HIV Myeloid Derived Suppressor Cells Inhibit the Protective Response to Cytomegalovirus

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Abstract

Background: HIV infection is associated with diminished protective responses against opportunistic infections including cytomegalovirus (CMV). In this study, we examined the effect of HIV expanded myeloid derived suppressor cells (MDSCs) on CMV specific effector cells.

Methods: Peripheral blood mononuclear cells (PBMCs) obtained from HIV seronegative, CMV seropositive healthy donors were cultured in presence of heat inactive HIV_{BaL}. After 5 days, cells were stained with anti-CD11b, anti-CD33, anti-CD14 and anti-HLA DR. $CD11b^+CD33^+CD14^+HLA\ DR^{hi}$ (DR^{hi} monocytes) and $CD11b^+CD33^+CD14^+HLA\ DR^{-/lo}$ (MDSCs) cell subsets were sorted using flow cytometry. Sorted cell fractions were cultured with freshly isolated autologous PBMCs in presence or absence of a peptide pool of CMV pp65 antigen (1 µg/ml). IFNγ and IL-10 were determined by ELISPOT and expressed as spot forming units (SFU). Sorted cell fractions were cultured in presence of LPS (100 ng/ml) and IFNγ (2 ng/ml). IL-12p70 was determined in culture supernatants by ELISA and surface expression of HLA DR and CD83 (maturation markers) by flow cytometry. CD150 and B7H4 (leukocyte activation markers) expression on sorted cell fractions was determined by flow cytometry. Data were analyzed using two-tailed, paired Student's *t* test.

Result: Culture of PBMCs with heat inactivated HIV resulted in a significant expansion of MDSCs as compared to control PBMCs (p=0.003). Expansion of MDSC and DR^{hi} monocyte cell populations were comparable in the presence of HIV (p=0.74). PBMCs stimulated with the CMV pp65 peptide pool and cultured with sorted autologous MDSCs had fewer IFNγ SFU compared to PBMCs without MDSCs (p=0.03); however, PBMCs cultured with DR^{hi} monocytes did not significantly differ (p=0.37). Furthermore, pp65 stimulated PBMCs produced increased IL-10 SFU when cultured with MDSCs compared to cultures with the DR^{hi} monocyte subset. The MDSC subset exhibited a 2-fold increase in expression of surface CD150 and 1.3-fold increased expression of B7H4 as compared to the expression on the DR^{hi} monocyte subset (p=0.03). HIV MDSCs maintained HLA DR^{lo} and $CD83^{lo}$ expression as compared to the DR^{hi} monocytes, even when cultured with LPS and IFNγ. Furthermore, these cells produced 4-fold less of the T_H1 polarizing cytokine IL12p70 as compared to DR^{hi} monocytes (p=0.02).

Conclusion: These findings suggest that HIV infection results in a significant expansion of MDSCs and that MDSCs are involved in suppression of the immune response directed against CMV. Understanding the mechanism(s) responsible for the suppressed immune response against CMV will assist in the development of treatment strategies designed to improve the immunity to CMV and other opportunistic infections associated with advanced HIV infection.

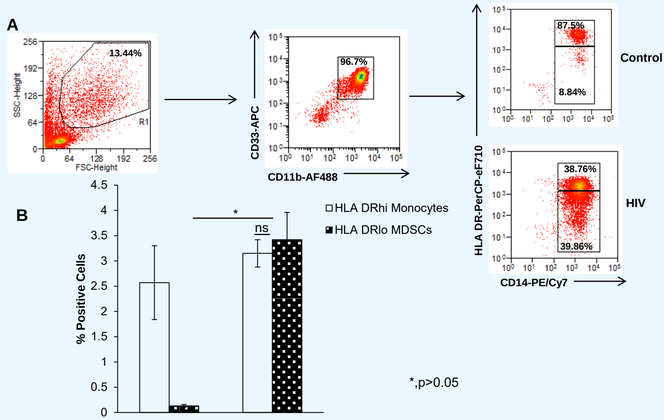
Background

Human Immunodeficiency Virus type-1 (HIV) infection causes profound immune suppression leading to loss of cellular immunity. The use of cART has contributed to increased life expectancy of HIV-1 survivors. Despite tremendous success of HAART leading to effective restoration of CD4+ T cell counts and reconstitution of the immune system; pharmacologically treated individuals continue to exhibit fewer Gag and CMV peptide specific IFN-γ producing, perforin+/CD107a+ and polyfunctional CD4+ T cells but produce elevated IL-10 responses. HIV infected person compared to uninfected lack robust protective immunity for intracellular pathogens such as CMV, EBV and Influenza and also exhibit diminished responses to vaccines. Recently, a subset of myeloid cells known as Myeloid derived suppressor cells (MDSCs) has been characterized that negatively regulate innate and adaptive immune functions. Previously, we have shown that MDSCs expand during HIV-1 infection and suppress T cell effector functions. We hypothesize these MDSCs may contribute to diminished immunity against opportunistic viral pathogens. In this study, we examined the effect of HIV-1 expanded MDSCs on CMV specific effector functions. Further, we investigated the mechanism by which HIV-1 MDSCs inhibit generation of T_H1 immunity.

Results

Figure 1

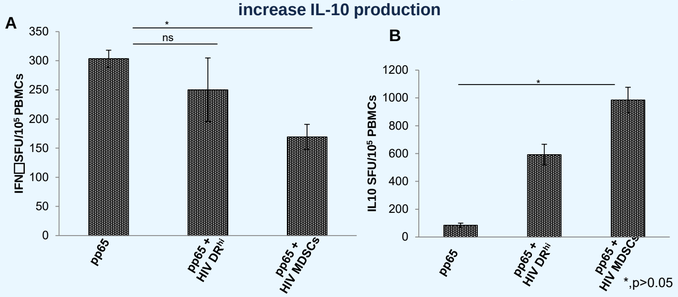
HIV-1 expands $CD33^+CD11b^+CD14^+HLA\ DR^{lo/-ve}$ MDSCs with comparable expansion of MDSC and DR^{hi} monocyte populations



PBMCs of CMV(+) HIV(-) healthy donors were cultured with or without inactive HIV for 5 days. $CD11b^+CD33^+CD14^+HLA\ DR^{lo}$ MDSCs and $CD11b^+CD33^+CD14^+HLA\ DR^{hi}$ monocyte cell expansion was determined by flow cytometry. (A) Representative dot plot is shown (B) Frequency of HLA DR^{lo} MDSCs and HLA DR^{hi} monocytes in total cell population is shown.

Figure 2

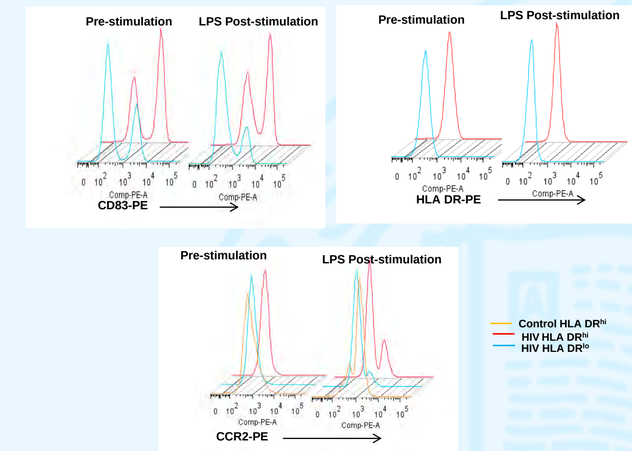
HIV-1 MDSCs cultured with PBMCs inhibit pp65 specific IFNγ production and increase IL-10 production



PBMCs of CMV(+) HIV(-) healthy donors were cultured with or without inactive HIV for 5 days. HLA DR^{lo} MDSCs and HLA DR^{hi} monocytes were flow sorted and cultured with freshly isolated PBMCs of same donor on ELISPOT plate coated with capture anti-IFNγ or anti-IL10 antibody in presence or absence of a peptide pool of CMV pp65 antigen (1 µg/ml). After 24-48 hrs, plate was washed and biotinylated detection anti-anti-IFNγ or anti-IL10 antibody was added for 2 hrs followed by addition of Streptavidin-ALP conjugate. Spots were developed using BCIP/NBT substrate, counted on automated ELISPOT reader (IMMUNOSPOT, CTL) and spot forming units (SFU) / 10^5 PBMCs were calculated.

Figure 3

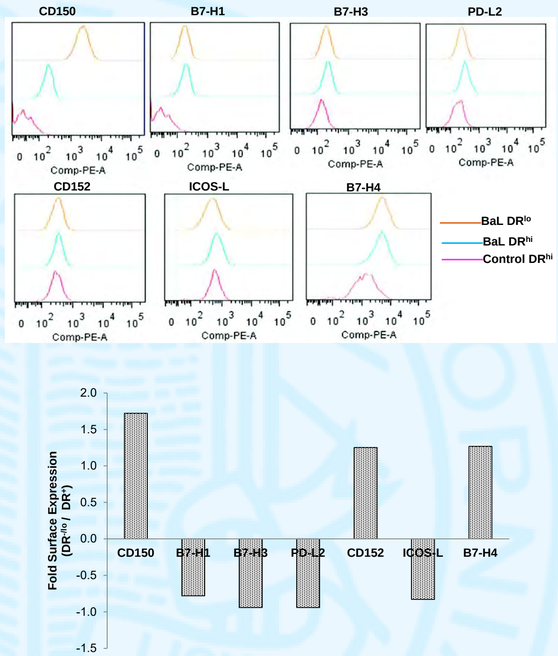
HIV-1 MDSCs exhibit down regulated T_H1 priming function



PBMCs of HIV(-) healthy donors were cultured with or without inactive HIV-1 for 5 days. HLA DR^{lo} MDSCs and HLA DR^{hi} monocytes were flow sorted and cultured with Lipopolysaccharide (LPS) and recombinant IFNγ for 24-48 hrs. (A) Cells were stained to analyze surface expression of maturation marker by flowcytometry (B) IL12p70 was determined in culture supernatants. (C) Sorted cells were cultured on ELISPOT plate coated with capture anti-IL12p70 antibody. After 24 hrs, plate was washed and biotinylated detection anti-IL12p70antibody was added for 2 hrs followed by addition of Streptavidin-ALP conjugate. Spots were developed using BCIP/NBT substrate, counted on automated ELISPOT reader (IMMUNOSPOT, CTL) and spot forming units (SFU) / 10^5 PBMCs were calculated.

Figure 4

HIV-1 MDSCs overexpress inhibitory ligands CD150, CD152 and B7-H4



PBMCs of HIV(-) healthy donors were cultured with or without inactive HIV for 5 days. HLA DR^{lo} MDSCs and HLA DR^{hi} monocytes were flow sorted. Cells were stained with PE-conjugated antibodies and expression of various inhibitory ligands was determined by flow cytometry.

Conclusions

- HIV-1 infection results in comparable expansion of HLA DR^{hi} monocytes and HLA DR^{lo} MDSC populations
- HIV-1 expanded MDSCs inhibit pp65 specific IFNγ and increase IL-10 from PBMCs of CMV (+) healthy donors
- HIV-1 MDSCs are poor inducers of T_H1 priming IL12p70 cytokine
- HIV-1 MDSCs overexpress inhibitory ligands that are known to down regulate T cell activity

Acknowledgements

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