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Alteration of Intracellular Calcium Flux and Impairment of Nuclear Factor-AT Translocation in T Cells During Acute *Toxoplasma gondii* Infection in Mice[1]

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Down-regulation of host immune response to *Toxoplasma gondii* is associated with the expression of specific cytokines, in particular IL-10, and the induction of $CD4^+$ T cell anergy. In the present study we report that the expression of both CD4 and CD2 antigen is down-regulated during the acute phase of infection. A decrease in the expression of CD2 was apparent during the acute phase of *T. gondii* infection in three genetically distinct strains of mice, CBA/J, C57BL/6, and BALB/c. The lymphoproliferative response induced by cross-linked anti-CD3 mAb or by Con A was markedly depressed. This suppressed response was associated with a reduction in the influx of Ca^{2+}. We have examined whether lymphocytes from *T. gondii* mice maintain NF-AT transcription factors in the nucleus where they participate in the Ca^{2+}-dependent induction of genes required for lymphocyte activation and proliferation. Immunofluorescence with confocal microscopy using an Ab to NF-ATc demonstrates a decrease in translocation of NF-ATc in T lymphocytes from acutely infected mice. Together, these results suggest that the defect in T cell expansion that occurs during acute murine toxoplasmosis is related to reduced activity of NF-AT, a calcium-dependent transcription factor required for T cell proliferation. *The Journal of Immunology,* **1998, 161: 6812–6818.**

The intracellular protozoan parasite *Toxoplasma gondii* is a major opportunistic pathogen of the newborn and those with AIDS. It has been demonstrated that suppression of lymphocyte proliferation occurs in response to parasite Ag and mitogen during acute toxoplasmosis in both humans and mice (1–3). In humans this down-regulatory response can be mediated in vitro by parasite-infected monocytes (4). In mice, both spleen-derived macrophages and T cells can elaborate this suppressive response (5–7).

A number of soluble cell products has been implicated in this down-regulation. In mice, the maximal immune suppression (day 7 postinfection) has been associated with a significant reduction in IL-2 secretion (6). In vitro, the immunosuppressive effect on IL-2 production is dependent upon the number of parasites used to infect macrophages (8). Both IL-10 and nitric oxide appear to play a role in manipulating the down-regulatory event. In addition to the well-recognized role for IFN-γ in host resistance to this parasite (9–11), in humans and perhaps mice this cytokine appears to partially mediate the release of an immune down-regulatory soluble factor (4). Acute toxoplasma infection in mice can induce a state of T cell unresponsiveness (12, 13). On day 7 postinfection, a partial reduction in the proliferative response of all $CD4^+$ T cells to mitogen or parasite Ag stimulation was observed, particularly in Vb5 cells. Addition of rIL-2 partially restored the $CD4^+$ T cell proliferative response in vitro. These studies suggested that the activation-induced $CD4^+$ T cell unresponsiveness may be an important immune down-regulatory event in the infected host (12, 13).

It has been observed that after T cell activation rapid changes in the function or expression of several membrane-associated molecules occur, including CD11a, CD2, and CD69 (14, 15). Activated T cells require a primary signal mediated via triggering of the Ag-specific TCR and a secondary accessory signal, such as CD2, CD4, CD8, and CD28 (16). It has been shown that anti-CD2 mAbs can block T cell activation in vitro, implying an important role for CD2 in T cell activation (17). In T lymphocytes, the binding of mAb to the CD3 complex mimics activation via the Ag receptor, resulting in the production of inositol 1,4,5-trisphosphate, an increased intracellular ionized Ca^+ concentration, and subsequent proliferation (18). Some microbial pathogens, including HIV, *Trypanosome cruzi*, and *Leishmania donovani* (19–21) have been associated with the defective regulation of $[Ca+]_i$[3] in response to extracellular stimuli. The calcium-mediated signaling event is essential for growth, death, differentiation, and function of immune cells (22, 23). Several Ca^{2+}-sensitive transcriptional regulators, NF-κB, JNK, and NF-AT, participate in the expression of genes that underlie these responses (24). Sustained high concentrations of Ca^+ are required to maintain NF-AT transcription factors in the nucleus, where they participate in Ca^{2+}-dependent induction of the genes required for lymphocyte activation and proliferation (25).

In this study we report that acute infection with toxoplasma in mice is able to alter the expression of several T cell membrane molecules, in particular CD2 and CD4. Furthermore, we observed that acute infection with this obligate intracellular parasite alters both the CD3-activated and mitogen-induced $[Ca+]_i$ response. The effect of this response on the Ca^{2+}-sensitive transcriptional regulator NF-AT was determined during acute murine infection with this parasite.

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[3] Abbreviations used in this paper: $[Ca^{2+}]_i$, intracellular Ca^{2+}; PE, phycoerythrin; LP, live tachyzoite of *Toxoplasma gondii* parasite; FFP, formalin-fixed tachyzoite of *Toxoplasma gondii* parasite.

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Materials and Methods

Parasites and mice

Female CBA/J (H-2k), BALB/c (H-2d), and C57BL/6 (H-2b) mice, 5–6 wk old, were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were housed in the accredited Animal Research Facility at Dartmouth Medical School (Hanover, NH) and maintained under the guidelines established by the institution for their use. The parental P strain of *T. gondii* (PLK) was used for our experiments. Parasites were maintained in our laboratory by in vitro passage in human foreskin fibroblasts at 37°C in MEM medium without calf serum. Parasites were purified from human fibroblast cell culture as previously described (6). Each mouse received 1.2×10^5 tachyzoites/i.p. injection.

T cell preparation and proliferation assays

Mice were killed on day 5 after infection, and spleens were removed and gently dissociated into single cell suspensions. RBCs were removed using lysing buffer (Sigma, St. Louis, MO). Cell suspensions were passed through nylon wool columns to enrich for T cells. These cells were >90% T cells. The cells were stained, and flow cytometric analysis was performed. T cell proliferation assays was performed as previously described (6, 26). Briefly, lymphocytes (2×10^5) were suspended in 200 μl of complete medium and cultured in 96-well microtiter plates in the presence or the absence of Con A and cross-linked anti-CD3 mAb. For the anti-CD3 mAb (PharMingen, San Diego, CA; 145-2C11 mAb)-driven proliferation assay, culture plate wells were precoated with goat anti-hamster IgG (14 μg of anti-hamster IgG; Jackson Immunology Research Laboratories, West Grove, PA) overnight at 4°C. After washing, the wells were incubated with different concentrations of anti-CD3 mAb at 37°C for several hours. Splenocytes were added to the wells and were cultured, and DNA synthesis was determined after 42 h by 6-h thymidine incorporation of [*methyl*-^{3}H]thymidine (ICN, Costa Mesa, CA). All cell culture and FACS analysis experiments were performed in 96-well plates.

Flow cytometric analysis

Abs directed against CD2 (mAb RM2-5, PE-conjugated), CD3 (mAb 145-2C11, FITC-conjugated), CD4 (mAb RM4.5, FITC-conjugated), CD8 (mAb 53-6.7, FITC-conjugated), and TCR (mAb H57-597, FITC-conjugated) were used in this experiment. All Abs directed against these epitopes were purchased from PharMingen (San Diego, CA). Direct immunofluorescence PE- or FITC-conjugated mAb was added to 1×10^6 cells, followed by a 45-min incubation on ice. Cells were then washed twice with PBS containing 1% BSA and fixed in 1% formaldehyde. Negative controls were stained with PE- or FITC-conjugated mouse Ig.

Measurement of $[Ca^{2+}]_i$

Spleen cells were obtained from mice on day 5 postinfection and from uninfected mice. Cell suspensions were passed through nylon wool columns to enrich for T cells. Changes in Ca^+ were measured by flow cytometry using indo-1/AM (Molecular Probes, Eugene, OR) on a HH/2150 flow cytometer (FACStar, Becton Dickinson, Mountain View, CA) as previously described (27). Briefly, after lysis of RBCs, cells were washed with serum-free medium and loaded with 5 μM indo-1 (Molecular Probes) for 45 min at 37°C. For each assay, indo-1-loaded cells were diluted to 1×10^5/ml with medium containing 5% FCS, equilibrated at 37°C, and analyzed by flow cytometry. All unstimulated cells were removed before analysis. Con A or anti-CD3 mAb was added 30 s before the beginning of the experiment. To analyze CD4-positive cells, lymphocytes were loaded with indo-1/AM and stained with FITC-conjugated CD8 mAb (18), and then FITC ($CD8^+$) fluorescent $CD8^+$ cells were excluded from analysis by electronic gating (18). The cells were analyzed at about 250 cells/s by means of dual-laser FACS. The calcium concentration was determined by the ratio between 485 nm and 405 nm emission with 355 nm excitation. Calibration was performed by measuring R_{min} and R_{max} in cells, and applying the equation described previously (28). Responses are reported as Ca^+ concentrations vs time.

Immunofluorescence for NF-AT visualization

For this assay, the Ab 7A6 (anti NF-ATc) were provided by Dr. Luika Timmerman (Stanford University School of Medicine, Palo Alto, CA). Cells from uninfected and day 5 postinfection animals were stimulated with ionomycin and PMA (1 μM and 10 ng/ml, respectively; for 20 min). All cells were prepared as described previously (25). Briefly, after centrifugation (Cytospin, Shandon, Pittsburgh, PA; 3 min at 300 rpm), cells were fixed in 4% paraformaldehyde in PBS for 10 min, permeabilized with 0.1% Triton X-100 in PBS for 10 min at room temperature, and rehydrated in PBS. The cells were then incubated overnight at 4°C with NF-AT-specific mAb 7A6 (1/500 in PBS) followed by anti-mouse biotin-conjugated (1/1000; Sigma) and avidin-FITC-conjugated (1/200; PharMingen, CA) Abs.

Table I. *Lymphocytes responses to different concentrations of cross-linked anti-CD3 mAb or Con A*[a]

Stimulus	[^{3}H]Thymidine Incorporation (cpm)	
	Uninfected[b]	*T. gondii* infected[c]
CD3 mAb/ml		
5.0 μg	122,520 ± 1,969	24,592 ± 654
2.0 μg	96,250 ± 5,449	11,877 ± 1,583
0.5 μg	77,365 ± 2,781	4,930 ± 245
ConA/ml		
5.0 μg	106,850 ± 3,039	13,575 ± 77
2.0 μg	74,299 ± 133	9,521 ± 77
0.5 μg	35,575 ± 1,376	5,286 ± 457

[a] Proliferative response to cross-linked anti-CD3 mAb or Con A of lymphocytes from CBA/J mice (n = 3/group) infected with *T. gondii* (PLK) tachyzoites (1.2×10^5/mouse) or age-matched uninfected mice. On day 5 after infection, lymphocytes from spleens of infected mice or control mice were cultured in quadruplicate (2×10^5 viable cells per well) in flat-bottom wells in RPMI 1640 plus 10% FCS. The cells were cultured in the presence of different concentrations of either anti-CD3 mAb (5, 2, and 0.5 μg/ml) or Con A (5, 2, and 0.5 μg/ml). Cells from uninfected and infected animals were cultured in medium only, and cpm in uninfected group is 2142 ± 145 and experimental group is 2250 ± 280. [^{3}H]Thymidine incorporation was determined on day 3 of culture. The results are expressed as means ± SD for quadruplicate. These results are representative of three separate experiments.

[b,c] Significant difference in the levels of lymphoproliferation between uninfected (b) and infected mice (c) (b vs c, $p < 0.005$).

Statistical analysis

Levels of significance of the differences between groups were determined by Student's t test. Statistical significance was set at $p < 0.05$ for all comparisons.

Results

T cell activation by cross-linking of the CD3 molecule and Con A

The cells from infected mice were assayed for their ability to initiate DNA synthesis in response to either CD3-mediated activation or mitogen-driven stimulation. For these experiments, spleen cells from mice infected with *T. gondii* for 5 days were cultured with either anti-CD3 mAb or Con A. Splenocytes from infected mice failed to proliferate in response to either anti-CD3 mAb or mitogen stimulation compared with those from control mice (Table I; $p < 0.005$). As previously reported, the addition of rIL-2 to the suppressed splenoyctes from infected mice increased the proliferative response to several stimuli, including mitogen, parasite Ag, and live parasites (data not shown) (6, 8).

Phenotypic analysis of mouse T cell subpopulations during acute infection

To determine the effect of *T. gondii* infection on the expression of T cell surface molecules, lymphocytes were isolated from day 5 postinfection CBA/J, BALB/c, and C57BL/6 mice, and the expression of T cell phenotypes was analyzed by FACS. As shown in Table II, a decrease in the expression of both CD2 and CD4 surface molecules was observed in all three strains of mice on day 5 postinfection. Compared with uninfected mice, this diminution in CD2 ($p < 0.05$) and CD4 ($p < 0.005$) molecules was statistically significant in all three strains of mice infected with *T. gondii*. The diminution in CD2 expression ranged from 11% (CBA/J) to 24% (C57BL/6). For CD4, the decrease in the number of cells expressing this phenotype fell approximately 27 ± 2% for all three strains of mice. There was no significant change in the expression of $CD8^+$, although a modest, but insignificant, rise in the CBA/J and

Table II. *Phenotypic analysis of mouse T cell populations*[a]

Cell-Surface Ag Expression	Percent Positive (mean fluorescence intensity)					
	Cells from uninfected mice			Cells from day 5 postinfected mice		
	CBA/J	BALB/c	C57BL/6	CBA/J	BALB/c	C57BL/6
CD2	92 ± 4.0 (345 ± 7.0)	99 ± 1.5 (270 ± 2.6)	92 ± 2.0 (363 ± 3.0)	82 ± 3.5 (290 ± 8.0)*	87 ± 2.0 (145 ± 30)*	70 ± 3.0 (265 ± 1.5)*
CD3	44 ± 1.0 (147 ± 1.4)	44 ± 1.0 (254 ± 2.0)	42 ± 1.0 (94 ± 4.5)	42 ± 3.0 (189 ± 10)	41 ± 2.0 (213 ± 10.0)	39 ± 1.0 (108 ± 3.0)
CD4	23 ± 1.0 (515 ± 10.0)	29 ± 1.0 (344 ± 3.0)	24 ± 2.0 (138 ± 4.0)	17 ± 1.5 (386 ± 3.0)*	21 ± 1.0 (251 ± 2.5)*	17 ± 1.0 (98 ± 2.0)*
CD8	13 ± 0.5 (235 ± 2.5)	10 ± 1.0 (476 ± 1.0)	14 ± 1.0 (192 ± 1.5)	15 ± 0.6 (162 ± 5)	12 ± 1.0 (270 ± 50)	8 ± 2.0 (135 ± 1.5)
TCR$\alpha\beta$	29 ± 6.0 (80 ± 4.0)	40 ± 4.0 (203 ± 5.0)	ND	35 ± 2.0 (62 ± 5.0)	32 ± 3.0 (165 ± 10.0)	ND

[a] Splenocytes were freshly obtained from mice (n = 2/group) infected for 5 days and cell-surface Ag expression was analyzed by FACS. The mean fluorescence intensity of the cell-surface Ag and the percentage of positive cells of 5000 cells analyzed were monitored with FITC or PE-conjugated mAbs and flow cytometry. Similar results were obtained in three repeated independent experiments.

*, Indicates satistically significant difference in percentage or in mean fluorescence intensity ($p < 0.05$) between cells derived from infected mice vs uninfected mice.

BALB/c mice was found. There was no difference in the expression of the $\alpha\beta$ TCR molecules in cells from any of the three strains of mice infected with *T. gondii.*

Modulation of $[Ca^{2+}]_i$ by indo-1 fluorescence

Splenocytes from infected mice were analyzed for calcium mobilization using the fluorescence indicator indo-1 in conjunction with flow cytometry following CD3-mediated activation or mitogen stimulation. Cells that were stimulated with either anti-CD3 mAb or Con A had an impaired $[Ca^{2+}]_i$ response compared with the control cells. In the response to anti-CD3 mAb (10 μg/ml) exposure, the $[Ca^{2+}]_i$ achieved a maximum level of 850 nM (Fig. 1*A*, uninfected) in cells from uninfected CBA/J mice. In contrast, the $[Ca^{2+}]_i$ of lymphocytes from infected CBA/J mice on day 5 postinfection reached 450 nM (Fig. 1*A*, LP infected). Similar results were obtained when the splenocytes from either infected or uninfected mice were stimulated with the T cell mitogen, Con A (Fig. 1*B*). There was only a nominal reduction compared with control cells when $[Ca^{2+}]_i$ mobilization was determined for splenocytes on day 2 postinfection (not shown). The difference in the degree of $[Ca^{2+}]_i$ mobilization between anti-CD3 mAb activation and mitogen stimulation for both infected and uninfected conditions appears insignificant. Of note, 35% of the control cells responded to anti-CD3 mAb stimulation, whereas only 12% of the cells from *T. gondii*-infected mice demonstrated a shift in $[Ca^{2+}]_i$ flux (data not shown). Parasite Ag was also used to assess the

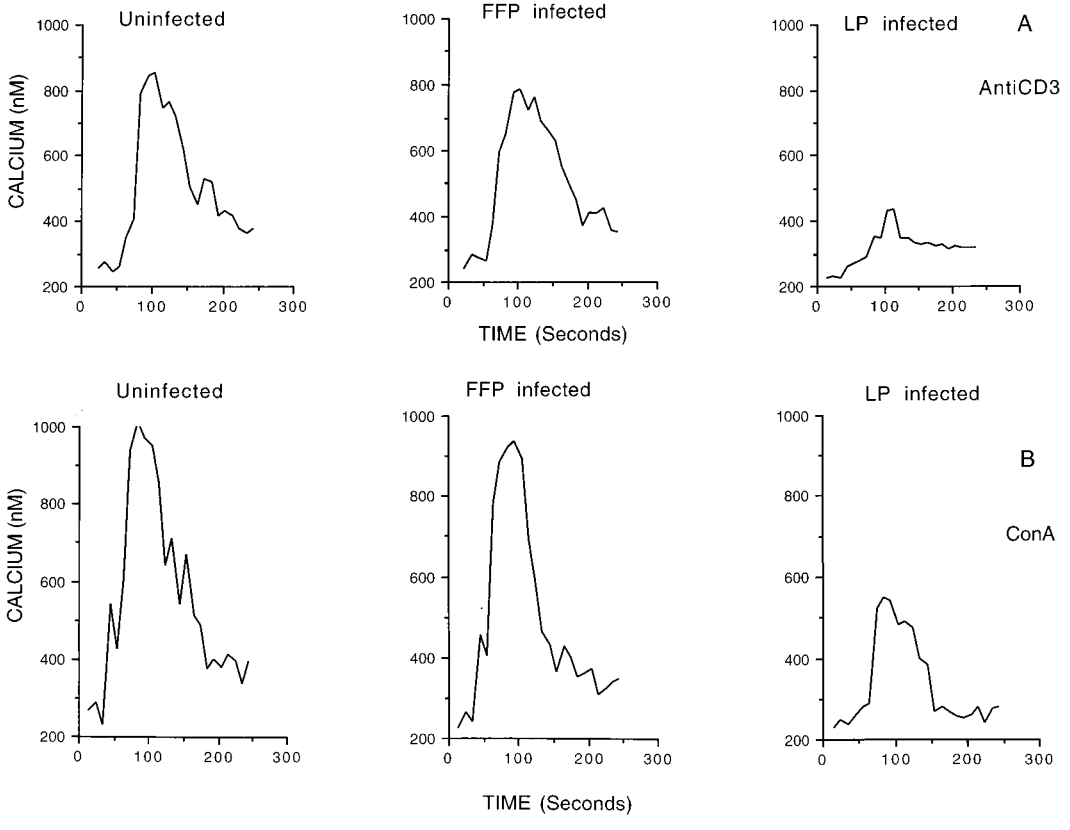

FIGURE 1. Effects of CD3 mAb (*A*) or Con A (*B*) stimulation on $[Ca^{2+}]_i$ of lymphocytes obtained from uninfected, FFP, or LP mice. Lymphocytes from uninfected, FFP infected (3×10^5), or LP infected (1.2×10^5) mice were loaded with indo-1 (45 min) and stimulated with anti-CD3 mAb (10 μg/ml) or Con A (10 μg/ml). CD3 mAb or Con A was added 30 s before the beginning of the experiment, and the cells were analyzed at 250 cells/s. Results are plotted as the mean calcium concentration vs time.

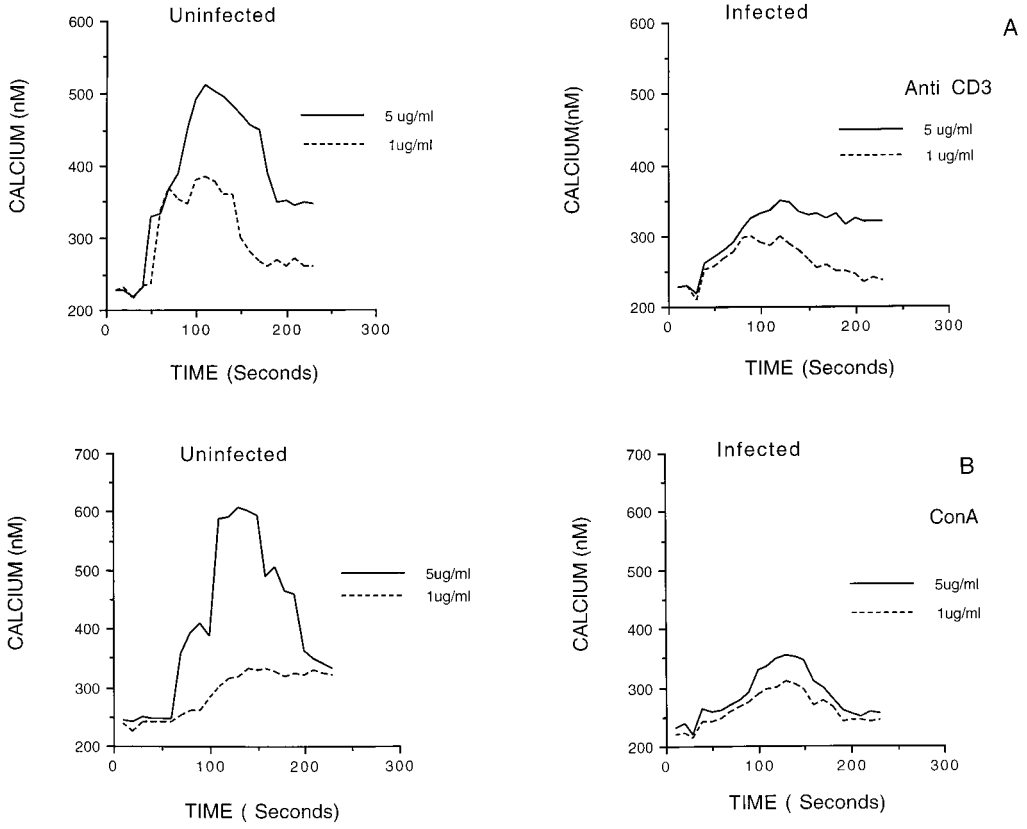

FIGURE 2. Effects of different concentrations of CD3 mAb (*A*) or Con A (*B*) stimulation on $[Ca^{2+}]_i$ of lymphocytes obtained from either uninfected or day 5 postinfected mice. Lymphocytes from either uninfected or day 5 postinfection mice were loaded with indo-1 (45 min) and stimulated with different concentrations (5 and 1 μg/ml) of either anti-CD3 mAb or Con A. CD3 mAb or Con A was added 30 s before the beginning of the experiment, and the cells were analyzed at 250 cells/s. Results are plotted as the mean calcium concentration vs time.

effect on $[Ca^{2+}]_i$ mobilization. In this assay, mice were treated with formalin-fixed parasites (FFP; 5×10^5 parasites/mouse). Five days later their splenocytes were isolated and stimulated with either anti-CD3 mAb or Con A. As shown in Fig. 1 (*A* and *B*, FFP infected), there was no significant reduction of the $[Ca^{2+}]_i$ mobilization in response to either stimulant following immunization with parasite Ag.

Although there was no difference in the level of $[Ca^{2+}]_i$ mobilization between mitogen and CD3 activation, the response was stimulant concentration dependent. As shown in Fig. 2, *A* and *B*, lymphocytes from uninfected or infected mice responded differentially to varying concentrations of either anti-CD3 mAb or Con A. A significant difference was apparent between cells from infected vs uninfected mice.

To better determine the specific T cell phenotype involved in the $[Ca^{2+}]_i$ alteration postinfection, the $[Ca^{2+}]_i$ level in $CD4^+$ T cells from infected or uninfected mice was investigated. The $CD4^+$ T cells were selected for study, since our data suggested that it was this population of T cells that was altered during acute infection (Table I). In these experiments, nylon wool-purified enriched T cells from both infected and uninfected mice were loaded with indo-1/AM. $CD8^+$ T cells were removed by FACS following Ab staining. The residual cells to be used were approximately 90% positive for expression of $CD4^+$ molecule. These cells were stimulated with either mitogen or anti-CD3 mAb, and the level of $[Ca^{2+}]_i$ mobilization was determined. As illustrated in Fig. 3, $CD4^+$ T cells obtained from infected mice showed a significant reduction in $[Ca^{2+}]_i$ mobilization in response to stimulation via CD3 activation or mitogen. In response to anti-CD3 mAb exposure, the $[Ca^{2+}]_i$ for these cells was reduced by one-third in the infected (320 nM) vs uninfected (480 nM) mice. Similarly, a 41% reduction was observed with Con A stimulation (uninfected, 550 nM; infected, 325 nM).

NF-AT translocation

We evaluated whether this alteration in calcium mobilization had an impact on the capacity to translocate NF-AT, an important transcription factor in activated T cells. Studies by others have shown, using an mAb (NF-ATc 7A6), that an elevation of intracellular calcium was required to maintain the NF-AT level in the nucleus. An immunofluorescence assay with this Ab was used to explore whether the reduced calcium flux in the lymphocytes from infected mice was sufficient for nuclear import of NF-AT (22, 29).

For this study mice were infected with *T. gondii* parasites, and their splenocytes were isolated on day 5 postinfection. The cells were stimulated with ionomycin and PMA, and the translocation of NF-AT was determined by fluorescence. When lymphocytes from uninfected mice were stimulated with ionomycin and PMA, NF-AT was imported into the nucleus (Fig. 4*B*). In contrast, NF-AT could not be localized in the nucleus of stimulated T lymphocytes from acutely infected mice (Fig. 4*E*). However, addition of a high concentration of $CaCl_2$ (10 mM) resulted in translocation of NF-AT in the nuclei of lymphocytes from infected mice (Fig. 4*F*).

Discussion

In this study we observed that acute murine infection with *T. gondii* results in generalized immunosuppression postinfection. During this period of immune unresponsiveness, splenocytes isolated from parasite-infected mice: 1) fail to proliferate in response to

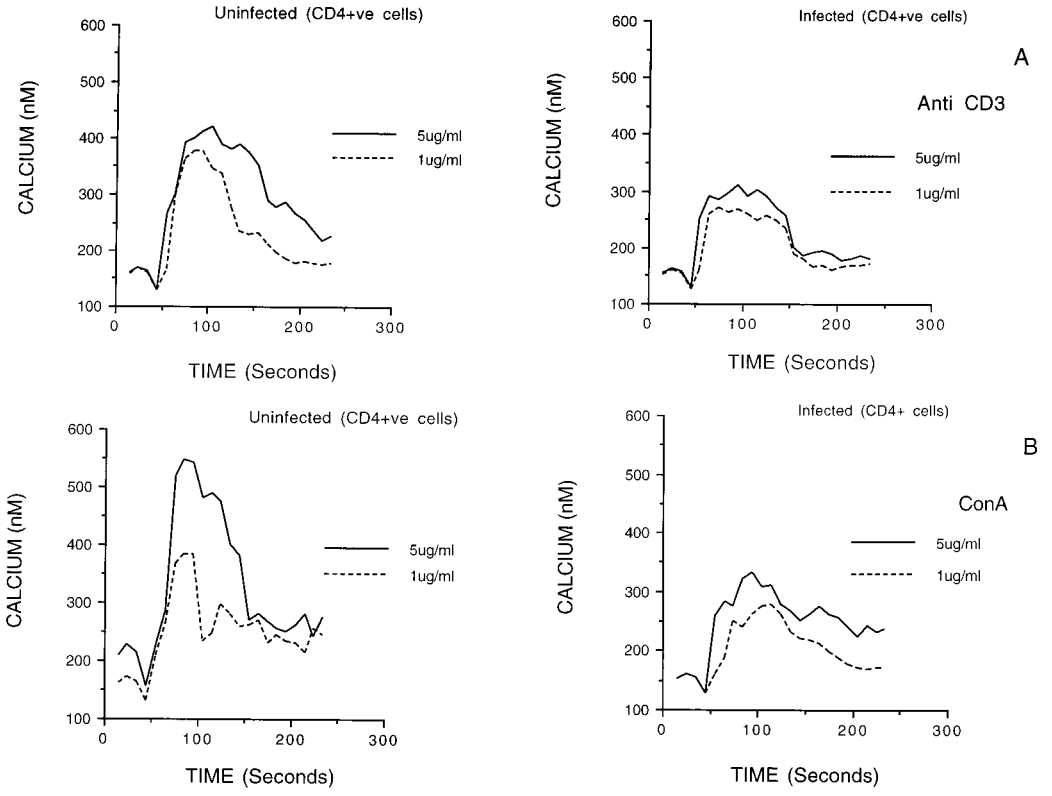

FIGURE 3. Effects of CD3 mAb (*A*) or Con A (*B*) stimulation on $[Ca^{2+}]_i$ of $CD4^+$ lymphocytes. Indo-1-loaded T lymphocytes (from uninfected or day 5 postinfection mice) were stained for 20 min with FITC-conjugated CD8 mAb. The cells were analyzed at 250 cells/s, and electronic gating was used to display the $FITC^-$ but $CD4^+$ T cells. Different concentrations (5 and 1 μg/ml) of CD3 mAb or Con A were added 30 s before the beginning of the experiment, and the cells were analyzed at 250 cells/s. Results are plotted as the mean calcium concentration vs time.

CD3 activation, 2) have diminished expression of both CD2 and CD4, 3) have an impaired $[Ca^{2+}]_i$ response to mitogen stimulation, and 4) exhibit insufficient NF-AT import into the nucleus.

Diminished T cell proliferation during acute murine toxoplasmosis in response to cross-linked anti-CD3 mAb may be attributed to alterations in the mechanism of T cell activation. This condition was associated with an impairment in IL-2 production (6). The failure to restore T cell responsiveness by addition of exogenous IL-2 may be partly due to local factors defined by culture conditions, such as altered balances of cytokines and the inhibitory role of IL-10 and nitric oxide (4, 6–8, 30). CD48 augments the proliferative response of spleen cells when cross-linked with anti-CD3 mAbs (costimulatory signal) (31). In mice, CD48 is a ligand of CD2. We observed that cells from 5-day-postinfected mice were only partially responsive upon exposure to anti-CD48 mAb (data not shown).

The involvement of the $CD4^+$ T cell subset during acute toxoplasma infection in mice has been investigated previously. Activation-induced programmed cell death may account for the unresponsive state of $CD4^+$ T cells during acute infection (13).

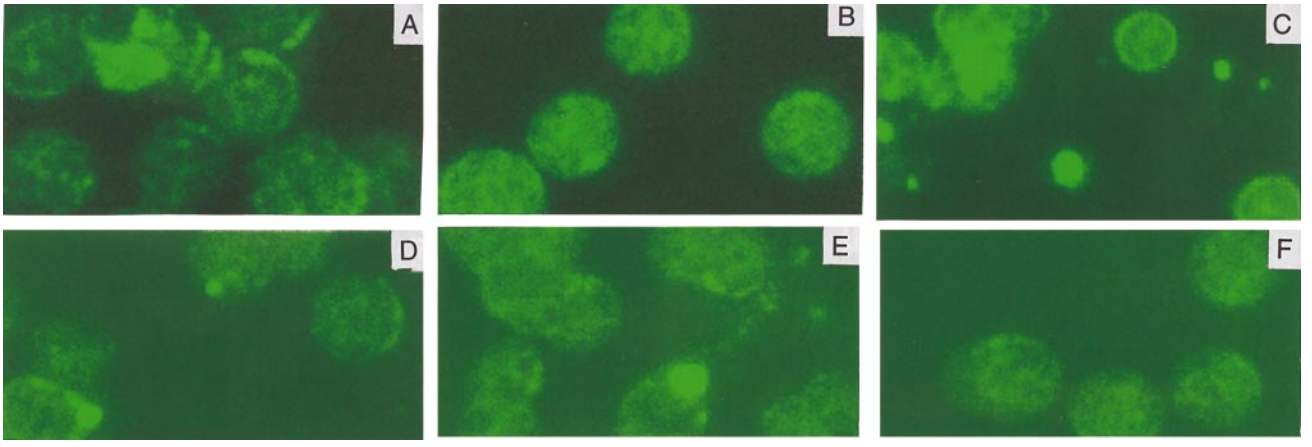

FIGURE 4. NF-ATc does not translocate in splenic cells obtained from day 5 postinfection animal. NF-ATc localization was assessed in splenocytes obtained from uninfected (*A–C*) and day 5 postinfection (*D–F*) animals by using NF-ATc mAb 7A6. Cells were cultured in medium alone for 60 min (*A* and *D*), stimulated with ionomycin (1 μM) and PMA (10 ng/ml) for 60 min (*B* and *E*), or stimulated with ionomycin (1 μM) and PMA (10 ng/ml) for 60 min but in the presence of 10 mM $CaCl_2$ (*C* and *F*). Staining with isotype-matched control Ab gave no visible fluorescence.

Alteration of the CD4/CD8 ratio during acute infection may preclude the progressive decline in the $CD4^+$ population as the unresponsive T cells are sequestered and eliminated from the peripheral circulation by the process of apoptosis during in vitro culture. We evaluated this response in vivo using a chromatin condensation assay with Hoechst 33342. In that study we did not find any significant differences in the number of apoptic cells from either infected or uninfected mice (data not shown). The results of the current study demonstrated that splenocytes from infected mice exhibited decreased expression of CD4 on day 5 postinfection. A reduction in the expression of this surface molecule was observed in T cells from all three strains of mice investigated (CBA/J, BALB/c, and C57BL/6). Several reports suggest that the CD4 molecule may serve to enhance or inhibit CD3-induced signaling, although we observed no changes in the expression of CD3 (32). Although the modulation of $CD4^+$ molecule was identical in the different strains of mice used in our experiments, alterations in CD2 were most apparent in the highly susceptible C57 mouse strain. The contribution of CD2 to the immune hyporesponsiveness in vivo has been difficult to assess due to the limitation of experimentation in animal models (33–35). Studies in HIV-infected cells demonstrate the persistence of CD2 mRNA expression despite a marked diminution in its surface expression (18). The importance of CD2 regulation in the development of immune unresponsiveness to toxoplasma infection remains uncertain and is currently under study.

In some parasitic protozoan infections such as *T. cruzi* (21, 36, 37), *Plasmodium falciparum* (38), and *Leishmania donovani* (19) infections, a role for Ca^+ in the process of host cell invasion has been suggested. These studies indicated changes in the cytosolic Ca^+ concentration in diverse host cells, such as in HUVEC, fibroblast cells, HeLa cells, and mononuclear phagocytes, following infections by these parasites. However, little information is available on the consequences of these changes on the host's immune responses. Few studies have provided information on calcium signal transduction in T cells during the course of parasitic infections. In the present study we report that during acute toxoplasma infection lymphocytes obtained from infected mice had a significant reduction in Ca^+ mobilization (50–53% reduction in the $[Ca+]_i$ response to both CD3 mAb and mitogen compared with control values) (Fig. 1). The importance of Ca^{2+} in T lymphocyte activation is evident from the effectiveness of the immunosuppressant cyclosporin A and the observations that individuals with lymphocytes defective in Ca^{2+} signaling suffer from primary immunodeficiency (39). HIV has been shown to alter the stores of intracellular free calcium and impair inositol phosphatase production (18). Our findings clearly demonstrate that *T. gondii*, like HIV, can affect stores of intracellular calcium and suggest that a reduced calcium flux could be implicated in the development of transient T cell hyporesponsiveness during acute infection.

We next wanted to determine whether the Ca^+ signaling defect in stimulated T cells from infected mice could regulate NF-AT, the transcription factor that stimulates early immune response genes such as cytokines (23, 40). A sustained rise in the intracellular Ca^{2+} concentration can activate calcineurin, a Ca^{2+}-dependent, cyclosporin A-sensitive serine/threonine phosphatase that dephosphorylates the transcription factor NF-AT (23). Once dephosphorylated, NF-AT migrates to the nucleus, where it associates with Jun and Fos to promote the transcription of a host of immunoregulatory genes (40). We have taken advantage of availability of a mAb to NF-ATc (7A6) (25), which is expressed exclusively in the lymphoid system and is induced upon lymphocyte activation. By using this mAb in confocal microscopy, we were able to evaluate calcium regulation of NF-AT translocation in the nucleus of T lymphocytes stimulated with ionomycin and PMA. Our data clearly showed that the calcium flux in the lymphocytes from infected mice was not sufficient for nuclear import of NF-AT (compare Fig. 4, *D* and *E* with *A* and *B*). However, NF-AT will translocate in the nucleus of these lymphocytes if $[Ca^{2+}]_i$ is artificially augmented and sustained by increasing concentrations of extracellular Ca^{2+} (Fig. 4*F*). Ca^+ signaling involves the mobilization of Ca^+ from intracellular stores and the extracellular medium (41). We and others have demonstrated that a decrease in both the production and the expression of IL-2 occurs during the hyporesponsive state associated with acute toxoplasma infection (6, 8, 42). Insufficient concentrations of intracellular free calcium may explain the defect in IL-2 production by T lymphocytes from acutely infected mice. It may be noted that sustained levels of Ca^+ are required to maintain NF-AT transcription factors in the nucleus, where they participate in Ca^+-dependent induction of genes required for IL-2 enhancement (23, 40). Together these findings indicate that during acute toxoplasma infection, NF-AT translocation is affected inside T lymphocytes by live parasite infection, rendering the host unable to respond in an immunologically competent fashion.

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