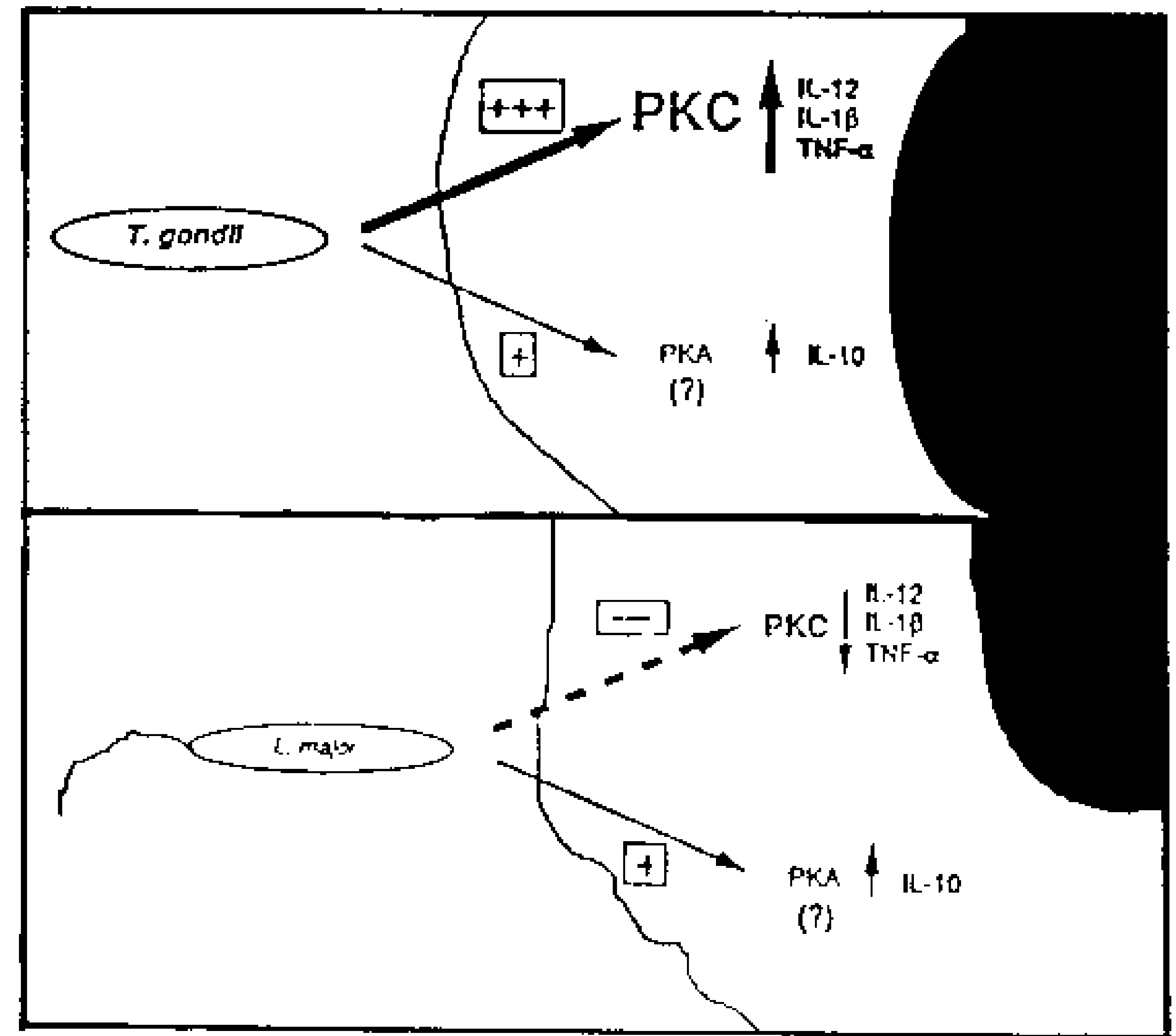
but not PKA activity are inhibited by infection with promastigote stages from *L. major.*

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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Fig. 1). Expression of the NEO gene allowed G418 selection of epimastigotes bearing the plasmid DNA. When the whole, supercoiled plasmid was trasfected 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 mIL-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 mIL-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 transfectants within six weeks, the supercoiled transfectants were deemed too unstable to 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) let do diminished ability of mammalian forms to grow *in vivo* and *in vitro*. Transfectants using this locus for gene replacement were too atenuated 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 P388D.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.

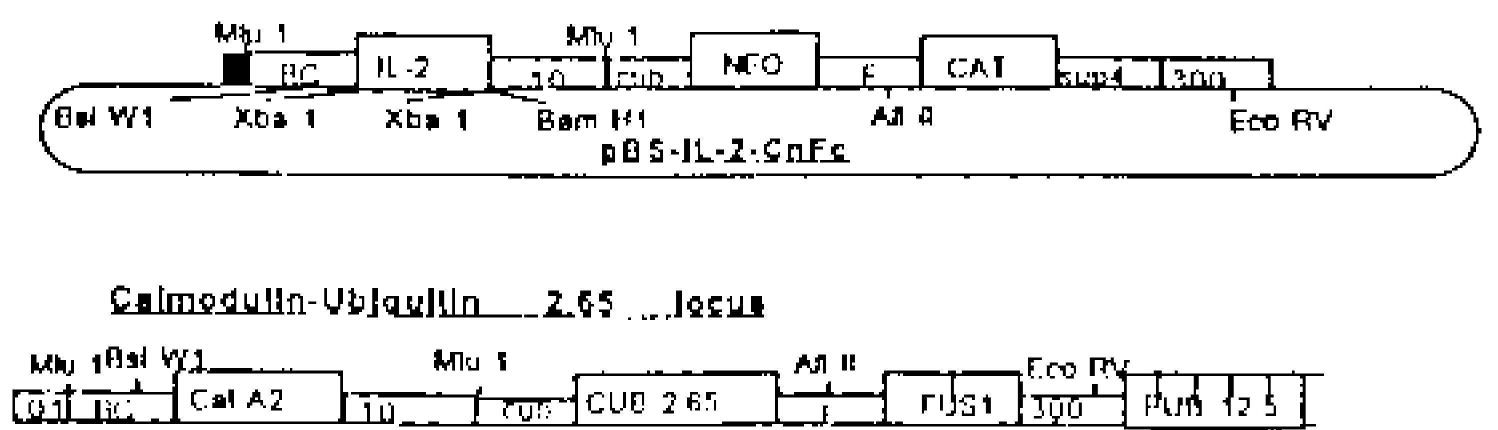

Fig. 1: at the top is plasmid construct for the plasmid vector (pBS-IL-2-CnFc) used to induce IL-2 secretion from *Trypanosoma cruzi* and at bottom a portion of the calmodulin-ubiquitin 2.65 gene locus. Genes and cDNAs are shown as tall rectangles, where as intergenic regions are shown as short rectangles. The plasmid is constructed such that the intergenic regions that normally flank the calmodulin A2 (Cal A2) gene flank IL-2 cDNA, flanking regions of calmodulin-ubiquitin associated gene (CUB 2.65) flank NEO, and flanking regions of ubiquitin fusion gene (FUS1) flank chloramphenicol acetyl transferase (CAT, used to detect transient expression).

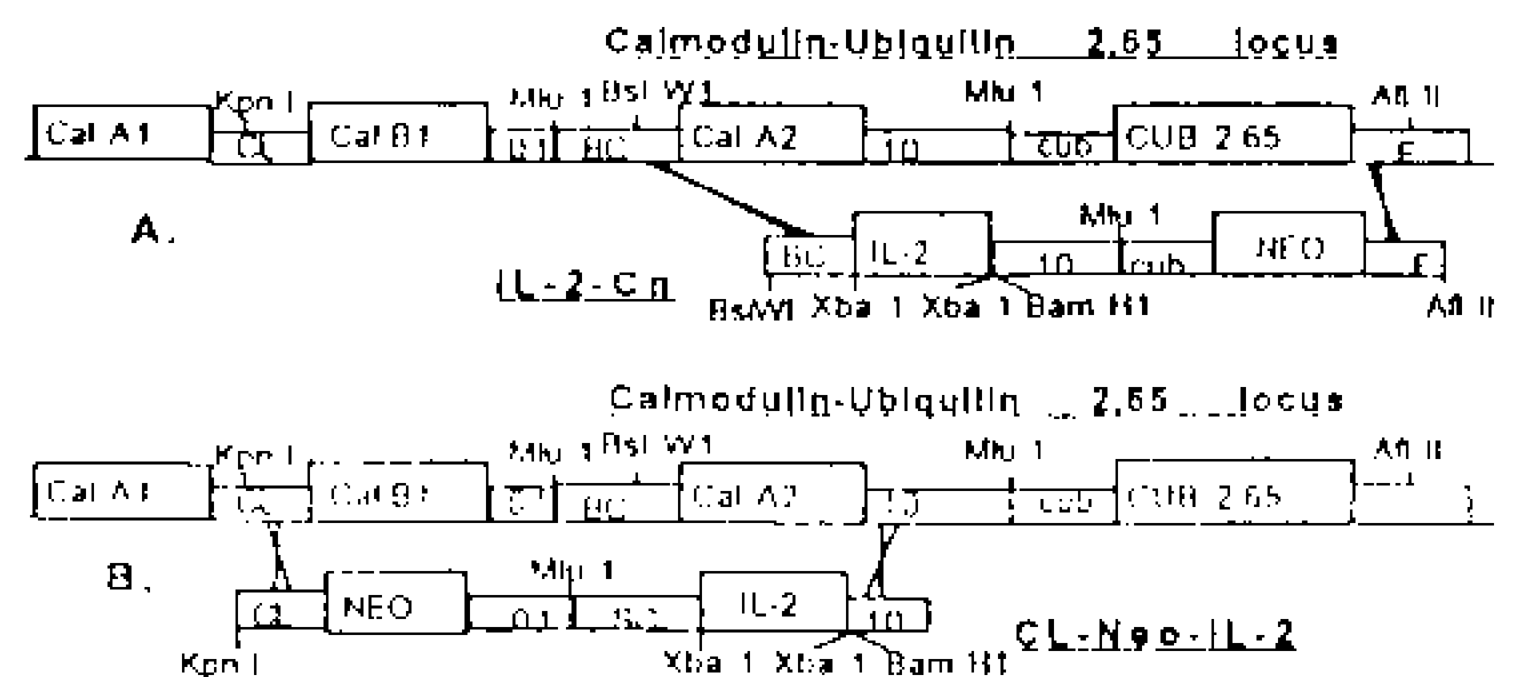

Fig. 2: two independent recombination events (A, B) detected with the linearized plasmid DNAs (bottom of A, B) and the calmodulin-ubiquitin locus (top of A, B). Note that the intergenic sequences appear to target the foreign genes precisely into the expected sites as a tandem array, and only rarely was NEO integration detected without cytokine expression.