Obesity-related Plasma CXCL10 Drives CX3CR1-dependent Monocytic Secretion of Macrophage Migration Inhibitory Factor

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ABSTRACT

Obesity is characterized by excessive body fat accumulation and comorbidities such as diabetes mellitus, cardiovascular disease, and obstructive sleep apnea syndrome (OSAS). Both obesity and OSAS are associated with immune disturbance, alterations of systemic inflammatory mediators, and immune cell recruitment to metabolic tissues. Chemokine CXCL10 is an important regulator of proinflammatory immune responses and is significantly increased in patients with severe obesity. This research project aims to investigate the impact of CXCL10 on human monocytes in patients with obesity. We studied the distribution of the CD14/CD16 monocyte subsets as well as their CX3CR1 expression patterns in whole-blood measurements from 92 patients with obesity and/or OSAS with regard to plasma CXCL10 values and individual clinical parameters. Furthermore, cytokine secretion by THP-1 monocytes in response to CXCL10 was analyzed. Data revealed significantly elevated plasma CXCL10 in patients with obesity with an additive effect of OSAS. CXCL10 was found to drive monocytic secretion of macrophage migration inhibitory factor via receptor protein CX3CR1, which significantly correlated with the individual body mass index. Our data show, for the first time, to our knowledge, that CX3CR1 is involved in alternative CXCL10 signaling in human monocytes in obesity-related inflammation. Obesity is a multifactorial disease, and further investigations regarding the complex interplay between obesity-related inflammatory mediators and systemic immune balances will help to better understand and improve the individual situation of our patients. *ImmunoHorizons*, 2024, 8: 19–28.

INTRODUCTION

Obesity is a dramatically increasing disease very often accompanied with obstructive sleep apnea syndrome (OSAS). Both increase the incidence of concomitant diseases such as hypertension, insulin resistance, cardiovascular diseases, and nonalcoholic fatty liver disease (1, 2). The synergistic impact of obesity and OSAS has been shown to trigger serious low-grade systemic inflammation, associated with peripheral blood cytokine shifts and immune cell alterations (3–5).

In adipose tissue, increased abundances of proinflammatory immune cells such as M1 macrophages and CD8+ T cells have been observed that secrete proinflammatory cytokines such as IL-1β, IL-6, IL-17, and IFN-γ (6–8). In particular, the CXCL subfamily of chemokines plays an important role in the development of certain comorbidities, such as atherosclerosis and cardiovascular disease (9). Plasma levels of chemokines CXCL10 and CXCL11 were found to be significantly increased in patients with severe obesity compared with healthy control subjects (10, 11), whereas CXCL10 is involved in the regulation of

proinflammatory immune responses via its established receptor protein CXCR3 (12, 13). CXCL10 is also known as IP-10 (IFN-γ–inducible protein 10 kDa) and was first identified after IFN-γ activation of monocytic U937 cells (14, 15). Moreover, CXCL10 can be induced by IFN-α, IFN-β, and LPS in activated T cells, monocytes, endothelial cells, and keratinocytes (16) and exerts chemotactic activity toward human peripheral blood monocytes and T lymphocytes (17). Interestingly, also human adipocytes could be significantly induced to secrete CXCL10 by IFN-γ but not in response to LPS, indicating distinct patterns of CXCL10 regulatory behavior in adipocytes and immune cells (18). In this context, several studies observed a lack of CXCR3 mRNA in monocytes (19), the absence of significant levels of cell surface CXCR3 expression (20), and also no effects of CXCR3 antagonists on CXCR3 ligand-induced monocyte responses, which also suggests the involvement of an alternative receptor protein (21).

Similarly, it has been shown that the absence of CXCR3 signaling in Tstem-l cells promotes the upregulation of other chemokine receptors, such as CXCR6 and CX3CR1 (22, 23). Monocytic CX3CR1 is known to be associated with atherosclerosis and vascular inflammatory processes (24, 25), and increased expression levels of CX3CR1 on classical and intermediate monocytes (IMs) have recently been observed in patients with obesity (26).

However, the immunological consequences of plasma CXCL10 in patients with obesity in terms of peripheral blood monocytes are poorly understood. In the present study, we analyzed the impact of CXCL10 on circulating CD14/CD16 monocyte subsets from 92 patients with obesity and/or OSAS as well as on THP-1 monocytic cells.

The study aimed to better understand the influence of obesity-related inflammatory mediators on individual immunological shifts and the clinical situation of our patients.

MATERIALS AND METHODS

Ethics statement and blood collection

All patients were clinically examined at the Department of Internal Medicine 1 or the Department of Otorhinolaryngology, University Hospital Schleswig-Holstein, Campus Luebeck, Germany. The study was approved by the local ethics committee of the University of Luebeck (approval number 21-183) and was conducted in accordance with the ethical principles for medical research formulated in the World Medical Association Declaration of Helsinki. All subjects have signed an informed written consent and were clarified about the aims of the study and the use of their samples. Blood samples were collected from healthy donors ($n = 10$, mean age 36.4 y, body mass index [BMI] 24.7 kg/m^2) and obese patients ($n = 82$, mean age 46.3 y, BMI 46.8 kg/m^2) and normal weight patients with OSAS ($n = 10$, mean age 48.7 y, BMI 25.7 kg/m^2). Blood was drawn by venipuncture into a sodium citrate–containing S-Monovette (Sarstedt; Nümbrecht, Germany).

Staining of monocyte subsets in whole blood

Within 4 h after blood collection, 20 μl citrate blood was diluted in 80 μl PBS. Blood cells were stained with following Abs: CD45-PE, CD14-FITC, CD16-BV-510, HLA-DR-allophycocyanin-Cy7, and CX3CR1-BV421 (all from BioLegend, San Diego, CA). After 25-min staining in the dark, 650 μl RBC lysis buffer (BioLegend) were added to the samples and incubated for another 20 min. Subsequently, the suspension was centrifuged at $400 × g$ for 5 min, and the supernatant was discarded. The cell pellet was resuspended in 100 μl fresh PBS and used for FACS analysis.

FACS analysis

Flow cytometry was performed with a MACSQuant 10 flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany), and data were analyzed using the FlowJo software version 10.0 (FlowJo, LLC, Ashland, OR). All Ab titrations and compensations were performed beforehand. For whole-blood measurements, at least 100,000 CD45$^+$ leukocytes were analyzed. Gating of monocyte subsets was performed as described before (27). In summary, CD45 was used as a pan-leukocyte marker to facilitate whole-blood measurement, and monocytes were first roughly gated by their forward scatter/side scatter characteristics and the positivity for CD14 and CD16. Neutrophil granulocytes and NK cells were excluded by their missing HLA-DR expression. Remaining B cells were excluded by the help of their lack of CD14 expression. Finally, remaining monocytes were subgated into CD14^{++}CD16$^-$ (classical monocytes [CMs]), CD14^{++}CD16$^+$ (IMs), and CD14^{dim+}CD16$^+$ (nonclassical monocytes [NCMs]).

THP-1 cells and culture conditions

For cell culture experiments, the nonadherent monocyte cell line THP-1 (Tohoku Hospital Pediatrics-1) was used. Cell culture was performed in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% sodium pyruvate, and 1% streptomycin/penicillin at 37°C and 5% CO_2 under a humidified atmosphere. Cells were subcultured every 3 d when they reached a maximum density of $1 × 10^6$ cells/ml. For stimulation experiments THP-1 cells were incubated with 100 pg/ml CXCL10 (R&D Systems, Minneapolis, MN).

Cytokine analysis

Plasma concentrations of cytokines CXCL10 and macrophage migration inhibitory factor (MIF) were assessed from citrate-plasma samples and were determined by ELISA according to the manufacturer's protocols (R&D Systems, Minneapolis, MN).

Comprehensive analysis of THP-1 cytokine expression patterns in responses to CXCL10 (R&D Systems) was performed using human cytokine arrays. Therefore, supernatants from cell cultures were collected after incubation and instantly preserved at −80°C until further processing. The Proteome Profiler Human XL cytokine array (R&D Systems) was hybridized with the cell culture medium as recommended by the supplier.

Statistical analysis

Statistical analyses were performed with GraphPad Prism version 7.0f. The mean and SEM are presented. The differences between groups were determined after testing for normal distribution and applying parametric (Student t test), or nonparametric one-way ANOVA with Bonferroni post hoc test. The correlation between parameters was calculated using multivariate regression with the Pearson correlation coefficient ($*p <$ 0.05, $**p < 0.01$, and $***p < 0.001$). Additional statistical details are given in the respective figure legends, when appropriate.

RESULTS

Obesity-related plasma CXCL10

Obesity is accompanied by individually changed levels of different inflammatory cytokines and chemokines. We detected significantly increased plasma levels of chemokine CXCL10 in patients with obesity compared with healthy donors using ELISA measurements (Fig. 1). Correlation analyses revealed significant correlations between the measured plasma CXCL10 values and years of age ($p = 0.0267$), but not with regard to the BMI or cholesterol values of our cohort (all BMI >35 kg/m^2) (Fig. 1). Moreover, obese patients were stratified by status of

OSAS, diagnosed by polysomnography and recordings of snoring and heart rate by a portable device. Our data revealed significantly increased plasma levels of CXCL10 in the OSAS-positive subcohort ($p = 0.0449$), but no significant correlation with regard to the apnea–hypopnea index values (Fig. 2A, 2B) or the individual diabetes status (Fig. 2C, 2D).

Obesity and OSAS occur simultaneously in the majority of patients, and both conditions are known to promote each other. To analyze the unambiguous impact of both conditions on plasma CXCL10, investigations were carried out with normal weight patients with OSAS and patients with obesity but without OSAS compared with healthy donors. Data revealed significantly increased plasma CXCL10 levels in patients with obesity without OSAS compared with healthy donors ($p = 0.0491$), but not in normal weight patients with OSAS (Fig. 3A). Correlation analyses revealed a significant positive correlation between plasma CXCL10 and BMI within the groups of healthy donors and patients with obesity, but not with apnea–hypopnea index values of patients with OSAS (Fig. 3B, 3C).

Impact of CXCL10 on monocyte characteristics

Because plasma CXCL10 expression was found to be elevated in patients with obesity, we next wanted to validate the association

FIGURE 1. ELISA measurements of plasma CXCL10.

(**A**) Measurements of plasma CXCL10 revealed significantly increased values in patients with obesity compared with healthy donors (HDs). Correlation analyses revealed no significant correlations between plasma CXCL10 and (**B**) BMI or (**C**) cholesterol (low-density lipoprotein [LDL]; mmol/L) values, but a significant correlation with patient age (**D**). The Pearson correlation coefficient (r) and p values are given. $p < 0.05$ was considered as significant. $*p < 0.05$.
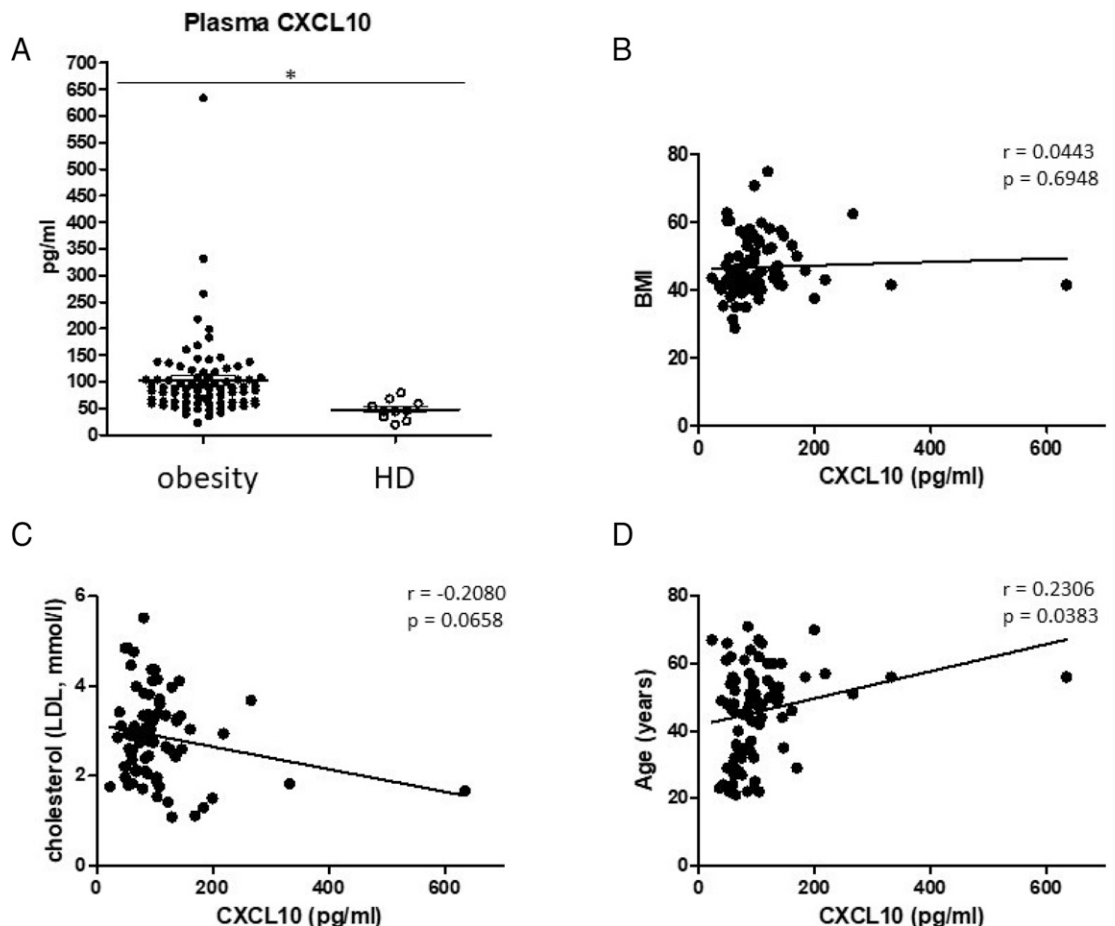
FIGURE 2. Plasma CXCL10 with regard to OSAS and diabetes.
(**A**) Patients with obesity and OSAS revealed significantly increased plasma CXCL10 values compared with patients with obesity but without OSAS. (**B**) There is no significant correlation between plasma CXCL0 and the corresponding apnea–hypopnea index (AHI) values. (**C**) Diabetes has no significant impact on plasma CXCL10 within our obesity cohort, and, correspondingly, there is no correlation between plasma CXCL10 and the corresponding values of glycated hemoglobin (HbA1c in percent) (**D**). The Pearson correlation coefficient (*r*) and *p* values are given. *p* < 0.05 was considered as significant. *p < 0.05.
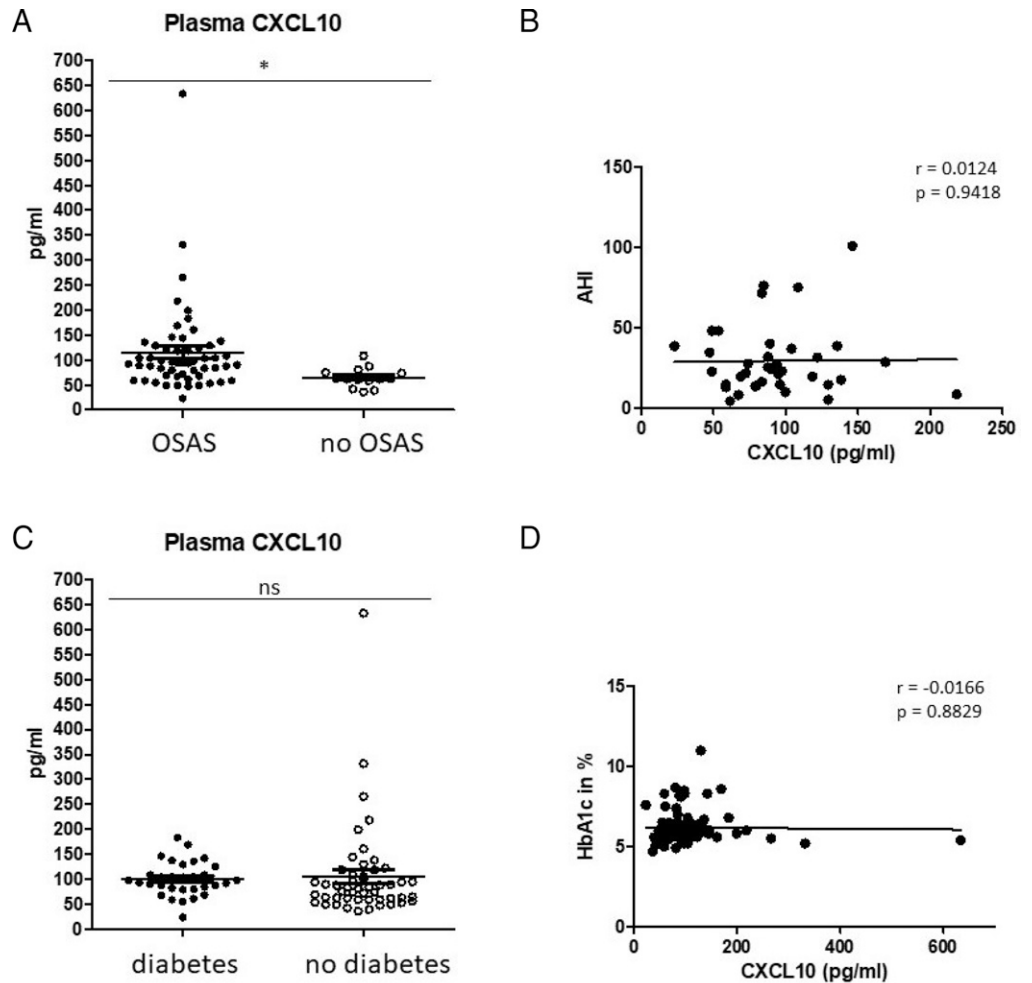
of plasma CXCL10 and alterations of circulating monocytes. Therefore, whole-blood measurements were performed using flow cytometry to analyze CD14/CD16-characterized monocyte subset abundances and CX3CR1 expression levels as previously described (27). Data corroborated significantly decreased abundances of CMs in patients with obesity accompanied by significantly
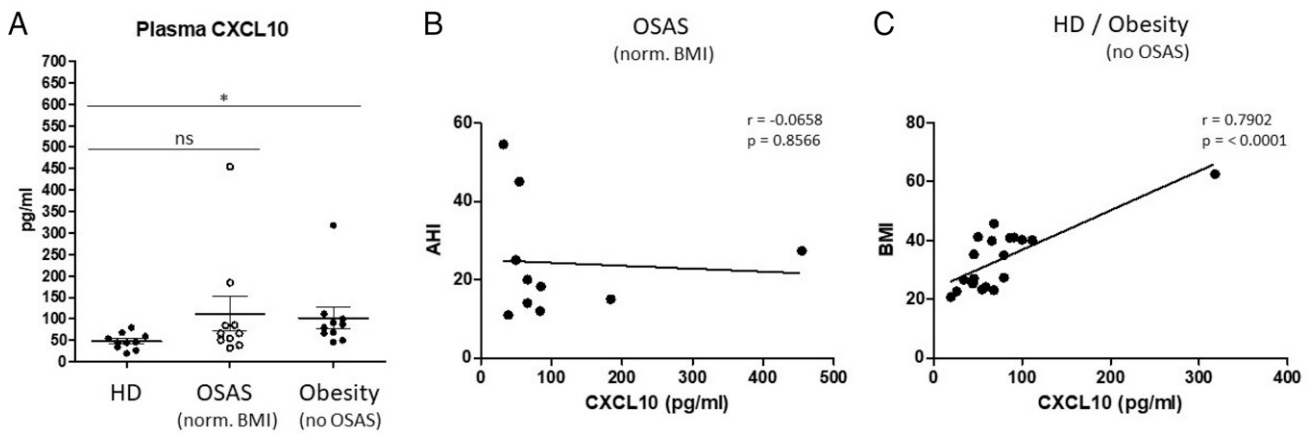

FIGURE 3. Distinguishing OSAS and obesity-related plasma CXCL10.
(**A**) ELISA measurements of plasma CXCL10 in healthy donors (HDs), patients with OSAS with normal BMI, and patients with obesity without OSAS. (**B**) Correlation analyses revealed no significant correlation between plasma CXCL10 and the corresponding apnea–hypopnea index (AHI) values. (**C**) Correlation analyses revealed a significant positive correlation between plasma CXCL10 and the corresponding BMI values. The Pearson correlation coefficient (*r*) and *p* values are given. *p* < 0.05 was considered as significant. *p < 0.05.
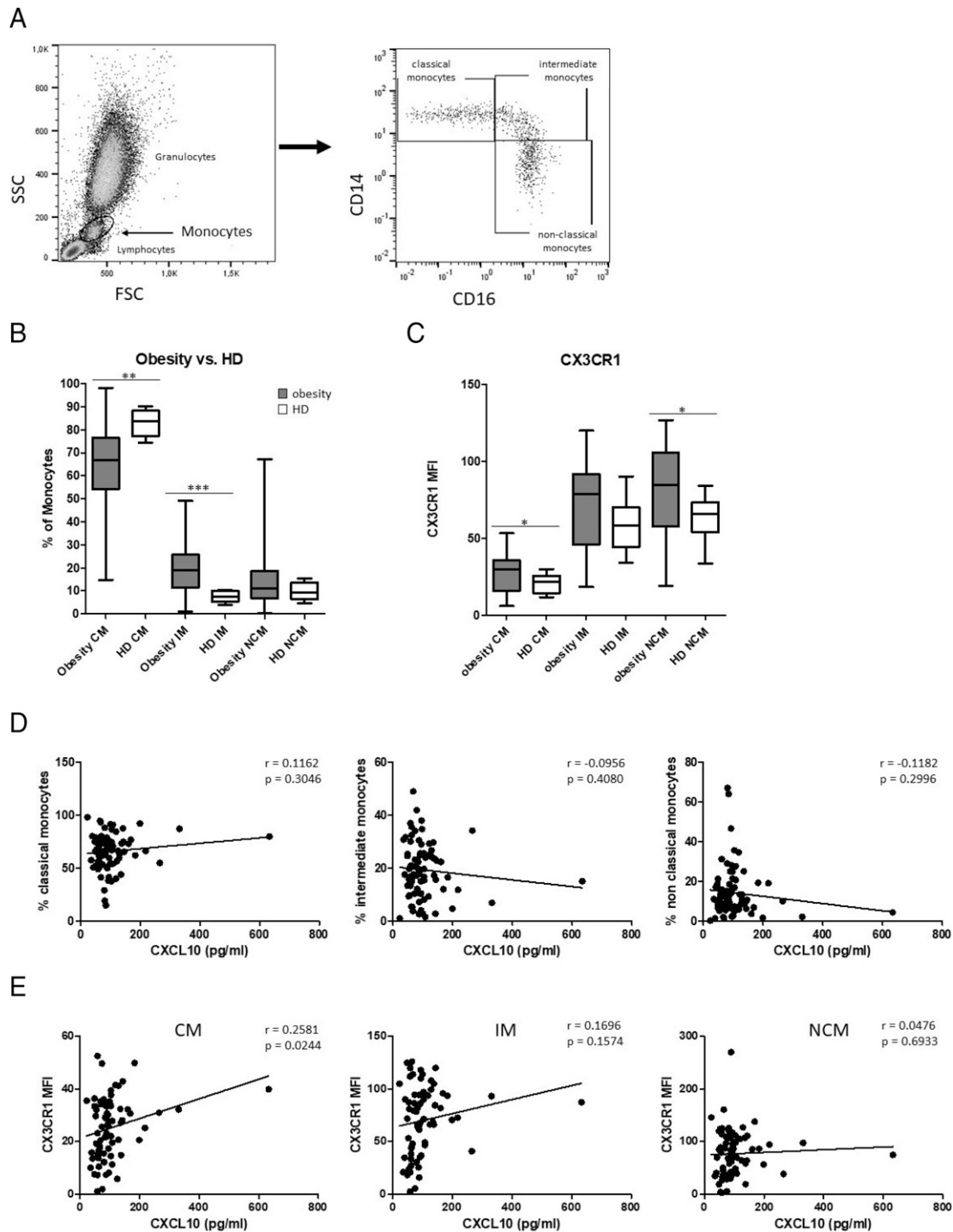
FIGURE 4. Plasma CXCL10-related monocyte subset characteristics.
(**A**) Representative example gating scheme of peripheral blood monocyte subset analysis using flow cytometry. (**B**) Flow cytometric whole-blood analysis revealed significantly decreased percentages of CMs accompanied by significantly increased percentages of IMs in patients with obesity compared with healthy donors (HDs). (**C**) Significantly increased CX3CR1 expression was found on CMs and NCMs from patients with obesity compared with HDs. (**D**) Data revealed no significant correlation between plasma CXCL10 and the percentages of CMs, but not IMs and NCMs. (**E**) There is a significant correlation between plasma CXCL10 and CX3CR1 mean fluorescence intensity (MFI) on CMs. The Pearson correlation coefficient (*r*) and *p* values are given. $p < 0.05$ was considered as significant. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

increased percentages of the IM and NCM subsets, but no significant correlation with regard to the corresponding plasma CXCL10 levels (Fig. 4A, 4B). Furthermore, surface expression of CX3CR1 was measured and revealed significantly increased CX3CR1 expression levels on CMs ($p = 0.0205$) and NCMs ($p = 0.0223$) from patients with obesity compared with healthy donors (Fig. 4C). A significant correlation was found between plasma CXCL10 and CX3CR1 expression levels on CMs ($p = 0.0244$), but not IMs ($p = 0.1574$) or NCMs ($p = 0.6933$) (Fig. 4C).

To determine cytokine secretion patterns of THP-1 monocytes in responses CXCL10 treatment, secretion of 105 different cytokines and chemokines in supernatants of the treated monocyte cell cultures were screened using a human cytokine Ab array. Semiquantitative analyses were performed by measuring the density of the resulting dots (Fig. 5A). Data revealed increased secretion levels of CD31 (PECAM-1), MIF, and CD147 (basigin) in response to CXCL10 stimulation compared with the internal medium control. The addition of inhibitory anti-CX3CR1 Abs could significantly inhibit CXCL10-driven secretion of these cytokines (Fig. 5B). Particularly, the expression of MIF has been associated with obesity and its comorbidities. Therefore, ELISA

measurements were carried out to verify and quantify these findings. Data corroborated significantly increased MIF secretion by THP-1 monocytes in response to CXCL10 treatment ($p = 0.0229$) and its significant inhibition by anti-CX3CR1 Abs ($p = 0.0179$) (Fig. 5C).

Obesity-related plasma MIF and monocytic CX3CR1

Plasma MIF levels were found to be significantly increased in patients with obesity compared with healthy donors (Fig. 6A). Further comparative analyses revealed significantly higher plasma MIF levels in patients with obesity and without OSAS compared with normal weight patients with OSAS ($p \leq 0.001$) and healthy donors ($p = 0.0043$), but also significantly increased MIF values in normal weight patients with OSAS compared with healthy control subjects (Fig. 6B). Plasma MIF significantly correlated with patients' BMI values of healthy donors and patients with obesity (Fig. 6C). Moreover, data revealed a significant positive correlation between plasma MIF concentrations and monocytic CX3CR1 expression in CMs ($p = 0.0283$), IMs ($p = 0.0300$), and NCMs ($p = 0.0277$) (Fig. 6D), which is consistent with the CX3CR1-dependent MIF secretion by THP-1 cells in
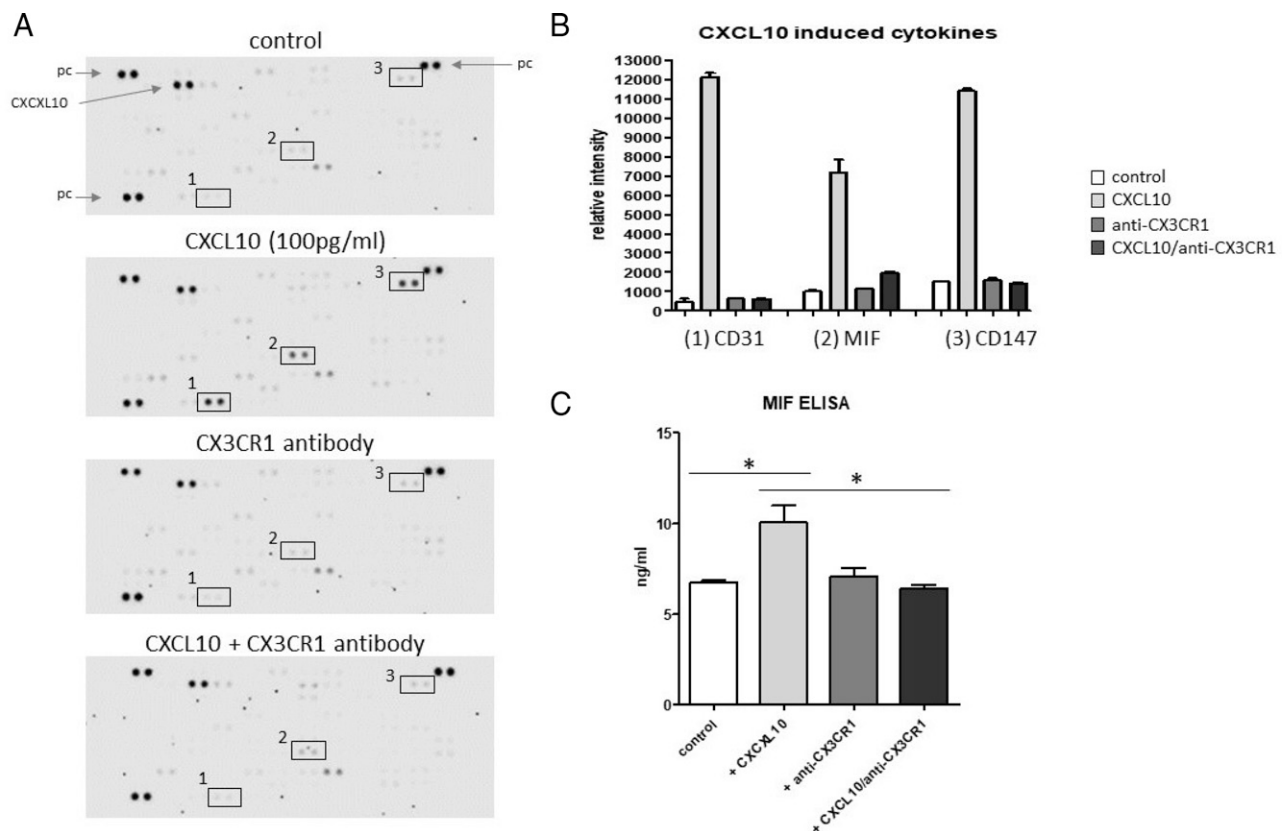

FIGURE 5. Impact of CXCL10 on cytokine secretion of THP-1 monocytes.

(**A**) Raw images of cytokine arrays of THP-1 cell culture supernatants after 24 h of treatment with CXCL10 (100 pg/ml) and/or inhibitory anti-CX3CR1 Ab. Numbers indicate differential densities of bands of certain cytokines (1, CD31; 2, MIF; 3, CD147). (**B**) Semiquantitative analysis was performed by measuring the density of the dots and revealed differential secretion patterns of different cytokines (CD31, MIF, CD147) in response to CXCL10, which could be prevented by the addition of anti-CX3CR1 Abs. (**C**) ELISA measurements verified significantly increased secretion of MIF in response to CXCL10 treatment and the inhibitory effect of CX3CR1 blockade. *$p < 0.05$. Internal positive control (pc) and CXCL10 dots are indicated.
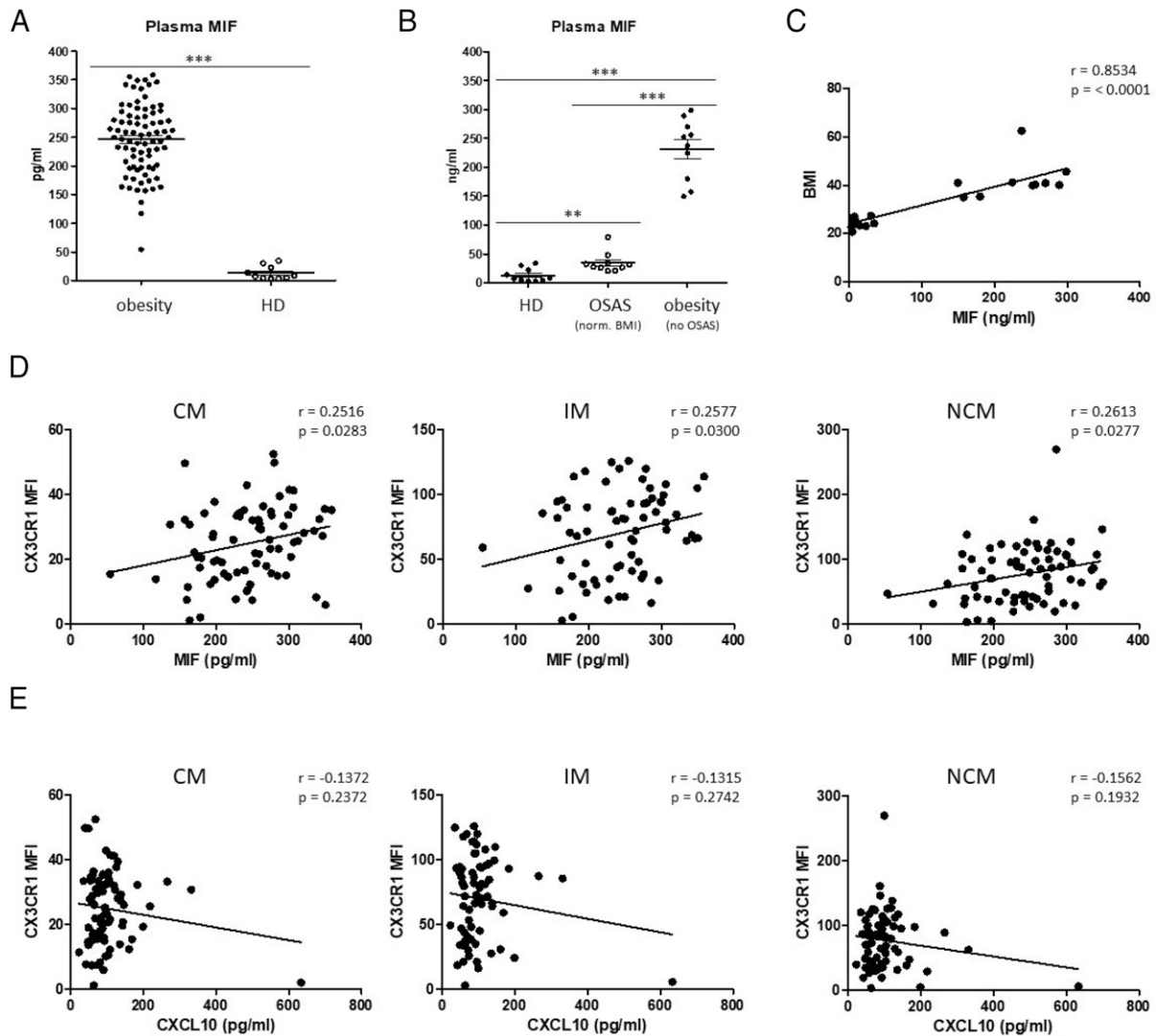
FIGURE 6. MIF in patients with obesity.

(**A**) ELISA measurements of plasma MIF in patients with obesity and healthy donors (HDs). (**B**) Measurements of plasma MIF in healthy donors, patients with OSAS with normal BMI, and patients with obesity without OSAS. (**C**) Correlation analyses revealed significant correlations between plasma MIF and the corresponding BMI values. (**D**) Data revealed significant correlations between plasma MIF and CX3CR1 mean fluorescence intensity (MFI) on CMs, IMs, and NCMs, but (**E**) no significant correlation between plasma CXCL10 and CX3CR1 expression values. The Pearson correlation coefficient (*r*) and *p* values are given. $p < 0.05$ was considered as significant. ***p < 0.001.

response to CXCL10. Correlation analysis between plasma CXCL10 values and CX3CR1 expression levels of the different monocyte subsets revealed no significant correlation (Fig. 6E).

DISCUSSION

Obesity-related plasma CXCL10

CXCL10 is involved in the regulation of different biological functions, such as chemotaxis, activation, and differentiation of inflammatory cells in innate and adaptive immunity. Our data revealed significantly increased plasma CXCL10 concentrations in patients with obesity compared with healthy donors, which was even significantly elevated in patients with an additional

OSAS but not influenced by the individual diabetes status. Moreover, the presence of additional unknown possible confounders, such as cardiovascular disease and stroke, might influence the individual immunologic situation and might have to be further investigated. A significant positive correlation was found between plasma CXCL10 and the age of the patients, which corroborates data from earlier studies, where increased serum CXCL10 levels have been found in association with aging (28, 29).

Besides its secretion in response to IFN-α, IFN-β, or LPS (16), it has been shown that increased circulating IFN-γ and TNF-α induce synergistically the secretion of CXCL10 by podocytes, attracting activated macrophages into kidney tissue (30). In human THP-1 monocytes, TNF-α revealed even more

potential than IFN-γ to induce CXCL10 in an NF-κB–dependent manner (31).

In contrast to THP-1 monocytic cells, human peripheral blood monocyte subsets can phenotypically be distinguished into CMs (CD14^{++}CD16$^−$), IMs (CD14$^+$CD16$^+$), and NCMs (CD14^{dim+}CD16$^+$), based on their CD14 and CD16 surface expression levels (32–34). Both IMs and NCMs are the main sources of proinflammatory cytokines (32, 35, 36), and elevated abundances have been associated with inflammatory diseases, such as rheumatoid arthritis, asthma, and also OSAS and obesity (26, 27, 37–40).

Human circulating monocytes were identified as a major source of CXCL10 secretion in the peripheral blood of patients with malaria (41); in turn, however, CXCL10 has also been identified as a regulator of monocyte cytokine production (42).

Our investigations revealed increased secretion patterns of cytokines CD147 (basigin) and CD31 (PECAM-1) by THP-1 monocytes in response to CXCL10 stimulation. CD147 has been shown to mediate platelet–monocyte interactions and monocyte recruitment to the vascular wall (43), and elevated monocytic CD147 has been associated with monocyte invasion in rheumatoid arthritis (44). CD31 (PECAM-1) is a member of the Ig superfamily; is involved in the regulation of leukocyte transmigration; and can be found on platelets, monocytes, or neutrophils (45, 46).

Moreover, our data revealed increased secretion levels of MIF by human monocytes in response to CXCL10, which was found to be dependent on receptor molecule CX3CR1. MIF is expressed in mature adipocytes and macrophages and is associated with obesity and insulin resistance (47). It has been shown that MIF deficiency results in a reduction of monocyte adhesion and macrophage accumulation in adipose tissues (47–49).

In humans, there are 16 representatives of CXC motif chemokines and at least 6 different CXC motif receptor proteins with different specificities, all of which are involved in immune regulation (50). Although there is some evidence to suggest the involvement of CX3CR1 in alternative CXCL10 signaling, it is not yet fully understood, and also the involvement of additional receptors has to be further elucidated.

Both human and mouse CD16$^+$ monocyte subsets have been shown to express high levels of the chemokine receptor CX3CR1 and to respond to CX3C chemokine ligand 1 (51). An upregulation of CX3CR1 in monocyte subpopulations from patients with obesity was also shown in a previous study (39). CX3CR1 is associated with atherosclerosis and vascular inflammatory processes (24, 25) and contributes to the accumulation of tumor-associated macrophages in patients with skin cancer (52). We have shown significant correlations between plasma MIF and CX3CR1 expression on all three monocyte subsets in patients with obesity. These data suggest that CX3CR1/MIF signaling might participate in the accumulation of obesity-associated macrophages in adipose tissues, although only little is known about CX3CR1 in terms of the differentiation pattern of human macrophage subsets (53). Further investigations on primary monocyte subsets in larger cohorts in correlation with adipose tissue-infiltrating macrophages are needed to unravel the impact of monocytic CXCL10/CX3CR1/

MIF signaling on obesity-related systemic inflammation and its concomitant diseases.

DISCLOSURES

The authors have no financial conflicts of interest.

ACKNOWLEDGMENTS

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