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Impact of Opportunistic *Mycobacterium tuberculosis* Infection on the Phenotype of Peripheral Blood T Cells of AIDS Patients

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While the detrimental consequences of opportunistic tuberculosis (TB) in the course and outcome of HIV-1 infection are well studied, little information about the impact of the mycobacterial infection on the phenotype of T lymphocytes is available. In this study we analyzed by cytofluorimetry the peripheral blood T cell phenotype of 13 patients with AIDS, 23 HIV-1 negative patients with active pulmonary TB, nine HIV-1/*Mycobacterium tuberculosis* coinfected individuals, and 21 age- and sex-matched healthy controls. CD4+ T cells were equally depleted in AIDS and coinfection ($P<0.001$). The findings suggest a rescuing effect of the added mycobacterial infection. CD3 T cell loss was not observed in coinfection, whereas it was severe in AIDS ($P<0.001$). Similar (albeit less striking) effects were observed with other markers (CD45RA, CD45RO, and CD27) that were diminished in CD4+ T cells of AIDS patients. Apparent detrimental effects of the added mycobacterial infection were the increased expression of the proapoptotic molecule CD95 on CD4+ T cells, and decreased expression of the major costimulatory molecule CD28 on CD8+ T cells. In this work we show that *M. tuberculosis* infection modifies the T cell phenotype of the HIV-1 infected individual. J. Clin. Lab. Anal. 20:80–86, 2006.

Key words: HIV-1; AIDS; tuberculosis; flow cytometry; T cells

INTRODUCTION

The synergistic interaction of *Mycobacterium tuberculosis* (Mtb) and human immunodeficiency virus type 1 (HIV-1) poses an important challenge to public health, particularly in less-developed countries. The impact of the HIV epidemic on the incidence of tuberculosis (TB) is important: 9% of the 8.3 million new TB cases and 12% of the 1.8 million TB deaths in 2000 were HIV-related (1). On the other hand, TB aggravates the course of HIV-1 infection and is the cause of 11% of all adult acquired immunodeficiency syndrome (AIDS) deaths (1). The superimposed Mtb infection, by further activating the immune system, favors cell infection and intracellular viral replication (2).

The high susceptibility of patients with AIDS to Mtb infection is probably related to a progressive decline of CD4+ T cells (3), which play a central role in the immune response to Mtb (4). Moreover, CD4+ T cells are dysfunctional even before depletion occurs, with

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poor in vitro blastogenic responses (5–7), diminished cytotoxic activity (8), increased apoptosis (9,10), and defective T cell costimulation, which is associated with decreased expression of CD28 and CD40L on CD4+ T cells (11–13). High levels of soluble CD27 and CD30 in serum that correlate with high viral loads in plasma and CD4+ T cell loss have also been reported (14,15). Related to the above T cell defects, particularly CD4+ T cell depletion, HIV-1 infection is frequently complicated by opportunistic infections, including TB (3). AIDS/TB coinfected patients present higher viral loads and follow a poor clinical course that is related to several factors (16). Lymphocyte activation is crucial for viral infection and replication (17,18), and mycobacteria are potent activators of CD4+ T cells (4). In addition, the T cells of TB patients produce high levels of tumor necrosis factor alpha and interleukin 6, which promote viral replication (2,19). On the other hand, a defective T cell response is frequently observed in TB, which is manifested by CD4+ T cell lymphopenia and anergy (20,21). In addition, as observed in AIDS, abnormal apoptosis of T cells and dysregulation of the cytokine network with predominance of a Th2 response are frequently observed in TB patients (22,23).

In view of the above data, we considered it possible that in dually infected patients the impact of mycobacterial antigens on the immune system of HIV-1-infected individuals might modify the phenotype of peripheral blood T cells. To test this hypothesis, we investigated the phenotype of peripheral blood T cells in patients with AIDS, patients with pulmonary TB, and AIDS/TB-coinfected individuals, with an emphasis on the expression of T cell costimulatory molecules.

MATERIALS AND METHODS

Study Population

This study included 13 AIDS patients, 23 HIV-1-negative patients with active pulmonary TB, and nine AIDS/TB-coinfected individuals. Patients were recruited at the Centro Estatal de Información sobre SIDA in San Luis Potosí, México, and the Hospital de Infectología, Centro Médico La Raza, Instituto Mexicano del Seguro Social, in Mexico City. The HIV-1 diagnosis was done by a standard screening enzyme-linked immunosorbent assay (ELISA; UmElisa HIV 1+2 Reconvinant, La Havana, Cuba) and confirmed by immunoblotting (Grupo Roche Syntex, Mexico City, Mexico). At the time of bleeding, patients were taking medications such as zidovudine (AZT), didanosine (ddI), and zalcitabine (ddC). The TB diagnosis was based on clinical and chest X-ray data and confirmed by sputum culture and/or by demonstrating acid-fast bacilli on sputum smears. For controls, blood samples were obtained from 21 individuals who were HIV-1-negative and clinically healthy. The AIDS patients (two females and 11 males) varied in age from 28 to 51 years (mean = 34 ± 6 years). The AIDS/TB-coinfected patients (two females and seven males) varied in age from 22 to 56 years (mean = 35 ± 13 years). The TB patients (10 females and 13 males) ranged in age from 29 to 78 years. The average age was 55 ± 14 years, and six patients (26%) were elderly (≥ 65 years old). Of the 23 TB patients, 10 who matched the age of the AIDS and coinfected groups were chosen for comparative statistical analysis. These 10 HIV-1-negative TB patients (five females and five males) varied in age from 29 to 49 years (mean = 41 ± 7 years). The healthy controls (11 females and 10 males) varied in age from 21 to 64 years (mean = 34 ± 15 years).

Monoclonal Antibodies

Monoclonal antibodies (mAb), unlabeled or labeled with fluorescein isothiocyanate, R-phycoerythrin, or R-phycoerithrin-Cy5, were obtained from various laboratories. mAb to CD3 (clone UCHT1, IgG1), CD45RA (clone F8-11-13, IgG1), CD30L (clone MB1, IgG2b), CD40L (clone TRAP1.3.6, IgG1), and CD27 (clone LT27, IgG2a) were purchased from Serotec (Raleigh, NC). mAb to CD4 (clone RPA-T4, IgG1), CD28 (clone CD28.2, IgG1), CD95 (clone DX2, IgG1), and CD45RO (clone UCHL1, IgG2b) were obtained from Pharmingen (San Diego, CA). mAb against CD4 (clone MT310, IgG1), CD8 (clone DK25, IgG1), and CD30 (clone Ber-H2, IgG1) were purchased from the Dako Corporation (Carpinteria, CA). Appropriate isotype control antibodies were employed.

Flow Cytometry Assay

Blood samples were obtained from patients and controls by venipuncture in Vacutainer tubes (Becton Dickinson, San Jose, CA) with EDTA as anticoagulant. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation in Histopaque-1077 gradient (Sigma, St. Louis, MO). PBMC (5×10^5) were stained with mAbs or isotype controls for 30 min in the dark at 4°C, and washed in PBS with 1% fetal bovine serum and 0.1% sodium azide. The cells were then incubated with the indicated labeled secondary antibodies for 30 min. After the cells were rinsed they were fixed with 1% paraformaldehyde in PBS. At least 10,000 cells were analyzed in a two-color FACScan™ (Becton Dickinson, San Jose, CA) operating with Cellquest software and a 488-nm argon laser. The cells were gated according to typical forward and right angles to establish a lymphocyte gate that excluded blasts, cell debris, and cell aggregates.

Statistical Analysis

To establish statistical differences between groups, we analyzed the data with a one-way analysis of variance (ANOVA) and Tukey post test using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, CA).

RESULTS

The statistically significant changes observed in 23 TB patients were as follows: decreased CD3+ T cell counts ($P<0.05$), severe depletion of CD4+ T lymphocytes ($P<0.001$), and low CD8+ T cell counts ($P<0.01$). CD4+/CD45RO+ and CD4/CD30 T cell subsets were increased in percentage ($P<0.01$ and $P<0.05$, respectively). The expression of the CD28 T cell costimulatory molecule on CD4+ and CD8+ T cells was severely diminished ($P<0.001$).

In the AIDS patients, flow cytometry results revealed a severe CD3 T cell loss (controls: $1{,}518\pm19/mm^3$; AIDS: $720\pm417/mm^3$) that was highly significant ($P<0.001$). CD3 T cell counts in AIDS/TB-coinfection ($911\pm582/mm^3$) and TB patients ($1209\pm676/mm^3$) were not significantly different from those of controls.

Table 1 and Figs. 1 and 2 show the results of the flow cytometry analysis of CD4+ T cells. As expected, in AIDS patients CD4+ T cells were severely diminished in both percentage and absolute numbers ($P<0.001$). In AIDS/TB coinfection the loss of CD4+ T cells was also very severe (percentage and absolute numbers, $P<0.001$). HIV-1-negative pulmonary TB also exhibited CD4+ T cell loss, but of lesser significance (percentage and absolute number, $P<0.01$). Regarding the expression of the CD45RA naïve T cell marker, the CD4+/CD45RA+ T cell subset was severely diminished in AIDS ($P<0.001$), while in coinfection the loss of this subset was less significant ($P<0.01$). Markedly reduced CD4+/CD45RO+ T cell counts were observed in AIDS patients ($P<0.001$), whereas these cells did not differ significantly between coinfected individuals and controls. As regards the proapoptotic CD95 molecule, in AIDS the absolute number of CD4+ T cells that expressed this marker was decreased ($P<0.01$) and the percentage was unchanged. In AIDS/TB-coinfection the absolute number of CD4+/CD95+ T cells was also diminished ($P<0.05$), but the percentage was higher than that of controls ($P<0.05$). We also analyzed the expression of the T cell costimulatory molecules CD27, CD30, CD30L, and CD40L, which belong to the TNF family (24), and of CD28, a member of the immunoglobulin superfamily (25). In AIDS patients the CD4/CD27 T cell subset was diminished in absolute numbers ($P<0.05$), whereas in AIDS/TB-coinfected individuals these cells were within the normal range. In HIV-1-negative TB patients CD27 expression was not altered. Contrary to findings in the literature (15), in this study the expression of CD30 and CD30L on T cells of AIDS patients was no different from that of controls. These cells were also within the normal range in coinfected and TB patients. In all study groups the expression of CD40L on CD4+ T cells was within control values. As regards the major T cell costimulatory molecule CD28, its expression in CD4+ T cells of AIDS patients was severely decreased in absolute numbers ($P<0.001$). In HIV-1-negative TB patients the CD4+/CD28+ T cell subset was also diminished ($P<0.05$). In AIDS/TB coinfection, the diminished expression of CD28 on CD4+ T cells was similar to that observed in AIDS ($P<0.001$).

Table 2 shows the results of the flow cytometry analysis of CD8+ T cells. In all study groups the

TABLE 1. The peripheral blood CD4+ T cell phenotype in HIV-1/*Mycobacterium tuberculosis* coinfected patients*

	Controls (n = 21)		AIDS (n = 13)		HIV/TB (n = 9)		TB (n = 10)	
Marker	%	#	%	#	%	#	%	#
CD3/CD4	37±11	884±392	8±6[c]	118±117[c]	10±10[c]	176±215[c]	24±8[b]	488±195[b]
CD4/CD45RA	25±13	237±146	27±35	34±64[c]	39±28	224±229[b]	35±24	181±143
CD4/CD45RO	59±19	463±145	59±25	70±67[c]	66±20	139±167	66±18	311±167
CD4/CD95	37±26	410±349	58±25	59±57[b]	72±38[a]	99±133[a]	59±39	316±267
CD4/CD27	37±36	400±449	26±29	23±28[a]	38±30	66±102	57±43	293±240
CD4/CD30	1±2	9±12	3±8	2±6	0±0	0±0	4±4	20±19
CD4/CD30L	2±30	146±183	45±28	74±59	24±17	59±62	16±16	105±99
CD4/CD40L	9±21	61±107	33±38	31±39	22±18	29±33	2±4	7±10
CD4/CD28	69±30	676±378	16±29	47±49[c]	54±39	136±232[c]	59±42	337±313[a]

*Media, absolute cell counts and percentage±standard deviation of flow cytometry results. Statistically significant differences between groups were determined by one-way ANOVA with Tukey post test.
[a]Significantly different from the control group ($P<0.05$).
[b]Significantly different from the control group ($P<0.01$).
[c]Significantly different from the control group ($P<0.001$).

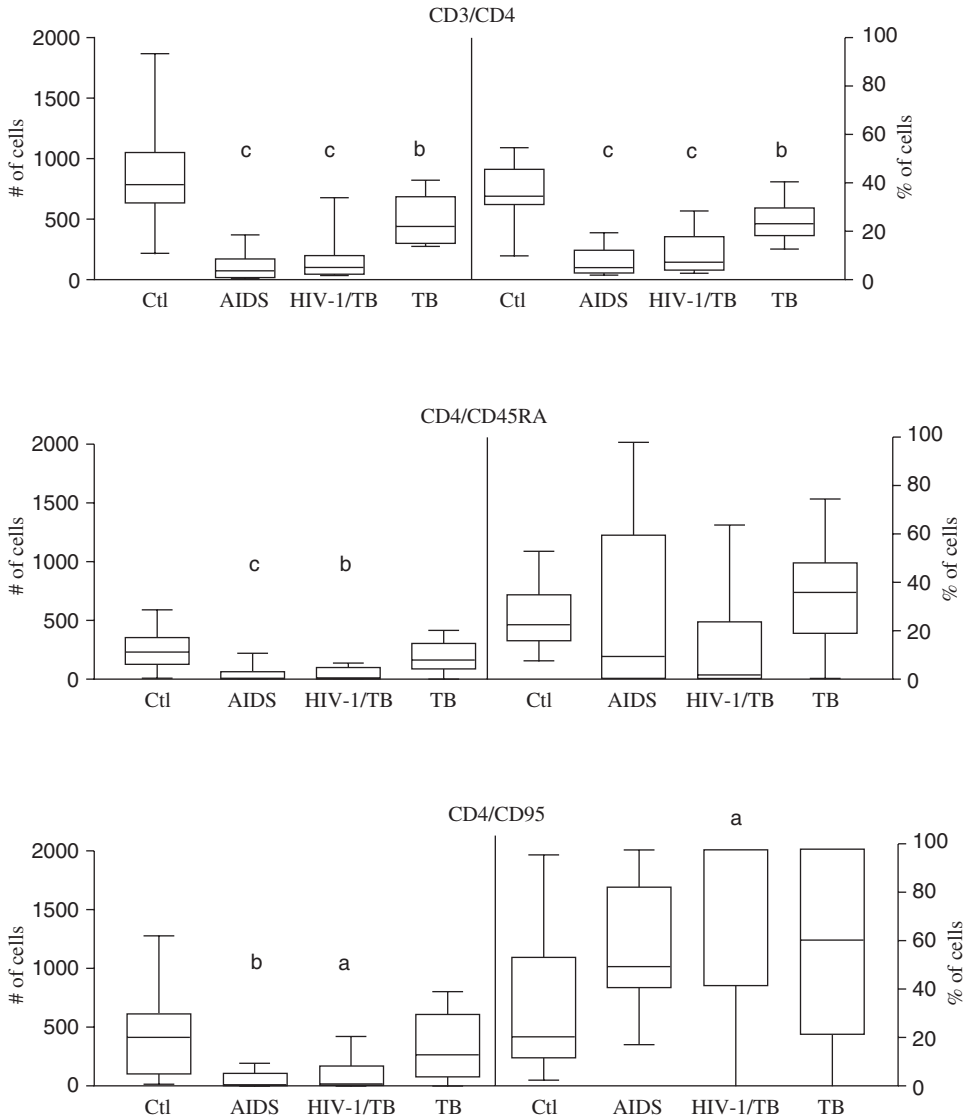

Fig. 1. Flow cytometry analysis of peripheral blood cells from 13 AIDS patients, nine HIV-1/*M. tuberculosis*-coinfected patients, and 10 HIV-1-negative patients with pulmonary TB. Significant results are shown in absolute numbers and percentage of CD4+, CD4+/CD45RA+, and CD4+/CD95. The box plot represents the median, 25/75 percentile, and extreme values. **a:** Significantly different from control group ($P<0.05$). **b:** Significantly different from control group ($P<0.01$). **c**: Significantly different from control group ($P<0.001$).

percentages and absolute numbers of these cells did not differ from those of controls. However, CD8+ cells showed a tendency to increase (AIDS/TB: $592\pm430/mm^3$; AIDS: $458\pm343/mm^3$). Expression of CD45RA and CD45RO on CD8+ T cells of all study groups was not altered. Expression of CD95 on CD8+ T cells of AIDS, AIDS/TB coinfection, and HIV-1-negative TB groups was also within control values. Regarding the T cell costimulatory molecules, the expression of CD27, CD30, CD30L, and CD40L on CD8+ T cells was within control values in all study groups. In contrast, in AIDS patients the number of CD8+/CD28 T cells was reduced in both absolute numbers and percentage ($P<0.01$). In HIV-negative TB patients the absolute numbers of these cells was also reduced ($P<0.01$). In AIDS/TB coinfection, CD28 expression on CD8+ T cells was severely diminished in percentage but not in absolute numbers ($P<0.001$). Figures 1 and 2 show a graphic representation of the statistically significant results.

DISCUSSION

The consequences of opportunistic TB on the clinical course and outcome of HIV-1 infection have been well studied (1). In contrast, information regarding the impact of the mycobacterial infection on the T cell

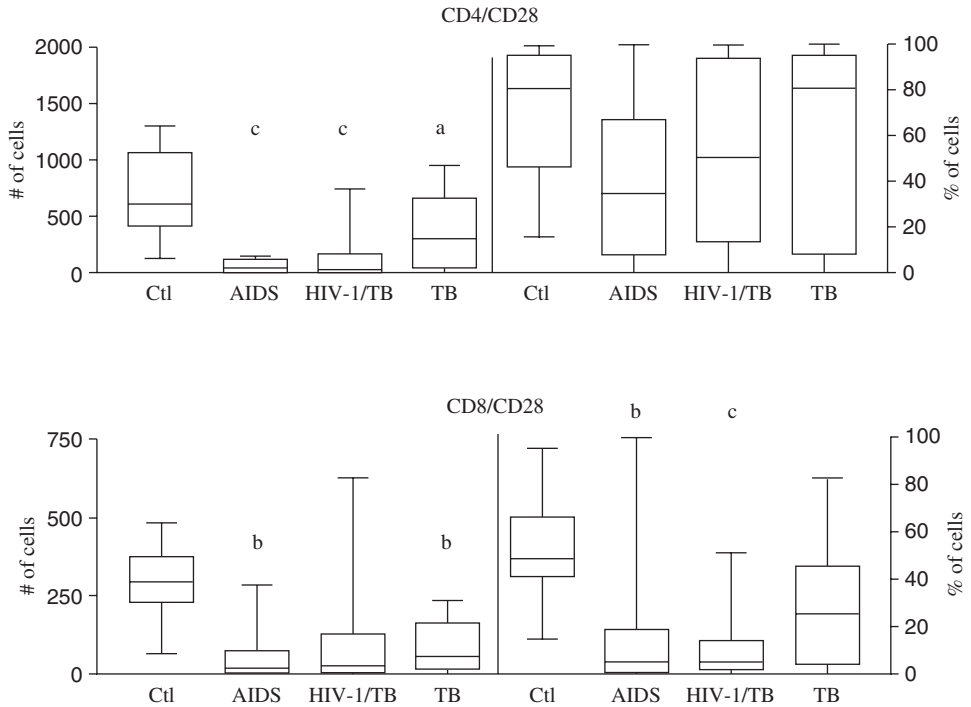

Fig. 2. Flow cytometry analysis of peripheral blood cells from 13 AIDS patients, nine HIV-1/*M. tuberculosis*-coinfected patients, and 10 HIV-1-negative patients with pulmonary TB. Significant results are shown in absolute numbers and percentage of CD4+/CD28+, and CD8+/CD28+ T cells. The box plot represents the median, 25/75 percentile, and extreme values. **a:** Significantly different from control group ($P<0.05$). **b:** Significantly different from control group ($P<0.01$). **c:** Significantly different from control group ($P<0.001$).

TABLE 2. The peripheral blood CD8+ T cell phenotype in HIV-1/*Mycobacterium tuberculosis* coinfected patients*

	Controls (n = 21)		AIDS (n = 13)		HIV/TB (n = 9)		TB (n = 10)	
Marker	%	#	%	#	%	#	%	#
CD3/CD8	20±8	473±216	31±24	4558±343	37±19	592±430	13±8	244±142
CD8/CD45RA	55±27	302±182	42±39	194±247	27±17	183±153	48±21	192±170
CD8/CD45RO	47±26	273±174	39±28	267±216	36±12	224±229	60±27	168±137
CD8/CD95	42±26	251±181	36±36	190±245	48±39	443±436	45±36	169±183
CD8/CD27	32±31	146±138	25±29	66±67	13±13	104±128	33±33	92±100
CD8/CD30	6±22	16±55	1±2	7±14	0±0	0±0	7±8	17±23
CD8/CD30L	26±39	110±160	27±45	19±26	4±5	27±38	13±8	32±21
CD8/CD40L	10±25	38±64	22±37	58±156	2±3	18±34	7±9	14±14
CD8/CD28	53±21	302±110	16±29[b]	58±90[b]	11±16[c]	111±201	28±26	77±79[b]

*Media, absolute cell counts and percentage±standard deviation of flow cytometry results. Statistically significant differences between groups were determined by one-way ANOVA with Tukey post test.
[a]Significantly different from the control group ($P<0.05$).
[b]Significantly different from the control group ($P<0.01$).
[c]Significantly different from the control group ($P<0.001$).

phenotype of the HIV-1-infected individual is scanty. Here we analyzed by flow cytometry the peripheral blood T cells of AIDS patients with and without pulmonary TB. In AIDS patients opportunistic TB did not further decrease CD4+ T cell counts. This was somewhat unexpected since two conditions characterized by CD4+ T cell loss converge in the coinfected individual. In HIV-1 infection, CD4+ T cell loss is progressive and severe and predicts a bad outcome (3). In TB, CD4 lymphopenia is less frequent and severe, is not always associated with a bad outcome, and may be corrected with antituberculous chemotherapy (21).

Our observations suggest a rescuing effect of the superimposed mycobacterial infection. The reversal of

the severe CD3+ T cell loss in AIDS patients to healthy control values in coinfected individuals is noteworthy. This finding could be relevant since CD3+ T cell loss may predict the development of AIDS independently of CD4 counts (26).

CD4+ T cells that express the cell-surface isoforms CD45RA and CD45RO are major targets in HIV-1 infection (27). The CD45RA marker characterizes naïve cells that differentiate into CD45RO memory cells after antigen activation (28). In this work we found that the diminished expression of CD45RA and CD45RO on CD4+ T cells was partially reversed in coinfected individuals. A similar (albeit less striking) rescuing effect was observed in the diminished expression of CD27 and CD95 on CD4+ T cells. The latter is an interesting finding because HIV-1 infection is known to increase the expression of CD95 on T cells, which is associated with decreasing peripheral blood CD4+ T cell counts (9,10,29).

The interaction of CD28 with its ligand B7 plays a crucial role in T cell activation (25). In HIV-1 infection CD28 plays both positive and negative roles. CD4+ T cell activation through TCR and CD28 promotes viral transcription (30). CD28 costimulation may also inhibit HIV-1 infection by downregulating chemokine receptor expression (31). Decreased expression of CD28 on both CD4+ and CD8+ T cells has been observed in HIV-1 infection (11,32), and depletion of the CD8+/CD28+ T cell subset was reported in a previous study of TB patients (33). In our TB patients we observed a severely decreased expression of CD28 on both CD4+ and CD8+ T cells. We also found a decreased expression of CD28 on CD4+ T cells of AIDS patients that was not modified in the coinfected patients. The CD8+/CD28+ T cell subset was similarly reduced and, interestingly, depletion of this subset was higher in AIDS/TB coinfection. The effects of CD8+/CD28+ T cell loss in the course of HIV-12 infection are unknown. CD8+ CD28– T cells differ from CD28+CD8+ T cells in their high catalytic activity, elevated IFN-γ production, and increased tendency to apoptosis (32,34). The diminished expression of CD28 on CD8+ T cells has been attributed to repeated antigenic stimulation (11,32), as observed in AIDS and TB.

In summary, in this study we found that the T cell phenotype of AIDS patients is impacted by additional *M. tuberculosis* infection inducing both negative and positive effects on peripheral blood T cells. A prospective follow-up study is needed to determine whether these changes influence the course and outcome of the AIDS/TB-coinfected individual. It is possible, however, that the increased expression of the proapoptotic molecule CD95 on CD4+ T cells, and the decreased expression of the major costimulatory molecule CD28 on CD8+ T cells related to the additional mycobacterial infection further compromise T cell function in the HIV-1-infected patient.

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