Expression profiling of human macrophages and atherosclerotic plaques to identify genes and mechanisms that modulate the development of atherosclerosis

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Macrophages play an important role in atherosclerosis, a disease that affects large and medium size arteries and causes clinical manifestations such as myocardial infarction or stroke. The aim of this thesis was to identify genes that are important in the development of atherosclerosis. Genes that have their major site of expression in macrophages or in atherosclerotic plaques, or are differently expressed in macrophages from subjects with atherosclerosis compared with macrophages from control subjects may affect atherogenesis.

By comparing DNA microarray expression profiles of macrophages and atherosclerotic plaques with expression profiles from major tissues and cell types, macrophage and plaque specific genes were identified. The macrophage specific anti-inflammatory cytokine interleukin 1 receptor antagonist (IL1RN) was down regulated by oxidized low-density lipoprotein (LDL), suggesting a novel pro-inflammatory role of oxidized LDL. Immunohistochemistry showed that the plaque specific gene chemokine CC motif ligand 18 (CCL18) co-localized with macrophages in the plaques. In addition, macrophages from subjects with atherosclerosis had more than two-fold higher gene expression of CCL18 than macrophages from subjects without atherosclerosis. CCL18 is chemotactic for leukocytes and may therefore contribute to plaque inflammation. A promoter region polymorphism of the CCL18 gene was associated with increased macrophage CCL18 gene expression, but not with an increased risk of coronary heart disease (CHD).

Comparison of macrophage expression profiles from subjects with atherosclerosis and control subjects identified 27 genes with an altered expression. Among these genes, CD44 and insulin receptor substrate 2 (IRS2) were both expressed at higher levels in macrophages from subjects with atherosclerosis compared with macrophages from control subjects. Immunohistochemistry showed that IRS2, an intracellular signaling molecule important in metabolism, was expressed in macrophages and endothelial cells in human carotid plaques. The C allele of the -765C→T SNP in the promoter region of the IRS2 gene was associated with increased macrophage expression of IRS2, and subjects homozygous for the C allele had 40% increased risk of coronary heart disease. The receptor CD44 mediates adhesion of monocytes to the vascular wall, a crucial step in atherosclerosis. CD44 expression correlated with secretion of interleukin 6 (IL-6) in macrophages, and IL-6 augmented CD44 expression in macrophages. In addition, CD44 deficient mice had lower circulating IL-6 than wild type mice. This suggests a positive feed-back loop between IL-6 and CD44, and that CD44 may affect atherosclerosis progression by modulating the inflammatory response.

In conclusion, IRS2 might be a new susceptibility gene for atherosclerosis and CHD. CCL18, IL1RN and CD44 may play important roles in the development of atherosclerosis.

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II. Hägg DA, Olson FJ, Kjelldahl J, Jernås M, Thelle DS, Carlsson LMS, Fagerberg B, Svensson P-A. *Expression of chemokine (C-C motif) ligand 18 in human macrophages and atherosclerotic plaques.* Atherosclerosis **2008** Accepted for publication.

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IV. Hägg DA, Sjöberg S, Hultén LM, Fagerberg B, Wiklund O, Rosengren A, Carlsson LM, Borén J, Svensson P-A, Krettek A. *Augmented levels of CD44 in macrophages from atherosclerotic subjects: a possible IL-6-CD44 feedback loop?* Atherosclerosis **2007** 190(2):291-7.