Obesity Alters B Cell and Macrophage Populations in Brown Adipose Tissue

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Objective: The prevalence of obesity continues to rise, and it is understood that regulation of white adipose tissue (WAT) function is important to systemic metabolic homeostasis. Immune cells play a central role in the maintenance of WAT, and their compositions change in number and inflammatory phenotype with the progression of obesity. Because of its energy-burning capabilities, brown adipose tissue (BAT) has become a focus of obesity research. Although novel studies have focused on the function of brown adipocytes in thermogenesis, the tissue as a whole has not been immunologically characterized.

Methods: BAT immune cell populations were analyzed by flow cytometry and immunohistochemistry in mice with diet-induced obesity (3, 8, or 16 weeks of diet) and in aged mice (1, 6-7, and 10-15 months).

Results: The data confirmed the presence of macrophages and eosinophils, as previously reported, and showed that 20% to 30% of the immune cells in BAT were B cells. The number of B cells and eosinophils increased with diet-induced obesity, whereas macrophages decreased. There was no change in number of any immune cell quantified with age.

Conclusions: These studies reveal a novel finding of B220+ B cells in BAT and show that BAT immune cell populations change in response to diet-induced obesity.

Introduction

Properly functioning white adipose tissue (WAT) is vital for systemic metabolic homeostasis. Studies of brown adipose tissue (BAT) function in metabolism have come to the forefront because BAT is capable of producing energy through nonshivering thermogenesis (1). BAT responds to sympathetic nerve signaling through activation of UCP1. This process can be activated by the catecholamine norepinephrine, cold exposure, and adenosine (2). A goal of investigators is to manipulate white adipocytes to resemble brown adipocytes, a process termed “beiging” (1,3). Although this strategy shows obvious health benefits, there is not enough known about the physiology of BAT and how the tissue environment is involved in thermogenesis.

There are a variety of immune populations resident in WAT in lean animals, and the immune repertoire in WAT changes in obesity (4,5). Macrophages make up about 12% of WAT cells in lean mice, and this can increase to 41% in mice with obesity (6). Other innate immune cells, such as eosinophils, neutrophils, and dendritic cells, as well as adaptive immune cells, are also involved in regulation of WAT health (7).

Immune signaling in BAT has not been systematically studied. Studies show that there is evidence of the presence of various immune cell types in BAT, such as eosinophils (8), macrophages and monocytes (9), and regulatory T cells (10), but many of these studies were carried out as supporting data to studies on WAT. Because of the lack of a systematic understanding of the BAT immune cell repertoire, we used an immunophenotyping approach to determine which cell types are present. This information will be vital for further comparing the function of BAT in normal and diseased states.
Methods

Mouse studies

Animal procedures were performed following Institutional Animal Care and Use Committee approval. Male and female C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, Maine) and aged or fed special diets in the Vanderbilt facility. Mice were maintained on a chow diet for the aging studies (1, 6-7, or 10-15 months) and on a 10% fat low-fat diet (LFD) or 60% fat high-fat diet (HFD) (Research Diets, Inc., New Brunswick, New Jersey) for 3, 8, or 16 weeks for the obesity studies. Mice were fed ad libitum. Upon termination of the study, BAT was harvested by cutting away connecting tissue that looked more like WAT.

Flow cytometry

Stromal vascular fraction (SVF) of the total BAT from one animal per sample was collected following collagenase digestion and centrifugal separation, as previously described (11). Briefly, BAT was digested for 4 hours with 2 mg/mL of collagenase type 4 (Worthington Biochemical Corp., Lakewood, New Jersey; Lot #45H15909). The tissue was pressed through a 100-µm filter. The isolated SVF was incubated with Fc Block for 5 minutes on ice, washed, and incubated with fluorophore-conjugated antibodies for 20 minutes at 4°C. The following antibodies were used: B220-FITC, CD3-APC/Cy7, CD45-PE/Cy7, F4/80-APC, and SiglecF-PE (BD Biosciences, Franklin Lakes, New Jersey). DAPI was used for viability staining. Flow cytometry was carried out on the entire volume from each sample at the Vanderbilt Flow Cytometry Shared Resource by using a BD Biosciences special order research product called LSRFortessa. Please see Supporting Information Figure S1 for the gating scheme.

Histology and imaging

BAT tissue was fixed in 10% formalin overnight and was paraffin embedded, sectioned, and stained by the Vanderbilt Translational Pathology Shared Resource for hematoxylin and eosin, CD11b (macrophages), B220 (B cells), and major basic protein (eosinophils). Images were captured and analyzed through the Vanderbilt Digital Shared Resource and Leica Digital Image Hub software.

Statistical analysis

All statistics were performed by using GraphPad Prism software. BAT mass changes and all LFD to HFD cell population comparisons were analyzed by using an unpaired t test. Aging-associated differences and body weight curves were analyzed by one-way and two-way analysis of variance (ANOVA), respectively.

Results

Metabolic parameters of mice on diet

Male and female wild-type C57BL/6J mice gained weight on an HFD (Figure 1A-1B). In addition to WAT expansion, BAT mass was also increased and was significantly greater in mice fed an HFD for 8 and 16 weeks (Figure 1C-1D).
Flow cytometry of BAT SVF

BAT SVF was analyzed for the presence of B cells, macrophages, and eosinophils by flow cytometry (see Supporting Information Figure S1 for gating scheme). CD45+ leukocytes represented less than 5% of all live cells. In general, there was a higher frequency of B220+ B cells (Figure 2A-2B) and a lower frequency of F4/80+ macrophages (Figure 2C-2D) in HFD- compared with LFD-fed mice. Concomitantly, there was a lower frequency of SiglecF+ eosinophils in animals with diet-induced obesity (Supporting Information Figure S2). The data showed that 13.5% ± 2.1 of live leukocytes were SiglecF+ in male mice fed an HFD, whereas 6.9% ± 1.5 (P < 0.05) were SiglecF+ in mice fed an LFD for 8 weeks. The very low prevalence of B220 + B cells, CD11b + macrophages (Figure 3), and major basic protein + eosinophils (Supporting Information Figure S2) was confirmed by immunohistochemical staining of BAT from male wild-type animals fed an LFD or HFD for 8 weeks.

Analysis of BAT immune cells with aging

Additionally, the effect of aging on the BAT immune cell repertoire was investigated. The animals’ body weights increased with age, and mice that were 6 to 7 months and 10 to 15 months old were significantly heavier than mice that were only 1 month old (P < 0.0001; Supporting Information Figure S3A-S3B). BAT mass increased with age in the male mice, but not in the female mice, in a pattern similar to that displayed with body weight (P < 0.01; Figure S3C-S3D). The SVF was analyzed by flow cytometry, and there were no observed changes in B cell or macrophage repertoires in response to aging (Supporting Information Figure S3E-S3F).

Discussion

Our data showed that macrophages, eosinophils, and B cells are present in BAT, cumulatively making up about 75% of the CD45+ immune cell population. Although previous reports have shown that eosinophils (8) and macrophages (9) are present in BAT, to our knowledge, this is the first report of the presence of B cells.
We showed a higher frequency of B cells in mice with obesity than in lean mice, whereas the opposite trend was shown for macrophages and eosinophils. These trends are present in both sexes, but the males do not appear to have a diet-driven effect that is as strong.

Previous studies have assessed macrophages in BAT by flow cytometry with markers such as Cx3Ccr1, F4/80, CD14, CD64, MerTK, and MHCII (12). During the preparation of our manuscript, Wolf et al. beautifully showed that, during development, most BAT macrophages are Cx3Ccr1+MHCII+, but that they progressively form four distinct populations based upon Cx3Ccr1 and MHCII expression (12). Although our data showed a reduction in the percentage of macrophages in BAT in mice with obesity, we did not see changes during aging. The understanding of aging WAT is that it becomes more inflammatory, with a phenotype similar to obese adipose tissue (13). With these studies, however, we cannot rule out phenotypic changes associated with aging, as we did not assess the gene expression patterns of Cx3Ccr1, MHCII levels, or polarization markers. The underlying tone of some work on BAT macrophages has been that they exist in relatively high numbers in BAT and that they play an important role in BAT physiology. However, in our studies, we showed that macrophages make up only 30% of the BAT immune cell population, which is already less than 5% of all live cells. Published studies showing histology for macrophages in BAT have also shown low numbers. Buettner and colleagues reported that Mac-2-positive macrophages exist in only small numbers in BAT (14), and Czech and colleagues did not observe any F4/80+ macrophages in BAT (15). It has also been reported that the inflammatory nature of macrophages in diet-induced obesity is lower in BAT than in WAT, and we in fact detected a reduction in the BAT macrophage percentage in mice with obesity. Furthermore, Dowal et al. showed that the coculture of macrophages with brown adipocytes had little impact on their inflammatory nature (16). The function of BAT macrophages in homeostasis or in pathogenesis is not yet clear. A role for BAT macrophages in catecholamine synthesis and adaptive thermogenesis was suggested by one group (17), but this was recently disputed (14). Certainly, macrophages are important cells for tissue homeostasis; however, given their very low numbers, the lack of changes in gene expression of macrophages cocultured with brown adipocytes, the absence of change with obesity or aging, and the recent publication showing no effect of M2 macrophages on catecholamine synthesis or adaptive thermogenesis (14), the physiological function of BAT macrophages is unclear.

Interestingly, we found that 20% to 30% of leukocytes in BAT are B cells. Although B cells have traditionally been studied for their role in antigen processing and antibody secretion, their immunoregulatory role has surfaced in the context of inflammatory disease. B cell phenotypic plasticity is influenced by both norepinephrine (18) and adenosine (19), which are also known to activate thermogenesis in BAT. Additionally, norepinephrine is released in response to antigen presentation and is necessary for normal expression of immunoglobulin G (20). Further studies are needed to characterize BAT B cells and their role in this tissue.

Because of the paucity of immune cells in BAT, we limited our analyses to macrophages, eosinophils, and B cells. However, future studies could provide a more detailed analysis of their phenotype and reveal other immune populations as well. Despite these limitations, our work adds to the field’s quantification of immune populations in BAT, especially with the addition of B cells.

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