Human natural killer cells as immunological sensors of obesity-induced adipocyte stress during development of insulin resistance

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Background: Obesity-induced adipose tissue deregulation might result in a stress-response by adipocytes and stromal cells, in turn causing activation and accumulation of proinflammatory immune cells in adipose tissue. The ensuing low-grade, chronic inflammation reduces systemic insulin sensitivity and can cause type 2 diabetes.\(^1\)\(^-\)\(^3\) However, how adipose tissue stress is translated into a signal that activates the immune system is largely unknown. An important early step in adipose tissue inflammation is macrophage (MΦ) accumulation, activation, and polarization from anti-inflammatory M2-cells to pro-inflammatory M1-cells.\(^2\)\(^,\)\(^3\) These cells are considered the dominant producers of IL-1β and TNF, and, thus, pivotal for development of obesity-induced insulin resistance.\(^4\) Much is known about factors that yield MΦ accumulation, but it is still unclear how the overt M2-to-M1 transition is initiated. Natural killer (NK) cells are important sentinels in the body, surveying peripheral tissues, that specialize in recognizing “stressed” cells.\(^5\) This is achieved through an array of activating and inhibitory receptors on the surface of NK cells calibrated to detect different signs of pathology, while ensuring tolerance to self.\(^5\) Interestingly, NK cell-derived IFN\(\gamma\) is critical for M1 MΦ polarization and IFN\(\gamma\) ablation reduces obesity-induced insulin resistance in mice.\(^6\)\(^,\)\(^7\) In line with this, recent work in a HFD-model showed that murine NK-cells could react on adipose stress and cause inflammation and insulin resistance.\(^8\) However, the role of adipose tissue resident NK cells in development of type 2 diabetes in humans is still unclear.

Hypothesis: Human adipose tissue-resident NK cells are sensors that specifically recognize adipocyte stress and subsequently promote adipose tissue inflammation and insulin resistance.

Aims: I) To compare the immune composition of visceral- and subcutaneous adipose tissue (VAT/SAT) of obese insulin-resistant humans with that of obese humans without insulin resistance. II) To establish a co-culture system with primary adipocytes, adipose tissue derived NK cells, and MΦ for mechanistic studies. III) To evaluate the importance of adipocyte stress pathways on the ensuing NK-cell sensing and MΦ inflammatory response via genetic silencing of key genes in adipocytes.

Workplan: The current project is a collaborative project between the Björkström-group, the group of Prof. Mellgren, University of Bergen, experts on human adipocyte research.\(^9\)
and Ass. Prof. Stål, Senior Consultant in Hepatology at Karolinska University Hospital, expert on fatty liver disease.\textsuperscript{10} The Björkström-group are experts on human tissue-resident NK cells, detailed characterization of tissue immune compartments using 23-parameter flow cytometry, and assessments of immune cell function in advanced co-culture systems. The candidate will perform the main work in the Björkström-group with shorter visits anticipated to the University of Bergen. VAT and SAT will be obtained from 30 obese patients (BMI 35–45, 15 insulin-resistant and 15 insulin-sensitive) selected for elective bariatric surgery. As additional controls, SAT from lean individuals will be obtained. The patients will be clinically well characterized with glucose tolerance test, HOMA-A/B, liver biopsy during surgery, and blood lipid profile. Importantly, this study will control for NASH, a confounding disease and independent risk factor for diabetes development. In a parallel project, funded through a NovoNordisk Foundation Young Excellence Award to Björkström, we are currently investigating the role of human NK cells as initiators of liver inflammation in NASH. From VAT/SAT, immune cells will be freshly isolated and subjected to detailed phenotypic and functional analysis using high-resolution flow cytometry. Pre-adipocytes isolated from VAT and SAT will be matured to adipocytes \textit{in vitro}, exposed to metabolic (uric acid, ATP, acetylated HMGB1, palmitic acid) and gut-derived (LPS) danger signals, phenotyped by flow cytometry for surface expression of stress-induced ligands to NK-cell receptors (NKR), and put in co-culture systems with adipose tissue-resident NK cells and \( \text{M}\Phi \). Adipocyte-dependent NK-cell responses (intracellular detection of IFN\(\gamma\), TNF, and GM-CSF) and the capacity of NK cells to promote \( \text{M}\Phi \) polarization and activation will be evaluated using flow cytometry. The Mellgren-group has recently generated extensive microarray datasets on genes differentially regulated in adipocytes upon cellular stress. Using this data, the \textit{in vitro} system will be dissected on the adipocyte-side using either blocking antibodies against NKR-ligands or siRNA knockdown of components in stress pathways with the aim to identify key pathways responsible for NK-cell sensing of stressed adipocytes.

References for the project:


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