Interplay Between Human Adipocytes and T Lymphocytes in Obesity

CCL20 as an Adipochemokine and T Lymphocytes as Lipogenic Modulators

Carine Duffaut, Alexia Zakaroff-Girard, Virginie Bourlier, Pauline Decaunes, Marie Maumus, Patrick Chiotasso, Coralie Sengenès, Max Lafontan, Jean Galitzky, Anne Bouloumié

Objective—Adipose tissue (AT) plays a major role in the low-grade inflammatory state associated with obesity. The aim of the present study was to characterize the human AT lymphocytes (ATLs) and to analyze their interactions with adipocytes.

Methods and Results—Human ATL subsets were characterized by flow cytometry in subcutaneous ATs from 92 individuals with body mass index (BMI) ranging from 19 to 43 kg/m² and in paired biopsies of subcutaneous and visceral AT from 45 class II/III obese patients. CD3⁺ ATLs were composed of effector and memory CD4⁺ helper and CD8⁺ cytotoxic T cells. The number of ATLs correlated positively with BMI and was higher in visceral than subcutaneous AT. Mature adipocytes stimulated the migration of ATLs and released the chemokine CCL20, the receptor of which (CCR6) was expressed in ATLs. The expression of adipocyte CCL20 was positively correlated with BMI and increased in visceral compared to subcutaneous adipocytes. ATLs expressed inflammatory markers and released interferon gamma (IFNγ). Progenitor and adipocyte treatment with ATL-conditioned media reduced the insulin-mediated upregulation of lipogenic enzymes, an effect involving IFNγ.

Conclusions—Therefore, crosstalk occurs between adipocytes and lymphocytes within human AT involving T cell chemotraction by adipocytes and modulation of lipogenesis by ATLs. (Arterioscler Thromb Vasc Biol. 2009;29:00-00.)

Key Words: inflammation • visceral fat • macrophages • T lymphocyte subsets • CCR6

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human ATLs and mature adipocytes was studied in terms of adipocyte-derived T cell chemoattractant and ATL-mediated metabolic effects.

Materials and Methods

Materials

Chemicals were purchased from Sigma. Collagenase NB4 was purchased from Serva. Selection kits for CD34⁺, CD14⁺, and CD3⁺ cells were purchased from StemCell Technologies, and magnetic microbeads coupled with anti-CD31 antibodies were purchased from Dynal Biotech (Invitrogen). Culture media were from purchased Promocell. Antibodies for flow cytometry analysis were purchased from BD Biosciences.

Isolation of the Stroma-Vascular Fraction (SVF) and Mature Adipocytes

Human subcutaneous adipose tissue (AT) was obtained from healthy women undergoing elective procedures of fat removal for esthetic purposes. Their body mass index (BMI) ranged from 19 to 43 kg/m² (n = 92, mean BMI = 27.2 ± 0.6 kg/m², mean age = 42.6 ± 1.2 years). In addition, paired human omental and subcutaneous tissues were obtained from a group of healthy patients undergoing bariatric surgery (n = 45, 41 women/4 men, mean BMI = 43 ± 1 kg/m², mean age = 42 ± 1 years). The protocols of fat collection were approved by the Institutional Research Board of INSERM and Toulouse University Hospital Ethics committee. The cells from the stroma-vascular fraction (SVF) and mature adipocytes from human AT were obtained after collagenase digestion as previously described. For lipolysis measurement, mature adipocytes obtained after collagenase digestion of subcutaneous AT were plated in fibrin gels as previously described. After gel polymerization, basal medium, ATCM, or ATL-CM were added. After 24-hour incubation pharmacological agents (0.1 μmol/L isoproterenol [ISO] or 10 μmol/L noradrenaline [NA]) were added to the media for 90 minutes at 37°C. At the end of the incubation period, aliquots of the media were taken for glycerol and FFA determination (Sigma-Aldrich and Wako Unipath, respectively). Total lipid content was determined gravimetrically after solvent extraction. For gene expression analysis, mature adipocytes in fibrin gels were treated with basal medium in the presence or absence of 50 ng/mL human recombinant IFN-γ or with ATL-CM in the presence or absence of 2 μg/mL neutralizing antihuman IFN-γ antibody or its isotype control (BD Biosciences) or with ATCM. After overnight incubation, 0.1 μmol/L insulin was or not added for 24 hours. Media was then removed and adipocytes were lysed in Qiazol (Qiagen) (v/v) for mRNA extraction.

Isolation of Human Adipose Tissue Lymphocytes

The cells of the human AT-SVF were isolated using an immunoselection/depletion protocol as previously described with modifications to isolate the CD3⁺ ATLs. Briefly, after depletion of the progenitor cells (CD34⁺/CD31⁻) and AT macrophages (ATMs; CD34⁺/CD14⁺) from the AT-SVF, the CD3⁺/CD14⁻ cells were incubated with a CD3⁺ Stem Cell System positive cocktail (Grenoble; 15 minutes at room temperature). After the addition of magnetic selection beads (Myltenyi Biotec), and the removal of platelets by successive centrifugations, macrophages were depleted using magnetic CD14⁺ beads (Miltenyi Biotec), and the CD3⁺ cells were selected on the CD14 negative cell fraction with the same protocol described for the AT CD3⁺ cells. The blood CD3⁺ cells were then lysed (Qiagen) and stored at −20°C until mRNA extraction.

Flow Cytometry Analysis

Human SVF cells, ATLs, or blood cells (100 000 cells) were incubated with FITC- (CD4, CD8, CD25, CD62L) PerCP-Cy5.5 (CD45), PE-Cy7 (CD45-RO), PE- (CD3, CD14, CD45-RA), APC- (CD8, CD19, CD36) conjugated antibodies or respective isotype controls. For IFN-γ intracellular staining, ATLs were incubated in the presence or absence of brefeldin-A (BD Biosciences) and stained with FITC-CD4, PE-Cy7-IFN-γ and APC-CD8 antibodies or respective isotype controls. Analyses were performed using a FACSCalibur flow cytometer and CellQuest Pro software (BD Biosciences).

Confocal Fluorescence Microscopy Analyses

After 1-hour fixation in 4% paraformaldehyde and extensive washing steps in PBS, pieces of human subcutaneous AT was incubated for 30 minutes at room temperature in PBS/0.2% BSA followed by a 2-hour incubation with the rabbit polyclonal antibody directed against human CD3 (Dako) (1:20). After washing (PBS/0.2% Tween and PBS) and 1-hour incubation with anti-rabbit antibody coupled to AlexaFluor488 (Invitrogen) (1:200), the tissues were placed on slides and a 3-dimensional reconstruction was performed using a Zeiss LSM 510 META confocal microscope.

Lipolysis and Modulation of Lipogenic Enzyme Expression in Mature Adipocytes and Progenitor Cells

For lipolysis measurement, mature adipocytes obtained after collagenase digestion of subcutaneous AT were plated in fibrin gels as previously described. After gel polymerization, basal medium, ATCM, or ATL-CM were added. After 24-hour incubation pharmacological agents (0.1 μmol/L isoproterenol [ISO] or 10 μmol/L noradrenaline [NA]) were added to the media for 90 minutes at 37°C. At the end of the incubation period, aliquots of the media were taken for glycerol and FFA determination (Sigma-Aldrich and Wako Unipath, respectively). Total lipid content was determined gravimetrically after solvent extraction. For gene expression analysis, mature adipocytes in fibrin gels were treated with basal medium in the presence or absence of 50 ng/mL human recombinant IFN-γ or with ATL-CM in the presence or absence of 2 μg/mL neutralizing antihuman IFN-γ antibody or its isotype control (BD Biosciences) or with ATCM. After overnight incubation, 0.1 μmol/L insulin was or not added for 24 hours. Media was then removed and adipocytes were lysed in Qiazol (Qiagen) (v/v) for mRNA extraction.

Migration Assays

Human ATL migration was assessed on 3 μm-pore HTS FluoroBlock inserts (Falcon). After labeling for 60 minutes with 10 μmol/L Calcein AM, ATLs (20 000 cells in 300 μL ECBM/0.1% BSA) were incubated in the upper side of the insert and 800 μL mature adipocyte-conditioned media, or conditioned media without cells (control) was added to the companion plate under the insert. ATL migration was evaluated by counting the number of labeled lymphocytes attached to the lower surface of the wells after 4 hours.

Cytokine Detection

The RayBio Human Cytokine antibody Array V (RayBiotech Inc, Cliniscience) provides antibody array membranes to detect expression of 79 cytokines. The experiment was performed according to the manufacturer’s instructions. Briefly, 1 mL of conditioned medium by human mature adipocytes originating (n = 6) maintained in fibrin gels for 24 hours was added to the antibody-coated membrane and incubated on a plate shaker at 4°C overnight. After incubation with biotinylated antibodies and labeled streptavidin, the signal was detected from the membrane by chemiluminescence using Chemiatrack 3000 (Vilber LourmatFrance). Data analysis was performed using Bio1D software (Vilber Lourmat). Positive controls were used to normalize the results from different membranes.
RNA Extraction and Real-Time PCR

Total RNA was extracted from ATLs, adipocytes, and progenitor cells using the RNeasy kit (Qiagen). The RNA concentration was measured either by a spectrophotometer (nanodrop® ND-1000, Labtech) or by fluorometric assay (Ribogreen, Invitrogen). The RNA was reverse-transcribed using the “Superscript II” kit (Invitrogen). Reverse transcription was also performed without the superscript enzyme on RNA samples to ensure the absence of contaminating genomic DNA. Primers were from Applied Biosystems (Hs00174128_m1, Hs00174143_m1, Hs00174122_m1, Hs00174131_m1, Hs00169473_m1, Hs00381459_m1, Hs00176122_m1, Hs00176125_m1, Hs00176121_m1, Hs00174114_m1, Hs00174122_m1, Hs00176131_m1, Hs00169473_m1, Hs00381459_m1, Hs00188012_m1, Hs00176125_m1, Hs00176107_m1, respectively). The amplification reaction was done in duplicate on 15 ng of the cDNA samples in a final volume of 20 μL in 96-well reaction plates (Applied Biosystems) in a GeneAmp 7500 detection system (Applied Biosystems). All reactions were performed under the same conditions: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, 40°C for 1 minute. Results were analyzed with the GeneAmp 7500 software and all the values were normalized to the levels of 18S rRNA.

Statistical Analysis

Values are given as mean±SEM for (n) separate experiments. Correlations were performed using a Spearman rank correlation. Comparisons between groups were analyzed by ANOVA for experiments with more than 2 subgroups followed by post hoc tests and the nonparametric Mann–Whitney test or using the Student t test, when appropriate (Prism 4, GraphPad Software, USA). Differences were considered significant when P<0.05.

Results

Characterization of the Lymphocyte Subsets in the Human Subcutaneous Adipose Tissue

Flow cytometry analysis performed on the stroma-vascular fraction (SVF) obtained after collagenase digestion of human subcutaneous adipose tissue (AT) (n=92, mean BMI=27.2±0.6 kg/m²) showed 2 main CD45+ leukocyte populations. A predominant CD14+ macrophage population (19±1% of total SVF cells) was identified. The lymphocyte population was mainly composed of CD3+ T lymphocytes (6±0.5% of total SVF cells), a minor CD56+ NK cell population (1.5±0.1% of total SVF cells), and few CD19+ B-lymphocytes (Figure 1A). Three-dimensional reconstruction of confocal fluorescence microscopy analyses using anti-CD3 antibody revealed the presence of CD3+ cells organized in clusters around adipocytes (Figure 1B). Some individual CD3+ cells were identified in the stroma and few in the capillaries (data not shown). Flow cytometry analyses performed under the same conditions on the AT-SVF and peripheral blood showed that the CD3+ cells identified in the AT exhibited a lower size but a higher granularity than the blood CD3+ cells (supplemental Figure IA). Moreover, the expressions of the homing receptor CD62L and the naïve T cells CD45RA markers were markedly lower in the AT SVF CD3+ cells compared to peripheral blood (supplemental Figure IB). To analyze the subsets of human AT CD3+ T lymphocytes, CD3+ cells were immunoselected from freshly harvested SVF after depletion of the CD34 (progenitor and capillary endothelial cells) and CD14 (macrophages) positive cells. Helper CD4+ and cytotoxic CD8+ T cells were the major CD3+ T-lymphocyte subsets (Figure 1C). A minor subset of NKT cells (CD3+/CD56+) and few, if any, regulatory CD25+ and immature γδ T lymphocytes were detected (data not shown). The helper CD4+ T cells were mainly memory (CD45RO+) as well as effector T cells (CD45RO-/RA-), whereas the CD8+ T cells were mainly effector cells (Figure 1D).

Influence of the Degree of Adiposity and Fat Anatomic Location on the Number of Human Adipose Tissue T Lymphocytes

The number of AT lymphocytes (ATLs) determined by flow cytometry analysis of the SVF of the subcutaneous AT (n=92) increased with the degree of adiposity. Indeed, a statistically significant correlation was detected between the number of AT CD3+, CD4+, as well as CD8+ cells and the BMI of the individuals (data not shown). The patients were grouped according to BMI, and obese patients (BMI ≥30) exhibited a statistically significant higher numbers of AT CD3+, CD4+, and CD8+ T lymphocytes than lean patients (BMI ≤25;
Figure 2A through 2C. The number of CD56+/H11001-NK cells within the subcutaneous AT remained constant whatever the BMI (data not shown). The analysis of the SVFs of paired biopsies of subcutaneous and visceral ATs from class II/III obese patients (n/H11005=45, mean BMI=43.1 kg/m²) showed that the number of CD14+/H11001 cells (adipose tissue macrophages, ATMs) was slightly higher in visceral compared with subcutaneous AT (1.2-fold, P<0.05, Figure 3A). The number of AT CD3+/H11001 cells showed a marked increase in the visceral depot (3-fold, P<0.001, Figure 3A), because of a clear enhanced effector CD8+/H11001 cell number and a moderate increase in memory CD4+/H11001 cell numbers (Figure 3B through 3D).

Adipocyte Expression of the T Cell Chemoattractant CCL20

To assess whether adipocytes attracted ATLs, we studied the effects of the adipocyte-derived factors contained in the media from human mature adipocytes of subcutaneous AT included in fibrin gel for 24 hours on the migration of ATLs. As depicted in Figure 4A, ATLs exhibited a marked migratory responsiveness to human mature conditioned media (5.5-fold increase, P<0.01). The human mature conditioned media were analyzed on protein arrays allowing the simultaneous detection of 79 proteins (Figure 4B). As expected, strong positive signals were detected for the adipokine leptin and the cytokines and growth factors IL-6, vascular endothelial growth factor (VEGF), tumor growth factor beta 2 (TGFβ2), oncostatin M (OSM), as well as tissue inhibitor of matrix metalloproteinase 1 (TIMP-1). Concerning chemokines, positive signals were obtained for chemokines active on neutrophils (CXCL1, CXCL2, and CXCL8), for MCP-1/CCL2, RANTES/CCL5, and MCP-4/CCL13. Interestingly, the lymphocyte chemoattractant chemokine CCL20 was also identified. Notably, no signals above the baseline were detected for CCL1, CCL8, CCL17, CCL18, CXCL5, CXCL6, CXCL9, CXCL10, CXCL12, and CXCL13. To further characterize the adipocyte-derived CCL20, real-time RT-PCR analyses were performed on mature adipocytes from subcutaneous AT (n=18 women, age=44±2 years, BMI=27±1 kg/m²) and from subcutaneous and visceral AT from class II/III obese women (n=12, age=41±3 years, BMI=44.7±1.3 kg/m²). The levels of CCL20 transcripts in adipocytes from subcutaneous AT correlated with the adiposity degree assessed by the BMI (Spearman r=0.5, P<0.05) but not with age. Adipocytes originated from obese individuals exhibited 7-fold more CCL20 transcripts than adipocytes from lean individuals (Figure 4C). The effects of conditioned media (CM) originating from native immunoselected human ATLs and ATMs on adipocyte CCL20 expression were studied. Although treatment of adipocytes with ATL-CM or IFNγ did not modify CCL20 expression, ATM-CM induced a marked increase in the levels of CCL20 transcript (3.8-fold increase, P<0.05, Figure 4D). In class II/III obese

Figure 2. Influence of the degree of adiposity on the number of lymphocytes in the subcutaneous adipose tissue. The number of AT lymphocytes (normalized per gram of AT) was determined in the subcutaneous AT-SVF by flow cytometry analyses using (A) CD3-PE, (B) CD3-PE/CD4/FITC, (C) CD3-PE/CD8-APC antibodies in lean (open bars, n=41), overweight (hatched bars, n=26), and obese (filled bars, n=25) patients. *P<0.05, **P<0.01 obese vs lean.

Figure 3. Influence of fat mass location on the number of lymphocytes in class II/III patients. Multicolor FACS analyses were performed on the SVF obtained from paired biopsies from subcutaneous (open bars) and visceral (filled bars) AT of class II/III obese patients using CD14-PE, CD3-PE, CD4-PE/CD45RO-FITC, CD8-APC, and CD45RA-PE antibodies. A, Number of ATMs (CD14+) and ATLs (CD3+; n=45). B, Numbers of CD4+ and CD8+ ATLs (n=45; C and D) Number of memory (CD45RO−), naïve (CD45RA+), and effector (CD45RO-/RA-) CD4+ and CD8+ ATLs (n=19). Results are means±SEM. *P<0.05, **P<0.01 visceral vs subcutaneous.
exhibited a marked higher transcript level for IFN-γ (Figure 4A) and visceral AT of obese patients was too low. ATLs of cells recovered from the paired biopsies of subcutaneous AT of 6 lean and 6 obese patients. Results are mean ± SEM of n = 6. B, Representative photomicrograph of a cytokine antibody array membrane performed on conditioned media from pooled human mature adipocytes (n = 6). C, mRNA levels of CCL20 determined by real-time RT-PCR analyses on mature adipocytes from subcutaneous AT of 6 lean and 6 obese patients. Results are mean ± SEM. **P < 0.01. D, mRNA levels of CCL20 determined by real-time RT-PCR analyses in adipocytes (n = 4) treated or not (Control) with ATL-CM (n = 4), 50 ng/mL IFN-γ, or ATM-CM (n = 12). Results are expressed as percentage of control and are mean ± SEM. **P < 0.01 vs control. E, mRNA levels of CCL20 determined by real-time RT-PCR analyses on mature adipocytes isolated from paired biopsies of subcutaneous and visceral AT of class I/III obese patients (n = 12). **P < 0.01.

women, the expression of CCL20 in visceral adipocytes was higher than in the paired subcutaneous adipocytes (5-fold increase, P < 0.05, Figure 4E).

Phenotype of the Human Adipose Tissue Lymphocytes

Real-time RT-PCR analysis was performed on the native immunoselected ATLs and compared to immunoselected CD3+ cells from peripheral blood from different donors. The study was restricted to subcutaneous AT because the amount of cells recovered from the paired biopsies of subcutaneous and visceral AT of obese patients was too low. ATLs exhibited a marked higher transcript level for IFN-γ, TNFα, RANTES, IL-2, as well as perforin-1 (PRF-1) when compared with blood T lymphocytes (Table). The expression of IL-4 was not detected in ATLs. Interestingly, the expression of the CCL20 receptor CCR6 as well as the expression of the leptin receptor (Leptin-R) were markedly upregulated in human ATLs. The production of IFN-γ by ATLs was further analyzed by flow cytometry (Figure 5A). Both CD4+ and CD8+ cells produced IFN-γ (data not shown).

Specific Paracrine Effects of ATLs on Human Mature Adipocytes

The effects of conditioned media (CM) originating from native immunoselected human ATLs and ATMs on adipocyte metabolism were studied. Pretreatment of human mature adipocytes with ATL-CM or ATM-CM did not alter either basal lipolysis or the lipolytic activity stimulated with isoproterenol (ISO) or noradrenaline (NA), as assessed by the release of glycerol (data not shown) and free fatty acids (Figure 5B). Pretreatment of human mature adipocytes with AT-LCM altered the insulin responsiveness of the lipogenic enzymes fatty acid synthase (FAS) and lipoprotein lipase (LPL). Indeed, although ATL- and ATM-CMs did not alter the basal expression of FAS and LPL in human mature adipocytes (data not shown), ATL-CMs strongly inhibited the insulin-mediated upregulation of FAS and LPL transcript levels (Figure 5C and 5D). Furthermore, it was associated with the downregulation of a key component of the insulin-dependent intracellular pathway, the phosphoinositide-3-kinase, regulatory subunit 1 alpha (PIK3R1; Figure 5E). Such an insulin-mediated inhibitory effect on FAS, LPL, and PIK3R1 expression was reversed by a neutralizing IFN-γ antibody and mimicked by 50 ng/mL IFN-γ treatment. ATM-CM did not modulate the insulin responsiveness of the adipocyte under the same conditions (Figure 5D and 5E). Human AT progenitor cells (CD34+CD31+ cells) were treated with human ATL-conditioned media under adipogenic culture conditions. Human ATL-conditioned media led to a marked decrease of the expression of FAS and LPL that was associated with a lower triglyceride content (Figure 5F).

Discussion

The present study demonstrates that, in humans, the degree of adiposity is positively correlated with the accumulation of adipose tissue lymphocytes (ATLs). Flow cytometry analyses of the subcutaneous AT-SVF showed the presence of CD3+ T lymphocytes but few CD19+ B lymphocytes and innate lymphocytes (NK, NKT, and γδT-cells). These results clearly show that human AT and murine AT are different in terms of relative proportion of T lymphocyte subsets within the SVF,8,16 Among the T lymphocytes, CD4+ and CD8+ cells were characterized as memory (CD45RO+ ) and effector (CD45RO−/RA- ) cells. Compared to the blood cells, few naive CD45RA+ as well as CD62L T cells were identified in the human adipose tissue. Moreover, the mean size of the tissue lymphocytes was lower than the blood CD3+ cells,

Table. Gene Expression in Blood and Adipose Tissue T Cells

<table>
<thead>
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<th>Gene</th>
<th>Blood CD3+ Cells</th>
<th>Adipose CD3+ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>0.1±0.02</td>
<td>2.3±0.4*</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.2±0.06</td>
<td>8.1±1.3*</td>
</tr>
<tr>
<td>CCL5</td>
<td>12±2</td>
<td>47±6*</td>
</tr>
<tr>
<td>CCR6</td>
<td>5×10^{-3}±1×10^{-3}</td>
<td>47×10^{-3}±6×10^{-3}**</td>
</tr>
<tr>
<td>Leptin-R</td>
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<td>73×10^{-3}±10×10^{-3}**</td>
</tr>
<tr>
<td>IL-2</td>
<td>1×10^{-3}±0.2×10^{-3}</td>
<td>8×10^{-3}±0.3×10^{-3}**</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.6×10^{-5}±0.7×10^{-5}</td>
<td>Nondetectable</td>
</tr>
</tbody>
</table>

Gene expression was determined by real-time RT-PCR. Results were normalized to the levels of 18S rRNA and expressed as 2ΔΔCT×10 000, blood CD3+ cells (n = 5); adipose CD3+ cells (n = 30 for IFN-γ, TNFα, CCL5, PRF-1, n = 18 for CCR6 and Leptin-R, n = 7 for IL-2 and IL-4). Results are mean ± SEM. *P < 0.05, **P < 0.01.
whereas the higher granularity of the ATLs compared to the blood suggested distinct activation states. The direct comparison in the expression of several activation markers in the immunoselected blood and AT CD3+ cells confirmed that human ATLs exhibited higher expression of inflammatory markers. Taken together, the low levels of B cells and of CD62L and naïve T cells together with the high proportion of effector T cells demonstrate that the human ATLs exhibit a specific and distinct profile than the one found in peripheral blood. ATLs are therefore mostly infiltrated or resident cells. This was confirmed by confocal analyses that revealed that ATLs were localized in the stroma and more specifically surrounding some adipocytes. However, we cannot rule out the presence of a minor contamination from blood. Increased adiposity, assessed by BMI, was associated with a specific accumulation of CD4+ as well as CD8+ ATLs in subcutaneous AT, whereas the number of NK cells was not modulated. In addition to an adiposity-dependent increase of ATL numbers, an AT location-dependent pattern of ATLs was observed. Indeed, in paired biopsies from subcutaneous and visceral AT of class II/III obese patients, a marked increase in ATLs together with a modest increase in the number of adipose tissue macrophages (ATMs) was observed in visceral ATs. In mice, the AT infiltration of T lymphocytes during a high-fat diet has been speculated to involve several chemokines such as stromal-derived factor 1 alpha/CXCL12 and RANTES/CCL5. In the present study, ATLs exhibited an enhanced migration in response to adipocyte-conditioned media. Protein arrays performed on conditioned media from human native mature adipocytes showed no adipocyte production of CXCL12. Both CCL5 and CCL20 were detected in the adipocyte conditioned media. Because CCL20 release by adipocytes has not been reported previously, we further analyzed the adipocyte expression of CCL20. Mature adipocytes from subcutaneous AT of obese individuals expressed higher levels of CCL20 than adipocytes from lean individuals. In class II/III obese patients, adipocytes from visceral AT expressed higher levels of CCL20 than subcutaneous adipocytes. Moreover, ATLs expressed the CCL20 receptor, CCR6. The ligand-receptor pair CCL20-CCR6 has been described to be responsible for the chemotraction of effector/memory T-cells in skin and mucosal surfaces under homeostatic and inflammatory conditions, as well as in pathology, including cancer and rheumatoid arthritis. The present results suggest that the adipocyte CCL20 may also play a major role in the accumulation of T lymphocytes within the AT. Interestingly, CCL20 expression in adipocytes was increased in the presence of soluble factors originated from human ATMs, suggesting that ATMs may contribute via the stimulation of CCL20 expression in adipocytes to the accumulation of ATLs. Freshly immunoselected ATLs exhibited a proinflammatory profile of activation, with clear expression of TNFα, IFNγ, and RANTES, whereas the expression of IL-4 (the Th2-type cytokine) was not detected. The mechanisms involved in the promotion of the ATL phenotype remain to be established. However, because ATLs were found to express high transcript levels of the leptin receptor and considering that leptin is well described to promote T12 activation,18 we propose that leptin locally produced by human mature adipocyte may be involved in the activation of ATLs. The lack of B cells together with the accumulation of Th1 type effector T cells and the previously reported characterization of human ATMs that were strongly positive for MHC-II,7 suggest that a cell-mediated immune reaction takes place in the AT with obesity. However, additional experiments are clearly needed to define whether the T lymphocytes of human adipose tissue are selected toward specific antigens.

Because the lymphocyte clusters identified by immunohistochemistry were localized in the vicinity of adipocytes, paracrine effects of ATLs on adipocyte metabolism were investigated and compared to that of human ATMs. Although
ATLs as well as ATM-derived secretory products did not affect lipolysis, soluble factors originating from ATLs markedly inhibited the insulin-mediated upregulation of both lipogenic enzymes FAS and LPL. Such an effect was associated with the lower expression of PIK3R1 (encoding the 85kD regulatory subunit of PI3K), a key component in the insulin-stimulated pathway.21,22 Moreover, the effect was specific to lymphocyte-derived products because conditioned media from human ATMs had no effect. Because human ATLs were shown to produce IFNy, adipocytokines were treated by IFNy. IFNy, as ATL-conditioned media, reduced the insulin sensitivity of FAS, LPL, and PIK3R1 and its neutralization in ATL-conditioned media reversed the effects. Therefore, IFNy produced by ATLs is a paracrine mediator of the T-cells on adipocyte metabolism. The experiments performed on the human progenitor cells confirmed the effects of ATLs on the expression of both LPL and FAS. Moreover, because a decreased triglyceride accumulation was also formed on the human progenitor cells confirmed the effects of ATLs on the expression of both LPL and FAS. Moreover, a decreased triglyceride accumulation was also observed, it is suggested that ATLs interact with the insulin-mediated promotion of triglyceride storage. Accumulation of visceral AT is now well recognized to be associated with an increased risk of developing type 2 diabetes. Visceral AT exhibits marked differences compared with subcutaneous AT and increased risk of developing type 2 diabetes. Visceral AT is now well recognized to be associated with an increased accumulation of ATLs in human visceral AT and cytotoxic CD8+ ATLs increases in parallel with adiposity and has a location-dependent pattern. We report a new adipochemokine, CCL20, the expression of which is modulated by the degree of adiposity and the anatomic location of AT. We also show that ATLs are potent local regulators of adipocyte metabolism and more specifically insulin-mediated lipogenesis.

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**Disclosures**

None.

**References**

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Figure S1

A

CD3+ cell granularity (Mean SSC)

B

% CD3+ cells

CD62L CD45RA