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Phenotypic Profile and Functional Characteristics of Human Gamma and Delta T Cells during Acute Toxoplasmosis

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Gamma and delta (γδ) T-cell receptor lymphocytes are increased during acute toxoplasmosis. These cells are BB3$^+$ CD45RO$^+$ CD8$^-$. Purified γδ T cells failed to proliferate in response to *Toxoplasma gondii* antigen (stimulation index, 1.4 ± 0.6) but were responsive to phytohemagglutinin stimulation (stimulation index, 20.8 ± 1.9). Natural-killer-like cytotoxicity was strongly acquired only after in vitro culture of purified γδ T cells with recombinant interleukin 2 (40% ± 7% specific lysis). Our data show that γδ T-cell receptor T cells with a peculiar phenotype are increased during human acute *T. gondii* infection.

Two structurally distinct types of CD3-associated T-cell receptor (TCR) exist. Most peripheral blood T lymphocytes express the alpha-beta heterodimer, while a minor population is characterized by expression of the gamma-delta (γδ) heterodimer (4, 17).

γδ T cells in the peripheral blood express mainly the Vγ9-Vδ2-encoded TCR, while a minority of these cells bear the δ chain encoded by the Vδ1 gene segment (17). γδ T cells are usually CD4$^-$ CD8$^\pm$ CD45RO$^\pm$ CD25$^-$ (7, 13).

The antigenic response and the functional role of γδ T cells have been recently clarified. An increase of this subset has been found in the peripheral blood of patients with congenital or acquired immunodeficiencies (5, 14, 18); in vivo and in vitro studies suggest an important role of γδ T cells in generating an immune response to certain microbial antigens (6, 8, 12). A portion of this response may be directed against some bacterial or human peptides, such as heat shock proteins (2, 8) or the staphylococcal enterotoxin A microbial superantigen (12).

We will show here that infection with *Toxoplasma gondii* is characterized by the expansion of γδ T cells preferentially expressing the Vγ9-Vδ2 gene-encoded receptor.

The study group consisted of 12 subjects (six males and six females; mean age, 28 ± 8 years) with acute *T. gondii* infection. These patients were found to have evidence of acute *Toxoplasma* infection with high levels (titers from 1/128 to 1/1,024) of immunoglobulin M-specific antibodies by immunofluorescence assay. Immunoglobulin G titers ranged from 1/128 to 1/4,096. All of the patients (normal, nonimmunosuppressed hosts) had lymphadenopathy, while four also had fever and malaise. All of the laboratory investigations were performed within 3 weeks after the onset of symptoms. The control population consisted of healthy, sex- and age-matched donors without clinical or serological (immunoglobulin M positivity) signs of toxoplasmosis.

The peripheral blood was layered on Plasmagel for 30 min, and the leukocyte-rich buffy coat was then recovered. Fluorescein isothiocyanate- or phycoerythrin-conjugated CD4 (OKT4; Ortho), CD8 (OKT8; Ortho), CD45RO (UCHL1; Dako), CD45RA (Leu18; Becton Dickinson), CD25 (IL2R; Becton Dickinson), and TCRδ1 (T Cell Science) monoclonal antibodies were used. When nonconjugated BB3 or A13 (gifts of L. Moretta, Genoa, Italy) monoclonal antibodies were used, a second incubation with phycoerythrin-conjugated rat anti-mouse serum was performed. Fluorescence was measured on a FACScan flow cytometer. Controls included fluorescein isothiocyanate- or phycoerythrin-conjugated mouse immunoglobulins. Cells (10,000) were acquired in the list mode of the Consort 30 computer.

Mononuclear cells were separated after Ficoll-Paque centrifugation. Cells were incubated for 30 min at 4°C with appropriate amounts of anti-CD4, anti-CD8, anti-CD16, and anti-CD19 monoclonal antibodies; after being washed, the cells were incubated at 4°C with continuous mixing with rabbit anti-mouse immunoglobulin-coated magnetic beads (Dynabeads; Unipath). Rosetting cells were removed with a magnet (3). After separation, more than 90% of the cells were γδ$^+$ (TCRδ1$^+$).

Total mononuclear cells or γδ-enriched T cells plus autologous monocytes (10%) were incubated with *T. gondii* antigen (prepared from tachyzoites by sonication at 1.0, 0.1, or 0.01 μg/ml; Sorin Biomedica) or phytohemoagglutinin (PHA) (100, 30, or 10 μg/ml; Gibco) in microtiter wells at 10^6 cells per mm^3. [^{3}H]thymidine uptake was measured at day 3 of incubation. The stimulation index (S.I.) was calculated as follows: S.I. = Mean counts per minute for stimulated samples/Mean counts per minute for unstimulated samples.

A standard ^{51}Cr release was used to measure lytic activity against the K562 cell line. Target cells were labelled by incubation at 37°C for 1 h with $Na_2\,^{51}CrO_4$ (specific activity, 5.0 mCi/ml; Amersham). A portion of γδ T cells was cultured for 3 days in RPMI 1640 medium containing 200 IU of recombinant interleukin 2 (Boehringer B.R.).

Effector cells (resting and lymphokine activated) were incubated with labelled targets at a 25:1 effector/target ratio at 37°C overnight.

Percent cytolysis was calculated as follows: % Cytolysis = [(Experimental release − Spontaneous release)/(Total release − Spontaneous release)] × 100.

Total release was determined by addition of an HCl lysing solution.

Patients acutely infected with *T. gondii* show a distinctive phenotypic profile (10). The TCRδ1 antibody, a pan-γδ T-cell marker, recognized 10.3% ± 3% of total lymphocytes (321 ± 145 cells per mm^3; $P < 0.005$ compared with healthy controls) (Table 1). Similar results were obtained with patients irrespective of clinical symptoms or disease duration or chronicity.

γδ T cells were further analyzed by double immunofluo-

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TABLE 1. Percentages and absolute numbers of lymphocyte subpopulations defined by monoclonal antibody reactivity in patients with acute toxoplasmosis and in healthy controls

Monoclonal antibody	Mean % of total lymphocyte population (no.) ± SEM[a]	
	Patients with toxoplasmosis	Normal controls
CD4	34 ± 5 (976 ± 350)	45.2 ± 4 (980 ± 240)
CD8	38.2 ± 4[b] (1,126 ± 400)[b]	27.9 ± 4 (510 ± 210)
TCRδ1	10.3 ± 3[b] 321 ± 145)[c]	5.2 ± 1.9 (124 ± 53)
A13	1.7 ± 1 (44 ± 33)	1.0 ± 0.2 (33 ± 16)

[a] The data shown refer to a single bleeding for each patient.
[b] $P < 0.05$.
[c] $P < 0.005$.

rescence flow cytometric studies. BB3 cells preferentially coexpressed the CD45RO antigen (6.6 ± 0.7), while the percentage of CD45RA was not different from that of controls (Table 2). Only 0.3% ± 0.2% of the cells were $BB3^+$ $CD8^+$.

Total mononuclear cells from patients did proliferate (S.I., 9.7 ± 2; Table 3) in response to 1 μg of *Toxoplasma* antigen per ml, while no proliferation in response to the same antigen concentration was found in the γδ T-cell-enriched population from patients (S.I., 1.4 ± 0.6) and healthy controls (Table 3).

TCR γδ lymphocyte responses to the mitogen PHA were similar in acutely *Toxoplasma*-infected patients and normal controls (S.I., 20.6 ± 1.9 versus 9.9 ± 9.5; not statistically significantly different) (Table 3).

Freshly isolated γδ T cells from three patients with toxoplasmosis had a low degree of cytotoxicity (14.7% ± 9.1% versus 9.7% ± 11% cytotoxicity in normal controls) at a 25:1 effector/target ratio. After 48 h of culture with interleukin 2, γδ T cells from both *Toxoplasma*-infected patients and normal controls acquired a significant degree of cytotoxicity (40.2% ± 11.1% in patients and 29.7% ± 11.3% in normal controls; not statistically significantly different in patients versus controls).

Data obtained in vitro and in vivo indicate that cell-mediated immunity is important in the control of *T. gondii* infection (9–11).

We have analyzed the phenotypic and functional profile of γδ T cells in the peripheral blood of patients acutely infected with *T. gondii*. These cells are $BB3^+$ ($V\delta2^+$) $CD45RO^+$; this phenotype has been associated with a population of memory T cells (1, 13, 15), and a role of γδ T cells in the immune response to many pathogens has therefore been postulated (15).

TABLE 2. Percentages of BB3-positive cells and BB3 subsets, as defined by various monoclonal antibody combinations, in patients with toxoplasmosis and in healthy controls

Monoclonal antibodies	Mean % of positive cells ± SEM[a]	
	Patients with toxoplasmosis	Normal controls
BB3	7.8 ± 1[b]	3.5 ± 1.5
BB3-CD45RO	6.6 ± 0.7[b]	1.6 ± 0.2
BB3-CD45RA	1.2 ± 0.1	1.5 ± 0.3
BB3-CD8	0.2 ± 0.1	0.2 ± 0.3
A13-CD45RO	0.2 ± 0.1	0.4 ± 0.1
A13-CD45RA	1.2 ± 0.7[c]	0.4 ± 0.1

[a] This assay was performed simultaneously with the other flow cytometric studies.
[b] $P < 0.005$.
[c] $P < 0.05$.

TABLE 3. Proliferative responses of lymphomonocytes or γδ-enriched lymphocytes to PHA or *T. gondii* antigen from patients with toxoplasmosis and from healthy controls

Subjects and conditions	Mean cpm ± SEM	Mean S.I. ± SEM
Patients		
Total lymphomonocytes + *T. gondii* antigen	3,990 ± 410	9.7 ± 2[a]
γδ-enriched lymphocytes + *T. gondii* antigen	810 ± 171	1.4 ± 0.6
γδ-enriched lymphocytes + PHA	14,547 ± 1,837	20.8 ± 1.9
Controls		
γδ-enriched lymphocytes + *T. gondii* antigen	446 ± 499	0.3 ± 0.5
γδ-enriched lymphocytes + PHA	3,534 ± 4,369	9.9 ± 9.5

[a] $P < 0.05$.

Functional analysis of γδ T cells purified from patients with acute toxoplasmosis showed that they proliferated in response to PHA. These cells are not spontaneously cytolytic but do lyse K562 target cells after lymphokine stimulation, as do γδ T cells isolated from normal healthy donors.

Parenterally administered recombinant interleukin 2 protects mice against death due to acute toxoplasmosis (16); it may be that interleukin 2 functions by inducing lymphokine-activated killer cells, a population that is also composed of γδ T lymphocytes.

The inability of γδ T cells to respond in vitro to *T. gondii* antigen remains to be explained. Other experimental conditions may be required to obtain their proliferation.

These findings suggest that γδ T cells could participate in immune functions during acute *T. gondii* infection. Further definition of how this subset is engaged will probably add new insights into its role in the immune response.

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