Roles of Gamma Interferon and Other Cytokines in Suppression of the Spleen Cell Proliferative Response to Concanavalin A and Toxoplasma Antigen during Acute Toxoplasmosis

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Suppressed splenocyte proliferation in response to mitogen and toxoplasma lysate antigen (TLA) is observed in mice acutely infected with *Toxoplasma gondii*. Recently, we reported that *N*^G-monomethyl-L-arginine (NMMA), an inhibitor of reactive nitrogen intermediate (RNI) production, partially restored proliferative responses of splenocytes from infected mice. In the present study we have examined the effect of NMMA on production of cytokines by splenocytes from mice acutely infected with *T. gondii* and assessed the role of gamma interferon (IFN- γ) and interleukin-10 (IL-10) in the RNI-mediated suppression. Stimulation with concanavalin A (ConA) or TLA of splenocytes from CBA/Ca mice infected for 7 days resulted in increased production of IFN- γ , IL-4, and IL-10 but reduced levels of IL-2 when compared with cultures of splenocytes from uninfected mice. Whereas addition of NMMA did not alter levels of cytokines produced by splenocytes from uninfected mice, splenocytes from infected mice stimulated with ConA produced significantly higher levels of IL-10 and reduced levels of IL-2 and IL-4. Addition of anti-IFN- γ monoclonal antibodies to cultures of spleen cells from mice infected for 7 or 14 days remarkably decreased the levels of nitrite and resulted in a 47- and 4-fold increase in proliferation induced by stimulation with ConA or TLA, respectively. Anti-IL-10 did not reduce levels of nitrite produced in culture but did result in a fourfold increase in the proliferative response of splenocytes from mice infected for 14 days. In vivo administration of anti-IFN- γ or anti-IL-10 monoclonal antibodies to infected mice partially restored ex vivo spleen cell proliferative responses by approximately 40 and 15%, respectively. Our data indicate that IFN- γ is important in inducing the RNI-mediated immunosuppression, which, in turn, affects production of cytokines by splenocytes. Our data also demonstrate that IL-10 is involved in the suppression observed but that this activity is independent of RNI.

Suppression of lymphocyte function has been reported during acute toxoplasmosis in humans and in murine models (4, 6, 7, 15, 17, 18, 23, 32, 34). The mechanisms underlying this immunosuppression are incompletely understood. Macrophages from infected mice have been demonstrated to suppress lymphocyte proliferation of spleen cells from uninfected mice (33–35); this is mediated, in part, by their production of prostaglandins (4, 6, 24). Recently, we reported that the addition of *N*^G-monomethyl-L-arginine (NMMA), an inhibitor of reactive nitrogen intermediate (RNI) production, to cultures of splenocytes from infected mice reversed the suppressed proliferative responses of lymphocytes to concanavalin A (ConA) by approximately 50% (5). These findings with *Toxoplasma gondii* were similar to those in reports that described the immunosuppressive properties of RNI during infection with a number of pathogens including *Trypanosoma brucei* (27, 31), *Plasmodium vinckei* (26), and *Listeria monocytogenes* (14). The suppressive role of RNI during murine toxoplasmosis contrasts remarkably with the capacity of RNI to inhibit replication of *T. gondii* within macrophages (2).

To further characterize the events involved in the RNI-mediated suppression we observed, we have attempted to determine whether the inhibition of RNI production, by the ad-

dition of NMMA, alters the levels of interleukin-2 (IL-2), IL-4, IL-10, and gamma interferon (IFN- γ) produced by spleen cells from infected mice. In addition, we decided to characterize the role of IFN- γ and IL-10 in the RNI-mediated suppression. To determine the possible role of these cytokines in the immunosuppression observed, we assessed the effects of neutralizing antibodies to IFN- γ and IL-10 in vitro and in vivo on proliferation and nitrite production of spleen cells from mice acutely infected with *T. gondii*. These cytokines were chosen since they both are produced in vivo during the acute phase of infection with *T. gondii* (20) and IFN- γ is a stimulus of RNI production while IL-10 antagonizes this activity of IFN- γ (9a, 11).

MATERIALS AND METHODS

Toxoplasma strains. *Toxoplasma* cysts of the avirulent Me49 strain were prepared as previously described (4). Mice were infected orally with 20 cysts by gavage. The RH strain, maintained in female Swiss Webster mice, was used for preparation of toxoplasma lysate antigen (TLA) as previously described (28).

Mice. Female CBA/Ca mice were obtained from Simonsen Laboratories, Gilroy, Calif., and were used at 6 to 8 weeks of age. Each experimental group consisted of three or four mice.

Reagents. The acetate salt of NMMA (Calbiochem Novabiochem Corp., San Diego, Calif.) was used in cultures of spleen cells at a final concentration of 500 μ M. Murine recombinant IFN- γ (kindly provided by R. Shepard, Genentech, South San Francisco, Calif.) was supplied at a specific activity of 5.2×10^6 U/mg and used in vitro at concentrations of 1 to 1,000 U/ml. Murine recombinant IL-10 (rIL-10, kindly provided by Satish Menon and Kevin Moore, DNAX, Palo Alto, Calif.) was supplied at a specific activity of 0.5×10^7 to 1×10^7 U/mg and was used in vitro at concentrations of 0.2 to 200 U/ml. Murine monoclonal antibodies (MAb) with neutralizing activity specific for IL-10 (JES5-2A5) and IFN- γ (XMG1.2) and an isotype control MAb (*Escherichia coli* β -Gal, GL 113, and GL 117) were provided by J. Abrams, DNAX. Both the anti-IL-10 and anti-IFN- γ

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MAB have been used previously by this laboratory for in vivo neutralization of these cytokines (19). These antibodies were used in vitro at final concentrations of 0.00025 to 0.5 mg/ml or were administered intraperitoneally at 1 mg per mouse 6 h prior to infection and again 9 days postinfection.

Spleen cell proliferative assay. T-cell proliferative responses to ConA or TLA were studied by [3 H]thymidine ([3 H]TdR) incorporation. Spleen cells were prepared as previously described (4, 5). Briefly, spleens were disrupted in endotoxin-free RPMI 1640 (Sigma, St. Louis, Mo.) supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/ml), streptomycin (100 μ g/ml), and amphotericin B (0.025 μ g/ml) (Whittaker Bioproducts, Walkersville, Md.). Erythrocytes were lysed by treatment with 0.83% NH_4Cl in 0.01 M Tris-HCl (pH 7.2). Cells were washed twice in RPMI containing heat-inactivated fetal calf serum, counted, and added at a cell density of 4×10^5 cells per well of 96-well flat-bottom tissue culture plates (Costar, Cambridge, Mass.). Cells were stimulated with ConA (1.25 μ g/ml; Sigma) or TLA (1 μ g/ml). The concentrations of ConA and TLA that induced optimal spleen cell proliferation were determined in preliminary experiments. Experiments were performed in triplicate wells in a final volume of 200 μ l per well. After 48 h of incubation at 37°C under 5% CO_2 , cultures were pulsed for 18 h with 1 μ Ci of [3 H]TdR (1 mCi/ml; Amersham, Chicago, Ill.), and the cells were harvested (PHD cell harvester; Cambridge Technology, Inc., Watertown, Mass.). Incorporation of [3 H]TdR was determined with a scintillation counter. In all experiments in which the effect of NMMA on production of cytokines was investigated, parallel experiments were performed to ensure that the addition of NMMA enhanced [3 H]TdR incorporation of splenocytes from infected mice stimulated with ConA or TLA (5).

Nitrite production. Levels of nitrite in the culture supernatants were used as a direct measure of RNI production. The nitrite concentration in the cell culture supernatants was measured by the Griess assay (13). Briefly, 100 μ l of sample was added to each well of a 96-well plate, and 100 μ l of a 1:1 mixture of 1% sulfanilamide dihydrochloride in 2.5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride in 2.5% H_3PO_4 was then added to the samples. The A_{550} was determined with a microplate reader (Dynatech Laboratories, Chantilly, Va.) with reference to a standard curve for concentrations of sodium nitrite (Sigma) from 0.1 to 50 nmol.

Measurement of cytokine production in supernatants of spleen cell cultures. The levels of IFN- γ , IL-2, IL-4, and IL-10 in supernatants of spleen cell cultures collected at 48 h of culture were measured by enzyme immunoassays, as previously described (1). Cytokine levels were estimated by comparison with standard curves for recombinant murine IFN- γ , IL-2, IL-4, or IL-10.

Statistics. An unpaired two-tailed student *t* test was performed with INSTAT software. $P < 0.05$ was considered significant.

RESULTS

Cytokine production by spleen cell cultures. To determine whether the RNI-mediated suppression observed during acute toxoplasmosis affects the production of cytokines, we measured the levels of IFN- γ , IL-2, IL-4, and IL-10 in cultures of splenocytes stimulated with ConA or TLA alone or in the presence of NMMA. ConA stimulation of spleen cells from uninfected mice resulted in increased production of IFN- γ , IL-2, IL-4, and IL-10 (Fig. 1). TLA stimulation of spleen cells from uninfected mice did not alter the basal levels of IFN- γ , IL-2, IL-4, or IL-10 (Fig. 1). When compared with spleen cells from uninfected mice, spleen cells from infected mice stimulated with ConA or TLA produced significantly higher levels of IFN- γ , IL-4, and IL-10 ($P < 0.0001$) (Fig. 1). In contrast, production of IL-2 was decreased (ConA) ($P < 0.0001$) or unchanged (TLA) (Fig. 1).

Addition of NMMA to splenocytes from uninfected mice stimulated with ConA or TLA did not significantly alter production of IL-2, IL-4, or IFN- γ . However, a small but significant increase in the levels of IL-10 was noted in response to TLA in the presence of NMMA (Fig. 1). Addition of NMMA to splenocytes from infected mice stimulated with ConA or TLA did not significantly alter production of IFN- γ but did result in a significant ($P < 0.01$) decrease in levels of IL-2 and IL-4; in contrast, addition of NMMA resulted in elevated IL-10 production by ConA- or TLA-stimulated spleen cells ($P < 0.01$) (Fig. 1).

Effects of in vitro neutralization of IFN- γ on proliferative responses and nitrite production by spleen cells. Since IFN- γ is an important cytokine in stimulating the production of RNI by macrophages (11, 22) and is produced at high levels in

FIG. 1. In vitro production of IFN- γ , IL-2, IL-4, and IL-10 by spleen cells from uninfected mice (open bars) or mice infected for 7 days (solid bars). Cultures were stimulated with ConA or TLA alone (A to D) or in the presence of NMMA (E to H). Similar results were observed in two experiments.

culture supernatants of splenocytes from acutely infected mice (see above), we investigated the role of this cytokine in the RNI-mediated suppression of proliferative responses. Proliferation of spleen cells from uninfected mice stimulated with ConA was not altered by addition of MAb to IFN- γ (data not shown). In contrast, the proliferative response to ConA of spleen cells from infected mice increased remarkably and significantly ($P < 0.0001$) in the presence of anti-IFN- γ (Fig. 2). Similar results were observed when TLA was used (data not shown). Addition of anti-IFN- γ to unstimulated splenocytes from mice infected for 7 days resulted in a small but significant increase in [3 H]TdR incorporation when compared with controls ($15,712 \pm 563$ versus $1,691 \pm 888$ cpm). Maximal increases in proliferation of 47-fold and 3.4-fold were observed for ConA and TLA, respectively, at the highest concentrations

FIG. 2. Proliferative response to ConA and nitrite production after addition of anti-IFN- γ MAb to the cultures of splenocytes from mice infected for 7 days. The uptake by cells cultured in the medium alone was 1,691 cpm. Addition of anti-IFN- γ to unstimulated splenocytes from mice infected for 7 days resulted in a small but significant increase in [³H]TdR incorporation compared with controls ($15,712 \pm 563$ versus $1,691 \pm 888$ cpm). Similar results were observed in two separate experiments as well as with TLA-stimulated spleen cells.

of anti-IFN- γ used. Addition of anti-IFN- γ also resulted in decreased production of nitrite, and this effect was inversely related to the concentration of anti-IFN- γ used (Fig. 2). Similar effects of anti-IFN- γ on proliferation and nitrite production were observed with splenocytes from mice infected for 14 days (Table 1). These data indicate that IFN- γ has an important role in controlling the RNI-mediated suppression of proliferative responses.

Effects of in vitro neutralization of IL-10 on proliferative responses and nitrite production by spleen cells. IL-10 has several immunosuppressive activities (9a) and can inhibit production of RNI by macrophages (11). The presence of increased levels of IL-10 in culture supernatants of splenocytes from infected mice (see above) indicated a possible role of this cytokine in the parasite-induced immunosuppression. Addition of anti-IL-10 MAb to culture supernatants did not significantly alter proliferation or production of nitrite by spleen cells from uninfected or infected mice at day 7 postinfection. Enhanced proliferation was observed with spleen cells from mice infected 14 days earlier (Table 1); anti-IL-10 did not alter the levels of nitrite when compared with controls (Table 1). These data indicate that endogenous IL-10 has a strong suppressive activity at 14 days after infection but that this activity is probably independent of RNI.

Effects of exogenous IFN- γ on proliferative responses and nitrite production by spleen cells. Since the data presented above indicate that endogenous IFN- γ is responsible in part for the suppressed proliferative responses of spleen cells from infected mice, a series of experiments were performed to de-

termine whether exogenous IFN- γ affects proliferative responses or production of nitrite by spleen cells from infected or uninfected mice. Addition of exogenous IFN- γ (from 1 to 1,000 IFN- γ U/ml) did not alter the proliferation or nitrite production of either unstimulated or ConA- or TLA-stimulated spleen cells from uninfected or infected mice (data not shown).

Effects of exogenous IL-10 on proliferative responses and nitrite production by spleen cells. Addition of increasing concentrations of IL-10 (0.2 to 200 U/ml) did not affect the proliferative response of spleen cells from uninfected mice to ConA or TLA (data not shown). In contrast, addition of IL-10 (200 U/ml) significantly decreased the proliferative response to TLA ($P = 0.003$) but not to ConA ($P > 0.05$) of spleen cells from infected mice (Fig. 3). When the effect of IL-10 on production of RNI was examined, this cytokine did not alter production of nitrite by ConA-stimulated splenocytes from uninfected or infected mice. However, addition of IL-10 induced a significant decrease, in a dose-dependent manner, of nitrite production by TLA-stimulated splenocytes from infected mice (Fig. 4). These data indicate that the ability of exogenous IL-10 to reduce proliferative responses to TLA was not associated with increased RNI production.

Effect of IL-10 on proliferative responses in the absence of suppression due to RNI. To study the effect of exogenous IL-10 on splenocyte proliferation in the absence of the RNI-mediated suppression, we investigated the effect of IL-10 on proliferation of splenocytes in the presence of either anti-IFN- γ or NMMA. Addition of IL-10 (200 U/ml) plus anti-IFN- γ to splenocytes from uninfected mice significantly ($P < 0.0001$) reduced the proliferative response to ConA (39.5% decrease) (data not shown). Such inhibition was not observed in the presence of NMMA. Addition of IL-10 plus anti-IFN- γ to splenocytes from infected mice antagonized the proliferative response to ConA ($P < 0.0001$) (Fig. 3A) and TLA (Fig. 3B). Furthermore, IL-10 significantly antagonized the increased proliferation observed in the presence of NMMA in response to ConA ($P = 0.03$) but not to TLA ($P > 0.05$) (Fig. 3). These data indicate that in the absence of the RNI-mediated suppression, exogenous IL-10 can antagonize proliferation of splenocytes from infected mice.

Effects of in vivo administration of anti-IFN- γ MAb or anti-IL-10 MAb on proliferative responses and nitrite production by spleen cells. The in vitro data presented above indicate that both IFN- γ and IL-10 play important roles in mediating the immunosuppression observed during the acute stage of infection with *T. gondii*. To determine whether these data can be extrapolated to the infection in vivo, mice were treated with neutralizing antibodies to IFN- γ or IL-10 and the effects of these treatments on ex vivo spleen cell proliferation and nitrite production were assessed. Administration of anti-IFN- γ MAb to infected mice resulted in an increased ex vivo spleen cell

TABLE 1. Effects of anti-IFN- γ and anti-IL-10 MAb in ConA-stimulated proliferation of spleen cells from infected mice at days 7 and 14 postinfection

Day post-infection	Spleen cells from infected mice stimulated with ConA ^a					
	[³ H]TdR uptake (cpm) for:			Nitrite production (pmol/ml) for:		
	Isotype control	Anti-IFN- γ	Anti-IL-10	Isotype control	Anti-IFN- γ	Anti-IL-10
7	2,538 $\pm$ 2,241	182,830 $\pm$ 10,464	2,794 $\pm$ 943	10,329 $\pm$ 512	5,778 $\pm$ 491	10,448 $\pm$ 587
14	54,489 $\pm$ 13,638	209,177 $\pm$ 7,416	216,808 $\pm$ 10,708	9,735 $\pm$ 387	3,936 $\pm$ 245	8,694 $\pm$ 299

^a Spleen cells were cultivated for 72 h with 1 μ g of ConA per ml. [³H]TdR uptake was measured in the last 18 h and was expressed in counts per minute. Similar results were observed with TLA-stimulated spleen cells. Antibodies were used at a concentration of 0.5 mg/ml.

FIG. 3. Proliferative responses of splenocytes from mice infected for 7 days and stimulated with ConA (A) or TLA (B) in the presence of NMMA or anti-IFN- γ MAb after addition of exogenous IL-10 (see Materials and Methods). The uptake by cells cultured in medium alone was 2,512 cpm. Similar results were observed in a second experiment. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Symbols: $\square$, ConA or TLA alone; $\square$, plus NMMA; $\blacksquare$, plus anti-IFN- γ MAb.

proliferation in response to ConA or TLA (mean increase of three experiments, 41- and 12-fold for ConA and TLA, respectively) when compared with results in mice treated with a control isotype MAb (Fig. 5A). The increased proliferative responses were accompanied by a significant decrease in nitrite concentrations in the culture supernatants of splenocytes stimulated with TLA (Fig. 5B). A similar reduction in the level of nitrite was noted in the cultures of unstimulated splenocytes and in those stimulated with ConA (Fig. 5B). The data presented in Fig. 5B approached but did not reach statistical significance ($P = 0.07$). In two additional experiments, in vivo administration of anti-IFN- γ did result in a significant decrease in ex vivo production of nitrite in cultures of unstimulated splenocytes ($P < 0.05$) or splenocytes stimulated with ConA ($P < 0.001$) (data not shown).

Administration of anti-IL-10 MAb to infected mice significantly increased their ex vivo spleen cell proliferation in response to ConA ($P = 0.0001$; mean increase of three experiments, 5.2-fold) but not TLA when compared with controls treated with the control MAb (Fig. 5A). Levels of nitrite produced when spleen cells were stimulated with either ConA or TLA were not altered (Fig. 5B).

FIG. 4. Production of nitrite by splenocytes from mice infected for 7 days and stimulated with TLA after addition of exogenous IL-10. Similar results were observed in a second experiment. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

DISCUSSION

Our previous work had shown that suppression of spleen cell responsiveness to ConA and TLA observed in *T. gondii*-infected mice was due in part to production of RNI by macrophages (5). In the present study, we demonstrated that the addition of NMMA to splenocytes from infected mice to restore proliferative responses did not affect the levels of IFN- γ produced but did result in increased levels of IL-10 and decreased production of IL-2 and IL-4. It is unclear whether the alteration in production of these cytokines was due directly to

FIG. 5. Effect of in vivo administration of an isotype control (bars A), anti-IFN- γ (bars B), or anti-IL-10 (bars C) MAb (see Materials and Methods) on the proliferative response (A) to ConA and TLA and production of nitrite (B) by splenocytes from mice infected for 10 days. The uptake by cells cultured in medium alone was 3,032 cpm. ***, $P < 0.001$. Similar results were observed in two further experiments.

inhibition of RNI production or was secondary to the effects of lack of RNI on T-cell function. Another explanation may be that the reduced levels of IL-2 and IL-4 observed in the presence of NMMA is a result of increased consumption of these cytokines as a consequence of the augmented proliferation.

Stimulation of splenocytes from infected mice with ConA or TLA resulted in production of high levels of IL-10 and low but appreciable levels of IL-4, two cytokines associated with a Th2-type response. These data suggest the presence of a Th2-type response during acute toxoplasmosis. This contrasts with data demonstrating a strong Th1-type response in mice immunized with *T. gondii* or chronically infected mice (10, 12). The decreased production of IL-2 that we observed in the supernatants of spleen cell cultures from mice infected with *T. gondii* confirms previous work (6, 7) and is similar to results obtained from mice acutely infected with *Trypanosoma cruzi* (16, 21) and *Trypanosoma brucei* (29, 30). It is noteworthy that inhibition of the RNI-mediated suppression did not result in emergence of a predominant Th1- or Th2-type response as assessed by production of IL-2 or IL-4.

Our results reveal that treatment with anti-IFN- γ in vitro or in vivo causes decreased nitrite production by spleen cells from infected mice and partial restoration of their proliferative responses. Thus, it seems likely that whereas RNI produced by macrophages has cytostatic activity against *T. gondii* (2), IFN- γ -induced RNI production also mediates in large part the immunosuppression observed during acute *T. gondii* infection. Our data also suggest that the activation state of the macrophages present in the spleen may be important in this suppression. The production of high levels of IFN- γ by splenocytes from uninfected mice stimulated with ConA or the addition of exogenous IFN- γ did not result in a significant increase in nitrite production or suppression of the proliferative responses. Although IFN- γ is an important stimulus for production of RNI by macrophages, other cytokines, such as tumor necrosis factor alpha (22), are also required. Thus, it seems likely that the splenic macrophages from infected mice are already primed to produce RNI in response to IFN- γ . This might explain the inability of high levels of exogenous or endogenous IFN- γ to inhibit proliferation of splenocytes from uninfected mice.

It has recently been reported that neutralization of endogenous IFN- γ reverses the suppression of T-cell responsiveness to ConA or specific antigen observed in *Mycobacterium leprae*-infected (25) or *T. brucei*-infected (9) mice as well as in ConA-stimulated cocultures of macrophages and splenocytes (3). Thus, the inhibitory activity of IFN- γ that we observed appears to be a common regulatory mechanism observed during immune hyperactivity and may regulate the immune response in vivo. Of interest, mice without the IFN- γ gene exhibit uncontrolled splenocyte proliferation in response to ConA. This unrestricted response is restored to more normal levels by addition of IFN- γ (8). These data illustrate the importance of IFN- γ in the regulation of T-cell proliferative responses. Alternatively, the stimulation of high levels of IFN- γ may represent a strategy of infectious agents to suppress the specific immune response.

In our studies, increased levels of IL-10, a cytokine known to inhibit proliferation of T cells of mice and humans (31), were detected in the spleen cell supernatants of acutely infected mice. When we examined the role of this cytokine in the immunosuppression observed during acute toxoplasmosis, we observed that exogenous IL-10 inhibited the ability of anti-IFN- γ or NMMA to restore proliferative responses. These data indicate that in the absence of a strong RNI-mediated suppression of proliferative responses, the suppressive proper-

ties of IL-10 become apparent. Although we detected high levels of IL-10 in spleen cell culture supernatants of mice infected 7 days earlier, the addition of anti-IL-10 at this time point did not reverse the suppression we observed. However, addition of anti-IL-10 to spleen cell cultures of mice infected 14 days earlier partially reversed the suppression. Our data, which demonstrate that IL-10 inhibited T-cell proliferation of splenocytes from infected mice in the absence of the RNI-mediated suppression, may be related to this observation. We previously reported that by 14 days after infection with *T. gondii*, the RNI-mediated suppression of T-cell responses is significantly lower than that observed at day 7 of infection (5). Thus, the suppressive effects of IL-10 may be more apparent on day 14 than on day 7 of infection. In contrast to our data, Haque et al. (15) have recently reported that addition of anti-IL-10 to splenocytes from mice infected with a virulent strain of *T. gondii* for 7 days partially restored proliferative responses.

Interestingly, anti-IL-10 in vitro or in vivo did not alter the levels of RNI detected in culture supernatants. These data indicate that the IL-10-mediated suppression that we observed does not appear to act through RNI. However, addition of IL-10 reduced RNI production by spleen cells of infected mice when they were cultured in the presence of TLA plus NMMA. This confirms a previous observation by Gazzinelli et al. (11) that IL-10 inhibited production of RNI by macrophages activated with IFN- γ . Of interest is that we did not observe such down-regulation when ConA was used. When anti-IL-10 MAB were used in vivo, an increased proliferation was observed in response to ConA but not TLA by spleen cells from infected mice. This recovery of the proliferative response was far less pronounced than the remarkable recovery observed when anti-IFN- γ MAB were used. These data from ex vivo experiments are in agreement with our results obtained when these MAB were added in vitro, suggesting that IFN- γ plays a more significant role than IL-10 in immunosuppression of proliferative responses at day 7 of infection with an avirulent strain of *T. gondii*.

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