Biological response modifiers and parasitic infections: Experimental aspects of toxoplasmosis

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MH BEAMAN. Biological response modifiers and parasitic infections: Experimental aspects of toxoplasmosis. Can J Infect Dis 1994;5(Suppl A):47A-50A. Parasitic infections are important causes of disease in the developing world and, since the advent of AIDS, the developed world. Over the past decade, in vitro and in vivo studies have established the important role that biological response modifiers play in pathogenesis of parasitic disease. These basic studies have resulted in successful clinical trials of interferon gamma (IFN-γ) in human leishmaniasis. Toxoplasmic encephalitis is a major opportunistic infection in patients with AIDS, and current therapy is often problematic. IFN-γ has been shown in in vitro and in vivo animal studies to be critical for host defence against *Toxoplasma gondii*. Tumour necrosis factor alpha plays a critical role in mediating IFN-γ effect in vitro, but its role in vivo is under further study. Interleukin (IL)-6 and IL-10 have both recently been shown to enhance *T gondii* replication in vitro and to antagonize the beneficial effects of IFN-γ. In addition, in certain mouse strains, IL-6 has been shown to worsen mortality from *T gondii* infection. Future strategies for therapy of *T gondii* may include administration of exogenous IFN-γ or IL-12 with or without antibody to antagonistic cytokines such as IL-6 (or possibly IL-10).

Key Words: *Cytokines, Interferon gamma, Interleukin-6, Interleukin-10, Parasites, Tumour necrosis factor alpha,* Toxoplasma gondii

Modificateurs de la réponse biologique et infections parasitaires: aspects expérimentaux de la toxoplasmose

RÉSUMÉ : Les infections parasitaires sont d'importantes causes de maladies dans les pays en voie de développement et, depuis l'arrivée du SIDA, dans les pays industrialisés. Au cours de la décennie passée, des études *in vitro* et *in vivo* ont déterminé le rôle important des modificateurs de la réponse biologique dans la pathogenèse de la maladie parasitaire. Ces études fondamentales ont donné lieu à des essais cliniques réussis sur l'interféron gamma (IFN-γ) dans la leishmaniose humaine. L'encéphalite toxoplasmique est une infection opportuniste importante dans les cas de SIDA, et son traitement actuel est souvent problématique. L'IFN-γ s'est révélé important dans des études *in vitro* et dans des études animales *in vivo* pour la protection de l'hôte contre *Toxoplasma gondii*. Le facteur de nécrose tumorale alpha joue un rôle central de modulateur de l'effet de l'IFN-γ *in vitro*, mais son rôle *in vivo* doit être étudié davantage. L'interleukine-6 (IL-6) et IL-10 se sont toutes deux récemment révélées capables d'améliorer la réplication de *T. gondii in vitro* et d'inhiber les effets bénéfiques de l'IFN-γ. De plus, chez certaines espèces de souris, l'IL-6 s'est révélée apte à aggraver le taux de mortalité dû à l'infection à *T. gondii*. Les stratégies futures pour ce qui est du traitement de *T. gondii* pourrait inclure l'administration d'IFN-γ ou IL-12 exogène, avec ou sans anticorps anticytokines antagonistes, comme le sont l'IL-6, ou possiblement l'IL-10.

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PARASITIC INFECTIONS HAVE ALWAYS BEEN IMPORTANT causes of morbidity and mortality in the tropical world (1), but their importance has been rediscovered in the developed world with the advent of the AIDS pandemic (2). Treatment of some parasitic infections with antimicrobial agents alone may be hampered by serious toxicities and/or inadequate responses, and newer safe and effective regimens are urgently required. Over the past 10 years, numerous groups have established the critical role that biological response modifiers, particularly cytokines, play in host defence against parasitic infections (3-5). It has also been recognized that certain groups of patients, such as neonates (6) or adults with AIDS (7), have impaired production of cytokines known to be important in defence against some parasites. Such basic research has now led to successful clinical trials of interferon gamma (IFN-γ) as therapy for cutaneous (8) and visceral (9) leishmaniasis. It is clear, therefore, that an understanding of the role of cytokines in the pathophysiology of individual parasitic diseases has the potential for identifying novel therapies or agents useful as adjuncts to current therapies.

INFECTION WITH *TOXOPLASMA GONDII*

Toxoplasma gondii is an obligate intracellular pathogen with a universal distribution that causes serious and potentially fatal disease in neonates (10), patients with malignancies (11) or AIDS (12) and recipients of organ transplants (11). In AIDS, *T gondii* is a major cause of opportunistic infection; a recent study from Austria documented the occurrence of toxoplasmic encephalitis (TE) in 47% of such patients. In almost all cases, TE occurs as a result of reactivation of a latent infection (13). Clinical studies of AIDS patients have revealed a strong correlation between the occurrence of TE and depressed levels of circulating CD4+ T lymphocytes (14). Animal studies have identified the critical role played by cell-mediated immunity in host defence against *T gondii*, and the important contribution to this defence made by the activated macrophage (15) and CD8+ T lymphocytes (16).

CYTOKINE SECRETION IN RESPONSE TO *T GONDII*

The presence of an IFN in serum of mice infected with *T gondii* was first reported in 1966 by two independent groups (17,18). Shirihata et al (19) identified by bioassay a serum activity induced by *T gondii* infection as immune IFN, and noted differences in production between a mouse strain susceptible to infection, compared with resistant strains, suggesting that differences in cytokine generation may play a role in expression of disease phenotype. Using two-site double sandwich enzyme-linked immunoadsorbent assay, the strain-dependent differences in serum IFN-γ production during acute murine toxoplasmosis have been confirmed (20).

Tumour necrosis factor alpha (TNF-α) is detectable in the serum of mice dying of acute toxoplasmosis (21) but only late in the course of infection (20). Interleukin (IL)-6 is also detectable in significant quantities in the serum of mice dying of acute toxoplasmosis and appears to correlate with clinical signs of disease (20).

Human monocyte-derived macrophages (HMDM), when cultured in the presence of immune lymphocytes and toxoplasma lysate antigen (TLA), develop the ability to resist intracellular infection with *T gondii* (22). Supernatants from TLA-stimulated immune lymphocytes (or nonimmune lymphocytes stimulated with mitogens) could also induce such activity. Immune IFN was recognized as being present in such preparations and was capable of inducing inhibition of replication of *T gondii* (23). IFN-γ produced by immune Balb/c spleen cells stimulated with toxoplasma antigen was shown by Gazzinelli et al (24) to originate predominantly from CD4+ lymphocytes, with a smaller contribution from CD8+ T cells (24). Splenocytes of CBA/Ca mice (which are genetically predisposed to development of TE) secrete greater amounts of IFN-γ in response to stimulation with TLA than do mice resistant to TE (Balb/c) during the course of chronic infection with *T gondii* (25). CBA/Ca splenocytes also secrete larger amounts of IL-6 in the initial stages of chronic infection (when brain inflammation develops) than Balb/c mice do, raising the possibility that the latter cytokine may be associated with adverse sequelae of *T gondii* infection.

IN VITRO STUDIES OF CYTOKINES AND *T GONDII* INFECTION

Nathan et al (26) confirmed that IFN-γ is the major lymphokine that activates HMDM antitoxoplasma activity under experimental conditions in vitro. Studies have established the ability of recombinant murine (rMu) IFN-γ to activate murine peritoneal macrophages in vivo (27,28), alveolar macrophages in vitro and in vivo, and peritoneal macrophages in vitro (28) to kill *T gondii*. Suzuki et al (29) also noted that treatment of mice with a monoclonal antibody to IFN-γ before infection with *T gondii* prevented the activation of peritoneal macrophages that was observed in control antibody treated animals.

rMu TNF-α is unable to activate murine macrophages to inhibit the replication of *T gondii*, although this treatment is capable of activating macrophages to inhibit the intracellular replication of *Trypanosoma cruzi* (30). Chang et al (21) were also unable to activate murine macrophages against *T gondii* using TNF-α alone, but the combination of TNF-α and IFN-γ was synergistic, compared with IFN-γ administered alone. In addition, the ability of rMu IFN-γ to activate macrophages in vitro to kill *T gondii* was shown to be partially inhibited by a polyclonal neutralizing antibody against TNF-α, suggesting that TNF-α may be necessary for the optimal activation of macrophages. In fact, using stringent endotoxin-free conditions, IFN-γ appears to be unable to activate macrophages to kill *T gondii*, but this

ability is restored by the addition of small quantities of TNF-α (31).

IL-6 has been shown to enhance intracellular replication of *T gondii* markedly after invasion of macrophages in vitro (32). Of equal importance is the observation that the ability of rMu IFN-γ to activate macrophages to kill *T gondii* is ablated by recombinant human IL-6, when both cytokines are administered together before infection, suggesting that the balance between activating and inhibitory cytokines may be important during disease due to *T gondii*. Supporting this hypothesis is the observation of inhibition by IL-10 of the IFN-γ mediated activation of macrophages to kill *T gondii* (33). This effect is seen when IL-10 is administered before or at the same time as, but not after, IFN-γ.

IN VIVO STUDIES OF CYTOKINES AND *T GONDII* INFECTION

The administration of exogenous rMu IFN-γ to mice completely protects them from acute challenge with a virulent strain of *T gondii* (27). The importance of endogenous IFN-γ in resistance to *T gondii* infection was shown by Suzuki et al (34) in a study in which mice treated with a monoclonal antibody to IFN-γ before challenge with an avirulent strain of *T gondii* manifested a 100% mortality rate, compared with no deaths in the control mice. Administration of recombinant IFN-γ reduces the degree of TE in CBA/Ca mice infected with an avirulent strain of *T gondii*, which suggests a possible therapeutic role for IFN-γ in chronic, as well as acute, *T gondii* infection (35).

The effect of TNF-α on in vivo murine infection with *T gondii* is somewhat controversial, with enhanced mortality after TNF-α treatment of mice during acute infection being observed in some studies (36), as well as significant protection induced by TNF-α reported in another study (21). The reasons for these disparate findings are not obvious at present, but possibly relate to differences in cytokine preparations or methodology. Evidence supporting the involvement of TNF-α in TE includes the finding that treatment of C57BL/6J mice with a polyclonal antibody to TNF-α results in an increase in the number of brain cysts during the course of a chronic infection with the C56 strain, compared with control animals (37). This study also reported that administration of rMu TNF-α resulted in a decrease in the number of *T gondii* cysts, compared with controls. The number of cysts does not necessarily correlate with the degree of inflammation in TE, however, and further studies are needed to identify the effect of TNF-α on the degree of inflammation in TE.

Evidence for a significant role for IL-6 in *T gondii* infection comes from experiments that demonstrate improved survival of mice treated with an antibody to IL-6 before a lethal infection with *T gondii* (5). This antibody also protects immunodeficient mice with toxoplasmosis (38).

SUMMARY

These studies have documented a critical role for cytokines in experimental infections with *T gondii*. The major importance of IFN-γ is well established in resistance to the organism, and a clinical trial studying the effect of this cytokine in TE in AIDS patients is underway (personal communication). More recent animal studies have identified adverse immunological phenomena related to certain cytokines (IL-6, IL-10) during *T gondii* infection, suggesting that ablation of such cytokines (with antibodies or soluble cytokine receptors) should be evaluated further for potential therapeutic activity. IL-12 has recently been identified as an important protective mediator during toxoplasmosis in T cell deficient mice (39).

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