EXPRESSION OF T CELL RECEPTOR GENES IN HUMAN B CELLS

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The human T lymphocyte antigen receptor (Ti) 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 Tiα and Tiβ molecules have been cloned and sequenced (7–11). A third homologous rearranging gene, Tiγ, has also been cloned; its role in T lymphocyte recognition of antigen is unclear (12, 13).

Tiα and Tiβ 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 Tiβ variable region gene (11, 16). In the human Tiα gene, it is clear that V and J segments are involved in such rearrangements; whether Dα 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 Tiα and Tiβ transcripts that lack V regions, as well as full-length mRNAs. In mature peripheral T cells, only full-length Tiα and Tiβ 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 Dα and Dβ 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
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_{a} and Ti_{p} transcripts are indeed produced in small quantities in human B cell lines, and in the case of Ti_{p}, in freshly purified tonsillar B cells. However, rearrangements of Ti_{p} 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); Jot-7 is a B cell precursor line from an X-linked agammaglobulinemia patient, obtained from Shu Man Pu, The Rockefeller University, New York (31); RPMI 8226 is a λ-secretase 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_{a} probe was the Eco RI insert of pY14, which contains the full-length Ti_{a} cDNA from Jurkat (19). The Ti_{p} probe was the Bam HI–Xba I insert of pTβF-neo, which contains the full-length Ti_{p} cDNA from Jurkat (5). Hybridized blots were washed stringently; the final two washes were 45 min each at 68°C in 0.2x 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 [32P]dNTP (800 Ci/mmol, Amersham Corp., Arlington Heights, IL), 100 mM Tris (pH 8.3), 60 mM KCl, and 7 mM MgCl₂. RNA-DNA duplexes were tailed with terminal transferase (P-L Biochemicals, Milwaukee, WI) and dGTP, and second-strand cDNA synthesis was primed with oligo(dG)_{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 Agt10 arms, packaged in vitro, and amplified on Escherichia coli strain C600 hfl, as described (42).

The library was screened with nick-translated Ti and Ti probes by standard techniques (41). Positive clones were plaque purified, and inserts were subcloned into Eco RI-digested M13 mp8 (43). 32P and 35S 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 Cα and Cδ genes near the J–C junctions (nucleotides 622–641 of pY14, and nucleotides 488–507 of Y735; 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 32P-labeled, 4.0-kb genomic Eco RI fragment containing the human C02 gene and a 4.1-kb genomic Eco RI fragment containing the human J#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-aminoethylisothiouronium bromide (AET)-conjugated SRBC. Aliquots (2 × 10⁶ 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, cross-linked to the membrane by UV irradiation (45), prehybridized, and hybridized to 32P-labeled Tiα and Tiβ 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 Tiα and Tiβ 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 (Joshi-7) (29–32). By Southern and Northern blotting, we verified that all four cell lines were clonal, had rearranged their IgH and Igλ genes, and expressed Igλ 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 32P-labeled Tiα and Tiβ cDNA probes, and washed at high stringency. With the Tiα probe, Jurkat showed abundant levels of the expected 1.7-kb Tiα...
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) Ti₃ probe. (B) Ti₅ 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 Ti₃ transcripts. By densitometry, Josh-7 and RPMI 4265 contained 15% and 4%, respectively, of the amount of Ti₃ mRNA seen in Jurkat. Raji and RPMI 8226 displayed no detectable Ti₃ mRNA even after prolonged exposure. U-937 and Colo-320 were also totally negative for Ti₃ transcripts, whereas the myeloid cell line HL-60 showed barely detectable levels of 1.3- and 1.4-kb Ti₃ mRNA; this represented <1/200 of the levels seen in the T cell line. Failure to detect Ti₃ 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 Ti₅ 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 Ti₅ transcripts. By densitometry, the truncated Ti₅ 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 Ti₅ transcripts, while HL-60 expressed extremely low levels of 1.1-kb Ti₅ transcripts.

When similar Northern blots were probed with the Ti₇ 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 Ti₇ cDNA probe, we did not detect Ti₇ 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 Ti₃ and Ti₅ 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 Tiα and Tiβ probes. ~1 clone in 2,000 hybridized to the Tiα probe, and 1 in 10,000 to the Tiβ 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 Ca or Cβ. 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 Tiα 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 Jα segment, containing 19–21 codons. The putative amino acids that would be encoded by each of these Jα segments (if appropriate translation initiation codons existed [48]) are shown in Fig. 3. They show good homology to each other, and to a Jα 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 Tiα cDNA clones can be translated in the correct reading frame. Clones Rα2, Rα3, and Rα5 have AUG initiation codons in the 5'-flanking DNA or Jα segments, but all are in incorrect reading frames, and all are followed by a translation stop codon within 3–28 amino acids. For clones Rα1 and Rα4, the first AUG codon occurs with the Ca 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 Jα genes are not yet known, the sizes and sequences of these five clones strongly suggest that they are transcribed from promoters upstream of unrearranged Jα genes.

Two Tiβ cDNA clones from RPMI 4265 were sequenced. As shown in Fig. 2, clone Rβ1 contains the Jβ2.6 and Cβ2 segments (11). Clone Rβ2, interestingly, contains two different Jβ segments. Both Jβ2.4 and Jβ2.5 are present in this cDNA. The RNA splice donor site at the 3' end of Jβ2.4 is not used; instead, the Jβ2.5 splice donor site is spliced to Cβ2 (11). Comparison with the known genomic sequence of this region demonstrates conclusively that these Tiβ cDNAs are the product of unrearranged genes (11). Neither of these clones contains a translation initiation codon in the 5'-flanking or Jα regions. The first AUG codon lies in the Cβ2 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 Tiα cDNA Sequences from T and B Cells: Evidence for Dα*
FIGURE 2. DNA sequences of Ti. and Ti6 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.e4. in clone R52 are lightly underlined. The top five are Ti. cDNA clones; the bottom two are Ti6 cDNA clones.

Genes. While it is clear that Ti. transcripts in B cells contain only J and C segments, it is likely that D. segments or non-germline-encoded N regions contribute to the formation of full-length Ti. transcripts in T cells. This inference is based on the comparison of the DNA sequence of a single Ti. cDNA clone with its corresponding genomic Va and Ja sequences (9, 19). In this study we have been able to extend this conclusion. Since the B cell Ti. transcripts have heptamer-nanomer recombination signals at the 5' ends of their Ja segments, they almost certainly arise from unarranged genes. Thus, their sequences can be compared with the sequences of full-length transcripts in T cells to determine
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 Tα 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 Tα 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 Tα Genes in B Cell Lines. Because the Jα segments are spread out over >50 kb of genomic DNA, it is impossible to study Tα gene rearrangements by ordinary Southern blotting using Cα probes (9). However,
the Tiβ 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 Cβ and Jβ genomic probes, it is possible to detect all rearrangements involving either the Jβ1 or Jβ2 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 Tiβ genes. All of the B cell and nonlymphoid cell lines displayed the germline pattern of DNA bands. These data confirmed the
conclusion, from sequencing of cDNA clones, that the Tiβ 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 Tiα and Tiβ 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 FACSanalysis 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 Tiα and Tiβ probes. As seen in Fig. 6B, the T cells contained abundant full-length, 1.7-kb Tiα transcripts; no Tiβ mRNA was seen in the B cells. In contrast, low levels of truncated Tiβ 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 Ti₈ transcripts, but not Tiₐ transcripts, are expressed in tonsillar B cells.

Discussion

We have examined the expression of truncated mRNA transcripts of Tiₐ and Ti₈ genes in human B cells. Such transcripts are present at low levels in several B cell lines examined and, in the case of Ti₈, 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 Ti₈ transcripts, and the frequency of Ti₈ gene rearrangements, are highest in T cells, intermediate in B cells (and some myeloid cells), and absent in nonhematopoietic cells (51, 52).

**Ti₈ Transcripts are Found in Human B Cells.** We used Northern blot analysis to test for the presence of Ti₈ and Ti₈ poly(A) mRNA. We detected low-abundance, 1.0–1.1-kb Ti₈ 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 Xgt10 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 Ti₈ mRNA bands seen in the B cell lines are somewhat broad. We attribute this to heterogeneity in the population of truncated transcripts. Multiple J₈ promoters give rise to transcripts having different 5′ ends and slightly different lengths. Extremely low levels of 1.1-kb Ti₈ 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 Tiₐ Transcripts are Found in Some B Cell Lines.** Truncated Tiₐ transcripts were detected in two of the four B cell lines, and were not seen in tonsillar B cells. The presence of truncated Tiₐ 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 Tiₐ mRNA per cell. It is not clear why some of the B cell lines synthesize truncated Tiₐ transcripts while others do not. Since Josh-7, which has cell surface markers characteristic of immature B cells, expresses Tiₐ transcripts, while the fully differentiated plasma cell line RPMI 8226 does not, Tiₐ 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 subalpha 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 subalpha 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 subalpha genes, and probably many more, are simultaneously transcribed. In contrast, two other B cell lines contain no detectable Ti subalpha transcripts. This strongly suggests that these multiple J subalpha 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 subalpha and Ti subdelta cDNA clones from RPMI 4265 to determine their structures and potential protein coding domains. Of the five Ti subalpha and two Ti subdelta cDNA clones sequenced, all are apparently transcribed from promoters upstream of J subalpha or J subdelta 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 subalpha genes until the genomic sequences of these J subalpha 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 subalpha and Ti subdelta genes to be translated has precedents in other Ti and Ig systems. Truncated C subalpha and J-C subalpha H chain Ig transcripts from B cells are also generally "sterile," or incapable of encoding protein, as are some truncated C subdelta and Ti subdelta transcripts in T cells (54–56). Some of the D1-J-C subalpha transcripts seen in Abelson-transformed pre-B cells are translated (22). The functional role of such "D1" proteins is unknown, though they appear to be glycosylated and inserted into the endoplasmic reticulum (22).

D subalpha Segments of N Regions Contribute to Ti subalpha Gene Formation in T Cells. Evidence for D subalpha genes or N regions in the human Ti subalpha 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 subalpha 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 subalpha gene, one can delineate precisely which nucleotides in the T cell transcripts were derived from J subalpha genes. Similarly, by comparing the sequence of different T cell transcripts which use the same V subalpha but different J subalpha genes, one can determine which nucleotides were derived from V subalpha genes.

In the two cases where we were able to make such comparisons between our truncated B cell transcripts and the T cell Ti subalpha 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 subalpha or J subalpha genes. Therefore, by analogy with Ti subdelta
and Ig H chain genes, these 13–14 nucleotides must be derived either from D\(_{\alpha}\) 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\(_{\beta}\) and D\(_{\gamma}\) genes (16, 28), whereas most N regions are considerably shorter. Proof that those nucleotides are derived from D\(_{\alpha}\) genes will require the cloning of genomic D\(_{\alpha}\) genes.

**Low Frequency of Rearrangement of Ti Genes in B Cells.** For the Ti\(_{\beta}\) 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\(_{\beta}\) genes in the germline configuration.

While none of the B cell lines in our small survey had undergone Ti\(_{\beta}\) 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\(_{\beta}\) rearrangements (51). The precise nature of these rearrangements was not determined, but they may have included D\(_{\gamma}\)-J\(_{\beta}\) 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\(_{\beta}\) genes. The frequencies of Ti\(_{\beta}\) 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\(_{\beta}\) gene rearrangements (57). These three tumors expressed Ti\(_{\beta}\) transcripts.

Analysis of Ti\(_{\alpha}\) gene rearrangements is complicated by the great genomic distance between the C\(_{\alpha}\) gene and most of the J\(_{\alpha}\) genes. Standard Southern blotting using C\(_{\alpha}\) probes is generally uninformative. We attempted to analyze Ti\(_{\alpha}\) rearrangements using oligonucleotide probes complementary to three different J\(_{\alpha}\) segments, based on the sequence of the RPMI 4265 Ti\(_{\alpha}\) 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\(_{\alpha}\) segments were members of families of closely related J\(_{\alpha}\) 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\(_{\alpha}\) gene has already been reported (58). Reliable analysis of Ti\(_{\alpha}\) 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\(_{\alpha}\) cDNA clones from RPMI...
4265 has shown that most, and probably all, of the truncated Ti\(_a\) 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\(_{\alpha}\) RNA from unrearranged IgH genes, as well as D\(_{H\rightarrow J}\)-C\(_{\alpha}\) transcripts from genes that have undergone D\(_{H\rightarrow J}\) rearrangement but not V\(_{H\rightarrow D\rightarrow J}\) rearrangement (20-24). Similar truncated transcripts have been detected from Ti\(_a\) and Ti\(_\beta\) genes in T cells, thymocytes and, in the present study, B cells (17-19, 60). In particular, 1.0-kb truncated Ti\(_\beta\) transcripts, but not full-length transcripts, are detected in day-14 murine thymocytes (60). At this stage, the majority of thymocytes' Ti\(_\beta\) genes retain the germline configuration, while a few have undergone D\(_{p}\)-J\(_{\beta}\) rearrangement (60). Unrearranged V\(_{H}\) genes are transcribed in Abelson-transformed B cell precursors that are in the process of undergoing V\(_{H\rightarrow D\rightarrow J}\) 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\(_{\beta}\) and J\(_{\beta}\) genes occurs at extremely low frequency in Abelson-transformed murine B cells, rearrangement between D\(_{\beta}\) and J\(_{\beta}\) gene introduced by DNA transfection occurs quite frequently (62). The frequency of such D\(_{p}\)-J\(_{\beta}\) rearrangement is similar to that of transected D\(_{H\rightarrow J}\) constructs. Thus, the low frequency of endogenous Ti\(_\beta\) 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\(_\beta\) 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\(_{\beta}\) 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 $T_i$ transcripts occur in thymocytes, which rearrange $T_i$ 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 $T_i$ rearrangements even more rarely than B cells; and nonhematopoietic cells neither rearrange nor transcribe $T_i$ 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 $T_i$ 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 ($T_i$) genes $T_{i\alpha}$ and $T_{i\beta}$ 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 $T_i\beta$ transcripts, as did freshly purified tonsillar B cells. Two of the B cell lines and one of the myeloid lines also expressed truncated $T_i\alpha$ transcripts, while tonsillar B cells did not. Sequence analysis of cDNA clones from a B cell line demonstrated that these truncated $T_i\alpha$ and $T_i\beta$ transcripts were composed of unrearranged J
and C gene segments. Comparison of cDNA clones from T and B cells suggests that Dα genes or N regions contribute to the formation of Tα transcripts in T cells but not in B cells. None of the B cell or myeloid cell lines in this study showed evidence of Tβ gene rearrangements by Southern blotting. Our data, and other studies of gene rearrangements in human tumors, demonstrate that the level of Tβ transcriptional activity and the frequency of Tβ 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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