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Human Dendritic Cells Express the IL-18R and Are Chemoattracted to IL-18

Ralf Gutzmer, Katja Langer, Susanne Momment, Miriam Wittmann, Alexander Kapp, and Thomas Werfel

IL-18 is secreted by a variety of cells such as epithelial cells, macrophages, and dendritic cells (DC), in particular, in areas of chronic inflammation. The effects of IL-18 are complex and not fully understood thus far. We sought to explore human DC as a new target for IL-18, since IL-18R expression has been described on myeloid cells such as macrophages and DC are likely to get in contact with IL-18 at sites of inflammatory reactions. We demonstrate the expression of the IL-18R on human DC in peripheral blood and epidermis, as well as monocyte-derived dendritic cells (MoDC). On MoDC, IL-18R expression is up-regulated by IFN-γ. IL-18 strongly up-regulated CD54 on MoDC, whereas the effect on MHC class II, CD83, and CD86 was only moderate and the expression of CD40 and CD80 was not affected. MoDC primed with IL-18 did not increase their capacity to stimulate the proliferation or IFN-γ production of autologous T cells. However, IL-18 had a direct migratory effect on MoDC as indicated by induction of filamentous actin polymerization and migration in Boyden chamber experiments. In epidermal DC, IL-18 was also able to induce filamentous actin polymerization. Therefore, IL-18 might represent a novel mechanism to recruit DC to areas of inflammation, in particular under Th1 cytokine conditions where IFN-γ is increased such as psoriasis or inflammatory bowel diseases. The Journal of Immunology, 2003, 171: 6363–6371.

Interleukin-18 and its receptor, IL-18R, are structurally related to IL-1 and the IL-1R family. IL-18 is synthesized as an inactive precursor (pro-IL-18) and cleaved by caspase 1 (IL-1β converting enzyme) to yield biologically active IL-18. Similar to other members of the IL-1R family, IL-18R is composed of two chains, the binding chain (IL-1R related protein, IL-18Rα) and the signaling chain (IL-1R accessory protein-like, IL-18Rβ) (1–4).

Pro-IL-18 is produced by professional APCs (macrophages and dendritic cells (DC)) (5–8) and cells not primarily involved in immune responses such as keratinocytes (9), intestinal epithelial cells (10), osteoblasts, rheumatoid arthritis synovial cells (11), and adrenal cortex cells (reviewed in Refs. 1 and 12). The factors influencing IL-18 production and conversion from pro-IL-18 to mature IL-18 are poorly understood thus far (12). However, an increased IL-18 production at the site of inflammation has been described in a number of inflammatory disorders, such as (human) psoriasis (9, 13), (murine) contact hypersensitivity of the skin (14), (human and murine) chronic inflammatory bowel diseases (15–17), and (human) muscular sarcoidosis (8).

The IL-18R is expressed mainly on Th1-cells (18, 19), NK cells (20), and B cells (20, 21) but has also been described on a subset of synovial macrophages (11) and myelomonocytic leukemia cell lines KG-1, MOLM-6, and U937 (22).

Functionally, IL-18 induces in synergy with IL-12 high levels of IFN-γ from human and murine T cells and NK cells, and was therefore originally described as an IFN-γ inducing factor (2, 23). The picture of IL-18 as an inductor of a Th1-cytokine profile has been fostered by the deficiency of Th1-responses in IL-18R knockout mice (24). However, in recent years a number of reports indicate a more complex immunologic and nonimmunologic role for IL-18 (reviewed in Refs. 1, 25, and 26). IL-18 is able to induce Th2 cytokines such as IL-4, IL-5, and IL-13 and production of IgE in a variety of murine models (27–31). The targeted overexpression of mature IL-18 in the skin of transgenic mice resulted in atopic dermatitis-like lesions with an increase of mast cells, Th2 cytokine accumulation, and systemic elevation of IgE and histamine (32). In human PBMC, IL-18 induced the TNF-α production in T lymphocytes and NK cells and subsequently the production of IL-1β and IL-8 in monocytes (33).

Therefore, IL-18 exerts complex immunologic effects depending on the microenvironment where it is released. Furthermore, other effects have been ascribed to IL-18, such as induction of cytolytic activity of CD8+ T cells and NK cells (25), induction of chemokines like monocyte chemotactic protein 1 (33), suppression of angiogenesis (34), Fas ligand up-regulation (35, 36), blocking of osteoclast formation (37), and atherogenic properties (38, 39).

Since 1) the expression of the IL-18R has been described on myeloid cells such as macrophages and myelomonocytic leukemia cell lines, and 2) IL-18 expression is increased in loco in inflammatory (skin) diseases where DC are also present and thought to play a major (immuno-) regulatory role, we examined a possible expression of the IL-18R and potential effects of IL-18 on human DC. We show that the IL-18R is expressed on human DC. On monocyte-derived dendritic cells (MoDC), the IL-18R expression is up-regulated by IFN-γ. Striking effects of stimulation of MoDC with IL-18 were an up-regulation of CD54 and induction of filamentous actin (F-actin) polymerization as well as chemotactic migration.
Materials and Methods

Preparation of MoDC

MoDC were differentiated from PBMC as previously described (40). In brief, PBMCs were isolated by lymphoprep density gradient centrifugation of heparinized leukocyte-enriched buffy coats. After 2 h of plastic adherence, nonadherent cells were removed by vigorous washing times with PBS and the remaining adherent cells were cultured in IMDM supplemented with 4% heat-inactivated human serum, 250 U/ml IL-4 (R&D Systems, Wiesbaden, Germany), and 1000 U/ml GM-CSF (Essex Pharma, Munich, Germany). The cells were fed with fresh medium and cytokines on day 2 of culture. Nonadherent cells, thereafter called MoDC, were harvested on day 7. Before initiation of additional experiments, cell cultures were analyzed by double color flow cytometry for contaminating CD3+ T cells, CD20+ B cells, CD56+ NK cells, and CD16+ granulocytes as described below. Only preparations with less than 5% contaminating T cells, B cells, NK cells, or granulocytes were used in subsequent experiments (for RT-PCR experiments the contamination was required to be <2%).

Stimulation of MoDC and FACs analysis

Cells were either unstimulated or stimulated for various periods of time with IL-18 (200 U/ml; R&D Systems), IFN-γ (200 U/ml; R&D Systems), IFN-α2a (200 U/ml; Roche Molecular Biochemicals, Mannheim, Germany), TNF-α (200 U/ml; R&D Systems), IL-12 (1 μg/ml; R&D Systems), PGE2 (1 μg/ml; Sigma-Aldrich, Deisenhofen, Germany), LPS (500 ng/ml; Sigma-Aldrich). Then, cells (1–2 × 10^6) were washed and resuspended in PBS containing 0.2 μg/mL sodium azide, and 10 μg/ml heat-aggregated human IgG (Sigma-Aldrich) for 15 min. Subsequently, cells were incubated with fluorescent-labeled Abs on ice for 30 min (CD3-FITC, CD20-FITC, CD54-FITC, CD56-FITC, CD80-FITC, CD4-PE, CD16-PE, CD84-PE, CD83-PE, or isotype matched controls from Immunotech, Hamburg, Germany; IL-18R from R&D Systems, HLA-DR-FITC from BD Biosciences, Heidelberg, Germany). Stained cells were washed three times, fixed in PBS with 1% paraformaldehyde, and analyzed by flow cytometry (FACScan, BD Biosciences).

mRNA isolation, reverse transcription, and LightCycler PCR

mRNA was isolated from 10^7 MoDC using a mRNA isolation kit (Roche Molecular Biochemicals) according to the suppliers instructions. For RT-PCR analysis, RNA was subjected to first strand cDNA synthesis using oligo(dT)14 for full length cDNA synthesis. The RT reaction mixture contained final concentrations of 50 U Expand RT (Roche Molecular Biochemicals), 10 mM DTT, 1× first-strand RT buffer for Expand-RT, 0.5 μM of each dNTP (Roche Molecular Biochemicals), RNAinhibitor (Invitrogen, Karlsruhe, Germany), and 80 μM of oligo(dT)14 (Roche Molecular Biochemicals). To control for genomic DNA contamination, cDNA synthesis was performed in the absence of reverse transcriptase. First strand cDNA was stored at −20°C.

The following primers were used for PCR amplification: IL-18Rα chain sense 5’-TGG GAG TGT TGG TAT AAT GCA ACA G 3’ and IL-18Rα chain antisense 5’-TCC ACT GCA ACA TGG TTA AG, IL-18Rβ chain sense 5’-ATG CTC GCT TGT ATT CTT GGA 3’ and IL-18Rβ chain antisense 5’-CAT CTG TAC GAC ACA ACG TAC (based on a previous publication (2)). β-Actin sense 5’-AAG GAC AAC CGC GAG AAG ATG, β-actin antisense 5’-GGA AGA GTG CCT CAG GGC AGC G (40).

Real-time fluorescence PCR was performed using the LightCycler (Roche Molecular Biochemicals) as described previously (40). PCR was performed by rapid-cycling in a reaction volume of 20 μl with 0.5 μM of each primer and 4 μl cDNA. As a reaction buffer, the LightCycler DNA Master SYBR Green I (containing reaction buffer, TaqDNA polymerase, dNTPs (with dUTP instead of dTTP), MgCl2, and a calibrated amount of SYBR Green I dye (Roche Molecular Biochemicals)). Additional MgCl2 was added to the final concentration of 4 mM (IL-18Rα chain) and 5.5 mM (IL-18Rβ chain) for an initial denaturation step at 95°C for 30 s. Amplification was performed using 40 cycles (IL-18Rα chain) and 50 cycles (IL-18Rβ chain), respectively, of denaturation (95°C), annealing (53°C for IL-18Rα chain and 57°C for IL-18Rβ chain), and extension (72°C). Fluorescence was measured at the end of the annealing period of each cycle to monitor amplification.

After DNA extraction had been completed, a final melting curve was recorded by cooling the samples to 65°C at 20°C/s and then increasing the temperature to 95°C at 0.2°C/s. Fluorescence was measured continuously during the slow temperature rise to monitor the dissociation of the PCR product. The fluorescence signal was plotted in real-time against the temperature to produce melting curves of each sample. Melting curves were then converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature against temperature (−dF/dT vs T). Thus, each specific PCR product generates a specific signal, and therefore, a product specific melting peak.

After completion of LightCycler analysis, PCR products were subjected to electrophoresis on a 2% agarose gel, stained with ethidium bromide, visualized, and photographed under ultraviolet illumination. Expected band lengths were 421 for IL-18Rα and 428 for IL-18Rβ.

Real-time monitoring of amplification after each annealing period allows quantification of the samples during the log-linear phase of the PCR. For quantitative analyses, the second derivative maximum method was used (41). A four point standard (100, 50, 25, and 12.5%) was run with every PCR using dilutions of the corresponding positive control. Undiluted standard was defined as 100%, according to the standard curve the relative amount of target in the unknown samples was calculated and referred to the β-actin amounts using the LightCycler software. Amplification of the β-actin gene was performed in parallel to amplification of IL-18Rα and IL-18Rβ as previously reported (40, 42).

Autologous proliferation and cytokine secretion assay

MoDC were unstimulated or stimulated for 48 h with IL-18 (200 U/ml), IFN-γ (200 U/ml), IL-18 plus IFN-γ, or TNF-α (200 U/ml) as positive control. CD4+ autologous T cells were separated from nonadherent cells of the buffy coat using the CD4+ cells MACS isolation kit following the manufacturers’ instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of CD4+ was at least 95% as controlled by flow cytometry. The isolated T cells were labeled with CFSE (Molecular Probes, Eugene, OR) by incubating 1 × 10^6 cells/ml with 1 μM CFSE in the dark for 90 s. The reaction was quenched with the same volume of FCS for 1 min, afterward the cells were washed two times in IMDM and suspended in IMDM supplemented with 4% human serum.

The CD4+ autologous T cells were added to 10^6 prestimulated and washed MoDC per well in 96-well culture plates. A total of 10 μg/ml tetanus toxoid (TT) as Ag were added. Cocultures without TT were used as negative control.

T cells and MoDC were cocultured for 5 days, then cells were restimulated with 2.5 μg/ml anti-CD3 Ab (Pelicluster, Amsterdam, The Netherlands) and 1 μg/ml anti-CD28 Ab (Pelicluster) for 18 h. Afterward, a cytokine secretion assay to detect IFN-γ release with a PE-labeled Ab was performed using the cytokine secretion assay (Miltenyi Biotec) following exactly the recommendations of the manufacturer.

Assessment of F-actin polymerization

Nitroreuscin diazolone (NBD)-phallacidin (Molecular Probes) staining of MoDC was conducted by modifications of the method described by Howard and Meyer (43, 44). Briefly, cells were resuspended at a concentration of > 2 × 10^6 cells/ml in PBS-buffer lacking Ca 2+ and stimulated with the indicated stimulus for different periods of time at room temperature. Following stimulation, cells were fixed using 3.7% formaldehyde for 60 min. Lysoctophatidylcholine (20 μg/ml; Sigma-Aldrich) and 3.7 × 10^-5 M NBD-phallacidin were added to the sample and incubated for a period of 50 min in the dark. NBD-phallacidin-stained cells were analyzed on a BD Biosciences FACScan with a linear fluorescence channel (FL-1) where the fluorescence is proportional to F-actin content (43). Relative F-actin content is expressed as the ratio of the mean channel fluorescence (= integrated fluorescence) between stimulated and unstimulated cells.

Boyden chamber chemotaxis assays

Chemotactic activity of MoDC was determined using a modified Boyden chamber technique as described previously (45, 46). In brief, Boyden chambers (Nuclepore, Tübingen, Germany) were filled with stimuli (200 U/ml IL-18 or medium) and covered with polycarbonate filters (pore size 8 μm, Nuclepore). One hundred microliters of a MoDC suspension at a concentration of 1 × 10^6 cells/ml was added to each chamber. MoDC pretreated with IFN-γ to up-regulate IL-18 (200 U/ml for 48 h) were used in some chambers. After incubation for 90 min at 37°C, migrated cells in the lower part of the Boyden chambers were lysed by adding 0.1% Triton X-100, and β-glucuronidase activity in the lysates was determined photometrically using p-nitrophenyl β-D-glucuronide as a substrate (all from Sigma-Aldrich). Readings were performed with the EARA400AT reader from SLT Labinstruments (Crailsheim, Germany). For calculation of the number of migrated cells equivalent to β-glucuronidase activity determined in the lower parts of the Boyden chambers, values were calculated from a standard curve using known numbers of MoDC. Chemotactic activity was expressed as chemotactic index: quotient of the number of migrated cells in presence of stimulus/migrated cells in presence of medium.
Assessment of IL-18R expression on peripheral blood DC

PBMC isolated by Lymphoprep density gradient centrifugation were triple-stained for flow cytometric analysis. In the FL-1 channel, FITC-labeled Abs to CD3, CD14, CD16, and CD19 were used to detect T and B lymphocytes, monocytes, and NK cells.

In the FL-2 channel, PE-labeled Ab to IL-18R was used, and in the FL-3 channel PerCP-labeled Ab to HLA-DR (BD Biosciences). Cells expressing HLA-DR (i.e., positive for PerCP) but not expressing CD3, CD14, CD16, or CD19 (i.e., negative for FITC) were defined as peripheral blood DC and analyzed for IL-18R expression.

Preparation of epidermal cell suspensions and analysis of epidermal cells

Clinical normal human skin was obtained from patients obtaining breast plastic surgery. Epidermis and dermis were separated by overnight incubation in dispase (2.4 U/ml, Roche Molecular Biochemicals) at 4°C. The epidermis was detached from the dermis with fine forceps and incubated in Hank’s solution with 0.25% trypsin (Sigma-Aldrich) for 20 min at 37°C. The digestion was stopped by addition of FCS (Life Technologies, Karlsruhe, Germany).

The epidermal sheets were vigorously pipetted in and out of a pipette and filtered through sterile gauze. The epidermal cell suspension obtained by this procedure was short-term cultured in IMDM supplemented with 4% heat inactivated human serum for 1–3 h.

For determination of IL-18R expression on epidermal DC, epidermal cell suspensions were double-stained with FITC-labeled Ab to HLA-DR and PE-labeled Ab to IL-18R and analyzed by flow cytometry.

For determination of IL-18 induced F-actin polymerization, epidermal cell suspensions were stimulated with IL-18 and subjected to the F-actin polymerization assay as described above for MoDC. Next, cells were stained with PE-labeled Ab to HLA-DR. The following flow cytometric analysis allowed the detection of F-actin polymerization induced by IL-18 in epidermal DC by gating on PE-positive cells.

Statistical analysis

Statistical analyses were performed using the paired Student t test.

Results

Expression and regulation of the IL-18R on MoDC

IL-18R expression on MoDC was investigated on the protein level (Fig. 1) and mRNA level (Fig. 2). In a subset of 5–10% of immature MoDC, a clearcut IL-18R expression was detected by flow cytometry (Fig. 1A). Stimulation with IFN-γ resulted in a homogeneous up-regulation of IL-18R expression in the majority of cells as demonstrated in Fig. 1A. This effect was statistically significant (Fig. 1B) and observed in 20/22 consecutive experiments (Fig. 1C). IFN-α also up-regulated the IL-18R to some degree (Fig. 1A), however, this effect was statistically not significant (Fig. 1B). Other stimuli such as TNF-α, prostaglandin E2, LPS, IL-12 and IL-18 did not affect IL-18R expression on MoDC.

Real time LightCycler PCR assays were established for the two components of the IL-18R, IL-18Rα chain, and IL-18Rβ chain. On immature MoDC, mRNA for both chains was detected by LightCycler PCR melting curve analysis (Fig. 2A) and agarose gel electrophoresis of the PCR products (Fig. 2B). Stimulation with IFN-γ transiently up-regulated the mRNA for both chains with a maximum after 4 h (Fig. 2C).

Effects of IL-18 on surface molecule expression of MoDC

MoDC were stimulated for 24 h with IL-18, either prestimulated or not prestimulated with IFN-γ for 24 h to up-regulate IL-18R expression. IL-18 up-regulated most strikingly the expression of CD54 and much less (but regularly and, therefore, significantly) the expression of CD83, CD86, and HLA-DR. The expression of CD40 and CD80 was not significantly affected by IL-18 (Fig. 3).

Preincubation with IFN-γ augmented the effect of IL-18 on CD54, CD83, CD86, and HLA-DR expression (Fig. 3).

FIGURE 1. Expression and regulation of the IL-18R on MoDC at the protein level. IL-18R expression was detected by flow cytometry on unstimulated MoDC and after 48 h of incubation with various stimuli (A and B). The expression of the IL-18R was up-regulated by IFN-γ (200 U/ml) and to some degree by IFN-α but not by IL-12 (1 μg/ml), TNF-α (200 U/ml), IL-18 (200 U/ml), PGE₂ (1 μg/ml), or LPS (500 ng/ml). A, One representative experiment; B, mean (± SEM) fluorescence intensity of 11 independent experiments. Statistically, only IFN-γ significantly up-regulated IL-18R surface expression (B). This up-regulation of IL-18R by IFN-γ was observed in 20 of 22 consecutive experiments (C).
Effects of IL-18 primed MoDC on proliferation and IFN-γ production of autologous T cells

The effect of IL-18-primed MoDC on T cells was studied in an autologous system using TT as Ag in donors immunized to TT. T cells were labeled with CFSE before the coculture with MoDC. This dye allows flow cytometric determination of cell proliferation in the FL-1 channel. Moreover, IFN-γ producing T cells were detected flow cytometrically in the FL-2 channel by using the cytokine secretion assay from Miltenyi Biotec. Therefore, this system allowed us to assess cell proliferation (CFSE fluorescence, FL-1 channel) and IFN-γ production (FL-2 channel) in parallel as shown in Fig. 4. Furthermore, IFN-γ production was determined in the cell culture supernatant by ELISA.

Preincubation with IL-18 did not significantly alter the ability of MoDC to stimulate autologous T cell proliferation or IFN-γ production (Fig. 4, Table I). Preincubation of MoDC with IL-18 after up-regulation of the IL-18R with IFN-γ did also not increase their ability to stimulate proliferation or IFN-γ production in autologous T cells (Fig. 4, Table I). Instead, the number of IFN-γ secreting T cells was diminished in all three experiments, and the concentration of IFN-γ in the cell culture supernatant was significantly reduced (Table I).

Chemotactic effects of IL-18 on MoDC

The effect of IL-18 on the migratory capacity of MoDC was assessed by two different assays. First, by determination of F-actin polymerization induced by IL-18 as parameter for cytoskeleton reorganization and indirect marker for cell migration. Second, by determination of cell migration in Boyden chamber chemotaxis assays.

IL-18 induced F-actin polymerization in MoDC. The polymerization was increased after pretreatment of MoDC with IFN-γ to up-regulate the IL-18R (Fig. 5).

MoDC migrated toward an IL-18 gradient in Boyden chamber assays (Fig. 6). This effect was also increased after pretreatment of MoDC with IFN-γ (Fig. 6a) and migration of MoDC was dependent on the concentration of IL-18 (Fig. 6b).

Expression and function of the IL-18R on DC from epidermis and peripheral blood

To demonstrate expression of the IL-18R on naturally occurring DC, DC from epidermis and peripheral blood were studied by multicolor flow cytometry (Fig. 7). On both types of human DC, the IL-18R was expressed. Stimulation of epidermal DC with IL-18 resulted in F-actin polymerization (Fig. 8).

Discussion

IL-18 is produced by various cells such as DC and epithelial cells. Although the exact mechanisms of its regulation are not known, its overexpression has been described in a number of inflammatory disorders, such as psoriasis, chronic inflammatory bowel diseases, and rheumatoid arthritis. As cellular targets of IL-18, mainly T
cells and NK cells have been described. We were interested whether human DC might also represent a target for IL-18 as it has been shown before for IL-12 as another DC-derived cytokine (47). We demonstrate here that human DC express the IL-18R. On MoDC, this expression is up-regulated by IFN-γ/H9253. A number of studies reported the up-regulation of the IL-18R on T cells, NK cells, and B cells by IL-12 (20, 21, 48–51). IFN-γ/H9251 has also been reported to up-regulate the IL-18R on T cells and NK cells (51), TNF-α/H9251 induced the IL-18R on hematopoietic cell lines, i.e., KG-1 cells (myelomonocytic cell line), PEER cells (T cell line), and MOLT-16 cells (T cell line) (22). On macrophages, endothelial cells, and smooth muscle cells, the IL-18R was up-regulated by TNF-α, LPS, and IL-1β (39). In our study, IFN-γ was the only stimulus that significantly up-regulated the IL-18R on MoDC, all other stimuli tested did not significantly affect the IL-18R expression, i.e., IL-12, IFN-α, TNF-α, LPS, PGE₂, and IL-18. This is in contrast to B cells, where IFN-γ had no effect, but IL-12 significantly up-regulated the IL-18R (21).

The selective effect of IFN-γ on the IL-18R expression on MoDC is interesting, since IL-18 in synergy with IL-12 is known to be a strong inducer of IFN-γ production in T cells. Therefore, the up-regulation of the IL-18R on DC by IFN-γ might be a positive feedback loop, enhancing the effect of IL-18 on DC in a Th1-cytokine milieu.

**FIGURE 3.** Effect of IL-18 on the expression of surface molecules on MoDC. MoDC were stimulated for 24 h with IL-18 (200 U/ml), either not prestimulated or prestimulated with IFN-γ (IFN-γ, 200 U/ml) for 24 h to up-regulate IL-18R expression. Mean fluorescence intensity of a variety of surface molecules was determined by flow cytometry. Relative fluorescence intensity was calculated by division of the mean fluorescence intensity of the stimulated cells by the mean fluorescence intensity of the unstimulated cells (basal). Mean ± SEM of 11 independent experiments are shown. IL-18 increased mainly the expression of CD54 and to a lesser extent the expression of CD86, CD83, and HLA-DR but not the expression of CD40 and CD80 (n.s., not significant).

**FIGURE 4.** Effects of IL-18 on the T cell stimulatory capacity of MoDC. MoDC (1 × 10⁴ cells/well) were either not stimulated or stimulated with IL-18 (200 U/ml) with or without IFN-γ (200 U/ml) to up-regulate the IL-18R. After 48 h, MoDC were washed and autologous CFSE-labeled T cells (1 × 10⁵ cells/well) and TT (10 μg/ml) were added. After 5 days of coculture, cells were restimulated with mAbs against CD3 and CD28 overnight. Then IFN-γ-secreting cells were determined using the cytokine secretion assay from Miltenyi Biotec with bispecific, PE-labeled Abs. Cells were analyzed by flow cytometry, detecting CFSE fluorescence in the FL-1 channel as indicator for cell division and PE fluorescence in the FL-2 channel as indicator for IFN-γ production. MoDC prestimulated with TNF-α (200 U/ml) served as positive control, cocultures without TT served as negative control. One representative experiment out of three is shown; numbers correspond to the number of lymphocytes in each quadrant.
Next, we examined functional effects of IL-18 on the expression of surface molecules relevant for T cell stimulation on MoDC. CD54 (ICAM-1) expression was significantly up-regulated by IL-18. This effect was pronounced after preincubation of MoDC with IFN-γ. The up-regulation of CD54 by IL-18 has been described on other human cells, i.e., on peripheral blood monocytes (52), on KG-1 cells (53), on endothelial cells, and on rheumatoid arthritis synovial fibroblasts (54). Other surface molecules on MoDC were either not (CD40, CD80) or much less (CD83, CD86, HLA-DR) affected.

Both IL-18 and CD54 have been associated with Th1-type immune responses (55). We were therefore testing the hypothesis of whether the priming of MoDC with IL-18 increases their ability to induce IFN-γ-producing T cells and the concentration of IFN-γ detected in the supernatant. This difference was statistically significant (\( p = 0.015 \) comparing both values).

Looking for possible chemotactic effects of IL-18 on MoDC, we found an induction of F-actin polymerization and migration in Boyden chamber chemotaxis assays. These effects were increased after prestimulation of MoDC with IFN-γ. F-actin polymerization could also be demonstrated on epidermal DC as example for naturally occurring DC. Direct chemotactic effects have been described for other members of the IL-1 family. In Boyden chamber assays, IL-1β had a chemotactic effect on human Th cells (56) and on tracheal smooth muscle cells of dogs (57). IL-1α was found to be chemotactic for human keratinocytes in Boyden chamber assays (58). A very recent report showed that also IL-18 itself is a direct chemoattractant for human T-lymphocytes (59). Results from immunotherapy approaches using IL-18 in murine tumor models are also consistent with IL-18 being a chemotactic factor for cells of the immune system. CT26 murine colon cancer cells genetically modified to produce mature IL-18 resulted in significantly reduced tumor growth in syngeneic mice and a massive infiltration of tumors with mononuclear cells as compared with control CT26 cells (60). In another model, injection of an adenoviral vector expressing IL-18 into established B16 murine melanomas resulted in an infiltrate with neutrophils, lymphocytes, and monocytic cells (61). In contrast, injection of an adenoviral vector expressing IL-18 antisense RNA into inflamed intestine in a murine model of colitis resulted in a reduced concentration of IL-18 and decrease of the inflammatory infiltrate (62).

However, IL-18 has also been shown to have indirect effects on DC migration via induction of cytokines such as TNF-α, IL-1β, and on T cell migration via induction of IL-8 or via induction of chemokines (63). In a murine model, intradermal injection of IL-18 into ears resulted in a moderate reduction of Langerhans

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**Table 1. Effects of IL-18 on the T-cell stimulatory capacity of MoDC**

<table>
<thead>
<tr>
<th></th>
<th>% Divided Cells (CFSE Staining)</th>
<th>% IFN-γ Positive Cells (IFN-γ Secretion Assay)</th>
<th>IFN-γ Production (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC (NS)</td>
<td>34 ± 13</td>
<td>64 ± 9</td>
<td>1405 ± 305(^a)</td>
</tr>
<tr>
<td>DC (IL-18)</td>
<td>43 ± 16</td>
<td>61 ± 9</td>
<td>1208 ± 268</td>
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<tr>
<td>DC (IFN-γ)</td>
<td>36 ± 15</td>
<td>59 ± 9</td>
<td>1242 ± 273</td>
</tr>
<tr>
<td>DC (IFN-γ + IL-18)</td>
<td>39 ± 17</td>
<td>52 ± 12</td>
<td>1008 ± 353(^a)</td>
</tr>
<tr>
<td>DC (TNF-α)</td>
<td>47 ± 13</td>
<td>70 ± 7</td>
<td>2435 ± 639</td>
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<tr>
<td>DC + T cells without TT</td>
<td>9 ± 4</td>
<td>9 ± 8</td>
<td>30 ± 0</td>
</tr>
</tbody>
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\(^a\) CFSE-labeled MoDC were prestimulated and cocultured with autologous T cells and TT as indicated in Fig. 4. After 5 days of coculture, cells were restimulated with mAbs against CD3 and CD28 overnight. Then, IFN-γ production was measured in the cell culture supernatants by ELISA and cells were analyzed by flow cytometry as shown in Fig. 4. The percentage of divided cells includes the cells in the upper left and lower right quadrants, and the percentage of IFN-γ-positive cells in the upper left and right quadrants is as depicted in Fig. 4. Mean ± SEM values of three independent experiments are shown. Prestimulation of MoDC with IL-18 increased their ability to induce T cell proliferation; however, the difference was statistically not significant. Prestimulation of MoDC with IFN-γ and IL-18 decreased their ability to induce IFN-γ-producing T cells and the concentration of IFN-γ detected in the supernatant. This difference was statistically significant (\( p = 0.015 \) comparing both values).

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**FIGURE 5.** IL-18 induces F-actin polymerization in MoDC. For measurement of F-actin polymerization, MoDC were stimulated with IL-18 (200 U/ml) or buffer (as negative control) for the indicated period of times. MoDC were either immature or prestimulated with IFN-γ and then stimulated with IL-18. The percentage of divided cells includes the cells in the upper left and lower right quadrants, and the percentage of IFN-γ-positive cells in the upper left and right quadrants is as depicted in Fig. 4. Mean ± SEM values of three independent experiments are shown. Prestimulation of MoDC with IL-18 increased their ability to induce T cell proliferation; however, the difference was statistically not significant. Prestimulation of MoDC with IFN-γ and IL-18 decreased their ability to induce IFN-γ-producing T cells and the concentration of IFN-γ detected in the supernatant. This difference was statistically significant (\( p = 0.015 \) comparing both values).
cells within 4 h and a marked accumulation of DC in regional lymph nodes within 17 h. This effect was dependent on TNF-α and IL-1β (64). Therefore, direct and indirect effects of IL-18 on cell migration are complex and require further investigation.

In summary, we showed the expression of the IL-18R on human DC and its up-regulation by IFN-γ. As a novel effect of IL-18, we demonstrated that IL-18 has a direct chemotactic effect on DC, which might recruit them to the site of inflammation in diseases where IL-18 is increased. Furthermore, IL-18 increased the expression of CD54 on MoDC as described for other cells of myeloid

FIGURE 6. IL-18 induces chemotactic migration in Boyden chamber assays. For measurement of chemotaxis, MoDC were exposed to an IL-18 gradient in Boyden chambers for 90 min. A buffer, instead of IL-18, served as background control. MoDC were either immature or prestimulated with IFN-γ to up-regulate IL-18R. C5a served as positive control. Migrated cells in the lower part of the Boyden chambers were lysed and glucuronidase activity in the lysates was determined in a photometrical assay as described in Material and Methods. The chemotactic index was calculated as quotient of the number of migrated cells in the presence of stimulus/migrated cells in the presence of medium. Mean ± SEM of six (A) and five (B) independent experiments are shown. *, Significant differences in comparison to the medium control. IL-18 (200 U/ml) induced a significant cell migration of MoDC, which was increased by prestimulation of cells with IFN-γ (A). This migration was dependent on the concentration of IL-18 (B, cells were not prestimulated with IFN-γ).

FIGURE 7. Peripheral blood DC and epidermal DC express the IL-18R. PBMC were triple stained for CD3, CD14, CD16, and CD19 (FL-1 channel lineage markers), IL-18R (FL-2 channel), and HLA-DR (FL-3 channel). Cells expressing HLA-DR but negative for lineage markers were considered as peripheral blood DC. IL-18R expression could be demonstrated on this cell population in the FL-2 channel (A, one representative of seven experiments is shown). Epidermal cell suspensions were double stained for HLA-DR (FL-1 channel) and IL-18R (FL-2 channel). Cells expressing HLA-DR were considered as epidermal DC that also expressed the IL-18R (B, one representative of five experiments is shown).

FIGURE 8. IL-18 induces F-actin polymerization in epidermal DC. Epidermal cell suspensions were stimulated with IL-18 and subjected to the F-actin polymerization assay as described in Materials and Methods. To identify epidermal DC, suspensions were stained with a PE-labeled Ab to HLA-DR. By gating on PE-positive cells, F-actin polymerization in epidermal DC was determined. One representative of three experiments is shown.
References


48. Leukin (IL)-18 receptor.


