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7. J. M. C. Ribeiro *et al.*, unpublished data.
8. Rabbit antibody to human CGRP (Accurate Chemical and Scientific Corporation) was used at a dilution of 1:10,000. ^{125}I-Labeled human CGRP (Amersham) was added to give 20,000 cpm per vial. The antibody was precipitated out of solution with 0.5 mg of goat antibody to rabbit immunoglobulin G beads (Bio-Rad).
9. Sand flies were reared by the procedure of G. B. Modi and R. B. Tesh [*J. Med. Entomol.* **20**, 568 (1983)]. Salivary glands of 5- to 7-day-old female flies were removed, put into a solution containing 5 m*M* tris-Cl, *p*H 7.4, and frozen at −70°C until use.
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A New Cluster of Genes Within the Human Major Histocompatibility Complex

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A 435-kilobase (kb) DNA segment, which is centromeric to HLA-B in the human major histocompatibility complex, was isolated by chromosome walking with overlapping cosmids. Within the cloned region, the genes for the tumor necrosis factors (TNFs) α and β and HLA-B were 210 kb apart. The human homolog of a mouse gene, B*144*, was located next to TNFα. Moreover, the presence of additional genes was suggested by a large cluster of CpG islands. With cosmid probes, several distinct transcripts were detected in RNA samples from a variety of cell lines. Altogether, five novel genes were identified by isolation of corresponding complementary DNA clones. These "HLA-B–associated transcripts" (BATs) were mapped to different locations within a 160-kb region that includes the genes for TNFα and TNFβ. The presence of the genes for BAT1 to BAT5 in the vicinity of HLA-B again raises the question of which gene in this region determines susceptibility to ankylosing spondylitis.

THE HUMAN MAJOR HISTOCOMPATIBILITY complex (MHC) includes numerous genes involved in immune system responses. The class I (HLA-A, -B, and -C) and class II (DR, DQ, and DP) gene families encode highly polymorphic cell surface receptors that bind peptides derived from antigen and mediate T cell activation (*1–3*). The class III genes code for components of the complement system (C4A, C4B, Bf, and C2) and steroid 21-hydroxylase (*4*). These three regions are arranged in the order class II, class III, and class I, from centromere to telomere on the short arm of chromosome 6. In addition, the genes for TNFα and TNFβ are located within the MHC (*5*). A 3500-kb molecular linkage map of the entire MHC has been established by pulsed-field gel electrophoresis (PFGE) (*6–8*). The distance between the most telomeric class II locus, DR, and the class III region is about 400 kb. The class III gene C2 is separated from the proximal class I locus, HLA-B, by 600 kb. This interval includes the closely linked genes for TNFα and TNFβ.

Population studies suggest that the MHC region between DR and HLA-B may encode important information. Some HLA-B and DR alleles together represent haplotypes that prevail through strong linkage disequilibrium (*9, 10*). Moreover, susceptibility to a number of diseases is associated with certain HLA-B and DR alleles. Among the tightest correlations is that between HLA-B27 and the rheumatoid disease ankylosing spondylitis (*11, 12*).

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In the present study, the possibility that unidentified genes are located in proximity to HLA-B has been explored. Linkage data generated by PFGE in combination with cosmid cloning (this report) have localized the TNFα and TNFβ gene pair about 200 kb centromeric of HLA-B (*7*). The genes for the two TNFs are contained together within 6 kb and are in the same transcriptional orientation (*13*). TNFβ is nearest to HLA-B, and the 5′ ends of these two genes are directed toward each other (*7*). The genetic organization in the mouse H-2 complex is similar, except that the TNFβ gene is only 70 kb distant from H-2D, the murine homolog of HLA-B (*14*). These facts guided a bidirectional chromosome walk between the genes for the TNFs and HLA-B.

Cosmid libraries were screened with labeled TNFα cDNA and a locus-specific genomic probe for HLA-B, and isolated clones were analyzed by restriction mapping and DNA blot hybridization. The HLA-B gene was encoded in the cosmids M20A and O32A (Fig. 1). Its position and relative orientation were determined with probes for the 5′ and 3′ ends, respectively. The cosmids M2A, M31A, O19A, O30A, and O31A contained the genes for TNFα and TNFβ (Fig. 1). The arrangement of the two genes was inferred from hybridization experiments in which the corresponding probes were used individually. The results obtained were in accord with published data (*13*). To expand the cloned TNF and HLA-B regions toward each other, overlapping series of cosmids were isolated with walking probes (wp's) that were prepared from several locations upstream from the genes for TNFβ and HLA-B, respectively (*15*). Thus, the genes for the TNFs and HLA-B were linked within a continuous stretch of cosmids in a 210-kb distance (Fig. 1). This result was corroborated by data derived from PFGE (*7, 8*). Additional cosmids were cloned downstream from TNFα (*15*). Altogether, a total of 435 kb was obtained in a contiguous cosmid cluster, currently the largest cloned segment of the human genome. Upon alignment with a PFGE linkage map, the centromeric breakpoint of the cloned region was about 200 kb from the C2 gene in the MHC class III complement gene cluster (*8*).

In the mouse H-2 complex, the gene B*144*, which is transcribed specifically in B cells and macrophages, is located 10 kb downstream from TNFα (*16*). The human B*144* homolog was found in a corresponding position within the cosmid O30A by DNA blot hybridization with the mouse cDNA probe (Fig. 1).

Within the cloned region, an accumulation of Bss HII and Sac II sites, which are relatively rare in mammalian genomes, was

mapped to a 50-kb segment about 40 kb downstream from the TNFα gene (Fig. 1). Cleavage by Bss HII and Sac II frequently identifies CpG-rich sequences, which constitute a minor fraction in mammalian DNA and are often associated with the 5′ ends of genes (*17, 18*). This correlation was exemplified by the presence of three tightly spaced Bss HII and Sac II sites within the 5′ region of the HLA-B gene (Fig. 1). These observations raised the possibility that additional genes were encoded within the cloned region, most likely centromeric of TNFα.

RNA blots of fractionated samples of polyadenylated [poly(A)$^+$] RNA from the cell lines HPB-ALL (T cell), Raji (B cell), U937 (monocyte), HeLa, and M1XP (fibroblast) were probed with a selection of cosmid inserts representing the region between the cosmid R13B and the HLA-B gene. To prevent hybridization of probes to highly repeated sequences, we used a preannealing procedure. Two transcripts of about 1.7 and 6 kb were detected with the M9A and R13B cosmid probes, respectively. These "HLA-B–associated transcripts" 1 and 2 (BAT1 and BAT2) were found in all

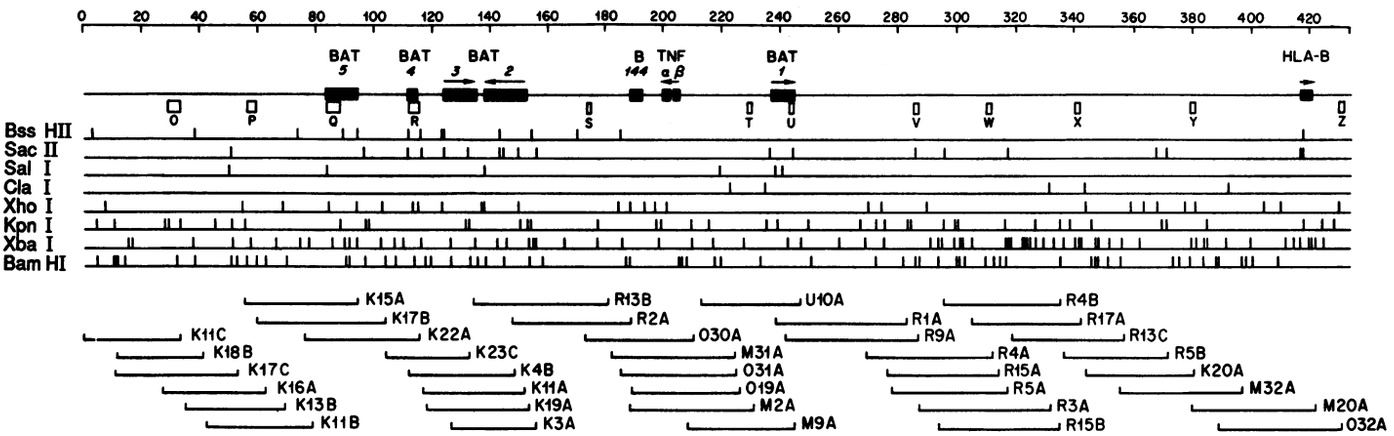

Fig. 1. Molecular structure of the 435-kb MHC region centromeric of HLA-B. This genomic segment is defined by a series of cosmids shown at the bottom. They were mapped by means of the restriction enzymes Bss HII, Sac II, Sal I, Cla I, Xho I, Kpn I, Xba I, and Bam HI. The top line gives the scale (in kilobases). Closed boxes refer to genes. Arrows show the direction of transcription of genes. The orientation of the genes for BAT1 to BAT3 is based on nucleotide sequence data (*20*). Open boxes indicate locations of walking probes (wp's). The cosmid library and the screening procedure have been described (*21*). DNA probes were labeled with [^{32}P]dCTP by the random hexamer priming procedure (*22*). The HLA-B locus-specific probe was the 885-bp Xba I fragment (3′ untranslated region) from a genomic subclone, B7-14 (*23*). A 665-bp Eco RI/Aha II fragment from the same source was used as a probe for the 5′ end of HLA-B. The TNFα cDNA probe was the 650-bp Pst I insert from phTNF5 (*24*). The TNFβ probe was the 380-bp Pst I/Eco RI fragment (exon IV) from a genomic 2.4-kb Eco RI subclone (*13*). A 350-bp Pst I/Bam HI mouse cDNA fragment was used as a probe for the B*144* gene (*16, 31*). After hybridization with the B*144* probe, the final wash was in 2× standard saline citrate (SSC), at 65°C. The wp's *S*, *T*, *U*, *V*, *W*, *X*, *Y*, *Z* were 200- to 600-bp Alu I, Hae III, or Rsa I fragments derived from the cosmids O30A, M2A, M9A, R9A, R5A, R17A, M20A, and O32A, respectively. These probes were subcloned and selected for absence of repeat sequences (*21*). By means of a modification of another protocol (*25*), the wp's *O*, *P*, *Q*, and *R* were prepared from 3- to 6-kb restriction fragments isolated from the cosmids K16A, K15A, K22A, and K23C, respectively. DNA samples were digested with Sau 3AI, labeled, heat denatured in the presence of sonicated human total genomic DNA (10 mg/ml; average size 300 to 800 bp) in 6× SSC, incubated on ice for 1 min, and preannealed for 10 min at 68°C. This procedure was also used to process entire 35- to 40-kb cosmid inserts for probing RNA blots and cDNA libraries. The two cDNA libraries used to obtain clones for BAT1 through BAT5 were from the human B and T cell lines JY and HPB-ALL, respectively (*26, 27, 31*).

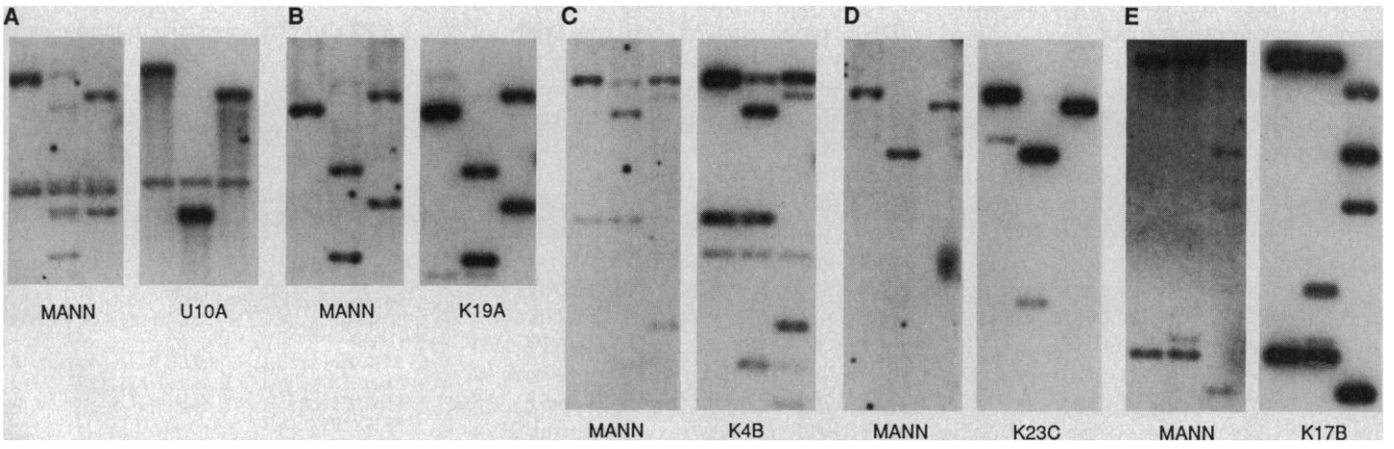

Fig. 2. Localization of the genes for BAT1 through BAT5 within cloned cosmids and detection in total genomic DNA by DNA blot analysis (*28*). In parallel experiments, total Mann cell DNA [left panel in (A) to (E)] and cosmid clones [right panel in (A) to (E)] were compared. (**A**) The BAT1-3 cDNA probe hybridized to the cosmid U10A and identified corresponding fragments in total genomic DNA. Digests were with Xba I, Xba I/Kpn I, and Xba I/Bam HI, respectively. In (B) to (E), all the bands displayed on the total genomic DNA blots with the BAT2 to BAT5 probes were matched by cosmid fragments. (**B**) Hybridization of the BAT2-5 cDNA probe to the cosmid K19A, digested with Bam HI, Bam HI/Xba I, and Xba I, respectively. (**C**) Hybridization of the BAT3-15 cDNA probe to the cosmid K4B, digested with Bam HI, Bam HI/Kpn I, and Bam HI/Xba I, respectively. (**D**) Hybridization of the BAT4-3 cDNA probe to the cosmid K23C, digested as in (B). (**E**) Hybridization of the BAT5-1 cDNA probe to the cosmid K17B, digested as in (C).

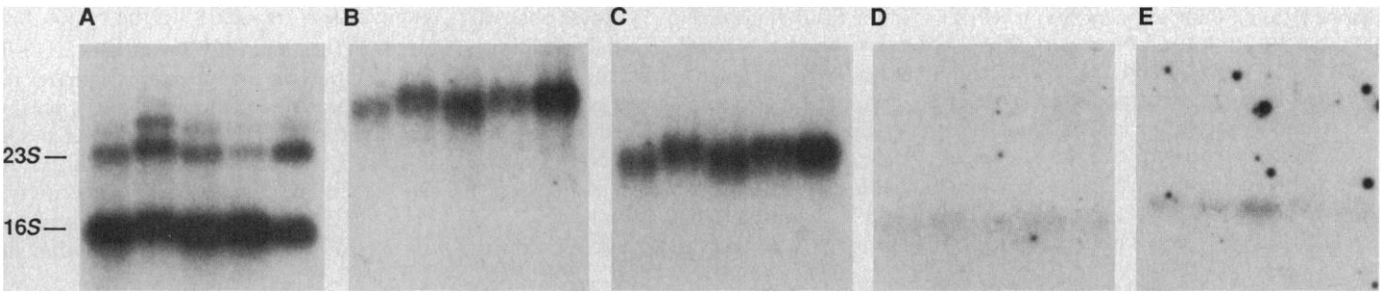

Fig. 3. Northern blot analysis of the expression of the genes for BAT1 through BAT5. Lanes 1 to 5 in (A) to (E) include poly(A)$^+$ RNA samples (2 μg each) from HPB-ALL, Raji, U937, Hela, and M1XP, respectively, separated by electrophoresis in 1% agarose-formaldehyde gels (*28–30*). (**A**) The BAT1-3 cDNA probe detected the corresponding 1.7-kb transcript as well as two additional mRNAs of 4 and 5 kb, respectively. (**B** and **C**) Single transcripts of 6 and 4 kb hybridized to the BAT2-5 and BAT3-15 cDNA probes, respectively. (**D** and **E**) The mRNAs hybridizing to the BAT4-3 and BAT5-1 cDNA probes were 1.6 and 2 kb in length, respectively, and were expressed at very low levels.

Table 1. Length of BAT1 to BAT5 cDNAs and mRNAs.

Gene	cDNA (kb)	mRNA (kb)
BAT1	1.7	1.7
BAT2	5.0	6.0
BAT3	3.8	4.0
BAT4	0.9	1.6
BAT5	2.0	2.0

of the five cell lines tested (see below).

A number of BAT1 cDNA clones were isolated from a JY library with the M9A cosmid probe. Most of the cDNAs were 1.7 kb long and had matching restriction maps. The clone BAT1-3 was used to localize the BAT1 gene within the overlapping cosmids M9A and U10A, 30 kb upstream from TNFβ (Fig. 1). A DNA blot of total genomic DNA displayed corresponding bands (Fig. 2A). However, some additional fragments hybridizing to the BAT1-3 probe were not accounted for by the cosmid U10A, although it included the complete 8-kb BAT1 gene. Moreover, these fragments were absent from the entire 435-kb cloned region. Thus, at least one additional gene closely related to BAT1 is encoded in the genome, most likely outside the MHC. This idea was supported by two more cDNAs, BAT1-2 and BAT1-36, which were distinct from BAT1-3. These 3-kb clones comprised a section hybridizing strongly to BAT1-3 as well as unique sequences.

BAT2 cDNA clones were obtained by screening a HPB-ALL library with the R13B cosmid probe. The 5-kb clone BAT2-5 was the longest of several homologous cDNAs with different truncation sites (Table 1). DNA blot analysis of the overlapping cosmids R13B and K19A next to total genomic DNA demonstrated that the BAT2 gene was a 13-kb locus 48 kb downstream from TNFα (Fig. 2B). To address the possibility that additional genes were encoded adjacent to BAT2, the HPB-ALL cDNA library was screened with a number of cosmid probes comprising the region between the BAT2 gene and the centromeric end of the cosmid cluster. Two distinct sets of cDNAs were isolated with the K4B cosmid probe. DNA blot analysis established that two genes, BAT3 and BAT4, were included in the cosmid K4B (Fig. 2, C and D). The longest BAT3 cDNA, BAT3-15, was 3.8 kb. The location of the 12-kb BAT3 gene was only 2.5 kb from BAT2 (Fig. 1). For BAT4, two 900-bp cDNAs were obtained. By means of the clone BAT4-3, the BAT4 gene was mapped within the overlapping cosmids K4B and K23C 9 kb from BAT3 (Fig. 2D). After another library screening, a single 2-kb cDNA, BAT5-1, was isolated with the K17B cosmid probe. DNA blot hybridization showed that the BAT5 gene, like the genes for BAT2, BAT3, and BAT4, was a single-copy sequence (Fig. 2E). The location of the 10-kb BAT5 gene was 18 kb from BAT4. No additional cDNA clones were identified upon screening the HPB-ALL library with the K16A and K11C cosmid probes.

RNA blot analysis with the BAT1-3 cDNA probe showed two transcripts of about 4 and 5 kb in addition to the 1.7-kb BAT1 mRNA (Fig. 3A). This result supported the data that suggested the existence of at least one more gene highly homologous to BAT1 (Fig. 2A). The BAT2 and BAT3 mRNAs were about 6 and 4 kb in length, respectively, and were expressed at levels comparable to BAT1 (Fig. 3, B and C, and Table 1). As inferred from cDNA cloning, the abundance of these mRNAs was about 0.02%. In contrast, the BAT4 (1.6 kb) and BAT5 (2 kb) transcripts were at most 1/20 as frequent (Fig. 3, D and E). All of the BAT mRNAs were present in all of the cell lines examined (Fig. 3, A to E), in agreement with the idea that most genes associated with CpG islands are ubiquitously expressed (*18*). Thus, BAT2 and BAT3, as well as BAT4 and BAT5, may represent gene pairs, with the rate of transcription coordinately regulated within each pair, and each pair coding for products that are related or complementary in function.

In conclusion, a cluster of eight genes is contained within a 160-kb MHC segment between the class I gene for HLA-B and the class III region. At present, the structure and function of the genes for BAT1 to BAT5 are unknown. Further investigation will determine whether they represent a family of genes with common structures, functions, or both, as are the class I, II, and III genes of the MHC. Their location in the vicinity of HLA-B raises the question of whether the association of ankylosing spondylitis with HLA-B27 is due to this allele or rather to a variant of another gene in this region. A number of restriction fragment length polymorphisms (RFLPs) have already been identified with probes, both wp's and cDNAs from different locations within the cloned 435-kb region (*19*). Segregation analysis of these RFLPs should resolve this controversy. Finally, virtually all of the human MHC genes are involved in the immune system. Therefore, the question of whether the products of the genes for BAT1 to BAT5 also play some role in immunity is of interest.

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15. From distal locations in the cosmids M2A and M20A, wp's *T* and *Y* were derived (Fig. 1). With wp *T*, M9A and U10A were cloned. Restriction mapping confirmed that these cosmids overlapped M2A. Similarly, M32A, which hybridized to wp *Y*, shared diagnostic restriction fragments with M20A. The chromosome walk was continued by extension of the TNF cosmid cluster; the cosmid library was successively screened four times. From M9A, wp *U* was prepared to clone R1A and R9A. The subsequent series of cosmids, R4A, R15A, and R5A was obtained with wp *V* from R9A. From R5A, wp *W* was derived to isolate R3A, R4B, R15B, and R17A. Finally, R13C and R5B were identified with wp *X* from R17A. They were matched by another cosmid, K20A, which had previously been found with wp *V* from R9A. Therefore, a DNA segment including wp *V* must be duplicated within the cloned region. Inspection of restriction maps revealed a definitive overlap among R5B, K20A, and M32A. Thus, the genes for the TNFs and HLA-B were linked. Cosmid clones downstream from TNFα were also sought. R2A and R13B were isolated with wp *S* from O30A. A 600-bp Xba I fragment from BAT2-5 cDNA was the probe for cloning K3A, K19A, K11A, and K4B. From K4B, wp *R* was prepared to isolate K23C and K22A. Screening with wp *Q* from K22A yielded K17B and K15A, and screening with wp *P* from K15A yielded K11B, K13B, and K16A. In the last walking step, K17C, K18B, and K11C were identified with wp *O* from K16A.
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Polymerase Chain Reaction with Single-Sided Specificity: Analysis of T Cell Receptor δ Chain

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In the polymerase chain reaction (PCR), two specific oligonucleotide primers are used to amplify the sequences between them. However, this technique is not suitable for amplifying genes that encode molecules where the 5′ portion of the sequences of interest is not known, such as the T cell receptor (TCR) or immunoglobulins. Because of this limitation, a novel technique, anchored polymerase chain reaction (A-PCR), was devised that requires sequence specificity only on the 3′ end of the target fragment. It was used to analyze TCR δ chain mRNA's from human peripheral blood γδ T cells. Most of these cells had a Vδ gene segment not previously described ($V_\delta 3$), and the δ chain junctional sequences formed a discrete subpopulation compared with those previously reported.

THE POLYMERASE CHAIN REACTION (PCR) is useful in the analysis of DNA or RNA sequences from minute quantities of starting material (*1*). PCR, however, requires sequence information on both sides of the region of interest, and thus is of limited use in the analysis of sequences that have variable termini, such as those of the T cell antigen receptor (TCR) or immunoglobulins. We now show that homopolymer tailing of the variable (V) region end of T cell receptor cDNA's and the attachment of an "anchor" sequence can effectively substitute for the unknown sequence information. With an anchor primer and primers complementary to the constant (C) or joining (J) regions, T cell receptor mRNA's from small amounts of total RNA can be rapidly amplified and analyzed. We term this procedure anchored polymerase chain reaction (A-PCR) and use it to analyze the diversity of TCR δ chain mRNA's derived from human peripheral blood lymphocytes.

Most T cells in higher organisms express the αβ TCR heterodimer and are of the helper or cytotoxic subsets (*2*). Another TCR heterodimer, γδ (*3*), whose genes have been isolated and characterized (*4–6*), is present on up to 10% (*3*) of normal human peripheral blood lymphocytes of unknown function. The γδ TCR has a limited number of germline V regions available, despite a large amount of junctional diversity (*2, 6, 7*). There are 11 human V_γ genes identified, versus 50 to 100 for V_β and V_α (*8*). Only two V_δ genes have been identified in humans. $V_\delta 1$ is the predominant gene used in thymocytes, but the monoclonal antibody (MAb) TCSδ1 for $V_\delta 1$ stains only a small fraction of peripheral blood γδ T cell lines (*9*). Thus, peripheral blood γδ bearing T cells present an interesting target population for A-PCR analysis.

In this A-PCR procedure (Fig. 1), mRNA is first transcribed with reverse transcriptase and a poly(dG) tail is added to the 3′ end of the strand with terminal deoxynucleotidyl transferase (TdT). The product is then amplified with a specific 3′ primer (in this case a $J_\delta 1$ oligonucleotide) and another oligonucleotide consisting of a poly(dC) tail attached to a sequence with convenient restriction sites, termed the "anchor" (*10*). In pilot experiments, A-PCR generated a single band with known cloned cDNA sequences that had either 12 or 22 G's at the 5′ end (due to the way they had been cloned). The first round of DNA synthesis was primed with the anchor-poly(dC) primer (ANpolyC), and subsequent amplification of the poly(dG)-bearing strand was performed with either ANpolyC or the anchor (AN) primer [without the poly(dC) tail] along with the specific 3′ primer. These gave roughly equivalent results. The procedure was then tested with total RNA (3 μg) from the Molt 13 and Peer cell lines, which express δ chain mRNA. First strand synthesis was primed with either a C_δ region primer or with poly(dT). The subsequent amplification was done with ANpolyC and a J region primer (Fig. 1). When the colonies containing the amplified cDNA's were screened with a $V_\delta 1$ region probe, approximately 10% were positive. DNA from several of these colonies were sequenced, and the sequences agreed with the published sequences (*11*). This demonstrated that A-PCR can isolate the receptors from cell lines, starting with relatively small amounts of total RNA (equivalent to 10^6 cells).

We then used total RNA (~5 μg) from a polyclonal cell line, established from interleukin-2 (IL-2)–stimulated peripheral blood

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