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***


Department of Medicine *Department of Pathobiology, University of Washington, SJ-10, Seattle, WA 98195, USA. **University of Tennessee, Memphis, USA

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;
Fig. 1). Expression of the NEO gene allowed G418 selection of epimastigotes bearing the plasmid DNA. When the whole, supercoiled plasmid was transfected into parasites, bioactive IL-2 secretion was demonstrated and the plasmid was found as an extrachromosomal element in multiple head to tail repeats. Higher IL-2 secretion could be induced by increasing G418 concentration and was associated with amplification of the number of extrachromosomal plasmid elements. This increased production of IL-2 allowed purification of T. cruzi derived mL-2 and determination of its amino terminal amino acids. The IL-2 was found to be cleaved six amino acids distal to the site determined for native mL-2, suggesting T. cruzi may follow different rules for signal sequence cleavage than mammals.

Use of the native cDNA for IFN-γ did not lead to secretion of IFN-γ, but IFN-γ was found trapped within the cell. Splicing the signal sequence for IL-2 to the mature sequences of IFN-γ, led to the secretion of IFN-γ, suggesting the signal sequence for IFN-γ was unable to promote secretion of IFN-γ in T. cruzi, but the IL-2 signal sequence repaired this deficit. This finding underscores that the signal sequence rules for T. cruzi are different from that of mammals.

Because removal of drug pressure (G418) led to loss of cytokine secretion in supercoiled transfecants within six weeks, the supercoiled transfecants were deemed too unstable to be used in vivo.

Linearized DNA of the cytokine-NEO plasmids, when transfected into T. cruzi, led to precise tandem gene replacements within the calmodulin-ubiquitin locus (Fig. 2). These constructs have been stable for over six months in the absence of G418. Replacement of one of the two copies of the calmodulin-ubiquitin associated gene (CUB; Fig. 2A) led to diminished ability of mammalian forms to grow in vivo and in vitro. Transfectants using this locus for gene replacement were too attenuated to use for in vivo studies. Replacement of two calmodulin genes (there are at least eight calmodulin genes in T. cruzi) did not attenuate growth of T. cruzi in epimastigote or mammalian forms. Thus, a construct was created to replace a tandem array of two calmodulin genes with the NEO gene and cytokine cDNA (Fig. 2B). Transfection with linear DNA from this construct was found to cause secretion of IL-2 or IFN-γ in epimastigote and mammalian forms in the absence of G418 pressure. The IL-2 and IFN-γ produced retain bioactivity, as assayed by stimulation of IL-2 dependent CTLL T cell proliferation and stimulation of IFN-γ dependent P88D.1 expression of Class II MHC antigen. The effects of the secretion of these cytokines by T. cruzi on acute and chronic infection are now being tested.

![Diagram](image-url)