**Mycobacterium tuberculosis** mannose-capped lipoarabinomannan can induce NF-κB-dependent activation of human immunodeficiency virus type 1 long terminal repeat in T cells

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Tuberculosis has emerged as an epidemic, extended by the large number of individuals infected with human immunodeficiency virus type 1 (HIV-1). The major goal of this study was to determine whether the mycobacterial cell wall component mannose-capped lipoarabinomannan (ManLAM) of *Mycobacterium tuberculosis* (*M. tuberculosis*) could activate transcription of HIV-1 in T cells with the use of an in vitro cell culture system. These experiments are of prime importance considering that CD4-expressing T lymphocytes represent the major virus reservoir in the peripheral blood of infected individuals. Using the 1G5 cell line harbouring the luciferase reporter gene under the control of the HIV-1 LTR, it was first found that culture protein filtrates (CFP) from *M. tuberculosis* or purified ManLAM could activate HIV-1 LTR-driven gene expression unlike similarly prepared CFP extracts devoid of ManLAM. The implication of protein tyrosine kinase(s), protein kinase A and/or protein kinase C was highlighted by the abrogation of the ManLAM-mediated activation of HIV-1 LTR-driven gene expression using herbimycin A and H7. It was also determined, using electrophoresis mobility shift assays, that *M. tuberculosis* ManLAM led to the nuclear translocation of the transcription factor NF-κB. *M. tuberculosis* ManLAM resulted in clear induction of the luciferase gene placed under the control of the wild-type, but not the κB-mutated, HIV-1 LTR region. Finally, the ManLAM-mediated activation of HIV-1 LTR transcription was found to be independent of the autocrine or paracrine action of endogenous TNF-α. The results suggest that *M. tuberculosis* can upregulate HIV-1 expression in T cells and could thus have the potential to influence the pathogenesis of HIV-1 infection.

Introduction

Tuberculosis remains one of the leading causes of death in humans, with an estimated 3 million deaths annually worldwide (Murray *et al*., 1990). This disease is due to *Mycobacterium tuberculosis* and causes enormous human and economic costs. The advent of AIDS in the early 1980s has dramatically changed the epidemiological data of tuberculosis. Indeed, there has been an increase in the incidence of infection with *M. tuberculosis* (Barnes *et al*., 1991), which was coincident with the human immunodeficiency virus (HIV) epidemic, the aetiological agent of AIDS.

The putative interactions between these two pathogens might reveal some clinical relevance considering that, in many developing countries, tuberculosis has emerged as the most common opportunistic disease associated with HIV-1 infection (Barnes *et al*., 1991; Bloom & Murray, 1992). Indeed, a direct interaction between both agents may occur since *M. tuberculosis*, which is a facultative intracellular parasite, infects the macrophage that also represents the predominant cell type productively infected with HIV-1 in tissues such as in the central nervous system, lymph nodes and lungs (Meltzer *et al*., 1990). The possibility that HIV-1 infection may promote the pathogenesis of *M. tuberculosis* is supported by several clinical studies in which HIV-1 has been shown to be predisposed to
the development of active tuberculosis following the initial mycobacterial infection (Daley et al., 1992) and to reactivate a latent infection (Selvyn et al., 1989). The cofactor role played by HIV-1 with regard to the pathogenesis of M. tuberculosis infection could be probably linked with the ability of HIV-1 to destroy the immune system and, more specifically, to impair cell-mediated immune responses.

Reciprocally, M. tuberculosis could also be seen as a potential cofactor for HIV-1 replication. In fact, results from clinical studies (Daley et al., 1992; Martin et al., 1995; Pape et al., 1993; Toossi et al., 1993; Wallis et al., 1993) and in vitro experiments (GollaPudi et al., 1994; Lederman et al., 1994; Peterson et al., 1995; Shatlock et al., 1994), in which viral gene expression was promoted in lymphoid cells following incubation with either purified protein derivatives (PPD) or phagocytosis of M. tuberculosis, have led to the idea that M. tuberculosis may accelerate HIV-1 disease progression in dually infected individuals. In addition, one of the major cell wall components of M. tuberculosis, the mannosace-capped lipoarabinomannan (ManLAM), has been shown to induce expression of cytokines such as tumour necrosis factor-α (TNF-α) in cells of monocytoid origin (Barnes et al., 1990; Moreno et al., 1989; Wallis et al., 1990). Interestingly, TNF-α has been reported to induce HIV-1 expression in cells of both T cell and monocytoid origin (Poli et al., 1990). Several in vitro studies have shown that TNF-α, a 17 kDa cytokine produced mainly by activated monocytes and macrophages, plays a pivotal role in the pathogenesis of HIV-1 (Fauci, 1990). More recently, ManLAM has been shown to activate transcription of the regulatory elements of HIV-1 (LTR) in monocytoid cells primarily via the transcription factor NF-κB (Zhang et al., 1995).

To the best of our knowledge, the effect of M. tuberculosis ManLAM on HIV-1 expression in T cells has not been previously investigated. Indeed, much of the effort into studying the existing interactions between M. tuberculosis and HIV-1 has been focused on cells of monocytoid origin. These experiments are crucial considering that CD4-positive T cells have been shown to be the major reservoir for HIV-1 in human peripheral blood of infected persons (McElrath et al., 1989; Schnittman et al., 1989; Spear et al., 1990). The aim of our study was thus to determine whether M. tuberculosis ManLAM could activate expression of HIV-1 in T cells. We found that incubation of T lymphoid cells with ManLAM led to activation of HIV-1 LTR-dependent gene expression. Such ManLAM-mediated activation of gene expression in T cells was mediated by the transcription factor NF-κB and was not associated with the secretion of the cytokine TNF-α.

Methods

Mycobacterial preparations. ManLAM, crude culture filtrate proteins (CFP) and ManLAM-free CFP (CFP-LAM) were isolated from M. tuberculosis virulent strain Erdman as previously described by Hunter et al. (1986) (kindly provided by J. T. Belisle, Colorado State University, Fort Collins, CO, USA). The ManLAM preparations contained less than 17.7 pg endotoxin/μg test reagents as determined by the Limulus ameboocyte assay.

Cell lines and plasmids. The human lymphoid T cell line Jurkat E6-1 (Weiss et al., 1984) was obtained from the ATCC (Rockville, MD, USA). 1G5 is a derivative of Jurkat E6-1 that contains a stably integrated HIV-1LTR–luciferase construct (Aguilar-Cordova et al., 1994). This cell line was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH (Rockville, MD, USA). Cells were maintained in complete culture medium made of RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS; Hyclone Laboratories), glutamine (2 mM), penicillin G (100 U/ml) and streptomycin (100 μg/ml). pLTRLUC and mutated NF-κB plasmids were kindly provided by K. L. Calame (Columbia University, NY, USA) and contain the luciferase reporter gene under the control of wild-type (GGGACTTTCC) or NF-κB-mutated (CTCAGCTTTC) HIV-1LTR, respectively (Henderson et al., 1995).

Transfection and cell treatments. To minimize variations in plasmid transfection efficiencies, 24 h after transfection, transiently transfected cells were pooled and separated into various treatment groups. Jurkat E6-1 cells (5–10 x 10⁶) were first washed once in a TS buffer (25 mM Tris–HCl pH 7.4, 5.0 mM KCl, 0.6 mM Na₂HPO₄, 0.5 mM MgCl₂ and 0.7 mM CaCl₂) and resuspended in 0.5–1 ml TS containing 1.0 μg of each plasmid (pLTRLUC or pmoBLTRLUC) and 500 μg/ml DEAE–dextran (final concentration). The cells/TS/plasmid/DEAE–dextran mix was incubated for 25 min at room temperature. Thereafter, cells were diluted at a concentration of 1 x 10⁶/ml using complete culture medium supplemented with 100 μM chloroquine (Sigma). After 45 min of incubation at 37 °C, cells were centrifuged, resuspended in complete culture medium and incubated at 37 °C for 24 h. Transiently (Jurkat E6-1) and stably (1G5) transfected cells were seeded at a density of 10⁶ cells per well (100 μl) in 96-well flat-bottom plates. Next, cells were left untreated or were treated for 72 h at 37 °C with ManLAM (1 and 10 μg/ml), CFP-LAM (1 and 10 μg/ml) and purified PHA (1 μg/ml; Wellcome). In some experiments, cells were pretreated for 60 min at 37 °C with subcytostatic and subcytotoxic concentrations of herbicym A (0.01, 0.1 and 1 μM; Gibco BRL). H7 (5 and 10 μM; Calbiochem) and anti-human NF-κB polyclonal neutralizing antibody (0.02, 0.2 and 2 μg/ml; R&D Systems). In most experiments, cells were incubated at 37 °C in a 5% CO₂ atmosphere for 72 h, after which luciferase activity was monitored. In appropriate experiments, cell proliferation was estimated by the MTS assay as previously described (Buttke et al., 1993).

Luciferase analysis. Luciferase activity was monitored as described previously (Bénubé et al., 1996). Briefly, following the appropriate incubation period, 100 μl of cell-free supernatant was withdrawn from each well and 25 μl of cell culture lysis buffer (25 mM Tris phosphate pH 7.8, 2 mM DTT, 1% Triton X-100 and 10% glycerol) was added before incubation at room temperature for 30 min. An aliquot of cell extract (20 μl) was mixed with 100 μl luciferase assay buffer (20 mM Tricine, 1.07 mM (MgCO₃)₂,Mg(OH)₂₅H₂O, 2.67 mM MgSO₄, 0.1 mM EDTA, 270 μM coenzyme A, 470 μM luciferin, 530 μM ATP and 33.3 mM DTT). The sample was introduced into the counting chamber of a standard liquid scintillation counter equipped with a single-photon monitor software (Beckman Instruments). Total photo events over 30 s were measured.

Preparation of nuclear extracts. 1G5 cells were either left untreated or were incubated for 72 h at 37 °C with purified PHA (1 μg/ml), CFP-LAM (10 μg/ml) and ManLAM (10 μg/ml). Incubation of 1G5 cells with various stimuli was terminated by the addition of ice-
cold PBS and nuclear extracts were prepared according to the microscale preparation protocol (Andrews & Faller, 1990). In brief, sedimented cells were resuspended in 400 μl of cold buffer A (10 mM HEPES pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT and 0.2 mM PMSF). After 10 min on ice, the lysate was vortexed for 10 s and samples were centrifuged for 10 s at 12000 g. The supernatant fraction was discarded and the cell pellet was resuspended in 100 μl of cold buffer B (20 mM HEPES-KOH pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT and 0.2 mM PMSF) and incubated on ice for 20 min. Cellular debris was removed by centrifugation at 12000 g for 2 min at 4 °C and the supernatant fraction was stored at −70 °C until assayed.

### EMSA

The electrophoresis mobility shift assay (EMSA) was performed with 7 μg of nuclear extracts. Protein concentrations were determined by the bicinchoninic assay with a commercial protein assay reagent (Pierce). Nuclear extracts were incubated for 30 min at 23 °C in 15 μl buffer C [100 mM HEPES pH 7.9, 40% glycerol, 10% Ficoll, 250 mM KCl, 10 mM EDTA, 250 mM NaCl, 2 μg poly(dI-dC), 10 μg nuclease-free BSA fraction V] containing 0.8 ng of 32P-5′-end-labelled dsDNA oligonucleotide. dsDNA (100 ng) was labelled with [γ-32P]ATP and T4 polynucleotide kinase in a kinase buffer (Biolabs). This mixture was incubated for 30 min at 37 °C and the reaction was stopped with 5 μl of 0.2 M EDTA. The labelled oligonucleotide was extracted with phenol–chloroform and passed through a G-50 spin column.

The dsDNA oligonucleotide, which was used as a probe or as a competitor, contained the consensus NF-xB binding site corresponding to the sequence 5′ ATGTGAGGGAGCTTCCAGGC 3′. A dsDNA oligonucleotide containing a mutated NF-xB binding site (in bold and underlined) was also used (5′ ATGTGACTCAGCTTCCAGGC 3′). Oligonucleotides were purchased from Santa Cruz Biotechnology. DNA–NF-xB complexes were resolved from free labelled DNA by electrophoresis in native 4% (w/v) polyacrylamide gels containing 50 mM Tris–HCl (pH 8.3), 200 mM glycine and 1 mM EDTA. The gels were subsequently dried and autoradiographed. Cold competitor assays were carried out by adding 1-, 10- and 100-fold molar excess of homologous, unlabelled dsDNA NF-xB oligonucleotide simultaneously with the labelled probe.

### Statistical analysis

Results shown are expressed as means ± standard deviations of triplicate samples. Statistical analysis of the differences between groups was first performed by ANOVA. If P values were less than 0.05, group comparisons were done using the Fisher least-significance difference post-hoc test. A P value less than 0.05 was considered significant. Calculations were made using Statview software.

## Results

### Activation of HIV-1 transcription in T cells by M. tuberculosis ManLAM

To examine directly the effect of *M. tuberculosis* CFP and ManLAM on transcription from the HIV-1 regulatory elements, we evaluated its capacity to induce reporter gene activity in 1G5, a T lymphoid cell line stably transfected with an HIV-1 LTR-driven luciferase construct (Aguilar-Cordova et al., 1994). The measurement of luciferase activity in 1G5 cells represents a convenient and sensitive indicator of inducible HIV-1 LTR promoter activity as the basal level of transcription of the luciferase gene is very low and a high responsiveness to several different stimuli such as mitogens and bacterial superantigens can be seen (Aguilar-Cordova et al., 1994; Schwartz et al., 1990).

We first set out to determine whether CFP from *M. tuberculosis* could directly activate HIV-1 LTR-driven gene transcription in the T cell line 1G5. As initially determined in monocytic-derived cell lines, no increase in HIV-1 LTR activity was apparent when CFP-treated 1G5 cells were measured in terms of luciferase activity within 48 h (data not shown). However, a weak but reproducible increase in luciferase activity in extracts of 1G5 cells treated with CFP for 72 h was apparent and showed a dose-dependent response giving optimal induction at concentrations of 50 and 100 μg/ml (1.4- and 1.6-fold induction, respectively; Fig. 1 A). Most interestingly, this induction was not observed in similarly pre-treated 1G5 cells when CFP samples devoid of ManLAM (CFP-LAM) were used instead (Fig. 1 B). To further substantiate the role played by ManLAM in the increased HIV-1 LTR activity obtained by treatment of 1G5 cells with CFP, a kinetic analysis was carried out to determine whether purified *M. tuberculosis* ManLAM could activate HIV-1 LTR transcription in 1G5 cells by measuring luciferase activity at different time-points. As
These experiments have been done several times. Results shown are the means ± standard deviations of triplicate samples.

**Fig. 2.** Activation of HIV-1 LTR transcription by M. tuberculosis ManLAM. 1G5 cells were either left untreated (●), or treated with PHA (1 µg/ml; ▲) or ManLAM (10 µg/ml; △) for the indicated time periods. The cell extracts were evaluated for luciferase activity using a scintillation counter.

**Fig. 3.** ManLAM-induced activation of HIV-1 LTR activity requires the participation of protein tyrosine kinases and PKA and/or PKC. (A) 1G5 cells were pretreated with several concentrations of herbimycin A (0·01, 0·1 and 1 µM) and were next incubated for 72 h in the absence or the presence of 10 µg/ml of ManLAM. (B) Cells were pretreated with H7 (5 and 10 µM) for 1 h prior to incubation for 72 h with ManLAM (10 µg/ml). The cell extracts were evaluated for luciferase activity using a scintillation counter. These experiments have been done several times. Results shown are the means ± standard deviations of triplicate samples. Asterisks indicate significant differences from untreated 1G5 cells (P < 0·01).

depicted in Fig. 2, stimulation of HIV-1 LTR-driven reporter gene activity decreased over time following treatment with purified PHA. On the other hand, a significant enhancement of HIV-1 LTR-driven reporter gene activity was only seen after 72 h of treatment with 10 µg/ml M. tuberculosis ManLAM. These data thus suggested that ManLAM was inducing HIV-1 LTR activity in an identical time kinetic to the induction driven by CFP.

**ManLAM-induced upregulation of HIV-1 LTR-driven gene expression is mediated via protein tyrosine kinases and PKA and/or PKC**

Next, to identify the intracellular second messengers responsible for the observed ManLAM-mediated activation of HIV-1 transcription, similar experiments were carried out in the presence of herbimycin A, a protein tyrosine kinase inhibitor which possesses a wide spectrum of activity. Treatment of 1G5 with different subcytostatic and subcytotoxic concentrations of herbimycin A (0·01, 0·1 and 1 µM), prior to the addition of ManLAM, resulted in a dose-dependent inhibition of ManLAM-induced activation of HIV-1 transcription (Fig. 3A). This suggests that protein tyrosine kinases are key elements in the signalling cascade initiated by M. tuberculosis ManLAM which results in an enhancement of HIV-1 LTR-driven gene expression. To further study the biochemical events generated in T cells by ManLAM, 1G5 cells were also treated with different subcytostatic and subcytotoxic concentrations of H7, a selective serine/threonine kinase inhibitor that can inhibit both protein kinase A (PKA, K_i = 3·0 µM) and protein kinase C (PKC, K_i = 6·0 µM). The ManLAM-induced activation of HIV-1 transcription was totally abolished by a pretreatment with H7 at concentrations sufficient to inhibit PKA, as well as PKC (5 and 10 µM) (Fig. 3B).

**NF-κB binding sites are necessary for M. tuberculosis ManLAM-induced activation of HIV-1 transcription**

Several studies have suggested that most of the agents that are potent inducers of HIV-1 replication act via activation of NF-κB (Clouse et al., 1989; Duh et al., 1989; Folks et al., 1987; Pomerantz et al., 1990a, b). Therefore, to investigate the potential contribution of the transcription factor NF-κB to the ongoing M. tuberculosis ManLAM-induced activation of HIV-1 transcription, the parental Jurkat E6-1 cell line was transiently transfected either with an HIV-1 LTR molecular construct linked to the luciferase reporter gene (HIV-1 LTR–LUC) or with its respective plasmid in which the two κB sites were mutated (HIV-1 mvBLTR–LUC). Treatment of transfected Jurkat E6-1 cells with M. tuberculosis ManLAM (10 µg/ml) resulted in the induction of luciferase activity when the cells were transfected with the HIV-1 LTR–LUC wild-type construct (Fig. 4), confirming our previous observations in 1G5 cells. The levels of HIV-1 LTR activation induced by PHA, TNF-α and M. tuberculosis ManLAM were comparable. However, none of the agents used to treat Jurkat E6-1 transfected with the deleted κB construct, including M. tuberculosis ManLAM, could activate HIV-1 LTR-mediated
Fig. 4. Requirement of the HIV-1 LTR NF-κB binding site for the ManLAM-induced effect. The luciferase-encoding HIV-1 plasmids containing wild-type (filled bars) or NF-κB-mutated HIV-1 LTR (open bars) were transfected into Jurkat E6-1 cells. After incubation for 72 h, cells were either left untreated or treated with PHA (1 µg/ml), TNF-α (2 ng/ml) or ManLAM (10 µg/ml). The cell extracts were evaluated for luciferase activity using a scintillation counter. These experiments have been done several times. Results shown are the means ± standard deviations of triplicate samples. Asterisks indicate significant differences from untreated 1G5 cells (P < 0.01).

Fig. 5. ManLAM induces nuclear translocation of NF-κB. Nuclear proteins were extracted from 1G5 cells incubated for 72 h with PHA, CFP-LAM or ManLAM and then incubated with 32P-labelled NF-κB probe to be finally resolved on a 4% polyacrylamide gel. Lane 1, free probe; lane 2, RAJI cells (first positive control); lane 3, 1G5 cells stimulated with 5 µg/ml PHA (second positive control); lane 4, untreated 1G5 cells (negative control); lane 5, 1G5 cells treated with 10 µg/ml CFP-LAM; lanes 6–10, 1G5 cells treated with 10 µg/ml ManLAM in the absence (lane 6) or the presence of a 1-, 10- and 100-fold molar excess of a cold wild-type NF-κB competitor (lanes 7–9). As a control, nuclear extracts from ManLAM-treated 1G5 cells were equally incubated with a radiolabelled oligonucleotide bearing a mutated NF-κB binding site (lane 10). The position of the specific complex bound by the κB site probe is indicated by an arrow.

Luciferase activity. Therefore, results from these experiments clearly indicate that activation of the HIV-1 LTR elements by \( \textit{M. tuberculosis} \) ManLAM is mediated via a signalling pathway involving the transcription factor NF-κB.

**ManLAM leads to the activation of NF-κB in T cells**

Next, we performed band-shift assays to confirm the participation of the transcriptional factor NF-κB. At 72 h after exposure to \( \textit{M. tuberculosis} \) ManLAM, 1G5 cells were harvested, nuclear proteins were extracted, and EMSA analysis was performed with labelled NF-κB oligomers. As shown in Fig. 5, the NF-κB complex was absent from untreated (lane 4) and CFP-LAM-treated 1G5 cells (lane 5), while it was present in PHA- (lane 3) and ManLAM-stimulated cells (lane 6). This band was shown to co-migrate with a similar signal observed in RAJI cells (lane 2) and could be successfully competed by increasing levels of free wild-type oligonucleotides (lanes 7–9). As a control, nuclear extracts from ManLAM-treated 1G5 cells were equally incubated with a radiolabelled oligonucleotide bearing a mutated NF-κB binding site (lane 10). The position of the specific complex bound by the κB site probe is indicated by an arrow.

Induction of HIV-1 transcription by ManLAM is not mediated through endogenous TNF-α secretion

\( \textit{M. tuberculosis} \) ManLAM has been reported to induce the release of TNF-α by mononuclear phagocytic cells (Barnes et
ManLAM, the purified form of the cell wall component of tuberculosis when using an history of PPD positivity, but not from PPD-negative donors tuberculosis was shown to enhance replication of HIV-1 only in LTR activity indicates that TNF-α induction.

Discussion

Several studies have led to speculation that M. tuberculosis can act as a cofactor by increasing the likelihood of progression from the asymptomatic carrier state to HIV-1-related illness. This postulate is based on the observation that M. tuberculosis is associated with T cell activation and cytokine production (Flesch & Kaufman, 1993), two components of the immune response that have been shown in vitro to promote HIV-1 replication (Fauci, 1993). The concept that M. tuberculosis plays an important role in the pathogenesis of HIV-1 infection has further been confirmed by several laboratory and clinical studies. For example, incubation with M. tuberculosis or PPD, a culture filtrate of M. tuberculosis, resulted in activation of HIV-1 transcription and expression in T lymphoid and monocyteid cells (Lederman et al., 1994; Shatlock et al., 1993). Zhang et al. (1995) have reported that live M. tuberculosis and, to a lesser extent, its cell wall component (ManLAM) can upregulate HIV-1 replication in monocyteid cell lines, primarily via an NF-κB-dependent signalling pathway. In another report, M. tuberculosis was shown to enhance replication of HIV-1 only in primary mononuclear cells from healthy donors with a previous history of PPD positivity, but not from PPD-negative donors when using an in vitro acute virus infection model (Goletti et al., 1996). In addition, human monocytes from patients with tuberculosis have been demonstrated to be more susceptible to productive HIV-1 infection (Toossi et al., 1993). An observational study suggested that active tuberculosis may promote progression of HIV-1. Indeed, immune activation markers were found to be increased in HIV-1-infected individuals with active tuberculosis as compared with virally infected persons without active tuberculosis, or HIV-1-seronegative subjects with active tuberculosis (Wallis et al., 1993). In support of this notion, Goletti et al. (1996) reported a 5–160-fold increase in plasma virus load in HIV-1-infected individuals during the acute phase of M. tuberculosis. Altogether, results from these studies are very indicative of the potentiating effect on HIV-1 replication mediated by M. tuberculosis.

Our experiments were designed to determine whether ManLAM, the purified form of the cell wall component of M. tuberculosis, can regulate HIV-1 LTR activity in T cells. Results from our experiments showed that ManLAM increases HIV-1 LTR-driven gene expression. The induction observed with ManLAM was found to be more drastic than the one measured with complete CFP, requiring 10-fold higher contents of extract and resulting in a weaker signal, although both were found to optimally activate HIV-1 LTR at 72 h after the start of treatment. This is most likely a consequence of lower ManLAM levels in these extracts and thus provides further evidence for the direct implication of ManLAM. Accordingly, we found that ManLAM-free CFP failed to stimulate HIV-1 LTR activity in all tested concentrations at all measured time-points. Intracellular second messengers were scrutinized and protein tyrosine kinases were found to be key elements in the ManLAM-mediated activation of HIV-1 LTR transcription. Moreover, we determined that PKA and/or PKC were also involved in the ManLAM-mediated activation of HIV-1 LTR transcription.

Inhibitory concentrations of antibodies against TNF-α could be responsible for the activation of HIV-1 replication induced by ManLAM in T cells. In Fig. 6, a comparison between TNF-α- and ManLAM-induced HIV-1 LTR activity indicates that TNF-α retains a higher level of induction.

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clinical relevance of this cytokine in HIV-1-infected individuals has been provided by the detection of increased levels of TNF-α secretion in HIV-1-infected individuals (Reddy et al., 1988); and, finally, (5) *M. tuberculosis* has been reported to act as a strong inducer of TNF-α production following infection of human and murine macrophages (Barnes et al., 1992; Moreno et al., 1989; Roach et al., 1993; Zhang et al., 1993). To our surprise, we found that ManLAM-induced activation of HIV LTR-driven luciferase activity was independent of the secretion of TNF-α by T cells (Barnes et al., 1992; Moreno et al., 1989; Roach et al., 1993; Zhang et al., 1993). A similar observation was made by Lederman et al. (1994) who showed an NF-κB-mediated TNF-α-independent activation of HIV-1 expression in T lymphoid cells. However, it should be emphasized that these experiments were carried out in the presence of the viral transactivating protein Tat (as opposed to our studies) and used a culture filtrate of *M. tuberculosis* (i.e. PPD), which might contain other components than ManLAM. Furthermore, the maximal activation of the regulatory elements of HIV-1 was seen after 24 h of incubation with PPD as opposed to a 72 h incubation period in our experiments with ManLAM. These discrepancies might be attributed to the viral Tat protein and/or differences in *M. tuberculosis*-derived preparations (PPD vs ManLAM).

In summary, the results of these in vitro studies support the concept that *M. tuberculosis* activates HIV-1 replication in T cells. The clinical relevance of the findings of this work is highly pertinent to HIV-1-infected patients with tuberculosis as it suggests that this mycobacteria may activate latently infected T cells and thus promote the pathogenesis of HIV-1. However, the relative importance of ManLAM-mediated upregulation of HIV-1 LTR activity, compared to that induced by an activated T cell response to *M. tuberculosis*, remains to be defined with regard to the overall pathogenesis of the disease. Although ManLAM has been previously shown to induce the release of TNF-α by cells of monocytoid origin, we did not find evidence suggesting that this is the case in the studied T lymphoid cells. We are currently identifying the *M. tuberculosis* ManLAM-induced factor(s) in T cells that is responsible for the upregulation of HIV-1 LTR activity. Further studies are essential to supplement our knowledge of the complex uni- or bidirectional interactions existing between *M. tuberculosis* and HIV-1 in dually infected patients. This may lead to the development of more appropriate and efficient therapeutic strategies aimed at controlling the dual infection.

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