Caspase 1 and caspase 8 in HIV infected patients with and without tuberculosis

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ABSTRACT

Background: Caspase 8 is involved in apoptosis mediated by Fas and p55 tumor necrosis factor receptor ligation in HIV infection. Apoptosis is partially mediated by interleukin-1beta-converting enzyme (caspase-1).

Aims: We determined apoptosis, using caspase-1 and caspase-8, among patients with HIV infection, with and without tuberculosis (TB), those with TB alone and healthy individuals.

Setting and design: Cross-sectional analysis of caspase-1 and caspase-8 among patients with HIV infection, with and without TB, those with TB alone and healthy individuals.

Materials and Methods: Nineteen HIV infected patients with TB (HIV+/TB+) and 20 with HIV infection without TB (HIV+/TB-) were studied. Fifteen individuals with TB alone were disease controls (HIV-/TB+) and 20 were healthy controls (HIV-/TB-). Caspases were measured by single-step ELISA using commercially available monoclonal antibodies.

Statistical Analysis: Two-way ANOVA and Pearson’s correlation coefficient.

Results: Mean CD4 counts of HIV+/TB+ were lower than HIV+/TB- (p<0.05). OD value of caspase 1 in HIV+/TB+ was 0.295±0.05, while that in HIV+/TB- it was 0.302±0.18. It was 0.293±0.07 in HIV+/TB+ and in HIV-/TB- the values were 0.287±0.06. OD value of caspase 8 in HIV+/TB+ was 0.307±0.07, lower than HIV+/TB- (0.927±0.25). It was 0.008±0.03 in HIV+/TB+ and in HIV-/TB-, 0.074±0.004. Values of caspase 8 in patients with HIV infection (with/without TB) were higher than those with TB alone or healthy individuals (p<0.01). Levels of caspase 8 in HIV+/TB- were higher than patients with HIV+/TB+ (p<0.01).

Conclusion: Levels of caspase-1 are not different irrespective of presence or otherwise of TB and HIV infection. Fas-related apoptosis is higher in HIV infection. With concomitant TB, levels of caspase 8 were lower as compared with those without TB.

KEY WORDS: HIV, Tuberculosis, Caspase1, Caspase 8, apoptosis

Depletion of CD4+ T cells is largely responsible for the immunodeficiency that occurs during the late stages of HIV disease. Since only a very small proportion of CD4+ T cells are productively infected at any given time and a large number of cells are getting destroyed continuously, several mechanisms must exist whereby HIV-1 might induce the depletion of CD4+ cells, including uninfected CD4+ T cells. An important basis of T-cell depletion in patients infected with HIV is increased apoptosis of CD4 and CD8 T cells.

Fas ligand (FasL) and tumour necrosis factor (TNF) bind to their receptors to initiate apoptosis. In the case of FasL and TNF, membrane-associated proteins may be cleaved by the action of matrix metalloproteases to release soluble ligands that maintain their biologic activity. Ligation of these death receptors results in sequential activation of a family of cysteine proteases that cleave at aspartate residues, or caspases. Caspase 8 is involved in apoptosis mediated by Fas and p55 TNF receptor ligation. Activated caspases activate downstream effector caspases, which activate various cellular proteases and endonucleases to cleave host cell structural and regulatory proteins and host nuclear DNA, ultimately causing the cell to undergo the morphologic and biochemical changes that occur in apoptosis. Apoptosis is partially mediated by Fas-Receptor (Fas-R, CD95) and by interleukin-1beta (I-1b)-converting enzyme (ICE; caspase 1).

Protective immunity against the intracellular pathogen Mycobacterium tuberculosis is dependent on the activation of T cells. Like other intracellular pathogens, mycobacteria induce Ag-specific, major histocompatibility complex-restricted CD4+ and CD8+ cytotoxic T lymphocytes (CTL). Most M. tuberculosis-specific cytolytic activity is mediated by CD4+ CTL, and the killing by CD4+ CTL clones of Mycobacterium-infected human macrophages can inhibits the growth of the bacteria, probably by destroying the intracellular habitat of the microorganisms. Since CD4+ CTL kill target cells via FasL-induced apoptosis, we determined the level of caspase 8 in
patients with TB with and without HIV infection. In addition, since there is a role of release of macrophage-activating cytokines, we also measured the levels of caspase 1 in these patients.

Materials and Methods

Patients and controls: Nineteen consecutive patients with HIV infection who were diagnosed in the Department of Internal Medicine, PGIMER, Chandigarh on the basis of positivity on a panel of three ELISAs (Genedia, Korea, Lab Systems, Finland, Xcyton Diagnostics Ltd, India) and diagnosed to have active TB (HIV+/TB+) as shown by acid fast bacilli positivity were included in the study. Twenty patients with HIV infection but with no opportunistic infection (HIV+/TB-) were studied as disease controls. Twenty healthy individuals served as healthy controls (HIV-/TB-). These are the same set of patients that have been reported in an earlier study where we reported nitric oxide production before and after four weeks of chemotherapy.13 Fifteen individuals with pulmonary tuberculosis only were studied as another group of disease controls (HIV+/TB+). The study protocol was approved by the Ethics Committee of the Institute.

To determine CD4+ T cell population in the HIV infected population, 10 μl of anti-human CD4-FITC mAb (Sigma, USA) was added to 100μl of whole blood and incubated at room temperature for 15 min. At the end of the incubation, lysis solution was added and incubated for 10 min at room temperature. The washed and fixed cells were then analyzed on the flowcytometer (FACSscan, Becton Dickinson, Mountain View, CA). Ten thousand cells were counted and analyzed using Cell Quest program (Becton Dickinson, Mountain View, CA) after gating for lymphocytes. Dead cells were excluded by forward and side scatter gating. Since we used anti-human CD4-FITC, which was of mouse origin and IgG1 subclass, we used irrelevant goat anti-mouse IgG1-FITC as the isotype control antibody. Absolute values were calculated by determining the percent CD4 cells from the absolute lymphocyte counts (from Coulter counter).

Caspase levels were measured by us recently.15 In this study we measured these levels, in the serum, using a double antibody sandwich ELISA. Unlabelled polyclonal antibody to Caspase-1 or 8 (Santa Cruz Biotechnology, USA) was added to 96 well plate as 50 ul of antibody solution (20 ug/ml in PBS) and incubated for 2 h at room temperature (RT). After incubation, the wells were washed with PBS-Tween 20 washing buffer and 100 ul blocking buffer (5% BSA / PBS + 0.02% Sodium azide) was added to all the wells and left for 2 h at RT. This was followed by another washing step and addition of 50 ul of sample which was incubated at RT for 2h. This was washed and 50 ul of the polyclonal antibody to Caspase 1 or 8 was added to the respective plates. After a 2h incubation at RT the Horseradish peroxidase labeled antibody was added to the wells (anti-rabbit –HRP for Caspase-1 and anti-goat HRP for Caspase-8, Sigma, USA). This was again incubated for 2h at RT and washed at the end of incubation. The substrate ortho phenylene diamine (OPD, Sigma, USA) was used as a substrate. Anti-human Caspase-1 used is non-cross reactive with caspase-1 p10 or caspase-1 p20. It does not detect caspase-1 of mouse or rat origin. Anti-human Caspase-8 used is known to react with the carboxy terminal prodomain of Caspase-8 (also designated as pro Mch5, MACH alpha 1 or FLICE) of human origin.

Statistical Analysis: The results were analyzed by two-way analysis of variance (ANOVA). Pearson’s correlation coefficient was calculated to determine correlation between OD value and CD4 counts for caspase 1 and 8. P<0.05 was considered significant.

Results

Mean CD4 counts of HIV/TB coinfected were significantly lower (P = 0.02) than those of patients with HIV infection alone. They were well matched for age and sex (Table I). OD value of caspase 1 (Figure 1) was not significantly different among the four groups. The two HIV infected groups showed no correlation between the CD4 counts and the OD values of caspase 1 (r=0.24).

Mean OD value of caspase 8 in HIV+/TB+ (Figure 2) was 0.683±0.04, which was significantly lower (P<0.01) than that in HIV-/TB- in which it was 0.74±0.04. Its value was 0.655±0.04 in HIV+/TB+ and in HIV-/TB- it was 0.545±0.03. Values in the two groups with HIV infection (with and without TB) were higher (P<0.01) than those in patients with TB alone or healthy individuals. Caspase 8 levels in patients with HIV infection alone were higher than the corresponding levels in those with dual infection, i.e. HIV+/TB+ (P<0.01). There was no correlation between the CD4 counts and the OD values for caspase 8 of the two HIV-infected groups (r=0.35).

Discussion

Apoptosis is an important mechanism of lymphocyte death among patients with HIV infection.19,20 In this study, levels among the four groups were similar for caspase-1 but caspase-8 levels were higher in HIV infected patients, more so if there was no TB.

Protective immune response to TB involves activation of infected macrophages by antigen-specific T cells, and killing of intracellular tubercle bacilli.21 IL-1 contributes to host defense mechanisms in mycobacterial infection.22 Since it is seldom found in the circulation of patients, it has been suggested that

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<td><strong>Sex</strong></td>
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<td><strong>CD4 count (/mm³)</strong></td>
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ND – not done, *P<0.01 compared to HIV+/TB+
serum concentrations of endogenous inhibitors of this cytokine may indirectly reflect its activity. While IL-1 receptor antagonist(s) might be useful as markers of inflammatory activity in TB alone, IL-1, per se, might not be as useful. In a recent study, the authors investigated the possibility that Mycobacterium tuberculosis-induced apoptosis could be associated with pro-inflammatory cytokine production. It was shown that Mycobacterium tuberculosis-induced apoptosis is inhibited by a caspase-1 inhibitor and is associated with the maturation of IL-1β. There might be a relation between the stage of HIV infection (and, consequently, host immune response) and its ability to respond to apoptotic signals.

Caspase-8 levels were higher in HIV infection. Since Fas mediated apoptosis is an important mediator of CD4 cell death in HIV infection, higher levels of caspase-8 are expected. The levels in patients with HIV infection without TB were significantly higher than those with TB. Mycobacterium tuberculosis can evade apoptosis of host macrophages by release of TNF receptor-2 (TNF-R2). Mycobacterium tuberculosis can escape killing in human macrophages by specific CD4+ CTL that could induce in vivo FasL-dependent macrophage apoptosis, not only depriving mycobacteria from their growth environment but also reducing viable bacterial counts during apoptosis. CTLs may complement the effector functions of IFNγ -secreting effector cells by specific killing of infected cells. The advantage is that mycobacteria within apoptotic macrophages remain intracellular and prone to phagocytosis in apoptotic debris by freshly attracted monocytes, preventing bacterial dissemination. In turn, the Mycobacterium tuberculosis can modulate the Fas expression and the susceptibility of infected macrophages to FasL-induced killing. This may explain lower caspase-8 among patients with HIV/TB coinfection as compared with those with HIV alone. In addition, patients with HIV infection alone had higher CD4 counts as compared with those who had HIV/TB coinfection and were at a less advanced stage of disease. It is likely that response to apoptotic signals might be affected by the ability of the host to mount an immune response and at a higher CD4 count this might reflect in significantly higher Caspase 8 levels.

Our findings show that apoptosis mediated by caspase 1 levels are not significantly different among patients with or without TB and those with or without HIV infection. However, apoptosis mediated by caspase 8 is greater among individuals with HIV infection. If there is concomitant TB infection then levels are lower as compared with the corresponding levels in those without TB. The bacillus may have devised this method for protection against the host to perpetuate its survival.

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