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RT-002

Leishmania major and *Toxoplasma gondii* have Opposite Effects on Cytokine Synthesis by Macrophages

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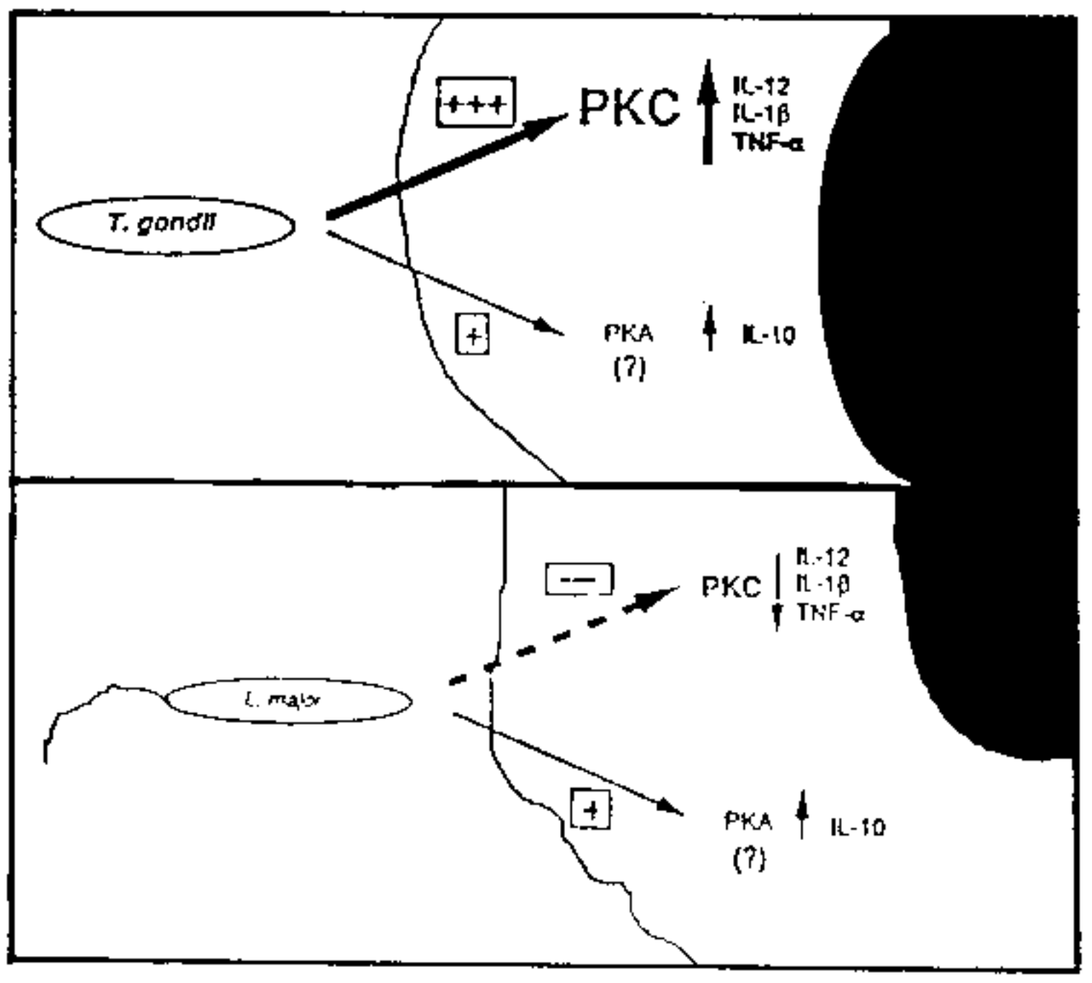
Our previous studies show that *Toxoplasma gondii* non-specifically trigger macrophages to produce different cytokines, such as IL-1β, IL-12, TNF-α and IL-10 (RT Gazzinelli et al. 1993 *Proc Natl Acad Sci USA 90:* 6115-6119, A Sher et al. 1993 *J Immunol 150:* 3982, RT Gazzinelli et al. 1993 *J Immunol 151:* 3672). Activation of macrophages by *T. gondii* also induces protein tyrosine phosphorylation as early as 5 to 10 min reaching a maximum by 15 to 20 min post stimulation (ZY Li et al. 1994 *Inf Immun 62:* 3434). To further investigate the role of protein posphorylation on macrophage activation by *T. gondii*, we used different derivatives of isoquinoline sulfonamide (H7, H8 and HA-1004) as well as SS, which inhibit protein kinases (PK) differentially due to the different binding affinities for these enzymes (M Chiaramonte et al. see Abstracts). These experiments were performed by measuring TNF-α protein using a bioassay, or TNF-α, IL-12, IL-1β and IL-10 mRNAs using a semi-quantitative technique of RT-PCR. Our results show that H7 and SS which have high affinity for PKCs were the best inhibitors of TNF-α, IL-12 and IL-1β. Interestingly, expression of the down-regulatory cytokine IL-10 was preferentially inhibited by H8 and HA-1004, which have higher affinity of cyclic nucleotide-dependent PK, but low affinity for PKCs (AD Politis & SN Vogel 1990 *J Immunol 145:* 3788). In spite of the lower levels of cytokine synthesis in response to live parasites, similar results were obtained with the different inhibitors.

In contrast to *T. gondii*, the promastigote stage of *Leishmania major* has been shown to evade cytokine induction by macrophages (SL Reiner et al. 1994 *J Exp Med 179:* 447). In fact, different studies demonstrate that infection of machrophages with *L. donovani* results in impairment of PKC but not PKA activity, resulting in down-regulation of certain macrophage functions (NE Reiner 1987 *J Immunol 138:* 1919, A Descoteaux et al. 1992 *J Immunol 149:* 3008, KJ Moore et al. 1993 *J Immunol 150:* 4457). To investigate the ability of *L. major* to inhibit monokine synthesis, we used bone marrow macrophages (BMMØ) cultured *in vitro* for seven days in the presence of 30% L929 fibroblast supernatants as a source of GM-CSF. After macrophage maturation in vitro, uninfected BMMØ or BMMØ infected with *L. major* were activated with either LPS, heat killed *Mycobacterium tuberculosis* or *T. gondii* antigens, and cytokine protein synthesis or cytokine mRNA expression measured. These experiments demonstrate that infection of BMMØ with *L. major* had a selective inhibitory effect on cytokine synthesis. *L. major* infection resulted in a complete inhibition of IL-12 synthesis, and partial inhibition of TNF-α synthesis and IL-1 mRNA expression. In contrast, expression of IL-10 mRNA induced by different stimuli was augmented during BMMØ infection with *L. major.*

These results raised the possibility that the inhibitory activity on cytokine synthesis in infected BMMØ was mediated by IL-10. In order to answer this question we performed the same experiments using BMMØ from IL-10 knockout (IL-10KO) mice and wild type controls. Interestingly, infection of BMMØ from IL-10KO with *L. major* promastigotes also resulted in inhibition of IL-12, TNF-α and IL-1β synthesis, indicating that the inhibition must occur by an IL-10 independent mechanism. Based on the results presented here and studies performed by different groups showing the inhibitory activity of Lipophosphoglicans (LPG) from *L. donovani* on PKCs (Reiner, Decoteaux et al., Moore et al. *loc. cit.*), we postulate that monokine synthesis dependent on PKC

but not PKA activity are inhibited by infection with promastigote stages from *L. major*.

Monokine synthesis by macrophage accessory cells has been shown to have an important role in the establishment of a strong cell mediated immunity (Gazzinelli et al. *Proc Natl Acad Sci USA 90*, SC Hsieh et al 1993 *Sci 260:* 547, CS Tripp et al. 1993 *Proc Natl Acad Sci USA 90:* 3725-3730). Thus, *in vitro* and *in vivo* experiments suggest that the early production of IL-12 is responsible for driving the parasite specific T cell response in the Th1 direction. Additionally the early production of IFN-γ by NK cells and T lymphocytes in response to IL-12, would suppress Th2 expansion thereby contributing to the subsequent selection of Th1 CD4+ lymphocytes. Together with the studies showing the importance of different monokynes in resistance to parasite infections, the results presented here suggest that induction and regulation of different PK pathways by intracellular parasite may have important implication in the establishment of the cell-mediated immune response and/or disease development.



Opposite effects of *Leishmania major* and *Toxoplasma gondii* on protein kinases results in different profile of cytokine synthesis by macrophages. *T. gondii* induces IL-12, TNF-α and IL-1β production in a PKC dependent manner, whereas *L. major* inhibits induction of this pathway by *T. gondii* or different microbial products.

MC-006

Engineering Cytokine Secretion from *Trypanosoma cruzi*

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Deficient secretion of interleukin-2 (IL-2) and interferon-γ have been theorized to interfere with the ability of the immune response to clear *Trypanosoma cruzi* infection. To test whether this hypothesis is true, as well as to bolster the immune response to *T. cruzi*, we engineered *T. cruzi* to secrete IL-2 and IFN-γ. It was reasoned that cytokine secreting *T. cruzi* would deliver cytokines directly to the microenvironment of infection, where the high levels of cytokines may stimulate an efficient immune response. If a more effective immune response to *T. cruzi* (i.e. sterile immunity) is found, the basis for this immunity will be dissected to predict vaccine and immunomodulator treatment strategies.

A plasmid vector was created using the intergenic regions of the *T. cruzi* calmodulin-ubiquitin locus to flank the murine IL-2 cDNA and the neomycin phosphotransferase II gene (NEO;

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