Bad company: monocytes in HIV and atherosclerosis

David Rohde[1,2] and Matthias Nahrendorf [1,2,3,4]*

[1]Center for Systems Biology, Massachusetts General Hospital and Harvard Medical School, 185 Cambridge Street, Boston, MA 02114, USA; [2]Department of Radiology, Massachusetts General Hospital and Harvard Medical School, 185 Cambridge Street, Boston, MA02114, USA; [3]Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, 185 Cambridge Street, Boston, MA02114, USA; and [4]Department of Internal Medicine I, University Hospital Wuerzburg, Oberduerrbacher Str 6, 97080 Wuerzburg, Germany

This editorial refers to 'Classical monocyte transcriptomes reveal significant anti-inflammatory statin effect in women with chronic HIV' by E. Ehinger et al., pp. 1166–1177.

Atherosclerosis, the underlying cause of myocardial infarction, ischaemic stroke and limb ischaemia, is a chronic inflammatory disease characterized by the progressive accumulation of lipoproteins and leucocytes in the arterial vessel wall.[1] Monocytes, precursors of macrophages, contribute critically to all phases of atherosclerosis and the outcome of its complications. Upon production and release from the bone marrow, inflammatory blood monocytes, characterized as Ly6C^{high} in mice and CD14^{++}CD16$^-$ in humans,[2] fuel atherosclerosis lesion formation by binding to activated endothelium, migrating into the vessel wall, and differentiation into macrophages.[3] Circulating CD14^{++}CD16$^-$ blood monocytes numbers correlate closely with disease progression and the cardiovascular event rate in patients.[4]

About 40 years after the observation of the first cases of acquired immunodeficiency syndrome (AIDS), 1.7 million people become newly infected with the disease-causing human immunodeficiency virus (HIV) every year.[5] Of 38 million people living with HIV, 26 million are accessing antiretroviral therapy (ART). While CD4$^+$ T cells represent the primary target of HIV, myeloid cells can also be infected by HIV.[6] In patients with undetectable plasma virus loads under ART, blood monocytes are reservoirs for replication-competent HIV.[7] Patients with persistent HIV infection, despite effective viral suppression under ART, display a more than two-fold increased risk for cardiovascular disease.[8] The increased prevalence of atherosclerosis in people living with HIV has been associated with blood monocyte CD11b and CX3CR1 surface marker abundance[9] and their potential to form disease-promoting foam cells *ex vivo*.[10]

In their article entitled 'Classical monocyte transcriptomes reveal significant anti-inflammatory statin effect in women with chronic HIV', Ehinger et al[11] report the results of whole transcriptome sequencing (RNA-Seq) performed on classical blood monocytes (CM) obtained from participants of the Women's Interagency HIV Study (WIHS). The investigators took advantage of this unique resource by selecting blood samples from a total of 92 study participants, which were equally divided into four study cohorts based on HIV status (H$^+$ or H$^-$) and the presence of carotid artery plaque as assessed by ultrasound examination (C$^+$ or C$^-$): H$^-$C$^-$, H$^+$C$^-$, H$^-$C$^+$, and H$^+$C$^+$. Cryopreserved blood samples were processed in a standardized fashion and CM were flow-sorted as live CD3$^-$ CD19$^-$ CD56$^-$ CD66b$^-$ CD14^{++} CD16$^-$, before each CM sample was subjected to bulk RNA-Seq (*Figure 1*).

The authors found significantly altered gene expression patterns in CM from both H$^+$C$^-$ and H$^-$C$^+$ cohorts when compared to H$^-$C$^-$, the control group. Displaying high consistency within each group and clearly outweighing down-regulated genes, 204 and 179 genes were significantly overexpressed in CM from H$^+$C$^-$ and H$^-$C$^+$, respectively. Enabled by their resourceful study design, Ehinger et al. found striking similarities between these two groups, with 42 genes up-regulated in both groups, including pro-inflammatory cytokines like Interleukin 1α and Interleukin 6 (IL6). Accordingly, subsequent pathway and gene regulation analysis prominently featured IL6, High Mobility Group Box protein 1 (HMGB1), and NF-κB signalling in H$^+$C$^-$ and H$^-$C$^+$ groups in a comparable fashion. This compelling concordance in blood monocyte transcriptomes found in patients with either HIV infection or early stages of atherosclerosis points towards a shared myeloid cell activation pattern. The fact that 91% of HIV patients without carotid artery plaque (H$^+$C$^-$) were on ART and 61% had undetectable HIV loads illustrates that the pro-inflammatory circulating monocyte phenotype is persistent under effective pharmacological suppression of virus replication.

Next, the investigators compared the transcriptomes of CM from study participants with HIV and carotid artery plaque (H$^+$C$^+$) to the H$^-$C$^-$ reference group. Unexpectedly, the authors found only modest changes in CM gene expression with just 9 differentially regulated genes in H$^+$C$^+$ compared to H$^-$C$^-$. As 43% of the H$^+$C$^+$ cohort received statin medication (compared to only 22% in the H$^-$C$^+$ group), Ehinger et al. hypothesized that statin treatment may be a potential confounder. Indeed, a comparison of H$^+$C$^+$ study participants without statin use (H$^+$C$^+$S$^-$) to the H$^-$C$^-$ control group revealed 118 significantly overexpressed genes. Upstream regulatory pathway analysis showed activation of pro-inflammatory pathways like IL6, HMGB1, and NF-κB signalling as previously seen in samples from H$^+$C$^-$ and H$^-$C$^+$ patients. Of note, the pattern of up-regulated genes in CM from H$^+$C$^+$S$^-$ was overall distinct from the two other disease groups, but also overlapped with a panel of 27

*Corresponding author. Tel: +1 617 643 0500; fax: +1 617 726 5708, E-mail: mnahrendorf@mgh.harvard.edu
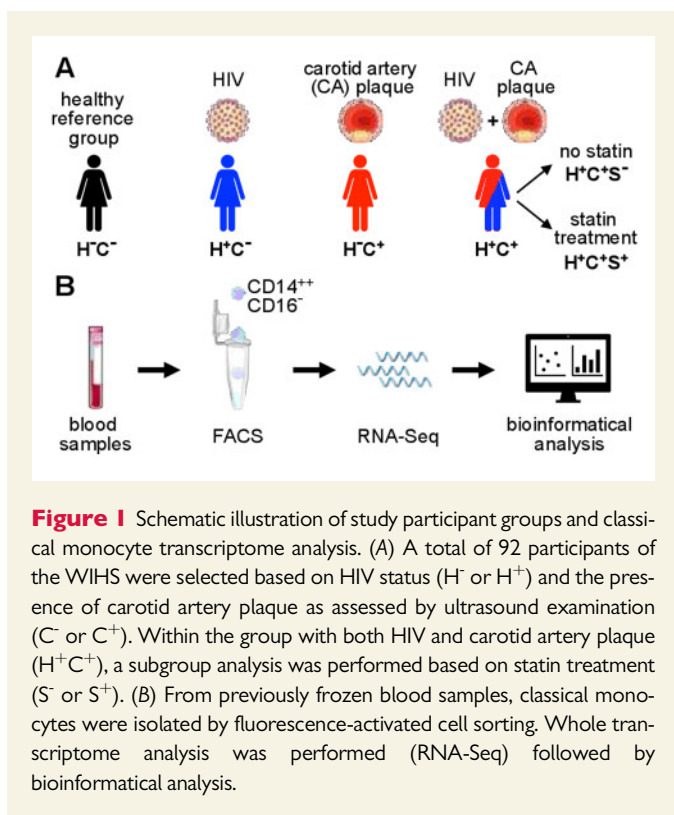
Figure 1 Schematic illustration of study participant groups and classical monocyte transcriptome analysis. (*A*) A total of 92 participants of the WIHS were selected based on HIV status (H⁻ or H⁺) and the presence of carotid artery plaque as assessed by ultrasound examination (C⁻ or C⁺). Within the group with both HIV and carotid artery plaque (H⁺C⁺), a subgroup analysis was performed based on statin treatment (S⁻ or S⁺). (*B*) From previously frozen blood samples, classical monocytes were isolated by fluorescence-activated cell sorting. Whole transcriptome analysis was performed (RNA-Seq) followed by bioinformatical analysis.

genes that were significantly increased in all three cohorts (H⁺C⁻, H⁻C⁺, and H⁺C⁺S⁻). In contrast to samples from patients with HIV and atherosclerosis without statin use (H⁺C⁺S⁻), CM from H⁺C⁺ study participants with ongoing statin treatment showed no relevant gene expression changes when compared to the H⁻C⁻ reference group, further arguing in favour of statin medication as a main confounder.

Finally, the authors identified a gene cluster, comprising the cytokine CXCL2 and Tissue Inhibitor of Metalloproteinases 1, among others, which was up-regulated in CM from H⁺C⁺S⁺ when compared to H⁺C⁺S⁻. Overexpression of these genes, referred to as cluster 9, has previously been associated with acute cardiovascular events like acute myocardial infarction and plaque rupture, suggesting a potentially lower event risk in the statin-treated subgroup with HIV and atherosclerosis.

In their thoughtful gene expression study on human blood monocytes, the authors shed light on inflammatory myeloid cells at the intersection of infection and cardiovascular disease. Over the past years, we have learned that modifiable lifestyle factors, including unhealthy diet, psychosocial stress, sleep deprivation, and lack of physical activity, negatively impact atherosclerosis and cardiovascular event risk by triggering inflammatory myeloid cell abundance.[12] Growing evidence suggests that this concept can be expanded to viral infections like influenza and hepatitis C.[13,14] The striking similarities in gene expression of circulating monocytes from patients with either chronic HIV infection or early-stage atherosclerosis, as reported by Ehinger *et al.*, point towards a common activation pattern of myeloid cells. Of note, this finding emerged from whole transcriptome analysis, preventing investigator bias towards established mechanisms. Inflammatory monocyte activation was lacking in patients with HIV and atherosclerosis (H⁺C⁺) on statin treatment, indicating anti-inflammatory action of statins in this cohort. As there was

no relevant difference in blood cholesterol levels between H⁺C⁺ patients with or without statin use, this effect might be assigned to pleiotropic statin effects, potentially targeting endothelial cells as well as leucocytes.[15] End-point oriented clinical studies will ultimately have to resolve the question if statin treatment can lower the burden of cardiovascular disease in HIV patients.[16]

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