# Apoptosis in Atherosclerosis: A Mini-Review

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**Abstract:** Apoptosis in atherosclerotic lesions is triggered by inflammatory processes, both *via* cell-cell contact and by cytokines and oxidized lipids. The role of apoptosis in atherogenesis is dual, depending on the stage of the plaque: In early stages, apoptotic death of smooth muscle - and inflammatory cells, such as lymphocytes and macrophages, may delay atherosclerotic process. However, once the plaque is formed, apoptosis may lead to plaque rupture and thrombosis.

**Key Words**: Atherosclerosis, apoptosis, pathogenesis.

## INTRODUCTION

Until the first Virchow classic descriptions there was only one type of cell death: necrosis. Walter Flemming described another type of cell death which had different characteristics, called chromatolysis [1]. It was in 1972 that Kerr, Wyllie and Currie introduced the term apoptosis to distinguish a special form of cell death different from necrosis [1]. However, apoptotic morphology was typically described by embryologists at the beginning of the previous century, who recognized it as a mechanism to counterbalance the excess cellular proliferation during the development of organs and limbs [2].

Apoptosis and necrosis are two main types of cell death (Table **1**): Cell death may occur in a disorganized and chaotic energy-independent manner, associated with swelling, in response to lethal injury, a way known as necrosis, or more properly oncosis [3, 4]. In contrast, cell death by apoptosis occurs in a highly organized and coordinated manner that involves shrinkage. Apoptosis requires the expression of certain groups of genes which are critical for signal transduction and metabolism. Thus, apoptosis constitutes a major mechanism by which tissues remove unwanted, damaged and aged cells. Because of the ordered sequence of the apoptosis' events, investigators often refer to it as programmed cell death. Different cell types undergoing apoptosis share very similar morphological features, such as chromatin compaction and margination, as well as nuclear condensation and fragmentation. These morphological alterations, which are different from those seen in oncosis, reflect the self-directed catabolism of cytoskeleton and other intracellular molecules. Complex interactions between extracellular microenvironmental factors and intrinsic gene products occur before apoptosis begins. However, once activated, apoptosis can progress in the absence of extracellular insults. What happens to apoptotic cells and bodies? They usually retain an intact cellular membrane and undergo removal by macrophages or adjacent cells, so that damage of neighboring tissue and possible inflammation are avoided.

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Anoikis is another type of apoptosis which seems to be involved in pathological remodeling of cardiovascular tissues, including cardiac myocyte detachment in heart failure, deendothelialisation and plaque rupture in atherosclerosis [5]. The term comes from the ancient greek word for homelessness and was first proposed by Frish and Francis in 1994 [6]. Anoikis is defined as programmed cell death induced by loss of cell/matrix interactions. Adhesion to structural glycoproteins of the extracellular matrix is essential for differentiated adherent cells survival in the cardiovascular system [5]. Anoikis could be one of the mechanisms which lead to vascular cell disappearance and thus, it is involved in vascular pathology. Disappearance of endothelial cells could initiate atherosclerosis [5].

In the last few years autophagy, also called type II programmed cell death has been described in various pathophysiological situations, such as neurodegenerative diseases and cancer [7]. Autophagy involves the formation of autophagic vacuoles, called autophagosomes, which contain portions of the cytoplasm and organelles which are typically surrounded by two membrane layers. Autophagosomes fuse with pre-existing lysosomes and transform into autolysosomes, where lysosomal hydrolases degrade their content [8]. Under normal conditions, autophagy is suppressed in most cells. Various conditions of stress, however, such as starvation and cellular injury may induce this process [8]. Recent observations suggest that autophagy occurs in atherosclerosis, but its specific role and regulation need to be furthermore investigated [8].

Imai and Thomas studied diet-induced lesions in cerebral atherosclerosis in swine some 40 years ago. They examined the atherosclerotic lesions extensively using transmission electron microscopy and documented smooth musle cell death [9]. It is now accepted that it is not only cell proliferation that holds the key to atherogenesis, but apoptosis as well, in an ongoing struggle between cell division and cell death [3]. The present review aims to clarify the role of apoptosis in atherosclerosis and its complications.

## MECHANISMS OF APOPTOSIS

The intracellular signaling pathways regulating apoptosis in many cell types are very similar to those of apoptosis in atherosclerosis [3]. There are basically two signaling path-

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**Table 1. Major Characteristics of the Two Processes of Cell Death: Apoptosis Versus Oncosis**

| | Apoptosis | Oncosis |
|---|---|---|
| Stimulation | External/Internal | External |
| Death Process | Programmed | Accidental |
| Cell Bodies | Shrinking | Swelling |
| Plasma Membranes | Intact | Broken |
| Lysosomes | Intact | Leaking |
| Mitochondria | Intact with Cytochrome-c release and ATP synthesis | Broken without ATP synthesis |
| Nuclei | Fragmented and shrinking | Broken and enlarged |
| Chromatin | Condensation | Clumping |
| DNA | Fragmented | Fragmented |
| Caspases | Present, early activation | Uncertain |
| Inflammation | Rare | Common |

ways regulating cellular apoptosis [10]. First, specific death ligands bind to their respective cell surface receptors activating downstream pathways which include the recruitment of adapter molecules through death domain interactions [11]. These molecules recruit a cysteine protease, called caspase 8, which, in turn, cleaves further caspases whose final aim is DNA degradation through activation of various intracellular substrates.

One of the most popular mediators of this category is the Fas/Fas ligand (FasL) system [12]. Fas (APO 1, CD95) is a 45 kDa type I transmembrane receptor protein that belongs to the tumor necrosis factor (TNF) receptor/nerve growth factor receptor family [10, 13, 14]. sFas is the soluble inactive form of the membrane-bound Fas and it is produced either by alternative splicing of the Fas mRNA or by protease cleavage of the extra-membrane part of Fas [15]. Circulating sFas has been positively associated with coronary artery disease [16, 17] and with peripheral arterial occlusive disease in patients with end-stage renal failure [17]. Recently, Blanco-Colio *et al.* have showed that circulating sFas is increased in subjects with high cardiovascular risk compared to healthy individuals [18]. Fas can activate apoptosis, when it is bound by its ligand, FasL [19, 20] The central adaptor molecule for Fas is Fas-associated death-domain (FADD) [11]. FasL (CD95R) is a 37 kDa type II transmembrane protein that belongs to the TNF and CD40 ligand family [21]. Fas is expressed in various immune and non-immune tissues, while FasL is expressed in a more restricted pattern. More specifically, FasL is expressed by activated T cells, natural killer (NK) cells and in more immunoprivileged tissues, such as testis and the eye. Additionally, FasL expression by normal endothelium is believed to play an atheroprotective role by inhibiting leukocyte extravasation and inducing apoptosis of mononuclear cells invading the vessel wall.

Activation of TNF receptor 1 (TNF-R1) requires an adaptor protein, TRADD (TNF-R1-associated death domain). When TNFa is bound to TNF-R1, then TRADD is recruited. TRADD is followed by FADD, which induces activation of caspase-8 [11]. Furthermore, TNF-related apoptosis inducing ligand (TRAIL) has two receptors, DR4 and DR5. Cell killing from those receptors occurs due to recruitment of FADD, as well, which in turn recruits the pro form of caspase 8 [11, 22].

The second signaling pathway detected in apoptosis does not require the activation of death receptors. It is a mitochondrial mediated pathway, as it depends on the release of mitochondrial cytochrome c (cyt c) and other pro-apoptotic molecules into the cytoplasm because of the loss of mitochondrial membrane integrity.

Cyt c is an abundant hemoprotein which is concentrated in the intermembrane space of mitochondria [23, 24]. Cyt c functions not only as an effective shuttle of electrons between respiratory complexes III and IV, but also as an effector molecule in apoptosis reactions *via* its catalytic properties [24]. The association of cyt c with an adaptor molecule, called apoptotic protease-activating factor-1 (Apaf-1) and caspase 9 activates effector caspases, which lead in DNA fragmentation [10, 24, 25]. Recently two additional functions of cyt c in apoptosis have been discovered that are carried out *via* its interactions with anionic phospholipids: a mitochondria specific phospholipid, called cardiolipid (CL) and a plasma membrane phospholipid, called phosphatidyleserin (PS). Cyt c interacts with CL and acts as CL oxygenase during apoptosis. This CL oxygenation is required for the release of pro-apoptotic factors from mitochondria. Furthermore, cyt c catalyzes the oxidation of PS. This peroxidized PS facilitates its externalization, essential for the recognition and clearance of apoptotic cells by macrophages in atherogenesis [24, 26]. Thus, soluble cyt c may serve as an easily assessed apoptotic marker [27].

In the nucleus of eukaryotic cells, DNA is associated with several protein components forming complexes, which

are known as nucleosomes. During apoptosis, endonucleases are activated that cleave chromatin into oligo- and mononucleosomes. These nucleosomes are packed into apoptotic bodies that are engulfed by macrophages. In case of mitochondria mediated apoptosis, nucleosomes are released into the circulation, as mitochondrial membrane's integrity is lost. Thus, nucleosomes can be detected in plasma and they may serve as a circulating marker of apoptosis [27-29].

It is certain that the death receptor and the mitochondrial pathways are distinct. However, one apoptotic pathway can activate the other. For example, activated caspase 8 can convert Bid, a member of a growing family of both pro-apoptotic (eg Bcl-2) and anti-apoptotic (eg Bid) regulatory gene products [30], into a pro-apoptotic molecule that promotes the mitochondrial pathway through cyt c release [10].

## EVALUATION OF APOPTOSIS: QUANTITIVE ASPECTS

No single validated marker exists for apoptosis. DNA fragmentation, eg *in situ* labeling of DNA fragments or terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL), has been used to detect apoptosis in athrosclerosis [3].

Annexin V and propidium iodide (PI) dual staining can be used to identify the stage of apoptosis. Annexin V:FITC and PI are added to the cellular suspension and cells are analysed by flow cytometry. Cells which are Annexin V:FITC positive/ PI negative are identified as early apoptotic, whereas cells Annexin V:FITC positive/ PI positive belong to late apoptosis or are necrotic [31]. Intracellular ROS production, usually observed in apoptosis, can be monitored by flow cytometry using a method based on reactive oxygen species conversion of 2',7'-dichlorofluorescin (DCFH) to 2',7'-dichlorofluorescein (DCF) [32]. Active caspase-3 and cleaved polymerase poli(ADP) ribose [PARP] can be measured in the cell lysate by the formation of sandwich complexes similarly to ELISA. The array of reagents contains a bead population with distinct fluorescence intensities coated with capture antibodies which are specific for active caspase-3 and PARP [31].

Nevertheless, the standard technique for apoptosis detection in tissue sections is the TUNEL technique. Various studies have used this technique to prove apoptosis in atherosclerosis and other diseases [33]. According to Zapolska-Downer *et al.* the cells in suspension are fixed with 1% paraformaldehyde in phosphate-buffered saline (PBS), washed in PBS, and suspended in 70% (v/v) ice-cold ethanol. The cells are stored in ethanol at -20° C. The controls and the samples are stained with FITC-dUTP by incubation in terminal deoxynucleotidyl transferase buffer. The cells are finally analysed by flow cytometry. Results are given in percentage of TUNEL positive cells [31].

Beyond tissue or cellular detection, the measurement of circulating markers of apoptosis, such as sFas, FasL, caspases, soluble cyt c and nucleosomes, offers an easy and effective way to detect apoptosis. Receptor mediated apoptosis can be assessed by measurement of sFas, FasL and caspases [34, 35], while mitochondria - mediated apoptosis by measurement of circulating cyt c and nucleosomes, using ELISA in both cases. The major limitation of biochemical assessment is that it does not enable the localization of apoptotic activity, since these markers are not tissue-specific.

## EFFECT OF APOPTOSIS IN DIFFERENT STAGES OF ATHEROSCLEROSIS

The earliest recognizable event in atherosclerosis is the increased recruitment of macrophages into the arterial subendothelium [36, 37]. Macrophages first play a protective role by removing LDL from the environment, but when cholesterol is in excess, macrophages are converted into foam cells [38, 39]. These cells accompanied by T cells from fatty streaks secrete chemokines and growth factors, which induce smooth muscle cells (SMCs) to migrate from the media to the intima and to proliferate within the neointima of the plaques under development. After this point, SMCs form the majority of foam cells and become the predominant cell type in the plaque [40].

Atherosclerotic plaque, therefore, is a complex structure that consists of SMCs, macrophages, lymphocytes, microvessels and different collagen types. The plaque often contains a central necrotic core that is separated from the vascular lumen by a fibrous cap, called "plaque cap". This cap is composed of SMCs and interstitial collagen fibers. When the mechanical stresses in the fibrous cap exceed a critical level, plaque rupture occurs. Factors predisposing to plaque rupture are cap thinning because of collagen and cell loss, a large lipid-rich necrotic core, narrow lumen and the fluidity of the lipid pool [41-43].

The significance of apoptosis in atherosclerosis depends on the stage of the plaque and on the cell types involved. The cells undergoing apoptosis are endothelial cells (ECs), smooth muscle cells (SMCs) and macrophages.

### Apoptosis of Endothelial Cells

Vascular endothelial cells form the inner lining of the vessels. Formation of new vessels and regression of pre-existing ones depends on the balance between endothelial cells' proliferation and apoptosis [44]. Additionally, in mature vessels endothelial cell turnover is controlled by proliferation and apoptosis. It is clear that endothelial cell apoptosis and dysfunction may initiate atherosclerosis [44]. More specifically, ECs in lesion-prone regions, where atherosclerotic plaques preferentially develop, present enhanced turnover suggesting increased apoptosis [33, 45]. *In vivo*, endothelial cell apoptosis results in endothelial dysfunction [46]. Interestingly, Kotani *et al.* demonstrated that FasL mRNA levels of circulating leukocytes reflect endothelial dysfunction in patients with hyperlipemia [47]. Regarding endothelial apoptosis' involvement in thrombogenicity, Giesen *et al.* reported that arterial thrombosis is considered to be induced when tissue factor (TF) in the vascular wall interacts with platelets and coagulation factors in circulating blood. Giesen *et al.* suggest that blood-borne TF is thrombogenic and that thrombus formation is initiated by vascular injury and blood exposure to vessel-wall TF [48].

### Apoptosis of Smooth Muscle Cells (SMCs)

Smooth muscle cells are significant components of atherosclerotic plaques and appear to play an important role on

the plaque stability [49]. In normal arteries SMCs apoptosis is 'silent', as they can withstand cell loss, whereas it renders atherosclerotic plaques more vulnerable to plaque rupture [49].

### Apoptosis of Macrophages

Monocytes/macrophages are considered important in atherogenesis, however their role in established plaques is still under investigation. Stoneman *et al.* developed CD11b-diphtheria toxin (DT) receptor (DTR) transgenic mice, where DT selectively induces apoptosis in monocytes/macrophages. In atherogenesis, DT significantly delayed plaque formation, suggesting that monocytes/macrophages are critical in this stage. However, in mice with established plaques, there was no evidence of significant sequelae of macrophage apoptosis with acute or chronic DT treatment, suggesting that formed plaques are resistant to monocytes reduction [50]. Interestingly, a large body of evidence suggests that plaque macrophages undergo apoptosis due to accumulation of free cholesterol (FC). Macrophages are known to accumulate large amounts of FC *in vivo* (forming foam cells) and cultured FC loaded macrophages appear to undergo cell death [51]. I. Tabas *et al.* used a cell culture model to explore the cellular mechanism of that process. It was demonstrated that during early stages of FC loading of macrophages a fraction of cells exhibited biochemical changes indicative of apoptosis, 50% of which was mediated by FasL activation and triggering of the Fas pathway [51]. In another study, Yao *et al.* proved that FC loading of macrophages triggers cyt c release and activation of caspase-9 and the effector caspases perhaps *via* Bax and/or cholesterol overloading of mitochondria [52].

The above findings are important from the clinical point of view, as macrophage death in advanced atherosclerotic lesions results in plaque rupture and acute vascular occlusion [52]. Selective removal of macrophages in atherosclerotic plaques with pharmacological means in order to stabilize rupture-prone lesions, is a novel concept in cardiovascular research [7]. However, macrophages are phagocytic cells and thus their additional role is that of scavenging modified lipoproteins, unwanted and apoptotic cells and debris *via* their phagocytic activity [8]. In early lesions, macrophage foam cells undergo apoptosis and then are rapidly scavenged by neighboring phagocytic macrophages [53]. In late lesions, macrophages also undergo apoptosis, but recent evidence suggests that phagocytosis by macrophages is severely impaired [8]. Thus postapoptotic necrosis of macrophages occurs, leading to increased inflammation, plaque instability and acute thrombosis [53].

To conclude, early in atherogenesis, apoptotic loss of macrophages and SMCs may delay the development of atherosclerotic plaque [54]. Furthermore, apoptosis may inhibit leukocyte extravasation and thus suppress intimal migration of monocytes and their transformation into tissue macrophages[13]. Once the plaque is formed, however, apoptosis can be detrimental for plaque stability and increase the risk of thrombosis [13, 33]. The apoptotic loss of SMCs leads to reduction of collagen type I, which is important for the tensile strength of the fibrous cap. Furthermore, plaque SMCs undergoing apopotosis increase the plaque thrombogenicity, as they have the same potency to generate thrombin as platelets [55]. In addition to this, apoptosis of macrophages can induce intraplaque thrombosis since the apoptotic bodies can activate thrombin if not scavenged [33].

## REGULATION OF APOPTOSIS IN ATHEROSCLEROSIS

Vascular SMCs and ECs are responsive to various environmental and internal factors, such as mechanical force [56-58], oxidative stress [59], radiation [60, 61], reactive oxygen or nitrogen species [62], lipids [61, 63], infectious agents [64], inflammatory cytokines [3, 64], immunological factors [64] and growth factors [30, 65], all of which are involved in mediating apoptosis. In this chapter the major determinants of apoptosis in atherosclerotic process are discussed.

### Lipids

Atherosclerotic lesions contain modified lipoproteins, especially oxLDL, which induces vascular cell apoptosis. While cholesterol and its esters have little proapoptotic action, oxidatively modified cholesterol has cytotoxic properties. High levels of cholesterol oxides in the cell membrane trigger foam cell death by apoptosis [66]. The mechanisms by which oxLDLs and oxysterols trigger apoptosis remain uncertain. Activation of caspases [67] and sphingomyelinase [68], attenuation of Bcl-2 [67] and suppression of the nuclear transcription factor-kB are considered to induce oxLDL-mediated apoptosis.

### Reactive Oxygen Species

Beyond oxidatively modified lipids, there is accumulating evidence that increased reactive oxygen species (ROS) production plays an important role in a wide array of clinical disorders, including atherosclerosis [69-72]. ROS can damage cells by oxidizing membrane phospholipids, proteins and nucleic acids. ROS' damaging effects are usually kept under control by endogenous antioxidants, such as glutathione, ascorbic acid and enzymes, such as superoxide desmutase (SOD), catalase and glutathione peroxidase. Oxidative stress occurs when antioxidants are overwhelmed by ROS.

NADPH oxidase on the plasma membrane, and cytoplasmic enzymes such as xanthine oxidase and nitric oxide synthase can generate superoxide anion ($H_2O_2$). Additionally, mitochondria are a major source of ROS, because superoxide anion is produced by the electron transport chain on the inner mitochondrial membrane. In mitochondria, where SOD is present, superoxide anion can be converted to hydrogen peroxide ($H_2O_2$). In case of high iron concentration, $H_2O_2$ can form hydroxyl radical ($OH^{\cdot}$), which is highly reactive. $O_2^{\cdot -}$ can also react with nitric oxide to form the reactive peroxynitrite ($ONOO^-$).

Mitochondria are vulnerable to oxidative stress as long as they are constantly exposed to high levels of ROS. Mitochondrial DNA undergoes oxidative damage. Lipid peroxidation, protein oxidation and nitration results in altered function of many mitochondrial enzymes. Furthermore, lipid peroxidation may lead to alterations in cell permeability. Finally, peroxidation of cardiolipin, through which cyt c is normally bound to mitochondrial membrane, leads to cyt c release into the cytosol [73-75]. Cyt c activates caspase-9, which triggers the caspase cascade resulting in apoptosis [72,

75]. Dussmann *et al.* also suggest that mitochondria increase their superoxide production during apoptosis after release of intermembrane proteins [76].

A particularly important consequence of oxidative stress is loss of endothelium-derived nitric oxide (NO), which has anti-atherosclerotic properties: Nitric oxide inhibits cell growth, leukocyte adhesion and platelet adherence and aggregation [77-79]. Reactive oxygen species reduce endothelial NO availability *via* at least 3 pathways: Firstly, superoxide reacts directly with NO leading to loss of NO bioactivity [80]. Additionally, increased vascular reactive oxygen species production promotes the oxidative degradation of endothelial NO synthase's (eNOS) cofactor, tetrahydrobiopterin resulting in eNOS 'uncoupling' [81-85]. Finally, Cooke has recently shown that there is redox-sensitive inhibition of the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which increases the levels of the endogenous NO-synthase inhibitor asymmetric dimethylarginine (ADMA) and as a result, reduces eNOS activity [86].

### Inflammation

The inflammatory factor dominates all phases of atherosclerotic cascade [87-89]. Endothelial cell (EC) injury due to turbulent blood flow, hypertension, hyperlipidemia and diabetes is associated with increased expression of proinflammatory cytokines [90, 91], vascular cell adhesion molecules (VCAMs) [90-93], and chemoattractant cytokines [94]. VCAMs promote the adherence of monocytes and T-lymphocytes on EC surface and their translocation into the subintimal space. Monocyte chemoattractant protein-1 (MCP-1) and regulated upon activation, normal T-cell expressed and secreted (RANTES) are members of the CC subfamily of chemokines, which mediate monocyte and T-lymphocyte migration respectively through endothelial cell-cell junctions [89, 90].

The CD8 positive T lymphocyte, effector of acquired immunity, can kill cells by apoptosis *via* the production of perforin, granzyme and FasL. However, mediators of innate immunity participate in this process. For example, a "cocktail" of cytokines, including interleukin(IL)-1β , TNF-α and INF-γ induce apoptosis [3]. In addition, cytokines of innate immunity can sensitize SMCs to kill by Fas engagement. Thus, innate and acquired immunity cooperate in causing apoptosis [95]. The activated macrophages and T cells produce cytokines, which synergistically induce apoptosis *via* activation of the sphingomyelin-ceramide signaling pathway [95] and the L-arginine-NO pathway [3].

The inflammatory and vascular cells, such as SMCs and ECs, express Fas in atheroma. Although vascular SMCs express Fas, they do not undergo apoptosis under normal conditions, even if FasL or agonistic antibodies are present. Under an inflammatory environment, however, the cytokines TNF-α, IL-1 and IFN-γ promote SMCs apoptosis, enhance the expression of Fas and provide additional death-promoting signals for the apoptotic cascade in plaque cells. Furthermore, T lymphocytes, especially the CD8 cells, produce effector molecules, such as FasL, granzymes and perforin that can promote the apoptosis of adjacent plaque cells. Interestingly, some vascular cells may autoactivate themselves for apoptosis by expressing FasL [3].

### Growth Factors

Vascular endothelial growth factor (VEGF) is known to have several functions on ECs, the most important of which is the induction of proliferation and differentiation [65]. Gerber *et al.* found that VEGF or a mutant potently prevents apoptosis and displays high levels of survival activity through the phosphatidylinositol 3'-kinase/ Akt signal transduction pathway [65]. VEGF has also been found to prevent apoptosis by inducing the expression of the anti-apoptotic proteins Bcl-2 and A1 in vascular ECs [30].

Angiopoietin-1, when bound to its receptor Tie-2 has been shown to have anti-apoptotic effect through the phosphatidylinositol 3'-kinase/ Akt signal transduction pathway, as that of VEGF [44, 96]. Basic fibroblast growth factor (FGF-2) acts as intravascular survival factor for endothelial cells *in vivo* [44, 97], while it has been shown *in vitro* to induce the anti-apoptotic protein Bcl-2 [98].

## INTERACTIONS BETWEEN LIPID LOWERING AND APOPTOSIS/ ATHEROSCLEROSIS

Recent studies on the effect of statins on apoptosis and atherosclerosis progression seem to have controversial results. Henrich *et al.* clearly proved that high dosage of simvastatin reduces TNF-a-induced apoptosis in endothelial progenitor cells, whereas the IL-1beta-mediated apoptosis was slightly reduced [99]. Interestingly, lovastatin has recently been proved to reduce apoptosis *via* its anti-inflammatory effect (inhibition TNF-a-induced apoptosis) independent of its lipid-lowering action in cerebral vascular endothelial cells [100]. Ahn *et al.*, however, demonstrated that simvastatin potentiates TNF-a-induced apoptosis *via* down-regulation of NF-kappa-B-regulated anti-apoptotic gene products that mediate cell proliferation, cell survival (e.g., Bcl-2, inhibitor of apoptosis protein 1 and inhibitor of apoptosis protein 2, surviving), invasion and angiogenesis (eg VEGF) [101]. Vascular smooth muscle cells apoptosis may be induced by atorvastatin in a dose- and time-dependant manner, while the mechanism seems to be mainly associated with down-regulation of survivin expression in SMCs [102]. Besides simvastatin, pitavastatin appears to enhance oxidant-induced vascular SMCs' apoptosis [103]. Finally, Park *et al.* demonstrated that simvastatin interfered with angiogenesis *via* the inhibition of the small GTP-binding protein, RhoA and suggested that this antiangiogenic effect of statins might prevent the atherosclerosis progression *via* inhibition of plaque angiogenesis.

## CONCLUSION

Apoptosis is mediated *via* two distinct mechanisms, the death ligand- and the mitochondria-mediated pathways, each of which is regulated by various environmental and endogenous factors, the major being the inflammatory process. Apoptosis plays a dual role in atherosclerosis: In early stages, apoptosis of SMCs and inflammatory cells may delay atherosclerotic process, while in late stages it may render the atherosclerotic plaque vulnerable to rupture.

Various circulating molecules, such as sFas, FasL, caspases, soluble cyt c and nucleosomes have already been used to detect or to quantify apoptosis. Although apoptosis is best assessed by histopathological techniques, biochemical

estimation of circulating apoptotic markers constitutes a considerably simpler and more convenient way to study apoptotic activity.

## ABBREVIATIONS

| | | |
|---|---|---|
| FasL | = | Fas ligand |
| TNF | = | Tumor necrosis factor |
| NK | = | Natural killers |
| TNF-R1 | = | TNF receptor 1 |
| FADD | = | Fas-associated death-domain |
| TRADD | = | TNF-R1-associated death domain |
| TRAIL | = | TNF-related apoptosis inducing ligand |
| TF | = | Tissue factor |
| cyt c | = | Cytochrome c |
| Apaf-1 | = | Apoptotic protease-activating factor-1 |
| CL | = | Cardiolipid |
| PS | = | Phosphatidyleserin |
| CE | = | Capillary electrophoresis |
| TUNEL | = | Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling |
| LIF | = | Laser-induced fluorescence detector |
| PI | = | Propidium iodide |
| DCFH | = | Dichlorofluorescin |
| DCF | = | Dichlorofluorescein |
| PARP | = | Polymerase poli(ADP)ribose |
| PBS | = | Phosphate-buffered saline |
| SMCs | = | Smooth muscle cells |
| FC | = | Free cholesterol |
| ROS | = | Reactive oxygen species |
| SOD | = | Superoxide desmutase |
| NO | = | Nitric oxide |
| eNOS | = | Endothelial NO synthase |
| ECs | = | Endothelial cells |
| DDAH | = | Dimethylarginine dimethylaminohydrolase |
| ADMA | = | Asymmetric dimethylarginine |
| VCAMs | = | Vascular cell adhesion molecules |
| MCP-1 | = | Monocyte chemoattractant protein-1 |
| RANTES | = | Regulated upon activation, normal T-cell expressed and secreted |
| VEGF | = | Vascular endothelial growth factors |
| FGF-2 | = | Fibroblast growth factor |
| DT | = | Diphtheria toxin |
| DTR | = | Diphtheria toxin receptor |

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