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Review

The complement system and toll-like receptors as integrated players in the pathophysiology of atherosclerosis

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ABSTRACT

Despite recent medical advances, atherosclerosis is a global burden accounting for numerous deaths and hospital admissions. Immune-mediated inflammation is a major component of the atherosclerotic process, but earlier research focus on adaptive immunity has gradually switched towards the role of innate immunity. The complement system and toll-like receptors (TLRs), and the crosstalk between them, may be of particular interest both with respect to pathogenesis and as therapeutic targets in atherosclerosis. Animal studies indicate that inhibition of C3a and C5a reduces atherosclerosis. In humans modified LDL-cholesterol activate complement and TLRs leading to downstream inflammation, and histopathological studies indicate that the innate immune system is present in atherosclerotic lesions. Moreover, clinical studies have demonstrated that both complement and TLRs are upregulated in atherosclerotic diseases, although interventional trials have this far been disappointing. However, based on recent research showing an intimate interplay between complement and TLRs we propose a model in which combined inhibition of both complement and TLRs may represent a potent anti-inflammatory therapeutic approach to reduce atherosclerosis.

1. Atherosclerosis – an inflammatory disease

Atherosclerosis is a common disorder and a leading cause of morbidity and mortality worldwide. In many cases, individuals are asymptomatic and the disease is therefore not recognized until an acute thrombotic manifestation like myocardial infarction (MI), stroke or sudden death occurs. Moreover, the prevalence of atherosclerotic disease and its related costs are expected to increase not only in the industrialized but also in developing countries [1]. It remains a huge challenge to solve this global clinical problem.

Inflammation is a major component of atherosclerosis and considered to play a role in all developmental stages of the disease [2,3]. Illustratively, cholesterol and inflammation have been described as two partners in crime during atherogenesis [4]. Lipoproteins that are trapped and retained by matrix proteoglycans in the intimal layer of the arterial wall easily undergo oxidative modifications, and this event is followed by an immediate innate immune response [5,6]. The bidirectional interaction between inflammation and lipids will lead to an accumulation of lipid-filled macrophages in the intima and eventually form a lipid core not only including lipid-filled cells but also apoptotic and necrotic cells, cell

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debris and cholesterol crystals. Provided a local cytokine profile favoring smooth muscle cell proliferation and synthesis of extracellular matrix proteins, the lesion will acquire a stable but narrowing phenotype in relation to lumen diameter characterized by the central lipid core and a thick surrounding layer of smooth muscle cells and fibrous connective tissue, a so-called fibrous cap.

However, atherosclerosis is a dynamic process and the stable lesion may be transformed into an unstable, rupture-prone lesion. In contrast to the stable plaque, a large lipid core and a thin fibrous cap characterize the unstable plaque. In addition there is consistent evidence for an imbalance between pro- and anti-inflammatory mediators towards larger infiltrates of T cells and activated macrophages, higher apoptotic rates and increased expression of pro-inflammatory cytokines, chemokines and proteolytic enzymes in unstable plaques. Despite this increasing knowledge of plaque characteristics, the complex and multifactorial mechanisms behind plaque destabilization are far from clarified.

Several types of immune cells are involved in the inflammatory arm of atherosclerosis. Overexpression of T helper 1 (Th1)-derived cytokines, including interferon (IFN)-γ and tumor necrosis factor (TNF), has been associated with advanced and unstable plaque phenotypes [7,8]. An excessive Th1 activity is thus considered to drive the development towards plaque destabilization. On the other hand, regulatory T cells seem to have atheroprotective properties by exerting anti-inflammatory and Th1 suppressive effects. Recently, B cells have also been shown to be involved in atherogenesis elicting both pro- and anti-atherogenic activities [9–11]. Thus, while B2 B cells seem to have pro-atherogenic effects, B1 B cells appear to attenuate the atherosclerotic process at least partly by secreting interleukin (IL)-10.

Macrophages, prototypical cells in the innate immune system, have for several years been known to play a key role in lipid accumulation and inflammation during atherogenesis. These cells have now been divided into inflammatory (M1) and resolving (M2) phenotypes [12,13]. Thus while LPS through TLR4 activation and in combination with IFN-γ, released from Th1 cells, promotes M1 polarization, IL-4 and IL13, released from Th2 cells promote M2 polarization of macrophages. More recently, additional subdivision of M2 macrophages has been performed, i.e. M2a, M2b, M2c and M2d macrophages [12,13]. A functional classification refers to these M2a macrophages as 'wound-healing macrophages'. M2b macrophages are induced upon combined exposure to immune complexes and TLR ligands or IL-1 receptor agonists, producing both inflammatory (e.g., IL-6 and TNF) and anti-inflammatory cytokines (IL-10), M2c macrophages are induced by IL-10 and glucocorticoids [14]. These M2c macrophages, together with M2b macrophages, are also referred to as "regulatory macrophages". Finally, M2d macrophages are induced by co-stimulation with TLR and adenosine A2A receptor agonists, characterized by high levels of IL-10 and vascular endothelial growth factor (VEGF), potentially playing a role in angiogenesis. In the atherosclerosis field, additional forms have been described including the Mhem macrophage, consistent with their presence in regions of haemorrhage [15], and M4 macrophages that are induced by CCL4 showing high expression of matrix metalloproteinases associated with plaque destabilization in carotid plaques [16]. M1 polarization is induced by TLR2 and TLR4 activation in combination with lipids. Th2 related cytokines and not TLR activation seem to be of importance for M2 macrophage polarization. Like TLRs, complement activation has been linked to M1 polarization and C3 deficient mice have been shown to have fewer M1 macrophages and more M2 macrophages [17].

Indeed several components of innate immunity including the complement system and TLRs, as mentioned above, have increasingly been targeted in atherosclerosis research [3,18]. Oxidatively modified lipoproteins in the arterial wall are potentially dangerous stressors. The innate immune system is initiating and orchestrating the elimination of these particles. In this "first line defence" a variety of pattern-recognition receptors (PRRs) are used including cellular PRRs such as scavenger receptors and TLRs, and soluble PRRs such as complement components and germline naturally occurring IgM antibodies. The innate immune response not only involves immediate pro-inflammatory actions, but also initiation of adaptive immunity and resolution of inflammation and tissue repair. The production of natural IgM antibodies to oxidation-specific epitopes by naïve B cells is one potential atheroprotective effect generated by the innate immune system [19,20].

A chronic exposure to stressors in the arterial wall may eventually lead to a loss of immune homeostasis. TLRs and complement are mediators bridging danger sensing further to adaptive immunity, thereby acting as key regulators in the maintenance of immune homeostasis. The complement system has important regulatory effects on both B cells and T cells [21,22]. Previous reviews have either addressed the interaction between TLRs and atherosclerosis [23–26] or between complement and atherosclerosis [27–29]. However, recent research indicates an extensive crosstalk between TLRs and complement, thus proposing a complex interplay between these pathways of innate immunity in atherogenesis. As discussed in the present review, this may open up for therapeutic strategies favoring the repair process and stabilization of atherosclerotic lesions.

2. The complement system

The complement system (Fig. 1) is part of our innate defence against infections, and was initially described in the late 19th century [30]. It consists of more than 40 membrane bound and soluble proteins, the latter mainly being secreted by hepatic cells, monocytes and macrophages [31,32]. The traditional view of complement as being predominantly a host defence system against microbes has expanded markedly the last decades to our current knowledge that complement is a surveillance system that quickly can be activated by sensing any danger to the host and thereby contribute to maintaining tissue homeostasis and promote tissue regeneration and repair [33]. On the other hand, undesired or uncontrolled activation of the system can induce tissue damage and organ dysfunction in the host. Forty years ago the interplay between atherosclerosis and the complement system was suggested [34], and the theory has later been maintained [29,35].

2.1. Activation pathways

Traditionally there are three known ways through which the complement system is activated (Fig. 1). The classical pathway (CP) is activated by C1q binding to antibodies when bound to their antigen, or antibody independent by other recognition molecules like the pentraxins including C-reactive protein (CRP), serum amyloid component P (SAP) and long pentraxin 3 (PTX3). The lectin pathway (LP) is activated when proteins like mannose-binding lectin (MBL), the ficolins (−1, -2 and −3) and collectin-11 recognize their ligands like sugar molecules on microbes, on dying host cells or on a subendothelial matrix [36,37]. The alternative pathway (AP) is continuously undergoing a low-grade activation due to hydrolysis of the internal C3 thiol-ester bond, and further activated when there is an imbalance between activation and inhibition e.g. on foreign surfaces or structures lacking complement regulatory proteins.

The different activation pathways lead to the common pathway with activation of C3 and C5 (Fig. 1). From this point the cascade continues to the terminal pathway with release of the biologically