THE ROLE OF THE NFκB PATHWAY IN ATHEROSCLEROSIS

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Introduction

Cardiovascular diseases are the leading cause of morbidity and mortality in Western countries. Although coronary thrombosis is the final event in acute coronary syndromes, there is increasing evidence that inflammation also plays a role in development of atherosclerosis and its clinical manifestations, such as myocardial infarction, stroke, and peripheral vascular disease. Many inflammatory genes relevant to atherosclerosis are influenced by the transcriptional regulator nuclear factor κ B (NFκB).

The NFκB Signalling Pathway

NFκB plays a pivotal role in co-ordinating the expression of genes involved in the immune and inflammatory response, for example cytokines, including tumor necrosis factor α (TNFα), chemokines such as monocyte chemoattractant protein-1 (MCP-1) and interleukin (IL)-8, matrix metalloproteinase enzymes (MMP), and genes involved in cell survival. Mammalian cells contain homo- and hetero-dimers of NFκB subunits, generally sub-divided into those synthesized as mature forms (relA or p65, relB, and c-rel) and those made as large precursors (p105 and p100), which undergo proteolysis to produce mature DNA binding proteins (p50 and p52 respectively) [1,2]. In the so-called “canonical” activation pathway, IκB kinase (IKK)-2 (IKKβ) phosphorylates IκBα, leading to its ubiquitination and degradation, allowing NFκB to translocate to the nucleus [3]. This pathway is thought to be crucial for activation of innate immunity and inflammation in response to mediators such as TNFα. However, an alternative or “non-canonical” pathway has been described, under the control of NFκB-inducing kinase (NIK) and IKKα/IKK-1. In response to stimuli such as lymphotoxin β, NIK has been shown to activate IKK-1 leading to inducible processing of p100 with preferential nuclear translocation of p52-RelB dimers. This “non-canonical” pathway has been proposed to have a role in B-cell-mediated responses and adaptive humoral immunity [4].

Atherosclerosis, the common pathological substrate underlying cardiovascular diseases, has been proposed to be an “inflammatory disease” [5]. Activated T-cells are present in human atherosclerotic plaques [6], and expression of pro-inflammatory cytokines, such as TNFα [7] and IL-1 [8], and chemokines, such as IL-8, RANTES, and MCP-1 [9,10] has been described. Cells
present in atherosclerotic lesions, including macrophages, endothelial cells, and smooth muscle cells, express MHC class II antigens, and CD4+ T cells and macrophages co-localize, mainly at the site of plaque rupture [11]. In parallel, clinical studies have highlighted the fact that elevated levels of C-reactive protein [12] and its main IL-6 [13], are associated with adverse prognosis in unstable angina patients.

A complex array of mechanisms, including T cell activation, leukocyte extravasation, tissue factor expression, MMP expression and activation, as well induction of cytokines and chemokines, have been implicated in atherosclerosis, and many of these events are regulated by NFκB [14].

**NFκB Activation in Atherosclerosis: The Evidence**

A number of studies have suggested that the NFκB pathway plays a key role in development of atherosclerosis. Many relevant stimuli have the potential to activate NFκB, including low density lipoprotein (LDL), infectious agents, and cytokines. For example, minimally oxidized LDL stimulate endothelial cells to produce NFκB-dependent chemokines and adhesion molecules [15]. Infectious agents such as Chlamydia pneumoniae, postulated to play a role in atherosclerosis, express molecules such as lipopolysaccharide (LPS), which activates NFκB in many cells, including endothelial cells and macrophages. Nuclear translocation of relA (p65) in the intima and media of human atherosclerotic plaques has been reported [16,17] and nuclear NFκB binding activity has been found in peripheral blood mononuclear cells [18] of patients with unstable angina.

Furthermore, there is evidence for the importance of NFκB in development of atherosclerosis from animal models of disease. Activated NFκB was detected in coronary arteries of pigs fed a hypercholesterolemic diet [19] and in arterial smooth muscle cells of a rat balloon injury model [20]. In LDL receptor knock-out mice, expression of relA, IkBα, and IkBβ was considerably higher in a region of ascending aorta and arch predisposed to atherosclerotic lesion formation. However, nuclear translocation of relA was only found after initiation of an atherogenic diet, or after systemic injection of LPS, and even then, only in regions predisposed to atherosclerosis [21]. More recent studies have utilized LDL receptor knock-out mice with a macrophage-restricted deletion of IKK-2. Somewhat surprisingly, such animals showed an increased atherosclerotic lesion size [22].

To overcome some of the limitations of animal models, we have developed a short-term culture system of dissociated cells from human atherosclerotic tissue. These cells consist of a mixture of CD3+ lymphocytes, CD68+ macrophages, and smooth muscle cells. Using this short-term culture system, we reported constitutive NFκB DNA-binding activity present in the nucleus of human atherosclerotic plaque cells. This activity was found to consist of p65, c-Rel, and p50, but not relB and p52. Since relB and p52 are thought to be involved in the “non-canonical” pathway, these results suggest that it the canonical pathway of NFκB activation is induced in atherosclerosis [23].

**NFκB Activation in Atherosclerosis: The Consequences**

Expression of NFκB in the atherosclerotic milieu is likely to have a number of potentially harmful consequences. For example, loss of fibrous cap integrity is likely to involve over-
expression of MMP. In human and rabbit smooth muscle cells, IL-1 activates NFκB to upregulate expression of MMP-1, -3, and -9 [24]. Oxidized LDL increases macrophage MMP-9, associated with increased nuclear binding of NFκB and AP-1 [25]. We have reported that human atherosclerotic plaque cells produce MMP-1, -3, and -9, as well as tissue inhibitor of MMP (TIMP)-1, and that expression of MMP-1, -3, and -9, but not TIMP-1, was strongly inhibited following adenovirally mediated over-expression of IκBα and a dominant-negative form of IKK-2 [23]. Similarly, expression of tissue factor, the major initiator of the coagulation cascade, is regulated by NFκB [26]. In atherosclerotic plaque cells, tissue factor antigen and activity were inhibited following over-expression of IκBα and dominant-negative IKK-2, but not by dominant negative IKK-1 or NIK, supporting the concept that activation of the “canonical” pathway upregulates pro-thrombotic mediators involved in disease.

Many of the cytokines and chemokines which have been detected in human atherosclerotic plaques are also regulated by NFκB. Over-expression of IκBα inhibits release of TNFα, IL-1, IL-6, and IL-8 in macrophages stimulated with LPS and CD40 ligand (CD40L) [27]. Interestingly, dominant negative IKK-2 adenovirus inhibited cytokine release in response to CD40L, but not LPS [27]. Secretion by macrophages of CXC chemokines in response to TNFα, but not LPS, was also suppressed following over-expression of IκBα, whereas expression of CC chemokines induced by either stimulus was reduced by IκBα [28]. These studies highlight the aspect that NFκB utilization depends on the cell type and stimulus. We have observed that expression of pro-inflammatory cytokines TNFα, IL-6, and IL-8, but not the anti-inflammatory cytokine IL-10, in human atherosclerotic plaque cells is NFκB-dependent and involves IKK-2, but not IKK-1 or NIK [23]. In contrast, deletion of IKK-2 in murine macrophages was associated with inhibition of both pro-inflammatory cytokines and IL-10, and was associated with increased atherosclerotic lesion size [22]. Differences between human and murine cells probably explain these discrepancies.

NFκB is also a central player in cell survival, which is regulated, among others, by caspases and members of the Bcl-2 and inhibitors of apoptosis (IAP) families. To date, six IAP have been identified in humans, including HIAP-1 and -2, XIAP, and survivin. Induction of XIAP has been shown to be NFκB-dependent in TNFα-activated endothelial cells [29]. Moreover, the key angiogenic stimulus vascular endothelial growth factor (VEGF), which is expressed in atherosclerosis, upregulates Bcl-2, surviving, and XIAP in endothelial cells. We have shown that VEGF activates NFκB to induce Bcl-2 and survivin, via IKK-2. However, NFκB activation occurred without degradation of IκB, and is likely to involve tyrosine, rather than serine, phosphorylation of IκBα. Activation of NFκB in response to VEGF may be a key signaling event in regulating endothelial survival. In smooth muscle cells, proliferation induced by fibroblast growth factor has been shown to involve NFκB, and induction of HIAP-1 has been found to be NFκB-dependent [30]. Furthermore, adenoviral gene transfer of IκBα inhibited smooth muscle cell proliferation induced by cytokines [31].

**Conclusion**

Atherosclerosis and its acute complications are a major cause of mortality and morbidity around the world. NFκB activation has been observed in animal models of and in human disease tissue. Our own data demonstrate for the first time that the “canonical” pathway of NFκB activation is activated in human atherosclerosis and results in selective upregulation of major pro-
inflammatory and pro-thrombotic mediators of the disease. Whether inhibition of the NFκB inhibition in atherosclerosis and its complications is a potential therapeutic option remains to be determined.

Acknowledgements

The Kennedy Institute of Rheumatology receives a core grant from the Arthritis Research Campaign.

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