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Interleukin-6 Protects Human Macrophages from Cellular Cholesterol Accumulation and Attenuates the Proinflammatory Response*[S]

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Cholesterol-laden monocyte-derived macrophages are phagocytic cells characteristic of early and advanced atherosclerotic lesions. Interleukin-6 (IL-6) is a macrophage secretory product that is abundantly expressed in atherosclerotic plaques but whose precise role in atherogenesis is unclear. The capacity of macrophages to clear apoptotic cells, through the efferocytosis mechanism, as well as to reduce cellular cholesterol accumulation contributes to prevent plaque progression and instability. By virtue of its capacity to promote cellular cholesterol efflux from phagocyte-macrophages, ABCA1 was reported to reduce atherosclerosis. We demonstrated that lipid loading in human macrophages was accompanied by a strong increase of IL-6 secretion. Interestingly, IL-6 markedly induced ABCA1 expression and enhanced ABCA1-mediated cholesterol efflux from human macrophages to apoAI. Stimulation of ABCA1-mediated cholesterol efflux by IL-6 was, however, abolished by selective inhibition of the Jak-2/Stat3 signaling pathway. In addition, we observed that the expression of molecules described to promote efferocytosis, *i.e.* c-mer proto-oncogene-tyrosine kinase, thrombospondin-1, and transglutaminase 2, was significantly induced in human macrophages upon treatment with IL-6. Consistent with these findings, IL-6 enhanced the capacity of human macrophages to phagocytose apoptotic cells; moreover, we observed that IL-6 stimulates the ABCA1-mediated efflux of cholesterol derived from the ingestion of free cholesterol-loaded apoptotic macrophages. Finally, the treatment of human macrophages with IL-6 led to the establishment of an anti-inflammatory cytokine profile, characterized by an increased secretion of IL-4 and IL-10 together with a decrease of that of IL-1β. Taken together, our results indicate that IL-6 favors the elimination of excess cholesterol in human macrophages and phagocytes by stimulation of ABCA1-mediated cellular free cholesterol efflux and attenuates the macrophage proinflammatory phenotype. Thus, high amounts of IL-6 secreted by lipid laden human macrophages may constitute a protective response from macrophages to prevent accumulation of cytotoxic-free cholesterol. Such a cellular recycling of free cholesterol may contribute to reduce both foam cell formation and the accumulation of apoptotic bodies as well as intraplaque inflammation in atherosclerotic lesions.

The retention and accumulation of modified LDL and apoptotic cells in the arterial intima represent critical steps in the formation of cholesterol-rich vulnerable atherosclerotic plaques; such plaques feature an immunoinflammatory process in which monocyte-derived macrophages play a central role (1, 2). Indeed, intimal macrophages endocytose modified LDL via scavenger receptors (SR-A, CD36), thereby favoring intracellular lipid accumulation with foam cell formation. Cholesterol-laden macrophages may equally result from phagocytosis of apoptotic cells via the process of efferocytosis (3); however, such a massive influx of cholesterol may prove cytotoxic if cholesterol recycling is disrupted in macrophage phagocyte (4).

The ATP binding cassette A1 (ABCA1)[3] transporter plays a central role in maintaining macrophage cholesterol homeostasis by preventing cellular lipid accumulation as a result of its capacity to efflux cellular free cholesterol to apoAI (5). Thus, ABCA1 may promote elimination of cholesterol derived from ingestion of either modified lipoproteins or apoptotic cells (6). Recent studies clearly highlight the close link between inflammation and cholesterol homeostasis in macrophages through mechanisms in which ABCA1 appears to be a major actor (7). Thus, increased intracellular free cholesterol concentration in ABCA1 KO macrophages is accompanied by enhanced proinflammatory response upon LPS induction (8–10). Moreover, the enhanced Toll-like receptors signaling observed in ABCA1/ABCG1 double KO macrophages is associated with free cholesterol accumulation and up-regulated expression of proinflammatory genes (11). Reciprocally, activation of Toll-like receptors 3 and 4 inhibits the induction of LXR target genes, such as ABCA1, in macrophages and strongly reduces cholesterol efflux (12). Although those studies suggest that the inflammatory property of ABCA1 results from its ability to modulate free cholesterol levels and distribution in plasma membrane, recent

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[3] The abbreviations used are: ABCA1, ATP binding cassette A1; HMDM, human monocyte-derived macrophage; acLDL, acetylated LDL; Ctrl, control; THBS1, thrombospondin-1; TG2, transglutaminase 2; PPAR, peroxisome proliferator-activated receptor; LXR, liver X receptor.

studies propose that macrophage ABCA1 may act as an anti-inflammatory receptor through activation of Jak2/Stat3 pathway after apoAI binding (13, 14).

Among the cytokines present in atherosclerotic tissue, interleukin-6 (IL-6) is prominent and, moreover, is abundantly produced by free cholesterol-loaded macrophages in advanced lesions (15). Importantly, interleukin-6 has been identified as an independent risk factor for premature coronary artery disease (16), and moreover, elevated levels of IL-6 are associated with an increased risk for myocardial infarction in healthy men (17). The Offspring Cohort of the Framingham Heart Study indicated that IL-6 levels were associated with internal carotid artery intima-media thickness and stenosis (18). The relative contribution of IL-6 as a causative factor, as a consequence, or as a marker of atherosclerosis is unclear. Indeed, experimental evidence in genetically modified mouse models susceptible to atherosclerosis highlight opposite functions of IL-6 (19). Indeed, treatment of mice with recombinant mouse IL-6 increased lesion size in both C57BL/6 and apoE$^{-/-}$ mice fed a high fat/cholate diet (20). However, IL6$^{-/-}$ mice developed larger fatty streak lesions than control mice when fed an atherogenic diet for 15 weeks (21). Similar studies in IL-6/LDL receptor double KO mice failed to detect a significant effect of IL-6 deficiency on lesion size (22). Finally, a potential atheroprotective role for IL-6 was described in IL-6/apoE double KO mice maintained for 1 year on a chow diet (23, 24). Taken together, these findings suggest that IL-6 may exert opposing actions in the inflammatory dimension of the atherosclerosis process.

To evaluate this question further, we explored the impact of IL-6 on the capacity of human macrophages to regulate cholesterol homeostasis in the lipido-inflammatory context of atherosclerosis. We presently report that disruption of cellular lipid homeostasis leading to cholesterol accumulation in human macrophages was accompanied by an increased secretion of IL-6. Moreover, IL-6 reduced macrophage lipid accumulation derived from ingestion of either modified lipoproteins or apoptotic cells by stimulating ABCA1-mediated free cholesterol efflux through activation of the Jak-2/Stat3 signaling pathway. In addition, IL-6 enhanced the capacity of human macrophages to ingest apoptotic cells and attenuated the proinflammatory phenotype of human cholesterol-loaded human macrophages. Our findings, therefore, suggest that the autocrine action of macrophage-secreted-IL-6 may contribute to reduce the formation of inflammatory foam cells and apoptotic macrophages in atherosclerotic lesions.

EXPERIMENTAL PROCEDURES

Preparation and Culture of Human Macrophages

Monocytes were isolated from the blood of individual healthy normolipidemic donors (Etablissement Français du Sang) on Ficoll gradients (Ficoll-Paque PLUS, GE Healthcare) and subsequently differentiated into human monocyte-derived macrophages (HMDM) on plastic Primaria plates (Falcon) over a period of 10 days of culture in RPMI 1640 medium supplemented with 10% heat-inactivated human serum, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 20 ng/ml human macrophage colony-stimulating factor. The human THP-1 monocytic cell line was obtained from American Type Culture collection (ATCC) and maintained in culture in 5% CO_2 at 37 °C in RPMI medium containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, and 80 units/ml penicillin, 80 μg/ml streptomycin. THP-1 monocytes were seeded onto 24- or 6-well plates at density 1×10^6 or 4×10^6 cell/well, respectively, in the presence of 50 ng/ml phorbol 12-myristate 13-acetate for 3 days to induce differentiation into macrophage-like cells.

Cellular Cholesterol Efflux Assays

Human macrophages (THP-1 and HMDM) were cholesterol-loaded with 50 μg/ml acetylated LDL (acLDL) labeled with 1 μCi/ml [^{3}H]cholesterol for 24 h in the presence or in the absence of 50 ng/ml recombinant human IL-6 (R&D Systems) in an RPMI medium containing 2 mM glutamine, 50 mM glucose, and 0.2% BSA (RGGB). All cell culture reagents were certified as endotoxin-free by the manufacturers. As described in our previous studies (25, 26), pyrogen-free commercial plastic was used at all critical steps during LDL isolation to prevent endotoxin contamination. Under these conditions, endotoxin content was $<$1 pg/μg LDL protein as monitored by the *Limulus* amebocyte lysate chromogenic assay (Biogenic) (26). After the acetylation procedure, the endotoxin content of acLDL was $<$0.5 pg/μg of acLDL protein (25), such endotoxin levels being not able to induce a detectable change in cytokine secretion, including that of IL-6 (27). Human macrophages were then equilibrated in RGGB in the presence or absence of 50 ng/ml recombinant human IL-6 and 1 μM TO901317 (Sigma) for an additional 24-h period. Cellular cholesterol efflux to 5 μg/ml lipid-free apoA-I (Biodesign) or HDL (density = 1.063–1.21 g/ml; 15 μg/ml PL) isolated from normolipidemic plasma by preparative ultracentrifugation (28) was assayed in serum-free medium for a 4-h chase period in the presence or absence of 50 ng/ml recombinant human IL-6 and 1 μM TO901317. Finally, culture media were harvested and cleared of cellular debris by brief centrifugation. Cell-associated radioactivity was determined by extraction in hexane-isopropanol (3:2), evaporation of the solvent, and liquid scintillation counting (Wallac Trilux 1450 Microbeta). The percentage of cholesterol efflux was calculated as 100 $\times$ (medium cpm)/(medium cpm + cell cpm). ApoA-I-specific cholesterol efflux was determined by subtracting nonspecific cholesterol efflux occurring in apoA-I-free medium.

When required, a cell-permeable Stat3 inhibitor peptide (100 μM Stat3-I, Calbiochem) or a selective inhibitor of either the Jak-2 protein-tyrosine kinase (25 μM AG490, Sigma) or the Jak-3 protein-tyrosine kinase (10 μM ZM39923, Sigma) or the Jak-2/Stat3 signaling pathway (2 μM cucurbitacin I, Sigma) was added together with IL-6.

Determination of Cellular Cholesterol Mass

The masses of esterified and free cholesterol in human macrophages was quantified using the Amplex Red cholesterol assay kit (Molecular Probes) as previously described (29).

Western Blot Analysis

Cell protein extracts from human THP-1 macrophages were prepared from 6-well plates pretreated or not with 50 ng/ml recombinant human IL-6 in the presence or the absence of 1 μM TO901317 for 24 h in RGGB medium. Total cell protein was extracted in M-PER mammalian protein extraction reagent (Thermo) containing 10% protease inhibitor mixture (Roche Applied Science). Protein concentration was determined using a BCA protein assay (Pierce). Equal amounts of protein (50 μg) were loaded onto a 3–8% Tris acetate polyacrylamide gel (Invitrogen) and transferred onto a nitrocellulose membrane using iBlot technology (Invitrogen). The membrane was blocked with 5% skim milk (in PBS, 0.1% Tween) for 2 h, and ABCA1 was detected by incubation overnight at 4 °C with a rabbit anti-human ABCA1 (Novus) at 1:500 and horseradish peroxidase-conjugated goat ant-rabbit secondary antibody at 1:5000. The signal was revealed with an enhanced chemiluminescence Immobilon Western substrate (Millipore). Quantification of Western blots was performed using a Kodak Image Station 440 CF with Kodak 1D Image Analysis software. Quantification of ABCA1 protein levels was normalized to matching annexin1 (Zymed Laboratories Inc.) levels.

Phagocytosis of Apoptotic Cells

Generation of Apoptotic Cells—Free cholesterol-induced apoptotic THP-1 macrophages were produced by incubation with 100 μg/ml acLDL and 10 μg/ml concentrations of an acyl-CoA:cholesterol acyltransferase inhibitor (S58035, Sigma) for 24 h in serum-free medium as previously described (30). UV-induced apoptotic human Jurkat cells were generated by UV exposition (312 nm, 6 × 15 Watts) for 15 min followed by a 4-h incubation at 37 °C in a 5% CO_2 atmosphere. Apoptosis was quantified by both annexin V and propidium iodide (PI) staining (Beckman Coulter) using flow cytometry (Beckman FC500). The degrees of apoptosis in Jurkat apoptotic cells were ~74% in early apoptosis (annexin V-positive only), <7% in late apoptosis (annexin V + PI-positive), and <2% in necrosis (PI-positive only).

Phagocytosis of Apoptotic Cells—Apoptotic Jurkat cells were labeled with 5 μM calcein-AM (Invitrogen) and added to THP-1 macrophage phagocyte cells (Ratio 3:1) previously treated in the presence or absence of 50 ng/ml recombinant human IL-6 ± 1 μM TO901317. After 30 min of contact, which has been reported to be a sufficient period to completely engage and internalize the apoptotic cells (6), non-ingested apoptotic cells were removed by extensive washing with PBS. Subsequently, THP-1 phagocytes were detached from the plates and then subjected to flow cytometry (Beckman FC500) to quantify positive fluorescent phagocytic cells that had ingested calcein-AM-labeled apoptotic Jurkat cells. Positive fluorescent phagocytic cells were visualized by fluorescence microscopy to ensure that the detected fluorescence was localized within the phagocytes. Data are expressed as a phagocytic index corresponding to the mean fluorescence in positive phagocytes.

Cellular [^{3}H]Cholesterol Efflux from THP-1 Phagocytes after Ingestion of [^{3}H]Cholesterol-labeled THP-1 Apoptotic Cells—As originally described by Cui *et al.* (30) in mouse peritoneal macrophages, monolayers of [^{3}H]cholesterol-labeled apoptotic THP-1 macrophages were extensively washed with PBS, detached from 100-mm bacterial dishes, and added to THP-1 phagocytes (Ratio 5:1) for 30 min at 37 °C in medium containing 10 μg/ml S58035. Non-ingested apoptotic cells were removed by extensive washing with PBS, and efflux of [^{3}H]cholesterol from THP-1 phagocytes, derived from the ingestion of [^{3}H]cholesterol-labeled apoptotic cells, was assayed in the presence of lipid-free apoAI for 4 h in a medium containing 10 μg/ml S58035 as described above.

RNA Interference (RNAi)-mediated ABCA1 Silencing Using Small Interfering RNA (siRNA)

Silencing of ABCA1 expression was performed by application of siRNA oligonucleotides (Dharmacon) targeted to the cDNA sequence of the human *ABCA1* gene (GenBank™ #NM_005502). THP-1 macrophages were grown in 24-well plates and transfected with 50 nM control siRNA (Dharmacon) or siRNA targeting human ABCA1 using Lipofectamine RNAiMax (Invitrogen) according to the manufacturer's instructions.

RNA Extraction, Reverse Transcription, and Quantitative-PCR

Human THP-1 macrophages were incubated with or without 50 ng/ml recombinant human IL-6 in the presence or the absence of 1 μM TO901317 for 24 h at 37 °C. Cells were then washed twice with cold PBS, and total RNA was extracted using a NucleoSpin RNA II kit (Macherey-Nagel) according to the manufacturer's instructions. Then, 1500 ng of RNA was reverse-transcribed with 75 ng of random hexamer using 200 units of Moloney murine leukemia virus reverse transcriptase. An initial denaturation step for 5 min at 68 °C was followed by an elongation phase of 1 h at 42 °C; the reaction was completed by a 5-min incubation at 68 °C.

Real time quantitative PCR was performed using a LightCycler LC480 (Roche Applied Science). The reaction contained 2.5 ng of reverse transcribed total RNA, 150 pmol of forward and reverse primers, and 5 μl of Master Mix SYBR Green in a final volume of 10 μl. Samples underwent the standard PCR protocol. Crossing point values for genes of interest were normalized to housekeeping genes (human δ-aminolevulinate synthase and human α-tubulin). Expression data were based on the crossing points calculated with the software for LightCycler data analysis and corrected for PCR efficiencies of the target and the reference gene. Data were expressed as a -fold change in mRNA expression relative to control values.

Analysis of Cytokine Secretion Profile

Human THP-1 macrophages were incubated in serum-free media with 50 ng/ml human recombinant IL-6 for 24 h, and secreted cytokines in the culture media were quantified using a semiautomated Biochip Array Technology analyzer (Evidence Investigator, Randox).

Statistical Analysis

Data are shown as the mean ± S.E. Experiments were performed in triplicate, and values correspond to the mean from at least three independent experiments. Comparisons of two

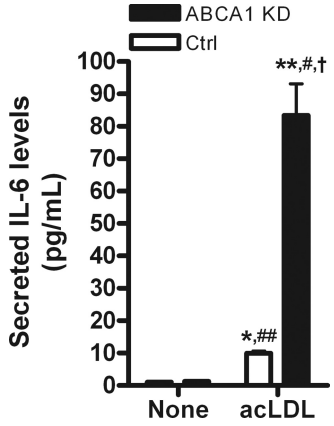

FIGURE 1. **Secretion of IL-6 from human macrophages is enhanced in response to lipid loading.** Human Ctrl and ABCA1 KD THP-1 macrophages were cholesterol-loaded or not with acLDL for 24 h in serum-free media, and the secretion of IL-6 cytokine in the culture media was quantified on a semi-automated Biochip Array analyzer (Randox). *, $p < 0.01$; **, $p < 0.001$ *versus* unloaded Ctrl cells (*None*). #, $p < 0.01$; ##, $p < 0.001$ *versus* unloaded ABCA1 KD cells; †, $p < 0.001$ *versus* cholesterol-loaded Ctrl cells. Values are the means ± S.E. of three independent experiments performed in triplicate.

groups were performed by a two-tailed Student's *t* test, and comparisons of three or more groups were performed by analysis of variance with the Newman-Keuls post-test. All statistical analyses were performed using Prism software from GraphPad (San Diego, CA).

RESULTS

Secretion of IL-6 Was Markedly Increased from Human Macrophages in Response to Lipid Loading—Analysis of secreted IL-6 levels by human macrophages (Fig. 1) revealed that the secretion of IL-6 by cholesterol-loaded human THP-1 macrophages (16.9 ± 0.3 *versus* 12.9 ± 0.4 μg of cholesterol/mg of protein in cholesterol-loaded and control cells, respectively, $p < 0.005$) was ~10-fold ($p < 0.01$) more elevated than that observed in non-loaded macrophages, thereby suggesting that foam cells contribute to a major degree to the determination of IL-6 amounts produced in human atherosclerotic plaques. Interestingly, disruption of lipid homeostasis in cholesterol-loaded macrophages through silencing of ABCA1 expression using RNA interference (ABCA1 KD) (supplemental Fig. 1) led to an exacerbated secretion of IL-6 (8.4-fold, $p < 0.001$) as compared with control cholesterol-loaded cells (Ctrl). Taken together, those results indicate that the lipid accumulation in human macrophages is accompanied by a marked elevation of secreted IL-6 amounts. To determine whether this increased IL-6 production may constitute a compensatory response from human macrophages to cholesterol loading, we next examined the effect of IL-6 on the capacity of human macrophages to eliminate the excess of cholesterol resulting from either the uptake of modified LDL or the ingestion of apoptotic cells.

Enhanced ABCA1-mediated Cholesterol Efflux to ApoAI from Human Macrophages in Response to IL-6—To determine whether IL-6 may modulate cellular free cholesterol efflux from cholesterol-loaded human macrophages after incubation with modified LDL (acLDL), we analyzed the impact of IL-6 on cholesterol efflux from HMDM and from THP-1 macrophages to

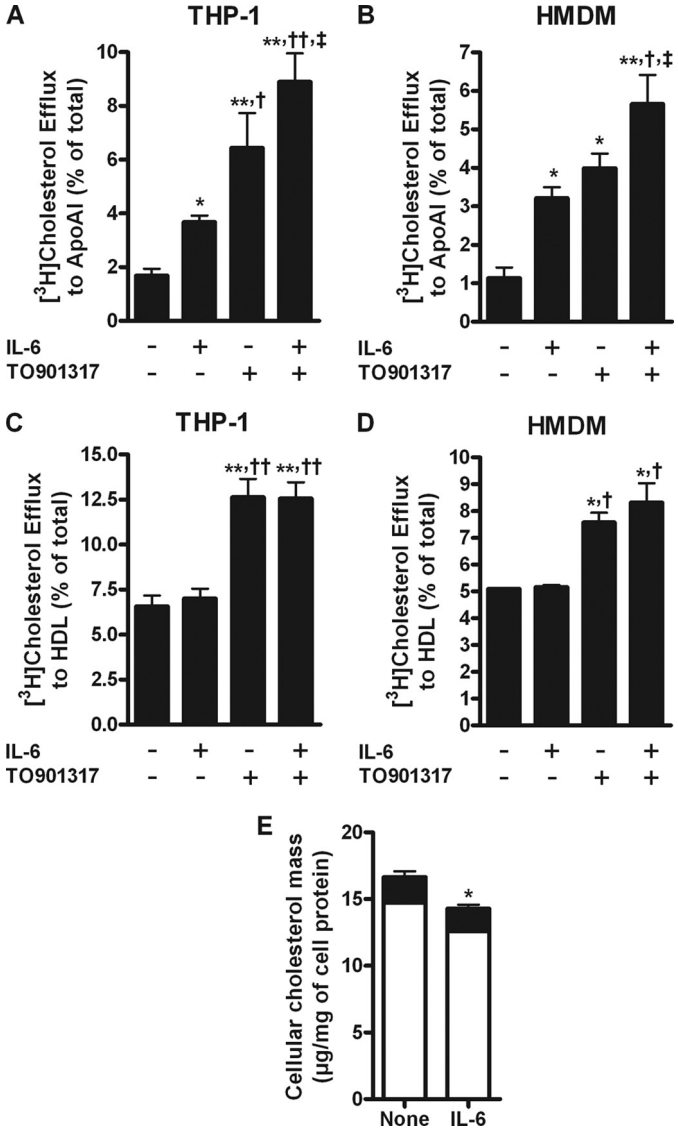

FIGURE 2. **Interleukin-6 stimulates ABCA1-mediated cholesterol efflux to apoAI in human macrophages.** Human THP-1 macrophages (*A* and *C*) and HMDM (*B* and *D*) were cholesterol-loaded with acLDL and treated with or without IL-6 (50 ng/ml) for 24 h. After an additional 24-h period with or without TO901317 (1 μM) to induce ABCA1, cholesterol efflux to lipid-free apoAI (*A* and *B*) or HDL (*C* and *D*) from human macrophages was assayed over a period of 4 h. Values are the means ± S.E. of three independent experiments. *, $p < 0.05$; **, $p < 0.001$ *versus* untreated control cells; †, $p < 0.05$; ††, $p < 0.001$ *versus* IL-6-treated cells; ‡, $p < 0.05$ *versus* TO901317-stimulated cells. *E*, free (□) and esterified (■) cholesterol mass in cholesterol-loaded human THP-1 macrophages upon stimulation or not with IL-6. *, $p < 0.001$ *versus None*.

both lipid-poor apoAI and to HDL particles. As shown in Fig. 2, ABCA1-mediated cholesterol efflux to apoAI from THP-1 (Fig. 2*A*) and HMDM (Fig. 2*B*) was stimulated upon incubation with IL-6 (2.2- and 2.8-fold, respectively, $p < 0.05$). When human macrophages were stimulated with a synthetic LXR agonist (TO901317), which markedly induced ABCA1-mediated cholesterol efflux to apoAI subsequent to marked up-regulation of ABCA1 mRNA expression (31), elevation in rates of cholesterol efflux to apoAI in response to IL-6 was also observed (THP-1, +38%; HMDM, +42%, $p < 0.05$). However, IL-6 did not affect cellular cholesterol efflux to HDL from both THP-1 macrophages and HMDM treated in the presence or in the absence of

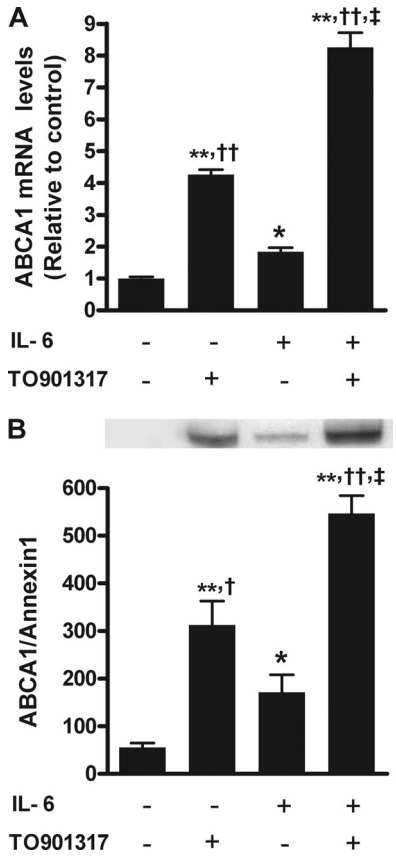

FIGURE 3. **ATP binding cassette A1 transporter expression is induced by IL-6 in human THP-1 macrophages.** Human THP-1 macrophages were treated with or without IL6 (50 ng/ml) ± TO901317 (1 μM) for 24 h, and ABCA1 expression was assessed by quantification of mRNA by real-time quantitative PCR (*A*) and protein levels by Western blot (*B*). Levels of ABCA1 mRNA were normalized to housekeeping genes (δ-aminolevulinate synthase and human α-tubulin) and ABCA1 protein to matching annexin1 levels. Values are the means ± S.E. of three independent experiments. *, $p < 0.05$; **, $p < 0.001$ *versus* untreated control cells; †, $p < 0.05$; ††, $p < 0.001$ *versus* IL-6-treated cells; ‡, $p < 0.001$ *versus* TO901317-stimulated cells.

LXR agonists (Fig. 2, *C* and *D*), thereby indicating that the effect of IL-6 on cellular cholesterol efflux mechanisms is specific to ABCA1-mediated cholesterol efflux. Consistent with the induction of ABCA1-mediated cholesterol efflux to apoAI, cellular cholesterol mass was significantly reduced (−14%, $p < 0.001$) in cholesterol-loaded human macrophages when incubated with IL-6 (Fig. 2*E*).

We next determined whether activation of ABCA1-mediated cholesterol efflux by IL-6 may result from stimulation of ABCA1 gene expression in human macrophages. As shown in Fig. 3*A*, IL-6 significantly up-regulated ABCA1 mRNA levels in THP-1 macrophages when acting alone (+84%, $p < 0.01$) or in combination with the synthetic LXR agonist TO901317 (+94%, $p < 0.001$); such induction of ABCA1 by IL-6 was confirmed at the protein level (Fig. 3*B*). By contrast, mRNA levels of other genes known to modulate cellular cholesterol efflux mechanisms (ABCG1, ABCA7, Cla-1, apoE, LXRα, Retinoid X Receptor α, PPARα,γ) were not affected by IL-6 (Table 1). Those findings, therefore, suggest that stimulation of ABCA1-mediated cholesterol efflux to apoAI by IL-6 likely results from induction of ABCA1 gene expression.

Interleukin-6 Stimulates ABCA1-mediated Cholesterol Efflux to ApoAI through Activation of the Jak-2/Stat3 Signaling Pathway—As IL-6 has been described to exert its cellular action by activation of the Jak/Stat pathway (32), we analyzed the effect of the inhibition of the Jak/Stat signaling pathway on stimulation of cholesterol efflux to apoAI by IL-6 using specific inhibitors targeting either Jak-2 or Jak-3 or Jak-2/Stat3. As shown in Fig. 4, the inhibition of the Jak-2 protein-tyrosine kinase by a specific inhibitor (AG490) completely abolished the induction of cholesterol efflux to apoAI in response to IL-6 in both non-stimulated (Fig. 4*A*) and TO901317-stimulated human macrophages (Fig. 4*B*), whereas the inhibition of the Jak-3 protein-tyrosine kinase (ZM39923) was without effect. Because Jak-2 preferentially phosphorylates and activates Stat3, we then tested the possible implication of Stat3 in this mechanism by use of a selective inhibitor of the Jak-2/Stat3 signaling pathway, cucurbitacin I (33). Treatment of human THP-1 macrophages with cucurbitacin I led to a complete lack of the activation of cholesterol efflux to apoAI by IL-6, indicating that the activation of Jak-2/Stat3 signaling is required for the stimulatory effect of IL-6. Our data, therefore, demonstrate that IL-6 stimulates ABCA1-mediated cholesterol efflux to apoAI in human macrophages through activation of the Jak-2/Stat3 signaling pathway.

Interleukin-6 Favors ABCA1-mediated Elimination of Apoptotic Cell-derived Cholesterol within Phagocytes—Phagocytosis of apoptotic cells by macrophages, *i.e.* efferocytosis, results in the cellular uptake of large amounts of lipids that contribute to accumulation of cholesterol into phagocytes (3). Recent studies demonstrated that engulfment of apoptotic cells by phagocytes led to elevated cholesterol efflux from phagocytes to extracellular acceptors (6, 30), a mechanism in which ABCA1 has been demonstrated to play a major role (6).

ABCA1 has been also proposed to promote the engulfment of apoptotic cells by phagocytes (34–36). Thus, we first examined the possibility that the increased ABCA1 expression in macrophages upon IL-6 stimulation may enhance their capacity to phagocyte apoptotic cells. Indeed, the incubation of human THP-1 macrophages with IL-6 was significantly associated with an increased capacity of phagocytes to engulf apoptotic Jurkat cells (Fig. 5*A*; +80%, $p < 0.05$). In addition, treatment of THP-1 macrophages with the synthetic LXR agonist TO901317 led to a 2.8-fold elevation ($p < 0.001$) in the ingestion of apoptotic Jurkat cells; this effect was further increased upon incubation with IL-6 (+40%, $p < 0.01$). However, siRNA-mediated silencing of ABCA1 expression in LXR-induced THP-1 macrophages was without effect on the stimulatory effect of IL-6 on efferocytosis (Fig. 5*B*), suggesting the involvement of an ABCA1-independent pathway. Notably the expression of factors known to facilitate the recognition and uptake of apoptotic cells by macrophages, such as c-mer proto-oncogene-tyrosine kinase (*MERTK*, Fig. 5*C*), TG2 (Fig. 5*D*), and THBS1 (Fig. 5*E*), were found significantly induced (+21, +58, and +100%, respectively) in THP-1 macrophages treated by IL-6 for 24 h, whereas the expression of complement C1q and milk fat globule-epidermal growth factor-8 was not affected (data not shown).

Finally, to evaluate the impact of IL-6 on the capacity of human macrophage-phagocytes to eliminate the excess of chol-

TABLE 1

Gene expression profile in human macrophages upon stimulation with interleukin-6

Quantification of mRNA levels by real-time QPCR in THP-1 macrophages and HMDM. Amounts of mRNA levels were normalized to housekeeping genes (δ-aminolevulinate synthase, α-tubulin, and hypoxanthine phosphoribosyltransferase 1). Values are the means ± S.E. of four independent experiments. NS, not significant; RXR, Retinoid X Receptor; LXR, Liver X Receptor; LPL, lipoprotein ligase; CEH, cholesteryl ester hydrolase.

	THP-1		HMDM	
Human macrophages	**-Fold increase (IL-6 *vs.* control)**	***p***	**-Fold increase (IL-6 *vs.* control)**	***p***
Lipid homeostasis				
ABCG1	1.2 ± 0.2	NS	1.1 ± 0.1	NS
ABCA7	1.1 ± 0.3	NS	1.0 ± 0.2	NS
Cla-1	1.0 ± 0.2	NS	1.1 ± 0.3	NS
ApoE	1.0 ± 0.1	NS	0.9 ± 0.1	NS
LPL	0.9 ± 0.3	NS	1.0 ± 0.2	NS
CEH	1.0 ± 0.2	NS	0.8 ± 0.1	NS
ACAT-1	1.1 ± 0.3	NS	1.0 ± 0.0	NS
HMG-CoA receptor	1.1 ± 0.2	NS	1.1 ± 0.1	NS
LDL receptor	1.1 ± 0.3	NS	1.2 ± 0.1	NS
SR-A	0.9 ± 0.2	NS	1.1 ± 0.2	NS
CD36	1.0 ± 0.2	NS	1.2 ± 0.1	NS
LRP	1.0 ± 0.1	NS	0.9 ± 0.1	NS
Gene regulation				
PPARα	1.2 ± 0.1	<0.05	1.0 ± 0.1	NS
PPARδ	1.4 ± 0.2	<0.05	1.9 ± 0.1	<0.00005
PPARγ	1.2 ± 0.2	NS	0.9 ± 0.2	NS
RXRα	1.0 ± 0.1	NS	1.0 ± 0.1	NS
LXRα	0.9 ± 0.2	NS	0.9 ± 0.1	NS
Bcl-3	2.3 ± 0.8	<0.05	2.5 ± 0.5	<0.05
Cell survival				
Bcl-2	1.2 ± 0.2	NS	1.0 ± 0.2	NS
Bcl-xL	1.1 ± 0.1	NS	1.0 ± 0.1	NS
Jak/Stat pathway				
Stat1	1.1 ± 0.1	NS	1.2 ± 0.1	NS
Stat3	1.7 ± 0.2	<0.0005	1.7 ± 0.2	<0.0005
SOCS3	3.1 ± 1.1	<0.05	9.4 ± 1.4	<0.0005
Oxidative stress				
HO-1	1.1 ± 0.1	NS	0.9 ± 0.1	NS

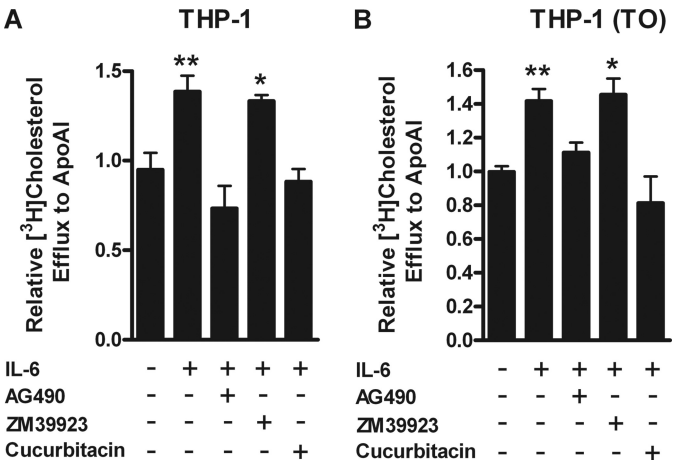

FIGURE 4. **Interleukin-6 stimulates ABCA1-mediated cholesterol efflux to apoAI through activation of the Jak-2/Stat3 pathway.** Human THP-1 macrophages were cholesterol-loaded and treated with or without IL6 (50 ng/ml) in the presence or in the absence of a specific inhibitor of either the Jak-2 protein-tyrosine kinase (AG490, 25 μM) or the Jak-3 protein-tyrosine kinase (ZM39923, 10 μM) or the Jak-2/Stat3 signaling pathway (cucurbitacin I, 2 μM) for 24 h. After an additional 24-h period with (*B*) or without TO901317 (*TO*; *A*) to induce ABCA1, cholesterol efflux to lipid-free apoAI from human macrophages was assayed over a period of 4 h. Cholesterol efflux to apoAI from THP-1 macrophages treated with IL-6 or inhibitors of the Jak/Stat pathway is expressed relative to cholesterol efflux to apoAI from nontreated cells. Values are the means ± S.E. of three independent experiments. *, $p < 0.05$; **, $p < 0.01$ *versus* untreated control cells.

esterol subsequent to the phagocytosis of apoptotic cells, cholesterol efflux to apoAI was examined in THP-1 macrophages (phagocytes) that had been previously incubated with apoptotic-free [^{3}H]cholesterol-loaded THP-1 cells. After the engulfment of free [^{3}H]cholesterol-loaded apoptotic cells, THP-1 phagocytes eliminated the ingested free [^{3}H]cholesterol through ABCA1-mediated cholesterol efflux to apoAI, a pathway that was abolished in the presence of cytochalasin D, a specific inhibitor of phagocytosis (37) (data not shown). Consistent with a role of ABCA1 in this mechanism, treatment of THP-1 phagocytes with the synthetic LXR agonist TO901317 led to an elevated efflux of cholesterol derived from apoptotic cells to apoAI (Fig. 5*F*; +44%, $p < 0.01$). Interestingly, this effect was further increased upon incubation with IL-6 (+32%, $p < 0.01$). Our findings, therefore, indicate that in addition to a role in favoring the clearance of apoptotic cells by macrophages, IL-6 prevents the accumulation of cytotoxic free cholesterol in phagocytes resulting from the ingestion of apoptotic cells via stimulation of ABCA1-mediated cholesterol efflux to apoAI.

Attenuation of the Proinflammatory Macrophage Response upon IL-6 Stimulation—Numerous studies have reported the close link between cholesterol homeostasis and inflammation in macrophages. Thus lipid accumulation leading to foam cell formation together with a proinflammatory status are features frequently observed in macrophages foam cells in atherosclerotic lesions. The role of ABCA1 in those mechanisms emerged from several studies which indicate that ABCA1 is a major actor at the crossroad of lipid homeostasis and inflammation in macrophages (7).

Analysis of macrophage cytokine secretion profile using biochips revealed that cholesterol loading in human THP-1 macrophages upon incubation with acLDL was accompanied by a marked increase of secreted IL-10 (4.2-fold, $p < 0.0005$;

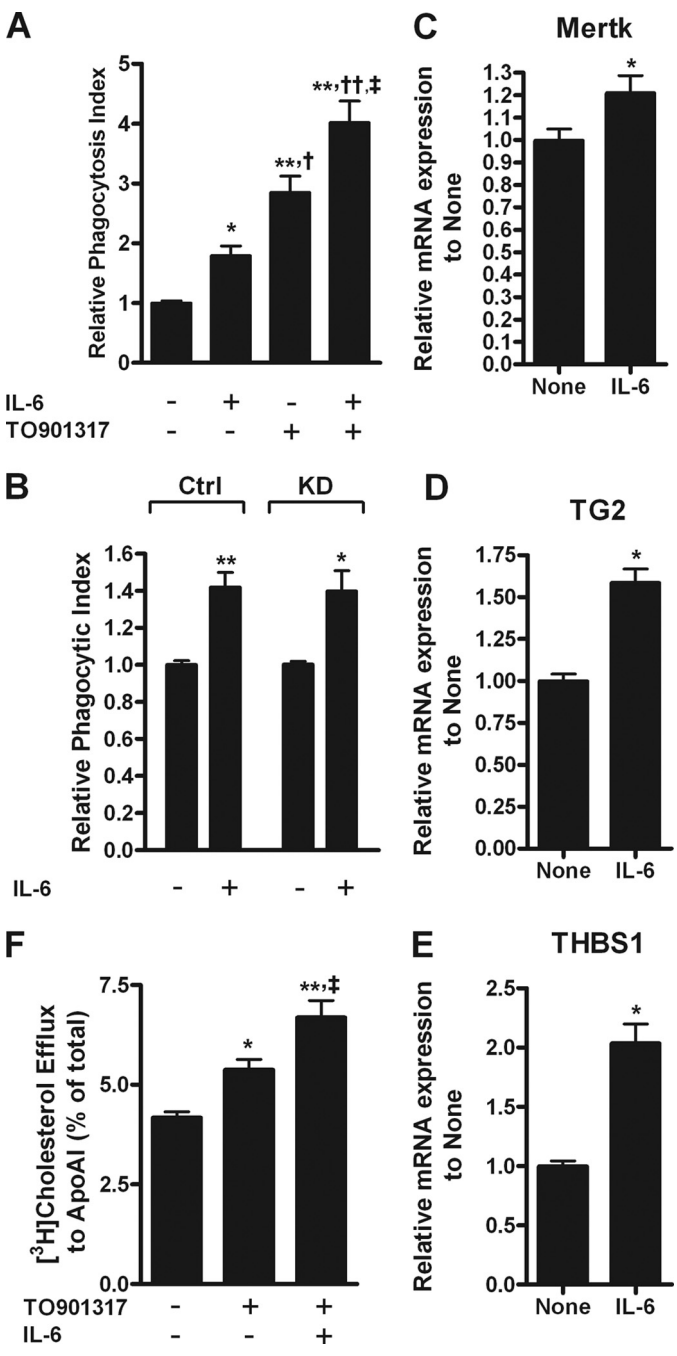

FIGURE 5. **Interleukin-6 increases efferocytosis and favors the capacity of human phagocytes to eliminate cholesterol derived from apoptotic cells through stimulation of ABCA1-mediated cholesterol efflux to apoAI.** *A*, human THP-1 macrophages (phagocytes) were treated with or without IL-6 (50 ng/ml) in the presence or in the absence of TO901317 (1 μM) for 24 h. Phagocytes were incubated together with calcein-AM-labeled apoptotic Jurkat cells for 30 min, and positive fluorescent phagocytes that had ingested calcein-AM-labeled apoptotic cells were quantified by flow cytometry. Values are the means ± S.E. of three independent experiments and are expressed as relative to control cells. *, $p < 0.05$; **, $p < 0.001$ *versus* control cells; †, $p < 0.01$; ††, $p < 0.001$ *versus* IL-6-treated cells; ‡, $p < 0.01$ *versus* TO901317-stimulated cells. *B*, phagocytosis of apoptotic cells in Ctrl and ABCA1 KD THP-1 macrophages upon stimulation with TO901317 is shown. Values are the means ± S.E. of three independent experiments and are expressed relative to respective control cells. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0001$ *versus* control cells. Quantification of c-mer proto-oncogene-tyrosine kinase (*MERTK*; *C*), TG2 (*D*), and THBS1 (*E*) mRNA levels in THP-1 macrophages upon stimulation with IL-6 is shown. Amounts of mRNA levels were normalized to housekeeping genes (δ-aminolevulinate synthase, α-tubulin, and hypoxanthine phosphoribosyltransferase 1). Values are the means ± S.E. of four independent experiments performed in triplicate. *, $p < 0.05$ *versus None*. *F*, human THP-1 macrophages (Phagocytes) were treated with or without IL-6 (50 ng/ml) in the presence of TO901317 (1 μM) for 24 h. Phagocytes were incubated together with apoptotic [^{3}H]cholesterol-loaded THP-1 macrophages for 30 min, and cholesterol efflux to lipid-free apoAI from phagocytes was assayed for 4 h. Values are the means ± S.E. of two independent experiments. *, $p < 0.05$; **, $p < 0.001$ *versus* non-stimulated cells; ‡, $p < 0.01$ *versus* TO901317-stimulated cells.

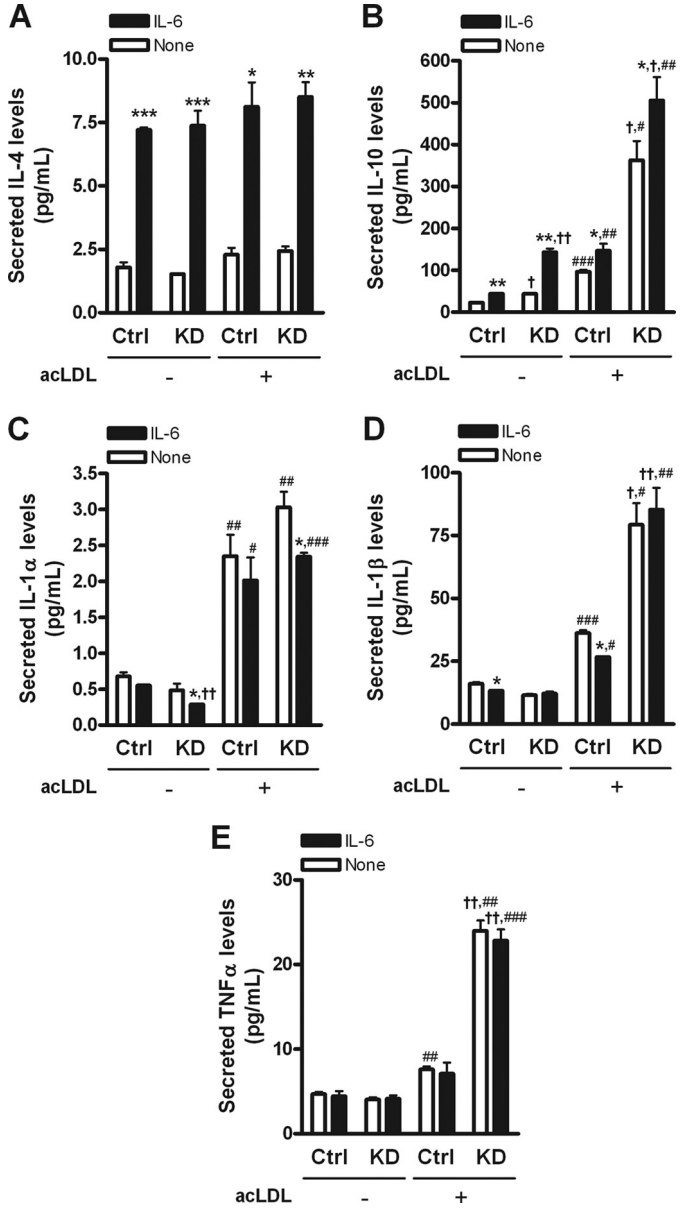

FIGURE 6. **Interleukin-6 induces an anti-inflammatory macrophage phenotype.** Human Ctrl and ABCA1 KD THP-1 macrophages were cholesterol-loaded or not with acLDL and incubated in the absence or in the presence of IL-6 (50 ng/ml) for 24 h. The secretion of IL-4 (*A*), IL-10 (*B*), IL-1α (*C*), IL-1β (*D*), and TNFα (*E*) cytokines in the culture media was quantified on a semiautomated Biochip Array analyzer (Randox). *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$ *versus* respective cells nonstimulated by IL-6; #, $p < 0.05$; ##, $p < 0.005$, ###, $p < 0.0005$ *versus* noncholesterol-loaded respective cells; †, $p < 0.05$; ††, $p < 0.005$; †††, $p < 0.005$ *versus* respective Ctrl cells. Values are the means ± S.E. of three independent experiments performed in triplicate.

Fig. 6*B*), IL-1α (3.5-fold, $p < 0.005$; Fig. 6*C*), IL-1β (2.4-fold, $p < 0.0005$; Fig. 6*D*) and TNFα (1.6-fold, $p < 0.005$; Fig. 6*E*) levels in the culture media thereby highlighting that transformation of

human macrophages into foam cells was concomitant with the establishment of a proinflammatory macrophage status. It is noteworthy that secretion of IL-6 was by far the most up-regulated (~10-fold, $p < 0.01$; Fig. 1) by lipid loading. When ABCA1 expression was knocked down in unloaded macrophages (ABCA1 KD), a significant ~2-fold increase of the secretion of the anti-inflammatory IL-10 cytokine was detected (Fig. 6*B*, $p < 0.05$), whereas that of IL-4, IL-1α, IL-1β, and TNFα was unaffected. By contrast, the silencing of ABCA1 expression in cholesterol-loaded human macrophages strongly elevated the amounts of IL-10 (3.4-fold, $p < 0.005$), IL-1β (+2.2-fold, $p < 0.05$), and TNFα (+3.2-fold, $p < 0.0005$) cytokines secreted in the culture media without any affect on those of IL-4 and IL-1α. Thus, disruption of lipid homeostasis in human macrophages caused by the knocking down of ABCA1 expression exacerbated the proinflammatory response subsequent to cholesterol loading.

Strikingly, a sustained stimulation by IL-6 for 24 h allowed a ~4-fold increase of the secreted IL-4 amounts by both Ctrl and ABCA1 KD human THP-1 macrophages whatever the lipid loading status (Fig. 6*A*). In addition, secretion of the anti-inflammatory IL-10 cytokine in the culture media was also found increased by IL-6 treatment from macrophages when cholesterol-loaded (+50%, $p < 0.05$) or not (+90%, $p < 0.005$) in the presence of modified LDL, such an induction being also observed in ABCA1 KD macrophages (Fig. 6*B*). Finally, a significant reduction in IL-1β secretion (-15%, $p < 0.05$) from THP-1 macrophages incubated in the presence or in the absence of acLDL was detected upon IL-6 treatment, this latter being no longer observable in ABCA1 KD macrophages (Fig. 6*D*), suggesting that the action of IL-6 on the secretion of IL-1β was dependent on ABCA1 expression. By contrast, silencing of ABCA1 expression in human macrophages (ABCA1 KD) conferred to IL-6 an inhibitory effect on IL-1α secretion when loaded or not with cholesterol (-40%, $p < 0.05$), whereas such an effect was not effective in control macrophages (*Ctrl*) (Fig. 6*C*). Taken together, these results clearly indicate that IL-6 attenuates the proinflammatory response from human macrophages through reduction of the secretion of proinflammatory IL-1β cytokine concomitant to induction of those of anti-inflammatory IL-4 and IL-10.

DISCUSSION

For the first time we provide experimental evidence that IL-6 enhances the capacity of human macrophages to maintain cholesterol homeostasis subsequent to cholesterol loading after uptake of modified LDL or phagocytosis of apoptotic cells through induction of ABCA1-mediated cholesterol efflux. In addition to a protective role from lipid accumulation, we report here that IL-6 concomitantly attenuates the proinflammatory response in human macrophage-foam cells. Our findings, therefore, suggest that the induction of IL-6 secretion in cholesterol-loaded human macrophages constitutes a protective mechanism that contributes to prevent cellular cholesterol accumulation through the autocrine action of IL-6 onto macrophages (Fig. 7).

Cholesterol efflux to apoAI from human macrophages was induced by IL-6 via activation of the Jak2/Stat3 pathway, in all likelihood as a result of the stimulation of ABCA1 expression. Those data are concordant with a previous study that identified a well conserved Stat3 element located in the first intron of the human ABCA1 gene as essential to its regulation (38). Thus, stimulation of human macrophages by IL-6 led to reduction in cellular cholesterol accumulation and potentially foam cell formation. In addition to the crucial role of ABCA1 in preventing foam cell formation by virtue of its capacity to drive efflux of cellular cholesterol derived from modified LDL uptake, ABCA1 was equally reported to exert a determinant role in mechanisms that allow macrophages to handle cytotoxic membrane cholesterol delivered by phagocytosis of apoptotic cells (6). Moreover, this mechanism was identified as a survival response in macrophages that have ingested apoptotic cells (30), as cytotoxic cholesterol may accumulate in phagocytes and, hence, trigger free cholesterol-induced apoptosis. Thus, stimulation of ABCA1-mediated free cholesterol efflux to apoAI by IL-6 in human macrophages may not only prevent foam cell formation but may also reduce the formation of apoptotic macrophages; indeed, we observed that IL-6 enhanced the ABCA1-mediated efflux of cholesterol derived from the ingestion of free cholesterol-loaded apoptotic macrophages. Our results are consistent with the observation that infusion of a humanized monoclonal antibody to the IL-6 receptor in patients with active Crohn disease led to an increased number of apoptotic mononuclear cells, thereby suggesting that inhibition of IL-6 may increase apoptosis (39); such an effect may, however, also result in part from defective clearance of apoptotic cells.

Interestingly, we observed that IL-6 enhanced the phagocytosis of apoptotic cells, *i.e.* efferocytosis, by THP-1 phagocytes. Silencing of ABCA1 expression in LXR-stimulated human THP-1 macrophages had no impact on the enhanced phagocytosis of apoptotic cells in response to IL-6, indicating that this effect occurs through an ABCA1-independent mechanism. An additional member of the ABC transporter family, ABCA7, was shown to be implicated in the phagocytosis of apoptotic cells (40, 41). However, ABCA7 mRNA levels were not increased in human macrophages upon stimulation with IL-6 (Table 1), suggesting that ABCA7 was in all likelihood not responsible for the IL-6-mediated enhancement of the engulfment of apoptotic cells. Indeed, our results rather suggest that induction of efferocytosis by IL-6 results from induction of THBS1, c-mer proto-oncogene-tyrosine kinase, and TG2 expression as mRNA levels of those molecules known to facilitate the recognition and uptake of apoptotic cells by macrophages were found increased upon IL-6 incubation. It is of note that TG2 was previously reported to be a key factor in the clearance of apoptotic cells by human macrophages (42). The relevance of IL-6 to phagocytosis of apoptotic cells is strengthened by our observation that PPARδ expression was also stimulated by IL-6 in human macrophages (Table 1). Indeed, a recent study has identified PPARδ as the key sensor in the clearance of apoptotic cells through its role in the stimulation of opsonins (C1qb, milk fat globule-epidermal growth factor-8, and Thbs1) and c-mer proto-oncogene-tyrosine kinase in bone marrow-derived mouse macrophages after ingestion of apoptotic cells (43). Although an elevation of C1qb and milk fat globule-epidermal growth factor-8 mRNA levels was not detected in human

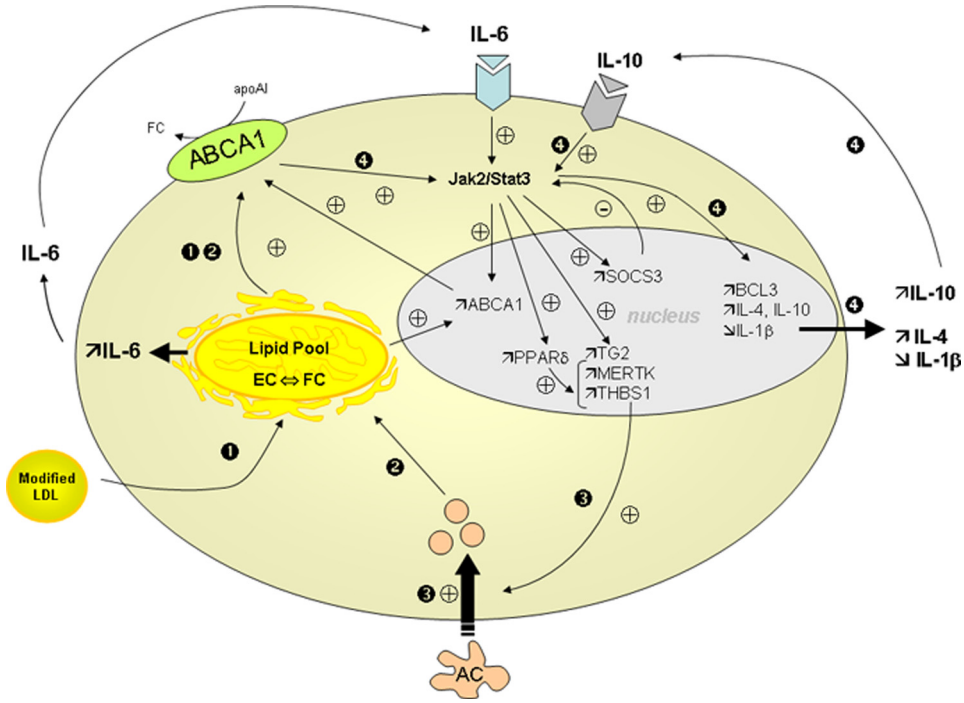

FIGURE 7. **Schematic representation of intracellular pathways implicated in the regulation of cholesterol homeostasis and inflammatory response in human macrophages by IL-6.** In lipid-loaded human macrophages secreting high amounts of IL-6, the autocrine action of IL-6 induces ABCA1 gene expression and enhances ABCA1-mediated efflux of cholesterol derived from ingestion of either modified lipoproteins (❶) or apoptotic cells (❷) through activation of the Jak-2/Stat3 pathway, thereby reducing foam cell formation and accumulation of cytotoxic free cholesterol, respectively. Moreover, IL-6 induces the expression of PPARδ, TG2, c-mer proto-oncogene-tyrosine kinase (*MERTK*), and THBS1 and enhances the clearance of apoptotic cells by human macrophages phagocytes (❸). Finally stimulation of human macrophages by IL-6 led to an attenuation of the proinflammatory response (❹) by the increase of IL-4 and IL-10 secretion and the reduction of that of IL-1β. Elevation of secreted IL-10 amounts upon IL-6 stimulation may contribute to maintain Stat3 activation and then to abolish the repression operated by induction of SOCS3 expression. It is of note that the interaction of apoAI with ABCA1 was previously reported to activate the Jak-2/Stat3 pathway (❹) and to participate to the establishment of the anti-inflammatory response in human macrophages (13, 14). Taken together, the autocrine action of IL-6 on lipid-laden human macrophages constitutes a survival response by protecting from lipid accumulation and by attenuating the proinflammatory response. *FC*, free cholesterol; *EC*, esterified cholesterol; *AC*, apoptotic cells.

macrophages when treated with IL-6, we cannot exclude the possibility that IL-6 up-regulated the expression of THBS1, c-mer proto-oncogene-tyrosine kinase, and TG2 through induction of PPARδ. Thus, the induction of PPARδ expression may constitute a pathway by which IL-6 enhances the clearance of apoptotic cells by human macrophages.

In the present study IL-6 not only favored the elimination of excess cholesterol in human macrophages but also contributed to attenuate the proinflammatory macrophage response. Indeed, the sustained stimulation of human macrophage-foam cells with IL-6 led to elevation in IL-10 and IL-4 secretion, which are implicated in establishment of the anti-inflammatory Th2 response (44) together with a reduction of secreted IL-1β levels. Silencing of ABCA1 expression in human macrophages indicates that only the action of IL-6 on IL-1β secretion may be dependent on ABCA1 as this latter was abolished in ABCA1 KD macrophages. Previous studies described ABCA1 as an anti-inflammatory receptor through the interaction of apoAI with ABCA1 and subsequent activation of the Jak2/Stat-3 signaling pathway (13, 14). Although human macrophages do not express apoAI, such an effect occurs *in vitro* when THP-1 macrophages are incubated in the presence of apoAI (14). Thus, the addition of exogenous apoAI to THP-1 macrophages strongly attenuated the stimulation of proinflammatory cytokines secretion, such as IL-1β, observed upon LPS treatment (14). The requirement of ABCA1 in the inhibitory effect of IL-6 on IL-1β secretion may, therefore, result from the induction of ABCA1 expression by IL-6 in THP-1 macrophages, which would favor interaction of ABCA1 not with apoAI, which was not present in our experimental conditions, but potentially with apoE, which is abundantly expressed by human macrophages and was largely described to interact with ABCA1 for promoting cellular cholesterol efflux. This potential mechanism remains, however, to be explored.

In agreement with the enhanced production of IL-10 observed in human macrophages upon IL-6 stimulation, the expression of the proto-oncogene bcl-3 gene, a key mediator in the anti-inflammatory response of IL-10, was equally induced (Table 1). Stimulation of bcl-3 gene expression by IL-10 through activation of Stat3 in human macrophages has been proposed to underlie the cytokine-suppressive effects of IL-10 (45). However, bcl-3 was also induced by IL-6 via specific activation of Stat3 (46), and we observed that IL-6, just as for IL-10, may stimulate bcl-3 expression in human macrophages. We cannot, however, exclude the possibility that stimulation of bcl-3 gene expression may have resulted initially from IL-6-mediated activation of Stat3 and that this latter effect was subsequently relayed by IL-10. Activation of Stat3 by IL-6 may be rapidly deactivated by SOCS3 induction (47), such induction of SOCS3 being observed in human macrophages activated by IL-6 (Table 1). Increase in IL-10 secretion induced by IL-6 could, therefore, be required to maintain Stat3 activation in

human macrophages, as phosphorylation of Stat3 mediated by IL-10 through the specific IL-10R receptor was not repressed by SOCS3 (48). Taken together with findings from Yin *et al.* (14), our data, therefore, suggest that stimulation of Jak2/Stat3 constitutes a pivotal signaling pathway in cholesterol-loaded human macrophages present in atherosclerotic lesions for attenuating both the cellular lipid accumulation and the proinflammatory response.

These findings illustrate that although IL-6 is primarily considered as a major proinflammatory cytokine, it may also act as a key anti-inflammatory mediator in the control of inflammatory responses (49); indeed such an inflammatory role of IL-6 is observed in response to exercise through enhanced secretion of IL-10 (50). More strikingly and in agreement with our data, the infusion of recombinant human IL-6 in young healthy volunteers induced an increase in anti-inflammatory IL-10 levels without affecting those of TNFα (51). This effect initially appears to contradict the widespread view that IL-6 contributes exclusively to the deleterious effects associated with chronic inflammatory diseases such as atherosclerosis. However, recent studies suggest that the role of IL-6 in inflammatory mechanisms appears more complex. Thus, the hepatocyte-specific IL-6 receptor α deficiency in mice caused an unexpected exaggerated inflammatory response during euglycemic hyperinsulinemic clamp analysis (52).

Our present findings, integrated schematically in Fig. 7, support an autocrine action of IL-6 that promotes clearance of apoptotic cells while protecting human macrophages from cellular lipid accumulation. Indeed, the sustained exposure to modified LDL or apoptotic cells compels macrophages to handle high amounts of lipid that may be cytotoxic and thus lead to apoptosis if not recycled properly. We here propose that IL-6 may favor the recycling of cholesterol by human macrophages and thus contribute to the resolution of local inflammation by eliminating the proinflammatory material from the site of inflammation. Such a protective effect may be indeed beneficial in inflammatory atherosclerotic lesions in coronary artery disease patients. However, the action of IL-6 described in our study may be also operative on macrophages present in the adipose tissue or on specialized macrophage-phagocytes such as Kupffer cells in the liver, a key target tissue for IL-6. Indeed, Kupffer cells as macrophages in atheroma plaques are able to recognize modified LDL, to ingest apoptotic cells, and to secrete inflammatory cytokines, these latter contributing to interactions with hepatocytes and other liver cells (53). Thus, the recycling of cholesterol by tissue macrophages may constitute a pathway by which IL-6 regulates metabolic homeostasis. Indeed, it is noteworthy that IL-6$^{-/-}$ mice developed mature-onset obesity and glucose intolerance (54), a result that is consistent with the observation that the treatment of Castleman disease patients with tocilizumab, a humanized IL-6 receptor-inhibiting monoclonal antibody, led to an increase of body weight in addition to hypertriglyceridemia (55). Moreover, the regulatory action of IL-6 on lipid metabolism is highlighted by the increase of plasma total cholesterol observed in rheumatoid arthritis patients upon tocilizumab therapy (56). Taken together with our study, those findings suggest that IL-6 is an inflammatory cytokine that may exert a Janus role in metabolic diseases, and further studies are, therefore, required to elucidate the complete spectrum of its pathophysiological actions.

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