Downregulated CD36 and oxLDL uptake and stimulated ABCA1/G1 and cholesterol efflux as anti-atherosclerotic mechanisms of interleukin-10

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Abstract

Objective: Marked anti-atheromatous effects of the anti-inflammatory cytokine interleukin-10 (IL-10) were observed in several lipid-driven animal models of arteriosclerosis. We therefore investigated whether IL-10 affects macrophage cholesterol handling.

Methods: Human THP-1 cells and peripheral monocytes served as macrophage models. Specific mRNA was quantified by real-time RT-PCR, protein expression by flow cytometry and Western blotting. Cellular cholesterol handling was studied by lipoprotein-facilitated uptake and efflux assays. IL-10 effects were also studied in cells transfected with liver X receptor alpha (LXRα)-siRNA or a LXRα response element (LXRE) reporter construct.

Results: Picomolar IL-10 suppressed basal and peroxisome proliferator-activated receptor gamma (PPARγ)-stimulated transcription of the scavenger receptor CD36 due to reduced PPARγ protein expression. In contrast, IL-10 stimulated transcription of the active cellular cholesterol exporters ATP-binding cassette transporters A1 and G1 (ABCA1, ABCG1) and the LDL receptor, whereas scavenger receptor-BI (SR-BI) was unchanged. The reduction of CD36 and stimulation of ABCA1 expression was confirmed in human monocytes. Thereby, IL-10 prevented cellular cholesterol overloading from oxidized LDL (oxLDL) and enhanced efflux to apoA-containing particles initiating reverse cholesterol transport. Experiments with inhibitors, LXRα silencing and the LXRE reporter gene construct supported the proximal transmission of the IL-10 effect on ABCA1 by the IL-10 receptor/signal transducer and activator of transcription 3 (STAT3) pathway and distal cross-talk to the LXRα and PPARα/retinoic acid X receptor (RXR) and cAMP/protein kinase A (PKA) pathways.

Conclusions: In addition to immune and anti-inflammatory actions, IL-10 redirects macrophage cholesterol handling towards reverse cholesterol transport, which contributes to its anti-atherosclerotic action.

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Keywords: Cytokines; CD36; ABCA1; Reverse cholesterol transport; Atherosclerosis

1. Introduction

Current concepts of atherogenesis assume that diverse noxious stimuli, like radicals generated by smoking, hyperlipidemia or diabetes, chronic infection or hemodynamic stress may irritate endothelial cells leading to reduced endothelial dependent vasodilatation, increased lipoprotein insudation and adherence and transmigration of monocytes [1]. Whether these defects will resolve again or progress to an advanced atherosclerotic plaque with infiltration, lipid and matrix deposition and cellular proliferation largely depends on the local formation of cytokines.

In contrast to most cytokines that act as strong paracrine amplifiers of the immuno-inflammatory process in the vessel wall, IL-10 secreted by activated monocytes/macrophages and T-cells has pronounced immunosuppressive and anti-inflammatory effects. The inflammatory component of atherosclerosis therefore offers a rewarding target for IL-10. In fact, IL-10 deficiency aggravated [2] and IL-10 overexpression [3] attenuated experimental atherosclerosis. Even in lipid driven models like cholesterol feeding [2] and apoE [4–6] or LDL-receptor knockout mice [7,8] IL-10 pro-
foundly reduced the lipid accumulation in the vessel wall. We hypothesized that IL-10 enhances reverse cholesterol transport out of the vessel wall. This requires the uptake of extracellular lipid depots into macrophages by scavenger receptors like CD36, SR-A or SR-BI and the efflux of internalized cholesterol to apoAI containing particles via cholesterol exporters like ABCA1 and ABCG1 [9–11]. We therefore studied the effects of IL-10 on key receptors of macrophage cholesterol homeostasis, on the nuclear transcription factors PPARγ and LXRα regulating their expression and on macrophage cholesterol handling.

2. Materials and methods

2.1. Reagents and cell incubations

IL-10 was purchased from PromoCell, piceatannol from Calbiochem, 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) from Cayman and Ro 31-8220 from Upstate Biotechnology. All other reagents were from Sigma. Human monocyteid THP-1 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum, penicillin (200 U/ml) and streptomycin (200 µg/ml), and differentiated to macrophage-like cells by pre-incubation with phosphor-myristrate-acetate (PMA, 160 nM, 72 h). In accordance with the Declaration of Helsinki, healthy volunteers were asked to donate 80 ml blood for the preparation of peripheral mononuclear cells (PBMCs). Cells were isolated by ficoll gradient centrifugation and cultured in RPMI 1640 medium. Human HepG2 cells were cultured in DMEM medium supplemented as above plus 1% non-essential amino acids.

Cells were incubated with IL-10 (10 ng/ml) or agonists like 15d-PGJ2 (2 µM), indomethacin (10 µM), 22-hydroxycholesterol (22-OHC, 10 µM), 9-cis-retinoic acid (RA, 2 µM) or fenofibrate (100 µM) for 3 to 72 h. Inhibitors of the PKA (Ro 31-8220, 900 nM) or STAT3 pathways (piceatannol, 50 µM) were added 30 min before incubation with IL-10 for 3 h. cAMP was measured as described [12]. No toxicity was detected by cell count, trypan-blue exclusion and quantitative real-time RT-PCR of β-actin or GAPDH.

2.2. RNA isolation and real-time RT-PCR

Total RNA was extracted by the Quantum Prep AquaPure Kit (BioRad) and treated with DNase (Promega). Quantitative real-time RT-PCRs were run on an iCycler (BioRad) using ready-to-go RT-PCR beads (Amer- sham Biosciences), validated primers (Metabion GmbH) for β-actin, GAPDH, CD36, ABCA1, ABCG1, LXRα, SR-BI, LDL-receptor [12,13] and SybrGreen I (Molecular Probes) for detection. The cycle number, when the fluorescence first reaches a preset threshold \( C_i \), allows the quantification of the specific template concentration. Transcripts of the housekeeping genes β-actin or GAPDH in the same incubations were used for normalization. Effects of treatment are reported as fold change of specific receptor mRNA in cells incubated with IL-10 (and agonists or inhibitors) versus control cells incubated in parallel with carrier (and agonists or inhibitors). It was calculated by the double delta method as fold change \( = 2^{(-\Delta \Delta C_i)} \) with \( \Delta C_i = C_i^{(specific transcript)} - C_i^{(housekeeping transcript)} \) and \( \Delta \Delta C_i = \Delta C_i^{(treatment)} - \Delta C_i^{(control)} \) [14]. Expression in concurrent controls is by definition at any time represented by a horizontal line through fold change =1 and was omitted from figures.

2.3. Protein extraction and Western blot

Identical amounts of cell lysate proteins [15] (Dc Protein Assay, BioRad) were separated on 10% (LXRα, 64 kDa) or 6% SDS PAGE gels (ABCA1, 220 kDa). After transfer, membranes were incubated with specific antibodies against LXRα (Dianova) or ABCA1 (Novus Biochemicals). Anti-rabbit IgG-HP antibody (Santa Cruz) and chemiluminescence (Pierce) were used for detection. β-Actin (Amersham Biosciences) served as loading control.

2.4. Flow cytometry

Cells were washed with Hank’s buffer complemented with 10 mM HEPES, 1 mM Ca\(^{2+}\), 1 mM Mg\(^{2+}\) and 0.5% bovine serum albumin, permeabilized with ice-cold methanol (40 s), incubated with 5% human serum for 30 min and with isotype (MOPC-21, Sigma) or anti-PPARγ (E-8, Santa Cruz) for 45 min on ice. Then cells were incubated with FITC-labeled anti-mouse (Sigma), fixed with 3.7% formaldehyde and analyzed by flow cytometry (FACScan, Becton Dickinson). For FACS analysis of cell surface CD36, cells were directly incubated with anti-CD36 (E-8, Santa Cruz) for 45 min on ice. Then cells were incubated with FITC-labeled anti-mouse antibody.

2.5. Lipoprotein preparation and uptake/efflux assay

Lipoproteins were prepared from 80 ml blood from fasting healthy donors by gradient ultracentrifugation [16]. The HDL3 sub-fraction was prepared in the density range 1.125–1.210 g/ml [10], concentrated with Centriflo Cones (BioRad) (2200 rpm, 20 min, 4 °C) and washed twice with PBS buffer. Purity of preparations was confirmed by lipoprotein-gel-electrophoresis (Helena Titan Gel System).

LDL was desalted using Econo-Pac 10 DG columns (Amersham Biosciences), diluted to 0.2 mg/ml protein and oxidized with 10 µM CuCl\(_2\) for 16 h at 37 °C [16]. Oxidation was monitored at 234 nm [17] and stopped after 16 h with 24 mM EDTA. OxLDL was concentrated and washed twice. HDL was delipidated [18] with 12 volumes ice-cold methanol and 28 volumes ice-cold diethyl ether. After centrifugation (1000 rpm, 5 min, 4 °C), the pellet was washed, dried with nitrogen and dHDL was reconstituted in buffer.
For uptake and efflux assays, cells were pre-incubated for 24 h with IL-10 (10 ng/ml) or carrier. Then cells were either exposed to oxLDL (100 μg/ml) for 48 h or dHDL (20 μg/ml) or HDL3 (20 μg/ml) for 24 h or the corresponding buffer. Fresh IL-10 (10 ng/ml) or carrier was added at 0 h and 24 h. At the end, cells were washed twice, lipids were extracted from the pelleted cells and total cellular cholesterol was quantified as previously described [12].

2.6. Transfection for LXRα silencing and LXRE reporter gene assay

For knock-down experiments, THP-1 cells were transfected using the Cell Line Nucleofector™ Kit V (Amaxa) for 24 h and the pSilencer™ 1.0-U6 vector (Ambion). The LXRα specific small interference (si) RNA sequences used were: forward 5'-ACT GAA GCG GCA AGA GGA GTT CAA GAG ACT CCT TGT GCC GCT TCA GTT TTT TT-3' and reverse 5'-AAT TAA AAA AAC TGA AGC GGC AAG AGG AGT CTC TTG AAC TCC TCT TGC CGC TTC AGT GGC C-3'.

For assaying LXRα activation HepG2 cells were magnetofected [19] with 0.5 μg of reporter plasmid (CMV-hLXRα) and 0.125 μg of LXRα plasmid (tk-LXREX3-luc) for 48 h. The plasmids were a gift of Dr. Mangelsdorf (HHMI, Dallas). After 48 h, wells were incubated with IL-10 (10 ng/ml), 22-OHC (10 μM), RA (1 μM) or carrier control for 3 h. The Luciferase Assay System from Promega was used and luminescence normalized to protein amount.

3. Statistical analysis

Means of multiple experimental conditions were compared by Kruskal–Wallis H analysis of variance by ranks (*p<0.05, **p<0.01, ***p<0.001). Then a single pair comparison of the IL-10 effect versus the corresponding control was made by Mann–Whitney U-test (p<0.05, ** p<0.01). Data are plotted as mean±S.E.M.

4. Results

The expression of CD36, the quantitatively most important scavenger receptor, was studied during incubation of PMA-differentiated THP-1 cells with IL-10 (Fig. 1A). Compared to cells incubated with carrier only, IL-10 caused a rapid and sustained suppression of CD36 mRNA by more than 50%. CD36 expression was stimulated about threefold by incubation with the PPARγ agonist 15d-PGJ2. IL-10 attenuated this stimulation of CD36 by about 50%. Therefore, IL-10 cannot only reduce baseline CD36 expression, but also attenuate the feed forward induction of CD36 by PPARγ.

Next, ABCA1, a cellular cholesterol exporter, was studied (Fig. 1B). Incubation of cells with IL-10 caused a rapid and sustained enhancement of ABCA1 expression by about 120%. In contrast, the marked stimulation of ABCA1 by 15d-PGJ2 was significantly reduced by co-incubation with 15d-PGJ2 alone (grey line). (B) Time course of ABCA1 mRNA expression in cells stimulated as described above. (C) LDL-receptor and SR-BI mRNA in cells incubated with IL-10 (10 ng/ml). Effects of specific treatments are always represented as fold change compared to control cells concurrently incubated with carrier only (means±S.E.M., n>4; *<0.05, **<0.01, ***<0.001).

Fig. 1. (A) Time course of CD36 mRNA expression in PMA-differentiated THP-1 cells incubated with IL-10 (dashed black line), in cells incubated with IL-10 and 15d-PGJ2 (2 μM) (dashed grey line), and in cells stimulated with 15d-PGJ2 alone (grey line). (B) Time course of ABCA1 mRNA expression in cells stimulated as described above. (C) LDL-receptor and SR-BI mRNA in cells incubated with IL-10 (10 ng/ml). Effects of specific treatments are always represented as fold change compared to control cells concurrently incubated with carrier only (means±S.E.M., n>4; *<0.05, **<0.01, ***<0.001).
supporting the relevance of these IL-10 effects in vivo. The expression of the bidirectional cellular cholesterol transporter SR-BI was not changed by IL-10, whereas IL-10 enhanced the expression of the apoB specific LDL-receptor (Fig. 1C).

The negative interference of IL-10 with the PPARγ agonist 15d-PGJ2 was explained by the reduction of PPARγ protein expression in monocytoid cells incubated with IL-10 (Fig. 2A). Similar to the effect on the mRNA level, IL-10 also reduced CD36 protein cell surface expression and abolished its stimulation by the PPARγ agonist indomethacin (Fig. 2B).

ABCA1 antibodies available are not suitable for flow cytometry. Using western blot, IL-10 was shown to stimulate ABCA1 protein expression several fold with the potent LXRα agonist 22-OHC serving as positive control (Fig. 2C, upper insert). LXRα protein expression was moderately stimulated by IL-10 after 24 h (Fig. 2C, lower insert). LXRα mRNA expression was increasingly stimulated by IL-10 for at least 48 h (Fig. 2D). The effects of IL-10 on CD36, PPARγ, ABCA1 and LXRα protein paralleled the effects on the mRNA level.

To elucidate the mechanisms involved, known signaling pathways of CD36 and ABCA1 regulation were studied. ABCA1 stimulation by IL-10 was abrogated by co-incubation with piceatannol, the most proximal inhibitor available for the IL-10-receptor-STAT3 pathway. Basal ABCA1 expression remained unchanged (Fig. 3A). Co-incubation of cells with IL-10 amplified the stimulation of ABCA1 by 22-OHC and RA, permissive activating ligands of the heterodimeric LXRα/RXR transcription factor (Fig. 3B). Conversely, transfection of cells with a specific LXRα-siRNA sequence, but not random-siRNA abrogated the IL-10 stimulation of ABCA1 (Fig. 3C). In cells transfected with LXRα and a LXRE-luciferase reporter gene construct, IL-10 alone induced a threefold increase of luciferase activity over carrier control corresponding to about a third of the signal of massive LXRα/RXR stimulation with 22-OHC and RA (Fig. 3D).

Inhibition of PKA by Ro 31-8220 reduced baseline ABCA1 expression and abrogated the stimulation of ABCA1 by IL-10 (Fig. 3E). The involvement of the cAMP/PKA pathway in the ABCA1 stimulation by IL-10 was supported by an increase of cAMP levels (30 min: +28%±9%, n.s.). In contrast to the attenuation of the PPARγ pathway, IL-10 had an additive effect on the stimulation of ABCA1 by the PPARα agonist fenofibrate (Fig. 3F). Thus, mechanistic experiments demonstrated the activation of the LXRα pathway by IL-10 and cross-talk to the PPARα and cAMP/PKA pathways contributing to ABCA1 stimulation by IL-10.

The functional consequences of the IL-10 effects on CD36 and cholesterol exporters were assayed as lipoprotein facilitated uptake and efflux. Incubation with IL-10 alone always tended to reduce total cellular cholesterol

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Fig. 2. (A) Time course of PPARγ protein expression in THP-1 cells after stimulation with IL-10 (10 ng/ml) measured by flow cytometry after specific staining of permeabilized cells (means±S.E.M., n>4; ***<0.001). (B) Time course of CD36 protein expression measured by flow cytometry on cells stimulated with indomethacin (10 µM) (grey line), stimulated with indomethacin and incubated with IL-10 (10 ng/ml) (grey dashed line) or incubated with IL-10 alone (solid black line) (means±S.E.M., n>4; ***<0.001). (C) Western blot of ABCA1 (upper insert) and LXRα protein expression (lower insert) in extracts from cells after incubation with carrier (Co), IL-10 (10 ng/ml) or 22-OHC (10 µM) for 24 h. (D) Time course of LXRα mRNA expression in cells incubated with IL-10 (10 ng/ml) (means±S.E.M., n=8; ***<0.001).
content. OxLDL increased cellular cholesterol, but co-incubation with IL-10 more than compensated for the cholesterol accumulation from oxLDL (Fig. 4A). dHDL as cholesterol acceptor from ABCA1 reduced cellular cholesterol content and co-incubation of cells with IL-10 and dHDL further enhanced cellular cholesterol depletion (Fig. 4B). Native HDL3 reduced cellular cholesterol content and co-incubation with IL-10 tended to enhance cellular cholesterol loss to HDL3, the preferred acceptor for ABCG1[10] (Fig. 4C). In concordance, ABCG1 specific mRNA was time-dependently stimulated by IL-10 (Fig. 4D). In summary, the functional assays supported the relevance of the IL-10 effects on CD36, ABCA1 and ABCG1 for macrophage lipid handling.

5. Discussion

If noxious stimuli irritate endothelial cells severely enough to initiate an inflammatory reaction, this still represents a physiologic attempt of repair. However, the inflammatory response in the vessel wall has to be shut down again to avoid progression to advanced lesions. IL-10 can counteract inflammatory amplification loops by redirecting the immunologic orientation and cytokine production of infiltrating mononuclear cells toward a TH2 response. However, marked vascular lipid accumulations in IL-10 knockout mice [2] and resistance to atherosclerosis in IL-10 overexpressing mice [3] pointed to an additional anti-atherogenic effect of IL-10 in the vessel wall. We
therefore studied the effects of IL-10 on the lipid handling of macrophages.

We found a reduced expression of the scavenger receptor CD36 already by low concentrations of IL-10 in monocyted cells and after macrophage-like differentiation and in freshly prepared peripheral monocytes. The IL-10 effect on CD36 was more marked on the mRNA than on the protein level, what can be explained by the storage of preformed CD36 protein in intracellular pools of cells and receptor recycling [20]. Oxidation products of fatty acids in modified LDL particles internalized by macrophages via scavenger receptors like CD36 can activate PPARγ. PPARγ further stimulates CD36 expression [21], potentially leading to foam cell formation, cell death, release of lipids and matrix-metalloproteinases, all features of unstable plaques. IL-10 also delayed and attenuated CD36 expression after stimulation with endogenous and pharmacologic PPARγ agonists and reduced PPARγ protein. Therefore, the inhibition of CD36 by IL-10 was at least in part mediated by interference with PPARγ thus counteracting the deleterious autamplification loop of CD36 expression stimulated by its ligand oxLDL.

Scavenger receptor mediated internalization by macrophages is required for removal of modified lipoproteins from the vessel wall. Cholesterol outflow to the reverse transport pathway has to keep pace with uptake by scavenger receptors to avoid transformation of macrophages to foam cells. ABCA1 and ABCG1 have been characterized as the rate limiting unidirectional cellular cholesterol exporters transferring cholesterol onto lipid poor apoAI and lipidated HDL particles, respectively [10,22,23]. In contrast to the inhibition of CD36, IL-10 stimulated ABCA1 and ABCG1 expression, explaining the marked increase in HDL cholesterol in transgenic mice overexpressing IL-10 [2].

Cellular cholesterol depletion normally leads to down-regulation of ABCA1 expression [24]. The stimulation of ABCA1 by IL-10 appears to escape the feed-back suppression of ABCA1 by cholesterol depletion. PPARγ induces ABCA1 expression [25,26]. In fact, the endogenous PPARγ agonist 15d-PGJ2 alone stimulated ABCA1 expression, but co-incubation with IL-10 attenuated the stimulation of ABCA1 by the PPARγ agonist. This negative interaction of the two positive stimuli was explained by the inhibition of PPARγ protein expression by IL-10. Thus, PPARγ cannot transmit the stimulation of ABCA1 by IL-10.

IL-10 binds to a member of the cytokine receptor family that signals through the Jak/STAT3-pathway [27]. It finally transmits the anti-inflammatory and immune effects of IL-10 by induction of suppressor of cytokine signalling-3 and -5 (SOCS-3 and -5) [27]. SOCS-3 inhibits the transcription of pro-inflammatory cytokines and genes stimulated by them. In addition, metabolic effects like hepatosteatosis and insulin resistance could be induced by hepatic overexpression of SOCS-1 and SOCS-3 in mice [28]. Direct blockers of the IL-10-receptor are not available. We therefore used piceatannol, a specific inhibitor of the proximal Jak1/Tyk2/STAT3/5 pathway [29,30]. Piceatannol abolished the stimulation of ABCA1 by IL-10, but did not affect baseline expression of
ABCA1. Thus, the IL-10 stimulation of ABCA1 is proximally transmitted via the classic IL-10 pathway, but IL-10 is not relevant for baseline ABCA1 expression in macrophages. A STAT-binding site in the ABCA1 promoter at −350 bp (www.genomatix.de) offers a potential direct link from the IL-10-receptor pathway to ABCA1 expression. But this binding motif is not STAT3 specific and may integrate signals from several cytokine receptors.

We also studied the potential cross-talk to pathways known to regulate ABCA1. LXRα, an oxysterol-activated nuclear transcription factor that heterodimerizes with RXR, stimulates ABCA1 [31,32]. IL-10 stimulated LXRα mRNA and protein in our cells. Co-incubation with IL-10 enhanced the stimulation of ABCA1 by RA as well as by 22-OHC. Conversely, transfection of cells with a specific LXRα-siRNA prevented the IL-10 stimulation of ABCA1 and transfection with a LXRα/LXRE-luciferase construct directly demonstrated LXRα activation by IL-10. This supports a major role of cross-talk to the LXRα/RXR pathway in the IL-10 effect on ABCA1.

The cAMP/PKA [22] and the PPARα/RXR pathways also induce ABCA1 expression [25,33]. Co-incubation of macrophages with the specific PKA inhibitor Ro 31-8220 blocked the stimulation of ABCA1 by IL-10. Ro 31-8220 appeared to withdraw a contribution of the cAMP/PKA pathway to baseline ABCA1 expression. A moderate stimulation of the cellular cAMP level by IL-10 was directly confirmed. cAMP may integrate the signals from several adenylate cyclase coupled membrane receptors on ABCA1 expression. Finally, co-incubation with IL-10 enhanced the stimulation of ABCA1 by a PPARα agonist in an additive manner. Taken together, the cross-talk from the classical IL-10 pathway to the LXRs/RXR, cAMP/PKA and PPARα/RXR pathways contributes to the IL-10 stimulation of ABCA1.

The selectivity of the IL-10 effects on ABCA1 and CD36 expression is underlined by the absence of IL-10 effects on SR-BI. SR-BI, structurally related to CD36, functions as a bidirectional transporter equilibrating cellular cholesterol with plasma lipoproteins. SR-BI serves as HDL receptor in the liver completing reverse cholesterol transport. SR-BI can be induced by the PPARγ, PPARα and LXRα pathways [34–36], but its regulation is incompletely understood as SR-BI is also inversely correlated with ABCA1 expression [37,38].

Earlier studies in cholesterol fed animals and in apoE and IL-10 double knockouts [5] reported no change of total cholesterol levels by IL-10, but IL-10 overexpression reduced total cholesterol, LDL and very low density lipoprotein (VLDL) sub-fractions [3]. If IL-10 causes a similar increase of LDL-receptor expression in the liver as we observed in macrophages, this can well explain the reduction in LDL. An inhibition of hydroxy-methyl-glutaryl-CoA (HMG-CoA) reductase expression by IL-10 has been reported in HepG2 cells [39]. In contrast to the sterol-regulatory-element-binding-protein (SREBP) pathway that stimulates LDL-receptor and HMG-CoA reductase in parallel in response to cellular cholesterol depletion, IL-10 would favor net hepatic cholesterol clearance.

On the functional level, the IL-10 effects on CD36, ABCA1 and ABCG1 were shown to attenuate cholesterol accumulation in macrophages exposed to oxLDL and to enhance cellular cholesterol efflux towards reverse cholesterol transport. However, some of our findings are at variance with a recent report also studying IL-10 effects on THP-1 cells and peripheral PBMC differentiated into macrophages [40]. IL-10 enhanced neutral lipid accumulation from oxLDL (mostly measured as oil red O reextractable from stained cells) in THP-1 cells and in macrophages obtained from patients with unstable angina, but reduced lipids in macrophages from healthy controls. The authors demonstrated an anti-apoptotic effect of IL-10 on oxLDL exposed macrophages and discussed a benefit from IL-10 by plaque stabilization.

Taken together, our findings in human cells offer a mechanism for the potent anti-atheromatous action of IL-10 in animal models [2,3]. The evidence from cross-sectional observations in man is less clear cut. In unstable angina, both decreased [41,42] and increased levels of immunoreactive IL-10 [43] have been reported. Anti-CMV-IgG positivity was more often associated with high IL-10 levels in coronary heart disease patients than in controls [44]. IL-10 is highly expressed in advanced plaques, which could elevate systemic levels, but may be a counter regulatory response to pro-inflammatory cytokines [45]. Noteworthy, all studies reporting increased IL-10 levels in unstable angina had measured it in serum, which will contain IL-10 secreted by mononuclear cells during clotting. In contrast, plasma levels of IL-10 more accurately reflect the in vivo situation, are much lower and were decreased in patients with stable as well as unstable angina [42].

In summary, IL-10 at low picomolar concentrations, which exert its well known anti-inflammatory and immune effects, attenuates macrophage CD36 expression and enhances ABCA1 and ABCG1 expression. This prevents macrophage cholesterol overloading and stimulates reverse cholesterol transport. These additional effects of IL-10 on key effectors of macrophage cholesterol handling can explain the marked anti-atheromatous action of IL-10 seen in several animal models of arteriosclerosis. The effects of IL-10 on CD36, ABCA1, ABCG1 and LDL-receptor complement its anti-inflammatory effects. Thereby, IL-10 could promote plaque stabilization and regression. As the general pharmacologic safety profile of IL-10 in phase III trials in other inflammatory conditions [46] appeared promising, and low IL-10 levels indicated an excess mortality in unstable angina [47], the potential benefit from IL-10 in situations were rapid plaque stabilization is required deserves further evaluation.
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