Role of the adipocyte-specific NF-κB activity in the regulation of IP-10 and T cell migration

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MATERIALS AND METHODS

OBESITY IS CHARACTERIZED AS a state of chronic low-grade inflammation with elevated plasma levels of proinflammatory adipokines (7). Chemokines came into the focus of diabetes research, since studies in mouse models and in humans have shown that obesity is associated with an enhanced infiltration of macrophages (4, 20) and T cells, especially Th1 (T helper) polarized T cells, into adipose tissue (AT) (6, 11, 22). Recent research revealed a functional link between specific T cell subpopulations and obesity-associated insulin resistance (5, 13, 21). However, the molecular mechanism involved in T cell infiltration in AT is still poorly understood.

There is growing evidence that local chemokine secretion promotes lymphocyte infiltration into AT. The chemokines regulated upon activation, normal T cell expressed and secreted (RANTES)/chemokine CC motif ligand 5, stromal cell-derived factor 1α/chemokine CXC motif ligand 12, and macrophage inflammatory protein 3α/chemokine CC motif ligand 20 (CCL20) seem to play a role in T cell recruitment into AT (5, 11, 22). Yet, except for CCL20, their cellular origin is unknown. One further potent chemoattractant for T cells, particularly Th1 cells, is the CXC chemokine interferon-γ-induced protein 10 kDa (IP-10). The serum concentrations of IP-10 correlate positively with the incidence of type 2 diabetes (9). In addition, it is produced by freshly isolated mature human adipocytes from various depots with a positive correlation to body mass index (BMI) (10). Thus it may be hypothesized that IP-10 is also involved in the chronic inflammatory process observed in obesity and type 2 diabetes. In different cell types, IP-10 was shown to be regulated mainly by NF-κB and/or Jak-Stat activation in a highly cell type-specific manner (14, 18, 23). NF-κB is a major regulator of inflammatory processes with an important function in the pathogenesis of obesity-related insulin resistance (24). However, its specific function for the accumulation of immune cells in AT remains elusive.

The aim of the present study was 1) to investigate whether NF-κB is involved in the regulation of IP-10 in preadipocytes and adipocytes and 2) to characterize the potential role of NF-κB activity and IP-10 for T cell infiltration into AT.

Subjects. For the isolation of preadipocytes, adipocytes, and stromal vascular fraction (SVF), human subcutaneous AT was obtained from healthy women and men undergoing elective abdominal surgery; no selection was made for BMI, age, or gender. Patients with diabetes, cancer, serious diseases, acute infection, malignancies, or any other consuming disease were excluded. Informed consent was obtained from all subjects. The study was approved by the ethical committee of the Technische Universität München, Germany.

Mice. For all experiments, male C57BL/6J mice were housed in a temperature-controlled (25°C) facility with a 12:12-h light-dark cycle. Mice were fed a standard chow D12450B (%kcal: 20% protein, 70% carbohydrate, and 10% fat) or a high-fat diet D12492 (%kcal: 20% protein, 20% carbohydrate, and 60% fat) from Sniff (Soest, Germany). Experimental feeding was started at the age of 10 wk and then for 16 wk (Fig. 1A) or for 10 wk (Fig. 1B). Besides these experiments,
we obtained cDNA from mouse experiments published earlier (11) for additional analysis of gene expression. Male C57BL/6j mice were fed a high-fat diet for 10 wk vs. a control diet, and AT samples were collected after 0, 5, and 10 wk. All animal procedures were in accordance with institutional guidelines with national law and were approved.

**Cell culture.** Freshly isolated primary mature adipocytes and preadipocytes/SVF were isolated and cultured as previously described (10). Isolation of SVF and adipocytes from murine gonadal fat tissue was performed according to the protocol published by Weisberg et al. (19). 3T3-L1 cells (Xantos Biomedicine, Munich, Germany) and the retroviral packaging cell line Phoenix-Eco provided by B. Baumann (University of Ulm, Ulm, Germany) were cultured in basal medium containing DMEM (Invitrogen, Karlsruhe, Germany), 10% FCS (Invitrogen), and 50 μg/ml gentamycin (Roth, Karlsruhe, Germany) at 37°C, 5% CO₂. Differentiation of 3T3-L1 cells was performed as described (12). Cells were incubated in the presence or absence of the following agents: IL-1β (R&D, Wiesbaden, Germany), lipopolysaccharide (LPS; Sigma-Aldrich, Munich, Germany), Bay117082 (NF-κB inhibitor; Sigma-Aldrich), U-0126 [mitogen-activated protein kinase kinase 1 (MAP2K1) inhibitor; Promega], LY-294002 [phosphoinositide 3-kinase (PI 3-kinase) inhibitor; Sigma-Aldrich], U-0126 [mitogen-activated protein kinase kinase 1 (MAP2K1) inhibitor; Promega], LY-294002 [phosphoinositide 3-kinase (PI 3-kinase) inhibitor; Sigma-Aldrich], goat anti-IP-10 antibody (R&D), or the respective control goat IgG (R&D). All agents were not cytotoxic at the concentrations used and as demonstrated in a cell viability assay (data not shown).

**Conversion of cells.** 3T3-L1 cells were transfected on various days of differentiation using Lipofectamine 2000 transfection reagent (Invitrogen). DNA (1.5 μg) and 3 μl of reagent were mixed and added to the cells for 4 h. After transfection (24 h), luciferase activity was measured using the dual-luciferase assay (Promega, Mannheim, Germany). In all transfections, 0.3 μg of ubiquitin-promoter Renilla luciferase vector was cotransfected to normalize for transfection efficiency. Ubiquitin-promoter Renilla luciferase vector and 6xFKBP-luciferase vector were provided by B. Baumann (University of Ulm) and IP-10 promoter-luciferase vector by D. Haller (Technische Universität München, Munich, Germany).

**Retroviral infection of 3T3-L1.** To inhibit NF-κB in 3T3-L1 cells, a dominant-interfering IκBα mutant protein (IκBα-mut, serine 32/36 mutated to alanine, resulting in a nondegradable repressor) or an empty vector as control (mock) was stably integrated into 3T3-L1 cells using retroviral gene transfer. For virus production, the pCFG5-IκBα-mut retroviral vector (provided by B. Baumann, University of Ulm) was transfected using calcium phosphate transfection in the ecotropic Phoenix virus-producer cell line. Supernatant containing the retrovirus was collected 48 and 72 h after transfection and used to infect 3T3-L1 preadipocytes in the presence of 8 μg/ml diethylaminoethyl. After a second infection (4 h), the selection with zeocin was started for 5 days until all cells were 100% positive for green fluorescent protein.

**ELISA.** 3T3-L1 cells were stimulated with IL-1β or LPS for 20 h. The supernatant was immediately frozen at −80°C. IP-10 protein level was determined by a mouse-specific DuoSet ELISA assay, according to the manufacturer’s instructions (R&D).

**Quantitative RT-PCR.** Total RNA was isolated using the Nucleospin RNAII kit (Macherey-Nagel, Düren, Germany) and reverse transcribed using the cDNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany). PCR for detection of human/mouse IP-10, chemokine CXC motif receptor 3 (CXCR3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and mouse adiponectin were performed using qPCR-SYBR-Green (ABgene; Epsom) or for primers ordered from MWG (Ebersberg, Germany) were designed according to the manufacturer’s instructions (R&D). Quantification of gene regulation was performed by the ΔΔCt method; results were corrected for GAPDH expression.

**Western blot and electrophoretic mobility shift assay.** For Western blot analysis, 50 μg of total protein extracts were separated on 12.5% polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Millipore, Schwabach, Germany). Membranes were blocked (PBS, 0.1% Tween, and 2% Ambersham blocking reagent), stained

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**Fig. 1. Interferon-γ-induced protein 10 kDa (IP-10) expression in mouse and human adipose tissue and adipose tissue cell types.** Total mRNA was isolated from inguinal (ing) and epididymal (epi) AT of 4 male C57BL/6j mice fed a standard chow compared with mice fed a high-fat diet for 16 wk (A); from primary stromal vascular fraction (SVF) and adipocytes (Ad), freshly isolated from adipose tissue of 6 male mice/group either fed a high-fat diet (HFD) or control mice fed a standard chow diet (CD) for 10 wk (B); from freshly isolated primary human preadipocytes (Pre) and mature human adipocytes (Ad) of adipose tissue from subjects (C); and from 3T3-L1 preadipocytes and adipocytes (n = 3) (D). mRNA was reverse transcribed, and IP-10 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression was determined by qRT-PCR. The expression level of one sample CD/ing AT (A) and of quantitative RT-PCR (qRT-PCR) one sample CD/SVF (B) was set to one. Results are means ± SE. *P < 0.05 and **P < 0.01.
with anti-1α-2β integrin (Santa Cruz Biotechnology, Heidelberg, Germany) followed by incubation with a horseradish secondary antibody (Dianova, Hamburg, Germany), and visualized by enhanced chemiluminescence (Amersham Bioscience, Freiburg, Germany). As a loading control, stripped membrane was incubated with anti-β-actin (Santa Cruz Biotechnology). Electrophoretic mobility shift assays were performed as previously described (3).

**Migration assay.** For the analysis of the ability of splenocytes to migrate toward a chemokine gradient established by adipocytes, 3T3-L1 cells were differentiated as described. Adipocytes were stimulated as indicated in DMEM medium containing 2% heat-inactivated FCS. After 18 h, the supernatant was taken and filled in the bottom chambers of Costar Transwells (Costar, Cambridge, MA). Lymphocyte-M (Cedarlane Laboratories, Hornby, ON, Canada) purified splenocytes (10^6/sample in DMEM medium containing 2% FCS) were assayed for transmigration with 5-μm-pore-size Costar Transwell culture inserts. The migration occurred at 37°C in a humidified atmosphere with 5% CO₂ over 3 h. After incubation, cells that migrated to the lower chamber were harvested, stained with anti-CD8-FITC (eBioscience, Frankfurt, Germany) and anti-CD4-PerCP (Peridinin Chlorophyll Complex Protein; BD Biosciences, Heidelberg, Germany), counted by Becton-Dickinson FACS-LSRII (all other experiments), and analyzed by FACS-Diva software 6.1.

**Statistical analysis.** Values for all experiments are expressed as means ± SD or means ± SE of a minimum of three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey’s test, Student’s t-test, or one-sample t-test, with P < 0.05 and P < 0.01 indicated.

**RESULTS**

**High-fat diet-induced weight gain increases IP-10 mRNA expression levels in epididymal and inguinal white AT of C57BL/6J mice.** To investigate IP-10 expression in AT under a high-fat diet, we performed a first set of experiments with male mice fed a high-fat diet for 16 wk. These mice showed a significant increase in body weight and inguinal/epididymal fat pad weight (Supplemental Fig. 1, A and B). Real-time PCR analysis revealed a 5.7- and 9.6-fold increase in IP-10 mRNA expression in inguinal (subcutaneous) and epididymal (visceral) AT, respectively, in mice fed the high-fat diet compared with control mice fed a standard chow (Fig. 1A). This upregulation was significantly higher in epididymal compared with inguinal AT.

**IP-10 is differentially expressed in different AT cell types in mice and humans.** AT is composed of adipocytes and the so-called “SVF” that in turn includes preadipocytes, but also macrophages and T cells. To further elucidate the origin of AT IP-10, we isolated SVF and adipocytes from lean mice as well as from high-fat diet-fed mice. In lean mice, SVF expressed 2.5-fold higher levels of IP-10 mRNA (P < 0.05) than adipocytes, whereas, in overweight mice (high-fat diet fed), no difference was detectable because of the increase of IP-10 expression in adipocytes (Fig. 1B). Hence, upon weight gain, adipocytes are the cell type that contributes to the higher IP-10 mRNA expression in AT. Similar to overweight mice, in samples derived from overweight human subjects, a comparable expression level of IP-10 in isolated SVF and adipocytes was observed (Supplemental Fig. 2).

**Primary human and 3T3-L1 adipocytes express higher levels of IP-10 mRNA than preadipocytes.** Next, the cellular origin of IP-10 synthesis in AT was investigated in more detail. Human subcutaneous AT samples were used for the isolation of preadipocytes and adipocytes. mRNA expression of IP-10 was considerably higher in adipocytes compared with undifferentiated preadipocytes (Fig. 1C). The same results were obtained using mouse 3T3-L1 adipocytes compared with undifferentiated preadipocytes (Fig. 1D). On average, adipocytes expressed eightfold more IP-10 mRNA than preadipocytes in both models.

**IL-1β and LPS induce IP-10 protein secretion in 3T3-L1 cells.** Next, we asked if IP-10 secretion by adipocytes is further enhanced by stimuli that mimic an inflammatory status. 3T3-L1 adipocytes were exposed to IL-1β or LPS. Both stimuli are known to mediate their inflammatory response through NF-κB. An increase in IP-10 release was observed at concentrations of 10 ng/ml IL-1β or 1 μg/ml LPS (Supplemental Fig. 3).

**IP-10 expression/secretion and promoter activity increase during adipogenesis.** To further explore the mode of IP-10 regulation in adipocytes, IP-10 mRNA expression/secretion and IP-10 promoter activity were analyzed during 3T3-L1 adipogenesis. IP-10 mRNA expression was initially low in 3T3-L1 cells (on days 1-6 after induction of adipogenesis) but increased steadily during adipogenesis and reached a maximal eightfold increase in freshly isolated mature adipocytes on day 14 of adipocyte differentiation compared with day 0 preadipocytes (Fig. 2A). To verify whether the increase of IP-10 mRNA expression during differentiation is accompanied by an enhanced protein secretion, IP-10 protein release was analyzed during differentiation. IP-10 protein secretion was detectable exclusively in fully differentiated adipocytes, whereas, in preadipocytes, no protein release could be observed (Fig. 2B).

In parallel, we assessed the mRNA expression level of adiponectin and glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity as markers of adipogenesis (Supplemental Fig. 4, A and B). These data indicate that IP-10 expression increases with the level of adipocyte differentiation to mature adipocytes.

To study the molecular mechanisms involved in the expression of IP-10 in adipocytes, a reporter containing 673 bp of the proximal murine IP-10 promoter (containing 2 kB sites) in front of the luciferase gene was transfected into 3T3-L1 cells. In parallel, an NF-κB-responsive luciferase-reporter construct containing six kB consensus sites was transfected to determine NF-κB activity. The activities of both the IP-10 promoter (Fig. 2C) and the synthetic NF-κB-responsive reporter (Fig. 2D) behave exactly the same, with a slight decrease after induction of differentiation and an overall increase in luciferase activity over the 14-day differentiation period.

**NF-κB binds to IP-10 promoter kB sites and inhibition of NF-κB decreases IP-10 expression in primary human adipocytes and secretion in 3T3-L1 adipocytes.** Because IP-10 is known to be regulated by two kB sites in its promoter, we analyzed NF-κB DNA-binding activity to these sites. Electrophoretic mobility shift assays revealed an increased NF-κB binding activity to the IP-10 promoter kB sites upon IL-1β stimulation in 3T3-L1 adipocytes (Fig. 3). To further investigate the role of NF-κB for the expression of IP-10 in AT, we used an NF-κB inhibitor (Bay117082). Mouse 3T3-L1 adipocytes and freshly isolated mature human adipocytes were preincubated for 1 h with or without Bay117082 (1 and 5 μM), followed by incubation with IL-1β (10 ng/ml) for 20 h. Bay117082 (1 and 5 μM) significantly prevented IL-1β stimula-
tion of IP-10 protein release from 3T3-L1 adipocytes (Fig. 4A) and of IP-10 mRNA expression in freshly isolated mature human adipocytes (Fig. 4B) in a dose-dependent manner. The Jak/Stat inhibitor AG-490 reduced protein release in 3T3-L1 cells significantly by twofold only at a high dose. MAP2K1 (U-O126) and PI 3-kinase inhibitor (LY-294002) reduced IL-1β-induced IP-10 protein release in 3T3-L1 adipocytes; however, these effects were not statistically significant (Supplementary Fig. 5). Treatment of cells with IL-1β stimulation or Bay117082 inhibitor did not affect cell viability (Supplementary Fig. 6).

Stable overexpression of a dominant-negative IκBα mutant inhibits NF-κB activity in 3T3-L1 cells. To block the NF-κB pathway more specifically and to further elucidate the contribution of NF-κB signaling to IP-10 regulation in 3T3-L1 preadipocytes and adipocytes, we used retroviral gene transfer to express a dominant-negative interfering IκBα-mut or an empty vector as control (mock). IκBα-mut overexpression was confirmed by Western blot (Fig. 5A). In control cells (mock), IL-1β-induced IκBα degradation started after 10 min (Fig. 5B); however, no degradation occurred in cells expressing the mutant protein (Fig. 5C). Adipogenic differentiation capacity was monitored morphologically and by measuring the specific activity of GPDH, a lipogenic marker enzyme. 3T3-L1-IκBα-mut cells showed no changes in GPDH activity (data not shown) and Oil red O staining vs. the control cells (Supplementary Fig. 7).

Inhibition of NF-κB signaling in preadipocytes and adipocytes leads to a significant decrease in basal and stimulated IP-10 expression and secretion. IP-10 expression and secretion by preadipocytes and adipocytes were measured depending on the overexpression of a dominant-negative mutant of IκBα. Basal and stimulated IP-10 mRNA expression (Fig. 5D) and protein secretion (Fig. 5E) were higher in mock adipocytes compared with mock preadipocytes. This reduced level of IP-10 mRNA expression correlates with the observation that almost no IP-10 protein is released by preadipocytes. Moreover, in adipocytes, the overexpression of IκBα-mut significantly reduced basal and stimulated IP-10 mRNA expression and protein release compared with control cells. These results demonstrate the central role of NF-κB in the regulation of IP-10 in 3T3-L1 adipocytes rather than in preadipocytes in which the reduction of IP-10 expression and secretion in cells overexpressing IκBα-mut was less evident.

IL-1β- and LPS-induced T cell migration is inhibited by overexpression of IκBα-mut. NF-κB regulates the expression of several chemokines in many cell types. To elucidate the relevance of the NF-κB pathway for adipocyte-induced T cell migration, we performed migration experiments using conditioned medium from the here-established cell lines. In a migration assay using isolated mouse spleen leukocytes, minimal
reanalyzed samples from a recently published study (11) show-

ing T lymphocyte infiltration in visceral AT as a primary event in
AT inflammation. In AT from mice fed a high-fat diet for 0,
5, or 10 wk or a chow control diet for 10 wk, a slight
upregulation of IP-10 mRNA level after 5 wk and a significant
threefold upregulation after 10 wk of a high-fat diet compared
with initial levels (week 0) was observed (Fig. 8A). In the same
tissues, expression of the IP-10 receptor CXCR3 was signifi-
cantly upregulated at week 5 (1.7-fold) and week 10 (2.5-fold)
(Fig. 8B). Both IP-10 and CXCR3 expression increased in
parallel to T cell infiltration shown recently (11). These data
give further evidence that IP-10 secreted from AT plays a role
in the recruitment of T cells in obese AT.

**DISCUSSION**

In the present study, we could demonstrate that IP-10 is
upregulated in AT of a diet-induced obese mouse model. In
addition, adipocytes rather than SVF and preadipocytes signif-
ificantly contribute to elevated IP-10 levels in obesity. Our data
clearly show that the NF-κB pathway plays a central role for
the regulation of IP-10 in 3T3-L1 and primary human adipi-
cyes. These results also provide evidence that NF-κB target
genes, especially IP-10, are major activators of AT CD4+ and
CD8+ T cell migration.

Upon feeding a high-fat diet, IP-10 expression was signifi-
cantly stronger upregulated in epididymal (visceral) AT com-
pared with subcutaneous (inguinal) AT. This observation is in
line with the hypothesis that visceral AT contributes to a larger
extent to obesity-associated inflammation compared with
subcutaneous AT. In humans, a weak correlation of IP-10 secre-
tion from adipocytes with BMI was reported (10), sustaining
the function of IP-10 in the pathophysiology of obesity.

Adipokines are expressed by several different cell types in
AT (7). It has been postulated that AT stromal vascular cells,
especially preadipocytes, are the main source of proinflam-
atory adipokines. However, some data also indicate an active
role of freshly isolated mature adipocytes in obesity-related
inflammation. Compared with preadipocytes, IP-10 was pre-
dominantly expressed and secreted in fully differentiated
adipocytes in parallel with the late differentiation marker adi-
ponecitin. The preadipocyte/adipocytes difference in IP-10
mRNA expression was more pronounced in the stably infected
vs. naïve 3T3-L1 cells, indicating an influence of infection on
IP-10 expression. However, the amount of secreted IP-10
protein was similar comparing infected vs. naïve 3T3-L1. There-
fore, we can assume that infection and selection of cells
influence the expression of IP-10, but only marginal its protein
secretion. The here-observed predominant expression and se-
cretion of IP-10 in adipocytes and its upregulation in obesity
provides evidence that adipokines released from freshly iso-
lated mature adipocytes contribute to local inflammation in
obesity.

Furthermore, besides adipocytes, SVF cell types other than
preadipocytes strongly contribute to IP-10 expression. The
higher IP-10 expression in SVF of lean mice is turned to an
equal expression level when comparing SVF vs. adipocytes in
obese mice. This shows the strong contribution of adipocytes
to the obesity-dependent increased expression of IP-10. How-
ever, the molecular mechanisms regulating its expression and
their contribution to the mild inflammation observed in obese
AT are still topics that require further investigations.
It was shown that the secretion of IP-10 by adipocytes is enhanced by stimulation with interferon γ (IFNγ) (10). Our experiments reveal that proinflammatory stimuli like IL-1β and LPS, known activators of the NF-κB pathway, are also able to induce IP-10 secretion by adipocytes. In cell types other than adipocytes, IP-10 gene expression is controlled in a complex manner with interrelated roles of NF-κB or interferon regulatory factor, and several studies suggested that the regulation of IP-10 by immunological stimuli is highly cell type-specific (14, 18, 23). In our experiments, the Jak-Stat pathway showed only a weak influence on IP-10 secretion from adipocytes. However, IP-10 regulation was largely dependent on NF-κB activity in 3T3-L1 and primary human adipocytes. Both basal and stimulated IP-10 expression and secretion were reduced in 3T3-L1 cells stably overexpressing a transdominant IκBα protein. However, no changes could be observed in preadipocytes.

Fig. 5. Stable overexpression of IκBα mutant protein (IκBα-mut) in 3T3-L1 cells inhibits NF-κB activity and IP-10 expression/secretion. Protein from 3T3-L1 cells was harvested, and 50 μg protein were used for Western blot with anti-IκBα antibody. β-Actin was assayed as an internal loading control (A). 3T3-L1-mock (B) and 3T3-L1-IκBα-mut (C) adipocytes were incubated with IL-1β (10 ng/ml). Protein of cells was harvested at the indicated time points, and Western blot was performed as described above. RNA and supernatant from stably infected 3T3-L1 preadipocytes and adipocytes were harvested 20 h after adding fresh media with or without IL-1β (10 ng/ml) or lipopolysaccharide (LPS, 1 μg/ml) stimulation. mRNA was reverse transcribed, and IP-10 and GAPDH mRNA expression was determined by qRT-PCR (D). IP-10 protein concentration was measured from supernatants using ELISA (E). Results are means ± SD (*P < 0.05 and **P < 0.01).

Fig. 6. Migration of T cells in response to 3T3-L1 adipocyte-conditioned medium is inhibited by overexpression of IκBα-mut. Stably infected 3T3-L1 preadipocytes (A and B) and adipocytes (C and D) were induced for 20 h by adding fresh media with or without IL-1β (10 ng/ml, striped bar) or LPS (1 μg/ml, black bar). Conditioned media (CM) were harvested and transferred to the lower chamber of Costar Transwells. Freshly isolated mouse splenocytes were placed in Costar Transwell permeable supports. Migration of CD4+ T cells (A and C) and CD8+ T cells (B and D) toward conditioned media was assayed by fluorescence-activated cell sorter (FACS) analysis. Results are means ± SE (n = 9). *P < 0.05 and **P < 0.01.
In obese AT, a shift toward an inflammatory phenotype of T cells was observed, with an increase in CD8<sup>+</sup> and Th1 polarized T cells and a decrease in regulatory T cells. Furthermore, AT T cells from obese mice produce large amounts of the Th1 cytokine IFNγ (5, 15). As discussed by Duffaut et al. (5), the molecular mechanisms leading to the trafficking of T cells into AT are so far poorly understood. We could show that conditioned media from 3T3-L1 adipocytes can induce CD4<sup>+</sup> and CD8<sup>+</sup> T cell migration when cells were stimulated by LPS or IL-1β in an NF-κB-dependent manner. This finding was substantiated by the observation that T cell migration was reduced to basal levels by overexpression of dominant-negative IkBα in 3T3-L1 adipocytes, clearly indicating the importance of the pathway for the migration process. We can conclude that NF-κB-regulated adipokines are of central importance for the recruitment of T cells. Indeed, for most of the chemokines secreted by AT (CCL20, RANTES, and IP-10), a contribution of NF-κB to their promoter activity could be observed (2). Our data support the strong molecular link of NF-κB to inflammation, metabolic dysregulation in adipocytes, and finally diabetes, which has been shown by analyzing inhibitor of nuclear factor κB kinase β-subunit and salicylate-treated mice (24). Even more, in a recent work, an activation of NF-κB in primary human AT of obese subjects has been observed (5a).

By specifically inhibiting IP-10 activity with an anti-IP-10 antibody, the migration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells toward the media from 3T3-L1 adipocytes was reduced. These data suggest that IP-10 is an important factor for recruitment of T cells in AT. Moreover, we could show that expression of both IP-10
and its receptor CXCR3 increases during weight gain in mouse AT in parallel to T cell infiltration shown recently (11). Notably, CXCR3 is highly expressed on activated T cells (16), mainly on TH1 cells, and recent publications indicate that AT infiltrating T cells represent TH1 cells (5, 6, 15, 21).

Taken together, these observations highlight the role of IP-10 secreted from adipocytes as a new candidate for the recruitment of T cells in AT. CXCR3 is also expressed on myeloid cells; however, the inflammatory process leading to an accumulation of monocytes/macrophages is characterized by low CXCR3 and high chemokine receptor 2 levels, which is mainly responsible for the capacity of monocytes/macrophages to migrate into inflamed sites (17). Therefore, it is rather likely that IP-10 produced by adipocytes mainly recruits TH1-primed T cells into AT. As recently shown, the balance between regulatory and effector CD4+ T cell subsets is a crucial factor of obesity-associated insulin resistance. Moreover, in atherogenesis, it was shown that IP-10 modulates the balance of effector and regulatory T cells. In IP-10-deficient mice, diminished effector T cell trafficking switches the lesional balance to a regulatory phenotype (8). Because atherosclerosis and obesity share common pathophysiological features, a similar functional role for IP-10 to modulate the local balance of effector and regulatory T cells in obese AT can be postulated. An IP-10 knockout model could provide additional insight into the physiological relevance of IP-10 in the context of AT inflammation.

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DISCLOSURES

The authors declare that there is no duality of interest associated with this manuscript.

REFERENCES