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Adiponectin stimulates release of CCL2, -3, -4 and -5 while the surface abundance of CCR2 and -5 is simultaneously reduced in primary human monocytes

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ABSTRACT

The adipokine adiponectin is well known to affect the function of immune cells and upregulation of CCL2 by adiponectin in monocytes/macrophages has already been reported. In the current study the effect of adiponectin on CCL2, -3, -4, and -5 and their corresponding receptors CCR1, CCR2, and CCR5 has been analyzed. Adiponectin elevates mRNA and protein of the CC chemokines in primary human monocytes. Simultaneously the surface abundance of CCR2 and CCR5 is reduced while CCR1 is not affected. Downregulation of CCR2 by adiponectin is blocked by a CCR2 antagonist although expression of the CCL2 regulated genes CCR2 and TGF-beta 1 is not altered in the adiponectin-incubated monocytes. CCL2, -3, and -5 concentrations measured in supernatants of monocytes of normal-weight (NW), overweight (OW), and type 2 diabetic (T2D) patients positively correlate with BMI and are increased in obesity and T2D. In contrast CCL4 is similarly abundant in the supernatants of all of these monocytes. The degree of adiponectin-mediated induction of the chemokines CCL3, -4, and -5 negatively correlates with their basal levels and upregulation of CCL3 and CCL5 is significantly impaired in OW and T2D cells. Serum concentrations of these chemokines are almost equal in the three groups and do not correlate with the levels in monocyte supernatants. In conclusion these data demonstrate that adiponectin stimulates release of CCL2 to CCL5 in primary human monocytes, and induction in cells of overweight probands is partly impaired. Adiponectin also lowers surface abundance of CCR2 and CCR5 and downregulation of CCR2 seems to depend on autocrine/paracrine effects of CCL2.

1. Introduction

Chemokines of the CC family attract mononuclear cells to sites of inflammation [1]. CCL2 (Chemokine CC Motif Ligand 2, also known as Monocyte Chemoattractant Protein-1, MCP-1) is the most extensively studied chemokine so far [1]. CCL2 deficiency is associated with a higher susceptibility to infections and impaired immune response [2,3]. However, concentrations of this chemokine are also increased in metabolic diseases and contribute to systemic and tissue inflammation [4]. In atherosclerotic lesions higher CCL2 production promotes infiltration of monocytes thereby accelerating foam cell formation [5]. In obesity CCL2 attracts monocytes to fat tissues increasing local and systemic inflammation [6]. CCL2 mRNA expression is also found induced in peripheral monocytes of type 2 diabetic patients [7]. CCL2 is the ligand of the G-protein coupled receptor CCR2 whose expression is increased in monocytes of hypercholesterolemic and type 2 diabetes patients [8,9]. Blockage of CCR2 or CCR2 deficiency protects from obesity, inflammation, and insulin resistance in rodent models of obesity [6,10].

The role of other CC chemokines including Macrophage Inflammatory Protein (MIP)-1α (CCL3), MIP-1β (CCL4), and CCL5 (Regulated on Activation Normal T-cell Expressed And Secreted, RANTES), and their corresponding receptors CCR1 (CCL3, CCL5) and CCR5 (CCL3, CCL4 and CCL5) has been less well characterized in metabolic diseases [1]. These chemokines are all released by monocytes and their synthesis is enhanced by lipopolysaccharide stimulation [11]. Systemic CCL5 is increased in serum of type 2 diabetes patients [12]. Similar to CCL2 this chemokine enhances the recruitment of blood monocytes to adipose tissue. Furthermore, CCL5 protects adipose tissue resident macrophages from apoptosis thereby further elevating fat inflammation [13]. In contrast CCL3 deficiency is not associated with altered accumulation of macrophages in adipose tissue [14]. CCL4 has been mainly analyzed for its role in autoimmune diseases and it protects from insulitis [15].

Adiponectin is an adipocyte-derived adipokine with immunoregulatory, antidiabetic, and antiatherosclerotic properties [16,17]. Adiponectin exerts anti-inflammatory effects in activated monocytes and suppression of LPS-mediated IL-6 and CXCL8

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synthesis has been shown [18,19]. In non-stimulated cells these proteins are even induced by adiponectin suggesting an immunoregulatory function of this adipokine [20,21]. Upregulation of CCL2 by adiponectin has been demonstrated in cultured human microvascular endothelial cells, colonic epithelial cells, and monocytes/macrophages [20–22]. Adiponectin mediated induction of this chemokine is even essential for the appropriate expression of CCL2 in response to *Listeria monocytogenes* infection and immune defense [23].

So far it has not been analysed whether chemokine receptors are altered upon adiponectin incubation. Monocytes express CCR1, CCR2, and CCR5, and mRNA expression is reduced upon LPS treatment [24]. LPS further induces internalisation and subsequent degradation of CCR2 [25]. Short-term incubation of monocytes with CCL2 causes rapid CCR2 internalization but prolonged incubation with this chemokine induces CCR2 expression [26,27]. Therefore, it may be speculated that at least abundance of CCR2 is altered in adiponectin-treated monocytes because of paracrine/autocrine effects due to increased CCL2 concentrations.

In the current study it was analysed whether adiponectin regulates CCL2, CCL3, CCL4 and CCL5 and their corresponding receptors CCR1, -2, and -5 in primary human monocytes.

2. Materials and methods

2.1. Subjects

The study protocol was approved by the local ethics committee and the investigation conforms with the principles outlined in the Declaration of Helsinki (1997). Each proband gave written informed consent to participate in the study.

Monocytes were isolated from the blood of 11 male T2D patients, 12 male controls with a BMI > 25 kg/m^2 (overweight control group, OW) and 10 male controls with a BMI $\leqslant$ 25 kg/m^2 (normal-weight controls, NW). T2D patients were treated with drugs like metformin. Further two patients were treated with ACE inhibitors, one patient with lipid lowering drugs, and two patients with β-blockers. In the OW group one patient was treated with lipid lowering drugs. In the NW group one patient got ACE inhibitors and one patient was treated with lipid lowering drugs. The characteristics of the study group are summarized in Table 1. This cohort includes patient samples of recently described study groups [28,29].

2.2. Material

Recombinant human adiponectin expressed in a mammalian cell line, CCL2-, CCL3-, CCL4-, and CCL5 ELISAs were from R&D Systems (Wiesbaden-Nordenstadt, Germany). Antibodies to detect CCR1, CCR2, and CCR5 by flow cytometry were from R&D Systems (Wiesbaden-Nordenstadt, Germany). Immunoblot analysis was done using antibodies for CCR1, -2 and -5 from Biozol (Eching, Germany). Anti-mouse PE-conjugated IgG antibody was from DAKO (Glostrup, Denmark). CCR2 antagonist RS-102895 was from Sigma Bioscience (Deisenhofen, Germany) and 20 μM were used.

2.3. Real-time polymerase chain reaction (PCR)

Real-time PCR was performed using LightCycler FastStart DNA Master SYBR Green I from Roche (Mannheim, Germany) as described elsewhere [21,30]. The primer sequences are listed in Table 2. Data were normalized to β-actin mRNA levels. The specificity of the PCRs was confirmed by sequencing of the PCR fragments (Geneart, Regensburg, Germany).

2.4. Isolation and culture of primary blood monocytes

Peripheral blood leukocytes were isolated from 40 ml of whole blood using Vacutainer CPT systems (Becton Dickinson, Franklin Lakes, NJ) as described [31]. Primary human monocytes were purified by magnetic separation with CD14 beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Serum was coagulated with Thromborel S (Roche, Mannheim, Germany) and $CaCl_2$ (Calbiochem-Merck, Darmstadt, Germany) and was dialyzed two times against phosphate buffered saline (PBS) for 2 h each. Flow cytometry analysis revealed that about 95% of the isolated cells were monocytes [31]. 3×10^6 monocytes were cultivated in 3 ml RPMI supplemented with 10% autologous serum for 24 h. Subsequently, the medium was replaced with 1 ml fresh medium. For adiponectin stimulation monocytes were incubated for 24 h with 10 μg/ml adiponectin until indicated otherwise. Cells and supernatants were collected 24 h later and used for ELISA and western blot analysis.

2.5. Elisa

ELISAs were performed as recommended by the distributor. Supernatants were diluted 10-fold to measure CCL2, 4-fold to measure CCL3, 2-fold to measure CCL4, and 5-fold to measure CCL5. CCL5 serum levels are in the ng/ml range and concentrations added to the monocyte culture medium were subtracted before performing calculations. Serum was used undiluted for CCL2 and CCL4 determinations, 2-fold diluted for CCL3 and 100-fold diluted for CCL5 determinations.

2.6. Immunoblot

Cells were solubilized in radioimmunoprecipitation assay lysis buffer. Proteins (10–20 μg) were separated by SDS–PAGE and were transferred to polyvinylidene difluoride membranes (Bio-Rad, Mu-

Table 1
Anthropometrical and biochemical characteristics of the study groups used for the isolation of blood monocytes (all males).

	T2D	OW	NW	*p*-value
Probands (*n*)	*n* = 11	*n* = 12	*n* = 10	
Age	62 (42–76)	56 (40–63)	55 (49–67)	
BMI (kg/m^2)	32.7 (27.3–45.9)	30.4 (26.8–36.5)	22.8 (20.1–24.4)	<0.001[2,3]
WHR	1.07 (0.91–1.10)	1.00 (0.89–1.13)	0.92 (0.83–0.99)	0.001[2,3]
Glucose (mg/dl)	143 (100–171)	105 (74–101)	97 (84–98)	<0.001[1,3]
Chemokine and adiponectin serum concentrations				
CCL2 (pg/ml)	30.5 (13.0–70.1)	25.0 (18.7–71.2)	26.0 (12.5–67.1)	
CCL3 (pg/ml)	325.6 (0.5–788.5)	326.1 (0.3–536.9)	317.7 (0.4–660.9)	
CCL4 (pg/ml)	19.8 (14.5–39.7)	30.5 (10.8–36.3)	26.7 (12.7–74.0)	
CCL5 (ng/ml)	55.0 (7.5–120.0)	64.7 (7.9–139.0)	65.5 (9.0–144.0)	
Adiponectin (μg/ml)	1.6 (0.6–3.8)	2.5 (1.0–5.4)	2.4 (1.1–4.4)	

Body mass index (BMI), normal-weight controls (NW), overweight controls (OW), type 2 diabetes (T2D), waist to hip ratio (WHR).
Significance of T2D versus OW = 1, of OW versus NW = 2, of T2D versus NW = 3.

Table 2
Sequences of the primers used for real-time PCR.

Gene	Universe primer 5′ → 3′	Reverse primer 5′ → 3′
CCL2	TTCTGTGCCTGCTGCTCAT	CACCAATAGGAAGATCTCAGTGC
CCL3	GCAACCAGTTCTCTGCATCA	GCTTCGCTTGGTTAGGAAGA
CCL4	CCCAGCCAGCTGTGGTAT	AGGAACTGCGGAGAGGAGTC
CCL5	CCTCATTGCTACTGCCCTCT	ACACACTTGGCGGTTCTTTC
CCR1	TCTGAGTCCCAGAGCCAATC	CCAACCCCCTATCAGCTACA
CCR2	GGAGAGTTTGGGAACTGCAA	CCACCCCCTTCTCATAATCA
CCR5	GACAGAGCTGGTTGGCAAGA	TCCCTCCTTCCCATCCTTAC
TGFbeta	ACTGCAAGTGGACATCAACG	GGGTTATGCTGGTTGTAC
β-Actin	CTACGTCGCCCTGGACTTCGAGC	GATGGAGCCGCCGATCCACACGG

nich, Germany). Incubations with antibodies (dilution 1:1000) were performed in 1.5% BSA in PBS and 0.1% Tween overnight. Detection of the immune complexes was carried out with the enhanced chemiluminescence Western blot detection system (Amersham Pharmacia, Deisenhofen, Germany). Quantification was done using Optiquant software.

2.7. Flow cytometry

Monocytes of four (CCR1 measurement) to six (CCR2 and CCR5 analysis) different donors were isolated and 5×10^6 cells were cultivated on petriPERM dishes (Sarstedt, Nürnbrecht, Germany) for 24 h as described above. Medium was replaced and cells were incubated with or without recombinant adiponectin (10 μg/ml) for 24 h. For cell removal, petriPERM dishes were placed on a cold metal block for 10 min and cells were collected in cold PBS. After centrifugation, cells were incubated on ice for 1 h with 25 μg/ml CCR1, CCR2 or CCR5 antibody or the respective isotypes, IgG1 (isotype for CCR2 and CCR5 antibodies) and IgG2b (isotype for CCR1 antibodies). Following, cells were washed with cold PBS, resuspended in 100 μl ice-cold PBS and incubated with anti-mouse R0439 PE-conjugated antibody (1:50-fold diluted) for 45 min on ice in the dark. Subsequently cells were washed with ice-cold PBS and resuspended in 200 μl ice-cold PBS. Fourcolor flow cytometric analysis was performed using a FACSCalibur flow cytometer (BD, Heidelberg, Germany). Data were acquired with CELL-Quest software (BD, Heidelberg, Germany).

2.8. Statistics

Data are presented as the median values and the range of the values (PASW Statistics 17.0). Data in Fig. 1 are shown as mean ± standard error of the mean because data of only two different experiments using monocytes of two donors are shown. Statistical differences were analyzed by two-tailed Mann–Whitney *U* Test or Studentś *t*-test for paired samples and a value of $p < 0.05$ was regarded as significant. The Spearman-Rho correlation was calculated using the PASW Statistics 17.0 software.

3. Results

3.1. Adiponectin upregulates chemokine mRNA and protein in human monocytes

Monocytes were isolated from the blood of two normal-weight donors and were incubated with different concentrations of adiponectin for 24 h. Release of CCL2, -3, -4 and -5 was dose-dependently increased when 2.5, 5.0, 7.5 and 10 μg/ml adiponectin were used (Fig. 1A–D). Higher concentrations of 12.5 and 15 μg/ml did not further elevate CCL3 and CCL4 whereas CCL2 and CCL5 even tended to be decreased (Fig. 1A–D). Therefore, 10 μg/ml adiponectin was used for further studies.

Chemokines were measured in the supernatants of monocytes isolated from the blood of eight normal-weight donors and subsequently cultivated with or without 10 μg/ml adiponectin for 24 h. CCL3, -4, and -5 were all significantly increased in the supernatants of adiponectin-treated cells (Fig. 2B–D), as well as CCL2 which has already been described to be induced by adiponectin [17]

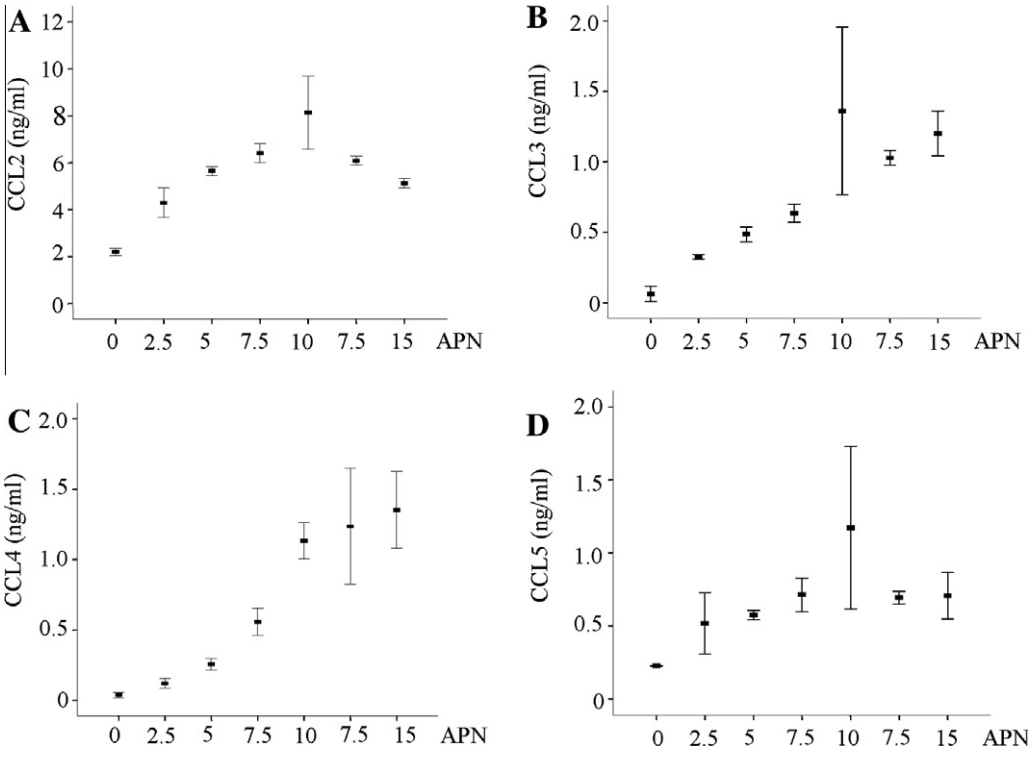

Fig. 1. Dose-dependent upregulation of CCL2 to -5 by adiponectin in human monocytes (A) CCL2, (B) CCL3, (C) CCL4, and (D) CCL5 in the supernatants of monocytes incubated with PBS as control or different concentrations of adiponectin (APN) for 24 h. Data of two independent experiments are shown as mean ± standard error of the mean.

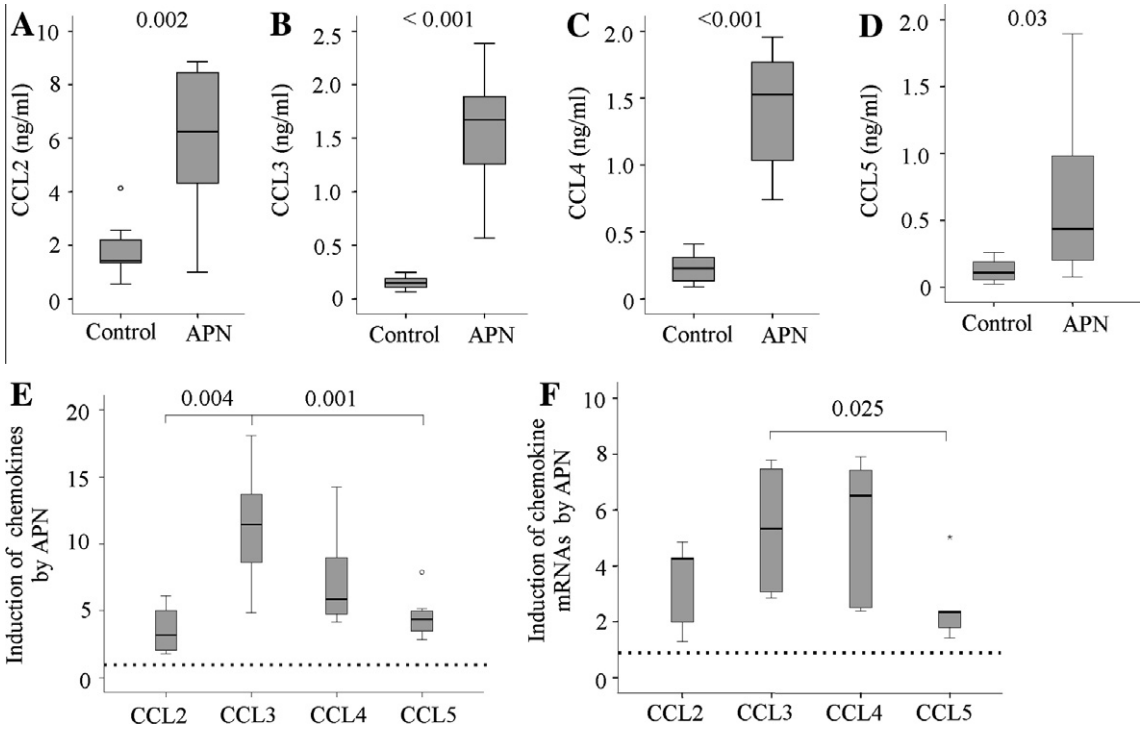

Fig. 2. Adiponectin induces CCL2 to -5 in human monocyte (A) CCL2, (B) CCL3, (C) CCL4, and (D) CCL5 in the supernatants of monocytes of eight donors incubated with PBS as control or adiponectin (APN) for 24 h. Number in the figures indicate the *p*-values. (E) Fold induction of chemokines in the supernatant of adiponectin incubated cells compared to PBS incubated monocytes of the identical donor. The dotted line indicates "no induction". (F) Fold induction of chemokine mRNA in adiponectin incubated cells compared to PBS incubated monocytes of the identical donor. The dotted line indicates "no induction".

(Fig. 2A). CCL3 was significantly more induced than CCL2 and CCL5 and tended to be more efficiently upregulated compared to CCL4 whereas the effect of adiponectin on CCL2, CCL4, and CCL5 was similar (Fig. 2E). The mRNA expression of these chemokines was analysed by real-time RT-PCR in monocytes isolated from the blood of five different donors and was found significantly enhanced in the adiponectin-incubated cells ($p = 0.034$ for CCL2, $p = 0.017$ for CCL3, $p = 0.026$ for CCL4 and $p = 0.030$ for CCL5). The effect on CCL3 mRNA levels was higher compared to CCL5 induction (Fig. 2F).

3.2. Adiponectin lowers abundance of chemokine receptors on the cell surface

Surface abundance of CCR1, CCR2, and CCR5 was analysed by flow cytometry and representative results are shown in Fig. 3A. In monocytes of four different donors incubated with adiponectin for 24 h surface levels of CCR1 were not altered (Fig. 3B). CCR2 and CCR5 were significantly reduced when monocytes of six different donors were analysed and CCR2 was significantly more suppressed than CCR5 (Fig. 3B). When monocytes of three different donors were preincubated with the CCR2 receptor antagonist RS-102895 for 2 h adiponectin-mediated downregulation of CCR2 was abrogated (Fig. 3C). Further, this antagonist significantly reduced surface CCR2 abundance (Fig. 3C). Chemokine receptor protein was analysed in the cell lysates of monocytes of 10 donors (Fig. 3D and data not shown) and were not reduced upon adiponectin incubation (Fig. 3E). Chemokine receptor mRNA expression was determined in monocytes of eight different donors and was not significantly changed (Fig. 3F).

3.3. Chemokine levels in cells of normal-weight, overweight, and type 2 diabetes patients

Chemokine levels were also measured in the supernatants of monocytes isolated from the blood of normal-weight controls (NW, 10 donors), overweight donors (OW, 12 donors), and T2D patients (11 donors) (Table 1). The levels of CCL2, CCL3, and CCL5 were all increased in cells of OW and T2D probands compared to NW (Fig. 4A, B and D). CCL5 was even higher in the supernatants of T2D compared to OW cells (Fig. 4D). CCL4, however, was similarly concentrated in the supernatants of all of these cells (Fig. 4C). Because chemokine levels were highly variable the log(10) levels were used for further calculations and a positive correlation of logCCL2 ($r = 0.576$, $p < 0.001$), logCCL3 ($r = 0.578$, $p = 0.001$) and logCCL5 ($r = 0.686$, $p < 0.001$) but not logCCL4 ($r = 0.217$, $p = 0.225$) with BMI was identified (Fig. 4E–H). Furthermore, levels of all chemokine were correlated to each other even when adjusted for BMI (Table 3) but not to systemic adiponectin concentrations (data not shown).

3.4. Effect of adiponectin on chemokine levels in cells of normal-weight, overweight, and type 2 diabetes patients

The effect of adiponectin to induce these chemokines was also tested. Here, induction of CCL3 and CCL5 was significantly impaired in monocytes of OW compared to NW donors ($p = 0.009$ for CCL3 and $p = 0.036$ for CCL5) and in cells of T2D compared to NW monocytes ($p < 0.001$ for CCL3 and $p = 0.006$ for CCL5). Adiponectin-mediated upregulation of the chemokines was similar in OW and T2D monocytes (Fig. 5A and B). The degrees of adiponectin-induced upregulation of CCL3 and CCL5 showed a strong negative correlation to BMI ($r = -0.599$, $p < 0.001$ for CCL3 and $r = -0.399$, $p = 0.021$ for CCL5) and were positively correlated to each other ($r = 0.668$, $p < 0.001$) suggesting that similar regulatory pathways are involved (Fig. 5C and data not shown). The latter association was even highly significant after adjusting for BMI ($r = 0.638$, $p < 0.001$). The degree of adiponectin-induced upregulation of CCL4 was negatively associated with basal logCCL4 levels ($r = -0.720$, $p < 0.001$, Fig. 5D). Upregulation of CCL2 by adiponectin was neither associated with its basal levels nor BMI of the blood donors.

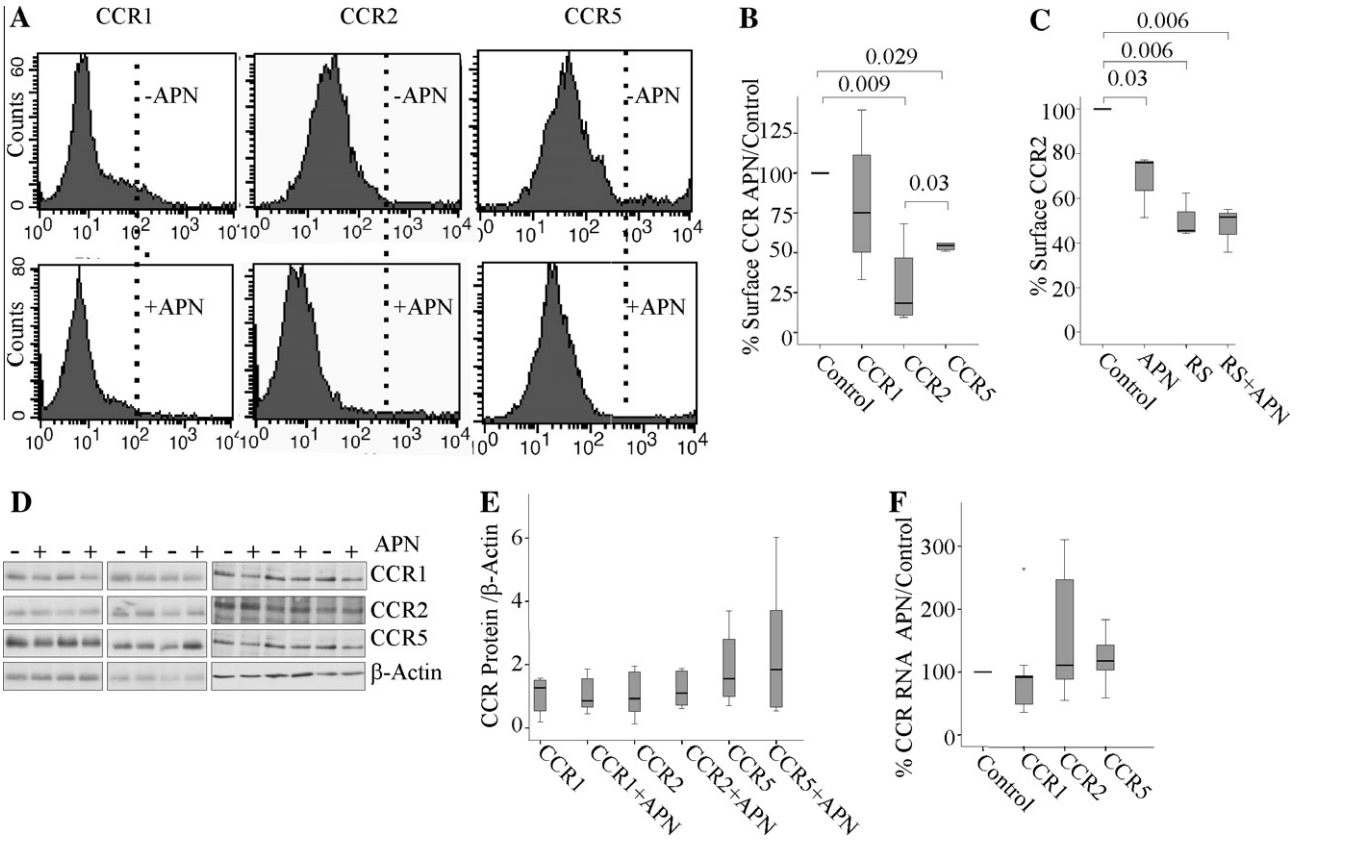

Fig. 3. Surface levels of CCR2 and -5 are reduced in adiponectin incubated cells (A) Flow cytometric analysis of CCR1, CCR2 and CCR5 in monocytes incubated with PBS as control or adiponectin for 24 h. (B) Surface abundance of CCR1, CCR2 and CCR5 in monocytes incubated with PBS as control or adiponectin for 24 h. Number in the figures indicate the *p*-value. (C) Surface abundance of CCR2 in control-incubated monocytes, in cells incubated with adiponectin (APN), with the CCR2 receptor antagonist RS-102895 (RS) or both. (D) CCR1, CCR2 and CCR5 protein in monocytes incubated with PBS as control or adiponectin (APN) for 24 h. (E) Quantification of the data of 10 independent experiments partly shown in C. (F) CCR1, CCR2 and CCR5 mRNA normalized to β-actin mRNA in monocytes of eight different donors incubated with PBS as control or adiponectin (APN) for 24 h.

3.5. Chemokine levels in serum of normal-weight, overweight, and type 2 diabetes patients

Chemokines measured in the serum of these probands (Table 1) did not correlate with concentrations in the supernatants of the respective monocytes (data not shown). Systemic levels of CCL2, CCL3, CCL4, and CCL5 did not correlate with age, BMI, waist to hip ratio, serum adiponectin or fasting glucose of the patients. There was a modest positive correlation of systemic CCL3 and CCL5 ($r = 0.386$, $p = 0.026$).

4. Discussion

In the current study we confirmed previous data showing that adiponectin upregulates CCL2 in primary human monocytes [20,32]. Further, induction of CCL2 mRNA is demonstrated suggesting that transcriptional mechanisms are involved. Besides CCL2, the mRNA and protein levels of the chemokines CCL3, -4, and -5 are also significantly induced by adiponectin. CCL2 is the ligand of CCR2, CCL3 and CCL5 are ligands of CCR1 whereas CCL3, CCL4, and CCL5 bind to CCR5 [1]. Neither mRNA nor total protein levels of these receptors are altered by adiponectin incubation. However, surface abundance of CCR2 is about 4-fold reduced and CCR5 is about 2-fold lower in adiponectin incubated cells. CCR1 surface levels are not reduced by adiponectin. Lower surface levels of at least CCR2 seem to be secondary effects of ligand binding because its downregulation by adiponectin is completely blocked by a CCR2 antagonist. Reduced numbers of cell-surface localized chemokine receptors may desensitize those cells for further chemokine-associated signaling events. Interestingly this antagonist significantly lowers CCR2 surface levels in control- and adiponectin-incubated cells.

Recently it has been shown that CCL2 upregulates CCR2 and TGF-beta 1 mRNA in monocytes [27] but neither CCR2 nor TGF-beta 1 (own unpublished data) expression are increased in the adiponectin incubated cells. Cells studied herein were incubated for 24 h with adiponectin whereas Sakai et al. mainly analysed monocytes which were cultivated with CCL2 for 48 h, and shorter incubation time used herein may partly explain these different findings.

Although adiponectin levels have been found reduced in obesity and metabolic syndrome [33,34] systemic concentration are similar in the cohort analysed herein. Recent work has demonstrated higher adiponectin in patients with impaired renal function and adiponectin has been identified to even predict an increased risk for coronary heart disease [35,36]. Therefore, adiponectin levels are affected by renal dysfunction and coronary heart disease and some of the probands studied herein may have suffered from these complications. Further, the cohorts studied may have been too small to demonstrate lower adiponectin in obesity and type 2 diabetes.

Adiponectin concentrations used in the in vitro studies herein are four- to five-fold higher compared to median systemic levels of the study cohort analysed. Dose–response curves demonstrate a maximal effect when 10 μg/ml recombinant adiponectin is used. When analysing primary cells varying basal chemokine levels and distinct responses to adiponectin are expected. This led us to use

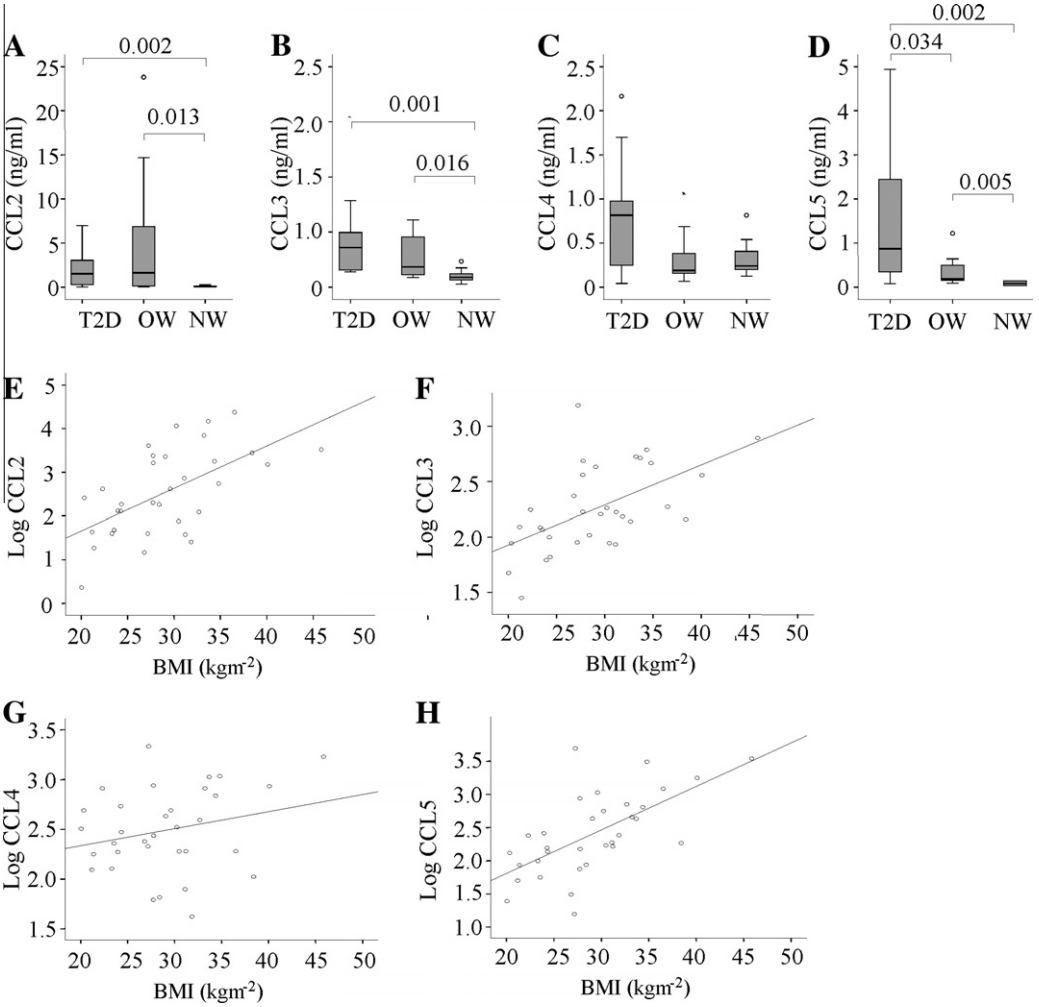

Fig. 4. CCL2 to -5 in the supernatants of monocytes isolated from the blood of type 2 diabetic patients (T2D), overweight (OW) and normal weight controls (NW). (A) CCL2 (B) CCL3 (C) CCL4, and (D) CCL5 in the supernatants of monocytes of T2D, OW and NW. Correlation of BMI with the log(10) of monocyte CCL2 (E), CCL3 (F), CCL4 (G) and CCL5 (H) in the whole study group.

Table 3
Correlation of log(10) chemokine levels in the supernatants of monocytes isolated from the blood of male normal-weight controls, overweight controls and type 2 diabetics. Correlation coefficients after adjusting for BMI are listed, *p*-values are given in brackets.

	log CCL2	log CCL3	log CCl4	log CCL5
log CCL2	1	0.664 (<0.001)	0.510 (0.002)	0.718 (<0.001)
log CCL3	0.664 (<0.001)	1	0.602 (<0.001)	0.634 (<0.001)
log CCL4	0.510 (0.002)	0.602 (<0.001)	1	0.613 (<0.001)
log CCL5	0.718 (<0.001)	0.634 (<0.001)	0.613 (<0.001)	1

the most effective concentration of adiponectin to assure that the cells of all donors respond to this adipokine. Several studies have shown that effects of adiponectin are impaired in obesity and type 2 diabetes [30,37–40] and higher adipokine concentrations may be necessary to stimulate the cells.

Impaired adiponectin-mediated upregulation of CCL3 and CCL5 is also detected in T2D and OW cells compared to NW monocytes. The induction of CCL3 and CCL5 by adiponectin shows a strong positive correlation to each other and is even negatively associated with BMI of the respective blood donors. These findings suggest that monocyte CCL3 and CCL5 synthesis are similarly regulated by adiponectin and this pathway is partly blocked by factors affected in cells of overweight and obese blood donors. In addition, a positive correlation of CCL3 and CCL5 levels is also found in the monocyte supernatants and in serum of the blood donors.

CCL2 and CCL4 are similarly induced by adiponectin in the monocytes of NW, OW and T2D patients. Whereas the induction of CCL2 by adiponectin is not related to its basal levels or BMI of the donors, a strong negative correlation of basal CCL4 and adiponectin mediated upregulation of CCL4 independent of BMI has been identified. This might indicate that monocytes with a higher basal release of CCL4 are more resistant to adiponectin-mediated upregulation of CCL4. However, neither the exact pathways involved in adiponectin-induced chemokine induction nor the reason for higher basal monocyte CCL4 synthesis have been analysed so far and future studies are needed to evaluate the underlying mechanisms.

The data discussed above indicate that different signal transduction pathways are involved in the upregulation of the chemokines studied herein. Furthermore, these data demonstrate that there is no general resistance to adiponectin effects in state of obesity or type 2 diabetes. In fact only specific signal transduction pathways seem to be affected. Similar results have recently been obtained in fat-loaded hepatocytes where only certain actions of adiponectin are blocked [41].

Adiponectin binds to different receptors, AdipoR1 and AdipoR2, which are both expressed in monocytes [30,42] and signaling of

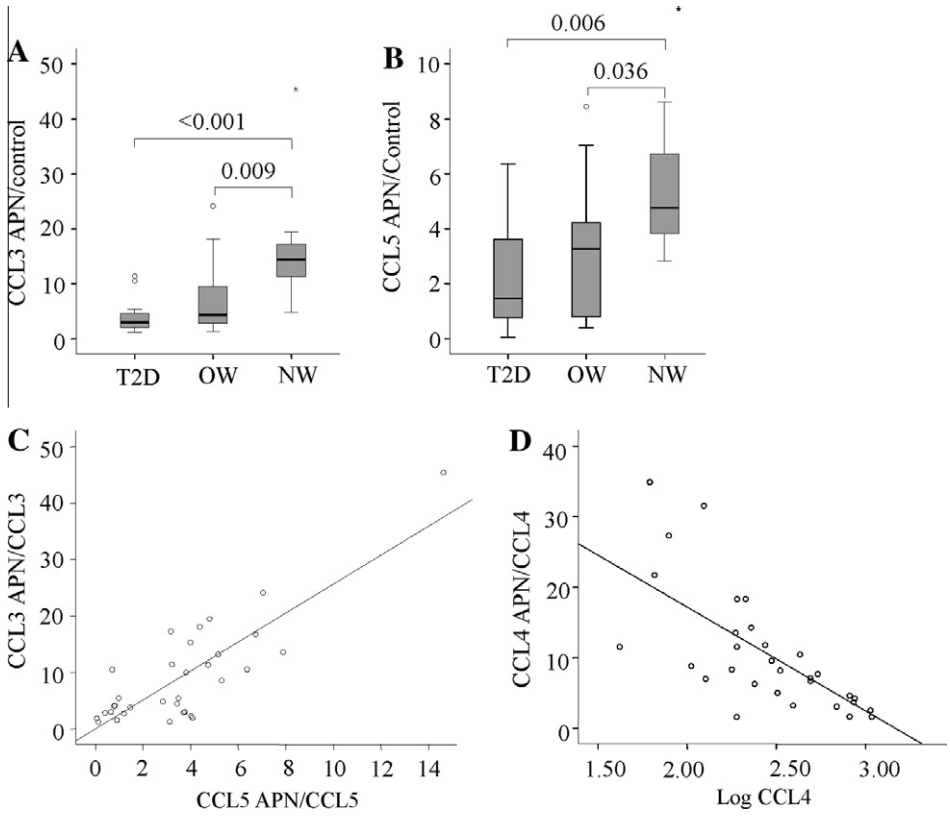

Fig. 5. Impaired upregulation of CCL3 and CCL5 in monocytes of type 2 diabetic patients (T2D) and overweight controls (OW) compared to normal-weight controls (NW). (A) Adiponectin-mediated CCL3 upregulation in T2D, OW and NW monocytes. (B) Adiponectin-mediated CCL5 upregulation in T2D, OW and NW monocytes. (C) Correlation of the degrees of adiponectin-mediated upregulation of CCL3 and CCL5. (D) Correlation of log(10) CCL4 levels with the degrees of adiponectin-mediated CCL4 induction.

just one pathway may be disturbed in obesity. Various adiponectin receptor downstream effectors including p38 MAPK, ERK1/2, AMP-activated protein kinase and NFκB have been identified [42–44] and activity of specific molecules may be differentially affected in obesity.

Recently we described that LPS-induced release of these chemokines is similar in monocytes of controls and T2D patients [11] suggesting that anthropometrical and disease-related factors affect only some pathways as described for adiponectin herein.

Basal levels of CCL2, CCL3, and CCL5 are all increased in the supernatants of OW and T2D monocytes indicating that higher synthesis is related to BMI and not to type 2 diabetes. Interestingly, levels of these chemokines in the supernatants of the monocytes correlates positively with BMI of the blood donors. CCL4, however, is similarly abundant in all of the supernatants and not associated with BMI.

None other than CCL5 is modestly higher in T2D cells compared to monocytes of BMI-matched controls. CCL5 at least in serum has been suggested to be involved in the development of T2D independent of classical risk factors like BMI and dyslipidemia [12]. Low-dose insulin infusion in T2D significantly reduces mononuclear cell CCL5 but not CCL2 expression suggesting that impaired insulin activity may contribute to increased monocyte CCL5 in T2D patients [45]. CCL5 and CCL2 serum levels are reduced upon insulin infusion indicating that at least systemic CCL2 is not influenced by its synthesis in mononuclear cells [45].

Levels of CCL2 and CCL5 have been found increased in serum of type 2 diabetes patients when analyzing rather large cohorts [9,12], and the low number of patient samples studied herein may explain why this increase has not been confirmed. Further, chemokines in serum do not correlate with the respective chemokines in the monocyte supernatants. The chemokines analysed herein are synthesized by various cells and tissues [46–48], and therefore, it is unlikely that monocyte released protein considerably affect systemic levels.

In summary this study shows that adiponectin stimulates synthesis of CCL2 to CCL5 in primary human monocytes, and release of CCL3 and -5 is impaired in obesity. Surface abundance of CCR2 and -5 is reduced in adiponectin-incubated monocytes suggesting that this adipokine regulates CC-chemokine activity at the ligand and receptor level.

Duality of interest statement

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

Author contributions

Study concept and design: CB, HB, AS. Aquisition of data: MN, SB, AK, KE, RW, SA. Drafting of the manuscript: CB Critical revision of the manuscript HB, AS, MN, SB.

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