Autoaggressive Myocytotoxic T Lymphocytes Expressing an Unusual γ/δ T Cell Receptor

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Summary

Polymyositis mediated by γ/δ T cells is a unique disease in which autoaggressive T lymphocytes surround, invade, and destroy muscle fibers. Histochemically, the vast majority of muscle-infiltrating T cells in a patient with polymyositis were reactive with a pan-γ/δ T cell receptor (TCR)-specific monoclonal antibody (TCR-δ1+), but unlike >90% of peripheral blood γ/δ T cells, these lymphocytes did not react with Vδ1- or Vγ9-specific antibodies (A13− and TiγA−, respectively). Differential reactivity with two different Vδ2-specific monoclonal antibodies (BB3−/T(Vδ2+) indicated that the infiltrating T cells express a Vδ2-containing TCR with unusual additional structural features. Using conventional and anchored polymerase chain reaction for the analysis of TCR transcripts, we found a striking predominance of one unusual Vδ2-Jδ3 recombination and one Vγ3-Jγ1 recombination. Both the unusual phenotype (TCR-δ1+/A13−/TiγA−/BB3−/T(Vδ2+) and the dominance of distinct TCR transcripts are compatible with the assumption that one T cell clone, which expresses a Vγ3-Jγ1-Cγ1/Vδ2-Jδ3-Cδ disulfide-linked TCR, dominates among the infiltrating T cells of the polymyositis muscle specimen analyzed.

Although γ/δ T cells are suspected to be involved in autoimmune immunity, there is little direct evidence to support this hypothesis in human autoimmune disease. One notable exception is a recently described inflammatory muscle disease that represents a variant of polymyositis (1). In this disease autoaggressive γ/δ T cells can be demonstrated to surround, invade, and destroy muscle fibers (1). Our analysis of TCR transcripts of the myocytotoxic T cells provides the first complete structural description of a TCR used by autoaggressive human γ/δ T cells.

Materials and Methods

Clinical Material. A biopsy specimen of the left triceps muscle of a patient previously described (1) was taken before treatment and was divided for immunocytochemical studies and for RNA extraction.

Immunohistology. 4-μm cryostat sections of muscle were used for single and paired immunofluorescence studies and for RNA extraction.

Analysis of TCR Transcripts. Total RNA was prepared from a sample (25 mg) of the muscle biopsy specimen by the acid guanidinium thiocyanate phenol-chloroform method (2) and its integrity verified by running a portion of the material on an agarose gel. First-strand cDNA was prepared from total RNA using a poly(dT) primer and AMV reverse transcriptase (Boehringer, Mannheim, Germany) (3). PCR was carried out as described previously (3, 4) with 0.5 μM of oligonucleotide primers and 1 U of Taq polymerase (Bethesda Research Laboratories, Gaithersburg, MD) using incubation times of 30 s at 93°C, 1 min at 50°C, and 1 min at 72°C in a reaction volume of 100 μL. DNA bands of the expected size were isolated from 1% agarose gels and blunt-end cloned into the Smal site of Bluescript KS (Stratagene, La Jolla, CA) (3). Double-
stranded plasmid DNAs were sequenced directly with the Sequenase kit (U.S. Biochemical Co., Cleveland, OH).

PCR amplification of δ chain cDNA was accomplished by use of a Cδ antisense primer (GACAAGCGACATTTGGTCCCA) in combination with either a Vδ2 sense primer (CAAGAAACCTGGGCTGATC) or a set of Vδ3 sense primers (Vδ3: GCAGCGCTGAGACACAGAT or with a Jδ3 (GGCTCACGAAGATTTGGTAC) antisense primer. In one experiment the relative abundance of Vδ2-Jδ1 and Vδ2-Jδ3 rearrangements in cDNA derived from the muscle biopsy, and from Ficoll-purified PBMC of a healthy donor, were compared. Here amplifications were done using a Vδ2 sense primer (GCAGGAGTCATGTCAGCCAT) either in combination with a Jδ1 (GGTTCCACAGTCACACGGGT) or with a Jδ3 (GGCTCACGAAGATTTGGTAC) antisense primer.

For the analysis of the junctional regions of γ chain transcripts, cDNA derived from the muscle biopsy was amplified using one-sided PCR carried out by a modification (4) of a published method (5). cDNA was size fractionated by Bio-Gel A-5m (Bio-Rad Laboratories, Richmond, CA) column chromatography and an oligo(dG)-tail was added to the 3' end of cDNAs of appropriate size using terminal deoxynucleotide transferase (Bethesda Research Laboratories) (4). Two successive amplifications of the oligo(dG)-tailed cDNA were accomplished with an oligo(dC) primer (dC16) in combination with one of two different Cγ antisense primers (GGGTAGGTCATGTCAGCCAC or with the nested Cγ primer AAGGAAAGAAAATAGGGC). Analysis of γ chain transcripts by conventional PCR was done with Cγ (exon 1) antisense primers (AAGGAAAGAAAATAGGGC) in combination with a set of Vγ sense primers (Vγ subgroup I: TGCTTCTAGCTTTCCTGG; Vγ9: ATCAACGCTGGCAGTCC; Vγ10: TCTCTCCTGGCTCCTGA; Vγ11: TCTCTCCTGGCTCCTGG) or with Cγ (exon 3) antisense primers (TGAGGAGCAGGAGTGAC) in combination with a Vγ subgroup I sense primer (TGAGGAGCAGGAGTGAC). Since the different Vγ gene segments of subgroup I are highly homologous (6), it was possible to design universal primers for the amplification of rearrangements with all members of this subgroup.

Results and Discussion

For the analysis of the structure and heterogeneity of TCR transcripts from muscle-infiltrating γ/δ T cells, RNA was isolated from a polymyositis biopsy specimen that had previously been characterized by immunohistology (1). RNA was transcribed into cDNA, and TCR-specific cDNA was amplified either by conventional or by anchored PCR. PCR products of expected size were cloned and the sequences of independently isolated clones analyzed. A set of sense primers for the most commonly used Vδ gene segments, Vδ1, Vδ2, and Vδ3 (7), was used in combination with a Cδ antisense primer for the amplification of cDNA derived from TCR δ chain transcripts. Only Vδ2 PCR products were obtained and only one unique rearrangement was found when randomly chosen clones were analyzed (Fig. 1 A). One Dδ gene segment and nongermline-encoded nucleotides at the V-D and the D-J junctions contributed to the junctional region of this dominant transcript. While both ends of the Dδ gene segment were shortened, the entire coding sequences of the Vδ and the Jδ gene segments were conserved in this rearrangement. To rule out the possibility that the dominance of this sequence was the result of an accidental preferential amplification of one particular cDNA, we performed a second independent amplification using a different Vδ2-specific sense

Table 1

| Vδ2 | PN | Dδ3 | PN | Jδ3 | Frequency |
|-----|----|------|----|-----|------------|
| A   | TGT GCC TGT GAC ACC | GT | TGTGCCGAAG | CCTCAAAAGAG C TCC TGG GAC | 9/10 |
|     | TGT GCC TGT GAC ACC | GT | TGTGCCGAAG | CCTCAAAAGAG C TCC TGG GAC | 1/10 |
| B   | TGT GCC TGT GAC ACC | GT | TGTGCCGAAG | CCTCAAAAGAG C TCC TGG GAC | 4/6 |
|     | TGT GCC TGT GAC ACC | GT | TTGGGGGAA | CCTCAAAAGAG C TCC TGG GAC | 1/6 |
|     | TGT GCC TGT GAC ACC | GT | CAAGGGGAA | CCTCAAAAGAG C TCC TGG GAC | 1/6 |

Germline

Vδ2 | TGT GCC TGT GAC ACC | GT | TGTGCCGAAG | CCTCAAAAGAG C TCC TGG GAC

Dδ3 | AAGGAAAGAAAATAGGGC

Jδ3 | AAGGAAAGAAAATAGGGC

Figure 1. Junctional regions of δ chain cDNA sequences from muscle-infiltrating γ/δ T cells. The 3' and the 5' ends of the V, D, and J regions were determined from genomic clones (8, 9). PCR amplification of the δ chain cDNA was accomplished by use of a Cδ antisense primer in combination with a set of sense primers for Vδ1, Vδ2, and Vδ3 (4), or a second different Vδ2 sense primer (B).
Table 3. Junctional regions of γ chain cDNA sequences from muscle-infiltrating γδ T cells. The 3' and the 5' ends of the V and J regions, respectively, were determined from genomic clones (6, 14). Five Jγ segments have been identified, with Jγ1 being identical in amino acid sequence to Jγ2. Jγ1 is located upstream of Cγ1, whereas Jγ2 is located between Cγ1 and Cγ2 (14, 15). cDNA was amplified by single-sided PCR (A), by conventional PCR using a Cγ3 (exon 1) antisense primer in combination with a set of sense primers for the different Vγ families (B), or by conventional PCR using a Cγ3 (exon 3) antisense primer in combination with a Vγ subgroup I sense primer (C).

| Vγ | PIN | Jγ | Frequency |
|----|-----|----|-----------|
| A  | TGT GCC ACC TGG GAC | AT TAT TAT AAG AAA | 14/14 |
| B  | TGT GCC ACC TGG GAC | AT TAT TAT AAG AAA | 5/5 |
| C  | TGT GCC ACC TGG GAC | AT TAT TAT AAG AAA | 10/13 |
|    | TGT GCC ACC TGG GAC | AT TAT TAT AAG AAA | 1/13 |
|    | TGT GCC ACC TGG GAC | AT TAT TAT AAG AAA | 2/13 |

primer. All clones derived from this second amplification product were identical to the rearrangement observed in the first experiment (Fig. 1 B). A few of the analyzed junctional sequences differed from the others by a single nucleotide exchange. Since the fidelity of the Thermus aquaticus (Taq) DNA polymerase in DNA amplification is relatively low, such polymerase-induced point mutations tend to accumulate during PCR amplification (10).

A high proportion of γδ T cells in peripheral blood express a limited receptor repertoire: Vδ2-Jβ1-Cδ usually pairs with Vγ9-Jγ4-Cγ1 (11, 12). Vδ2-Jβ3 rearrangements such as the one observed here have previously been described in γδ T cell clones (13), but are rare in peripheral blood T cells. To further verify the dominance of Vδ2-Jβ3 transcripts in the muscle biopsy, PCR was performed with a Vδ2 sense primer in combination with either a Jβ3 or a Jβ1 antisense primer. Whereas only a Vδ2-Jβ3-PCR band was observed when cDNA from the muscle biopsy was used as a template, a Vδ2-Jβ1-band was dominant in reactions with cDNA derived from PBL (Fig. 2). This demonstrates that the majority of γδ T cells in the biopsy represent a unique subpopulation rather than a polyclonal infiltrate of peripheral T cells. This conclusion is further supported by the observation that >90% of the γδ T cells present in the blood at the time of biopsy had a different phenotype (BB3', Tγ1A') than the infiltrating γδ T cells (BB3', Tγ1A') (1).

For the analysis of TCR γ chain transcripts a modification of the single-sided PCR amplification method (4, 5) was used that requires sequence information only from the 3' end of the target receptor cDNA (constant gene segment) and therefore allows V segment sequence-independent amplification. PCR products in the predicted size range were cloned and the sequences of a set of randomly chosen isolates were determined. All 14 sequences analyzed were identical, representing a Vγ3-Jγ1 recombination with addition of four nongermline-encoded nucleotides and shortening of both the V and the J coding region by imprecise joining (Fig. 3 A). The same sequence was obtained when an independent second amplification was performed by conventional PCR with a set of sense primers for all eight functional human Vγ gene segments (Fig. 3 B).

Of the 14 Vγ gene segments that have been identified in the human genome, six are considered to be pseudogenes. The functional genes fall into four distinct subgroups (15). Whereas five functional genes (Vγ2, Vγ4, Vγ5, and Vγ8, in addition to the Vγ3 gene segment found in the transcripts analyzed here) belong to subgroup I, subgroups II, III, and IV each consist of a single gene (Vγ9, Vγ10, Vγ11, respectively). The degree of variation of the γ chains is increased by the existence of polymorphic γ constant region gene segments (Cγ). The Cγ1 gene segment consists of three exons (ex1, ex2, ex3), whereas Cγ2 contains in some cases two and in others three copies of exon 2 (15, 16). Exons 1 and 3 of both gene segments are virtually identical. The Cγ1 gene products possess a cysteine residue in the second exon that is responsible for disulfide bond formation with the δ chain, while the corresponding exon 2 of Cγ2 lacks the cysteine. It is not known whether these structural differences between...
the Cγ gene segments affect the functional properties of the TCR. It is known, however, that the Vγ and the Jγ segments associated with Cγ1 and Cγ2 differ at least in peripheral blood γ/δ T cells, where Cγ1 is preferentially rearranged with Vγ9/JγP and Cγ2 with Vγ2/Jγ1 (17). To analyze the Cγ gene usage in the muscle-invading T cells, we performed PCR with a Vγ subgroup I sense primer in combination with V3'9/J'yP and C'y2 with Vqr2/J3d (17). To address this problem, we used another Vδ2-specific mAb, T,V-Vδ2 (19), for additional immunohistochemical studies. All the T cells surrounding and invading muscle fibers reacted with T,V-Vδ2 (Fig. 4), indicating that these cells express Vδ2, although in a form that is not recognized by antibody BB3. Hence, both sequence analyses and antibody reactivities are consistent with the assumption that the autoaggressive lymphocytes express a Vγ3-J'y1-C'y1/Vδ2-Jδ3-Cδ TCR that is unusual both with respect to its Vδ-Jδ recombination and its specific Vy/Vγ combination.

In conclusion, our results provide the first complete description of a human γ/δ TCR expressed by autoaggressive T cells. A striking predominance of one clone of T cells was observed in the infiltratory muscle infiltrate. This clone uses an unusual combination of TCR V and J segments and is associated with a TCR phenotype that has not previously been described among the major populations of human γ/δ T cells. It should be noted that Mycobacterium and heat shock protein-responsive γ/δ T cells, which have been postulated to participate in autoimmune processes (20), express dominantly Vγ9-J'yP/Vδ2-Jδ1 TCR variable regions (21–23). We anticipate that the molecular characterization of the Vγ3-J'y1-C'y1/Vδ2-Jδ3-Cδ TCR associated with the myocytotoxic T cells, which share only the Vδ2 segment with the known heat shock protein-reactive T cells, may eventually help to identify the unknown autoantigen in this disease.

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