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Effects of cytokines and prolactin on the replication of Toxoplasma gondii in murine microglia

Abstract

In the central nervous system, cytokine-activated microglia play a crucial role in host defence against Toxoplasma gondii infections. In this study, the effect of recombinant tumor necrosis factor (rTNF)-a and prolactin (PRL) on T. gondii infection in microglia was examined. Pretreatment of microglia with rTNF-a and PRL induced toxoplasmastatic activity, the intracellular killing of T. gondii and the release of interleukin (IL)-1b IL-3 and IL-6: 50% of the intracellular killing was abrogated by anti-ICAM-1 monoclonal antibodies, whereas more than 54 or 87%

of toxoplasmastatic activity was reversed by anti-IL-3 or IL-6 monoclonal antibodies. In addition, the treatment of microglia with either rIL-3 or rIL-6, in the absence or presence of rTNF-a significantly limited T. gondii replication. Inasmuch as either NMA or S-M-ITU affected cytokine-activated toxoplasmastatic activity during the infection phase, the NO-dependent pathway itself appears not to be directly involved in the parasitostatic activity. These findings suggest that TNF-a and PRL up-regulate the expression of ICAM-1 and the production of endogenous IL-6 and IL-3 by microglia, which could induce anti-parasitic functions against T. gondii infection in the brain.

Full text

INTRODUCTION

Toxoplasma gondii is an opportunistic, obligate intracellular protozoan found in the central nervous system (CNS) of the infected fetus [1] and of certain groups of immunocompromised patients, especially those with acquired immunodeficiency syndrome (AIDS) [2]. The role of the somatic immune system in host defence against *Toxoplasma gondii* is well known. Human mononuclear phagocytes activated by interferon-gamma (IFN-gamma) [3], and murine macrophages activated by IFN-gamma and lipopolysaccharide (LPS) or tumor necrosis factor-alpha (TNF-alpha) [4, 5] or other stimuli [6] appear to be key cell types that prevent intracellular replication of this parasite. The cell types and mediators involved in host defence within the CNS, however, have not been clearly defined. Recent *in vitro* studies, to elucidate the role of glial cells, such as microglial cells and astrocytes in host defense against *T. gondii* have been carried out using enriched murine microglial or astrocyte cultures activated with cytokines [7-9]. Microglia, the resident macrophages of the CNS [10], are generally considered to have a mesodermal origin and to be derived from the macrophage precursors originating in the bone marrow, that infiltrate the brain during fetal development to establish a resident population [11]. Resting microglia form a neuron-glia network with potential macrophage-like functions [12], including expression of major histocompatibility complex class II ag (MHC class II ag) [13], intercellular adhesion molecule 1 (ICAM-1) [14], phagocytosis of microorganisms [7, 8, 12, 15], production of cytokines upon stimulation [15-22] and free radicals (*i.e.* nitric oxide (NO) and reactive oxygen intermediates (RO)) [8, 18, 19]. On the other hand, microglia represent a sensor for pathological events in the CNS [23]. During the development of inflammatory processes,

microglia migrate, differentiate, proliferate and display ameboid shape, and phagocytose degenerating cells at sites of inflammation. This type of cell, also named activated microglia, plays an important role in immune host defence [7, 8, 15] and apoptotic neuronal death [24] in the CNS, although these cells may also contribute to neuronal injury [18, 19]. Previous studies have reported that murine microglia are suitable host cells for *T. gondii* but, when stimulated by IFN-gamma and LPS are able to inhibit parasite multiplication in an NO-dependent pathway [8]. Conversely, microglia derived from human fetuses activated by TNF-alpha or interleukin-6 (IL-6) in the presence or absence of IFN-gamma appear to inhibit parasite multiplication through an NO-independent mechanism [15]. It is well known that the immune system influences the neuroendocrine system and vice versa, *via* a bioregulatory feedback loop that permits reciprocal signalling. This signal is carried by cytokines produced in the immune system, which exert bioregulatory actions on the neuroendocrine tissues, and by the hormones produced in the neuroendocrine and peripheral endocrine organs, which biomodulate the activity of immunoeffector cells [25]. The major hormones involved in this loop are prolactin (PRL) and growth hormone [26]. In cultured astrocytes, PRL induces the expression of TNF-alpha, IL-1alpha and transforming growth factor-alpha (TGF-alpha) cytokines, which could synergize with PRL in promoting astrocyte proliferation and secretory activity [27]. In addition, *in vitro* PRL induces the production of NO and IL-1, both inflammatory mediators, in murine peritoneal macrophages [28] and enhances additively with IFN-gamma, both the NO_2^- release and inducible nitric oxide synthase (iNOS) expression in rat C6 glioma cells [29]. Recently, the *in vivo* immunoprotective effect of exogenous PRL in restoring the lymphocyte functions and macrophage tumoricidal activity in hypoprolactinemic mice, as well as the immunoregulatory role of PRL and its receptor in the induction of bactericidal activity and protection against *Salmonella typhimurium* infection has been reported [30, 31]. In our previous work carried out on murine toxoplasmosis, we showed that administration of PRL alone or in combination with rIFN-gamma or rTNF-alpha increases survival or delayed time to death of mice challenged with a lethal dose of *T. gondii* [32]. The immunoprotective effect of PRL plus TNF-alpha appeared to be correlated with a decreased number of bradyzoite cysts in the brain of surviving mice [32]. Since the mechanism whereby TNF-alpha and PRL or other cytokines participate in defence against *T. gondii* cerebral infection is unknown, the aim of this study was to increase the understanding of the effect of TNF-alpha or other cytokines on *T. gondii* replication in murine microglia, and to clarify the role of PRL in host defence against *T. gondii* in the brain.

MATERIALS AND METHODS

Reagents. Recombinant (r) mouse tumor necrosis factor-alpha (rTNF-alpha), rIL-1beta, rIL-3, rIL-6 and rIFN-gamma were purchased from Genzyme (MA, USA). Monoclonal antibodies (mAbs), hamster anti-mouse IL-1beta, rat anti-mouse IL-3 and IL-6 and control antibodies from non-immunised animals were purchased from Genzyme. Murine mAbs specific for intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were purchased from Bender Med System (Vienna, Austria). Monoclonal antibodies to neural cell adhesion molecules (N-CAM) were purchased from Biochemicals (Milan, Italy). Ovine prolactin was purchased from Sigma (Italy).

The presence of endotoxin in reagents was checked by the *Limulus amebocyte* lysate test [33] and all results were negative (*i.e.* < 0.05 EU of LPS/ml of sample).

Microglial cell cultures (MGC). Cell cultures were prepared as previously described by Chao *et al.* [7]. Briefly, cerebral cortical cells from 1-day-old BALB/c mice were dissociated after a 30 min trypsinization (0.25%), and plated in 75 cm^3 Falcon culture flasks (Nunclon, Denmark) in RPMI 1640 medium (Seromed, Italy), containing 20% heat-inactivated fetal calf serum (FCS; Seromed), 2 mM L-glutamine (Seromed), 20 mM HEPES (Seromed), 100 U/ml penicillin (Sigma), 100 mg/ml streptomycin (Sigma). The medium was replenished 1 and 4 days after plating. On day 8 of culture, plates were shaken for 20 min, at a speed of 200 rpm in an orbital shaker to remove oligodendrocytes. On day 12 of culture, plates were shaken again for 2 hours at a speed of 180 to 200 rpm. Harvested cells were filtered through a 20 mum nylon mesh, plated onto a 60 mm Petri dish, and incubated for 15 min at 37° C. After extensive washing with culture medium, adherent cells (microglia) were collected with a rubber policeman and centrifuged at 200 *g* for 10 min. Isolated microglia were plated onto glass cover slips previously coated with poly-L-lysine (Sigma) and placed in wells of 24-well plates. Purified microglia were composed of a cell population, > 98% of which stained with MAC-1 antibodies (Boerhringer-Mannheim, Biochemical, Milan, Italy) and < 2% stained with antibodies specific to glial fibrillary acid protein (Sigma), an astrocyte marker.

Preparation of T. gondii. The tachyzoite suspension of the highly virulent RH strain of *T. gondii* maintained by twice-a-week intraperitoneal inoculation of parasites into female Swiss mice (Charles River, Italy) was harvested from the

peritoneal fluids, as previously described [34]. For the *in vitro* infection of MGC, tachyzoites were added to the MGC cultures within 3 hours.

Effects of rTNF-alpha or PRL in MGC infected with T. gondii. To determine the effects of rTNF-alpha or PRL on *T. gondii replication*, in preliminary experiments MGC at different concentrations, were incubated with medium alone (control) or containing different concentrations of rTNF-alpha, IFN-gamma or PRL alone or together at various times. Inasmuch as maximal antiparasitic activity was observed with rTNF-alpha and PRL and which was also in agreement with our previous studies [32], we chose these agents for our subsequent experiments. The concentrations and optimal times turned out to be about 100-1,000 U/ml and 24 hours for rTNF-alpha, and 20-100 ng/ml and 24-48 hours for PRL. Therefore, to evaluate the dose-effect relationship of rTNF-alpha or PRL on *T. gondii* multiplication, after the addition of 100 U/ml superoxide dismutase (SOD; 0_2-scavenger, Sigma), MGC cultures (5×10^4 cells/well) were primed with or without various concentrations of rTNF-alpha in the presence of 20-100 ng/ml PRL or with or without different concentrations of PRL in the presence of 100-1,000 U/ml rTNF-alpha. After incubation for 24 hours at 37° C in 5% CO_2, the cells were extensively washed with prewarmed phosphate-buffered saline (PBS) and reincubated for an additional 1 hour under normal culture conditions in a medium containing living *T. gondii* tachyzoites (parasite/host cell ratio 2:1), followed by three washes with warm PBS to remove non-ingested parasites. The cells of one cover slip were fixed in methanol and marked with Diff Quik (Baxter Scientific Products, McGaw Park, IL, USA) stain, for determination of the number of ingested tachyzoites and the percentage of infected MGC at the start (time 0) of the assay. Other replicates were incubated in the presence of fresh medium with or without cytokine or hormone plus SOD for another 20 hours (time 20) under normal culture conditions, then fixed and stained as above. The course of intracellular infection was assessed microscopically by enumerating the percentage of infected cells and the number of toxoplasmas/100 cells in Diff Quik stained preparations. If the percentage of infected cells at 20 hours of the assay had increased by more than 7% relative to the percentage of infected cells at the start of the assay, the results were discarded. Intracellular multiplication, an index of toxoplasmastatic activity, was expressed as fold increase (FI), *i.e.*, the ratio of the mean number of parasites per 100 MGC after 20 hours (T20) of infection and the mean number of parasites per 100 MGC at 1 hour (T0) of the assay. Intracellular killing of *T. gondii* was defined as the decrease in the

percentage of infected cells after T20 of incubation, relative to the percentage of infected MGC at T0 of the assay.

Effect of antibodies against ICAM-1, VCAM-1 or N-CAM on intracellular killing of T. gondii by activated MGC. To investigate the involvement of specific cell adhesion molecules (CAMs) in the intracellular killing of *T. gondii*, MGC were incubated for 24 hours with medium alone or containing different concentrations of rTNF-alpha or PRL or rTNF-alpha plus PRL in the absence or presence of mAbs for human ICAM-1, VCAM-1, N-CAM. Preliminary experiments were carried out to determine the concentration giving the greater inhibitory effect. The optimal concentration of mAbs, which turned out to be 5-10 mug/ml, was then used in our experiments. Monoclonal antibodies (5 mug/ml) were added to MGC in the first 1 hour of the activation phase with cytokine or hormone alone, or in combination. The cells were washed and then infected with tachyzoites for 1 hour and 20 hours under normal culture conditions, as above. Anti-toxoplasma function was analysed by the intracellular killing of tachyzoites.

Effect of antibodies against IL-1beta, IL-3 or IL-6 on toxoplasmastatic activity in MGC. To investigate the implication of specific cytokines in the induction of parasitostatic activity, MGC were treated with medium alone or containing rTNF-alpha (100 U/ml) plus PRL (20 ng/ml) in the presence of mAbs (5 mug/ml) specific for IL-1beta, IL-3 or IL-6. The concentration of antibodies used for this experiment was based on our preliminary studies, which had shown that this antibody concentration completely abolished the bioactivity of 5 ng/ml of each cytokine. Antibodies were added to microglial cell cultures between 6 hours and 20 hours after rTNF-alpha and PRL. Toxoplasmastatic activity in MGC was then evaluated at T20 of infection by microscopic examination.

Determination of cytokine production by and effect of rIL-3 or rIL-6 on T. gondii multiplication in MGC. To determine whether the toxoplasmastatic activity of activated rTNF-alpha + PRL microglia was associated with release of endogenous cytokines, supernatant media were removed from cell cultures (5 x 10^4 cells/well) and analysed for IL-beta, IL-3 or IL-6 production, at various times during the 24 hours activation phase. All measurements were carried out using immunoenzymatic methods. IL-beta cytokine was determined by monoclonal hamster anti-mouse IL-1beta (enzyme-linked immunosorbent assay [ELISA test]; Genzyme). Whereas both IL-3 and IL-6 were measured by monoclonal rat anti-mouse IL-3 and IL-6 (ELISA test; Genzyme).

To assess whether specific cytokines could replace PRL as a triggering signal in the induction of anti-toxoplasma functions, MGC were incubated with or without various concentrations of rIL-3 or rIL-6, in the absence or presence of a fixed dose of rTNF-alpha (100 U/ml) for 24 hours, before challenge with tachyzoites, and then incubated for 1 hour and 20 hours at normal culture conditions. Intracellular multiplication in MGC was evaluated as above.

Measurement of NO_2^- production by activated MGC and correlation with toxoplasmastatic activity. The presence of nitrite (NO_2^-) was determined in culture supernatants of MGC incubated for 24 hours without medium (negative control [NC]) or with rTNF-alpha (100 U/ml) plus PRL (20 ng/ml), before challenge with *T. gondii,* and in culture supernatants collected during the 20 hours of infection by Griess reaction, as previously described [35]. As positive control (PC) of NO_2^- release, MGC were also incubated as in our previous study [35] with IFN-gamma (100 U/ml) plus LPS (1 mug/ml; Sigma).

To analyse the involvement of NO_2^- in the induction of toxoplasmastatic activity, 10-100 muM of S-methylisothiourea (S-M-ITU: an inhibitor of the enzyme iNOS; Sigma) or 100-800 muM of NG-monomethyl-L-arginine (NMA: a competitive inhibitor of L-arginine; Amersham) were added, immediately, during the 24 hours incubation period of the MGC with rTNF-alpha plus PRL, before infection (activation phase), and during the 20 hours challenge period with *T. gondii* (infection phase).

Statistical analysis. Each set of results presented is the mean ± standard deviation (SD) of triplicate independent measurements of four separate, repeated experiments. Differences were considered to be significant if $p < 0.05$, determined by the Student' s *t*-test.

RESULTS

*Effect of rTNF-*alpha *and PRL on the intracellular replication of T. gondii in MGC*. In preliminary experiments, our results showed that MGC treated with 50 U/ml rTNF-alpha or 10 ng/ml PRL alone inhibited the intracellular replication of *T. gondii* slightly but not significantly (FI = 6.1 ± 1.0 or 6.4 ± 0.6 respectively *versus* 7.4 ± 1.0 in control cultures, p = ns), whereas MGC primed with either 100 U/ml rTNF-alpha or 20 ng/ml PRL alone exhibited a low but significant toxoplasmastatic activity (FI = 4.3 ± 0.7 or 4.5 ± 0.6 respectively *versus* 7.4 ± 1.0 in control MGC, p <

0.05). The presence of polymyxin B added to bind free endotoxin did not increase toxoplasmastatic activity; in fact, the values obtained from MGC treated with either 100 U/ml rTNF-alpha or 20 ng/ml PRL plus 30 mug/ml of polymyxin B were (FI = 3.8 ± 0.8 or 4.3 ± 0.5 respectively) similar to those in MGC treated with 100 U/ml rTNF-alpha or 20 ng/ml PRL alone; further, MGC activated with 1,000 U/ml rTNF-alpha or 100 ng/ml PRL alone did not lead to greater inhibition of *T. gondii* replication (data not shown). These data indicate that the subsequent lack of dose-response for higher concentrations is probably linked to the blocking of either TNF-alpha or PRL receptor activation.

As a result of this, we determined the dose-effect relationship of rTNF-alpha or PRL on toxoplasmastatic activity, therefore, MGC were primed with various concentrations of rTNF-alpha in the presence of 20 ng/ml PRL or with various concentrations of PRL in the presence of 100 U/ml rTNF-alpha. In the presence of 100 U/ml rTNF-alpha, 20 ng/ml PRL markedly inhibited ($p < 0.01$ *versus* control) intracellular *T. gondii* replication. The antiparasitic effect of rTNF-alpha was additive in the presence of a fixed concentration of PRL and vice versa (Figure 1). In addition, MGC activated with 100-500 U/ml rIFN-gamma plus 20 ng/ml PRL did not induce greater antiparasitic activity than rTNF-alpha plus PRL (data not shown).

*Effect of rTNF-*alpha*, PRL and rTNF-*gamma*/PRL, in the absence or presence of antibodies against ICAM-1, VCAM-1 or N-CAM, on intracellular killing of T. gondii by MGC*. Intracellular killing of *T. gondii* was determined by microscopic examination of activated-MGC at T0 and T20 of infection. Our findings (Figure 2) showed that MGC primed with either 100 U/ml rTNF-alpha or 20 ng/ml PRL alone induced only slight intracellular killing. This was proven by a decrease of the percentages of *T. gondii*-infected cells at T20 of infection period, relative to the values at T0 of the assay ($p < 0.05$ each *versus* control). Moreover, neither rTNF-alpha nor PRL alone influenced the percentage of infected cells at T0 of the assay; the values obtained were included between 54.5 ± 6.8 and 60 ± 5.7% (p = ns each *versus* control), while the values obtained at T20 of infection corresponded well to indices of toxoplasmastatic activity presented in Figure 1. However, neither concentrations of 1,000 U/ml rTNF-alpha nor 100 ng/ml PRL alone affected the percentage of infected cells at T0 and did not lead to greater intracellular killing (Figure 2). Both a priming synergistic effect (Figure 2), with a decrease of the percentage of infected-cells already at T0 ($p < 0.05$ *versus* control), and an additive effect at T20 ($p < 0.01$ *versus* control) of infection were

observed when MGC were primed with rTNF-alpha (100 U/ml) and PRL (20 ng/ml). The anti-parasitic effect afforded by rTNF-alpha and PRL appeared in a dose-dependent fashion. These results suggested that activation of MGC with TNF-alpha plus PRL leads to a decrease in the percentage of *T. gondii*-infected cells after 20 hours of infection by reducing the entry of parasites into cells at T0. In a separate series, experiments were carried out to detect if this anti-parasitic activity was linked to specific CAMs. Our findings (Figure 2) showed that MGC activated with rTNF-alpha (100 U/ml) and PRL (20 ng/ml), in the presence of 5 mug/ml mAbs specific for ICAM-1 (but not for VCAM-1 or N-CAM) abrogated by 50% the induction of intracellular killing, leading to an increase of *T. gondii*-infected cells at T0 and T20 ($p = ns$ or $p < 0.05$ and $p < 0.05$ *versus* control or rTNF-alpha + PRL-treated cells, respectively). These findings suggested that ICAM-1 expressed on the microglia-cell surface could be implicated in the cell host-parasite interaction, probably by controlling the entry of the parasite into microglial cells.

Effect of neutralizing antibodies against IL-1beta, IL-3 or IL-6 on toxoplasmastatic activity. The implication of specific cytokines in the induction of toxoplasmastatic activity was evaluated using mAbs specific for IL-1beta, IL-3 or IL-6. Incubation of MGC (Figure 3) with rTNF-alpha (100 U/ml) and PRL (20 ng/ml) in the presence of 5 mug/ml mAbs specific for IL-3 or IL-6 (but not for IL-1beta) blocked more than 54 or 87%, the induction of toxoplasmastatic activity ($p < 0.05$ or $p = ns$ and $p < 0.01$ each *versus* control or rTNF-alpha + PRL-treated cells, respectively), and further reduced the intracellular killing of tachyzoites, although did not affect the percentage of infected cells at T0 of infection (data not shown). Both anti-IL-3 and IL-6 mAbs had maximal inhibitory effect when added at 12 hours after cytokine and hormone (Figure 3), which corresponded with the periods during which the activated microglia produced significant levels of both IL-3 and IL-6 (Figure 4). Moreover, anti-IL-6, in combination with anti-IL-3 mAbs, did not show an additive effect. The same blockage was seen in activated-MGC in the presence of anti-IL-6 mAbs alone or in the combination with anti-IL-3 (data not shown).

Determination of cytokine production by, and effect of IL-6 or IL-3 on T. gondii multiplication in MGC. These results suggested that both endogenous IL-3 and IL-6 cytokines released during the activation phase, before infection with *T. gondii*, participate in the subsequent inhibition of *T. gondii* multiplication. Therefore, we measured the levels of all three IL-1beta, IL-3 and IL-6 cytokines in

culture supernatants, taken every 3 hours during the activation phase of MGC with cytokine-hormone. Incubation of MGC (Figure 4) with rTNF-alpha (100 U/ml) and PRL (20 ng/ml) for 24 hours released significant amounts of the three cytokines. Both IL-3 and IL-6 cytokines showed similar kinetic patterns and peaked soon in the course of the activation phase with significant levels already at 12 hours of the activation period ($p < 0.01$ *versus* control). Maximal amounts for both cytokines were then observed at 24 hours of the activation phase ($p < 0.001$ *versus* control). IL-1beta was undetectable in the course of the incubation period although small but slightly significant levels were observed at 24 hours of incubation phase ($p < 0.05$ *versus* control).

To investigate whether IL-6 or IL-3 could replace PRL as a triggering signalling for toxoplasmastatic activity, microglia cultures were treated with each of the above-mentioned cytokines at various concentrations, in the absence or presence of 100 U/ml rTNF-alpha for 24 hours, before challenge with *T. gondii*. Figure 5 shows that MGC primed with 0.03 or 20 U/ml rIL-6 alone did not show an antiparasitic effect (p = ns and $p < 0.01$ *versus* untreated or rTNF-alpha + PRL-treated cells, respectively), whereas MGC activated with 100 or 500 U/ml rIL-6 inhibited dose-dependently ($p < 0.05$ *versus* untreated or rTNF-alpha + PRL-treated cells, respectively) *T. gondii* replication. An additive anti-parasitic effect appeared when 100 or 500 U/ml IL-6 were combined with 100 U/ml rTNF-alpha ($p < 0.01$ each *versus* control). Similarly to IL-6 no anti-toxoplasma effect was observed when MGC were treated with 0.03 or 20 U/ml rIL-3 (p = ns and $p < 0.01$ each *versus* control or rTNF-alpha + PRL-treated cells, respectively). Anti-parasitic but no additive effect (Figure 6) was then seen when microglial cells were exposed to 100 or 500 U/ml rIL-3 in the absence or presence of 100 U/ml rTNF-alpha ($p < 0.05$ *versus* control or rTNF-alpha + PRL cells, respectively). Analogously, anti-toxoplasma activity was also observed when microglia were primed with 100 U/ml of IL-3 or IL-6 in the presence of 20 ng/ml PRL (data not shown).

Production of NO_2^- by activated MGC and correlation with toxoplasmastatic activity. To determine if cytokine-hormone treatment induced the release of NO_2^- by MGC, both at 24 hours of the activation phase with rTNF-alpha (100 U/ml), and PRL (20 ng/ml), and at 20 hours of the infection phase, supernatant media were saved and analysed spectrophotometrically. The results of this analysis (Figure 7A and 7B) showed that in both phases, rTNF-alpha + PRL-

treatment induced NO_2^- release ($p < 0.01$ and $p < 0.05$ *versus* negative control, respectively) by microglial cells.

To investigate the involvement of NO_2^- in the induction of toxoplasmastatic activity, MGC were activated with rTNF-alpha and PRL in the absence or presence of various concentrations of NMA or S-M-ITU added during the 24 hours period of activation phase, before infection with *T. gondii,* and during the 20 hours period of infection phase. We found, in agreement with our previous studies (Figure 7A and 7C), that the presence of 300 muM NMA or 50 muM S-M-ITU during the activation phase of MGC, before infection, inhibited both the release of NO_2^- (p = ns or $p < 0.01$ *versus* negative or positive control, respectively) and toxoplasmastatic activity (p = ns or $p < 0.01$ *versus* NC or PC, respectively); while, the presence of NMA or S-M-ITU during the infection phase induced a slight inhibition ($p < 0.05$ *versus* NC or PC, respectively) of toxoplasmastatic activity, but completely abrogated the release of NO_2^- by these cells (p = ns or $p < 0.05$ *versus* NC or PC, respectively). These data suggest that NO itself influences the microbicidal functions only during the early stage of the *T. gondii* infection phase.

DISCUSSION

TNF-alpha [4-7, 9, 15, 32, 36-41], IL-6 [9, 15, 37, 38] and IL-3 [42-44] cytokines, as well as PRL hormone [31, 32] play an important role in immunity to parasites. The importance of cytokines and microglia in regulating protective immunity to *T. gondii* infection in the brain is well established, but the effect of PRL on *T. gondii* replication in microglia is not yet known. Based on our previous studies of murine toxoplasmosis [32], we anticipated that microglial cells, PRL and TNF-alpha or other cytokines would be involved in the parasiticidal cerebral activity. From the present study it can be concluded that both rTNF-alpha and PRL, alone or together, can directly prime murine microglia to induce anti-toxoplasmal functions in these cells. This conclusion is based on the following observations. Firstly, low but activating concentrations of either rTNF-alpha or PRL alone slightly induces both the toxoplasmastatic activity and the intracellular killing of *T. gondii* tachyzoites in microglia, whereas combinations of both agents prime microglia optimally, result-ing a complete inhibition of *T. gondii* multiplication and enhanced intracellular killing. Secondly, neutralization of ICAM-1 by specific monoclonal antibodies partially reduces the intracellular killing of *T. gondii*, whereas neutralization of either endogenous IL-6 or IL-3 by specific monoclonal

antibodies inhibits, totally or partially, the toxoplasmastatic activity. These findings demonstrate clearly that rTNF-alpha additively with PRL limits the outcome of *T. gondii* growth in microglia. The priming effect of rTNF-alpha or PRL on microglia was not due to contamination with LPS, because incubation of microglia with cytokine or hormone in the presence of polymixin B gave results similar to those observed in the absence of polymixin B. Although the manner in which rTNF-alpha and PRL induce anti-parasitic effects in microglia appears to be not yet understood, it is well known that the action of cytokines or hormones requires bindings of the cytokine or hormone to its specific cell surface receptors, which activate the cells. Thus, activated cells can display receptors for the cytokine or hormone that stimulate or regulate these cells. Because either rTNF-alpha or PRL alone slightly activate microglia, and both agents are required for an effective activation, it is conceivable that binding of either rTNF-alpha or PRL to its receptor alone is not sufficient to trigger the intracellular signal transduction pathways implicated in the induction of anti-toxoplasmal capacities. Therefore, a second stimulus (*e.g.*, low concentrations of either PRL or TNF-alpha) is needed for induction of intracellular signalling events that convert the initial interaction of the cytokine or hormone with its receptor into pathways leading to the biological functions. Because concentrations of 100 U/ml for TNF-alpha and 20 ng/ml PRL are required to prime the microglial cells in order to get a significant protective effect, and higher concentrations do not lead to greater protection, it is likely that the subsequent lack of dose-response for higher concentrations is linked to the blocking of either TNF-alpha or PRL receptor activation. It is well known the IFN-gamma appears to be the most active cytokine in controlling the replication of *T. gondii* [45], and that TNF-alpha is required to trigger anti-toxoplasmal activity of IFN-gamma-primed macrophages [4, 5]. Nevertheless, our data provide strong evidence that IFN-gamma is not the sole endogenous regulator of macrophage-like cell activation, because the cell microglial cultures were pure (> 98%) and the source of IFN-gamma appears to be confined to T and NK cells (but not to microglia). It is conceivable that PRL could have an activity similar to IFN-gamma and together with TNF-alpha operates as key signalling in the triggering of endogenous-IFN-gamma-mediated-like effects, probably by the induction of interferon-gamma receptor (IFN-gammaR) on microglia. This hypothesis is supported by recent reports that have shown IFN-gammaR signalling to be the major pathway for the activation of microglia in murine toxoplasmic encephalitis (TE) [46], and that PRL affects the expression of the IFN-gammaR gene leading to increased IFN-gammaR mRNA levels and IL-6 release [47]. Our findings also show that rTNF-alpha, in synergy with PRL, increases the intracellular killing by a decrease in the

number of infected microglial cells at T0. The blockade of about 50% of the intracellular killing by anti-ICAM-1 (but not anti-VCAM-1 or N-CAM) monoclonal antibodies added to rTNF-alpha + PRL-treated microglia, suggests that ICAM-1 molecules play a role in controlling the portal of entry of the parasite into the cell, probably by a competitive binding of *T. gondii* to adhesion receptor ICAM-1. Future studies should evaluate the role of ligands coating *T. gondii* tachyzoites and CAMs or chemokine receptors on microglia in the cell host-parasite interaction. The *in vitro* anti-toxoplasma effects of rTNF-alpha alone or together with PRL are in agreement with our previous *in vivo* studies and with other reports [32, 38], which have shown that systemic administration of PRL alone or in combination with rTNF-alpha increases survival or delay time to death of mice challenged with *T. gondii* [32], and that anti-TNF-alpha mAbs affect the severity or progression of the infection [38]. In particular, PRL in combination with rTNF-alpha is implicated in controlling the intracerebral spread of the parasite and the number of bradyzoite cysts in the brain of mice with toxoplasmosis [32]. Our findings also suggested that in the induction of anti-toxoplasma activity by rTNF-alpha and PRL, other cytokines produced endogenously, such as IL-3 and IL-6 (but not IL-1beta) could, through a complex network, participate in an autocrine or paracrine manner in this activity. This hypothesis was supported by the abolishment by more than 54 or 87% of the toxoplasmastatic activity by either anti-IL-3 or anti-IL-6 (but not anti-IL-1beta) monoclonal antibodies when added to rTNF-alpha + PRL-primed microglia. The results with anti-IL-6 antibodies are in agreement with *in vitro* findings for human microglia [15], but in contrast with *in vivo* studies, which have reported that anti-IL-6 antibodies reduce inflammation and the numbers of cysts in brains of mice with TE [48]. Consistent with our research, we found that murine microglia, primed with rTNF-alpha and PRL, released considerable levels of IL-6 and IL-3 (but only small amounts of IL-1beta) proinflammatory monokines. In fact, in the early stages of TE, the immune response to *T. gondii* is characterized by an up-regulation in IFN-gamma and IL-2 Th1 cytokine mRNAs accompanied by increases in TNF-alpha GM-CSF, IL-1, IL-6 and IL-10 monokine mRNAs, as well as MHC class II ag, iNOS and Mag-1 mRNA levels, all markers of macrophage activation [38]. Therefore high levels of IL-6 are detectable in cerebrospinal fluids and serum of infected *T. gondii* mice and correlate with systemic and intracerebral disease activity [37]. Conversely, in the later stages of TE, the immune response is dominated by a dramatic down-regulation of IFN-gamma, TNF-alpha, GM-CSF, IL-1, IL-6 and IL-10, Mag-1 and iNOS mRNA levels, which is associated with enhanced necrosis and neutrophilic infiltrates in the brain [38]. The up-regulation of TNF-alpha or other inflammatory mediators, such as IL-12 cytokines and chemokine macrophage-

inflammatory protein-1alpha (MIP-1alpha) and MIP-1beta [41] is a key event in the initiation of immunity to *T. gondii* [41]. Given that during inflammation within the CNS, microglia secrete a number of proinflammatory cytokines, such as TNF-alpha [15, 16, 20, 22], IL-1beta [16], IL-3 [17], IL-6 [15, 16, 20], IL-8 [21] and as well as anti-inflammatory cytokines, such as TGF-beta [22] and IL-10 [20] for defense against microorganisms and neuronal injury, there is emerging evidence that in cerebral toxoplasmosis, TNF-alpha and PRL, *via* a bio-feedback loop, up-regulate the release of proinflammatory (*e.g.*, IL-1beta, IL-3 and IL-6) and probably anti-inflammatory cytokines too, which may induce microbicial activity in microglia and limit inflammation-associated brain injury. Further support for the implication of both IL-6 and IL-3 in the anti-parasitic activity of murine microglia was provided by the findings that both rIL-6 and rIL-3 alone or in combination with rTNF-alpha dose-dependently reduced the intracellular growth of *T. gondii*. In human microglia and murine astrocytes, IL-6 causes a 50 to 75% inhibition of *T. gondii* growth [9, 15]. Similarly, IL-3 limits the spread of parasites, such as *Stronglyoides venezuelensis* and *Mycobacterium tuberculosis* [43, 44], and regulates IgE responses in *Trichinella spiralis* infection [42]. The mechanism whereby cytokine treatment induces anti-toxoplasma activity in microglia is not completely understood. In murine microglia and human astrocytes [8, 49], the most important effector mechanism directed against *T. gondii* is the production of NO *via* the induction of the enzyme NO synthase. Conversely, microglial cells derived from human fetus [15], and murine astrocytes derived from syngeneic mice deficient in the enzyme iNOS [9], appear to inhibit parasite replication through an NO-independent pathway. Although, our data show that the priming effect of rTNF-alpha and PRL on microglia lead to a significant release of NO_2^-, the cytokine-induced inhibition of *T. gondii* was found to be NO-dependent only during the early stage of *T. gondii* infection, as demonstrated by the ability of NMA or S-M-ITU to completely abrogate the toxoplasmastatic activity when added during the activation phase of the microglia, but not when added during the infection phase. It is possible that at a later stage of infection, NO affects the toxoplasmastatic activity *via* triggering of other anti-parasitic effector mechanisms (*e.g.*, IDO indolamine 2,3-dioxygenase-dependent pathway).

CONCLUSION

In conclusion, the results of this *in vitro* study may be more directly pertinent to understanding the immunopathogenesis of TE. The accomplishment of this

protective intracerebral mechanism should be very important for resistance to congenital or acquired toxoplasmosis, because the brain is a major site of *T. gondii* infection in the fetus and immunocompromised patients. Therefore, the ability of cytokines and PRL to prime microglia and limit growth of *T. gondii* may also be implicated in recrudescences of toxoplasmosis in immunocompromised subjects. In patients with hypoprolactinemia or AIDS, for example, a switch from Th1 to Th2 cytokines, which is accompanied by decreases in IFN-gamma , TNF-alpha , IL-6, could represent a dramatic event in the development of TE. Anyway, the relevance of these data to the situation *in vivo* remains to be elucidated. Future studies may be carried out with trials adding another batch of regulatory factors to infected microglial cells, aimed at blocking the intracerebral invasion of *T. gondii*, and thus contributing to the prevention of toxoplasmic encephalitis.

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References

1. Cook G C. 1990. *Toxoplasma gondii* infection: a potential danger to the unborn fetus and AIDS sufferer. (Review) *Q. J. Med.* 74: 3.

2. Luft B J, Remington J S. 1992. Toxoplasmic encephalitis in AIDS. (Review) *Clin. Infect. Dis.* 15: 211.

3. Murray H W, Teitelbaum R F. 1992. L-arginine-dependent reactive nitrogen intermediates and the antimicrobial effect of activated human mononuclear phagocytes. *J. Infect. Dis.* 165: 513.

4. Sibley L D, Adams L B, Fukutomi Y, Krahenbuhl J L. 1991. Tumor necrosis factor-alpha triggers anti-toxoplasmal activity of IFN-gamma primed macrophages. *J. Immunol.* 147: 2340.

5. Langermans J A, van der Hulst M E, Nibbering P H, Hiemstra P S, Fransen L, van Furth R. 1992. IFN-gamma-induced L-arginine-dependent toxoplasmastatic

activity in murine peritoneal macrophages is mediated by endogenous tumor necrosis factor-alpha. *J. Immunol*. 148: 568.

6. Peetermans W E, Raats C J, van Furth R, Langermans J A. 1995. Mycobacterial 65-kilodalton heat shock protein induces tumor necrosis factor-alpha and interleukin 6, reactive nitrogen intermediates, and toxoplasmastatic activity in murine peritoneal macrophages. *Infect. Immun*. 63: 3454.

7. Chao C C, Hu S, Gekker G, Novick W J, Remington J S, Peterson P K. 1993. Effects of cytokines on multiplication of *Toxoplasma gondii* in microglial cells. *J. Immunol*. 150: 3404.

8. Chao C C, Anderson W R, Hu S, Gekker G, Martella A, Peterson P K. 1993. Activated microglia inhibit multiplication of *Toxoplasma gondii via* a nitric oxide mechanism. *Clin. Immunol. Immunopathol.* 67: 178.

9. Halonen S K, Chiu F C, Weiss L M. 1998. Effect of citokines on growth of *Toxoplasma gondii* in murine astrocytes. *Infect. Immun.* 66: 4989.

10. Perri V H, Gordon S. 1989. Resident macrophages of the central nervous system: modulation of phenotype in relation to a specialized microenvironment. In: Goetzel E J, Spector N H, eds. *Neuroimmune networks: physiology and diseases*. New York: Alan R. Liss: 119.

11. Cuadros M A, Navascues J. 1998. The origin and differentiation of microglial cells during development. (Review) *Prog. Neurobiol*. 56: 173.

12. Nakajima K, Kohsaka S. 1993. Functional roles of microglia in the brain. (Review) *Neurosci. Res*. 17: 187.

13. Sedgwick J D, Schwender S, Gregersen R, Dörries R, ter Meulen V. 1993. Resident macrophages (ramified microglia) of the adult brown norway rat central nervous system are constitutively major histocompatibility complex class II positive. *J. Exp. Med*. 177: 1145.

14. Zielasek J, Archelos J J, Toyka K V, Hartung H P. 1993. Expression of intercellular adhesion molecule-1 on rat microglial cells. *Neurosci. Lett*. 153: 136.

15. Chao C C, Gekker G, Hu S, Peterson P K. 1994. Human microglial cell defense against *Toxoplasma gondii*. The role of cytokines. *J. Immunol.* 152: 1246.

16. Lee S C, Liu W, Dickson D W, Brosnan C F, Berman J W. 1993. Cytokine production by human fetal microglia and astrocytes. Differential induction by lipopolysaccharide and IL-1beta. *J. Immunol.* 150: 2659.

17. Gebicke-Haerter P J, Appel K, Taylor G D Schobert A, Rich I N, Northoff H, Berger M 1994. Rat microglial interleukin-3. *J. Neuroimmunol.* 50: 203.

18. Chao C C, Hu S, Peterson P K. 1996. Glia: the not so innocent bystanders. *J. Neurovirol.* 2: 234.

19. Hu S, Sheng W S, Peterson P K, Chao C C. 1995. Cytokine modulation of murine microglial cell superoxide production. *Glia.* 13: 45.

20. Sheng W S, Hu S, Kravitz F H, Peterson P K, Chao C C. 1995. Tumor necrosis factor alpha upregulates human microglial cell production of interleukin-10 *in vitro*. *Clin. Diagn. Lab. Immunol.* 2: 604.

21. Ehrlich L C, Hu S, Sheng W S, Sutton R L, Rockswold G L, Peterson P K, Chao C C. 1998. Cytokine regulation of human microglial cell IL-8 production. *J. Immunol.* 160: 1944.

22. Chao C C, Hu S, Sheng W S, Tsang M, Peterson P K. 1995. Tumor necrosis factor-alpha mediates the release of bioactive transforming growth factor-beta in murine microglial cell cultures. *Clin. Immunol. Immunopathol.* 77: 358.

23. Kreutzberg G W. 1996. Microglia: a sensor for pathological events in the CNS. (Review) *Trends Neurosci.* 19: 312.

24. Toku K, Tanaka J, Yano H, Desaki J, Zhang B, Yang L, Ishihara K, Sakanaka M, Maeda N. 1998. Microglial cells prevent nitric oxide-induced neuronal apoptosis *in vitro*. *J. Neurosci. Res.* 53: 415.

25. Blalock J E. 1994. The syntax of immune-neuroendocrine communication. (Review) *Immunol. Today* 15: 504.

26. Weigent D A. 1996. Immunoregulatory properties of growth hormone and prolactin. (Review) *Pharmacol. Ther.* 69: 237.

27. DeVito W J, Avakian C, Stone S, Okulicz W C, Tang K, Shamgochian M. 1995. Prolactin induced expression of interleukin-1alpha, tumour necrosis factor-alpha, and transforming growth factor-alpha in cultured astrocytes. *J. Cell Biochem.* 57: 290.

28. Kumar A, Singh S M, Sodhi A. 1997. Effect of prolactin on nitric oxide and interleukin-1 production of murine peritoneal macrophages: role of Ca^{2+} and protein kinase C. *Int. J. Immunopharmacol.* 19: 129.

29. Raso G M, Meli R, Gualillo O, Pacilio M, Di Carlo R. 1999. Prolactin induction of nitric oxide synthase in rat C6 glioma cells. *J. Neurochem*. 73: 2272.

30. Bernton E W, Meltzer M S, Holaday J W. 1988. Suppression of macrophage activation and T lymphocyte function in hypoprolactinemic mice. *Science* 239: 401.

31. Meli R, Raso G M, Bentivoglio C, Nuzzo I, Galdiero M, Di Carlo R. 1996. Recombinant human prolactin induces protection against *Salmonella typhimurium* infection in the mouse: role of nitric oxide. *Immunopharmacology* 34: 1.

32. Benedetto N, Folgore A, Galdiero M, Meli R, Di Carlo R. 1995. Effect of prolactin, rIFN-gamma or rTNF-alpha in murine toxoplasmosis. *Path. Biol.* (Paris) 43: 395.

33. Yin E T, Galanos C, Kinsky S, Bradshow R A, Wessler S, Luderitz O, Sarmiento M E. 1972. Picogram sensitive assay for endotoxin: gelation of *Limulus polyphemus* blood cell lysate induced by purified lipopolysaccharide and lipid A from Gram-negative bacteria. *Biochem. Biophys. Acta.* 261: 284.

34. Benedetto N, Auriault C, Darcy F, Lando D, Watier H, Capron A. 1991. Effect of rIFN-gamma and IL-2 treatments in mouse and nude rat infections with *Toxoplasma gondii*. *Eur. Cytokine Netw.* 2: 107.

35. Benedetto N, Folgore A, Ferrara C, Molitierno M, Galdiero F. 1997. Effects of alpha-adrenergic agonists on *Toxoplasma gondii* replication in human umbilical vein endothelial cells. *Path. Biol.* (Paris) 45: 9.

36. Freund Y R, Sgarlato G, Jacob C O, Suzuki Y, Remington J S. 1992. Polymorphisms in the tumor necrosis factor alpha (TNF-alpha) gene correlate with murine resistance to development of toxoplasmic encephalitis and with levels of TNF-alpha mRNA in infected brain tissue. *J. Exp. Med.* 175: 683.

37. Schluter D, Deckert-Schluter M, Schwendemann G, Brunner H, Hof H. 1993. Expression of major histocompatibility complex class II antigens and levels of interferon-gamma, tumour necrosis factor, and interleukin-6 in cerebrospinal fluid and serum in *Toxoplasma gondii*-infected SCID and immunocompetent C.B-17 mice. *Immunology* 78: 430.

38. Gazzinelli R T, Eltoum I, Wynn T A, Sher A. 1993. Acute cerebral toxoplasmosis is induced by *in vivo* neutralization of TNF-alpha and correlates with the down-regulated expression of inducible nitric oxide synthase and other markers of macrophage activation. *J. Immunol*. 151: 3672.

39. Däubener W, Remscheid C, Nockemann S, Pilz K, Seghrouchni S, Mackenzie C, Hadding U. 1996. Anti-parasitic effector mechanisms in human brain tumor cells: role of interferon-gamma and tumor necrosis factor-alpha. *Eur. J. Immunol.* 26: 487.

40. Fischer H G, Nitzgen B, Reichmann G, Hadding U. 1997. Cytokine responses induced by *Toxoplasma gondii* in astrocytes and microglial cells. *Eur. J. Immunol*. 27: 1539.

41. Bliss S K, Marshall A J, Zhang Y, Denkers E Y. 1999. Human polymorphonuclear leukocytes produce IL-12, TNF-alpha, and the chemokines macrophage-inflammatory protein-1alpha and -1beta in response to *Toxoplasma gondii* antigens. *J. Immunol*. 162: 7369.

42. Korenaga M, Watanabe N, Abe T, Hashiguchi Y. 1996. Acceleration of IgE responses by treatment with recombinant interleukin-3 prior to infection with *Trichinella spiralis* in mice. *Immunology* 87: 642.

43. Lantz C S, Boesiger J, Song C H, Mach N, Kobayashi T, Mulligan R C, Nawa Y, Dranoff G, Galli S J. 1998. Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* 392: 90.

44. Byrd T F. 1998. Multinucleated giant formation induced by IFN-gamma/IL-3 is associated with restriction of virulent *Mycobacterium tuberculosis* cell to cell invasion in human monocyte monolayers. *Cell Immunol*. 188: 89.

45. Suzuki Y, Conley F K, Remington J S. 1989. Importance of endogenous IFN-gamma for prevention of toxoplasmic encephalitis in mice. *J. Immunol*. 143: 2045.

46. Deckert-Schluter M, Bluethmann H, Kaefer N, Rang A, Schluter D. 1999. Interferon-gamma receptor-mediated but not tumor necrosis factor receptor type 1- or type 2-mediated signaling is crucial for the activation of cerebral blood vessel endothelial cells and microglia in murine *Toxoplasma* encephalitis. *Am. J. Pathol*. 154: 1549.

47. Sedo A, van Weyenbergh J, Rouillard D, Bauvois B. 1996. Synergistic effect of prolactin on IFN-gamma-metiated growth arrest in human monoblastic cells: correlation with the up-regulation of IFN-gamma receptor gene expression. *Immunol. Lett*. 53: 125.

48. Suzuki Y, Yang Q, Conley F K, Abrams J S, Remington JS. 1994. Antibody against interleukin-6 reduces inflammation and numbers of cysts in brains of mice with toxoplasmic encephalitis. *Infect. Immun*. 62: 2773.

49. Peterson P K, Gekker G, Hu S, Chao C C. 1995. Human astrocytes inhibit intracellular multiplication of *Toxoplasma* by a nitric oxide-mediated mechanism. *J. Infect. Dis*. 171: 516.

Figures

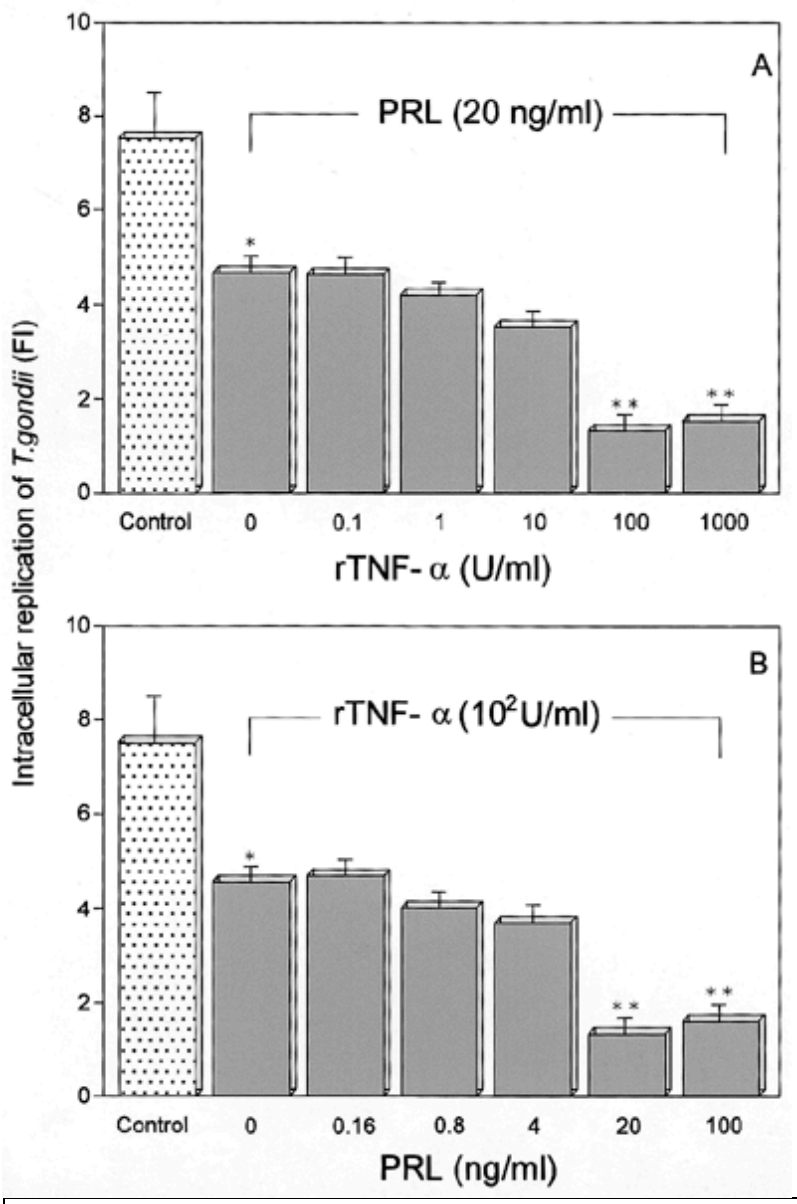


Figure 1

Dose-effect relationship of rTNF-alpha or PRL on inhibition of *Toxoplasma gondii* multiplication.

Microglial cell (MGC) cultures at a concentration of 5×10^4 cells/well were treated with medium alone (control) or medium containing the indicated concentrations of (A) rTNF-alpha in the presence of 20 ng/ml of PRL or (B) PRL in the presence of 100 U/ml of rTNF-alpha for 24 hours and then challenged for 1 hour and 20 hours with *T. gondii* tachyzoites. Intracellular replication of *T. gondii*, an index of toxoplasmastatic activity, was expressed as fold increase (FI) in the number of tachyzoites per 100 MGC at

20 hours of infection. Values are the means ± SD of triplicate counts of four separate experiments. Significant differences *versus* control (* $p < 0.05$; ** $p < 0.01$).

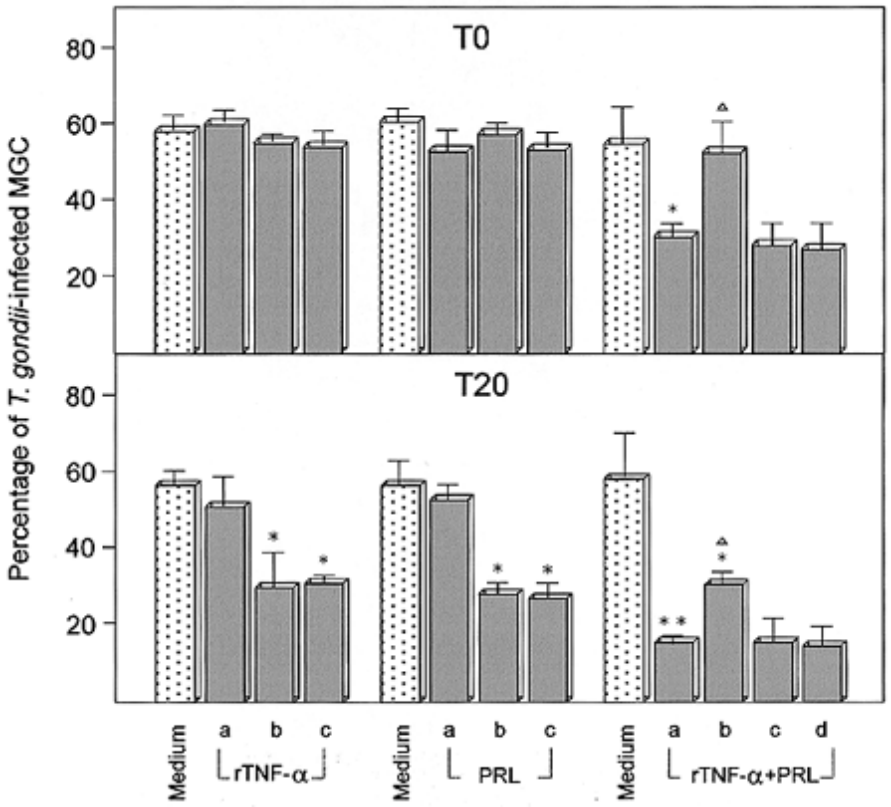


Figure 2

Effect of rTNF-alpha, PRL or rTNF-alpha + PRL in the absence or presence of monoclonal antibodies (mAbf) for CAMs on intracellular killing of *T. gondii* by MGC.

Cell cultures (5×10^4) were incubated with medium alone or containing (a) 10, (b) 100 and © 1,000 U/ml of rTNF-alpha or (a) 4, (b) 20 and © 100 ng/ml of PRL or (a) 100 U/ml of rTNF-alpha plus 20 ng/ml of PRL in the absence or presence mAb specific for (b) I-CAM-1, © VCAM-1 or (d) N-CAM for 24 hours. Monoclonal antibodies (5 mug/ml) were added to MGC in the first 1 hour of the activation phase with cytokine or hormone. The cells were then infected with tachyzoites for 1 hour (T0) and 20 hours (T20).

Intracellular killing of *T. gondii* was proven by a decrease in the percentage of infected MGC from 1 to 20 hours after infection. Values are the means ± SD of triplicate counts of four experiments. *: significant difference *versus* control; delta: significant difference *versus* rTNF-alpha + PRL-treated cells (*, delta $p < 0.05$; **, deltadelta $p < 0.01$).

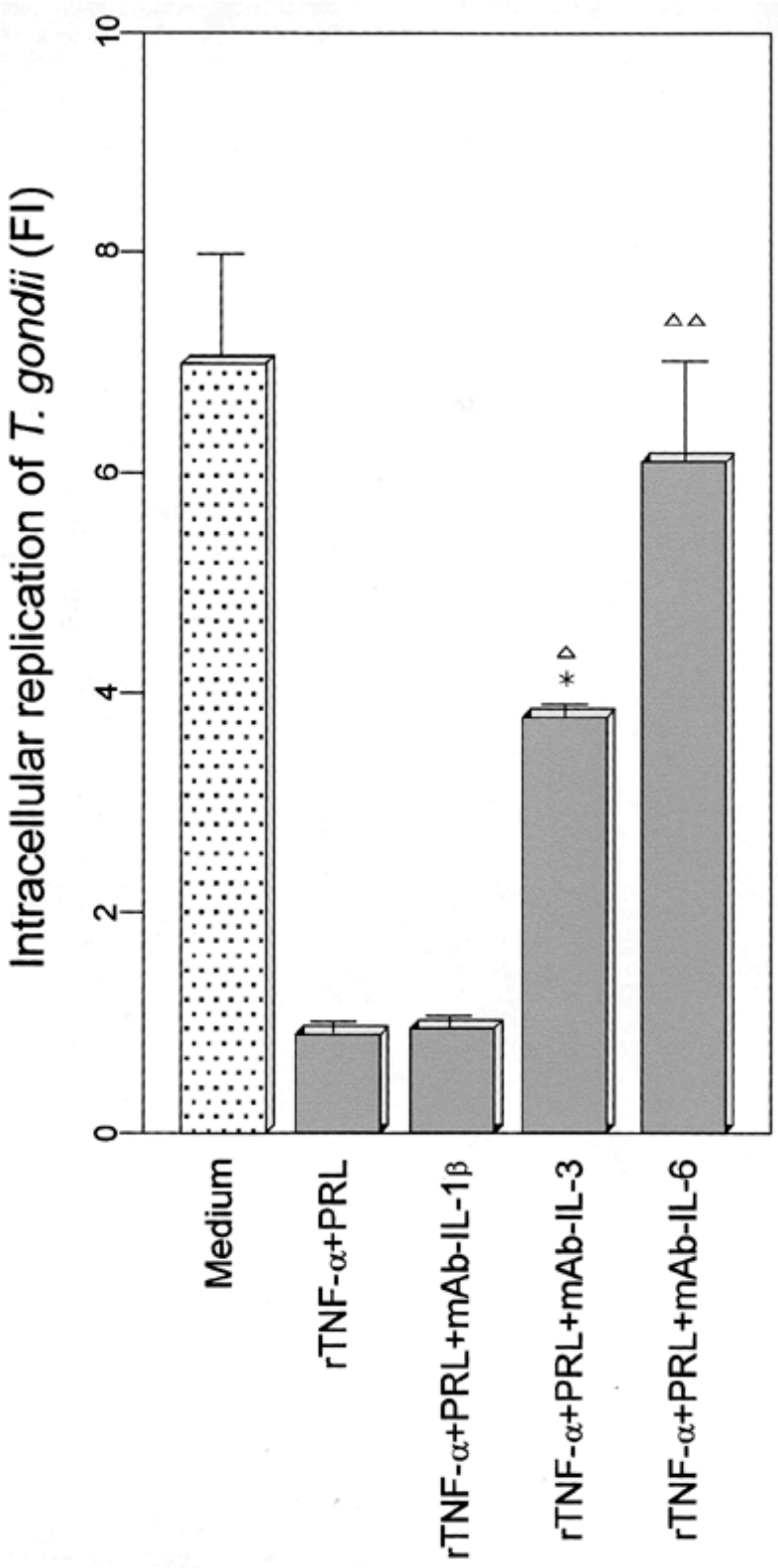


Figure 3

Effect of mAb against IL-1beta, IL-3 or IL-6 on toxoplasmastatic activity in MGC.

Cell cultures (5×10^4) were incubated with medium alone or containing rTNF-alpha (100 U/ml) plus PRL (20 ng/ml) for 24 hours. At 12 hours of incubation, neutralizing mAb (5 mug/ml) specific for IL-1beta, IL-3 or IL-6 were added under normal condition cultures. MGC were then challenged with tachyzoites. Intracellular replication of *T. gondii* was determined after 20 hours of infection as above. Data are the means ± SD of triplicate counts of four experiments. Significant difference *versus* control; delta: significant difference *versus* rTNFalpha + PRL cells (*, delta $p < 0.05$; **, deltadelta $p < 0.01$).

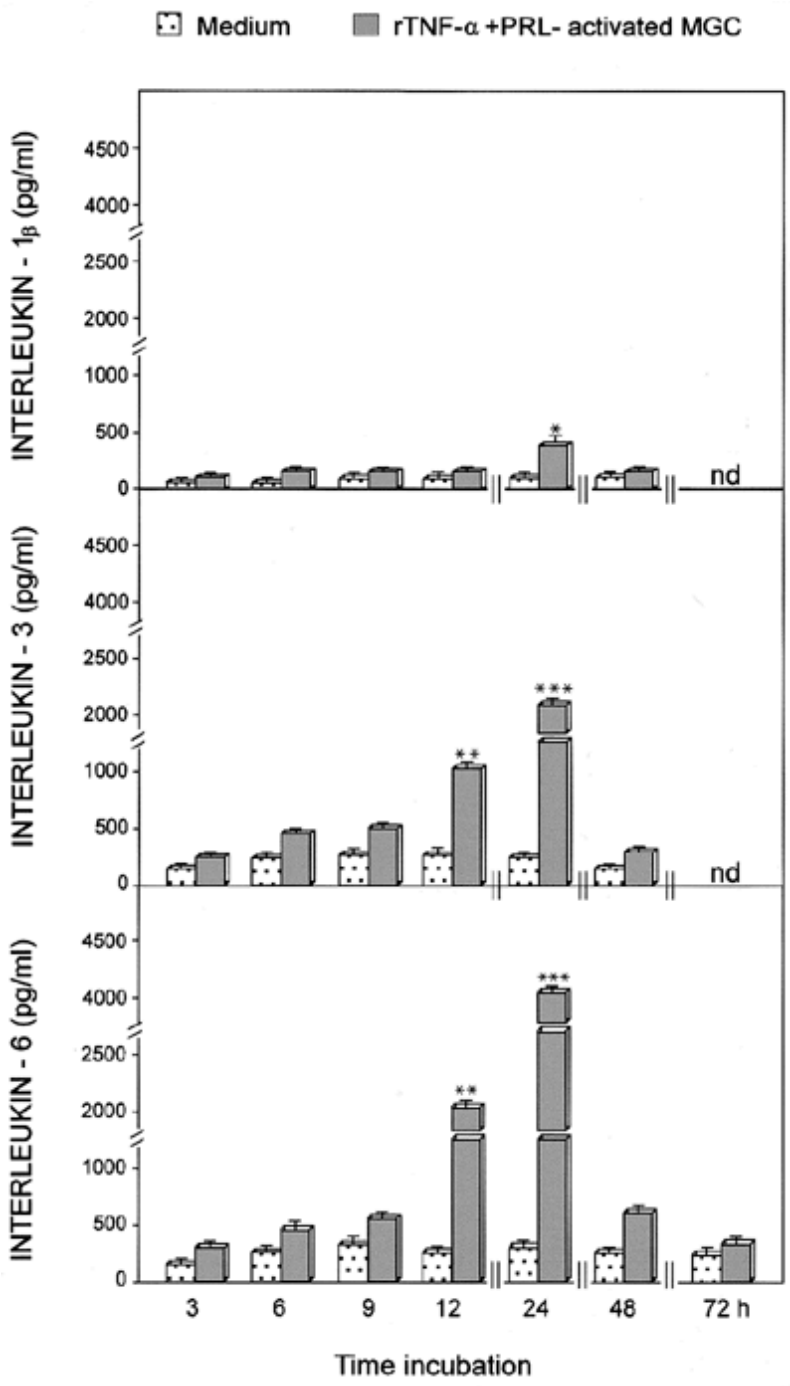


Figure 4

Course of the release of IL-1beta, IL-3 and IL-6 by MGC during incubation with rTNF-alpha + PRL.

MGC cultures (5 x 10^4 cells) were incubated with medium alone or containing rTNF-alpha (100 U/ml) plus PRL (20 ng/ml). At various time points during the activation phase, the culture supernatant was collected and the levels of IL-1beta, IL-3 and IL-6 were measured by ELISA methods. At

72 hours no IL-1beta or IL-3 could be detected; nd: not detected. Values are the means ± SD of three determinations of four experiments. Significant difference *versus* control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

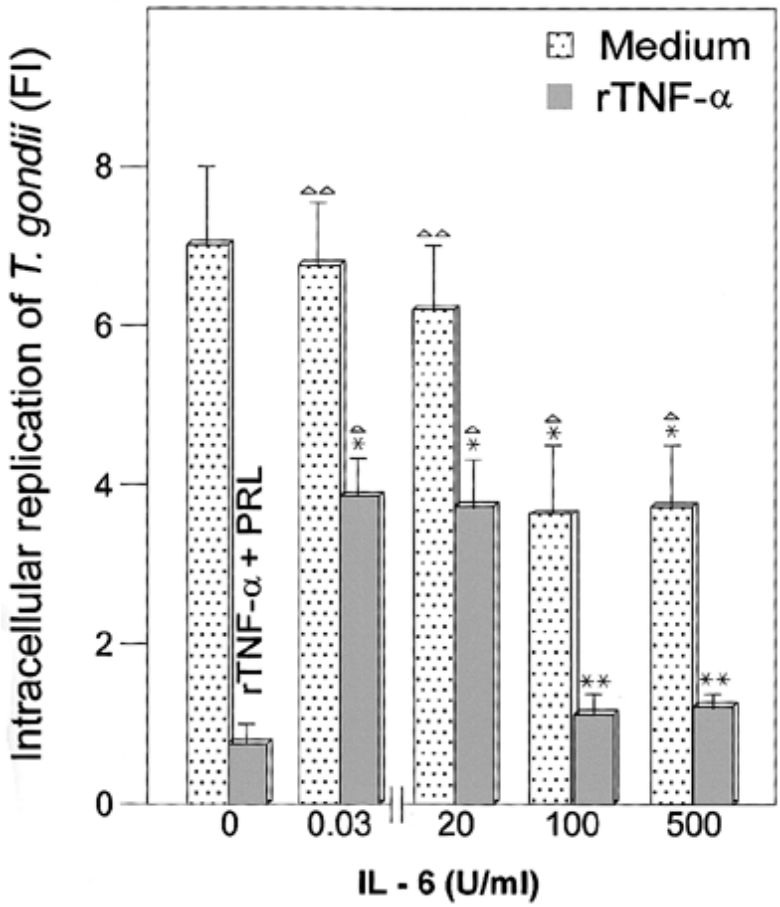


Figure 5

Dose effects of rIL-6 on *T. gondii* replication.

MGC cultures (5×10^4) were incubated (for 24 hours) with medium alone or containing rIL-6, at the indicated concentrations, in the absence or presence of rTNF-alpha (100 U/ml), before infection with *T. gondii* tachyzoites for 1 hour and 20 hours. Intracellular replication was determined at T20 of infection as above. Values are the means ± SD of triplicate counts of four separate

experiments. Significant difference *versus* control; delta: significant difference *versus* rTNF-alpha + PRL-treated cells (*, delta p < 0.05; **, deltadelta p < 0.01).

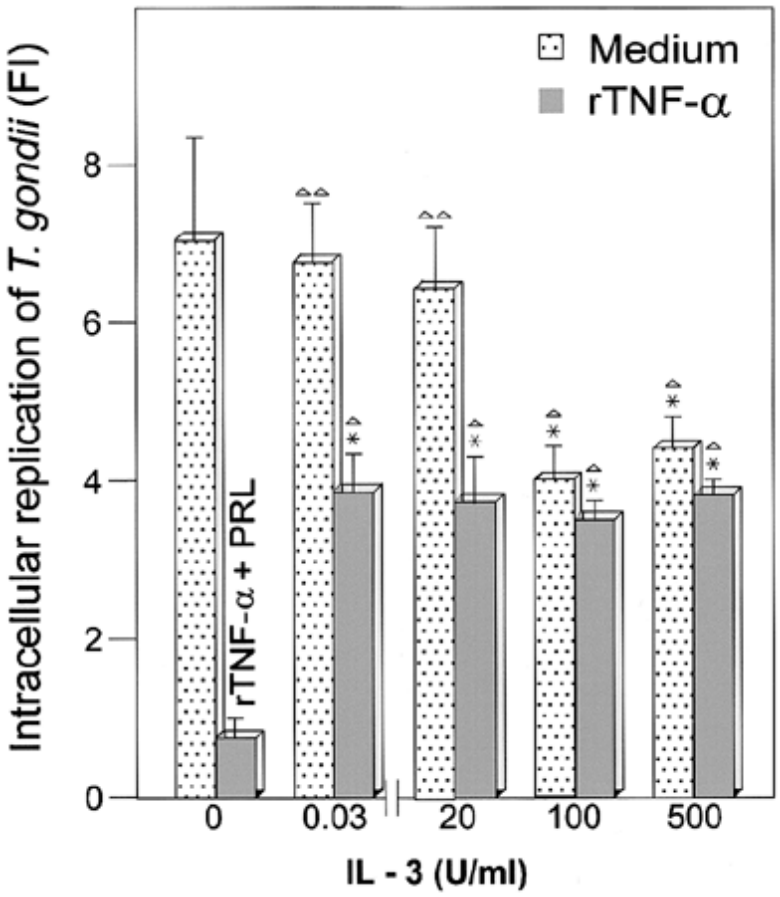


Figure 6

Dose effects of rIL-3 on *T. gondii* growth.

Cultures (5×10^4) were incubated for 24 hours with medium alone or containing rIL-3, at the indicated concentrations, in the absence or presence of rTNF-alpha (100 U/ml), before challenge with tachyzoites at 1 hour and 20 hours. Intracellular replication was determined at T20 of infection. Values are the means ± SD of triplicate counts of four separate experiments. Significant difference *versus* control; delta: significant difference

versus rTNF-alpha + PRL cells (*, delta p < 0.05; **, deltadelta p < 0.01).

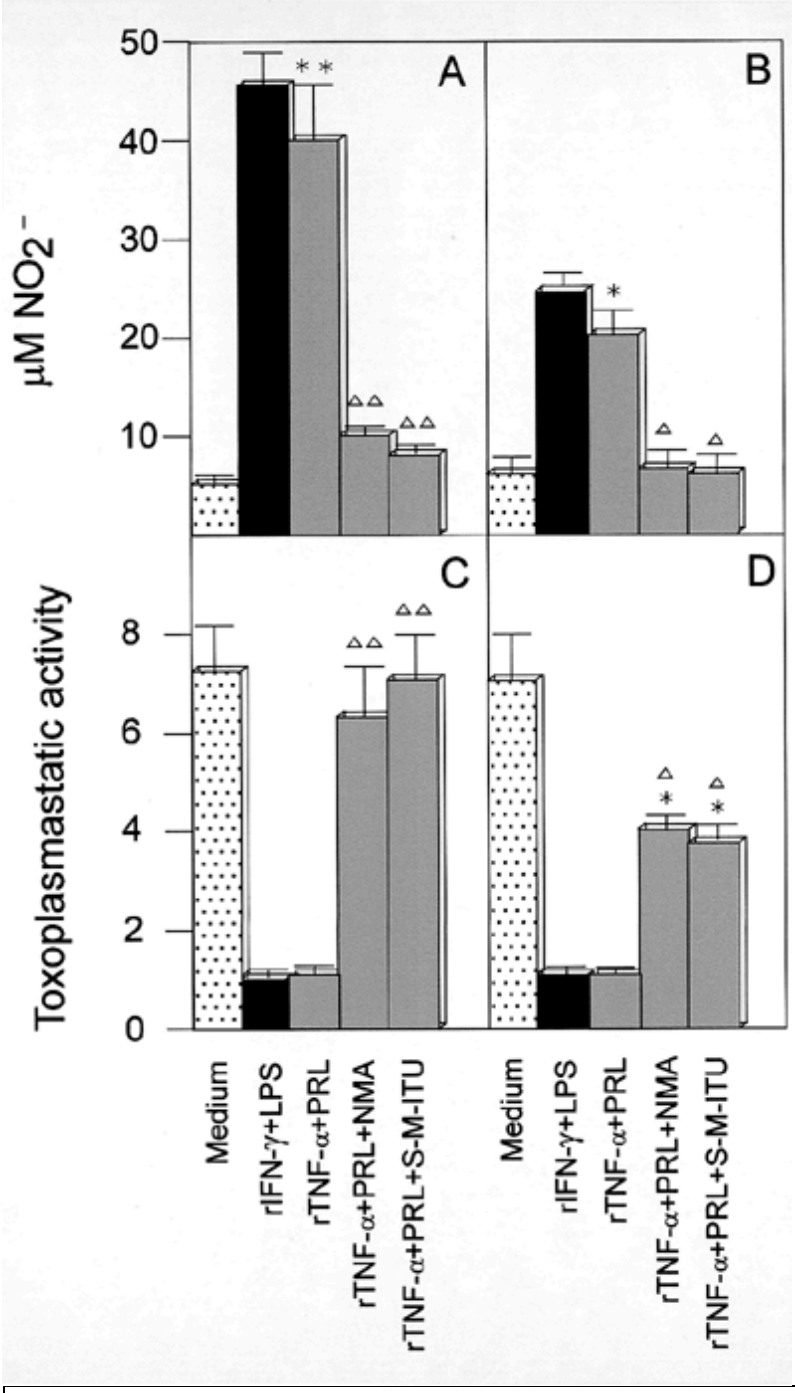


Figure 7

Effect of NMA or S-M-ITU on release of NO_2^- by and toxoplasmastatic activity in MGC.

Cell cultures were treated with medium alone (negative control) or containing 100 U/ml IFN-gamma plus 1 mug/ml LPS (positive control) or 100 U/ml rTNF-alpha plus 20 ng/ml PRL (20 ng/ml) in the absence or presence of 300 muM of NMA or 50 muM of S-M-ITU added during the 24 hours activation phase, before infection with *T. gondii*, and during the 20 hours infection phase. **A:** The amount of NO_2^- released by 5 x 10^4 MGC was measured in the culture supernatant collected after the 24 hours activation phase with or without rTNF-alpha plus PRL in the absence or presence of NMA or S-M-ITU, before infection with *T. gondii*. **B:** The amount of NO_2^- produced by 5 x 10^4 cells incubated with or without rTNF-alpha plus PRL in the absence or presence of NMA or S-M-ITU was determined in the culture supernatant collected after 20 hours of infection with tachyzoites. **C** and **D:** Toxoplasmastatic activity expressed as fold increase in the number of toxoplasmas per 100 MGC at T 20 of infection. In **(A)** and **(C)** NMA or S-M-ITU was added during the 24 hours activation phase, before infection with *T. gondii* tachyzoites. In **(B)** and **(D)** NMA or S-M-ITU was added during the 20 hours infection phase with tachyzoites. Values are the means ± SD of triplicate determinations of four experiments. Significant difference *versus* negative control; delta: significant difference *versus* positive control (*, delta $p < 0.05$; **, deltadelta $p < 0.01$).

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In this issue

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Fibronectin increases the migration induced by stromal cell-derived factor-1a (SDF-1a) in pre-B acute lymphoblastic leukemia cells

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acute lymphoblastic leukemia, SDF-1a, CXCR-4, VLA-4, VLA-5, fibronectin.

Hematopoietic growth factors in patients receiving intensive chemotherapy for malignant disorders: studies of granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3) and Flt-3 ligand (Flt3L)

O. Bruserud, B. Foss, H. Petersen
chemotherapy, acute leukemia, G-CSF, GM-CSF, IL-3, Flt3L.

Can interleukin-2 reverse anthracyclin chemoresistance in metastatic soft tissue sarcoma patients. Results of a prospective phase II clinical trial

G. Gravis, M. Mousseau, J.Y. Douillard, T. Dorval, M. Fabbro, B. Escudier, L. Mignot, P. Viens
immunotherapy, soft tissue sarcoma, interleukin-2, chemoresistance, anthracyclin.

Activation of two distinct anti-proliferative pathways, apoptosis and p38 MAP kinase-dependent cell cycle arrest, by tumor necrosis factor in human melanoma cell line A375

T. Hattori, H. Hayashi, T. Chiba, K. Onozaki
melanoma, tumor necrosis factor, apoptosis, cell cycle arrest, p38 MAPK.

Impaired interleukin-5 (IL-5) production by T cells as a prognostic marker of disease progression in human immunodeficiency virus type 1 (HIV-1)-infected children

S. Resino, S. Sanchez-Ramon, J.M. Bellon, J.L. Jimenez, M.A. Munoz-Fernandez
HIV-infected children, cytokine production, interleukin-5, PBMC, prognostic markers.

Cytokine-mediated inflammatory hyperalgesia limited by interleukin-13

B.B. Lorenzetti, S. Poole, F.H. Veiga, F.Q. Cunha, S.H. Ferreira
inflammatory hyperalgesia, interleukin-13, tumour necrosis factor alpha, interleukin-1b, interleukin-8, prostaglandin E2.

Expression of thrombopoietin by umbilical vein endothelial cells

A. El Andaloussi, P. Duchez, J. Rieffers, J. Ripoche, C. Grosset
thrombopoietin, umbilical vein endothelial cells.

Production of cytokines by immature erythroid cells derived from human embryonic liver

S.V. Sennikov, S.V. Krysov, T.V. Injelevskaya, A.N. Silkov, V.A. Kozlov
erythroid progenitor, IL-1b, IL-2, IL-4, IL-6, fetal liver.

Inflammatory cytokine production in interferon-g-primed mice, challenged with lipopolysaccharide. Inhibition by SK&F 86002 and interleukin-1b-converting enzyme inhibitor

S. Spinelle-Jaegle, P. Devillier, S. Doucet, S. Millet, C. Banissi, A. Diu-Hercend, E. Ruuth
interferon-g, lipopolysaccharide, interleukin-1b, cytokine, interleukin-1b-converting enzyme inhibitors.

The effect of Sambucol, a black elderberry-based, natural product, on the production of human cytokines: I. Inflammatory cytokines

V. Barak, T. Halperin, I. Kalickman
Sambucol, inflammatory cytokines, IL-1b, TNF-a, IL-6, IL-8.

Interferon (IFN)-g and Herpes simplex virus/tumor necrosis factor-a synergistically induce nitric oxide synthase 2 in macrophages through cooperative action of nuclear factor-kB and IFN regulatory factor-1

S.R. Paludan, L. Malmgaard, S. Ellermann-Eriksen, L. Bosca, S.C. Mogensen

macrophages, NOS2, Herpes simplex virus, NF-kB, IRF-1.

Negative regulation of onconstatin M signaling by suppressor of cytokine signaling (SOCS-3)

F. Magrangeas, O. Boisteau, S. Denis, Y. Jacques, S. Minvielle
cytokine signaling, inhibition, Jak-STAT pathway.

Increased expression of interleukin-8 and aminopeptidase N by cell-cell contact: interleukin-8 is resistant to degradation by aminopeptidase N/CD13

A. Kehlen, I. Egbert, K. Thiele, K. Fischer, D. Riemann, J. Langner
cell co-culturing, leucine aminopeptidase, plasma membrane preparations, CD13, proteolysis of IL-8, interleukin-8.

Functional characterization of the mouse lymphotoxin-beta receptor promoter

P. Muller, D.N. Männel, T. Hehlgans
LTbR-promoter, transcriptional activation, dexamethasone.

Glucose potentiates interleukin-1b (IL-1b)-induced p38 mitogen-activated protein kinase activity in rat pancreatic islets of Langerhans

A.M.E. Sprinkel, N.A. Andersen, T. Mandrup-Poulsen
interleukin-1b, p38, ERK1/2, JNK, nitric oxide, diabetes mellitus.

Sustained parasite burden in the spleen of Leishmania infantum-infected BALB/c mice is accompanied by expression of MCP-1 transcripts and lack of protection against challenge

D. Rousseau, S. Demartino, F. Anjuère, B. Ferrua, K. Fragaki, Y. Le Fichoux, J. Kubar
murine leishmaniasis, acquired immunity, inflammation, chemokines, spleen cell subpopulations.

Effects of cytokines and prolactin on the replication of Toxoplasma gondii in murine microglia

N. Benedetto, A. Folgore, C. Romano-Carratelli, F. Galdiero
T. gondii, cytokine, PRL, MGC.

Strip test for bedside detection of interleukin-6 in cervical secretions is predictive for impending preterm delivery

H. Trébéden, F. Goffinet, G. Kayem, F. Maillard, E. Lemoine, D. Cabrol, B. Weill, F. Batteux
cytokine, IL-6, prematurity, cervicovaginal secretions, strip test.

Serum levels of interleukin-18 in patients with uncomplicated Plasmodium falciparum malaria

D. Torre, M. Giola, F. Speranza, A. Matteelli, C. Basilico, G. Biondi
interleukin-18, malaria, Plasmodium falciparum, gamma interferon.

The ecdysone inducible gene expression system: unexpected effects of muristerone A and ponasterone A on cytokine signaling in mammalian cells

S. Constantino, R. Santos, S. Gisselbrecht, F. Gouilleux
ecdysone, PI 3-kinase, AKT, survival.

Presence of estrogen receptors in human myeloid monocytic cells (THP-1 cell line)

M. Cutolo, B. Villaggio, A. Bisso, A. Sulli, D. Coviello, J.M. Dayer
THP-1 cells, macrophages, estrogen receptors, estrogens, monocytes.

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