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$\alpha\beta$ T Cell Response to *Toxoplasma gondii* in Previously Unexposed Individuals[1]

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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 $\alpha\beta$ T cells from *T. gondii*-seronegative individuals. Resting $\alpha\beta$ 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 $CD4^+$ $\alpha\beta$ T cells. Purified $CD4^+$ $\alpha\beta$ T cells but not $CD8^+$ $\alpha\beta$ T cells proliferated in response to these *T. gondii* preparations. Both $CD4^+$ $\alpha\beta$ T cells with naive ($CD45RA^+$) and memory ($CD45RO^+$) phenotypes from adults as well as $\alpha\beta$ T cells from *T. gondii*-seronegative newborns proliferated after incubation with *T. gondii*. This $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ T cells. $\alpha\beta$ T cells secreted significant amounts of IFN-γ after incubation with *T. gondii*-infected monocytes. This rapid and remarkable $\alpha\beta$ T cell response may play an important role in the early events of the immune response to *T. gondii*. *The Journal of Immunology,* 1998, 160: 3403–3411.

It is well recognized that T cell-mediated immunity plays a central role in the host response to intracellular pathogens (1). The generation of a T cell response against conventional microbial Ags requires processing of these Ags and their presentation in association with MHC molecules on the surface of Ag-presenting cells (2). This would be followed by Ag recognition at the level of the highly variable complementarity determining region (CDR)[3] 3 of the TCR and clonal expansion of Ag-specific T cells (3). In general, prior in vivo priming to the Ag is required to detect in vitro T cell responses to conventional Ags (4).

Certain microbial Ags can induce a T cell response without following the pattern of events described above. The best characterized example are the so-called superantigens. Superantigens directly bind MHC class II molecules and the TCR in regions outside the peptide-binding groove and the CDR3, respectively (4–6). Provided that the T cells bear the appropriate β- or γ-chain of the TCR, superantigens can stimulate $CD4^+$, $CD8^+$, and $\gamma\delta$ T cells in vitro without prior in vivo priming (6). However, there appear to be other situations in which microbial Ags induce an in vitro response by T cells from previously unexposed hosts following mechanisms that remain to be fully characterized (7–9).

Toxoplasma gondii is an obligate intracellular protozoan against which T cell-mediated immunity can confer protection (10, 11). Indeed, *T. gondii* has become an important opportunistic pathogen in patients with deficiencies in cell-mediated immunity (12). We previously demonstrated that human peripheral blood T cells from both *T. gondii*-seropositive and *T. gondii*-seronegative individuals proliferate in response to the parasite (13). This response was accompanied by activation and expansion of $\gamma\delta$ T cells (13). However, given the fact that $\gamma\delta$ T cells represent a small subset of the T cell population present in peripheral blood (14), we considered that the remarkable levels of *T. gondii*-mediated T cell proliferation observed with T cells from seronegative individuals might be due to a concomitant response of other subsets of T cells. In the present study, we demonstrate in vitro reactivity to *T. gondii* of presumably unprimed $CD4^+$ $\alpha\beta$ T cells from *T. gondii*-seronegative adults and newborns. In addition, we demonstrate that $\alpha\beta$ T cells produce IFN-γ in response to *T. gondii*, an effector function that may be critical to the early immune response to the parasite. This rapid and remarkable $\alpha\beta$ 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

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[3] Abbreviations used in this paper: CDR3, complementarity determining region 3; CBMC, cord blood mononuclear cells; SEB, staphylococcal enterotoxin B; TLA, toxoplasma lysate Ag; TSST-1, toxic shock syndrome toxin-1; SI, stimulation index.

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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 × *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, Annandale, NJ), anti-CD56 (Becton Dickinson, San Jose, CA), anti-CD19 (Coulter Cytometry, Hialeah, FL), and anti-γδ TCR (anti-TCRδ1, generous gift from Dr. Michael Brenner). Anti-glycophorin A mAb (10F7 MN, American Type Culture Collection (ATCC), Rockville, MD) was added to remove erythroblasts present in CBMC. To obtain purified populations of $CD4^+$TCR-$\alpha\beta^+$ and $CD8^+$TCR-$\alpha\beta^+$ T cells, anti-CD8 (OKT8, ATCC) and anti-CD4 (OKT4, ATCC) were added, respectively, to the combination of mAb mentioned 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 at a ratio of 10 beads per cell. Rosetting cells were removed with a magnet (Dynal). Addition of magnetic beads was repeated once for purification of $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ cells. These protocols resulted in populations that were either >99% $CD3^+$TCR-$\alpha\beta^+$, >98% $CD4^+$TCR-$\alpha\beta^+$, >98% $CD8^+$TCR-$\alpha\beta^+$, >98% $CD4^+CD45RA^+$TCR-$\alpha\beta^+$, or >98% $CD4^+CD45RO^+$TCR-$\alpha\beta^+$ cells as determined by flow cytometry.

Purification 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-CD56, anti-CD66b (Immunotech), and anti-glycophorin A. After addition of magnetic beads coated with anti-mouse IgG (Dynal), rossetting 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 (Limbro; ICN Pharmaceuticals, Costa Mesa, CA) at 5×10^5 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 cells: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 (0.25 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% CO_2; 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 as described (16). Briefly, after washing to remove serum, cells were resuspended in 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 [^{3}H]thymidine during the last 18 h of in vitro stimulation and harvested as previously described (13). Results are presented as mean cpm ± SD of triplicate wells. Stimulation indices were 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-αβ, anti-TCR-γδ, anti-CD3, anti-CD4, anti-CD8, anti-CD16, anti-CD56 (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 phycoerythrin (PE)-conjugated anti-CD4 or PE-conjugated anti-CD8 mAb and one of the following FITC-conjugated mAb 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, Vβ13.1, Vβ13.6, Vβ16, Vβ18, Vβ21.3, Vβ22. Samples were analyzed on a FACScan cytofluorometer (Becton Dickinson). Isotype control mAb 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 (1×10^6/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

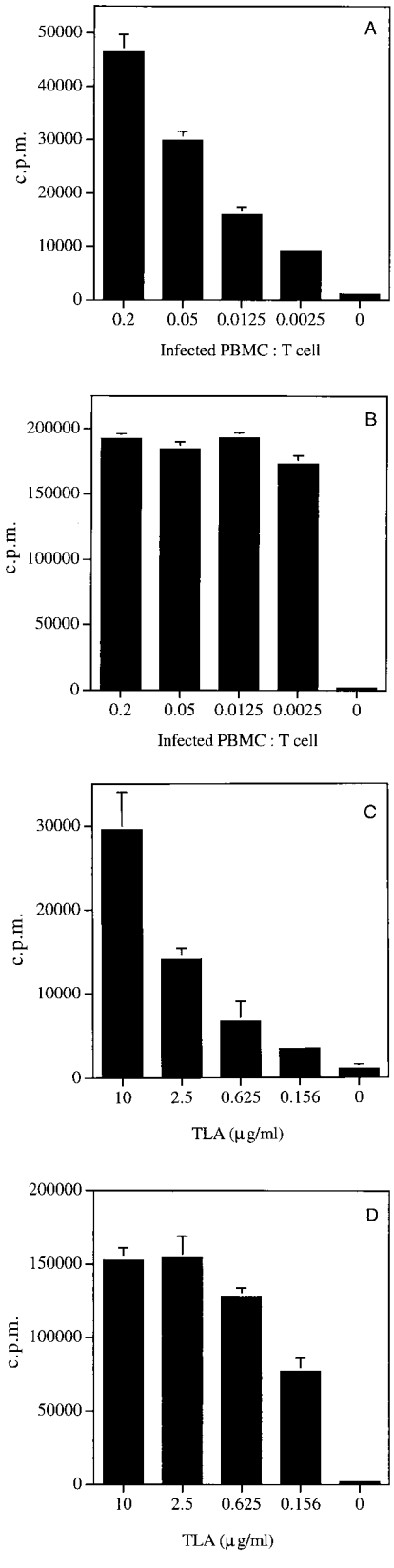

FIGURE 1. $\alpha\beta$ T cells from both *T. gondii*-seronegative (*A* and *C*) and *T. gondii*-seropositive (*B* and *D*) individuals proliferate in response to *T. gondii*. $\alpha\beta$ T cells were incubated with either decreasing concentrations of *T. gondii*-infected PBMC (decreasing infected PBMC:T cell ratios) (*A* and *B*) or uninfected PBMC plus decreasing concentrations of TLA (*C* and *D*). Results are representative of three independent experiments.

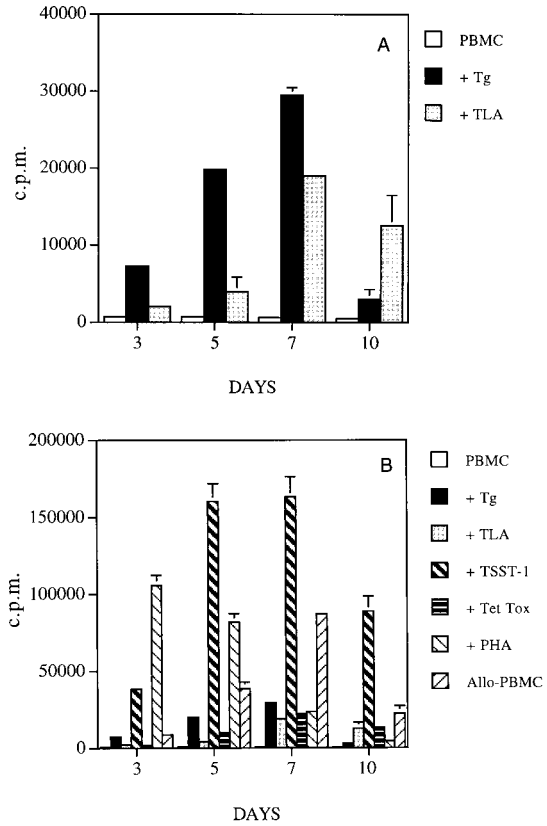

FIGURE 2. Time course of proliferation of $\alpha\beta$ T cell from *T. gondii*-seronegative individuals in response to *T. gondii*, exogenous superantigen (TSST-1), recall Ag (tetanus toxoid), mitogen (PHA), or allogeneic PBMC (Allo-PBMC). *A*, $\alpha\beta$ T cells were stimulated with autologous PBMC that were either infected with *T. gondii* (PBMC + Tg), incubated with TLA (PBMC + TLA), or used as controls (PBMC). *B*, $\alpha\beta$ T cells were also incubated with autologous PBMC plus either TSST-1 (PBMC + TSST-1), tetanus toxoid (PBMC + Tet Tox), or PHA (PBMC + PHA), or with allogeneic PBMC (Allo-PBMC). Results are representative of three independent experiments.

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, $\alpha\beta$ T cells were incubated with parasite Ag preparations for 3, 5, 7, and 10 days. Maximal proliferation of $\alpha\beta$ T cells to PBMC infected with *T. gondii* or pulsed with TLA was observed on day 7 of in vitro stimulation (Fig. 2*A*). In parallel experiments, the kinetics of $\alpha\beta$ 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 2*B*, maximal proliferation occurred on day 3 for PHA, day 5 for TSST-1, day 7 for tetanus toxoid, and day 7 for allogeneic PBMC.

$CD4^+$ $\alpha\beta$ T cells preferentially respond to T. gondii

To determine whether a particular subpopulation of $\alpha\beta$ T cells preferentially responds to *T. gondii*, cytofluorometric analyses of purified resting $\alpha\beta$ T cells from seronegative individuals were performed before and after in vitro stimulation with *T. gondii*. Compared with unstimulated $\alpha\beta$ 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 $\alpha\beta$ T cell blasts obtained after in vitro stimulation

Table I. *Phenotypic composition of $\alpha\beta$ T cells stimulated with either* T. gondii *or PHA*[a]

		T. gondii-Seronegative		*T. gondii*-Seropositive	
		$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)
Unstimulated		52.0	48.3	71.5	28.5
Stimulated	Tachyzoites	87.1 (blasts: 94.2)[b]	12.4 (blasts: 6.0)	71.7 (blasts: 66.4)	28.3 (blasts: 33.6)
	TLA	89.3 (blasts: 96.0)	10.1 (blasts: 3.7)	86.2 (blasts: 93.5)	13.8 (blasts: 6.5)
	PHA	49.1	50.4	46.6	53.7

[a] Values are derived by two-color flow cytometry using mAb specific for $\alpha\beta$ 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.
[b] T cell blasts were gated according to forward scatter. In the case of PHA, all T cells had blast characteristics as determined by cytofluorometric analysis.

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 $\alpha\beta$ 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 $\alpha\beta$ T cells from chronically infected individuals with cells that contained intracellular tachyzoites induced a significant $CD8^+$ $\alpha\beta$ T cell blast population (Table I).

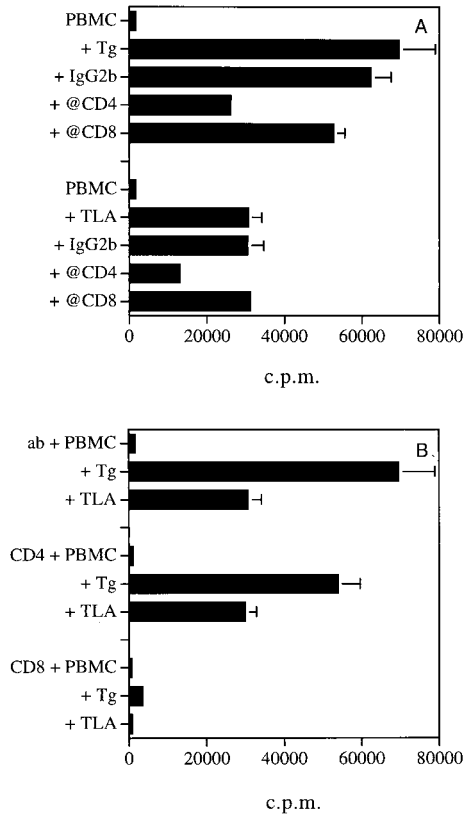

FIGURE 3. $CD4^+$ T cells are the subset of $\alpha\beta$ T cells from *T. gondii*-seronegative individuals that preferentially responds to the parasite. *A*, $\alpha\beta$ T cells were incubated with *T. gondii*-infected PBMC (PBMC + Tg), PBMC plus TLA (PBMC + TLA), or control PBMC in the presence of either isotype control mAb ($IgG2_b$), anti-CD4 mAb, or anti-CD8 mAb. *B*, $\alpha\beta$ $CD4^+$, $\alpha\beta$ $CD8^+$, or unseparated $\alpha\beta$ 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.

To further analyze the response to *T. gondii* of subsets of $\alpha\beta$ 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 3*A*, anti-CD4 mAb induced significant inhibition of the proliferation of $\alpha\beta$ T cells to PBMC infected with *T. gondii* or pulsed with TLA ($p \leq 0.01$; $n = 3$). 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 ($p \geq 0.2$) ($n = 3$). Finally, to further prove that $CD4^+$ T cells were the subset of $\alpha\beta$ T cells that preferentially responds to *T. gondii*, purified $CD4^+$TCR-$\alpha\beta^+$ and purified $CD8^+$TCR-$\alpha\beta^+$ T cells were stimulated with *T. gondii*. Figure 3*B* 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 $\alpha\beta$ T cells that preferentially responds to the parasite in vitro.

MHC class II molecules are required for T. gondii*-mediated $\alpha\beta$ 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 $\alpha\beta$ 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%; $p \leq 0.0003$) of the proliferation in response to infected cells and 83.8% inhibition (range, 71.8–100%; $p \leq 0.01$) 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 $\alpha\beta$ T cells to PHA (data not

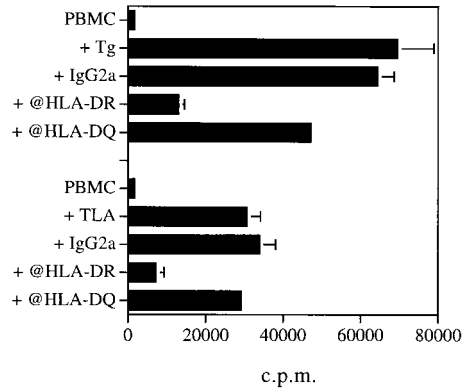

FIGURE 4. Anti-MHC class II mAb inhibit proliferation of $\alpha\beta$ T cells in response to *T. gondii*. $\alpha\beta$ T cells were incubated with either *T. gondii*-infected PBMC (PBMC + Tg), PBMC plus with TLA (PBMC + TLA), or control PBMC in the presence of either anti-HLA-DR, anti-HLA-DQ, or isotype control mAb (IgG2a). Results are representative of three independent experiments.

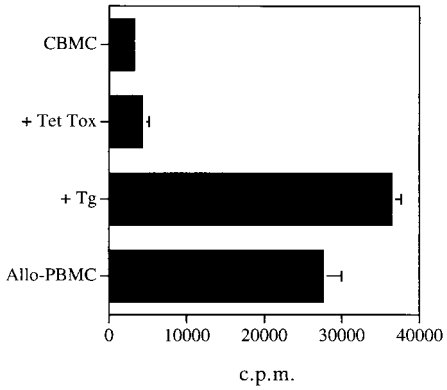

FIGURE 6. $\alpha\beta$ T cells from *T. gondii*-seronegative healthy newborns proliferate in response to *T. gondii*. T cells were incubated with either *T. gondii*-infected autologous CBMC (CBMC + Tg), CBMC plus tetanus toxoid (CBMC + Tet Tox), control CBMC, or allogeneic PBMC (Allo-PBMC). Results are representative of three independent experiments.

shown). Thus, these results demonstrate that the proliferative response of $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ T cells from *T. gondii*-seronegative individuals respond to *T. gondii*, proliferation assays were performed using $\alpha\beta$ T cells isolated from neonatal cord blood. These samples of blood were obtained from healthy *T. gondii*-seronegative newborns. In accordance to previous reports (18, 19), phenotypic analysis of cord blood $\alpha\beta$ T cells indicated that they were >99% $CD45RA^+$ and <1% $CD45RO^+$, thus confirming their naive phenotype. In addition, cord blood $\alpha\beta$ T cells did not proliferate in response to tetanus toxoid (Fig. 6). Stimulation with *T. gondii*-infected autologous CBMC resulted in a significant and reproducible proliferative response of cord blood $\alpha\beta$ T cells. However, compared with the *T. gondii*-induced proliferation of $\alpha\beta$ T cells from adults, the proliferative response of neonatal $\alpha\beta$ T cells appeared to be less prominent (SI 9.3; range, 5.5–11.3). This may be due to the lower proliferative response of neonatal T cells to T cell stimulants (19). In this regard, we observed that, compared with $\alpha\beta$ T cells from adults, neonatal $\alpha\beta$ T cells had a lower proliferative response to alloantigens (mean SI = 13.5 and range, 8.6–18.5, vs mean SI = 70.5 and range, 26.2–146.5) (n = 3).

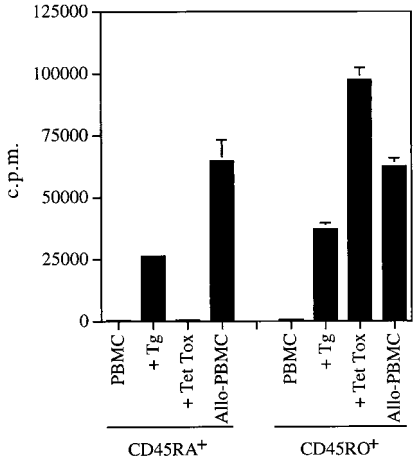

FIGURE 5. Both $CD45RA^+CD4^+$ and $CD45RO^+CD4^+$ $\alpha\beta$ T cells from *T. gondii*-seronegative adults proliferate in response to *T. gondii*. T cells were incubated with autologous PBMC that had been either infected with *T. gondii* (PBMC + Tg), incubated with tetanus toxoid (PBMC + Tet Tox), or used as controls (PBMC) or with allogeneic PBMC (Allo-PBMC). Results are representative of three independent experiments.

Processing of T. gondii *Ag(s) is required for $\alpha\beta$ T cell proliferation*

To determine whether processing of *T. gondii* Ag(s) is necessary to induce an $\alpha\beta$ T cell response, proliferation assays were conducted using either paraformaldehyde-fixed or untreated PBMC (Fig. 7). The complete inhibition of $\alpha\beta$ 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. 7*A*). However, despite the use of fixed PBMC, $\alpha\beta$ 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 $\alpha\beta$ 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. 7*B*). In parallel experiments, $\alpha\beta$ 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. 7*B*). In addition, stimulation with paraformaldehyde-fixed *T. gondii*-infected PBMC has allowed us to generate *T. gondii*-reactive $\alpha\beta$ T cell lines (21). Taken together, our results indicate the need for processing of *T. gondii* Ags for induction of an $\alpha\beta$ T cell response.

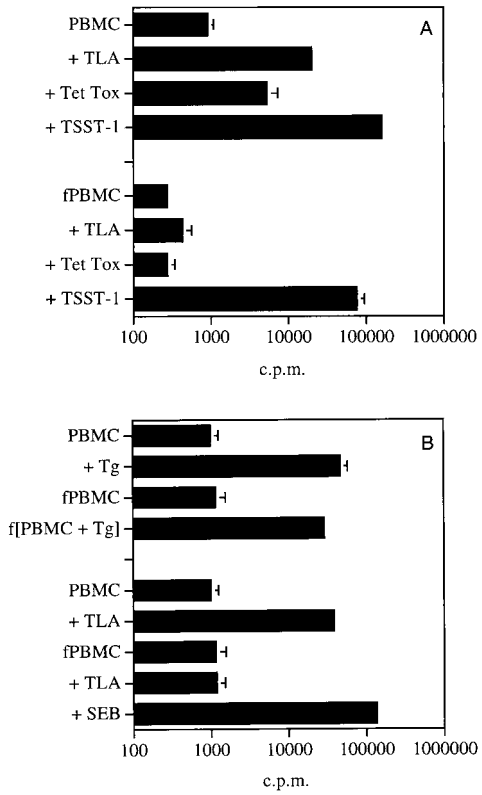

FIGURE 7. *T. gondii* Ags require processing by APCS to induce proliferation of αβ T cells from *T. gondii* seronegative individuals. *A*, T cells were incubated with either unfixed PBMC or paraformaldehyde-fixed PBMC (fPBMC) plus either TLA, tetanus toxoid (Tet Tox), or TSST-1. *B*, T cells were incubated with either unfixed PBMC, *T. gondii*-infected PBMC (PBMC + Tg), or PBMC plus TLA, or with paraformaldehyde-fixed PBMC (fPBMC), fixed *T. gondii*-infected PBMC (f[PBMC + Tg]), or fixed PBMC plus either TLA or SEB. Results are representative of three independent experiments.

Stimulation with T. gondii *induces populations of T cell blasts heterogeneous in TCR Vβ expression*

The effects of incubation of PBMC with either *T. gondii* tachyzoites, TLA, SEB, or PHA on TCR Vβ expression was analyzed by flow cytometry. Stimulation with PHA did not selectively expand any of the Vβ studied, since comparison of PHA-induced T cell blasts with freshly isolated T cells showed similar patterns of Vβ expression (Table II). As previously described (22), SEB induced expansion of Vβ3.1- and Vβ12-bearing T cells. Both PHA and SEB had similar effects on $CD4^+$ and $CD8^+$ αβ T cells. Thus, for each of the Vβ studied, the percentage of $CD4^+$ and $CD8^+$ αβ T cells remained unchanged (data not shown). In contrast, only 1 to 2% of the blast populations induced after incubation with either tachyzoites or TLA were $CD8^+$TCR-$\alpha\beta^+$. Therefore, we concentrated our analysis on $CD4^+$ αβ T cells.

Analysis of $CD4^+$ αβ T cell blasts induced by stimulation with *T. gondii* tachyzoites or TLA indicated that, with occasional exceptions, all of the Vβ families studied were represented in these populations (Table II). Comparison of TCR Vβ expression on *T. gondii*-induced $CD4^+$ αβ T cell blasts with those on T cells before stimulation or on T cells that remained in a resting state (as assessed by flow cytometry) after incubation with the parasite, indicated that stimulation with *T. gondii* induced an increase (as high as threefold) in the percentage of T cell blasts bearing certain TCR Vβ (Table II). However, there were variations from donor to donor in regard to the subpopulations of TCR Vβ bearing cells that increased in percentage after stimulation with *T. gondii*. Thus, when the results obtained from the donors tested were pooled, no pattern of preferential increase of a particular TCR Vβ-bearing subpopulation was observed. The percentages of cells that stained with anti-TCR Vβ mAb were not significantly different when comparing *T. gondii*-induced T cell blasts, PHA-induced T cell blasts, or T cells prior in vitro stimulation ($p \geq 0.2$; data not shown). In contrast, the superantigen SEB significantly stimulated selective expansion of Vβ3.1- and Vβ12-bearing T cells, which was consistent from donor to donor ($p \leq 0.005$). In addition, only TCR $V\beta3.1^+$ and TCR $V\beta12^+$ cells were detected to a significant extent in the T cell blast populations induced by SEB (Table II). Taken together, these data indicate that, in regard to the TCR Vβ-chains studied, there is no evidence for a restriction in TCR Vβ usage during *T. gondii* stimulation of αβ T cells from seronegative individuals.

T. gondii *stimulates production of IFN-γ by resting αβ T cells from seronegative individuals*

To study the production of cytokines by αβ T cells from *T. gondii*-seronegative donors, either uninfected or *T. gondii*-infected purified autologous monocytes were incubated with resting αβ T cells. Whereas incubation with uninfected monocytes did not result in production of IFN-γ, significant amounts of this cytokine were

Table II. *Effects of stimulation with* T. gondii *on TCR Vβ repertoire*[a]

Stimulus		Percentage of $CD4^+$ T Cells Bearing Vβ												
		Vβ2	Vβ3.1	Vβ5.1	Vβ5.2	Vβ6.7	Vβ8	Vβ12	Vβ13.1	Vβ13.6	Vβ16	Vβ18	Vβ21.3	Vβ22
Unstimulated	Resting	9.3	5.3	8.0	1.1	1.4	4.2	2.6	0.9	1.8	1.0	0.4	3.2	4.3
PHA	Blasts	10.2	5.8	6.8	0.8	1.6	3.4	2.2	2.6	1.8	1.4	0.4	3.2	2.0
Tachyzoites	Resting	7.0	5.2	7.6	0.7	1.3	5.0	2.0	0.4	2.3	0.8	0	2.2	3.8
	Blasts	5.1	8.3	5.8	0.6	1.6	3.9	0	4.2	3.7	3.2	1.4	0.6	5.0
TLA	Resting	7.9	5.4	7.3	0.5	0.9	4.3	1.6	0.3	2.2	1.1	0.4	2.3	3.6
	Blasts	6.7	4.5	7.7	0	2.7	6.2	1.6	2.1	1.3	4.2	0.4	2.2	3.3
SEB	Resting	9.2	0.3	9.3	1.0	1.5	5.3	0.3	0.6	1.9	1.0	0.4	2.8	4.3
	Blasts	1.2	23.2	0.4	0.1	0	0.1	8.8	0.1	0.2	0.1	0.1	0.2	0.3

[a] PBMC (2.5×10^6/ml) were stimulated with either UV-attenuated *T. gondii* tachyzoites (2.5×10^6/ml), TLA (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.

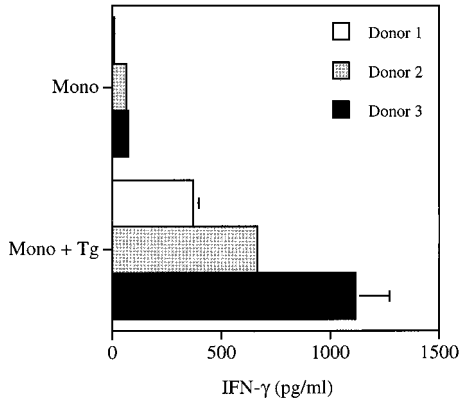

FIGURE 8. $\alpha\beta$ T cells from *T. gondii*-seronegative donors produce IFN-γ in response to *T. gondii*. $\alpha\beta$ T cells were incubated with either *T. gondii*-infected autologous monocytes (Mono + Tg) or uninfected monocytes (Mono). After 72 h, supernatants were collected and IFN-γ was measured by ELISA. Results are representative of three independent experiments.

produced after stimulation with infected monocytes (Fig. 8). Incubation of $\alpha\beta$ 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 $\alpha\beta$ 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 $CD4^+$ $\alpha\beta$ T cells from individuals previously unexposed to *T. gondii* proliferated when incubated with the parasite in vitro. Presumably, unprimed $\alpha\beta$ T cells reacted to *T. gondii* in vitro, since $CD4^+CD45RA^+$ T cells from adults and $CD45RA^+$ $\alpha\beta$ T cells from newborns proliferated when incubated with *T. gondii*. Moreover, $\alpha\beta$ T cells appeared to recognize *T. gondii* Ags in a manner similar to that of conventional Ags, since $\alpha\beta$ T cell response required processing of parasite Ags. Finally, we demonstrate that *T. gondii*-reactive $\alpha\beta$ T cells from *T. gondii*-seronegative individuals secreted IFN-γ when incubated with parasite-infected monocytes.

Our initial demonstration that $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ T cells to *T. gondii* strongly argues against prior exposure to epitopes that cross-react with *T. gondii* as the mechanism responsible for $\alpha\beta$ T cell reactivity to the parasite.

Another potential explanation for our results was that $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ T cells from previously unexposed individuals remains to be identified. It is interesting to note that many features of this response resemble that to alloantigens. 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 allorecognition, 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 allorecognition (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 alloantigens. Arguments used to support the molecular 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 alloantigens (38). Of interest in this regard is our demonstration that both $CD45RA^+$ and $CD45RO^+$ $CD4^+$ $\alpha\beta$ T cells from unexposed individuals responded in vitro not only to alloantigens but also to *T. gondii*. It remains to be determined whether molecular mimicry is responsible for the in vitro reactivity to *T. gondii* by $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 $\alpha\beta$ 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 toxoplasmic 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 $\alpha\beta$ 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

1. Kaufmann, S. H. E. 1993. Immunity to intracellular bacteria. *Annu. Rev. Immunol. 11:129.*
2. Neefjes, J. J., and F. Mombourg. 1993. Cell biology of antigen presentation. *Curr. Opin. Immunol. 5:27.*
3. Jorgensen, J. L., U. Esser, B. Fasakas de St. Groth, P. A. Reay, and M. M. Davis. 1992. Mapping T-cell receptor-peptide contacts by variant peptide immunization of single-chain transgenic. *Nature 355:224.*
4. Corradin, G., H. M. Etlinger, and J. M. Chiller. 1977. Lymphocyte specificity to protein antigens. I. Characterization of the antigen-induced in vitro T cell-dependent proliferative response with lymph node cells from primed mice. *J. Immunol. 119:1048.*
5. Fleischer, B. 1994. Superantigens. *APMIS 102:3.*
6. Kotzin, B. L., D. Y. M. Leung, J. Kappler, and P. Marrack. 1993. Superantigens and their potential role in human disease. *Adv. Immunol. 54:99.*
7. Goodier, M., P. Fey, K. Eichmann, and J. Langhorne. 1992. Human peripheral blood $\gamma\delta$ T cells respond to antigens of *Plasmodium falciparum*. *Int. Immunol. 4:33.*
8. Munk, M. E., B. Schoel, S. Modrow, R. W. Karr, R. A. Young, and S. H. E. Kaufmann. 1988. T cell responses of normal individuals towards recombinant protein antigens of *Mycobacterium tuberculosis*. *Eur. J. Immunol. 18:1835.*
9. van Voorhis, W. C. 1992. Coculture of human peripheral blood mononuclear cells with *Trypanosoma cruzi* leads to proliferation of lymphocytes and cytokine production. *J. Immunol. 148:239.*
10. Subauste, C. S., and J. S. Remington. 1993. Immunity to *Toxoplasma gondii*. *Curr. Opin. Immunol. 5:532.*
11. Gazzinelli, R. T., E. Y. Denkers, and A. Sher. 1993. Host resistance to *Toxoplasma gondii*: model for studying the selective induction of cell-mediated immunity by intracellular parasites. *Infect. Agents Dis. 2:139.*
12. Wong, S. Y., and J. S. Remington. 1994. *Toxoplasmosis in the Setting of AIDS*. Williams and Wilkins, Baltimore.
13. Subauste, C. S., J. Y. Chung, D. Do, A. H. Koniaris, C. A. Hunter, J. G. Montoya, S. Porcelli, and J. S. Remington. 1995. Preferential activation and expansion of human peripheral blood $\gamma\delta$ T cells in response to *Toxoplasma gondii* in vitro and their cytokine production and cytotoxic activity against *T. gondii*-infected cells. *J. Clin. Invest. 96:610.*
14. Brenner, M. B., J. McLean, D. P. Dialynas, J. L. Strominger, J. A. Smith, F. L. Owen, J. G. Seidman, F. Rosen, and M. S. Krangel. 1986. Identification of a putative second T-cell receptor. *Nature 322:145.*

15. Subauste, C. S., L. Dawson, and J. S. Remington. 1992. Human lymphokine-activated killer cells are cytotoxic against cells infected with *Toxoplasma gondii*. *J. Exp. Med. 176:1511.*
16. Geppert, T. D., and P. E. Lipsky. 1987. Dissection of defective antigen presentation by interferon-γ-treated fibroblasts. *Immunol. 138:385.*
17. Kappler, J., B. Kotzin, L. Herron, E. W. Gelfan, R. D. Bigler, A. Boylston, S. Carrel, D. N. Posnett, Y. Choi, and P. Marrack. 1989. Vβ-specific stimulation of human T cells by staphylococcal toxins. *Science 244:811.*
18. Roncarolo, M. G., M. Bigler, E. Ciuti, S. Martino, and P. A. Tovo. 1994. Immune responses by cord blood cells. *Blood Cells 20:573.*
19. Harris, D. T., M. J. Schumacher, J. Locascio, F. J. Besencon, G. B. Olson, D. de Luca, L. Shenker, J. Bard, and E. A. Boyse. 1992. Phenotypic and functional immaturity of human cord blood T lymphocytes. *Proc. Natl. Acad. Sci. USA 289:10006.*
20. Fleischer, B., and H. Schrezenmeier. 1988. T cell stimulation by staphylococcal enterotoxins: clonally variable response and requirement for major histocompatibility complex class II molecules on accessory or target cells. *J. Exp. Med. 167:1697.*
21. Montoya, J. G., K. E. Lowe, C. Clayberger, D. Moody, D. Do, J. S. Remington, S. Talib, and C. S. Subauste. 1996. Human $CD4^+$ and $CD8^+$ T lymphocytes are both cytotoxic to *Toxoplasma gondii*-infected cells. *Infect. Immun. 64:176.*
22. Choi, Y., B. Kotzin, L. Herron, J. Callaha, and P. Marrack. 1989. Interaction of *Staphylococcus aureus* toxin "superantigens" with human T cells. *Proc. Natl. Acad. Sci. USA 86:8941.*
23. Beverly, P. C. L. 1990. Is T-cell memory maintained by crossreactive stimulation? *Immunol. Today 11:203.*
24. Bell, E. B., and S. M. Sparshott. 1990. Interconversion of CD45R subsets of CD4 T cells in vivo. *Nature 348:163.*
25. Hensen, E. J., and B. G. Elferink. 1979. Primary sensitization and restimulation of human lymphocytes with soluble antigen in vitro. *Nature 277:223.*
26. Mehta-Damani, A., S. Markowicz, and E. G. Engleman. 1995. Generation of antigen-specific $CD4^+$ T cell lines from naive precursors. *Eur. J. Immunol. 25: 1206.*
27. Denkers, E. Y., P. Caspar, and A. Sher. 1994. *Toxoplasma gondii* possesses a superantigen activity that selectively expands murine T cell receptor Vβ5-bearing $CD8^+$ lymphocytes. *J. Exp. Med. 180:985.*
28. Leggard, P. K., R. D. Legrand, and M. L. Misfeldt. 1991. The superantigen *Pseudomonas* exotoxin A requires additional functions from accessory cells for T lymphocyte proliferation. *Cell. Immunol. 135:372.*
29. Marrack, P., and J. Kappler. 1990. The staphylococcal enterotoxins and their relatives. *Science 248:705.*
30. Herman, A., J. W. Kappler, P. Marrack, and A. N. Pullen. 1991. Superantigens: mechanisms of T-cell stimulation and role in immune responses. *Annu. Rev. Immunol. 9:745.*
31. Boitel, B., M. Ermonval, P. Panina-Bordignon, R. A. Mariuzza, A. Lanzavecchia, and O. Acuto. 1992. Preferential Vβ gene usage and lack of junctional sequence conservation among human T cell receptor specific for a tetanus-derived peptide. *J. Exp. Med. 175:765.*
32. Barth, C., M. Pavlakis, P. Lavery, T. B. Strom, and R. Loertscher. 1996. Restricted TCR Vβ repertoire in the human mixed lymphocyte reaction is determined by the TCR on the responder cell and not by the stimulating antigen on the stimulator cell. *Transplantation 61:133.*
33. Gaschet, J., M. A. Trevino, M. Cherel, R. Vivien, A. Garcia-Sahuquillo, M. M. Hallet, M. Bonneville, J. L. Harrousseau, R. Bragado, N. Milpied, and H. Vie. 1996. HLA-target antigens and T-cell receptor diversity of activated T cells invading the skin during acute graft-versus-host disease. *Blood 87:2345.*
34. Dietrich, P. Y., A. Caignard, A. Lim, V. Chung, J. L. Pico, C. Pannetier, K. P., T. Hercend, J. Even, and F. Triebel. 1994. In vivo T-cell clonal amplification at time of acute graft-versus-host disease. *Blood 84:2815.*
35. Lechler, R. I., G. Lombardi, J. R. Batchelor, N. Reinsmoen, and F. H. Bach. 1990. The molecular basis of alloreactivity. *Immunol. Today 11:83.*
36. Lombardi, G., S. Sidhu, M. Daly, J. R. Batchelor, W. Makgoba, and R. I. Lechler. 1989. Are primary alloresponses truly primary? *Int. Immunol. 2:9.*
37. Lombardi, G., S. Sidhu, J. R. Batchelor, and R. I. Lechler. 1989. Allorecognition of DR1 by T cells from a DR4/CRw13 responder mimics self-restricted recognition of endogenous peptides. *Proc. Natl. Acad. Sci. USA 86:4190.*
38. Merkenschlager, M., L. Terry, R. Edwards, and P. C. L. Beverly. 1988. Limiting dilution analysis of proliferative responses in human lymphocyte populations defined by the monoclonal antibody UCHL1: implications for differential CD45 expression in T cell memory formation. *Eur. J. Immunol. 18:1653.*
39. Currier, J., J. Sattabangkot, and M. F. Good. 1992. "Natural" T cells responsive to malaria: evidence implicating immunological cross-reactivity in the maintenance of TCR $\alpha\beta^+$ malaria-specific responses from non-exposed donors. *Int. Immunol. 4:985.*
40. Suzuki, Y., M. A. Orellana, R. D. Schreiber, and J. S. Remington. 1988. Interferon-γ: the major mediator of resistance against *Toxoplasma gondii*. *Science 240:516.*
41. Suzuki, Y., F. K. Conley, and J. S. Remington. 1989. Importance of endogenous IFN-γ for prevention of toxoplasmic encephalitis in mice. *J. Immunol. 143:2045.*
42. Gazzinelli, R., Y. Xu, S. Hieny, A. Cheever, and A. Sher. 1992. Simultaneous depletion of $CD4^+$ and $CD8^+$ T lymphocytes is required to reactivate chronic infection with *Toxoplasma gondii*. *J. Immunol. 149:175.*
43. Seder, R. A., R. Gazzinelli, A. Sher, and W. E. Paul. 1993. Interleukin 12 acts directly on $CD4^+$ T cells to enhance priming for interferon γ production and diminishes interleukin 4 inhibition of such priming. *Proc. Natl. Acad. Sci. USA 90:10188.*
44. Soong, L., C. H. Chang, J. Sun, B. J. Longley, N. H. Ruddle, R. A. Flavell, and D. McMahon-Pratt. 1997. Role of $CD4^+$ T cells in pathogenesis associated with *Leishmania amazoniensis* infection. *J. Immunol. 158:5374.*
45. Titus, R. G., I. Muller, P. Kimsey, A. Cerny, R. Behin, R. M. Zinkernagel, and J. A. Louis. 1991. Exacerbation of experimental murine cutaneous leishmaniasis with $CD4^+$ *Leishmania major*-specific T cell lines or clones which secrete interferon-gamma and mediate parasite-specific delayed-type hypersensitivity. *Eur. J. Immunol. 21:559.*
46. Teixeira, A. R. L., G. Teixeira, V. Macedo, and A. Prata. 1978. Acquired cell-mediated immunodepression in acute Chagas disease. *J. Clin. Invest. 62:1132.*
47. Strickland, G. T., and P. C. Sayles. 1977. Depressed antibody responses to a thymus-dependent antigen in toxoplasmosis. *Infect. Immun. 15:184.*
48. Khan, I. A., T. Matsuura, and L. H. Kasper. 1996. Activation-mediated $CD4^+$ T cell unresponsiveness during acute *Toxoplasma gondii* infection in mice. *Int. Immunol. 8:887.*
49. Remington, J. S., and L. O. Gentry. 1970. Acquired toxoplasmosis: infection versus disease. *Ann. NY Acad. Sci. 174:1006.*
50. McCabe, R. E., R. G. Brooks, R. F. Dorfman, and J. S. Remington. 1987. Clinical spectrum in 107 cases of toxoplasmic lymphadenopathy. *Rev. Infect. Dis. 9:754.*
51. Canessa, A., V. Pistoia, S. Roncella, A. Merli, G. Melioli, A. Terragna, and M. Ferrarini. 1988. An in vitro model for *Toxoplasma* infection in man: interaction between $CD4^+$ monoclonal T cells and macrophages results in killing of trophozoites. *J. Immunol. 140:3580.*
52. Saavedra, R., and P. Herion. 1991. Human T-cell clones against *Toxoplasma gondii*: production of interferon-γ, interleukin-2, and strain cross-reactivity. *Parasitol. Res. 77:379.*