Inflammatory monocytes contribute to inflammageing

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Introduction:

- There is increased chronic low-grade systemic circulating inflammation termed inflammageing Elevated inflammation has been shown to be detrimental to a functioning immune system.
- Mechanisms of inflammageing initiation are numerous however monocytes are a key cell driving this phenomenon.
- However the effect of ageing on monocyte phenotype and function is less well understood.

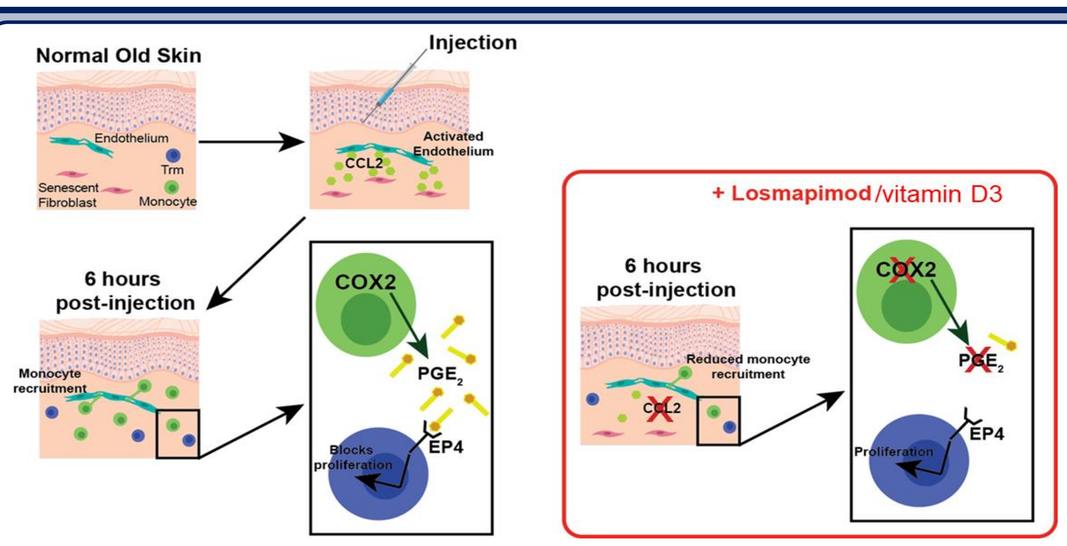

Figure 1: Schematic demonstrating how inflammatory monocytes inhibit antigen-specific immunity in older adults
Older adults (≥ 65 years) are injected with antigen. This results in CCL2 productions from senescent fibroblasts and activation of the endothelium. Six hours later inflammatory monocytes are recruited to the site of injection, these inflammatory monocytes express COX2, secrete prostaglandin E2 (PGE2) which binds to EP4 receptor on T resident memory (Trm) cells in the skin and blocks antigen-specific immunity. Importantly blockade of the inflammatory monocytes using either the p38-MAPK or vitamin D3 results in restoration of the inflammatory monocytes. (**ES Chambers et al Nature Aging 2021; ES Chambers et al Immunotherapy Advances 2021**).

Methods:

- Older (≥65 years) and Younger (≤40 years) healthy (with no declared autoimmune or inflammatory disease) adults donated peripheral blood after full informed consent (QMERC23.059).
- Monocytes were assessed ex vivo using flow cytometry and FACs sorted and assessed by proteomics mass spectrometry analysis
- Monocytes were cultured *in vitro* and cytokine production was assessed by cytometric bead array.

The **overarching aim of this project** is to understand the impact of ageing on monocyte phenotype and function to determine their contribution to the inflammageing phenomenon.

Results:

Increased frequency of CD16+ monocytes in older adults

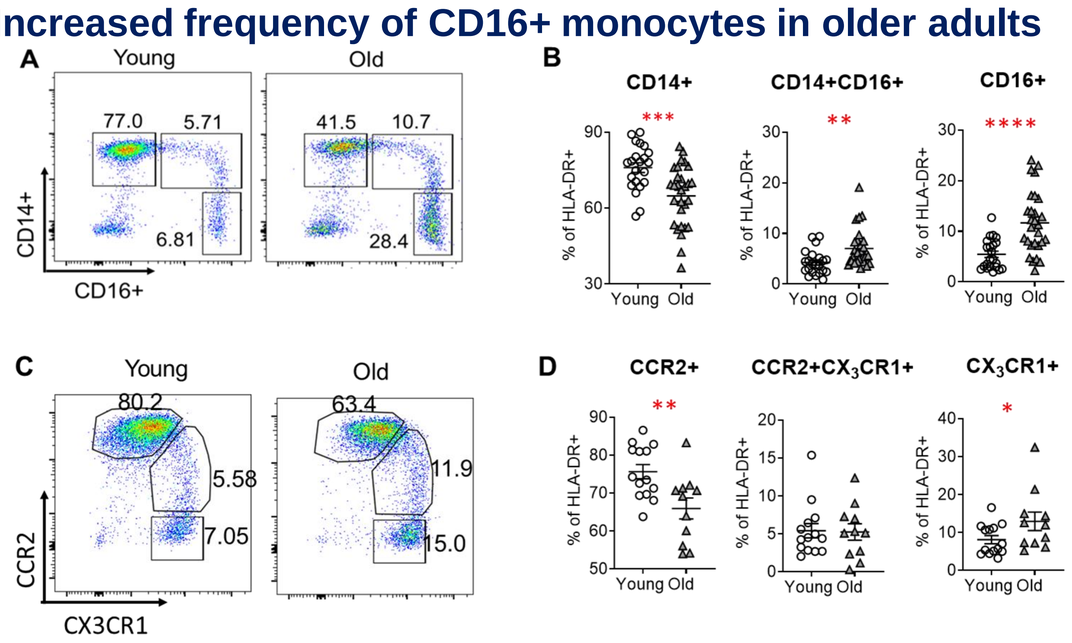

Figure 2: Whole blood was assessed by flow cytometry, monocytes were identified as being Live, Lineage negative (CD3, CD56, CD19, CD20) and HLA-DR+ within this monocyte gate cells were assessed for A and B CD14 and CD16 expression and C and D CCR2 and CX3CR1 expression. Data was assessed by t test. * = p<0.05; ** = p<0.01; *** = p<0.001; **** = p<0.0001.

Significant age-related changes in monocyte proteome

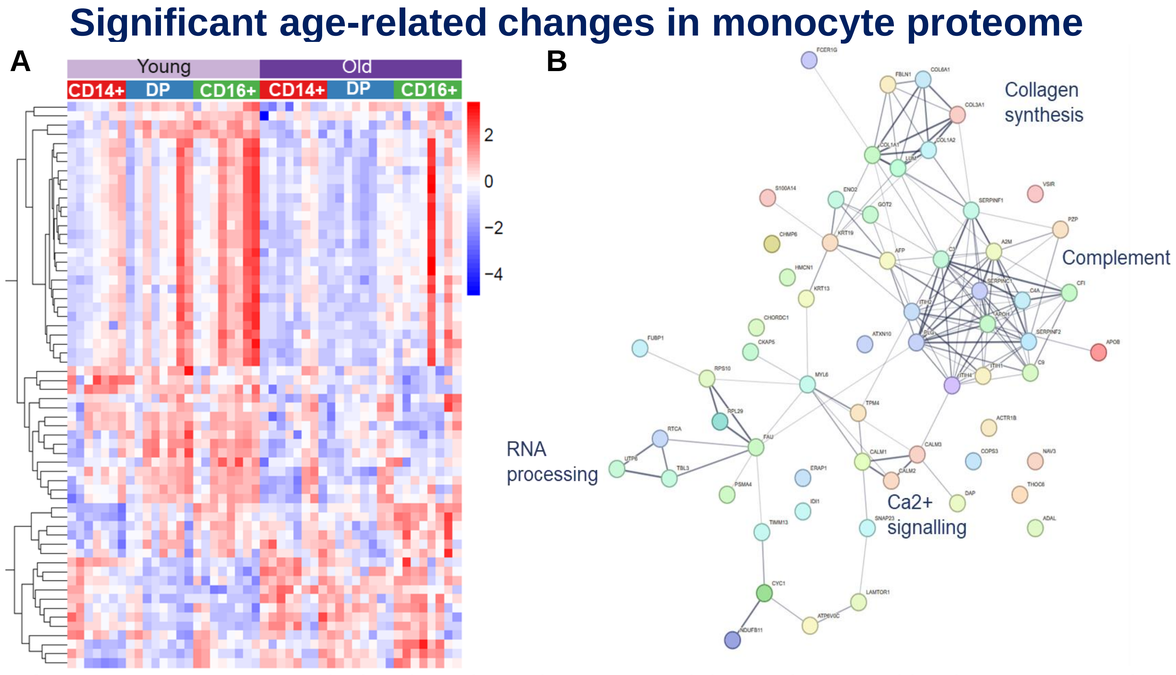

Figure 3: Monocytes were isolated ex vivo using FACs and the markers used in Figure 2A. Samples were snap frozen and protein was isolated and assessed by mass spectrometry. **A,** Heatmap showing the significantly differentially expressed proteins according to age sorted according to age (purple) and monocytes type classical (CD14+), intermediate (CD14+CD16+; DP) and non-classical (CD16+) **B,** String analysis of statistically differentially expressed proteins in aged monocytes.

Older monocytes produce significantly more inflammatory cytokines *in vitro* as compared to young

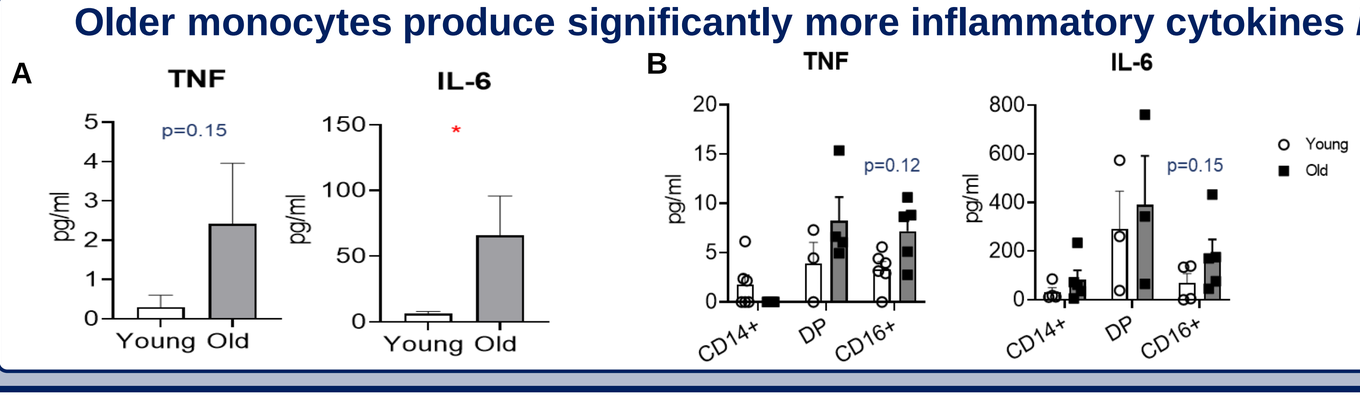

Figure 4: Monocytes were isolated from peripheral blood by magnetic isolation in A or FACS sorting in B. Cells were culture *in vitro* in the absence of any stimuli and supernatants were collected at 24 hours. Cytokines were assessed by cytometric bead array with the lower limit of detection being 2.5pg/ml. Data was assessed by Mann Whitney U statistical test and * = p<0.05.

Conclusions:

- Inflammatory monocytes can inhibit antigen-specific immunity in the skin of older monocytes – Chambers et al Nature Aging 2021
- There is an increased frequency of CD16+ monocytes in the peripheral blood of older adults as compared to young
- These CD16+ monocytes have a significantly altered proteome and secrete significantly elevated inflammatory cytokines
- Here we propose that therapeutically targeting inflammatory monocytes can reduce inflammageing

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