Inflammation: a role for NR4A orphan nuclear receptors?

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Abstract

Inflammation is paradoxical; it is essential for protection following biological, chemical or physical stimuli, but inappropriate or misdirected inflammation is responsible for tissue injury in a variety of inflammatory diseases. The polarization of immune cells is critical in controlling the stages of inflammatory response. The acute phase of inflammation is characterized by a T-lymphocyte:Th2 cytokine profile and involves a co-ordinated migration of immune cells to the site of injury where production of cytokines and acute-phase proteins brings about healing. However, persistent inflammation can result in inappropriate and prolonged T-lymphocyte:Th1 cytokine-mediated action and reaction of self-molecules, leading to a chronic phase in diseases such as RA (rheumatoid arthritis), Ps (psoriasis) and atherosclerosis. The inflammatory response is also controlled by activated macrophage cells, with classically activated (M1) cells producing a wide variety of pro-inflammatory mediators, while alternatively activated (M2) macrophages participate in anti-inflammatory response. Members of the NR4A subfamily (NR4A1/NUR77, NR4A2/NURR1 and NR4A3/NOR1) of orphan NRs (nuclear receptors) have emerged as key transcriptional regulators of cytokine and growth factor action in diseases affecting our aging population. As ligand-independent and constitutively active receptors, the activity of these transcription factors is tightly controlled at the level of expression, post-translational modification and subcellular localization. NR4A subfamily members are aberrantly expressed in inflamed human synovial tissue, psoriatic skin, atherosclerotic lesions, lung and colorectal cancer cells. Significantly, prolonged or inappropriate inflammatory responses contribute to the pathogenesis of these diseases. In activated cells, NR4A receptors are rapidly and potently induced, suggesting that these receptors may act as important transcriptional mediators of inflammatory signals. NR4A receptors may contribute to the cellular processes that control inflammation, playing a critical part in the contribution of chronic inflammation or they may have a protective role, where they may mediate pro-resolution responses. Here, we will review the contribution of the NR4A orphan NRs to integration of cytokine signalling in inflammatory disorders.

Chronic inflammatory diseases

Chronic inflammation and hyperplasia of the synovium are hallmarks of both RA (rheumatoid arthritis) and PsA (psoriatic arthritis) [1–5]. The synovium is a delicate tissue lining the joint capsule [3,4]. In arthritis, the synovium transforms into an aggressive, tumour-like structure called pannus [3–7]. Angiogenesis, the formation of new blood vessels, is an essential process required for pannus development and is one of the earliest histopathological findings in disease pathogenesis [4,5]. The vascular endothelium plays a leading role in blood vessel homeostasis, contributing significantly to the initiation of joint destruction. Under normal conditions, the endothelium is a quiescent tissue that regulates vessel permeability and vasodilation. During acute inflammation, the endothelium is activated, resulting in the release of cytokines and enhanced expression of cell-surface adhesion molecules, which facilitate the attraction of immune cells, including leucocytes and monocytes, to the site of inflammation. Following inflammatory resolution, the endothelium reverts to a quiescent normal state.

Chronic joint inflammation causes a state of continued endothelial cell activation, resulting in perpetuation of the inflammatory environment and immune cell recruitment [3–7]. In addition to the pro-inflammatory mediators secreted by the activated endothelium, macrophage-derived cytokines are potent mitogens for proliferating FLSs (fibroblast-like synoviocytes) in the vicinity of the affected cartilage that produce matrix-degrading molecules. FLSs and macrophages within the synovium orchestrate a self-perpetuating inflammatory response via the autocrine actions of cytokines [e.g. TNFα (tumour necrosis factor α), IL (interleukin)-1β], growth factors [e.g. bFGF (basic fibroblast growth factor) and VEGF (vascular endothelial growth factor)] and prostaglandins [e.g. PGE2 (prostaglandin E2)] [4,6,7]. It is the persistent, invasive and destructive growth of synovial tissue that ultimately leads to joint erosion [7,8]. The
long-term prognosis for RA suffers is poor, with 80% of affected patients presenting with significant joint damage and functional disability after 20 years. Life expectancy for these patients is strikingly reduced by an average of 3–18 years [9]. This increased mortality is predominantly associated with accelerated coronary artery disease and cerebrovascular atherosclerosis [9].

Ps (psoriasis), like RA, is an autoimmune disorder, with chronic dermatosis, which is also associated with significant morbidity [10–12]. Ps is characterized by accumulation of erythematous, scaly plaques, with characteristic histological changes of epidermal hyperplasia, inflammatory cell infiltrate and increased vascularity [10]. Increased production and release of cytokines and growth factors by activated T-lymphocytes and macrophage cells trigger basal stem cell keratinocyte proliferation, abnormal terminal differentiation and resistance to apoptotic signals [11,12]. These inflammatory mediators also support vascular changes and promote angiogenesis, which is a prominent and early event [13,14]. Over 2% of the population are affected by these disorders and approximately 15% of patients with Ps also develop a sero-negative polyarthritis-PsA [13].

Atherosclerosis is a chronic inflammatory condition that develops from de-regulation of the immune system and metabolic pathways [15–17]. As observed in RA, PsA and Ps, the vascular endothelium plays a paramount role in the initiation and development of atherosclerosis, facilitating immune cell ingress leading to increased production of pro-inflammatory cytokines. Macrophages have been identified as a critical cell type involved in the initiation, progression and rupture of atherosclerotic lesions. Ingestion of modified lipid particles transforms these macrophages into ‘foam cells’, resulting in the formation of a fatty streak on the vessel wall. These lipid-laden macrophages produce excess amounts of pro-inflammatory molecules and matrix-degrading enzymes, which perpetuate a continued state of chronic inflammation. Over time the number of foam cells increases and the lesion becomes unstable, finally cumulating in the rupture of the lesion [15].

Regulation of inflammation: NR4A subfamily

Extensive *in vitro* and *in vivo* studies have identified a critical role of cytokines and growth factors, as well as the participating cell types, that promote chronic inflammation [6,7]. What remains to be further explored is the control of transcriptional mediators downstream of these pro-inflammatory signals within these cell systems. Identifying these transcription factors and their co-regulatory proteins (co-activators and co-repressors) will facilitate the development of targeted and specific therapeutic approaches. The NR (nuclear receptor) superfamily has been proposed as key transcription factors capable of modulating both immune and metabolic pathways. Two subgroups within this superfamily, the NR1C/PPAR (peroxisome-proliferator-activated receptor) and NR1H/LXR (liver X receptor) receptors, have been identified as important transcriptional modulators of both inflammation and lipid homoeostasis [16,17].

Recent interest has focused on the NR4A subfamily of orphan NRs. The NR4A (NR4A1/NUR77, NR4A2/NURR1 and NR4A3/NOR1) subfamily has emerged as potential regulators of cytokine and growth factor action through regulating the inflammatory response underlying several diseases. NR4A transcription factors are aberrantly expressed in inflamed human synovial tissue, colorectal cancer cells, Ps, atherosclerotic lesions and multiple sclerosis [18–25]. In activated primary cells, NR4A receptors are rapidly and potently induced by a range of cytokines, suggesting that these receptors act as potential transcriptional mediators of inflammatory signals [19,26–28]. The transcriptional function of these receptors in response to inflammatory molecules is under intense investigation, and several NR4A target genes have recently been identified [21–28].

Modulation of inflammation: disease-modifying drugs

While NR4A activity does not appear to be regulated by endogenous ligands, pharmacological modulation of NR4A activity can be achieved with the antineoplastic agent 6-mercaptopurine [29]. In addition, altering expression, subcellular localization or post-translational modification of these receptors may be a feasible approach to modulate NR4A activity and target gene expression. Dexamethasone and MTX (methotrexate), used in the treatment of joint and skin diseases, can regulate NR4A2 expression *in vitro* and *in vivo* [26,30] (Figure 1). These studies suggest that the clinical benefits of these agents may be mediated through altered NR4A2 expression and activity.

Traditionally, therapies for chronic inflammation with non-specific disease-modifying medications, including MTX, have been limited by both inefficacy and the development of adverse drug reactions in patients [31,32]. MTX significantly reduces cellular infiltration via decreased expression of cell-surface adhesion molecules reducing the levels of soluble mediators of inflammation such as MMPs (matrix metalloproteinases) and cytokines [33,34]. Several lines of evidence now indicate that the immunosuppressive and anti-inflammatory actions of low dose MTX are not due to inhibition of folate metabolism and are mediated by its capacity to increase extracellular adenosine levels [35]. The molecular targets modulated by MTX and adenosine are being revealed through the creation and appropriate study of *in vivo* models of acute and chronic inflammation [36,37].

The importance of the adenosine A$_2A$ receptor in mediating inflammatory resolution and wound healing is clearly demonstrated in the adenosine A$_2A$ $^{-/-}$ receptor mice [38]. Promotion of angiogenesis is an integral component in the mechanism of repair and is necessary for the transport of oxygen, nutrients and inflammatory cells to promote healing. Interestingly, adenosine, acting at one or more of the four adenosine membrane receptors, may regulate angiogenesis through tilting the dynamic balance of angiogenic mediators. Acting
Figure 1 | Ps, PsA and inflammatory arthritis (A) are prevalent chronic inflammatory conditions

A number of common pathogenic features link skin and joint inflammatory processes. Dysregulated angiogenesis resulting in enhanced leucocyte infiltration appears to be a fundamental inflammatory response early in these diseases. Overlapping non-specific agents used to treat these conditions include glucocorticoids, MTX and cyclosporin. The development of TNFα inhibition has led to significant advances in the treatment of these chronic inflammatory diseases.

through the A2A receptor, adenosine stimulates endothelial cell migration and proliferation in vitro and increases secretion of the pro-angiogenic VEGF, while down-regulating the angiostatic matricellular protein thrombospondin [36,39]. In addition, adenosine regulates inflammation in cell types important for the repair of tissue damage, such as neutrophils, macrophages, endothelial cells and lymphocytes (Figure 2). Cronstein and co-workers have demonstrated that the A2A receptor is required for uniform formation of granulation tissue and revascularization in a model of wound repair [37].

The cell-specific signal transduction events and the transcriptional mediators that act to promote adenosine receptor-mediated responses during acute inflammation and tissue repair are largely unknown. Findings from our laboratory establish NR4A2 as a molecular target of MTX in vivo and correlate with changes in NR4A2 gene expression. Interestingly, it has been demonstrated that COX-2-derived PGE2 can rapidly and potently induce NR4A2 levels in primary human chondrocytes and cancer cells [19,43]. Furthermore, enhanced NR4A2 activity leads to elevated transcriptional repression of MMP gene expression in chondrocyte cells [43].

Cytokine blockade

The success of treating inflammatory joint and skin disease with non-specific disease-modifying drugs has been limited by both inefficacy and the development of adverse drug reactions in up to 50% of patients [31,32]. In recent years, targeted therapy with biological agents, which are highly specific and directed against pro-inflammatory molecules, such as TNF, has been shown to be highly efficacious in the treatment of Ps, PsA and RA [4,6,7,14,20]. Currently, the early treatment of joint and skin disease with anti-TNF agents alone, as well as combination therapies involving MTX, is a therapeutic approach under intense investigation [40–42]. Clinical results indicate that the early administration of such drug combinations may induce a sustained remission in up to 20% of patients. Thus the blockade of TNFα provides an important benchmark for the continued development of new therapies to treat chronic inflammatory conditions [7]. A more detailed understanding of the mediators that drive distinct stages of inflammatory disease may reveal biomarkers for the quantitative assessment of diagnosis and facilitate the introduction of appropriate and effective treatment(s) early in disease. Such studies may also provide new opportunities for the development of therapeutics to target specific mediators central to distinct phases of chronic inflammatory disease.

Given that the transcriptional function of NR4A receptors can be controlled at the level of expression and nuclear localization, we recently examined the expression of NR4A2 in Ps skin and monitored the biological effects on this receptor following inhibition of TNFα signalling [20]. Increased expression levels of NR4A2 mRNA and protein are present in involved Ps skin compared with uninvolved and normal skin, which correlates significantly with clinical measures of the Ps area and severity index [20]. Enhanced NR4A2 expression localizes to both the nucleus and the cytoplasm of cells of involved epidermis, blood vessels and inflammatory infiltrates, in contrast with predominant cytoplasmic distribution in uninvolved and normal skin. Importantly, following TNFα inhibition with infliximab or etanercept, NR4A2 expression in involved skin was significantly decreased and cytoplasmic distribution was restored [20].

Target genes and physiological functions of NR4A receptors are likely to be tissue- and context-dependent. Recent findings reveal that OA (osteoarthritis) joint tissues produce markedly increased levels of NR4A receptors compared with normal tissue [30,43]. In OA cartilage, relative to other family members NR4A1 and NR4A3, NR4A2 is expressed at the highest level. Consistent with advanced cartilage degradation and inflammation, COX-2 (cyclo-oxygenase-2) levels are also significantly elevated and correlate with changes in NR4A2 gene expression. Interestingly, it has been demonstrated that COX-2-derived PGE2 can rapidly and potently induce NR4A2 levels in primary human chondrocytes and cancer cells [19,43].

Cells that promote chronic inflammation: macrophage cells

Several cell types including mast cells, fibroblasts, neutrophils, T- and B-lymphocytes have been implicated in the initiation and progression of chronic inflammatory conditions. It is now emerging that a central cell player in
Figure 2 | Role of adenosine A2A receptors in acute and chronic inflammation

Regulation of the inflammatory response is controlled by alternatively activated macrophage cells with classically M1 macrophages producing a wide variety of pro-inflammatory mediators. In contrast, alternatively activated M2 macrophages participate in anti-inflammatory response and promote tissue repair. Adenosine A2A receptors regulate the processes involved in wound healing, including the transition of macrophages to the M2 phase [34–39]. The mechanism of switching macrophages to an M2 phenotype may be a prerequisite for resolution of inflammation. Tissue repair is characterized by three overlapping phases: inflammation, tissue formation and tissue remodelling. Models of acute and chronic inflammation will permit the contribution and physiological functions of NR4A receptors during the initiation and resolution phases of inflammation to be examined. EC, endothelial cell; TLR, Toll-like receptor; TSP1, thrombospondin 1.

Macrophage polarization: a role for NR4A receptors?

The critical contribution of macrophage cells in mediating the transition from an acute to a chronic state of inflammation in RA has been proposed recently [44]. Macrophage cell populations are more complex than initially thought, and there are reports demonstrating the extent of heterogeneity and plasticity within this cell type [47,48]. Studies indicate that regulation of the inflammatory response is controlled in part by activated macrophages, with classically activated...
(M1) macrophages producing a wide variety of pro-inflammatory cytokines [TNF<sub>high</sub>, IL-12<sub>high</sub>, IL-10<sub>low</sub> and iNOS (inducible nitric oxide synthase)] [2,16]. In contrast, alternatively activated (M2) macrophages participate in anti-inflammatory responses (TNF<sub>low</sub>, IL-12<sub>low</sub>, IL-10<sub>high</sub> and agranulocyte) and promote inflammatory resolution and tissue repair. Unlike T- or B-lymphocytes, macrophages display a high degree of plasticity, which offers the possibility of ‘switching’ pro-inflammatory M1 subtype to pro-resolution M2 subtype cells. Altering the macrophage polarization subtype using GM-CSF (granulocyte/macrophage colony-stimulating factor) or anti-GM-CSF antibody blockade may offer an exciting possibility of interfering and redirecting the inflammatory response, opening a new avenue of therapeutic intervention [49]. The role of NR4A receptors as transcriptional regulators during macrophage phenotypic differentiation is currently unknown and remains to be explored (Figure 2). It is possible that the polarization status of macrophage cells could predetermine the role NR4A receptors play in regulating and integrating the inflammatory response.

**Future directions**

Polarization and priming of immune cells are of significant importance in determining the course of the inflammatory response and outcome. The critical role of cytokines, such as IL-33 (recently implicated in mast cell activation), has shed new light on our understanding of the early events that determine the pathophysiology of inflammation [50]. These studies, together with the recent identification of T<sub>i</sub>17 and T<sub>i</sub>22 T-cell responses, raise compelling questions regarding the regulatory processes controlling the progression from acute to chronic inflammation. This new information is now challenging us to rethink our understanding of the molecular mechanisms underpinning chronic inflammatory disease.

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**References**


