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IL-13 pre-treatment of murine peritoneal macrophages increases their anti-*Toxoplasma gondii* activity induced by lipopolysaccharides

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Abstract

Th1 cytokines and microbial lipopolysaccharides (LPS) activate macrophages to produce inflammatory mediators and effector molecules. Althrough Th2 cytokines often have an opposite action to Th1 cytokines and down-modulate the inflammatory response of macrophages, they can induce a distinct alternative activation that is beneficial in host defence. In this study, we report that IL-13 enhances the anti-*Toxoplasma* activity of LPS-activated murine macrophages. The inhibition of parasite proliferation was not related to reduced *Toxoplasma gondii* penetration into the cells, nor to the conversion of tachyzoites into bradyzoites. Used alone, IL-13 triggers the polarisation of macrophages towards type 2. However, in LPS-activated macrophages, we show the priming capacity of this cytokine to enhance the expression of inducible nitric oxide synthase (iNOS), a major marker of type 1 macrophages. This effect of IL-13 was not dependent on the activation state of macrophages (resident versus thioglycolate-elicited) or the timing of pre-treatment. We demonstrate a correlation between the enhancement of NO production and upgrading of the microbicidal effectiveness of the macrophages. Thus, both Th2 and Th1 cytokines could activate macrophages to control infections.
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1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite that causes severe and often fatal disease in immunocompromised hosts and congenital infections in new-borns. Infection is characterised by an acute phase during which the rapidly proliferating tachyzoite disseminates throughout the host organism. In immunocompetent patients, chronic infection ensues and bradyzoites, the slowly dividing stage, form cysts mainly in the central nervous system, retina and muscles. The host effectively suppresses acute infection by initiating a strong cell-mediated immune response that restrains the growth and spread of the parasite, thereby initiating latency. Dormancy is then maintained for the life of the host unless an immunosuppressive event occurs, such as the acquisition of acquired immunodeficiency syndrome (AIDS) (Frenkel, 1988; Hunter and Remington, 1994; Carruthers, 2002; Gray et al., 2003) or in patients given immunosuppressive therapy for organ (Couvreur et al., 1992; Renoult et al., 1997; Campbell et al., 2006) or haematopoietic stem cell transplantation (Maschke et al., 1999; de Medeiros et al., 2000, 2001; Martino et al., 2000).

It is well established that cytokines are a determinant in the outcome of toxoplasmosis infection. IL-12 induces IFN-γ production and then activates effector functions of macrophages such as TNF-α and nitric oxide (NO) production. The induction of TNF-α production by macrophages has a profound anti-toxoplasmic activity that is mediated

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by inducible NO (Langermans et al., 1992b). Inducible NO synthase (iNOS) is responsible for the increased production of NO by macrophages after stimulation with microbial products such as lipopolysaccharides (LPS) and/or Th1 cytokines, in particular IFN-γ (Suzuki et al., 1988; Scharton-Kersten et al., 1997). IFN-γ pre-treatment increases the magnitude and the sensitivity of the response of macrophages to LPS. This effect, referred to as a priming effect, is well-demonstrated. The production of NO and the antimicrobial response are a hallmark of classically activated macrophages.

The role of Th2 cytokines (IL-4, IL-13) is less well-known but it is becoming clear that Th2 cytokines play an important role in innate immune responses. Although Th2 cytokines are able to down-modulate macrophage inflammatory activity and counteract the Th1 response (Bogdan et al., 1994) these cytokines, such as IL-4 and IL-13, can induce an alternative activation of macrophages. This activation differs in terms of the expression of membrane receptors, the production of cytokines and chemokines, and effector functions (Mantovani et al., 2002; Gordon, 2003). Th2 cytokines could have a positive effect in several infections. Indeed, Th2 cytokines have been described as playing a role in the control of helminths (Brombacher, 2000; Finkelman and Urban, 2001; Finkelman et al., 2004), *Plasmodium falciparum* (Serghides and Kain, 2001) and *Candida albicans* infections (Coste et al., 2003). The induction of membrane receptors as scavenger receptors (CD36) and the mannose receptor are strongly implicated in the control of *P. falciparum* and *C. albicans* infections, respectively.

Moreover, it has been shown that during *T. gondii* infection, Th2 cytokines are necessary to host survival. Indeed, IL-4 or IL-10 knock-out mice do not survive infection with the avirulent *T. gondii Beverley* strain (Roberts et al., 1996; Suzuki et al., 2000). The beneficial role of Th2 cytokines in this infectious response was described by Dimier-Poisson et al. (2003) who showed a protective mucosal Th2 immune response against *T. gondii* by murine mesenteric lymph node dendritic cells. This protection was independent of mouse strain and was closely related to the production of a mucosal Th2 cytokine profile including increased IL-13 production.

In light of these findings, we investigated the capacity of IL-13 to modulate macrophage activation induced by LPS against *T. gondii*. We have demonstrated that IL-13 treatment of macrophages reduces *T. gondii* proliferation in LPS-activated macrophages. We show that this enhancement of the anti-infectious functions of macrophages was mediated through an over-production of NO.

2. Materials and methods

2.1. Macrophage culture

Resident murine peritoneal cells were harvested from female Swiss mice (Janvier, France). Thioglycolate-elicited macrophages were isolated form the peritoneal cavity of mice injected with 3% Brewer's thioglycolate 3 days before harvest. Cells were obtained by injection of sterile NaCl (0.9%) into the peritoneal cavity. The collected cells were centrifuged and the cell pellet suspended in serum-free medium (SFM) optimised for macrophage culture (Gibco Invitrogen Corporation, France). Cells were allowed to adhere on 24-well culture plates over 2 h at 37 °C with 5% CO_2. Non-adherent cells were removed by washing with PBS (Gibco Invitrogen Corporation, France) and the remaining adherent cells were stimulated with 10 ng/mL of murine IL-13, 10 ng/mL of LPS and 2 ng/mL of murine IFN-γ (Sigma, France) as described in the figures. In some experiments, macrophages were pre-treated with 10 μM of 1400W dihydrochloride, a potent and selective inhibitor of iNOS (Sigma, France) 15 min before LPS treatment (Garvey et al., 1997) or 50 ng/mL of IL-4 mutant Y124D which has been characterised as a specific IL-4 receptor antagonist (Sanofi Synthelabo, France) (Zurawski et al., 1993) 15 min before IL-13 treatment. Work was performed in accordance with Approval No. A3155503 and all procedures for animal care and maintenance were conformed with French and European Regulations (Law 87-848 dated 19/10/1987 modified by Decree 2001-464 and Decree 2001-131 relative to European Convention, EEC Directive 86/609 dated 24/11/1986).

2.2. Toxoplasma gondii strain and determination of T. gondii proliferation

The virulent RH strain of *T. gondii* was maintained in mice by serial i.p. passages of 5×10^5 tachyzoites. The intracellular growth of *T. gondii* was measured following the incorporation of [5,6-^{3}H]-uracil (Pfefferkorn and Pfefferkorn, 1977). Macrophage monolayers were incubated with *T. gondii* at a *T. gondii*:macrophage ratio of 1:20 for 1 h. After 1 h of incubation, 4 μCi of [5,6-^{3}H]-uracil per well were added and incubated for 24 h at 37 °C and 5% CO_2.The parasite contains a significant amount of uracil phosphoribosyltransferase, an enzyme responsible for the conversion of uracil into uracil monophosphate (UMP), which is not normally present in the host cell. Controls were performed to evaluate the incorporation of radioactivity into uninfected macrophages. After two washes, the monolayers were disrupted with 1 N NaOH and HCl, and the radioactivity incorporated into the parasites was counted with biodegradable counting scintillant (BCS) fluid (Amersham) in a liquid scintillation counter (LKB 1217 Rackbeta, Pharmacia). Thus, the number of disintegrations per min (dpm) counted provided a direct assessment of *T. gondii* proliferation.

2.3. Penetration assay

Fluorescently labelled tachyzoites were obtained by incubation in fluorescein isothiocyanate (FITC) carbonate buffer (Sigma, France) for 40 min. After centrifugation, the

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