# Dual Antigenic Recognition by Cloned Human γδ T Cells

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## Abstract

**The function of γδ T cells is still elusive. The nature of the antigens that they recognize and the mode of presentation of these antigens are largely unknown. The majority of human peripheral γδ T cells bear a Vγ9/Vδ2 T cell receptor, and display nonclonal reactivity to mycobacteria, without restriction by MHC. It is unknown whether these cells have clonal antigenic specificity as well. Here we describe rheumatoid arthritis–derived Vγ9/Vδ2 T cell clones, displaying dual antigenic recognition: a nonclonal, MHC-unrestricted recognition of mycobacteria, and a clonal recognition of a short tetanus toxin peptide presented by HLA-DRw53, a nonpolymorphic class II MHC molecule associated with susceptibility to rheumatoid arthritis. This is the first evidence that Vγ9/Vδ2 T cells can recognize nominal antigenic peptides presented by class II MHC molecules. These results suggest that much like αβ T cells, Vγ9/Vδ2 cells may contribute to the immune response against foreign antigens in an antigen-specific and MHC-restricted manner. The reactivity of these γδ T cells to mycobacteria may represent a superantigen-like phenomenon. (*J. Clin. Invest.* 1992. 89:308–314.) Key words: mycobacteria • tetanus toxoid • superantigens • major histocompatibility complex • T cell receptor**

## Introduction

Nominal antigens are recognized by αβ T cells as short peptides presented by MHC molecules. Another type of recognition occurs with bacterial superantigens which bind to monomorphic regions of class II MHC molecules (1, 2). These superantigens activate αβ T cells expressing particular Vβ gene products, irrespective of their nominal antigenic specificity, in an MHC-unrestricted manner. The nature of the antigens recognized by γδ T cells and the mode of their presentation are still largely unknown. Previous studies have shown that the majority of adult human peripheral γδ T cells express T cell receptors (TCRs)[1] using the Vγ9 and Vδ2 gene product pairs (3–7). T cells of the Vγ9/Vδ2 subset have been found to respond to mycobacterial antigens uniformly, despite TCR V-(D)-J junctional diversity, in an MHC-unrestricted manner (8–10). This reactivity resembles the recognition of bacterial superantigens by αβ T cells. The extensive junctional sequence diversity of Vγ9/Vδ2 cells lends support to the hypothesis that these cells would likely recognize short antigenic peptides presented by MHC molecules, similar to the αβ lineage. However, thus far nominal antigen recognition by Vγ9/Vδ2 cells has not been reported.

We have previously described Vγ9/Vδ2 T cell clones which were isolated by the limiting dilution technique from the synovial fluid of a patient with early RA. These clones proliferated in response to the acetone precipitable fraction of *Mycobacterium tuberculosis* (AP-MT) in an MHC-unrestricted manner (8). The present report provides the first evidence that in addition to their nonclonal reactivity to mycobacteria, Vγ9/Vδ2 T cells are capable of specific recognition of short peptides presented by class II MHC molecules. The presenting molecule was identified as DRw53, a nonpolymorphic class II MHC molecule previously shown to be associated with susceptibility to RA.

## Methods

### *Antigens*

AP-MT was prepared from *M. tuberculosis* $H_{37}Ra$ (Difco Laboratories, Inc., Detroit, MI) as previously described (13). Tetanus toxoid (TT) was purchased from Massachusetts Department of Public Health, Jamaica Plains, MA. Biochemically purified fragment C of tetanus toxoid (98% purity) was purchased from Inland Laboratories, Inc., Austin, TX. Recombinant fragment C of tetanus toxoid was purchased from Boehringer Mannheim Corp., Indianapolis, IN, and PHA was purchased from Wellcome Diagnostics, Research Triangle Park, NC.

Synthetic peptides covering residues 872-885, 957-975, 1017-1030, 1065-1077, 1094-1104, 1120-1139, 1153-1174, 1229-1252, and 1270-1283 of the tetanus toxin sequence were prepared as described (14) and kindly donated by Drs. J. Halpern and W. Habig, FDA, Bethesda, MD. Additional peptides, corresponding to amino acids 1229-1240, 1235-1246, and 1241-1252, were synthesized in the University of Michigan Protein Core Facility using synthesizer model ABI-431A (Applied Biosystems, Inc., Foster City, CA) and purified by HPLC. Analysis of peptides included amino acid sequencing and mass spectroscopy.

### *Monoclonal antibodies*

Anti-Leu 4, anti-IL-2R, anti-leu16, anti-DR (L243), and anti-TCR-γ/δ-1 monoclonal antibodies were all purchased from Becton Dickinson, Mountain View, CA. Monoclonal antibody 7.3.19.1 was purchased from MeDiCa, Carlsbad, CA. TiγA was kindly donated by Dr. T. Hercend, Institut Gustave-Roussy, Villejuif, France. A13 and BB3 were provided by Dr. L. Moretta, National Institute for Cancer Research, Genova, Italy. TS1.22 was prepared by Dr. T. Springer, Harvard University, Boston, and provided by Dr. A. Krensky, Stanford University.

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1. *Abbreviations used in this paper:* AP-MT, acetone precipitable fraction of *Mycobacterium tuberculosis;* TCR, T cell receptor; TT, tetanus toxoid.

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109d6 was provided by Dr. P. Gregersen, Cornell University, New York.

### *T cell clones*

Clones 1.2, 1.3, 1.4, and 1.6 were isolated from the synovial fluid of a patient with early RA as described (8). Briefly, synovial fluid T cells were periodically stimulated with AP-MT in the presence of antigen-presenting cells in the form of irradiated autologous peripheral blood mononuclear cells, and subsequently cloned by the limiting dilution technique. The clonality of these γδ T cells was confirmed by Southern blot analysis and by γ-chain cDNA oligonucleotide sequencing data, which showed common use of Vγ9-Jp rearrangements with junctional diversity among clones. Only a single γ-chain transcript was found in each one of the three tested T cell clones, indicating that they were distinct clonal populations (Loh, E. Y., J. Holoshitz, and S. Strober, unpublished results). Clones of the BC series and SF series were isolated from the peripheral blood of a normal donor, and from the synovial fluid of a patient with RA, respectively, using magnetic sorting of anti-TCR-γ/δ-1 positive cells, and subsequent culture in limiting dilutions as described by Fisch et al. (11). The tetanus toxoid–specific αβ T cell clone TT44G was isolated from the peripheral blood of a normal individual, and donated by Dr. B. Richardson, University of Michigan.

TCR phenotypes were determined by indirect immunofluorescence and flow cytometry as previously described (8, 12), using first stage monoclonal antibodies against Vγ9 (TiγA), against Vδ1 (A13), and against Vδ2 (BB3).

### *Proliferation assays*

The [$^3$H]thymidine incorporation assay was done as described (8), by coculturing irradiated (3,000 R) peripheral mononuclear cells, $10^5$ per well, and $2 \times 10^4$ T cells in the presence or absence of antigens in supplemented RPMI 1640 medium containing 10% pooled normal human serum (North American Biologicals, Inc., Miami, FL). After 54 h, cultures were pulsed with [$^3$H]thymidine (1 μCi per well; Dupont NEN, Wilmington, DE) and harvested 18 h later and counted. Results are expressed as mean cpm, or stimulation index (the ratio between cpm obtained with test antigen to that obtained without antigen). Optimal antigen concentrations, predetermined by dose-response experiments were as follows: AP-MT; 10 μg/ml, tetanus toxoid; 1:500 dilution, PHA; 0.25 μg/ml, fragment C of tetanus toxin; 10 μg/ml, peptides; 10 μM.

HLA typing of antigen presenting cells was done by a standard microcytotoxicity assay.

### *Monoclonal antibody inhibition experiments*

Proliferation assays were carried out as above, in the presence or absence of 2 μg/ml of the following purified monoclonal antibodies: anti-Leu4, anti-Leu16, anti-IL-2R, and anti-DR, or 1:2,000 dilution of A13, BB3, or TS1.22 ascites fluids. Ascites fluids of monoclonal antibody 109d6 and 7.3.19.1 were used in final concentrations of 1:100 and 1:200, respectively. All antibodies were extensively dialyzed before use. Results are expressed as relative proliferation (stimulation obtained in the presence of test antibody, divided by the stimulation obtained in the absence of antibodies, ×100) (8).

## Results

### *Proliferative response to two antigens*

In a previous report (8) we have shown that four γδ T cell clones derived from the synovial fluid of a patient with early RA proliferated in response to the mycobacterial antigen AP-MT. Subsequently, we found that all four clones also recognized tetanus toxoid (Table I). As can be seen, the γδ clones responded comparably well to AP-MT and tetanus toxoid. A control CD4$^+$ CD8$^-$ αβ anti–tetanus toxoid clone (TT44G), which was derived from the peripheral blood of a normal donor, responded to tetanus toxoid, but not to AP-MT (Table I).

Proliferation of a clonal T cell population in response to two antigens could be due to cross-reactivity or could represent two distinct recognition mechanisms, similar to activation of antigen-specific αβ T cell clones by superantigens (15). To address these possibilities, we compared the MHC restriction patterns in which AP-MT and tetanus toxoid are recognized by using antigen presenting cells from HLA-typed donors. As can be seen in Table II, in contrast to the HLA unrestricted recognition of AP-MT, tetanus toxoid could be presented by donors of the HLA-DRw53 serotype only. DRw53 is a supertype shared by all individuals with the haplotypes DR4, DR7, or DR9. The DRw53 specificity is conferred by a nonpolymorphic DRβ chain which is encoded by the *DRB4*0101* gene.

### *Role of TCR and adhesion molecules*

Recognition of both antigens could be specifically blocked by anti-CD3 and anti-Vδ2 monoclonal antibodies, but not by anti-Vδ1 antibodies. These results implicate the TCR in the recognition of AP-MT and tetanus toxoid. Intercellular adhesion was also found to play a role in the reactivity to AP-MT and tetanus toxoid, as proliferation in response to both antigens could be blocked by anti-LFA 1α monoclonal antibodies (Fig. 1). While presentation of tetanus toxoid could be blocked by anti-DR antibodies, presentation of AP-MT could not be blocked by anti-DR (Fig. 1), anti-DP, or anti-DQ antibodies (8). Interestingly, anti-IL-2 receptor antibodies blocked activation by tetanus toxoid but not by AP-MT. This finding corroborates previous observations that γδ T cells stimulated with my-

*Table I. Proliferative Responses of γδ T Cell Clones and a Control αβ T Cell Clone*

| T cell clone | Phenotype | Proliferative response | | | |
|---|---|---|---|---|---|
| | | No antigen | AP-MT | TT | PHA |
| | | *cpm × 10$^{-3}$* | | | |
| 1.2 | CD4$^-$CD8$^-$γδ | 0.5 | 2.0 | 9.0 | 55.2 |
| 1.3 | CD4$^-$CD8$^-$γδ | 0.1 | 7.4 | 5.4 | 21.4 |
| 1.4 | CD4$^-$CD8$^-$γδ | 0.6 | 4.2 | 4.1 | 29.9 |
| 1.6 | CD4$^-$CD8$^-$γδ | 0.2 | 4.7 | 8.1 | 12.5 |
| TT44G | CD4$^+$CD8$^-$αβ | 0.4 | 0.5 | 75.5 | 37.2 |

Proliferation assays were carried out as described (8), using $2 \times 10^4$ T cells and $10^5$ irradiated (3,000 R) antigen presenting cells per well in the presence or absence of AP-MT, 10 μg/ml, TT, at 1:500 dilution, or PHA, 0.25 μg/ml. Cultures were pulsed with [$^3$H] thymidine at 54 h and harvested at 72 h. Results are presented as mean cpm. Standard deviations were less than 20% of the mean.

*Table II. HLA Restriction of Antigen Presentation to γδ Clone 1.4*

| APC | | HLA type of antigen presenting cells | | | | | Proliferative response to antigen | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | DR | DQ | No antigen | AP-MT | TT | PHA |
| | | | | | | | | *cpm × 10⁻³* | | |
| Autologous | | 2,– | 51,8 | 1,7 | 4,5 w52,w53 | w3,– | 0.6 | 4.2 | *3.8* | 28.6 |
| Heterologous | 1 | 2,24 | 35,44 | 4,– | 2,6 w52 | w1,– | 0.4 | 6.6 | 0.5 | 24.3 |
| | 2 | 1,3 | 35,53 | 4,– | 1,11 w52 | w1,w3 | 0.6 | 5.2 | 0.4 | 20.3 |
| | 3 | 1,3 | 8,38 | –,– | 3,6 w52 | w1,w2 | 0.7 | 3.8 | 0.9 | 41.2 |
| | 4 | 24,– | 54,– | –,– | 2,8 w52 | w1,– | 0.4 | 6.1 | 0.5 | 46.0 |
| | 5 | 23,30 | 18,44 | 5,– | 3,4 w52,w53 | w2,w3 | 0.7 | 9.7 | *3.3* | 28.7 |
| | 6 | 24,32 | 35,– | 4,– | 7,– w52,w53 | w2,w3 | 0.6 | 3.4 | *2.5* | 37.9 |
| | 7 | 2,30 | 13,50 | –,– | 3,7 w52,w53 | w2,w3 | 0.7 | 3.1 | *2.9* | 52.8 |
| | 8 | 2,3 | 49,60 | 3,– | 1,4 w53 | w1,w3 | 1.2 | 11.8 | *6.5* | 28.3 |
| | 9 | 2,24 | 7,14 | –,– | 2,4 w53 | w1,w3 | 0.3 | 7.7 | *3.8* | 39.1 |
| | 10 | 1,2 | 8,44 | –,– | 2,7 w53 | w1,w2 | 0.6 | 4.8 | *6.6* | 50.8 |
| | 11 | 2,11 | 44,60 | 3,5 | 4,– w53 | w3,– | 0.5 | 10.6 | *2.6* | 28.1 |
| | 12 | 11,30 | 13,53 | 4,– | 4,7 w53 | w2,w3 | 0.6 | 9.3 | *3.1* | 37.1 |
| | 13 | 2,29 | 39,44 | –,– | 7,15 w53 | w1,w2 | 1.1 | 13.9 | *2.0* | 39.8 |

Proliferative responses of γδ T cells to AP-MT, TT, and PHA were measured as in Table I using antigen-presenting cells obtained from different HLA-typed donors.

cobacteria do not secrete detectable levels of IL-2 (16). Production of other lymphokines by the γδ T cell clones is currently being studied in our laboratory. Taken together, these results suggest that although reactivity to both antigens involves the TCR and an adhesive interaction with antigen presenting cells, AP-MT and tetanus toxoid are presented by different antigen presenting molecules, and involve distinct activation mechanisms.

### *Mapping the epitope*

Tetanus toxoid is a crude extract which may contain nearly as much contaminating bacterial antigens as the toxin itself. To further define the antigenic specificity of our clones, we used purified fragment C of tetanus toxin. Results of these experiments (Fig. 2) indicate that our γδ T cells recognize an epitope on fragment C (the 450 carboxy-terminal amino acids of the tetanus toxin protein). The epitope was further defined by using synthetic peptides corresponding to various regions of fragment C of tetanus toxin. As Fig. 2 shows, peptide 1229-1252 was recognized by clone 1.4 cells. Synthetic peptides corresponding to eight other regions of fragment C failed to stimulate the γδ T cells.

The epitope was further defined by using three partially overlapping synthetic peptides covering the 1229-1252 sequence. Fig. 3 *A* shows that the epitope could be mapped to 12 amino acids within the 1235-1246 sequence. All three clones tested, 1.2, 1.3, and 1.4, recognized this peptide (Fig. 3 *B*). Recognition of the same peptide by junctionally diverse clones is surprising, although murine αβ T cell clones reacting to a single epitope, despite junctional diversity, have been described (17). It should be noted, however, that it is not clear whether our three γδ T cell clones respond to a single epitope on peptide

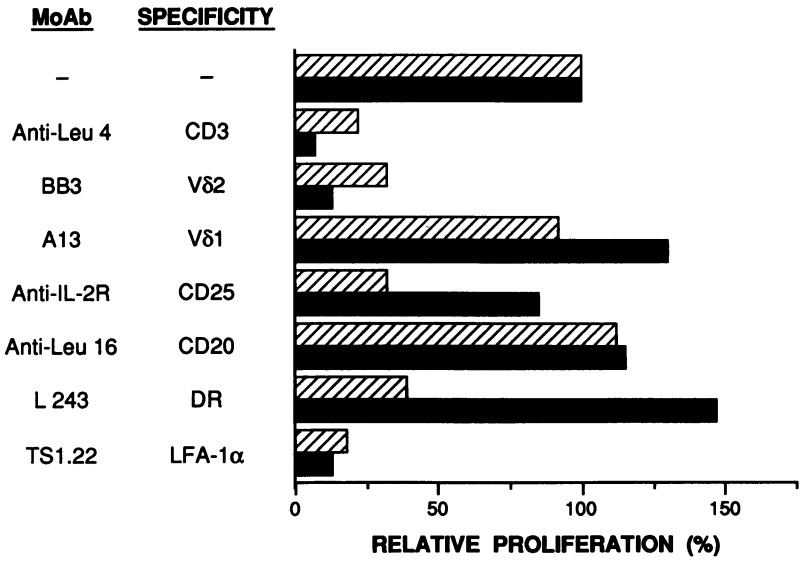


*Figure 1.* Antibody inhibition of proliferative responses of γδ T cells to AP-MT and tetanus toxoid. Proliferation of clone 1.3 cells in response to AP-MT could be blocked by anti-CD3, anti-Vδ2 and by anti-LFA1-α, but not by anti-DR, or anti-IL-2 receptor monoclonal antibodies. Proliferative response of these cells to tetanus toxoid could be blocked by anti-CD3, anti-Vδ2, anti-LFA1-α, anti-DR and anti-IL-2 receptor antibodies, but not by a control monoclonal antibody anti-CD20. ▨, TT; ■, AP-MT.

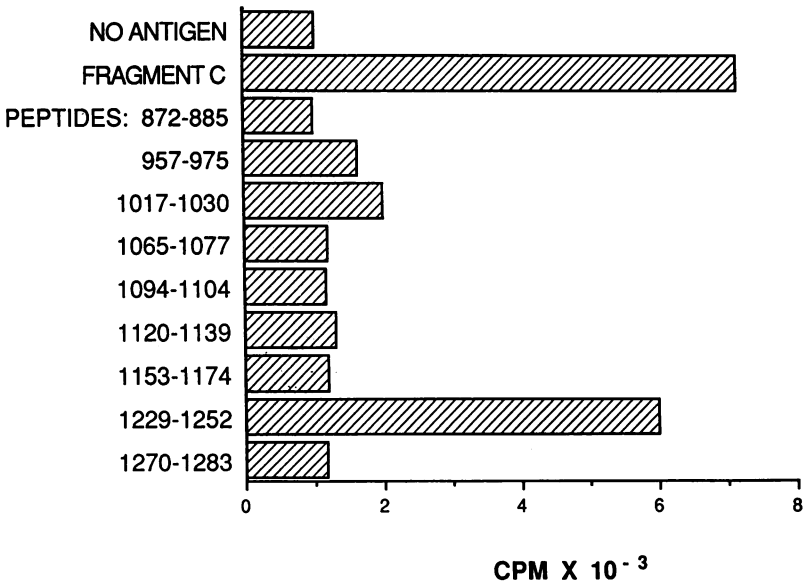


*Figure 2.* Proliferation of clone 1.4 to tetanus toxin peptides. Clone 1.4 cells were incubated in the presence of DRw53-positive peripheral blood mononuclear cells and biochemically purified fragment C of tetanus toxin, and nine synthetic peptides corresponding to different regions of fragment C. Peptides included residues number 872-885, 957-975, 1017-1030, 1065-1077, 1094-1104, 1120-1139, 1153-1174, 1229-1252, and 1270-1283. Results are presented as cpm obtained with 10 $\mu$g/ml of fragment C or 10 $\mu$M of the peptides. These concentrations were found to be optimal in dose-response experiments.

1235-1246. The possibility that peptide 1235-1246 was mitogenic was ruled out by studying control panels of $\gamma\delta$ T cell clones. As can be seen in Fig. 3 *B*, none of eight control clones of the V$\gamma$9/V$\delta$2, mycobacteria-reactive subset, or three $\gamma\delta$ clones with the V$\delta$1 TCR proliferated in response to the peptide. Thus, the reactivity of our $\gamma\delta$ T cell clones to peptide 1235-1246 represents a genuine antigen-specific recognition.

*Presentation of tetanus toxin peptide by DRw53*

Presentation of peptide 1235-1246 was restricted by DRw53. Peripheral blood mononuclear cells of DRw53 donors, but not of DRw52 donors, could present the peptide (Fig. 3 *C*). To directly study the role of the DR$\alpha$:DR$\beta$4 (DRw53) molecule in antigen presentation we took two approaches. First, we used DR$\alpha$:DR$\beta$4 (DRw53)-expressing transfectant L-cells. Using this murine cell line as antigen presenting cells we obtained only weak presentation (mean stimulation index in four consecutive experiments: 2.6, not shown). A possible explanation, as suggested by the results shown in Fig. 1, is that a costimulatory signal such as ICAM-1 is required. The requirement of coexpression of ICAM-1 on DR-transfected L-cells for efficient antigen presentation to $\alpha\beta$ T cells has been reported (18). As an alternative approach, we carried monoclonal antibody inhibition studies using 109d6, an anti-DRw53 monoclonal antibody. This antibody has been previously shown to specifically recognize the DR$\alpha$:DR$\beta$4 (DRw53) molecule (19), and has been useful in identifying DRw53 as the antigen presenting molecule for anti-chlamydial T cell clones (20). As can be seen in Fig. 3 *D*, antibody 109d6 could effectively block presentation of peptide 1235-1246 and fragment C of tetanus toxin, confirming that the DRw53 molecule is directly involved in presentation of peptide 1235-1246 to the $\gamma\delta$ T cells. Presentation of AP-MT and PHA was unaffected by this antibody. A control monoclonal antibody specific for DRw52 did not inhibit presentation of the toxin peptides (Fig. 3 *D*).

## Discussion

Considerable understanding of the development and tissue distribution of $\gamma\delta$ T cells has been achieved over the past several years. However, fundamental questions about their function remain unanswered. The enigma stems from the paucity of knowledge about their antigenic specificity and the identity of antigen presenting molecules. Particularly interesting is the human V$\gamma$9/V$\delta$2 subset, which displays extensive junctional sequence diversity, consistent with a versatile nominal antigenic specificity. Our data provide the first evidence that this subset can recognize nominal peptides presented by class II MHC molecules.

Current knowledge about the antigenic specificity of $\gamma\delta$ T cells in general is scarce. Some $\gamma\delta$ T cells have been found to react against cells expressing MHC class I molecules, MHC class II molecules, or class I-related molecules (21–24). The nature of the antigens recognized by $\gamma\delta$ T cells in those studies remains to be determined. Kozbor et al. reported human CD8$^+$ $\gamma\delta$ T cells of the V$\delta$1 TCR phenotype, a subset of $\gamma\delta$ T cells which constitute a small minority of peripheral $\gamma\delta$ T cells in adults. These cells responded to an undefined antigen in a tetanus toxoid extract presented by cells obtained from some, but not all DR4$^+$ individuals (25). Given the heterogeneous nature of tetanus extracts and the equivocal MHC restriction pattern, it is difficult to conclude whether those cells responded to the toxin, or to contaminating antigens, and what the identity of the presenting molecule was. Another recent study reported a single murine T cell hybridoma with mRNA expression of all four TCR chains ($\alpha$, $\beta$, $\gamma$, $\delta$) and evidence for cell surface expression of $\gamma$ and $\delta$ chains only (26). This hybridoma responded to the synthetic copolymer poly(Glu$^{50}$Tyr$^{50}$) in the context of Qa-1 cell surface products. Due to the unusual nature of the cells and the antigen used in that study, it is difficult to draw firm conclusions about the physiological function of $\gamma\delta$ T cells. The present results provide direct evidence that "physiological" $\gamma\delta$ T cells can recognize naturally occurring foreign peptides presented by class II MHC molecules. These results suggest that, much like $\alpha\beta$ T cells, V$\gamma$9/V$\delta$2 cells may contribute to the immune response against foreign antigens in vivo in an antigen-specific and MHC-restricted manner.

In addition to their DRw53-restricted reactivity to peptide 1235-1246, our clones proliferated in the presence of a mycobacterial extract AP-MT in an MHC-unrestricted manner. Such nonclonal reactivity has been previously shown to characterize the entire V$\gamma$9/V$\delta$2 subset. Our results showing that cells

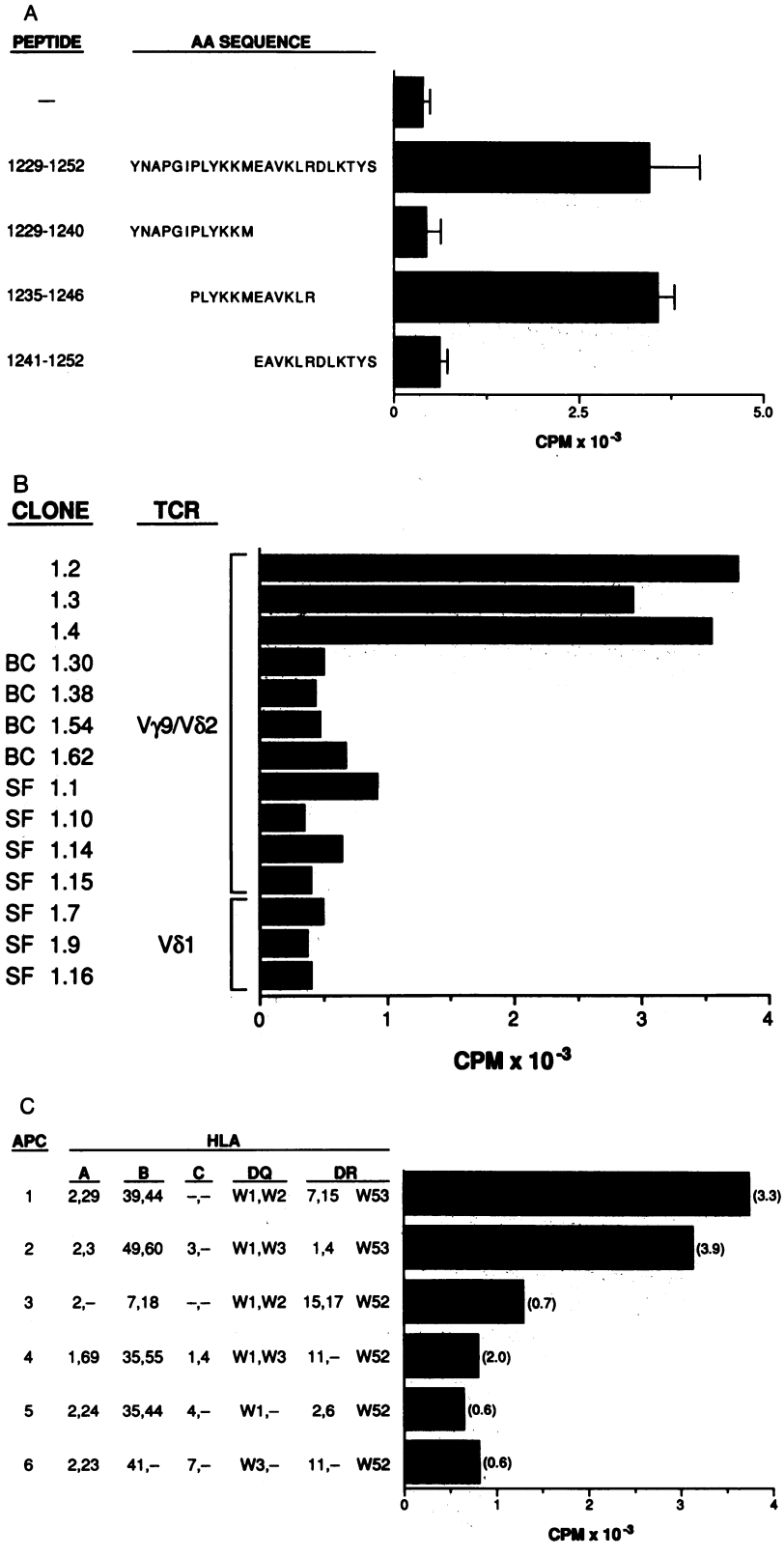


*Figure 3.* Fine specificity and MHC restriction of tetanus toxin peptide recognition by γδ T cells clones. (*A*) Mapping of the epitope to amino acids 1235-1246 of tetanus toxin. Proliferation assay with partially overlapping 12-mer synthetic peptides corresponding to the region 1229-1252 of tetanus toxin indicate that the epitope recognized by the γδ T cells is confined to the region within amino acid 1235-1246. (*B*) Control γδ T cell clones do not proliferate in response to peptide 1235-1246. Clones 1.2, 1.3, and 1.4 all recognized peptide 1235-1246, while 11 other γδ T cell clones did not respond. All clones responded comparably well to PHA and all Vγ9/Vδ2 clones proliferated in response to AP-MT (not shown). (*C*) Peptide 1235-1246 is presented by DRw53$^+$ antigen presenting cells, but not by DRW52$^+$ antigen presenting cells. Numbers in parentheses indicate stimulation index. (*D*) Presentation of peptide 1235-1246 can be blocked by anti-DRw53 specific monoclonal antibody 109d6. The inhibitory effect was specific, as no inhibition of presentation of AP-MT or PHA was found. A control monoclonal antibody 7.3.19.1 specific for DRw52 did not have any inhibitory effect. ▨, 7.3.19.1; ■, 109d6.

of this subset are also capable of nominal antigen recognition strengthen the notion that their reactivity to mycobacteria represent a superantigen-like phenomenon (27–30). The identity of the mycobacterial superantigenic moiety is still unclear. While some have proposed low molecular weight nonprotein components (31), we (8) and others (32, 33) have identified reactivity to a mycobacterial 65-kD heat shock protein. It is possible that mycobacteria contain more than one antigenic determinant for γδ T cells. Born et al. (34) identified an epitope on the mycobacterial 65-kD heat shock protein recognized by murine γδ T cell hybridomas. Our own attempts to map the epitope for our clones on that protein have been unsuccessful

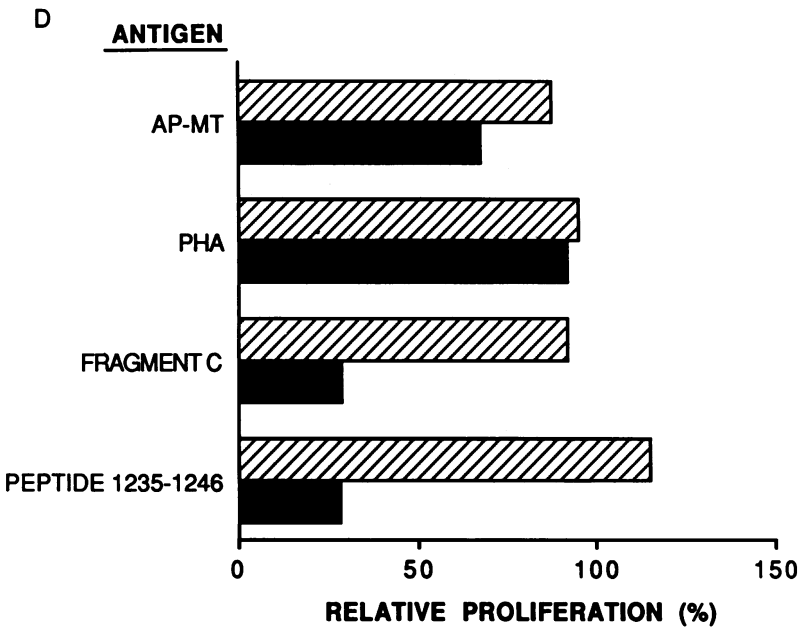


*Figure 3 (Continued)*

so far. Neither truncated recombinant polypeptides nor a panel of synthetic peptides covering the entire sequence could substitute for the native heat shock protein isolated biochemically from *Mycobacterium bovis* BCG (unpublished). It is noteworthy that bacterial superantigens for $\alpha\beta$ T cells have been shown to be presented as intact proteins only (reviewed in 1). Different from the previously described superantigenic recognition, however, class II MHC molecules do not seem to be required for presentation of the mycobacterial antigen. Presentation of AP-MT could not be blocked by anti-class II HLA antibodies (Fig. 1 and reference 8). In addition, xenogeneic and human cell lines expressing transfected class II HLA genes failed to present AP-MT to these cells. Moreover, we have recently identified a class II-negative cell line capable of presenting AP-MT (manuscript in preparation). Thus, unlike recognition of previously described superantigens by $\alpha\beta$ T cells, recognition of AP-MT by our $\gamma\delta$ cells appears to be HLA class II-independent. We are currently studying the role of non-MHC molecules in AP-MT presentation.

It is intriguing that four $\gamma\delta$ clones which were cloned and expanded by virtue of their nonclonal reactivity to AP-MT responded all to tetanus toxoid. Furthermore, the three clones tested to date all recognized a single peptide. This may suggest that the uncloned population contained a high frequency of $\gamma\delta$ T cells specific to the nominal antigen (either tetanus toxoid, or a different antigen with an epitope cross-reactive with peptide 1235-1246). The clones reported here have been isolated from the synovial fluid of an RA patient. It is therefore interesting that our cells recognized their antigen in association with DRw53. A determinant on DRw53, identified by the monoclonal antibody 109d6, has been proposed as a "shared epitope" which confers susceptibility to RA (35, 36). Thus, since the same antibody specifically blocked presentation of the nominal antigen to our $\gamma\delta$ T cells, it is tempting to speculate that these clones are pathogenically relevant.

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