Thalidomide Inhibits Lipoarabinomannan-Induced Upregulation of Human Immunodeficiency Virus Expression

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Received 30 June 1995/Returned for modification 5 September 1995/Accepted 5 October 1995

Mycobacterium tuberculosis accelerates the progression of human immunodeficiency virus type 1 (HIV-1) infection. The results of this study, which show that thalidomide inhibits the upregulation of HIV-1 expression in U1 cells stimulated with mycobacterial lipoarabinomannans, support the rationale behind conducting controlled trials of this immunomodulatory agent with patients dually infected with HIV-1 and M. tuberculosis.

Although it is well established that human immunodeficiency virus type 1 (HIV-1) infection promotes the pathogenesis of Mycobacterium tuberculosis, it also has been proposed that M. tuberculosis accelerates the progression of HIV-1 infection. This idea is supported by clinical studies (5, 9, 10, 18–20) and by in vitro experiments which have demonstrated that phagocytosis of tubercle bacilli by HIV-1-infected monocyte lines induces viral expression (4, 7, 17, 22). Also, in a recent study transcription of the HIV-1 long terminal repeat has been shown to be induced by the mycobacterial cell wall component lipoarabinomannan (LAM) (22). LAM is known to stimulate tumor necrosis factor alpha (TNF-α) production by mononuclear phagocytes (1, 2, 21), and this cytokine appears to play a part in the upregulation of HIV-1 expression by M. tuberculosis (7, 22).

Thalidomide (α-N-phthalimidoglutaramide), a sedative removed from the market because of its severe teratogenic effects, recently has been shown to be a potent inhibitor of TNF-α production by lipopolysaccharide (LPS)-stimulated blood monocytes (16) and by tissue macrophages stimulated with LAM (14). This anti-TNF-α property appears to underlie many of thalidomide’s immunomodulatory activities, including its therapeutic effect in aphthous ulcers in HIV-1-infected patients (11) and its antiviral activity in HIV-1-infected cell lines (8). On the basis of these observations with thalidomide and the proposed reciprocal relationship between M. tuberculosis and HIV-1, we tested the hypothesis that thalidomide inhibits the upregulation of HIV-1 expression in chronically infected promoncytoid (U1) cells stimulated with LAM.

Upregulation of HIV-1 expression by LAM. Initial studies were performed to characterize the effect of LAM on the upregulation of HIV-1 expression in U1 cells (a subclone of U-937 promonocyte cells chronically infected with HIV-1, provided by the National Institute of Allergy and Infectious Diseases Reference Reagent Program). U1 cells express low levels of virus constitutively but produce abundant amounts of HIV-1 when stimulated with TNF-α (15). U1 cells were cultured in RPMI medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with 2 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10% (vol/vol) heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, Utah) containing <1 ng of lipopolysaccharide per ml. Two mycobacterial LAMs were provided by Patrick J. Brennan and John Belisle: a mannansylated LAM (ManLAM) isolated from the virulent M. tuberculosis Erdman strain, and an arabinofuranosyl-terminated LAM (AraLAM) isolated from a rapidly growing, atypical Mycobacterium species (the LPS contamination of each of the LAM preparations was <25 pg/μg). ManLAM is capped at the nonreducing termini of the molecule, whereas AraLAM has been shown to be devoid of any mannose caps at the nonreducing termini (2). ManLAM and AraLAM were added to U1 cell cultures (5 × 10^6 cells), and after 4 days of incubation, supernatants were harvested for assessment of HIV-1 expression by quantifying the amount of HIV antigen by an enzyme-linked immunosorbent assay that detects mainly HIV-1 p24 antigen with a sensitivity of 30 pg/ml (Abbott Laboratories, North Chicago, Ill.) (12). Previous experiments have shown that reverse transcriptase inhibitors have no effect on upregulation of HIV-1 expression in U1 cell cultures, whereas viral expression is markedly suppressed by an HIV-1 protease inhibitor (13).

Both ManLAM and AraLAM induced the upregulation of HIV-1 expression in U1 cells in a dose-dependent manner (Fig. 1). Of the two LAMs tested, AraLAM was considerably more potent (approximately 20-fold) as a stimulus of viral expression (means ± standard errors of the mean, 1,400 ± 91 versus 44,700 ± 1,700 pg of p24 antigen per ml in U1 cell cultures stimulated with 200 μg of ManLAM and AraLAM, respectively). The finding that AraLAM stimulated the upregulation of viral expression in U1 cells is consistent with the recent report of induction of the transcription of the HIV-1 long terminal repeat in transfected THP-1 cells by LAM isolated from another atypical mycobacterial species (22). The marked difference in the potencies of ManLAM and AraLAM in the U1 cell culture system parallels the results of previous studies of the TNF-α-inducing capacities of these LAMs in macrophage cultures (1, 2, 21). That the effects of ManLAM and AraLAM on HIV-1 expression in U1 cells were not due to LPS contamination is supported by the finding that at 20 μg/ml, polymyxin B (Sigma Chemical Co., St. Louis, Mo.), an antibiotic which binds LPS, had no effect on the virus-inducing capacities of either LAM (data not shown). Also, LPS (catalog no. 0111B4; Sigma) at 1 μg/ml, a concentration greatly exceeding the levels found in both LAM preparations, had no appre-
ciable effect on HIV-1 expression in U1 cells, as has been reported previously (12).

To investigate the involvement of TNF-α in the upregulation of HIV-1 expression in LAM-stimulated U1 cells, anti-TNF-α antibodies (20 μg/ml; R & D Systems, Minneapolis, Minn.) were added to the U1 cell cultures 30 min prior to the addition of ManLAM (50 μg/ml) and AraLAM (2 μg/ml). AraLAM was used at a lower concentration than was ManLAM in this and all subsequent experiments in an attempt to achieve comparable levels of HIV-1 expression following stimulation by the two LAMs. In contrast to the absence of an effect of a nonspecific control antibody (20 μg/ml) and anti-interleukin-6 antibody (20 μg/ml; R & D Systems), anti-TNF-α inhibited LAM-induced HIV-1 expression (Fig. 2A). Furthermore, addition of a monoclonal antibody specific for CD14 receptors, the binding sites on monocytes for LAM-induced TNF-α production (21), completely blocked ManLAM- and AraLAM-stimulated HIV-1 expression in the U1 cell cultures (Fig. 2B). Neither anti-TNF-α nor anti-CD14 antibodies had an effect on U1 cell viability (assessed by trypan blue dye exclusion), indicating that the inhibitory activities of these antibodies on HIV-1 expression were not due to cytotoxic mechanisms. Thus, agents which interfere with the ability of LAM to induce TNF-α production in U1 cells are capable of suppressing the upregulation of HIV-1 expression.

**Inhibitory activity of thalidomide.** The hypothesis that thalidomide inhibits the upregulation of HIV-1 expression in LAM-stimulated U1 cells was tested by adding various concentrations (2.5, 25, and 100 μg/ml) of (±)-thalidomide (Research Biochemicals International, Natick, Mass.) simultaneously with ManLAM (50 μg/ml) or AraLAM (2 μg/ml) to U1 cultures. At 2.5 μg/ml, thalidomide had little effect on LAM-induced expression of HIV-1; however, at both of the higher concentrations, significant inhibition was observed (Fig. 3). At 25 μg/ml, thalidomide inhibited ManLAM- and AraLAM-stimulated viral expression by 66% and 51%, respectively; at 100 μg/ml, thalidomide completely suppressed the upregulation of HIV-1 by both LAMs. U1 cell cultures incubated for 4 days with ManLAM or AraLAM, in the presence or absence of thalidomide, showed no significant loss of cell viability compared with that of unstimulated control cells (assessed by trypan blue dye exclusion), suggesting that the antiviral activity of thalidomide was not due to a cytotoxic effect.

Taken together, the results of this in vitro study with ManLAM support the concept that *M. tuberculosis* promotes HIV-1 infection and that agents which suppress TNF-α production may intervene in this process. Although we did not directly demonstrate that the suppressive effect of thalidomide on LAM-induced upregulation of HIV-1 expression in U1 cells

![FIG. 1. Effects of LAMs on HIV-1 expression. ManLAM (A) and AraLAM (B) were added to U1 cell cultures at the indicated concentrations, and after 4 days of incubation, supernatants were assayed for HIV-1 p24 antigen levels. Data are means of values from duplicate samples and are representative of three separate experiments.](image)

![FIG. 2. Effect of TNF-α blockade on LAM-induced HIV-1 expression. (A) Antibodies (20 μg/ml) to TNF-α or interleukin-6 (IL-6) were added to U1 cell cultures stimulated with ManLAM (50 μg/ml) or AraLAM (2 μg/ml) for 4 days. (B) Antibodies (5 μg/ml) to CD14 receptors or nonspecific immunoglobulin (Ig) G isotype control antibodies were added to U1 cell cultures stimulated with ManLAM (50 μg/ml) or AraLAM (2 μg/ml) for 4 days. Data with medium alone reflect constitutive HIV-1 expression. Data are means of values from duplicate samples and are representative of three separate experiments.](image)
weakened due to the inhibitory effect of this drug on TNF-α production (8, 14, 16), this mechanism seems most likely. Thalidomide is presently under investigation for use in the treatment of HIV infection and tuberculosis. The clinical relevance of the findings in this study with a chronically infected promonocyte clone (U1 cells) is unknown. Levels of thalidomide in serum are generally less than 10 μg/ml, and we found that 2.5 μg/ml had no effect in the U1 cell culture system. It is possible, however, that thalidomide could reach inhibitory concentrations in critical tissue sites, such as the central nervous system. Also, the anti-HIV-1 effects of thalidomide on acutely infected mononuclear phagocytes were not examined, and these cells could react differently to LAM and thalidomide. While our mononuclear phagocytes were not examined, and these cells could react differently to LAM and thalidomide, it is possible that drugs which alter TNF-α production, such as thalidomide, would have an adjunctive therapeutic role in this dual infection.

We thank Stacey Larson for assistance in the preparation of the manuscript.

This study was supported in part by Public Health Service grant DA-04381 and NIAID contract N01-A1-25147.

REFERENCES


