

Brief Definitive Report

HUMAN TCR- γ^+/δ^+ , CD8⁺ T LYMPHOCYTES RECOGNIZE TETANUS TOXOID IN AN MHC-RESTRICTED FASHION

BY DANUTA KOZBOR,* GIORGIO TRINCHIERI,* DIMITRI S. MONOS,‡
MASAHARU ISOBE,* GIANDOMENICO RUSSO,* JO ANN HANEY,*
CHESTER ZMIJEWSKI,‡ AND CARLO M. CROCE*

From *The Wistar Institute, and ‡The Hospital of the University of Pennsylvania,
Philadelphia, Pennsylvania 19104

Although the TCR- γ/δ complex contains relatively few germline V gene segments (1, 2), the TCR- γ diversity is increased by N nucleotide incorporation at the V-J junction (1), and TCR- δ junctional diversity is high, with the use of at least two tandemly arranged D δ elements and extensive N nucleotide incorporation (2). Because adult TCR- γ/δ shows the most extensive dichotomy with respect to the spectrum of V region diversity compared with junctional diversity, Davis and Bjorkman (3) have suggested that the ligand for γ/δ heterodimer will consist of a peptide fragment of antigen in association with an MHC-like molecule. γ^+/δ^+ T cells with allogenic reactivity have been established (4, 5), indicating that the TCR- γ/δ is capable of self-non-self MHC discrimination. However, the contribution of γ^+/δ^+ T cells to an antigen-specific immune response has never been demonstrated. We have established human γ^+/δ^+ , CD8⁺ T cell clones that specifically responded to tetanus toxoid (TT) and autologous APC with proliferation and IFN- γ production, and the response was restricted by HLA-DR4-related element. These results represent the first experimental demonstration that γ^+/δ^+ T cells contribute to the immune response to a nominal antigen.

Materials and Methods

Cell Culture. Purified TT (lot TAS 239) was obtained from Connaught Laboratories Ltd. (Willowdale, Canada). Purity was confirmed by HPLC analyses. The TT-specific T cell line DF was obtained by stimulation with TT (10 μ g/ml) and irradiated (4,000 rad) autologous APC, and maintained in RPMI 1640 medium with 10% heat-inactivated FCS (Flow Laboratories, Inc., McLean, VA) and 20 U/ml of rIL-2 (Amgen Biologicals, Thousand Oaks, CA). Cells were restimulated with antigen and autologous APC every 3 wk.

Antigen-induced Proliferation and IFN- γ Production. T cells were seeded in flat-bottomed microtiter plates (Linbro; Flow Laboratories) at 2×10^4 cells/200 μ l in the presence of irradiated APC after plastic adherence of 4×10^5 cells/200 μ l. Cultures were stimulated for 36 h with 10 μ g/ml of TT or TNP-BSA. Production of IFN- γ was measured by RIA, using standard solutions of IFN- γ as controls. For proliferation, cultures were pulsed for 12 h with 1 μ Ci/well [³H]TdR (Amersham Corp., Amersham, UK).

Southern and Northern Blotting. DNA samples from DF, from EBV-transformed autologous

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B cell clone, B6, and from γ^+/δ^+ cloned cells were cleaved with restriction enzymes and subjected to electrophoresis in an agarose (Sigma Chemical Co., St. Louis, MO) gel. Cytoplasmic RNA from DF, from γ^+/δ^+ and α^+/β^+ clones, and from γ^+/δ^+ PEER cell line (6) was denatured, electrophoresed in a 1% agarose gel, transferred to nitrocellulose, and hybridized to ^{32}P -labeled TCR probes (6): the cDNA TCR- α ($T\alpha 2$), TCR- β ($C\beta$), TCR- δ (0-240), and T γ -1. The PMI19 genomic TCR- δ probe corresponds to a region 5–11 kb upstream of $C\delta$ (7).

Results and Discussion

TT-specific T Cell Line. The TT-specific T cell line DF was derived from lymphocytes of a hyperimmunized donor by in vitro stimulation with TT (10 $\mu\text{g}/\text{ml}$) and autologous APC. Two-color staining with anti-TCR $\delta 1$ (6) and anti-CD8 mAbs revealed a percentage of γ^+/δ^+ , CD8 $^+$ T cells that fluctuated between 30 and 50% of the total T cell number during the 2-yr culture period. No γ^+/δ^+ T cells were detected in three other TT-specific T cell lines obtained from unrelated donors (data not shown). Therefore the presence of γ^+/δ^+ T cells in the TT-specific cell line may be related to the immunization history of donor D.F., who had received seven TT inoculations. Lymphocytes of donor D.F. showed $\sim 1\%$ of T cells reacting with anti-TCR $\delta 1$ antibody, and an autologous T cell line specific for TNP-hapten did not contain γ^+/δ^+ T cells. Immunoprecipitation of ^{125}I -labeled γ^+/δ^+ DF cells with the anti-TCR $\delta 1$ antibody revealed a non-disulfide-linked 55-kD TCR- γ and a 40-kD TCR- δ chain after SDS-PAGE (not shown). Cloning of DF cells by limiting dilution yielded several α^+/β^+ T cell clones and three clones that were homogeneously positive for TCR- γ/δ , CD8 and CD2 surface antigens (not shown). One of the γ^+/δ^+ clones was analyzed for rearrangement and expression of the TCR- δ gene (Fig. 1). The Hind III digest of DNA revealed a germline configuration of the $C\beta 2$ complex (7.5 kb) in γ^+/δ^+ clone 4, whereas the same blot hybridized to the $J\delta$ genomic probe PMI19 (7) showed one rearranged band of 12 kb and two germline bands of 6.2 kb and 3.3 kb due to a Hind III site within the PMI 19 probe. An additional $J\delta$ rearrangement of 4.7 kb was detected in DF cells indicating presence of another γ^+/δ^+ T cell population (Fig. 1 A). TCR- δ transcripts of 2.2 and 1.5 kb, as well as a 1.6-kb TCR- γ message, were present in DF cells, in clone 4, and PEER cells (Fig. 1 B). The 1.5-kb TCR- α transcript was detected in clones 7 and 9 as well as the DF population. Analysis of TCR- β expression revealed 1.3- and 1.0-kb transcripts in DF cells

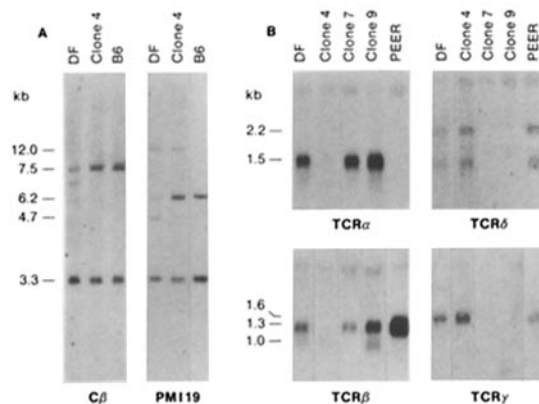


FIGURE 1. Southern (A) and Northern (B) blot analysis of the TCR complex in γ^+/δ^+ clone 4. (A) Southern blotting of Hind III-digested DNA (10 μg) with a probe for constant regions of TCR- β ($C\beta$), and $J\delta$ probe (PMI19). (B) Northern blotting of 10 μg of RNA/lane with probes for TCR- α ($T\alpha 2$), TCR- δ (0-240), TCR- β , and TCR- γ ($T\gamma$ -1). Clones 7 and 9 are α^+/β^+

and in clone 9, whereas clone 7 and PEER cells showed a 1.3-kb message. A trace of the 1.0-kb message was detected in the γ^+/δ^+ cells.

Antigenic Specificity and MHC Restriction of the γ^+/δ^+ T Cell Clones. Proliferation and IFN- γ production by DF cells and by the α^+/β^+ and γ^+/δ^+ , CD8⁺ T cell clones analyzed was optimal using 10 $\mu\text{g}/\text{ml}$ of TT (not shown). In α^+/β^+ T cells, stimulated with TT and autologous APC, [³H]TdR incorporation and IFN- γ production was ~10-fold and ~5-fold higher, respectively, than in the γ^+/δ^+ T cell clones (Fig. 2 A). The autologous TNP-specific T cell line used as a specificity control did not respond to stimulation with TT, and none of the TT-specific T cell clones responded to stimulation with TNP-BSA (10 $\mu\text{g}/\text{ml}$). No proliferation or IFN- γ production was detected when TT-specific T cell clones were stimulated with either TT or autologous APC only. Addition of IL-2 (10 U/ml) for 36 h to cultures of γ^+/δ^+ cells (2×10^4 cells/200 μl) containing TT (10 $\mu\text{g}/\text{ml}$) and autologous APC increased both proliferation and IFN- γ production (~3-fold) in three γ^+/δ^+ clones (Fig. 2 A). To determine whether the antigenic specificity of γ^+/δ^+ , CD8⁺ T cells was MHC restricted, DF T cells, α^+/β^+ clones 7 and 9 and γ^+/δ^+ clone 4 were stimulated with TT and allogeneic APC and assayed for proliferation and IFN- γ production. The D.F. donor

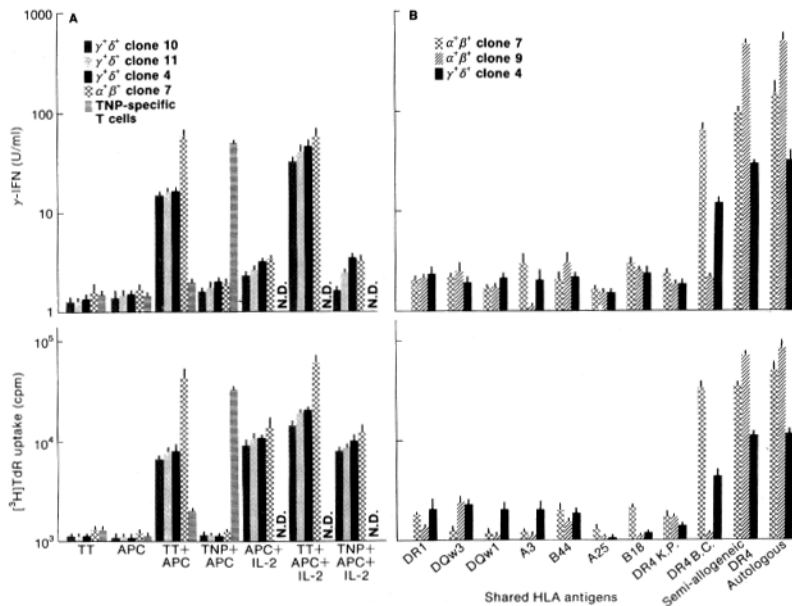


FIGURE 2. Antigen specificity (A) and HLA restriction (B) of α^+/β^+ and γ^+/δ^+ T cell clones. (A) 2×10^4 cells/well of α^+/β^+ T cell clone 7, γ^+/δ^+ T cell clones 4, 10, and 11, and a TNP-specific autologous T cell line were stimulated with TT, TNP-BSA, and autologous APC with or without IL-2 (10 U/ml) for 36 h. Proliferation of cells was monitored by [³H]TdR uptake (bottom panel), and IFN- γ was measured by RIA (top panel). (B) HLA restriction was studied by stimulation of the T cells with TT and APC from 14 DR4⁻ donors sharing one HLA-determinant with the autologous donor (two donors per case), from 5 DR4⁺ donors, and from a DR4⁺ sister of the autologous donor (semi-allogeneic DR4). The response was monitored by [³H]TdR uptake (background ~1,500 cpm; cultures with T cells and APC, or APC and TT, or T cells and TT), and IFN- γ production. DR4⁺ APC from donor B.C. were able to present TT to T cells, and APC from DR4⁺ donor K.P. stimulated no response.

HLA phenotype was: A3, A25, B18, B44, DR1, DR4, DQw1, and DQw3. Semi-allogeneic APC derived from a sister of the autologous donor with the following phenotype: A3, A26, B18, B49, DR1, DR4, DQw1, DQw3, presented TT to all α^+/β^+ and γ^+/δ^+ T cells, and the response was identical to that obtained with autologous APC (Fig. 2 B). When allogeneic APC from 19 donors, each sharing one class I or class II MHC antigen with D.F. were used, antigen presentation was detected only when T cells were stimulated with TT in the presence of DR4-matched APC. APC from all 14 DR4⁻ donors were unable to stimulate our T cells. Of the five DR4⁺ donors tested, donor B.C. gave the best response (60–70% of autologous response), three others stimulated T cells up to 20–40% of the autologous response (not shown), and one donor (K.P.) stimulated no response. Although APC obtained from donor B.C. stimulated DF cells, α^+/β^+ clone 7 and γ^+/δ^+ clone 4, they were unable to present antigen to α^+/β^+ clone 9. The association between the expression of DR4 on unrelated APC and the ability to present TT to the γ^+/δ^+ T cells in highly significant (χ^2 14.19, $n = 19$, $p < 0.0004$). Similar results were obtained with γ^+/δ^+ clones 10 and 11 stimulated with APC from autologous, semi-allogeneic and B.C. DR4⁺ donors, and with APC from a DR4⁻ donor (not shown). These results indicate that the TCR- γ/δ receptor can recognize non-MHC-encoded foreign antigens in a self-MHC-restricted fashion. There are at least two possible explanations for the variability among the APC derived from different DR4⁺ donors in presenting TT to the α^+/β^+ and the γ^+/δ^+ T cell clones. The restriction element for the clones might not be DR4 itself, but a gene closely associated and in linkage disequilibrium with it at the population level. Alternatively, these differences might reflect the well known molecular and structural heterogeneity within DR β chains isolated from a single serologically defined DR4 phenotype that presumably contribute to the dominant polymorphisms recognized by T cells in the MLR (8). The differential response of our T cell clones to TT presented on different DR4⁺ donors might reflect the discriminatory ability of the TCR- γ/δ . The response of all T cell clones was inhibited by >70% by an anti-HLA DR (DI.B6) antibody directed against a monomorphic determinant, but not by an anti-HLA-DQ (G2.53) mAb (not shown). This inhibition, although consistent with class II restriction, is not conclusive because mAbs against monomorphic determinants of HLA-DR antigens block the human MLR and an IL-2-dependent T cell line by direct interaction with these T cells. Although CD8 antigen expression is especially associated with class I MHC recognition, CD8⁺ T cell clones specific for class II MHC antigens have been described (9). Since anti-CD8 antibody did not affect the activity of class II MHC-specific CTL clones (9) and did not inhibit antigen-induced proliferation (10) these clones apparently interact with the target cells through the antigen-specific receptor and do not use the CD8 molecule as an associative recognition element. Similarly anti-CD4 and anti-CD8 antibodies did not inhibit proliferation and IFN- γ production, respectively, in our α^+/β^+ , CD4⁺ and γ^+/δ^+ , CD8⁺ clones (not shown).

Although the antigenic response of γ^+/δ^+ T cell remains largely unknown, our results indicate that the TCR- γ/δ heterodimer is a functionally distinct TCR, capable of more than the broad self-non-self discrimination. It is therefore possible that the physiological significance of γ^+/δ^+ T cells in the immune response against a foreign antigen resides in their regulatory ability.

Summary

We have analyzed the ability of human γ^+/δ^+ T cells to recognize a nominal antigen in association with MHC molecules. A TT-specific T cell line with $\sim 40\%$ γ^+/δ^+ T cells was established from a hyperimmunized donor, D.F., by stimulation with antigen and autologous APC. Three DF-derived γ^+/δ^+ clones were CD8⁺ as determined by immunofluorescence staining, and by Southern and Northern blotting with probes detecting δ chain rearrangement and δ and γ chain transcripts, respectively. The γ^+/δ^+ clones responded to stimulation with TT, but not TNP-BSA, and autologous APC by proliferation and IFN- γ production. No proliferation or IFN- γ production was detected when TT-specific T cell clones were stimulated with either TT or autologous APC only. The response to TT was enhanced by addition of exogenous IL-2. The use of allogeneic APC from 19 donors sharing one HLA-determinant with the autologous donor D.F., showed that the γ^+/δ^+ T cells responded to TT with HLA-DR4-related restriction as measured by proliferation and IFN- γ production. These results demonstrate that γ/δ receptors can recognize non-MHC-encoded foreign antigen in a self-MHC-restricted fashion.

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