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SHIV Infection Triggers Endothelial Dysfunction and Diminished Expression of Krüppel-Like Factor 2 (KLF2) in Non-human Primates

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Introduction

HIV infection both treated and untreated is linked to an increased risk of thromboembolic and cardiovascular (CV) co-morbidities. The determinants of the heightened inflammation/coagulation that are thought to drive CV risk are incompletely understood but among putative drivers are included exposure to translocated bacterial products and oxidized lipids that have been linked to vascular inflammation and endothelial dysfunction. Detailed characterization of vascular pathology in HIV infection is lacking. For this reason we examined the aortas of Rhesus macaques that were either uninfected or infected with the Simian Human Immunodeficiency Virus (SHIV 162P3). We sought here histopathologic evidence of vascular inflammation, expression of eNOS as a marker of endothelial function and expression of Krüppel like Factor 2 (KLF2) a nuclear factor found in endothelial cells that is the master regulator of endothelial function that induces an anti-atherogenic, anti-inflammatory and anti-coagulant phenotype. Overexpression of KLF2 inhibits pro-inflammatory and pro-thrombotic gene expression, and enhances the expression of eNOS and thrombomodulin.

This study was designed to identify any morphological alterations in the endothelium and sub-endothelial infiltration of inflammatory cells, to measure nitric oxide synthase (eNOS), and KLF2 expression as a direct evidence of endothelial dysfunction in the SHIV-infected animals. In parallel we also verified the protective effects of the HMG-CoA reductase inhibitor simvastatin against endothelial KLF2 downmodulation induced by exposure of human aortic endothelium to bacterial lipopolysaccharides (LPS) and oxidized low density lipoprotein (oxLDL) a bioactive oxidized lipid that we have found to be increased in plasma of HIV infected individuals.

Hypothesis

We hypothesize that SHIV infection will be associated with vascular inflammation, endothelial dysfunction as defined by low levels of eNOS and decreased endothelial expression of Krüppel-like factor 2 (KLF2) - a transcriptional master regulator of an anti-atherogenic, anti-inflammatory and anti-thrombotic endothelial environment.

Material and Methods

Tissue samples: Paraffin sections of thoracic aorta from 4 SHIV (SHIVSF162P3) infected, and age and sex matched 22 SHIV-uninfected *Rhesus macaques* (M, ~3yr) collected at necropsy on day ~80 of infection.

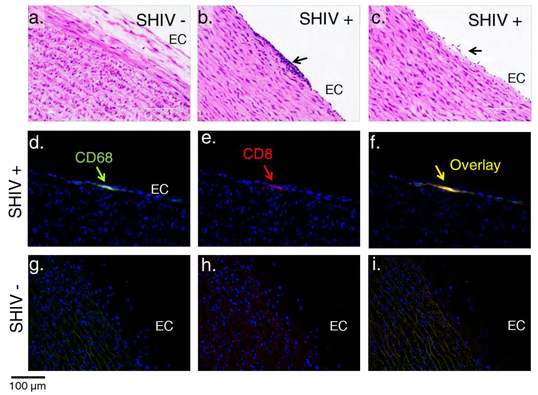
Staining: H&E, and immune-fluorescence staining for eNOS, KLF2, CD68, CD41, and CD8

Microscopy, and imaging: epi-fluorescent microscopy by EVOS®FL, (Life Technologies)

Image analysis: ImageJ v2.0-0.rc-24/1.49m (http://imagej.nih.gov/ij/) was used to measure target to nuclear fluorescence ratio (Fc/n) in the endothelium, calculated according to the formula: Fc/n = (Fn-Fb)/(Fc-Fb), where Fb is background auto-fluorescence.

Results

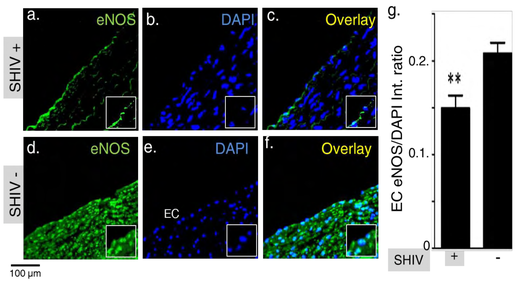
SIV infection is associated with focal endothelial proliferation and sub-endothelial migration of inflammatory cells.



Sections of thoracic aorta from (a, g-i) SIV un-infected, (b-f) SHIV infected Rhesus macaque were processed for H&E, and immunofluorescence (CD68-FITC, CD8-Rhodamine Red, and DAPI) staining. Representative images demonstrate sub-endothelial infiltration of CD68+ (monocytes) and CD8+ T-cells in the aortas of SHIV-infected animals only.

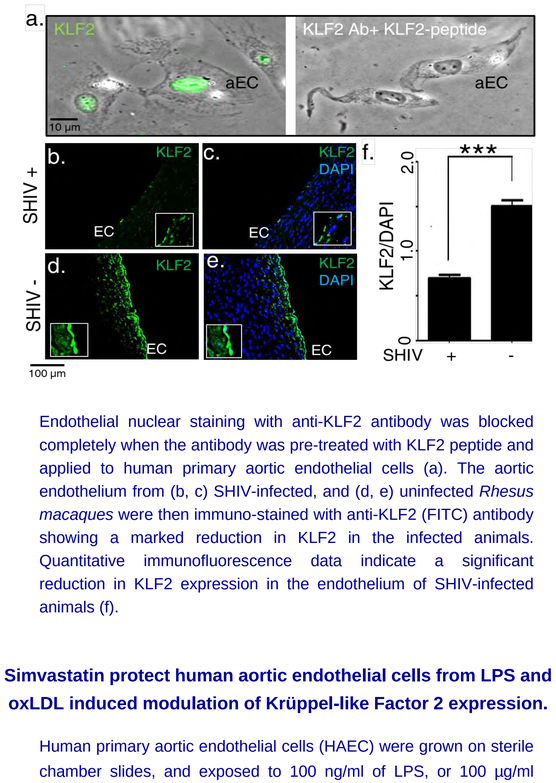
We also observed multiple areas where CD8+, CD68+ and CD41+ (platelet integrin-α_{IIb}) cells were either adhered, or infiltrated the vascular endothelium in the SHIV-infected animals (not shown).

The vascular impact of SIV infection in *Rhesus macaque* significantly reduces the endothelial eNOS expression



Sections from (a-c) SHIV-infected, (d-f) uninfected control *Rhesus macaque* aortas were immuno-stained with anti-eNOS (FITC). Representative data from epi-fluorescence microscopy, and summary data (g), show reduced eNOS expression in the endothelium of the infected animals.

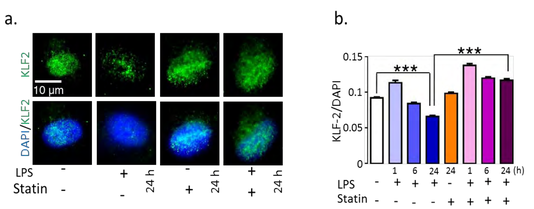
Endothelial Krüppel-like Factor 2 expression is reduced in the SIV infected Rhesus macaque aorta.



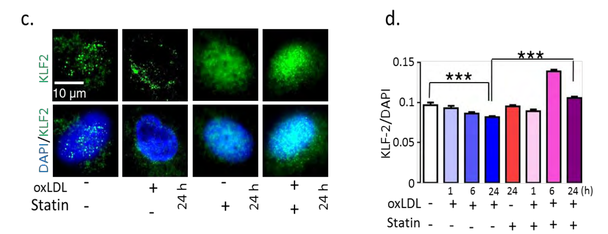
Endothelial nuclear staining with anti-KLF2 antibody was blocked completely when the antibody was pre-treated with KLF2 peptide and applied to human primary aortic endothelial cells (a). The aortic endothelium from (b, c) SHIV-infected, and (d, e) uninfected *Rhesus macaques* were then immuno-stained with anti-KLF2 (FITC) antibody showing a marked reduction in KLF2 in the infected animals. Quantitative immunofluorescence data indicate a significant reduction in KLF2 expression in the endothelium of SHIV-infected animals (f).

Simvastatin protect human aortic endothelial cells from LPS and oxLDL induced modulation of Krüppel-like Factor 2 expression.

Human primary aortic endothelial cells (HAEC) were grown on sterile chamber slides, and exposed to 100 ng/ml of LPS, or 100 µg/ml oxLDL, with or without 10 µM of simvastatin.

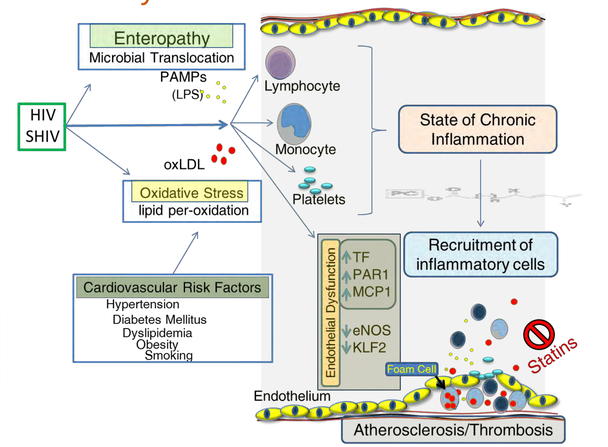


An initial surge of KLF2 expression was observed in the HAECs nuclei after the first hour of exposure to LPS followed by diminishing levels that were significantly lower than baseline by 6 - 24 hrs. Pretreatment with simvastatin protected the HAECs from KLF2 deregulation induced by LPS (a, b).



Exposure to oxLDL induced progressive reduction in nuclear KLF2 expression in HAEC; simvastatin blocks this effect.

Summary



Conclusions

- SHIV-infection triggers vascular inflammatory changes characterized by subendothelial infiltration of immune cells and platelets.
- We documented for the first time a significant reduction in eNOS, and KLF2 in the aortic endothelium of SHIV-infected animals
- The traditional HMG-CoA reductase inhibitor, simvastatin, it protects against LPS/oxLDL induced downmodulation of KLF2 expression in human vascular endothelium in vitro, an effect that is independent from the lipid lowering effect of the drug.

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