Regulation of lipoprotein trafficking in the kidney: role of inflammatory mediators and transcription factors

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Abstract

Inflammation and dyslipidaemia both play important roles in the development of glomerular atherosclerosis in renal diseases. We have demonstrated that inflammatory mediators induced Scr (scavenger receptor) expression and the formation of foam cells, and that AP-1 (activator protein 1)/ets were necessary transcriptional factors for Scr induction in HMCs (human kidney mesangial cells). Most cells are protected from excessive native LDL (low-density lipoprotein) accumulation by tight feedback regulation of the LDLr (LDL receptor). However, we observed that HMCs formed foam cells via the LDLr pathway when incubated with IL-1β (interleukin-1β; 5 ng/ml) and unmodified LDL (200 µg/ml), suggesting that inflammatory mediators may disrupt the cholesterol-mediated feedback regulation. This feedback involves cholesterol-mediated down-regulation of LDLr controlled by SCAP [SREBP (sterol responsive element-binding protein) cleavage-activating protein]. We have also demonstrated that both tumour necrosis factor α and IL-1β increased nuclear SREBP-1 levels by increasing SCAP mRNA expression, even in the presence of a high concentration of LDL. Since intracellular lipid content is governed by both influx and efflux mechanisms, we set out to examine the impact of inflammatory cytokines on cholesterol efflux, a process mediated by the protein ABCA1 (ATP binding cassette A1). IL-1/β inhibited [3H]cholesterol efflux from HMCs by inhibition of the peroxisome-proliferator-activated receptor/LXR (liver X receptor)/ABCA1 pathway. Taken together, our results suggest that inflammatory mediators increase lipid accumulation in HMCs not only by promoting increased lipoprotein uptake by Scr and LDLr, but also by inhibiting ABCA1-mediated cholesterol efflux to high-density lipoprotein.

Background

Inflammation, lipid accumulation and foam cell formation are recognized features of atherosclerosis. Lipid-mediated renal injury causing the progression of renal disease was first suggested by our group [1]. We proposed the term ‘glomerular atherosclerosis’ on the basis that atherosclerosis and glomerulosclerosis share common pathogenic mechanisms [1]. Others confirmed our findings that lipid-mediated renal injury is an important component of glomerulosclerosis [2]. Recently, we have been focusing on how inflammatory mediators affect cholesterol homoeostasis and increase lipid accumulation in HMCs (human kidney mesangial cells).

Conventionally, foam cells are thought to involve predominantly macrophages. However, other cell types, such as smooth muscle cells and HMCs, can be converted to foam cells. There are many lipoprotein receptors which mediate cholesterol uptake. Unlike the LDLr [LDL (low-density lipoprotein) receptor], the activity of type-A Scr (scavenger receptor) is not suppressed by rising intracellular cholesterol concentration, thus providing a mechanism for unregulated cholesterol uptake. Previous studies characterizing the expression of Scr on HMCs have been conflicting. In vitro, Gröne, Lee and co-workers reported that acetyl-LDL and oxidized LDL were not taken up specifically, suggesting no active Scr in cultures of HMCs [3,4]. However, Takemura and others demonstrated Scr on the membranes of mesangial cells of renal biopsy tissues from patients with several types of glomerular disease and that the Scr expression was increased in glomeruli with marked mesangial proliferation [5]. One explanation for these discrepancies is that growth conditions in culture led to alterations in Scr gene expression that would not occur in vivo. We demonstrated that an inducible form of Scr is present in HMCs [6].

The LDLr is the primary receptor for binding and internalization of plasma-derived LDL-cholesterol and regulates plasma LDL concentrations [7]. Brown and Goldstein observed that LDLr activity is under tight metabolic control by the intracellular cholesterol concentration through a feedback system [8]. Most cells are protected from native LDL accumulation by tight feedback regulation of LDLr.
Such feedback involves cholesterol-mediated down-regulation of LDLr controlled by SCAP [SREBP (sterol responsive element-binding protein) cleavage-activating protein]. Therefore, native LDL is ineffective in generating the lipid-rich foam cells under physiological conditions. However, our previous study indicated that inflammatory mediators caused dysregulation of LDLr expression [9]. In the present study, we investigated the effects of TNFα (tumour necrosis factor α) and IL-1β (interleukin-1β) on the regulation of LDLr in the presence of cholesterol and the underlying molecular mechanisms involved in the dysregulation of LDLr under the influence of inflammatory cytokines.

Since intracellular lipid content is governed by both influx and efflux, we set out to examine the impact of inflammatory cytokines on cholesterol efflux, a process mediated by the protein ABCA1 (ATP binding cassette A1). ABCA1, a member of the ABC transporter superfamily, is a cholesterol transporter [10]. The activation of ABCA1 seems to be the first step of the reverse cholesterol transport pathway and is therefore important in the control of plasma levels of high-density lipoprotein, which is an important factor in the development of atherosclerosis [11]. The present study examines the effects of the inflammatory cytokine IL-1β on ABCA1-mediated cholesterol efflux in HMCs and investigates the underlying molecular mechanisms.

It is our hypothesis that inflammatory mediators increase lipid accumulation in HMCs by inducing Scr, disrupting LDLr feedback regulation and reducing cholesterol efflux by ABCA1 pathways. An anti-inflammatory therapeutic strategy is therefore indicated in preventing lipid-induced renal injury.

Materials and methods

Cell culture

An established stable HMC line that had been immortalized by transfection with T-SV40 and H-ras oncogenes was used in all experiments (kindly donated by Dr J.D. Sraer, Hôpital Tenon, Paris, France) [12]. Recombinant IL-1β [(1.0–3.3) × 10⁶ units/mg] was obtained from R&D Systems Europe (Abingdon, Oxon, U.K.).

Preparation of lipoprotein

Plasma was collected from healthy human volunteers and LDL was isolated by sequential ultracentrifugation [9]. LDL labelled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI-LDL) was obtained from Biogenesis (Poole, Dorset, U.K.).

Morphological examination

HMCs were plated in chamber slides for tissue culture (Nunc, Naperville, IL, U.S.A.) and incubated in serum-free RPMI medium for 24 h with various treatments described in the Results section. The cells were washed, fixed for 30 min with 5% formalin solution in PBS, stained with Oil Red O for 30 min and counterstained with haematoxylin for another 5 min. Finally, the cells were examined by light microscopy.

Cholesterol efflux assay

Cholesterol efflux to apolipoprotein A was estimated as described in our previous publications [6,13]. Total cholesterol was also measured by enzymic assay (Dade Behring, Milton Keynes, Berks, U.K.).

Cell labelling and flow cytometric analysis

HMCs were incubated in serum-free medium with various treatments described above. The treated cells were labelled with 10 µg/ml DiI-labelled LDL (or DiI-acetyl-LDL) for 5 h at 37°C. The cells were fixed in 5% formalin solution in PBS and analysed by FACS using a flow cytometer (Coulter; EPICS XL-MCL).

Reverse transcriptase-PCR and Southern blot analysis

Total RNA (500 ng) was used as a template for reverse transcriptase-PCR using an RNA PCR kit from ABI (Applied Biosystems, Warrington, Cheshire, U.K.). The cDNA synthesized by reverse transcriptase was used for separate amplification of LDLr, SCAP, Scr, ABCA1, PPAR (peroxisome proliferator-activated receptor) α, PPARγ, LXR (liver X receptor) α and glyceraldehyde-3-phosphate dehydrogenase cDNAs using specific primers as described [6,13]. A 20 µl sample of each PCR reaction was subjected to electrophoresis in a 2% agarose gel. Nucleic acids were transferred to a nylon membrane (Boehringer Mannheim, Lewes, East Sussex, U.K.). The nylon membranes were probed with [γ-³²P]ATP-labelled oligonucleotides of LDLr, SCAP, Scr, ABCA1, LXRα and PPARs.

Western blot analysis for SREBP-1

The nuclear extracts were prepared from the treated HMCs. Identical amounts of total protein from nuclear extracts were denatured, and then subjected to electrophoresis. Western blot was performed by using rabbit anti-(human SREBP-1) polyclonal antibody (1 µg/ml; Santa Cruz Biotechnology) and goat anti-rabbit horseradish peroxidase-labelled antibody.

Data analysis

In all experiments, groups of data were evaluated for significance by one-way ANOVA using Minitab software. Data were considered significant at P ≤ 0.05.

Results

We demonstrated that both PMA and angiotensin II induced Scr expression in a dose-dependent manner, and formed foam cells visualized by Oil Red O staining in HMCs. Luciferase

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reporter constructs containing the full-length Scr promoter, one or three copies of the AP-1 (activator protein 1)/ets motifs or the corresponding sequences containing mutations in either the AP-1 or ets site were introduced upstream of a minimal promoter derived from the rat prolactin gene. Both PMA and angiotensin II increased Scr promoter activity. Increasing the copy number of AP-1/ets enhanced the response to PMA stimulation. Mutation of either the AP-1 or the ets motif decreased its ability to respond to PMA, suggesting that AP-1/ets motifs are necessary response elements for gene expression induced by PMA.

We observed that HMCs formed foam cells when incubated with TNFα (50 ng/ml) or IL-β (5 ng/ml) and unmodified LDL (200 μg/ml). This process was not inhibited by polyinosinic acid, which blocks Scr, but was prevented by heparin, which displaces LDL bound to its cell-surface receptors. We investigated SREBP-1 translocation from endoplasmic reticulum to the nucleus due to the action of these inflammatory cytokines. High concentrations of LDL decreased SREBP-1 levels in the nucleus. However, both TNFα and IL-β increased nuclear SREBP-1 levels, even in the presence of a high concentration of LDL. Furthermore, we demonstrated that IL-β affected SREBP cleavage by increasing SCAP mRNA expression, which may result in translocation of the SCAP–SREBP complex from the endoplasmic reticulum to the Golgi, where proteases cleave SREBP, even in the presence of a high concentration of LDL.

Since intracellular lipid content is governed by both influx and efflux mechanisms, we set out to examine the impact of inflammatory cytokines on cholesterol efflux, a process mediated by the protein ABCA1. IL-β (5 ng/ml) inhibited [3H]cholesterol efflux from HMCs which resulted from reduced expression of the ABCA1 gene and increased intracellular cholesterol concentration. To ascertain the molecular mechanisms involved, expression of PPARα, PPARγ and LXRα were examined. IL-β reduced the mRNA expression of PPARα, PPARγ and LXRα whether or not cells had been preloaded with lipid.

**Discussion**

We demonstrated that both PMA and angiotensin II increased Scr gene expression and function in HMCs. These results imply that there is limited expression of Scr in HMCs under physiological conditions, but when HMCs were stimulated by PMA and angiotensin II, they expressed an inducible Scr. This may explain why HMCs, which have not previously been shown to express Scr in normal culture, can express Scr in vivo [3,4]. Therefore, during inflammation, HMCs may express an inducible Scr through which cells can acquire lipids and convert to foam cells observed in glomerulosclerosis. Additionally, we focused on the molecular mechanism of Scr up-regulation by PMA and angiotensin II. The Scr 5′ upstream (~4.1 kb to +46) of genomic DNA contains several copies of binding motifs for AP-1/ets transcription factors. Reporter gene analysis showed that PMA induced Scr promoter activity by increasing binding of AP-1/ets transcription factors to the Scr promoter region and that AP-1/ets transcription factors are necessary for Scr expression induced by PMA in HMCs.

We have also demonstrated that TNFα and IL-β override the suppression of LDLr induced by a high concentration of LDL [13]. The process by which TNFα and IL-β increased the number of intracellular Oil Red O-stained lipid droplets in HMCs could not be inhibited by Poly I, which blocks Scrs, but was blocked by heparin, which removes LDL bound to the cell surface, implying LDLr pathway involvement and effectively excluding the participation of Scr. Additionally, all experimental incubation media contained the antioxidants EDTA and butylated hydroxytoluene, both of which powerfully prevent oxidation of LDL by HMCs. The electrophoretic mobility of LDL from the culture medium was the same as that of fresh LDL, indicating that no oxidation had taken place during the experiments. Therefore, there was no ligand for Scrs in the culture medium. Taken together, these results imply that foam cell formation occurred through the dysregulation of the LDLr.

We also investigated the molecular mechanisms by which inflammatory cytokines overrode the normal cholesterol-mediated suppression of the LDLr by examining the expression of human SCAP. Both TNFα and IL-β increased SCAP mRNA expression in the presence of high concentrations of LDL. Up-regulation of SCAP may compete for the limited amounts of endoplasmic reticulum retention factor Insig-1 [14], allowing the SCAP–SREBP complex to travel from the endoplasmic reticulum to the Golgi for cleavage.

Furthermore, our results, which demonstrated that IL-β inhibits ABCA1 mRNA expression, provide a plausible mechanism by which this inflammatory cytokine impairs cellular cholesterol efflux. Inhibition of the ABCA1 pathway by inflammatory cytokines might also explain the clinical observation that patients with an activated inflammatory response have low circulating levels of apolipoprotein A1 [15]. Interestingly, IL-β caused suppression of both PPAR (PPARα and PPARγ) and LXRα mRNA expression over a timescale that would be consistent with their role in regulating ABCA1 gene transcription, suggesting that IL-β reduced cholesterol efflux by inhibition of the PPARs/LXRα/ABCA1 pathway.

Taken together, our results suggest that inflammatory cytokines increase lipid accumulation, not only by promoting increased lipoprotein uptake by LDLr and Scrs, but also by inhibiting ABCA1-mediated cholesterol efflux to high-density lipoprotein in HMCs. These findings may also be relevant to the pathogenesis of atherosclerosis as well as to other disease processes characterized by intracellular lipid deposition, such as rheumatoid arthritis and other inflammatory diseases.

**References**


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