

Direct relation between monocyte gene expression, circulating count and apoptosis resistance following therapy interruption: Increased metallothionein gene (MT1) expression associated with functional resistance against apoptosis induction

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ABSTRACT

Background: Freshly recovered monocytes from patients chronically infected with HIV-1 (viremic) exhibit an apoptotic signature in which a variety of genes related to apoptosis, such as MT1 are differentially expressed and have been demonstrated to be associated with resistance to apoptosis-induced cell death (AICD). It is unknown how closely the observed *in vivo* apoptosis gene signature relates to regulation of AICD during an acute viremic episode associated with monocyte count changes *in vivo* or its relation to *in vitro* gene regulation following infection of macrophages.

Methods: Archived PBMCs derived from whole blood of patients within a cohort undergoing structured treatment interruption or from seronegative donors were tested for a) resistance to AICD (Cadmium (CdCl₂), measured by Caspase-3 activation in T-cells and monocytes, b) monocyte versus CD4 T cell count, and c) gene expression at the initiation and during a 4 week therapy interruption interval. Total RNA was isolated and amplified from CD14 monocytes or CD4 T cells. Gene expression as determined by quantitative real-time PCR was also compared between acute viral rebound (therapy interruption) and chronic viremia. Additionally, *in vitro* R5 HIV-1 infected monocyte-derived macrophages (MDM) from seronegative donors the *in vitro* gene modulation and the role of genes in constitutive AICD by siRNA were tested.

Results: Increased monocyte counts following therapy interruption (p=0.031; median at week 0 = 400 and at week 6 = 500; 25% difference) was associated with viral rebound, a drop in CD4 T cell count (p=0.065; 15% difference), increased resistance to AICD in monocyte but not in CD4 T cells, and increased MT1 expression. MT1 expression remained high before and after viral rebound and during chronic viremia in contrast to other apoptosis related genes examined. MT1 expression was elevated following HIV-1 infection of macrophages *in vitro* and its inhibition is shown to increase constitutive apoptosis.

Conclusions: We identified that *in vivo* changes in monocyte count can be associated with regulation of apoptosis and expression of MT1. This study shows a direct role for MT1 expression in resistance to induced apoptosis during HIV-1 infection *in vitro* and suggests a role for this gene in maintenance of monocyte/macrophage cells *in vivo* during HIV-1 infection.

INTRODUCTION

Although monocytes, like CD4⁺ T-cells are targets of HIV infection, apoptosis-related depletion of this cell population does not occur during chronic infection (reviewed in Lum and Bradley, 2003). This suggest that during HIV infection monocytes are resistant to apoptosis. Several microarray studies have already demonstrated that monocytes/macrophage infected *in vitro* with HIV up-regulate anti-stress and anti-apoptosis related genes (i.e. metallothioneins, IER3, PAI-2, and HSP70) that ultimately result in an overall "anti-apoptotic gene signature" (Giri et al 2006). This signature may be an important component in the establishment of monocyte viral reservoirs.

Metallothioneins (MTs) are small metal binding proteins that protect the cell against metal toxicity. Induction of MTs occur during metal-associated oxidative stress and cytokine/hormone release. Recently, MTs have been demonstrated to be integral to stress and immune responses. Additionally, MTs have been shown to regulate cellular susceptibility to apoptosis, the release of hormones and cytokine, and leukocyte chemotaxis. Cytokines IL1- α , IL1- β , TNF- α , and IFN- β have all been shown to induce MT gene transcription.

Like CdCl₂, HIV-1 gp120 and Tat induce oxidative stress resulting in apoptosis via the mitochondrial (intrinsic) pathway suggesting a potential dominant role for increased MT1 expression in the protection of monocytes from gp120/Tat-induced apoptosis. There are several isoforms of MT1 (A-H, and X) that are expressed in myeloid cells. During HIV infection, our cDNA microarray data showed isoforms G, F, and X as differentially up-regulated in freshly recovered monocytes from HIV infected patients. This suggested that these isoforms may be integral to monocyte resistance to stress/viral-induced apoptosis. Here we present preliminary data to further test the relationship between MT1 expression and monocyte resistance to apoptosis.

EXPERIMENTAL DESIGN AND METHODS

• Cross-sectional study cohort/design in which differential gene expression patterns of freshly adherent monocytes, derived from viremic/suppressed HIV-infected patients, were identified by cDNA microarray, as differentially expressed when compared to uninfected. Target genes were confirmed via real time PCR and then correlated with monocyte sensitivity/resistance to apoptosis.

• Longitudinal study cohort using monocytes derived from cryopreserved PBMCs from HIV+ patients undergoing antiretroviral treatment (ART) interruption at start (T₀) and 6 weeks after stopping ART (T₁) to examine the effects of acute viremia on monocyte gene expression and susceptibility to apoptosis on/off ART.

• Monocytes isolated from PBMCs of uninfected donors were infected *in vitro* with an R5 strain of HIV-1 and analyzed over time to directly examine metallothionein gene expression. siRNA against MT1 was also examined for effects on constitutive AICD.

Summary of Results

- Microarray analysis reveal that numerous genes associated with apoptosis are differentially expressed in monocytes of HIV infected patients. Differential expression of metallothionein genes was confirmed by real-time PCR (Figure 1)
- Monocytes derived from HIV+ patients are resistant to cadmium induced-apoptosis. (Figure 2)
- Monocyte count is directly correlated with increased viral load during antiretroviral treatment interruption. (Figure 3)
- Monocyte exhibit resistance to Cd-induced apoptosis in the presence of acute viremia. (Figure 4)
- Gene expression of MT1G and MT1X gene is increased in HIV-1 infection both before (T₀) and after ART interruption (T₁). (Figure 5).
- *In vitro* infection increases MT1 expression while silencing of MT1 (isoform F) increases monocyte sensitivity to apoptosis. (Figure 6)

Figure 1: Anti-apoptosis signature of monocytes in HIV-1 infection

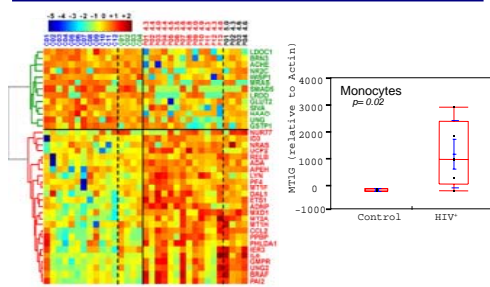


Figure 1: Microarray reveal anti-apoptosis signature in monocytes from HIV infected patients inclusive of Metallothionein gene expression. Left panel: Basic gene expression of freshly recovered adherent monocytes derived from peripheral blood mononuclear cells (PBMC) of HIV-infected patients were compared to uninfected control via cDNA microarray analysis. (A) Global mRNA expression in adherent monocytes freshly recovered from 12 HIV-infected patients and 13 uninfected controls were analyzed. Message RNA was isolated using Trizol and then used to generate tagged cDNA. Tagged cDNA was hybridized to in-house (Wistar) gene chips. Right panel: MT1G gene expression reconfirmed by RT-PCR.

Figure 2: Monocytes from HIV-infected patients resist Cd-induced apoptosis

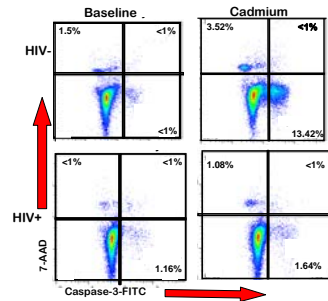


Figure 2: Monocytes from HIV-1 infected patients are resistant to cadmium-induced apoptosis. CD14⁺ cells derived from either uninfected controls (upper) or HIV-infected patients (lower) were either left untreated or treated with cadmium for 18 hours, harvested, and stained with 7-AAD and caspase-3-FITC antibodies. Flow cytometry analysis revealed no significant difference in the degree of spontaneous apoptosis in cell subsets of patients and controls (Baseline). Monocytes from HIV infected individuals were resistant to cadmium-induced apoptosis

RESULTS

Figure 3: Monocyte count increases along with viral load during STI

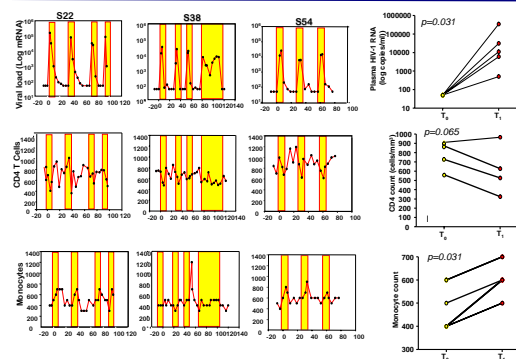


Figure 3: Monocyte count increases proportional to viral load and CD4 T cell count decreases during treatment interruption. Left panel: S22, S38 and S54 as representatives of patient cohort undergoing structured treatment interruption summarizing longitudinal data on viral load (Log mRNA, top), CD4 T cell count (middle) and monocyte counts (bottom). Right panel: Summary changes at start (T₀) and end of a 6 week interruption (T₁) of therapy showing viral load, CD4 T cell and monocyte changes. Note data summarized here reflects subjects analyzed in Figures 4 and 5 below.

Figure 4: Monocytes exhibit resistance to apoptosis during acute viremia

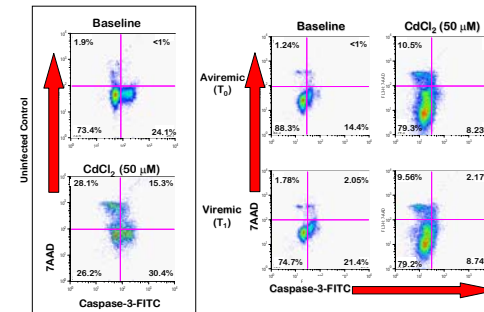


Figure 4: Monocytes from HIV-1 infected patients are resistant to cadmium-induced apoptosis during treatment interruption. CD14⁺ cells derived from either cryopreserved uninfected (left panels) or HIV-infected (right panels) subject's PBMC were either (left untreated or treated with cadmium stained with 7-AAD and caspase-3-FITC antibodies). Monocytes from HIV infected individual showed a retained resistance to cadmium-induced apoptosis both before or after acute viremia following ART interruption. The right panels are representative of donors shown in Figure 3.

Figure 5: MT levels remain high on ART and following ART interruption/viremia.

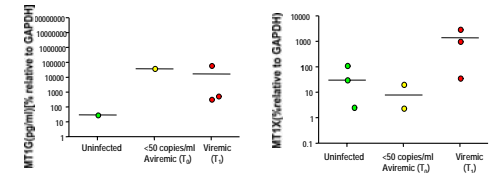


Figure 5: Monocytes from HIV-1 infected patients express high levels of MT1 before and after ART interruption. Total RNA was isolated from cryopreserved CD14⁺ cells derived from either uninfected controls or HIV-infected patients undergoing treatment interruption. Data summarizes gene expression in uninfected controls as compared to longitudinal samples from HIV-infected subjects interrupting ART. Left panel: MT1G expression relative to GAPDH. Right panel: MT1X expression relative to GAPDH.

Figure 6: Expression of MT1 increases in *in vitro* MDM infection and inhibition of MT1F increases MDM sensitivity to apoptosis

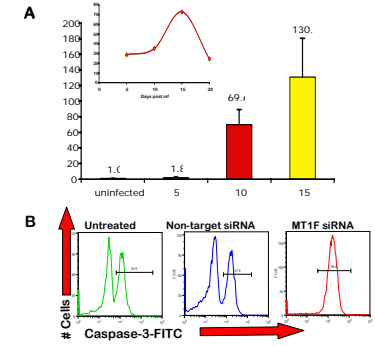


Figure 6: Monocytes infected *in vitro* with HIV-1 exhibit gene profiles similar to cells infected *in vivo*. (A) Human peripheral blood obtained from healthy donors was enriched for monocytes by elutriation, plated at a density of 566 cells/ 35 mm plate, and cultured at 37 °C for 4 days prior to infection with BeL-1 strain of HIV-1. Culture supernatants were harvested 5, 10, and 15 days post infection (dpi) and viral replication monitored by p24 ELISA. Cells harvested, total RNA extracted and prepared for real-time PCR analysis. Real-time PCR data normalized to the uninfected control. MT gene expression increased as the infection progressed. (B) Isoform F of MT silenced in uninfected monocytes increased sensitivity to apoptosis.

CONCLUSIONS

- Monocytes from HIV Infected individuals exhibit an anti-apoptotic signature in association with increased MT1 expression.
- Monocytes maintain an anti-apoptotic state before and after ART interruption and acute viremia in conjunction with increased monocyte circulating levels and MT1 gene expression.
- MT1 gene expression is increased following *in vitro* monocyte-derived macrophage (MDM) infection by R5 HIV-1.
- MT1 has a direct anti-apoptotic role on constitutive MDM apoptosis as evident in presence of siRNA against MT1F.

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