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3

The Innate Immune Response Mediated by TLRs in Atherosclerosis

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1. Introduction

The average life expectancy increased in the 20th Century, implying that important changes in disease and causes of death worldwide have occurred. Longevity increase and risk factors for chronic diseases have been combined to turn cardiovascular diseases into one of the main causes of death in the world (Libby, 2011). Heart disease and stroke are the first and third leading causes of death, respectively, in the United States. In 2006, cardiovascular disease was responsible for 31.7% of all deaths: 26.0% from heart disease and 5.7% from stroke (Heron et al., 2009). Deaths from coronary heart disease (425,425 deaths) comprise 67.4% of all deaths from heart disease (631,636 deaths) (Keenan et al., 2011). In developing countries such as Mexico, cardiovascular disease is the leading cause of death (Inegi, 2009). Atherosclerosis is a disease characterized by the accumulation of lipids, fibrous elements, cell proliferation and an inflammatory response that results in changes to the arterial wall (Libby, 2002). This disease has been observed in man throughout history, having been identified and reported in Egyptian mummies 3500 years old (Allam et al., 2009).

2. Risk factors associated with cardiovascular diseases

The risk factors associated with cardiovascular disease include the following: age, male gender, high serum levels of low-density lipoproteins (LDL), cholesterol, high-density lipoproteins, high serum cholesterol levels, diabetes mellitus, hypertension, smoking, family history of premature cardiovascular disease and infections by microorganisms such as *Chlamydia pneumoniae*. Furthermore, the combination of these risk factors is associated with a higher risk of cardiovascular disease (Ross, 1999, Garg, 2011).

3. Low-density lipoprotein structure

LDL is a spherical particle with a 22 nm diameter and a molecular weight of 2500 kDa. The particle consists of a hydrophobic nucleus of about 1600 cholesterol ester molecules and 170 triglyceride molecules surrounded by a superficial monolayer of 700 phospholipids

molecules (mainly phosphatidylcholine) and 600 molecules of free cholesterol. Apolipoprotein B-100 (apoB-100) is found embedded in a monolayer; it consists of 4536 residues of amino acids, with a molecular weight of 500 kDa (figure 1). The average half-life of circulating LDL is 2.5 days (Segrest et al., 2001). The main disposal mechanism for LDL in the blood is by endocytosis of nucleated cells through the LDL receptor, which is also the primary source of cholesterol used to maintain cell membranes (Jeon & Blacklow, 2005).

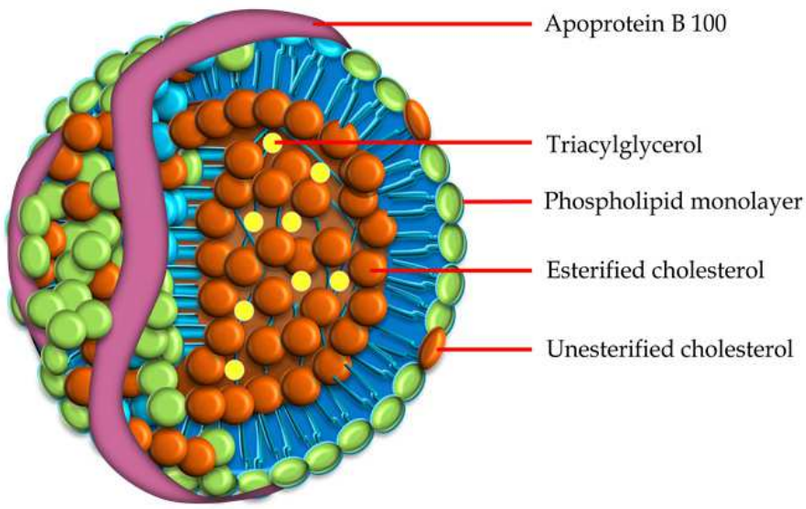

Fig. 1. Low-density lipoprotein structure. The LDL is a spherical particle consisting of the cholesterol ester, triglycerides, phospholipids, free cholesterol and apolipoprotein B-100.

4. Contribution of the low-density lipoprotein in atherosclerotic lesion development

An increase in plasma LDL levels leads to an increase in the adherence of circulating monocytes to arterial endothelial cells and, at the same time, to an increased rate of entry of LDL into the intima, resulting in a higher steady state concentration of LDL in the intima. Once incorporated, the LDL can undergo oxidative modification by endothelial cells, smooth muscle cells, or macrophages and this oxidation is a key step in the development of an atherosclerotic lesion (Steinberg, 1997). There is evidence demonstrating that oxidized LDL (oxLDL) is present in atherosclerotic plaques. Immunohistochemical analysis using antibodies against oxLDL has revealed oxLDL in atherosclerotic lesions of humans and hyperlipidemic rabbits (Damasceno et al., 2006). Likewise, oxLDL has also been obtained from atherosclerotic plaques of human arteries, and this molecule (oxLDL) presents the same properties and characteristics as oxLDL observed *in vitro*: a high electrophoretic mobility, high free cholesterol content, and a high proportion of sphingomyelin and lysophosphatidylcholine in the phospholipid fraction (Ylä-Herttuala et al., 1989). LDL may suffer a minimal oxidation and is known as minimally modified LDL (mmLDL) or complete (oxLDL); mmLDL increases adherence and penetration of monocytes, in part by stimulating the release of MCP-1 from endothelial cells (Cushing et al., 1990). mmLDL can also stimulate the release of macrophage colony-stimulating factor, which can induce differentiation of the

monocyte into a cell with the phenotypic pattern of a tissue macrophage, including increased expression of the scavenger receptor (SR) (Rajavashisth et al., 1990), which does not recognize mmLDL (Berliner et al., 1990). In contrast, oxLDL is itself directly chemotactic for monocytes and is the major ligand for SR and other receptors on the arterial macrophage that contribute to foam cell formation. These may be the basis for the contribution that cells make to the foam cell population. A centrally important point is that the fatty streak lesion, while being clinically silent itself, is the precursor of the more complex lesions that cause stenosis and limited blood flow. These complex lesions ultimately represent the sites of thrombosis leading to myocardial infarction (Steinberg, 1997).

5. Atherosclerotic plaque development

The atherogenic process starts with endothelial dysfunction, recently, have shown that endothelial dysfunction may be caused by increased production of free radicals causing oxidative damage in vascular endothelial cells, which may be due to unresolved inflammatory response or the loss of balance between tumorigenic [apoptosis ('Yin')] and tumoricidal [wound healing or resolution ('Yang')] of the acute inflammatory process, which represents one of the first stages in the pathogenesis of atherosclerosis (Khatami M, 2008, 2009, 2011). The first phase consists of a loss of homeostatic functions in the endothelium (anti-adhesive, anti-aggregating, anti-proliferative, anti-thrombotic, antioxidant, and vasomotor tone regulator), as well as an increase in the endothelial permeability to LDL, which retain the extracellular matrix (figure 2A) (Ross, 1999). The LDL is modified by lipoperoxidation in the sub-endothelial space by oxygen-derived compounds produced by endothelial cells (Steinberg, 1997, Libby, 2002). The increase of LDL particles in the sub-endothelial space initiates the formation of the atherosclerotic plaque (Ross, 1999, Steinberg, 1997, Libby, 2002). The lipolysis of LDL by phospholipase A2 and lipoperoxidation generates lysophosphatidylcholine, which increases the pro-inflammatory effect in the artery intima (Mehrabian & Allayee, 2003). As a consequence, the expression of adhesion molecules are increased, including platelet/endothelial cell adhesion molecule (PECAM)-1, intercellular cell adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1 (Davies et al., 1993). These adhesion molecules permit the interaction of T cells and circulating monocytes with endothelial cells (Ross, 1999, Libby, 2002).

Moreover, the endothelial and smooth muscle cells synthesize and secrete chemoattractants, such as monocyte chemotactic protein (MCP)-1 (Ross, 1999, Libby, 2002), thereby stimulating the migration and accumulation of monocytes to the lesion site (figure 2A) (Osterud & Bjorklid, 2003). Other cells that participate in the atherosclerotic plaque include macrophages and platelets, which adhere to proteins of the extracellular matrix, such as von Wilebrand factor and exposed collagen. The adherence of platelets to the exposed matrix is considered the first stage in the formation of a clot (Ross, 1999). Subsequently, activated platelets release vasoactive mediators that lead to the formation of a pro-inflammatory state during clot development (Shi & Morrell, 2011). The smooth muscle cells then migrate to the lesion (figure 3B), stimulated by growth factors, such as fibroblast growth factor, among other stimuli. In addition, T cells are recruited (figure 2B) and secrete tumor necrosis factor (TNF)-α, IL-2, and other molecules (Ross, 1999, Libby, 2002).

Monocytes and macrophages participate in the innate immune response and are essential effector cells during atherosclerosis. These cells express the cell surface scavenger receptors

(SR A type I and II, and CD36), which identify and internalize oxLDL particles (Mazzone, 2000). Upon internalization, oxLDL induces monocyte transformation into foam cells (figure 2B). These events precede the formation of the advanced lesion (figure 2C), which tends to form a fibrous cover in the walls of the lumen. The fibrous cover is characterized by an extracellular growth of lipids, especially cholesterol, cholesterol esters, and matrix proteins derived from smooth muscle cells. These lesions extend to the shoulders of the plaque (Ross, 1999). As a result, the activated macrophages in the plaque secrete pro-inflammatory cytokines (Takahashi et al., 2002), among which are interleukin (IL) -1β, IL-8, TNF-α, macrophage colony-stimulating factor, and MCP-1, resulting in further monocyte/macrophage recruitment and their accumulation (Ross, 1999). Within the plaque, macrophages increase their expression of the co-stimulatory molecules CD80/CD86 (Buono et al., 2004), CD40 (Phipps, 2000), and major histocompatibility (MHC) type II molecules, which modulate T cell activation (Buono et al., 2004). The activation of T cells favors the secretion of interferon-gamma and TNF-α, which act to amplify the inflammatory response. However, apoptosis or necrosis may be generated by the accumulation of lipids, promoting the advance of the necrotic nucleus to the plaque (figure 2C) (Ross, 1999). Moreover, damage to the lesion may be augmented by macrophages that produce TNF-α, IL-1β, and metalloproteinases (Ross, 1999, Libby, 2002). The atherosclerotic lesion may suffer a rupture in the fibrous layer (figure 2D) or ulceration, which leads to unstable angina syndromes or myocardial infarction (Ross, 1999). The vulnerability of the plaque originates from a thinning of the shoulders of the lesion, which happens when macrophages degrade the matrix of the fibrous layer by means of interstitial collagenase, gelatinase, and stromelysin. In addition, there is an inhibition in the secretion of the matrix proteins from smooth muscle cells by IFN-γ secreted by T cells. Degradation of the fibrous layer may lead to a hemorrhage (figure 2D). Alternatively, the activated platelets adhere to the injured artery and cause the formation of the clot and occlusion of the artery. These changes may also be accompanied by the production of pro-coagulant tissue factors, which enhances the possibility of thrombosis (Ross, 1999, Libby, 2002).

6. TLRs

Several lines of evidence have demonstrated that toll-like receptors (TLRs) play an essential role in inflammatory responses (Medzhitov, 2001, Trinchieri & Sher 2007) and may be important for the progression of atherosclerotic disease. The gene that encodes the Toll receptor was discovered early in the 1980s as an essential component in the path that establishes the dorsoventral axis in the early *Drosophila melanogaster* embryo (Anderson et al., 1985). In 1996, Lemaitre et al. documented the first Toll-like receptor involved in the anti-fungal immune response in *D. melanogaster*, and the discovery of the first human TLR4 was performed in 1997 by Medzhitov et al., which has since been identified as a crucial component of the innate and adaptive immune responses. In mammals, 13 TLRs have been described, (11 in humans), which are located at the cellular surface and in intracellular vesicles (Kawai, et al., 2010). The TLRs form a family of receptors that have been phylogenetically conserved, exhibiting three structural characteristics: 1) they have an extracellular region that is rich in leucine-rich repeats; 2) they have a short transmembrane region; and 3) they have a cytoplasmic region that is homologous to the IL-1 receptor, also called Toll/interleukin-1–receptor (TIR), which is required to initiate signaling cascades (Medzhitov, 2001).

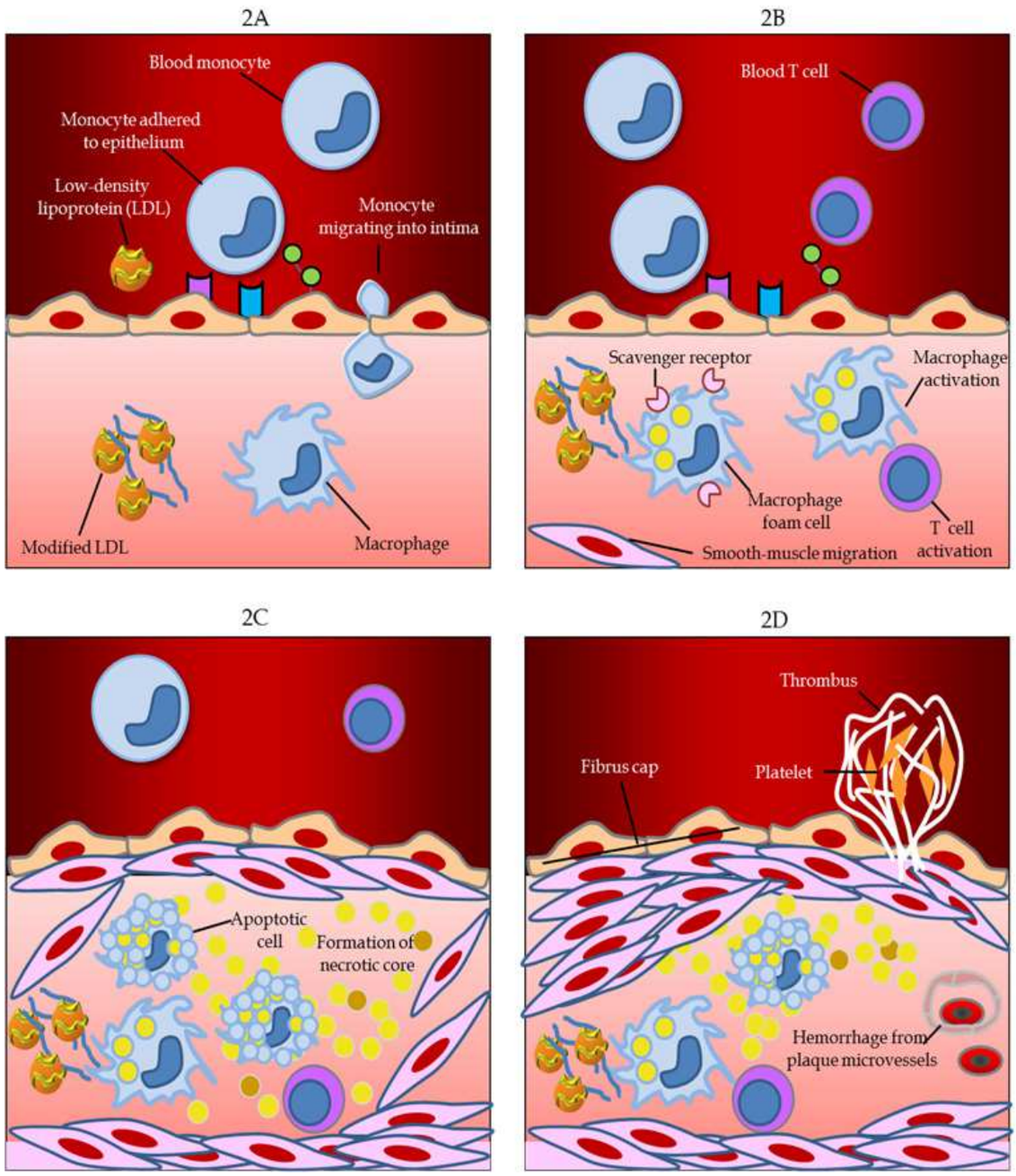

Fig. 2. Development of the atherosclerotic plaque. (2A) The lesion originates when damage to the endothelium increases the endothelial permeability and promotes leukocyte migration and adhesion. (2B) In the following stage of the lesion, smooth muscle cells migrate to the lesion, macrophages transform into foam cells, T cells are activated, platelets adhere to and accumulate at the lesion, and leukocytes continue to arrive. (2C) In the lesion, an accumulation of macrophages occurs, which subsequently die by apoptosis or necrosis, generating the necrotic nucleus and forming the fibrous layer. (2D) In the final stages, the lesion exhibits a thinning of the fibrous cap, and plaque rupture and bleeding of microvessels can ensue.

TLRs participate in the innate immune response, recognizing pathogen-associated molecular patterns (PAMPs), which are described in Table 1.

TLR	Ligand	Source ligand	
TLR1/TLR2	Tri-acyl lipopeptides	*Mycobacterium tuberculosis*	(Takeda et al., 2002)
TLR2	Peptidoglycan	*Staphylococcus aureus*	(Schwandner et al., 1999)
	Lipoarabinomannan	*Mycobacterium tuberculosis*	(Tapping & Tobias 2003)
	Phospholipomannan	*Candida albicans*	(Netea et al., 2002b)
TLR3	dsRNA	Viruses	(Alexopoulou et al., 2001)
TLR4	LPS	Gram-negative bacteria	(Chow et al., 1999)
	Envelope F protein	Respiratory syncytial virus	(Kurt-Jones et al., 2000)
	Glycoinositolphospholipids	*Trypanosoma cruzi*	(Oliveira et al., 2004)
TLR5	Bacterial flagellin	*Salmonella typhimurium*	(Andersen-Nissen et al., 2007)
TLR6/TLR2	Lipoteichoic acid	Group B streptococcus	(Henneke et al., 2005)
TLR7	ssRNA	Viruses	(Diebold et al., 2004)
TLR8	ssRNA	Viruses	(Heil et al., 2004)

Table 1. TLRs and some of their ligands

6.1 TLRs signaling

The activation of TLRs involves their dimerization, heterodimerization, or collaboration with other receptors, as well as a redistribution and aggregation at the cell surface (Husebye et al., 2006, Trianrafilou et al., 2006). Most TLRs use signaling pathways dependent on myeloid differentiation primary response protein 88 (MyD88). MyD88-dependent signaling starts in the TIR region, which then recruits the MyD88 adaptor molecule and promotes the association of IRAK (IL-1RI-associated protein kinase) 4 and IRAK1. During the formation of this complex, IRAK4 activates and phosphorylates IRAK1, which in turn interacts with TRAF (TNF receptor-associated factor) 6, thereby generating the IRAK1-TRAF6 complex that can interact with other molecules and induce the activation of the IKK complex. The IKK complex consists of IKKα and IKKβ, which catalyze phosphorylation of IkB. Phosphorylated IkB is then ubiquitinated and degraded by the proteosome, allowing the liberation and further translocation of NF-κB to the nucleus. The MyD88 independent pathway involves the TRIF protein (Toll/interleukin-1-receptor (TIR)-domain-containing adaptor protein inducing interferon (IFN)-β), which associates with the TANK-binding

kinase (TBK) 1. TBK1, in turn, induces the phosphorylation of the interferon-regulatory factor (IRF) 3 transcription factor and allows its translocation to the nucleus. Thus, the activation of TLR signaling pathways induces NF-κB and IRF translocation, which activate multiple inflammatory genes such as TNF-α, IL-1β, IL-6, IFN-β, CD80, CD86, ICAM-1, VCAM-1, IL-8, and MIP1-α, among other molecules (figure 3) (Akira et al., 2006, Medzhitov, 2001).

7. TLRs Expression in atherosclerosis

In human and mouse atherosclerotic lesions, TLR1, TLR2, and TLR4 have been shown to be over-expressed in endothelial cells and monocytes/macrophages (Edfeldt et al., 2002). For example, endothelial cells located within the atherosclerotic lesion express high levels of TLR1, TLR2, and TLR4, whereas endothelial cells from a normal artery exhibit lower expression levels of these TLRs (Edfeldt et al., 2002). *In vivo,* the endothelial cells of the coronary artery increase TLR2 expression under hyperlipidemia. This increase is also observed in regions where blood flow is altered, suggesting that TLR2 participates in the initial pro-inflammatory events and contributes to the early processes of atherosclerosis (Mullick et al., 2008).

Circulating monocytes in peripheral blood from patients with unstable angina and myocardial infarction express higher levels of TLR4 than patients with stable angina or healthy subjects (Methe et al., 2005). Monocytes from patients with cardiovascular disease present higher levels of TLR2 when compared to monocytes from healthy controls. Indeed, high TLR2 levels in patients are considered a risk factor for atherogenesis (Kuwahata et al., 2010) and reflect levels of infiltrated macrophages, which also predominantly overexpress TLR2 and TLR4, in the atherosclerotic plaque of humans (Edfeldt et al., 2002).

7.1 The role of TLRs in the development of atherosclerosis

The participation of TLRs in the development of atherosclerosis has been clearly demonstrated in studies using animal models. In Apo $E^{-/-}$/$TLR4^{-/-}$ mice, a reduction in atherosclerotic plaques has been found, and it has been associated with decreased levels of pro-inflammatory cytokines, such as IL-12 or MCP-1, as well as an alteration in the plaque composition, characterized by a decrease in the macrophage infiltrate in the lesion area (Michelsen et al., 2004). In a similar study using $LDLR^{-/-}$/$TLR2^{-/-}$ mice fed a diet high in fat, under pathogen-free conditions, TLR2-deficient mice exhibited a considerable decrease in atherosclerotic lesions when compared with $LDLR^{-/-}$/$TLR2^{+/+}$ control mice. These studies clearly establish a role for TLR2 in the development of atherosclerosis, suggesting the possibility that endogenous ligands activate TLR2. Another study demonstrated that the bone marrow (BM) from $TLR2^{+/+}$ or $TLR2^{-/-}$ mice did not impact the cellular expression of TLR2 in the aortic lesion when transplanted into $LDLR^{-/-}$ mice. This effect is attributed to the resident cells, such as endothelial cells, or cells from the smooth muscle and fibroblasts, but not to cells derived from the BM, such as monocytes and macrophages. Finally, it was found that the specific *in vivo* activation of TLR2 results in an increase in the formation of the atherosclerotic plaque in control mice (Mullick et al., 2005). Other studies that support the participation of TLRs in the development of atherosclerotic lesions have demonstrated that MyD88-deficient mice are somewhat protected from the development of atherosclerosis and have a reduction in the development of the atherosclerotic plaques, accompanied by a decrease in circulating levels of pro-inflammatory cytokines, such as IL-12 and MCP-1 (Michelsen et al., 2004, Björkbacka et al., 2004).

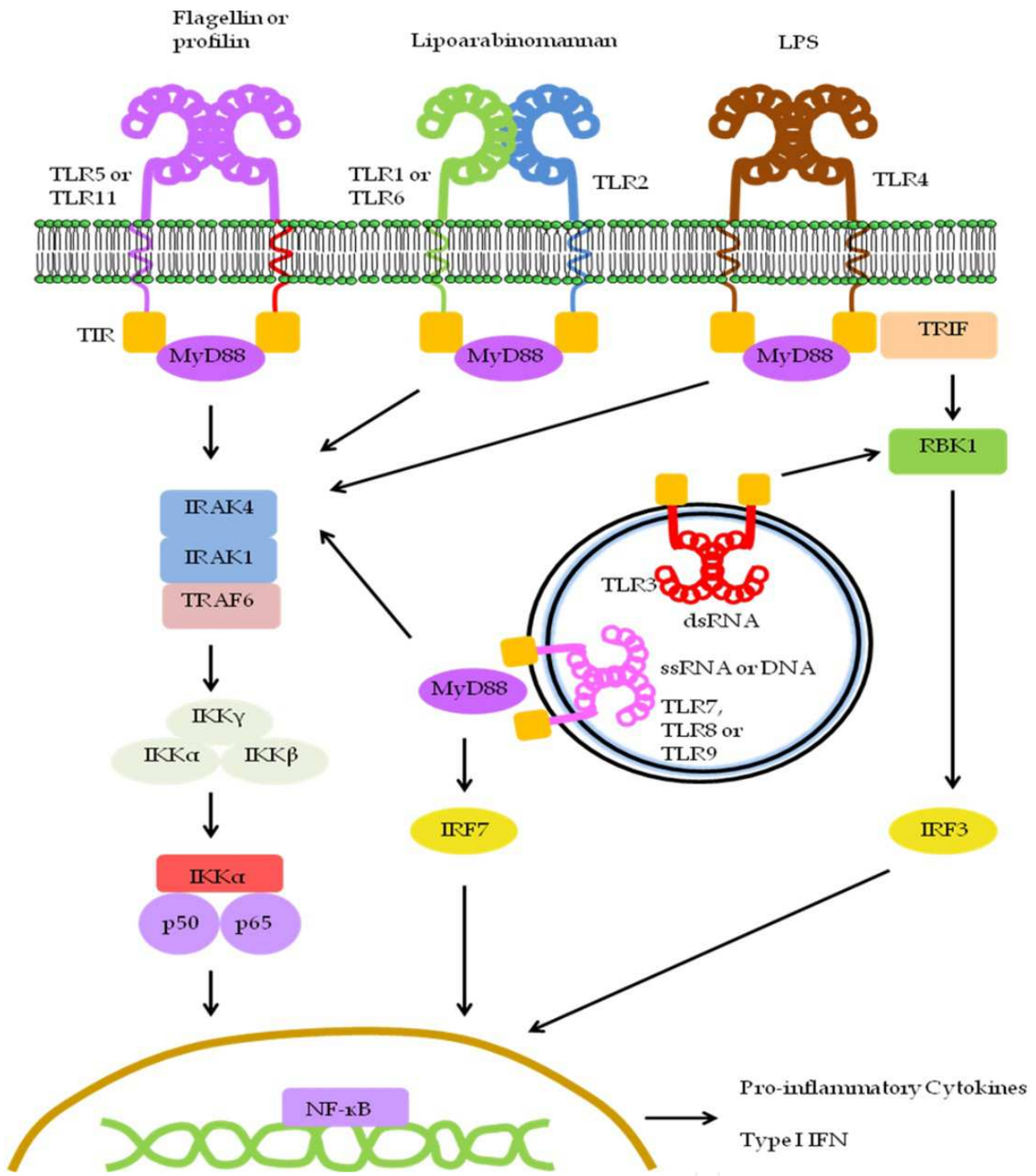

Fig. 3. Schematic representation of the TLR signaling pathway. The signaling of TLRs starts when they recognize their specific ligands. The TLRs signal through a MyD88-dependent pathway to initiate a complex signaling cascade that involves diverse proteins and culminates in the activation of NF-κB, which facilitates the expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, among others. Similarly, the MyD88-independent signaling pathway involves TRIF proteins, which are associated with the TANK binding kinase (TBK1); this induces phosphorylation of the transcription factor IRF3 and facilitates the expression of type I interferon. IkB, inhibitor of NF-κB; IRF, interferon-regulatory factor; MyD88, myeloid differentiation primary-response gene 88; TBK1, TANK-binding kinase; TRIF, Toll/interleukin-1-receptor (TIR)-domain-containing adaptor protein inducing interferon (IFN)-β.

7.2 TLR polymorphisms in atherosclerosis

The Asp299Gly polymorphism in TLR4 attenuates signaling of the receptor and decreases the inflammatory response to Gram-negative pathogens, which have been associated with a decrease in the risk for atherosclerosis (Kiechl et al., 2002). Some studies have reported this polymorphism as associated with cardiovascular disease (Ameziane et al., 2003, Boekholdt et al., 2003), although other groups have not found this association (Yang et al., 2003, Netea et al., 2004).

Some functional studies have shown that the Arg753Gln polymorphism in TLR2 results in a weak response to bacterial peptides (Lorenz et al., 2004), and it is associated with protection during restenosis (Hamann et al., 2005). However, there was no association with myocardial infarction (Labrum et al., 2007, Balistreri et al., 2008), similar to observations of the Thr1237Cys and Thr1486Cys polymorphisms from the TLR9 promoter, which suggested that these polymorphisms were not associated with atherogenesis or restenosis (Hamann et al., 2006).

7.3 The role of TLRs in response to infectious agents in atherosclerosis

One of the possible causes of inflammation in atherosclerosis is lipopolysaccharide exposure, which is a glycolipid present in the external wall of Gram-negative bacteria (Bryant et al., 2010), such as *C. pneumoniae* (Kuo et al., 1993), *Porphyromonas gingivalis* (Dorn et al., 1999), and *Helicobacter pylori* (Ameriso et al., 2001). These bacteria have been associated with atherosclerosis. Likewise, cytomegalovirus (CMV) has been associated with cardiovascular disease (Nieto et al., 1996).

For example, *C. pneumoniae* has been isolated from coronary arteries in patients with acute coronary syndrome (Saikku et al., 1988), and in experimental studies, it has been found that infection with *C. Pneumoniae* increases atherosclerotic plaque size in Apo $E^{-/-}$ mice compared to the controls. It has also been reported that the size of the aortic lesion and the expression of pro-inflammatory cytokines, such as MCP-1, IL-12p40, TNF-α, and IL-6, are reduced in ApoE$^{-/-}$TLR2$^{-/-}$, ApoE$^{-/-}$TLR4$^{-/-}$ and ApoE$^{-/-}$MyD88$^{-/-}$ mice when compared with the ApoE$^{-/-}$ controls infected with *C. Pneumoniae* (Naiki et al., 2008). Other studies have reported that HSP60 of *C. Pneumoniae* (cHSP60) reduces the expression and activity of nitric oxide synthase in the endothelial cells of the human coronary artery, which has also been associated with endothelial dysfunction. Moreover, the effect of cHSP60 on endothelial nitric oxide synthase deregulation is inhibited by blocking TLR2 and TLR4 (Chen et al., 2009). Additionally, the endogenous cHSP60 stimulates the proliferation of vascular smooth muscle cells (Hirono et al., 2003). Other studies have shown that *C. Pneumoniae* induces the formation of foam cells in the presence of oxLDL through TLR2 (Cao et al., 2007) and that this occurs through both MyD88-dependent or –independent pathways (Chen et al., 2009). Finally, infection of vascular smooth muscle cells with *C. Pneumoniae* mediates the persistent release of MCP-1 through the activation of TLR2 (Yang et al., 2005), and infection of mononuclear cells with *C. Pneumoniae* induces TLR2- dependent TNF and IL-1β secretion (Netea et al., 2002a), which may also contribute to the formation of the plaque.

Infection of ApoE$^{-/-}$ mice with *P. gingivalis* demonstrated an increase in the atherosclerotic plaque, characterized by an increase in levels of lipids, macrophages, and T cells (Hayashi et al., 2011). Moreover, *P. gingivalis* induced the expression of TLR2-dependent inflammatory mediators, such as IFN-γ, IL-1β, IL-6 and TNF-α (Hayashi et al., 2010). Another study demonstrated that *P. gingivalis* LPS increased TLR2 expression and induced IL-6 and TNF-α secretion in vascular secretion cells. It was also found in this study that a heterotypic

receptor complex is formed, comprised of TLR2, TLR1, CD36, and CD11b/CD18 (Triantafilou et al., 2007), resulting in an overregulation of ICAM-1 and VCAM-1 in endothelial cells, which facilitates the adhesion of mononuclear cells (Nakamura et al., 2008). Other data have demonstrated that the infection by CMV increases the size atherosclerotic plaque in ApoE$^{-/-}$ mice (Hsich et al., 2001). However, to date, no data exists regarding the role of the TLRs in response to a CMV infection in the context of atherosclerosis. It is important to mention that TLR7 and TLR9 may exhibit redundant roles in the production of IFN-α/β, IL-12p40, and TNF-α by plasmacytoid dendritic cells (pDC) during CMV infection (Zucchini et al., 2008). Likewise, pDCs infected with CMV are capable of triggering the proliferation of B cells and the production of antibodies in the presence of T cells (Varani et al., 2008). This evidence suggests that a role for CMV in the pathogenesis of atherosclerosis may exist, particularly because the atherosclerotic plaque contains pDC (Van Vré et al., 2011).

TLRs participate in the innate immune response in atherosclerosis, recognizing infectious agents, which are described in Table 2.

TLR	Ligand	Infectious agent	
TLR2		*C. pneumoniae*	(Naiki et al., 2008)
	HSP60	*C. Pneumoniae*	(Chen et al., 2009)
	LPS	*P. gingivalis*	(Nakamura et al., 2008)
TLR4	HSP60	*C. Pneumoniae*	(Chen et al., 2009)

Table 2. TLRs and some of their ligands in infectious agents.

7.4 Role of TLRs in response to PAMP in atherosclerosis

It has been clearly established that PAMPs promote various processes in atherosclerosis. Among these are endothelial cell activation, foam cell formation and the development of an atherosclerotic plaque (Erridge, 2008).

The endothelium maintains the vascular tone and blood flow with little or no expression of pro-inflammatory factors under homeostatic conditions (Hadi et al., 2005). However, LPS induces cell activation resulting in an increase in the expression of TLR2 and TLR4, as well as the secretion of IFN-γ, TNF-α (Faure et al., 2001), and MCP-1 (Yumoto, et al., 2005) in vascular endothelial cells. Cell activation also results in the expression of adhesion molecules, such as E-selectin, VCAM-1 and ICAM-1, which are involved in the adhesion of monocytes and T cells to the endothelium (Jersmann et al., 2001). In addition, LPS and histamine (acting via H1 receptors) synergistically induce the production of prostaglandin and IL-6 in endothelial cells (Raveendran et al., 2011). Coronary artery endothelial cells activation through TLR2 with lipoteichoic acid exocytose Weibel-Palade bodies is accompanied by the release of von Willebran factor, P-selectin, and IL-8 (Into et al., 2007). Additionally, TLR3 activation of endothelial cells impairs endothelium-dependent vasodilation, increases the production of reactive oxygen species, reduces re-endothelialization after carotid artery damage, and increases atherosclerotic plaque formation in ApoE$^{-/-}$ mice (Zimmer et al., 2011).

Macrophages play key roles in lipid metabolism and immune responses. However, macrophages are converted into foam cells during early and late stages of atherosclerosis

and contain massive amounts of cholesterol esters (Glass & Witztum, 2001). Stimulation of RAW264.7 macrophages through TLR2 with the ligand Pam3Cys in the presence of LDL leads to the formation of foam cells, and this effect is not observed in TLR2-deficient macrophages (Cao et al., 2007). The accumulation of cholesterol ester during atherogenesis reflects a balance between the internalization of lipids by scavenger receptors and cholesterol efflux. Alterations in this balance favoring the removal of lipids by efflux could limit the formation of foam cells, whereas interference with the efflux pathway would exacerbate the lesion. In this context, activation of macrophages with poly I:C, the ligand for TLR3, and lipid A, a TLR4 ligand, inhibit cholesterol efflux-dependent apoAI (Castrillo et al., 2003). These data suggest that signaling via TLR2, TLR3, or TLR4 is potentially an important modulator of cardiovascular disease, which is supported by studies in animal models that have revealed the role of PAMPs in the development of atherosclerosis. The administration of the TLR2 ligand PamCys to LDLR$^{-/-}$ mice, which are susceptible to developing atherosclerosis, showed a dramatic increase in the severity of atherosclerotic plaques (Mullick et al., 2005), while PamCys induced intimal hyperplasia in arteries of C57BL/6 mice (Schoneveld et al., 2005).

7.5 Role of TLRs in response to endogenous ligands during atherosclerosis

Most studies have focused on determining the involvement of TLRs in response to microorganisms or PAMPs. However, there is growing evidence showing that TLRs can signal through endogenous ligands, which are classified as damage-associated molecular patterns and are able to mount an inflammatory response in the absence of exogenous antigens (Chen & Nuñez, 2010).

During atherosclerosis, several endogenous ligands have the potential to activate TLRs. Initial studies indicate that the activation of macrophages with oxLDL induces the up-regulation of TLR4 mRNA in a dose-dependent manner, suggesting that a mechanism connecting lipids and TLRs exists (Xu et al., 2001). Subsequently, it was determined that mmLDL is capable of binding to CD14 and that, through the TLR4/MD2 complex, mmLDL causes actin polymerization and membrane spreading in macrophages (Miller et al., 2002). However, it has been shown that mmLDL induces secretion of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, in human monocytes and macrophages through CD14 (Chávez-Sánchez et al., 2010a, Chávez-Sánchez et al., 2010b), which is corroborated by the fact that the blockade of CD14 with anti-CD14 antibodies significantly reduces the concentration of pro-inflammatory cytokines, including IL-1β and IL-6, produced by macrophages in response to oxLDL (Pasini et al., 2007). The stimulation of human monocytes and macrophages with mmLDL induces the secretion of IL-1 β, IL-6, and TNF-α through a TLR4-dependent mechanism (Chávez-Sánchez et al., 2010a, Chávez-Sánchez et al., 2010b), which is similar to the mechanism by which end products of LDL glycosylation lead to the production of TNF-α (Hodgkinson et al., 2008a). Another study showed that mmLDL induces the secretion of MIP-2 in mice and that this secretion is TLR4/MyD88 dependent in mouse macrophages, whereas the secretion of MCP-1, TNF-α, and IL-6 was shown to be independent of TLR4/MyD88 (Miller et al., 2005). Discrepancies across these studies could be due to different types of mmLDL or differences in the types of cells that were used in each study. For example, Chávez-Sánchez et al. used copper-modified LDL whereas Miller et al. used LDL modified by fibroblasts that overexpressed 15-lipoxygenase.

mmLDL has also been shown to activate TLR2 and induce the secretion of IL-1β, IL-6, and TNF-α in human monocytes and macrophages (Chávez-Sánchez et al., 2010a, Chávez-

Sánchez et al., 2010b). Moreover, the stimulation of monocytes with mmLDL causes a redistribution of CD14 and TLR4 on the cell surface, as well as the colocalization of CD14 and TLR4. mmLDL also caused the redistribution and patching of TLR2 on the cell surface, suggesting that there is a close association between these cell surface receptors (Chávez-Sánchez et al., 2010a). Similar data show that TLR2 and TLR4 colocalized with oxLDL (Su et al., 2011). Notably, mmLDL increases the expression of TLR2, rather than the expression of TLR4, in human monocytes and macrophages (Chávez-Sánchez et al., 2010a), suggesting that mmLDL may induce cross-talk between the TLR2 and TLR4 pathways of activation, resulting in amplified secretion of pro-inflammatory cytokines (Fan et al., 2006). Similarly, it has been shown that oxLDL, which can induce foam cell transformation, can increase TLR2 and TLR4 transcript expression (Holvoet et al., 2006). Surprisingly, mmLDL induces mRNA synthesis of IL-10 (Bae et al., 2009) and the secretion of this cytokine in monocytes and macrophages (Chávez-Sánchez et al., 2010b), indicating that the activation of TLR2 and TLR4 also initiates regulatory mechanisms, including the production of anti-inflammatory cytokines such as IL-10 (Liew et al., 2005).

Endogenous ligands have been associated with atherosclerosis in recent studies, including elevated serum amyloid A , which can predict cardiovascular events (Kosuge et al., 2007) and has been postulated as a shared mediator of inflammation and cardiovascular disease (Wilson et al., 2008). Serum amyloid A can induce cellular activation through TLR2 (Cheng et al., 2008), and activation of smooth muscle cells by serum amyloid A lead to an increase in the incorporation of sulfate proteoglycan, which causes an increase in glycosaminoglycan chain size and a greater binding affinity of LDL (Wilson et al., 2008).

Another acute phase protein that is involved in cardiovascular disease is fibrinogen, which induces the secretion of MCP-1 in macrophages (Smiley et al., 2001), IL-8 in monocytes (Kuhns et al., 2007), and TNF-α, IL-6, MMP-1, and MMP-9, among other molecules (Hodgkinson et al., 2008b), through the activation of TLR4.

In mice and humans, atherosclerotic plaque-resident macrophages and foam cells express fibronectin with an extra domain A (EDA) (Tan et al., 2004). This EDA domain may act as a ligand for TLR4 (Okamura et al., 2001) and TLR2, which feeds back to increase the expression of TLR2, TLR4 and CD11b (Schoneveld et al., 2008). In the development of atherosclerosis, there is a marked increase in the number of macrophages producing high mobility group box 1 (HMGB1) protein (Kalinina et al., 2004). HMGB1 can activate the

TLR	Ligand	
TLR2	mmLDL	(Chavez-Sanchez et al., 2010)
	Serum amyloid A	(Cheng et al., 2008)
	EDA	(Okamura et al., 2001)
	HMGB1	(Park et al., 2004)
TLR4	mmLDL	(Miller et al., 2002, Chavez-Sanchez et al., 2010)
	AGE-LDL	(Hodgkinson et al., 2008)
	Fibrinogen	(Hodgkinson et al., 2008)
	EDA	(Okamura et al., 2001)
	HMGB1	(Park et al., 2004) (Yang et al., 2010)

Table 3. TLRs and some of their ligands in atherosclerosis.

receptor for advanced glycation end products, and it induces secretion of TNF following activation of TLR2 and TLR4 and downstream NF-κB (Park et al., 2004). HMGB1 in endothelial cells increases the expression of ICAM-1 and E-selectin, whereas the inhibition of TLR4 leads to a suppression of these molecules plus NF-κB (Yang et al., 2010).
TLRs participate in the innate immune response in atherosclerosis, recognizing endogenous ligands, which are described in Table 3.

7.6 The TLRs as therapeutic targets

TLR4 and TLR2 have a pathogenic role in cardiovascular disease; their ability to initiate and propagate inflammation makes them attractive therapeutic targets. Therefore, blocking antibodies directed against these TLRs and pharmacological inhibitors of their signaling pathways have been considered as potential therapeutics.
Eritoran (E5564) is an antagonist of lipid A that interferes with TLR4/MD2/LPS complex formation and attenuates the inflammatory response in myocardial ischemic reperfusion, as evidenced by a reduction in infarct size and a decrease in the expression of TNF-α, IL-1β, IL-6, MIP-1α, MIP-2, and MCP-1 (Shimamoto et al., 2006). This compound is in phase III clinical trials for sepsis, and its administration in patients undergoing surgery cardiac causes no cytotoxicity, but significantly reduces the incidence of any adverse action or postoperative systemic inflammation/organ dysfunction endotoxin (Bennett-Guerrero et al., 2007).
Similarly, the blockade of TLR2 with anti-TLR2 antibody (OPN-301) reduces myocardial ischemia-reperfusion and preserves cardiac function in vivo. OPN-301 prevents the activation of NF-κB and reduces the production of TNF-α, CD11b, and proapoptotic signals, as well as stinting the infiltration of leukocytes. Thus, OPN-301 is a good candidate for adjuvant therapy in patients undergoing percutaneous transluminal coronary angioplasty (Arslan et al., 2010).

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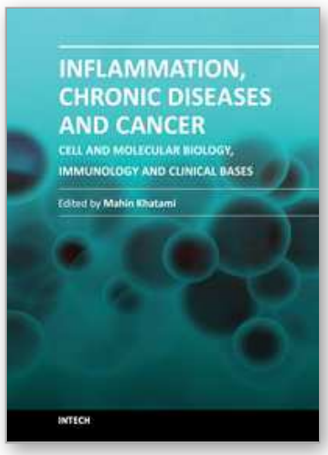

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This book is a collection of excellent reviews and perspectives contributed by experts in the multidisciplinary field of basic science, clinical studies and treatment options for a wide range of acute and chronic inflammatory diseases or cancer. The goal has been to demonstrate that persistent or chronic (unresolved or subclinical) inflammation is a common denominator in the genesis, progression and manifestation of many illnesses and/or cancers, particularly during the aging process. Understanding the fundamental basis of shared and interrelated immunological features of unresolved inflammation in initiation and progression of chronic diseases or cancer are expected to hold real promises when the designs of cost-effective strategies are considered for diagnosis, prevention or treatment of a number of age-associated illnesses such as autoimmune and neurodegenerative diseases as well as many cancers.

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