Lymphocytes bearing the γδ T-cell receptor in acute toxoplasmosis

F. SCALISE, R. GERLI,* G. CASTELLUCCI, F. SPINOZZI,* G. M. FABIETTI, S. CRUPI, L. SENSI, R. BRITTA, R. VACCARO & A. BERTOTTO *Departments of Paediatrics and *Internal Medicine, Perugia University Medical School, Perugia, Italy*

Accepted for publication 2 April 1992

SUMMARY

Although the relative and absolute numbers of $CD3^+$ cells (T lymphocytes) were similar in eight children with acquired *Toxoplasma gondii* infection and 10 uninfected age- and sex-matched healthy controls, the proportion of cells bearing the γδ T-cell receptor was significantly higher in the subjects with acute toxoplasmosis. The great majority of γδ T cells from the infected patients expressed covalently bound γδ chains on their surface, i.e. were $BB3^+$ lymphocytes. Since the γδ T-cell subsets exert both restricted and unrestricted major histocompatibility complex cytotoxicity, further research is needed to elucidate the role of γδ T cells in the control of this coccidian protozoan infection.

INTRODUCTION

A small percentage (~5%) of mature, predominantly double-negative ($CD4^-$ $CD8^-$) peripheral blood T lymphocytes bear both the signal transduction CD3 molecular complex and a T-cell receptor (TcR) composed of γ and δ subunits, which may be either disulphide- or non-disulphide-linked heterodimers, on their surface.[1] In contrast, the majority of circulating T cells, $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$, express covalently bound αβ chains. The γδ T-cell subset has been found to be increased in certain human infectious diseases, e.g. localized cutaneous, but not mucocutaneous, leishmaniasis; reversal reactions in leprosy; the area surrounding zones of necrosis in tuberculous lymphadenitis; the peripheral blood of patients with measles, Epstein–Barr virus-induced infectious mononucleosis, human immunodeficiency virus (HIV)-1 infection and *Plasmodium falciparum* malaria.[2]

We here report that children with acquired *Toxoplasma gondii* infection sampled during the acute phase of the illness have a higher proportion of γδ T cells in the bloodstream than age- and sex-matched healthy controls.

Eight children, six males and two females, seen as outpatients at the Dept. of Paediatrics, Perugia University Medical School, were studied. Their mean age was 9 years (range 3–14 years). All came from the rural Umbrian areas, where acute toxoplasmosis is still an endemic infectious disease. The presumed diagnosis, based on malaise, fever, generalized lymphadenopathy, maculopapular rash (3/8) and moderate hepatomegaly (5/8), was serologically confirmed by the Sabin–Feldman dye and IgM-IFA tests. Ten age- and sex-matched uninfected children who were undergoing venipuncture for routine child care served as controls. The monoclonal antibodies (mAb) used in this study were OKT3 (anti-CD3) (IgG2a; Ortho, Raritan, NJ), TcRδ1 (IgG1; T Cell Sciences, Inc., Cambridge, MA), a pan-reactive anti-γδ TcR-staining reagent, δTCS1 (IgG1, T Cell Sciences) and BB3 [IgG1, kindly supplied by Dr L. Moretta (Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy)] which recognize Vδ1 and Vδ2 encoded determinants normally expressed on lymphocytes bearing non-covalently bound γδ chains (Vδ1) or the disulphide-linked form of the γδ TcR (Vδ2).[3–5] Surface phenotyping was carried out by a previously described two-colour immunofluorescence staining technique.[6] Appropriate isotype-specific goat anti-mouse antibodies, extensively absorbed with human immunoglobulins (Southern Biotechnology Ass., Birmingham, AL) and conjugated with either fluorescein or phycoerythrin, were employed as developing reagents for all mAb. Negative controls for each experiment were performed by using a mouse mAb unreactive with human determinants, followed by the corresponding fluorochrome-conjugated goat anti-mouse antiserum or the latter reagent alone. The stained cells were fixed in 1% paraformaldehyde in phosphate-buffered saline and analysed by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA). Lymphocyte subsets were identified by gating analysis and fluorescence profiles obtained for 50,000 cells per sample.

Due to the abnormal distribution of data, the Kruskall–Wallis analysis of the variance was used for statistical evaluation of the results.

The relative and absolute numbers of $CD3^+$ cells (T lymphocytes) were similar in all blood samples tested, as was the distribution of the two major immunoregulatory T-cell subsets ($CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$) (data not shown). However, two-colour cytofluorimetric analysis demonstrated that the proportion of γδ T lymphocytes ($CD3^+$ TcR $\delta1^+$) from subjects with acute toxoplasmosis was significantly higher than in

Correspondence: Dr A. Bertotto, Dept. of Paediatrics, Perugia University Medical School, I-06100 Perugia, Italy.

Table 1. γδ T-cell subset distribution in blood from children with acquired toxoplasmosis (AT) and age- and sex-matched healthy controls

	No. of samples tested	CD3+ cells co-expressing*		
		TcRδ1	BB3	δTCS1
AT blood	8	9·4 (4·1–14·9)†‡	7·6 (3·0–14·4)‡	1·0 (0·5–2·6)
Normal blood	10	3·5 (0·5–5·9)	3·0 (0·5–6·6)	0·8 (0·5–2·5)

* Data obtained by indirect immunofluorescence followed by two-colour cytofluorimetric analysis.

† Relative percentage of positive cells expressed as mean and (range).

‡ $P < 0.005$ (Kruskall–Wallis analysis of the variance).

healthy children. The increase, mainly due to an overexpansion of cells with the BB3+ phenotype (Table 1), was not observed in the patients retested during convalescence, i.e. 1 month after the onset of symptoms. Furthermore, identical results were obtained in four additional experiments that compared the percentages of TcRδ1+ cells to those calculated by adding a mixture of BB3 and δTCS1 mAb (data not shown). No relationship was found between γδ T-cell numbers (TcRδ1+) and specific (IgG and/or IgM) serum antibody titres.

Although there are several lines of evidence that both classic (class I and class II) self- or allo-major histocompatibility complex (MHC) and non-classical MHC antigens are an important component of the γδ TcR ligands,[7] the putative role γδ T cells in the normal and pathological immune response has never been substantiated. Minoprio *et al.*[8] have shown that preferential expansion of CD3+ CD4− CD8− cells occurs at the early phase in the polyclonal response to murine *Trypanosome cruzi* infection. Moreover, Ohga *et al.*[9] have found that γδ T lymphocytes appear before αβ T cells in the peritoneal cavity of mice during intraperitoneal infection with *Listeria monocytogenes*. The same group has also recently demonstrated that murine mycobacteria-specific γδ T cells are present in inflammed sites during the primary stage of mycobacterial infection.[10] It would, therefore, seem that γδ T cells home to the infected areas of experimental animals before the αβ T lymphocytes, and that early appearing γδ T cells may serve as a first line of defence against at least some invading micro-organisms. In contrast, the late occurrence of the γδ T-cell responses observed in certain human and murine viral infections may be independent of the recognition of the pathogen itself, but rather due to host-derived stimuli, such as autologous heat-shock proteins.[2]

We found that γδ T cells are overexpanded in the blood of children with acquired toxoplasmosis, a systemic protozoan infection, examined at the time of diagnosis. The great majority of these lymphocytes were BB3+, i.e. expressed the disulphide-linked form of the γδ TcR. Interestingly, BB3+ cells bear the memory or primed T-cell marker CD45RO on their membrane and proliferate in response to nominal antigens, thereby suggesting that they are engaged in combating many human pathogens and infectious agents.[11] For example, there is mounting laboratory evidence that BB3+ lymphocytes are implicated in immunological reactions against the *Mycobacterium tuberculosis*.[12]

The present study extends previously published data by demonstrating that γδ T cells are precociously increased in the blood of patients with *Toxoplasma gondii* infection. The question of whether, in view of their lytic mechanism and lymphokine production,[10,13] these cells contribute to eradicating the protozoan infection has not yet received an answer. Our future investigations will be aimed at searching for the answer in both acquired and, if possible, congenital toxoplasmosis. These latter studies may be of interest as the number of γδ T cells, which are mostly δTCS1+, is very low in the blood of normal human newborns. In fact, the extrathymic expansion of BB3+ lymphocytes is presumably due to specific antigenic pressure, and this is the reverse of the situation encountered in the thymus where the δTCS1+ cells predominate throughout pre- and postnatal life.[14]

REFERENCES

1. Triebel F. & Hercend T. (1989) Subpopulations of human peripheral T gamma delta lymphocytes. *Immunol. Today*, **10**, 186.
2. Born W.K., Harshan K., Modlin R.L. & O'Brien R.L. (1991) The role of γδ T lymphocytes in infection. *Curr. Opin. Immunol.* **3**, 455.
3. Sturm E., Braakman E., Bontrop R.E., Chuchana P., van de Griend R.J., Koning F., Lefranc M.P. & Bolhuis R.L.H. (1989) Coordinated V_γ and V_δ gene segment rearrangements in human T cell receptor γ/δ+ lymphocytes. *Eur. J. Immunol.* **19**, 1261.
4. Casorati G., De Libero G., Lanzavecchia A. & Migone N. (1989) Molecular analysis of human γ/δ+ clones from thymus and peripheral blood. *J. exp. Med.* **170**, 1521.
5. De Libero G., Casorati G., Giachino C., Carbonara C., Migone N., Matzinger P. & Lanzavecchia A. (1991) Selection by two powerful antigens may account for the presence of the major population of human peripheral γ/δ T cells. *J. exp. Med.* **173**, 1311.
6. Gerli R., Bertotto A., Rambotti P., Barbieri P., Ciompi M.L. & Bombardieri S. (1988) T cell immunoregulation in rheumatoid synovitis. *Arthritis Rheum.* **31**, 1075.
7. Roberts K., Yokoyama W.M., Kehn P.J. & Shevach E.M. (1991) The vitronectin receptor serves as an accessory molecule for the activation of a subset of γ/δ T cells. *J. exp. Med.* **173**, 231.
8. Minoprio P., Bandeira A., Santos T. & Coutinho A. (1989) Preferential expansion of ly-1B and CD4− CD8− T cells in the polyclonal lymphocyte responses to murine *T. cruzi* infection. *Int. Immunol.* **1**, 176.
9. Ohga S., Yoshikai Y., Takeda Y., Hiromatsu K. & Nomoto K. (1990) Sequential appearance of γ/δ and α/β-bearing T cells in the peritoneal cavity during an i.p. infection with *Listeria monocytogenes*. *Eur. J. Immunol.* **20**, 533.

10. INOUE T., YOSHIKAI Y., MATZUZAKI G. & NOMOTO K. (1991) Early appearing γ/δ-bearing T cells during infection with Calmétte Guérin bacillus. *J. Immunol.* **146,** 2754.
11. MIYAWAKI T., KASAHARA Y., TAGA K., YACHIE A. & TANIGUCHI N. (1990) Differential expression of CD54RO (UCHL1) and its functional relevance in two subpopulations of circulating TCR-γ/δ^+ lymphocytes. *J. exp. Med.* **171,** 1833.
12. KABELITZ D., BENDER A., PROSPERO T., WESSELBORG S., JANSSEN O. & PECHHOLD K. (1991) The primary response of human γ/δ^+ T cells to *Mycobacterium tuberculosis* is restricted to Vγ9-bearing cells. *J. exp. Med.* **173,** 1331.
13. BRENNER M.B., STROMINGER J.L. & KRANGEL M.S. (1988) The $\gamma\delta$ T cell receptor. *Adv. Immunol.* **43,** 133.
14. PARKER C.M., GROH V., BAND H., PORCELLI S.A., MORITA C., FABBI M., GLASS D., STROMINGER J.L. & BRENNER M. (1990) Evidence for extrathymic change in the T cell receptor γ/δ repertoire. *J. exp. Med.* **171,** 1597.