

CONCISE COMMUNICATION

The Immunomodulatory Effects of Thalidomide on Human Immunodeficiency Virus–Infected Children

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The safety and immune effects of low-dose thalidomide treatment (3 mg/kg/day for 28 days) were evaluated in a study involving 8 South African human immunodeficiency virus (HIV)–infected children. The children were 7–69 months old and in disease stages A1–C3. Thalidomide therapy did not affect virus load, even though none of the children was receiving anti-retroviral therapy. Thalidomide stimulated CD8⁺ T cells in peripheral blood, which increased expression of the activation markers CD38 and human leukocyte antigen DR and of the memory cell marker CD45RO. The frequency of HIV gag–specific CD8⁺ T cells in peripheral blood increased in 3 of 4 children who were evaluated during treatment with thalidomide. Clinical adverse events were mild. In this study, thalidomide was found to be safe and well tolerated and caused significant immunomodulation at a low dose. This is the first report describing use of an oral drug that may enhance HIV-specific CD8⁺ T cell function in HIV-infected children.

Every year, thousands of infants worldwide vertically acquire human immunodeficiency virus (HIV) infection. Most of these children do not have access to antiretroviral therapy, which results in high morbidity and mortality. There is, therefore, a need for alternative interventions that target either HIV or HIV-related immune abnormalities and complications in these children.

Thalidomide improves the outcome of wasting syndrome and aphthous ulceration in HIV-infected adults [1–3]. The clinical efficacy of the drug may be due to its anti-inflammatory effects, as indicated by a reduction in plasma tumor necrosis factor (TNF)– α that occurs in thalidomide-treated adults with erythema

nodosum leprosum, a complication of leprosy [4]. Alternatively, T cell stimulatory properties may be responsible, as is suggested by the increase in peripheral blood CD8⁺ T cells and plasma interleukin (IL)–12 levels that is seen after thalidomide therapy in adults with HIV infection, severe tuberculosis, or scleroderma [5–7]. In vitro studies have shown that thalidomide acts primarily as a CD8⁺ T cell costimulant, resulting in increased T cell proliferation, IL-2 and interferon (IFN)– γ production, and enhanced cytotoxic activity [8].

Anecdotal reports have described the use of thalidomide to treat aphthous ulcers and Kaposi's sarcoma in HIV-infected children [9–11]. Thalidomide may also be useful in the management of specific complications of pediatric HIV infection, particularly growth failure. We therefore embarked on a pilot study of low-dose thalidomide treatment in HIV-infected South African children to evaluate the drug's safety and its effects on virus load and T cell function, before testing thalidomide's effect on HIV-associated growth failure.

Methods

Subject enrollment. HIV-infected children without acute or chronic complications who were attending clinics of the academic hospitals of the University of Cape Town were eligible for study enrollment.

Study drug administration. Thalidomide (Celgene) was administered in 1 oral dose (3 mg/kg/day; maximum dose, 200 mg/day) at night. The dose of thalidomide powder, which was obtained from tablets, was prepared individually for each subject and mixed into a spoonful of cereal, which was administered to the patient.

Received 23 May 2001; revised 12 July 2001; electronically published 21 September 2001.

Presented in part: Pediatric Academic Societies and American Academy of Pediatrics Joint Meeting, Boston, Massachusetts, May 2000 (abstract 1554); XIII International AIDS Conference, Durban, South Africa, July 2000 (abstract ThPeB5136).

Written informed consent was obtained from the parents or guardians of all study subjects. The human experimentation guidelines of the US Department of Health and Human Services were followed, and the Institutional Review Board of The Rockefeller University and the Research Ethics Committee of the University of Cape Town approved the protocol.

Financial support: South African Medical Research Council, Direct Effect, Celgene Corporation, and National Institutes of Health (AI-42056 to G.K.).

G.K. is on the Board of Directors of Celgene Corporation (Warren, NJ), which manufactures thalidomide.

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The Journal of Infectious Diseases 2001;184:1192–6

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 0022-1899/2001/18409-0015\$02.00

Each patient and parent or guardian was admitted to the hospital for days 1–3 of the study and educated about the safe administration of thalidomide and possible adverse effects. After discharge, parents or guardians administered the study drug at home until 28 days of therapy had been completed.

Clinical and laboratory safety monitoring. Patients were evaluated at baseline; on days 3, 7, 11, 14, 21, and 28 during therapy; and on days 34 and 42 after therapy was completed. At each visit, a physical examination was performed. At baseline and weekly thereafter until day 42, safety hematologic and chemistry tests were performed on blood obtained from the patients.

Pediatric AIDS Clinical Trials Group (PACTG) toxicity grading tables were used to classify adverse events [12]. Specific procedures that would follow adverse events, including criteria for discontinuing thalidomide therapy, were predefined.

HIV load monitoring. Virus load was determined at baseline and every 2 weeks thereafter by nucleic acid sequence–based amplification (Nuclisens; Organon Teknika). A confirmed 0.7-log increase in virus load from baseline, in the absence of another explanation for the increase (such as an intercurrent infection), was a safety end point for the study and required discontinuation of thalidomide.

Immunologic monitoring. Blood samples for use in immune studies were obtained at baseline and every 2 weeks thereafter. Plasma levels of soluble markers of immune activation were determined in batch on cryopreserved plasma, using ELISA. TNF- α (Biosource), IFN- γ (Genzyme), IL-12 (Endogen), soluble CD8 (sCD8; Endogen), and soluble IL-2 receptor (sIL-2R; Endogen) were assayed.

Leukocyte phenotyping was done on fresh whole blood within 6 h of collection, using fluorescence-activated cell-sorter (FACS) analysis (Coulter XL; Beckman Coulter and FACSCalibur; Becton Dickinson). Combinations of the following fluorescent-conjugated antibodies (all from Becton Dickinson) were used: anti-CD3, anti-CD4, anti-CD8, anti-CD38, anti-CD28, anti-CD45RO, and anti-HLA-DR.

HIV-specific CD8⁺ T cell function was evaluated in batch, using peripheral blood mononuclear cells (PBMC) isolated and cryopreserved at each immunologic blood specimen collection time point. A FACS-based assay of intracellular IFN- γ expression in CD8⁺ T cells was used as described elsewhere [13]. Four pools containing 10 overlapping 20-mer HIV-1 subtype C–specific gag peptides (10 μ M each peptide; AIDS Reagent Program, National Institutes of Health, Bethesda, MD) and a fifth pool containing 6 peptides were incubated with PBMC for 18 h. Brefeldin A (Sigma) was added for the last 5 h. Staphylococcal enterotoxin B (100 ng/mL; Sigma) was the positive control. Limited quantities of PBMC were available for the HIV-specific CD8⁺ T cell assay.

Statistical analysis. The nonparametric Kruskal-Wallis test was used to measure the statistical significance of differences in immune markers at study time points. $P < .05$ was considered to be significant.

Results

Patient characteristics. Eight children (4 boys and 4 girls) with a median age of 49 months (range, 7–69 months) were

enrolled. One child's weight was below the third percentile for age, and the height of 2 children was below the third percentile. One child had stage A1 HIV disease, 2 children had stage B1, 2 children had stage B2, 1 child had stage B3, and 2 children had stage C3 [14]. Median HIV load was 227,500 copies/mL (range, 8750–5,650,000 copies/mL). None of the children was receiving antiretroviral therapy, and most of the children, although relatively healthy at enrollment, had a history of typical HIV-associated complications.

Adverse events. Clinical adverse events were mild, except in 1 patient, who developed bronchiolitis that required hospitalization (table 1). An intercurrent viral infection was the most likely cause. Five other patients developed upper respiratory tract symptoms while receiving thalidomide (table 1); however, whether there was an increased frequency of these symptoms that would be suggestive of viral infections was difficult to assess, because there was no control group. Three patients who had a history of chronic parotid swelling, a typical chronic complication of pediatric HIV disease, had exacerbations while they were receiving thalidomide (table 1). Two patients developed the well-described skin rash associated with thalidomide treatment (table 1) [1, 6].

Adverse events found on laboratory studies were mostly mild (table 1). Hyperamylasemia occurred in 3 patients and did not coincide with clinical parotid swelling. Monocytosis also occurred in 3 patients; however, monocyte counts returned to the normal range after completion of thalidomide therapy, suggesting that the effect was drug induced. One patient presented with raised hepatic transaminase levels that were concurrent with fever and tonsillitis, suggesting a mononucleosis syndrome. A viral etiology was suspected but not confirmed. The patient recovered uneventfully.

Thalidomide therapy was not associated with any change in quality of life, as judged using a PACTG scoring system (data not shown).

Effect of thalidomide on virus load. Thalidomide did not affect HIV load. The median increase in virus load from baseline to after day 14 of thalidomide therapy was 0.03 log copies/mL (range, –0.84 to 0.53 log copies/mL; $P = .89$), and the median increase after day 28 of therapy was 0.11 log copies/mL (range, –0.74 to 0.64 log copies/mL; $P = .58$).

Effect of thalidomide on immune status. Changes in both soluble and cell-associated markers of immune activation and HIV-specific CD8⁺ T cell activity were monitored. Plasma levels of sIL-2R, a nonspecific soluble marker of immune activation, increased from a median baseline value of 1486 pg/mL (range, 465–3029 pg/mL) to 2576 pg/mL (range, 1080–6685 pg/mL; $P = .012$) after day 14 of therapy (figure 1A). The plasma level of sCD8, a marker of CD8⁺ T cell activation, also increased (figure 1B). The median baseline level of sCD8 was 865 U/L (range, 655–1732 U/L), and the level after day 14 of therapy was 1648 U/L (range, 1292–2383 U/L; $P = .018$; figure 1B). Increases in both sIL-2R and sCD8 levels appeared to be tran-

Table 1. Adverse clinical effects and laboratory findings for 8 human immunodeficiency virus–infected children receiving thalidomide therapy.

Adverse event (no. of patients)	Day(s) of therapy (no. of events ^a)	Description
Clinical event		
Bronchiolitis and fever (1)	7	Hospitalized for bronchiolitis requiring oxygen therapy. Course complicated by persistent fever, resulting in discontinuation of thalidomide on day 13, even though predetermined study end points were not reached. Recovered uneventfully.
Parotid gland enlargement (3)	4, 28 (2)	Asymptomatic in all 3 patients; all had a history of this complication before the study. Resolved in all after completion of therapy.
Skin rash (3)	2, 4, 7	Grade 1 papular urticaria developed in 1 patient on day 7 after commencement of amoxicillin treatment for otitis media; this rash abated within 1 day after discontinuation of amoxicillin, without additional therapy. Other patients experienced fine papular (grade 1) rashes.
Symptoms of upper respiratory tract infection (6)	2, 4 (2), 7 (2), 18	Mild in most, except in 1 patient, who developed bronchiolitis (see first row of this table). Two patients had exacerbations of chronic suppurative otitis while receiving thalidomide therapy.
Laboratory finding		
Hyperphosphatemia (5)	7 (2), 14 (2), 21	Grade 1 abnormality in all.
Hyperamylasemia (3)	7 (2), 21	Grade 1 abnormality in all.
Monocytosis (3)	7, 14, 21	Grade 1, except in 1 patient, who developed a grade 2 abnormality (monocytes, 1990 cells/ μ L; 28% of total leukocytes) after discontinuation of thalidomide therapy.
Anemia (1)	7	Grade 2; lowest hemoglobin level, 7.9 g/dL; baseline value, 9.1 g/dL.
Hyponatremia (1)	7	Grade 3; lowest sodium level, 127 mM. At baseline, this patient, who had intercurrent, severe cryptosporidial diarrhea, had grade 1 hyponatremia.
Increased levels of alanine and aspartate transaminases (1)	28	Grade 3; highest alanine transaminase level, 2091 U/L; highest aspartate transaminase level, 2197 U/L. Temporal relationship with tonsillitis, suggesting a diagnosis of infectious mononucleosis.
Neutropenia (1)	28	Grade 3; absolute neutrophil count, 204 neutrophils/ μ L.

NOTE. Adverse events were classified according to a Pediatric AIDS Clinical Trials Group grading system [12]. Classifications are as follows: Grade 1 is mild, not requiring intervention; grade 2 is moderate, not necessarily demanding discontinuation of the study drug but possibly requiring further evaluation and intervention; grade 3 is severe, commonly demanding withdrawal of the study drug and requiring active evaluation and intervention; and grade 4 is life threatening.

^a No. of events on each study day is 1, unless otherwise indicated.

sient (figure 1A and 1B). Although the absolute numbers of CD8⁺ T cells did not change, expression of the activation markers CD38 and HLA-DR on CD8⁺ T cells increased during thalidomide therapy (figure 1C and 1D). In a like manner, the fluorescence intensity of CD8⁺ T cell expression of the costimulatory marker CD28 increased by a median of 33% (range, 19%–213%; $P = .018$) from baseline to day 14. At the completion of therapy (day 28) and 2 weeks thereafter, levels of CD38 and HLA-DR expression were still higher than at baseline. These results suggest that thalidomide therapy caused significant CD8⁺ T cell activation.

To assess whether thalidomide treatment affected the expansion of memory CD8⁺ T cells, expression of CD45RO, a memory marker, was evaluated. Fluorescence intensity of CD45RO expression on CD8⁺ T cells increased by a median of 547% from baseline to day 14 (range, 151%–878%; $P = .018$; figure 1E), and absolute numbers of CD45RO-expressing CD8⁺ lymphocytes increased from a median of 663 cells/mm³ (range, 211–2069 cells/mm³) to 935 cells/mm³ (range, 397–3020 cells/mm³) during this period ($P = .23$). These results suggest that thalidomide preferentially activated and expanded antigen-primed memory CD8⁺ T cells (CD3⁺, CD8⁺, and CD45RO⁺).

We therefore determined whether HIV-specific CD8⁺ T cell function was enhanced during thalidomide treatment. HIV gag-specific CD8⁺ T cells were quantitated by a FACS-based assay of intracellular IFN- γ expression. Samples of PBMC from 4 patients were available. Over the course of treatment, 3 of these 4 patients demonstrated increases in CD8⁺ T cell IFN- γ expression induced by 1 of 5 gag pools. One of those 3 patients demonstrated increases in response to 2 pools: The frequency of IFN- γ -producing CD8⁺ T cells after stimulation with 1 pool was 0.03% at baseline, 0.07% on day 14, and 0.33% on day 28 during thalidomide therapy, and it was 0.22% on day 42, 2 weeks after treatment was discontinued. The other pool induced frequencies of 0.03%, 0.03%, 0.43%, and 0.07% at each time point, respectively. These results suggest that thalidomide might have enhanced HIV-specific CD8⁺ T cell activity.

Thalidomide did not affect the absolute numbers or percentages of CD4⁺ T cells in peripheral blood or expression of costimulatory, activation, and memory cell markers on these cells (data not shown). In addition, thalidomide therapy was not associated with changes in plasma IL-12, TNF- α , and IFN- γ during therapy (data not shown).

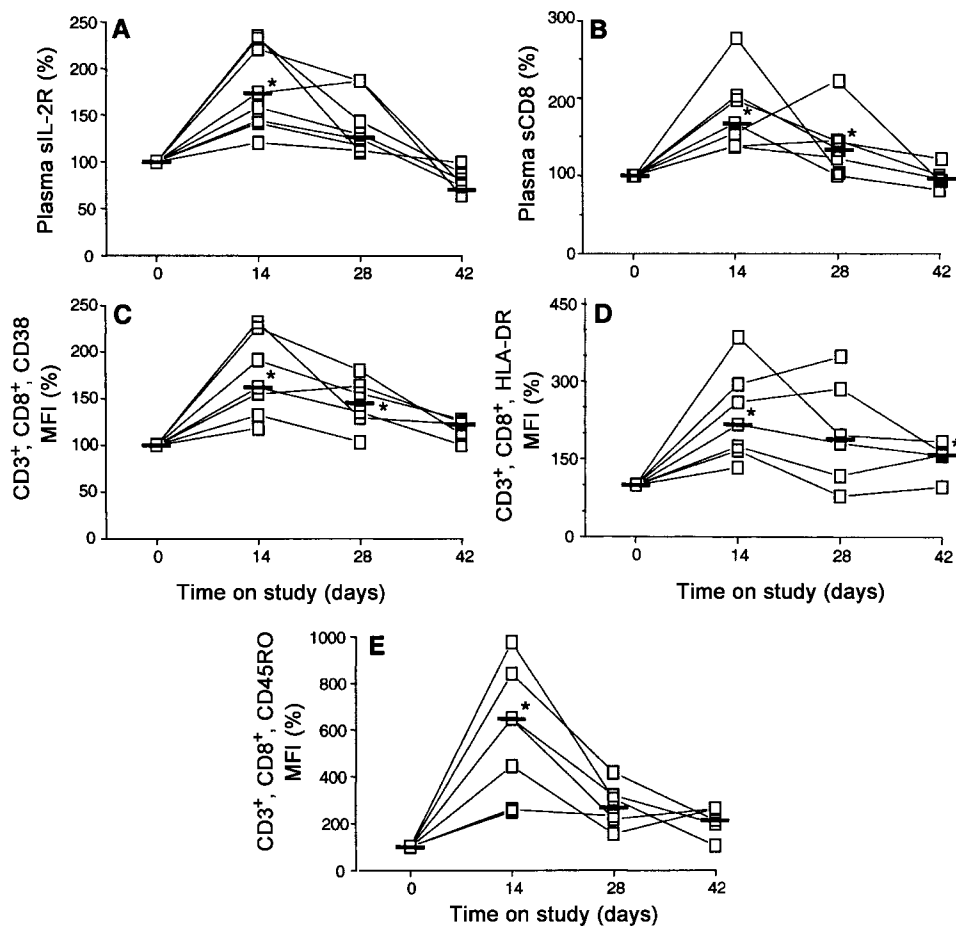


Figure 1. Effect of thalidomide on plasma soluble interleukin-2 receptor (sIL-2R) (A); plasma soluble CD8 (sCD8) (B); CD8⁺ T cell expression of CD38 (C); CD8⁺ T cell expression of HLA-DR (D); and CD45RO expression on CD8⁺ T cells (E). The percentage of change in individual patients' soluble marker levels and in median fluorescence intensity (MFI) of cell-associated marker expression are shown; thick black lines indicate the median percentage of change from baseline for each group. * $P < .05$.

Discussion

To our knowledge, this is the first report of a systematic evaluation of the safety and immune effects of thalidomide therapy for HIV-infected children. Thalidomide induced consistent immune activation, even at a low dose (3 mg/kg/day). As has been reported for adults, thalidomide preferentially activated CD8⁺ T cells [5, 8]. Transiently increased expression of activation markers, such as HLA-DR and CD38, and the costimulatory molecule CD28 was noted. In a similar manner, levels of the immune marker sCD8 also increased, which was suggestive of CD8⁺ T cell activation. Although the overall number or percentage of CD8⁺ T cells in peripheral blood did not change during thalidomide therapy, a relative expansion of memory CD8⁺ T cells was observed. This result suggests that thalidomide induced preferential activation of antigen-experienced CD8⁺ T cells. Indeed, an increase in the frequency of HIV gag-specific CD8⁺ T cells in the peripheral blood of 3 of 4 evaluated patients was demonstrated. These results suggest that thalidomide may enhance HIV-

specific immunity; however, larger controlled studies with a more comprehensive assessment of HIV-specific CD8⁺ T cell function are necessary to confirm this finding.

Thalidomide therapy was not associated with the increase in virus load that was expected in the face of generalized immune activation and the absence of antiretroviral therapy (which is not routinely available in South Africa). Activation of HIV-specific immunity in this relatively asymptomatic cohort may have prevented an increase in virus load. A mild increase in viremia after thalidomide therapy in adults with advanced HIV disease who were receiving concomitant antiretroviral therapy has been reported elsewhere [1, 2].

In general, thalidomide was safe and well tolerated. The immune-activating effects of thalidomide may have caused the mild adverse events that were seen, such as skin rash and parotid gland enlargement. Parotid gland enlargement has not been ascribed to thalidomide therapy; however, the CD8⁺ T cell-activating effects of the drug may have caused this com-

plication, because CD8⁺ cells have been shown to predominate in enlarged parotids in HIV-infected patients [15]. Immune activation may also have predisposed patients to the increased frequency of respiratory tract symptoms that was seen in our cohort. Hyperamylasemia, monocytosis, and hyperphosphatemia also have not been reported in association with thalidomide therapy. That plasma amylase levels were normal in the children with parotid enlargement is of interest. This finding suggests that thalidomide may cause subclinical parotitis or pancreatitis in HIV-infected children. This requires further study, because pancreatitis may have serious clinical implications when antiretrovirals such as didanosine, which is known to cause this complication, are used concomitantly.

This pilot study demonstrated that thalidomide may be administered safely in a controlled setting within a developing country. Thalidomide was safe and well tolerated and had potent immunomodulatory effects in HIV-infected children. To our knowledge, this is the first report describing use of an oral drug that may enhance HIV-specific CD8⁺ T cell function in children with HIV infection. This finding strongly favors further study of thalidomide therapy, particularly in areas where expensive antiretroviral agents may not be available.

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