

## Chemokine Receptor-8: Potential Role in Atherogenesis

Peter C. Harpel MD<sup>1</sup> and Nasreen S. Haque PhD<sup>1</sup>

<sup>1</sup>Division of Hematology, Department of Medicine, Mount Sinai School of Medicine, New York, NY, USA

**Key words:** atherosclerosis, chemokines, lipoproteins

### Abstract

Chemokines and their receptors play regulatory roles in inflammatory reactions. Lipoprotein(a) is an atherogenic lipoprotein, however the mechanisms of its actions are not defined. Our interest in chemokines and their receptors was stimulated by the finding that incubation of Lp(a) with human umbilical vein endothelial cells produced a conditioned medium that was chemotactic for human monocytes. Since infiltration of monocytes into the vessel wall is an early lesion in atherosclerosis, this finding provided a novel mechanism to explain the relationship between Lp(a) and atherosclerosis. The chemoattractant produced by HUVEC was identified as CCL1/I-309, a CC chemokine previously reported to be secreted by stimulated monocytes/macrophages and T lymphocytes. CCR8, the CCL1 receptor, was identified on endothelial cells, and CCL1 was found to be a chemoattractant for these cells. Most recently we demonstrated functional CCR8 on human vascular smooth muscle cells and found that the Lp(a)-HUVEC conditioned medium is a chemoattractant for these cells. CCL1 increased metalloproteinase-2 production by HUVEC, an activity that enables these cells to remodel the vascular matrix. These studies suggest that CCR8 may play an important role in arterial wall pathology.

*IMAJ 2002;4:1025-1027*

Amiram Eldor was a close friend. I shall miss him greatly.

### Lp(a) stimulates HUVEC to secrete a monocyte chemoattractant activity

Our studies have focused on lipoprotein(a) [Lp(a)] and the mechanisms by which this particle mediates atherosclerosis. Elevated blood levels of Lp(a) are an independent risk factor for atherosclerosis. Structurally, Lp(a) consists of a low density lipoprotein particle that is disulfide-linked to apolipoprotein(a), a glycoprotein of variable size that shares a partial homology with plasminogen [1]. We have demonstrated that the apo(a) portion of Lp(a) binds to plasmin-degraded fibrin or fibrinogen [2] and that homocysteine dramatically enhances this binding [3]. The attraction of monocytes to the injured arterial wall is an early event in atherogenesis, but the full spectrum of monocyte chemoattractants has not been established [4]. Oxidized low density lipoprotein was shown to stimulate both vascular smooth muscle cells and human umbilical vein endothelial cells to secrete monocyte chemoattractant protein-1, a CC chemokine chemotactic for monocytes [5]. We

have examined whether unoxidized, native Lp(a) would induce HUVEC to produce a monocyte chemoattractant activity [6]. Unoxidized Lp(a) or apo(a), the lipid-free portion of Lp(a), when incubated with HUVEC or with human coronary artery endothelial cells in tissue culture was found to produce monocyte chemoattractant activity. Granulocyte-monocyte colony stimulating factor antigen was not detected in the Lp(a)-conditioned medium, nor was MCP-1 mRNA induced in HUVEC by Lp(a). Since apo(a) induced chemoattractant activity, we have ruled out the possibility that the LDL portion of Lp(a) was responsible. Endotoxin was also shown not to be involved. Actinomycin D and cycloheximide inhibited the HUVEC response to Lp(a), indicating that protein and RNA synthesis were required.

### CCL1/I-309 is the chemoattractant induced

The identity of the agonist responsible for the monocyte chemoattractant activity induced by the conditioned medium from incubations of Lp(a) or apo(a) with HUVEC was found to be I-309/CCL1, a CC chemokine [7]. CC chemokines are a family of closely related proteins characterized by two adjacent cysteine residues that function as leukocyte activators and chemoattractants in inflammatory reactions [8]. CCL1 is secreted by activated monocytes and lymphocytes [9] and is a potent chemoattractant for both of these cell types [10]. Our finding that Lp(a) induced HUVEC to secrete a monocyte chemoattractant, CCL1, suggests that Lp(a) may stimulate the recruitment of monocytes to the vessel wall. This hypothesis is supported by histochemical analysis of human atherosclerotic plaques that showed that CCL1 was widely expressed. CCL1 antigen was associated with luminal endothelium, with monocytes/macrophages, and was identified in the extracellular matrix where CCL1 co-localized with apo(a) [7].

### CCR8 mediates chemotaxis of endothelial cells

CC chemokines bind to G-protein-coupled receptors that comprise a family of seven transmembrane-spanning domains. These receptors were first described on leukocytes, where they function to induce chemotaxis, proliferation and cell activation [8]. CC chemokine receptor-8 has been characterized and is expressed on monocytes and T lymphocytes [11]. CCR8 is a G-protein-coupled 7-transmembrane receptor that is the unique receptor for the human CC chemokine CCL1 and for viral monocyte inflammatory protein-1, vCCL1, a human chemokine homologue induced by human

Lp(a) = lipoprotein(a)

HUVEC = human umbilical vein endothelial cells

apo(a) = apolipoprotein(a)

MCP-1 = monocyte chemoattractant protein-1

LDL = low density lipoprotein

CCR8 = CC chemokine receptor-8

herpesvirus-8, which has been directly linked to Kaposi sarcoma [12]. We have found CCR8 mRNA in HUVEC [13]. CCR8 was found to be a functional endothelial receptor as it mediated endothelial chemotaxis in response to CCL1 and vCCL1, and this response was inhibited by antibody directed against CCR8 as well as by the Gi-protein inhibitor pertussis toxin. The conditioned medium from apo(a)-stimulated HUVEC induced endothelial chemotaxis that was inhibited by anti-CCR8 antibody. That endothelial cells contained CCR8 mRNA was shown by RNA blot analysis and by direct sequence analysis. Immunohistochemical studies identified CCR8 on the endothelium of human atherosclerotic plaques and in endothelial-derived spindle cells of Kaposi sarcoma. CCR8 was also identified on HUVEC by Bernardini et al. [14] and was shown to induce angiogenesis when stimulated by CCL1. These studies document that CCR8 is an endothelial receptor that may modulate endothelial function.

### CCR8 mediates vascular smooth muscle cell chemotaxis

Our most recent observations indicate that CCR8 is also expressed on human vascular smooth muscle cells (VSMCs) [submitted]. Atherosclerosis involves the migration of VSMCs, accumulation of these cells in the neointima, and extracellular matrix remodeling [4]. We found that CCL1 and vCCL1, unique ligands for CCR8, stimulate chemotaxis of cultured human VSMC. A monoclonal antibody produced against the N-terminal 26 peptides of human CCR8, and shown previously to inhibit the chemotaxis of human monocytes and HUVEC when stimulated by CCL1 [13], also inhibited CCL1-induced chemotaxis of VSMC (submitted). Pertussis toxin, a specific Gi-protein inhibitor, prevented VSMC chemotaxis induced by CCL1, providing further evidence that a CC chemokine receptor was responsible. We have identified CCR8 mRNA in VSMC.

The conditioned medium from HUVEC incubated with Lp(a) was found to induce chemotaxis of VSMC that was inhibited by antibodies against CCR8 and CCL1, indicating that CCR8 was the receptor involved. The induction of VSMC chemotaxis by CCL1-containing conditioned medium from Lp(a)-stimulated endothelial cells suggests a novel pathway whereby Lp(a) may induce VSMC activation and migration.

Secretion of matrix-degrading metalloproteinases by VSMC accompanies the formation of the neointima following vascular injury [15]. The matrix metalloproteinases are zinc-dependent endopeptidases that degrade extracellular matrix proteins. They are secreted primarily as latent pro-enzymes. MMPs include the interstitial collagenases, the stromelysins, the gelatinases, and the membrane-type [16]. Administration of a gelatinase inhibitor to rats following vascular injury caused a 97% reduction in the number of VSMC migrating into the intima, thereby implicating gelatinases in VSMC activation [17]. Gelatinases were identified in human atherosclerotic plaques [18] and are up-regulated following arterial wall injury in animals models [17]. Migration of VSMC has been shown to depend principally on MMP-2 [19].

Our new data also document that CCL1 induces human VSMC to secrete pro-MMP-2 (gelatinase A) but does not stimulate MMP-9 (gelatinase B) production (submitted). Production of MMP-2 by VSMC, as quantified by gelatinolytic zymography, was increased approximately twofold as compared to the constitutive MMP-2 activity. We found that anti-MMP-2 antibody inhibited chemotaxis across a fibronectin-coated membrane, whereas anti-MMP-9 antibody had only a minor inhibitory effect.

Percutaneous transluminal balloon angioplasty induces neointimal formation due to VSMC migration and proliferation. We identified CCR8 and CCL1 antigens in restenotic plaque atherectomy specimens. These antigens co-localize with VSMC, as identified by  $\alpha$ -actin staining in serial sections. These findings strongly suggest that CCR8 and CCL1 participate in the response of VSMC to arterial injury.

CCR8 expression by cells that participate in vessel wall response to injury now include endothelial and vascular smooth muscle cells, monocytes/macrophages, and T lymphocytes. These cells can also be stimulated to produce the CCR8 ligand, CCL1. As we have demonstrated, Lp(a) induces endothelial cells to secrete CCL1, which can modulate both endothelial and VSMC function by interaction with CCR8. These studies suggest that CCR8 and its ligand, CCL1, may play an important role in arterial vessel wall pathology.

**Acknowledgment.** This work was supported in part by National Institutes of Health grant HL-54469.

### References

- McLean JW, Tomlinson JE, Kuang WJ, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* 1987;330:132-7.
- Harpel PC, Gordon BR, Parker TS. Plasmin catalyzes binding of lipoprotein (a) to immobilized fibrinogen and fibrin. *Proc Natl Acad Sci USA* 1989;86:3847-51.
- Harpel PC, Chang VT, Borth W. Homocysteine and other sulfhydryl compounds enhance the binding of lipoprotein(a) to fibrin: a potential biochemical link between thrombosis, atherogenesis and sulfhydryl compound metabolism. *Proc Natl Acad Sci USA* 1992;89:10193-7.
- Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999;340:115-26.
- Cushing SD, Berliner JA, Valente AJ, et al. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc Natl Acad Sci USA* 1990;87:5134-8.
- Poon M, Zhang X, Dunsy KG, et al. Apolipoprotein(a) induces monocyte chemotactic activity in human vascular endothelial cells. *Circulation* 1997;96:2514-19.
- Haque N, Zhang X, French DL, et al. The CC chemokine I-309 is the principle monocyte chemoattractant induced by apolipoprotein(a) in human vascular endothelial cells. *Circulation* 2000;102:786-92.
- Luster AD. Chemokines – chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436-45.
- Miller MD, Wilson SD, Dorf ME, et al. Sequence and chromosomal location of the I-309 gene. Relationship to genes encoding a family of inflammatory cytokines. *J Immunol* 1990;145:2737-44.
- D'Ambrosio D, Iellem A, Bonocchi R, et al. Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. *J Immunol* 1998;161:5111-15.

VSMC = vascular smooth muscle cells  
MMP = metalloproteinase

11. Tiffany HL, Lautens LL, Gao JL, et al. Identification of CCR8: a human monocyte and thymus receptor for the CC chemokine I-309. *J Exp Med* 1997;186:165-70.
12. Dairaghi DJ, Fan RA, McMaster BE, et al. HHV8-encoded vMIP-I selectively engages chemokine receptor CCR8. Agonist and antagonist profiles of viral chemokines. *J Biol Chem* 1999;274:21569-74.
13. Haque NS, Fallon JT, Taubman MB, et al. The chemokine receptor CCR8 mediates human endothelial cell chemotaxis induced by I-309 and Kaposi sarcoma herpesvirus-encoded vMIP-I and by lipoprotein(a)-stimulated endothelial cell conditioned medium. *Blood* 2001;97:39-45.
14. Bernardini G, Spinetti G, Ribatti D, et al. I-309 binds to and activates endothelial cell functions and acts as an angiogenic molecule in vivo. *Blood* 2000;96:4039-45.
15. Jenkins GM, Crow MT, Bilato C, et al. Increased expression of membrane-type matrix metalloproteinase and preferential localization of matrix metalloproteinase-2 to the neointima of balloon-injured rat carotid arteries. *Circulation* 1998;97:82-90.
16. Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J Biol Chem* 1999;274:21491-4.
17. Bendeck MP, Zempo N, Clowes AW, et al. Smooth muscle cell migration and matrix metalloproteinase expression after arterial injury in the rat. *Circ Res* 1994;75:539-45.
18. Brown DL, Hibbs MS, Kearney M, et al. Differential expression of 92-kDa gelatinase in primary atherosclerotic versus restenotic coronary lesions. *Am J Cardiol* 1997;79:878-82.
19. Pauly RR, Passaniti A, Bilato C, et al. Migration of cultured vascular smooth muscle cells through a basement membrane barrier requires type IV collagenase activity and is inhibited by cellular differentiation. *Circ Res* 1994;75:41-54.

**Correspondence:** Dr. P.C. Harpel, Box 1079, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029, USA.  
 Phone: (1-212) 241-0546  
 Fax: (1-212) 996-1029  
 email: peter.harpel@mssm.edu

*What orators lack in depth they make up in length*

*Anonymous*

## Capsule

### **Vaccinating Alzheimer patients generates antibodies specific for beta-amyloid**

To characterize antibodies produced in humans in response to A42 vaccination, Hock et al. carried out immunohistochemical examinations of the brains of both transgenic mice and human patients with beta-amyloid pathology. The researchers collected sera from patients with Alzheimer disease who received a primary injection of pre-aggregated A42 followed by one booster injection in a placebo-controlled study. Antibodies in immune sera recognized beta-amyloid plaques, diffuse A deposits and vascular beta-amyloid in brain blood vessels. The antibodies did not

cross-react with native full-length beta-amyloid precursor protein or its physiologic derivatives, including soluble A42. These findings indicate that vaccination of Alzheimer patients with A42 induces antibodies that have a high degree of selectivity for the pathogenic target structures. Whether vaccination to produce antibodies against beta-amyloid will halt the cognitive decline in Alzheimer disease will depend upon clinical assessments over time.

*Nature Med* 2002;8:1270

## Capsule

### **Anti-rheumatic treatment and serum lipid levels**

Recent studies have shown an association between C-reactive protein levels, lipid levels, and coronary heart disease in patients without apparent inflammatory conditions. Park et al. recently observed that newly diagnosed rheumatoid arthritis (RA) patients also had unfavorable lipid profiles. They compared lipid profiles before commencement of RA treatment with profiles 1 year later. Patients who had either received treatment for RA or had conditions that could influence their lipid profiles were excluded. Patients were given standard treatments including prednisolone,

up to 10 mg/day. After 1 year, 27 of the 42 patients (64%) showed an improvement in RA according to the 20% improvement criteria of the American College of Rheumatology. In addition, high density lipoprotein (HDL) levels increased by 21% and apolipoprotein A-I by 23%, and the ratio of low density lipoprotein to HDL decreased by 13% without the use of lipid-lowering drugs. The remaining patients who did not respond to treatment did not show significant changes in their lipid profiles.

*Am J Med* 2002;113:188