Crosstalk between apoptosis and inflammation in atherosclerosis

Westra, M.M.

Chapter 1

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1 Atherosclerosis and cardiovascular disease

Atherosclerosis can be defined as a multifactorial, progressive disease of medium and large sized arteries which sets off already in childhood[1] and is characterized by accumulation of lipid material and fibrous components in the artery wall[2]. Atherosclerosis is the pathophysiological cause of the majority of cardiovascular disease including myocardial infarction, angina pectoris and stroke. Most clinical complications are caused by plaque disruption and subsequent thrombus formation[3,4]. Its onset and progression was seen to associate with both environmental risk factors like smoking, high-fat diet and lack of exercise and factors with a strong genetic component like hypertension, hyperlipidemia, diabetes and male gender[5-8]. Therapies are mostly based on reducing these risk factors, such as lowering serum lipid levels using statins, lowering blood pressure and life style changes or consist of surgical intervention such as bypass surgery, percutaneous transluminal coronary angioplasty (PTCA) and stenting although the effectiveness of the latter interventions is often impaired by the recurrent narrowing of the vessel, a process referred to as restenosis[9]. Despite the available treatments, atherosclerosis continues to be one of the main causes of death in the world.

2 Pathogenesis of atherosclerosis

2.1 Leukocyte adhesion and migration

In the normal, healthy arterial wall the endothelium covers a layer of smooth muscle cells and produces various factors controlling vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, inflammation of the vessel wall and vascular remodeling[10]. Atherosclerotic plaques start as fatty streaks at specific predilection sites within the arterial tree, such as bifurcations and branches[1,2]. The first step herein lies in dysfunction of the endothelium due to increased turbulence or decreased shear stress often combined with aspects of the above mentioned risk factors[1,2]. As a result the expression by endothelial cells of adhesion and inflammatory molecules, essential in the recruitment of leukocytes, is increased[11]. The initial tethering and rolling of circulating leukocytes (monocytes and lymphocytes) is mediated by selectins, L-selectin expressed on circulating leukocytes and P-selectin and E-selectin on the activated endothelium, resulting in further leukocyte activation[12,13]. Subsequently firm adhesion of leukocytes requires the engagement of β_1 and β_2 integrins, e.g. VLA4 and CD18/CD11, which interact with upregulated intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expressed by endothelial cells[14,15]. Functional roles for ICAM-1 and both E-selectin and P-selectin in atherogenesis have been confirmed by gene deletion studies in mouse models for atherosclerosis, the ApoE and LDLr deficient mouse[16,17]. Transmigration of leukocytes into the subendothelial space is the final step in plaque initiation, a process also known as diapedesis. Various endothelial cell expressed molecules facilitate transmigration, such as platelet/endothelial-cell

adhesion molecule 1 (PECAM1), junctional adhesion molecule A (JAM-A), endothelial cell-selective adhesion molecule (ESAM), ICAM2 and CD99[18-22]. In addition to adhesion molecules chemokines are critically involved in the adhesion and migration of leukocytes[23]. Regarding lesion initiation chemokine receptor CCR2 and its ligand monocyte chemoattractant protein 1 (MCP1) are considered the most important. Deletion of MCP1 in LDLr$^{-/-}$ mice and (leukocyte) CCR2 in ApoE$^{-/-}$ or ApoE3 Leiden mice all resulted in significantly reduced atherosclerosis development[24-26]. Once migrated into the intima, monocytes differentiate into macrophages in response to macrophage-colony stimulation factor (M-CSF) secreted by endothelial cells and vascular smooth muscle cells (vSMC) and contribute to plaque progression[2]. Figure 1 shows a schematic overview of the processes described above.

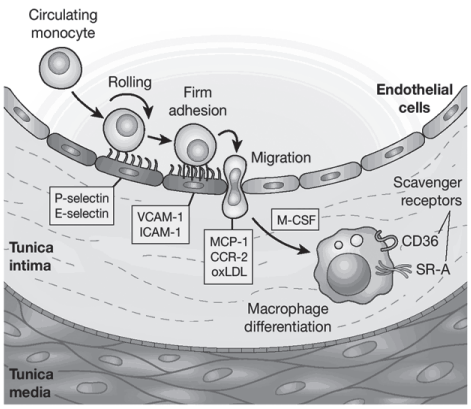

Figure 1. Atherosclerotic plaque initiation. Selectins mediate the first cell-cell interactions enabling capture, tethering and rolling of circulating monocytes. Once captured, integrins (interacting with ICAM-1 and VCAM-1) mediate the firm adhesion of monocytes to the endothelium after which they migrate into the subendothelial space along a chemokine gradient. Here they differentiate into macrophage under the influence of M-CSF and increase the expression of scavenger receptors. Adapted from Li and Glass[175].

2.2 Plaque progression and instability

Fatty streaks do not cause clinical symptoms but may progress to more complex plaques. They are characterized by continuous influx of inflammatory cells (macrophages and lymphocytes) and lipids into the vessel wall. Low-density-lipoprotein (LDL) within the intima can be modified by oxidation and aggregation[27-29]. In turn, these modified LDL particles and entrapped cholesteryl esters can be taken up by macrophages which have increased expression of scavenger receptors due to M-CSF stimulation[30]. As a result of this progressive accumulation of lipids, macrophages will convert into foam cells. Differentiated macrophages and infiltrated T lymphocytes will augment the inflammatory response by secreting growth factors and cytokines[31]. Formation of a more complex fibroatheromathous lesion involves the migration of vSMC from the vessel wall into the intima and vSMC proliferation under the influence of growth factors secreted by endothelial cells and macrophages. VSMC synthesize the bulk of the extracellular matrix such as collagen, elastin and proteoglycans within the plaque in response to transforming growth factor (TGF) β and platelet derived growth factor (PDGF). VSMC and extracellular matrix proteins form a fibrous cap overlying the lipid core[32]. Augmentation of the inflammatory response, vSMC migration and formation of a fibrous cap cause the initial fatty streak to develop into an advanced atherosclerotic lesion narrowing the

vessel lumen.
As the atherosclerotic plaque progresses a necrotic core is formed consisting of accumulated lipids and cell debris derived from apoptotic or necrotic cells. Whereas stable advanced lesions have a dense fibrous cap overlying this necrotic core, the potentially dangerous plaques, responsible for the majority of clinical manifestations, are unstable as a result of cap thinning which makes a plaque vulnerable to rupture and thrombus formation[33]. Several factors contribute to the progressive destabilization and thrombogenicity of atherosclerotic plaques. A large lipid core[34], accumulation of inflammatory cells[35], extracellular matrix degradation[36,37] and plaque cell death[38,39] comprise the most important contributors. In addition intraplaque hemorrhage has been proposed to be a critical factor in plaque destabilization[35]. Fibrous cap thinning and plaque inflammation in regard to lesion progression and destabilization will be discussed in more detail in the following sections.

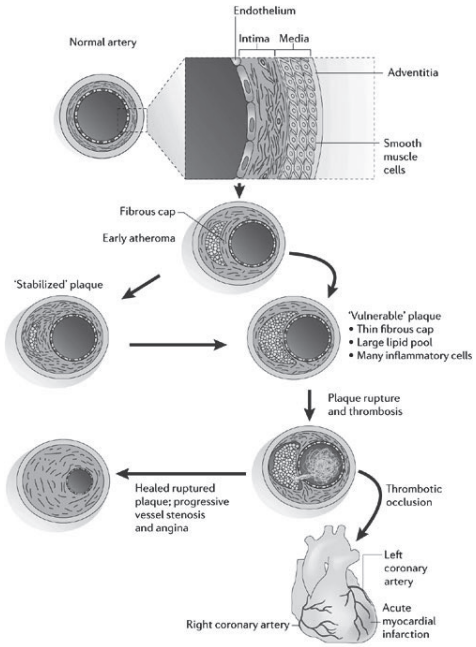

Figure 2.Atherosclerotic plaque progression from early atheroma to myocardial infarction. Early atheroma can progress into a stable fibrous plaque characterized by a small core and thick fibrous cap. Alternatively a vulnerable plaque develops with a large core containing lipids and cell debris, a high inflammatory cell content and a thin fibrous cap. Vulnerable plaques may rupture resulting in the formation of a thrombus. Ruptured plaques can either heal following vSMC migration and extracellular matrix production or result in myocardial infarction. Adapted from Watkins and Farrall[176].

3 The role of vascular smooth muscle cells in atherosclerosis

Vascular smooth muscle cells (vSMC) are one of the major cellular constituents of the atherosclerotic plaque. Evidence shows that intimal vSMC differ from medial vSMC in many aspects. Medial vSMC are predominantly of the contractile phenotype while most intimal vSMC have characteristics of the synthetic, migratory phenotype. This phenotypic switch can be induced by a variety of atherogenic stimuli like cytokines, shear stress, reactive oxygen species (ROS) and lipids. Synthetic vSMC migrate and proliferate better than contractile vSMC and synthesize more collagen[41]. VSMC migration can be triggered by various growth factors and chemokines secreted by

macrophages and T cells like platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and transforming growth factor (TGF) β, monocyte chemoattractant protein (MCP) 1 and stromal cell-derived factor (SDF) 1α[1,42,43].

VSMC, like macrophage, are able to ingest lipids and form foam cells. They express several receptors involved in (modified) lipoprotein uptake including the LDL receptor, CD36, type I and type II scavenger receptors and SR-PSOX[44-47]. Furthermore, adhesion molecules like vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) have been demonstrated to be expressed by vSMC, these may enable them to increase monocyte adherence and infiltration into the atherosclerotic lesion[48.] The mechanisms and consequences of adhesion of leukocytes to vSMC *in vivo* however are not well characterized. Furthermore, intimal vSMC have been reported to produce a wide variety of growth factors and cytokines, including PDGF, TGFβ, MIF and MCP-1, contributing to the pro-inflammatory environment of the atherosclerotic lesion[41].

VSMC play a crucial role in fibrous cap formation and preserving plaque stability. Unstable plaques prone to rupture contain a higher macrophage and lipid content and a thinned fibrous cap due to loss of vSMC and extracellular matrix. The strength of the fibrous cap seems to depend on a balance between collagen synthesis and breakdown and on the type of collagen. Expression of genes promoting collagen synthesis by vSMC and of matrix metalloproteinases (MMPs), important in the breakdown of extracellular matrix, can be influenced by inflammatory cytokines[49]. For instance, TGFβ enhances the ability of vSMC to produce collagen, while TNFα, IL1 and IFNγ suppress collagen content either directly or by inducing MMPs[50-52]. In addition MMP expression was shown to be elevated in atherosclerotic plaque in comparison to normal vessels, a result of both inflammatory cytokine production and oxidative stress[33]. MMP activity is balanced by tissue inhibitors of metalloproteinases (TIMPs), MMP specific inhibitors expressed by vSMC. Expression of TIMPs can be either constitutive or upregulated by TGFβ and PDGF[53].

Apart from MMPs, cathepsins which are cysteine proteases, can degrade the extracellular matrix[54]. Cathepsins are secreted by macrophages and their expression is increased in atherosclerotic lesions compared to healthy arteries[55]. Comparable with MMPs, cathepsin activity can be inhibited by a family of proteins, the cystatins of which cystatin C is best described. As opposed to cathepsins, expression of cystatin C is decreased in atherosclerotic lesions[55,56].

Another role for vSMC may lay in the healing of fibrous cap breaks that remain subclinical. Mediators released at sites of thrombosis, for example PDGF and TGFβ released by platelets, can stimulate vSMC migration, mitogenesis and production of collagen, thus promoting a fibrous lesion morphology[49]. A thrombus caused by plaque rupture that doesn't occlude the vessel is reorganized and incorporated into the plaque. Recurring incidents of plaque rupture and healing can be visible in plaques[57,58].

4 Inflammation in atherosclerosis

Monocyte infiltration contributes largely to plaque initiation. Stimulation with M-CSF secreted by endothelial cells and vSMC, causes the infiltrated monocytes to differentiate into macrophages and induces expression of scavenger receptors and cytokine production[59-60]. Macrophages are able to take up cell-activating modified LDL, mainly oxidized LDL (Ox-LDL) via several scavenger receptors including type 1 and 2 scavenger receptor A (SRA), CD36, CD86, MARCO (macrophage receptor with a collagenous structure), SR-PSOX (scavenger receptor that binds phosphatidylserine and oxidized lipoprotein) and lectin-like oxidized low density lipoprotein receptor 1 (LOX-1)[61-65]. Uptake of modified lipoproteins by scavenger receptors not only leads to the formation of foam cells but also results in macrophage activation. Subsequently, activated macrophages produce inflammatory cytokines, growth factors, proteases and reactive oxygen species influencing endothelial cell activation, vSMC migration, proliferation and collagen production and T cell activation[35]. Expression of scavenger receptors can be influenced by various cytokines present in the plaque including TNFα, IFNγ, IL4 and TGFβ[66-68]. TGFβ was shown to inhibit foam cell formation[68].
Uptake of modified lipoproteins via macrophage scavenger receptors can result in MHC restricted antigen presentation to T cells[69]. T cells are recruited into the lesion by mechanisms similar to the recruitment of monocytes. The majority of lesional T cells are CD4+ effector cells although CD8+ cells are present as well[70]. The role of lymphocytes in atherosclerosis has been studied using $RAG^{-/-}$ mice lacking T and B cells. In $ApoE^{-/-}$ mice lymphocyte deficiency results in the development of smaller lesions[71,72] while transfer of CD4+ T cells into immunodeficient (scid/scid) $ApoE^{-/-}$ mice aggravated atherosclerosis[73]. Several antigens have been associated with atherosclerosis. An important group of antigens consists of altered self molecules. T cells within the atherosclerotic lesions have been shown to respond to *Chlamydia pneumoniae* related antigens and stress-induced heat shock protein (HSP) 60[70]. Apart from Ox-LDL which is recognized by T cells present in human plaques[74] peptides derived from modified LDL components, for example apolipoprotein B and phospholipids can serve as antigens in atherosclerotic plaques[70]. CD4+ T cells can be subdivided in several T helper (Th) cell subsets based on their cytokine secretion profile, e.g. Th1 cells (which produce IFNγ and TNFα), Th2 cells (producing IL4, IL5 and IL13) and regulatory T cells (IL-10 and TGFbeta)[70]. Mouse and human studies have demonstrated a predominant pro-inflammatory Th1 cytokine pattern in atherosclerotic plaques[75,76]. IL2 and IFNγ were shown to be abundantly present whereas only small amounts of Th2 cytokines IL4 and IL5 have been found in plaques. Mouse studies have demonstrated that IL12 and IL18, both Th1 inducing cytokines, have pro-atherogenic properties[77-81] as do Th1 cytokines IFNγ[82,83] and TNFα [84,85], while the role of Th2 cytokines is less clear. IL4 was demonstrated to be atheroprotective[78,86] but deficiency of IL5 increased atherosclerosis[87].
Production of cytokines by macrophages and lymphocytes in the plaques does not only influence inflammatory processes but also modulates smooth muscle

cell activity. IFNγ inhibits smooth muscle cell proliferation[88] and the production of collagen, whereas TGFβ stimulates collagen production[89]. In addition TGFβ downregulates the expression of MMPs, collagen degrading proteins[90], while macrophages are stimulated to produce MMPs by TNFα and IL1[91]. Finally TNFα and IFNγ can promote the uptake of modified lipoproteins by smooth muscle cells leading to smooth muscle cell derived foam cells[92].

In addition to macrophages and T cells other inflammatory cell types have been demonstrated to be involved in atherosclerosis, including B cells, dendritic cells, mast cells and neutrophils. Although few B cells are present in the plaque the majority is located in the adventitia[70]. B cell associated immunity was shown to be protective in atherosclerosis as splenectomy increased plaque development in $ApoE^{-/-}$ mice while transfer of spleen derived B cells counteracted this effect[93]. Dendritic cells are the most potent antigen presenting cells. They are present in healthy vessels but accumulate during atherogenesis, being mainly localized in the rupture prone shoulder areas[94]. Skin dendritic cells have been shown to be activated by dislipidaemia with surprising inhibition of migration into lymph nodes suggesting that they contribute to local inflammation[95]. However a recent study by Packard *et al.*[96] found opposing results. Here, dendritic cells were demonstrated to maintain their antigen presenting function and ability to prime $CD4^+$ T cells in vitro under hypercholesterolemic conditions[96]. Mast cells are present in the atherosclerotic plaque and were shown to accumulate in the shoulder region[97]. Activated mast cells secrete cytokines and proteases and mast cell derived TNFα and IL6 were shown to promote atherosclerosis[98]. In addition mast cells have been demonstrated to be involved in intraplaque hemorrhage, macrophage apoptosis and vascular leakage, promoting plaque instability[99]. Neutrophils are thought to be pro-atherogenic as well. They are mainly present in the adventitia and the luminal area of mouse plaques[100] and in ruptured human coronary artery plaques[101]. Depletion of circulating neutrophils resulted in reduced plaque formation in $ApoE^{-/-}$ mice[100].

5 Apoptotic cell death

5.1 Signal transduction pathways

Removal of defective, damaged or dangerous cells is critical for normal development and tissue homeostasis of all organisms[102]. Death of these cells takes place via a process called apoptosis or programmed cell death[103]. Apoptosis is characterized by morphological changes like cell shrinkage, DNA fragmentation, condensation of chromatin and membrane blebbing. In contrast, features of passive, traumatic cell death or necrosis are cell swelling and loss of membrane integrity[104].

The executers of apoptotic cell death are a family of cysteine proteases known as caspases. Caspases proteolytically cleave proteins necessary for maintaining cellular structure like lamins[105] and focal adhesions kinase (FAK)[106] but also proteins that protect from cell death such as DFF45 (a nuclease inhibitor)[107] and Bcl-2 family members[108]. A cascade of caspases in which a pro-apoptotic signal activates initiator

caspases (e.g. caspases 1, 8, 9 and 10) which in turn activate effector caspases (caspases 3, 6 and 7) results in cellular breakdown[109]. There are two signaling pathways regulating apoptosis that share the same effector caspases. The extrinsic or death receptor mediated pathway is activated in response to ligation of death receptors (fig. 3). Binding of specific ligands to the cognate death receptor causes formation of a death-inducing signaling complex (DISC) in which various adaptor proteins like FADD and TRADD interact with death domains (DD) of the receptors[110]. Initiator caspase 8 is essential for death receptor induced apoptosis[111]. Death receptors belong to the tumor necrosis factor (TNF) receptor family and include TNF receptor 1 (TNFR1), FAS, death receptor (DR) 3, DR4 and DR5. Their ligands are TNF family members, including Fas ligand, TNFα, TWEAK (TNF-like weak inducer of apoptosis) and TRAIL (TNF related apoptosis inducing ligand)[110].
The intrinsic apoptosis signaling pathway requires the involvement of members of the Bcl-2 (B cell lymphoma 2) family of apoptosis regulators and mitochondria. Apoptotic stimuli activating this pathway include DNA damage, UV radiation, hypoxia and growth factor withdrawal[112]. Apoptosis signaling via the intrinsic pathway depends on the release of cytochrome c and other apoptosis regulating proteins like Smac/Diablo and apoptosis inducing factor (AIF) from the mitochondria (fig. 3). Once in the cytosol cytochrome c associates with an adaptor molecule called apoptotic protease-activating factor-1 (APAF-1) and pro-caspase 9 forming the so-called apoptosome. The subsequently activated caspase 9 is then able to activate effector caspases[113].

5.2 Bcl-2 family of apoptosis regulators

The intrinsic apoptosis pathway is mainly regulated by proteins of the Bcl-2 family. This family consists of both pro- and anti-apoptotic proteins sharing one or more Bcl-2 homology (BH) domains[114]. Anti-apoptotic proteins contain three or four BH domains and include Bcl-2, Bcl-w, Bcl-x_L, Bfl-1 and Mcl-1. There are two classes of pro-apoptotic Bcl-2 family proteins: proteins of the multidomain group comprising Bax, Bak and Bok which contain BH domains 1-3 and Bcl-2 proteins which carry only the BH-3 domain. The latter BH-3 only proteins include Bid, Bad, Bik, Bim, Noxa, Puma, Bmf, Blk and Hrk[114]. BH-3 only proteins initiate the apoptotic cascade[115], whereas Bax and Bak function downstream of BH-3 only proteins[116]. Bcl-2 family proteins Bak and Bax are thought to form pores in the outer mitochondrial membrane or change pore size thereby affecting of the mitochondrial permeability for cytochrome c[113]. Cytochrome c release from mitochondria takes place through these pores. Under non-apoptotic circumstances activity of BH3-only proteins is inhibited by Bcl-2 and other anti-apoptotic Bcl-2 proteins[112]. Following an apoptotic stimulus, BH-3 only proteins can either directly activate multidomain pro-apoptotic proteins (Bid and Bim) or interact with anti-apoptotic Bcl-2 proteins and prevent their binding to other pro-apoptotic proteins (Bim). Activity of BH3-only proteins can be regulated by phosphorylation (for example Bad and Bim[117,118]), transcriptional control (Puma and Noxa which are p53 targets[119,120]) or cleavage (Bid[121]). The pro-apoptotic protein

Bid, which functions in the intrinsic pathway, can also be activated by caspase-8 after stimulation of the extrinsic apoptosis pathway, thereby connecting both pathways[112].

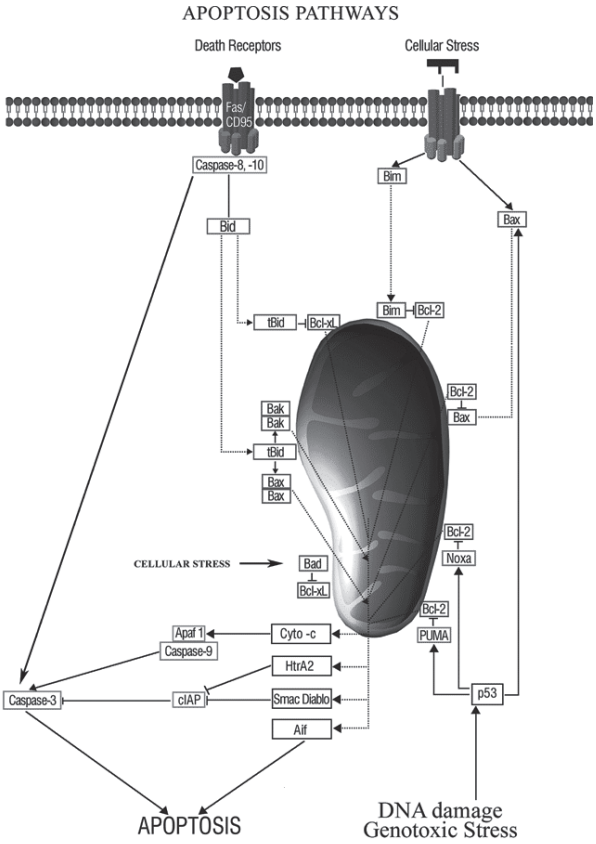

Figure 3. Apoptosis pathways. The death receptor (extrinsic) pathway is activated by ligation of death receptors. Subsequently initiator caspases activate effector caspases resulting in cell death. BH3-only proteins (e.g. Bim) initiate the mitochondrial or intrinsic pathway after apoptotic stimuli like DNA damage and oxidative stress, followed by activation of multidomain pro-apoptotic proteins (Bak and Bax) which form pores in the mitochondrial membrane. Apoptotic signaling is regulated by anti-apoptotic bcl-2 proteins (Bcl-2, Bcl-x_L, Mcl-1 etc). Cell death results from effector caspase activation and subsequent release of cytochrome c and other regulatory proteins from the mitochondria. Adapted from Kutuk and Basaga[112].

5.3 Apoptotic cell clearance

Apoptosis is followed by uptake of cellular remnants by professional phagocytes, macrophages, dendritic cells and granulocytes[122]. A wide range of receptors, ligands and adaptor molecules on both apoptotic cells and phagocytes are involved in the removal of apoptotic cells. One of the best described molecules in the recognition of apoptotic cells is phosphatidylserine (PS), which is translocated from the inner to the outer leaflet of the cell membrane early in the apoptotic process[123]. Other molecules implicated in the recognition and engulfment of apoptotic cells include scavenger receptors CD36, CD68 and SRA, Mer kinase, CD14 and integrins on the phagocyte membrane and bridging molecules such as milk fat globule epidermal growth factor 8 (Mfge8) and complement component C1q[122,124-127]. When removal of apoptotic cells is insufficient apoptotic cells may undergo secondary necrosis with leakage of cellular content. This may have pathological consequences since secondary necrotic cells and their debris can be taken up by antigen presenting cells and result in inflammation and autoimmunity[128].

6 Apoptosis and phagocytosis in the atherosclerotic plaque

Apoptosis occurs in atherosclerotic lesions affecting all major cell types, endothelial cells, macrophages, T cells and vSMC[129]. However, apoptosis increases with plaque progression, being virtually absent in initial lesions and increasingly present in advanced lesions[130]. Inducers of apoptotic cell death are abundant and include modified LDL, reactive oxygen species, cytokines with pro-apoptotic activity, hypoxia and death receptor ligation (Fas, TNFR1 and 2, DR4 and DR5)[131-137].

6.1 Endothelial cell apoptosis

Endothelial injury and apoptosis are late events in atherosclerosis[138]. Endothelial cells in lesion-prone regions in the vasculature have increased turnover due to increased apoptosis[139]. In endothelial cells in regions predisposed to atherosclerotic lesion development NF-κB signal transduction pathway was shown to be primed for activation[140] and NF-κB activation by various stimuli like hypoxia, IL18 and TNFα has been demonstrated to trigger apoptosis in endothelial cells[141-143]. Apoptosis is stimulated by exposure to oxidized LDL and oxidative stress among other factors. Nitric oxide (NO) may play a role in endothelial cell apoptosis in atherosclerosis as well. In healthy arteries NO derived from endothelial NO synthase (eNOS) acts protective against apoptosis[144]. In atherosclerotic lesion prone regions eNOS expression is decreased[145]. In addition, atherosclerotic plaque macrophages produce high amounts of inducible NOS (iNOS) which can generate peroxynitrite contributing to oxidative stress[146] which in turn can induce DNA damage and subsequent apoptosis in endothelial cells[138]. EC injury and apoptosis can have various consequences. Induction of EC apoptosis may promote thrombus formation followed by plaque erosion and leukocyte infiltration[147,148].

6.2 Vascular smooth muscle cell apoptosis

Apoptosis of vSMC has been shown to occur after injury in a rabbit balloon angioplasty model[149], in human abdominal aortic aneurisms[150] and in atherosclerotic lesions[21]. Surprisingly apoptosis of vSMC in atherosclerotic plaques can induce inflammation as shown *in vivo* in rat carotid arteries[151] where it triggered IL8 and MCP-1 expression together with massive macrophage infiltration after vSMC death. In $ApoE^{-/-}$ mice in which apoptosis was specifically induced in vSMC by diphtheria toxin (SM22α-hDTR / $ApoE^{-/-}$ mice) increased inflammation was observed after vSMC apoptosis as well[152]. Furthermore, vSMC apoptosis has been shown to lead to thrombin generation[153] and calcification[154] *in vitro*. In human atherosclerotic lesions apoptosis of both vSMC and macrophages was demonstrated to be elevated only in advanced lesions while in early lesions apoptosis was minimal[130]. In addition, human vSMC derived from coronary atherosclerotic plaques were shown to be more susceptible to cell death than vSMC from healthy coronary arteries *in vitro*[155] and vSMC may exhibit increased oxidative stress induced senescence[156]. VSMC senescence following ROS induced

DNA damage was shown to be mediated by p53 activation[156]. Abovementioned studies seem to support the general concept that apoptosis of vSMC promotes plaque vulnerability by thinning of the fibrous cap and also various studies in mice are in agreement with this concept. Induction of apoptosis by targeted overexpression of p53 into cap smooth muscle cells in advanced collar induced carotid artery plaques in ApoE$^{-/-}$ mice resulted in increased apoptosis of cap cells, reduced cap thickness, and in general a vulnerable plaque phenotype which was prone to phenylephrine induced rupture[157]. A comparable, vulnerable plaque phenotype was found after adenovirus mediated overexpression of the pro-apoptotic TNF family member Fas ligand in cap cells of ApoE deficient mice[158]. Plaques contained hemorrhage, buried caps and iron deposits, also indicating increased vulnerability. Recently, the above mentioned SM22α-hDTR / ApoE$^{-/-}$ mice were used to examine the impact of vSMC apoptosis on plaque phenotype and disease progression[152,159]. Induction of apoptosis in established atherosclerotic plaques resulted in plaque vulnerability as indicated by fibrous cap thinning, loss of collagen, accumulation of cell debris and increased inflammation[152]. In addition, persistent vSMC apoptosis throughout plaque development was seen to accelerate atherogenesis[159].

6.3 Macrophage apoptosis

Macrophage apoptosis occurs in both early and late stages of atherosclerosis and can be induced by a variety of stimuli including oxidized LDL, oxysterols, free cholesterol and hypoxia but also TNFα[160]. Apoptosis of macrophages has been demonstrated to be beneficial in early atherogenesis in several *in vivo* studies[161-164]. Inhibition of macrophage apoptosis due to leukocyte p53 deletion in ApoE3 Leiden transgenic mice[161] or LDLr$^{-/-}$ mice[162] and leukocyte Bax deletion in LDLr$^{-/-}$ mice[163], both pro-apoptotic factors, resulted in increased atherosclerotic lesion size. In addition deletion of pro-survival factor AIM (apoptosis inhibitor expressed by macrophages) in LDLr$^{-/-}$ mice led to increased macrophage apoptosis and decreased lesion area[164]. The consequences of macrophage apoptosis in advanced lesions are less clear. In advanced human lesions clearance of apoptotic cells was shown to be defective[165], suggesting that macrophage apoptosis will lead to secondary necrosis and accumulation of cell and lipid debris. This will translate in necrotic core expansion and elicit a pro-inflammatory response which could result in promotion of plaque instability[160]. However, others did not find such pronounced effects of macrophage apoptosis in advanced atherosclerotic plaques. For instance, Stoneman *et al.*[166] developed a model in which in ApoE$^{-/-}$ mice apoptosis could be induced specifically in macrophages with diphtheria toxin (DT), the CD11b-hDTR / ApoE$^{-/-}$ mouse[166]. Induction of apoptosis during early atherogenesis resulted in decreased plaque development together with reduced collagen content and necrotic core formation, confirming the atheroprotective effects of macrophage apoptosis in aforementioned studies regarding early atherogenesis. However in established plaques DT treatment induced macrophage apoptosis but this did not result in alterations in plaque size, cell composition or inflammation. In another

study macrophage apoptosis was achieved by LysM cre induced deletion of Bcl-2 in ApoE$^{-/-}$ mice[167]. Increased macrophage apoptosis was observed after 10 weeks of western type diet feeding but this resulted in a slight increase of 25% in necrotic core size only in female mice. No other characteristics of enhanced plaque instability were observed.

6.4 Phagocytosis of apoptotic cells

Phagocytosis of apoptotic cells in the atherosclerotic plaque limits plaque progression, inflammation and plaque instability as has been demonstrated by several gene deletion studies. Deficiency of leukocyte transglutaminase 2 (TG2) in LDLr$^{-/-}$ mice was seen to increase aortic valve lesion size and intimal macrophage infiltration[168]. LDLr$^{-/-}$ mice deficient in milk fat globule-EGF factor 8 (Mfge8) show accelerated atherosclerosis with increased necrotic core size and an elevated inflammatory status[169]. Finally, deletion of leukocyte Mer kinase in LDLr$^{-/-}$ mice led to increased accumulation of apoptotic cells, increased macrophage area and lymphocyte infiltration resulting in accelerated lesion development[170].

As mentioned in the previous section, phagocytic clearance of apoptotic cells is impaired at later stages of plaque progression[165]. Several mechanisms for defective phagocytosis have been proposed. First, Ox-LDL shares molecules involved in recognition by macrophages with apoptotic cells and as a result may compete with apoptotic cells for ingestion[171,172]. In addition auto-antibodies directed against Ox-LDL have been demonstrated to bind to apoptotic cells and inhibit their phagocytosis by macrophages[173]. Finally oxidative stress may inhibit the phagocytosis of apoptotic cells by macrophages as has been demonstrated in vitro for the oxidative stress mediators hydrogen peroxide (H2O2)[174] and peroxynitrite[165].

7 Thesis outline

In this thesis the role of several apoptosis regulating proteins in the development of atherosclerosis and atherosclerotic plaque stability is investigated. As many of these proteins also display immune-modulating features, we have particularly investigated effects of modulation of apoptosis regulating proteins on plaque and systemic inflammation. In chapter 2 current knowledge on pro- or anti-apoptotic proteins and their effects on inflammation in both murine and human atherosclerosis as well as the influence of pro- or anti-inflammatory mediators on apoptotic processes are reviewed.

Chapter 3 describes a study in which gene expression profiles of thin cap fibroatheroma are compared to those of thick cap fibroatheroma by micro-array technology in order to identify genes or pathways that are associated with plaque vulnerability. Two different mouse models for thin cap fibroatheroma are used to increase the significance of the findings.

In chapter 4 the relevance of Bim (Bcl-2 interacting mediator of cell death), a pro-apoptotic member of the Bcl-2 family identified as upregulated in both models in

the previous chapter, for atherosclerosis is investigated in LDLr$^{-/-}$ mice. Bim has been previously demonstrated to be an important regulator of B and T cell homeostasis. Therefore, apart from apoptotic processes relevant for atherosclerosis, we also assessed the role in disease associated innate and adaptive immunity. The pro-apoptotic activity of Bim is partly regulated by Mcl-1 (myeloid cell leukemia 1), an anti-apoptotic member of the Bcl-2 family. Mcl-1 is amongst others involved in proliferation and differentiation of monocytes and neutrophils and has been implicated in lipid accumulation by macrophages. In chapter 5 we therefore studied the impact of Mcl-1 deletion on cell death, lipid accumulation and inflammatory status of LDLr$^{-/-}$ mice.

Chapter 6 describes a study addressing the role of focal adhesion kinase (FAK), a kinase not only involved in cell death and proliferation, but particularly important in cell adhesion and migration, in atherosclerosis development and progression in ApoE$^{-/-}$ mice. Recently, FAK was shown to be involved in oxidized LDL mediated CD36 signaling. Thus, in chapter 6 the role of FAK in plaque apoptosis, inflammatory status and lipid metabolism in Western type diet fed ApoE$^{-/-}$ mice was investigated.

To conclude, in chapter 7 the main findings of the studies described in this thesis are summarized and discussed in relation to possible therapeutic approaches.

References

1. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999; 340(2):115-26.
2. Lusis AJ. Atherosclerosis. Nature. 2000; 407(6801):233-41.
3. Aikawa M, Libby P. The vulnerable atherosclerotic plaque: pathogenesis and therapeutic approach. Cardiovasc Pathol. 2004; 13(3):125-38.
4. Farb A, Tang AL, Burke AP, Sessums L, Liang Y, Virmani R. Sudden coronary death. Frequency of active coronary lesions, inactive coronary lesions, and myocardial infarction. Circulation. 1995; 92(7):1701-9.
5. Heitzer T, Ylä-Herttuala S, Luoma J, Kurz S, Münzel T, Just H, Olschewski M, Drexler H. Cigarette smoking potentiates endothelial dysfunction of forearm resistance vessels in patients with hypercholesterolemia. Role of oxidized LDL. Circulation. 1996; 93(7):1346-53.
6. McGill HC Jr, McMahan CA, Herderick EE, Zieske AW, Malcom GT, Tracy RE, Strong JP; Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Obesity accelerates the progression of coronary atherosclerosis in young men. Circulation. 2002; 105(23):2712-8.
7. Shepherd J, Packard CJ. Lipoprotein metabolism in familial hypercholesterolemia. Arteriosclerosis. 1989; 9(1 Suppl):I39-42.
8. Lusis AJ, Mar R, Pajukanta P. Genetics of atherosclerosis. Annu Rev Genomics Hum Genet. 2004; 5:189-218.
9. Yutani C, Imakita M, Ishibashi-Ueda H, Tsukamoto Y, Nishida N, Ikeda Y. Coronary atherosclerosis and interventions: pathological sequences and restenosis. Pathol Int. 1999; 49(4):273-90.
10. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007; 115(10):1285-95.
11. Quehenberger O. Thematic review series: the immune system and atherogenesis. Molecular mechanisms regulating monocyte recruitment in atherosclerosis. J Lipid Res. 2005; 46(8):1582-90.
12. Kansas GS. Selectins and their ligands: current concepts and controversies. Blood. 1996; 88(9):3259-87.
13. Krieglstein CF, Granger DN. Adhesion molecules and their role in vascular disease. Am J Hypertens. 2001; 14(6 Pt 2):44S-54S.
14. Walpola PL, Gotlieb AI, Cybulsky MI, Langille BL. Expression of ICAM-1 and VCAM-1 and monocyte adherence in arteries exposed to altered shear stress. Arterioscler Thromb Vasc Biol. 1995; 15(1):2-10.
15. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. Arterioscler Thromb Vasc Biol. 1998; 18(5):842-51.
16. Collins RG, Velji R, Guevara NV, Hicks MJ, Chan L, Beaudet AL. P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice. J Exp Med. 2000; 191(1):189-94.
17. Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, Wagner DD. The combined role of P- and E-selectins in atherosclerosis. J Clin Invest. 1998; 102(1):145-52.
18. Ostermann G, Weber KS, Zernecke A, Schröder A, Weber C. JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. Nat Immunol. 2002; 3(2):151-8.
19. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. J Exp Med. 1993; 178(2):449-60.
20. Schenkel AR, Mamdouh Z, Chen X, Liebman RM, Muller WA. CD99 plays a major role in the migration of monocytes through endothelial junctions. Nat Immunol. 2002; 3(2):143-50.
21. Vestweber D. Regulation of endothelial cell contacts during leukocyte extravasation. Curr Opin Cell Biol. 2002; 14(5):587-93.
22. Muller WA. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. Trends Immunol. 2003; 24(6):327-34.
23. Johnston B, Butcher EC. Chemokines in rapid leukocyte adhesion triggering and migration. Semin Immunol. 2002; 14(2):83-92.
24. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rollins BJ. Absence of monocyte

chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell. 1998; 2(2):275-81.
25. Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. Nature. 1998; 394(6696):894-7.
26. Guo J, Van Eck M, Twisk J, Maeda N, Benson GM, Groot PH, Van Berkel TJ. Transplantation of monocyte CC-chemokine receptor 2-deficient bone marrow into ApoE3-Leiden mice inhibits atherogenesis. Arterioscler Thromb Vasc Biol. 2003; 23(3):447-53.
27. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem. 1997; 272(34):20963-6.
28. Khoo JC, Miller E, McLoughlin P, Steinberg D. Enhanced macrophage uptake of low density lipoprotein after self-aggregation. Arteriosclerosis. 1988; 8(4):348-58.
29. Aviram M, Maor I, Keidar S, Hayek T, Oiknine J, Bar-El Y, Adler Z, Kertzman V, Milo S. Lesioned low density lipoprotein in atherosclerotic apolipoprotein E-deficient transgenic mice and in humans is oxidized and aggregated. Biochem Biophys Res Commun. 1995; 216(2):501-13.
30. Clinton SK, Underwood R, Hayes L, Sherman ML, Kufe DW, Libby P. Macrophage colony-stimulating factor gene expression in vascular cells and in experimental and human atherosclerosis. Am J Pathol. 1992; 140(2):301-16.
31. Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem. 2008; 54(1):24-38.
32. Raines EW, Ferri N. Thematic review series: The immune system and atherogenesis. Cytokines affecting endothelial and smooth muscle cells in vascular disease. J Lipid Res. 2005; 46(6):1081-92.
33. Halvorsen B, Otterdal K, Dahl TB, Skjelland M, Gullestad L, Øie E, Aukrust P. Atherosclerotic plaque stability--what determines the fate of a plaque? Prog Cardiovasc Dis. 2008; 51(3):183-94.
34. Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. Br Heart J. 1993; 69(5):377-81.
35. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005; 352(16):1685-95.
36. Jones CB, Sane DC, Herrington DM. Matrix metalloproteinases: a review of their structure and role in acute coronary syndrome. Cardiovasc Res. 2003; 59(4):812-23.
37. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest. 1994; 94(6):2493-503.
38. Geng YJ, Libby P. Evidence for apoptosis in advanced human atheroma. Colocalization with interleukin-1 beta-converting enzyme. Am J Pathol. 1995; 147(2):251-66.
39. Walsh K, Smith RC, Kim HS. Vascular cell apoptosis in remodeling, restenosis, and plaque rupture. Circ Res. 2000; 87(3):184-8.
40. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, Wrenn SP, Narula J. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol. 2005; 25(10):2054-61.
41. Doran AC, Meller N, McNamara CA. Arterioscler Thromb Vasc Biol. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. 2008; 28(5):812-9.
42. Kim WJ, Chereshnev I, Gazdoiu M, Fallon JT, Rollins BJ, Taubman MB. MCP-1 deficiency is associated with reduced intimal hyperplasia after arterial injury. Biochem Biophys Res Commun. 2003; 310(3):936-42.
43. Schober A, Knarren S, Lietz M, Lin EA, Weber C. Crucial role of stromal cell-derived factor-1alpha in neointima formation after vascular injury in apolipoprotein E-deficient mice. Circulation. 2003; 108(20):2491-7.
44. Ruan XZ, Moorhead JF, Tao JL, Ma KL, Wheeler DC, Powis SH, Varghese Z. Mechanisms of dysregulation of low-density lipoprotein receptor expression in vascular smooth muscle cells by inflammatory cytokines. Arterioscler Thromb Vasc Biol. 2006; 26(5):1150-5.
45. Lim HJ, Lee S, Lee KS, Park JH, Jang Y, Lee EJ, Park HY. PPARgamma activation induces CD36

expression and stimulates foam cell like changes in rVSMCs. Prostaglandins Other Lipid Mediat. 2006; 80(3-4):165-74.
46. Bickel PE, Freeman MW. Rabbit aortic smooth muscle cells express inducible macrophage scavenger receptor messenger RNA that is absent from endothelial cells. J Clin Invest. 1992; 90(4):1450-7.
47. Wågsäter D, Olofsson PS, Norgren L, Stenberg B, Sirsjö A. The chemokine and scavenger receptor CXCL16/SR-PSOX is expressed in human vascular smooth muscle cells and is induced by interferon gamma. Biochem Biophys Res Commun. 2004; 325(4):1187-93.
48. Braun M, Pietsch P, Schrör K, Baumann G, Felix SB. Cellular adhesion molecules on vascular smooth muscle cells. Cardiovasc Res. 1999; 41(2):395-401.
49. Libby P. The molecular mechanisms of the thrombotic complications of atherosclerosis. J Intern Med. 2008; 263(5):517-27.
50. Okada Y, Katsuda S, Watanabe H, Nakanishi I. Collagen synthesis of human arterial smooth muscle cells: effects of platelet-derived growth factor, transforming growth factor-beta 1 and interleukin-1. Acta Pathol Jpn. 1993; 43(4):160-7.
51. Zhu YK, Liu X, Wang H, Kohyama T, Wen FQ, Sköld CM, Rennard SI. Interactions between monocytes and smooth-muscle cells can lead to extracellular matrix degradation. J Allergy Clin Immunol. 2001; 108(6):989-96.
52. Amento EP, Ehsani N, Palmer H, Libby P. Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. Arterioscler Thromb. 1991; 11(5):1223-30.
53. Newby AC. Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates. Cardiovasc Res. 2006; 69(3):614-24.
54. Guo J, Bot I, de Nooijer R, Hoffman SJ, Stroup GB, Biessen EA, Benson GM, Groot PH, Van Eck M, Van Berkel TJ. Leucocyte cathepsin K affects atherosclerotic lesion composition and bone mineral density in low-density lipoprotein receptor deficient mice. Cardiovasc Res. 2009; 81(2):278-85.
55. Lutgens SP, Cleutjens KB, Daemen MJ, Heeneman S. Cathepsin cysteine proteases in cardiovascular disease. FASEB J. 2007; 21(12):3029-41.
56. Shi GP, Sukhova GK, Grubb A, Ducharme A, Rhode LH, Lee RT, Ridker PM, Libby P, Chapman HA. Cystatin C deficiency in human atherosclerosis and aortic aneurysms. J Clin Invest. 1999; 104(9):1191-7.
57. Burke AP, Kolodgie FD, Farb A, Weber DK, Malcom GT, Smialek J, Virmani R. Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. Circulation. 2001; 103(7):934-40.
58. Mann J, Davies MJ. Mechanisms of progression in native coronary artery disease: role of healed plaque disruption. Heart. 1999; 82(3):265-8.
59. de Villiers WJ, Fraser IP, Hughes DA, Doyle AG, Gordon S. Macrophage-colony-stimulating factor selectively enhances macrophage scavenger receptor expression and function. J Exp Med. 1994; 180(2):705-9.
60. van der Kooij MA, Morand OH, Kempen HJ, van Berkel TJ. Decrease in scavenger receptor expression in human monocyte-derived macrophages treated with granulocyte macrophage colony-stimulating factor. Arterioscler Thromb Vasc Biol. 1996; 16(1):106-14.
61. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density 3lipoprotein. J Biol Chem. 1993; 268(16):11811-6.
62. Ling W, Lougheed M, Suzuki H, Buchan A, Kodama T, Steinbrecher UP. Oxidized or acetylated low density lipoproteins are rapidly cleared by the liver in mice with disruption of the scavenger receptor class A type I/II gene. J Clin Invest. 1997; 100(2):244-52.
63. Yoshida H, Kondratenko N, Green S, Steinberg D, Quehenberger O. Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. Biochem J. 1998; 334 (Pt 1):9-13.
64. Moore KJ, Freeman MW. Scavenger receptors in atherosclerosis: beyond lipid uptake. Arterioscler Thromb Vasc Biol. 2006; 26(8):1702-11.

65. van Berkel TJ, Out R, Hoekstra M, Kuiper J, Biessen E, van Eck M. Scavenger receptors: friend or foe in atherosclerosis? Curr Opin Lipidol. 2005; 16(5):525-35.
66. de Villiers WJ, Fraser IP, Gordon S. Cytokine and growth factor regulation of macrophage scavenger receptor expression and function. Immunol Lett. 1994; 43(1-2):73-9.
67. Hsu HY, Twu YC. Tumor necrosis factor-alpha -mediated protein kinases in regulation of scavenger receptor and foam cell formation on macrophage. J Biol Chem. 2000; 275(52):41035-48.
68. Argmann CA, Van Den Diepstraten CH, Sawyez CG, Edwards JY, Hegele RA, Wolfe BM, Huff MW. Transforming growth factor-beta1 inhibits macrophage cholesteryl ester accumulation induced by native and oxidized VLDL remnants. Arterioscler Thromb Vasc Biol. 2001; 21(12):2011-8.
69. Nicoletti A, Caligiuri G, Törnberg I, Kodama T, Stemme S, Hansson GK. The macrophage scavenger receptor type A directs modified proteins to antigen presentation. Eur J Immunol. 1999; 29(2):512-21.
70. Hansson GK, Robertson AK, Söderberg-Nauclér C. Inflammation and atherosclerosis. Annu Rev Pathol. 2006; 1:297-329.
71. Reardon CA, Blachowicz L, White T, Cabana V, Wang Y, Lukens J, Bluestone J, Getz GS. Effect of immune deficiency on lipoproteins and atherosclerosis in male apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2001; 21(6):1011-6.
72. Dansky HM, Charlton SA, Harper MM, Smith JD. T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse. Proc Natl Acad Sci U S A. 1997; 94(9):4642-6.
73. Zhou X, Nicoletti A, Elhage R, Hansson GK. Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. Circulation. 2000; 102(24):2919-22.
74. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci U S A. 1995; 92(9):3893-7.
75. Zhou X, Paulsson G, Stemme S, Hansson GK. Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. J Clin Invest. 1998; 101(8):1717-25.
76. Frostegård J, Ulfgren AK, Nyberg P, Hedin U, Swedenborg J, Andersson U, Hansson GK. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. Atherosclerosis. 1999; 145(1):33-43.
77. Lee TS, Yen HC, Pan CC, Chau LY. The role of interleukin 12 in the development of atherosclerosis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol. 1999; 19(3):734-42.
78. Davenport P, Tipping PG. The role of interleukin-4 and interleukin-12 in the progression of atherosclerosis in apolipoprotein E-deficient mice. Am J Pathol. 2003; 163(3):1117-25.
79. Whitman SC, Ravisankar P, Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon-gamma. Circ Res. 2002; 90(2):E34-8.
80. Elhage R, Jawien J, Rudling M, Ljunggren HG, Takeda K, Akira S, Bayard F, Hansson GK. Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. Cardiovasc Res. 2003; 59(1):234-40.
81. Hauer AD, Uyttenhove C, de Vos P, Stroobant V, Renauld JC, van Berkel TJ, van Snick J, Kuiper J. Blockade of interleukin-12 function by protein vaccination attenuates atherosclerosis. Circulation. 2005; 112(7):1054-62.
82. Whitman SC, Ravisankar P, Elam H, Daugherty A. Exogenous interferon-gamma enhances atherosclerosis in apolipoprotein E-/- mice. Am J Pathol. 2000; 157(6):1819-24.
83. Whitman SC, Ravisankar P, Daugherty A. IFN-gamma deficiency exerts gender-specific effects on atherogenesis in apolipoprotein E-/- mice. J Interferon Cytokine Res. 2002; 22(6):661-70.
84. Brånén L, Hovgaard L, Nitulescu M, Bengtsson E, Nilsson J, Jovinge S. Inhibition of tumor necrosis factor-alpha reduces atherosclerosis in apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol. 2004; 24(11):2137-42.
85. Schreyer SA, Peschon JJ, LeBoeuf RC. Accelerated atherosclerosis in mice lacking tumor necrosis factor receptor p55. J Biol Chem. 1996; 271(42):26174-8.

86. King VL, Szilvassy SJ, Daugherty A. Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDL receptor-/- mice. Arterioscler Thromb Vasc Biol. 2002; 22(3):456-61.
87. Binder CJ, Hartvigsen K, Chang MK, Miller M, Broide D, Palinski W, Curtiss LK, Corr M, Witztum JL. IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis. J Clin Invest. 2004; 114(3):427-37.
88. Warner SJ, Friedman GB, Libby P. Immune interferon inhibits proliferation and induces 2'-5'-oligoadenylate synthetase gene expression in human vascular smooth muscle cells. J Clin Invest. 1989; 83(4):1174-82.
89. Amento EP, Ehsani N, Palmer H, Libby P. Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. Arterioscler Thromb. 1991; 11(5):1223-30.
90. Feinberg MW, Jain MK, Werner F, Sibinga NE, Wiesel P, Wang H, Topper JN, Perrella MA, Lee ME. Transforming growth factor-beta 1 inhibits cytokine-mediated induction of human metalloelastase in macrophages. J Biol Chem. 2000; 275(33):25766-73.
91. Yoo HG, Shin BA, Park JS, Lee KH, Chay KO, Yang SY, Ahn BW, Jung YD. IL-1beta induces MMP-9 via reactive oxygen species and NF-kappaB in murine macrophage RAW 264.7 cells. Biochem Biophys Res Commun. 2002; 298(2):251-6.
92. Li H, Freeman MW, Libby P. Regulation of smooth muscle cell scavenger receptor expression in vivo by atherogenic diets and in vitro by cytokines. J Clin Invest. 1995; 95(1):122-33.
93. Caligiuri G, Nicoletti A, Poirier B, Hansson GK. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. J Clin Invest. 2002; 109(6):745-53.
94. Bobryshev YV. Dendritic cells in atherosclerosis: current status of the problem and clinical relevance. Eur Heart J. 2005; 26(17):1700-4.
95. Angeli V, Llodrá J, Rong JX, Satoh K, Ishii S, Shimizu T, Fisher EA, Randolph GJ. Dyslipidemia associated with atherosclerotic disease systemically alters dendritic cell mobilization. Immunity. 2004; 21(4):561-74.
96. Packard RR, Maganto-García E, Gotsman I, Tabas I, Libby P, Lichtman AH. CD11c(+) dendritic cells maintain antigen processing, presentation capabilities, and CD4(+) T-cell priming efficacy under hypercholesterolemic conditions associated with atherosclerosis. Circ Res. 2008; 103(9):965-73.
97. Kaartinen M, Penttilä A, Kovanen PT. Accumulation of activated mast cells in the shoulder region of human coronary atheroma, the predilection site of atheromatous rupture. Circulation. 1994; 90(4):1669-78.
98. Sun J, Sukhova GK, Wolters PJ, Yang M, Kitamoto S, Libby P, MacFarlane LA, Mallen-St Clair J, Shi GP. Mast cells promote atherosclerosis by releasing proinflammatory cytokines. Nat Med. 2007; 13(6):719-24.
99. Bot I, de Jager SC, Zernecke A, Lindstedt KA, van Berkel TJ, Weber C, Biessen EA. Perivascular mast cells promote atherogenesis and induce plaque destabilization in apolipoprotein E-deficient mice. Circulation. 2007 May; 115(19):2516-25.
100. Zernecke A, Bot I, Djalali-Talab Y, Shagdarsuren E, Bidzhekov K, Meiler S, Krohn R, Schober A, Sperandio M, Soehnlein O, Bornemann J, Tacke F, Biessen EA, Weber C. Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. Circ Res. 2008; 102(2):209-17.
101. Naruko T, Ueda M, Haze K, van der Wal AC, van der Loos CM, Itoh A, Komatsu R, Ikura Y, Ogami M, Shimada Y, Ehara S, Yoshiyama M, Takeuchi K, Yoshikawa J, Becker AE. Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation. 2002; 106(23):2894-900.
102. Vaux DL, Strasser A. The molecular biology of apoptosis. Proc Natl Acad Sci U S A. 1996; 93(6):2239-44.
103. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972; 26(4):239-57.
104. Maino G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol. 1995; 146(1):3-15.

105. Orth K, Chinnaiyan AM, Garg M, Froelich CJ, Dixit VM. The CED-3/ICE-like protease Mch2 is activated during apoptosis and cleaves the death substrate lamin A. J Biol Chem. 1996; 271(28):16443-6.
106. Wen LP, Fahrni JA, Troie S, Guan JL, Orth K, Rosen GD. Cleavage of focal adhesion kinase by caspases during apoptosis. J Biol Chem. 1997; 272(41):26056-61.
107. Liu X, Zou H, Slaughter C, Wang X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell. 1997; 89(2):175-84.
108. Cheng EH, Kirsch DG, Clem RJ, Ravi R, Kastan MB, Bedi A, Ueno K, Hardwick JM. Conversion of Bcl-2 to a Bax-like death effector by caspases. Science. 1997; 278(5345):1966-8.
109. Thornberry NA, Lazebnik Y. Caspases: enemies within. Science. 1998; 281(5381):1312-6.
110. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science. 1998; 281(5381):1305-8.
111. Varfolomeev EE, Schuchmann M, Luria V, Chiannilkulchai N, Beckmann JS, Mett IL, Rebrikov D, Brodianski VM, Kemper OC, Kollet O, Lapidot T, Soffer D, Sobe T, Avraham KB, Goncharov T, Holtmann H, Lonai P, Wallach D. Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. Immunity. 1998; 9(2):267-76.
112. Kutuk O, Basaga H. Bcl-2 protein family: implications in vascular apoptosis and atherosclerosis. Apoptosis. 2006; 11(10):1661-75.
113. Ow YP, Green DR, Hao Z, Mak TW. Cytochrome c: functions beyond respiration. Nat Rev Mol Cell Biol. 2008; 9(7):532-42.
114. Marsden VS, Strasser A. Control of apoptosis in the immune system: Bcl-2, BH3-only proteins and more. Annu Rev Immunol. 2003; 21:71-105.
115. Huang DC, Strasser A. BH3-Only proteins-essential initiators of apoptotic cell death. Cell. 2000; 103(6):839-42.
116. Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. Genes Dev. 2001; 15(12):1481-6.
117. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell. 1997; 91(2):231-41.
118. Putcha GV, Le S, Frank S, Besirli CG, Clark K, Chu B, Alix S, Youle RJ, LaMarche A, Maroney AC, Johnson EM Jr. JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. Neuron. 2003; 38(6):899-914.
119. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell. 2001; 7(3):683-94.
120. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, Tokino T, Taniguchi T, Tanaka N. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science. 2000; 288(5468):1053-8.
121. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 1998; 94(4):491-501.
122. Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol. 2002; 2(12):965-75.
123. Fadok VA, de Cathelineau A, Daleke DL, Henson PM, Bratton DL. Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. J Biol Chem. 2001; 276(2):1071-7.
124. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. Nature. 2000; 407(6805):784-8.
125. Szondy Z, Sarang Z, Molnar P, Nemeth T, Piacentini M, Mastroberardino PG, Falasca L, Aeschlimann D, Kovacs J, Kiss I, Szegezdi E, Lakos G, Rajnavolgyi E, Birckbichler PJ, Melino G, Fesus L. Transglutaminase 2-/- mice reveal a phagocytosis-associated crosstalk between macrophages and apoptotic cells. Proc Natl Acad Sci U S A. 2003; 100(13):7812-7.
126. Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. Nature. 2002; 417(6885):182-7.

127. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK. Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature. 2001; 411(6834):207-11.
128. Silva MT, do Vale A, dos Santos NM. Secondary necrosis in multicellular animals: an outcome of apoptosis with pathogenic implications. Apoptosis. 2008; 13(4):463-82.
129. Björkerud S, Björkerud B. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. Am J Pathol. 1996; 149(2):367-80.
130. Lutgens E, de Muinck ED, Kitslaar PJ, Tordoir JH, Wellens HJ, Daemen MJ. Biphasic pattern of cell turnover characterizes the progression from fatty streaks to ruptured human atherosclerotic plaques. Cardiovasc Res. 1999; 41(2):473-9.
131. Kavurma MM, Tan NY, Bennett MR. Death receptors and their ligands in atherosclerosis. Arterioscler Thromb Vasc Biol. 2008; 28(10):1694-702.
132. Salvayre R, Auge N, Benoist H, Negre-Salvayre A. Oxidized low-density lipoprotein-induced apoptosis. Biochim Biophys Acta. 2002; 1585(2-3):213-21.
133. Geng YJ, Henderson LE, Levesque EB, Muszynski M, Libby P. Fas is expressed in human atherosclerotic intima and promotes apoptosis of cytokine-primed human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 1997; 17(10):2200-8.
134. Sata M, Walsh K. Oxidized LDL activates fas-mediated endothelial cell apoptosis. J Clin Invest. 1998; 102(9):1682-9.
135. Zhang L, Peppel K, Sivashanmugam P, Orman ES, Brian L, Exum ST, Freedman NJ. Expression of tumor necrosis factor receptor-1 in arterial wall cells promotes atherosclerosis. Arterioscler Thromb Vasc Biol. 2007; 27(5):1087-94.
136. Michowitz Y, Goldstein E, Roth A, Afek A, Abashidze A, Ben Gal Y, Keren G, George J. The involvement of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in atherosclerosis. J Am Coll Cardiol. 2005; 45(7):1018-24.
137. Sato K, Niessner A, Kopecky SL, Frye RL, Goronzy JJ, Weyand CM. TRAIL-expressing T cells induce apoptosis of vascular smooth muscle cells in the atherosclerotic plaque. J Exp Med. 2006; 203(1):239-50.
138. Kockx MM, Herman AG. Apoptosis in atherosclerosis: beneficial or detrimental? Cardiovasc Res. 2000; 45(3):736-46.
139. Dimmeler S, Hermann C, Zeiher AM. Apoptosis of endothelial cells. Contribution to the pathophysiology of atherosclerosis? Eur Cytokine Netw. 1998; 9(4):697-8.
140. Hajra L, Evans AI, Chen M, Hyduk SJ, Collins T, Cybulsky MI. The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. Proc Natl Acad Sci U S A. 2000; 97(16):9052-7.
141. Aoki M, Nata T, Morishita R, Matsushita H, Nakagami H, Yamamoto K, Yamazaki K, Nakabayashi M, Ogihara T, Kaneda Y. Endothelial apoptosis induced by oxidative stress through activation of NF-kappaB: antiapoptotic effect of antioxidant agents on endothelial cells. Hypertension. 2001; 38(1):48-55.
142. Chandrasekar B, Vemula K, Surabhi RM, Li-Weber M, Owen-Schaub LB, Jensen LE, Mummidi S. Activation of intrinsic and extrinsic proapoptotic signaling pathways in interleukin-18-mediated human cardiac endothelial cell death. J Biol Chem. 2004; 279(19):20221-33.
143. Hall JL, Wang X, Van Adamson, Zhao Y, Gibbons GH. Overexpression of Ref-1 inhibits hypoxia and tumor necrosis factor-induced endothelial cell apoptosis through nuclear factor-kappab-independent and -dependent pathways. Circ Res. 2001; 88(12):1247-53.
144. Polte T, Oberle S, Schröder H. Nitric oxide protects endothelial cells from tumor necrosis factor-alpha-mediated cytotoxicity: possible involvement of cyclic GMP. FEBS Lett. 1997; 409(1):46-8.
145. Won D, Zhu SN, Chen M, Teichert AM, Fish JE, Matouk CC, Bonert M, Ojha M, Marsden PA, Cybulsky MI. Relative reduction of endothelial nitric-oxide synthase expression and transcription in atherosclerosis-prone regions of the mouse aorta and in an in vitro model of disturbed flow. Am J Pathol. 2007; 171(5):1691-704.
146. Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, Harrison DG, Marsden PA. Expression

of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. Arterioscler Thromb Vasc Biol. 1997; 17(11):2479-88.
147. Durand E, Scoazec A, Lafont A, Boddaert J, Al Hajzen A, Addad F, Mirshahi M, Desnos M, Tedgui A, Mallat Z. In vivo induction of endothelial apoptosis leads to vessel thrombosis and endothelial denudation: a clue to the understanding of the mechanisms of thrombotic plaque erosion. Circulation. 2004; 109(21):2503-6.
148. Bombeli T, Karsan A, Tait JF, Harlan JM. Apoptotic vascular endothelial cells become procoagulant. Blood. 1997; 89(7):2429-42.
149. Pollman MJ, Hall JL, Gibbons GH. Determinants of vascular smooth muscle cell apoptosis after balloon angioplasty injury. Influence of redox state and cell phenotype. Circ Res. 1999; 84(1):113-21.
150. Henderson EL, Geng YJ, Sukhova GK, Whittemore AD, Knox J, Libby P. Death of smooth muscle cells and expression of mediators of apoptosis by T lymphocytes in human abdominal aortic aneurysms. Circulation. 1999; 99(1):96-104.
151. Schaub FJ, Han DK, Liles WC, Adams LD, Coats SA, Ramachandran RK, Seifert RA, Schwartz SM, Bowen-Pope DF. Fas/FADD-mediated activation of a specific program of inflammatory gene expression in vascular smooth muscle cells. Nat Med. 2000; 6(7):790-6.
152. Clarke MC, Figg N, Maguire JJ, Davenport AP, Goddard M, Littlewood TD, Bennett MR. Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. Nat Med. 2006; 12(9):1075-80.
153. Flynn PD, Byrne CD, Baglin TP, Weissberg PL, Bennett MR. Thrombin generation by apoptotic vascular smooth muscle cells. Blood. 1997; 89(12):4378-84.
154. Proudfoot D, Skepper JN, Hegyi L, Bennett MR, Shanahan CM, Weissberg PL. Apoptosis regulates human vascular calcification in vitro: evidence for initiation of vascular calcification by apoptotic bodies. Circ Res. 2000; 87(11):1055-62.
155. Bennett MR, Evan GI, Schwartz SM. Identification of a protective role for protein phosphatase 1cgamma1 against oxidative stress-induced vascular smooth muscle cell apoptosis. J Biol Chem. 2008; 283(32):22193-205.
156. Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, Goddard M, Bennett M. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. Circ Res. 2006; 99(2):156-64.
157. von der Thüsen JH, van Vlijmen BJ, Hoeben RC, Kockx MM, Havekes LM, van Berkel TJ, Biessen EA. Induction of atherosclerotic plaque rupture in apolipoprotein E-/- mice after adenovirus-mediated transfer of p53. Circulation. 2002; 105(17):2064-70.
158. Zadelaar AS, von der Thüsen JH, Boesten LS, Hoeben RC, Kockx MM, Versnel MA, van Berkel TJ, Havekes LM, Biessen EA, van Vlijmen BJ. Increased vulnerability of pre-existing atherosclerosis in ApoE-deficient mice following adenovirus-mediated Fas ligand gene transfer. Atherosclerosis. 2005; 183(2):244-50.
159. Clarke MC, Littlewood TD, Figg N, Maguire JJ, Davenport AP, Goddard M, Bennett MR. Chronic apoptosis of vascular smooth muscle cells accelerates atherosclerosis and promotes calcification and medial degeneration. Circ Res. 2008; 102(12):1529-38.
160. Tabas I. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. Arterioscler Thromb Vasc Biol. 2005; 25(11):2255-64.
161. van Vlijmen BJ, Gerritsen G, Franken AL, Boesten LS, Kockx MM, Gijbels MJ, Vierboom MP, van Eck M, van De Water B, van Berkel TJ, Havekes LM. Macrophage p53 deficiency leads to enhanced atherosclerosis in APOE*3-Leiden transgenic mice. Circ Res. 2001; 88(8):780-6.
162. Merched AJ, Williams E, Chan L. Macrophage-specific p53 expression plays a crucial role in atherosclerosis development and plaque remodeling. Arterioscler Thromb Vasc Biol. 2003; 23(9):1608-14.
163. Liu J, Thewke DP, Su YR, Linton MF, Fazio S, Sinensky MS. Reduced macrophage apoptosis is associated with accelerated atherosclerosis in low-density lipoprotein receptor-null mice. Arterioscler Thromb Vasc Biol. 2005; 25(1):174-9.

164. Arai S, Shelton JM, Chen M, Bradley MN, Castrillo A, Bookout AL, Mak PA, Edwards PA, Mangelsdorf DJ, Tontonoz P, Miyazaki T. A role for the apoptosis inhibitory factor AIM/Spalpha/Api6 in atherosclerosis development. Cell Metab. 2005; 1(3):201-13.
165. Schrijvers DM, De Meyer GR, Kockx MM, Herman AG, Martinet W. Phagocytosis of apoptotic cells by macrophages is impaired in atherosclerosis. Arterioscler Thromb Vasc Biol. 2005; 25(6):1256-61.
166. Stoneman V, Braganza D, Figg N, Mercer J, Lang R, Goddard M, Bennett M. Monocyte/macrophage suppression in CD11b diphtheria toxin receptor transgenic mice differentially affects atherogenesis and established plaques. Circ Res. 2007; 100(6):884-93.
167. Thorp E, Li Y, Bao L, Yao PM, Kuriakose G, Rong J, Fisher EA, Tabas I. Brief report: increased apoptosis in advanced atherosclerotic lesions of Apoe-/- mice lacking macrophage Bcl-2. Arterioscler Thromb Vasc Biol. 2009; 29(2):169-72.
168. Boisvert WA, Rose DM, Boullier A, Quehenberger O, Sydlaske A, Johnson KA, Curtiss LK, Terkeltaub R. Leukocyte transglutaminase 2 expression limits atherosclerotic lesion size. Arterioscler Thromb Vasc Biol. 2006; 26(3):563-9.
169. Ait-Oufella H, Kinugawa K, Zoll J, Simon T, Boddaert J, Heeneman S, Blanc-Brude O, Barateau V, Potteaux S, Merval R, Esposito B, Teissier E, Daemen MJ, Lesèche G, Boulanger C, Tedgui A, Mallat Z. Lactadherin deficiency leads to apoptotic cell accumulation and accelerated atherosclerosis in mice. Circulation. 2007; 115(16):2168-77.
170. Ait-Oufella H, Pouresmail V, Simon T, Blanc-Brude O, Kinugawa K, Merval R, Offenstadt G, Lesèche G, Cohen PL, Tedgui A, Mallat Z. Defective mer receptor tyrosine kinase signaling in bone marrow cells promotes apoptotic cell accumulation and accelerates atherosclerosis. Arterioscler Thromb Vasc Biol. 2008;28(8):1429-31.
171. Sambrano GR, Steinberg D. Recognition of oxidatively damaged and apoptotic cells by an oxidized low density lipoprotein receptor on mouse peritoneal macrophages: role of membrane phosphatidylserine. Proc Natl Acad Sci U S A. 1995; 92(5):1396-400.
172. Khan M, Pelengaris S, Cooper M, Smith C, Evan G, Betteridge J. Oxidised lipoproteins may promote inflammation through the selective delay of engulfment but not binding of apoptotic cells by macrophages. Atherosclerosis. 2003; 171(1):21-9.
173. Shaw PX, Hörkkö S, Tsimikas S, Chang MK, Palinski W, Silverman GJ, Chen PP, Witztum JL. Human-derived anti-oxidized LDL autoantibody blocks uptake of oxidized LDL by macrophages and localizes to atherosclerotic lesions in vivo. Arterioscler Thromb Vasc Biol. 2001; 21(8):1333-9.
174. Anderson HA, Englert R, Gursel I, Shacter E. Oxidative stress inhibits the phagocytosis of apoptotic cells that have externalized phosphatidylserine. Cell Death Differ. 2002 Jun;9(6):616-25.
175. Li AC, Glass CK. The macrophage foam cell as a target for therapeutic intervention. Nat Med. 2002; 8(11):1235-42.
176. Watkins H, Farrall M. Genetic susceptibility to coronary artery disease: from promise to progress. Nat Rev Genet. 2006; 7(3):163-73.