Soluble CD40 ligand contributes to dendritic cell mediated T-cell dysfunction in HIV infection

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Abstract

Background: Soluble CD40 ligand (sCD40L) levels are increased during human immunodeficiency virus (HIV) infection, likely due to heightened platelet activation, but it is unknown whether monomeric or multimeric forms predominate, and whether there are differential effects of these forms on dendritic cell function and dendritic cell-T cell interactions.

Methodology: Immunoblots were performed to delineate various forms of sCD40L present in plasma from HIV-infected donors. Monocyte-derived DCs (DCs) from seronegative donors were exposed to forms of sCD40L prior to Toll-like receptor (TLR) stimulation and DC function and T cell function was assessed *in vitro*. Levels of sCD40L in plasma from ART-treated HIV-infected donors before and after low dose aspirin therapy were measured via ELISA.

Results: Multiple forms of sCD40L were identified in plasma from ART-treated HIV-infected donors, including monomers and multimers. Though monomeric and multimeric forms of sCD40L had differential effects on DC activation when given alone, both strongly suppressed secretion of the Th1 skewing cytokine, IL-12, upon subsequent TLR stimulation. Furthermore, DC exposed to both monomeric and multimeric sCD40L induced T cell anergy and regulatory T cell formation. One week of low dose aspirin decreased sCD40L in ART-treated HIV infected subjects, suggesting that heightened sCD40L is due in part to platelet activation and is responsive to anti-platelet therapy.

Conclusion: Elevated sCD40L during HIV infection contributes to dendritic cell mediated T cell dysfunction. Anti-platelet agents that decrease sCD40L may provide immune modulatory benefits as adjunctive therapies to ART.

Methods

Experimental schema corresponding to figures:

Figures C, D, I
CD40L monomer or CD40L multimer or LPS or (-) → 1) CD86 expression (flow cytometry) 2) Cytokine secretion (IL-6, TNFα) 3) IDO expression

Figure E
CD40L monomer or CD40L multimer or (-) + Poly ICLC (TLR3 ligand) → IL-12 secretion

Figures F, G, J
CD40L monomer or CD40L multimer or LPS+IFNγ or (-) + → T cell proliferation, IFNγ secretion, Tregulatory cell formation

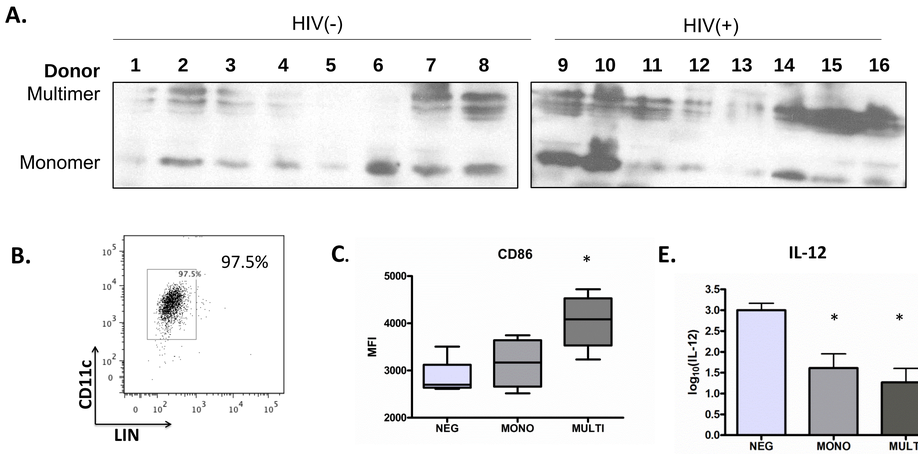
Figure H
CD40L monomer or CD40L multimer or (-) + Poly ICLC (TLR3 ligand) + → T cell proliferation, IFNγ secretion

Figure K
CD40L monomer or CD40L multimer or LPS or LPS+IFNγ or (-) + → 6-7 days → + R848 (TLR 7/8)-matured DCs → T cell proliferation

=DC =naïve CD4+ T cell

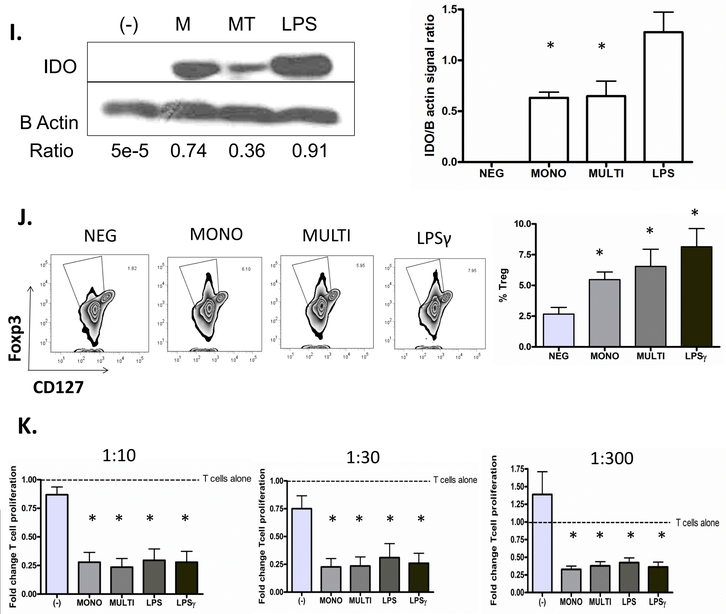
Results

Soluble CD40L circulates in plasma as monomers and multimers, which exert differential effects on DCs and on subsequent naïve CD4+ T cell skewing and proliferation.



(A) sCD40L forms in plasma from 8 HIV-infected donors and 8 healthy donors **(B)** DC purity **(C)** CD86 surface expression by FACS **(D)** IL-6 and TNF-α production by DCs in cell supernatant by cytokine bead array analysis (CBA). DCs incubated with sCD40L monomer (MONO), multimer (MULTI) or control DC (NEG) overnight then **(E)** stimulated overnight with Poly-ICLC and IL-12 production measured by CBA or **(F)** co-cultured with CFSE-labeled allogeneic naïve CD4 T cells where % CFSE dilution is represented as % proliferation and **(G)** culture supernatants were assayed for IFNγ **(H)** sCD40L-stimulated DCs were subsequently activated with Poly-ICLC and co-cultured with allogeneic naïve CD4+ T cells.

DCs exposed to both monomeric and multimeric sCD40L induce IDO expression, a regulatory T cell phenotype, and T cell anergy.



(I) DCs were incubated with sCD40L monomer (M), CD40L multimer (MT), LPS, or control (-) for 24 hours and IDO expression was measured via immunoblot, shown for 1 donor. The relative abundance of IDO is expressed as the ratio of IDO to B Actin signal intensity and is represented as a histogram on the right as the average of three donors. **(J)** DCs were incubated with sCD40L monomer (M), CD40L multimer (MT), LPS and interferon-γ, or control (-) for 48 hours, washed and then co-cultured with allogeneic naïve CD4 T cells at a ratio of 1:10. Phenotype of the day 7 T cell population is shown for one representative donor, gated on $CD4^+$ T cells and represented as a histogram as the average of three donors. **(K)** DCs were incubated with sCD40L monomer (MONO), CD40L multimer (MULTI), LPS, LPS γ, or control (-) for 48 hours, washed and then co-cultured with allogeneic naïve CD4 T cells at a ratio of 1:10 for 6-7 days, then T cells were stained with CFSE and were mixed at various ratios with allogeneic DCs, from the original DC donor, that had been matured overnight with R848 10uM. **(L)** Levels of sCD40L in plasma from ART-treated HIV-infected donors before and after 1 week of low dose aspirin therapy, measured by ELISA.

Conclusions

- sCD40L circulates in plasma in both monomeric and multimeric forms.
- While multimeric sCD40L is more DC-activating, both monomeric and multimeric forms contribute to dendritic cell-mediated T cell dysfunction.
- Anti-platelet agents that decrease sCD40L may provide immune modulatory benefits as adjunctive therapies to ART.