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αβ T Cell Response to Toxoplasma gondii in Previously Unexposed Individuals

Carlos S. Subauste, Franklin Fuh, Rene de Waal Malefyt, and Jack S. Remington

The mechanisms by which T cells from previously unexposed hosts respond in vitro to certain intracellular pathogens remain to be fully understood. We report and characterize the in vitro reactivity to Toxoplasma gondii of human αβ T cells from T. gondii-seronegative individuals. Resting αβ T cells from these individuals proliferated in response to PBMC infected with T. gondii or pulsed with T. gondii lysate Ags. This was accompanied by an increase in the percentage of CD4+ αβ T cells. Purified CD4+ αβ T cells but not CD8+ αβ T cells proliferated in response to these T. gondii preparations. Both CD4+ αβ T cells with naive (CD45RA+) and memory (CD45R0+) phenotypes from adults as well as αβ T cells from T. gondii-seronegative newborns proliferated after incubation with T. gondii. This αβ T cell response to the parasite was inhibited by anti-HLA-DR mAb and to a lesser degree by anti-HLA-DQ mAb. Use of paraformaldehyde-fixed PBMC completely abrogated the proliferation of αβ T cells, indicating the need for processing of T. gondii Ags. Analysis of the TCR Vβ expression did not show evidence for restriction in TCR Vβ usage during T. gondii stimulation of αβ T cells. αβ T cells secreted significant amounts of IFN-γ after incubation with T. gondii-infected monocytes. This rapid and remarkable αβ T cell response may play an important role in the early events of the immune response to T. gondii.


Materials and Methods

Study population

Buffy coats from heparinized blood of healthy volunteer donors were obtained from the Stanford Blood Bank (Stanford, CA). Samples of umbilical cord blood were obtained from placentas of healthy newborns at Stanford Children’s Hospital. Serologic tests for detection of T. gondii IgG and IgM Abs were performed in all samples of blood (15). Except when noted, samples used had no demonstrable T. gondii IgG or IgM Abs. In some experiments, blood from chronically infected but otherwise healthy adults was used (positive anti-T. gondii IgG and negative anti-T. gondii IgM).

T. gondii and infection

Tachyzoites of the RH strain were obtained from infected monolayers of human foreskin fibroblasts as well as from peritoneal fluid of mice as previously described (13). To obtain toxoplasma lysate Ags (TLA), mammalian cell-free RH tachyzoites released from infected human foreskin...
fibroblasts were resuspended in sterile distilled water and subjected to three freeze-thaw cycles. After reconstitution with sterile 10× PBS, cellular debris was pelleted by centrifugation at 900 x g for 20 min. Supernatant was collected and stored at −70°C and used as TLA. Antigenic preparations were devoid of detectable levels of endotoxin (<10 pg/ml) using a Limulus amebocyte lysate assay (Sigma). PBMC or monocytes were incubated overnight with UV-attenuated tachyzoites at a ratio of 2 or 4 parasites per cell, respectively. This resulted in a rate of infection of approximately 10% for PBMC and 40% for monocytes (mainly, one organism per cell).

**Purification of αβ T cells**

PBMC and cord blood mononuclear cells (CBMC) were isolated by centrifugation on Ficoll-Hypaque gradients (Pharmacia LKB Biotechnology, Piscataway, NJ). To purify αβ T cells, nylon wool-nonadherent PBL were incubated with saturating concentrations of the following mAb: anti-CD16 (Medarex, Hammastr, MD), anti-CD56 (Becton Dickinson, San Jose, CA), and anti-CD19 (Coulter Cytometry, Hialeah, FL), and anti-γδ TCR (anti-TCRδ, generous gift from Dr. Michael Brenner). Anti-glycoporin A mAb (10F7 MN, American Type Culture Collection (ATCC), Rockville, MD) was added to remove erythroblasts present in CBMC. To obtain purified populations of CD4+ γδ TCR-αβ and CD8+ γδ TCR-αβ T cells, anti-CD8 (OKT8, ATCC) and anti-CD4 (OKT4, ATCC) were added, respectively, to the culture medium used above. Anti CD45RO (UCHL-1, Immunotech, Westbrook, ME) and anti-CD45RA (ALB11, Immunotech) mAbs were used to obtain purified CD4+ CD45RA+ and CD4+ CD45RO+ T cells, respectively. Magnetic beads coated with anti-mouse IgG (Dynal, Great Neck, NY) were added as a ratio of 10 beads per cell. Rosetting cells were removed with a magnet (Dynal). Addition of anti-CD14 beads was repeated once for purification of CD4+ CD45RA+ and CD4+ CD45RO+ cells. These protocols resulted in populations that were either >99% CD3+ TCR-αβ+, >98% CD4+ TCR-αβ+, >98% CD8+ TCR-αβ+, >98% CD4+CD45RA+ TCR-αβ+, or >98% CD4+CD45RO+ TCR-αβ+ cells as determined by flow cytometry.

**Proliferation of monocytes**

PBMC were incubated with the following mAb (from Becton Dickinson except when indicated): anti-CD2, anti-CD3, anti-CD8, anti-CD19 (Coulter Cytometry), anti-CD16, anti-CD56, and anti-glycoporin A. After addition of magnetic beads coated with anti-mouse IgG (Dynal), rosetting cells were removed with a magnet. Populations obtained were >96% pure for monocytes by microscopic examination of Giemsa-stained cytocentrifuge preparations. In addition, cytofluorometric analysis indicated that >92% of the cells were CD14+, with <0.5% CD3−, <0.5% CD19−, <0.5% CD56−, and <2% CD66b+ cells.

**Proliferation assays**

αβ T cells were cultured in either 24-well or round-bottom 96-well plates (Linbro; ICN Pharmaceuticals, Costa Mesa, CA) at 5 × 10^4 cells/ml in complete medium consisting of RPMI 1640 supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 10% Sabin-Feldman dye test-negative pooled human AB serum (Irvine Scientific, Santa Ana, CA). In vitro stimulation of αβ T cells was conducted as previously described (13). Except when noted, αβ T cells were incubated with γ-irradiated (3000 rad) autologous PBMC that had been either infected with UV-attenuated tachyzoites of *T. gondii* (5 T. gondii:1 infected PBMC) (13) or incubated with previously determined optimal concentrations of TLA (10 μg/ml), tetanus toxoid (10 μg/ml; Massachusetts Public Health Biologic Laboratory, Boston, MA), staphylococcal enterotoxin B (SEB) (0.1 μg/ml; Toxin Technology, Sarasota, FL), toxic shock syndrome toxin-1 (TSST-1; 1 μg/ml; Toxin Technology), or PHA (0.5 μg/ml; Wellcome Diagnostics, Dartford, U.K.). Unless otherwise stated, when stimulated with T. gondii, tetanus toxoid, SEB, TSST-1, or allogeneic PBMC, αβ T cells were cultured for 7 days at 37°C, 5% CO2; they were cultured for 3 days when stimulated with PHA. In some experiments, PBMC incubated overnight with T. gondii or control PBMC were fixed with 1% paraformaldehyde in PBS for 5 min at 37°C. Reaction was stopped by adding cold 0.15 M glycine. After three washes, cells were resuspended in complete medium and incubated for at least 1 h at 37°C. This was followed by a final wash before incubation with αβ T cells.

Cells were pulsed with 1 μCi of [H]thymidine during the last 18 h of in vitro stimulation and harvested as previously described (13). Results are presented as net incorporation ± SD of triplicate wells. Stimulation index was calculated by dividing the cpm of cultures stimulated with *T. gondii* by cpm of cultures without *T. gondii*.

**Inhibition experiments using mAb**

In some experiments, αβ T cells were incubated with saturating concentrations of either anti-CD4 (OKT4; IgG2b, ATCC), anti-CD8 (OKT8; IgG2, ATCC), or isotype control mAbs (PharMingen, San Diego, CA) for 30 min on ice before adding PBMC and antigenic preparations. When anti-MHC class II mAbs were used, PBMC were incubated with saturating concentrations of either anti-HLA-DR (L243; IgG2a, ATCC), anti-HLA-DQ (SPV-L3; IgG2a, generous gift from Dr. Hans Yssel), or isotype control mAbs (PharMingen) for 30 min on ice before adding T cells.

**Analysis of Vβ expression**

PBMC (2.5 × 10^6/ml) were stimulated with either tachyzoites of *T. gondii* (1 tachyzoite/PBMC) or TLA (10 μg/ml) for 7 days; or SEB (100 ng/ml) or PHA (0.25 μg/ml) for 4 days. Cells were then washed and cultured for another 48 h in the presence of IL-2 (120 IU/ml; Chiron, Emeryville, CA) as previously described (17). Before culture, and after in vitro stimulation, cells were analyzed by two-color cytofluorometry as described below.

**FACS analysis**

To determine purity and phenotypic composition of T cell preparations, cells were incubated for 30 min at 4°C with the following mAbs in PBS containing 1% FBS and 0.1% sodium azide: anti-TCR-α, anti-TCR-γδ, anti-CD3, anti-CD4, anti-CD8, anti-CD16, anti-CD356 (all from Becton Dickinson), anti-CD45RA, and anti-CD45RO (Immunotech).

Flow cytometry was also used to analyze the TCR repertoire. This was done by staining cells with either phycocerythrin (PE)-conjugated anti-CD4 or PE-conjugated anti-CD8 mAb and one of the following FITC-conjugated mAbs directed against the following Vβ-chains (from Immunotech except when noted): Vβ2, Vβ3.1 (T Cell Diagnostics, Woburn, MA), Vβ5.1, Vβ5.2, Vβ8, Vβ12, Vβ3.1, Vβ13.6, Vβ16, Vβ21.3, Vβ22. Samples were analyzed on a FACScan cytofluorometer (Becton Dickinson). Isotype control mAbs were used to assess background fluorescence. Resting T cells and blasts were gated according to forward angle and 90° light scatter patterns.

**Cytokine assays**

Purified αβ T cells (1 × 10^5/ml) were incubated with either *T. gondii*-infected or uninfected purified monocytes (5 × 10^5/ml). Supernatants collected at 24, 48, and 72 h were used to measure concentrations of IL-2, IL-4, and IFN-γ, respectively, by ELISA (13). Data is presented as mean of triplicate wells ± SEM. None of the cytokines assayed were detected in supernatants obtained from wells that lacked T cells and contained only monocytes with or without *T. gondii* tachyzoites.

**Statistical analysis**

Statistical significance was assessed by unpaired Student’s t test.

**Results**

αβ T cells from *T. gondii*-seronegative individuals proliferate in response to the parasite

The proliferative response of resting αβ T cells to *T. gondii* was studied. Figure 1. A through D, shows that not only αβ T cells from healthy individuals chronically infected with *T. gondii* but also αβ T cells from *T. gondii*-seronegative donors proliferated when incubated with autologous PBMC infected with *T. gondii* or pulsed with TLA. However, whereas αβ T cells from both groups of donors exhibited a remarkable proliferative response at the higher doses of parasite Ags, only αβ T cells from seropositive individuals had significant proliferation at the lowest doses of parasite Ags. Proliferative response to *T. gondii* occurred regardless of whether tachyzoites were obtained from human foreskin fibroblasts or peritoneal cavities of infected mice (data not shown). Furthermore, no T cell proliferation was detected when either tachyzoite-free peritoneal lavage fluid or lysate from uninfected foreskin fibroblasts were used instead of *T. gondii* preparations (data not shown). Although αβ T cells from every seronegative donor tested (n = 10) proliferated in response to *T. gondii*, this response varied among individuals (*T. gondii*-infected cells: mean
SI = 68.0, range 17.8–199.1; TLA-pulsed cells: mean SI = 51.1, range 11.2–137.6).

To study the kinetics of *T. gondii*-mediated T cell proliferation in seronegative donors, αβ T cells were incubated with parasite Ag preparations for 3, 5, 7, and 10 days. Maximal proliferation of αβ T cells to PBMC infected with *T. gondii* or pulsed with TLA was observed on day 7 of in vitro stimulation (Fig. 2A). In parallel experiments, the kinetics of αβ T cell proliferation in response to optimal doses of a T cell mitogen (PHA), superantigen (TSST-1), recall Ag (tetanus toxoid), and alloantigen (allogeneic PBMC) were analyzed. As shown in Figure 2B, maximal proliferation occurred on day 3 for PHA, day 5 for TSST-1, day 7 for tetanus toxoid, and day 7 for allogeneic PBMC.

**CD4⁺ αβ T cells preferentially respond to T. gondii**

To determine whether a particular subpopulation of αβ T cells preferentially responds to *T. gondii*, cytofluorometric analyses of purified resting αβ T cells from seronegative individuals were performed before and after in vitro stimulation with *T. gondii*. Compared with unstimulated αβ T cells, stimulation with either PBMC infected with *T. gondii* or pulsed with TLA resulted in an increase in the percentage of CD4⁺ T cells (Table I). Furthermore, more than 94% of the αβ T cell blasts obtained after in vitro stimulation...
with parasite Ags were CD4\(^+\). These results were not caused by a nonspecific response of CD4\(^+\) T cells due to in vitro culture conditions, since stimulation of αβ T cells with PHA did not result in an increase in the percentage of CD4\(^+\) T cells. The lack of a significant CD8\(^+\) T cell response to *T. gondii* was not due to failure of the experimental conditions to provide stimulus to CD8\(^+\) T cells, since incubation of αβ T cells from chronically infected individuals with cells that contained intracellular tachyzoites induced a significant CD8\(^+\) αβ T cell blast population (Table I).

### Table I. Phenotypic composition of αβ T cells stimulated with either *T. gondii* or PHA

<table>
<thead>
<tr>
<th></th>
<th><em>T. gondii</em>-Seronegative</th>
<th><em>T. gondii</em>-Seropositive</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CD4(^+) (%)</td>
<td>CD8(^+) (%)</td>
</tr>
<tr>
<td>Unstimulated</td>
<td>52.0</td>
<td>48.3</td>
</tr>
<tr>
<td>Stimulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tachyzoites</td>
<td>87.1 (^b) (blasts: 94.2)</td>
<td>12.4 (^b) (blasts: 6.0)</td>
</tr>
<tr>
<td>TLA</td>
<td>89.3 (^b) (blasts: 96.0)</td>
<td>10.1 (^b) (blasts: 3.7)</td>
</tr>
<tr>
<td>PHA</td>
<td>49.1 (^b)</td>
<td>50.4</td>
</tr>
</tbody>
</table>

\(^b\) Values are derived by two-color flow cytometry using mAb specific for αβ TCR, CD4, and CD8 molecules. Cytofluorometric analysis was performed before or after a 7-day stimulation with PBMC that had been either infected with *T. gondii* tachyzoites or incubated with TLA or PHA. Results are representative of three independent experiments.

To further analyze the response to *T. gondii* of subsets of αβ T cells from seronegative donors, we studied the effects of anti-CD4 and anti-CD8 mAb on the *T. gondii*-mediated T cell proliferation. As shown in Figure 3A, anti-CD4 mAb induced significant inhibition of the proliferation of αβ T cells to PBMC infected with *T. gondii* or pulsed with TLA (p ≤ 0.01; n = 3). Anti-CD4 mAb induced a 51% inhibition (range, 42.4–59.6%) of the proliferation induced by *T. gondii*-infected cells, and a 66.1% inhibition (range, 60.7–71.5%) of the proliferation induced by TLA (n = 3). At the same time, anti-CD8 mAb did not inhibit the proliferative response to TLA (0% inhibition) and induced a minimal (15.8%; range, 15.6–18.8%) inhibition of the response to *T. gondii*-infected PBMC, which was nonstatistically significant (p ≥ 0.2; n = 3). Finally, to further prove that CD4\(^+\) T cells were the subset of αβ T cells that preferentially responds to *T. gondii*, purified CD4\(^+\) TCR-αβ\(^+\) and purified CD8\(^+\) TCR-αβ\(^+\) T cells were stimulated with *T. gondii*. Figure 3B shows that in response to *T. gondii*-infected PBMC, whereas CD4\(^+\) T cells exhibited remarkable proliferation, the proliferative response of CD8\(^+\) T cells was minimal. In addition, CD4\(^+\) T cells but not CD8\(^+\) T cells proliferated when stimulated with TLA. These differences were not due to a decreased capacity of CD8\(^+\) T cells to proliferate, since both CD4\(^+\) and CD8\(^+\) T cells exhibited remarkable proliferative responses to PHA (data not shown). Thus, these results demonstrate that, in *T. gondii*-seronegative individuals, CD4\(^+\) T cells are the subset of αβ T cells that preferentially responds to the parasite in vitro.

### MHC class II molecules are required for *T. gondii*-mediated αβ T cell proliferation

In view of the preferential response of CD4\(^+\) T cells, experiments were conducted to determine whether *T. gondii*-mediated T cell proliferation in seronegative individuals was dependent on MHC class II molecules. mAb to MHC class II molecules inhibited the proliferative response of αβ T cells to *T. gondii* (Fig. 4). The degree of inhibition varied depending on the mAb tested. Anti-HLA-DR mAb (L243) induced 89.9% inhibition (range, 81.9–100%; p ≤ 0.0003) of the proliferation in response to infected cells and 83.8% inhibition (range, 71.8–100%; p ≤ 0.01) of the proliferation in response to TLA. In contrast, anti-HLA-DQ (SPV-L3) induced 15% inhibition (range, 3.8–27.5%) (p range, 0.6–0.02) of the proliferation in response to infected cells and no inhibition (0%) of that in response to TLA (n = 3). These results were not due to a nonspecific inhibitory effect of the anti-MHC class II mAb used, since none of these mAb induced significant inhibition of the proliferation of αβ T cells to PHA (data not shown).

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**FIGURE 3.** CD4\(^+\) T cells are the subset of αβ T cells from *T. gondii*-seronegative individuals that preferentially responds to the parasite. A, αβ T cells were incubated with *T. gondii*-infected PBMC (PBMC + Tg), PBMC plus TLA (PBMC + TLA), or control PBMC in the presence of either isotype control mAb (IgG2a), anti-CD4 mAb, or anti-CD8 mAb. B, αβ CD4\(^+\), αβ CD8\(^+\), or unseparated αβ T cells were incubated with *T. gondii*-infected PBMC (PBMC + Tg), PBMC plus TLA (PBMC + TLA), or control PBMC. Results are representative of three independent experiments.
shown). Thus, these results demonstrate that the proliferative response of αβ T cells from seronegative donors to *T. gondii* requires MHC class II molecules, in particular HLA-DR molecules.

**CD4+ T cells with both naive (CD45RA+) and memory (CD45RO+) phenotypes respond to *T. gondii***

CD4+CD45RA+ and CD4+CD45RO+ T cells from seronegative donors were purified to determine their role in the αβ T cell proliferation to *T. gondii*. Whereas CD4+CD45RO+ T cells proliferated in response to a recall Ag (tetanus toxoid), CD4+CD45RA+ failed to proliferate, confirming their naive status (Fig. 5). Incubation with either *T. gondii*-infected PBMC or allogeneic PBMC induced proliferation of both CD4+CD45RA+ and CD4+CD45RO+ T cells.

To further determine whether naive αβ T cells from *T. gondii*-seronegative newborns respond to *T. gondii* Ags-MHC molecules due to the fixation protocol of fixation resulted in inhibition of Ag processing (Fig. 7A). However, despite the use of fixed PBMC, αβ T cells still exhibited a remarkable proliferative response to a superantigen (TSST-1) that does not require Ag processing. Use of fixed PBMC completely abrogated the proliferative response of αβ T cells to *T. gondii*. The lack of T cell proliferation in response to the parasite did not appear to be caused by impaired recognition of *T. gondii* Ags-MHC molecules due to the fixation protocol. T cell proliferation was observed after stimulation with either *T. gondii*-infected PBMC or PBMC preincubated with *T. gondii* and then fixed with paraformaldehyde (Fig. 7B). In parallel experiments, αβ T cells failed to proliferate in response to paraformaldehyde-fixed PBMC plus TLA but proliferated when incubated with fixed PBMC plus a superantigen (SEB) (Fig. 7B). In addition, stimulation with paraformaldehyde-fixed *T. gondii*-infected PBMC has allowed us to generate *T. gondii*-reactive αβ T cell lines (21). Taken together, our results indicate the need for processing of *T. gondii* Ags for induction of an αβ T cell response.

**Processing of *T. gondii* Ag(s) is required for αβ T cell proliferation**

To determine whether processing of *T. gondii* Ag(s) is necessary to induce an αβ T cell response, proliferation assays were conducted using either paraformaldehyde-fixed or untreated PBMC (Fig. 7). The complete inhibition of αβ T cell proliferation in response to tetanus toxoid induced by fixation of PBMC demonstrated that, as previously reported (20), the protocol of fixation resulted in inhibition of Ag processing (Fig. 7A). However, despite the use of fixed PBMC, αβ T cells still exhibited a remarkable proliferative response to a superantigen (TSST-1) that does not require Ag processing. Use of fixed PBMC completely abrogated the proliferative response of αβ T cells to *T. gondii*. The lack of T cell proliferation in response to the parasite did not appear to be caused by impaired recognition of *T. gondii* Ags-MHC molecules due to the fixation protocol. T cell proliferation was observed after stimulation with either *T. gondii*-infected PBMC or PBMC preincubated with *T. gondii* and then fixed with paraformaldehyde (Fig. 7B). In parallel experiments, αβ T cells failed to proliferate in response to paraformaldehyde-fixed PBMC plus TLA but proliferated when incubated with fixed PBMC plus a superantigen (SEB) (Fig. 7B). In addition, stimulation with paraformaldehyde-fixed *T. gondii*-infected PBMC has allowed us to generate *T. gondii*-reactive αβ T cell lines (21). Taken together, our results indicate the need for processing of *T. gondii* Ags for induction of an αβ T cell response.
Table II. Effects of stimulation with T. gondii on TCR Vβ repertoire

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Vβ2</th>
<th>Vβ3.1</th>
<th>Vβ5.1</th>
<th>Vβ5.2</th>
<th>Vβ6.7</th>
<th>Vβ8</th>
<th>Vβ12</th>
<th>Vβ13.1</th>
<th>Vβ13.6</th>
<th>Vβ16</th>
<th>Vβ18</th>
<th>Vβ21.3</th>
<th>Vβ22</th>
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<tbody>
<tr>
<td>Unstimulated</td>
<td>9.3</td>
<td>5.3</td>
<td>8.0</td>
<td>1.1</td>
<td>1.4</td>
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<td>2.6</td>
<td>0.9</td>
<td>1.8</td>
<td>1.0</td>
<td>0.4</td>
<td>3.2</td>
<td>4.3</td>
</tr>
<tr>
<td>PHA</td>
<td>10.2</td>
<td>5.8</td>
<td>6.8</td>
<td>0.8</td>
<td>1.6</td>
<td>3.4</td>
<td>2.2</td>
<td>2.6</td>
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<td>1.4</td>
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<tr>
<td>Tachyzoites</td>
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<td>7.6</td>
<td>0.7</td>
<td>1.3</td>
<td>5.0</td>
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<td>0</td>
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<td>SEB</td>
<td>7.9</td>
<td>5.4</td>
<td>7.3</td>
<td>0.5</td>
<td>0.9</td>
<td>4.3</td>
<td>1.6</td>
<td>0.3</td>
<td>2.2</td>
<td>1.1</td>
<td>0.4</td>
<td>2.3</td>
<td>3.6</td>
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</table>

* PBMC (2.5 × 10^6/ml) were stimulated with either UV-attenuated T. gondii tachyzoites (2.5 × 10^6/ml), LLA (10 µg/ml), PHA (0.25 µg/ml), or SEB (0.1 µg/ml) for 7 days. Cells were then washed and incubated with rIL-2 (120 IU/ml) for 2 days. For stimulated cells, FACS analysis was performed by gating on either large (blasts) or small (resting) lymphocytes. Unstimulated cells were analyzed by FACS, without gating for large cells. Results are representative of experiments performed with four different donors.
produced after stimulation with infected monocytes (Fig. 8). Incubation of αβ T cells with uninfected monocytes plus PHA resulted in production of significant amounts of IFN-γ and IL-2, while the production of IL-4 was either low or below detectable levels. Neither IL-2 nor IL-4 were detected after stimulation with infected monocytes (data not shown).

**Discussion**

Using an in vitro model of T. gondii infection, we examined the response to the parasite by human αβ T cells from T. gondii-seronegative donors as an approach to studying the early events in the induction of cell-mediated immunity to intracellular pathogens. In this report, we demonstrate that CD4+ αβ T cells from individuals previously unexposed to T. gondii proliferated when incubated with the parasite in vitro. Presumably, unprimed αβ T cells reacted to T. gondii in vitro, since CD4+CD45RA+ T cells from adults and CD45RA− αβ T cells from newborns proliferated when incubated with T. gondii. Moreover, αβ T cells appeared to recognize T. gondii Ags in a manner similar to that of conventional Ags, since αβ T cell response required processing of parasite Ags. Finally, we demonstrate that T. gondii-reactive αβ T cells from T. gondii-seronegative individuals secreted IFN-γ when incubated with parasite-infected monocytes.

Our initial demonstration that αβ T cells from seronegative individuals react to T. gondii in vitro suggested the possibility that the parasite might contain T cell mitogens. However, this hypothesis is not supported by our data. The inhibitory effect of anti-MHC class II mAb on the T. gondii-mediated αβ T cell proliferation, the requirement for Ag processing in order for these cells to respond to the parasite and the changes in the TCR Vβ repertoire induced by stimulation with the parasite argue against the presence of a T. gondii T cell mitogen in the experimental system we studied.

Despite the absence of serologic evidence of infection with T. gondii, we considered that prior in vivo exposure to cross-reactive T cells epitopes from other immunogens ubiquitously present in the environment could have resulted in clonal expansion of αβ T cells that could recognize T. gondii. This possibility was addressed by studying the in vitro reactivity of CD45RA+ and CD45RO+CD4+ T cells. The expression of these isoforms of the CD45 molecule has been associated with naive (CD45RA+) and memory (CD45RO−) CD4+ T cells (23). Our demonstration that both CD45RA+ and CD45RO+ cells proliferated in response to the parasite, whereas only CD45RO+ T cells responded to a recall Ag, does not support this hypothesis. However, it is important to keep in mind that the induction of expression of memory surface phenotype after T cell activation may not be permanent since reversion to a naive surface phenotype appears to occur (24). Nevertheless, the response of neonatal αβ T cells to T. gondii strongly argues against prior exposure to epitopes that cross-react with T. gondii as the mechanism responsible for αβ T cell reactivity to the parasite.

Another potential explanation for our results was that αβ T cells underwent in vitro priming during incubation with T. gondii. It has been reported that under certain conditions a primary T cell response can be generated in vitro (25, 26). However, there are significant differences between the T. gondii reactivity of αβ T cells that we demonstrated here and the T cell responses in the models in which in vitro priming occurred. Purified dendritic cells instead of PBMC were required as APCs to induce primary T cell sensitization, which translated into Ag-specific proliferation detected 7 days after in vitro stimulation (26). In other models in which PBMC could successfully induce in vitro priming, T cell proliferation was detected only after antigenic restimulation (25). In contrast, our studies using PBMC as APC demonstrate that remarkable αβ T cell proliferation was already detectable 3 days after in vitro stimulation with T. gondii-infected cells. Thus, it appears that the mechanism(s) responsible for the in vitro response to T. gondii of αβ T cells from unexposed individuals are likely to differ from those that lead to primary in vitro T cell sensitization.

Our initial results revealing the requirement of MHC class II molecules for αβ T cell proliferation suggested that these cells might be responding to a T. gondii superantigen. In fact, it has been reported that stimulation of nonimmune murine splenocytes with T. gondii results in an expansion of CD8+ T cells that bear Vβ5 TCR (27). These results led the investigators to propose that the parasite contains a superantigen for murine T cells (27). Several of our observations indicate the stimulus for the in vitro response of human αβ T cells from seronegative humans does not behave like a typical exogenous superantigen. Although there is some controversy regarding the requirement for the processing of certain exogenous superantigens by APCs (28), the large body of evidence indicates that exogenous superantigens do not require processing by APCs to stimulate T cells (20, 29). This conclusion is in sharp distinction to our data, which demonstrates the need for processing of T. gondii Ags to trigger αβ T cell proliferation. Whereas exogenous superantigens generally stimulate both CD4+ and CD8+ T cells (20, 30), here we demonstrate that T. gondii stimulates only human CD4+ T cells. The recognition of a superantigen by T cells depends on the expression of the appropriate TCR Vβ sequence, with little contribution by other variable components of the TCR (22). The central role that Vβ regions have on the T cell stimulation mediated by superantigens is reflected in our demonstration that the T cell blast populations generated by an exogenous superantigen (SEB) had a restricted expression of Vβ regions, which was consistent in every donor tested. In contrast, the T cell blast populations induced by stimulation with T. gondii were quite heterogeneous in regard to Vβ expression, and there was no consistent pattern of Vβ expansion among the different donors tested. The fact that incubation with T. gondii increased the percentages of CD4+ T cells that expressed certain Vβ regions does not necessarily indicate the presence of a superantigen in the parasite preparations, since imbalances in the Vβ repertoire can occur during the immune response to conventional Ags (31). Our results indicate that there is a mechanism(s) involved in the in vitro response...
of αβ T cells from *T. gondii* seronegative humans that differs from that induced by typical exogenous superantigens. The discrepancies between the results obtained after *T. gondii* stimulation of T cells from unexposed humans and those obtained using T cells from naive mice may suggest that some of the early immunologic events that occur in mice after exposure to *T. gondii* do not reflect those that occur in humans.

The explanation for the remarkable in vitro reactivity to *T. gondii* exhibited by αβ T cells from previously unexposed individuals remains to be identified. It is interesting to note that many features of this response resemble that to allografts. Mixed lymphocyte reaction is characterized by proliferation of CD4+ T cells that is driven by MHC-class II molecules. Using methodology similar to that employed in our studies, the changes in the TCR Vβ repertoire during mixed lymphocyte reaction were reported to differ from individual to individual (32). Moreover, studies of TCR repertoire during graft-vs-host disease, an illness triggered by allore cognition, have demonstrated a T cell response that is oligoclonal and not TCR Vβ restricted (33, 34). Molecular mimicry has been proposed as a mechanism that may explain allore cognition (35). According to this view, a resemblance between allogeneic MHC molecules and nominal Ag-self MHC complexes would result in self-MHC-restricted T cells recognizing allografts. Arguments used to support the mimicry theory (36) include the demonstration of T cell clones with this type of dual specificity (37) and the observation that T cells previously primed in vivo as defined by expression of CD45RO molecules (presumably self-MHC-restricted T cells) recognize allografts (38). Of interest in this regard is our demonstration that both CD45RA+ and CD45RO+ CD4+ αβ T cells from unexposed individuals responded in vitro not only to allografts but also to *T. gondii*. It remains to be determined whether molecular mimicry is responsible for the in vitro reactivity to *T. gondii* by αβ T cells from unexposed individuals. Of potential relevance to this hypothesis is the demonstration that *Plasmodium falciparum*-reactive T cell clones recognize bacterial, viral, fungal, and protozoan Ags (39).

IFN-γ plays a critical role in the immune response against *T. gondii*. This cytokine has been shown to confer protection during both the acute and chronic phases of infection (11, 40, 41). Our results indicate that during the early stages of the immune response, not only NK cells but also αβ T cells, the predominant subset of T cells, are an important source of IFN-γ. It has been proposed that protective immunity to *T. gondii* is associated with induction of a Th1-type T cell response (42). Thus, the early production of IFN-γ may confer protection to the host not only because of the direct effects of this cytokine on the growth of intracellular tachyzoites (40) but also because IFN-γ appears to play a role in promoting the generation of a Th1 cytokine pattern (43). Therefore, the innate capacity of humans to control *T. gondii* infection may be due, at least in part, to the remarkable early αβ T cell response that we have demonstrated.

There is evidence that microbial Ags can elicit an immune response without conferring protection against the offending pathogen (44, 45). Although acute infection with *T. gondii* is usually uneventful in humans, *T. gondii* successfully avoids elimination from the host, leading to a chronic (quiescent) infection, despite the strong early T cell response elicited by the parasite. Thus, it is conceivable that the early αβ T cell response triggered by *T. gondii* may not be directed against Ags that lead to the elimination of the parasite. Moreover, given that potent polyclonal T cell proliferation can be associated with reduced response to neoantigens (46), the induction of such a massive αβ T cell response may interfere, at least temporarily, with the development of protective cell-mediated immunity, allowing the micro-organism to “escape” by forming tissue cysts. In addition, such a response may be involved in the induction of immunosuppression observed during recently acquired *T. gondii* infection (47, 48). It is also possible that under certain circumstances this αβ T cell response may be implicated in some of the manifestations of the disease caused by *T. gondii* (toxoplasmosis). Approximately 10% of humans acutely infected with the parasite develop a self-limiting illness, usually manifested by lymphadenopathy (49). This form of toxoplasmosis presents with pathologic changes in lymph nodes that may be difficult to distinguish from lymphoproliferative disorders such as lymphoma (50). It remains to be determined whether an exaggerated T cell response or the failure to control this response is involved in the pathogenesis of toxoplastic lymphadenopathy.

Previous reports on the in vitro response of human T cells to *T. gondii* did not demonstrate proliferation of T cells from seronegative individuals (51, 52). This apparent discrepancy with our results can be explained by our demonstration of remarkable differences between the proliferative response of T cells from seropositive individuals and that of seronegative individuals to varying concentrations of *T. gondii* Ag preparations. Studies of T cell-mediated immunity in *T. gondii*-infected humans and, in particular, in vitro studies aimed at the identification of parasite Ags recognized specifically by T cells from these individuals should be performed using carefully chosen doses of *T. gondii* Ag preparations. A detailed understanding of the early response of αβ T cells to *T. gondii*, including the identification of the Ag(s) responsible for triggering this phenomenon, is important to the effort to identify protective *T. gondii* Ags using in vitro assays of T cell function.

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References


