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Oxidized LDL Immune Complexes Stimulate Collagen IV Production in Mesangial Cells via Fc Gamma Receptors I and III

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Abstract

Diabetic nephropathy is characterized by progressive mesangial expansion. Although we have reported that circulating oxidized LDL-containing immune complexes (oxLDL-ICs) are associated with abnormal levels of albuminuria, the underlying mechanisms have not been investigated. In this study, we have studied the effect of oxLDL-ICs on collagen IV expression by mesangial cells. We found that oxLDL-ICs markedly stimulated collagen IV expression in a concentration- and time-dependent fashion while oxLDL only had moderate effect. We also found that oxLDL-ICs stimulated collagen IV expression by engaging Fc gamma receptor (FcγR) I and III, but not FcγRII, and that p38 MAPK, JNK and PKC pathways were involved in collagen IV expression. Furthermore, we found that oxLDL-ICs stimulated FcγRI expression, suggesting a positive feedback mechanism involved in oxLDL-IC-stimulated collagen IV expression. Taken together, this study showed that oxLDL-ICs stimulated collagen IV in mesangial cells via FcγRI and FcγRIII, and the expression of FcγRI was increased by oxLDL-ICs.

Keywords

Collagen; Low-density Lipoprotein; Immune Complexes; Nephropathy; Mesangial Cells

INTRODUCTION

Diabetic nephropathy is the most common cause of end stage renal disease [1; 2]. It is characterized by both structural and functional changes, including albuminuria, decreased glomerular filtration rate (GFR), increased thickness of glomerular basement membrane (GBM), mesangial cell hypertrophy, and expansion of mesangial matrix [3; 4; 5]. Mesangial matrix expansion has been shown to be inversely correlated with the capillary filtering surface area density [6]. Proliferation of glomerular cells and accumulation of extracellular matrix proteins have been linked to sclerosis and progressive renal failure in a large number

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of animal models [7; 8]. Consequently, factors that affect mesangial growth and matrix accumulation are thought to play a central role in the pathogenesis of diabetic nephropathy [6; 9; 10].

Mesangial cells are located in the intercapillary space of the glomerular tufts. They are bone marrow-independent mesenchymal cells related to vascular smooth muscle. Mesangial cells are primary producers of mesangial extracellular matrix constituents. Functionally, they are responsible for preservation of the structural integrity of the glomerulus and regulation of the glomerular filtration [11]. Therefore, mesangial cells play an important role in most pathological processes of the renal glomerulus [12; 13]. The major components of mesangial matrix are collagen types IV and V, fibronectin, laminin, proteoglycans, heparan sulfate and chondroitin-dermatan sulfates [14]. Of these molecules, the metabolism of collagen type IV in diabetic mesangial lesions has been studied extensively. In pathologic states, there is progressive accumulation of extracellular matrix [15]. Several studies have reported substantial increase in the mesangial content of collagen IV and I [16; 17]. Although the precise mechanisms leading to matrix expansion are not known, there is growing evidence that oxidized low-density lipoprotein (oxLDL), growth factors and cytokines released by infiltrating leukocytes, platelets and resident glomerular cells play an important role [7; 18].

It has been shown that oxLDL is present mainly in the lesions of glomerulosclerosis and mesangial areas in human renal biopsies [19]. Results from an in vitro study showed that oxLDL stimulated collagen expression by mesangial cells [20]. Furthermore, it is well known that oxLDL is immunogenic and that it stimulates the synthesis of antibodies, predominantly of the pro-atherogenic subtypes IgG1 and IgG3 [21; 22; 23]. Thus, oxLDL-containing immune complexes (oxLDL-IC) are formed by binding of oxLDL to anti-oxLDL IgG1 or IgG3. We have recently shown in a large subset of the DCCT/EDIC cohort (type 1 diabetes) that oxLDL-IC are associated with the progression of microalbuminuria or/and macroalbuminuria as well as with the progression of carotid intima medial thickening [24]. This clinical study suggests that not only oxLDL, but also oxLDL-ICs may play important role in nephropathy. To understand how oxLDL-ICs are associated with the progression of microalbuminuria or/and macroalbuminuria, it is important to compare the effect of oxLDL-ICs with that of oxLDL on collagen expression by mesangial cells.

It has been shown previously that immune complexes can be involved in mesangial matrix expansion [25]. IC deposition and/or formation in the glomerular region have been implicated in the pathogenesis of glomerulonephritis. Previous studies have demonstrated that mesangial cells have receptors for IgG, and that stimulation of those cells by soluble or insoluble IgG-containing IC (IgG-IC) induce the release of different inflammatory mediators like platelet activating factor, oxygen radicals and prostaglandins [26; 27; 28]. Also, exposure of mesangial cells to IgG-IC has been shown to lead to a significant increase in the synthesis of matrix proteins [29].

In this study, we aimed at the comparison of effects of oxLDL-IC and oxLDL on the production of collagen IV by human mesangial cells. We also investigating the mechanisms potentially involved in the regulation of collagen IV by oxLDL-IC.

MATERIAL AND METHODS

Cell Culture

Human mesangial cells were a gift from Dr. Hanna Abboud at the University of Texas Health Science Center (San Antonio, Texas). The cells were maintained in medium containing a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 supplemented with 17% fetal bovine serum, 26 µg/ml insulin, and antibiotics including 100

units/ml penicillin and 100 µg/ml streptomycin at 37 °C in a humidified 5% CO₂ atmosphere. The cells were subcultured weekly and used for experiments between 8th and 12th passages.

Preparation of Human OxLDL and OxLDL-IC

OxLDL was prepared by incubating freshly isolated human LDL at 1.5 mg/ml with CuCl₂ at 0.04 µmol/ml as described previously [30]. The degree of oxidation was measured by fluorescence emission at 430 nm during a total of 12–18 h. Only preparations reaching an emission fluorescence value of 1.1 units or more during that time frame were used, because such preparations have been shown to react optimally with human anti-oxLDL antibodies and best absorb human purified oxLDL antibodies [30]. Insoluble oxLDL-IC were prepared with human oxLDL and human anti-oxLDL antibodies. Anti-oxLDL IgG was isolated from whole human serum using a combination of Protein G-Sepharose 4 fast flow chromatography (Amersham-Pharmacia Biotech Inc., Piscataway, NJ) to isolate IgG, and affinity chromatography in Sepharose-linked human oxLDL to isolate anti-oxLDL antibodies of the IgG isotype, as described previously [31]. The amounts of oxLDL and anti-oxLDL IgG in preparation of insoluble oxLDL-IC were determined empirically by performing a precipitin curve, constructed by incubating aliquots of the IgG with varying amounts of oxLDL. The empirical measurements indicated that a ratio of 150 mg of oxLDL protein vs. 500 mg of IgG produced peak precipitation.

Preparation of KLH-anti-KLH IC and IgG-anti-IgG IC

KLH-anti-KLH IC were prepared with IgG isolated by Protein G-Sepharose 4 fast flow affinity chromatography [22] from aliquots of human serum containing polyclonal anti keyhole limpet hemocyanin (KLH) [30] generously provided to us more than 20 years ago by an investigator from the University of Lisbon. The serum was aliquoted and stored at –70°C until fractionation. The isolated IgG reacted with KLH by enzyme immunoassay but did not cross react with human serum proteins or lipoproteins. The optimal proportion of KLH (Calbiochem, San Diego, CA) to anti-KLH IgG for preparation of insoluble human KLH-IC was 200/250 µg (w/w). Human IgG immune complexes (IgG-ICs) were prepared with human IgG and rabbit anti-human IgG antiserum as described previously [32]. All ICs were prepared under sterile conditions, washed and resuspended in PBS.

Immunoblotting

Cells were washed twice with ice-cold PBS before being treated with lysis buffer containing 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS and protease inhibitors including 1 mM PMSF, 2 mM EDTA, 1 µg/ml leupeptin, and 1 µg/ml aprotinin. Protein concentration in cell lysates was determined using a Bradford assay kit (BioRad, Hercules, CA). For detection of collagen, protein extracts were subjected to 4–20% gradient SDS PAGE and transferred to Immobilon-P membranes (Millipore, Bedford, MA). Membranes were blocked with 5% milk in a buffer containing 20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20 (vol/vol) for 1 h at room temperature and then incubated with primary antibodies including CD32, CD36 or CD16 blocking antibodies (Abcam, Cambridge, MA); human collagen IV antibody, human CD16 or CD32 FITC-conjugated antibodies (Invitrogen, Carlsbad, CA) for 16 h at 4°C. After incubation with horseradish peroxidase-conjugated secondary antibody for 1 hour, immunoreactive bands were detected by exposing to chemiluminescence (ECL) (Santa Cruz Biotechnology, Santa Cruz, CA) and X-ray films. The X-ray films were scanned using an Epson scanner (Perfection 1200U), and the density of bands on the images was quantified using Adobe Photoshop version 10.1.01.

Flow Cytometry

Flow cytometry analysis was performed to determine the surface expression of FcγRs. Confluent human mesangial cells (1×10^6 cells per sample) were incubated with fluorescein isothiocyanate (FITC)-labeled anti-FcγR antibodies (R&D System, Minneapolis, MA) for 25 min on ice. After one wash with fatty acyl-CoA (FACS) solution, a second wash was performed by adding 10% (w/v) propidium iodide. Cells were then resuspended in 300 μl of FACS solution and immediately processed using a FACS Calibur (Becton Dickinson, San Jose, CA), and the fluorescence emission profile was analyzed by the CellQuest program (Becton Dickinson).

Real-time PCR

Total RNA was isolated from cells using the RNeasy minikit (Qiagen, Santa Clarita, CA). First strand complementary DNA (cDNA) was synthesized with the iScript™ cDNA synthesis kit (Bio-Rad) using 20 μl of reaction mixture containing 0.25 μg of total RNA, 4 μl of 5× iScript reaction mixture, and 1 μl of iScript reverse transcriptase. The complete reaction was cycled for 5 min at 25 °C, 30 min at 42 °C, and 5 min at 85 °C using a PTC-200 DNA Engine (MJ Research, Waltham, MA). The reverse transcription reaction mixture was then diluted 1:10 with nuclease-free water and used for PCR amplification of cDNA in the presence of the primers. The Beacon designer software (PREMIER Biosoft International, Palo Alto, CA) was used for Fc gamma receptor I (FcγRI) primer designing. The FcγRI primers (forward: CAAGCCTAGCCTGATAATCC; reverse: TGTCTCCCTGAAATCTACCC, accession number: NM000566) were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA), and real time PCR was performed in duplicate using 25 μl of reaction mixture containing 1.0 μl of reverse transcription mixture, 0.2 μM both primers, and 12.5 μl of iQ™ SYBR Green Supermix (Bio-Rad). Real time PCR was run in the iCycler™ real time detection system (Bio-Rad) with a two-step method. The hot start enzyme was activated (95 °C for 3 min) and cDNA was then amplified for 40 cycles consisting of denaturation at 95 °C for 10 s and annealing/extension at 53 °C for 45 s. A melt curve assay was then performed (55 °C for 1 min and then the temperature was increased by 0.5 °C every 10 s) to detect the formation of primer-derived trimers and dimers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a control. Data were analyzed with the iCycler iQ™ software. The average starting quantity of fluorescence units was used for analysis. Quantification was calculated using the starting quantity of the cDNA of interest relative to that of GAPDH cDNA in the same sample.

FcγR Blocking study

Human mesangial cells were incubated without or with 50 μg/ml of IgG1, 10 μg/ml of CD32, CD36, or CD16 blocking antibodies for 5 min before being exposed to 50 μg/ml of oxLDL-ICs for 24 h. After the treatment, cellular proteins were extracted and subjected to immunoblotting to detect collagen IV as described above.

Statistic Analysis

Data are presented as mean ± SD. Student *t* tests were performed to determine the statistical significance of cytokine expression among different experimental groups. A value of *P* < 0.05 was considered significant.

RESULTS

OxLDL-ICs Stimulate Collagen IV Expression by Mesangial Cells

The effects of oxLDL-IC, anti-oxLDL antibodies and oxLDL on collagen IV expression in human mesangial cells were compared first. Results showed that the stimulatory effect of

oxLDL-IC on collagen IV expression was significantly stronger than either oxLDL antibodies or oxLDL (Fig. 1). Results further showed that oxLDL-ICs stimulated collagen IV expression in a concentration- (Fig. 2A) and time-dependent (Fig. 2B and 2C) manner. It appeared that the priming with interferon gamma (IFN γ) had no significant effect on oxLDL-IC-stimulated collagen IV expression (Fig. 2A). The time course study showed that the IFN γ priming only increased oxLDL-IC-stimulated collagen IV expression at 8 h, but had no effect at 18 h and 24 h (Fig. 2B and 2C). The stimulatory effect of oxLDL-IC on collagen IV expression reached plateau at 30 μ g/ml (Fig. 2A).

Fc γ RI and Fc γ RIII Are Responsible for OxLDL-IC-stimulated Collagen IV Expression

To investigate the mechanisms involved in oxLDL-IC-stimulated collagen IV expression by mesangial cells, we treated cells with oxLDL-IC in the presence of either monomeric human IgG1, known to block Fc γ RI, or blocking antibodies to Fc γ RII or Fc γ RIII. The specificity of these antibodies has been demonstrated in some of our previous studies [29; 30; 32]. We have also conducted similar experiments in the presence of anti-CD36, a major scavenger receptor for oxLDL [33]. Results showed that blocking with monomeric IgG1, which prevents the binding of oxLDL-IC to Fc γ RI, or with anti-CD16, which inhibits binding to Fc γ RIII, effectively reduced collagen IV expression (Fig. 3). In contrast, CD32 (Fc γ RII) and CD36 blocking antibodies had no effect on oxLDL-IC-stimulated collagen IV expression. These results indicate that Fc γ RI and Fc γ RIII, but not Fc γ RII and CD36, are responsible for the stimulation of collagen IV expression by oxLDL-IC.

Previous studies by Wilson et al. have shown that priming of macrophages with IFN γ increased Fc γ RI expression [34]. We thus determined the effect of IFN γ priming on the surface expression of Fc γ Rs by mesangial cells. Our results showed that exposure of mesangial cells to IFN γ increased Fc γ RI (CD64) and Fc γ RIII (CD16) expression in a time-dependent manner and a plateau was reached at 48 h (Figs. 4A and 4B). In contrast, IFN γ had no effect on Fc γ RII (CD32) and CD36 expression (Figs. 4A and 4B).

OxLDL-IC Upregulate Fc γ RI Expression

To explain why IFN γ priming increased Fc γ RI and Fc γ RIII expression, but failed to enhance the stimulation of collagen IV expression by oxLDL-IC (Fig. 3), we hypothesized that oxLDL-IC, like IFN γ , may stimulate Fc γ RI or/and Fc γ RIII expression and the addition of both stimuli does not lead to further expression of receptors. To test this hypothesis, we determined the effect of oxLDL-ICs on Fc γ RI, Fc γ RII, and Fc γ RIII expression. Results showed that oxLDL-IC stimulated Fc γ RI expression in a time-dependent manner and a 7.5-fold maximal increase for Fc γ RI mRNA expression was achieved after exposure to oxLDL-ICs for 6 h (Fig. 5). In contrast, oxLDL-IC had no effect on Fc γ RII and Fc γ RIII expression (data not shown).

Involvement of p38 MAPK, JNK and PKC pathways in oxLDL-IC-stimulated collagen IV expression

To determine which signaling pathways are involved in oxLDL-IC-stimulated collagen IV expression, we treated mesangial cells with oxLDL-IC in the presence of different inhibitors that block specific signaling pathways. Results showed that SP600125, an inhibitor of JNK pathway, SB203580, an inhibitor of p38 MAPK pathway, and calphostin C and staurosporine, inhibitors of PKC pathways, inhibited the stimulation of collagen IV expression by oxLDL-ICs significantly (Figs. 6A and 6B). In contrast, AG490, an inhibitor of JAK/STAT pathway, and PD98059 and U-126, inhibitors of ERK pathway, did not have significant effect (Figs. 6A and 6B). Furthermore, we studied the effect of oxLDL-ICs on the activation of different signaling pathways in mesangial cells. Results showed that oxLDL-IC stimulated ERK, JNK, and p38 MAPK at 5 min in a concentration-dependent

manner (Fig. 6C). In addition, oxLDL-IC also stimulated PKC β 2 signaling (Fig. 6C), but not PKC α and PKC β 1 (data not shown). Interestingly, although the ERK phosphorylation was stimulated by oxLDL-ICs (Fig. 6C), it was not involved in oxLDL-IC-stimulated collagen IV expression, revealing the specificity of the JNK, p38 MAPK and PKC pathways involved in collagen IV expression by mesangial cells in response to oxLDL-IC.

DISCUSSION

Previous studies by our group have shown that plasma concentrations of oxLDL-IC were significantly increased in diabetic patients with macroalbuminuria as compared to those with normal albuminuria [35; 36], indicating a positive correlation between oxLDL-ICs and diabetic nephropathy. Diabetic nephropathy is characterized by mesangial expansion with a strong inflammatory component. Previous studies conducted by our group have clearly shown that oxLDL-IC have proinflammatory properties, as reflected by their capacity to activate the complement system and to induce the release of proinflammatory cytokines such as IL-1 β , IL-6, and IL-18, as well as TNF α from MonoMac 6 cells and primary human macrophages [30]. Thus, oxLDL-IC may contribute to the release of inflammatory cytokines known to be involved in the development and progression of diabetic nephropathy [37].

Interestingly, our present study has shown that, in addition to the proinflammatory effects on monocytes and macrophages that contribute to diabetic nephropathy, oxLDL-IC are able to stimulate mesangial expansion by promoting the release by mesangial cells of increased amounts of collagen IV, a major matrix protein involved in diabetic nephropathy [38]. It appears that oxLDL-IC play a pathological role in diabetic nephropathy by not only stimulating inflammation via monocytes and macrophages, but also increasing matrix production from mesenchymal mesangial cells.

Our current study also showed that not only oxLDL-IC but also oxLDL stimulated collagen IV production by mesangial cells, which is in agreement with a previous report by Lee et al. [20]. However, our study clearly showed that the stimulatory effect of oxLDL-IC on collagen IV production was more potent than that of oxLDL. Clearly, after forming complexes with anti-oxLDL antibodies, the capacity of oxLDL in stimulating collagen IV production from mesangial cells is markedly enhanced.

To explore the mechanisms by which oxLDL-IC exert such a remarkable stimulation on collagen IV production by mesangial cells, we first determined the involvement of Fc γ Rs in oxLDL-IC-stimulated collagen IV production from mesangial cells. Our previous studies have shown that Fc γ RI is the major isotype of Fc γ Rs engaged by oxLDL-IC in macrophages and the ERK signaling pathway was responsible for upregulating gene expression [30]. Interestingly, the present study showed that both Fc γ RI and Fc γ RIII were engaged by oxLDL-IC in mesangial cells (Fig. 3). Thus, it is likely that oxLDL-IC, by binding to both Fc γ RI and Fc γ RIII, trigger activation of multiple signaling pathways, leading to a marked upregulation of collagen IV production. This hypothesis is supported by our signal transduction studies showing that not only the p38 MAPK and JNK, but also PKC pathways were involved in oxLDL-IC-stimulated collagen IV production (Fig. 6).

Another important finding from this study that also explains why oxLDL-ICs were so potent in stimulation of collagen IV production by mesangial cells was that oxLDL-IC stimulated Fc γ RI expression (Fig. 5). This is a positive feedback mechanism involved in the Fc γ RI expression. Time course study showed that the time to reach the peak (7.5-fold) of stimulation of Fc γ RI expression by oxLDL-ICs was 6 h. This relatively rapid upregulation of Fc γ RI in response to oxLDL-ICs facilitates more interaction between mesangial cells and

oxLDL-IC after the initial engagement and thus leads to a marked stimulation of collagen IV production.

Our study showed that both FcγR I and III are required for oxLDL-IC-stimulated collagen IV expression since blocking either of the receptors will preclude the stimulation of collagen IV production (Fig. 3). However, it is possible that FcγR I engagement is the trigger for collagen production. Alternatively, collagen production may plateau at certain level of cell stimulation. Since the degree of stimulation of FcγR I by oxLDL-ICs is so markedly high, it is possible that engaging FcγR I by oxLDL-ICs can induce the maximal collagen production and therefore the effect of IFN-γ cannot be additive.

It is known that oxLDL binds to CD36 [33]. Interestingly, our study showed that anti-CD36 blocking antibodies did not inhibit the stimulation of collagen IV production by oxLDL-ICs (Fig. 4). There are two possible mechanisms that may explain this observation. One possible explanation is that steric hindrance of critical regions of the oxLDL bound to anti-oxLDL antibodies to form oxLDL-IC although not preventing the binding of oxLDL to CD36 would prevent the interaction of oxLDL with the areas of CD36 responsible for cell signaling. The second explanation is that oxLDL-ICs bind predominantly to FcγRI and III and because of their size they are very likely to simultaneously bind and cross-link multiple FcγRs of those two types [39]. The net result would be high avidity binding to Fc receptors successfully competing with CD36, and blocking CD36 would be inconsequential.

Studies have shown that the ERK signaling pathway is responsible for CD36-mediated gene expression [40] while the PKC pathways are the major signaling cascades for FcγRI-mediated gene expression [41; 42]. Our current signaling studies seems to confirm that binding to FcγRI not CD36 is involved in the production of collagen since PKC, but not the ERK, pathways were involved in FcγRI and FcγRIII-mediated collagen IV production. Given the difference of receptors and receptor-linked signaling pathways activated by oxLDL and oxLDL-ICs, it is not surprising to find that the potency in regulation of collagen IV production by oxLDL and oxLDL-ICs is different.

In conclusion, the present study showed for the first time that oxLDL-ICs stimulated collagen IV expression in mesangial cells through FcγRI and FcγRIII and p38 MAPK, JNK and PKC signaling pathways. Our present work also suggests that increased production of collagen by mesangial cells is not a specific property of oxLDL-IC but it may be a general characteristic of ICs. Further and more detailed studies will be needed to address this issue. The study also showed that oxLDL-ICs were capable of upregulating FcγRI expression, revealing a positive feedback mechanism that may be involved in the oxLDL-IC-stimulated collagen IV production. All these data suggest that oxLDL-ICs may play an important role in diabetic nephropathy.

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Figure 1.

The effect of oxLDL-ICs, anti-oxLDL antibodies and oxLDL on collagen IV production by mesangial cells. Human mesangial cells were treated with 50 $\mu\text{g/ml}$ of oxLDL-ICs, 30 $\mu\text{g/ml}$ of anti-oxLDL antibody or 20 $\mu\text{g/ml}$ of oxLDL for 24 h. After the treatment, cellular proteins were extracted and subjected to immunoblotting to detect collagen IV and β -actin. The blot is representative of at least 3 separate experiments showing similar results.

Figure 2.

Concentration- and time-dependent stimulation of collagen IV production by oxLDL-ICs. Human mesangial cells were treated with different concentrations of oxLDL-ICs (A) for 24 h or with 50 μ g/ml of oxLDL-ICs for different time periods (B). After treatment, cellular proteins were extracted and subjected to immunoblotting to detect collagen IV. The image from the time course study was analyzed using densitometry after normalization of collagen IV to β -actin (C). The blot is representative of at least 3 separate experiments showing the similar results.

Figure 3.

The effects of monomeric IgG1 and blocking antibodies to Fc γ RII (CD32), III (CD16) or CD36 on collagen IV production. Human mesangial cells were treated with 50 μ g/ml of oxLDL-ICs in the absence or presence of 50 μ g/ml of IgG1, 10 μ g/ml of CD32, CD36, or CD16 blocking antibodies for 24 h. After the treatment, cellular proteins were extracted and subjected to immunoblotting to detect collagen IV. The blot is representative of at least 3 separate experiments showing similar results.

Figure 4.

Time course of FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16) and CD36 surface expression in mesangial cells treated with IFN γ . Human mesangial cells were treated with 100 ng/ml of IFN γ for different time periods (2–72 h) and the surface expression of FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16) and CD36 was determined at each time point using flow cytometry as described in Methods. A. Plots from flow cytometry analysis for FcγRI, FcγRII and FcγRIII surface expression recorded before and after IFN γ treatments. B. Quantification of the data from flow cytometry analysis. The data presented is representative of at least 3 separate experiments showing similar results.

Figure 5. Stimulation of FcγRI gene expression in mesangial cells by oxLDL-ICs. Human mesangial cells were treated with 50 μg/ml of oxLDL-ICs for different time periods (1–12 h). After treatment, FcγRI mRNA was quantified using real-time PCR and normalized to GAPDH mRNA. The data presented is representative of at least 3 separate experiments showing similar results.

Figure 6.

The effect of signaling pathway inhibitors on oxLDL-IC-stimulated collagen IV production by mesangial cells. A and B: Human mesangial cells were treated with 50 $\mu\text{g/ml}$ of oxLDL-ICs in absence or presence of different concentrations of SB203580 (SB), SP600125 (SP), calphostin C (Calph), staurosporine (Staur), AG490 (AG), PD98059 (PD) or U0126 for 24h. After the treatment, cellular proteins were extracted and subjected to immunoblotting to detect collagen IV (A), and collagen IV was quantified using densitometric scanning (B). C: Mesangial cells were treated with different concentrations of oxLDL-ICs for 5 min and cellular protein was extracted after the treatment. The phosphorylated ERK, JNK, p38 MAPK and PKC β 2 were detected using specific anti-ERK, JNK, p38 MAPK and PKC β 2

antibodies. For control, GAPDH was detected using anti-GAPDH antibody. The blot was representative of at least 3 separate experiments showing similar results.