Interleukin-10 enhances the oxidized LDL-induced foam cell formation of macrophages by antiapoptotic mechanisms

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Abstract Interleukin (IL)-10 may have a therapeutic potential in atherosclerosis, but its mechanisms of action have not been clarified. Foam cell formation is a key event in atherogenesis, and apoptosis of these lipid-laden cells may promote plaque destabilization. We sought to explore whether IL-10 could have plaque-stabilizing properties in acute coronary syndromes (ACS). We studied the effect of IL-10 on oxidized low density lipoprotein (oxLDL)-stimulated THP-1 cells and monocyte-derived macrophages from ACS patients and healthy controls using different experimental approaches. Our main findings were: *i*) IL-10 enhances lipid accumulation in oxLDL-stimulated THP-1 macrophages, at least partly by counteracting oxLDL-induced apoptosis; *ii*) This antiapoptotic effect of IL-10 involves increased expression of the antiapoptotic genes Bfl-1 and Mcl-1, accompanied by protective effects on mitochondria function; *iii*) By silencing Bfl-1 and Mcl-1 genes using siRNAs, we were able to abolish this IL-10-mediated effect on lipid accumulation; *iv*) IL-10 also induced lipid accumulation in oxLDL-stimulated macrophages from patients with ACS, but not in macrophages from healthy controls; *v*) In ACS patients, this enhancing effect of IL-10 on lipid accumulation was accompanied by enhanced Mcl-1 expression. No such antiapoptotic effect was seen in macrophages from healthy controls.—These findings suggest a new mechanism for the effect of IL-10 in atherosclerosis, possibly contributing to plaque stabilization.—Halvorsen, B., T. Wæhre, H. Scholz, O. P. Clausen, J. H. von der Thüsen, F. Müller, H. Heimli, S. Tonstad, C. Hall, S. S. Frøland, E. A. Biessen, J. K. Damås, and P. Aukrust. **Interleukin-10 enhances the oxidized LDL-induced foam cell formation of macrophages by antiapoptotic mechanisms.** *J. Lipid Res.* 2005. **46:** 211–219.

Supplementary key words foam cell macrophages • acute coronary syndromes • atherosclerosis • apoptosis

Atherosclerosis is a multifactorial disease with elements of both lipid deposition and inflammation. Exposure of macrophages to oxidized low density lipoprotein (oxLDL), a major component of human atherosclerotic plaques, appears to be a key event in this process, promoting both inflammation and intracellular cholesterol deposition with formation of lipid-laden foam cells (1). In addition to their role in atherogenesis, foam cells may also be involved in plaque destabilization. Thus, vulnerable atherosclerotic lesions are characterized by accumulation of foam cells, and destabilization of such lipid-rich areas may induce plaque rupture, leading to thrombus-mediated acute coronary syndromes (ACS) (1).

Interleukin (IL)-10, a prototypical antiinflammatory cytokine, seems to be involved in atherogenesis (2, 3). Thus, studies in IL-10-transgenic and IL-10-deficient mice models suggest an important protective role for this cytokine in both the formation and the stabilization of atherosclerotic lesions (2, 4, 5). Moreover, we have recently shown that IL-10 inhibits the release of inflammatory cytokines, tissue factor, and matrix metalloproteinases from periph-

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Abbreviations: ACS, acute coronary syndromes; CAD, coronary artery disease; CRP, C-reactive protein; IL, interleukin; LOX-1, lectin-like oxLDL receptor-1; oxLDL, oxidized low density lipoprotein; PBMC, peripheral blood mononuclear cell; PI, propidium iodine; PMA, phorbol myristate acetate; RPA, RNase protection assay; TNF, tumor necrosis factor.

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eral blood mononuclear cells (PBMCs) in patients with ACS, potentially promoting plaque stabilization (6). However, the exact mechanisms of action of IL-10 in atherogenesis have not been fully clarified and its role in plaque stabilization is far from clear. In particular, no reports, as far as we know, have addressed the role of IL-10 in foam cell formation.

Although the pathologic and clinical significance of apoptosis in atherogenesis remains controversial, it has been proposed that apoptotic cell death may promote plaque instability (7, 8). However, the cellular mechanisms and the role of apoptosis in the various stages of atherogenesis are still not clear. Furthermore, the ability of cytokines and growth factors to modify apoptosis in foam cells and other cellular components of atherosclerotic lesions has not been clarified.

IL-10 seems to have antiapoptotic properties in various cell types (9, 10), and we hypothesized that IL-10 could modulate foam cell formation and plaque stability, at least partly, through antiapoptotic mechanisms. In the present study, we investigated this hypothesis by examining the effect of IL-10 on oxLDL-induced foam cell formation in a human monocytic leukemia cell line (THP-1) and in monocyte-derived macrophages from patients with ACS and healthy controls.

MATERIALS AND METHODS

Patients and controls

When the effects of IL-10 on monocyte-derived macrophages from ACS patients were studied, cells were isolated from patients who had experienced ischemic chest pain at rest within the preceding 48 h (i.e., Braunwald class IIIB) but who had no evidence of myocardial necrosis according to enzymatic criteria. Transient ST-T segment depression and/or T-wave inversion were present in all cases. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities invalidating ST segment analyses, concomitant inflammatory diseases such as infections and autoimmune disorders, and liver or kidney disease. Coronary angiography was performed by standard techniques within 1–2 days of admission, and the diagnosis of coronary artery disease (CAD) was confirmed by at least one vessel disease, defined as >75% narrowing of luminal diameter, in all patients. Controls in the study were healthy health care workers.

Cell culturing

The human monocytic cell line THP-1 (American Type Culture Collection, Rockville, MD) was cultured in RPMI-1640 with 10% fetal calf serum (FCS) (Sigma; St. Louis, MO), penicillin (50 U/ml)-streptomycin (50μg/ml), and 2 mM L-glutamine (Sigma). Experiments were carried out in 6-well trays (1.5×10^6 cells/ml; Costar, Cambridge, MA) with and without oxLDL, IL-10 (R and D Systems; Minneapolis, MN), neutralizing antibodies against IL-10 (human IL-10-specific goat IgG, final concentration 1.6 μg/ml; R and D Systems), or a combination thereof in growth medium without FCS. Control cells were given PBS (vehicle). Prior to all experiments, the THP-1 cells were differentiated into macrophages by incubation for 72 h with 100 nM phorbol myristate acetate (PMA) (Sigma). In the experiments involving ACS patients, PBMCs and monocyte-derived macrophages were isolated and cultured as described (11). After culturing for 7 days, these macrophages were incubated in the THP-1 culturing medium for 48 h with 2.5% FCS and oxLDL (10 μg/ml) in the presence or absence of IL-10 (20 ng/ml). LDL was isolated from human endotoxin-free heparin plasma and oxidatively modified by Cu^{2+} ions (12). Endotoxin levels in all media, buffers, and stimulants were <10 pg/ml (limulus amoebocyte lysate test).

Lipid staining (Oil-Red-O) and lipid mass quantification

After 48 h, the cells were washed once in ice-cold PBS, followed by formaldehyde fixation (2% in PBS) for 30 min at room temperature. Neutral lipids were stained using 0.5% Oil-Red-O (Sigma) in isopropanol for 60 min. The Oil-Red-O-stained lipids were measured spectrophotometrically after isopropanol extraction, or morphologically evaluated by microscopy. Cellular content of total cholesterol and triacylglycerols was measured colormetrically by an enzymatic assay (BioMérieux; Marcy-l'Etoile, France) after hexane-isopropanol extraction (13).

Quantification of apoptosis

Propidium iodine and Hoechst staining. The cells were cultured on slides for 24 h, followed by propidium iodine (PI) and Hoechst 33342 (HO342) staining (14).

Flow cytometry. After culturing for 48 h, DNA fragmentation in apoptotic cells was assessed by the TUNEL assay (15).

Nucleosomal DNA fragmentation. Measuring of the nucleosomal DNA ladder in agarose gel was performed as described elsewhere (16) and by using an apoptotic DNA-ladder kit (Roche Applied Science; Indianapolis, IN).

mRNA analyses

Total RNA was extracted from cells using the Trizol method (Gibco-Invitrogen, Carlsbad, CA). RNase protection assay (RPA) was performed with the multiprobes hAPO-1b and hAPO-2c (Pharmingen, San Diego, CA) (17). Real-time quantitative RT-PCR was performed using the ABI Prism 7700 (Applied Biosystems; Foster City, CA) and sequence-specific PCR primers and TaqMan probes for CD36 [probe: (5′-TCCAACTGGCATTAGAATACCT-CCAAACACA-3′) and primers: (5′-CCAGGAGTTTGCAAGAA-ACA-3′ and 5′-TCTTCGAGGACAACTTGCTTT-3′)] and lectin-like oxLDL receptor-1 (LOX-1) (probe: 5′-CACCAGAATCTGA-ATCTCCAAGAAACACTGAAGA-3′ and primers: 5′-CTGGAGG-GACAGATCTCTAGC-3′ and 5′-CGGACAAGGAGCTGAACAAT-3′) (18). The gene products of Bfl-1 (primers: 5′-ATTTACAG-GCTGGCTCAGGA-3′ and 5′-AGCACTCTGGACGTTTTGCT-3′), Mcl-1 (primers: 5′-AGCGACGGCGTAACAAACT-3′ and 5′-CGA-TGCAGCTTTCTTGGTTT-3′), and IL-10 (primers: 5′-CTACGG-CGCTGTCATCGAT-3′ and 5′-TGGAGCTTATTAAAGGCATTC-TTCA-3′) were quantified using SYBR Green assays (Applied Biosystems) (18). mRNA signal from β-actin was used for normalization (Applied Biosystems).

Mitochondrial function

Cells were cultured for 48 h and during the last 6 h, coincubated with 50 μl XTT-labeling reagent (final concentration, 0.3 mg/ml; Boehringer Mannheim, Mannheim, Germany). The colored complex of formazan was monitored spectrophotometrically (Labsystems Multiscan; Helsinki, Finland), significantly correlating with the overall mitochondrial dehydrogenase activity (19).

Preparation and transfection of siRNA

siRNAs with the following sense and antisense sequences were used: Bfl-1, first strand (sense), 5′-G-G-U-G-A-G-C-C-A-G-C-U-C-A-A-G-A-C-U-dT-dT-3′, second strand (antisense), 5′-A-G-U-C-U-U-G-A-G-C-U-G-G-C-U-C-A-C-C-dT-dT-3′; Mcl-1, first strand (sense),

5′-G-C-U-C-A-U-C-G-A-A-C-C-A-U-U-A-G-C-C-dT-dT-3′, second strand (antisense), 5′-G-C-U-A-A-U-G-G-U-U-C-G-A-U-G-C-A-G-C-dT-dT-3′, all from Eurogentec (Belgium). The siRNA duplexes were prepared as recommended by the manufacturer. For transfection, cationic lipid complexes of siRNA duplexes and Oligofectamine (Gibco-Invitrogen) were prepared at a ratio of 70 pmol/3 µl in OptiMem with glutamax-1 (Gibco-Invitrogen) and added to the THP-1 cells at a final volume of 200 µl. After overnight incubation, the cells were grown for 6 h in the presence of 10% FCS before the start of the experiment. Controls in the siRNA transfection were either oligofectamine-treated cells (mock) or sense oligo-transfected cells. The toxicity after siRNA transfections was examined routinely for lactate dehydrogenase leakage using a cytotoxicity detection kit (Roche Applied Science) and presented as optical density at 500 nm.

Miscellaneous

The cellular content of Mcl-1 was determined by Western blotting using mouse anti-human Mcl-1 (Santa Cruz Biotechnology, Santa Cruz, CA) as previously detailed (20). All blots were stripped and reprobed with antibody against β-actin (Sigma). Levels of tumor necrosis factor (TNF)α were measured by enzyme immunoassay (R and D Systems).

Ethical aspects

In the human study, informed consent was obtained from all subjects. The study was conducted according to the ethical guidelines of our hospital, according to the Helsinki Declaration, and was approved by the hospital's authorized representative.

Statistical analysis

Data are presented as mean ± SEM. Statistical comparisons between groups were made using the Student t-test. $P < 0.05$ was considered statistically significant.

RESULTS

IL-10 enhances macrophage foam cell formation

We found that IL-10 (20 ng/ml) significantly promotes accumulation of neutral lipids in oxLDL-treated macrophages after incubation for 48 h, as measured by lipid mass quantification (**Fig. 1**) and Oil-Red-O staining (**Fig. 2A–C**). This enhancing effect of IL-10 was also seen at lower (i.e., ≥1 ng/ml), but with only a minor increase at higher concentrations, reaching a plateau at 20 ng/ml (Fig. 2A). Although the addition of neutralizing antibody against IL-10 to cell cultures had no effect on lipid accumulation, either on its own or in combination with oxLDL, it completely abolished the IL-10-mediated accumulation of lipids, demonstrating the specificity of this effect (Fig. 2A–C). Moreover, oxLDL-treated macrophages did not significantly alter the gene expression of IL-10, as compared with unstimulated cells (mRNA IL-10/β-actin; 0.973 ± 0.333 versus 0.803 ± 0.117, oxLDL-stimulated and unstimulated, respectively), suggesting that the IL-10-mediated effect on lipid accumulation was mediated through exogenous IL-10. We further examined whether the effect of IL-10 on lipid accumulation was secondary to increased expression of the receptors for oxLDL. However, IL-10 did not significantly influence the oxLDL-induced gene expression of either CD36 or LOX-1 (mRNA


Lipid mass
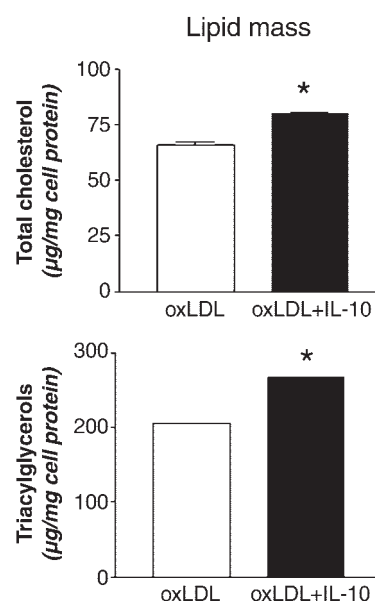
Fig. 1. Interleukin (IL)-10 enhances oxidized low density lipoprotein (oxLDL)-induced total cholesterol and triacylglycerol accumulation of THP-1 cells. THP-1 cells were incubated for 48 h in the presence of oxLDL (20 µg/ml) and oxLDL (20 µg/ml) + IL-10 (20 ng/ml). Data are presented as mean ± SEM of four separate cell cultures. *$P < 0.05$ versus oxLDL alone.

CD36/β-actin: 0.803 ± 0.107 versus 0.897 ± 0.107 and mRNA Lox-1/β-actin; 0.677 ± 0.074 versus 0.577 ± 0.097, oxLDL and oxLDL + IL-10, respectively), suggesting that other mechanisms are operating.

IL-10 protects against oxLDL-induced foam cell apoptosis

We next examined whether the enhancing effect of IL-10 on lipid accumulation in THP-1 macrophages was related to altered apoptosis. Using flow cytometry (TUNEL assay detecting DNA fragments generated by apoptosis-associated endonucleases), we found that IL-10 (20 ng/ml) protected DNA against oxLDL-induced DNA fragmentation (6.7%, 7.8%, and 13.5% in apoptotic cells; control cells and oxLDL-stimulated cells with and without IL-10, respectively; $P < 0.05$, comparing oxLDL with and without IL-10). In contrast, no differences in cell death as visualized by PI staining were seen between the different culture conditions, suggesting that the main effect of IL-10 is on the early phase of apoptosis. We found a similar pattern, with apoptotic effects of oxLDL and antiapoptotic effects of IL-10, when the degree of apoptosis was examined by Hoechst 33342 staining visualizing the presence of apoptotic nuclei and DNA laddering visualizing the degree of nucleosomal DNA fragmentation (data not shown). Moreover, several studies suggest that mitochondria play a major role in the promotion of apoptosis (21, 22), and notably, by using the overall dehydrogenase activity as a marker of mitochondria function, we found that IL-10 totally reversed the oxLDL-induced reduction in dehydrogenase activity, further supporting antiapoptotic effects of IL-10 (**Fig. 3**). However, the IL-10-mediated effect on oxLDL-induced apoptosis was relatively low compared with
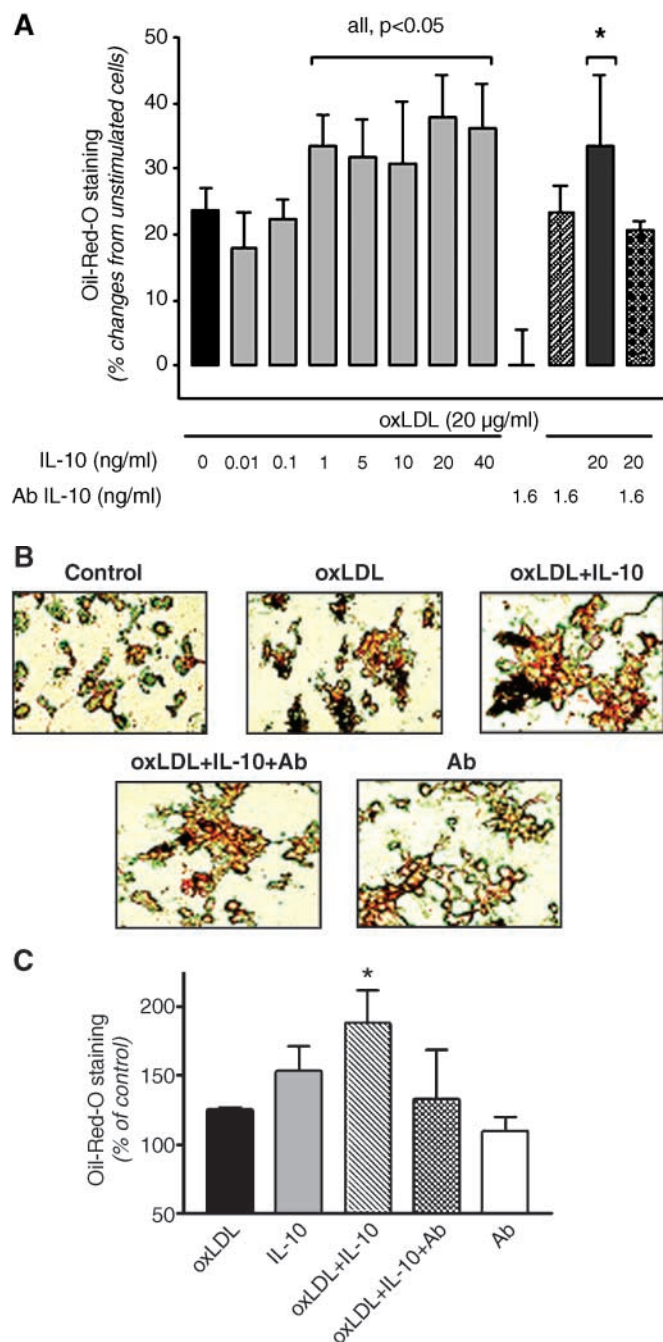
Fig. 2. IL-10 enhances oxLDL-induced lipid loading of THP-1 cells as assessed by Oil-Red-O staining. THP-1 cells were incubated for 48 h in the presence of either vehicle (control), oxLDL (20 µg/ml), IL-10 [20 ng/ml (B, C)], neutralizing IL-10 antibody [1.6 µg/ml Ab (A–C)], or a combination thereof. A: Data are presented as mean ± SEM of four to eight separate experiments. Concentration-dependent effect of IL-10 on lipid loading. B: Morphologic evaluation of one representative experiment (microscopic magnification 40×). C: Quantitative data (color-complex extraction) from six separate experiments (mean ± SEM) of the lipid accumulation shown in B. *$P < 0.05$ versus oxLDL alone.

its effect on oxLDL-induced lipid accumulation, suggesting that IL-10 could more directly modulate lipid accumulation in macrophages in addition to its antiapoptotic properties, and such IL-10-mediated effects on lipid me-

tabolism have recently been suggested in LDL receptor knock-out mice overexpressing IL-10 (23).

Effects of IL-10 on pro- and antiapoptotic genes

To further elucidate the potential antiapoptotic properties of IL-10, we examined the effect of oxLDL and IL-10 on the expression of a wide range of pro- and antiapoptotic genes in THP-1 macrophages using RPA. As shown in **Fig. 4A–C**, IL-10 selectively enhanced the expression of the two antiapoptotic genes, Mcl-1 and Bfl-1, and even more importantly, counteracted the oxLDL-mediated decrease in the expression of these genes. Moreover, IL-10 down-regulated the oxLDL-mediated enhancement of caspase mRNA expression as assessed by RPA (caspase-1, -3, and -8), although these suppressive effects did not reach statistical significance (data not shown).

Silencing of Bfl-1 and Mcl-1 decreases IL-10-induced lipid accumulation

To further link the IL-10-mediated effects on lipid accumulation in oxLDL-induced foam cells to the upregulation of Bfl-1 and Mcl-1 expression, we silenced both these genes using the siRNA transfection technique. We found successful silencing of these genes as assessed by real-time RT-PCR (∼60%) and Western blotting [∼80% for Mcl-1; Bfl-1 data are lacking owing to difficulties with antibodies, as also have been reported by others (24)] 48 h posttransfection (**Fig. 5A**), and importantly, through silencing of Bfl-1 and Mcl-1, we were able to reduce the IL-10-induced lipid loading by ∼50% (Fig. 5B). No significant changes in lactate dehydrogenase leakage were seen between the siRNA- and mock-transfected cells after incubation with either oxLDL or oxLDL + IL-10.

The effect of IL-10 on foam cells in human atherosclerotic disease

We next examined the relevance of our findings to human atherosclerotic disorders by comparing cells from ACS patients (n = 7) with those from healthy controls (n = 6). Comparable to the effect in THP-1 macrophages, IL-10 also increased lipid loading in oxLDL-stimulated macrophages from ACS patients (**Fig. 6A**). In contrast, IL-10 decreased lipid accumulation in oxLDL-stimulated macrophages from healthy controls (Fig. 6A). Notably, this increase in IL-10-induced lipid accumulation in macrophages from ACS patients was accompanied by a marked increase in Mcl-1 expression (Fig. 6B). In contrast, no such increase was seen in IL-10-stimulated macrophages from healthy controls (Fig. 6B). As for caspases and Bfl-1 expression, the differences in IL-10-mediated effects between healthy controls and ACS patients did not reach statistical significance (data not shown). Moreover, when examining the effect of IL-10 on gene expression in PBMCs, we found a significantly different response pattern for Bfl-1 and Mcl-1 between ACS patients and healthy controls, with a significantly enhanced expression in ACS patients compared with no change in controls (**Fig. 7A**, **B**). Also the response patterns to caspase-1 and -3, but not to caspase-8, were statistically different between the two
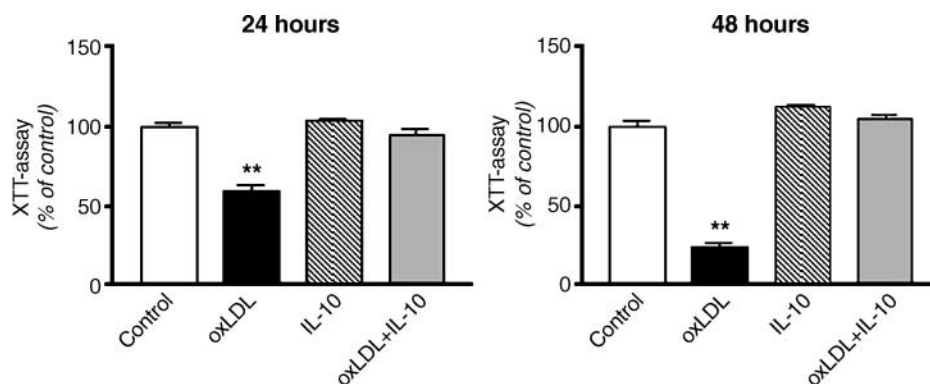
Fig. 3. IL-10 protects mitochondria against oxLDL-mediated toxicity. Data show total dehydrogenase activity (XTT assay) in THP-1 cells in the presence of vehicle (control), oxLDL (20 μg/ml), IL-10 (20 ng/ml), or a combination thereof. In a separate set of experiments (n = 6), we showed that neutralizing IL-10 antibodies totally abolished the protective effect of IL-10 on mitochondrial function (not shown). Data are presented as mean ± SEM of six separate experiments. **$P < 0.01$ versus oxLDL + IL-10.

groups of individuals, but the differences were more modest (Fig. 7C–E). Taken together, these data suggest that although the IL-10-mediated enhancement of lipid accumulation in oxLDL-stimulated macrophages from ACS patients may involve antiapoptotic mechanisms, the IL-10-mediated decrease in lipid accumulation in macrophages from healthy controls seems not to be related to enhanced apoptosis or cell death, inasmuch as no significant IL-10-mediated alteration in the expression of pro- and antiapoptotic genes was seen in these individuals (Figs. 6, 7), nor were IL-10-mediated changes in LDH release from oxLDL-stimulated macrophages (data not shown). Importantly, the ACS

patients had significantly higher ($P < 0.01$) serum levels of C-reactive protein (CRP, 19 ± 3.6 mg/l) than did controls (all <5 mg/l), and in line with this, unstimulated macrophages from ACS patients expressed markedly higher levels of TNFα than did corresponding control cells (Fig. 6C), suggesting preactivation of cells from ACS patients.

DISCUSSION

IL-10 is proposed to have a therapeutic potential in various inflammatory diseases, including atherosclerosis (2,
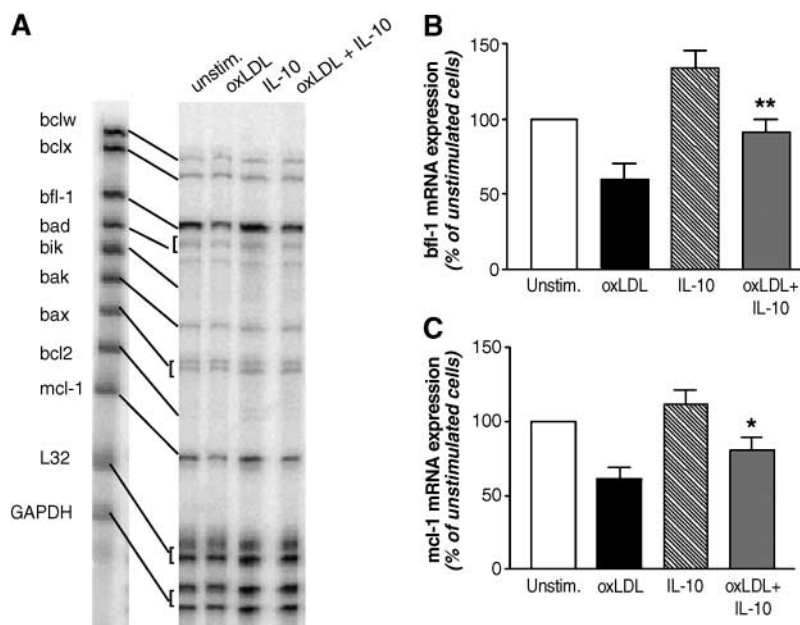
Fig. 4. IL-10 stimulates the mRNA expression of antiapoptotic genes. Total RNA and proteins were extracted from THP-1 cells after incubation for 24 h with vehicle (unstim/control), oxLDL (20 μg/ml), IL-10 (20 ng/ml), or a combination thereof, and assessed by RNase protection assay (RPA) (A) and quantitative real-time RT-PCR (B, C). B, C: Expression of Bfl-1 and Mcl-1 in relation to the control gene β-actin (percentage of unstimulated cells). Data are given as mean ± SEM of five experiments. * $P < 0.05$, **$P < 0.01$ versus oxLDL alone.
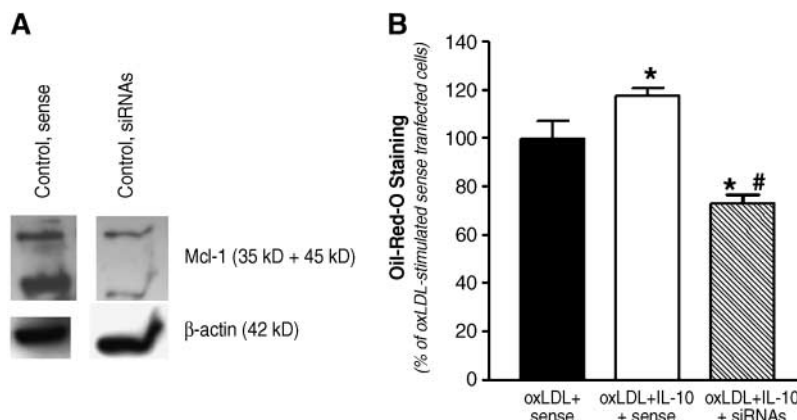
Fig. 5. siRNA silencing of Bfl-1 and Mcl-1 in THP-1 cells reduces the IL-10-stimulating effect on lipid loading. Transfected cells with silencing RNAs for both Bfl-1 and Mcl-1 or sense strands (sense) were incubated in the presence of oxLDL (20 μg/ml) or oxLDL (20 μg/ml) + IL-10 (20 ng/ml). A: Western blot of Mcl-1 demonstrates ∼80% reduced protein level after silencing of Bfl-1 and Mcl-1. B: The IL-10-mediated effect on lipid loading (Oil-Red-O staining) in oxLDL-stimulated THP-1 cells shows ∼50% decrease after silencing of Bfl-1 and Mcl-1. Data are given as mean ± SEM of three experiments. *$P < 0.05$ versus oxLDL + sense-transfected cells; # $P < 0.05$ versus oxLDL + IL-10 + sense-transfected cells.

25). Macrophages are central for both inflammation and lipid deposition during atherogenesis, and we therefore performed a detailed study to investigate the involvement of IL-10 in macrophage foam cell formation. We were able to demonstrate that IL-10 enhances lipid accumulation in oxLDL-stimulated THP-1 macrophages, at least in part by counteracting oxLDL-induced apoptosis, which involves increased expression of the antiapoptotic genes Bfl-1 and Mcl-1. A similar pattern was seen in macrophages from ACS patients, but not in cells from healthy controls. These findings suggest a new mechanism for the effect of IL-10 in atherosclerosis, potentially promoting plaque stabilization.

The mechanisms leading to foam cell apoptosis have not been fully clarified. We show that oxLDL stimulation of macrophages downregulates the expression of Mcl-1 and Bfl-1, two antiapoptotic members of the Bcl-2 family, and this is accompanied by enhanced mitochondria toxicity. Even more importantly, these proapoptotic events were counteracted by IL-10. Bfl-1 appears to act by inhibit-
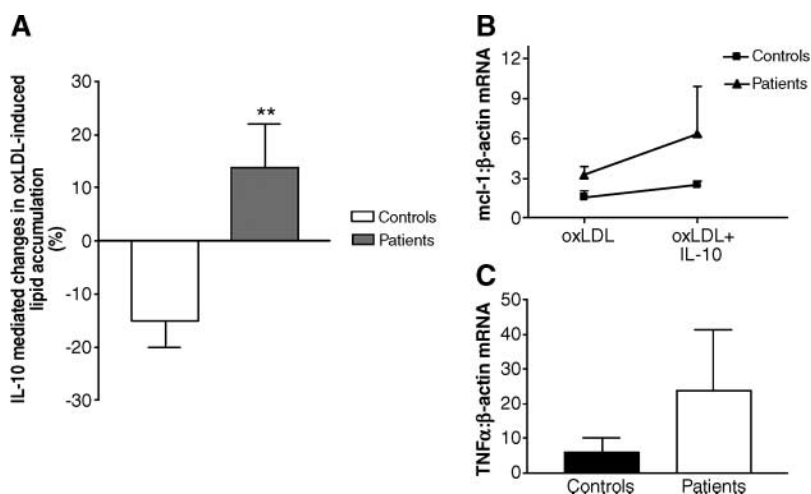
Fig. 6. Opposite effects of IL-10 on oxLDL-induced lipid loading in monocyte-derived macrophages from acute coronary syndromes (ACS) patients and healthy controls. Monocyte-derived macrophages from healthy controls (controls, n = 6) and ACS patients (patients, n = 7) were incubated for 48 h in the presence of oxLDL (10 μg/ml) or oxLDL (10 μg/ml) + IL-10 (20 ng/ml), and accumulation of neutral lipid was quantified using Oil-Red-O staining after extraction of color. A: Although IL-10 increases oxLDL-mediated lipid accumulation in ACS patients, this cytokine decreases the lipid filling in healthy controls (mean ± SEM). **$P < 0.01$ versus IL-10-induced changes in healthy controls. Gene expression of Mcl-1 (B) and tumor necrosis factor (TNF)α (C) in monocyte-derived macrophages from healthy controls and ACS patients after incubation for 12 h under the same conditions as described for A [Mcl-1, (B)] or without any stimulants [TNFα, (C)]. mRNA levels were quantified by real-time quantitative RT-PCR (mean ± SEM).
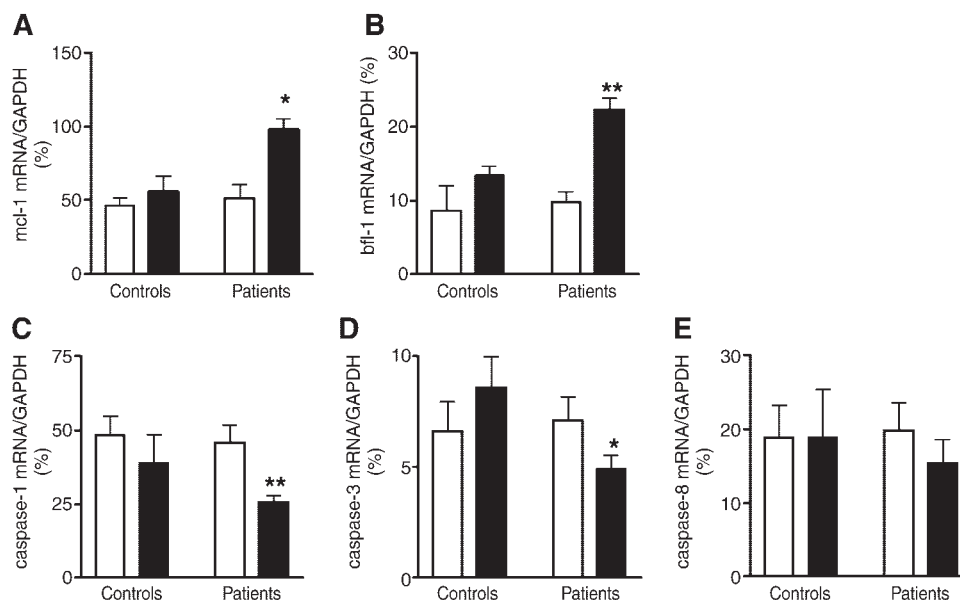
Fig. 7. Peripheral blood mononuclear cells from patients with ACS (n = 7) and healthy controls (n = 6) were incubated for 7 h alone (white bars) or in the presence of IL-10 (5 ng/ml, black bars). Relative expression of the antiapoptotic genes Mcl-1 (A) and Bfl-1 (B) and the pro-apoptotic genes caspase-1 (C), caspase-3 (D), and caspase-8 (E) in relation to the control gene, GAPDH, as assessed by RPA. The data are presented as mean ± SEM. * $P < 0.05$ or **$P < 0.01$ versus unstimulated condition.

ing the proapoptotic proteins Bid, Bax, and Bak in the induction of cytochrome c release from mitochondria (26), and the antiapoptotic properties of Mcl-1 seem also to involve mitochondria-related mechanisms (27). Herein we show that IL-10 may exert antiapoptotic actions by upregulating the gene expression of these two members of the Bcl-2 family, leading to protective effects on mitochondria function.

The literature presents discrepant results concerning the effect of IL-10 on apoptosis. In fact, IL-10 has been reported to both promote (e.g., resting B cells) (28) and inhibit (e.g., T cells) (29) apoptosis of various cells. These discrepancies may at least partly depend on co-stimuli and the timing of IL-10 addition. Thus, although in the present study we show antiapoptotic effects of IL-10 in THP-1 macrophages that are co-stimulated with oxLDL, Schmidt et al. (30) show apoptotic effects of IL-10 in resting monocytes from healthy individuals. It is tempting to hypothesize that such differing degrees of pre- or coactivation may contribute to the different effects of IL-10 on lipid filling in macrophages from healthy controls and ACS patients. In fact, the enhanced IL-10-mediated lipid accumulation in macrophages from ACS patients was accompanied by increased spontaneous release of TNFα, and these patients also had increased serum levels of CRP, suggesting that macrophages from ACS patients are preactivated in vivo, as compared to cells from healthy controls. As for the THP-1 macrophages, these cells are PMA-differentiated macrophages, and it is possible that PMA preactivation could contribute to the similarities between THP-1 macrophages and macrophages from ACS patients. Such an association between the degree of preactivation

and the response to IL-10 stimulation has also been suggested by others (31).

The pathological and clinical significance of apoptosis in atherosclerosis remains controversial. On the one hand, enhanced foam cell apoptosis could contribute to plaque rupture and the development of ACS (7, 8). Hence, while apoptotic macrophages in fatty streaks are rare, macrophage apoptosis is a prominent feature in advanced atherosclerotic plaques (32). Moreover, apoptosis of foam cell macrophages may induce the formation of a necrotic core during atherogenesis, possibly contributing to plaque destabilization (7, 32). Furthermore, apoptosis in oxLDL-stimulated macrophages seems to promote increased procoagulant activity predisposing to thrombus formation and ACS (1). It might be argued that foam cell apoptosis could enhance plaque stability by removal of inflammatory cells from the atherosclerotic lesions. However, inhibition of apoptosis induced by ischemia-reperfusion was recently shown to prevent inflammation in a murine model, challenging the concept of antiinflammatory effects of apoptosis (33). Nevertheless, decreased apoptosis may not necessarily be beneficial in CAD. Thus, although decreased apoptosis could lead to plaque stabilization, it could also increase cell build-up in the intimal compartment, leading to narrowing of the lumen, and promote atherogenesis, particularly in the early stage of this disorder (7).

Several lines of evidence suggest that our findings in THP-1 cells may be of potential relevance to the in vivo situation. First, we found that IL-10 also induced lipid accumulation in oxLDL-stimulated macrophages from ACS patients, and enhanced the expression of Bfl-1 and Mcl-1 in PBMCs; and as for Mcl-1, also in macrophages from

these patients. Second, previous studies of human atherosclerotic plaques have revealed an inverse association between the presence of IL-10 and TUNEL staining, supporting a link between IL-10 and decreased apoptosis (34), and such an association has also recently been demonstrated in LDL-receptor mice overexpressing IL-10 (35). Finally, although several studies have shown increased levels of inflammatory cytokines such as TNFα in ACS, we and others have recently reported normal IL-10 levels in these patients, suggesting an inflammatory imbalance (6, 36–38). However, the clinical consequences of the IL-10-mediated enhancement of foam cell formation is far from clear and will need to be further elucidated before any firm conclusion can be drawn.

Although the mechanisms of action have not been clarified, IL-10 has been shown to impair atherosclerosis in various animal models (4, 34) and has been proposed as an "immunological scalpel" in the atherosclerotic process (2, 38). Herein we show that IL-10 increases lipid accumulation in oxLDL-stimulated macrophages from ACS patients, at least partly through antiapoptotic mechanisms. Although the exact role of the IL-10-mediated inhibition of foam cell apoptosis must be examined further, it is possible that the combination of its antiapoptotic effects on foam cells and antiinflammatory effects on T-cells within the atherosclerotic plaque (35) may prevent plaque rupture and development of ACS. However, the harmful effects of enhanced foam cell formation cannot be excluded, particularly in the initiation of atherosclerosis. These issues must be further elucidated before any therapeutic role for IL-10 in atherosclerotic diseases can be established.⬛

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