PCSK9 and inflammation: in vitro study on THP-1 derived macrophages

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Chronic inflammation is directly linked to several metabolic diseases, such as atherosclerosis, and obesity, where inflammatory cells infiltrate adipose tissue and release pro-inflammatory mediators, such as TNF-a, IL-6, leptin and resistin, contributing to generate a state of chronic low-grade sub-clinical inflammation.
Resistin is a 12.5kDa polypeptide, secreted primarily by monocytes and macrophages in humans and it interacts with adenylyl cyclase-associated protein 1 (CAP1) receptor, with its carboxy-terminal region. In obese subjects, resistin levels are increased and it strongly correlate with the developing of atherosclerotic cardiovascular diseases. In vivo, resistin induces vascular inflammation by stimulating monocytes, causing an aggravation of atherosclerosis and it is also responsible for the increase of adhesion molecules expression on endothelial cells (like ICAM-1, VCAM-1 and MCP-1) and of the increase of TNF-a production. Moreover resistin is shown to promote foam cells formation, further emphasizing its role in the initiation of atherosclerosis.
In recent study, it has been observed a positive correlation between circulating resistin levels and fasting serum triglycerides. Moreover, this protein directly stimulates VLDL production in human hepatocites, by inducing VLDL synthesis through the induction of apoB100, triglycerides and cholesterol. Resistin is able to increase the microsomal triglyceride transfer protein (MTP), a crucial enzyme in the maintaining of apoB100 stability and in preventing its degradation. More recently, resistin has been shown to induce the degradation of the LDLR with a mechanism that involves both a PCSK9-dependent and a PCSK9-independent way.
PCSK9 is a ~62 kDa protein mainly produced and secreted by liver but also by in other tissues, like kidney and intestines, and it is the most relevant regulator of the LDL-cholesterol levels, by promoting LDLR degradation. PCSK9 has been included in autosomal dominant hypercholesterolemia causes, with LDLR and apoB. Some genetic studies shown that patients with gain-of-function mutation of PCSK9 exhibit consistently elevated VLDL triglycerides and apoB100 levels (due to an increase in synthesis and secretion rate and to a reduced degradation of apoB100), attributed to an increased hepatic VLDL production. PCSK9 levels are also significantly correlated with fasting serum triglycerides levels.
PCSK9 and resistin share their carboxy-terminal region structure and we know that these two protein share also some common biological activities. Very little is known about the possible role of PCSK9 in the inflammatory process, for this reason, in this work we wanted to understand if PCSK9 could have a pro-inflammatory action, maybe with a resistin-like mechanism.
We decided to use THP-1 derived macrophages and we threated them for 24h with increasing concentrations of PCSK9 (0.25, 0.5, 1.0 and 2.5 μg/ml) and resistin (20 and 50ng/ml) and we evaluated pro-inflammatory cytokines mRNA levels. We observed a dose dependent increase in IL1β mRNA levels but, more surprisingly, IL-6, TNF-Î±, MCP-1 and MIP-2Î± mRNA levels show a very strong increase in response to the 2.5 μg/ml of PCSK9 (~65 fold for IL6, ~160 fold for TNF-a, ~17 fold for MCP-1, and ~42 fold for MIP2Î±). Moreover, through ELISA assay, we observed a significantly higher amount of TNF-a and IL-6 protein in the conditioned media, after treatment with 2.5 μg/ml of PCSK9. We also observed an increase in cAMP levels, in response to the higher concentration of resistin and PCSK9, indicating the activation of NF-kB pathway by both the treatments. These results support a possible pro-inflammatory activity of PCSK9, potentially with a resistin-like activity. In the future, it will be determined the involvement of either the LDL receptor or the CAP1 receptor, both expressed in macrophages, on the pro-inflammatory response.