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Predominant Expansion of Vγ9/Vδ2 T Cells in a Tularemia Patient

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We describe a 58-year-old man with tularemia and expanding γδ T cells in his peripheral blood lymphocytes (PBL) (32.7% of total PBL). In the present work, we analyzed the T-cell receptor Vγ/Vδ repertoire of these cells by making use of the polymerase chain reaction and flow cytometry and found that they were mostly $CD4^-$ $CD8^-$ $CD3^+$ $Vγ9/Vδ2^+$. The sequence analysis of 16 cDNA clones encoding the Vγ9-J region revealed that the Vγ9-Jp combination was strikingly overrepresented but that the junctional (N) region was heterogeneous. This suggested that the γδ T cells in PBL from a patient with tularemia were polyclonally expanded.

The majority of peripheral T cells express an αβ T-cell receptor (TCR) which recognizes antigen and the major histocompatibility complex expressed on antigen-presenting cells and either the CD4 or CD8 molecule (2). An additional small subset of T cells expresses a second type of TCR, γδ (8). γδ T cells do not express CD4 or CD8 molecules (6, 7, 9), and the recognition specificity of the γδ TCR is not clear. Some γδ T cells recognize mycobacterial antigens and can proliferate to mycobacterial heat shock protein or purified protein derivative (4, 15, 16, 19, 27, 29). γδ T cells also accumulate in human leprosy skin lesions and American cutaneous leishmaniasis, rheumatoid arthritis synovial fluid, and peripheral blood lymphocytes (PBL) in patients with sarcoidosis (5, 16, 27, 36). Tularemia is an infection produced by the gram-negative organism *Francisella* (*Pasteurella*) *tularensis*. The organism is acquired by humans from infected animals and insect bites and invades the lymphoid tissue. The incubation period is 2 to 7 days. Histopathological findings revealed involvement of macrophages, and there is some analogy to findings in tuberculosis (35). Tularemia results in the development of $CD4^+$ T cells, which may play a protective role in development of the disease (34, 35). Here, we report that TCR Vγ9/Vδ2-positive T cells predominantly expanded in peripheral blood lymphocytes (PBL) from a man with tularemia. The sequence analysis showed that the Vγ9-Jp combination was strikingly overrepresented but that the junctional (N) region was heterogeneous. The possible role of these cells in the pathogenesis of tularemia is discussed.

A 58-year-old Japanese man presented with fever and was admitted to the hospital in January 1990. His body temperature reached 38°C 10 days after contact with a dead rabbit. Lymph nodes in his bilateral axillas and arms were enlarged. These lymph nodes were 1 to 3 cm in diameter. Anemia, icterus, and eruption were absent. The serum level of glutamic oxalacetic transaminase was 103 mU/ml; glutamic pyruvic transaminase was 195 mU/ml; lactic dehydrogenase was 477 IU/liter; and C-reactive protein was 3.4 mg/dl. A skin reaction against tularemia antigen was strongly positive (30 by 30 mm), and the aggregation reaction test against tularemia antigen was also positive (1:1,240). A diagnosis of tularemia was made on day 10 of hospitalization, and then 100 mg of Minomycin (Takeda Chemical Industries, Ltd., Osaka, Japan) (minocycline hydrochloride) and an additional 1 g of streptomycin sulfate were given orally daily. On day 7 after treatment, the patient became afebrile and his serum transaminase level reverted to normal. Swelling in the lymph nodes disappeared by April.

The γδ T cells in PBL from this patient with tularemia were analyzed before and after treatment by using flow cytometry with phycoerythrin-coupled anti-CD3 (Leu4) monoclonal antibody (MAb) and fluorescein isothiocyanate (FITC)-conjugated anti-TCR γδ MAb (TCR δ1). PBL from the tularemia patient were isolated by Ficoll-Paque separation (Pharmacia Fine Chemicals, Piscataway, N.J.). For two-color staining, the PBL were incubated for 1 h on ice with phycoerythrin-coupled anti-CD3 (Leu4) MAb or anti-CD4 (Leu3a) plus anti-CD8 (Leu2a) MAbs (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) and FITC-conjugated MAb to γδ TCR (TCR δ1; T Cell Sciences, Inc., Cambridge, Mass.). After incubation, the cells were washed and resuspended at a concentration of 10^6 cells per ml and then analyzed by FACScan (Becton Dickinson). Figure 1a shows that $CD3^+$ γδ T cells composed 32.7% of the PBL from this patient before treatment. The normal amount of γδ T cells in PBL from 10 healthy subjects in our clinics was 2.62 ± 1.57% (mean ± standard deviation). The number of γδ T cells in this tularemia patient decreased from 32.7 to 9.6% after treatment with minocycline hydrochloride for 1 month, and a level 10 to 15% higher than that in healthy individuals was retained for several months (Fig. 1b). To examine the expression of CD4 and CD8 molecules, we stained PBL obtained from the patient 1 month after treatment with phycoerythin-coupled anti-CD4 (Leu3a) and anti-CD8 (Leu2a) MAbs plus FITC-conjugated anti-TCR γδ MAb. Most of the $CD3^+$ γδ T cells did not stain with MAbs against CD4 and CD8 (Fig. 1b), thereby indicating that the phenotype of the γδ T cells expanded in tularemia was $CD3^+$ $CD4^-$ $CD8^-$.

To analyze Vγ gene usage on the γδ T cells increased in tularemia, we examined the TCR Vγ repertoire by polymerase chain reaction (PCR) with primers specific for eight different Vγ genes and the primer for the Cγ gene. PBL (10^7) were collected from 20 ml of blood from this patient after treatment. Total RNAs (10 μg) were prepared from PBL by using RNA zol solution (Biotecx Laboratories, Inc., East Houston, Tex.). cDNAs were synthesized from 5 μg of total

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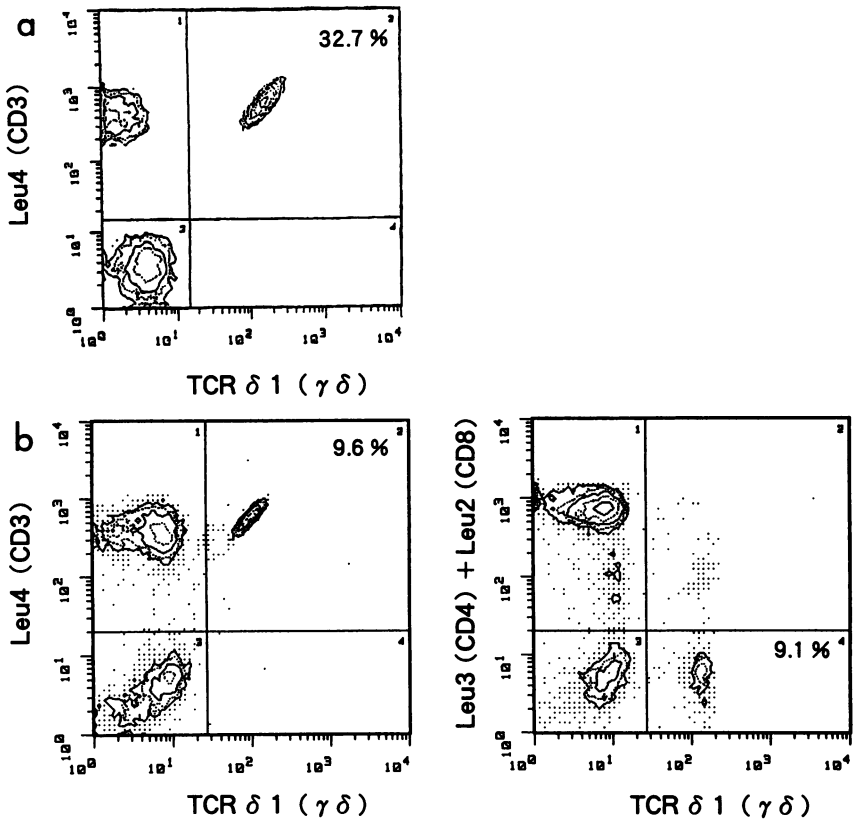

FIG. 1. γδ T cells on PBL from a tularemia patient. PBL from a patient before (a) and after (b) treatment were stained with phycoerythrin-coupled anti-CD3 (left panel) or anti-CD4 plus anti-CD8 (right panel) and FITC-conjugated anti-TCR γδ MAbs.

RNA in a 20-μl reaction mixture containing oligo(dT) primer by using avian myeloblastosis virus reverse transcriptase. Amplification was performed with *Taq* polymerase in 50 μl of standard buffer with 0.2 μl of cDNA (corresponding to 50 ng of total RNA). Eight different Vγ primers (Vγ2, 5'-GCAAGCACAAGGAACAACTTG-3'; Vγ3, 5'-ACACCCA GGAGGTGGAGCTGG-3'; Vγ4, 5'-TATGACTCCTACAC CTCCAGC-3'; Vγ5, 5'-ACACCGAGGAGGTGGAGCTGG-3'; Vγ8, 5'-AGCACAGGGAAGAGCCTTAAA-3'; Vγ9, 5'-TTGAGGTGGATAGGATACCTG-3'; Vγ10, 5'-ACTCTCA CTTCAATCCTTACC-3'; Vγ11, 5'-TGCTCAGGTGGGAA GACTAAG-3') and Cγ (5'-AGTGGGCTTGGGGGAAACA TC-3') or 5'-Cγ (5'-ATAAACAACTTGATGCAGATG-3') and 3'-Cγ (5'-CATGTATGTGTCGTTAGTCTT-3') primers were selected from previously published data (13, 17, 23, 24). Oligonucleotides were synthesized by a DNA synthesizer (Applied Biosystems Co., Ltd., Foster City, Calif.). The denaturing step was done at 94°C for 1.5 min, the annealing step was done at 60°C for 1 min, and the extension step was done at 72°C for 1 min for 30 cycles on a DNA Thermal Cycler (Zymoreactor V2; Atto Co., Ltd., Tokyo, Japan). One-fifth of each sample was loaded on a 2% agarose gel and hybridized with the ^{32}P-labeled 165-bp Cγ-region cDNA probe. This probe was synthesized by PCR with 5'-Cγ and 3'-Cγ primers and purified by electroelution. Southern blot analysis of the PCR products showed a strong radioactive band encoding Vγ9-JCγ gene segments (Fig. 2a). The other seven Vγ genes were not evident, even with a longer exposure. To investigate usage of the Vδ gene on the expanded γδ T cells, PBL obtained from the patient after treatment were stained with MAb against the Vδ2 molecule (BB3) (11) and analyzed by fluorescence-activated cell sorting. As shown in Fig. 2b, most of the TCR δ1-positive T cells (11%) were Vδ2$^+$ T cells (10.7%); hence, the increased T cells in PBL from the patient with tularemia were Vγ9/Vδ2 positive.

To analyze the clonality of expanded Vγ9/Vδ2 T cells, the cDNAs encoding the Vγ9 gene were amplified by using PCR, cloned, and sequenced. cDNA (0.2 μl) prepared from PBL from the patient after treatment were used for PCR together with the following primers: for Vγ9, a Vγ9 primer with an *Eco*RI cutting site (5'-TCTAGAATTCTTGAGGTGGAT AGGATACCT-3'), and a Cγ primer with an *Eco*RI cutting site (5'-TCTAGAATTCAGACAAAGGTATGTTCCAGC-3'). The reaction was performed under the same conditions as mentioned above. PCR products were purified by phenol extraction and precipitated with ethanol and then restriction enzyme digested in excess amounts of *Eco*RI. Fragments of the expected sizes for the cDNAs were enriched by preparative low-melting-point agarose gel electrophoresis. The recovered DNA fragments were ligated to M13mp19 plasmids obtained by *Eco*RI digestion. Bacteriophages were grown on *Escherichia coli* TG-1 cells. After hybridization with the Cγ probe, single phages were picked and cultivated and recombinant phage DNA was purified for DNA sequence determinations. Sequencing reactions were done manually by using the Sequenase sequencing system (United States Biochemical Corporation, Cleveland, Ohio). The GenBank libraries were used to search for homologies (12). As shown in Table 1, 14 (88%) of 16 Vγ9 clones sequenced

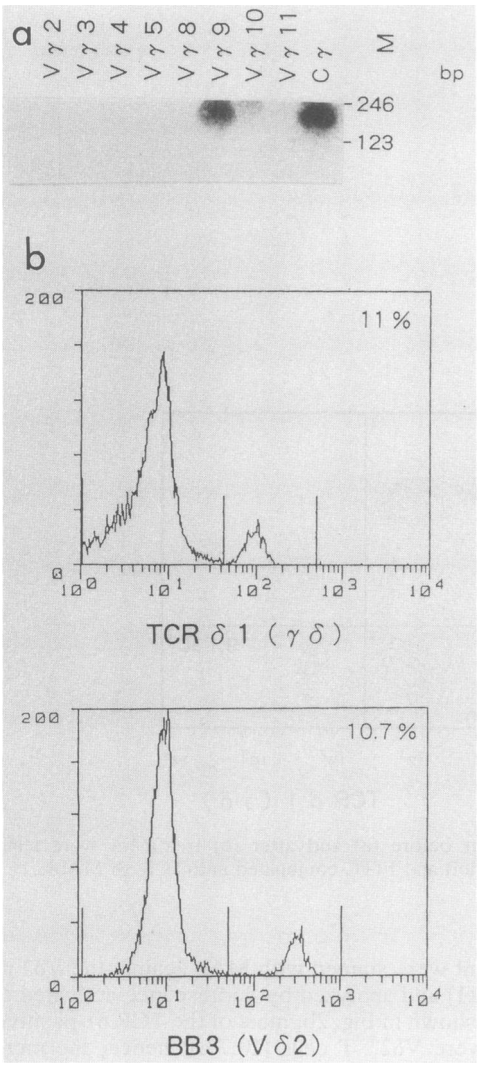

FIG. 2. (a) Analysis of Vγ usage on γδ T cells in PBL from a patient with tularemia after treatment, using PCR. Amplified PCR products yielded a band of about 200 bp. (b) Vδ2-positive T cells on PBL from a patient with tularemia after treatment. PBL were stained with FITC-conjugated anti-TCR γδ (TCR δ1) (upper panel) or anti-Vδ2 (BB3) (lower panel) MAb.

were productively rearranged. Two sets (clones 8 and 9, clones 15 and 18) of 14 productively rearranged clones had the same rearrangement and proved to be the same clone. The sequences of the other 10 clones differed. We conclude that the Vγ9 cDNA clones had different V-J junction sequences, that Vγ9-Jγ diversed extensively in the junctional regions, and that the Vγ9-positive T cells were polyclonally expanded. In addition, 11 productively rearranged clones were combined with the Jp segment and the other 3 were combined with the J1 segment. Thus, the Vγ9-Jp combination was strikingly overrepresented in our patient with tularemia.

We have described herein the first evidence that γδ T cells are present in large numbers in PBL from tularemia patients. Several investigators have established γδ T-cell clones or T-cell hybridomas and have noted that some T-cell clones responded specifically to mycobacterial antigens, purified protein derivative, heat shock protein, tetanus toxoid, and staphylococcal enterotoxin A (15, 16, 22, 29, 32). It was also clarified that γδ T cells accumulated in the granulomatous region of humans with leprosy (27) and American cutaneous leishmaniasis (16), synovial fluid of rheumatoid arthritis patients (5), and PBL of sarcoidosis patients (36). These findings suggested that TCR γδ cells or their secreted products play a role in development of the lesions.

Although human αβ T cells carry a broad diversity of antigen recognition by a large number of genetic elements (V, D, J), the human TCR γδ repertoire is limited since there are only eight functional Vγ and five Jγ gene segments (9, 30). In healthy individuals, more than 60% of TCR γδ cells utilize the Vγ9 segment (37). In addition, analyses of Vγ9 T-cell clones showed that the Vγ9 gene is mostly combined to Jγp and Cγ1 and paired with Vδ2 elements (38). The specificity of γδ T cells depends on extensive junctional sequence diversity generated by the addition of N-region nucleotides to the junctional ones (31).

Tamura et al. (36) found that junctional sequences associated with Vγ9 gene segments in PBL from sarcoidosis patients were limited. They also mentioned that the limitation of junctional region diversity observed in sarcoidosis might imply a response to a small number of epitopes by the oligoclonal population of γδ T cells. In contrast, Sioud et al. (33) reported that Vδ1-expressing T cells in patients with rheumatoid synovitis were polyclonal, as determined by junctional sequence analysis. We found, using flow cytometry and PCR, that most of the expanded γδ T cells in a tularemia patient were $CD4^-$ $CD8^-$ $V\gamma9/V\delta2^+$ T cells. However, sequences of Vγ9 cDNA clones demonstrated that these expanded T cells had different V-J junctional sequences, hence the broad diversity of Vγ9-positive T cells in subjects with this pathology.

A collection of superantigen, which binds to class II major histocompatibility complex and engages T cells bearing particular Vβ chains, regardless of the sequences of other variable components of the TCR, has been found in mice (18, 26). Two groups of superantigens have been described so far. One of the superantigens is minor lymphocyte stimulation (Mls) antigen (14, 21, 25) and may consist of molecules encoding mammary tumor virus (1, 10), and the other is a bacterial product such as staphylococcal enterotoxins (39). In humans, the enterotoxins of *Staphylococcus aureus* bind to class II molecules and stimulate Vβ2-positive T cells (20). It is tempting to postulate that heat shock protein and bacterial products activate γδ T cells with a particular TCR as well as αβ T cells. Therefore, the predominant expansion of $V\gamma9/V\delta2$ T cells bearing the different V-J junctions in patients with tularemia may support the notion that unique epitopes on *F. tularensis* act as a superantigen and induce the expansion of a limited γδ T-cell population, without preference for junctional region sequences.

Sjostedt et al. (34, 35) reported that a 17-kDa protein of *F. tularensis* was the major T-cell-reactive membrane protein. Further, gene mapping experiments showed that peripheral CD4-positive T cells recognized several epitopes of the 17-kDa protein (35). Peptides from the 17-kDa protein of *F. tularensis* induced $CD4^+$ T-cell proliferation and the production of interleukin-2 and gamma interferon, thereby suggesting that $CD4^+$ T cells are relevant to the host defense against *F. tularensis*, since gamma interferon enhanced antimicrobial activity in an intracellular bacterial infection (3, 28). Although the recognition specificity and the functional role of the $CD4^-$ $CD8^-$ γδ T cells in tularemia are not known, these cells might play an important role in the primitive

TABLE 1. Junctional sequences of cDNA encoding the Vγ9 gene[a]

Clone	Vγ9	N	J	Jγ region	In frame[b]
1	GCCTTGTGGGA	T	CAAGAGTTGGGC	Jp	+
2	GCCTTGTGGGAG	TA	GCAAGAGTTGGGC	Jp	+
3	GCCTTGTGGGAGG	AGGG	TTATAAGAAA	J1	+
5	GCCTTGTGGGAGGTG	CG	AAGAGTTGGGC	Jp	−
6	GCCTTGTGGGAG		CAAGAGTTGGGC	Jp	+
7	GCCTTGTGGGAGGTG	GG	AGAGTTGGGC	Jp	+
8[c]	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	Jp	+
9[c]	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	Jp	+
10	GCCTTGTGGGAGG		AAGAGTTGGGC	Jp	+
11	GCCTTGTGGGAG	CAGGG	AGAGTTGGGC	Jp	+
12	GCCTTGTGGGAGG	AGG	AGTTGGGC	Jp	+
13	GCCTTGTGGGAG	TGCAAGGTAACGG	AAGAGTTGGGC	Jp	+
15[d]	GCCTTGTGGGAGG	AGGG	TTATAAGAAA	J1	+
16	GCCT	GGTGTGGACCCGAGG	CTCTTTGGC[e]	J1	−
18[d]	GCCTTGTGGGAGG	AGGG	TTATAAGAAA	J1	+
22	GCCTTGTGGGAGG	C	GCAAGAGTTGGGC	Jp	+

[a] Sequences shown include the 3′ region of the Vγ9 element (Vγ9), the N region, the 5′ region of the Jγ element (J), and the specific Jγ element (Jγ).
[b] +, productive transcript; −, nonproductive transcript.
[c] Clones 8 and 9 were identical.
[d] Clones 15 and 18 had the same sequence.
[e] 5′ portion of Jγ1 gene segment was deleted.

immune defense against *F. tularensis* in collaboration with $CD4^+$ T cells.

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REFERENCES

1. **Acha-Orbea, H., A. N. Shakhov, L. Scarpellino, E. Kolb, V. Muller, A. Vessaz-Shew, R. Fuchs, K. Blochlinger, P. Rollini, J. Billotte, M. Sarafidon, H. R. MacDonald, and H. Diggelmann.** 1991. Clonal deletion of Vβ14-bearing T cells in mice transgenic for mammary tumor virus. Nature (London) **350:**207–211.
2. **Allison, J. P., and L. Lanier.** 1987. The structure, serology and function of the T-cell antigen receptor. Annu. Rev. Immunol. **5:**503–540.
3. **Anthony, L. S. D., E. Ghadirian, E. P. Nestel, and P. A. L. Kongshavin.** 1989. The requirement for gamma interferon in resistance of mice to experimental tularemia. Microb. Pathog. **6:**421–424.
4. **Augustin, A., R. Kubo, and G.-K. Sim.** 1989. Resident pulmonary lymphocytes expressing the γδ T-cell receptor. Nature (London) **340:**239–241.
5. **Balbi, B., D. R. Moller, M. Kirby, K. J. Holroyd, and R. G. Crystal.** 1990. Increased numbers of T lymphocytes with γδ-positive antigen receptors in a subgroup of individuals with pulmonary sarcoidosis. J. Clin. Invest. **85:**1353–1361.
6. **Bank, I., R. A. DePinho, M. B. Brenner, J. Cassimeris, F. W. Alt, and L. Chess.** 1987. A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. Nature (London) **322:**179–181.
7. **Borst, J., R. J. van de Griend, J. W. van Oostveen, S.-L. Aug, C. J. Melief, J. G. Seidman, and R. L. H. Bolhuis.** 1987. A T-cell receptor γ/CD3 complex found on cloned functional lymphocytes. Nature (London) **325:**683–688.
8. **Brenner, M. B., J. McLean, D. P. Dialynas, J. L. Strominger, J. A. Smith, F. L. Owen, J. G. Seidman, S. Ip, F. Rosen, and M. S. Krangel.** 1986. Identification of a putative second T-cell receptor. Nature (London) **322:**145–149.
9. **Brenner, M. B., J. L. Strominger, and M. S. Krangel.** 1988. The γδ T cell receptor. Adv. Immunol. **43:**133–192.
10. **Choi, Y., J. W. Kappler, and P. Marrack.** 1991. A superantigen encoded in the open reading frame of the 3′ long terminal repeat of mouse mammary tumor virus. Nature (London) **350:**203–207.
11. **Ciccone, E., S. Ferrini, C. Bottino, O. Viale, I. Prigione, G. Pantaleo, G. Tambussi, A. Moretta, and L. Moretta.** 1988. A monoclonal antibody specific for a common determinant of the human T cell receptor γ/δ directly activates $CD3^+$ $WT31^-$ lymphocytes to express their functional program(s). J. Exp. Med. **168:**1–11.
12. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
13. **Forster, A., S. Huck, N. Ghanem, M.-P. LeFranc, and T. H. Rabbitts.** 1987. New subgroups in the human T cell rearranging Vγ gene locus. EMBO J. **6:**1945–1950.
14. **Happ, M. P., D. Woodland, and E. Palmer.** 1989. A third T-cell receptor β-chain variable region gene encodes reactivity to Mls-1a gene products. Proc. Natl. Acad. Sci. USA **86:**6293–6296.
15. **Haregewoin, A., G. Soman, R. C. Hom, and R. W. Finberg.** 1989. Human $γδ^+$ T cells respond to mycobacterial heat-shock protein. Nature (London) **340:**309–312.
16. **Holoshitz, J., F. Koning, J. E. Coligan, J. DeBruyn, and S. Strober.** 1989. Isolation of $CD4^-$ $CD8^-$ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. Nature (London) **339:**226–229.
17. **Huck, S., P. Dariavach, and M.-P. LeFranc.** 1988. Variable region genes in the human T-cell rearranging gamma (TRG) locus: V-J junction and homology with the mouse genes. EMBO J. **7:**727–738.
18. **Janeway, C. A., Jr., J. Yagi, P. J. Conrad, M. Katz, S. Vroegop, and P. Marrack.** 1989. T-cell responses to Mls and to bacterial proteins that mimic its behavior. Immunol. Rev. **107:**61–88.
19. **Janis, E. M., S. H. E. Kaufmann, R. H. Schwartz, and D. M. Pardoll.** 1989. Activation of γδ T cells in the primary immune response to *Mycobacterium tuberculosis*. Science **244:**713–716.
20. **Kappler, J., B. Kotzin, L. Herron, E. W. Gelfand, R. D. Bigler, A. Boylston, S. Carrel, D. N. Posnett, Y. Choi, and P. Marrack.** 1989. Vβ-specific stimulation of human T cells by staphylococcal toxins. Science **244:**811–813.
21. **Kappler, J. W., U. Staerz, J. White, and P. Marrack.** 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature (London) **332:**35–40.
22. **Kozbor, D., G. Trinchieri, D. Monos, M. Isobe, G. Russo, J. A. Haney, C. Zmijewski, and C. M. Croce.** 1989. Human TCR-$γ^+$/

γ^+, $CD8^+$ T lymphocytes recognize tetanus toxoid in an MHC-restricted fashion. J. Exp. Med. **169:**1847–1851.
23. **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:**237–246.
24. **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:**9596–9600.
25. **MacDonald, H. R., R. Schneider, R. K. Lees, R. C. Howe, H. Acha-Orbea, H. Festenstein, R. M. Zinkernagel, and H. Hengartner.** 1988. T-cell receptor Vβ use predicts reactivity and tolerance to Mls^a-encoded antigens. Nature (London) **332:**40–45.
26. **Marrack, P., and J. W. Kappler.** 1990. The staphylococcal enterotoxins and their relatives. Science **248:**705–711.
27. **Modlin, R. L., C. Pirmez, F. M. Hofman, V. Torigian, K. Uyemura, T. H. Rea, B. R. Bloom, and M. B. Brenner.** 1989. Lymphocytes bearing antigen-specific γδ T-cell receptors accumulate in human infectious disease lesions. Nature (London) **339:**544–548.
28. **Nash, T. W., D. M. Libby, and M. A. Horwitz.** 1988. IFN-γ-activated human alveolar macrophages inhibit the intracellular multiplication of *Legionella pneumophila*. J. Immunol. **140:** 3978–3981.
29. **O'Brien, R. L., M. P. Happ, A. Dallas, E. Palmer, R. Kubo, and W. K. Born.** 1989. Stimulation of a major subset of lymphocytes expressing T cell receptor γδ by an antigen derived from *Mycobacterium tuberculosis*. Cell **57:**667–674.
30. **Raulet, D. H.** 1989. The structure, function and molecular genetics of the γ/δ T cell receptor. Annu. Rev. Immunol. **7:**175–207.
31. **Rellahan, B. L., J. A. Bluestone, B. A. Houlden, M. M. Cotterman, and L. A. Matis.** 1991. Junctional sequences influence the specificity of γ/δ T cell receptors. J. Exp. Med. **173:**503–506.
32. **Rust, C. J. J., F. Verreck, H. Vietor, and F. Koning.** 1990. Specific recognition of staphylococcal enterotoxin A by human T cells bearing receptors with the Vγ9 region. Nature (London) **346:**572–574.
33. **Sioud, M., O. Forre, and J. B. Natvig.** 1991. T cell receptor δ diversity of freshly isolated T lymphocytes in rheumatoid synovitis. Eur. J. Immunol. **21:**239–241.
34. **Sjostedt, A., G. Sandstrom, and A. Tarnvik.** 1990. Several membrane polypeptides of the live vaccine strain *Francisella tularensis* LVS stimulate T cells from naturally infected individuals. J. Clin. Microbiol. **28:**43–48.
35. **Sjostedt, A., G. Sandstrom, A. Tarnvik, and B. Jaurin.** 1990. Nucleotide sequence and T cell epitopes of a membrane protein of *Francisella tularensis*. J. Immunol. **145:**311–317.
36. **Tamura, N., K. J. Holroyd, T. Banks, M. Kirby, H. Okayama, and R. G. Crystal.** 1990. Diversity in junctional sequences associated with the common human Vγ9 and Vδ2 gene segments in normal blood and lung compared with the limited diversity in a granulomatous disease. J. Exp. Med. **172:**169–181.
37. **Triebel, F., F. Faure, M. Graziani, S. Jitsukawa, M.-P. LeFranc, and T. Hercend.** 1988. A unique V-J-C-rearranged gene encodes a γ protein expressed on the majority of $CD3^+$ T cell receptor α/β^- circulating lymphocytes. J. Exp. Med. **167:**694–699.
38. **Triebel, F., F. Faure, F. Mami-Chouaib, S. Jitsukawa, A. Griscelli, C. Genevee, S. Roman-Roman, and T. Hercend.** 1988. A novel human γδ gene expressed predominantly in the TiγA fraction of γ/δ^+ peripheral lymphocytes. Eur. J. Immunol. **18:**2021–2027.
39. **White, J., A. Herman, A. M. Pullen, R. Kubo, J. W. Kappler, and P. Marrack.** 1989. The Vβ-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. Cell **56:**27–35.