CCL5 Promotes Macrophage Recruitment and Survival in Human Adipose Tissue

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Objectives—To examine the role of adipose-produced chemokine, chemokine ligand (CCL) 5, on the recruitment and survival of macrophages in human white adipose tissue (WAT).

Methods and Results—CCL5 levels measured by enzyme immunoassay in serum and by real-time polymerase chain reaction in WAT were higher in obese compared to lean subjects. CCL5, but not CCL2, secretion was higher in visceral compared to subcutaneous WAT. CCL5 mRNA expression was positively correlated with the inflammatory macrophage markers as CD11b, tumor necrosis factor-α, and IL-6 in visceral WAT (n=24 obese subjects), and was higher in macrophages than other WAT cells. We found that CCL5 triggered adhesion and transmigration of blood monocytes to/through endothelial cells of human WAT. Whereas in obese WAT apoptotic macrophages were located around necrotic adipocytes, we demonstrated that CCL5, but not CCL2, protected macrophages from free cholesterol-induced apoptosis via activation of the Akt/Erk pathways.

Conclusions—CCL5 could participate in the inflammation of obese WAT by recruiting blood monocytes and exerting antiapoptotic properties on WAT macrophages. This specific role of CCL5 on macrophage survival with maintenance of their lipid scavenging function should be taken into account for future therapeutic strategies in obesity-related diseases. **(*Arterioscler Thromb Vasc Biol*. 2010;30:39-45.)**

Key Words: apoptosis ■ chemokines ■ human adipose tissue ■ macrophage ■ obesity

Obesity is considered a chronic low-grade inflammatory state, an important determinant shared with other associated pathologies like type 2 diabetes and atherosclerosis.[1,2] White adipose tissue (WAT) of obese subjects produces inflammatory factors like cytokines (IL-6, tumor necrosis factor-α) and chemokines (IL-8, chemokine [C-C motif] ligand [CCL] 2), which originate predominantly from the nonadipocyte cell fraction. This inflammatory state is linked with macrophage accumulation in human WAT and related to fat mass expansion.[3,4] WAT macrophages profoundly affect pre-adipocyte and adipocyte biology, leading particularly to a proinflammatory state of these cells.[5]

Blood monocytes, which are proinflammatory in obese subjects,[6] are thought to be prone to migrate and differentiate into macrophages in hypertrophied WAT. Macrophages are mainly classified as classically (M1) or alternatively (M2) activated states.[7,8] The precise phenotype of human WAT macrophages remains to be defined and probably varies according to the development stage and the degree of obesity. For example, human WAT macrophages were reported to exhibit the M2 phenotype with a significant production of M1 inflammatory mediators in overweight and moderately obese subjects.[9,10] The mechanisms underlying macrophage accumulation, at least in human WAT, are poorly defined. Previous studies in mice have shown a key role for CCL2 because mice deficient for CCL2 or CCR2, its receptor, showed a decrease in macrophage accumulation in adipose tissue.[11,12] In addition, overexpressing CCL2 in rodents stimulates macrophage accumulation and insulin resistance.[13] Nevertheless, another study in CCL2 knockout mice showed they had a similar accumulation of macrophages as their wild-type counterparts. Thus, the role of CCL2 in this process is debated.[14] Because chemokines are known to act in concert, an important goal is the precise identification of chemokines participating in macrophage recruitment in human obese WAT.

Using a cDNA microarray analysis, we previously identified CCL5 to be among the most overexpressed genes in human pre-adipocytes treated with macrophage-secreted factors (http:/corneliu.henegar.info/projects/FunCluster/mol_endocrinol_2008/). Whereas its role and its target receptors in human WAT are unknown, this chemokine is involved in

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blood mononuclear cell recruitment to inflammatory sites by binding to the G-protein-coupled receptors CCR1, CCR3, and CCR5. Moreover, CCL5 production by fibroblasts, platelets, and monocytes/macrophages is a particular feature of inflammatory disorders such as atherosclerosis.[15] In fact, CCL5, through CCR1 and CCR5, contributes to transendothelial migration of monocytes and T cells in atherogenic lesions.[16] Macrophages in these lesions accumulate large amounts of free cholesterol (FC), which in turn serve as a potent inducer for macrophage apoptosis.[17] In hypertrophied WAT, which represents a reservoir of FC, macrophages have been shown to scavenge lipids released by necrotic adipocytes.[18] In mice, apoptosis of virus-infected macrophages was prevented by CCR5/CCL5. As such, CCL5 provides antiapoptotic signals via the Akt and Erk1/2 pathways, which could then favor the scavenging role of tissue macrophages.[19]

Considering these findings from different cell and tissue models, we tested the hypotheses in human WAT that CCL5 may participate with other chemokines, such as CCL2, in the recruitment of monocytes and may act as a pro-survival factor protecting WAT macrophages from FC-induced apoptosis in human WAT.

Materials and Methods

Subjects and Biochemical Analysis

The method of recruitment and clinical and biochemical parameters of 24 morbidly obese women are presented (supplemental Table 1). Paired subcutaneous (SC) and visceral WAT samples and venous blood samples were obtained from all subjects. Thirteen of these patients were age-matched to 13 lean women enrolled in this study, who also provided WAT biopsy specimens. The clinical and biochemical parameters are presented in supplemental data (supplemental Table 2). Informed personal consents were obtained from all subjects. All clinical investigations were performed according to the Declaration of Helsinki and approved by the ethics committees of Hôtel-Dieu (Paris, France).

RNA Preparation and Real-Time Polymerase Chain Reaction

RNA extraction, reverse-transcription, and real-time polymerase chain reaction were performed as previously described.[5] Primers for the tested genes are listed in supplemental data (supplemental Table 3). Values were normalized to 18S expression.

Culture of Human Adipose Tissue Explants

Adipokine secretions in paired SC and visceral WAT from six obese subjects were obtained. WAT explants (100 mg) were incubated in triplicate in 1 mL of endothelial cell basal medium containing 1% bovine serum albumin, penicillin (100 U/mL), and streptomycin (100 mg/mL) under aseptic conditions. After 18-hour incubation, supernatants were collected and stored at −80° until required. Preliminary experiments indicated that the production of CCL5 was linear during 24-hour incubation.

Preparation of Adipose Tissue Macrophages and Endothelial Cells

Isolation of adipose tissue macrophages and adipose tissue endothelial cells (AT-EC) from stromavascular function (SVF) of human WAT was performed as described in the supplementary data.

Preparation of Human Blood Monocyte-Derived Macrophages and FC Loading

Plasma blood mononuclear cells isolation from the blood of women and their differentiation to macrophages were performed as previously described.[20] Macrophage FC loading was performed as described in the supplementary data.

Detection of Apoptosis by TUNEL Assay

FC-loaded macrophages were treated with CCL5 recombinant protein (1 ng/mL), CCL2 recombinant protein (1 ng/mL), the specific Akt inhibitor (10^{-5}M), or UO126 (10^{-5}M) for 18 hours. In some experiments, macrophages were treated with IL-4 (10 ng/mL) or lipopolysaccharides (100 ng/mL) for 24 hours to induce, respectively, M2 and M4 phenotypes before FC loading. Then, cells were fixed with 4% paraformaldehyde and the TUNEL assay was performed using an in situ Cell Death Detection Kit (Roche Diagnostics) according to manufacturer's instructions. Five fields were counted in each experimental condition.

Adhesion and Transmigration Assays

Human blood monocytes were labeled for 40 minutes with 10 μmol/L calcein acetoxymethyl ester.[21] AT-EC were grown to confluence for 5 to 6 days on a fluorobock insert system of 3-μm pore size coated with fibronectin (Costar).[3]

For adhesion assays, confluent AT-EC were incubated with recombinant proteins (1 ng/mL) or with control media. In another set of experiments, confluent AT-EC were incubated in conditioned media from obese visceral WAT with or without neutralizing antibodies. After incubation, labeled monocytes were added to AT-EC for 1 hour at 37°C, and the number of adherent monocytes was counted in 5 different fields.

In transendothelial migration assays, labeled monocytes were added to the top chamber and recombinant proteins (1 ng/mL) or control media were added to the bottom compartment. In another set of experiments, conditioned media from obese visceral WAT with or without neutralizing antibodies were added to the bottom compartment. After 4 hours at 37°C, the monocytes attached to the lower side of the wells were counted in 5 fields.[3]

Statistical Analysis

Data are expressed as the mean±SEM. Differences in clinical and biochemical parameters between lean and obese women were determined using the Wilcoxon unpaired nonparametric test. Spearman coefficients were computed to examine correlations. The cellular experiments were performed at least 3 times. Statistical analysis was performed using Student t test. Comparisons between >2 groups were performed using 1-way ANOVA analysis followed by post hoc test, in which $P<0.05$ was considered statistically significant.

Results

Serum CCL5 Increases in Obesity

The clinical and biochemical characteristics of 13 lean and 13 obese women at baseline are presented in the supplemental data (Table II). As expected, obese subjects exhibited a proinflammatory profile, as illustrated by increased circulating levels of IL-6. In contrast, adiponectin serum levels were lower in obese women (Table II). Those of CCL5 were significantly higher in obese subjects than in lean age-matched subjects ($P<0.05$; Figure 1A) and were in the same range as those recently reported.[22]

CCL5 Increases in Human Obese WAT and Is Related to the Degree of Inflammation

In accordance with the serum profile, mRNA levels of CCL5 were significantly increased in SC WAT of obese subjects compared to lean ones ($P<0.05$) to the same extent as the expression of the macrophage marker CD11b, which correlated with body mass index ($R=0.47$; $P=0.029$). Increases in IL-6 and leptin mRNA expression were also seen in SC WAT of obese subjects (Figure 1B).

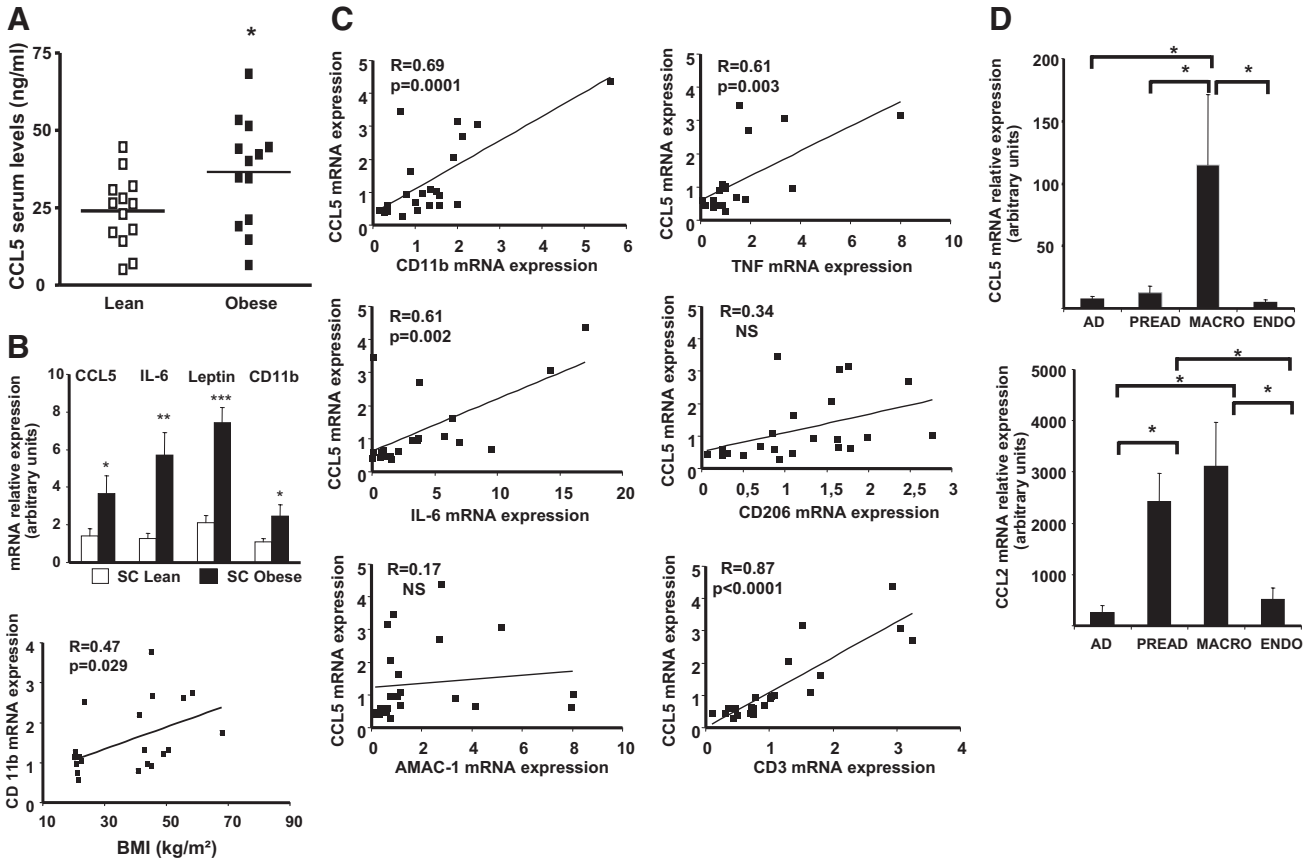

Figure 1. CCL5 gene expression in adipose tissue and adipose cells of lean and obese subjects. A, Serum levels of CCL5 in 13 lean and 13 obese subjects. B, CCL5, IL-6, leptin, and CD11b gene expression were quantified by real-time polymerase chain reaction in SC WAT of 9 lean and 12 age-matched obese subjects. C, CCL5, CD11b, tumor necrosis factor-α, IL-6, CD206, AMAC-1, and CD3 gene expression were quantified by real-time polymerase chain reaction in visceral WAT of 24 obese subjects. D, Adipocytes, pre-adipocytes, macrophages, and endothelial cells were isolated from SC WAT from 6 obese subjects. CCL5 expression and CCL2 expression were quantified by real-time polymerase chain reaction. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Next, CCL5 secretion was investigated in paired SC and visceral WAT samples from 6 obese subjects. CCL5 secretion was significantly higher in visceral samples compared to SC samples, as IL-6 secretion was. As expected, leptin secretion was higher in SC WAT. CCL2 secretion was similar in visceral and SC adipose tissues; however, CCL2 secretion by visceral WAT explants was ≈4-fold higher than CCL5 secretion ($P<0.001$; n=6). The same pattern of secretion was observed in SC explants in which CCL2 levels were ≈5-fold higher than CCL5 ones ($P<0.001$; n=6; Table).

We further explored the association between the expressions of CCL5 and macrophage markers. In visceral WAT from 24 obese subjects, CCL5 mRNA was strongly and positively correlated with CD11b ($R=0.69$; $P=0.0001$) and the M1 markers tumor necrosis factor-α ($R=0.61$; $P=0.003$) and IL-6 ($R=0.61$; $P=0.002$), but not with the M2 markers CD206 and AMAC-1.[23] CCL5 mRNA was also highly correlated with the T-cell marker CD3 ($R=0.87$; $P<0.0001$; Figure 1C). To gather information on the cell types present in WAT, the expressions of macrophage and lymphocyte markers are compared in adipose tissue macrophages and visceral WAT. The data presented in supplemental Figure I clearly indicate that macrophages are more numerous than lymphocytes in this tissue.

Table. Adipokine Secretions by SC and Visceral WAT From Obese Subjects

Adipokine, ng/mL	SC	Visceral
CCL5	0.28±0.08	0.43±0.11*
CCL2	1.54±0.13	1.79±0.11
IL-6	3.75±0.54	8.27±0.63†
Leptin	2.28±0.55	1.03±0.10*

*$P<0.05$.
†$P<0.01$.

Next, we studied the expression of the CCL5 receptors, namely CCR1, CCR3, and CCR5, by reverse-transcription polymerase chain reaction. CCR1 was the most highly expressed receptor in human monocytes, adipose tissue macrophages, and visceral WAT (supplemental Table 4).

Next, we studied CCL5 immunoreactivity in visceral WAT biopsy samples from obese subjects (supplemental Figure II). Cells positive for CCL5 were found in crown-like structures around adipocytes (Figure IIA, IIB) and in blood vessels (Figure IID). Some adipocytes also appeared to express CCL5 (Figure IIC). To establish the contribution of the different cell types, we determined expression of CCL5 and CCL2 by reverse-transcription polymerase

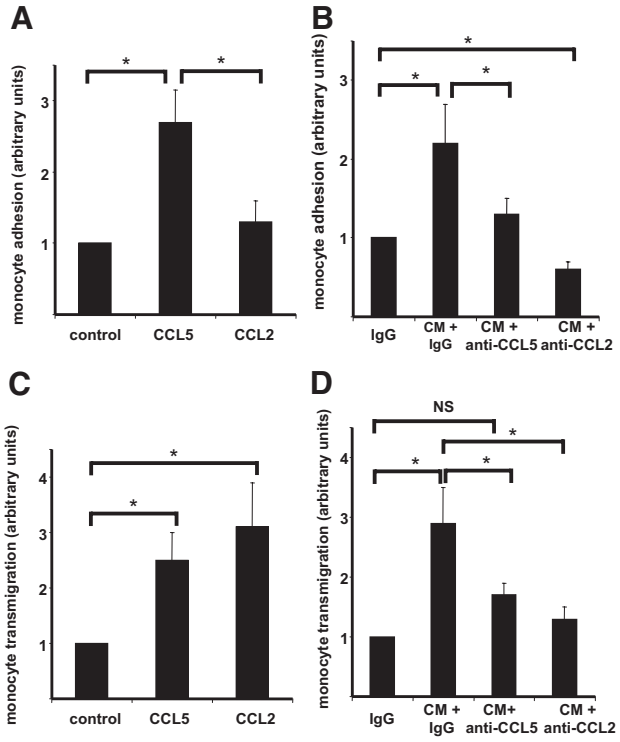

Figure 2. CCL5, adhesion, and transmigration of human monocytes. A, Adhesion of human monocytes labeled with calcein to AT-EC, either pretreated or not with recombinant proteins (CCL5 or CCL2, 1 ng/mL). B, Adhesion of human monocytes labeled with calcein to AT-EC, either pretreated or not with conditioned media from visceral WAT from obese subjects (conditioned media) in the presence of nonimmune IgG (1 μg/mL), anti-CCL5 neutralizing Ab (IgG anti-CCL5 Ab, 1 μg/mL), or anti-MCP1–1 neutralizing Ab (IgG anti-CCL2 Ab, 1 μg/mL). C, Transmigration of human monocytes through AT-EC. Calcein-labeled monocytes were added to the top compartment containing the AT-EC monolayer. The bottom compartment contained recombinant proteins (CCL5 or CCL2, 1 ng/mL). D, Transmigration of human monocytes through AT-EC. Calcein-labeled monocytes were added to the top compartment containing the AT-EC monolayer. The bottom compartment contained conditioned media from visceral WAT from obese subjects (conditioned media) in the presence of nonimmune IgG (1 μg/mL), anti-CCL5 neutralizing Ab (IgG anti-CCL5 Ab, 1 μg/mL), or anti-MCP1-1 neutralizing Ab (IgG anti-CCL2 Ab, 1 μg/mL). Data are the mean$\pm$SEM of 5 separate experiments, each using different AT-EC preparations. *$P<0.05$.

chain reaction in adipocytes, pre-adipocytes, macrophages, and endothelial cells isolated from obese WAT. As shown in Figure 1D, CCL5 was more highly expressed in macrophages than in other cell types, whereas CCL2 was more highly expressed in macrophages and pre-adipocytes than in adipocytes and endothelial cells.

CCL5 Stimulates Adhesion and Transmigration of Human Blood Monocytes

To determine if CCL5 was able to recruit blood monocytes, we tested its effect on the adhesion and transmigration of monocytes to/through endothelial cells isolated from human WAT (AT-EC). The recombinant protein CCL5 significantly increased adhesion of labeled monocytes to AT-EC (+250%; $P<0.05$; Figure 2A), whereas CCL2 did not. Conditioned media from visceral WAT of obese subjects increased adhesion of labeled monocytes to AT-EC. This effect was suppressed in the presence of CCL5 and CCL2 neutralizing antibodies (Figure 2B). The paradoxical effect of the CCL2 neutralizing antibody was previously explained by a significant inhibition of macrophage migration inhibitory factor (MIF)-induced monocyte recruitment.[16] The fact that CCL2 and MIF are both oversecreted by human WAT in obesity[24,25] strongly supports this hypothesis.

The transmigration of labeled monocytes through AT-EC layers was enhanced by the recombinant proteins CCL5 and CCL2 ($\approx$+300%; Figure 2C). The same stimulatory effect was observed with conditioned media from visceral WAT of obese subjects, whereas neutralizing antibodies of CCL5 and CCL2 abolished it (Figure 2D).

CCL5 Protects Macrophages From Apoptosis

To examine the hypothesis that FC induces apoptosis in WAT macrophage, we performed TUNEL staining in obese WAT macrophages found in crown-like structures around necrotic adipocytes. As shown (supplemental Figure III), overlay experiments using the macrophage markers Ham 56 and CD68 demonstrated TUNEL-positive macrophages in crown-like structures.[26] In contrast, the rare macrophages dispersed in the parenchyma of lean WAT were TUNEL-negative.

In our in vitro model, FC loading was observed in $\approx$80% of the monocyte-derived macrophages and $\approx$20% of these cells were apoptotic (data not shown). As depicted in Figure 3A, TUNEL assays show that incubation with CCL5 recombinant protein reduced apoptosis of FC-loaded macrophages, whereas CCL2 did not. M2 macrophages that exhibited higher apoptosis levels than M1 macrophages were sensitive to antiapoptotic effects of CCL5 recombinant protein, whereas M1 macrophages were not (Figure 3B). The fact that M1 macrophages secreted high concentrations of CCL5 compared to M2 macrophages (495$\pm$42 vs 15.5$\pm$1.1 pg/mL; n=4; $P<0.001$) could explain that M1 macrophages were protected from FC-induced apoptosis through their endogenous production of CCL5.

Moreover, FC-induced apoptosis of macrophages isolated from WAT was reduced after CCL5 addition (Figure 3C) and was increased by antibody neutralization of CCL5 (Figure 3D).

CCL5 is known to exert its antiapoptotic function via the Erk and Akt pathways in viral-infected macrophages from mice. Here, we verified that CCL5 stimulated macrophage Erk and Akt activities in a dose-dependent manner, with a half-maximal effect at 0.1 ng/mL CCL5 (supplemental data, Figure IV). This activation was rapid (5 minutes) and sustained for 30 minutes for both Erk and Akt (data not shown). CCL2 did not activate these two enzymes (supplemental data, Figure V).

To confirm the involvement of Erk and Akt, specific inhibitors of these pathways were tested in FC-loaded macrophages. The Akt inhibitor and UO126, the selective MEK inhibitor,[27,28] completely blocked the antiapoptotic effect of CCL5 (Figure 3E).

Discussion

This study explored the role of CCL5 in the biology of human adipose cells known to be modified by inflammation. In

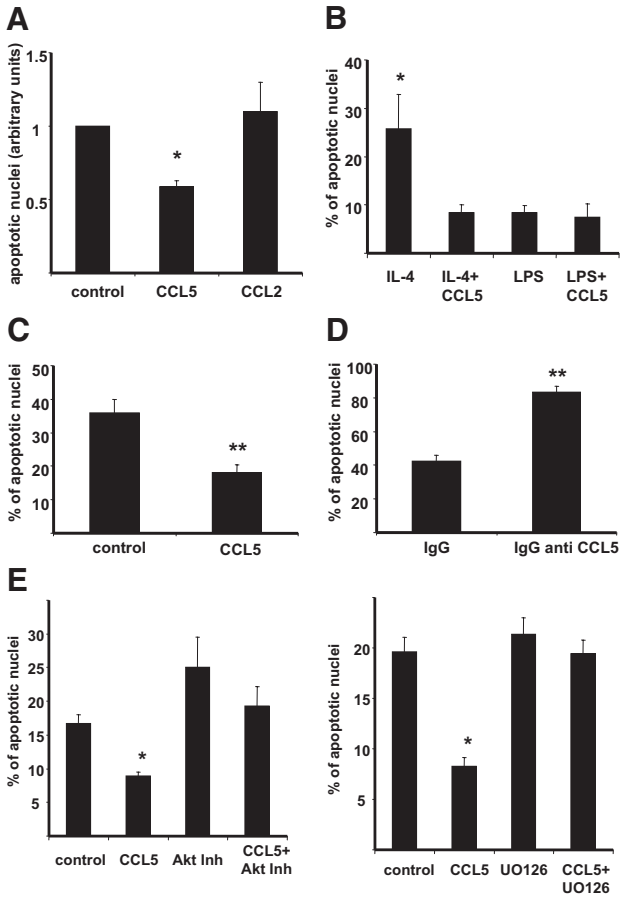

Figure 3. CCL5 and antiapoptotic effect in human macrophages. A, Blood monocyte-derived macrophages were incubated for 18 hours in RPMI 1% fetal bovine serum with FC (10 μg/mL) and acyl conenzyme A: cholesterol acyl tranferase (ACAT) inhibitor 58035 (2 μg/mL) in the presence or the absence of CCL5 (1 ng/mL) or CCL2 (1 ng/mL). B, Blood monocyte-derived macrophages were incubated for 24 hours in the presence of IL-4 (10 ng/mL) or lipopolysaccharides (100 ng/mL) before 18 hours in RPMI 1% fetal bovine serum with FC (10 μg/mL) and ACAT inhibitor 58035 (2 μg/mL) in the presence or the absence of CCL5 (1 ng/mL). C, WAT macrophages were incubated with FC and ACAT inhibitor 58035 in the presence or absence of CCL5 (1 ng/mL). D, WAT macrophages were incubated with FC and ACAT inhibitor 58035 in the presence or absence of nonimmune IgG (1 μg/mL) or anti-CCL5 neutralizing Ab (IgG anti-CCL5 Ab, 1 μg/mL). E, Blood monocyte-derived macrophages were incubated for 18 hours in RPMI 1% fetal bovine serum with FC and ACAT inhibitor 58035 in the presence or absence of CCL5 (1 ng/mL), Akt inhibitor (10 μmol/L), and UO126 (10 μmol/L). The cells were then fixed for TUNEL staining. Quantitative data from 5 fields for each condition in 5 separate experiments. Data are the mean$\pm$SEM of 5 separate experiments except for WAT macrophages, for which a representative experiment from 3 was presented. *$P<0.05$; **$P<0.02$.

agreement with previous clinical studies, we confirmed increases in the CCL5 secretion and gene expression in WAT with obesity. These human studies showed that the deregulation of CCL5 is observed both in obese men and women, whereas this was only exhibited in obese male mice.[29,30]

Moreover, the secretion levels of CCL5 were higher in obese visceral adipose depots compared to that in SC; however, this pattern was not observed for CCL2. The adipose depot site-specific secretion of CCL5 could be attributed to higher macrophage accumulation and vessel density in visceral compared to SC human obese WAT.[31,32] We observed that CCL5 gene expression was strongly correlated with T-lymphocyte markers in visceral WAT, as previously reported;[30] however, it should be noted that in contrast with rodent WAT, T lymphocytes poorly infiltrate obese human WAT in comparison to macrophages (Figure I). This does not exclude that CCL5 could participate in lymphocyte action in human WAT.

Our study focused on the putative contribution of CCL5 on WAT macrophage function. CCL5 expression in visceral WAT strongly correlated with gene markers characterizing M1, but not M2, macrophages. M1 macrophages from obese WAT are likely the major cellular source of CCL5, as supported by the fact that CCL5 is a tumor necrosis factor-α target,[30] and the fact that in vitro-induced M1 macrophages secrete higher levels of CCL5 compared to M2 cells.

The interactions with the vessel wall during tissue monocyte infiltration occur in sequential steps, namely selectin-mediated rolling, integrin-dependent arrest, and transendothelial diapedesis triggered by chemokines. CCL2 and CCL5 are candidate chemokines involved in this phenomenon and are already well-described in the development and progression of atherogenesis. In addition, chemokines like CCL5 can also mediate monocyte arrest.[33] Their contribution to this process in human adipose tissue is unknown. To characterize the role of CCL5 in WAT macrophages, we addressed the monocyte chemotaxis response to CCL5 in human models. We showed that recombinant CCL5, at a range of concentrations close to that present in secretion media from visceral WAT explants, triggered adhesion and transmigration to/through endothelial cells. As shown by neutralizing antibody experiments, the CCL5 produced by obese visceral WAT was also effective in stimulating monocyte chemotaxis. The CCL5 effect occurred to a similar extent as that observed with CCL2, which has been shown to contribute, in some mice models, to macrophage accumulation in WAT.[11,13] *De facto*, blood monocytes from obese subjects displaying proinflammatory properties[6] mainly express receptors of CCL2 and CCL5, namely CCR2 and CCR1, and thus are prone to migrate in obese WAT.

Because of the contribution of CCL5 in facilitating the diapedesis of monocytes/macrophages in adipose tissue, therapeutic approaches aimed at blocking CCL5 are conceptually intriguing.[34] As such, elucidating the role of CCL5 in the local biology of the adipose tissue is mandatory. Here, we demonstrate an antiapoptotic role for CCL5, a property not shared with CCL2. In hypertrophied human WAT, necrotic adipocytes are surrounded in crown-like structures by macrophages that have been positioned to clean up these "dead" adipocytes.[18] Adipocytes are an important site of FC storage,[35] which needs to be eliminated after adipocyte death. In such a context, macrophages that scavenge lipids are exposed to high levels of cytotoxic FC, a phenomenon well-demonstrated by foam cells in atherosclerotic plaques.[36] Our in vitro studies clearly demonstrated the antiapoptotic action of CCL5 at concentrations close to those found in secretion media of

WAT explants on FC-loaded M2 macrophages, but not in M1 cells, probably because of the protective endogenous CCL5 produced by the M1 cells.

The antiapoptotic effect of CCL5 appears to be mediated via the Erk/Akt pathways, as also observed with viral infections.[19] As such, a parallel could be established between a viral infection and the metabolic stress seen in obesity that, in both cases, CCL5 has, to an extent, a protective role in tissue macrophages that allows them to perform their scavenging function. It is tempting to speculate that, at some stages of in vivo adipose tissue expansion, macrophages might be overwhelmed by the lipid efflux from adipocytes. This could serve to explain the identification of apoptotic macrophages, which are only present in obese WAT (ie, estimated to be ≈40% of the total macrophage population) in crown-like structures, as reported by another group.[26] In addition, it should be noted that apart from its effects on recruitment and survival, CCL5 did not influence, at least in vitro, differentiation and scavenging properties of macrophages (data not shown).

The subtype of receptors involved in the cellular actions of CCL5 remains to be determined in human WAT. We suggest that CCL5 could act through CCR1, which is the most highly expressed of the 3 CCL5 receptors (CCR1>CCR5>CCR3) in human monocyte/macrophage and WAT. However, different roles have been attributed to CCR1 and CCR5, both of which have been linked to transendothelial migration. CCR1 predominantly mediated CCL5-induced arrest of human monocytes.[37] However, in mice models, CCR5 deficiency reduced macrophage infiltration in advanced atherosclerotic plaques, leading to their more stable phenotype.[38] Concerning the CCL5 action on macrophage apoptosis, in a rat model of renal injury associated with necrosis and fibrosis, CCR1, which participates in monocyte recruitment, was not involved in apoptosis.[39]

Importantly, distinct roles of CCR1 and CCR5 have been attributed in mice hepatic fibrosis; CCR1 is associated with mediating macrophage migration and CCR5 is associated with the profibrogenic effects of the resident liver cells.[40] As described for fibrosis in human obese WAT,[41] it is tempting to speculate that CCL5 and its receptors also participate in several steps of the fibrogenesis process in this tissue.

In conclusion, because of the importance of WAT inflammation in the associated complications of obesity, therapeutic approaches acting on chemokines and their receptors to prevent macrophage accumulation are of considerable interest. For example, mouse models of obesity have unraveled an important role for CCL2/CCR2 in macrophage accumulation and metabolic complications. Antagonists or deletion of CCR2 have demonstrated a reduction in macrophage accumulation and, subsequently, WAT inflammation and obesity metabolic complications.[42] In this study, we show that it is paramount to dissect the physiological role of such chemokines in the biology of expanded adipose tissue. Whereas CCL5 in WAT might be another important molecular player in the self-perpetuating inflammation associated with metabolic and vascular complications, this chemokine appears to have an important role in preserving the lipid scavenging role of macrophages. As such, CCL5 receptors should be considered a potential target for controlling low-grade inflammation in obesity.

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Disclosure

For cellular studies, ethical authorization was obtained from CPP Hôtel-Dieu, Paris. Human adipose tissue biopsy samples were obtained thanks to the Clinical Research Contract (Assistance Publique/Direction de la Recherche Clinique, AOR 02076).

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