Increased CD74 expression in human atherosclerotic plaques: contribution to inflammatory responses in vascular cells

José Luis Martín-Ventura1*, Julio Madrigal-Matute1, Begoña Muñoz-García1, Luis Miguel Blanco-Colio1, Melany Van Oostrom1, Guillermo Zalba2, Ana Fortuño2, Carmen Gomez-Guerrero1, Luis Ortega3, Alberto Ortiz1, Javier Diez2,4, and Jesús Egido1

1Renal and Vascular Research Lab, Fundación Jimenez Diaz, Autonoma University, Madrid, Spain; 2Division of Cardiovascular Sciences, Center for Applied Medical Research University of Navarra, Pamplona, Spain; 3Anatomy Pathology Services, Hospital Clínico de San Carlos, Universidad Complutense, Madrid, Spain; and 4Department of Cardiovascular Surgery, University Clinic, University of Navarra, Pamplona, Spain

Received 2 December 2008; revised 13 April 2009; accepted 27 April 2009; online publish-ahead-of-print 7 May 2009

Time for primary review: 13 days

1. Introduction

CD74 (invariant polypeptide of major histocompatibility complex, MHC, HLA-DR gamma) is a non-polymorphic type II integral membrane protein which was thought to function mainly as an MHC class II chaperone, although 2–5% is expressed independent of MHC class II at the cell membrane.1 In this respect, CD74 has been recently shown to have a role as an accessory-signalling molecule. Stimulation of surface CD74 induces a signalling cascade resulting in AKT and nuclear factor-kB (NF-kB) activation, as well as cell proliferation.2 Interestingly, NF-kB can be activated by the intracellular domain of CD74, which is liberated from the membrane.3 This process of intramembrane cleavage followed by nuclear translocation and transcriptional activation is known as regulated-intramembrane proteolysis (RIP).4 The initial step of ectodomain shedding is carried out by one or more metalloproteases (ADAM or MMPs), yielding a fragment which is substrate for another intramembrane proteases (γ-secretase/presenilins or SPP-type aspartic proteases). In addition, CD74 was found to be a putative receptor for macrophage inhibitor factor (MIF), implicating CD74 in MIF-induced activation of the MAP kinase cascade, cell proliferation, and prostaglandin E2 production in macrophages.5 More recently, chemokine receptors (CXCR2 and CXCR4) have been identified as new

* Corresponding author.
E-mail address: jlmartin@fjd.es

Keywords
Atherosclerosis; Inflammation; Biomarkers; Carotid stenosis

Aims The purpose of this study was to analyse the expression of CD74 in human atherosclerotic plaques and peripheral blood mononuclear cells (PBMC) as well as its potential participation in proinflammatory responses in cultured human vascular smooth muscle cells (VSMC).

Methods and results CD74 expression was analysed in human atherosclerotic plaques (immunohistochemistry), PBMC (real-time PCR), and human aortic VSMC (real-time PCR and western blotting). Nuclear factor-κB (NF-κB) activation was assessed by southwestern histochemistry and electrophoretic mobility shift assay. Monocyte chemoattractant protein-1 (MCP-1) levels were studied by both real-time PCR and enzyme-linked immunosorbent assay. CD74 immunostaining was increased in the inflammatory vs. the fibrous region of atherosclerotic plaques (n = 70, 18.2 ± 1.3 vs. 7.8 ± 0.6% positive staining/mm², P < 0.001). CD74 colocalized with the transcription factor NF-κB in both VSMC and macrophages. In cultured VSMC, CD74 expression was induced by interferon γ (IFNγ). Incubation with an agonistic anti-CD74 antibody or with IFNγ elicited MCP-1 expression, which was prevented by AKT and γ-secretase inhibitors. Moreover, CD74 small-interfering RNA decreased NF-κB activation and MCP-1 production induced by IFNγ in VSMC. Finally, CD74 mRNA levels in PBMC from patients with carotid stenosis were higher than in healthy subjects (n = 20, 3 ± 0.5 vs. 2 ± 0.5 AU, P < 0.001). Additionally, a linear trend between CD74 mRNA expression tertiles and intima-media thickness (IMT) was observed in PBMC from asymptomatic subjects (n = 185, P < 0.001).

Conclusion CD74 levels are increased in plaques and PBMC from patients with carotid stenosis and are associated with IMT in subjects free from clinical cardiovascular diseases. CD74 could be a novel therapeutic target to decrease the inflammatory response in atherosclerosis.
functional receptors for MIF. In this regard, inflammatory cell recruitment induced by MIF relied on both CXCR2 and CD74, although independent effects of CD74 were also suggested. Furthermore, Helicobacter pylori was shown to interact with CD74, stimulating the production of the chemokine interleukin-8. All these data support a potential role of CD74 in inflammatory diseases.

The early events of atherosclerosis include the recruitment of lipoproteins and inflammatory cells to the vessel wall and the proliferation of vascular cells. In these processes, there are many important mediators, such as interferon gamma (IFN-γ) and monocyte chemoattractant protein-1 (MCP-1). Among other proatherogenic properties, IFN-γ is able to induce vascular smooth muscle cell (VSMC) activation, and MCP-1 plays a crucial role in monocyte recruitment to the vascular wall. In this respect, the vulnerable region of human atherosclerotic plaques is characterized by an increase in macrophage infiltration, NF-κB activation, and MCP-1 expression. Since CD74 has been previously related with monocyte infiltration in experimental atherosclerosis and increased CD74 mRNA levels in circulating blood cells have been recently observed in different pathologies where inflammation plays an important role, we firstly performed an observational study to address the presence and localization of CD74 in human atherosclerotic plaques. To test the hypothesis that CD74 could be directly linked to monocyte recruitment in atherothrombosis, further studies in human vascular cells were performed addressing the involvement of CD74 in MCP-1 induction, analysing the potential mechanisms implicated. Finally, on the basis of the importance of inflammation in atherogenesis, recent work has focused on whether circulating blood cells have been recently observed in different pathologies where inflammation plays an important role. Since CD74 has been previously related with monocyte infiltration in experimental atherosclerosis and increased CD74 mRNA levels in circulating blood cells have been recently observed in different pathologies where inflammation plays an important role,

2. Methods

2.1 Patients

The local committees on human research approved the studies, which were performed in accordance with the Declaration of Helsinki, and all participants gave written informed consent.

Seventy consecutive patients undergoing carotid endarterectomy in Fundacion Jimenez Diaz were included. From the 70 atherosclerotic plaques (Stary stages V–VI) analysed, 27 of 70 were haemorrhagic and 34 of 70 has a lipid rich core. These plaques were fixed with paraformaldehyde and embedded in paraffin. Blood samples were also collected from 20 patients before anaesthesia on the day of endarterectomy and from 20 healthy volunteers not significantly different for sex and age. Peripheral blood mononuclear cells (PBMC) were isolated as described previously.

In addition, PBMC from a group of 185 asymptomatic subjects in whom global risk assessment was performed in the course of a general health check-up by Internal Medicine Department (University Clinic of Navarra, Spain) were also studied. In all subjects, absence of history of coronary disease, stroke, or peripheral arterial disease was recorded; additional exclusion criteria were the presence of severely impaired renal function, arthritis, connective tissue diseases, alcohol abuse, or use of non-steroidal, anti-inflammatory drugs in the 2 weeks before entering the study. The following conventional cardiovascular risk factors were defined as previously described: arterial hypertension and/or use of anti-hypertensive drugs; dyslipidaemia and/or use of cholesterol lowering drugs, obesity, smoking, and diabetes and/or use of pharmacological treatment. In all subjects, carotid ultrasonography was performed to determine IMT, as previously described. Subjects were examined by the same two sonographers blinded to all clinical information. The reproducibility of IMT measurements between and within sonographers had previously been checked in 20 individuals who returned 2 weeks later for a second examination. The between-observer intraclass correlation coefficient was 0.76 (P < 0.001) and the between subject repeatability was 0.82 (P < 0.001). The corresponding coefficients of variance were 5% and 10%, respectively.

2.2 RNA extraction and real-time quantitative-polymerase chain reaction

Total RNA was isolated from PBMCs, THP-1, and VSMC using TRIzol Reagent (Invitrogen); 1 μg of RNA was used to perform the reverse transcribed with High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR reactions were performed on an ABI Prism 7500 sequence detection PCR system (Applied Biosystems) according to manufacturer’s protocol using the Delta-Delta Ct method. Expression levels are given as ratios to 18S. Pre-developed primer and probe assays were obtained for human 18S and CD74 from Applied Biosystems.

2.3 Immunohistochemistry

Paraffin-embedded carotid arteries were cross sectioned into 4 μm thick pieces, dewaxed, and rehydrated. Mouse monoclonal anti-human macrophage (HAM-56, DAKO), monoclonal anti α-smooth muscle actin (HHF-35, Sigma), and rabbit anti-human CD74 (FL-296, SC-20082, Santa Cruz Biotechnology) antibodies were applied. Secondary antibodies and ABComplex/HRP were added and sections were stained with 3,3′-diaminobenzidine and mounted in Pertex. For colocalization studies with VSMC and macrophages, immunohistochemistry for CD74, HAM-56, and α-actin was carried out in serial sections. For colocalization of nuclear NF-κB and CD74, immunohistochemistry for CD74 was carried out directly from the final wash of Southwestern Histochromy as described. Sirius red and Masson trichrome were performed following manufacturer’s instructions. Morphometric analysis with the Olympus semiautomatic image analysis system with Micro Image™ software (version 1.0 for windows) was performed by a pathologist (L.O.) as described. Results are expressed as percentage of positive staining per mm².

2.4 Reagents

Recombinant human IFN-γ and tumour necrosis-alfa (TNF-α) were purchased from PeproTech. Recombinant MIF was provided by R&D systems. Anti-CD74 antibody (specific for the extracellular domain of CD74, C-16, SC-5438) was from Santa Cruz Biotechnology. LDLS were obtained as...
2.5 Cell culture

Human aortic VSMCs (ATCC; CRL-1999) were maintained in Ham’s F-12 medium supplemented with 10% FBS, penicillin-streptomycin, insulin-transferrin-sodium selenite, and 30 μg/mL endothelial cell growth supplement at 37°C in 5% CO₂. Cells were used between passages 3 and 7 as described.18 Human aortic VSMCs (ATCC; CRL-1999) were maintained in 5% CO₂. Cells were used between passages 3 and 7 as described.18 DAPT and LY294002 were from Sigma. Z-Phe-Leu-COCHO was from Calbiochem.

2.6 Transfection of small-interfering RNA

VSMCs were grown to 50% confluence and transfected with a mixture composed 2.5 mol/L CaCl₂, 25 nmol small-interfering RNA (siRNA) (CD74 siRNA, SC-35023, or control siRNA, SC-37007), and calcium phosphate buffer in serum-free medium (Invitrogen) as previously described.19 After 18 h, cells were washed and serum-depleted for 24 h before stimulation.

2.7 Electrophoretic mobility shift assay

Electrophoretic mobility shift assay for NF-κB binding activity was performed with nuclear protein extracts from VSMC as described.10 The specificity of the assay was tested with a 100-fold excess of unlabelled NF-κB consensus oligonucleotide added to the 32P-labelled probe-binding reaction.

2.8 Western blot

Equal amount of total protein was separated on 12.5% sodium dodecyl sulphate–poly-acrylamide gel electrophoresis (SDS–PAGE). Subsequently, membranes were blocked and incubated with rabbit polyclonal anti-CD74 (SC-20082, Santa Cruz Biotechnology) or mouse monoclonal anti-α-tubulin (Sigma-Aldrich). Proteins were visualized by ECL Western Blotting Detection Reagents (Amersham Biosciences) according to manufacturer’s instructions.

2.9 Enzyme-linked immunosorbent assay

Soluble MCP-1 levels were measured in the supernatants of VSMC after different experimental conditions with a commercially available ELISA kit (R&D systems). Hundred micro-litre of supernatant samples were assayed in parallel to known MCP-1 recombinant concentrations.

2.10 Statistical analysis

Statistics were performed using GraphPAD InStat (GraphPAD Software). In vitro experiments were performed at least three times. Results are expressed as mean ± SEM and were analysed by the Mann–Whitney non-parametric or Student’s t-tests when appropriate (two-tailed, significant differences at P < 0.05). CD74 in PBMC of asymptomatic subjects is expressed as medians and interquartile ranges and was analysed by the Mann–Whitney U test. Univariate association was performed by Pearson correlation test. Multivariate linear regression analysis was conducted with carotid IMT as dependent variable, including in the model the traditional risk factors and those variables that were significant in the univariate analysis.

3. Results

3.1 CD74 is expressed in human atherosclerotic plaques

We firstly assessed the presence of CD74 in human carotid atherosclerotic plaques, analysing its localization in the different regions of the plaques: the shoulder area, characterized by a high macrophage accumulation, and the fibrous region, characterized by increased smooth muscle cell and collagen content (Figure 1A). Quantification of CD74 immunostaining in 70 human plaques showed an increased CD74 expression in the shoulder region in relation to the fibrous area (18.2 ± 1.3 vs. 7.8 ± 0.6% positive staining/mm², P < 0.001, Figure 1A). To determine the cell types contributing to CD74 expression in atherosclerotic plaques, serial sections were stained for CD74, VSMC, and macrophages. We observed that CD74 was expressed by both types of cells (Figure 1B). Moreover, colocalization of nuclear NF-κB and CD74 expression was noted, suggesting the potential association between these proteins in vivo.

3.2 CD74 is upregulated in vascular cells in response to inflammatory stimuli

We investigated the expression of CD74 in cultured vascular cells in response to several factors known to participate in atherogenesis. Western blot analysis demonstrated a time- and dose-dependent increase of CD74 protein in human VSMC (Figure 2A) and monocytes (Figure 2B) in response to IFN-γ. In contrast, neither oxLDL nor TNF-α had any effect on CD74 protein levels. In addition, real-time PCR analysis showed a similar upregulation of CD74 mRNA in VSMC (Figure 2C) and monocytes (data not shown) in response to IFN-γ.

3.3 CD74 induces MCP-1 expression in VSMC

Since CD74 has been previously related to monocyte recruitment,6 we studied the involvement of CD74 on the chemokine MCP-1. Incubation of VSMC with an anti-CD74 antibody (which binds the CD74 extracellular domain) or IFN-γ induced MCP-1 mRNA expression. In contrast, no effect was observed in the presence of the CD74 ligand, MIF, or an isotype control antibody (Figure 3A). CD74 signalling involves the activation of AKT and NF-κB pathways in B cells.7 In our studies, preincubation with the PI3/AKT-inhibitor LY294002 prevented the MCP-1 induction by anti-CD74 or IFN-γ (Figure 3B and C). In addition, since intramembrane processing of CD74 is dependent on γ-secretase,3 we used the γ-secretase/presenilin1 inhibitor DAPT in our experimental conditions. Preincubation of VSMC with DAPT abolished MCP-1 overexpression induced by anti-CD74 (Figure 3B). DAPT preincubation was only able to partially reverse the induction of MCP-1 by IFN-γ (Figure 3C). Finally, since Maubach et al.20 reported that IFN-γ increases cathepsin S activity, we preincubated the cells with the synthetic cathepsin S inhibitor Z-Phe-Leu-COCHO,21 which was also able to partially reverse the MCP-1 induction by IFN-γ (Figure 3C).

3.4 CD74 siRNA decreases IFN-γ-induced MCP-1 levels in VSMC

To further elucidate whether CD74 could directly participate in proinflammatory actions in vascular cells, we evaluated...
the effect of CD74 silencing in VSMC. For that purpose, cells were transfected with a specific CD74 siRNA before IFNγ stimulation. As shown in Figure 4A, CD74 siRNA blocked CD74 overexpression induced by IFNγ. No effect was observed when a control siRNA was used (data not shown). Accordingly, a significant decrease in IFNγ-induced NF-κB activation and MCP-1 secretion was observed in siRNA transfected VSMC compared with non-transfected cells (Figure 4B and C). In contrast, no effect of MIF incubation on secreted MCP-1 levels was observed, similar to the results obtained at the mRNA level. Our data suggest that CD74 may participate in monocyte recruitment through the modulation of MCP-1 secretion.

3.5 CD74 expression in PBMC of human subjects

Finally, we aimed to evaluate the potential role of CD74 as a biomarker in atherosclerosis. We analysed CD74 expression in PBMCs in a training group of 20 patients with carotid atherosclerosis and 20 sex- and age-matched healthy controls. CD74 mRNA levels in PBMCs from carotid atherosclerosis patients were higher than in those of control subjects (3 ± 0.5 vs. 2 ± 0.5 AU, P < 0.001, Figure 5A). In addition, we analysed CD74 expression in a test population of 185 asymptomatic subjects in which IMT has been measured. Characteristics of the studied population are summarized.
Table 1. When we analysed tertiles of CD74 mRNA, there was a linear trend between the levels of CD74 mRNA and the increase in carotid IMT (Figure 5B). In addition, a univariate analysis showed a positive correlation between CD74 mRNA and IMT ($r = 0.37; P < 0.001$). No correlations between CD74 expression and other clinical parameters were observed (Table 2).

Interestingly, CD74 mRNA positively correlated with MCP-1 mRNA ($r = 0.187; P = 0.015$). The association between CD74 and carotid IMT remained significant after adjusting for traditional risk factors (Table 3).

4. Discussion

It is well established that the breakdown of atherosclerotic plaques occurs more frequently at points where the fibrous cap is thinner and where there is a great amount of inflammatory cells, such as macrophages and T lymphocytes. Studies on coronary arteries of patients suffering myocardial infarction demonstrated that the rupture of atheroma usually takes place in the shoulder region, an area characterized by a high-inflammatory content, NF-kB activation, and MCP-1 expression. In this study, we observed that CD74 immunostaining is augmented in the shoulder region of human atherosclerotic plaques, and it was expressed by both macrophages and VSMC. CD74 is expressed not only by immune cells but also by fibroblasts and podocytes. We showed colocalization of CD74 with NF-kB, suggesting that the association between both proteins could also takes place within atherosclerotic plaques. Among the different mediators involved in atherogenesis, we have observed that stimulation of vascular cells with IFNγ, but not with TNF-α or ox-LDL, dose- and time-dependently induced the expression of CD74 (mRNA and protein). This is in agreement with previous papers describing CD74 upregulation by IFNγ in other cell types.

The role of CD74 had been initially described as a chaperone that facilitates transport of MHC class II proteins from the endoplasmic reticulum to the Golgi complex. However, it is known that a portion of CD74 protein is expressed on the cell surface independently of class II molecules. CD74 was described as a putative receptor for the inflammatory cytokine MIF. Similarly, chemokine receptors (CXCR2 and CXCR4) have been recently identified as new functional receptors for MIF. In this regard, inflammatory cell recruitment induced by MIF relied on CXCR2 and CD74. However,
blocking CD74 in MIF<sup>−/−</sup> mice prevented inflammatory cell recruitment, suggesting that CD74 could participate, independently of MIF, in atherogenic cell recruitment. In this respect, the role of cell-surface CD74 and the identity of its natural ligand in VSMC and other cells are still unknown. Stimulation of CD74 with an anti-CD74 antibody, which binds the CD74 extracellular domain, results in the activation of AKT and NF-κB pathways in B cells. We have observed that both anti-CD74 and IFNγ, but not MIF, induced the expression of the NF-κB-regulated gene MCP-1 in VSMC, and this effect was prevented by an AKT-inhibitor. NF-κB is activated by the intracellular domain of CD74, which is released from the membrane after γ-secretase cleavage. We have shown that the γ-secretase/presenilin1 inhibitor DAPT blocks anti-CD74-induced MCP-1 expression in VSMC. In addition, both γ-secretase and cathepsin S inhibitors partially reduced MCP-1 induction by IFNγ. In this regard, CD74 silencing significantly decreased NF-κB activation and MCP-1 secretion induced by IFNγ. In contrast, no effect of MIF incubation on secreted MCP-1 levels was observed. Our results suggest a novel mechanism by which IFNγ induces MCP-1 expression and secretion implicating CD74 (Figure 6). However, since CD74 silencing was not able to completely block this effect, we cannot discard that other mechanisms of MCP-1 induction by IFNγ could also take place. In this regard, cellular responses to IFNγ such as MCP-1 expression are mainly mediated by JAK–STAT pathway. Whether CD74 signalling is linked to STAT activation, as previously shown for AKT, is unknown at present and will require further investigations.

There are several potential explanations for these results. First, IFNγ may directly induce CD74 intramembrane cleavage and release to the nucleus, as recently shown for IL-8, or indirectly via Cathepsin S upregulation or by other yet unknown mechanisms. Secondly, CD74 may recruit other proteins to its proximity to initiate signalling cascades. In this respect, CD74 can interact with CD44, angiotensin AT1-receptor or nitric-oxide synthase. Ectodomain shedding is regulated by PKC activity and signalling events initiated by IFNγ involve activation of PKC and calcium–calmodulin protein kinases. We have observed that blocking PKC with staurosporin also decreases IFNγ-induced MCP-1 expression in our experimental conditions (unpublished results). Thirdly, CD74 processing could be ligand dependent or constitutive, as shown for other RIP proteins.
Being able to predict who is at risk of an acute thrombotic event is at present one of the major challenges of cardiovascular medicine. Increases in the thickness of the intima and media of the carotid artery, as measured non-invasively by ultrasonography, are directly associated with an increased risk of myocardial infarction and stroke. While non-invasive imaging techniques are being investigated to improve characterization of the size and morphology of the atherosclerotic plaques, other field of growing interest is the search of blood diagnostic/prognostic biomarkers. In this regard, several inflammatory proteins involved in the pathophysiology of atherothrombosis have been evaluated. Among them, NF-κB activity is elevated in circulating cells from patients with unstable angina, patients with carotid stenosis and during acute coronary syndromes. These studies suggest that circulating monocytes are activated before entering the vascular wall. In a recent study, the changes in the gene expression profile of primary monocytes after constitutive adhesion to endothelial cells have shown an upregulation of CD74, suggesting that CD74 overexpression could be an early biomarker of the initiation of the inflammatory process.

### Table 1 Baseline clinical characteristics of the studied population

<table>
<thead>
<tr>
<th>Total population (n = 185)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, year</strong></td>
</tr>
<tr>
<td><strong>Gender, male/female</strong></td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
</tr>
<tr>
<td><strong>SBP, mmHg</strong></td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
</tr>
<tr>
<td><strong>Arterial hypertension, yes/no</strong></td>
</tr>
<tr>
<td><strong>Diabetes mellitus, yes/no</strong></td>
</tr>
<tr>
<td><strong>Obese, yes/no</strong></td>
</tr>
<tr>
<td><strong>Glucose, mg/dL</strong></td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
</tr>
<tr>
<td><strong>HDL cholesterol</strong></td>
</tr>
<tr>
<td><strong>LDL cholesterol</strong></td>
</tr>
<tr>
<td><strong>Triglycerides, mg/dL</strong></td>
</tr>
<tr>
<td><strong>C-reactive protein, mg/dL</strong></td>
</tr>
<tr>
<td><strong>Fibrinogen, mg/dL</strong></td>
</tr>
<tr>
<td><strong>vWF, %</strong></td>
</tr>
<tr>
<td><strong>eGFR, mL/min/1.73 m²</strong></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM and number of subjects. BMI, body mass index; DBP, diastolic blood pressure; IMT, intima-media thickness; SBP, systolic blood pressure; vWF, von Willebrand factor; eGFR, estimated glomerular filtration rate; MCP-1, monocyte chemoattractant protein-1.

### Table 2 Correlation coefficients of left carotid intima-media thickness and CD74 with clinical and laboratory parameters in the studies population

<table>
<thead>
<tr>
<th>CD74</th>
<th>IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, year</strong></td>
<td>0.047 0.052 0.336 &lt;0.001</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>0.012 0.869 0.076 0.305</td>
</tr>
<tr>
<td><strong>SBP, mmHg</strong></td>
<td>0.053 0.475 0.162 0.028</td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
<td>0.028 0.704 0.130 0.077</td>
</tr>
<tr>
<td><strong>Glucose, mg/dL</strong></td>
<td>0.086 0.246 0.060 0.589</td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
<td>0.056 0.445 0.040 0.589</td>
</tr>
<tr>
<td><strong>HDL cholesterol</strong></td>
<td>0.062 0.404 0.012 0.873</td>
</tr>
<tr>
<td><strong>LDL cholesterol</strong></td>
<td>0.048 0.520 0.025 0.735</td>
</tr>
<tr>
<td><strong>Triglycerides, mg/dL</strong></td>
<td>-0.028 -0.702 0.034 0.647</td>
</tr>
<tr>
<td><strong>C-reactive protein, mg/dL</strong></td>
<td>-0.098 -0.196 -0.099 -0.194</td>
</tr>
<tr>
<td><strong>Fibrinogen, mg/dL</strong></td>
<td>-0.136 0.067 -0.054 0.480</td>
</tr>
<tr>
<td><strong>vWF, %</strong></td>
<td>-0.135 0.700 -0.026 0.723</td>
</tr>
<tr>
<td><strong>eGFR, mL/min/1.73 m²</strong></td>
<td>0.830 0.290 -0.120 0.874</td>
</tr>
<tr>
<td><strong>MCP-1, AU</strong></td>
<td>0.187 0.014 0.044 0.574</td>
</tr>
<tr>
<td><strong>CD74, AU</strong></td>
<td>- - - -</td>
</tr>
<tr>
<td><strong>Left carotid IMT, mm</strong></td>
<td>0.374 &lt;0.001 - -</td>
</tr>
</tbody>
</table>

Correlations and P-values from Pearson correlation coefficient.
differential program of monocytes into macrophages. In our pilot study, we have observed that CD74 expression is significantly increased in PBMCs from carotid atherosclerosis patients compared with healthy individuals. Furthermore, we have shown that CD74 expression correlates with IMT in a test population of 185 asymptomatic subjects. Interestingly, correlation of CD74 with IMT remained significant after adjusting for some potentially confounding classical risk factors indicating that CD74 levels could be an independent biomarker of atherosclerosis. Future studies are needed to confirm the potential role of CD74 as a biomarker of sub-clinical atherosclerosis.

On the whole, our data suggest a role for CD74 in the inflammatory cascade during atherogenesis. Its biological functions, its presence in vulnerable regions of human plaques, and its association with surrogate markers such as IMT could implicate CD74 as a potential therapeutic target in atherosclerosis.

Conflict of interest: none declared.

Funding

This work was supported by the Spanish Ministerio de Ciencia y Tecnología (SAF2007/63648), Educación y Ciencia (SAF2007-62533), Fundación Ramon Areces, CAM (SA060/GEN-0247), Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III, Redes RECAVA (RD06/0014/0035; RD06/0014/0008) REDinREN/RD06/0016 and European Network (HEALTH F2-2008-200647), and European Union (InGenious HyperCare, grant LSHM-CT-2006-307093). Programa Intensificación Actividad Investigadora Sistema Nacional de Salud ISCIII/CMI to A.O. This project was also funded through the agreement between the Foundation for Applied Medical Research (FIMA) and ‘UTE project CIMA’.

References

