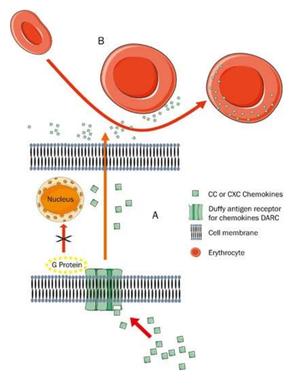
African Ancestry and HIV promote Systemic Inflammation and Endothelial Dysfunction

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Background

- Globally, over 39 million people are living with HIV.
- Despite effective cART, HIV derived proteins persist in circulation and contribute to inflammation and CVD.
- Individuals of African descent face a disproportionate burden of HIV and CVD.
- A major ancestry-linked genetic factor is the Duffy Antigen Receptor for Chemokine (DARC), leading to the DARC-negative (DARC-) genotype.
- DARC is a non-signaling chemokine receptor expressed on red blood cells and venular endothelial cells.
- DARC- is present in 95% of individuals of African origin and is associated with increased systemic inflammation.



S. Apostolakis et al.Acta Pharmacologica Sinica(2011)

Question

What is the link between DARC-/-, HIV derived proteins, and heightened cardiovascular risk in PLWH?

Hypothesis

Expression of HIV-derived proteins and DARC deficiency independently and synergistically contribute to endothelial dysfunction and hypertension

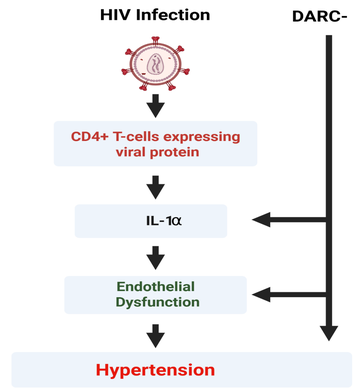
Mouse models of HIV

1) HIV-1 Tg26 transgenic mice (C57Bl/6 Bkgd)
- Expresses 7 of the 9 HIV viral proteins

2) DARC Knockout Mouse (DARC-/-)
- Global Knockout of the DARC gene
- mimics the Duffy-null genotype, highly prevalent in the African ancestry population

3) Tg26DARC Knockout Mouse (Tg26DARC-/-)
- Combines chronic HIV protein expression with DARC deficiency

4) Bone Marrow Transplant
- To investigate the role of RBC-expressed DARC and immune cell-derived viral proteins in endothelial dysfunction



Kress et al., Circulation, 2025

Results I.

HIV-Derived Protein Expression Increases Mean Arterial Pressure Independent of DARC Deficiency

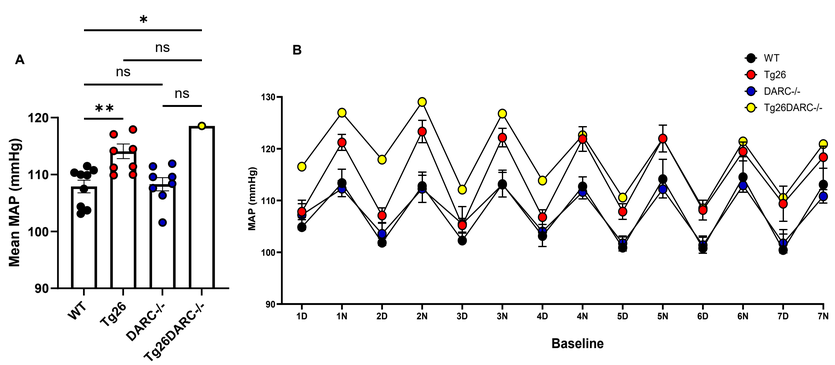


Figure 1: Mean Arterial pressure (MAP) measured by radiotelemetry in WT, Tg26, DARC-/- and Tg26DARC– mice at baseline. Tg26 and Tg26DARC-/- exhibit elevated MAP compared with WT, while DARC-/- display similar MAP compared to WT. N=1-9 per group. *p < 0.05 **p < 0.01

Results II.

HIV Proteins and DARC Deficiency Impair Endothelial Function without Additive Effects.

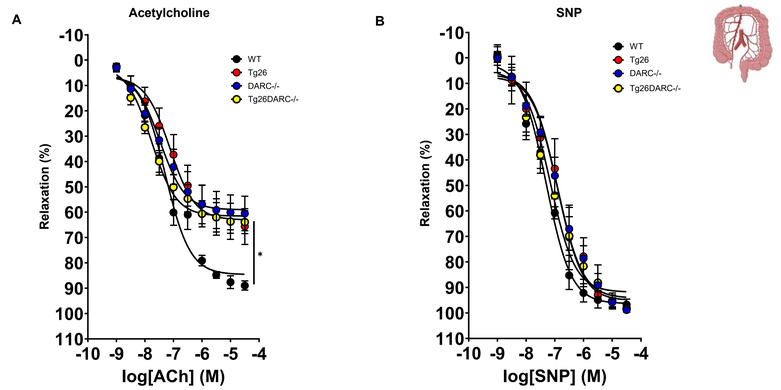


Figure 2: (A) Endothelial-dependent relaxation to ACh is significantly impaired in Tg26 and DARC-/- mice compared to WT, with no further impairment observed in Tg26DARC-/- mice. (B) Relaxation to sodium nitroprusside (SNP) was similar across all groups, indicating no dysfunction in vascular smooth muscle cells. N =4- 8 per group. *p < 0.05.

Plasma IL-1α and Endothelial NOX1 are Reduced in DARC-Deficient Mice

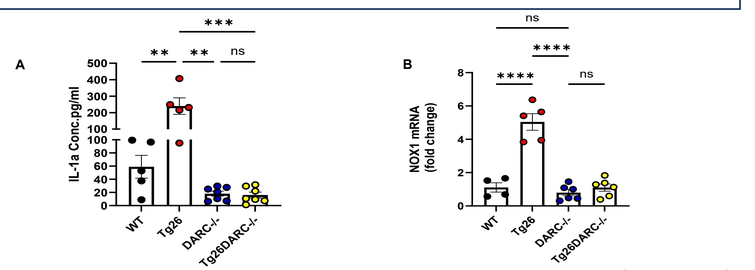


Figure 3:(A.) Plasma IL-1α levels were measured using the BioLegend mouse inflammation panel in WT, Tg26, DARC-/-, and Tg26DARC-/- mice. (B) Endothelial NOX1 mRNA measured by qPCR in the thoracic aorta. Data are presented as mean ± SEM; N=5-7 per group **p < 0.01, ***p < 0.001

DARC Deficiency Reduces Plasma Levels of the DARC Ligands CXCL1 and CCL11

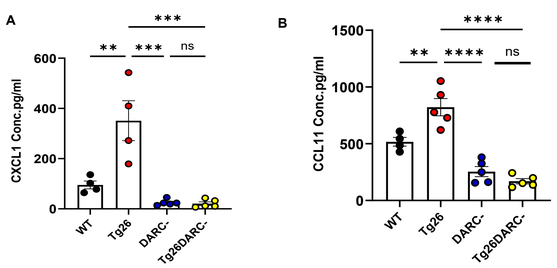


Figure 4: (A.) Plasma levels of CXCL1 (A) and CCL11 (B) DARC ligands were measured using the BioLegend mouse inflammation panel in WT, Tg26, DARC-/-, and Tg26DARC-/- mice. (Data are presented as mean ± SEM; N=4-5 per group **p < 0.01, ***p < 0.001

DARC Deficiency Shifts Inflammatory Profile Towards Elevated Plasma IL-17A, IL-1β, IL-6, and TNF-α.

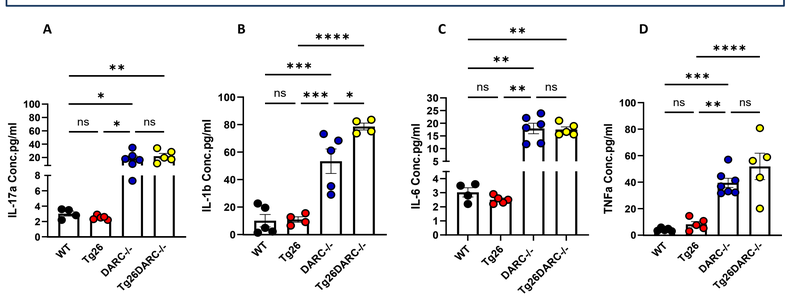


Figure 5: Plasma levels of IL-17A, IL-6, IL-1β, and TNF-α measured using the BioLegend Mouse Inflammation Panel in WT, Tg26, DARC-/-, and Tg26DARC-/- mice (A-D). Data are presented as mean ± SEM; N=5-10 per group. ***p < 0.001.

Results III.

HIV Proteins and DARC Deficiency Do Not Alter Circulating Immune Cells.

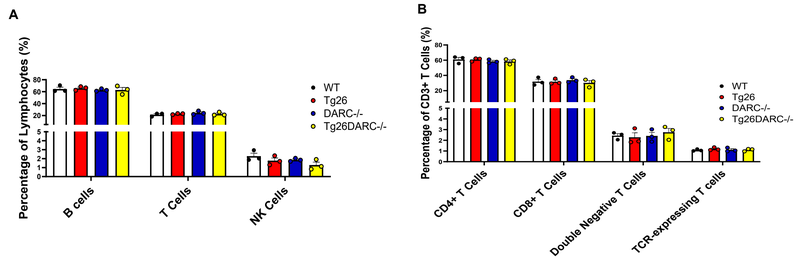


Figure 6: Flow cytometry analysis of peripheral blood from WT, Tg26, DARC-/-, and Tg26DARC-/- mice demonstrated no significant differences in overall lymphocyte population or major CD4+ T cell subset (A and B). Data are presented as mean ± SEM; N =3.

Loss of RBC-Associated DARC is Sufficient to Impair Endothelial Function

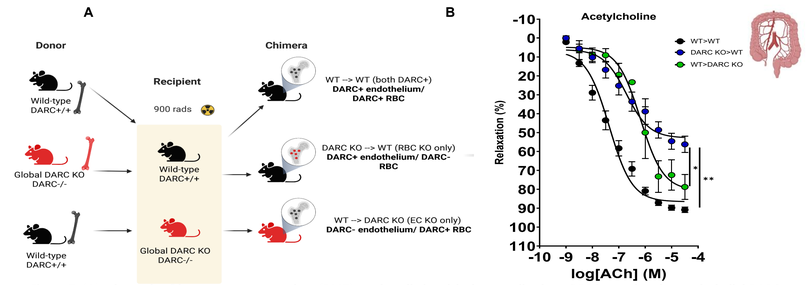


Figure 7: (A) schematic of bone marrow transplant (BMT) used to distinguish the contribution of RBC-associated vs endothelial DARC. (B) Endothelial-dependent relaxation to ACh is significantly impaired in mice lacking RBC-associated DARC (DARC KO-WT) compared to WT-WT controls. In contrast, preservation of RBC-associated DARC in WT-DARC KO chimeras restored endothelial-dependent relaxation, indicating loss of RBC-associated DARC is sufficient to impair endothelial function. N =4- 13 per group. **p < 0.01

RBC-Associated IL-1α is Elevated in the Absence of DARC

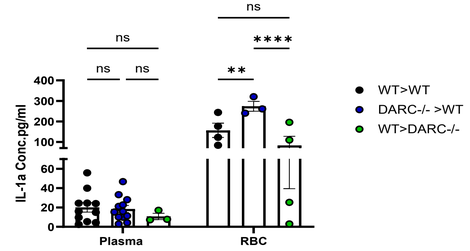


Figure 8: Plasma and RBC IL-1α levels were measured in BMT mice using the BioLegend Mouse Inflammation Panel. Loss of RBC-associated DARC resulted in elevated IL-1α associated with RBCs, whereas preservation of RBC-associated DARC reduced RBC-associated IL-1α levels. Plasma IL-1α levels remained comparatively low across all groups. Data are presented as mean ± SEM N = 3-13 per group

**p < 0.01, ***p < 0.001.

Summary and Conclusion

- DARC deficiency and HIV protein expression independently promote endothelial dysfunction with no additive effects.
- DARC-/- induces a shift in inflammatory profile towards elevated IL-17A, IL-1β, IL-6, and TNF-α.
- Bone marrow transplant studies suggest that RBC-associated DARC is sufficient to drive endothelial dysfunction.

Together, our data identifies RBC-associated DARC as a potential ancestry-linked regulator of inflammatory vascular risk in people living with HIV

Funding

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