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.

Keywords

Inflammation; NKG2D; CD56

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 manifest an activated phenotype relative to PBNK and in obese relative to lean subjects.

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Methods

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 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 were 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%).

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 units/ml in PBS + 2% BSA, Life Technologies Inc., Carlsbad, CA, USA) for 60 minutes at 37°C followed by centrifugation at 200g. 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..

Flow cytometry

Cells were incubated with antibodies (CD158a/h-FITC (clone HP-MA4), CD16-PE (clone 3G8), NKp46-PE (clone 9E2), NKG2D-PE/Cy7 (clone 1D11), CD56-APC (clone HCD56), CD3-APC/Cy7(clone UCHT1) (Biolegend, SanDiego, CA, USA); CD45-PE/Cy5.5(clone HI30), CD27-PE/Cy7 (clone 0323) (eBiosciences Inc., San Diego, CA, USA) for 30 minutes, washed with PBS, 0.5%, BSA, 0.1% NaN$_3$, fixed with Cytofix/Perm solution and analyzed on an LSR II flow cytometer (Becton Dickinson Inc., Franklin Lakes, NJ, USA). Data were analyzed using FlowJo software (Tree Star Inc., Ashland, OR, USA). Post-acquisition compensation, isotype controls, and fluorescence minus one gating were used to determine gates. After exclusion of doublets and nonviable cells using viable dye, a large forward and side scatter gate was used to include all viable cells, followed by gating on the pan-leukocyte marker CD45, followed by gating on cell populations of interest.

Results

Flow cytometry was used to characterize the phenotype of human ATNK and PBNK (Figure 1A). In obese and lean subjects, CD45+ cells and CD3+ cells (T-cells) comprised a greater percentage of total PBMC than of VAT or SAT SVF, while frequencies of CD3+CD56+ (NKT) cells and CD3−CD56+ (NK cells) were similar between VAT, SAT, and PBMC (Figure 1B, E). The frequency of CD56$_{bright}$ cells within the total NK cell population was markedly higher, while the frequency of CD56$_{dim}$ cells was lower, in VAT and SAT SVF relative to PBMC from obese subjects (Figure 1C) but not in lean subjects (Figure 1F). The

frequencies of CD158+, NKG2D+, $CD16_{dim}$, NKp46, and CD27+ cells within the NK cell population were increased, while the frequency of $CD16_{bright}$ cells was decreased, in VAT SVF relative to PBMC in obese subjects (Figure 1D). In lean subjects, the frequencies of $CD16_{bright}$ and $CD16_{dim}$ populations within the total NK cell population were similarly decreased and increased respectively in VAT SVF compared to PBMC, but, in contrast to obese subjects, no differences were observed between VAT SVF and PBMC with respect to expression of CD158, NKG2d, NKp46, or CD27 within the NK cell population (Figure 1G).

The frequency of CD45+ cells and T-cells was increased in obese compared to lean subjects in VAT SVF, while no such differences were observed for NKT or NK cells (Figure 1H). The frequency of $CD56_{bright}$ cells within the total NK cell population was higher, while the frequency of $CD56_{dim}$ cells was lower in VAT SVF from obese compared to lean subjects (Figure 1I). Expression levels of the NK cell activation marker NKG2D were increased within the NK cell population in VAT SVF from obese compared to lean subjects (Figure 1J).

Discussion

Much attention has focused on ATM, T-cells, and NKT cells in obesity-associated adipose tissue inflammation, but the role of NK cells in this inflammatory process remains unknown. PBNK cytotoxic function is attenuated in obesity and these changes are reversed with weight loss [5, 7] while PBNK from obese rats demonstrate signaling defects [8, 9]. Few studies of human ATNK exist. We present the first detailed phenotypic characterization of human adipose tissue NK cells.

$CD56_{dim}$ NK cells predisposed to cytotoxicity predominate in peripheral blood. $CD56_{bright}$ cells, in contrast, are a minority of human PBNK cells, express tissue homing molecules, are increased in tissues, and are predisposed to IFN- secretion [10–11]. Expression of the Fc receptor CD16 inversely correlates with CD56 expression in humans, with $CD56_{br}CD16_{dim}$ and $CD56_{dim}CD16_{br}$ subpopulations described. We confirm a similar reciprocity of expression of these markers in human ATNK in lean and obese patients. Furthermore, we demonstrate that in obese subjects, ATNK, compared to PBNK, are comprised of a greater percent of $CD56_{br}$ cells, consistent with prior reports demonstrating a similar predominance of $CD56_{br}$ NK cells in other tissues [12, 13]. We also demonstrate that ATNK in obese subjects, compared to PBNK, express higher levels of activation markers, including CD158, NKG2D, NKp46, and CD27. In contrast, in lean subjects, expression levels of CD158, NKG2D, NKp46, and CD27 were similar between ATNK and PBNK. Furthermore, expression of the NK cell activating marker NKG2D was increased in ATNK from obese compared to lean subjects, suggesting that the inflammatory state in obese adipose tissue promotes NK cell activation. Finally, the observed relative increased expression of the NK cell markers CD158 and NKp46, the latter an activation marker, in VAT relative to SAT within obese subjects is consistent with the established increased inflammatory state of the former depot [14], and further suggests a link between ATNK cells and inflammation.

Prior data by our group demonstrate that IFN- promotes ATM inflammation and insulin resistance in obesity, and that ATNK are a source of IFN- [15, 16]. Our present data demonstrate increased of $CD56_{bright}$ NK cells within adipose tissue, a cell population prone to cytokine secretion [10–11]. Others demonstrate that NK cells regulate macrophage inflammatory responses in murine obesity and other inflammatory diseases [17, 18]. Taken together these observations suggest a role for ATNK in promoting adipose tissue inflammation. Precedent exists for this concept, as others have implicated IFN- -expressing $CD56_{bright}$ NK cells in other inflammatory diseases [13, 18].

Our data contribute to increasing literature demonstrating that adaptive immune cells, including canonical and regulatory T-cells, NKT cells, and B-cells, contribute to inflammation in obesity (2, 4, 19). Crosstalk occurs between regulatory T-cells and B-cells in adipose tissue (19), and between NK cells and macrophages in the liver (20). Our data suggest that NK cells contribute to similar complex leukocyte interactions in obese adipose tissue.

Limitations in tissues and cell yield, exacerbated by the fact that NK cells represent a small fraction of SVF and PBMC, precluded functional analysis or correlation of NK cell phenotype with clinical variables such as diabetes. These data nevertheless provide the first detailed phenotypic characterization of human adipose tissue NK cells, and demonstrate that ATNK manifest an activated phenotype in obesity, suggesting a role of NK cells in adipose tissue inflammation in metabolic disease and providing avenues for further study.

Acknowledgments

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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A

SVF   **PBMC**

Activation markers within the
CD3-CD56+$_{total}$ gate

VAT SVF

CD45+ gate
within all SVF cells

CD3+ and CD3- populations
within the CD45+ gate

CD56$_{bright}$, CD56$_{dim}$, and CD56-
populations within the
CD45+CD3- gate

CD56br

CD56dim

Peripheral blood

CD56br

CD56dim

CD16br
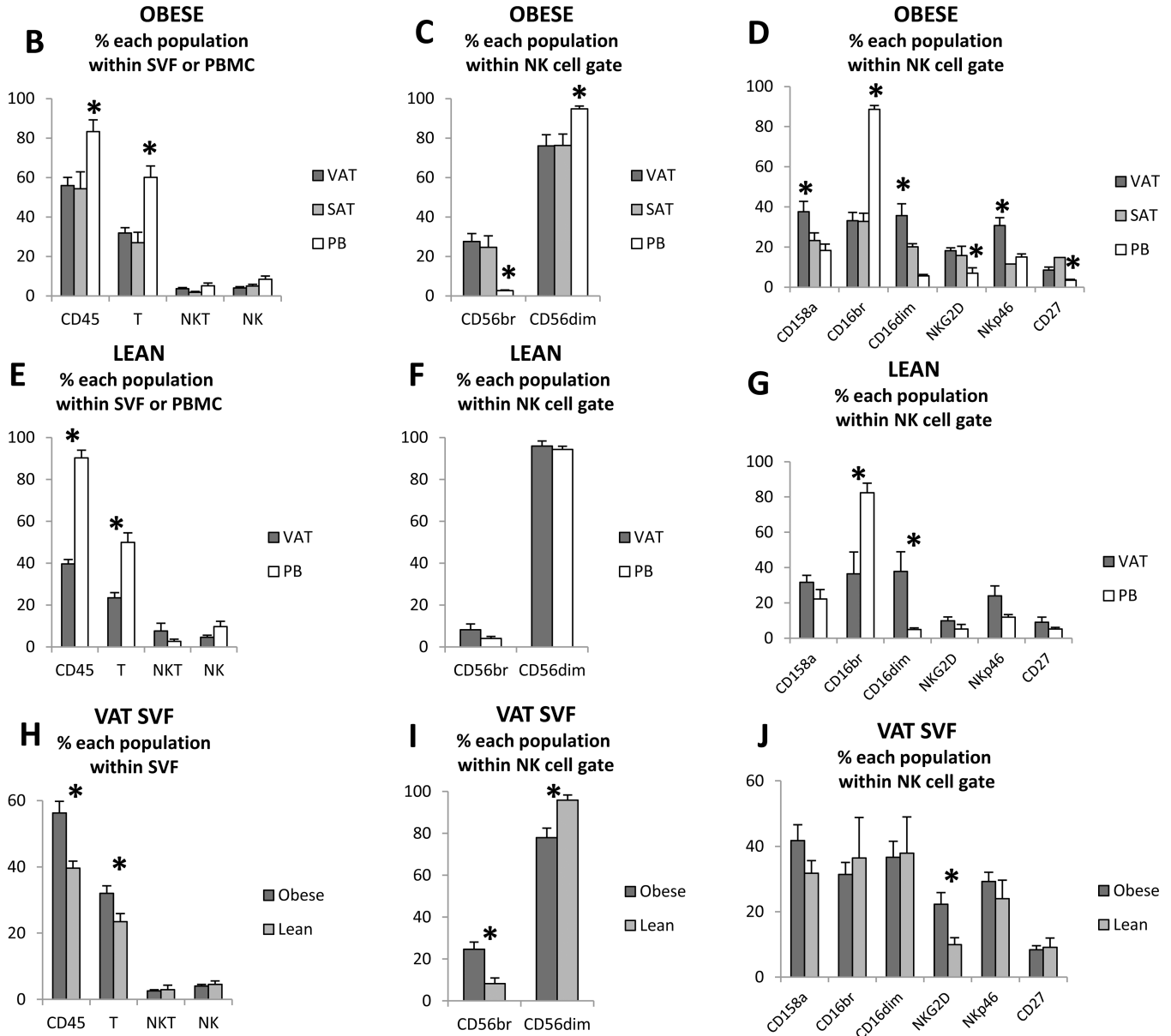
CD16dim

CD16br

CD16dim
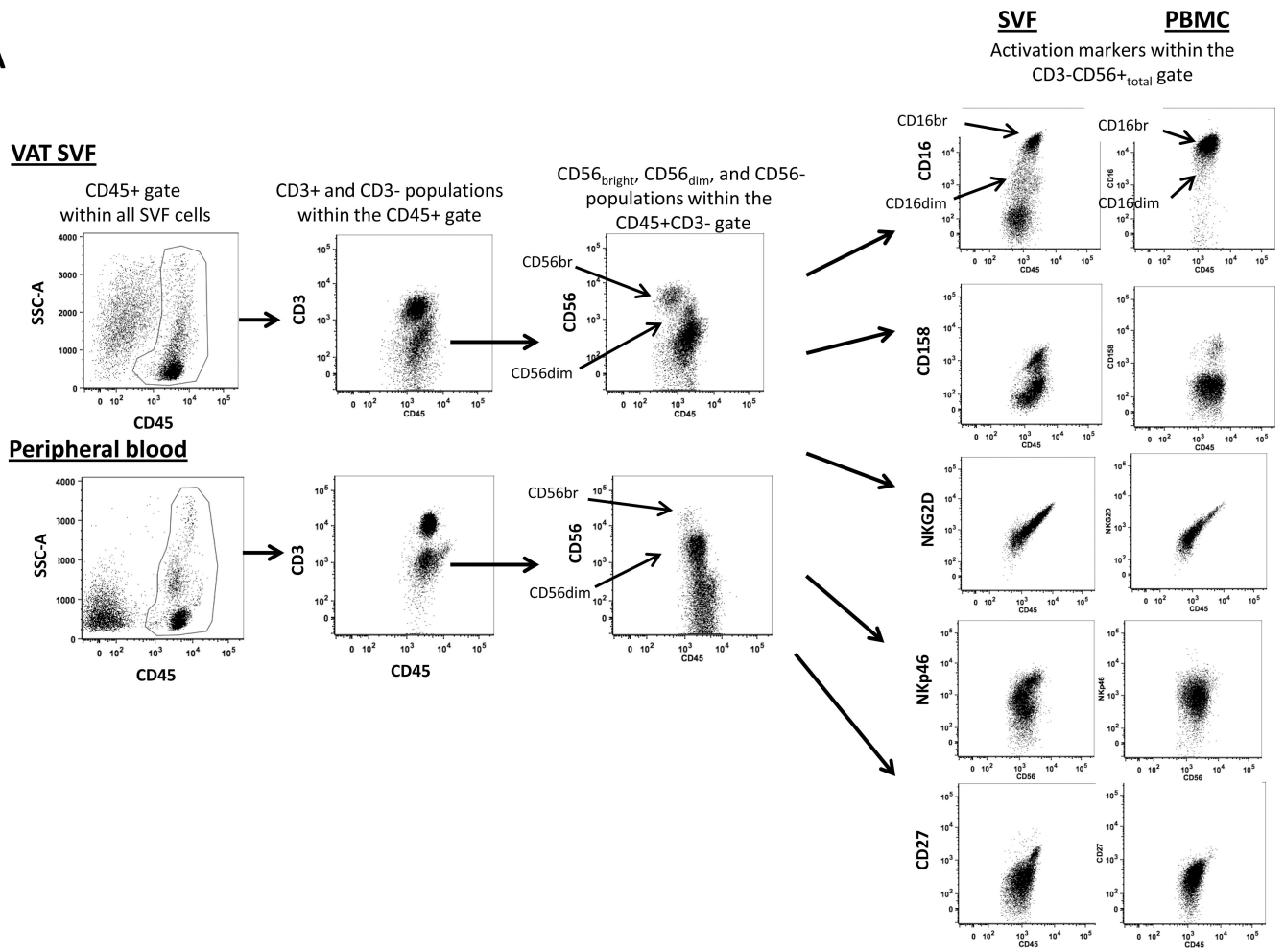
Figure 1. Flow cytometry phenotype of human ATNK

1A: 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.

1B–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, Figures 1B–G) or independent t-test (comparing lean and obese groups, Figure H–J).