ImmunoTools *special* Award 2013

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Induction of specific transcriptional programs in human macrophage subsets by free fatty acids

Systemic low-grade inflammation in obesity as well as type II diabetes is associated with elevated levels of pro-inflammatory cytokines, like IL-6 and TNFα. Yet, the source of inflammation is not clear. The adipose tissue might contribute to the early steps of obesity-associated inflammation by the release of adipokines and free fatty acids (FFAs) from adipocytes into the blood. Unsaturated fatty acids such as palmitate can induce the production of pro-inflammatory cytokines in myeloid cells. Yet the overall impact of fat-associated activation of these important immune cells in obesity and diabetes has not been revealed. Our current research aims to determine FFA-mediated pro-inflammatory transcriptional changes in a diverse subset of macrophages using palmitate as the model and to establish a link between metabolic programs and immune function in these important immune cells.

In obesity monocytes can be recruited by inflammatory mediators, like MCP-1 to the adipose tissue and differentiate there to mature macrophages. Still the phenotype and function of these adipose tissue macrophages (ATMs) is under intense debate, since flow cytometric analysis revealed discrepant data suggesting classically IFNγ (M1) and alternatively IL-4 (M2) activated macrophages being predominant in fat tissue. As outlined in previous reports a model of phenotypic switch tries to explain this discrepancy: there are only few IL-4 activated macrophages present in normal fat tissue, but with the enlargement of adipose tissue in obesity, increasing amounts of IFNγ activated macrophages are recruited to the adipose tissue. This influx results in a shift of the balance between the IFNγ and IL-4 induced phenotype towards IFNγ-like macrophages. It is assumed that the increased number of IFNγ activated macrophages is due to the enormous influx rather than to a conversion of present IL-4 activated macrophages into classically activated macrophages. However, this classical model might be oversimplistic, since increasing evidence suggests that it does not reflect macrophage biology in obesity.

In fact, elevated systemic levels of FFAs are a hallmark of obesity and might be a major factor influencing the cellular program of local tissue resident macrophages in obesity. There is accumulating evidence that not only macrophages become locally activated by inflammatory mediators, like adipokines, FFAs and triglycerides, which are released by bursting adipocytes due to lipotoxicity, but also circulating monocytes in the blood system might mature upon FFAs encounter. For example it has been

shown, that the FFA palmitate in co-operation with insulin is able to induce the production of IL-6 and TNFα in monocytes and immature macrophages. In addition, monocytes and macrophages have been shown to take up FFAs into lipid droplets inducing a pro-inflammatory cellular program. Data derived from murine model systems clearly indicate that the obese phenotype induced by high fat diet critically depends on monocyte and macrophage activation. In essence, preliminary data exist suggesting monocyte activation by FFAs in obesity might already occur prior influx of these cells into adipose tissue and these cells might not follow the classical pathways of macrophage activation (either M1- or M2-like cells).

So far we have investigated the transcriptional programs of FFA activated macrophages in comparison to a diverse spectrum of macrophage subtypes encompassing classically and alternatively activated macrophages as well as macrophages correlating to several chronic inflammatory disease states, e.g. artheriosclerosis and granulomatosis. FFA activated macrophages showed a distinct and specific transcriptome independent of the suggested M1 phenotype from the model of phenotypic switch.

In the view of macrophage plasticity, we are currently interested in the question, if already activated macrophages are able to take up FFA and develop a pro-inflammatory phenotype as observed in our previously performed transcriptome analysis. Therefore we will activate immature human macrophages by stimulation with ImmunoTools recombinant cytokines: GM-CSF, M-CSF, IL-4, IFNγ, IFNα, IFNβ, TNFα, IL-6, IL-8, IL-10, IL-13, VEGF and further culture this matured immune cells in the presence of FFA to survey transcriptomic changes by deep sequencing approaches and translate the findings into a mouse model.

We hope that the results will lead to a better understanding of human myeloid cell activation by FFAs in obesity, which is required to develop novel innovative diagnostic and therapeutic options for patients with obesity.