Distribution of Naive and Memory/Effector CD_4^+ T Lymphocytes and Expression of CD_{38} on CD_8^+ T Lymphocytes in AIDS Patients With Tuberculosis

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CD_4^+ and CD_8^+ T lymphocyte counts, naive and memory/effector CD_4^+ T subpopulations, and the expression of CD_{38} on CD_8^+ T lymphocytes were evaluated in four groups: AIDS patients with tuberculosis (HIV/TB, n=14), HIV-1 infected patients (HIV, n=10), HIV-1 negative patients with tuberculosis (TB, n=20) and healthy controls (CTL, n=17). TB and HIV had fewer CD_4^+ T cells than CTL, with the lowest values observed in TB/HIV ($p<0.001$). No difference between groups was observed in the percentage of naive and memory/effector subpopulations in CD_4^+ T lymphocytes. TB (355 cells/µL) and HIV (517 cells/µL) had diverging effects on CD_8^+ T cell counts, with a marked depletion observed in HIV/TB (196 cells/µL). TB and HIV up-regulated CD_{38} expression on CD_8^+ T cells, a finding also present in TB/HIV. While the decrease of CD_4^+ T cell counts in HIV/TB may be attributed to HIV and tuberculosis, the decrease of CD_8^+ T cell counts is likely to be due to tuberculosis.
Key Words: Tuberculosis, AIDS, CD_{38}, CD_4^+, CD_8^+ T lymphocytes.

Since the 90's, the global burden of tuberculosis (TB) has been markedly influenced by the acquired immunodeficiency syndrome (AIDS) epidemic. HIV infection has been claimed as one of leading causes of the recrudescence of tuberculosis in developed countries. Likewise, an increasing proportion of tuberculosis is occurring in HIV-infected patients worldwide [1].

Co-infection with *Mycobacterium tuberculosis* and HIV leads to alteration in the clinical course of both diseases [2,3]. It has been demonstrated that HIV-infected persons have a much higher susceptibility to *M. tuberculosis* disease. Clinical manifestations of tuberculosis in HIV-patients are usually more severe, with diffuse pulmonary involvement, and frequent extra-pulmonary dissemination [4]. In addition, *M. tuberculosis* has been shown to increase HIV-1 replication [5,6], and possibly progression to AIDS [7,8].

Thus, it is conceivable that the immune derangement found in co-infected patients differs from that of patients infected with either of these pathogens alone. It has been shown that co-infected patients have decreased proliferative response to *M. tuberculosis* antigens, and reduced production of IL-2 and IFNγ, compared to patients with tuberculosis and no HIV [8].

T lymphocytes play a pivotal role in host responses to *M. tuberculosis* and HIV-1 infections. Immunophenotyping of peripheral T lymphocytes and expression of surface activation markers in HIV-infected patients has demonstrated an imbalance of naive and memory/effector cells on CD_4^+ cells [9] and enhanced expression of activation surface markers, such as CD_{38}, on the CD_8^+ cells [10]. We have recently shown how *M. tuberculosis* infection and disease affect these parameters [11]. However, there is little information on these parameters in patients co-infected with *M. tuberculosis* and HIV-1. Most investigations conducted on patients with both *M. tuberculosis* and

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HIV-1 fail to estimate the contribution of each infection *per se* in the immune derangement seen in co-infected patients. We evaluated CD_4^+ and CD_8^+ T cell counts, the CD_4^+ T lymphocytes subpopulations of naive and memory/effector cells, and the expression of CD_{38}, a surface activation marker, on CD_8^+ T lymphocytes in patients co-infected with both *M. tuberculosis* and HIV and in patients infected either with *M. tuberculosis* or HIV.

Materials and Methods

Patients and Healthy Volunteers. Institutional Review Board approved written informed consent was obtained from all participants, according to the Brazilian Ministry of Health Guidelines. Four groups of volunteers were enrolled: (1) the active TB group consisted of HIV-1 negative patients with recently diagnosed active pulmonary tuberculosis, defined by a medical history and clinical findings compatible with pulmonary tuberculosis, a thoracic roentgenogram showing lung involvement suggestive of tuberculosis, and isolation of *M. tuberculosis* from a respiratory tract specimen; (2) the asymptomatic HIV group consisted of HIV-1-infected individuals, as determined by an enzyme-linked immunosorbent assay (ELISA) and a confirmatory Western Blot or Indirect Immunofluorescence assay. All patients had CD_4 T lymphocyte counts of less than 350 cells/µL) and had no evidence of active opportunist disease; (3) the co-infected group consisted of HIV-1-infected patients who presented with tuberculosis, based on clinical and laboratory findings, as defined above, enrolled prior to tuberculosis treatment; (4) the control group consisted of healthy volunteers, who had two consecutive non-detectable delayed-type hypersensitivity intradermal reactions to a purified protein derivative, and had no evidence of active respiratory disease.

Monoclonal Antibodies and Sample Preparation. CD_{27} phycoerythrin (PE, clone L128), CD_{45}RA FITC (clone L48), CD_8 peridin chlorophyll protein (PerCP, clone SK1), CD_4 PerCP (clone SK3), and CD_3 allophycocyanin (APC, clone UCHT1) monoclonal antibodies were obtained from Becton Dickinson Immunocytometry Systems (BDIS, San Jose, CA), and CD_{38} FITC (clone HIT2) was obtained from Pharmingen (San Diego, CA). One hundred microliters of EDTA-treated blood were incubated at room temperature with a combination of monoclonal antibodies for 15 minutes in the dark, and then treated with hemolysis buffer for a further 10 minutes. Cells were washed and resuspended in phosphate saline buffer supplemented with 0.1% sodium azide for cytometric analysis. Cell samples were analyzed on a FACSCalibur flow cytometer (BDIS). The subpopulation of naive (as defined by $CD_{45}RA^+/CD_{27}^+$) and effector/memory cells ($CD_{45}RA^-$) was analyzed as the percentage of cells expressing these surface markers on the CD_4^+ T lymphocytes. The activation of CD_8^+ T lymphocytes was determined by the percentage of expression of CD_{38}, after establishing the quadrants in samples with fluorescence labeled isotype control antibodies. For blood T CD_4^+ and CD_8^+ lymphocyte absolute counts, a TriTest and TrueCount reagent kit (BDIS) was used according to the manufacturer's instructions. Samples were acquired and analyzed using Multiset and CellQuest software (BDIS).

Statistical Analysis. Statistical analysis used Statistica (StatSoft, Tulsa, OK, USA) and NCSS (Keysville, UT, USA) software. Group results were compared using a Kruskal-Wallis one way ANOVA on ranks test. For those variables identified as having a $p<0.05$, multiple pair-wise group comparisons were further performed, using a Z value calculated with the Bonferroni correction as a threshold for statistical significance.

Results

From August, 1999, to July, 2000, 61 subjects were enrolled in the four defined groups: active TB (n=20), asymptomatic HIV+ group (n=10), co-infected group (n=14), and healthy volunteers group (n=17). Age in years (mean± standard deviation) was nearly the same in the four groups (33±11, 30±6, 29±6 and 32±13, respectively).

Figure 1. Peripheral blood numbers of CD_4^+ T lymphocytes in co-infected, asymptomatic HIV+ patients (HIV+), active tuberculosis (TB) and healthy volunteers. Box plot represents the median, 25th/75th percentile and extreme values. Figure 1A represents the absolute peripheral blood numbers of CD_4^+ T lymphocytes and Figure 1B shows the percentage participation of the naive subpopulation ($CD_{45}RA^+/CD_{27}^+$) among T CD_4^+ cells. Significant differences between groups was observed in CD_4^+ T counts in all pairwise comparisons, except between co-infected and the HIV+ asymptomatic group.

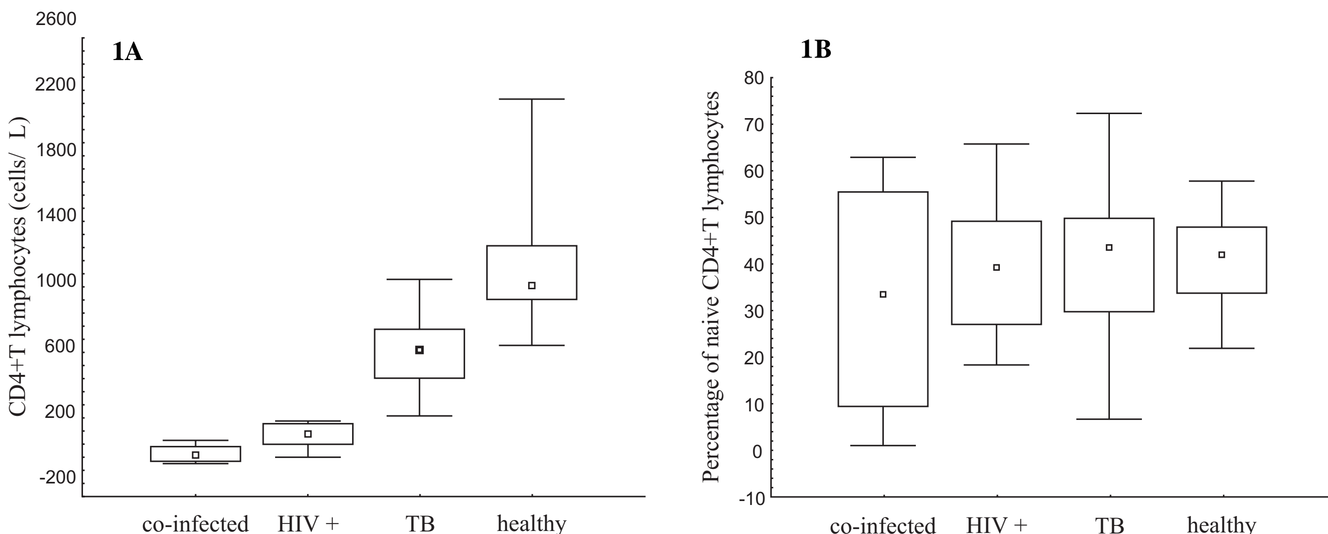
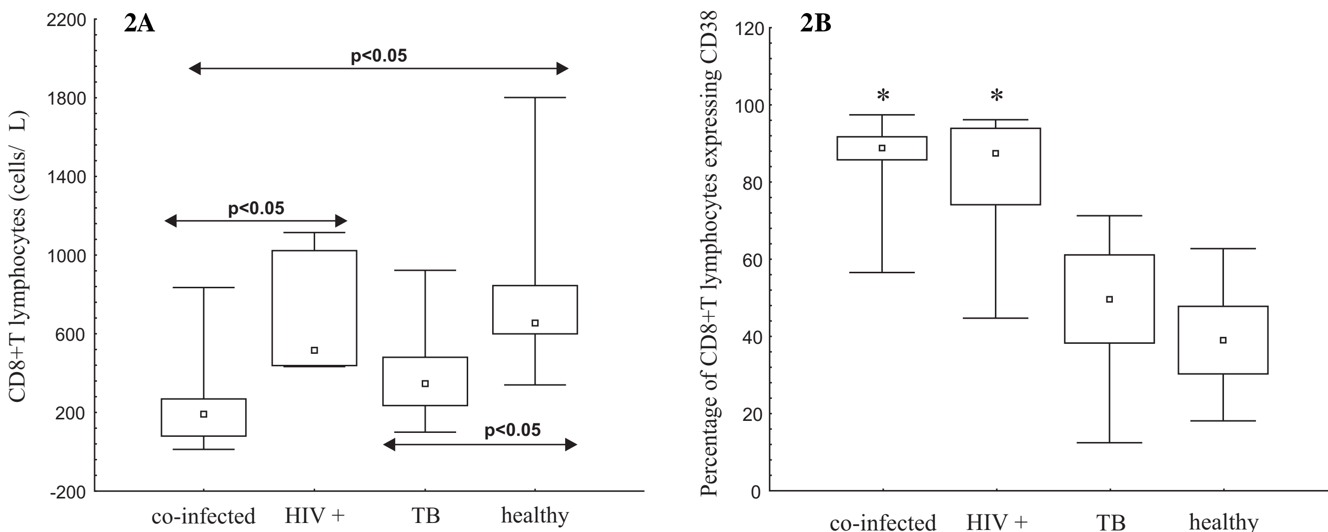


Figure 2. Peripheral blood CD_8^+ T lymphocytes in co-infected, asymptomatic HIV+ patients (HIV+), active tuberculosis (TB) and healthy volunteers. Box plot represents the median, 25th/75th percentile and extreme values. Figure 2A represents the absolute peripheral blood numbers of CD_8^+ T lymphocytes and Figure 2B shows the percentage of CD_8^+ T lymphocytes expressing CD_{38}.



*$p<0.05$ compared to TB and healthy volunteers.

CD_4^+ T lymphocyte counts were significantly different among the four groups ($p<0.001$), with the highest cell counts in the healthy volunteers (median 1,153 cells/µL, range 715 to 2,218 cells/µL), and the lowest in HIV/TB patients (median 53.5 cells/µL, range 2 to 194 cells/µL). According to multiple-comparison analyses by the Bonferroni test, the CD_4 T cell counts were higher in TB patients (median 690 cells/µL, range 291 to 1,124 cells/µL) than in the HIV asymptomatic (median 178 cells/µL, range 38 to 262 cells/µL) and HIV/TB patients (Figure 1A). The percentage of CD_4^+ T lymphocyte subpopulations of naive cells did not differ among the groups ($p=0.76$), nor did memory/effector subpopulations ($p=0.67$) (Figure 1B).

The number of CD_8^+ T lymphocytes was significantly different among the four groups ($p<0.001$). TB and HIV infection exerted differential effects on CD_8^+ T cell counts (median 355 cells/µL, range 138 to 917 cells/µL, and 517 cells/µL, range 438 to 1,108 cells/µL respectively), with co-infection resulting in a marked depression of these cells (median 196 cells/µL, range 10 to 1,009 cells/µL) (Figure 2A).

The percentage of T CD_8 expressing CD_{38} was different among the four groups ($p=<0.001$), with the lowest values presented by the healthy volunteers (Figure 2B). Asymptomatic HIV-1 patients had higher expression than tuberculosis patients, and HIV/TB patients were similar to asymptomatic HIV-1 patients, but had a higher expression than TB-patients ($p<0.05$).

Discussion

HIV-infected patients with tuberculosis had lower CD_4^+ and CD_8^+ T lymphocytes counts than patients with single TB or HIV infections, respectively. Both HIV-infected and TB patients presented lower CD_4^+ T lymphocyte counts than healthy controls. Low CD_4^+ T cell counts was an inclusion criterion for HIV patients (less than 350 cells/µL). This criterion, along with a lack of an ongoing opportunistic infection and antiretroviral therapy, was designed to enroll asymptomatic HIV-patients with established HIV-induced immune alterations. It is not unexpected that HIV-patients with opportunistic infections have lower CD_4^+ T cell counts than asymptomatic patients. Thus, the tendency towards lower cell numbers seen in co-infected patients may reflect more advanced HIV disease in such individuals. However, one may consider a role for TB as a further factor for the decrease of T CD_4^+ lymphocytes in such susceptible patients. This makes sense when we examine the depression of CD_4^+ T lymphocyte counts found in the group of patients with tuberculosis and not infected by HIV [11].

The decrease of CD_4^+ T lymphocytes was found to be differentially distributed in naive, memory and effector subpopulations, with the differences in absolute cell numbers in each subpopulation reflecting what was observed in total T CD_4^+ cells (data not shown). Although one may expect a change in the distribution of these subpopulations in the HIV/TB setting, as well as in each disease independently, we did not find differences in this study, which may be due to the small numbers of subjects in each group. A decrease in naive CD_4^+ cells has been described in HIV-infected patients with advanced disease [9]. We recently evaluated CD_4^+ T cell subpopulations in persons with TB infection (PPD positive skin test) and patients with disease, before and after treatment, and found a major impact on T CD_4^+ cells, but again changes in the percentages of naive and memory/effector cells were not significantly different [11].

We found that TB and HIV infection have different effects on CD_8^+ T cell counts. In TB patients there was a decreased number of CD_8^+ T lymphocytes, while the cell numbers were not altered in HIV-infected patients. In general these cells are found in enhanced numbers in HIV-infected patients during long periods of the natural progression of HIV infection [12]**.** The occurrence of TB in AIDS patients results in a strong reduction of CD_8^+ T lymphocytes, compared to HIV-infection alone, with a trend towards absolute counts even lower than in patients with tuberculosis. This finding, coupled with the extremely low CD_4^+ T cell counts, may contribute to the impaired cellular immunity seen in co-infected patients.

Expression of CD_{38} on CD_8^+ T lymphocytes is a useful tool to evaluate the state of cellular activation, which is higher in HIV-infected patients than in negative controls, increases as the disease advances, and has

been shown to be a predictor of progression of this disease [13]. We have previously reported enhanced expression of CD_{38} in patients with TB, compared to treated patients [11]. Our results support the hypothesis of augmented expression of CD_{38} secondary to HIV infection, at a proportion as high as 89% of CD8+T lymphocytes expressing CD_{38} in HIV/TB patients, demonstrating the high state of activation of these cells. Thus, whereas HIV/TB resulted in a decrease of CD_8^+ T cell counts, the marked upregulation of CD_{38} remains as high as in HIV-infected patients, as a result of both HIV infection and TB.

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