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Adipose tissue NK cells manifest an activated phenotype in human obesity

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ABSTRACT

Objective. Adipose tissue inflammation is a cause of obesity-related metabolic disease. Natural killer (NK) cells are an understudied cell type in the context of obesity. The goal of this study was to determine the phenotype of human adipose tissue NK cells.

Methods. We used flow cytometry phenotyping to study adipose tissue and peripheral blood NK cells from obese and lean humans.

Results. Human adipose tissue NK cells, relative to peripheral blood NK cells, express increased levels of activation markers. Adipose tissue NK cells also demonstrate an activated phenotype in obese relative to lean subjects, with increased expression of the activating receptor NKG2D.

Conclusions. These data are the first detailed phenotypic characterization of human adipose tissue NK cells, and suggest a role for NK cells in adipose tissue inflammation in obesity.

1. Introduction

Obesity is associated with a state of systemic inflammation that is based in adipose tissue and underlies the pathogenesis of metabolic disease. While adipose tissue macrophages (ATM) are central mediators of this inflammatory process, other leukocytes are involved, including T-cells and NKT cells [1-4]. Others have reported aberrations in peripheral blood NK cells (PBNK) in obesity [5-7]. Few published data study human adipose tissue NK cells (ATNK). The goal of this research was to describe the phenotype of human ATNK. We demonstrate that human ATNK cells manifest an activated phenotype relative to PBNK and in obese relative to lean subjects.

2. Methods

2.1. Subjects, tissue

Obese (BMI > =30 kg/m^2) and lean (BMI < =25) subjects undergoing abdominal surgery were enrolled and consented with Institutional Review Board approval consistent with institutional and governmental regulations. Peripheral blood (PB) and visceral (greater omentum) adipose tissue (VAT) were collected and processed immediately. Subcutaneous (abdominal wall) adipose tissue (SAT) was collected from obese subjects. Thirteen obese subjects undergoing laparoscopic bariatric surgery and 7 lean subjects undergoing laparoscopic

Abbreviations: ATNK, adipose tissue NK cells; ATM, adipose tissue macrophages; PB, peripheral blood; PBMC, peripheral blood mononuclear cells; PBNK, peripheral blood NK cells; SAT, subcutaneous adipose tissue; SVF, stromovascular cell fraction; VAT, visceral adipose tissue.

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abdominal surgery for benign disease (6, fundoplication for gastroesophageal reflux disease, 1, cholecystectomy for gallstones) were enrolled. For obese subjects, mean age was 51 years (standard deviation (SD), 12), mean BMI 52 (SD, 6), 85% were female, the prevalence of diabetes, hypertension, sleep apnea, and hyperlipidemia was 46%, 69%, 62%, and 62% respectively, and medications included angiotensin converting enzyme inhibitor (31%), statin (31%), proton pump inhibitor (23%), beta blocker (38%), metformin (38%), and aspirin (23%). For lean subjects, mean age was 52 years (SD, 18), mean BMI 24 (SD, 6), 71% were female, and 86% (n = 6) had gastroesophageal reflux disease, and 14% (n = 1) had biliary colic. No lean subjects had diabetes, sleep apnea, or hyperlipidemia, one (14%) had hypertension, and medications included angiotensin converting enzyme inhibitor (14%), proton pump inhibitor (86%), and aspirin (29%).

2.2. *Cell isolation*

Media and reagents were certified to have endotoxin levels less than 0.030 EU/ml. Vessels were dissected from adipose tissue, which was washed in PBS + 2% BSA, minced, and digested with Type II collagenase (175 U/ml in PBS + 2% BSA, Life Technologies, Carlsbad, CA, USA) for 60 min at 37 °C followed by centrifugation at 200 g. The stromovascular cell fraction (SVF) cell pellet was retrieved and washed in PBS. Peripheral blood mononuclear cells (PBMC) were isolated from blood with Ficoll gradient, treated with ammonium chloride, and washed in PBS.

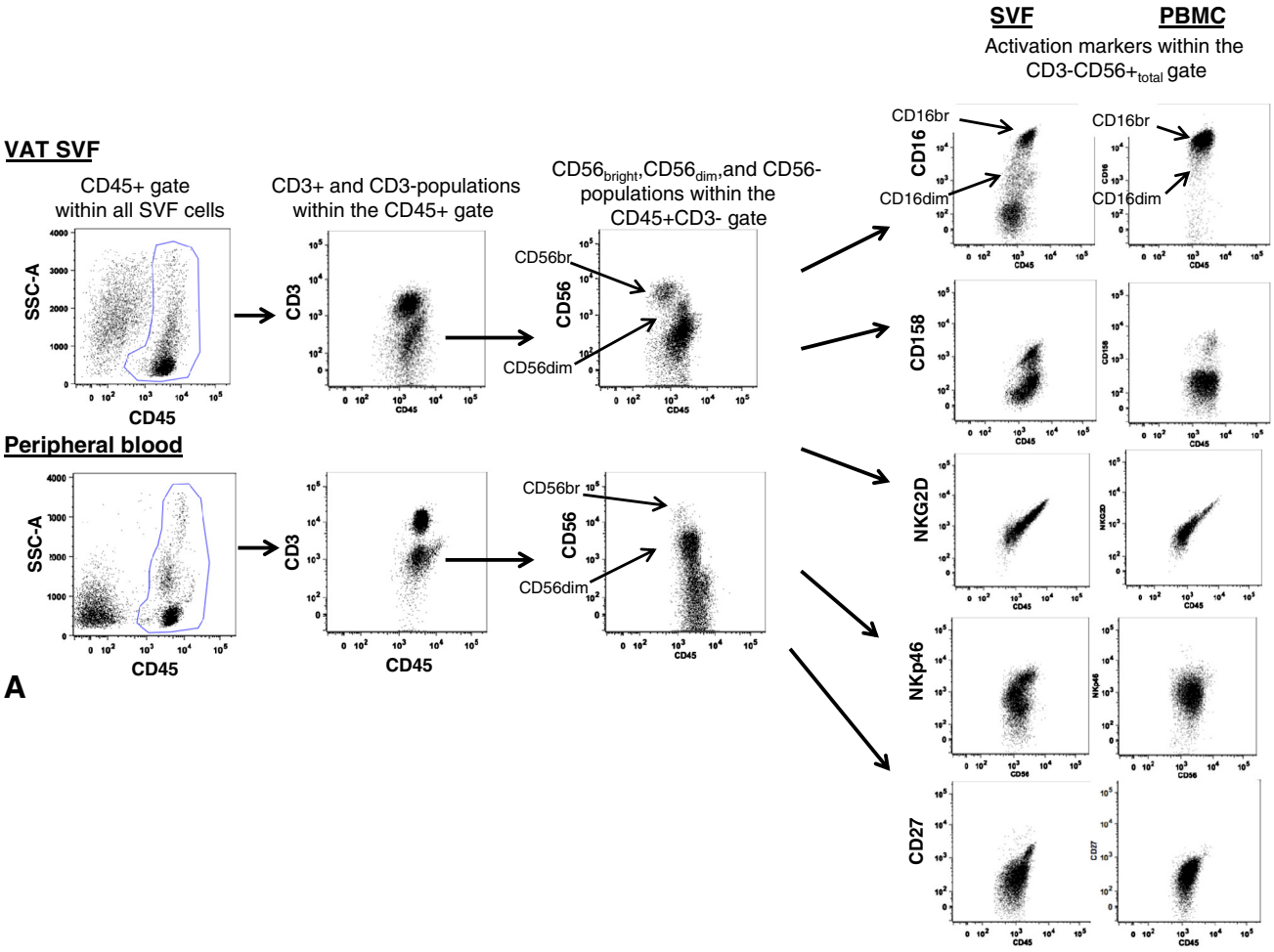

Fig. 1 – Flow cytometry phenotype of human ATNK. (A) Representative flow cytometry scatter-plots of NK cell-related subpopulations in human visceral adipose tissue SVF and peripheral blood mononuclear cells from an obese subject. T-cells were defined as CD45+CD3+. NK cells were defined as CD45+CD3–CD56+ cells. After exclusion of doublets and non-viable cells, CD45+ cells were gated and then divided into CD3+ and CD3– gates. The CD3– gate was further divided into $CD56_{bright}$ and $CD56_{dim}$ gates, as well as a $CD56_{total}$ gate that encompassed all CD3–CD56+ cells (bright and dim). Further phenotyping was performed on the $CD56_{total}$ population ($CD56_{bright}$+$CD56_{dim}$) for NK cell activation markers. (B–J) Frequencies of named subpopulations (CD45 cells i.e. leukocytes, T-cells (CD45+CD3+), NKT cells (CD45+CD3+CD56+), and NK cells (CD45+CD3–CD56+)) within total SVF or PBMC populations (B, E, H), and of NK cell subpopulations within the total NK cell gate (C, D, F, G, I, J) in VAT, SAT, and PB from obese (B–D, H–J) and lean (E–G, H–J) subjects. Asterisks denote $p < 0.050$ for the indicated data groups using paired t-test (comparing VAT, SAT, and PB within obese or lean subject groups, B–G) or independent t-test (comparing lean and obese groups, Figure H–J).