B Lymphocytes in Human Subcutaneous Adipose Crown-Like Structures

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Accumulation of macrophages and T cells within crown-like structures (CLS) in subcutaneous adipose tissue predicts disease severity in obesity-related insulin resistance (OIR). Although rodent data suggest the B cell is an important feature of these lesions, B cells have not been described within the human CLS. In order to identify B cells in the human subcutaneous CLS (sCLS) in obese subjects and determine whether the presence of B cells predict insulin resistance, we examined archived samples of subcutaneous and omental fat from 32 obese men and women and related findings to clinical parameters. Using immunohistochemistry, we identified B (CD19+) and T cells (CD3+) within the sCLS and perivascular space. The presence and density of B cells (B cells per high-power field (pHPF), T cells pHPF, and B cell:T cell (B:T) ratio) were compared with measures of insulin resistance (homeostasis model assessment (HOMA)) and other variables. In 16 of 32 subjects (50%) CD19+ B cells were localized within sCLS and were relatively more numerous than T cells. HOMA was not different between subjects with CD19+ vs. CD19− sCLS (5.5 vs. 5.3, P = 0.88). After controlling for diabetes and glycemia (hemoglobin A1c (HbA1c)), the B:T ratio correlated with current metformin treatment (r = 0.89, P = 0.001). These results indicate that in human OIR, B cells are an integral component of organized inflammation in subcutaneous fat, and defining their role will lead to a better understanding of OIR pathogenesis and potentially impact treatment.

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INTRODUCTION

Obesity-related insulin resistance (OIR) is a highly prevalent metabolic disorder that contributes to increased mortality through multiple diseases, including type 2 diabetes (T2D), cardiovascular disease, and malignancies (1). In addition to high body mass and impaired insulin action, OIR is associated with persistently elevated blood levels of inflammatory cytokines, which are thought to be largely derived from adipose tissue (2).

Many investigators have proposed that visceral adipose tissue (e.g., mesenteric, omental, epididymal) becomes inflamed as a primary event in OIR, a hypothesis initially raised by the presence of adipose-associated lymphoid structures identified in rodent omental fat (3,4). Several studies have now confirmed the presence of organized accumulations of immune cells in human adipose, forming “fat-associated lymphoid clusters” (5) in visceral depots and macrophage-predominant “crown-like structures” (CLS) in both visceral and subcutaneous depots (5,6). In rodents, the progressive accumulation of macrophages into these CLS is associated with insulin resistance (7,8). Our group and others have shown that macrophage infiltration and CLS burden in both subcutaneous and visceral fat predict the severity of insulin resistance in humans (7,9–11), as well as systemic endothelial dysfunction in the peripheral vasculature (6).

Although the CLS has been described as housing macrophages and T lymphocytes to coordinate local inflammation (12–18), the B lymphocyte has not as yet been described as an integral, or “resident,” feature of these lesions despite their described prominence in analogous immunologic structures, such as “milky spots” in rodent mesenteric fat depots (2). The few human studies investigating the presence of adipose tissue lymphocytes have shown rare B cells in mesenteric fat by flow cytometry using an antibody to CD19, a pan B-cell marker (16), or have not included B cell–specific antibodies in CLS immunohistochemistry (14). Plasma cells (large B cells that actively produce antibody) have been identified, but are rare and interspersed in visceral fat (19). Recently, Winer et al. proposed that B cells are pathogenic in OIR, showing that a B-cell null rodent model lacks pathogenic immunoglobulins that contribute to insulin resistance (20,21). Although the CLS has been described as housing macrophages and T lymphocytes to coordinate local inflammation (12–18), the B lymphocyte has not as yet been described as an integral, or “resident,” feature of these lesions despite their described prominence in analogous immunologic structures, such as “milky spots” in rodent mesenteric fat depots (2). The few human studies investigating the presence of adipose tissue lymphocytes have shown rare B cells in mesenteric fat by flow cytometry using an antibody to CD19, a pan B-cell marker (16), or have not included B cell–specific antibodies in CLS immunohistochemistry (14). Plasma cells (large B cells that actively produce antibody) have been identified, but are rare and interspersed in visceral fat (19). Recently, Winer et al. proposed that B cells are pathogenic in OIR, showing that a B-cell null rodent model lacks pathogenic immunoglobulins that contribute to insulin resistance (20,21).
insulin resistance (20). Members of our group and others have identified aberrant expression of pathogen-recognition Toll-like receptor (TLR) 4 and 2 on circulating B cells, which produce copious inflammatory cytokines in inflammatory states (21–23). In contrast to these findings, others have shown a protective role of B lymphocytes in tissue models of atherosclerotic disease (24). In light of this emerging and conflicting data, we sought to identify B cells in or around the human subcutaneous CLS (sCLS) in an obese population and determine whether their presence is associated with specific clinical parameters.

METHODS AND PROCEDURES

Study subjects

As previously described, we enrolled consecutive obese men and women with a BMI ≥30 kg/m² (range 32–78 kg/m²), age ≥18 years, receiving care at the Boston Medical Center Nutrition and Weight Management Center. All subjects gave written, informed consent and the study was approved by the Boston University Medical Center institutional review board. All subjects had subcutaneous adipose tissue collected either via percutaneous needle biopsy or during gastric bypass surgery. The subcutaneous adipose tissue collection methodology has been described previously (6). For the present analysis, we identified 32 subjects with evidence of CLS in adipose tissue (mean 3.6 per high-power field (pHPF), range 1–12) and complete clinical data for this study (see adipose immunohistochemistry below). In a subset of 13 subjects, omental fat was collected in addition to subcutaneous fat during bariatric surgery. Subjects with T2D were included, but those with more advanced diabetes (insulin-requiring) were excluded from analysis.

Adipose tissue flow cytometry

B cells were isolated from adipose tissue (n = 7) by gentle homogenization of minced tissues, rather than by collagenase degradation of the tissue, to avoid contamination of blood cells. A single-cell suspension was obtained by filtering the homogenate over a 70 μm cell strainer. Lymphocytes were purified from the cell suspension by Ficoll-density gradient, washed, and labeled with fluorescently labeled antibodies to CD19 (BD Pharmingen, San Jose, CA) TLR4 (eBioscience, San Diego, CA) and IgM (BD Pharmingen). Cells were analyzed with standard flow cytometric methods using isotype controls (BD Pharmingen) and forward scatter/side scatter settings that separate live cells from dead cells and debris (25).

Adipose tissue immunohistochemistry

Immunohistochemistry was performed in the Department of Anatomic Pathology at Boston Medical Center. Macrophages were identified using cell-specific stains targeted to CD68 (predilute antibodies from DakoCytomation, Carpinteria, CA). As described in a previous study, samples were initially evaluated in a blinded fashion by a pathologist for the presence (+) or absence (−) of macrophage CLS (6). CLS status was first assessed following examination of all fields available per slide at HPF magnification using light microscopy. Each subject-specific adipose sample yielded a mean of 15 ± 7 HPFs for analysis per slide. Subjects were dichotomously categorized as being sCLS+ if distinct adipose tissue macrophage clusters were present in any examined HPF, or sCLS− if clusters were completely absent in all histological fields for a given subject. For this study, we identified a subset of sCLS adipose samples (n = 32 subjects) for additional immunohistochemical (IHC) analysis targeted to B lymphocytes. B cells were identified using cell-specific antibodies to both pan B-cell markers, CD20 and CD19. We also performed IgG and IgM staining on adipose tissue.

T lymphocytes were identified using anti-CD3. Antibody to TLR4 was used to identify presence of this receptor within the CLS and on individual cells. B- and T-cell densities were measured by counting the number of cells per HPF following examination of all fields available per slide at high-power. Cells found within CLS were quantified as total number of cells per HPF that were located within a CLS. The ratio of B-cell density to T-cell density (B:T ratio) was calculated. Cell counts of zero (0) were designated as 0.1 to allow inclusion of samples without T cells present in the ratio analysis.

Anthropometric and metabolic measures

Clinical parameters include blood pressure, heart rate, height, weight, BMI, diabetes status, and waist circumference. Medications were recorded for each subject if they stated they were actively taking the medications as prescribed. Biochemical analyses including homeostasis model assessment (HOMA) as a measure of insulin resistance, hemoglobin A1c (HbA1c) as a measure of chronic glycemia, lipids, and glucose were quantified from blood samples collected in a fasting state using standard methods provided by the Boston Medical Center clinical chemistry laboratories.

Statistical analysis

Data are shown as means ± s.d. unless stated otherwise. Statistical analysis was performed using SAS, version 9.1.3 (SAS Institute, Cary, NC). The primary outcome was to determine whether there was a difference in insulin resistance severity, as measured by HOMA, between subjects with and without B cells present within the sCLS. Variable distributions were compared between subjects with B cell (CD19+) sCLS vs. B cell (CD19−) within the sCLS and total, including areas around the sCLS, using an unpaired t-test with Welch’s correction. Rank analysis of covariance was used to analyze non-normally distributed variables in a multivariate model. Spearman’s correlation was used to test relationships between B:T ratio. P values <0.05 were considered statistically significant.

RESULTS

All subjects (n = 32) had class 3 obesity (BMI ≥40 kg/m²) with an average BMI of 46 kg/m². Approximately 40% of subjects had T2D, defined by having the diagnosis on the clinic problem list or being treated with a diabetes medication. Thirty (13%)% of subjects were taking metformin; 28% were taking an HMG-coA reductase inhibitor (Statin); and 6% were taking a thiazolidinedione. Subjects taking insulin were excluded. Clinical and metabolic parameters of all subjects are displayed in Table 1 according to CD19 (+B cell) status, described below.

Subcutaneous fat

As an initial exploratory step, a small population of CD19+ cells that express TLR4 were identified by flow cytometry (Figure 1) performed on homogenized subcutaneous fat from 12 sCLS’ subjects, validating the presence of B lymphocytes in human subcutaneous fat. In one representative sample, 51% of the CD19+ cells expressed IgM (Figure 1c). Following this, IHC analysis was performed on all 32 samples. The relative densities of T cells and B cells within sCLS were estimated by counting cells on the selected slides. The mean number of sCLS pHPF was 3.55 (range 1–12). Specific staining for CD20 by IHC was negative, an expected result as B-cell activation results in decreased surface levels of CD20 (ref. 26; Figure 2b). Antibody to CD19 was then utilized to identify B cells in tissues. In 16 of 32 subjects (50%) a prominent CD19+ B-cell population was localized within a sCLS (mean= 5 cells pHPF, range 1–13) in
Table 1 Differences in subject characteristics and other lymphocyte patterns in subjects with B cell\(^+\) CLS vs. B cell\(^-\) CLS

| Subject characteristics | sCLS CD19\(^+\) (N = 16) | sCLS CD19\(^-\) (N = 16) | P value |
|-------------------------|--------------------------|--------------------------|---------|
| Age (years)             | 43.69 ± 2.807            | 41.40 ± 3.180            | 0.59    |
| Female (%)              | 11 (68.75)               | 13 (86.67)               | 0.24    |
| Waist circumference (in)| 54.16 ± 2.171            | 50.37 ± 1.520            | 0.16    |
| BMI (kg/m\(^2\))        | 46.14 ± 2.518            | 45.07 ± 1.804            | 0.73    |
| Type 2 diabetes\(^+\) (%)| 7 (43.75)                | 6 (40.00)                | 0.84    |
| LDL (mg/dl)             | 127.3 ± 10.63            | 122.9 ± 10.86            | 0.78    |
| HDL (mg/dl)             | 45.38 ± 2.389            | 49.64 ± 4.348            | 0.40    |
| Glucose (mg/dl)         | 119.4 ± 9.533            | 113.1 ± 11.45            | 0.68    |
| Systolic blood pressure (mm Hg) | 130.1 ± 2.706 | 132.1 ± 3.479 | 0.66 |
| Diastolic blood pressure (mm Hg) | 73.75 ± 3.351 | 74.90 ± 3.332 | 0.81 |
| Hypertension\(^\#\) (%) | 3 (75.00)                | 8 (80.00)                | 0.87    |
| Insulin level (\(\mu\)U/ml) | 19.25 ± 7.565      | 23.50 ± 6.276            | 0.68    |
| Hemoglobin A\(_{1c}\)  (%) | 6.588 ± 0.3305          | 6.980 ± 0.5315           | 0.54    |
| HOME\(_{A1c}\)         | 5.523 ± 0.8504           | 5.325 ± 0.9471           | 0.88    |
| Metformin\(^\#\) (%)   | 4 (25)                   | 0 (0)                    | 0.04    |
| Statin\(^\#\) (%)       | 5 (31)                   | 4 (25)                   | 0.79    |
| TZD\(^\#\)             | 1 (6)                    | 1 (6)                    | 1.00    |
| FMD (%)                 | 8.342 ± 1.290            | 9.166 ± 1.751            | 0.71    |
| hsCRP (mg/l)            | 8.433 ± 3.675            | 7.145 ± 1.516            | 0.76    |

Lymphocyte patterns

| +CD19 perivascular subcutaneous (%)\(^\#\) | 14 (87.50) | 5 (31.25) | <0.01 |
| +CD19 perivascular and omental (%)\(^\#\) | 2 (15.39) | 2 (14.29) | 0.94 |
| +CD3 CLS (%)\(^\#\) | 2 (15.39) | 1 (7.69) | 0.56 |
| +CD3 perivascular (%)\(^\#\) | 1 (7.69) | 1 (7.14) | 0.96 |
| +CD3 any location (%)\(^\#\) | 16 (100.00) | 5 (41.67) | <0.01 |
| +CD3 omental (%)\(^\#\) | 0 (0) | 0 (0) | 1.00 |

\(^{\#}\)Type 2 diabetes was defined by medical history or by fasting glucose >126 mg/dl on two or more occasions; hypertension was defined by medical history or by blood pressure >140/90 mm Hg. \(^{\#}\)Percentage of subjects who reported taking this medication, and verified by medication list in electronic medical record. \(^{\#}\)Percentage of subjects with the designated cell pattern within the sCLS. CD3, T cells; CD19, B cells; sCLS, subcutaneous crown-like structures; FMD, flow-mediated vasodilation, as previously described in ref. (7); HDL, HDL cholesterol; HOMA\(_{A1c}\), homeostasis model assessment of insulin resistance (fasting glucose (mg/dl) × fasting insulin (\(\mu\)U/ml)/405); hsCRP, highly sensitive C-reactive peptide; LDL, LDL cholesterol; Statin, drug class HMG-coA reductase inhibitor; TZD, drug class thiazolidinediones.

The presence of B and T cells and the relationship with clinical parameters

Forty percent of the population had T2D, which was generally well-controlled with a mean HbA\(_{1c}\) of 6.78 ± 1.7%, and diabetes prevalence did not differ between subjects characterized as having CD19\(^+\) vs. CD19\(^-\) within sCLS (Table 1). There was no difference in the primary outcome (HOMA) detected between subjects with sCLS CD19\(^+\) vs. sCLS CD19\(^-\) samples, and this did not depend on adipose depot. However, metformin treatment was more common in the CD19\(^+\) group (P = 0.04). Using unadjusted comparisons, there were no statistically significant differences related to the B-cell density and the collected clinical parameters. The same was true for T cells. However, when the total number of lymphocytes pHFP was in and around the sCLS were considered, differences were identified. After adjusting for diabetes status, T-cell number and glycemic control (by HbA\(_{1c}\)), the number of B cells pHFP was higher in subjects treated with close proximity to macrophages (Figure 2a). Fourteen of the subjects with CD19\(^+\) cells (89%) also had B cells (mean = 3 cells pHFP, range 1–14) surrounding a vessel (indicated by the presence of red blood cells in a typical morphologic structure) within the adipose tissue (Figure 2d). In five subjects, rare T cells (mean = 3 cells pHFP, range 1–4) in sCLS were identified by CD3 positivity (Figure 2c), and these slides also exhibited perivascular T cells (mean = 3 cells pHFP, range 1–5). Overall, among the 32 subjects, based on cell density by IHC, B cells were more prevalent (89%) than T cells (19%) within sCLS and perivascular spaces. The presence of T cells in the CLS was tied to B-cell presence, as all CD19\(^+\) sCLS samples had T cells present in or around the sCLS (Table 1), but not vice versa (P = 0.002). Some individual crown structures appeared to have a relatively higher B-cell density, with nearby lymphatic vessels containing >10 CD19\(^+\) lymphocytes pHFP (Figure 3). In addition, we performed IgG and IgM staining of representative CLS (Figure 4). Overall we identified more lymphocytes staining positive for IgG than IgM in or near CLS, although high background staining in the IgG IHC limited its technical accuracy. High background IgG staining was expected given IgG binding to Fc receptors on both macrophages and lymphocytes. No cells had morphologic characteristics consistent with plasma cells (basophilic cytoplasm, eccentric nucleus, characteristic perinuclear, and nuclear features). All identified sCLS stained positive for TLR4, and cells with lymphocyte features commonly stained positive for TLR4.

Omental fat

Thirteen of the 32 subjects provided omental adipose samples archived for IHC. Each of these samples had at least three CLS pHFP (mean = 6 CLS pHFP, range 3–13). Only four of these samples (28%) had CD19\(^+\) cells (B cells) within CLS (mean = 9 cells pHFP, range 3–23) with two including perivascular B cells, whereas CD3\(^+\) cells (T cells) were not present in any of the omental CLS nor perivascular area. There were no clinical differences between subjects with omental B cells vs. those without omental B cells (data not shown).
metformin than those who were not (12.6 vs. 0.22, \( P = 0.002 \)), and diabetes status was insignificant in this model (\( P = 0.9242 \)). In a similar model but adjusting for B cells pHPF, the number of T cells pHPF was also higher in metformin-exposed subjects (6.75 vs. 0.25, \( P = 0.001 \)), but this may have been due the presence of diabetes (\( P = 0.04 \)). When B-cell density was considered in relation to T-cell density, expressed as the ratio of B cells to T cells pHPF, an increased B-cell prevalence correlated with metformin therapy after adjustment for diabetes and Hba1c (\( r = 0.89, P = 0.001 \)). We compared this ratio across groups according to diabetes status and metformin exposure, and we found that the B:T cell ratio in an around the sCLS was significantly different in subjects with diabetes treated with metformin vs. subjects without diabetes and untreated with metformin (median = 200, range 110 vs. 2.8, range 90, \( P = 0.04 \); Figure 5).

**Discussion**

Accumulation of macrophages and T cells in adipose tissue present in the form of CLS in subcutaneous and visceral depots is a common finding in obesity and predicts the severity of both insulin resistance and endothelial dysfunction in humans (6). In rodent visceral fat, B lymphocyte migration to adipose tissue heralds immune cell infiltration before the onset of insulin resistance (13–14,16), yet recent literature supports either a beneficial (27,28) or maladaptive (20,29) nature of adipose B cells. Here, we present the novel finding in humans that B cells (CD19+) are present in relatively higher numbers than T cells (CD3+) within sCLS and that B-cell predominance (B:T cells) relates to exposure to the drug metformin, a highly effective treatment for OIR (30). Although our data support the hypothesis formed by rodent literature that the B cell in adipose tissue plays an active role in human adipose inflammation, it also poses more questions about whether this role is pathogenic or protective.

Our prior data associating the sCLS with abnormal metabolic and vascular phenotypes may on the surface implicate...
the sCLS B cell as pathogenic. However, the current literature is conflicted regarding the function of tissue-specific B cells in metabolic disease. As noted above, Winer et al. have found that B cells accumulate in adipose tissue of diet-induced obese mice and facilitate the activation of proinflammatory macrophages through production of specific, possibly pathogenic, IgG antibodies (20). This contrasts with in vitro data showing that incubation of adipocytes with the Fc moiety of IgG reduced expression of interleukin-1β and interleukin-6 (19), and with the finding that B cells identified in pre-atherosclerotic lesions may protect against atherosclerosis in rodents (24), possibly explained by the production of antibody reactive to modified low-density lipoproteins (27).

The complexity of the B cell could partially explain this conflict. Based on recent reviews on the interplay between immune cell types in adipose tissue and recent work reflecting this hypothesis (2,13,20,29). However, B cells in humans, particularly in chronic inflammatory diseases, do not necessarily recapitulate murine B-cell biology (21,31,32). In humans, circulating B cells express functional TLR4 and TLR2 in inflammatory diseases, whereas B-cell TLR expression in healthy controls is nearly absent (21).

By contrast, mice B-cell TLR responsiveness is constitutive in the healthy state. Moreover, we found that TLR ligands produce disease-specific responses from B cells, which can be either pro- or anti-inflammatory (32). Adipose lymphocytes express TLR4 as well (data not shown), suggesting that the B cell may be one of the cell types found in adipose tissue to affect downstream nuclear factor-κB activation and upregulated cytokine release via TLR activation (or suppression; ref. 33). This potential “alternative” function of B cells, via TLRs (either pro- or anti-inflammatory), has yet to be explored in adipose tissue. Although B-cell function in human adipose remains unknown, based on our finding and that in rodents, the B cell likely contributes to the complex immune environment described in adipose tissue in response to pathologic stimuli and tissue “remodeling” (8,34,35).

The unexpected finding that B cells are present in larger numbers than T cells could reflect the timing of adipose infiltration in our patient population, which is difficult, if not impossible, to control in human models of obesity. Duffaut first showed

**Figure 3** CD19+ B-cell variability in sCLS. (a) A representative sCLS double stained with CD68 and CD19. A single CD19+ lymphocyte (arrow) is identified among macrophages within one section of an sCLS (note: subject not on metformin treatment). Nuclei are stained with hematoxylin. (b) Shown are >10 CD19+ lymphocytes (bottom left arrows) within a lymphatic vessel near a portion of an sCLS containing two CD19+ lymphocytes (top right arrows) (note: subject on metformin treatment). sCLS, subcutaneous crown-like structure.

**Figure 4** IgG and IgM immunohistochemistry of sCLS. (a) Two IgG-positive lymphocytes (arrow) are present within an sCLS. There is significant background staining. (b) A single IgM-positive lymphocyte (arrow) is present within an sCLS. sCLS, subcutaneous crown-like structure.
that in mice fed a high-fat diet, B-cell accumulation occurred as early as 3 weeks, and 9 weeks before T-cell infiltration, before the onset of insulin resistance (16). The authors postulate that perhaps the high-fat diet in mice induces an early cell-mediated immune reaction (B cells) in adipose tissue that is followed by maladaptive changes (T cells). Because nearly all of our subjects were severely obese, reflecting long-term excess adiposity and overnutrition, our finding of a common high B:T ratio phenotype may depict a later stage in the natural history of adipose inflammation. It may be that B-cell infiltration is earliest, but is persistent over time, making the high B:T sCLS phenotype as a manifestation of chronic overnutrition. Overall, however, it is premature to hypothesize whether chronic B-cell presence is beneficial or maladaptive, and if that changes with time.

There were fewer lymphocytes overall in omental tissue compared with subcutaneous adipose, which although of interest, is not surprising given flow cytometry performed by others on mesenteric fat tissue has yielded very small B-cell fractions (16). This is of interest, as the study of adipose inflammation thus far has been largely dedicated to mesenteric depots, which tend to have more CLS and expression of inflammatory cytokines. It is possible that lymphocytes, specifically B lymphocytes, traffic preferentially to subcutaneous depots under certain conditions, and based on our preliminary findings, this may be a clinically important phenomenon and warrants further study.

Knowing at the outset that the adipose B-cell population was very small (<4% cells by our fluorescence-activated cell sorting (FACS)), we sought to identify a morphologic correlate with CLS. We acknowledge that the IHC technique does not serve to quantify cells due mostly to the fact that cells are viewed on slides in one dimension, and in one section of an adipose CLS. For this reason, we have reported cell density as others have done (19,36), and emphasized the relative presence of B cells when compared with T cells. Flow cytometric techniques (FACS) may be more accurate for cell quantification, which overcomes the obvious limitations of IHC and is also able to more specifically identify and characterize cells that express more than one identifying surface marker by using several antibodies simultaneously. However, as illustrated well by B cells in the sCLS, FACS cannot localize cells within a tissue bed, so potentially integral cells in relatively low numbers may be unquantifiable (15). In addition, because lymphocytes are usually isolated from the stromal–vascular fraction of adipose tissue for FACS, the final isolate is likely to include cells originating from the bloodstream due to the degradation of the endothelium. Thus, blood cells can “dilute out” cells originating from adipose, causing FACS to overestimate adipose lymphocyte numbers if care is not taken to isolate only tissue cells.

The independent relationship of sCLS lymphocyte prevalence and the B:T ratio with metformin therapy is preliminary given our small patient sample and cross-sectional study design, yet it is consistent with emerging data on the immunomodulatory effects of this drug. Metformin is the optimal first-line treatment for T2D (30) as its traditional mechanism of action is lowering blood glucose by impairing hepatic glucose secretion and improving insulin sensitivity in peripheral tissues (37). In overweight and obese cohorts with T2D, metformin therapy is associated with lower mortality (38). Metformin is also known to have pleotropic effects, including effects on inflammation and cellular apoptosis, thought largely mediated by modulation of adenosine monophosphate-kinase activity (39). Relevant to our findings, metformin has been found to inhibit the proliferation of T cells, potentially through its effect on fatty acid oxidation and reduction in oxidative stress (40). Whether metformin is partly responsible for an increased ratio of B cells to T cells in adipose tissue, or whether it reflects unmeasured variable(s) in our cohort, requires further investigation.

Despite its novelty, the design of this study limits the ability to draw conclusions about pathophysiology or B-cell function. We did identify a population of IgG+ B cells in the CLS, which may represent the pathologic B cells described similarly in rodents (20), although this is inconclusive given technical limitations and lack of functional data. We additionally did not identify any plasma cells, which actively secrete antibody and are rare in subcutaneous adipose (19). The sample size is likely too small to detect some differences that may be present in a larger population. Moreover, our group was relatively homogenous (all CLS) without a lean control group, which may have limited our ability to find relationships. For example, the HOMA and BMI did not vary (nearly all patients had BMI >40 and the standard deviation was ~2), making it difficult to evaluate these variables as relevant parameters.

In conclusion, we found that B cells are prominent in subcutaneous fat CLS in severely obese humans, and relative predominance appears to vary among individuals. The meaning of this finding is unclear, but a relative predominance of identified B cells over T cells pHPF correlated with metformin therapy, a drug that improves insulin resistance and may have an effect on immune function. It has only recently been accepted that the development of obesity has immunologic consequences, or origins, both systemically and in adipose tissue. What remains poorly understood is how the natural history of the adipose tissue sCLS relates to disease mechanism or severity of obesity.
Our findings provide a clue that B cells are not only integral in the sCLS natural history, but also that immunomodulation through B cells may be a target of OIR treatment. Although its relationship with metformin needs to be confirmed, the B cell is sufficiently complex that careful study of lymphocytes isolated from blood, CLS and lymphoid tissues will be required to link this cell to metabolic and cardiovascular disease in humans.

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DISCLOSURE
The authors declared no conflict of interest.

See the online ICMJE Conflict of Interest Forms for this article.

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