Quantification and classification of inflammatory leukocytes in a murine femoral artery wire injury model of neointimal hyperplasia: a high-parameter flow cytometry method

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Background

- Murine femoral artery wire injury (FAWI) is the primary model for studying neointimal hyperplasia, an inflammatory process resulting in restenosis and failure of surgery for arterial occlusive disease.
- To date, there have been no detailed studies of the composition, quantification, and dynamics of leukocyte infiltration in the arterial wall following FAWI.

Research Objective

Develop a flow cytometric method to assess time course, identify sub-populations, and quantify leukocyte infiltration following murine femoral artery wire injury.

Methods

- Designed an 18-fluorochrome flow cytometry panel for leukocyte identification in digested femoral artery (FA) samples
- C57BL/6 mice underwent left FAWI followed by euthanasia 7 days post-injury
- Injured left femoral arteries and uninjured right femoral arteries enzymatically digested to produce single cell suspensions
- Cell suspensions stained and analyzed on a BD FACSymphony A5 flow cytometer.

Results

Quantification of Leukocyte Sub-populations in Uninjured vs Injured Femoral Artery Samples

- Injured femoral artery samples demonstrated much higher levels of live leukocytes than uninjured samples (34,226 cells / FA vs. 625 cells / FA, p < .001)
- All leukocyte sub-populations were similarly present in higher numbers in injured samples than in uninjured samples

Conclusions

- Our high-parameter flow cytometry method allows for broad phenotyping of highly defined leukocyte sub-populations infiltrating in the arterial wall following FAWI in a single staining panel.
- This methodology has numerous downstream applications, including time-series analyses, cell sorting for gene expression analyses, identification of inflammatory surrogate markers (in conjunction with cytokine analysis), and correlation with vascular lesion formation.

Neointimal Hyperplasia

Histological cross section demonstrating neointimal hyperplasia

Flow Cytometry Gating Strategy

Demonstration of a viable gating strategy used on femoral arteries undergoing FAWI with identification of T/B lymphocytes, neutrophils, eosinophils, NK cells, monocytes, dendritic cells, and macrophage sub-populations

Fraction of Leukocyte Sub-populations Present in Injured vs. Uninjured Femoral Artery Samples at 7-days post-injury

- Injured femoral arteries had a notably higher numbers of neutrophils as a fraction of all live leukocytes 7-days post injury compared to uninjured femoral artery samples (light blue wedge, above)

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