Elevated soluble CD40 ligand during HIV infection suppresses dendritic cell function

Meagan O'Brien[1], Elizabeth Miller[1], Ramya Gopal[1], Vanessa Valdes[2], Jeffrey S. Berger[2], Nina Bhardwaj[1]
[1]Mount Sinai School of Medicine, New York, NY USA, [2]NYU School of Medicine, NY, NY

Meagan.O'Brien@mssm.edu
Mount Sinai School of Medicine
1470 Madison Avenue , 5th floor
NY NY 10029
Tel. 212-824-9245

National Institute of Allergy and Infectious Diseases

Introduction

Soluble CD40 ligand (sCD40L) levels in plasma are increased in various inflammatory states, including human immunodeficiency virus (HIV) infection[1-4]. sCD40L is shed from activated T cells and platelets. While expressed on the cell surface as a functional trimer, sCD40L is thought to circulate in plasma predominantly in its monomeric form. We have previously shown that platelets are hyper-activated during HIV infection and that one week of aspirin attenuates platelet activation, markers of immune activation and improves innate immune responses to Toll-like receptor (TLR) activation[5]. Here we evaluated whether plasma levels of sCD40L from our previous aspirin study also decreased after a week of aspirin and whether recombinant sCD40L might suppress dendritic cell responses to TLR agonists and stimulate Indoleamine-pyrrole 2,3-dioxygenase (IDO), thus potentially contributing to immune suppression during HIV infection, and suggesting a mechanism for how aspirin therapy might be immune modulating.

[1]*Diabetes* 2006, **55:**774-779; [2]*Blood*. 2012 Oct 11;120(15):3030-8; [3]*Clin Diagn Lab Immunol*. 2002 May;9(3):558-61;Plos One 2012, 7(3):e33925; [5]*JAIDS 2013* 63(3). Aspirin attenuates platelet activation and immune activation in HIV-1-infected subjects on antiretroviral therapy: a pilot study.

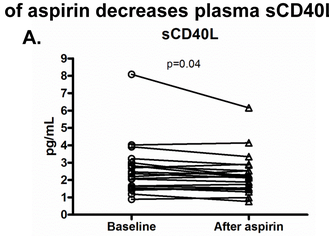
Methods

sCD40L was measured by ELISA in plasma of subjects from our previous aspirin pilot study. Plasma from HIV(-) healthy controls and from HIV(+) subjects on ART with suppressed viremia were evaluated by western blot for the forms of circulating sCD40L. Proteominer protein enrichment kits were used to enrich plasma for small proteins. Plasma proteins were quantified by BCA protein assay (Pierce) and normalized for protein concentration before separating on SDS-PAGE. The effects of monomeric versus multimeric sCD40L (Enzo) on monocyte-derived DC (DC) were compared after overnight stimulation and cytokine expression following subsequent overnight Toll-like receptor (TLR) stimulation via FACS analysis and cytokine bead arrays. T cell proliferation and Th1-skewing capacity of DCs exposed to monomeric versus multimeric sCD40L was evaluated following co-culture with carboxyfluorescein succinimidyl ester (CFSE) labeled naïve CD4+ T cells. Expression of IDO using western blot by DCs was performed after incubation with monomeric versus multimeric sCD40L and evaluated whether IDO was functional by examining differences in kynurenine production.

The aspirin pilot study was conducted in accordance with policies of the New York University Langone Medical Center Institutional Review Board, Bellevue Hospital Center, and the central office of the New York City Health and Hospital Corporation.

Results

Figure 1: A. One week of aspirin decreases plasma sCD40L in HIV infected subjects



B. sCD40L circulates in plasma as both monomeric and multimeric forms

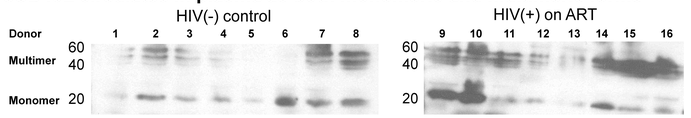
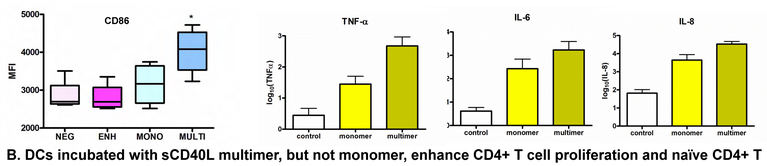


Figure 1 (A) sCD40L (Bender MedSystems) levels decrease in plasma after one week of aspirin therapy, p=0.04, 2 tailed paired t-test, n=20. (B) Plasma proteins (100ug) were separated by SDS-PAGE, transferred to PVDF membranes, and probed with mouse anti-CD40L Mab(R&D) followed by horse anti-mouse horseradish peroxidase (HRP)-conjugated secondary antibody (Cell signaling). The blots were visualized using HRP substrate (Pierce) on a AGFA Curix 60 film processor. Both multimers (dimer 40 kDa,trimer 60 kDa) and monomers 20 kDa circulate in the plasma of both HIV infected and uninfected).

Figure 2. Differential effects of sCD40L monomer versus multimer on DCs

A. sCD40L multimer matures DCs and stimulates them to produce more inflammatory cytokines than monomer



B. DCs incubated with sCD40L multimer, but not monomer, enhance CD4+ T cell proliferation and naïve CD4+ T cell Th-1 skewing

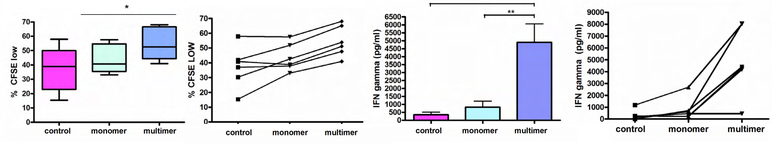


Figure 2 Peripheral PBMCs were purified from buffy coats from uninfected donors purchased from the New York Blood Center (Queens, NY) and plated at 50 × 10^6cells/10 mL per dish in RPMI with 5% pooled human serum(PHS). Cells were allowed to adhere for 1–2 hours at 37°C. Nonadherent cells were removed by washing with RPMI. The adherent monocyte-enriched fraction was supplemented with 100 UI/mL rhGM-CSF and 300 UI/mL rhIL-4 (R&D Systems) on days 0 and 2. On day 5 of culture, DCs were harvested, washed, and resuspended in RPMI supplemented with 5% PHS. (A) DCs were incubated overnight with 2ug/mL sCD40L monomer or multimer and were stained with CD86 and analyzed by FACS. Culture supernatants were evaluate by CBA for cytokine production. (B) After overnight incubation with sCD40L forms, DCs were added to CFSE-labeled allogenic naive CD4 T cells at a ratio of 1:20 for 6 days, proliferation was measured by % CFSE low and Th-1 skewing to allo-antigens was measured by IFN gamma production in culture supernatants.

Figure 3: IL-12 production and Th-1 skewing after subsequent Poly ICLC stimulation is suppressed by both sCD40L multimer and monomer

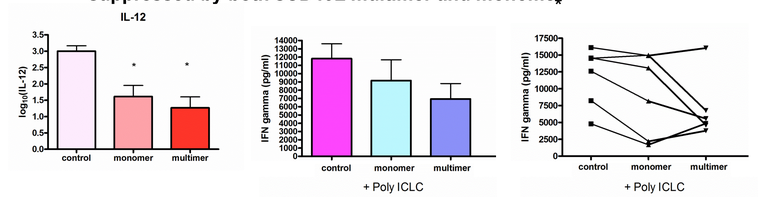
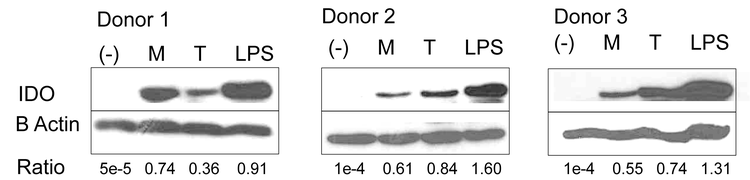


Figure 3 DCs were incubated with sCD40L forms overnight and then Poly ICLC was subsequently added (2ug/mL) for 24 hours and DCs were evaluated for IL-12 production by CBA. These differentially activated DCs were also added to allogenic naive CD4 T cells for 6 days and Th-1 skewing to allo-antigens was evaluated by IFN gamma production in culture supernatants.

Results

Figure 4. sCD40L stimulate functional IDO expression by DCs

A. Both sCD40L monomer and multimer activate DCs to express IDO



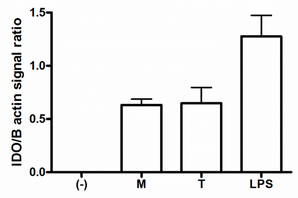


Figure 4 (A) 1 million DCs were incubated overnight with sCD40L forms (2ug/mL) or LPS (100ug/mL) and were then lysed in MPER and protease inhibitor cocktail. Lysates were separated by SDS-PAGE and transferred to PVDF membranes, and probed with mouse anti-IDO (Cell Signaling), mouse anti-B actin (Santa Cruz), followed by horse anti-mouse HRP-conjugated secondary antibody. The blots were visualised using HRP substrate (Pierce) on a AGFA Curix 60 film processor. Ratios = IDO/B-actin loading control, shown as a bar graph for 3 donors.

B. sCD40L stimulated DCs express functional IDO, as measured by kynurenine

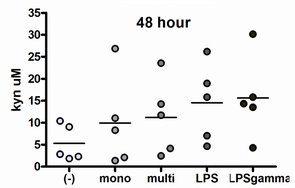


Figure 4 (B) 2e5 DCs were incubated for 48 hours with sCD40L forms (2ug/mL) or LPS (100ug/mL) or LPS/IFN gamma (2e3IU/mL) and culture supernatants were tested for kynurenine using a spectrophotometric assay. Briefly, 50uL of 30% TCA was added to 100uL of culture supernatant, vortexed, and centrifuged at 8000g for 5 minutes, then 75uL of the supernatant was added to 75uL Ehrlich reagent in a 96 well microtiter plate and optic density was measured at 492 nm, as compared to a standard curve of defines kynurenine concentration for analysis of unknowns.

Conclusion

- Recombinant multimeric sCD40L activates DCs to become potent antigen presenting cells and subsequently downmodulates the ability of DCs to be stimulated with TLR ligands after initial activation
- Recombinant monomeric sCD40L partially activates DCs to become suboptimal antigen presentation cells and also subsequently downmodulates the ability of DCs to be stimulated by TLR ligands
- Both sCD40L forms stimulate functional IDO expression by DCs
- Elevated sCD40L forms in HIV infection may contribute to DC dysfunction and immune suppression
- Aspirin therapy may improve dendritic cell dysfunction during HIV infection through decreasing sCD40L levels.