Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 5974–5978, July 1993
Medical Sciences

Thalidomide inhibits the replication of human immunodeficiency virus type 1

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Communicated by Zanvil A. Cohn, March 17, 1993

ABSTRACT **Thalidomide, a selective inhibitor of tumor necrosis factor α (TNF-α) synthesis, suppresses the activation of latent human immunodeficiency virus type 1 (HIV-1) in a monocytoid (U1) line. The inhibition is dose dependent and occurs after exposure of the cells to recombinant TNF-α, phorbol myristate acetate, lipopolysaccharide, and other cytokine combinations. Associated with HIV-1 inhibition is a reduction in agonist-induced TNF-α protein and mRNA production. Thalidomide inhibition of virus replication in the phorbol myristate acetate- and recombinant TNF-α-stimulated T-cell line ACH-2 is not observed. The presence of thalidomide also inhibits the activation of virus in the peripheral blood mononuclear cells of 16 out of 17 patients with advanced HIV-1 infection and AIDS. These results suggest the use of thalidomide in a clinical setting to inhibit both virus replication and the TNF-α-induced systemic toxicity of HIV-1 and opportunistic infections.**

The human immunodeficiency virus type 1 (HIV-1) infects T cells and monocytes in both a latent and productive manner (1–3). Replication of HIV-1 in cell lines and in cells from infected patients often requires an agonist to activate the cell and its latent virus (4). Cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin (IL) 3, and IL-6 upregulate virus expression, whereas tumor necrosis factor α (TNF-α) is a particularly potent activator (5–9).

To better understand the role of TNF-α in HIV-1 activation, we have studied the effects of a selective inhibitor of this cytokine. We have recently shown that thalidomide (N^{α}-pthalimidoglutarimide) selectively inhibits the production of human peripheral blood mononuclear cell (PBMC) TNF-α when these cells are induced *in vitro* with lipopolysaccharide (LPS) and other agonists (10). Thalidomide has also been shown to reduce serum TNF-α levels in lepromatous leprosy patients with erythema nodosum leprosum, in association with the disappearance of the clinical symptoms (11).

Studies were conducted on chronically HIV-1-infected, promonocytic U1 (12) and T-lymphocytic ACH-2 (8, 13) cell lines, as well the peripheral blood cells of HIV-1$^+$ patients. In this manuscript, HIV-1 activation in response to a number of stimuli was evaluated and correlated with the production of TNF-α by these cells. We demonstrate that thalidomide inhibits TNF-α mRNA levels and TNF-α protein as well as the expression of HIV-1 in infected cell lines. Thalidomide was also shown to inhibit HIV-1 activation in PBMCs of infected patients.

MATERIAL AND METHODS

Chronically HIV-1-Infected Cell Lines. The promonocytic cell line U1 and the T-lymphocytic cell line ACH-2 were obtained from the AIDS Research and Reference Reagent Program (AIDS Research and Reference Reagent Program Catalog, ERC Bio Services, Rockville, MD). The cell line U1 was produced by infection of U937 cells with HIV-1 (LAV) (12). The ACH-2 cell line was produced by infection of A3.01 cells with HIV-1 (8, 13). Cells were grown in RPMI 1640 medium (Mediatech, Cellgro, Herndon, VA) supplemented with 10% (vol/vol) fetal bovine serum (FBS) (GIBCO), 2 mM glutamine, penicillin at 100 units/ml, and streptomycin at 100 μg/ml (GIBCO) (complete medium).

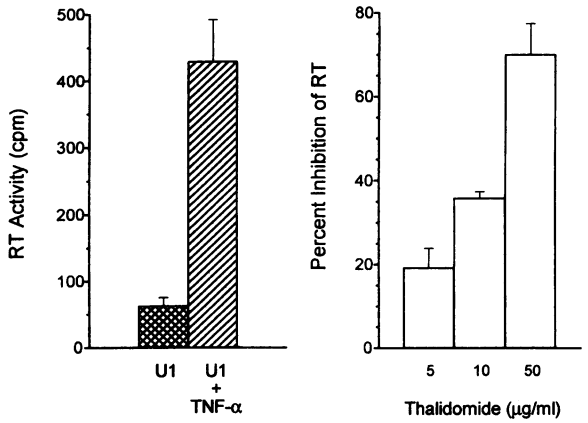

FIG. 1. Inhibition of HIV-1 activation in U1 cells by thalidomide. HIV-1 activation was induced by incubating U1 cells with recombinant TNF-α at 1 ng/ml. Thalidomide was added in RPMI with 0.2% DMSO. Results are expressed as mean cpm ± SEM for the stimulant (▨) and a medium/DMSO control (▩) (*Left*) and percent inhibition of RT in the presence of different concentrations of thalidomide (□) (*Right*) in five different experiments. When U1 cells (2×10^5/ml) were cultured for 48 h in RPMI/FCS with 0.2% DMSO alone or with thalidomide (50 μg/ml), no difference in numbers of cells ($112 \pm 2 \times 10^5$ vs. $125 \pm 4 \times 10^5$ cells per ml), [^{3}H]thymidine incorporation into cells (1.5×10^5 vs. 1.4×10^5 cpm), as well as viability (98% ± 3% vs. 95% ± 5%), was observed (mean of four experiments).

HIV-1 Induction and Inhibition in Cell Lines. U1 or ACH-2 cells in logarithmic growth were washed with RPMI medium and resuspended at 2×10^5 cells per ml in complete medium with 10 mM Hepes and 50 μM 2-mercaptoethanol. The cells were stimulated with inducers (see below) for 48 h. When the drug was tested for inhibitory activity, it was added to the culture 1 h before stimulation and once more after overnight incubation (24 h). Supernatants were harvested after 48 h of

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Abbreviations: Ag, antigen; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; GM-CSF, granulocyte–macrophage colony-stimulating factor; HIV-1, human immunodeficiency virus type 1; IL, interleukin; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; PMA, phorbol 12-myristate 13-acetate; RT, reverse transcriptase; TNF-α, tumor necrosis factor α.

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Table 1. Effect of thalidomide on agonist-induced RT activity in U1 cells

Stimulator*	*n*	RT activity,† cpm		% inhibition of RT in the presence of thalidomide‡		
		− stim	+ stim	5 μg/ml	10 μg/ml	50 μg/ml
PMA	4	58 ± 8	673 ± 63	32.9	48.4	66.7
rGM-CSF + LPS	5	49 ± 3	265 ± 65	28.5	30.1	51.0
rGM-CSF + rIL-6	4	54 ± 6	311 ± 108	20.9	40.3	59.6
rIL-6 + LPS	4	54 ± 6	270 ± 48	−11.2	6.3	44.9
rIL-6 + rIL-3	3	55 ± 8	184 ± 48	18.5	53.1	67.9

n, Number of experiments; r, recombinant.
*The concentrations of stimulators are given in *Materials and Methods*. Recombinant GM-CSF, recombinant IL-6, recombinant IL-3, and LPS alone did not induce HIV-1 activation. RT activity was only observed when the cytokines and bacterial cell wall products were combined as shown.
†RT activity expressed as the mean cpm ± SEM obtained in the number of experiments indicated in the absence (− stim) or presence (+ stim) of stimulator.
‡Thalidomide inhibition of agonist-induced RT activity at the drug concentrations shown. Results are expressed as the mean percent inhibition for the number of experiments indicated.

total incubation and tested for the presence of Mg^{2+}-dependent reverse transcriptase (RT) activity.

HIV-1 Inducers. Phorbol 12-myristate 13-acetate (PMA; 0.1 μM) (Sigma), recombinant TNF-α (1 ng/ml) (Genzyme), recombinant human IL-6 (200 units/ml) (Genzyme), recombinant IL-3 (1000 units/ml) (Genzyme), recombinant GM-CSF (450 units/ml) (Sandoz Pharmaceutical), and LPS (1 μg/ml) (List Biological Laboratories, Campbell, CA) were used as stimulators. Stock solutions were diluted with 10% FBS in RPMI 1640 to optimal concentrations.

Inhibitor. Thalidomide [the racemic mixture D- (+) and L- (−) forms] (lot no. 1055/8) was obtained from Grunenthal GMBH (Stolberg, Germany). The compound was dissolved in dimethyl sulfoxide (DMSO) (Sigma) at 20 mg/ml, and further dilutions were made in sterile RPMI with 10% or 20% FBS at pH 4.0.

Viral RT Assay. The RT activity of culture supernatants was measured by the method of Willey *et al.* (14). Briefly, 10 μl of culture supernatant was mixed with 50 μl of an RT reaction mixture containing 5 units of template primer poly(rA)·$(dT)_{12-18}$ (Pharmacia LKB) per ml in 50 mM Tris, pH 7.8/7.5 mM KCl/2 mM dithiothreitol/5 mM $MgCl_2$/0.05% Nonidet P-40/^{32}P-labeled dTTP (Amersham) at 10 μCi/ml (1 Ci = 37 GBq). The mixture was incubated for 90 min at 37°C and spotted onto DE81 ion-exchange chromatography paper (Whatman), and the radioactivity was measured as described (14). The percent inhibition of RT = 100 × [1 − (cpm of stimulator with inhibitor − cpm of medium with inhibitor)/(cpm of stimulator without inhibitor − cpm of medium without inhibitor)].

Table 3. Inhibition of RT activity and TNF-α production in agonist-induced U1 cells

Stimulator	Thalidomide, μg/ml	RT activity*		TNF-α†	
		cpm	%‡	pg/ml	%§
PMA	0	851	100	780	100
	5	593	70	300	38
	10	458	54	0	0
	50	335	39	0	0
rIL6 + LPS	0	251	100	200	100
	5	268	108	170	85
	10	235	92	100	50
	50	156	60	10	5

r, Recombinant.
*RT activity in triplicate culture supernatants was evaluated at 48 h. The RT value (mean ± SEM) in the absence of agonist stimulation was 52 ± 41 cpm.
†TNF-α activity in triplicate culture supernatants evaluated at 20 h.
‡Percent of maximal RT activity.
§Percent of maximal TNF-α production.

Viral p24 Antigen (Ag) Assay. Cell culture supernatants were assayed for p24 Ag activity by using the DuPont HIV-1 p24 core profile ELISA kit (DuPont/NEN). The absorbance of each sample was determined at 490 nm with an MR 700 microplate reader (Dynatech). Optical densities (OD) for duplicate samples were averaged. Percent inhibition of p24 Ag = 100 × [1 − (mean OD with DMSO and inhibitor/mean OD with DMSO)].

TNF-α Assay. Cell culture supernatants were assayed in triplicate for the amount of TNF-α by using an ELISA kit (Endogen, Boston) as described by the manufacturer.

HIV-1$^+$ Patients. After informed consent was obtained, a total of 17 HIV-1-seropositive adult participants were seen either as outpatients or inpatients at The Rockefeller University Hospital or as inpatients at Bellevue Hospital or New York Hospital. The patients were predominantly male, aged 25–49, and in Centers for Disease Control class II–IV (15, 16). Patients with lymphomas were excluded. Most patients were on the antiretroviral agents zidovudine, didanosine, or both (see Table 4).

Isolation of PBMCs. Approximately 60 ml of heparinized blood was obtained from each patient, and PBMCs were separated on Ficoll-Paque gradients (Pharmacia LKB). The cells were suspended in complete medium with 5% (vol/vol) IL-2 (Pharmacia LKB) and 20% FBS.

HIV-1 Culture and Quantitation. HIV-1 culture and quantitation were done by a modification of previously described methods (17–19). Normal lymphoblasts were prepared by cultivating normal PBMCs isolated from a buffy coat (The New York Blood Center) in complete medium with 20% FBS and 1% phytohemagglutinin P (Difco) at 10^6 cells per ml for 48 h. Freshly isolated patient PBMCs (4×10^6) were cocultured with 2×10^6 normal lymphoblasts, at a final concen-

Table 2. Effect of thalidomide on agonist-induced RT activity in ACH-2 cells

Stimulator	*n*	RT activity in the presence of thalidomide,* cpm			
		0 μg/ml	5 μg/ml	10 μg/ml	50 μg/ml
rTNF-α	4	725 ± 37	634 ± 52 (13)	586 ± 49 (19)	612 ± 30 (16)
PMA	3	708 ± 32	713 ± 113 (0)	642 ± 54 (9)	582 ± 76 (18)
None	4	73 ± 7	74 ± 11	77 ± 11	77 ± 13

n, Number of experiments; r, recombinant.
*RT activity is expressed as mean cpm ± SEM. The percent inhibition of RT activity is shown in the parentheses. ACH-2 cells cultured for 48 h in RPMI/FBS with 0.2% DMSO alone or with thalidomide at 50 μg/ml demonstrated similar numbers (13.1×10^5 vs. 15.0×10^5 cells per ml), similar [^{3}H]thymidine incorporation (19.0×10^5 vs. 24.0×10^4 cpm), and similar viability (100% vs. 99%) (mean of four experiments).

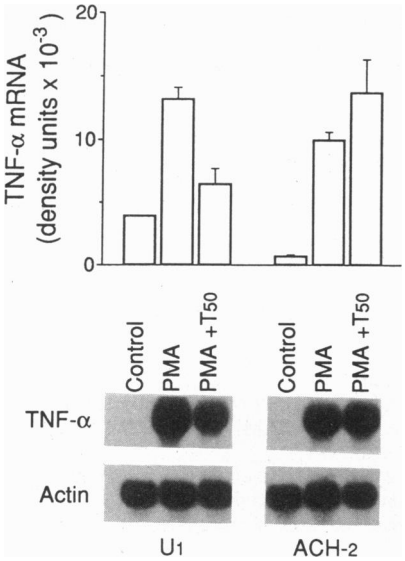

FIG. 2. Effect of thalidomide on TNF-α mRNA expression in U1 and ACH-2 cells stimulated with 0.1 μM PMA as analyzed by Northern blot hybridization. Cells were incubated for 10 h without PMA (Control), with PMA alone (PMA), or in the presence of PMA and thalidomide at 50 μg/ml (PMA + T_{50}). Gels were loaded with 20 μg of RNA per lane. The autoradiography of the Northern blot (*Lower*) and the densitometric analysis of the gel (*Upper*) are shown. The density of the TNF-α mRNA was standardized to the density of β-actin mRNA for each lane in the same blot.

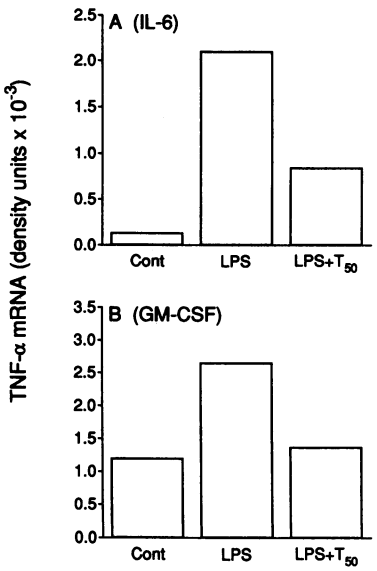

FIG. 3. Effect of thalidomide on TNF-α mRNA levels in U1 cells induced with IL-6 (200 units/ml) plus LPS (1 μg/ml) for 6 h (*A*) or with GM-CSF (450 units/ml) for 24 h and then with LPS (1 μg/ml) for 6 h (*B*). Control cells (Cont) were incubated in medium alone. Agonist-stimulated cells were incubated with the growth factor and LPS alone (LPS) or with thalidomide (50 μg/ml) added to the cultures (LPS + T_{50}). All cultures contained 0.25% DMSO. Results are densitometric analyses of the Northern blots. TNF-α mRNA density was standardized to β-actin mRNA density for each lane in the same blot.

tration of 10^6 cells per ml in six-well plates (Falcon). Cultures were set up without DMSO or inhibitor, with 0.2% DMSO alone, and with 0.2% DMSO in combination with thalidomide at concentrations of 10 and 20 μg/ml. Cultures were incubated at 37°C with 5% CO_2 for 7 days and sampled every 24 h, and supernatants were stored at −70°C until assayed. TNF-α ELISAs were performed on 24-h (day 1) supernatants; p24 Ag ELISAs were performed on day 3 and 4 supernatants and, if not positive, on day 5, 6, and 7 supernatants. RT assays were done on day 6 and 7 supernatants. Extensive cell death was recorded from day 8 of cocultures, which interfered with the evaluation of cell viability in the presence of the inhibitors. Therefore, assays were not carried out for longer than 7 days. Three patients with $CD4^+$ T-cell counts of >450/mm^3 of blood did not give positive p24 Ag ELISAs during the first 7 days of culture. These patients were not included in the results presented in the study.

Cell Survival. The possible toxic effects of thalidomide on PBMCs, phytohemagglutinin-stimulated blasts, U1 cells, or ACH-2 cells were evaluated by (*i*) estimation of total number of cells counted directly in a hemacytometer, (*ii*) percentage of viable cells estimated by the trypan blue dye (0.4%; Sigma) exclusion staining method, and (*iii*) proliferative activity using [^{3}H]thymidine uptake (1 μCi per well) for 16 h.

Northern Blot Hybridization. Total cellular RNA from stimulated monocytes was isolated by using the guanidine thiocyanate/phenol/chloroform method, size-fractionated by formaldehyde/agarose gel electrophoresis, and transferred to nylon membranes (Bio-Rad). The membranes were treated overnight at 42°C with prehybridization solution [50% (vol/vol) formamide/5× standard saline citrate (SSC)/5× Denhardt's solution/0.02 M $NaHPO_4$, pH 6.5/heat-denatured sheared salmon sperm DNA (100 μg/ml)/10% dextran sulfate]. This solution was then replaced with one containing heat-denatured ^{32}P-labeled random-primed cDNA (Stratagene) at 10^6 cpm/ml, TNF-α (1.3-kb *Pst* I fragment, gift of A. Cerami, The Picower Institute for Medical Research, Manhasset, NY), and β-actin (1.2-kb *Eco*RI–*Xho* I fragment). The filters were hybridized at 42°C overnight. The membranes were washed twice for 15 min at room temperature with 2× SSC/0.1% SDS, 30 min at RT with 0.1× SSC/0.1% SDS, and 30 min at 60°C with 0.1× SSC/0.1% SDS. After air drying, the blots were exposed to x-ray film for 12–24 h at −70°C and developed in a Kodak X-Omat processor stand. Densitometry was performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA), and results were expressed as density units.

RESULTS

Thalidomide Inhibits HIV-1 Activation in Promonocytic U1 Cells. Culture of U1 cells leads to little virus expression unless the cells are stimulated with an agonist such as TNF-α. More than a 5-fold increase in RT activity was noted with TNF-α at 1 ng/ml (Fig. 1). The addition of thalidomide at 5–50 μg/ml inhibited the TNF-α-induced release of RT by as much as 70%. The inhibitory effect of thalidomide on RT activity did not result from cytotoxicity since neither the proliferation nor the viability of U1 cells was influenced (Fig. 1 legend).

The inhibition of virus expression was observed only when thalidomide was present during the period of agonist-induced activation with TNF-α. If the cells were preincubated with the drug and washed before stimulation, inhibition did not occur. A similar effect of thalidomide was observed with the other stimuli shown in Table 1.

Effect of Thalidomide on HIV-1 Activation of the T-Cell Line ACH-2. Activation of latent HIV-1 in the T-cell line ACH-2 is easily achieved by culture of the cells with TNF-α as well as with PMA. However, as shown in Table 2, thalidomide at 5–50 μg/ml failed to inhibit viral expression by >20%. The drug did not influence ACH-2 cell number or viability (Table 2 legend).

The Effect of Thalidomide on TNF-α mRNA and Protein Production by U1 Cells. The stimulation of U1 cells by PMA or IL-6 plus LPS induced extensive production of TNF-α. Table 3 indicates that, after PMA or IL-6 plus LPS stimulation, thalidomide inhibited viral activation as well as the production of TNF-α by U1 cells. PMA stimulation of the U1

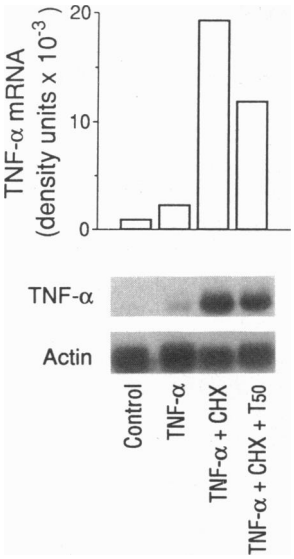

FIG. 4. Effect of thalidomide on TNF-α mRNA expression in U1 cells stimulated with TNF-α (1 μg/ml) as analyzed by Northern blot hybridization. Cells were incubated for 2 h with TNF-α followed by 2 additional h in the presence of TNF-α alone (TNF-α), TNF-α with cycloheximide at 10 μg/ml (TNF-α + CHX), or TNF-α with cycloheximide and thalidomide at 50 μg/ml (TNF-α + CHX + T_{50}). Control cells were incubated for 4 h with medium alone (Control). All cultures contained 0.25% DMSO. Gels were loaded with 20 μg of RNA per lane. The autoradiography of the Northern blot (*Lower*) and the densitometric analysis of the gel (*Upper*) are shown. The density for TNF-α mRNA was standardized to the density of β-actin mRNA for each lane in the same blot.

cells induced TNF-α mRNA, and the level of this message was inhibited by thalidomide at 20 and 50 μg/ml (Fig. 2). Although ACH-2 cells treated with PMA also demonstrated enhanced TNF-α mRNA, this mRNA production was not inhibited by thalidomide. The thalidomide suppression of RT in the cells stimulated with IL-6 plus LPS and GM-CSF plus LPS was also correlated with decreased TNF-α mRNA (Fig. 3).

The ability of TNF-α to induce endogenous TNF-α mRNA could be demonstrated only in the presence of cycloheximide. Under these conditions, TNF-α mRNA was also inhibited by the presence of thalidomide (Fig. 4). Therefore endogenously synthesized TNF-α may play a role in viral activation, and its inhibition by thalidomide could inhibit virus production (10, 11).

The Effect of Thalidomide on the PBMCs of Patients with HIV-1 Infection and AIDS. The studies on cell lines were extended to the circulating cells of HIV-1-infected individuals. A dose-dependent inhibition by thalidomide of both the p24 Ag and RT activity was noted in PBMCs from six patients with low blood $CD4^+$ T-cell counts (9–41 cells per mm^3) (data not shown). Table 4 demonstrates the inhibitory effect of thalidomide on HIV-1 activation in PBMCs obtained from 17 HIV-1^+ donors, evaluated by p24 Ag ELISA. In most individuals, an inhibition of HIV-1 activation was observed. In some donors with higher $CD4^+$ T-cell counts, HIV-1 activation and p24 Ag expression took longer than 7 days, and thalidomide inhibition could not be analyzed because of reduced cell viability (Table 4 legend).

DISCUSSION

Thalidomide, a selective inhibitor of TNF-α production by monocytes, significantly reduces HIV-1 replication in PBMCs from HIV-1-infected hosts and in agonist-stimulated latently infected cell lines. In both situations, either an agonist or amplifying mechanisms are necessary to activate cells with latent virus. Thalidomide has a pronounced inhibitory effect on TNF-α and TNF-α mRNA production by the U1 monocytic line activated with recombinant TNF-α or with a group of other agonists, which include PMA, other cytokines, and endotoxin. This suggests that HIV-1 activation

Table 4. Thalidomide inhibition of p24 ELISA in HIV-1^+ patients

Patient no.	$CD4^+$ T-cell count	Day of assay	p24 Ag ELISA, pg/ml	% inhibition of p24 Ag ELISA	Age, years	Race/ Sex*	AZT/DDI	Other medications†
1	9	4	4129	99‡	40	W/M	DDI	3, 4, 5, 6
2	10	3	4643	35§	31	B/M	?	1
3	10	4	607	98‡	35	H/M	?	2, 4, 7, 8
4	11	4	22	80§	30	H/M	AZT	1a, 1d, 1e, 1f, 1g, 3, 5, 9
5	20	3	38	72§	42	B/M	AZT	1a, 5, 10
6	36	4	1381	91§	25	B/M	AZT	1a, 1b, 1d, 3, 11
7	40	4	55	75§	41	W/M	AZT/DDI	3, 11
8	42	3	1414	40§	34	B/F	?	1
9	84	5	65	75§	38	W/M	AZT/DDI	3, 4
10	84	3	666	66§	41	B/M	AZT	1a, 1b, 1c, 1d
11	149	5	5	69§	49	H/M	AZT/DDI	3, 4, 5
12	204	5	127	None	44	W/M	AZT	4, 12, 13
13	256	3	53	57‡	45	W/M	None	None
14	295	5	207	42§	28	W/M	AZT	None
15	382	5	7	31‡	46	W/M	DDI	4
16	456	7	25	21‡	46	B/M	AZT	1a, 1b, 1c, 1d
17	458	6	<1	74§	33	B/F	AZT	None

Viability of the cells in the presence of 0.2% DMSO, thalidomide (10 μg/ml) in 0.2% DMSO, or thalidomide (20 μg/ml) in 0.2% DMSO was the same (±1%) for each time point; in 0.2% DMSO, it was 95%, 89%, 85%, and 79% on days 1, 3, 5, and 7, respectively. After day 7, the viability of the cells dropped lower, and the experiments were stopped. ?, Data not available; AZT, 3'-azido-3'-deoxythymidine; DDI, didanosine.

*B, black; H, hispanic; W, white; M, male; F, female.

†Other medications: 1, unspecified antituberculosis drugs; 1a, isoniazid plus vitamin B6; 1b, rifampicin; 1c, ethambutol; 1d, pyrazinamide; 1e, ethionamide; 1f, cycloserine; 1g, ciprofloxacin; 2, pentamidine; 3, trimethoprim/sulfamethoxazole; 4, acyclovir; 5, fluconazole; 6, interferon α; 7, omeprazole; 8, ondansetron hydrochloride; 9, trazodone; 10, nafcillin; 11, clotrimazole troches; 12, prednisone; 13, compound Q (GLQ23, experimental drug with stimulatory effects on ribosomal activity).

‡Inhibition demonstrated at a thalidomide concentration of 20 μg/ml.

§Inhibition demonstrated at a thalidomide concentration of 10 μg/ml.

under these *in vitro* conditions could proceed by way of a common TNF-α-dependent pathway. Studies on the mechanism of thalidomide action in monocytes (20) indicate that the drug functions by selectively accelerating the decay of the TNF-α mRNA.

The situation is less clear when one considers the T-cell line ACH-2. Here viral activation is readily accomplished with recombinant TNF-α as well as PMA, yet thalidomide inhibition never exceeds 20%. TNF-α mRNA production by these cells is, however, easily demonstrated. These differences in T-cell and monocytoid cell lines may reflect separate pathways necessary for viral activation, as reported (20, 21). In addition, the mechanism whereby TNF-α influences these pathways may also be distinct. It is of interest that pentoxifylline, another TNF-α inhibitor that blocks TNF-α mRNA transcription, also fails to inhibit HIV-1 replication in lymphoid cells but does so in monocytoid cells (ref. 22; G.K., unpublished results). This suggests that the thalidomide inhibition of viral activation in infected PBMCs is expressed through the monocyte population.

The role of TNF-α in infections and inflammatory processes depends, in part, upon its concentration in the blood and tissues. Low levels are sufficient and necessary to promote host resistance (23, 24). However, higher levels of TNF-α lead to systemic toxicity and even death. Considering the application of these results to the therapy of human HIV-1 infections, two positive results might be anticipated. First, thalidomide and/or its congeners may suppress viral replication and decrease viral burden. Second, it may enhance patient well-being by reducing TNF-α-induced fever, malaise, muscle weakness, and the cachexia of the immunodepressed host.

Dr. P. Cameron provided extensive advice during the entire project; Dr. W. N. Rom referred some of the patients included in the study. Thanks are due to J. C. Adams for graphic work and to S. Dennehy for technical help and M. Nulty for efficient secretarial help. These studies were supported in part by U.S. Public Health Service Grants AI-22616 and AI-24775 and the Celgene Corporation.

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