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Inflammation and atherosclerosis: direct versus indirect mechanisms

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It is now widely accepted that the development of atherosclerotic lesions involves a chronic inflammatory response that includes both innate and adaptive immune mechanisms. However, it is still unclear precisely what induces the inflammatory response. Furthermore, inflammation within the blood vessel can be divided into direct mechanisms where the primary inflammatory events occur within the intima of the blood vessel and contribute to both the initiation and progression of the plaques and indirect mechanisms where inflammation at nonvascular sites can contribute to the progression of the lesions. The direct mechanisms include lipid deposition and modification, influx of lipoprotein associated factors and microparticles derived from many different cell types, and possibly bacterial and viral infection of vascular cells. Indirect mechanisms derive from inflammation related to autoimmune diseases, smoking, respiratory infection, and pollution exposure, and possibly periodontal disease and gastric infection. The mechanisms include secretion of cytokines and other inflammatory factors into the circulation with subsequent uptake into the plaques, egress and recruitment of activated inflammatory cells, formation of dysfunctional HDL and crossreactive autoantibodies.

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Introduction

The connection between inflammation and atherosclerosis is not of recent origin. Microscopic observations beginning in the 19th century described the presence of inflammatory reddening and inflammatory cells within human plaques [1,2]. Today it is acknowledged that development of atherosclerosis is a unique type of chronic inflammatory response that involves both innate and adaptive immune mechanisms [3]. The development of animal models with cholesterol feeding, blood vessel injury and systemic infection has further supported the role of inflammation in the initiation and progression of atherosclerosis. The development of immunocytochemical and flow cytometric approaches with inflammatory cell type specific antibodies has now clearly established that atherosclerotic plaques at all stages of development contain multiple types of inflammatory cells including macrophages, dendritic cells, CD4 and CD8 positive T lymphocytes, B lymphocytes, mast cells and occasionally neutrophils and other granulocytes [4,5]. There is also a rapidly emerging new paradigm that suggests that several types of inflammatory cells within plaques have multiple phenotypes and can even change phenotype after entry into the plaques depending on the exposure to specific micro-environmental cues [6]. In this short review the focus will be on distinguishing between direct inflammatory mechanisms that occur within the wall of the blood vessel and are thought to be responsible for the initiation of the disease process and indirect mechanisms where inflammation at nonvascular sites contributes to the progression and destabilization of the plaques.

Direct mechanisms: pro-inflammatory factors that initiate the disease process

Because of page restrictions, I will not review the natural history of the development of atherosclerotic plaques but refer the reader to a number of excellent recently published reviews [5,7,8]. However, what is still unknown is precisely what enters the normal human blood vessel that sets in motion the subsequent activation of the endothelium to express adhesion molecules and leads to the recruitment of inflammatory cells into the intima. Today, lipids are still the leading candidates and the lipid retention hypothesis has been supported by much experimental evidence [9]. Although Anitschkow first demonstrated that cholesterol feeding to rabbits led to the formation of lipid loaded cells within the intima [10], more definitive evidence was provided by experiments demonstrating that LDL particles that were labeled with a nondegradable radioactive probe accumulated at lesion susceptible branch points within days of initiating cholesterol feeding in rabbits [11,12]. However, those experiments did not clarify whether the simple accumulation of LDL particles trapped by interactions with matrix molecules [9] is sufficient to activate the inflammatory response or whether the LDL particles must be modified by oxidation of the fatty acids, phospholipids, and cholesterol or by other modifications. There is experimental evidence showing that oxidized lipids induce expression of adhesion molecules by endothelial cells [13,14] and that oxidation specific epitopes can be localized in the intima

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coincident with endothelial expression of adhesion molecules [15,16]. Furthermore, lipoproteins with characteristics of those oxidized in vitro can be isolated from human plaques [17]. However, free cholesterol itself in a nonoxidized crystalline state, can also induce a similar degree of endothelial activation [18•]. Furthermore, there is now evidence for a role of free cholesterol in the induction of the NLRP3 inflammasome with consequent release of IL-1B [19••]. It is also probable that other factors that associate with the LDL particle or with HDL or remnant particles that enter the intima can induce an inflammatory response. These include endotoxin [20] complement factors [21] or enzymes such as myeloperoxidase [22,23•] that if catalytically active can oxidize proteins that then become pro-inflammatory. Another possibility is microparticles (also referred to as microvesicles) that have been reported to enter the artery wall [24••,25]. These microparticles are derived from platelets, neutrophils, macrophages, lymphocytes, endothelial cells and other cell types. The microparticles bear the signature of the cell type of origin including membrane proteins and bioactive lipids [24••,25]. Microparticles have been shown to activate endothelial expression of adhesion molecules and thus could play a role in the initiation of lesions [26•].

Finally, there is evidence that a number of different infectious agents populate human atherosclerotic lesions [27] and that infection of mice and rabbits can accelerate lesion development [27]. T cell clones have been isolated from human plaques that proliferate in response to *Chlamydia pneumoniae* antigens [28,29]. Whether infectious agents play a role in the initiation of lesions is not clear as we have reported that *C. pneumoniae* infection of nonhyperlipidemic mice does not initiate development of lesions but requires prior or simultaneous hyperlipidemia [30]. It is also unclear whether these infectious agents directly infect cells within the blood vessel or whether the bacteria are carried by cells that have been infected at other locations. For example, there is evidence that monocytes or macrophages that are infected by *C. pneumoniae* in the lungs can migrate to the aorta [31]. It is also unclear whether there is active infection within atherosclerotic plaques. In most cases, it has not been possible to re-isolate viable organisms from human or experimental plaques [27]. However, active infection is not a requirement for eliciting an immune or inflammatory response as even heat killed organisms can activate toll-like receptors on the plasma membrane of endothelial cells, macrophages and dendritic cells [32]. Inflammatory cells within human plaques express a number of different toll-like and NOD receptors and deficiency of TLR-2 or TLR-4 impairs the development of lesions in hyperlipidemic mice and reduces the accelerated development of lesions in response to *C. pneumoniae* infection [33–35]. Engagement of toll-like receptors activates a number of different signal transduction pathways leading to the activation of NFkB and other transcription factors that regulate the expression of pro-inflammatory cytokines [36,37].

As noted, there appear to be multiple phenotypes of inflammatory cells that populate the atherosclerotic plaque. In hyperlipidemic mice there are monocytes expressing both high and low levels of the Ly6C antigen coupled with the CCR2+ chemokine receptor and low levels of the CX3CR1 receptor that are recruited into the plaque. The monocytes expressing high levels of Ly6C are thought to be precursors of inflammatory macrophages that secrete pro-inflammatory cytokines [38–40]. There are also subsets of monocytes and dendritic cells in humans such as monocytes expressing high levels of CD14 and low levels of CD16 that may be the source of pro-inflammatory macrophages and dendritic cells in human plaques [41]. There are also both immature circulating blood DC antigen positive (BDCA 1+) myeloid versus (BDCA 2+) plasmacytoid dendritic cells that are not of monocyte origin [42]. The sources of both the monocytes and dendritic cells are controversial as it is not clear that they are all bone marrow derived. Some cells may also be recruited from lymph nodes and the spleen following some degree of maturation [43•,44,45••]. Mature dendritic cells and macrophages can also be recruited from the lesions back to lymph nodes and spleen during regression of plaques [46,47] and are thought to present plaque derived autoantigens to naïve T and B cells [48]. In fact, this may be the primary mechanism for how mature plaques regress as studies of plaque composition of regressed plaques where regression was induced by a variety of different approaches and in a number of different species show consistent reductions in the numbers of macrophages and macrophage-derived foam cells [46,49–52].

Primarily on the basis of in vitro observations, it is now thought that recruited monocytes can differentiate into both pro-inflammatory M1 and anti-inflammatory M2 phenotypes dependent on the combination of cytokines that they encounter [53•] and cells expressing markers of both M1 and M2 macrophages are found in both human and mouse lesions [54•,55]. Furthermore, these M1 and M2 macrophages can have a similar impact on the phenotype of T lymphocytes by stimulating formation of CD4+ T helper 1 versus T helper 2 cells or T regulatory cells from naïve T cells again dependent on antigen presentation and the types of cytokines they secrete [56]. A similar paradigm is thought to exist for dendritic cells and may be mediated by infection with *C. pneumoniae* [57]. However, this is an oversimplification of what is likely to occur in human plaques at different stages of lesion development as there are probably additional phenotypes of macrophages and dendritic cells than just pro-inflammatory and anti-inflammatory [58,59••]. Furthermore, it is still unclear precisely what induces macrophage and lymphocyte polarization within the plaques as factors

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