

Atherosclerotic plaque development: Disease Pathogenesis and emerging treatment options

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Abstract

Atherosclerosis is a process of plaque formation and it manifest into different cardiovascular diseases. Basic mechanism that helps in Artherogenesis are Trans-migration of different granulocytes in the intimal layer of the artery with the help of adhesion molecules expressed in the outer layer of the activated endothelium. Excess circulatory Low Density Lipoprotein (LDL) enters the intimal layer via dysfunctional endothelium and gets oxidized into Oxidized-Low Density Lipoprotein (OX-LDL) and further entrapment of OX-LDL leads to macrophage to foam cell conversion on a gradual feeling on the OX-LDL in the intima. Foam cell formation leads to series of enzymatic Reactive Oxygen Species (ROS), scavenging receptors and chemokine's production and ultimately leading towards the oxidative pathways which end up in the inflammation and further complications leading towards several different cardiovascular diseases.

Keywords: Atherosclerosis, Plaque, Cardiovascular, Artherogenesis, Macrophage, Chemokine, inflammation.

Introduction

Atherosclerosis is a gateway to several cardiovascular diseases (CVDs) and it is a major cause of deaths throughout the globe (Murray and Lopez, 1997). By 2015 there were estimated 17.7 million deaths reported due to CVDs which corresponds to 31 % of all global deaths. Out of all the different CVDs major deaths were due to stroke (6.7 million deaths) and due to coronary heart disease (WHO CVDs report, 2017). Atherosclerosis means hardening and at the same time narrowing of the arteries and the major factor behind this is atherosclerotic plaque formation. Atherosclerotic plaques are basically deposits which are made up of cholesterol, fatty substances, cellular waste products, calcium and fibrin (a clotting material in the blood) (Zaman *et al.*, 2000). Coronary arteries supply oxygen to heart with the help of circulatory system. When there is a plaque buildup in the arterial wall it leads to less oxygen supply to the cardiac muscle which initially leads to vomiting, anxiety, angina, coughing and feeling faintness. On a regular interval of time due to the lack of oxygen supply to the cardiac muscle cell may lead to myocardial ischemia, which results in the myocardial cell death and ultimately give rise to cardiac arrest (Libby, 2002). Plaque built up in the carotid artery may give rise to less blood supply to the brain. It may causes weakness, dyspnea, headache, facial numbness, paralysis and it can turn out to be as lethal as stroke (Elkind, 2006). The coronary artery wall consists

of three layers. The inner layer is called intima, the middle layer is called media, and the outer one is denoted the adventitia (Davis *et al.*, 1988). According to the hypothesis of endothelial dysfunction, Elevated amount of circulatory LDL (Low Density Lipoprotein) in the lumen enters the intimal layer which is the middle layer in the arterial wall with the help of dysfunctional endothelial cells and it return the dysfunctional endothelial cells release several Reactive Oxygen Species (ROS) as well as several metalloprotease results in the oxidation of LDL to OX-LDL (Oxidized-LDL) which in turn leads to the activation of the endothelial cells (Incalza *et al.*, 2017). Activated endothelial cell express receptors/adhesion molecules for the white blood cells (WBC's) on their surface and this helps in the migration of monocytes and T-helper cells into the intimal layer (Schnoor *et al.*, 2015). After migration of the Monocytes into the intimal layer in get differentiated into macrophage and starts to feed on OX-LDL and gradual accumulation of OX-LDL gives rise to the foam cell formation. Foam cells significantly contribute in the pathogenicity of atherosclerosis in many different ways and the most important role involves the release of Insulin-like growth factor-1 (IGF-1) which helps in the migration of the smooth muscle cells (SMC's) from the tunica media to the intimal layer and its proliferation (Sukhanov *et al.*, 2015). Foam cells secrete pro-inflammatory cytokines such as interleukins: IL-1, IL-6; tumour necrosis factor (TNF); chemokines: chemokines ligand 2, CCL5, CXCL1; as well as macrophage retention factors to the lumen which helps attract more macrophages to enter the intimal layer (Fatkhullina *et al.*, 2016). High number of SMC's indirectly promotes the synthesis of collagen formation which in turn leads to the hardening of the atherosclerotic plaque itself (Rocnik *et al.*, 1998). High numbers of foam cells accumulation and its gradual death leads to high lipid content release. The foam cell debris involves the genetic material which majorly attracts neutrophil cells (Döring, Y. *et al.*, 2015). Simultaneously due to released lipids, pro-inflammatory cytokines and ROS which leads to inflammation in the plaque area of the artery. The vasa vasorum is a network of small blood vessels that increase the blood supply to the layer of the tunica intima (Xu *et al.*, 2015). Meanwhile the T-cells exploits the adhesion receptors present in the endothelial cells and get activated by the residing macrophages and release substances such as Interferon gamma (IFN γ) which enhances the level of inflammation and attracts more white blood cells by further activation of the endothelial cells (Moss and Ramji, 2015). As the atherosclerotic plaque grows bigger in size it generates tremendous pressure in the arterial walls which ultimately results in the plaque rupture which is also known as thrombosis (Schnoor, *et al.*, 2015). Thrombosis is a process in which the coagulation process gets activated to stop the plaque from spilling out into the lumen and gives rise to thrombus or a clot which restrains the normal blood circulation of the artery (Otsuka *et al.*, 2016).

Atherosclerosis risk factors and indicators

The exact causes and risk factors of atherosclerosis are unknown; however, certain conditions, traits, or habits may raise the chance of developing atherosclerosis. Most risk factors including high cholesterol and LDL, low level of high density lipoprotein (HDL)

in the blood, hypertension, tobacco smoke, diabetes mellitus, obesity, inactive lifestyle, Age can be controlled and atherosclerosis can be delayed or prevented. (Ross, 1993; Owen *et al.*, 2011; Weber *et al.*, 2011)

Cholesterol increase

Cholesterol is a hydrophilic lipid that is progenitor of steroid hormones such as corticosteroids, sex hormones, bile acids, and vitamin D. Cholesterol is one of the major component of cell membrane. Half of the body's cholesterol is provided by synthesis, mainly in the liver of mammals while all tissues containing nucleated cells are able to synthesize cholesterol. (Ohara *et al.*, 1995; Corsini *et al.*, 1996; Nasri, 2013).

Homocysteine

There is a substantial evidence to prove increased plasma homocysteine levels as a risk factor for atherosclerotic vascular disease (Mayer *et al.*, 1996). Homocysteine is a thiol-containing amino acid intermediate formed during the metabolism of methionine, an essential amino acid. In healthy persons, plasma homocysteine levels are between 5 and 15 $\mu\text{mol/L}$ in the fasting state (Ueland *et al.*, 1993). Increased homocysteine plasma levels have been associated with aging, (Kang *et al.*, 1986) menopause (Jacobsen *et al.*, 1994) chronic renal insufficiency (Chauveau *et al.*, 1993), low plasma levels of vitamin cofactors (B_6 , B_{12} , and folate) (Ubbink *et al.*, 1993) and cardiac transplantation (Berger *et al.*, 1995).

Impaired fibrinolysis

Fibrinolytic system consists of plasminogen, which is converted to its active form plasmin by plasminogen activators, including Tissue plasminogen activator (tPA). One of the Inhibitors of this system is plasminogen activator inhibitor type 1 (PAI-1) and plasmin inhibitor such as α_2 -antiplasmin. In the Northwick Park Heart Study, fibrinolytic activity was measured by dilute blood clot lysis time at study entry in 1382 men, age 40 to 64 years, of which 179 subsequently experienced episodes of CAD during a mean follow-up of 16.1 years (Meade *et al.*, 1993). In the men who were 40 to 54 years old, impaired fibrinolysis was associated with a significantly increased risk of CAD ($P=.002$), even after adjustment for plasma fibrinogen. No significant association was noted in older men (Ridker *et al.*, 1994).

Diabetes

Elevated glucose levels may contribute to the development of atherosclerosis in people with diabetes, independent of other risk factors (Selvin *et al.*, 2005). Atherosclerosis is the cause of a majority of cardiovascular events, and atherosclerosis is accelerated by diabetes and the metabolic syndrome. Many risk factors are associated with the metabolic syndrome and help explain the increased cardiovascular disease (CVD) in that condition (Reilly *et al.*, 2003). Because the metabolic syndrome occurs in most people with type-2 diabetes, its presence likely accounts for most of the increased incidence of CVD in type-

2 diabetes (Alexander *et al.*, 2003). However, the presence of diabetes increases the risk of CVD beyond that seen with the metabolic syndrome alone (Alexander *et al.*, 2003). Moreover, CVD risk is increased in type 1 diabetes (Dorman *et al.*, 1984), in which the presence of the metabolic syndrome and these other risk factors is less common. Type-1 diabetes (T1DM) and obesity are main risk factors for cardiovascular events (Burke *et al.*, 2008; Margeirsdottir *et al.*, 2008). In particular, young adults with T1DM have an increased risk of early asymptomatic atherosclerosis and consequent cardiovascular morbidity and mortality (Jørgensen *et al.*, 2005; Larsen *et al.*, 2005; Orchard *et al.*, 2006). Similarly, childhood obesity has been reported associated with biochemical and inflammatory factors that affect vascular endothelial function and that might confer a premature atherogenicity (Aggoun *et al.*, 2007). Moreover, the insulin resistance, key feature of obesity, metabolic syndrome and type-2 diabetes, results in an array of metabolic and vascular events which finally promote the development of atherosclerosis (Cubbon *et al.*, 2009).

Hypertension

Hypertension is a major risk factor in cardiovascular diseases and stroke. These complications are generally caused by high diastolic blood pressure. Hypertension damages endothelium by increasing the hemodynamic pressure on endothelium and may increase the permeability of arterial walls for lipoproteins. Elevated angiotensin II concentration stimulates SMC growth, increases inflammation and finally accelerates LDL oxidation in such patients. (Asgary *et al.*, 2013; Asgary *et al.*, 2014). Arterial chronic hypertension (HTN) is one of the established cardiovascular risk factors for development of atherosclerosis (Dzau, 1990) and an increased incidence of peripheral vascular disease (Murabito *et al.*, 1997), cerebrovascular disease (Lewington *et al.*, 2002). Although, the complications of hypertension were formerly attributed to diastolic blood pressure, there is much evidence showing that systolic blood pressure plays a role as well.

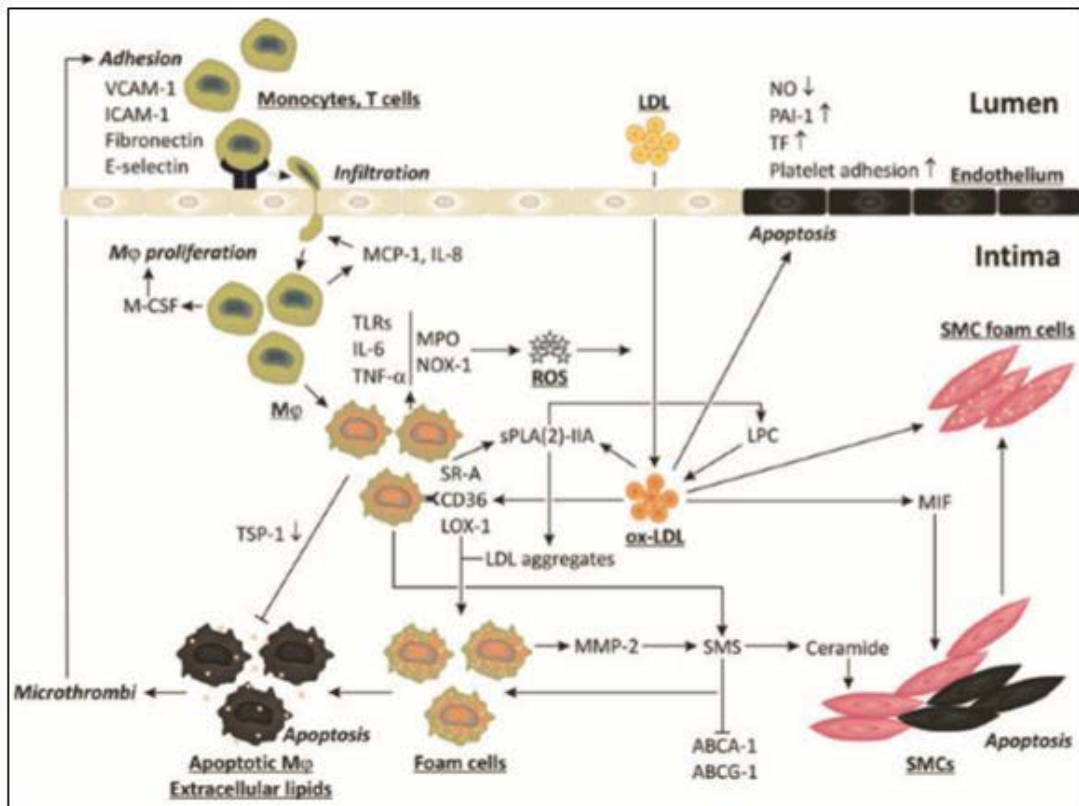


Figure 1: Molecular mechanisms involved in both oxidative as well as inflammatory pathways in the atherogenesis. (Picture courtesy: (Hulsmans and Holvoet, 2010))

The mechanism with which hypertension can accelerate atherosclerosis is still unknown; however, in animals fed with high fat, hypertension accumulates the fatty substances inside the arterial walls. (Khajehdehi *et al.*, 2012; Asgary *et al.*, 2013; Madihi *et al.*, 2013; Asgary *et al.*, 2014; Hajivandi, 2014).

Molecular mechanisms of oxidative stress in atherosclerotic plaques

Activated endothelial cells express adhesion molecules such as ICAM-1, VCAM-1, E-selectin and fibronectin leads to transcytosis and activation of inflammatory cells in to the intimal layer and in turn it helps in the expression of enzymes such as Myeloperoxidase, xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and nitric oxide synthase (Burtenshaw *et al.*, 2019). The consequence for this results in the production of ROS which in turn oxidises the phospholipids and the LDL into OX-LDL and its entrapment into the intimal layer. This sudden outburst of oxidative stress leads to further expression of chemokine's such as Monocyte chemo attractant protein-1 and Interleukin 8 which attracts primarily neutrophils and other granulocytes (Hulsmans and Holvoet, 2010). The existing macrophage in the intimal layer release macrophage colony-stimulating factor and help in its proliferation and simultaneously

express the scavenging receptors such as cluster of differentiation 36 (CD36), Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and macrophage scavenger receptor (SR-A). These receptors help in the further accumulation of the OX-LDL & cholesterol by the macrophage and get converted to foam cells, finally resulting in apoptotic macrophages and exposure of thrombogenic lipids. Deficient TSP-1 expression is associated with a decreased phagocytosis of dead cells. Matrix metalloproteinases (MMPs) & Spingomyelinases (SMS) are expressed by the foam cells induces cell death of the smooth muscle cells by excess production of Ceramide (Bioactive lipid molecule). ATP-binding cassette transporter (ABCA-1) & ATP binding cassette subfamily G member 1 function get altered by the SMS results in the impairment in the cholesterol and lipid efflux from foam cells. Sheer pressure at the site of plaque formation also promotes the production and release of ROS (Leopold and Loscalzo, 2008). Oxidative stress in humans with coronary artery disease is also exacerbated by a reduction of vascular extracellular superoxide dismutase, normally an important protective enzyme against the superoxide anion (Madamanchi *et al.*, 2005).

Role of inflammation in atherosclerosis

Inflammation works in all the different stages of atherosclerotic plaque development and rapture. Ox-LDL induces TLRs of which the ligands enhance the expression of inflammatory mediators IL-6 and TNF-. Inflammation has its significant role in giving rise to a vulnerable plaque from its stable form by weakening the atherosclerotic fibrous cap and allowing the plaque to rapture quickly (Stefanadis *et al.*, 2017).

Table 1: Critical Enzymes involved in pathogenesis of atherosclerosis

Sl#	Gene name	Enzyme name	Pathogenecity in atherosclerosis	References
1	<i>MMP3</i>	matrix metallopeptidase 3	K/O-Restricts VSMC migration	(Johnson <i>et al.</i> , 2016)
2	<i>MMP9</i>	matrix metallopeptidase 9	k/O- Restricts VSMC migration and plaque rapture	(Johnson <i>et al.</i> , 2016)
3	<i>PLAU</i>	plasminogen activator, urokinase	activate plasmin & MMP9	(Johnson <i>et al.</i> , 2016)
4	<i>NOS3</i>	Nitric Oxide Synthase 3	oxidative stress in vascular wall by NO	(Channon <i>et al.</i> , 2000)
5	<i>MAPK1</i>	Mitogen-activated protein kinase 1	p38 MAPK pathway activation: oxidative stress & Inflammation	(Reustle and Torzewski, 2018)
6	<i>PONI</i>	Paraoxonase 1	Reduce macrophage related oxidative stress	(Farid and Horii, 2012)
7	<i>MTHFR</i>	methylenetetrahydrofolate reductase	677C-T mutation MTHFR is related to plasma total homocysteine levels	(Verhoef <i>et al.</i> , 1997)
8	<i>mTORC1</i>	mechanistic target of Rapamycin kinase C1	Inhibition leads to reduced chemokines & HIF 1 α production	(Kurdi <i>et al.</i> , 2015)
9	<i>iNOS</i>	Inducible NO synthase	Inhibition leads to reduced inflammation	(Detmers <i>et al.</i> , 2000)
10	<i>AKT1</i>	AKT serine/threonine kinase 1	loss of AKT1 reduces eNOS phosphorylation, Nitric oxide release, VSMC migration	(Hernando <i>et al.</i> , 2007)
11	<i>MPO</i>	Myeloperoxidase	induces endothelial dysfunction, LDL oxidation to OX-LDL	(Kamanna <i>et al.</i> , 2013)
12	<i>PARP1</i>	Poly(ADP-ribose) polymerase 1	Pharmacological inhibition leads to reduction in AS development	(Xu <i>et al.</i> , 2014)
13	<i>SOD2</i>	Superoxide Dimutase 2	Protect from oxidative stress in AS	(Vendrov <i>et al.</i> , 2017)
14	<i>DPP4</i>	Dipeptidylpeptidase-4	Inhibition of DPP4 reduces atherosclerosis and CVD risk	(Aroor <i>et al.</i> , 2018)
15	<i>SRC</i>	Src Tyrosine Kinase	lipid oxidation and AS	(Reddy <i>et al.</i> , 2009)
Abbreviations : K/O- knockout for the gene, AS- Atherosclerosis , CVD- Cardiovascular Disease, VSMC- Vascular Smooth Muscle Cells,				

Ox-LDL induces migration inhibitory factor that stimulates SMC migration. The uptake of ox-LDL by SMCs leads to the production of SMC foam cells and secretion of MMPs that degrade the extracellular matrix proteins rendering the plaque more prone to

rupture. OxLDL stimulates platelet adhesion and aggregation by decreasing endothelial production of nitric oxide, and enhances the pro-coagulant activity of endothelium by inducing the release of tissue factor. Ox-LDL reduces the fibrinolytic activity of endothelium by increasing the release of plasminogen activator inhibitor-1. Finally, ox-LDL induces apoptosis in endothelial cells (black) contributing to plaque erosion and rupture (Hulsmans and Holvoet, 2010). Lipid-laden macrophages in atherosclerotic plaques express MMPs. various extracellular stimuli, including reactive oxygen species, plasmin, and thrombin, contribute to enzymatic activation of these enzymes that is required for their proteolytic activity (Libby, 2013).

Key players involved in both oxidative stress & Inflammation in atherosclerosis

Oxidative stress and inflammation are the key factor in orchestrating atherosclerosis and ultimately leads its way to cardiovascular complications. *National Center for Biotechnology Information* (NCBI) is one of the largest sources for biological information. 1142 genes were considered which are involved in atherosclerosis and have a significant role in inflammation (www.ncbi.nlm.nih.gov/gene/?term=atherosclerosis+and+inflammation) similarly for oxidative stress and its role in atherosclerosis 672 genes were considered (www.ncbi.nlm.nih.gov/gene/?term=atherosclerosis+and+oxidative+stress). Out of which there are 444 genes which work in both atherosclerosis as well as inflammation (Figure 2). Among the common link between both atherosclerosis as well as inflammation there are several regulatory protein, proteins involved in different stress pathways, wound healing, immune responses, and have several different other biological functions but out of the of them 125 major enzymes that play significant role in the pathogenicity process were found. Either up regulation or down regulation of these enzymes may lead to significant change in the disease state, hence these can be potential target for future drugs development for CVD. Out of those 125 enzymes the most significant ones are depicted in the Table 1.

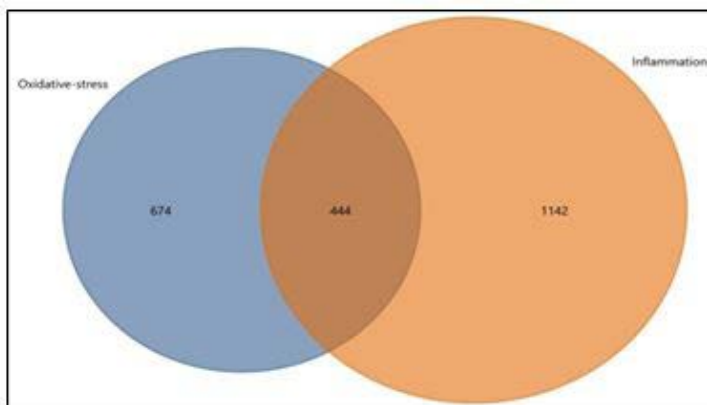


Figure 2: Venn diagram for the set of genes which are involved in oxidative stress & atherosclerosis in blue (674) and the set of genes involved in inflammation & atherosclerosis are in orange (1142) and the intersection of genes (common one's) which are involved in both oxidative stress as well as inflammation (444).

Treatment options and emerging therapy for atherosclerosis

Among the established therapies, the use of Statins and Astrovastatin leads to primary and secondary control of the disease through inhibition of cholesterol synthesis and acting as an anti-inflammatory agent. Aspirin, clopidogrel, prasugrel and ticagrelor inhibits platelet aggregation, thereby controlling the secondary progression of the disease. Among the emerging therapies, HDL mimetics, for example, apoA1-Milano, promotes cholesterol efflux and acts as an anti-inflammatory agent. The drugs are in Clinical phase 1 and 2 trials. Methotrexate acts as an immunosuppressive and this drug is currently under Clinical phase 3 trials. Again, there are a number of novel therapies that are showing promise, for example blocking the CD40-TRAF6 interaction site limits the atherosclerosis of unstable phenotype in mice. CCL17 inhibition interestingly attenuates lesion formation in mice (Table 2).

Table: 2: Treatment options for atherosclerosis and their outcome

Compound or Method	Mechanism Involved	Outcome	References
Established Therapies			
Statins, for example, atorvastatin and rosuvastatin	Inhibit cholesterol synthesis, anti-inflammatory	Primary and secondary prevention	Ray and Cannon (2005); Nissen (2005) and Ridker <i>et al.</i> (2009)
Nicotinic acid (niacin)	Inhibits fat breakdown in adipose tissue and increases HDL cholesterol, anti-inflammatory	Secondary prevention	Taylor <i>et al.</i> (2009); Lukasova (2011)
Aspirin, clopidogrel, prasugrel, ticagrelor	Inhibit platelet aggregation	Secondary prevention	Wallentin <i>et al.</i> (2009); von Hundelshausen and Weber (2007)
β-blockers	Antihypertensive	Secondary prevention	Sipahi <i>et al.</i> (2007)
Renin-angiotensin system inhibitors	Antihypertensive	Secondary prevention	Yusuf <i>et al.</i> (2008)
Emerging Therapeutic approaches			
HDL mimetics, for example, apoA1-Milano	Promote cholesterol efflux, anti-inflammatory	Clinical phase 1 and 2	Nissen <i>et al.</i> (2003); Navab <i>et al.</i> (2010)
Darapladib (selective Lp-PLA2 inhibitor)	Decreases atherogenic lipid production	Clinical phase 3	Wilensky <i>et al.</i> (2008); Wilensky <i>et al.</i> (2008)
IL-1ra (IL-1 receptor antagonist)		Clinical phase 2	Klingenberg and Hansson (2009)
Methotrexate	Immunosuppressive	Clinical phase 3	Klingenberg and Hansson (2009)

Novel experimental Strategies			
Blocking the CD40-TRAF6 interaction site	Impaired recruitment of Ly6C ⁺ monocytes to the arterial wall, and polarization of macrophages toward an antiinflammatory regulatory M2 signature	Limits atherosclerosis of unstable phenotype in mice	Lutgens <i>et al.</i> (2010)
Blocking MIF receptor binding	Impaired monocyte adhesion to the arterial wall in atherosclerosis-prone mice	Induces lesion stabilization and regression in mice	Bernhagen <i>et al.</i> (2007); Weber <i>et al.</i> (2008); Kraemer <i>et al.</i> (2011)
Maraviroc (CCR5 antagonist)	Blocking CCR5	Approved for US and European markets for HIV treatment	Koenen and Weber (2010)
MLN1202 (CCR2-specific antibody)	Blocking CCR2	Clinical phase 2	Gilbert <i>et al.</i> (2011)
Nonagonistic CCL2-competing mutant PA508	Nonagonistic plus increased proteoglycan Affinity	Attenuates lesion formation in mice	Liehn <i>et al.</i> (2010)
Dominant-negative CCL5 mutant [44AANA47]	Creates dimers devoid of proteoglycan binding	Attenuates lesion formation in mice	Braunersreuther <i>et al.</i> (2008)
Mkey (ct-2009)	Disrupts CCL5-CXCL4 heteromerization	Attenuates lesion formation in mice	Koenen, <i>et al.</i> (2009)
Immunization	Protective antibody generation, Treg cell induction	Can attenuate lesion formation in mice	Ludewig <i>et al.</i> (2000); Johansson <i>et al.</i> (2010); Habets <i>et al.</i> (2010); Hermansson <i>et al.</i> (2011); Klingenberg <i>et al.</i> (2010)
CCL17 inhibition	Supports Treg cell homeostasis	Supports Treg cell homeostasis	Weber <i>et al.</i> (2011)

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