Pro- and anti-inflammatory substances modulate expression of the leukotriene B_4 receptor, BLT1, in human monocytes

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Abstract: The high-affinity leukotriene B_4 (LTB_4) receptor, BLT1, is a chemotactic receptor involved in inflammatory responses. In this study, we have explored the regulation of BLT1 expression in human monocytes by pro- and anti-inflammatory cytokines, lipopolysaccharide (LPS), and dexamethasone. We found that proinflammatory mediators, such as interferon-γ (IFN-γ), tumor necrosis factor-α, and LPS, down-regulated expression, whereas the anti-inflammatory cytokine, interleukin-10, and dexamethasone up-regulated BLT1 mRNA expression. The effect of IFN-γ on BLT1 mRNA expression was rapidly detectable (<4 h) and concentration-dependent (1–50 ng/ml) and seems to be exerted through a block in transcriptional activity. Alterations in mRNA expression were accompanied by changes in BLT1 surface expression, and receptor down-modulation following IFN-γ stimulation resulted in a diminished chemotactic response to LTB_4. The regulation of BLT1 mRNA and receptor protein expression was similar to the regulation of the monocyte chemoattractant protein-1 chemokine receptor, CC chemokine recptor 2 (CCR2). Flow cytometric analysis of fresh peripheral blood cells revealed that classical ($CD14^{++}CD16^{-}$) monocytes express high levels of BLT1 and CCR2 and that both receptors are down-regulated on $CD14^{+}CD16^{+}$ monocytes. Apart from providing insight into the regulation of BLT1 in human monocytes, our results reveal a parallel expression and regulation of BLT1 and CCR2, which may help to understand monocyte trafficking during pathophysiological conditions. *J. Leukoc. Biol.* 77: 1018–1025; 2005.

Key Words: *mononuclear phagocytes · LTB_4 · IFN-γ · inflammatory mediators*

INTRODUCTION

Directed leukocyte migration is controlled by local production of chemotactic signals and the dynamic expression of chemotactic cell-surface receptors [1]. Chemotactic signals, chemoattractants, can be grouped into two families: the chemotactic cytokines (chemokines) and the classical chemoattractants, which include complement factors, formyl peptides, and arachidonic acid (AA) metabolites such as leukotriene B_4 (LTB_4) [2]. The chemical signals are released in tissues in response to inflammatory events and subsequently induce activation and mobilization of specific subsets of immune cells [1].

It is now well established that pro- and anti-inflammatory cytokines as well as microbial products modulate the release of chemotactic factors and the expression of leukocyte chemokine receptors [3–5]. For example, several proinflammatory cytokines induce the production of chemotactic signals, such as the CC chemokine receptor 2 (CCR2) ligand monocyte chemoattractant protein-1 (MCP-1) [3]. This leads to the local recruitment of CCR2-expressing leukocyte subsets, including monocytes, from the bloodstream. As the leukocytes enter the inflammatory site, the same cytokines will prevent further MCP-1-induced chemotaxis through the down-regulation of CCR2 [6, 7]. In this way, the same signals that participate in the mobilization of leukocytes also induce their arrest at the right location. These findings have added important information about how the local cytokine environment can regulate leukocyte trafficking in inflammation.

LTB_4 is a potent lipid inflammatory mediator derived from AA metabolism through the 5-lipoxygenase (5-LO) pathway [8]. Similar to the peptide chemokines, LTB_4 induces activation and chemotaxis of specific leukocyte subsets [9]. Two cell-surface receptors for LTB_4 have been identified: the high-affinity receptor, BLT1 [10, 11], and a low-affinity receptor, BLT2 [12–15]. Like chemokine receptors, they both belong to the G-protein-coupled receptor seven transmembrane domain superfamily. Whereas the role for BLT1 in LTB_4-induced leukocyte migration and activation has been well-characterized, the relevance of BLT2 expression needs further exploration. BLT1 is mainly expressed in granulocytes, monocytes, and to a lesser extent, in subgroups of lymphocytes [16–20]. As the high-affinity receptor for LTB_4, BLT1 is needed for the generation of an efficient immune response to invading microbes [21]. Furthermore, LTB_4/BLT1 interactions seem to be

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important for the pathogenesis of certain inflammatory diseases [22–25], and BLT1 may act as a coreceptor for human immunodeficiency virus type 1 (HIV-1) [26]. Recently, several investigators, using transgenic mice models, have also found convincing evidence for a role of BLT1 in the development of atherosclerosis [27, 28].

Previously, we and others have characterized the regulation of BLT1 expression in granulocytes [19, 29–31]. So far, information is scarce regarding the dynamics of BLT1 expression in human mononuclear phagocytes, the key players in several inflammatory conditions, including atherosclerosis. In this study, we show that pro- and anti-inflammatory substances in vitro modulate BLT1 surface expression and mRNA expression in human monocytes as well as chemotactic responsiveness to LTB_4. Flow cytometric analysis of fresh peripheral blood monocytes revealed a lower BLT1 surface expression on $CD14^+$, $CD16^+$ monocytes as compared with $CD14^{++}$, $CD16^-$ monocytes, possibly reflecting the in vivo relevance of our findings. In monocytes, the regulation of BLT1 by inflammatory mediators is similar to what can be seen for the chemokine receptor CCR2. Together with previous work that has revealed a cross-talk between LTB_4 and MCP-1 [28, 32, 33], our findings further indicate a functional relationship for these mediators and their corresponding receptors in human monocytes.

MATERIALS AND METHODS

Monocyte isolation

Monocytes were isolated from buffy coats according to Repnik et al. [34]. Briefly, buffy coats were mixed (1:2) with RPMI 1640 (Invitrogen, Copenhagen, Denmark), layered on a Ficoll density gradient (Amersham Pharmacia Biotech, Uppsala, Sweden), and centrifuged for 15 min at 950 *g*. The mononuclear cells were collected, washed, counted, and diluted to $50–70 \times 10^6$ cells/ml before they were placed on a hyperosmotic Percoll density gradient (Amersham Pharmacia Biotech) and centrifuged for 15 min at 580 *g*. The monocyte-enriched fraction was collected, washed, and diluted to 2×10^6 cells/ml in macrophage-serum-free medium (SFM) medium (Invitrogen). This is a medium designed for human monocytes and macrophages, which was used to minimize possible stress and activation caused by isolation. Cell viability was >99% as determined by trypan blue exclusion, and monocyte purity was 75–90% as determined by flow cytometric analysis and ocular inspection. Cells used for real-time polymerase chain reaction (PCR) quantification were further purified by incubating the monocyte-rich cell suspension during 1 h to let the monocytes adhere. The nonadherent cells were aspirated, and new medium was added. This resulted in a monocyte purity of >95%. All ingredients used during isolation and subsequent stimulation were endotoxin-free as determined by the Limulus amebocyte lysate test.

Stimulation conditions

To give the monocytes time to recover from possible isolation stress and activation induced by plastic adherence, they were incubated overnight (16–20 h) at 37°C and 7% CO_2 before stimulation with the following reagents: interferon-γ (IFN-γ; R&D Systems, Abingdon, UK) 1–50 ng/ml, interleukin (IL)-10 (R&D Systems) 10 ng/ml, IL-4 (R&D Systems) 20 ng/ml, lipopolysaccharide (LPS; Fluka, Stockholm, Sweden) 100 ng/ml, tumor necrosis factor-α (TNF-α; R&D Systems) 20 ng/ml, and dexamethasone (Sigma, Stockholm, Sweden) 10^{-7} M. In the chemotaxis experiments, monocytes were kept in suspension in 50 ml polypropylene tubes (Falcon, Stockholm, Sweden) on a rotating device during stimulation. All stimulations were performed at 37°C and 7% CO_2.

Flow cytometric analysis

Monocytes were incubated with cold (4°C) washing buffer [phosphate-buffered saline without Ca^{2+} and Mg^{2+} (Invitrogen), supplemented with 0.5% bovine serum albumin (BSA; Sigma) and 0.05% NaN_3 (Sigma)] before gentle removal with a cell-scraper. Cells ($\sim 5 \times 10^5$) were suspended in 100 μl washing buffer and stained with the appropriate antibodies, namely: BLT1 antibody 14F11-phycoerythrin (PE), 14F11-fluorescein isothiocyanate (FITC; raised in-house [35]), CCR2 antibody conjugated to PE (clone #48607), immunoglobulin G2b (IgG2b)-PE (R&D Systems), IgG1-FITC (Becton Dickinson, Stockholm, Sweden), CD14-allophycocyanin, CD16-FITC, CD19-FITC, IgG1-PE (PharMingen, Stockholm, Sweden), or CD3-FITC (Diatec, Oslo, Norway). The cells were incubated for 30 min at 4°C in the dark, washed, and analyzed using a FACSCalibur flow cytometer (Becton Dickinson) and the CellQuest software (10,000 cells were counted/sample).

Chemotaxis

Monocytes were stimulated with 50 ng/ml IFN-γ for 6–8 h and then suspended in macrophage–SFM medium (Invitrogen) to a concentration of 2×10^6 cells/ml. LTB_4 (Sigma), at concentrations ranging from 10^{-11} to 10^{-7} M, was added to the wells (28 μl/well) in a chemotaxis chamber (ChemoTx plate, NeuroProbe, Gaithersburg, MD) together with medium as negative control and cell suspension as positive control. A polycarbonate filter with 5 μm pores (NeuroProbe) was placed over the wells, and 50 μl cell suspension with nonstimulated or IFN-γ-stimulated cells was placed on the filter and incubated at 37°C, 7% CO_2, for 1 h. At the end of the incubation, the filter was removed, and the cells that had migrated into the wells were counted using an inverted microscope (Olympus IX70) equipped with a digital camera (Olympus Camedia C-3040ZOOM). The cells in three high magnification fields at the center of each well were counted.

cDNA preparation and real-time, quantitative PCR analysis

All reagents were purchased from Sigma, unless otherwise stated. Cells were lysed in 200 μl lysis/binding buffer [100 mM Tris-HCl, pH 8, 500 mM LiCl, 10 mM EDTA, 1% lithium dodecyl sulfate, and 5 mM dithiothreitol (DTT)], and the cell lysate was further processed with a QIAshredder (Qiagen, Stockholm, Sweden) to reduce viscosity. PolyA+ RNA was captured from the cell lysate with 50 μg Seramag Oligo dT14 paramagnetic beads (Serva, Gothenburg, Sweden). The beads were washed twice with 200 μl buffer A (100 mM Tris-HCl, pH 8, 150 mM LiCl, 1 mM EDTA, and 0.1% lithium dodecyl sulfate) and once with 100 μl buffer B (100 mM Tris-HCl, pH 8, 150 mM LiCl, and 1 mM EDTA). PolyA+ RNA was eluted in 10 μl water, and first-strand synthesis was carried out for 50 min at 50°C in a final volume of 20 μl containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 10 mM DTT, 5 mM $MgCl_2$, 0.5 mM deoxyunspecified nucleoside 5′-triphosphate (dNTP), and 0.5 μg Oligo (dT)12–18, 30 units RNaseOUT (Invitrogen), and 50 units Superscript III reverse transcriptase (Invitrogen). PCR primers (MWG, Ebersberg, Germany) were designed with Oligo 4 (National Bioscience, Oslo, Norway), and their sequences are as follows, with expected product size in parenthesis: BLT1 5′-GTTTTGGACTGGCTGGTTGC, 3′-GGTACGCGAGGACGGGTGTG (216 bp); CCR2 5′-CAGAAATACCAACGAGAGCG, 3′-GGCCACAGACATAAACAGAATC (545 bp).

Real-time, quantitative PCR was performed in a LightCycler system (Roche, Stockholm, Sweden) using the Sybr Green I detection method. The reactions were performed in a total volume of 10 μl containing 2 μl diluted (1:20) cDNA or external PCR standard, 50 mM Tris-HCl, pH 8.3, 10 mM KCl, 50 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 200 μM each dNTP, 0.5 μg/μl BSA, 1:30,000 dilution of SYBR Green I, 0.5 μM each primer, and 0.5 units FastStart Taq DNA polymerase (Roche). After an initial denaturation step at 95°C for 10 min, temperature cycling was initiated. A total of 45 cycles was run, and each cycle consisted of 10 s at 95°C, 10 s at 55°C, and 10 s at 72°C (BLT1) or 15 s at 95°C, 5 s at 54°C, and 22 s at 72°C (CCR2). At the end of each run, melting curve profiles were recorded, and the specificity of the amplification product was further verified by electrophoresis on a Visigel (Stratagene, Stockholm, Sweden) using GelStar (Cambrex, Stockholm, Sweden) DNA staining. To generate external standards, specific PCR products for each gene were gel-purified using QIAquick gel extraction kit (Qiagen). The copy number was calculated based on the measured concentration at 260 nm, and serial tenfold dilutions were made in ultra-pure water.

mRNA decay studies

Monocytes were incubated with or without 50 ng/ml IFN-γ for 4 h before the addition of 5 μg/ml Actinomycin D (Sigma). At 0, 2, 4, 6, and 8 h, cells were lysed, and mRNA was extracted for subsequent cDNA synthesis and real-time PCR quantification as described previously.

Data analysis

The data obtained from stimulating cells with different IFN-γ concentrations were fitted to an exponential equation (using the Prism software), which was used to calculate an effective concentration $(EC)_{50}$ value.

RESULTS

Pro- and anti-inflammatory agents affect BLT1 mRNA expression

Human monocytes were stimulated for 4 h with various inflammatory mediators, and BLT1 mRNA levels were examined using real-time, quantitative PCR. As shown in **Figure 1**A, LPS and the proinflammatory cytokines, IFN-γ and TNF-α, induced varying degrees of mRNA down-regulation. This was most pronounced for LPS, which decreased the mRNA levels by almost 50%. IFN-γ and TNF-α down-regulated BLT1 mRNA levels to ~77% of control values, and a combination of the two had a synergistic effect. The anti-inflammatory cytokine, IL-10, and the prototypic glucocorticoid, dexamethasone, up-regulated BLT1 mRNA levels, whereas IL-4 had no obvious effect.

The results from the stimulation with pro- and anti-inflammatory agents on BLT1 mRNA levels were compared with the effect on CCR2 mRNA (Fig. 1B) and were found to be similar: LPS, IFN-γ, TNF-α, and IFN-γ + TNF-α down-regulated CCR2 mRNA expression, although more pronounced (13–15% of control levels) than for BLT1. As for BLT1, IL-10 and dexamethasone up-regulated CCR2 expression. IL-4 lowered CCR2 mRNA expression slightly in one of two experiments. To assure that the BLT1 mRNA expression in nonstimulated cells did not change over the 4-h incubation, mRNA levels were compared before and after incubation, and they were found to be stable.

BLT1 mRNA alterations correlate with receptor protein expression at the cell surface

We next examined whether cytokine-induced alterations of BLT1 mRNA levels in human monocytes were reflected by changes in BLT1 surface expression. For this purpose, monocytes were stimulated with IFN-γ, IL-10, TNF-α, or dexamethasone for 8–12 h before flow cytometric analysis. IFN-γ (**Fig. 2, A** and **B**) and TNF-α (Fig. 2, E and F) down-regulated BLT1 and CCR2 expression, whereas IL-10 up-regulated both receptors (Fig. 2, C and D). In line with the aforementioned mRNA findings, the cytokine-induced alterations of receptor protein expression were more pronounced for CCR2. Dexamethasone stimulation gave varying results. In three out of five experiments, an up-regulation of BLT1 expression was seen, whereas in the remaining two, stimulated and nonstimulated cells did not differ (data not shown).

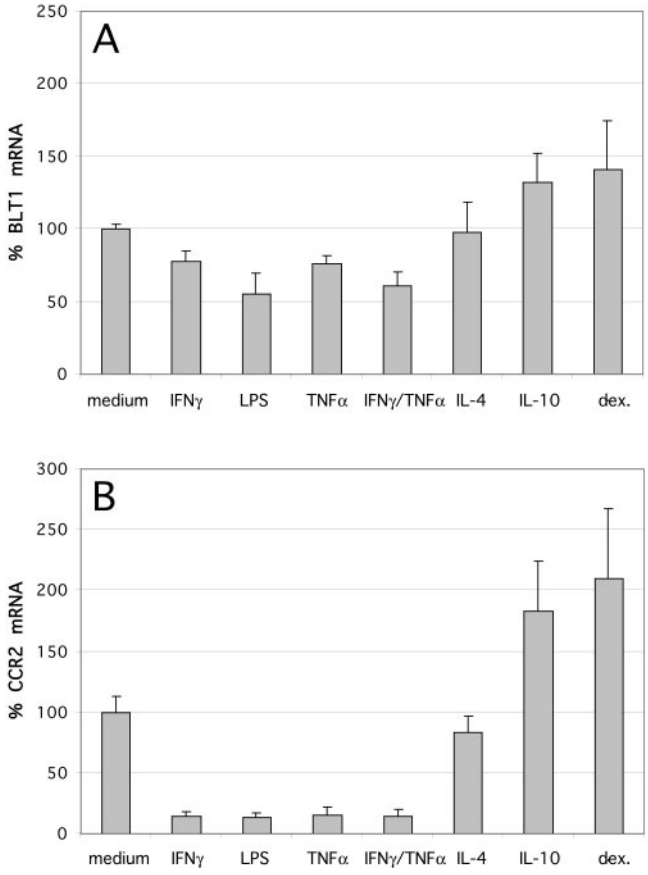

Fig. 1. (A) The effect of pro- and anti-inflammatory mediators on BLT1 mRNA levels. Monocytes rested for 16–20 h before 4 h stimulation, followed by lysis, mRNA harvest, and quantification by real-time PCR. The proinflammatory agents LPS (100 ng/ml), IFN-γ (50 ng/ml), TNF-α (20 ng/ml), and a combination of IFN-γ and TNF-α down-regulated BLT1 mRNA expression. IL-4 (20 ng/ml) had no significant effect, whereas the anti-inflammatory substances IL-10 (10 ng/ml) and dexamethasone (dex.; 10^{-7} M) up-regulated the levels of monocyte BLT1 mRNA. Experiments were conducted in triplicates, and bars represent results from at least three donors, except for IFN-γ/TNF-α (two donors). Results are presented as percentage of copy number in nonstimulated cells. (B) The effect of pro- and anti-inflammatory mediators on CCR2 mRNA levels. As for BLT1 mRNA expression, proinflammatory cytokines and LPS induced a down-modulation of CCR2 mRNA levels, whereas the anti-inflammatory IL-10 and dexamethasone up-regulated CCR2 mRNA expression. IL-4 stimulation lowered CCR2 mRNA levels slightly. Experiments were conducted in triplicates, and bars represent results from at least two donors, except for dexamethasone (one donor). Results are presented as percentage of copy number in nonstimulated cells.

Further analysis of IFN-γ-induced effects on BLT1 message levels

Subsequent studies on BLT1 mRNA expression were focused on the down-modulatory effects of IFN-γ.

BLT1 down-regulation by IFN-γ is concentration-dependent

To obtain the optimal stimulation conditions for further analyses, human monocytes were stimulated for 8 h with different concentrations of IFN-γ, ranging from 1 to 50 ng/ml, and BLT1 mRNA was quantified using real-time, quantitative PCR. A decrease of BLT1 mRNA was first seen after stimulation with 2.5 ng/ml (**Fig. 3**). Increasing IFN-γ concentrations gave a progressive down-regulation of BLT1 mRNA, which was max-

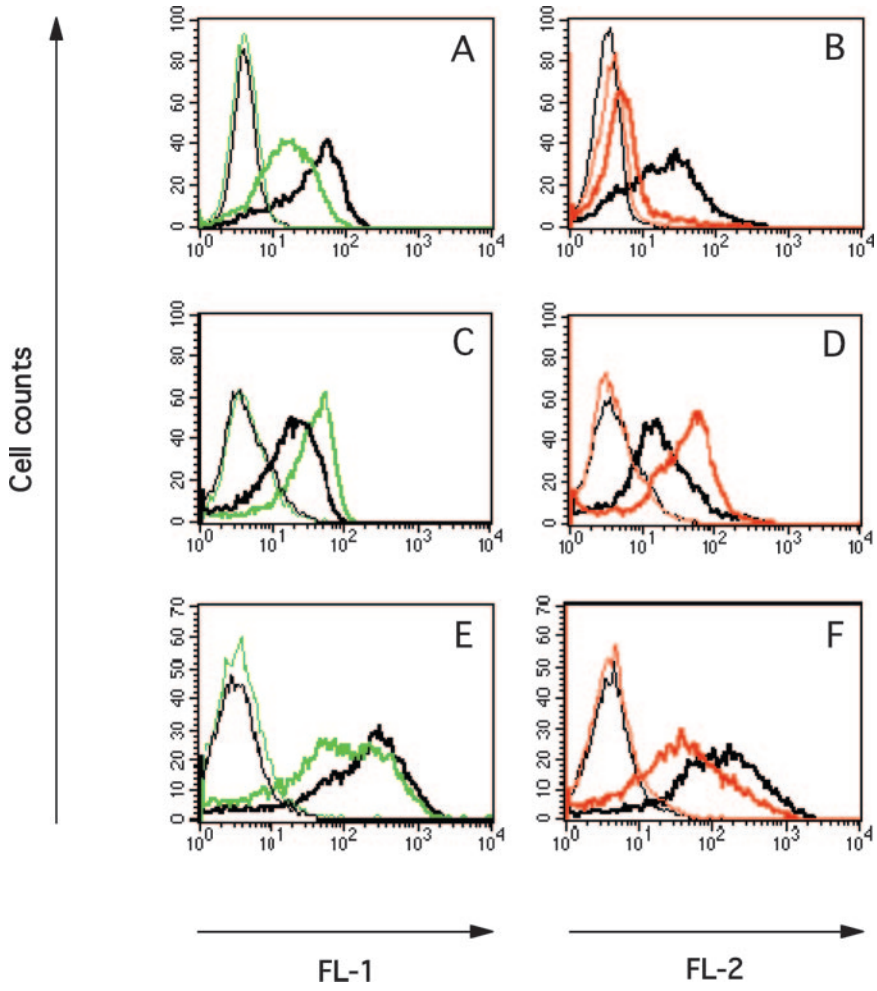

Fig. 2. Flow cytometric analysis of BLT1 and CCR2 expression on monocytes (10,000 cells counted/sample) after stimulation with 50 ng/ml IFN-γ (A, B), 10 ng/ml IL-10 (C, D), and 20 ng/ml TNF-α (E, F) during 8–12 h. The shift in BLT1 expression (green lines) is similar to that of CCR2 (red lines). Compared with nonstimulated, control cells (black lines), IFN-γ and TNF-α induced a down-regulation of BLT1 and CCR2. In contrast, IL-10 induced an up-regulation of receptor expression. Thin lines indicate isotype controls. Panels show a representative of at least two independent experiments.

imal at 25–50 ng/ml. The data yielded an EC_{50} value of 4 (2–11) ng/ml (mean and 95% confidence interval). Studies of CCR2 mRNA levels following stimulation with different IFN-γ concentrations (data not shown) indicated a similar potency as for BLT1. Our EC_{50} for CCR2 agreed with previous data [7]. The maximal inhibition, however, was less when measuring BLT1 transcripts (60% reduction) than for CCR2 (82% reduction).

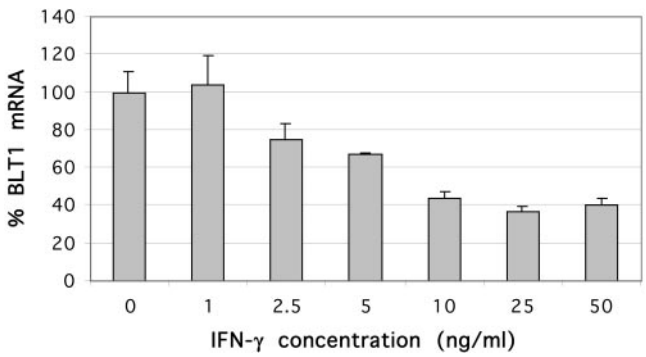

Fig. 3. IFN-γ lowers BLT1 mRNA levels in a concentration-dependent manner. Human monocytes were stimulated with 1–50 ng/ml IFN-γ for 8 h. The lowest levels of BLT1 mRNA were obtained after stimulation with 25–50 ng/ml IFN-γ. The approximate EC_{50} value was 4 (2–11) ng/ml (mean and 95% confidence interval). The figure shows triplicates from a representative of three independent experiments, and the results are presented as percentage of copy number in nonstimulated cells.

IFN-γ induced BLT1 mRNA down-regulation over time

Having defined the IFN-γ concentration that provided the optimal analytical window of BLT1 mRNA down-regulation, the effect over time was studied. Human monocytes were stimulated with 50 ng/ml IFN-γ, and mRNA was extracted at 0, 2, 4, 8, and 16–24 h, followed by real-time PCR quantification. There was a 20% down-regulation of BLT1 mRNA after 2 h (**Fig. 4**). At 4 h, mRNA levels had decreased by 25%, and the decrease amounted to almost 60% after 8 h. Stimulation for longer time-periods (16–24 h) gave no further decrease.

mRNA decay studies

mRNA levels may be altered by shifts in transcript stability or by changes in transcription rate. We evaluated the effects of IFN-γ on mRNA stability by inhibiting transcription with Actinomycin D, which was added to monocytes in the presence or absence of 50 ng/ml IFN-γ, after which mRNA was extracted at the indicated time-points for subsequent PCR quantification (**Fig. 5**). The half-life of BLT1 mRNA was ~5 h, and IFN-γ was found not to alter transcript stability. In agreement with previous studies [4, 7, 36], the half-life of CCR2 mRNA transcripts was ~2 h, which was reduced upon stimulation with IFN-γ (data not shown).

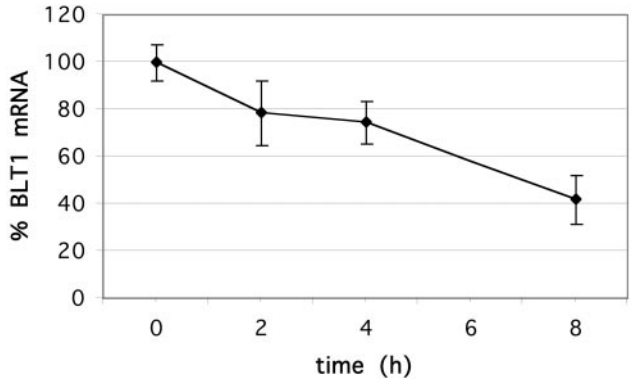

Fig. 4. IFN-γ regulation of BLT1 mRNA expression over time. Monocytes were stimulated with 50 ng/ml IFN-γ for 0–24 h. A down-regulation was detected at 2 h, reaching lowest levels of BLT1 mRNA at 8 h. After longer incubations (16–24 h), there was no further down-regulation of BLT1 mRNA levels. Figure shows triplicates or more from at least three donors, and the results are presented as percentage of copy number at 0 h.

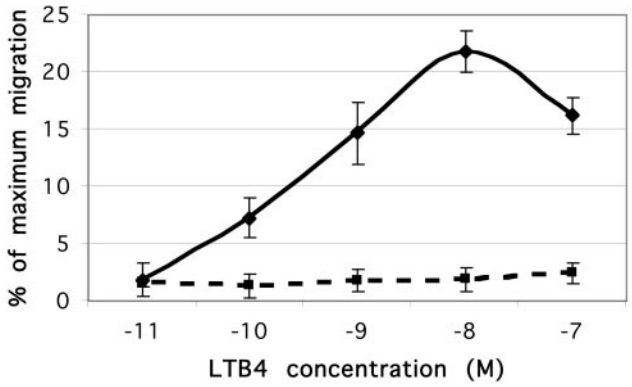

Fig. 6. IFN-γ inhibits LTB_4-induced monocyte chemotactic activity. Monocytes were stimulated with 50 ng/ml IFN-γ (dotted line) for 6–8 h or prior to challenge with LTB_4 (10^{-11} to 10^{-7} M) for 1 h. Compared with nonstimulated cells (solid line), IFN-γ-stimulated cells were practically irresponsive to LTB_4 challenge. The figure shows the results from three independent experiments with at least triplicates in each. Results are presented as percent of the highest possible amount of migrated cells, i.e., cell suspension added directly to wells. The cells in three high magnification fields at the center of each well were counted.

IFN-γ inhibits LTB_4-mediated monocyte chemotaxis

To determine if the IFN-γ-promoted down-regulation of BLT1 mRNA and cell-surface receptor protein expression affected functional responses to LTB_4, we performed a chemotaxis assay. Monocytes were incubated with 50 ng/ml IFN-γ for 6–8 h and were then evaluated for chemotactic responsiveness to a gradient of LTB_4 ranging from 10^{-11} to 10^{-7} M. Chemotactic responses to LTB_4 were almost completely abolished in cells pretreated with IFN-γ (**Fig. 6**).

Differential expression of BLT1 surface protein on human monocyte subpopulations

The last set of experiments included a three-color flow-cytometric analysis of two subpopulations of monocytes to study BLT1 and CCR2 expression. Whole blood was stained with antibodies recognizing CD14, CD16, and BLT1 or CCR2. During the analysis, a gate was set for monocytes based on forward- and side-scatter in addition to CD14 and CD16 expression. Two monocyte subsets expressing high levels of CD14 ($CD14^{++}CD16^{-}$) or low levels of CD14 and high levels of CD16 ($CD14^{+}CD16^{+}$) were defined. **Figure 7** illustrates that $CD14^{+}CD16^{+}$ monocytes express less BLT1 and CCR2 protein than $CD14^{++}CD16^{-}$. The differences between $CD14^{++}CD16^{-}$ and $CD14^{+}CD16^{+}$ were more pronounced for CCR2 than for BLT1. As $CD14^{+}CD16^{+}$ monocytes tend to be smaller and less granular than $CD14^{++}CD16^{-}$ monocytes, they are located close to the lymphocytes in a forward- and side-scatter diagram. To exclude the presence of $CD16^{+}$ natural killer cells, which express CD56, we analyzed the cells in the currently set gate for CD56 expression, but these cells were found to comprise less than 1% of the analyzed population (data not shown).

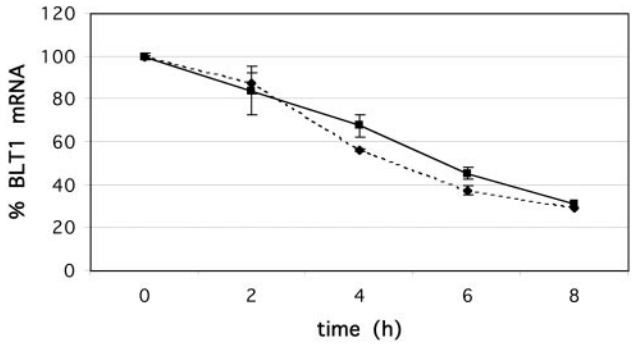

Fig. 5. BLT1 mRNA decay studies with Actinomycin D. Monocytes, nonstimulated (dotted line) or stimulated with 50 ng/ml IFN-γ (continous line) for 4 h, were exposed to Actinomycin D (5 μg/ml) for 0–8 h. The diagram reveals that BLT1 mRNA has a half-life of ~5 h and that IFN-γ did not affect BLT1 transcript stability. The figure shows a representative of two independent experiments performed in triplicates, and the results are presented as percentage of copy number at 0 h.

DISCUSSION

An important feature of the immune system is the directed migration of specific leukocyte subsets during inflammatory

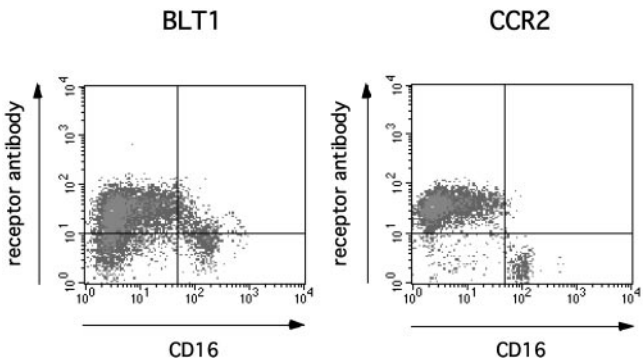

Fig. 7. Differential expression of BLT1 and CCR2 surface proteins in subpopulations of human monocytes in vivo. Whole blood was stained with CD14, CD16, and BLT1 (left panel) or CCR2 antibody (right panel). A gate was set for monocytes, and 10,000 cells were counted/sample. $CD14^{+}CD16^{+}$ monocytes showed a decreased expression of BLT1 and CCR2 as compared with $CD14^{++}CD16^{-}$ monocytes. Panels show a representative of three independent experiments.

events. This immune cell trafficking is orchestrated by chemotactic signals and their corresponding surface-bound receptors [1, 2]. The high-affinity receptor for LTB_4, BLT1, is expressed by subsets of immune cells and is needed for an efficient immune response to invading pathogens [16–21]. Furthermore LTB_4/BLT1 interactions are emphasized in the pathogenesis of various inflammatory conditions such as rheumatoid arthritis (RA), asthma, psoriasis, inflammatory bowel disease [22–25], and recently, atherosclerosis [27, 28]. Although BLT1 is highly expressed in human monocytes, information about its regulation in these cells has been lacking.

In the present study, we have analyzed the regulation of BLT1 mRNA and receptor surface expression in human monocytes by pro- and anti-inflammatory signals. We show that BLT1 mRNA is down-modulated by the proinflammatory cytokines IFN-γ, TNF-α, and LPS. In contrast, the anti-inflammatory cytokine IL-10 and the prototypic corticosteroid dexamethasone up-regulated BLT1 mRNA expression. Flow cytometric analyses, using highly specific monoclonal antibodies [35], revealed concomitant, positively correlated changes in BLT1 surface expression following monocyte exposure to IFN-γ, TNF-α, and IL-10. In line with previous studies [5, 7], chemokine receptor CCR2 mRNA and surface expression were regulated in a similar manner.

IFN-γ is a pleiotropic cytokine, important for T helper cell type 1-oriented inflammatory responses. It acts as a potent activator of mononuclear phagocytes and has well-characterized effects on CCR2 expression [6, 7]. Further exploration of BLT1 mRNA modulation therefore focused on IFN-γ. In these experiments, we found that IFN-γ lowered BLT1 mRNA expression in a time- and concentration-dependent manner, reaching maximal responses at 8 h and 25–50 ng/ml, respectively.

Steady-state mRNA levels may be reduced by inhibiting transcription or by decreasing mRNA stability. It has been reported that inflammatory mediators regulate levels of chemokine receptors by changing mRNA stability. For example, CCR2 mRNA half-life is reduced in monocytes treated with IFN-γ or LPS [4, 7, 37]. In the present work, we show that BLT1 mRNA transcript half-life is unaffected by IFN-γ stimulation and that the transcripts are relatively stable (5–6 h) when compared with those for several chemokine receptors (1–2 h) [36]. We therefore conclude that IFN-γ reduces BLT1 mRNA levels through an inhibition of BLT1 mRNA transcription. In addition, BLT1 mRNA levels were reduced in a similar manner over time in IFN-γ- (Fig. 4) and Actinomycin D-treated (Fig. 5) monocytes, which is in agreement with this conclusion. Based on the relative stability of BLT1 mRNA transcripts, the transcriptional rate of BLT1 in our experiments is probably low, and this may explain why IFN-γ modulation of BLT1 seems to be slower than what has previously been shown for CCR2 [7].

The functional relevance of lowered BLT1 expression was reflected in our chemotaxis experiments, where IFN-γ stimulation rendered monocytes irresponsive to LTB_4 challenge. As concomitant flow cytometric analyses revealed the presence of BLT1 receptors, although at a lower density, additional mechanisms seemingly contribute to this finding. To exclude the involvement of ligand-induced receptor desensitization as an explanation for this discrepancy, we measured LTB_4 levels in culture supernatants from IFN-γ-stimulated and nonstimulated monocytes. However, LTB_4 levels were barely detectable and were not elevated by IFN-γ stimulation (data not shown), which is in agreement with previous reports [38–40]. It is, however, possible that IFN-γ stimulation, through other mechanisms, render monocytes less responsive to LTB_4 by uncoupling BLT1 receptors from subsequent signal transduction pathways. In a study by D'Amico et al. [41], uncoupling several chemokine receptors was demonstrated in monocytes and dendritic cells stimulated with a combination of IL-10 and LPS/IFN-γ.

In recent years, the heterogeneity of human blood monocytes has received increasing attention. Two major populations can be detected based on differences in the expression of CD14 and CD16 [42, 43]. In healthy individuals, 85–95% of circulating monocytes express high levels of CD14 and no CD16 ($CD14^{++}CD16^{-}$), whereas 5–15% express CD16 and low levels of CD14 ($CD14^{+}CD16^{+}$) [44–46]. $CD14^{+}$ $CD16^{+}$ cells may represent monocytes that have been preactivated by inflammatory cytokines or microbial products. This is supported by the dramatic increase in $CD14^{+}CD16^{+}$ monocytes seen in various inflammatory conditions, such as sepsis, HIV-1 infection, and active RA [45–48]. Recent studies have revealed that these monocyte subsets have different migratory properties and chemokine receptor expression patterns. For example, although $CD14^{++}CD16^{-}$ cells are CCR2-positive and readily migrate toward a gradient of MCP-1, $CD14^{+}CD16^{+}$ monocytes lack CCR2 expression and display an attenuated chemotactic response to MCP-1 stimulation [44]. In the present study, we show that $CD14^{++}$ $CD16^{-}$ monocytes express high protein levels of BLT1 in addition to CCR2. In contrast, $CD14^{+}CD16^{+}$ monocytes were only weekly positive for BLT1 and as previously shown, lacked CCR2 expression. Thus, BLT1 and CCR2 are not only regulated in a similar manner by immunomodulating mediators but also share expression profiles in human monocytes.

It has been suggested that a down-modulation of chemotactic receptors by inflammatory mediators and microbial products may represent a mechanism of retaining immune cells at sites of inflammation [49]. In this way, locally produced cytokines can control leukocyte tissue infiltration through the regulation of chemotactic ligands and by inducing leukocyte arrest at the appropriate location.

The down-regulation of chemotactic receptors by proinflammatory signals may also represent a mechanism for avoiding excessive accumulation of immune cells at inflammatory sites. For instance, generation of high levels of proinflammatory mediators in severely inflamed tissue will leak out into the circulation, where a preactivation of leukocytes and down-modulation of chemotactic receptors will prevent further monocyte recruitment to the site of inflammation. Geissmann et al. [50] demonstrated that the mouse counterpart to the human $CD14^{+}CD16^{+}$ monocyte was excluded from inflamed tissues in vivo, and in light of our findings, this may be explained by a reduced expression of CCR2 and BLT1 on these cells. Furthermore, in a murine peritonitis model, injection with LPS resulted in the down-regulation of monocyte CCR2 expression and loss of macrophage infiltration in the peritoneum [51]. A concomitant down-modulation of BLT1 may occur and could

help to explain the completely abolished monocyte recruitment in this model. Finally, the same mechanism may contribute to the seemingly paradoxical, disease-limiting effects caused by systemic IFN-γ that have been observed in models of experimental autoimmune diseases (reviewed in ref. [52]).

It is interesting that MCP-1/CCR2 and LTB_4/BLT1 interactions seem to cooperate in the mobilization of immune cells during inflammation. In a study by Matsukawa et al. [33], exogenous MCP-1 stimulation induced the release of LTB_4 from mononuclear phagocytes in a murine model of peritonitis, and recently, Huang et al. [32] demonstrated a strong increase in MCP-1 production and release in primary human monocytes stimulated with LTB_4. This receptor cross-talk may represent an amplification loop of importance for leukocyte recruitment during inflammatory events, such as atherosclerosis. The striking similarities between BLT1 and CCR2 regulation and expression in monocytes may reflect this functional relationship.

Increased understanding of atherosclerosis as an inflammatory process has highlighted mononuclear phagocytes as well as inflammatory cytokines such as IFN-γ and TNF-α as major participants in this pathology [53, 54]. In recent years, studies of apolipoprotein E $(apoE)^{-/-}$ and low-density lipoprotein receptor $(LDLR)^{-/-}$ transgenic mice have indicated that not only MCP-1 and its receptor CCR2 but also LTB_4/BLT1 interactions substantially contribute to the development of atherosclerotic lesions. For example, treatment of $LDLR^{-/-}$ and $apoE^{-/-}$ mice with a BLT1 antagonist significantly inhibits atherosclerotic plaque formation and reduces monocyte infiltration of the lesions [27]. Deletion of BLT1 from the $apoE^{-/-}$ murine genome also attenuated the initial pathogenesis of atherosclerosis [28]. Furthermore, the 5-LO gene, which is involved in the formation leukotrienes including LTB_4, contributes profoundly to the development of atherosclerosis susceptibility in mice [55]. Recently, in a population-based study, Helgadottir et al. [56] found that a single nucleotide polymorphism in the gene encoding a 5-LO-activating protein was associated with an increased risk of myocardial infarction and found that this correlated with an increased production of LTB_4. Hence, mounting evidence points to a critical role for LTB_4 and BLT1 during different stages of atherosclerotic disease.

In conclusion, we show that BLT1 expression in human monocytes is modulated by inflammatory mediators in a similar manner to the chemokine receptor CCR2. Our findings could shed further light on the complex mechanisms that regulate monocyte trafficking during inflammation. Information about BLT1 regulation in human monocytes may also increase our understanding of inflammatory diseases that involve LTB_4/BLT1 interactions.

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