ImmunoTools *special* Award 2014

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To characterize cells in the adipose tissue of obese patients

Metabolic syndrome that includes type 2 diabetes, non-alcoholic fatty liver disease and atherosclerosis is the next epidemic facing the health care. While chronic inflammation induced by the free circulating fat has been established as a major culprit, there are no specific pathways or targets that have been identified to play a definite role in the process. *In fact, infiltration of adipose tissue by macrophages is one of the robust markers for obesity-related morbidity*. Our data from mouse models have identified certain inflammatory cytokines as important players that promote inflammation in the adipose tissue of mice. We will validate and extend our observations to human visceral and subcutaneous adipose tissue obtained from obese patients undergoing bariatric surgery. We will use subcutaneous and visceral adipose tissue obtained from age- and sex-matched obese diabetic and obese non-diabetic patients undergoing bariatric surgery at the Sherbrooke University hospital. All the required approval have been obtained.

The adipose tissues of lean individuals express adiponectin, TGFβ, IL-10, IL-4 and IL-13, while the obese adipose tissue shows increased expression of TNFα, IL-6, leptin, resistin and plasminogen activator inhibitor 1. A majority of obese individuals develop insulin-resistance, while about 25% remain insulin-sensitive. The factors that distinguish insulin-sensitive and insulin-resistant obese individuals are not yet clear. A comparison of adipose tissues from these two groups of patients will help to identify factors that may contribute to insulin resistance. In omental (VAT) and subcutaneous (SAT) depots from *insulin-resistant* individuals genes associated with inflammation are elevated. In mice, regulatory T cells that express the FOXP3 transcription factor (Tregs) exert a beneficial effect in obesity. However, in humans the results are not conclusive. Similarly, there are conflicting reports on the contribution of natural killer T (NKT) cells that are activated by lipid ligands in obesity. Thus, the factors that promote activation of pro-inflammatory macrophages, and those that regulate the balance between inflammatory and regulatory T cells, in human obese adipose tissues are not yet clear.

Even though adipose tissue associated macrophages (ATM) from obese human patients express genes associated with inflammation, there is limited evidence for the classical M1-M2 macrophage paradigm. $CD11c^{+}206^{+}$ ATMs are localized at a higher density to form crown-like structures surrounding necrotic adipocytes and express high levels of pro-inflammatory cytokines that induce insulin resistance in adipocytes. Whereas adipose tissues from healthy individuals show negligible numbers of granulocytes, cytotoxic T cells and B cells, visceral and subcutaneous adipose tissue from obese individuals show increased infiltration of $CD4^{+}$ and $CD8^{+}$ T cells.

We will carry out flow cytometry analysis of stromal vascular (SVC) fractions isolated from fresh tissues of obese patients. When available, we will use adipose tissue samples

from healthy lean subjects, although the cell yield will be very low. The SVCs will be analyzed for the frequency and numbers of CD4, CD8, B and NK cell subsets as well as macrophages, using lineage specific markers along with anti-CD45 as a marker for hematopoietic lineage. The use of CD45 marker will permit the quantification of the different subsets of infiltrating leukocytes. CD4 and CD8 T cell subsets will be further characterized using markers such as CD45RO, CD45RA, CD62L and CD44 that distinguish memory and effector subsets. We will characterize the macrophages for their phenotype based on CD11c, CD14 and CD206 markers. We will determine if they express increased levels of MHC class I, class II and co-stimulatory molecules (CD80, CD86) that may predict their ability to activate T lymphocytes. Most of the antibodies mentioned above are available from ImmunoTools. Their support will be invaluable for the inititation and the progression of this project.

ImmunoTools *special* AWARD for **Sheela Ramanathan** includes 25 reagents

FITC - conjugated anti-human CD1, CD14, CD45RA, CD56, CD69, CD80, CD86, HLA-ABC, HLA-DP, HLA-DR,

PE - conjugated anti-human CD11b, CD44, CD45, CD45RB,

PerCP - conjugated anti-human CD3, CD8, CD45,

APC -conjugated anti-human CD3, CD4, CD11c, CD14, CD25, CD44, CD62L, CD69,

DETAILS