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REVIEW

Chemokine receptor CCR5: from AIDS to atherosclerosis

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There is increasing recognition of an important contribution of chemokines and their receptors in the pathology of atherosclerosis and related cardiovascular disease. The chemokine receptor CCR5 was initially known for its role as a co-receptor for HIV infection of macrophages and is the target of the recently approved CCR5 antagonist maraviroc. However, evidence is now emerging supporting a role for CCR5 and its ligands CCL3 (MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES) in the initiation and progression of atherosclerosis. Specifically, the CCR5 deletion polymorphism CCR5delta32, which confers resistance to HIV infection, has been associated with a reduced risk of cardiovascular disease and both CCR5 antagonism and gene deletion reduce atherosclerosis in mouse models of the disease. Antagonism of CCL5 has also been shown to reduce atherosclerotic burden in these animal models. Crucially, CCR5 and its ligands CCL3, CCL4 and CCL5 have been identified in human and mouse vasculature and have been detected in human atherosclerotic plaque. Not unexpectedly, CC chemokines have also been linked to saphenous vein graft disease, which shares similarity to native vessel atherosclerosis. Distinct roles for chemokine–receptor systems in atherogenesis have been proposed, with CCR5 likely to be critical in recruitment of monocytes to developing plaques. With an increased burden of cardiovascular disease observed in HIV-infected individuals, the potential cardiovascular-protective effects of drugs that target the CCR5 receptor warrant greater attention. The availability of clinically validated antagonists such as maraviroc currently provides an advantage for targeting of CCR5 over other chemokine receptors.

Abbreviations

AD, Alzheimer's Disease; EC, endothelial cell; ELISA, enzyme-linked immunosorbent assay; HBMEC, human brain microvessel endothelial cell; HCAEC, human coronary artery endothelial cell; HCC, haemofiltrate CC chemokine; HFD, high fat diet; HUAEC, human umbilical artery endothelial cell; HUVEC, human umbilical vein endothelial cell; ICC, immunocytochemistry; IFN, interferon; IL, interleukin; ISH, *in situ* hybridization; LPS, lipopolysaccharide (endotoxin); LV, left ventricle; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PAH, pulmonary arterial hypertension; RANTES, regulated on activation normal T cell expressed and secreted; RPA, RNase protection assay; SV, saphenous vein; TNF, tumour necrosis factor; VSMC, vascular smooth muscle cell; WT, wild-type

Introduction

Chemokines and their G-protein coupled receptors have been increasingly recognized as key mediators in the pathology of cardiovascular disease. The chemokine receptor CCR5 is best known for its role as a co-receptor for HIV-1 infection and is of particular interest owing to the availability of small molecule highly selective and potent antagonists developed for clinical use against HIV. This review will focus on the emerging evidence supporting a role for CCR5 and its ligands CCL3, CCL4 and CCL5 in atherosclerosis.

CCR5 is a co-receptor for HIV infection

Following the discovery of the human CCR5 receptor (Combadiere *et al.*, 1996; Raport *et al.*, 1996; Samson *et al.*, 1996a) it was identified as a co-receptor for macrophage-tropic (or R5) HIV-1 (Alkhatib *et al.*, 1996; Choe *et al.*, 1996; Deng *et al.*, 1996; Doranz *et al.*, 1996; Dragic *et al.*, 1996). Subsequently, a deletion polymorphism in CCR5, known as CCR5delta32, was recognized and found to lead to near complete resistance to HIV-1 infection in the homozygous state, and slower progression to AIDS in heterozygotes (Dean *et al.*, 1996; Huang *et al.*, 1996; Liu *et al.*, 1996; Samson *et al.*, 1996b; Zimmerman *et al.*, 1997). The age of this polymorphism is still in dispute (Stephens *et al.*, 1998; Hummel *et al.*, 2005), as are the selection pressures that lead to the maintenance of this polymorphism (Novembre *et al.*, 2005; Cohn and Weaver, 2006; Hedrick and Verrelli, 2006; Faure, 2008; Faure and Royer-Carenzi, 2008). This 32 base pair deletion causes a shift in the reading frame, the creation of an early stop codon and the production of a truncated protein that is retained in the endoplasmic reticulum. Without CCR5 on the cell surface, R5 HIV-1, the usual infecting strain, is unable to gain entry to macrophages or other cells expressing CCR5, such as immature dendritic cells and $CD4^+$ T cells. Thus, proof-of-concept for blocking CCR5 in HIV-1 infection was provided by nature, together with supporting safety data from the lack of a deleterious phenotype in individuals homozygous for the CCR5delta32 allele (Dean *et al.*, 1996; Huang *et al.*, 1996; Liu *et al.*, 1996; Samson *et al.*, 1996b; Zimmerman *et al.*, 1997). This prompted the successful search for CCR5 antagonists as novel antiretroviral therapeutics with the first drug maraviroc (Dorr *et al.*, 2005) approved for use in both treatment-experienced and treatment-naive patients (FDA Expanded Indication for Selzentry, 2009). A limitation to treatment of HIV with CCR5 antagonists is that these drugs are ineffective against X4 (or T-cell-tropic) HIV strains that use CXCR4 as a co-receptor. Current guidelines therefore state that tropism tests must be carried out prior to use of CCR5 antagonists. However, development of drug resistance may occur as viral mutants emerge in R5-tropic individuals that allow viral entry via the CXCR4 receptor or even the CCR5–drug complex (Trkola *et al.*, 2002), the latter of which may occur to some extent in drug-naive patients (Pfaff *et al.*, 2010). As a consequence drugs that target novel sites on the viral envelope and therefore inhibit both the R5 and X4 strains are in development (Murray *et al.*, 2010), although interestingly it is suggested that viral resistance may be associated with a reduction in viral replication capacity that would be clinically beneficial (for review see Clementi and Lazzarin, 2010).

CCR5 was initially paired with three ligands on its discovery, macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4) and regulated on activation normal T cell expressed and secreted (RANTES, CCL5). In support of a role for CCR5 in HIV-1 cell entry, these ligands were reported to act as inhibitors of infection (Cocchi *et al.*, 1995). Since then further ligands have been suggested to bind and in some cases activate CCR5, listed in Table 1, although some of these ligands have low affinities for CCR5, and are therefore unlikely to be physiologically relevant. Of note, monocyte chemoattractant protein (MCP)-3 (CCL7) is an antagonist at CCR5 (Blanpain *et al.*, 1999). Studies on a non-allelic variant of CCL3, known as CCL3L1, indicate it is the most potent agonist at CCR5 yet described (Nibbs *et al.*, 1999). Many of these

Table 1

List of reported CCR5 ligands (Charo *et al.*, 2009 IUPHAR-DB; Alexander *et al.*, 2009) and their nomenclature (Zlotnik and Yoshie, 2000)

Standard nomenclature	Selected previous name(s)	Pairing reference
CCL2	MCP-1; JE	Blanpain *et al.*, 1999
CCL3	MIP-1α; LD78α	Combadiere *et al.*, 1996; Raport *et al.*, 1996; Samson *et al.*, 1996a
CCL3L1	MIP-1αP; LD78β	Nibbs *et al.*, 1999
CCL4	MIP-1β; LAG-1; ACT-2	Combadiere *et al.*, 1996; Raport *et al.*, 1996; Samson *et al.*, 1996a
CCL4L1	CCL4L (mature peptide identical to CCL4)	
CCL5	RANTES	Combadiere *et al.*, 1996; Raport *et al.*, 1996; Samson *et al.*, 1996a
CCL7 (antagonist)	MCP-3	Blanpain *et al.*, 1999
CCL8	MCP-2	Ruffing *et al.*, 1998
CCL11	Eotaxin	Blanpain *et al.*, 1999
CCL13	MCP-4	Ruffing *et al.*, 1998
CCL14	HCC-1 (only CCL14[9–74] is active)	Napier *et al.*, 2005; Blain *et al.*, 2007
CCL16	HCC-4	Alexander *et al.*, 2009

CCL, CC chemokine ligand; HCC, haemofiltrate CC chemokine; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T cell expressed and secreted.

chemokines are promiscuous and are also capable of binding to other CC chemokine receptors; however, CCL4 is unusual in that it seems to be an agonist at CCR5 only (Wells *et al.*, 2006), although it has been reported to act as an antagonist at recombinant CCR1 (Chou *et al.*, 2002). The HIV-1 R5 envelope protein gp120 is also a ligand for CCR5, and is able to signal through this receptor (Weissman *et al.*, 1997).

CCR5 and atherosclerosis

Atherosclerosis is a disease affecting large and medium elastic and muscular arteries and underlies a large proportion of cardiovascular disease morbidity and mortality. It is increasingly recognized to be an inflammatory disease, where an initially protective response continues to excess and becomes detrimental, known as the 'response-to-injury' hypothesis (Ross, 1999). Key cell types in disease pathology include monocytes/macrophages, T cells and vascular smooth muscle cells. Briefly, endothelial dysfunction increases vascular permeability, allowing circulating monocytes and T cells to migrate into the subendothelial space. The continued presence of immune cells, together with migration and proliferation of vascular smooth muscle cells, serves to perpetuate the immune response and promote atherosclerotic plaque formation. An advanced atherosclerotic plaque typically contains a mixture of proliferating smooth muscle cells, macrophages and T cells, and connective tissue, overlaid with a fibrous cap comprised of smooth muscle cells and matrix (Weber *et al.*, 2008). Rupture of a plaque may lead to clinical consequences including myocardial infarction and stroke.

The critical role of inflammation and immune cells in the aetiology of atherosclerosis makes it unsurprising that many chemokines and chemokine receptors have been linked to this disease. Much attention has been focussed on CCL2, CCL5, CX_3CL1 and their receptors CCR2, CCR5 and CX_3CR1, which seem to have important yet distinct roles, as discussed below. It is likely that many members of the chemokine family contribute to atherogenesis; however, CCR5 is particularly noteworthy given the availability of an approved antagonist.

Unusually for a G-protein coupled receptor, the naturally occurring CCR5delta32 variant allows the effect of knock-down of the human CCR5 gene on different diseases to be determined. Using this rare opportunity, genetic epidemiology has examined associations between CCR5 and human cardiovascular disease, although a definitive picture from this research has not yet emerged. The CCR5delta32 allele has been linked with reduced susceptibility to coronary artery disease (Szalai *et al.*, 2001; Afzal *et al.*, 2008), reduced early onset of coronary heart disease in women (Pai *et al.*, 2006) and protection against myocardial infarction (Gonzalez *et al.*, 2001; Balistreri *et al.*, 2008). In contrast, other studies have found no effect of the CCR5delta32 polymorphism on coronary artery disease or myocardial infarction in other populations (Simeoni *et al.*, 2004; Petrkova *et al.*, 2005; Apostolakis *et al.*, 2007; Ghilardi *et al.*, 2008; Sharda *et al.*, 2008). These results may reflect differences in the populations investigated, including variations in the frequency of the CCR5delta32 allele. Nevertheless, these findings provide the impetus for investigating a role for CCR5 in human atherosclerosis. Also of interest, Hyde *et al.* (2010) have found the CCR5delta32 polymorphism to be associated with higher plasma high density lipoprotein (HDL) cholesterol and lower plasma triglycerides, both beneficial lipid effects that would be expected to reduce the risk of cardiovascular disease. Indeed, maraviroc treatment was associated with better lipid profiles and less requirement for lipid-lowering therapy than standard initial HIV therapy (combivir plus efavirenz) in the Maraviroc versus Efavirenz Regimens as Initial Therapy trial (DeJesus *et al.*, 2008).

Further investigation of CCR5 as a target in atherosclerosis has been carried out using mouse models of atherosclerosis, with findings summarized in Table 2. A role for CCR5 has been verified in studies of both receptor antagonism (Schober *et al.*, 2002; Veillard *et al.*, 2004; van Wanrooij *et al.*, 2005; Tacke *et al.*, 2007) and genetic deletion (Kuziel *et al.*, 2003; Luckow *et al.*, 2004; Potteaux *et al.*, 2006; Zernecke *et al.*, 2006; Braunersreuther *et al.*, 2007; Quinones *et al.*, 2007). Two of these studies suggested that CCR5 may be more important in later stage plaque development (Kuziel *et al.*, 2003; Quinones *et al.*, 2007). Recently, combined inhibition of three chemokine–receptor systems, MCP-1 (CCL2)/CCR2, fractalkine (CX_3CL1)/CX_3CR1 and CCL5/CCR5, was reported to abolish development of atherosclerosis in an $ApoE^{-/-}$ mouse model (Combadiere *et al.*, 2008), supporting non-redundancy of these chemokines with regard to monocyte mobilization in atherosclerosis.

Owing to differences in atherosclerotic disease between humans and mouse models (Zadelaar *et al.*, 2007; deLuna, 2008; Potteaux *et al.*, 2008), studies in human cells and tissue are critical in substantiating a role for CCR5 in disease development. In particular, CCR5 message and protein has been identified in arterial (Schecter *et al.*, 2000; Jones *et al.*, 2007) and venous (Jones *et al.*, 2007) smooth muscle. CCR5 immunoreactivity was also detected in human atherosclerotic plaque (Schecter *et al.*, 2000) with up-regulation of CCR5 mRNA expression found in unstable carotid atherosclerotic plaque in comparison with stable plaque (Papaspyridonos *et al.*, 2006). A summary of CCR5 vascular localization in human cells and tissue, together with relevant mouse models, is presented in Table 3. These data add further support to the hypothesis that this receptor has a role in the development of atherosclerosis.

CCR5 ligands and atherosclerosis

CC chemokines have been widely implicated in atherosclerotic plaque development and broad-spectrum CC chemokine blockade has also been found to reduce atherosclerosis in $ApoE^{-/-}$ mice (Bursill *et al.*, 2004). Studies investigating single chemokines in mouse models have revealed CCR5 ligands CCL3 and CCL5 to be linked with atherosclerotic plaque progression. For example, knock-down of CCL5 expression in vascular smooth muscle was found to reduce neointimal thickening and macrophage infiltration in an $ApoE^{-/-}$ mouse model, but not in mice lacking the CCR5 receptor, demonstrating this effect was mediated by CCL5 acting at CCR5 rather than its other receptors (Krohn *et al.*, 2007). Use of [^{44}AANA^{47}]-RANTES, which prevents CCL5 interaction with glycosaminoglycans and so inhibits its function, was found to lessen progression of established atherosclerotic plaque

Table 2

Strategies to identify a role for CCR5 in atherosclerosis using animal models

Receptor blockade Antagonist	Target receptor	Animal model	Outcome	Reference
Met-RANTES	CCR5 CCR1	$ApoE^{-/-}$ mice High fat diet Wire injury	40%↓ carotid plaque size ↓ in plaque monocyte/macrophage content	Schober *et al.*, 2002
Met-RANTES	CCR5 CCR1	$Ldlr^{-/-}$ mice Western diet Spontaneous atherosclerosis	↓ aortic sinus and thoracoabdominal plaque size ↓ inflammatory cell infiltrate	Veillard *et al.*, 2004
Met-RANTES	CCR5 CCR1	$ApoE^{-/-}$, $CX_3CR1^{-/-}$, $CCL2^{-/-}$ mice Normal diet Spontaneous atherosclerosis	75%↓ in aortic sinus plaque size ↓ circulating monocytes	Combadiere *et al.*, 2008
TAK-779	CCR5 CXCR3	$Ldlr^{-/-}$ mice Western diet Vascular silastic collar	↓ size and leukocyte accumulation in carotid plaque	van Wanrooij *et al.*, 2005
TAK-779	CCR5 CXCR3	$ApoE^{-/-}$ mice Angiotension-II treated	↓ in circulating monocytes and vascular macrophage infiltration	Major *et al.*, 2009
Anti-CCR5 mAb	CCR5	$ApoE^{-/-}$ mice Normal or high fat diet Spontaneous atherosclerosis	50%↓ in monocyte recruitment	Tacke *et al.*, 2007

CCR5 gene deletion Animal model	Outcome	Reference
$ApoE^{-/-}$ $CCR5^{-/-}$ or $ApoE^{-/-}$ mice Normal diet, spontaneous atherosclerosis	No effect on aortic sinus plaque size over so many weeks studied	Kuziel *et al.*, 2003
$CCR5^{-/-}$ or WT mice Normal diet, carotid allograft	↓ neointima formation and T-cell accumulation in allograft plaque	Luckow *et al.*, 2004
$ApoE^{-/-}$ $CCR5^{-/-}$ or $ApoE^{-/-}$ mice Western diet, wire injury	↓ neointima formation ↓ inflammatory cell infiltrate	Zernecke *et al.*, 2006
$Ldlr^{-/-}$ mice, bone marrow transplant from WT or $CCR5^{-/-}$ mice at 15 weeks Western diet, spontaneous atherosclerosis	↓ in aortic sinus and thoracic aortic plaque macrophage accumulation trend to ↓ in lesion size ↑ cholesterol in $CCR5^{-/-}$ bone marrow recipients	Potteaux *et al.*, 2006
$ApoE^{-/-}$ $CCR5^{+/+}$ or $ApoE^{-/-}$ $CCR5^{-/-}$ mice High fat diet, spontaneous atherosclerosis	↓ aortic root and thoracoabdominal plaque size ↓ mononuclear cell infiltration	Braunersreuther *et al.*, 2007
$ApoE^{-/-}$ $CCR5^{-/-}$ or $ApoE^{-/-}$ mice Normal or high fat diet, spontaneous atherosclerosis	↓ in aortic sinus and coronary plaque size and macrophage accumulation	Quinones *et al.*, 2007

ApoE, apolipoprotein E; ldlr, low density lipoprotein receptor; mAb, monoclonal antibody; WT, wild-type.

and promoted a stable plaque phenotype in $ldlr^{-/-}$ mice fed a high cholesterol diet (Braunersreuther *et al.*, 2008). Disruption of CCL5 and platelet factor (PF)-4 (CXCL4) heteromers led to a reduction in atherosclerotic lesions in $ldlr^{-/-}$ mice and transfer of $CCL5^{-/-}$ bone marrow to $ApoE^{-/-}$ mice also attenuated atherosclerosis (Koenen *et al.*, 2009). Evidence for an involvement of CCL3 in atherogenesis was provided by the observation that reduction of atherosclerosis by angiotensin II receptor AT_1 antagonism was accompanied by a decrease in CCL3 expression (Dol *et al.*, 2001). In contrast, CCL3 gene deletion did not protect against angiotensin II-mediated neointimal thickening in a mouse carotid ligation model (Zhang *et al.*, 2007), supporting claims that CCL5 may be the most important CCR5 ligand in development of atherosclerosis.

Data from both human tissue and mouse models show vascular expression of chemokines, detailed in Table 4. CCL5 mRNA expression has been detected in human carotid, aorta and iliac arteries from donors (Hayes *et al.*, 1998) and expression of CCL3, CCL4 and CCL5 has been detected in the medial layer of human arteries and vein and localized to smooth muscle cells (Jones *et al.*, 2008). CCL3, CCL4 and

Table 3

CCR5 expression and localization in the cardiovascular system

Localization	Technique(s)	Reference
Vascular endothelial cells:		
CCR5 present on endothelia to variable extents in several vascular beds.	ICC and flow cytometry on tissue from human and macaques	Rottman *et al.*, 1997
CCR5 present on cultured EC and native endothelia. Coronary artery EC showed strong chemotactic response to CCL3, CCL4 and CCL5.	ICC and flow cytometry on HCAECs, HUVECs and coronary endothelia from sections of human heart tissue and transmigration assay	Berger *et al.*, 1999
Expressed CCR5 mRNA; no CCR5 protein detected on cell surface.	RT-PCR and flow cytometry on HBMECs	Kanmogne *et al.*, 2000
No CCR5 detected in EC.	RNA blot hybridization on primary HUVECs	Schecter *et al.*, 2000
Inhibition of CCL3 binding with unlabelled CCL3, CCL4 and CCL5; bound CCL3 internalized by microvessel EC.	Radioligand binding using labelled CCL3 on human brain microvessels	Andjelkovic and Pachter, 2000
Expressed CCR5 mRNA; no CCR5 protein on cell surface.	RT-PCR and flow cytometry on human lung microvascular endothelial cells	Kanmogne *et al.*, 2001
Vascular smooth muscle cells:		
CCR5 present on vascular smooth muscle in several vascular beds.	ICC and flow cytometry on tissue from human and macaques	Rottman *et al.*, 1997
No CCR5 mRNA detected.	Semi-quantitative RT-PCR on saphenous vein derived cultured VSMC	Hayes *et al.*, 1998
CCR5 expressed by VSMC; CCL4-dependent VSMC signaling abolished by antibodies to CCR5.	RNA blot hybridization, RT-PCR and ICC on human coronary artery- and aorta-derived VSMC	Schecter *et al.*, 2000
CCR5 mRNA detected in medial vascular smooth muscle from human aorta, coronary artery and saphenous vein.	RT-PCR on human artery and vein medial smooth muscle	Jones *et al.*, 2007
Atherosclerotic and diseased vasculature:		
CCR5 immunoreactivity detected in atheroma; staining of adjacent sections suggested co-localization to VSMC and macrophages. No CCR5 in normal coronary artery.	ICC on human coronary artery atherosclerotic plaque	Schecter *et al.*, 2000
CCR5 expression strongly induced in grafts. Aortic grafts showed greater absolute levels.	ICC and real time RT-PCR on rat cardiac and aortic grafts (transplant vasculopathy)	Horiguchi *et al.*, 2002
CCR5 expressed in atherosclerotic plaque. Binding sites detected all along aorta, highest at atheroma in $ldlr^{-/-}$, weaker binding in normal.	RPA and [^{125}I]Met-RANTES binding on aorta of WT and $ldlr^{-/-}$ mice	Veillard *et al.*, 2004.
11.7-fold increase in CCR5 in unstable plaque compared with stable regions.	Gene expression (Affymatrix gene chip analysis) of human carotid endarterectomy specimens comparing unstable regions and stable region of the same specimen as an internal control	Papaspyridonos *et al.*, 2006
Immunoreactivity present on some plaque VSMC.	Quantitative ICC on the aortic roots of $ApoE^{-/-}$ mice	Braunersreuther *et al.*, 2007
Initially maximal expression in perivascular tissue and EC, then shifted to media and adventitia followed by neointima and adventitia. Expression low at 14 days.	ISH and real time RT-PCR on balloon-injured porcine coronary arteries (model of angioplasty)	Jabs *et al.*, 2007
Increased expression of CCR5 mRNA in saphenous vein graft compared with control vein. Localization of CCR5 protein to smooth muscle cells within the vein atherosclerotic plaque. CCR5 mRNA and protein localized to neointima of human cultured saphenous vein.	RT-PCR and ICC on human saphenous vein graft retrieved from explant heart and on normal saphenous vein maintained in culture for 14 days	Jones *et al.*, 2009

ApoE, apolipoprotein E; EC, endothelial cell; HBMEC, human brain microvessel endothelial cell; HCAEC, human coronary artery endothelial cell; HUVEC, human umbilical vein endothelial cell; ICC, immunocytochemistry; ISH, *in situ* hybridization; ldlr, low density lipoprotein receptor; mRNA, messenger ribonucleic acid; RPA, RNase protection assay; RT-PCR, reverse transcription polymerase chain reaction; VSMC, vascular smooth muscle cell; WT, wild-type.

Table 4

Expression and localization of CCL3, CCL4 and CCL5 in the cardiovascular system

Chemokine	Localization	Technique(s)	Reference
Vascular endothelial cells:			
CCL3, CCL4	CCL3 and CCL4 not released even following stimulation with cytokines.	ELISA on release by pulmonary artery EC	Lukacs *et al.*, 1995
CCL5	Weak expression and little production on stimulation with individual cytokines, combining TNFα and IFNγ led to strong CCL5 expression and release.	ISH and ELISA on HUVECs	Marfaing-Koka *et al.*, 1995
CCL4, CCL5	Untreated cells expressed low amounts CCL4 and CCL5; increased after stimulation with cytokines/LPS. Only CCL5 mRNA detected in HUVECs.	RT-PCR and ICC on primary cultures of HBMECs and HUVECs	Shukaliak and Dorovini-Zis, 2000
CCL5	CCL5 mRNA and release induced in HCAECs after stimulation, less in HUVECs.	RPA and ELISA on HCAECs and HUVECs	Briones *et al.*, 2001
CCL3, CCL5	CCL5 released from HUAECs and to a lesser extent from HUVECs following stimulation. No release of CCL3.	ELISA on angiotensin II stimulated HUAECs and HUVECs	Mateo *et al.*, 2006
CCL5	Angiotensin II induced CCL5 release from HUAECs; inhibited by TNFα neutralization.	ELISA on release by HUAECs	Mateo *et al.*, 2007
CCL3	Oxidative stress induced increases in CCL3 expression and release.	RT-PCR and ELISA on human brain EC	Tripathy *et al.*, 2007
CCL5	Oxidative stress induced CCL5 expression and release.	RT-PCR and ELISA on rat brain EC	Tripathy *et al.*, 2010
Vascular smooth muscle cells:			
CCL5	No CCL5 expression under basal conditions; induced following treatment with IL-1α/TNFα.	ELISA and northern blot on human VSMC derived from SV and aorta	Jordan *et al.*, 1997
CCL3, CCL4	Release of CCL3 and CCL4 induced following stimulation with cytokines.	ELISA on release by pulmonary artery VSMC	Lukacs *et al.*, 1995
CCL5	CCL5 mRNA and release in transfected human VSMC. Increased CCL5 mRNA in neointimal vs. medial rat VSMC.	Real time PCR and ELISA on human coronary artery VSMC transfected with YB-1 (CCL5 transcriptional promoter) and rat VSMC from media and neointima of balloon-injured aorta	Krohn *et al.*, 2007
Vasculature:			
CCL5	CCL5 expressed in EC of pulmonary artery from PAH patients; little staining in control vasculature. Similar results from ICC; staining of consecutive sections suggested co-localization with infiltrating leukocytes. VSMC of muscular arteries negative.	ISH and ICC on lung biopsies from human PAH patients and controls	Dorfmüller *et al.*, 2002
CCL5	CCL5 expression in aorta increased with age.	RT-PCR on aorta of WT mice aged 1–9 months	Martin *et al.*, 2004

Table 4

Continued.

Chemokine	Localization	Technique(s)	Reference
CCL5	Greatest CCL5 staining in arterioles, less in venules.	ICC on mesenteric arterioles and post-capillary venules from angiotensin II-treated rats	Mateo *et al.*, 2006
CCL3	Higher levels of CCL3 mRNA and greater release in brain microvessels from AD patients.	RT-PCR and ELISA on brain microvessels from age-matched controls and patients with AD	Tripathy *et al.*, 2007
CCL5	Aortic expression induced following stimulation with angiotensin II.	Real time PCR on WT mouse aorta	Guzik *et al.*, 2007
CCL3, CCL4, CCL5	CCL3, CCL4 and CCL5 mRNA present in human vasculature free from disease. ICC localized all three ligands to EC and smooth muscle in human coronary artery and SV.	RT-PCR on medial layer of human arteries and vein and dual labelling ICC with cell markers on sections of human normal arteries and vein	Jones *et al.*, 2008
CCL3, CCL4, CCL5	No expression detected in normal SV, expression of CCL3, CCL4 and CCL5 in varicose vein.	RPA on normal SV and varicose vein	del Rio Solá *et al.*, 2009
CCL5	CCL5 expressed and released by normal brain microvessels.	RT-PCR and ELISA on human brain microvessels from normal and AD brains	Tripathy *et al.*, 2010
Human atherosclerosis:			
CCL3, CCL5	CCL5 and CCL3 in plaque, on 5% and 13% cells, respectively, compared with mRNA on <1% of medial cells for these in non-diseased arteries. Serial sections suggested macrophage or T-cell co-localization.	ISH on human atherosclerotic plaques (carotid endarterectomy)	Wilcox *et al.*, 1994
CCL3, CCL5	CCL3 mRNA not present in normal aorta but present in atherosclerotic vessels. CCL5 mRNA in all normal and atherosclerotic vessels.	Semi-quantitative RT-PCR on human aorta (normal and atherosclerotic) and atherosclerotic iliac and carotid artery	Hayes *et al.*, 1998
CCL4	Immunoreactivity detected in atheroma, staining of adjacent sections suggested co-localization with VSMC and macrophages.	ICC on sections of human atherosclerotic plaque	Schecter *et al.*, 2000
CCL5	Detected in intima and media but most staining on luminal surface of carotid arteries.	ICC on human carotid atherectomy specimens	von Hundelshausen *et al.*, 2001
CCL3, CCL4, CCL5	CCL3, CCL4 and CCL5 localized to smooth muscle and atherosclerotic plaque of human diseased coronary artery.	Dual labelling ICC on sections of human atherosclerotic coronary artery	Jones *et al.*, 2008
CCL5	CCL5 mRNA up-regulated in atherosclerotic compared with control artery. Immunoreactivity detected in lipid rich core of atherosclerotic plaque.	Microarray analysis and ICC on human carotid plaque and control iliac artery	Breland *et al.*, 2010
Mouse models of atherosclerosis:			
CCL3	CCL3 mRNA expressed in atherosclerotic lesions, two-fold reduction following treatment.	ApoE$^{-/-}$ mice treated with AT_1 antagonist to reduce lesion size	Dol *et al.*, 2001

Table 4

Continued.

Chemokine	Localization	Technique(s)	Reference
CCL5	Detected in intima and media but most staining on luminal surface of carotid arteries. No staining in WT untreated mice.	ICC on wire-injured carotid arteries from ApoE$^{-/-}$ mice fed a HFD, or TNFα-treated WT mice	von Hundelshausen *et al.*, 2001
CCL5	CCL5 detected on luminal surface and in neointimal cells with a VSMC phenotype.	ICC on wire-injured carotid arteries from ApoE$^{-/-}$ mice fed a HFD	Schober *et al.*, 2002
CCL5	Not detected in normal aortic root but highly expressed in atheroma. Staining of consecutive sections indicated localization to macrophages and T cells.	ICC on aorta from WT and ldlr$^{-/-}$ mice	Veillard *et al.*, 2004
CCL3, CCL5	CCL3 increased in lesions from 4-month-old mice onward; CCL5 significantly induced in 6- to 9-month-old mice only.	RT-PCR on aorta of ApoE$^{-/-}$ mice aged 1–9 months	Martin *et al.*, 2004
CCL3, CCL4, CCL5	CCL5 up-regulated in early atherosclerosis and possibly down-regulated in more advanced disease. CCL3 and CCL4 up-regulated in advanced atherosclerosis. ICC showed CCL3 to be mostly in foam cells of advanced plaques.	mRNA microarray analysis, real time RT-PCR and ICC on aortic atherosclerotic plaque from ApoE$^{-/-}$ mice fed chow and Western diets	Lutgens *et al.*, 2005
Transplant associated and other vascular injury:			
CCL5	Detected in EC of arteries undergoing accelerated atherosclerosis, not in normal coronary arteries.	ISH and ICC on coronary artery from patients with accelerated atherosclerosis during re-transplantation	Pattison *et al.*, 1996
CCL5	CCL5 mRNA and release from aorta significantly increased following elastase perfusion; no CCL5 detected in normal mouse aorta by ICC. Elastase exposure induced CCL5 expression and release from VSMC.	RT-PCR, ELISA and ICC on mouse aorta and VSMC. Mice underwent elastase perfusion of the abdominal aorta to induce development of aneurysm. Cultured mouse aortic VSMC exposed to elastase.	Colonnello *et al.*, 2003
CCL5, CCL4	VSMC produced CCL5 and CCL4. Chemokine mRNA mostly in vascular cells, both intima and media. CCL5 immunoreactivity detected in media.	ICC and real time RT-PCR on transplanted coronary artery (human coronary artery transplanted into immune-deficient mice as a mouse model of chronic transplant arteriosclerosis)	Burns *et al.*, 2005
CCL3, CCL4, CCL5	Increased expression of CCL4 and CCL5 mRNA in human SV graft compared with control SV. CCL3, CCL4 and CCL5 localized to neointimal smooth muscle of SV graft and cultured SV.	RT-PCR and dual labelling ICC on retrieved human SV graft from explant heart and normal SV maintained in culture for 14 days	Jones *et al.*, 2009
CCL5	Acute up-regulation of CCL5 by medial VSMC dependent on STAT3 and NF-κB. CCL5 production initiated by TNFα, but not by IL-6.	qRT-PCR and immunofluorescence on femoral artery following arterial injury of WT mice and WT murine VSMC	Kovacic *et al.*, 2010

AD, Alzheimer's Disease; ApoE, apolipoprotein E; EC, endothelial cell; ELISA, enzyme-linked immunosorbent assay; HBMEC, human brain microvessel endothelial cell; HFD, high fat diet; HCAEC, human coronary artery endothelial cells; HUVEC, human umbilical vein endothelial cells; ICC, immunocytochemistry; IFN, interferon; IL, interleukin; ISH, *in situ* hybridization; ldlr, low density lipoprotein receptor; LPS, lipopolysaccharide (endotoxin); LV, left ventricle; mRNA, messenger ribonucleic acid; PAH, pulmonary arterial hypertension; RPA, RNase protection assay; RT-PCR, reverse transcription polymerase chain reaction; SV, saphenous vein; TNF, tumour necrosis factor; VSMC, vascular smooth muscle cell; WT, wild-type.

CCL5 have been reported to be present in human atherosclerotic plaque (Wilcox *et al.*, 1994; Schecter *et al.*, 2000; Jones *et al.*, 2008; Breland *et al.*, 2010) and localized to synthetic smooth muscle cells and CD3-positive T cells. In support, genetic profiling of ApoE$^{-/-}$ mice identified up-regulation of CCL3, CCL4 and CCL5 mRNA in atherosclerotic plaque (Lutgens *et al.*, 2005) and angiotensin II stimulation was also found to induce mouse aortic expression of CCL5 (Guzik *et al.*, 2007).

There have been numerous studies investigating whether chemokine serum levels are associated with cardiovascular disease, although it should be noted that levels of circulating chemokines do not necessarily correspond to levels in tissues, and as such plasma levels may not accurately reflect disease pathology. It has been proposed that chemokines could be evaluated as potential biomarkers for cardiovascular disease, although greater validation is required (Aukrust *et al.*, 2007; 2008). For example, high circulating levels of CCL5 may be a marker for refractory unstable angina pectoris (Kraaijeveld *et al.*, 2007) and CCL3 plasma levels may be prognostic for ischaemic events (de Jager *et al.*, 2008). Furthermore, levels of CCL3 and CCL5 have been shown to correlate with congestive heart failure (Aukrust *et al.*, 1998) and myocardial infarction (Parissis *et al.*, 2002; Nomura *et al.*, 2003; Kobusiak-Prokopowicz *et al.*, 2005; 2007), while CCL3, CCL4 and CCL5 have all been linked to coronary atherosclerosis (Waehre *et al.*, 2003; Ardigo *et al.*, 2007; Jang *et al.*, 2007; DiPalma *et al.*, 2008; Gurbel *et al.*, 2008). In addition, an association between the CCL5 G403A polymorphism and increased risk of cardiovascular disease has been reported (Simeoni *et al.*, 2004; Boger *et al.*, 2005; Jang *et al.*, 2007; Ghilardi *et al.*, 2008; Vogiatzi *et al.*, 2009); however, this has not been found by all groups (Szalai *et al.*, 2001; Tereshchenko *et al.*, 2008). In contrast, lower serum CCL5 levels were found to be associated with coronary heart disease (Rothenbacher *et al.*, 2006) and cardiovascular mortality following coronary angiography referrals (Cavusoglu *et al.*, 2007), and no association was found between CCL5 and intima-media thickness (Magyar *et al.*, 2007), which may suggest further investigation is warranted. A better strategy may involve the use of a panel of chemokines, including CCL5, alongside other markers to aid predictions of prognosis for patients with acute coronary syndrome (Correia *et al.*, 2010).

Contribution of CCR5 and its ligands to development of atherosclerosis

It is becoming increasingly clear that many chemokines and their receptors play a role in the development of atherosclerosis, although there are certain 'key players' apparent, whose functions can be separated (Zernecke and Weber, 2010). In particular, evidence is emerging for important and distinct roles for CCL2/CCR2, CX_3CL1/CX_3CR1 and CCL5/CCR5 (Karshovska and Schober, 2008; Weber *et al.*, 2008). Although care must be taken in extrapolating results from mouse models, owing to differences in atherosclerotic disease (Zadelaar *et al.*, 2007; deLuna, 2008; Potteaux *et al.*, 2008), in monocyte subsets (Ingersoll *et al.*, 2010), and species differences in the complement of chemokines (Zlotnik and Yoshie, 2000) these models have provided many important insights into the interaction between chemokines, receptors and various inflammatory cells (Weber *et al.*, 2008). CCL5 acting at CCR5 is considered to be crucial to monocyte recruitment during development of atherosclerosis, while CCL2 is critical for monocyte adhesion and vascular smooth muscle cell proliferation and fractalkine acting at CX_3CR1 seems to sustain chronic monocyte adhesion and survival within the plaque (Karshovska and Schober, 2008; Gautier *et al.*, 2009). These distinct roles are supported by the *in vivo* finding that inhibition of CCL2, CX_3CR1 and CCR5 showed additive effects in reducing atherosclerosis, and that targeting of all three systems was required for almost complete abolition of disease in an atherosclerotic mouse model (Combadiere *et al.*, 2008). Despite this, there may also be a significant overlap between these functions; Tacke *et al.* (2007) demonstrated that 'classical' CCR2^{+}Ly-6C^{hi} monocytes, the dominant monocyte subset entering developing plaques, required CCR2, CCR5 and CX_3CR1 for plaque infiltration in ApoE$^{-/-}$ mice. CCR5 is also implicated in the entry of 'non-classical', or Ly-6C^{lo}, monocytes into lesions (Gautier *et al.*, 2009) and the recruitment of T cells into established plaques (Koenen and Weber, 2010). Interestingly, in human monocytes and human coronary artery endothelial cells it has recently been shown that reconstituted HDL decreased the expression of CCL5 suggesting that reduction of CCR5 function may contribute to the atheroprotective properties of HDL (Bursill *et al.*, 2010).

In addition to the important role for monocytes in atherogenesis, there is emerging evidence of a significant interaction between chemokines and platelets in the development of atherosclerosis (Weber, 2005; Gleissner *et al.*, 2008). This is particularly relevant to CCL5, which is released from platelet α-granules together with PF-4/CXCL4 (von Hundelshausen *et al.*, 2005). Indeed, CCL5 delivery appears to be critical in platelet-mediated atherogenic monocyte recruitment (Weber, 2005). Experiments in atherosclerotic mouse models have revealed that thrombin-activated platelets deposit CCL5 on the surface of inflamed microvascular and aortic endothelium, where it is immobilized and triggers subsequent monocyte and T-cell arrest (von Hundelshausen *et al.*, 2001; Schober *et al.*, 2002). This delivery of CCL5 is platelet P-selectin-dependent (Schober *et al.*, 2002) and may be mediated by platelet-derived microparticles (Mause *et al.*, 2005). Platelet-derived CCL5 can also be deposited on the surface of monocytes, and can increase adhesion molecule expression on endothelium, increasing the atherogenic potential (Weber, 2005; Aukrust *et al.*, 2008). It has been shown *in vivo* that injection of activated platelets enhances atherosclerotic lesion formation, attributed to endothelial deposition of CCL5 and CXCL4 (Huo *et al.*, 2003). Interactions between CCL5 and CXCL4 have also been observed to enhance the activity of CCL5 in promoting monocyte arrest on activated endothelium (von Hundelshausen *et al.*, 2005). The release of chemokines including CCL3 and CCL5 in a developing atherosclerotic plaque may also encourage platelet activation (Aukrust *et al.*, 2008). In addition to a role in plaque development, chemokines such as CCL5 may be mediators of plaque destabilization through activation of or release from platelets; however, the limitations of mouse models of plaque rupture restrict research in this area (Aukrust *et al.*, 2008).

It is also possible that CCL5 may mediate a link between two common risk factors for cardiovascular disease, hypertension and obesity, with the development of atherosclerosis. Angiotensin II, which has an established role in hypertension (Kim and Iwao, 2000; Rosendorff *et al.*, 2007), has been shown to stimulate CCL5 mRNA expression in circulating T cells, as well as increasing CCR5-expressing T-cell infiltration into the aorta in mice (Guzik *et al.*, 2007). Antagonism of CCR5 with Met-CCL5 reduced the hypertensive response to angiotensin II in mice and preserved endothelial function, supporting a role for this system in angiotensin II-induced hypertension and vascular dysfunction (Budzyn *et al.*, 2008). Similarly, CCL5 released from epicardial adipose tissue was found to correlate with cardiovascular risk factors (Surmi and Hasty, 2010).

A role for CCL3 and CCL4 acting at CCR5 in atherogenesis is less well defined, but it is likely that these chemokines are also important in atheroma progression and inflammatory cell recruitment into plaques (Reape and Groot, 1999). However, findings from animal models seem to indicate that CCL5 plays a greater role in atherosclerotic plaque development than other CCR5 ligands. Nevertheless, peripheral blood mononuclear cells in patients with coronary artery disease have increased expression of CCL3 and CCL4, which is reduced by statin therapy (Waehre *et al.*, 2003). Gene deletion of CCL3 or CCR5 reduces macrophage expression of MMP-9, a critical enzyme released from macrophages, which is present in atherosclerotic plaques and contributes to atherogenesis (Wu *et al.*, 2009). The alternative receptor for both CCL3 and CCL5, CCR1, does not seem to have a pro-atherogenic role (Zernecke *et al.*, 2006; Braunersreuther *et al.*, 2007), rather it may even be athero-protective (Potteaux *et al.*, 2005). Any role for the third CCL5 receptor, CCR3, in atherosclerosis has yet to be elucidated (Zernecke and Weber, 2010).

Saphenous vein graft disease

Atherosclerotic vascular disease is also a significant clinical problem following coronary artery bypass grafting. Revascularization using aortocoronary grafting is a surgical solution to atherosclerotic heart disease that is successful in alleviation of symptomatic angina, and in prolonging life expectancy for patients with coronary artery disease (Eagle *et al.*, 1999). This operation commonly utilizes the long saphenous vein; however, this conduit is limited by the development of vein graft disease, both early failure caused by thrombosis or later failure as a result of intimal thickening and subsequent atheroma (Motwani and Topol, 1998). After 10 years approximately 60% of grafts remain patent and reoperation is needed in about 10–15% of patients (Sarjeant and Rabinovitch, 2002). Although alternative graft vessels such as the mammary artery are in use, a high rate of multiple vessel disease in patients undergoing surgery necessitates use of the saphenous vein (Eagle *et al.*, 1999). Saphenous vein graft atheroma shares similarities with native vessel atheroma, but is characterized by increased inflammatory cell infiltrate, more diffuse atheroma, little calcification, a poorly developed fibrous cap and greater vulnerability to plaque rupture (Motwani and Topol, 1998; Safian, 2002). Disease progression is also more rapid, leading to the label of 'accelerated atherosclerosis' (Motwani and Topol, 1998). It is therefore plausible that chemokines and their receptors might have a role in vein graft disease and this has begun to be addressed. Broad-spectrum CC chemokine blockade reduced neointima formation in mouse (Ali *et al.*, 2005) and rabbit (Puhakka *et al.*, 2005) models of vein grafting, supporting a role for CC chemokines. More specifically, antagonism of CCR5 and inhibition of the related system CCL2/CCR2 have both been shown to reduce saphenous vein intimal thickening *in vitro* (Schepers *et al.*, 2006; Jones *et al.*, 2009). Furthermore, CCR5 and its ligands CCL3, CCL4 and CCL5 are expressed in retrieved human saphenous vein graft tissue, and localize to plaque smooth muscle cells (Jones *et al.*, 2009). *In vivo*, antisense blockade of the CCL2/CCR2 system has been shown to reduce vein graft disease in mice (Saiura *et al.*, 2004; Schepers *et al.*, 2006; Tatewaki *et al.*, 2007; Eefting *et al.*, 2009). Together, these data indicate a role for CCR5 and its ligands CCL3, CCL4 and CCL5, alongside CCR2 and its ligand CCL2, in the development of vein graft disease. Further support for this theory comes from the observation that CCL2, CCL4 and CCL5 circulate at increased levels in patients undergoing bypass surgery (Castellheim *et al.*, 2008), providing evidence that this system is active at the time of vein implant. Taken together with data showing CCL4 to be a potent constrictor of human saphenous vein, with responses abolished by the CCR5 antagonist maraviroc (Maguire *et al.*, 2008), we speculate that CCR5 ligands could be implicated in the rare postoperative venospasm that can occur following coronary artery bypass graft surgery (Victor *et al.*, 1981).

Potential of the CCR5 receptor as a drug target for cardiovascular disease

Despite emerging evidence for CCR5 in the pathogenesis of atherosclerotic vascular disease, proof-of-concept for CCR5 antagonism has not yet been established in man. Small molecule CCR5 antagonists have been developed as antiretroviral therapeutics, with maraviroc approved for use in treatment-naive and treatment-experienced HIV-infected patients, and other CCR5 antagonists are in development (Dorr and Perros, 2008). CCR5 antagonists are the first anti-HIV drug class to target a host protein rather than a viral molecule, which led to some concerns over the safety of long-term receptor blockade. CCR5 gene knock-out mice have minor defects in their inflammatory response to certain infections (Telenti, 2009), which may be of relevance in immune-compromised HIV patients; however, this is not supported by the reports that homozygous deletions in CCR5 (CCR5delta32) are not detrimental (Dean *et al.*, 1996; Huang *et al.*, 1996; Liu *et al.*, 1996; Samson *et al.*, 1996b; Zimmerman *et al.*, 1997).

The availability of a clinically validated antagonist and accompanying safety data provide a current significant advantage for targeting CCR5 over other chemokine receptors. Although there is much literature to support important roles for other chemokine receptors in atherosclerosis, in

particular CCR2 and CX_3CR1 (Karshovska and Schober, 2008; Weber *et al.*, 2008; Zernecke and Weber, 2010), antagonists for these receptors have yet to be approved for clinical use. Although combined blockade of CCL2, CX_3CR1 and CCR5 was required for almost complete inhibition of atherosclerosis in animal models (Combadiere *et al.*, 2008), studies show that inhibition of CCR5 alone can halt atherosclerotic plaque development in these animals. Given the higher risk of cardiovascular disease in patients with HIV infection (Boccara, 2008), monitoring the cardiovascular effects of CCR5 antagonists such as maraviroc in this group should yield insights into the potential benefits of dual targeting of HIV infection and atherosclerosis with this drug class in man.

The availability of CCR5 antagonists may also facilitate validation of this receptor system as a mediator of vein graft disease. The *ex vivo* nature of vein grafting provides an exciting opportunity to target the initial stages of intimal thickening with the aim of limiting or delaying later disease development. It is clear from the expanding literature that many mediators and pathways are involved in the pathogenesis of vein intimal thickening; therefore, determining the best target(s) for intervention is likely to require further investigations; however, CCR5 is a promising candidate.

It has been suggested that chemokines themselves may provide better disease targets than their receptors, potentially allowing 'rewiring' of the chemokine/receptor network without compromising physiological immunity (Ezerzer and Harris, 2007; Koenen and Weber, 2010). Examples of chemokine modulators used in animal models include the CCL3 binding protein Evasin-1 (Castor *et al.*, 2010), antagonists of CCL5-glycosaminoglycan binding (Braunersreuther *et al.*, 2008) and antagonism of CCL5/CXCL4 heteromers (Koenen *et al.*, 2009). Indeed, cyclic peptide antagonists of the CCL5/CXCL4 interaction are currently undergoing toxicity tests prior to entry into phase I clinical trials (Weber, 2010). Evidence from these studies supports CCL5 as the key CCR5 ligand to target, although Westerweel *et al.* (2008) found that CCL5 was critical in the angiogenic response to ischaemia, suggesting that impaired healing could be an unwanted consequence of chemokine inhibition. The large chemokine/receptor network has led to the suggestion that broad-spectrum chemokine blockade may be the most effective therapeutic strategy (Bursill *et al.*, 2004; 2009), although this risks greater immunological side effects. It remains to be seen whether this approach yields future therapies for human inflammatory diseases.

Conclusion

There is emerging evidence for a role for CCR5 in human cardiovascular disease from both animal models and human studies. The availability of small molecule potent and selective CCR5 antagonists provides an immediate advantage for targeting this receptor over other chemokine receptors, and should facilitate further investigation into this receptor as a drug target for cardiovascular disease. The current use of CCR5 antagonists to treat HIV infection, a condition associated with an increased occurrence of cardiovascular disease, should also be exploited to determine any beneficial cardiovascular effects of this drug class. It is anticipated that the study of cardiovascular parameters, as well as collection of long-term safety data, in this enriched patient group, will inform the potential of CCR5 as a drug target for atherosclerosis in the wider population.

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Conflict of interest

The authors declare no conflict of interest.

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