

FUNCTIONAL  $\gamma$  CHAIN-ASSOCIATED T CELL RECEPTORS  
ON CEREBROSPINAL FLUID-DERIVED NATURAL KILLER-  
LIKE T CELL CLONES

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The antigen receptor of most T cells consists of three components, T cell receptor (TCR)- $\alpha$  and - $\beta$  chains and the CD3 (T3) polypeptides (1). Recently, another receptor has been found (2) on the surface of a small fraction (1–3%) of normal adult peripheral blood T cells. This receptor also consists of three components, TCR- $\gamma$  and - $\delta$  chains and the CD3 polypeptides. The function and distribution of T cells bearing the TCR- $\gamma$  protein, termed TCR- $\gamma$  cells, is presently unknown. To further define the role of TCR- $\gamma$  cells we have asked whether these cells are found in another tissue besides the peripheral blood (3) and adult thymus (4), and if they bear a functional CD3-TCR- $\gamma$  protein complex on their cell surfaces. We chose to study the population of T cells in the cerebrospinal fluid (CSF) of a patient with a chronic viral infection of the central nervous system.

Using a single T cell cloning method (5), we have derived 33 T cell clones from the CSF of a patient with subacute sclerosing panencephalitis (SSPE). In SSPE, the immune response in the central nervous system is directed against measles virus (6). TCR- $\gamma$  cells in the CSF of this patient represented 6% (2 of 33) of the T cell clones. The TCR- $\gamma$  protein on both CSF clones existed as part of an 85-kD disulphide-linked dimer on the cell surface, noncovalently associated with the CD3 polypeptides. We also demonstrate here that these CSF clones have NK-like activity similar to TCR- $\gamma$  clones in the peripheral blood (3). Both CSF TCR- $\gamma$  clones proliferated in response to anti-CD3 mAb coupled to Sepharose beads and/or IL-2. Furthermore, stimulation of one of the CSF TCR- $\gamma$  clones with anti-CD3 mAb resulted in a rapid rise in intracellular calcium. These data suggest that the CD3-associated TCR- $\gamma$  protein complex is functional. The

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identification of significant numbers of functional TCR- $\gamma$  cells in the CSF of a patient with SSPE suggests that these are mature cells that play a role in the human immune response.

### Materials and Methods

*CSF T Cell Isolation.* CSF was obtained by lumbar puncture from a 16-yr-old female with SSPE, diagnosed on the basis of clinical examination and characteristic electroencephalogram findings and CSF anti-measles antibody titers. The procedure for the isolation and cloning of T cells from the CSF have been previously described (7).

*mAbs and T Cell Phenotyping.* Phenotypic analysis of T cell clones was performed by indirect immunofluorescence using mAbs and fluorescein-conjugated goat anti-mouse IgG (Tago Inc., Burlingame, CA) on a fluorescence-activated flow cytometer, as previously described (7). mAb WT-31 (8) was a generous gift of Dr. S. Ip, T Cell Sciences, Boston, MA. mAbs anti-CD3, anti-CD4, and anti-CD8 were from Coulter Immunology, (Hialeah, FL). Leu 7 and Leu 11 were from Becton Dickinson & Co. (Mountain View, CA). NKH-1 was kindly provided by Dr. J. Ritz, Dana-Farber Cancer Institute, Boston, MA.

*Immunoprecipitation.*  $10^6$  cells were iodinated by the lactoperoxidase method, lysed in Tris-buffered saline (pH 8) containing 0.3% 3-[(3-cholamidopropyl)-dimethylammonio] 1-propane-sulfonate) (Chaps; Sigma Chemical Co., St. Louis, MO) and immunoprecipitated as described previously (2). Anti-CD3 mAb UCHT-1 used in immunoprecipitation was kindly provided by Dr. P. Beverley, University College, London, United Kingdom (9). The rabbit human TCR- $\gamma$  antiserum, termed anti-C $\gamma$ , was made against amino acids 117-136 of the TCR- $\gamma$  protein sequence (2, 10).

*Cytotoxic Assays.* The NK-like activity of the cloned T cells was measured by their ability to release  $^{51}\text{Cr}$  from target cells at an effector/target ratio of 10:1 using the methods described previously (7). Blocking experiments were performed in triplicate at an effector/target ratio of 10:1 using anti-CD3 mAb at a dilution of 1:100, as previously described (11).

*Analysis of Intracellular Calcium.* Cells were loaded at a final density of  $10^7$  cells/ml with indo-1 acetoxymethyl ester (Molecular Probes, Junction City, OR) at  $10\ \mu\text{g/ml}$  in RPMI 1640 medium containing 10% pooled AB serum, 2 mM L-glutamine and penicillin/streptomycin. After 30 min of incubation at  $37^\circ\text{C}$ , cells were centrifuged and resuspended in the same medium supplemented with  $\text{CaCl}_2$  to a final concentration of 2 mM. Measurement of indo-1 fluorescence was performed by flow cytometry using an argon-ion ultraviolet laser excitation source, and simultaneous recording of long-wavelength fluorescence and short-wavelength fluorescence. The ratio of 515:400 nm fluorescence was recorded for each cell and displayed as a function of time. Typical runs involved processing  $10^6$  cells/ml at a flow rate of 1,000 cells/second. After base-line determination (30 s), sampling was interrupted briefly for the addition of anti-CD3 mAb (2Ad2A2). 2Ad2A2 was kindly supplied by Drs. J. Ritz and S. Schlossman, Dana-Farber Cancer Institute.

*Proliferation Assays.* Reactivity against measles virus was assayed using whole mononuclear cells infected with measles (kindly provided by Drs. S. Jacobson and H. McFarland, National Institutes of Health, Bethesda, MD) as previously described (12).

The ability of the anti-CD3 mAb coupled to Sepharose beads to stimulate T cell clones to proliferate was determined as described previously (13).  $\Delta\text{cpm}$  were calculated by subtracting incorporation of [ $^3\text{H}$ ]thymidine into unstimulated T cells from the results obtained with stimulated T cells.

### Results and Discussion

To define the TCR- $\gamma$  cells in a tissue during an immune response, we have cloned T cells from the CSF of a patient with SSPE. The analysis of T cells isolated from the CSF is difficult because only small numbers of cells are available. By using a single-cell cloning technique in which cells are cloned before other *in vitro* manipulation, we were able to get an accurate representation of the T cell

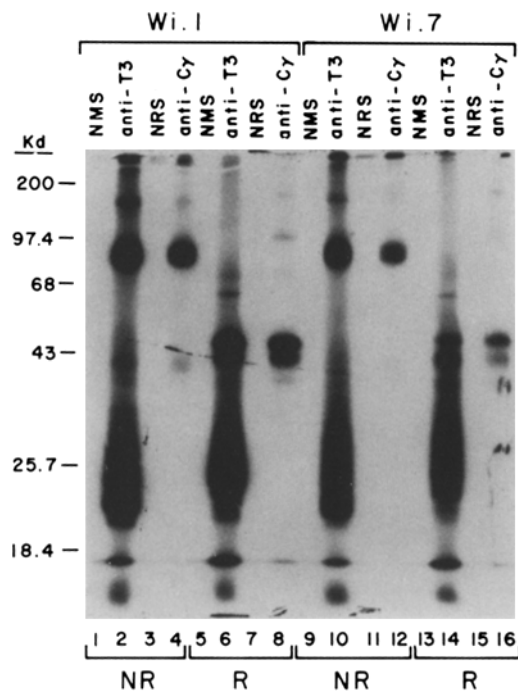


FIGURE 1. T3 and TCR- $\gamma$  chain polypeptides immunoprecipitated from clones Wi.1 and Wi.7.  $^{125}$ I-labeled Wi.1 (1-8) and Wi.7 (9-16) cell lysates were precipitated with normal mouse serum (NMS) (1, 5, 9, and 13), anti-CD3 (T3) antibody, UCHT-1 (2, 6, 10, and 14), normal rabbit serum (NRS) (3, 7, 11, and 15), and with anti-C $\gamma$  serum (4, 8, 12, and 16), and then were fractionated on SDS-polyacrylamide gels under nonreducing (1-4 and 9-12) or reducing (5-8 and 13-16) conditions.

population. We and others (5, 7) have shown that most peripheral blood and CSF T cells can be grown in vitro in the presence of PHA, IL-2, and irradiated mononuclear "feeder" cells. In these experiments the cloning efficiency was 25%. 33 independent T cell clones were isolated.

The biochemical nature of the TCR on the surface of these cloned T cells was determined by a two-step procedure that assumes that all TCR- $\gamma$  cells also bear CD3 but lack TCR- $\alpha$  or TCR- $\beta$  chains. First, cells from each T cell clone were tested for their ability to bind WT-31, which recognizes the TCR- $\alpha/\beta$  heterodimer. Screening the cells with the rabbit anti-C $\gamma$  serum was not possible because the serum recognizes only denatured protein. 2 of the 33 CSF clones, Wi.1 and Wi.7, did not stain with WT-31, although they were CD3 $^{+}$  (data not shown). These cells appeared to be good candidates for cells expressing a TCR- $\gamma$  protein on their surfaces. Phenotypic analyses indicated that these clones were also CD4 $^{-}$ , CD8 $^{-}$ , NKH1 $^{-}$ , Leu-7 $^{-}$  and Leu-11 $^{-}$  (data not shown). These WT-31 $^{-}$  T cells were the only T cells that we isolated from the CSF that lacked both the CD4 and CD8 antigens (data not shown).

Second, the two WT-31 $^{-}$  clones were examined for the expression of TCR- $\gamma$  protein by iodinating the cells and immunoprecipitation with the anti-C $\gamma$  serum. Both of these cells bear the TCR- $\gamma$  protein on their surfaces (Fig. 1, lanes 4, 8, 12, and 16).

The structure of the TCR- $\gamma$  protein-associated TCR was further characterized in the CSF clones. To demonstrate that these cells bear a TCR- $\gamma$  protein noncovalently associated with CD3 polypeptides on their surfaces, the  $^{125}$ I-labeled lysates were reacted with an anti-CD3 mAb, UCHT-1. The anti-CD3 mAb identified CD3 polypeptides as well as disulphide-linked dimers of 85 kD in clones Wi.1 and Wi.7. This disulphide-linked dimer was also precipitated by the

TABLE I  
*NK-like Activity and Proliferative Assays of CSF Clones*

CSF clones	NK-like activity and blocking effect of soluble anti-CD3 mAb (specific lysis)		Effect on proliferation of clones ( $\Delta$ cpm, 72 h) with:			
	K562	K562 + anti-CD3	Medium alone	IL-2	Anti-CD3 Sepharose	Anti-CD3 Sepharose + IL-2
	%					
Wi.1	61	1.5	176	1,594	99	28,066
Wi.7	52	0	83	1,038	133	3,742
Wi.P	0	0	68	6,415	17,249	76,301
Wi.Q	53	0	21	4,584	25,772	69,936

anti-C $\gamma$  serum (Fig. 1). Upon reduction, the 85-kD structure resolved into two chains of 45 and 40 kD. Previous studies (2–4) have demonstrated that the TCR- $\gamma$  protein is found as either a disulphide-linked molecule or a non-disulphide-linked molecule associated with CD3.

The molecular events that are mediated via the CD3-TCR- $\gamma$  protein complex are not well studied. To more precisely define these events, different functional analyses of the T cell clones bearing the TCR- $\gamma$  protein were performed and compared with TCR- $\alpha/\beta$  clones. Wi.1 and Wi.7 and the TCR- $\alpha/\beta$  clone, Wi.Q (CD4<sup>-</sup>, CD8<sup>+</sup>) exhibited NK-like activity, as assessed by lysis of the K562 target cell but not of the EBV-transformed B cell line, LaZ 509 (13). Clone Wi.P (CD4<sup>+</sup>, CD8<sup>-</sup>) demonstrated no NK-like activity (Table I). Anti-CD3 mAb was able to completely inhibit the NK-like activity in both sets of clones (Table I), suggesting that the CD3-TCR- $\gamma$  protein complex is functional. No inhibition of NK-like activity was observed with the mAb 4F2 (11) (1:100; data not shown). Additionally, neither of the CSF TCR- $\gamma$  clones proliferated to measles virus (data not shown).

One of the TCR- $\gamma$  clones, Wi.7, was stimulated with anti-CD3 mAb and changes in intracellular calcium were measured using the fluorescent indicator dye, indo-1. Relative fluorescence, which represents the ratio of 515:400 nm fluorescence, decreases as indo-1 binds calcium ions (Ca<sup>2+</sup>). Using this methodology, Wi.7 and a control TCR- $\alpha/\beta$  clone (Wi.Q) resulted in a rapid rise of intracellular Ca<sup>2+</sup> with anti-CD3 mAb but not with medium alone (Fig. 2).

We then examined whether the CSF TCR- $\gamma$  clones could be induced to proliferate by crosslinking the CD3-TCR- $\gamma$  complex with anti-CD3 mAb. These T cell clones were stimulated with anti-CD3 mAb coupled to Sepharose beads and/or with exogenous IL-2. As previously described for certain T cell clones (13), both CD4<sup>+</sup>, CD8<sup>-</sup> and CD4<sup>-</sup>, CD8<sup>+</sup> T cell clones with the TCR- $\alpha/\beta$  heterodimer could be stimulated without exogenous IL-2 by anti-CD3 mAb coupled to Sepharose beads. The CD4<sup>-</sup>, CD8<sup>-</sup>, CSF TCR- $\gamma$  clones exhibited no stimulation with anti-CD3 mAb coupled to Sepharose beads alone, and minimal stimulation with IL-2 alone (Table I). Proliferation in response to IL-2 was significantly increased when the CSF TCR- $\gamma$  clones were incubated with anti-CD3 mAb coupled to Sepharose beads and IL-2. Therefore, crosslinking of the CD3-TCR- $\gamma$  complex renders the CSF TCR- $\gamma$  clones more responsive to IL-2.

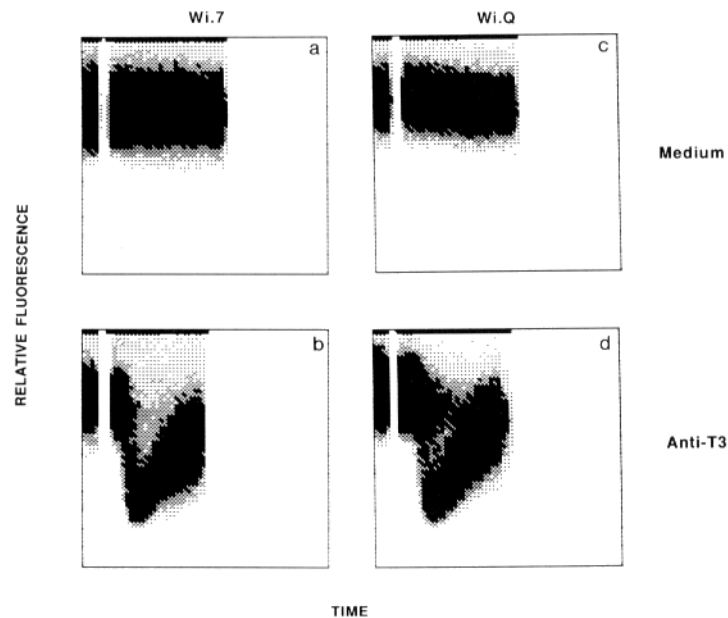


FIGURE 2. Increase in intracellular  $\text{Ca}^{2+}$  caused by anti-CD3 mAb binding to Wi.7 and Wi.Q cells. CSF clones, Wi.7 and Wi.Q, were incubated with either medium (a and c) or with anti-CD3 mAb (1:500 ascites dilution) (b and d). Relative fluorescence represents the ratio of linear fluorescence at 515:400 nm. Each sample was run for a total of 5 min. The breaks in the histograms represent removal of sample for the addition of antibody.

In summary, these studies strongly indicate that the CD3-associated TCR- $\gamma$  protein complex is functional.

Our results demonstrate that TCR- $\gamma$  cells can be found in tissues other than the thymus and blood. Both of the CSF TCR- $\gamma$  clones that we have identified in these studies are  $\text{CD4}^-$ ,  $\text{CD8}^-$ , while the other 31 T cell clones that we isolated from the CSF are either  $\text{CD4}^+$  or  $\text{CD8}^+$ . We suggest that these cells are members of the same T cell subset that has recently been identified in the peripheral blood (3). The identification of significant numbers of functional TCR- $\gamma$  cells in the CSF of a patient with SSPE suggests that they are mature cells that play a role in the immune response. Future studies will focus on identifying TCR- $\gamma$  cells in other tissues and determining their antigen specificity.

### Summary

We have derived 33 independent T cell clones from the cerebrospinal fluid (CSF) of a patient with subacute sclerosing panencephalitis using a single T cell cloning method. 6% (2 of 33) of these clones express the T cell receptor  $\gamma$  (TCR- $\gamma$ ) protein and are called CSF TCR- $\gamma$  clones. Phenotypic analyses of the CSF TCR- $\gamma$  clones indicate that they are  $\text{WT-31}^-$ ,  $\text{CD3}^+$ ,  $\text{CD4}^-$ , and  $\text{CD8}^-$ . The TCR- $\gamma$  protein exists on the cell surface as part of an 85-kD disulphide-linked dimer noncovalently associated with the CD3 polypeptides. The CSF TCR- $\gamma$  clones have NK-like activity that can be inhibited by anti-CD3 mAbs. Both CSF TCR- $\gamma$  clones proliferated in response to anti-CD3 mAbs coupled to Sepharose beads and/or IL-2. Furthermore, stimulation of one of these clones with anti-CD3 mAbs results in a rapid rise in intracellular calcium. These data suggest

that T cells bearing the CD3-TCR- $\gamma$  protein complex are functional and play a role in the human immune response.

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