Brief Definitive Report

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$_i$V-δ2$^+$) 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$_i$V-δ2$^+$) 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 autoimmunity, 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. The following antibodies were used: anti-CD3 (5 μg/ml; Dako Corp., Carpenteria, CA); anti-TCR-δ1 (5 μg/ml; T Cell Sciences, Cambridge, MA); TiγA (5 μg/ml; T. Hercend, Institute Gustave Roussy, Villejuif, France); T$_i$V-δ2 (2 μg/ml; T. Hercend); A13 and BB3 (6 μg/ml; L. Moretta, Istituto Nazionale Per La Ricerca Sul Cancro, Genova, Italy). All antibodies were mouse mAbs except anti-CD3, which was an affinity-purified rabbit polyclonal antibody. The primary mouse mAbs were detected using a biotin-labeled second antibody followed by treatment either with rhodamine-avidin or with FITC-avidin. The rabbit anti-CD3 primary antibody was detected using rhodamine-labeled goat anti-rabbit IgG. Isotype-matched IgG at identical concentrations was substituted for primary antibodies for negative controls. Smears of PBMC and thymocytes were used as positive controls for antibodies specific for subsets of γ/δ T cells. Tissue samples used as controls included sections from patients with different inflammatory myopathies and from normal control subjects with no evidence of muscle disease.

Analysis of TCR Transcripts. Total RNA was prepared from a sample (25 mg) of the muscle biopsy specimen by the acid guanidinium thyocyanate 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 SmaI site of Bluescript KS (Stratagene, La Jolla, CA) (3). Double-

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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 (GACAAGCGACATTTGTTCCA) in combination with either a Vδ2 sense primer (CAAAGAACCTGGCTGTAC) or a set of Vδ sense primers (Vδ1: TACTCTGGATCAAGTGTGGC; Vδ2: GCAGGAGTCATGTCAGCCAT; Vδ3: GGCACGCTGTGTGACAAAGT). 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 (GGCTCCACGAAGAGTTTGAT) antisense primer.

For the analysis of the junctional regions of γ chain transcripts, cDNA derived from the muscle biopsy was amplified using single-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 (dC_{16}) in combination with one of two different Cγ (exon 1) antisense primers (GGTATGTTCCAGCCTTCTGG or the nested Cγ primer AAGGAAGAAAAATAGTGGGC). Analysis of γ chain transcripts by conventional PCR was done with Cγ (exon 1) antisense primers (AAGGAAGAAAAATAGTGGGC) in combination with a set of Vγ sense primers (Vγ subgroup I: TGCTTCTAGCTTTCCTG; Vγ9: ATCAACGCTGGCAGTCC; Vγ10: TCCTCCTGGGCTCCTGA; Vγ11: TTCTTCTCCCTCTGGGT) or with Cγ (exon 3) antisense primers (TGAGGAGCAGGAGGAGGTAC) in combination with a Vγ subgroup I sense primer (TGCAGCCAGTCAGAAATCTT). 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.

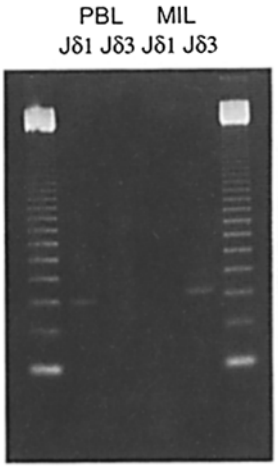

Figure 2. Vδ2-Jδ1 and Vδ2-Jδ3 TCR gene rearrangements expressed in muscle-infiltrating lymphocytes (MIL) and PBL. Agarose gel analysis of ethidium bromide–stained PCR amplification products. cDNA obtained from the muscle biopsy or from Ficoll-purified mononuclear cells from a healthy donor was amplified with a Vδ2 sense primer in combination with either a Jδ1 or a Jδ3 antisense primer.

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

	Vδ2	P/N	Dδ3	P/N	Jδ3	Frequency
A						
	TGT GCC TGT GAC ACC	GT	CTGGGGGA	CCTCAAAGAG	C TCC TGG GAC	9/10
	TGT GCC TGT GAC ACC	GT	CTGGGGGA	CCTCTAAGAG	C TCC TGG GAC	1/10
B						
	TGT GCC TGT GAC ACC	GT	CTGGGGGA	CCTCAAAGAG	C TCC TGG GAC	4/6
	TGT GCC TGT GAC ACC	GT	TTGGGGGA	CCTCAAAGAG	C TCC TGG GAC	1/6
	TGT GCC TGT GAC ACC	GT	CAGGGGGA	CCTCAAAGAG	C TCC TGG GAC	1/6
germline						
Vδ2	TGT GCC TGT GAC ACC cacoctg					
Dδ3			cactgtg ACTGGGGGATACG cacagtg			
Jδ3					taatgtg C TCC TGG GAC	

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 (*A*), or a second different Vδ2 sense primer (*B*).

	Vγ3	P/N	Jγ1	Frequency
A				
	TGT GCC ACC TGG GAC	GGTT	AT TAT TAT AAG AAA	14/14
B				
	TGT GCC ACC TGG GAC	GGTT	AT TAT TAT AAG AAA	5/5
C				
	TGT GCC ACC TGG GAC	GGTT	AT TAT TAT AAG AAA	10/13
	TGT GCC ACC TGG GAC	GGTT	AT TAT TAT AAG AAG	1/13
	TGT GCC ACC TGG GAC A	CAGAAG	AT TAT AAG AAA	2/13
germline	TGT GCC ACC TGG GAC AGGcacagtg		cactgtgG AAT TAT TAT AAG AAA	

Figure 3. Junctional regions of γ chain cDNA sequences from muscle-infiltrating γ/δ 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 Cγ (exon 1) antisense primers in combination with a set of sense primers for the different Vγ families (*B*), or by conventional PCR using a Cγ (exon 3) antisense primers in combination with a Vγ subgroup I sense primer (*C*).

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 γ/δ T cells in peripheral blood express a limited receptor repertoire: Vδ2-Jδ1-Cδ usually pairs with Vγ9-JγP-Cγ1 (11, 12). Vδ2-Jδ3 rearrangements such as the one observed here have previously been described in γ/δ T cell clones (13), but are rare in peripheral blood T cells. To further verify the dominance of Vδ2-Jδ3 transcripts in the muscle biopsy, PCR was performed with a Vδ2 sense primer in combination with either a Jδ3 or a Jδ1 antisense primer. Whereas only a Vδ2-Jδ3-PCR band was observed when cDNA from the muscle biopsy was used as a template, a Vδ2-Jδ1-band was dominant in reactions with cDNA derived from PBL (Fig. 2). This demonstrates that the majority of γ/δ 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 γ/δ T cells present in the blood at the time of biopsy had a different phenotype ($BB3^+$, $Ti\gamma A^+$) than the infiltrating γ/δ T cells ($BB3^-$, $Ti\gamma A^-$) (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

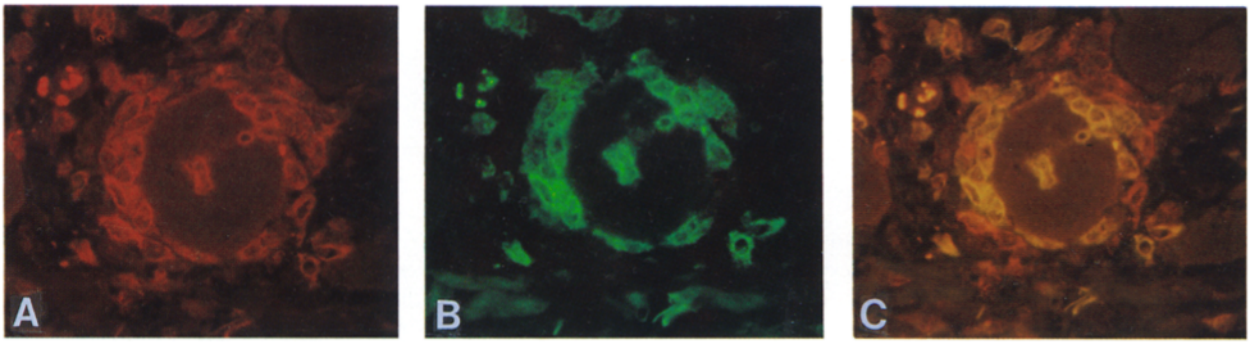

Figure 4. Localization of CD3 antigen with red rhodamine fluorescence (*A*) and of the T_iV-δ2 antigen with green FITC fluorescence. (*A*) $CD3^+$ T cells focally surround and invade a muscle fiber. (*B*) Demonstration that most of these cells are T_iV-$\delta2^+$ cells. (*C*) Double exposure of *A* and *B*, revealing $CD3^+$ T_iV-$\delta2^+$ cells in yellow. *A* and *B*, ×410; *C*, ×365.

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 a Cγ exon 3 antisense primer, and sequenced the bracketed DNA. This analysis revealed an association of the dominant Vγ3-Jγ1 rearrangement with Cγ1. In addition, we observed one additional unique Vγ3 rearrangement (Fig. 3 *C*) associated with a Cγ2 gene segment that contained three copies of exon 2. The fact that the very same sequences were found in the products of several independent amplifications using different amplification techniques and primers rules out the possibility that the dominance of the Vδ2-Jδ3 and Vγ3-Jγ1 rearrangements are merely PCR artifacts. Furthermore, a preliminary PCR analysis of TCR transcripts from another small portion of the biopsy had also yielded the dominant Vδ2-Jδ3 and Vγ3-Jγ1 rearrangements. Here Vδ2-Jδ1 and Vγ9-JγP rearrangements that are commonly found in peripheral γ/δ T cells and thus presumably derived from contaminating PBMC were obtained in addition.

Taken together, the above results suggest that one T cell clone expressing a Vδ2-Jδ3-Cδ/Vγ3-Jγ1-Cγ1 disulfide-linked TCR dominates the population of infiltrating T cells of the analyzed muscle specimen. The phenotype of the vast majority of γ/δ T cells in this specimen was previously established as $CD2^+$, $CD3^+$, $CD4^-$, $CD8^-$, TCR-$\delta1^+$, $Ti\gamma A^-$, $A13^-$, $BB3^-$ (1). While TCR-δ1 stains all γ/δ T cells, the mAbs TiγA and A13 are specific for subsets Vγ9 or Vδ1, respectively. While the observed TCR-$\delta1^+$, $Ti\gamma A^-$, $A13^-$ staining pattern was expected for a Vγ3/Vδ2-expressing T cell population, the $BB3^-$ phenotype seems to be incompatible with the genetic data, since BB3 is considered specific for Vδ2 (18). One possible explanation for the discrepancy was that the rare Vδ2-Jδ3 rearrangement and/or combination with Vγ3 eliminates the epitope recognized by mAb BB3. To address this problem, we used another Vδ2-specific mAb, T_iV-δ2 (19), for additional immunohistochemical studies. All the T cells surrounding and invading muscle fibers reacted with T_iV-δ2 (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-Jγ1-Cγ1/Vδ2-Jδ3-Cδ TCR that is unusual both with respect to its Vδ-Jδ recombination and its specific Vγ/Vδ 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 inflammatory 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 *Mycobacteria*- and heat shock protein–responsive γ/δ T cells, which have been postulated to participate in autoimmune processes (20), express dominantly Vγ9-JγP/Vδ2-Jδ1 TCR variable regions (21–23). We anticipate that the molecular characterization of the Vγ3-Jγ1-Cγ1/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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References

1. Hohlfeld, R., A.G. Engel, K. Ii, and M.C. Harper. 1991. Polymyositis mediated by T lymphocytes that express the γ/δ receptor. *N. Engl. J. Med.* 324:877.
2. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156.
3. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. Pluschke, G., G. Ricken, H. Taube, S. Kroninger, I. Melchers, H.H. Peter, K. Eichmann, and U. Krawinkel. 1991. Biased T cell receptor Vα region repertoire in the synovial fluid of rheumatoid arthritis patients. *Eur. J. Immunol.* 21:2749.
5. Loh, E.Y., J.F. Elliott, S. Cwirla, L.L. Lanier, and M.M. Davis. 1989. Polymerase chain reaction with single-sided specificity: analysis of T cell receptor δ chain. *Science (Wash. DC).* 243:217.

6. LeFranc, M.-P., A. Forster, R. Baer, M.A. Stinson, and T.H. Rabbitts. 1986. Diversity and rearrangement of the human T cell rearranging γ genes: nine germ-line variable genes belonging to two subgroups. *Cell.* 45:237.
7. Hata, S., M. Clabby, P. Devlin, H. Spits, J.E. de Vries, and M.S. Krangel. 1989. Diversity and organization of human T cell receptor δ variable gene segments. *J. Exp. Med.* 169:41.
8. Takihara, Y., D. Tkachuk, E. Michalopoulos, E. Champagne, J. Reimann, M. Minden, and T.W. Mak. 1988. Sequence and organization of the diversity, joining, and constant region genes of the human T-cell δ-chain locus. *Proc. Natl. Acad. Sci. USA.* 85:6097.
9. Dariavach, P., and M.-P. Lefranc. 1989. First genomic sequence of the human T-cell receptor $\delta 2$ gene (TRDV2). *Nucleic Acid Res.* 17:4880.
10. Keohavong, P., and W.G. Thilly. 1989. Fidelity of DNA polymerases in DNA amplification. *Proc. Natl. Acad. Sci. USA.* 86:9253.
11. Moretta, L., E. Ciccone, S. Ferrini, P.G. Pelicci, M.C. Mingari, J. Zeromski, C. Bottino, C. Grossi, and A. Moretta. 1991. Molecular and cellular analysis of human T lymphocytes expressing $\gamma\delta$ T-cell receptor. *Immunol. Rev.* 120:117.
12. Porcelli, S., M.B. Brenner, and H. Band. 1991. Biology of the human $\gamma\delta$ T-cell receptor. *Immunol. Rev.* 120:137.
13. Casorati, G., G. de Libero, A. Lanzavecchia, and N. Migone. 1989. Molecular analysis of human γ/δ^+ clones from thymus and peripheral blood. *J. Exp. Med.* 170:1521.
14. Quertermous, T., W. Strauss, C. Murre, D.P. Dialynas, J.L. Strominger, and J.G. Seidman. 1986. Human T-cell γ genes contain N segments and have marked junctional variability. *Nature (Lond.).* 322:184.
15. Lefranc, M.-P., and T.H. Rabbits. 1991. Genetic organization of human T cell receptor gamma locus. *Curr. Top. Microbiol. Immunol.* 173:3.
16. Lefranc, M.-P., A. Forster, and T.H. Rabbitts. 1986. Genetic polymorphism and exon changes of the constant regions of the human T-cell rearranging gene γ. *Proc. Natl. Acad. Sci. USA.* 83:9596.
17. Fagioli, M., E. Ciccone, C. Bottino, B. Falini, F. Grignani, L. Moretta, and P.G. Pelicci. 1990. The Cγ1-encoded disulphide-linked and the Cγ2-encoded nondisulphide-linked forms of the gamma/delta heterodimer use different variable regions. *Blood.* 76:279.
18. Triebel, F., F. Faure, F. Mami-Chouaib, S. Jitsukawa, A.L. Griscelli, C. Genevee, S. Roman-Roman, and T. Hercend. 1988. A novel human V delta gene expressed predominantly in the TigammaA$^+$ fraction of gamma/delta$^+$ peripheral lymphocytes. *Eur. J. Immunol.* 18:2021.
19. Miossec, C., F. Faure, L. Ferradini, S. Roman-Roman, S. Jitsukawa, S. Ferrini, A. Moretta, F. Triebel, and T. Hercend. 1990. Further analysis of the T cell receptor γ/δ^+ peripheral lymphocyte subset: the $\delta 1$ gene segment is expressed with either Cα or Cδ. *J. Exp. Med.* 171:1171.
20. Kaufmann, S.H.E. 1990. Heat shock proteins and the immune response. *Immunol. Today.* 11:129.
21. Panchamoorthy, G., J. McLean, R.L. Modlin, C.T. Morita, S. Ishikawa, M.B. Brenner, and H. Band. 1991. A predominance of the T cell receptor Vγ2/Vδ2 subset in human mycobacteria-responsive T cells suggests germline gene encoded recognition. *J. Immunol.* 147:3360.
22. Ohmen, D.J., P.F. Barnes, K. Uyemura, S. Lu, C.L. Grisso, and R.L. Modlin. 1991. The T cell receptors of human $\gamma\delta$ T cells reactive to *Mycobacterium tuberculosis* are encoded by specific V genes but diverse V-J junctions. *J. Immunol.* 147:3353.
23. de Libero, G., G. Casorati, C. Giachino, C. Carbonara, N. Migone, P. Matzinger, and A. Lanzavecchia. 1991. Selection by two powerful antigens may account for the presence of the major population of human peripheral $\gamma\delta$ T cells. *J. Exp. Med.* 173:1311.