TNF Drives Foam Cell Formation by Monocytes from HIV+ Donors in an *In Vitro* Model of Early Atherosclerotic Events.

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Background

Increased risk of Cardiovascular Disease in HIV infection

- HIV+ individuals have a 2-fold increased risk of cardiovascular disease (CVD) irrespective of treatment with combination antiretroviral therapy (cART).
- Increased risk of CVD is independent of traditional CVD risk factors such as smoking and dyslipidemia, suggesting additional, undefined mechanisms contribute to CVD in HIV infection.
- Atherosclerosis (the major cause of CVD) is an inflammatory disease which is more prevalent in HIV+ individuals and progresses at a faster rate.
- Monocytes play a critical role in the initiation and progression of atherosclerosis via migration into fatty streaks and development into lipid-laden **foam cells** (see Fig 1).
- Chronic inflammation and immune activation persist in HIV+ individual despite cART and are thought to contribute to increased CVD risk, but the mechanism is unknown.

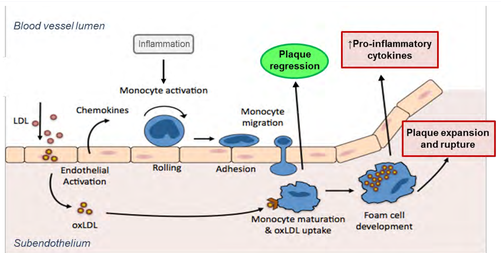

Figure 1: Monocytes play a critical role in atherosclerotic plaque progression. Monocytes are attracted to the activated endothelium via chemokines, where they roll, attach and subsequently migrate across the endothelial layer. Within the subendothelial space, they differentiate into macrophages, ingest lipids and can either reverse migrate to the blood vessel lumen (associated with plaque regression) or develop into lipid-laded, foam cells (associated with plaque progression)

Methods

The potential of purified monocytes from HIV+ (n=16, 75% receiving cART) or seronegative (n=7) individuals to undergo forward and reverse transendothelial migration (TEM) and form foam cells was determined using an *in vitro* model of early atherosclerotic events (see Figure 2).

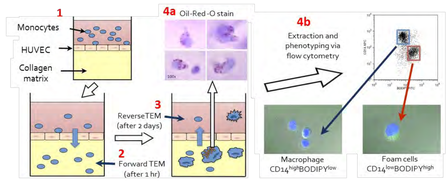

Figure 2: Model of monocyte migration/foam cell formation. Purified monocytes are added to a primary endothelial cell monolayer (human umbilical vein endothelial cells, HUVEC) cultured onto collagen matrix (1). Monocytes undergo forward TEM in response to chemokines released by the HUVEC (2). After 2 days, cells which have undergone reverse TEM (3) are collected from the media. Cells remaining in the collagen are identified as either foam cells (large, lipid-laden) or monocytes/early macrophages (smaller, low lipid) via Oil-Red-O staining of lipids and microscopy (4a). Resident cells are recovered using collagenase and phenotyped via CD14 and BODIPY (lipid) staining and flow cytometry (4b).

Hypothesis

Chronic activation of monocytes in HIV+ individuals increases their potential to form inflammatory foam cells which hastens the development of atherosclerosis.

Results

1. Monocytes from HIV+ individuals show decreased reverse migration and increased foam cell formation which is potentiated by HIV+ serum

In the presence of control human serum, monocytes from HIV+ donors showed an increased propensity to form foam cells and a decreased ability to reverse migrate compared to control monocytes. Autologous serum from HIV+ donors heightened this effect. Forward monocyte migration was not significantly altered.

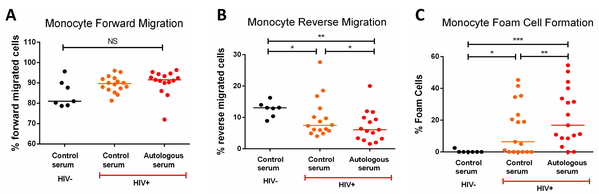

Figure 3 Forward **(A)** and Reverse **(B)** transendothelial migration of monocytes from HIV+ and seronegative donors as indicated (% of total monocytes added). **(C)** Foam cell formation by migrated monocytes (as a % of cells retained in the gel). Experiments conducted in presence of control (HIV-) serum or autologous serum from HIV+ donors as indicated. Bars indicate medians; * p<0.05.

2. Foam cells show increased inflammatory properties

Staining of migrated cells with CD14 and Bodipy (lipid stain) revealed two distinct populations with morphology consistent with foam cells and monocytes/macrophage. Foam cells contained increased intracellular concentrations of TNF and decreased surface expression of the anti-inflammatory molecule CD11b as compared to migrated monocytes/macrophages.

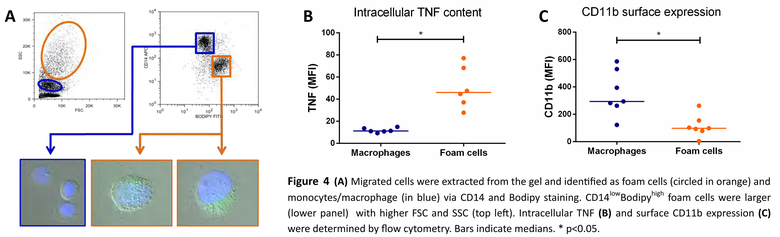

Figure 4 (A) Migrated cells were extracted from the gel and identified as foam cells (circled in orange) and monocytes/macrophage (in blue) via CD14 and Bodipy staining. CD14^{low}Bodipy^{high} foam cells were larger (lower panel) with higher FSC and SSC (top left). Intracellular TNF **(B)** and surface CD11b expression **(C)** were determined by flow cytometry. Bars indicate medians. * p<0.05.

Results (cont.)

3. TNF is elevated in HIV+ plasma and increases foam cell formation

Soluble TNF levels were elevated in HIV+ donors (Fig 5A). Blocking TNF activity ***after*** monocyte forward migration reduced foam cell formation by monocytes from HIV+ individuals, suggesting TNF acts on migrated cells to alter their atherogenic potential.

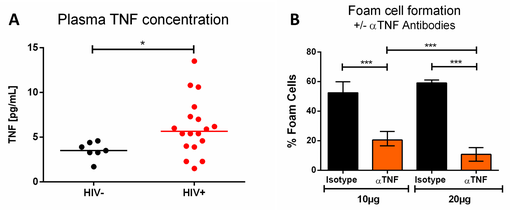

Figure 5 (A) TNF concentration in the plasma of HIV– and HIV+ participants measured by ELISA. **(B)** Foam cell formation (% of migrated cells) by monocytes in the presence of 10 or 20μg of antibodies to TNF receptors I and II or isotype control antibodies. Median and IQR shown. * p<0.05, ***p<0.001.

4. Monocytes from HIV+ donors show decreased motility post-migration

Live-video microscopic analysis of monocytes post migration showed that cells from HIV+ donors moved at a slower average speed than those from controls, and showed a restricted migration trajectory around a fixed point. This is consistent with decreased motility due to foam cell formation.

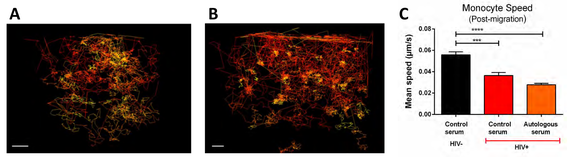

Figure 6 The movement of migrated cells was analysed for 48hrs at 37°C via live video fluorescent microscopy. Images from a representative experiment from a control (A) and HIV+ donor (B). Colours indicate different times (red=early, yellow=late). Concentration of yellow colour tracks in B indicate restricted movement around a fixed point. (C) Mean speed of individual monocyte tracks was calculated from 2 experiments for control and HIV+ donors as indicated.

Conclusions

- Monocytes from a cohort of largely virologically suppressed HIV+ individuals showed an increased potential to form atherosclerosis-promoting foam cells in our model.
- HIV+ serum contains factors including TNF which potentiate foam cell formation.
- This is some of the first mechanistic data potentially explaining increased atherosclerosis and CVD in HIV+ individuals.
- Ongoing work is focussing on elucidating the relationship between inflammation, chronic monocyte activation and foam cell formation in HIV+ individuals and identifying agents which inhibit this process.