Atlas of the immune cell repertoire in human atherosclerotic plaques characterized by single cell RNA-sequencing and multi-color flow cytometry

H. Horstmann[1], A. Lindau[1], S. Hansen[1], P. Stachon[1], I. Hilgendorf[1], C. Bode[1], A. Zirlik[2], D. Wolf[1]

[1]*Universitaetsklinikum Freiburg, Freiburg im Breisgau, Germany;* [2]*Medical University of Graz, Cardiology, Graz, Austria*

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Rationale: Atherosclerosis is a chronic inflammatory disease that is driven by the accumulation of pro- and anti-inflammatory leukocytes in the intima of affected arteries. Yet, the cellular composition of human atherosclerotic plaques is only poorly understood. Here, we characterized immune cells to human carotid atherosclerotic plaques by multi-color flow cytometry and scRNAseq.

Methods and results: First, we compared a set of previously reported digestion protocols to liberate leukocytes from human carotid plaques after surgical thrombendarteriectomy. One digestion cocktail, containing Collagenase IV and DNase I, was superior regarding cell survival and cell surface marker preservation. Second, leukocytes from 56 surgical specimen were characterized by flow cytometry with a set of 16 parameters and cell surface markers capable of identifying principal hematopoietic leukocyte lineages. This protocol allowed to extract and analyze on average $4x10^3$ viable CD45+ leukocytes from a mean of 988 mg plaque tissue. Surprisingly, we found that atherosclerotic plaques were dominated by T cells with 33.7±2.2% CD4+ T-helper cells and 25.6±2.5% CD8+ cytotoxic T cells. CD11b+ myeloid cells, including monocytes and macrophages, represented only 20.2±4.0% of all CD45+ leukocytes. CD19+B cells and CD56+ NK-cells accounted for 3.9±1.2 and 3.3±0.5%, respectively. TCR-g/d+ T cells and neutrophils were undetectable in atherosclerotic plaques. This cellular composition differed significantly from peripheral blood, but was not relevantly changed between different plaque locations, indicating that macrophage-rich necrotic cores mostly contain dead cells. We confirmed the principal composition of human plaques by single-cell RNA-sequencing from six patients. To allow an estimation of cellular heterogeneity independent of classical cell surface marker assignment, we performed an unsupervised cluster detection algorithm by t-distributed stochastic neighbor embedding (tSNE) and found more than 16 leukocyte clusters with unique cell surface marker expression, suggesting an unexpected high diversity of plaque leukocytes.

Conclusion: We developed an immune cell phenotyping protocol optimized for human carotid plaques. The definition of phenotypes and frequencies in atherosclerotic plaques will allow to build clinical associations between the immune cell composition and clinical outcomes in future.