shorter than 2 kb in the λgt10 system. The low abundance of these transcripts (up to 50-fold less abundant in B cell lines than in T cell lines) explains why they have not been previously detected; indeed, we could detect them only after using relatively large amounts of poly(A)-enriched RNA and highly radioactive DNA probes. The Ti$_\beta$ mRNA bands seen in the B cell lines are somewhat broad. We attribute this to heterogeneity in the population of truncated transcripts. Multiple J$_\beta$ promoters give rise to transcripts having different 5′ ends and slightly different lengths. Extremely low levels of 1.1-kb Ti$_\beta$ transcripts, roughly an order of magnitude less abundant than in the B cell lines, were also detected in one of two myeloid leukemia lines studied.

Truncated Ti$_\alpha$ Transcripts are Found in Some B Cell Lines. Truncated Ti$_\alpha$ transcripts were detected in two of the four B cell lines, and were not seen in tonsillar B cells. The presence of truncated Ti$_\alpha$ transcripts in some but not all human B cell lines has also been noted by others (19). We estimate that the B cell lines RPMI 4265 and Josh-7 contain 10–20 copies of Ti$_\alpha$ mRNA per cell. It is not clear why some of the B cell lines synthesize truncated Ti$_\alpha$ transcripts while others do not. Since Josh-7, which has cell surface markers characteristic of immature B cells, expresses Ti$_\alpha$ transcripts, while the fully differentiated plasma cell line RPMI 8226 does not, Ti$_\alpha$ transcription may be confined to early stages

of B cell development. A more comprehensive survey of B cell lines in various stages of development would be needed to resolve this question. One of two myeloid leukemia lines studied expressed 1.3- and 1.4-kb Ti_α transcripts, at a level at least an order of magnitude lower than that seen in the two B cell lines.

It is striking that multiple J_α promoters, spread over more than 50 kb of genomic DNA, appear to be coordinately regulated. In the B cell line RPMI 4265, at least five different J_α genes, and probably many more, are simultaneously transcribed. In contrast, two other B cell lines contain no detectable Ti_α transcripts. This strongly suggests that these multiple J_α promoters are switched on and off in concert, in response to specific positive and/or negative transcription factors, in a tissue- and stage-specific manner. The precise start site of transcription, and the location of regulatory elements involved in Ti transcriptional control, are not known. In Ig H chain J genes, where similar truncated transcripts are seen, multiple transcription initiation sites are involved (53).

Structure of Ti Transcripts in B Cells. We sequenced several representative Ti_α and Ti_β cDNA clones from RPMI 4265 to determine their structures and potential protein coding domains. Of the five Ti_α and two Ti_β cDNA clones sequenced, all are apparently transcribed from promoters upstream of J_α or J_β genes, and contain J and C coding segments. In each case, the J segment is flanked at its 5' end by a heptamer-nanomer recombination signal (47). All of these cDNAs are transcribed from unrearranged genes (though this cannot be formally proven for the Ti_α genes until the genomic sequences of these J_α genes are known), and cannot encode Ti proteins, since no in-frame AUG codons are present. We cannot exclude the possibility that other truncated transcripts, which were not sequenced in this study and which use different J segments, may be translated to yield truncated Ti proteins.

The inability of truncated J-C transcripts from Ti_α and Ti_β genes to be translated has precedents in other Ti and Ig systems. Truncated C_μ and J-C_μ H chain Ig transcripts from B cells are also generally "sterile," or incapable of encoding protein, as are some truncated C_μ and Ti_β transcripts in T cells (54–56). Some of the D-J-C_H transcripts seen in Abelson-transformed pre–B cells are translated (22). The functional role of such "D_μ" proteins is unknown, though they appear to be glycosylated and inserted into the endoplasmic reticulum (22).

D_α Segments of N Regions Contribute to Ti_α Gene Formation in T Cells. Evidence for D_α genes or N regions in the human Ti_α gene complex has been scanty and inferential, based on the comparison of genomic and cDNA sequences for a single cell line, Jurkat (9, 19). We have extended this observation by comparing Ti_α cDNA sequences from T and B cells. By comparing sequences from full-length T cell transcripts and truncated B cell transcripts that use the same J_α gene, one can delineate precisely which nucleotides in the T cell transcripts were derived from J_α genes. Similarly, by comparing the sequence of different T cell transcripts which use the same V_α but different J_α genes, one can determine which nucleotides were derived from V_α genes.

In the two cases where we were able to make such comparisons between our truncated B cell transcripts and the T cell Ti_α transcripts described by Yoshikai et al. (49), we found that 13–14 nucleotides of the full-length T cell transcripts were not contributed by either V_α or J_α genes. Therefore, by analogy with Ti_β

and Ig H chain genes, these 13–14 nucleotides must be derived either from D_α genes or from non–germline-encoded elements (N regions) thought to be added by terminal transferase during the rearrangement process (50). The length of 13–14 nucleotides corresponds closely to the length of known D_β and D_H genes (16, 28), whereas most N regions are considerably shorter. Proof that those nucleotides are derived from D_α genes will require the cloning of genomic D_α genes.

Low Frequency of Rearrangement of Ti Genes in B Cells. For the Ti_β genes, analysis of gene rearrangement is readily accomplished by standard Southern blotting (14, 51, 52). Using a combination of DNA probes that can detect any rearrangement involving either the $J_{\beta1}$ or $J_{\beta2}$ cluster, we found that the four B cell lines and five myeloid and nonhematopoietic cell lines examined had all retained their Ti_β genes in the germline configuration.

While none of the B cell lines in our small survey had undergone Ti_β gene rearrangements, larger studies performed by others (51, 52) suggest that such rearrangements do occur occasionally. In one such series, 5 of 33 B cell leukemias and lymphomas had undergone Ti_β rearrangements (51). The precise nature of these rearrangements was not determined, but they may have included D_β-J_β rearrangements and, in a few cases, more complex rearrangements. In another study, 39 non–T, non–B acute lymphoblastic leukemias were examined for Ig and Ti gene rearrangements (52). All had undergone Ig H chain gene rearrangements; of these, 10 of 39 had also rearranged or deleted their Ti_β genes. The frequencies of Ti_β rearrangement observed in these B cell tumors (15%) and presumptive lymphocyte precursors (26%) resemble the frequency of the converse event, i. e., D-J rearrangement of Ig H chain genes in T cells (20, 28, 51). T or B lymphocytes that have rearranged both Ig and Ti genes nevertheless retain a "pure" T or B phenotype, as determined by lineage-specific cell surface antigen expression (51). Thus, occasional rearrangement of an inappropriate antigen receptor gene is unlikely to have functional consequences. Finally, it is notable that in a survey of myeloid tumors, only 3 of 24 (12%) were found to have undergone Ti_β gene rearrangements (57). These three tumors expressed Ti_β transcripts.

Analysis of Ti_α gene rearrangements is complicated by the great genomic distance between the C_α gene and most of the J_α genes. Standard Southern blotting using C_α probes is generally uninformative. We attempted to analyze Ti_α rearrangements using oligonucleotide probes complementary to three different J_α segments, based on the sequence of the RPMI 4265 Ti_α cDNA clones. When these probes were hybridized to Southern blots of DNA from various cell lines, multiple bands were detected even at relatively high hybridization stringencies, indicating that the cloned J_α segments were members of families of closely related J_α genes (data not shown). While minor differences in band patterns were noted between different cell lines, it was impossible to ascertain whether they represented rearrangements or merely DNA polymorphisms, especially since a restriction fragment length polymorphism linked to the human C_α gene has already been reported (58). Reliable analysis of Ti_α gene rearrangements in B cells will require Southern blotting of DNA electrophoresed on pulse-field gels (59). In any case, the sequencing of five Ti_α cDNA clones from RPMI

4265 has shown that most, and probably all, of the truncated Ti_α transcripts in this B cell line arise from unrearranged genes.

Relationship Between Transcription and Rearrangement of Antigen Receptor Genes. Transcription of unrearranged antigen receptor gene segments is a widespread phenomenon. Truncated Ig H chain transcripts have been noted in many B cells and some T cells. These transcripts consist of J_H-C_μ RNA from unrearranged IgH genes, as well as D_H-J_H-C_μ transcripts from genes that have undergone D_H-J_H rearrangement but not V_H-DJ_H rearrangement (20–24). Similar truncated transcripts have been detected from Ti_α and Ti_β genes in T cells, thymocytes and, in the present study, B cells (17–19, 60). In particular, 1.0-kb truncated Ti_β transcripts, but not full-length transcripts, are detected in day-14 murine thymocytes (60). At this stage, the majority of thymocytes' Ti_β genes retain the germline configuration, while a few have undergone D_β-J_β rearrangement (60). Unrearranged V_H genes are transcribed in Abelson-transformed B cell precursors that are in the process of undergoing V_H-DJ_H rearrangement (26). Recently, Ig C region genes were shown to be transcriptionally active in B cells undergoing Ig class switching (27, 61). Thus, transcription of gene segments before their rearrangement appears to be a common theme.

Others (26, 27) have proposed a model that relates the phenomena of antigen receptor gene transcription and rearrangement. They suggested that rearrangement of receptor gene segments requires not only that a recombinase be present, but also that the chromatin surrounding the gene in question be "accessible" to this enzyme. This accessibility is accompanied by transcription, and is demonstrable by increased sensitivity of the affected regions to chemical and enzymatic probes of chromatin structure, such as DNase I (27). Transcription could either play a causative role in forming a more open chromatin structure, or it could simply be a secondary manifestation of increased accessibility caused by some other primary mechanisms. The mechanisms by which such changes in chromatin structure might be brought about are not understood.

The accessibility model is supported by the observation that while recombination between endogenous D_β and J_β genes occurs at extremely low frequency in Abelson-transformed murine B cells, rearrangement between D_β and J_β gene introduced by DNA transfection occurs quite frequently (62). The frequency of such D_β-J_β rearrangement is similar to that of transfected D_H-J_H constructs. Thus, the low frequency of endogenous Ti_β gene rearrangement in Abelson-transformed murine lymphocytes cannot be due to an inherent inability of the recombinase to catalyze this reaction, but rather must be due to some property of the endogenous Ti_β genes or their surrounding chromatin, such as a "closed" chromatin structure that is inaccessible to the enzyme. A more direct validation of the accessibility model has come from recent studies by Alt et al. (27), where high-frequency rearrangement of stably integrated transfected genes occurred only when local transcriptional activity was high, and surrounding chromatin was DNase-sensitive.

Our data indicate that low-level transcription from J_β promoters occurs in human B cells and in some myeloid cell lines. Such transcription is accompanied by rearrangement only about one-fifth of the time (51, 52). Thus, transcriptional levels and rearrangement frequencies are roughly correlated. High levels of

truncated Ti_β transcripts occur in thymocytes, which rearrange Ti_β genes with high frequency; low levels are found in human B cells, which rearrange much less frequently; some myeloid lines have still lower levels of transcription, and such cells show Ti_β rearrangements even more rarely than B cells; and nonhematopoietic cells neither rearrange nor transcribe Ti_β genes. This rule may apply only to the initial step (D-J rearrangement) in Ti gene rearrangement; the tissue specificity of later steps is probably more tightly controlled (27).

Because Northern blot hybridization measures the total accumulation of transcripts in a population of cells, our data cannot distinguish between uniform low-level Ti transcription, and high-level transcription by a small subpopulation of cells. Either of these possibilities is consistent with the data and with the predictions of the accessibility model. However, in the latter case one might expect to see variations over time in the level of Ti gene transcription, due to possible differential growth rates of Ti-transcribing and nontranscribing subclones. In addition, according to the model, the Ti-transcribing cells might have a tendency to rearrange their Ti genes. Since no significant variations in Ti mRNA levels or Southern blot band patterns were observed over several months of cell culture, we believe this possibility is somewhat unlikely. The possibility of cell-to-cell variation in Ti transcription levels within these B cell lines could be addressed by analysis of several subclones of each line after limiting dilution.

Our data thus support the notion that transcription of unrearranged gene segments is a consistent accompaniment, and perhaps a necessary prerequisite, for gene rearrangement. Why then are truncated Ig and Ti transcripts synthesized, albeit at low levels, in inappropriate cell types? We favor the idea that such inappropriate transcripts and rearrangements do not have a direct functional role, but rather reflect the inherent "leakiness" of the regulatory mechanisms that govern antigen receptor gene rearrangement and expression. T and B lymphocytes use very similar machinery for gene rearrangement, as evidenced by the virtually identical heptamer-nanomer recombination signals used by Ti and Ig genes, and by the ability of Abelson-transformed pre-B cells to rearrange transfected Ti_β genes (62). Furthermore, both T and B cells can use some cis-acting Ig gene regulatory elements (63). Thus, it is not surprising that transcription and rearrangement of Ti genes in B cells, and of Ig genes in T cells, do occur. These events occur at levels low enough not to interfere with normal lymphocyte function and the rearrangement of appropriate receptor genes (51). The study of these lineage-inappropriate transcripts may shed light on the mechanisms of normal expression and rearrangement of antigen receptor genes.

Summary

We analyzed the transcription and rearrangement of the T cell antigen receptor (Ti) genes Ti_α and Ti_β in human B cell, T cell, and myeloid cell lines, as well as in purified tonsillar B and T cells. All four B cell lines examined, as well as one of two myeloid cell lines, expressed low levels of truncated Ti_β transcripts, as did freshly purified tonsillar B cells. Two of the B cell lines and one of the myeloid lines also expressed truncated Ti_α transcripts, while tonsillar B cells did not. Sequence analysis of cDNA clones from a B cell line demonstrated that these truncated Ti_α and Ti_β transcripts were composed of unrearranged J

and C gene segments. Comparison of cDNA clones from T and B cells suggests that D_α genes or N regions contribute to the formation of Ti_α transcripts in T cells but not in B cells. None of the B cell or myeloid cell lines in this study showed evidence of Ti_β gene rearrangements by Southern blotting. Our data, and other studies of gene rearrangements in human tumors, demonstrate that the level of Ti_β transcriptional activity and the frequency of Ti_β gene rearrangements are correlated in all cell types examined. Thus, our data support the accessibility model of antigen receptor gene rearrangement, whereby the susceptibility of gene segments to recombination enzymes is correlated with their transcriptional activity.

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