Crosstalk between apoptosis and inflammation in atherosclerosis

Westra, M.M.

Citation

Westra, M. M. (2010, January 26). *Crosstalk between apoptosis and inflammation in atherosclerosis*. Retrieved from https://hdl.handle.net/1887/14616

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/14616

Note: To cite this publication please use the final published version (if applicable).

Chapter 2

Apoptosis Associated Inflammation in Atherosclerotic Plaque Progression and Stability

Marijke M. Westra[1], Theo J.C. van Berkel[1] and Erik A.L. Biessen[1,2]

[1] Division of Biopharmaceutics, Leiden Amsterdam Centre for Drug Research, Leiden University, Leiden, The Netherlands
[2] Experimental Vascular Pathology group, Department of Pathology, Maastricht University Medical Center, Maastricht, The Netherlands

Abstract
Inflammation and apoptosis are regarded key processes in the development, progression and instability of atherosclerotic plaques. Although originally considered anti-inflammatory, recent insights promote the notion that apoptosis may under conditions prevalent in atheromathous tissue promote inflammation. Vice versa several pro-inflammatory mediators that contribute to atherogenesis can have pro- or anti-apoptotic effects. In this review we shall describe recent advances in our understanding of the crosstalk between apoptotic and inflammatory signaling pathways in the context of atherosclerosis.

1 Introduction

Immunoregulatory effects of cell death

Cells can die through two major processes, traumatic cell death ("necrosis") and or programmed cell death ("apoptosis"). Features of necrotic cell death are cell swelling and loss of membrane integrity, whereas apoptosis is characterized by a completely different repertoire of morphological changes including cell shrinkage, DNA fragmentation and membrane blebbing[1]. Necrosis will elicit a pro-inflammatory response. In contrast, apoptotic cells maintain their membrane integrity and in addition are readily taken up by phagocytes including macrophages and dendritic cells. Both measures will prevent the release of cell contents and as a result, apoptotic death generally does not cause an inflammatory response. In fact, apoptotic cells and phagocytes ingesting apoptotic remnants both can act anti-inflammatory by secreting transforming growth factor β1 (TGFβ1), interleukin (IL) 10 and prostaglandin E2 (PGE2) and by decreasing the secretion of pro-inflammatory cytokines such as TNF-α, IL1 and IL12[2,3,4,5]. However, recent studies have shown that apoptosis not always acts inflammation-neutral or anti-inflammatory (for reviews see 6 and 7). For example, various inducers of apoptosis, among which Fas, can influence cytokine secretion or immune cell behaviour[8]. Adequate elimination of apoptotic remnants to prevent secondary necrosis and the ensuing pro-inflammatory response is therefore of great importance.

Inflammation and apoptosis in atherogenesis

Atherosclerosis, a chronic inflammatory disease, is initiated by endothelial dysfunction and accumulation of lipoprotein and low-density lipoprotein (LDL) derived lipids in the vessel wall. Expression of adhesion molecules in the endothelium results in adherence and migration of T lymphocytes and monocytes into the intima, after which monocytes differentiate into macrophages[9]. Intimal leukocytes release a whole range of pro- and anti-inflammatory cytokines, chemokines and growth factors[10]. Moreover reactive oxygen species (ROS) are generated in the intima, which act pro-apoptotic on the one hand and modify (phospho)lipids and LDL promoting foam cell formation on the other hand[11]. Modified LDL can also cause further endothelial activation and dysfunction[10]. Furthermore lesional vSMC are also able to take up lipids to form foam cells and secrete adhesion molecules and cytokines, thereby contributing to inflammation[12].

The relevance of apoptosis to plaque progression and instability has been established in numerous studies. Clearly, both rate and consequences of apoptotic cell death are dependent on the actual cell type and on lesion stage. VSMC apoptosis increases with plaque progression. Differences in expression patterns of pro- or anti-apoptotic proteins and survival cytokines like decreased insulin-like growth factor 1 (IGF-1) and increased p53 levels and alterations in expression of Bcl-2 family members may cause increased sensitivity of intimal vSMC to apoptosis compared to medial vSMC[13]. In murine atherogenesis persistent apoptosis of vSMC was seen to result in accelerated lesion development[14] and both persistent vSMC apoptosis and induction

of vSMC apoptosis in established lesions leads to plaque vulnerability as indicated by increased necrotic core and fibrous cap thinning[14,15]. In addition, in established lesions increased intimal inflammation was detected[15].
Macrophage death occurs at all stages of atherosclerotic lesion development[16], although in human lesions macrophage death was elevated mainly in advanced lesions compared to healthy vessels[17]. Apoptosis of macrophages can be induced by various stimuli present in the plaque including reactive oxygen species (ROS), oxidized LDL (ox-LDL), free cholesterol, TNF-α and Fas ligand[16]. Experimental studies in mice revealed that macrophage apoptosis may be beneficial in early atherosclerosis, limiting lesion size and cellularity. On the contrary in late stage atherosclerosis macrophage death leads to increased lesion size and necrotic core expansion. The latter is possibly attributable to insufficient removal of apoptotic cells and resulting secondary necrosis. Apoptosis of the various plaque cell types in atherosclerosis and its consequences for lesion progression and stability has been extensively reviewed elsewhere[13,16,18]. Recent studies, which be will reviewed here, suggest that several apoptotic proteins are involved in inflammatory processes while inflammatory mediators can influence apoptosis and that the association of apoptosis and inflammation might be of great significance in atherosclerotic lesion progression and stability. This review will discuss this link between apoptosis and inflammation in the context of atherosclerosis elaborating on pro-inflammatory effects of plaque cell apoptosis on the one hand and on pro- or anti-apoptotic effects of the foremost inflammatory mediators in atherosclerotic lesions on the other hand.

2 Immunomodulatory effects of intraplaque apoptosis

2.1 Inflammatory consequences of apoptotic protein activity

2.1.1 Apoptosis inducing TNF family proteins

Fas/Fas ligand

Fas (CD95) is a death receptor of the TNF receptor super family that stimulates cells to undergo apoptosis after ligation of Fas ligand. The Fas /Fas ligand system is key to the elimination of immune cells (T cells) and thus in the regulation of adaptive immune responses. In fact *lpr* en *gld* mice, which have inactivating mutations in Fas and Fas ligand respectively, both develop autoimmune disease very reminiscent of lupus erythematosis with overt lymphadenopathy and splenomegaly[19,20].
In several studies Fas triggered apoptosis has been implicated in neointima formation and atherogenesis. Adenoviral Fas ligand gene delivery inhibit neointima formation in balloon-injured rat carotid arteries[21], while in advanced atherosclerotic lesions it reduced the number of vSMC in the cap inducing intraplaque hemorrhage and buried cap formation. Moreover overexpression in carotid artery plaques was seen to promote leukocyte adhesion, endothelial leakage and intraplaque bleeding[22]. Several other studies have explored the impact of Fas function in atherosclerosis on inflammatory status. Yang *et al*[23] describe that Fas induced apoptosis of endothelial cells has anti-inflammatory properties. Transgenic mice overexpressing Fas ligand

on vascular endothelial cells showed decreased plaque macrophage and CD8+ T cell content and overall decreased aorta lesion size[23]. Conversely Fas signaling in vSMC had rather pro-inflammatory effects. Overexpression of inducible Fas-associated death domain (FADD), a downstream signal transducer of Fas, in a rat vSMC line increased apoptosis[23]. Seeding of these FADD overexpressing vSMC on rat carotid arteries led to Fas ligand induced intimal vSMC apoptosis resulting in MCP-1 and IL-8 secretion and monocyte recruitment to adventitia and neointima. The link between auto-immune disease and atherosclerosis has been addressed in Fas ligand defective *gld* mice, backcrossed to ApoE$^{-/-}$ mice. *gld.*ApoE$^{-/-}$ double transgenics not only had increased atherosclerotic lesion size and apoptotic cell content compared to ApoE$^{-/-}$ mice, but also increased lymphadenopathy, splenomegaly and formation of autoantibodies compared to *gld* mice[25], implying that autoimmune effects associated with Fas ligand deficiency is exacerbated by the proinflammatory milieu in ApoE$^{-/-}$ mice. Finally, Fas has been implicated in oxidative stress and ox-LDL induced cell death[26]. Ox-LDL induced apoptosis of T lymphocytes has been demonstrated to be preceded by an increased expression of Fas and membrane associated and soluble Fas ligand, and apoptosis could be inhibited by blocking of the Fas/Fas ligand dyad[26]. The relevance of Fas signaling for oxidative apoptosis in atherosclerosis is illustrated by colocalisation of TUNEL positive apoptotic cells in human carotid plaques with Fas expression on T cells and that of Fas ligand on macrophages. Interestingly both cell types showed increased iNOS expression, implicating Fas in iNOS induced cell death[27].

The above mentioned studies identify Fas as an important factor in atherosclerotic lesion apoptosis, including Ox-LDL and ROS induced cell death, of several cell types while both pro- and anti-inflammatory effects have been reported.

TRAIL

TNF related apoptosis inducing ligand (TRAIL), another member of the TNF super family, was originally described as a protein inducing apoptosis exclusively in transformed and infected cells. TRAIL acts by binding to signaling (i.e. death receptors or DR's) and non-signaling receptors (osteoprotegerin and decoy receptors) and displays both pro-apoptotic and immunoregulatory capacity[28]. TRAIL expression on immune cells is upregulated in response to type I interferons IFNα and IFNβ[29]. IFNα induced TRAIL upregulation on T cells has also been shown in atherosclerotic lesions via colocalization. The enhanced TRAIL expression enabled these T cells to induce apoptosis in coronary SMC[30]. TRAIL colocalizes with CD3 and ox-LDL in human stable lesions with increased expression in vulnerable plaques[31]. Secchiero *et al*[32] showed that systemic adenoviral TRAIL delivery reduces atherosclerotic lesion size in diabetic ApoE$^{-/-}$ mice, while increasing apoptosis of infiltrating macrophages within the lesion and increasing plaque vSMC content.

CD40

CD40 and CD40 ligand (CD154) are expressed by all cell types of human atherosclerotic

lesions. CD40 activation acts proliferative and pro-apoptotic and stimulates the secretion of pro-inflammatory cytokines and chemokines[33]. Genetic or antibody based CD40 signaling blockage decreased atherosclerotic lesion size in ApoE$^{-/-}$ and LDLr$^{-/-}$ mice[34,35] and led to a more stable phenotype[35]. Leukocyte CD40 ligand deletion however did not affect lesion size at all[36] suggesting that pro-atherogenic activity of CD40 ligand can be attributed to non-hematopoietic cells. This is supported by the finding that human umbilical vein endothelial cells (HUVECs) stimulated with CD40 ligand undergo massive apoptosis along with VCAM-1 and ICAM-1 release[37], suggesting that stimulation of CD40 on endothelial cells may promote monocytes/macrophages recruitment and thereby lesion development.

TWEAK

TNF-like weak inducer of apoptosis (TWEAK) has various biological functions including induction of inflammation, activation of cell growth, and stimulation of apoptosis upon binding its receptor Fn14[38]. Fn14 and TWEAK are expressed by macrophages, foam cells and smooth muscle cells in human carotid atherosclerotic plaques and in human aortic SMC[39,40]. Fn14 expression is upregulated after stimulation with IL1β and IFNγ[39], and TWEAK induced pro-inflammatory cytokines IL-6, MCP-1 and IL-8 in THP-1 human macrophages[40], suggesting that this pro-apoptotic TNF family member may function in apoptosis and inflammation in atherosclerotic plaques. Experimental proof to support this is lacking to date.

2.1.2 Bcl-2 family

Bcl-2 family members, which can be either pro- or anti-apoptotic, are the major regulators of both extrinsic and intrinsic apoptosis signaling pathways. In the vasculature these proteins are expressed in all major cell types and regulate apoptosis in response to oxidation and inflammation. Expression of Bcl-2 family members has been studied in human carotid endarterectomy samples showing increased expression of pro-apoptotic Bax and Bak in lipid laden macrophages, vSMC and apoptotic cells, absence of anti-apoptotic Bcl-2 and Bcl-XL in apoptotic cells and expression of the latter in non-apoptotic vSMC. Expression patterns of Bcl-2 family members in atherosclerotic lesions have been reviewed in detail by Kutuk and Basaga[41].

Inhibition of Bcl-XL by antisense oligonucleotides was shown to induce apoptosis in intimal cells of rabbit vascular lesions, an effect that was absent in medial SMC and in control vessels. Increased apoptosis led to a reduction in intimal lesion size[42].

Bax is another Bcl-2 family member which has been studied in an atherosclerotic mouse model. Liu *et al* showed reduced macrophage apoptosis in hematopoietic LDLr$^{-/-}$ chimeras with Bax deficiency leading to an increase in atherosclerotic lesion size in the aorta[43].

Increasing evidence indicates that Bcl-2 family members are involved in apoptosis induced by ROS, Ox-LDL, and inflammation. Ox-LDL induced apoptosis in U937 human monocytes and in human differentiated endothelial progenitor cells was

shown to involve ROS generation with concomitant mitochondrial Bax translocation and activation[44,45]. Translocation of Bax from cytoplasm to mitochondria in these cells could be inhibited by Bcl-2 overexpression, which did not prevent ROS generation suggesting that Bax acts downstream of ROS[44]. In endothelial cells Bax activation and translocation was mediated by an increase in p53[45]. Similarly minimally oxidized LDL induced apoptosis in human coronary endothelial and vSMC was mediated by Fas and TNF receptor domains and, in endothelial cells, accompanied by an increase in pro-apoptotic Bad and a decrease in anti-apoptotic Bcl-2[46]. In addition, activation of Bim and Bad and downregulation of Bcl-XL was observed in oxysterol (25-hydroxycholesterol and 7-ketocholesterol) induced cell death in P388D1 murine macrophages, probably by promoting AKT degradation[47]. Finally Badrichani *et al* showed that Bcl-2 and Bcl-XL overexpression not only protects bovine aortic EC's from TNFα induced apoptosis but also inhibits TNFα dependent upregulation of pro-inflammatory genes like E-selectin and IL-8 by inhibiting NF-κB[48].
Bcl-2 family members appear to be involved in apoptosis signaling pathways induced by both pro-apoptotic proteins and inflammatory mediators Ox-LDL and ROS and in addition are able to affect inflammatory responses in vascular cells.

2.1.3 P53 and p21

Tumor suppressor p53 is a key regulator of cellular homeostasis exerting various functions such as cell-cycle arrest, senescence, differentiation and apoptosis. Downstream regulators of p53 in apoptosis and survival include Bax, NOXA, PUMA, PTEN, Fas, DR5 and p21[49]. Vascular p53 expression was reported to be increased in advanced atherosclerosis. Various studies have been undertaken to elucidate the role of p53 in atherosclerosis investigating the effect of (cell type specific) p53 deficiency and p53 overexpression on lesion development in animal models (reviewed in 50). From these data p53 function in atherosclerotic lesion appears to be complex and stage-, context- and cell-dependent. For instance p53 protected from apoptosis and stimulated proliferation in atherosclerotic lesions in one study[51], whereas its overexpression in advanced atherosclerosis was seen to promote vSMC apoptosis and destabilize plaques[52]. In macrophages p53 induced apoptosis. Generally, deficiency of p53 increased atherosclerotic lesion size[50]. Recently p53 was implicated in Ox-LDL induced apoptosis in endothelial cells via the pro-apoptotic Bcl-2 member Bax[45]. Ox-LDL led to activation and mitochondrial translocation of Bax and subsequent apoptosis, processes that could be prevented by siRNA induced p53 knockdown. Earlier, induction of apoptosis by mildly oxidized LDL in human coronary endothelial and vSMC has also been shown to increase p53 expression together with that of multiple other pro-apoptotic proteins[53]. In a human colorectal cancer cell line (DLD-1) p53 induced apoptosis was preceded by increased expression of several ROS generating genes[54], identifying p53 as a pro-oxidant factor. In comparison, relatively low levels of p53 were able to protect DNA from oxidation and damage by ROS[55].
An important p53 target, p21 cyclin-dependent kinase inhibitor, also known as WAF1

and CIP1, can upon activation inhibit apoptosis[49]. Recombinant p21 was reported to inhibit lymphocyte proliferation and expression of pro-inflammatory cytokines IL2, TNFα and IFNγ[56] as well. Adenoviral gene delivery of p21 into balloon injured porcine arteries inhibited the development of intimal hyperplasia[57]. In addition, overexpression of a p21 mutant with increased biological activity, reduced restenosis in $ApoE^{-/-}$ mice[58]. This was attributed to attenuated vSMC proliferation and macrophage infiltration and increased apoptosis of vascular cells. In contrast, both systemic and leukocyte p21 deficiency in $ApoE^{-/-}$ mice protected against atherosclerosis and led to increased apoptosis rates. In these mice systemic p21 deficiency reduced VCAM-1 expression[59]. Furthermore p21 deficient peritoneal macrophages expressed lower levels of pro-inflammatory cytokines such as macrophage inflammatory proteins (MIP) 1 and 2 and displayed increased phagocytosis of apoptotic cells.
The role of p53 in atherosclerotic lesions is stage-, context- and cell-dependent. P53 is implicated in apoptosis induced by various factors like Ox-LDL and ROS involving different Bcl-2 family members as well. Several studies indicate that p53 responsive protein p21, like p53, has a complex role in atherogenesis.

2.2 Phagocytosis of apoptotic cells

Apoptotic cell death was originally considered to be immunologically neutral or anti-inflammatory[6]. In general apoptotic cells are rapidly removed by phagocytes such as macrophages and dendritic cells, which is mediated by dedicated eat-me signals including phosphatidyl serine (PS) exposure on the surface of apoptotic cells.
Various molecules participate in recognition and engulfment of apoptotic cells including CD14, TG2, calreticulin, Mer receptor tyrosine kinase, lactadherin, complement components (C1q, CR3 and CR4), integrins and scavenger receptors CD36, CD68 and SRA[60-64]. Although considerable redundancy in uptake and signaling mechanisms exists, these mechanisms may differ between the various phagocytic cells. Moreover inflammatory responses after apoptotic cell recognition and uptake may depend on specific receptors and proteins implicated as demonstrated in knockout mouse models[60,61,65-67].
Anti-inflammatory effects are attributable to the apoptotic cells themselves as well as to the remnant ingesting phagocytes[61]. For example phagocytic DC's exhibit decreased secretion of the pro-inflammatory cytokine TNFα after exposure to apoptotic cells[68] but secretion of anti-inflammatory mediators like TGFβ, IL10, and IL1 receptor antagonist is promoted[61]. In addition apoptotic lymphocytes were shown to release TGFβ[61]. Apoptotic cell death can also be pro-inflammatory. For example Fas induced apoptosis *in vivo* resulted in hepatic inflammation and neutrophil recruitment[8]. Furthermore, apoptotic cells may undergo secondary necrosis when not readily cleared by phagocytes, to subsequently evoke an inflammatory response. Figure 1 shows potential immunomodulatory effects of apoptotic cell death.
Defective phagocytosis by plaque macrophages has been demonstrated by Schrijvers *et al*[69]. Analysis of human endarterectomy samples revealed a higher ratio of free

to phagocytised apoptotic cells when compared with human tonsils[69]. Tabas has postulated the hypothesis that in early lesions phagocytotic capacity is sufficient to clear cellular debris implying that macrophage apoptosis will result in decreased lesion progression. In more advanced lesions phagocytosis is impaired, which not only dampens immunoregulatory feedback normally observed after phagocytosis but also favors proinflammatory secondary necrosis promoting lesion progression and instability[16].

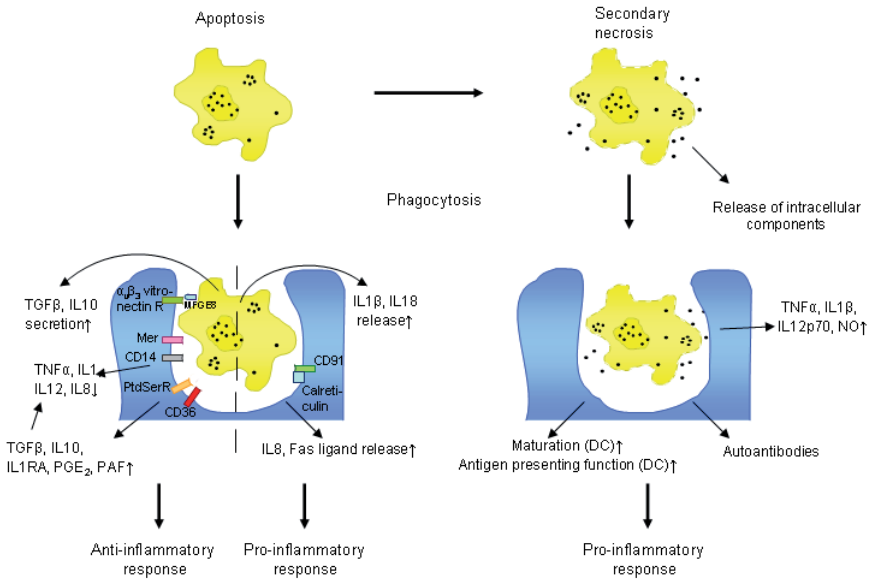

Figure 1. Modulation of inflammatory response through apoptotic cells and phagocytosis. Phagocytosis of apoptotic cells generally results in an anti-inflammatory response via increased release of anti-inflammatory molecules and inhibition of that of pro-inflammatory molecules by both apoptotic cells and phagocytes. However, apoptosis and subsequent phagocytosis can also be pro-inflammatory. A number of receptors and factors involved in apoptotic cell recognition and engulfment were shown to be associated with activation of pro- as well as with anti-inflammatory pathways, suggesting that a shifted pattern of apoptotic cell handling will translate in an altered inflammatory profile. Defective engulfment of apoptotic cells may lead to secondary necrosis. In contrast to apoptotic cells necrotic cells lose membrane integrity and their cellular content is released resulting in a pro-inflammatory response. TGFβ, transforming growth factor β; IL, interleukin; TNFα, tumor necrosis factor α; IL1RA, interleukin 1 receptor antagonist; PGE2, prostaglandin E2; PAF, platelet activating factor; NO, nitric oxide; DC, dendritic cell.

The relevance of phagocytosis to atherosclerosis was subject to various recent studies. Aprahamian *et al* [25] observed that the induced atherogenic response in Fas ligand deficient *gld*.ApoE$^{-/-}$ mice was associated with decreased apoptotic cell clearance and increased macrophage and lymphocyte infiltration. Even deficiency of ApoE itself may already affect phagocytosis[70]. *In vitro* ApoE deficient macrophages ingest less apoptotic thymocytes and in ApoE$^{-/-}$ mice apoptotic macrophage content is increased in various tissues compared with wild-type mice resulting in a pro-

inflammatory state as witness the elevated plasma TNFα levels in the former mice. One of the first macrophage receptors to be implicated in phagocytosis of apoptotic remnants is CD36, a class B scavenger receptor[61]. Ox-LDL, an established substrate for this receptor, was suggested to compete with apoptotic cells for ingestion by macrophages[71,72]. In keeping Khan *et al* [73] reported impaired uptake but not binding of apoptotic vSMC and fibroblast by thioglycolate elicited mouse peritoneal macrophages in the presence of excess Ox-LDL. In addition, release of IL6 and MCP-1, inhibited by phagocytosis without Ox-LDL, was no longer suppressed[72]. A similar apoptotic cell engulfment reducing effect of Ox-LDL was noted in peritoneal macrophages by Schrijvers *et al* [71]. Interestingly, minimally modified LDL, although not recognized by scavenger receptors, also can inhibit phagocytosis of apoptotic cells by binding to CD14, while increasing uptake of Ox-LDL[73]. Altogether these studies suggest partially shared but non-identical recognition sites for Ox-LDL and apoptotic cells. A second mechanism underlying the impaired apoptotic cell handling in atherosclerosis may involve Ox-LDL targeted auto-antibodies, which are present in both human and murine atherosclerotic lesions. These auto-antibodies were shown to bind to apoptotic cells and inhibit their phagocytosis by macrophages[74].

The role of several key genes in apoptotic cell removal on vascular disease progression has been recently addressed. Deficiency of leukocyte transglutaminase 2 (TG2), which mediates apoptotic cell uptake and TGFβ secretion by macrophages[62], in $LDLr^{-/-}$ mice was seen to increase aortic valve lesion size and intimal macrophage penetration[65]. In another study the effect of leukocyte milk fat globule-EGF factor 8 (also known as Mfge8 or lactadherin) deficiency was assessed on atherogenesis in $LDLr^{-/-}$ mice[66]. Mfge8 binds to PS exposed by apoptotic cells and enhances their uptake by macrophages[63]. Atherosclerosis was accelerated in $Mfge8^{-/-}LDLr^{-/-}$ mice and, importantly, lesions showed larger necrotic cores. In addition, Mfge8 deficiency appeared to favor proatherogenic Th1 type immune responses, with reduced IL10 production by spleen and increased IFNγ levels in both spleen and atherosclerotic arteries[66]. Another critical factor involved in phagocytosis of apoptotic cells is Mer receptor tyrosine kinase (Mer). Macrophages with dysfunctional Mer had defective phagocytosis of apoptotic thymocytes[64]. Moreover, Mer was shown to be necessary for apoptotic cell induced inhibition of NF-kB activation and pro-inflammatory cytokine secretion in dendritic cells[68]. Mer has been implicated in phagocytosis in atherosclerosis as well. Apoptosis induced in macrophages by free cholesterol loading elicited an inflammatory response in phagocytes, consisting of TNFα and IL1β production. This inflammatory response was constrained by Mer as shown by the use of Mer deficient phagocytes[67]. Similar to TG2 and Mfge8, deletion of leukocyte Mer in $LDLr^{-/-}$ mice also led to increased accumulation of apoptotic cells, increased macrophage area and lymphocyte infiltration resulting in accelerated lesion development[75].

3 Pro- and anti-apoptotic effects of immunomodulators in atherogenesis

3.1 Pro-inflammatory cytokines

IFNγ

Interferon gamma (IFNγ) is a pro-inflammatory cytokine produced by T and NK cells and macrophages. It is highly expressed in atherosclerotic lesions[76]. IFNγ has been shown to induce apoptosis in endothelial cells, SMC and macrophages in culture. Several pro-apoptotic proteins were suggested to be responsible for IFNγ induced cell death. In microvessel endothelial cells (EC) IFNγ synergizes with TRAIL to induce apoptosis, but not in large-vessel EC[77]. In agreement with the last finding, IFNγ lowers expression of death receptors DR4 and DR5 in HUVECs which is accompanied by decreased apoptosis[78]. In contrast, Li *et al* proposed a key role for Fas rather than TRAIL in IFNγ induced apoptosis in HUVECs[79]. Anti-Fas antibodies were able to completely block the pro-apoptotic effects of IFNγ. Likewise pro-apoptotic effects of IFNγ in vSMC were attributed to Fas[80] as well as to the pro-apoptotic X-linked inhibitor of apoptosis associated factor-1 (XAF1) and Bcl-2 family member Noxa[81] while in THP-1 macrophages upregulation of TNFR1 appeared to be implicated in IFNγ induced apoptosis[82].

Animal studies do support a pro-atherogenic role of IFNγ, but leave unanswered whether IFNγ dependent apoptosis could in part be held accountable for this effect. In IFNγ deficient ApoE$^{-/-}$ mice atherosclerotic lesion size and T lymphocyte content was shown to be reduced[83,84] while intraperitoneally administered recombinant IFNγ increases lesion size accompanied by an increase in lesional T lymphocytes[85]. A similar, reduced lesion size was observed in LDLr$^{-/-}$ mice lacking IFNγ[86].

Tumor Necrosis Factor alpha

Tumor Necrosis Factor alpha (TNFα) is highly expressed by activated macrophages and other immune cells in murine as well as human lesions. It exerts its, mainly pro-inflammatory, effects through two receptors, TNFR1 (p55) and TNFR1 (p75). For instance TNFα promotes monocyte/macrophage recruitment by inducing expression of E-selectin, VCAM-1 and ICAM-1 in endothelial cells via TNFR2 activation[87]. TNFα can induce apoptosis via TNFR1, which in contrast to TNFR2 contains a death domain, although TNFR1 is also capable of transducing survival signals[88-90]. TNFα induces apoptosis in endothelial cells and SMC, both on its own account and in response to various other pro-inflammatory cytokines like IFNγ and IL1β which are able to enhance expression of TNFα[91-93]. Boyle *et al* showed that in coculture macrophage derived TNFα acts proapoptotic on SMC via TNFR1[92]. Possibly, p73β, a protein closely related to p53, may mediate the pro-apoptotic effects of TNFα on vSMC as shown for rat aorta SMC by Tang *et al* [93]. The role of TNFα and TNF receptors in atherogenesis has been studied in various mouse models. The results are rather contradictory, but most data are supportive of a proatherogenic effect[76]. Two of the studies examining the effect of TNFα deletion assessed apoptotic cell content in atherosclerotic lesions. In ApoE*3 Leiden transgenic mice TNFα deletion resulted in decreased necrosis area but paradoxically an increased number of apoptotic cells[94]. In contrast, in ApoE$^{-/-}$ mice, TNFα deletion led to decreased apoptotic cell content[95].

Interleukins

Pro- and anti-inflammatory interleukins are regarded key mediators in vascular inflammation and atherogenesis[76,96]. More recently several interleukins such as IL1β, IL4 and IL10 have been shown to affect apoptotic processes as well, while others like IL6 and IL12 and IL18 promote proliferation[97,98]. IL1β can promote apoptosis of endothelial cells and SMC, an effect which could in human (HUVEC) and mouse lung EC be abrogated by overexpression of the naturally occurring interleukin 1 receptor agonist IL1-RA[99]. IL1β induced vSMC apoptosis however was shown to be TNFα dependent[91]. IL1β expression appears to regulate, and in turn is regulated, by various apoptotic proteins, including TWEAK receptor Fn14 and bcl-2 family members Bcl-2 and Bcl-XL[39,100]. The role of caspase-1 (Interleukin 1 converting enzyme, ICE) in pro-IL1β and pro-IL18 cleavage to their active form[101,102] further underlines the intertwined relationship between apoptosis and inflammation. Apart from IL1β, IL2[103] and IL4[104] have been implicated in apoptosis of vascular cells, at which the former acts in an indirect, TNFα and NO dependent manner.

Anti-apoptotic properties have been attributed to the anti-inflammatory IL10. In THP-1 macrophage IL10 was shown to decrease Ox-LDL induced apoptosis by upregulating anti-apoptotic Bcl-2 family members Bfl-1 and Mcl-1[105]. Interestingly plaque targeted and systemic overexpression of IL10 in LDLr$^{-/-}$ mice both inhibited atherosclerosis and resulted in decreased apoptosis of lesional macrophages[106]. An anti-apoptotic activity of this cytokine was supported by the finding that in advanced human atherosclerotic plaques high IL10 expression was seen to associate with decreased macrophage death[107].

Macrophage inhibitory factor

The pro-inflammatory macrophage inhibitory factor (MIF), which is increasingly expressed during atherogenesis, has recently been identified as an important contributor to inflammation in atherosclerotic lesions. MIF deletion in LDLr$^{-/-}$ mice resulted in reduced aortic atherosclerosis[108] while in ApoE$^{-/-}$ mice MIF blockade by neutralizing antibodies reduced macrophage infiltration and expression of adhesion and inflammatory molecules ICAM-1, MMP-2, TNF, IL-12, and CD40L[109]. Antibody blockade of MIF in LDLr$^{-/-}$ mice led to increased apoptosis in carotid arteries after endothelial denudation, pointing to a regulatory role of MIF in apoptosis[110]. In another study the anti-apoptotic properties of MIF in macrophages in culture were shown to be caused by inhibition of p53 expression[111].

3.2 Oxidative stress

During atherogenesis reactive oxygen species (ROS) and oxidized LDL (Ox-LDL) will accumulate in the vessel wall which leads to progressively increased oxidative stress. Ox-LDL, generated by *in situ* oxidation of extravasated LDL, can be taken up by macrophages leading to foam cell formation and promotion of inflammatory responses. Moreover, as discussed above, Ox-LDL can induce apoptosis in endothelial cells, vSMC and macrophages[112] and interfere with apoptotic cell handling, often

favoring secondary necrosis. ROS are thought to be pro-atherogenic by, amongst others, inducing cell proliferation and apoptosis, recruitment of leukocytes and expression of pro-inflammatory cytokines like TNFα, IL1β and IFNγ (reviewed in 113 and 114). ROS can also cause degradation of cellular components including (mitochondrial) DNA, proteins and lipids. Sources of ROS in the vasculature include cyclooxygenases, lipoxygenases, NO synthase and NADPH oxidase. Most of the atherosclerotic risk factors (hypercholesterolemia, diabetes, hypertension, smoking and aging) were seen to increase ROS production[114]. In inflammatory cells and in particular in phagocytes, NADPH oxidase, which is activated by phagocytic neutrophils and macrophages[114], is the major source of ROS. The importance of NADPH oxidase has been established in animal models. ApoE$^{-/-}$ that lack p47phox, an essential subunit of NADPH oxidase, showed reduced levels of O_2^- production and smaller atherosclerotic lesions in the aorta[115]. A similar protective effect was seen after pharmacological inhibition of NADPH oxidase in balloon injured rat carotid arteries[116]. Lipoxygenases (LO) are another class of ROS producing enzymes expressed in leukocytes and vSMC. Inhibition of 12/15–LO in vSMC reduces growth factor induced migration, proliferation and matrix production[117] and 5-lipoxygenase pathway products leukotriens are potent vascular inflammation inducing mediators[118]. In ApoE$^{-/-}$ mice 12/15-LO gene disruption led to substantially smaller aortic artery lesions together with decreased circulating levels of auto-antibodies to Ox-LDL[119] while pharmacological inhibition of the receptor for Leukotriene B4, a product of the 5-lipoxygenase pathway of arachidonic acid metabolism, in both ApoE$^{-/-}$ and LDLr$^{-/-}$ mice reduced monocyte infiltration and foam cells in atherosclerotic lesions[120].

In the vasculature nitric oxide (NO), produced by endothelial NO synthase (eNOS) and inducible NO synthase (iNOS), is instrumental in controlling vascular tone. At physiological concentrations it is suggested to be anti-apoptotic and atheroprotective. Under inflammatory conditions as apparent during atherogenesis, catalytically active iNOS will be upregulated and substrate as well as cofactors may become rate limiting favoring the production of the pro-oxidant nitroperoxide at the expense of a reduced NO production[114]. This translates in augmented synthesis of pro-inflammatory cytokines TNFα, MCP-1 and IFNγ and adhesion molecules VCAM-1 and ICAM-1, promoting recruitment, infiltration and activation of monocytes and macrophages. INOS has been shown to protect EC against apoptosis[121]. The critical importance of a proper NO/ROS balance for endothelial cell function was illustrated by a study in which antisense oligonucleotide induced downregulation of iNOS expression in EC was seen to lead to endothelial dysfunction and ROS mediated cell death[118]. Furthermore, foam cells show increased iNOS expression resulting in toxic levels of NO. Fas associated cell death may be involved in this toxicity[122].

Ox-LDL promotes generation of ROS in vascular and atherosclerotic lesion cells, which can in turn induce oxidation of LDL. In endothelial cells Ox-LDL stimulates expression of adhesion molecules and growth factors leading to monocyte recruitment[112]. Both mildly and extensively oxidized LDL can induce apoptosis of endothelial cells,

vSMC and macrophages including foam cells, although exposure of macrophages to low concentrations of oxidized LDL also was reported to stimulate proliferation[123]. Mildly oxidized LDL results not only in activation of Fas and TNF receptor signaling but also in an increased pro-apoptotic and decreased anti-apoptotic Bcl-2 family member expression in human coronary endothelial and SMC[53]. Furthermore Ox-LDL induces various transcription factors involved in oxidative stress pathways including p53 and NF-κB. Another mechanism by which oxidative stress influences Ox-LDL induced apoptosis and inflammation involves the endothelial oxidized LDL receptor LOX-1. Lectin-like oxidized low density lipoprotein receptor (LOX-1) activation by Ox-LDL stimulates NF-κB and subsequent adhesion molecule and pro-inflammatory gene expression. In endothelial cells Ox-LDL has been shown to reduce eNOS and increase LOX-1 expression thereby inhibiting endothelial cell proliferation and function[124]. In keeping, a recent study showed that LOX-1 disruption in LDLr$^{-/-}$ mice had reduced aortic atherosclerotic lesion size and pro-inflammatory markers within the lesions[125]. Furthermore LOX-1 expression has been linked to apoptosis in human endothelial cells[126]. Figure 2 gives a summary of the effects of ROS on apoptosis and inflammation.

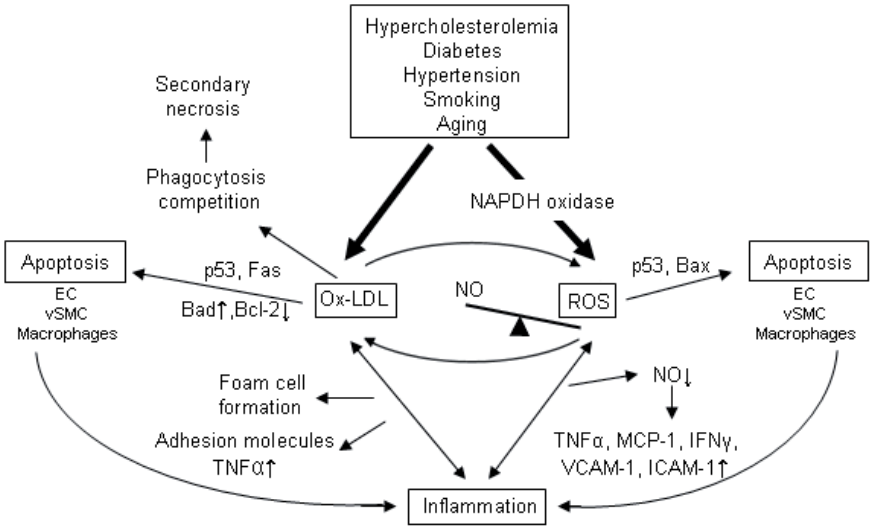

Figure 2. Generation of reactive oxygen species and its effects on inflammation, apoptosis and atherogenesis. During early atherogenesis ROS are generated in the vascular wall, reducing NO production and resulting in an unfavorable NO-ROS balance. ROS promotes endothelial activation, oxidation of LDL, apoptosis and inflammation. Ox-LDL in turn promotes generation of ROS and exerts pro-apoptotic and pro-inflammatory effects as well. Ox-LDL, oxidized low-density lipoprotein; NO, nitric oxide; ROS, reactive oxygen species; EC, endothelial cell; vSMC, vascular smooth muscle cell, TNFα, tumor necrosis factor α; MCP-1, monocyte chemoattractant protein 1; IFNγ, interferon γ; VCAM-1; vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1.

4 Summary and conclusions

Inflammatory and apoptotic processes in atherogenesis are closely intertwined. This notion has been conclusively demonstrated in various recent *in vitro* studies in cell types relevant to atherosclerosis as well as in *in vivo* studies in atherosclerosis prone animal models. First many of the proapoptotic members of the TNF super family, Fas, CD40 and Fn14, can promote secretion of inflammatory cytokines like MCP-1 and IL8 and/or adhesion molecules accompanying induction of apoptosis. Reactive oxygen species and Ox-LDL, accumulating in the vessel wall during plaque progression, are able to induce cell death in endothelial cells, vSMC and macrophages by upregulating several apoptotic proteins including Fas, TNFα, p53 and Bcl-2 family proteins. In addition, several key cytokines present in atherosclerotic lesions, apart from being induced in apoptosis, are able to induce apoptosis themselves. Vice versa, apoptotic cells were seen to modulate inflammatory responses either on their own account by elaborating amongst others cytokines and proteases or after uptake and processing by phagocytes. Impaired and altered phagocytosis, as apparent during progressive atherosclerosis, will, by eliciting a proinflammatory response and by promoting secondary necrosis, further aggravate inflammation leading to lesion progression and instability. Although several pro- or anti-apoptotic proteins and inflammatory mediators are attractive targets for therapeutic strategies, crosstalk between and the complex consequences of inflammatory, apoptotic and phagocytic processes have to be accounted for.

References

1. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol. 1995; 146(1):3-15.
2. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature. 1997; 390(6658):350-1.
3. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest. 1998; 101(4):890-8.
4. Chen W, Frank ME, Jin W, Wahl SM. TGF-beta released by apoptotic T cells contributes to an immunosuppressive milieu. Immunity. 2001; 14(6):715-25.
5. Chung EY, Kim SJ, Ma XJ. Regulation of cytokine production during phagocytosis of apoptotic cells. Cell Res. 2006; 16(2):154-61.
6. Rock KL, Kono H. The inflammatory response to cell death. Annu Rev Pathol. 2008; 3:99-126.
7. 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.
8. Faouzi S, Burckhardt BE, Hanson JC, Campe CB, Schrum LW, Rippe RA, Maher JJ. Anti-Fas induces hepatic chemokines and promotes inflammation by an NF-kappa B-independent, caspase-3-dependent pathway. J Biol Chem. 2001; 276(52):49077-82.
9. Libby P. Inflammation in atherosclerosis. Nature. 2002; 420(6917):868-74.
10. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999; 340(2):115-26.
11. Madamanchi NR, Hakim ZS, Runge MS. Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. J Thromb Haemost. 2005; 3(2):254-67.
12. Doran AC, Meller N, McNamara CA. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. Arterioscler Thromb Vasc Biol. 2008; 28(5):812-9.
13. McCarthy NJ, Bennett MR. The regulation of vascular smooth muscle cell apoptosis. Cardiovasc Res. 2000; 45(3):747-55.
14. 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.
15. 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.
16. 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.
17. 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.
18. Littlewood TD, Bennett MR. Apoptotic cell death in atherosclerosis. Curr Opin Lipidol. 2003; 14(5):469-75.
19. Nagata S, Suda T. Fas and Fas ligand: lpr and gld mutations. Immunol Today. 1995; 16(1):39-43.
20. Nagata S. Human autoimmune lymphoproliferative syndrome, a defect in the apoptosis-inducing Fas receptor: a lesson from the mouse model. J Hum Genet. 1998; 43(1):2-8.
21. Sata M, Perlman H, Muruve DA, Silver M, Ikebe M, Libermann TA, Oettgen P, Walsh K. Fas ligand gene transfer to the vessel wall inhibits neointima formation and overrides the adenovirus-mediated T cell response. Proc Natl Acad Sci U S A. 1998; 95(3):1213-7.
22. 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.
23. Yang J, Sato K, Aprahamian T, Brown NJ, Hutcheson J, Bialik A, Perlman H, Walsh K. Endothelial overexpression of Fas ligand decreases atherosclerosis in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2004; 24(8):1466-73.
24. 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.
25. Aprahamian T, Rifkin I, Bonegio R, Hugel B, Freyssinet JM, Sato K, Castellot JJ Jr, Walsh K. Impaired

clearance of apoptotic cells promotes synergy between atherogenesis and autoimmune disease. J Exp Med. 2004; 199(8):1121-31.
26. Alcouffe J, Therville N, Ségui B, Nazzal D, Blaes N, Salvayre R, Thomsen M, Benoist H. Expression of membrane-bound and soluble FasL in Fas- and FADD-dependent T lymphocyte apoptosis induced by mildly oxidized LDL. FASEB J. 2004; 18(1):122-4.
27. Esaki T, Hayashi T, Muto E, Kano H, Kumar TN, Asai Y, Sumi D, Iguchi A. Expression of inducible nitric oxide synthase and Fas/Fas ligand correlates with the incidence of apoptotic cell death in atheromatous plaques of human coronary arteries. Nitric Oxide. 2000; 4(6):561-71.
28. Almasan A, Ashkenazi A. Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy. Cytokine Growth Factor Rev. 2003; 14(3-4):337-48.
29. Kayagaki N, Yamaguchi N, Nakayama M, Eto H, Okumura K, Yagita H. Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs. J Exp Med. 1999; 189(9):1451-60.
30. Niessner A, Sato K, Chaikof EL, Colmegna I, Goronzy JJ, Weyand CM. Pathogen-sensing plasmacytoid dendritic cells stimulate cytotoxic T-cell function in the atherosclerotic plaque through interferon-alpha. Circulation. 2006; 114(23):2482-9.
31. 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.
32. Secchiero P, Candido R, Corallini F, Zacchigna S, Toffoli B, Rimondi E, Fabris B, Giacca M, Zauli G. Systemic tumor necrosis factor-related apoptosis-inducing ligand delivery shows antiatherosclerotic activity in apolipoprotein E-null diabetic mice. J Am Coll Cardiol. 2005; 45(7):1018-24.
33. Xu Y, Song G. The role of CD40-CD154 interaction in cell immunoregulation. J Biomed Sci. 2004; 11(4):426-38.
34. Lutgens E, van Suylen RJ, Faber BC, Gijbels MJ, Eurlings PM, Bijnens AP, Cleutjens KB, Heeneman S, and Daemen MJ. Atherosclerotic plaque rupture: local or systemic process? Arterioscler Thromb Vasc Biol. 2003; 23: 2123–30.
35. Mach F, Schonbeck U, Sukhova GK, Atkinson E, and Libby P. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. Nature. 1998; 394: 200–3.
36. Smook ML, Heeringa P, Damoiseaux JG, Daemen MJ, de Winther MP, Gijbels MJ, Beckers L, Lutgens E, Tervaert JW. Leukocyte CD40L deficiency affects the CD25(+) CD4 T cell population but does not affect atherosclerosis. Atherosclerosis. 2005; 183(2):275-82.
37. Xia M, Ling W, Zhu H, Ma J, Wang Q, Hou M, Tang Z, Guo H, Liu C, Ye Q. Anthocyanin attenuates CD40-mediated endothelial cell activation and apoptosis by inhibiting CD40-induced MAPK activation. Atherosclerosis (2008), doi:10.1016/j.atherosclerosis.2008.04.005.
38. Wiley SR, Winkles JA. TWEAK, a member of the TNF superfamily, is a multifunctional cytokine that binds the TWEAKR/Fn14 receptor. Cytokine Growth Factor Rev. 2003; 14: 241–249.
39. Muñoz-García B, Martín-Ventura JL, Martínez E, Sánchez S, Hernández G, Ortega L, Ortiz A, Egido J, Blanco-Colio LM. Fn14 is upregulated in cytokine-stimulated vascular smooth muscle cells and is expressed in human carotid atherosclerotic plaques: modulation by atorvastatin. Stroke. 2006; 37(8):2044-53.
40. Kim SH, Kang YJ, Kim WJ, Woo DK, Lee Y, Kim DI, Park YB, Kwon BS, Park JE, Lee WH. TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinas-9 in macrophages. Circ J. 2004; 68(4):369-9.
41. Kutuk O, Basaga H. Bcl-2 protein family: implications in vascular apoptosis and atherosclerosis. Apoptosis. 2006; 11(10):1661-75.
42. Pollman MJ, Hall JL, Mann MJ, Zhang L, Gibbons GH. Inhibition of neointimal cell bcl-x expression induces apoptosis and regression of vascular disease. Nat Med. 1998; 4(2):222-7.
43. 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.
44. Ermak N, Lacour B, Drüeke TB, Vicca S. Role of reactive oxygen species and Bax in oxidized low density lipoprotein-induced apoptosis of human monocytes. Atherosclerosis. 2008; 200(2):247-56.
45. Cheng J, Cui R, Chen CH, Du J. Oxidized low-density lipoprotein stimulates p53-dependent activation of

proapoptotic Bax leading to apoptosis of differentiated endothelial progenitor cells. Endocrinology. 2007; 148(5):2085-94.
46. Napoli C, Quehenberger O, De Nigris F, Abete P, Glass CK, Palinski W. Mildly oxidized low density lipoprotein activates multiple apoptotic signaling pathways in human coronary cells. FASEB J. 2000; 14(13):1996-2007.
47. Rusiñol AE, Thewke D, Liu J, Freeman N, Panini SR, Sinensky MS. AKT/protein kinase B regulation of BCL family members during oxysterol-induced apoptosis. J Biol Chem. 2004; 279(2):1392-9.
48. Badrichani AZ, Stroka DM, Bilbao G, Curiel DT, Bach FH, Ferran C. Bcl-2 and Bcl-XL serve an anti-inflammatory function in endothelial cells through inhibition of NF-kappaB. J Clin Invest. 1999; 103(4):543-53.
49. Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat Rev Cancer. 2002; 2(8):594-604.
50. Mercer J, Bennett M. The role of p53 in atherosclerosis. Cell Cycle. 2006; 5(17):1907-9.
51. Guevara NV, Kim HS, Antonova EI, Chan L. The absence of p53 accelerates atherosclerosis by increasing cell proliferation in vivo. Nat Med. 1999; 5(3):335-9.
52. 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.
53. Napoli C, Quehenberger O, De Nigris F, Abete P, Glass CK, Palinski W. Mildly oxidized low density lipoprotein activates multiple apoptotic signaling pathways in human coronary cells. FASEB J. 2000; 14(13):1996-2007.
54. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. Nature. 1997; 389(6648):300-5.
55. Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The antioxidant function of the p53 tumor suppressor. Nat Med. 2005; 11(12):1306-13.
56. Khanna AK, Plummer M, Nilakantan V, Pieper GM. Recombinant p21 protein inhibits lymphocyte proliferation and transcription factors. J Immunol. 2005; 174(12):7610-7.
57. Yang ZY, Simari RD, Perkins ND, San H, Gordon D, Nabel GJ, Nabel EG. Role of the p21 cyclin-dependent kinase inhibitor in limiting intimal cell proliferation in response to arterial injury. Proc Natl Acad Sci U S A. 1996; 93(15):7905-10.
58. Condorelli G, Aycock JK, Frati G, Napoli C. Mutated p21/WAF/CIP transgene overexpression reduces smooth muscle cell proliferation, macrophage deposition, oxidation-sensitive mechanisms, and restenosis in hypercholesterolemic apolipoprotein E knockout mice. FASEB J. 2001; 15(12):2162-70.
59. Merched AJ, Chan L. Absence of p21Waf1/Cip1/Sdi1 modulates macrophage differentiation and inflammatory response and protects against atherosclerosis. Circulation. 2004; 110(25):3830-41.
60. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. Nature. 2000; 407(6805):784-8.
61. 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.
62. 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.
63. 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.
64. 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.
65. 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.
66. 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.
67. Li Y, Gerbod-Giannone MC, Seitz H, Cui D, Thorp E, Tall AR, Matsushima GK, Tabas I. Cholesterol-induced apoptotic macrophages elicit an inflammatory response in phagocytes, which is partially attenuated by the Mer receptor. J Biol Chem. 2006; 281(10):6707-17.
68. Sen P, Wallet MA, Yi Z, Huang Y, Henderson M, Mathews CE, Earp HS, Matsushima G, Baldwin AS Jr, Tisch

RM. Apoptotic cells induce Mer tyrosine kinase-dependent blockade of NF-kappaB activation in dendritic cells. Blood. 2007; 109(2):653-60.
69. 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.
70. Grainger DJ, Reckless J, McKilligin E. Apolipoprotein E modulates clearance of apoptotic bodies in vitro and in vivo, resulting in a systemic proinflammatory state in apolipoprotein E-deficient mice. J Immunol. 2004; 173(10):6366-75.
71. 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.
72. 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.
73. Miller YI, Viriyakosol S, Binder CJ, Feramisco JR, Kirkland TN, Witztum JL. Minimally modified LDL binds to CD14, induces macrophage spreading via TLR4/MD-2, and inhibits phagocytosis of apoptotic cells. J Biol Chem. 2003; 278(3):1561-8.
74. 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.
75. 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.
76. Kleemann R, Zadelaar S, Kooistra T. Cytokines and atherosclerosis: a comprehensive review of studies in mice. Cardiovasc Res. 2008; 79(3):360-76.
77. Stefanescu R, Bassett D, Modarresi R, Santiago F, Fakruddin M, Laurence J. Synergistic interactions between interferon-gamma and TRAIL modulate c-FLIP in endothelial cells, mediating their lineage-specific sensitivity to thrombotic thrombocytopenic purpura plasma-associated apoptosis. Blood. 2008; 112(2):340-9.
78. Tanaka F, Kawakami A, Tamai M, Nakamura H, Iwanaga N, Izumi Y, Arima K, Aratake K, Huang M, Kamachi M, Ida H, Origuchi T, Eguchi K. IFN-gamma/JAK/STAT pathway-induced inhibition of DR4 and DR5 expression on endothelial cells is cancelled by cycloheximide-sensitive mechanism: novel finding of cycloheximide-regulating death receptor expression. Int J Mol Med. 2005; 15(5):833-9.
79. Li JH, Kluger MS, Madge LA, Zheng L, Bothwell AL, Pober JS. Interferon-gamma augments CD95(APO-1/Fas) and pro-caspase-8 expression and sensitizes human vascular endothelial cells to CD95-mediated apoptosis. Am J Pathol. 2002; 161(4):1485-95.
80. Rosner D, Stoneman V, Littlewood T, McCarthy N, Figg N, Wang Y, Tellides G, Bennett M. Interferon-gamma induces Fas trafficking and sensitization to apoptosis in vascular smooth muscle cells via a PI3K- and Akt-dependent mechanism. Am J Pathol. 2006; 168(6):2054-63.
81. Bai Y, Ahmad U, Wang Y, Li JH, Choy JC, Kim RW, Kirkiles-Smith N, Maher SE, Karras JG, Bennett CF, Bothwell AL, Pober JS, Tellides G. Interferon-gamma induces X-linked inhibitor of apoptosis-associated factor-1 and Noxa expression and potentiates human vascular smooth muscle cell apoptosis by STAT3 activation. J Biol Chem. 2008; 283(11):6832-42.
82. Inagaki Y, Yamagishi S, Amano S, Okamoto T, Koga K, Makita Z. Interferon-gamma-induced apoptosis and activation of THP-1 macrophages. Life Sci. 2002; 71(21):2499-508.
83. Gupta S, Pablo AM, Jiang X, Wang N, Tall AR, Schindler C. IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. J Clin Invest. 1997; 99(11):2752-61.
84. 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.
85. 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.
86. Buono C, Come CE, Stavrakis G, Maguire GF, Connelly PW, Lichtman AH. Influence of interferon-gamma on the extent and phenotype of diet-induced atherosclerosis in the LDLR-deficient mouse. Arterioscler Thromb Vasc Biol. 2003; 23(3):454-60.

87. Chandrasekharan UM, Siemionow M, Unsal M, Yang L, Poptic E, Bohn J, Ozer K, Zhou Z, Howe PH, Penn M, DiCorleto PE. Tumor necrosis factor alpha (TNF-alpha) receptor-II is required for TNF-alpha-induced leukocyte-endothelial interaction in vivo. Blood. 2007; 109(5):1938-44.
88. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 2003; 3(9):745-56.
89. 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.
90. Sugano M, Tsuchida K, Tomita H, Makino N. Increased proliferation of endothelial cells with overexpression of soluble TNF-alpha receptor I gene. Atherosclerosis. 2002; 162(1):77-84.
91. Niemann-Jönsson A, Ares MP, Yan ZQ, Bu DX, Fredrikson GN, Brånén L, Pörn-Ares I, Nilsson AH, Nilsson J. Increased rate of apoptosis in intimal arterial smooth muscle cells through endogenous activation of TNF receptors. Arterioscler Thromb Vasc Biol. 2001; 21(12):1909-14.
92. Boyle JJ, Weissberg PL, Bennett MR. Tumor necrosis factor-alpha promotes macrophage-induced vascular smooth muscle cell apoptosis by direct and autocrine mechanisms. Arterioscler Thromb Vasc Biol. 2003; 23(9):1553-8.
93. Tang V, Dhirapong A, Yabes AP, Weiss RH. TNF-alpha-mediated apoptosis in vascular smooth muscle cells requires p73. Am J Physiol Cell Physiol. 2005; 289(1):C199-206.
94. Boesten LS, Zadelaar AS, van Nieuwkoop A, Gijbels MJ, de Winther MP, Havekes LM, van Vlijmen BJ. Tumor necrosis factor-alpha promotes atherosclerotic lesion progression in APOE*3-Leiden transgenic mice. Cardiovasc Res. 2005; 66(1):179-85.
95. Canault M, Peiretti F, Poggi M, Mueller C, Kopp F, Bonardo B, Bastelica D, Nicolay A, Alessi MC, Nalbone G. Progression of atherosclerosis in ApoE-deficient mice that express distinct molecular forms of TNF-alpha. Pathol. 2008; 214(5):574-83.
96. von der Thüsen JH, Kuiper J, van Berkel TJ, Biessen EA. Interleukins in atherosclerosis: molecular pathways and therapeutic potential. Pharmacol Rev. 2003; 55(1):133-66.
97. Viedt C, Vogel J, Athanasiou T, Shen W, Orth SR, Kübler W, Kreuzer J. Monocyte chemoattractant protein-1 induces proliferation and interleukin-6 production in human smooth muscle cells by differential activation of nuclear factor-kappaB and activator protein-1. Arterioscler Thromb Vasc Biol. 2002; 22(6):914-20.
98. Coma G, Peña R, Blanco J, Rosell A, Borras FE, Esté JA, Clotet B, Ruiz L, Parkhouse RM, Bofill M. Treatment of monocytes with interleukin (IL)-12 plus IL-18 stimulates survival, differentiation and the production of CXC chemokine ligands (CXCL)8, CXCL9 and CXCL10. Clin Exp Immunol. 2006; 145(3):535-44.
99. Dewberry RM, King AR, Crossman DC, Francis SE. Interleukin-1 receptor antagonist (IL-1ra) modulates endothelial cell proliferation. FEBS Lett. 2008; 582(6):886-90.
100. Bruey JM, Bruey-Sedano N, Luciano F, Zhai D, Balpai R, Xu C, Kress CL, Bailly-Maitre B, Li X, Osterman A, Matsuzawa S, Terskikh AV, Faustin B, Reed JC. Bcl-2 and Bcl-XL regulate proinflammatory caspase-1 activation by interaction with NALP1. Cell. 2007; 129(1):45-56.
101. Li P, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C, McDowell J, Paskind M, Rodman L, Salfeld J, et al. Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. Cell. 1995; 80(3):401-11.
102. Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K, Fleming MA, Hayashi N, Higashino K, Okamura H, Nakanishi K, Kurimoto M, Tanimoto T, Flavell RA, Sato V, Harding MW, Livingston DJ, Su MS. Activation of interferon-gamma inducing factor mediated by interleukin-1beta converting enzyme. Science. 1997; 275(5297):206-9.
103. Choi KS, Song EK, Yim CY. Cytokines secreted by IL-2-activated lymphocytes induce endogenous nitric oxide synthesis and apoptosis in macrophages. J Leukoc Biol. 2008; 83(6):1440-50.
104. Lee YW, Kühn H, Hennig B, Toborek M. IL-4 induces apoptosis of endothelial cells through the caspase-3-dependent pathway. FEBS Lett. 2000; 485(2-3):122-6.
105. Halvorsen B, Waehre T, Scholz H, Clausen OP, von der Thüsen JH, Müller F, Heimli H, Tonstad S, Hall C, Frøland SS, Biessen EA, Damås JK, Aukrust P. Interleukin-10 enhances the oxidized LDL-induced foam cell formation of macrophages by antiapoptotic mechanisms. J Lipid Res. 2005; 46(2):211-9.
106. Pinderski LJ, Fischbein MP, Subbanagounder G, Fishbein MC, Kubo N, Cheroutre H, Curtiss LK, Berliner JA, Boisvert WA. Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-

deficient Mice by altering lymphocyte and macrophage phenotypes. Circ Res. 2002; 90(10):1064-71.
107. Mallat Z, Heymes C, Ohan J, Faggin E, Lesèche G, Tedgui A. Expression of interleukin-10 in advanced human atherosclerotic plaques: relation to inducible nitric oxide synthase expression and cell death. Arterioscler Thromb Vasc Biol. 1999; 19(3):611-6.
108. Pan JH, Sukhova GK, Yang JT, Wang B, Xie T, Fu H, Zhang Y, Satoskar AR, David JR, Metz CN, Bucala R, Fang K, Simon DI, Chapman HA, Libby P, Shi GP. Macrophage migration inhibitory factor deficiency impairs atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation. 2004; 109(25):3149-53.
109. Burger-Kentischer A, Göbel H, Kleemann R, Zernecke A, Bucala R, Leng L, Finkelmeier D, Geiger G, Schaefer HE, Schober A, Weber C, Brunner H, Rütten H, Ihling C, Bernhagen J. Reduction of the aortic inflammatory response in spontaneous atherosclerosis by blockade of macrophage migration inhibitory factor (MIF). Atherosclerosis. 2006; 184(1):28-38.
110. Chen Z, Sakuma M, Zago AC, Zhang X, Shi C, Leng L, Mizue Y, Bucala R, Simon D. Evidence for a role of macrophage migration inhibitory factor in vascular disease. Arterioscler Thromb Vasc Biol. 2004; 24(4):709-14.
111. Mitchell RA, Liao H, Chesney J, Fingerle-Rowson G, Baugh J, David J, Bucala R. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. Proc Natl Acad Sci U S A. 2002; 99(1):345-50.
112. Martinet W, Kockx MM. Apoptosis in atherosclerosis: focus on oxidized lipids and inflammation. Curr Opin Lipidol. 2001; 12(5):535-41.
113. Napoli C, De Nigris F, Palinski W. Multiple role of reactive oxygen species in the arterial wall. J Cell Biochem. 2001; 82(4):674-82.
114. Singh U, Jialal I. Oxidative stress and atherosclerosis. Pathophysiology. 2006; 13(3):129-42.
115. Barry-Lane PA, Patterson C, van der Merwe M, Hu Z, Holland SM, Yeh ET, Runge MS. p47phox is required for atherosclerotic lesion progression in ApoE(-/-) mice. J Clin Invest. 2001; 108(10):1513-22.
116. Jacobson GM, Dourron HM, Liu J, Carretero OA, Reddy DJ, Andrzejewski T, Pagano PJ. Novel NAD(P)H oxidase inhibitor suppresses angioplasty-induced superoxide and neointimal hyperplasia of rat carotid artery. Circ Res. 2003;92(6):637-43.
117. Reddy MA, Kim YS, Lanting L, Natarajan R. Reduced growth factor responses in vascular smooth muscle cells derived from 12/15-lipoxygenase-deficient mice. Hypertension. 2003; 41(6):1294-300.
118. Osher E, Weisinger G, Limor R, Tordjman K, Stern N. The 5 lipoxygenase system in the vasculature: emerging role in health and disease. Mol Cell Endocrinol. 2006; 252(1-2):201-6.
119. Cyrus T, Witztum JL, Rader DJ, Tangirala R, Fazio S, Linton MF, Funk CD. Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. J Clin Invest. 1999; 103(11):1597-604.
120. Aiello RJ, Bourassa PA, Lindsey S, Weng W, Freeman A, Showell HJ. Leukotriene B4 receptor antagonism reduces monocytic foam cells in mice. Arterioscler Thromb Vasc Biol. 2002; 22(3):443-9.
121. Hemmrich K, Suschek CV, Lerzynski G, Kolb-Bachofen V. iNOS activity is essential for endothelial stress gene expression protecting against oxidative damage. J Appl Physiol. 2003; 95(5):1937-46.
122. Esaki T, Hayashi T, Muto E, Kano H, Kumar TN, Asai Y, Sumi D, Iguchi A. Expression of inducible nitric oxide synthase and Fas/Fas ligand correlates with the incidence of apoptotic cell death in atheromatous plaques of human coronary arteries. Nitric Oxide. 2000; 4(6):561-71.
123. Hamilton JA, Myers D, Jessup W, Cochrane F, Byrne R, Whitty G, Moss S. Oxidized LDL can induce macrophage survival, DNA synthesis, and enhanced proliferative response to CSF-1 and GM-CSF. Arterioscler Thromb Vasc Biol. 1999; 19(1):98-105.
124. Ma FX, Zhou B, Chen Z, Ren Q, Lu SH, Sawamura T, Han ZC. Oxidized low density lipoprotein impairs endothelial progenitor cells by regulation of endothelial nitric oxide synthase. J Lipid Res. 2006; 47(6):1227-37.
125. Mehta JL, Sanada N, Hu CP, Chen J, Dandapat A, Sugawara F, Satoh H, Inoue K, Kawase Y, Jishage K, Suzuki H, Takeya M, Schnackenberg L, Beger R, Hermonat PL, Thomas M, Sawamura T. Deletion of LOX-1 reduces atherogenesis in LDLR knockout mice fed high cholesterol diet. Circ Res. 2007; 100(11):1634-42.
126. Li D, Mehta JL. Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells: evidence from use of antisense LOX-1 mRNA and chemical inhibitors. Arterioscler Thromb Vasc Biol 2000; 20:1116–1122.