Chapter 2

Krüppel-Like Factor 2 (KLF2) in Limiting Inflammation in Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is an autoimmune disease characterized by severe inflammation and damage to joints. At present, its etiology is idiopathic and no definitive cure exists. A number of cellular and molecular factors are at play in disease progression, and many of these are the targets of existing therapies. B cells, T cells, and macrophages are among the cells critically important in RA pathogenesis; the inflammatory factors and the intricacies of molecular expression that characterize their role in the disease are an ever-unfolding area of study. In this chapter, a novel therapeutic approach is proposed which targets Krüppel-like factor 2 (KLF2), a transcription factor with important effects on many relevant signaling pathways. The anti-inflammatory qualities of KLF2 have been demonstrated to have a positive effect in various disease states and show considerable promise in regard to the treatment of RA.

Introduction

Rheumatoid arthritis (RA) is a degenerative disease in which the joints are subject to attack by the body's own immune cells, resulting in severe inflammation and subsequent damage to the affected tissues. The triggering events and pathogenesis of this disease are incompletely understood, and the complete picture of the causal relationships among cellular and molecular factors and physiological presentations of disease has yet to be elucidated [1]. The

presence of certain molecules and pathological signs are used to diagnose and monitor disease progression.

RA cannot be cured at present; it is imperative that pain be managed and that inflammation and damage to joints be slowed. The drugs currently used to manage RA target a variety of molecular pathways to alleviate symptoms and prevent damage to joints and bones [2]. KLF2 is a transcription factor that has numerous anti-inflammatory applications in a variety of disease contexts. It promotes T cell quiescence as these cells circulate in the bloodstream before being presented with their cognate antigen. In monocytes, it suppresses pro-inflammatory gene expression [3]. In endothelial cells, the anti-inflammatory and antithrombotic effects of KLF2 are effected via positive regulation of the enzyme nitric oxide synthase [4]. Because of the strong evidence for its ability to influence the recruitment and activation of immune cells and its effects on a number of other signaling pathways, it is proposed to be a promising novel therapeutic target in the treatment of RA.

Rheumatoid Arthritis

RA is a symmetric polyarticular arthritis that, though a systemic disease, primarily affects the small synovial (diarthrotic) joints of the hands and feet. It is an autoimmune disease in which the patient's immune cells, namely the T cells, attack the synovial membrane lining the joints, causing inflammation and consequent damage to the bones and articular cartilage. The inflamed synovium thickens and the synovial fluid that fills the joint accumulates therein. The resulting pressure causes pain and tenderness. The synovial membrane produces an abnormal granulation tissue called the pannus, which adheres to the cartilage capping bones and can lead to its erosion. Destroyed cartilage is replaced by fibrous tissue that joins the bone ends and ossifies so that the joint is ultimately immobile [5, 6]. Though its effects are most immediate in the joints, extra-articular manifestations of RA may involve hepatic, gastrointestinal, nervous, or ocular complications, among others. The risk for ischemic heart disease and atherosclerosis, for instance, is elevated in RA patients. Extra-articular symptoms are indicative of aggressive RA and pose a heightened risk of premature death [7].

RA is diagnosed according to the clinical manifestation of synovitis, the presence or absence of the rheumatoid factor (RF) autoantibody and anti-citrullinated protein antibody (ACPA), and markers of inflammation [8]. The diagnosis of RA must be made in such a way that excludes the possibility of other conditions with similar symptoms, such as osteoarthritis. Because the damage inflicted on bones and joints is irreversible, it is important to diagnose RA early in its progression so that patients can derive the greatest benefit from the treatments available. In clinical practice, serum C-reactive protein (CRP) and

erythrocyte sedimentation rate (ESR) are monitored as indicators of disease activity.

Causes for Rheumatoid Arthritis

RA is an autoimmune disease of unknown etiology. Numerous environmental factors, susceptibility genes, and epigenetic modifications are implicated in its development. An estimated 1% of the population worldwide is affected. The prevalence of RA is greater among women than it is among men. It occurs at the highest rates in Pima and Chippewa American Indians and at the lowest among Chinese and Japanese people, suggesting the possibility of genetic involvement. Furthermore, twin studies show concordance rates of 15% to 30% between monozygotic twins and 5% between dizygotic twins, further suggesting the importance of genetic factors [8,9]. Environmental and lifestyle factors such as periodontitis and cigarette smoking are likewise associated with increased risk of developing RA [10].

Cellular and Molecular Factors Involved in Rheumatoid Arthritis

The Role of T Cells in Pathogenesis

RA is classically categorized as a delayed T cell-mediated hypersensitivity. Helper T cells express CD4 surface protein and recognize major histocompatibility antigen

(MHC) class II molecules expressed on the surface of antigen presenting cells (APC) such as macrophages, dendritic cells, and B lymphocytes. The association between a certain allelic variant at the HLA-DBR1 locus and RA is among the strongest pieces of supportive evidence for the involvement of CD4+ T cells in the pathogenesis of this disease. The functional basis for this association remains to be elucidated. This polymorphism may impact T cell receptor (TCR) recognition, either through the differential selection of epitopes for presentation or through direct effects on MHC/TCR association [11]. T cell activation in RA occurs by a different pathway than classical antigen-driven expansion. Th1 cells in the RA synovium express IFN-γ but generally express cytokines at low constitutive levels; this anomaly is attributed to the inflammatory, hypoxic local environment, which is known to impair TCR responsiveness [12]. In established disease, synovial T cells express these cytokines at low levels, including IL-10, IL-2, and IL-4 [13].

Of interest in the study of disease pathogenesis is the role of helper T cells expressing IL-17, known as Th17 cells. In murine models, collagen-induced arthritis is markedly reduced in IL-17 deficient mice, and spontaneous arthritis in animals deficient in IL-1 receptor antagonist (IL-1Ra) can be prevented in the absence of IL-17 [14]. IL-17 is produced spontaneously in cultures of RA synovial cells and has been identified by immunohistochemistry in T-

cell rich zones [15]. Furthermore, IL-23, which evidence suggests may promote the expansion and survival of Th17 cells, is also detectable in RA synovia. T cell derived IL-17 promotes the production by monocytes and fibroblasts of pro-inflammatory cytokines, prostaglandins, MMPs, and osteoclast differentiating factors [13].

The Role of Macrophages in Pathogenesis

In the RA synovium, macrophages are numerous and show signs of activation, as they overexpress signaling molecules associated with inflammation. Monocytes immigrate into the synovial membrane and differentiate into macrophages. Macrophages may further differentiate into stimulatory or inhibitory subpopulations that affect T-cells differently; they may be responsible for the synthesis of pro-inflammatory or regulatory cytokines and be important in the process of angiogenesis [16, 17]. Macrophages overexpress MHC class II molecules, pro-inflammatory cytokines and growth factors, TNF-α, and metalloproteinases, among others. Though it is unlikely that macrophages play a role in the causation of RA, their pro-inflammatory, destructive, and remodeling activity contribute considerably to the damage done to the joint [18].

Protein Citrullination

The first molecular manifestations of RA involve altered post-transcriptional regulation and self-protein citrullination. Citrulline is a noncoding, deiminated form of arginine. Protein citrullination is a post-translational modification in which arginine residues are modified by the enzyme peptidylarginine deiminase (PAD). This brings about the loss of the positive peptidylarginine charge, significantly changing the biochemical and antigenic properties of the peptide. The activity of PAD is dependent on the calcium concentration and pH of the local environment. Under normal physiological conditions, several proteins, such as myelin basic protein and fibrinogen are citrullinated; histones are also subject to citrullination by the isozyme PAD4, implying that the enzyme plays a role in transcriptional regulation. Furthermore, biological events such as inflammation, apoptosis, and trauma increase post-translational citrullination. Though it appears to be a normal process in dying cells, its role remains unknown [19].

There is evidence which suggests that in the RA synovium, arginine residues of fibrin and its precursor fibrinogen, among other proteins, undergo local citrullination [20]. Both intracellular and extracellular citrullinated proteins have been discovered in RA synovial tissue. Because PAD cannot citrullinate intracellular proteins under normal physiological conditions due to the low concentration of Ca^{2+} concentration within the cell, it is suggested that apoptosis or terminal differentiation are critical for Ca^{2+} influx to activate PAD [21].

Rheumatoid Factors and Anti-Citrullinated Protein Antibodies

Rheumatoid factor (RF) was the first discovered among a number of autoantibodies that characterize RA. They bind the Fc region of IgG and thereby induce an immune response [22]. The autoantigens recognized by RF and related autoantibodies include enzymes, heat shock proteins, nuclear proteins, cartilage components, and citrullinated proteins. They are produced by B cells in the inflamed synovium in a T-cell driven manner. RF has been observed in various other autoimmune conditions as well as in states of chronic infection and old age. RFs in RA patients are distinct from those in healthy individuals in that the former exhibit high affinity maturation rather than polyreactivity and low affinity [23]. RF may have a role in the pathogenesis of RA; however, no clear causal relationship has been established [24].

Current clinical practice assesses anti-citrullinated protein antibody (ACPA) levels as a diagnostic tool due to the high prevalence and specificity of this molecule to the RA disease state [25]. Though citrullination must occur for ACPA production to follow, it does not always induce it. Certain polymorphisms in human leukocyte antigen (HLA)-DRB1 alleles appear to affect the susceptibility of an individual to RA as well as the severity of disease when it does present. In patients with certain HLA-DBR1 conserved sequences in the hypervariable region or 'shared

epitope', citrullinated antigens form which are not recognized as self. As a consequence, the autoantibody ACPA is produced [8]. Clinical evidence suggests, however, that systemic production of autoantibodies and inflammation precedes the formation of adhesion molecules and inflammation in the synovium by up to fifteen years, implying that a later trigger may be necessary to involve the synovium in RA [26].

Both RF and ACPA are present in the sera of RA patients prior to the onset of clinical manifestations; furthermore, the risk of RA development is highest when both autoantibodies are present together [27]. They are poor prognostic factors of joint destruction, however.

Tumor Necrosis Factor

Tumor necrosis factor (TNF) is a cytokine with various roles in inflammation [28]. It is produced primarily by macrophages and to a lesser extent by lymphocytes, natural killer cells, and mast cells as well as endothelial cells and fibroblasts. Among the targets of TNF activation are macrophages, synovial cells, chondrocytes, and bone cells. TNF receptors can activate NF-κB and MAP kinase signal transduction pathways [29].

In murine models, overexpression of TNF greatly exacerbates collagen-induced arthritis (CIA), while inhibition of TNF reduces but does not completely block CIA. While its role in arthritis is supported by experimental

evidence, it does not appear to have as profound of an effect as other signaling molecules such as granulocyte macrophage colony stimulating factor (GM-CSF) or NF-κB. This suggests that TNF has profound pro-inflammatory rather than destructive effects, though anti-TNF therapies have shown to be effective in ameliorating damage to joints [30]. Other laboratory and clinical evidence, however, suggests that monoclonal antibodies against TNF-α do reduce the production of GM-CSF and IL-1 in the RA synovium [31, 32]. Furthermore, administration of drugs that inhibit TNF have dramatic effects in patients unresponsive to other standard treatments. They are especially effective in reducing the disabling fatigue that often accompanies RA [33].

Nuclear Factor-kB

Nuclear Factor (NF)-κB is a family of transcription factors involved in inflammation as well as cell survival, proliferation, and differentiation. They are involved in myriad cellular responses to stress, cytokines, free radicals, heavy metals, UV radiation, oxidized low-density lipoprotein, and foreign antigens [34]. NF-κB transcription factors are found in the cytosol of unstimulated cells bound to an inhibitor IκB. A variety of extracellular signals can stimulate the activation of IκB kinase (IKK), which phosphorylates the inhibitor, resulting in its dissociation from NF-κB and subsequent ubiquitination and proteasomal degradation. NF-κB is then translocated into the nucleus,

where it promotes or, in some cases, represses the transcription of certain genes.

NF-κB is activated in the pathogenesis of RA. It plays a major regulatory role in the transcription of TNF-α, suggesting that it may act as a "master regulator" of inflammatory cytokines implicated in the inflammation that characterizes RA [35]. Experimental knockout of the P50 subunit of NF-κB, furthermore, resulted in a complete loss of humoral response, which severely impedes T cell proliferation and induces resistance to arthritis, implying that this subunit, presumably as a P50/P65 heterodimer, plays a pivotal role in RA inflammation [36].

Interleukins

Interleukins are a large group of cytokines that are important stimulators of immune responses, including inflammation. Interleukin (IL)-1 is a key mediator of inflammation and cell differentiation and proliferation. It initiates and potentiates inflammatory and immune responses and is therefore implicated in autoimmune conditions as well as tissue destruction [37]. The IL-2 family of cytokines is involved primarily in the growth and proliferation of mature cells, but also plays a role in the differentiation of progenitor cells [38]. Members of this family have important roles in T-cell development and activation, and it has been demonstrated that certain polymorphisms in the genes encoding them are associated with increased susceptibility to more destructive forms of RA [39, 40].

IL-6 and related interleukins are pro-inflammatory cytokines involved in the pathogenesis of RA as mediators of the acute phase response [41]. Certain polymorphisms in the chemokine IL-8 are associated with earlier age of RA onset [42].

Local Expression of Inflammatory Molecules

Other molecules that are upregulated in the RA synovium include the cyclooxygenases COX-1 and COX-2. Both synthesize prostanoids and are central to the inflammatory response; the former is constitutively expressed while the latter is induced by stress. Both COX-1 and COX-2 promote angiogenesis, a hallmark of the RA synovium [43].

The production of matrix metalloproteases (MMPs) is stimulated by cytokines such as IL-1β and TNF-α. MMPs can degrade all components of the extracellular matrix (ECM). MMP-1 and MMP-13 are especially important in RA pathogenesis, as they are the rate-limiting factor in collagen degradation. MMP-1 is produced by the cells of the synovium and MMP-13 is secreted by the chondrocytes. MMP-2, -3, and -9 are also upregulated in the RA synovium and contribute to the degradation of non-collagen elements of the ECM [44].

Current Therapies

The goals of RA treatment are to stop inflammation, alleviate symptoms, and prevent long-term damage to joints and organs. Nonsteroidal anti-inflammatory drugs

(NSAIDs) are used to relieve pain and inflammation associated with RA. Corticosteroids such as prednisone or its active metabolite prednisolone are immunosuppressant in nature and are therefore used to mitigate inflammation. Used at low doses, they help patients manage pain and stiffness; higher doses are prescribed to manage inflammatory flare-ups [45].

Disease-Modifying Anti-rheumatic Drugs

Disease-modifying antirheumatic drugs (DMARDs) aim to protect joints and achieve remission. Because RA cannot be cured, it is important to control inflammation in order to prevent irreversible damage to tissues. DMARDs are classified either as non-biologic drugs that have broad effects on the immune system or as biologic agents that work by blocking specific molecular pathways [46].

Methotrexate is a common DMARD with anticancer and immunosuppressive properties; it is the gold standard of RA treatment against which the efficacy of other drugs is compared [47]. As a chemotherapeutic agent, it acts as an inhibitor of dihydrofolate reductase and therefore halts thymine synthesis, preventing the replication of DNA and the proliferation of actively dividing cells. At the lower dose used to treat RA, it continues to reduce the growth of rapidly dividing cells, including those of the immune system. The specifics of its mechanism of action in autoimmune diseases, however, are not entirely understood [48].

Cyclophosphamide is another drug with joint chemotherapeutic and DMARD properties. It is an alkylating

drug that interferes with DNA replication by forming DNA cross-links, thereby preventing cell replication. Its toxicity is severe, however, due to the production of the metabolite acrolein; its use is associated with symptoms that include leukopenia and thrombocytopenia due to its antineoplastic properties [49].

Sulfasalazine is a drug made in combination of salicylate and a sulfa antibiotic effective against pain and inflammation as well as in the prevention of joint damage. Among its reported mechanisms of action is inhibition of the NF-κB molecule, specifically by the prevention of NF-κB translocation into the nucleus by inhibition of IκB degradation [50, 51]. Furthermore, it has been shown to inhibit the pro-inflammatory cytokines IL-1 and IL-2. It is often administered in conjunction with methotrexate and hydroxychloroquine.

Hydroxychloroquine is an antimalarial effective in the treatment of RA. Its mechanism of action differs significantly from that of other RA medications. By increasing pH inside intracellular vacuoles, hydroxychloroquine alters protein degradation, macromolecule assembly, and posttranslational modifications. The ability of antimalarial drugs to interfere with the cellular components needed to assemble MHC class II proteins prevents the stimulation of CD4+ T cells and the immune response against autoantigenic peptides, making these drugs efficacious antirheumatic medications [52].

Leflunomide is an immunosuppressive drug, which blocks the formation of DNA, thereby preventing the proliferation of immune cells. Its immunosuppressive qualities reduce inflammation and disease progression. It is thought to inhibit the enzyme dihydroorotate dehydrogenase, which plays a critical role in *de novo* pyrimidine synthesis [53]. Its major effects are T cell-related; it preferentially inhibits the activation of pro-inflammatory Th1 cells and promotes the differentiation of anti-inflammatory Th2 cells [54].

Biologics are a subset of DMARDs that, in general, work more quickly and with fewer side effects than non-biologic agents. They target specific signaling molecules involved in inflammation. Etarnecept, for instance, is a TNF type II receptor linked to an IgG1-Fc moiety that binds to and inactivates TNF, a key cytokine in RA pathogenesis [55]. Clinical studies have shown that it works more quickly, is as effective, and is less toxic than methotrexate [56]. Studies reveal, however, that dual therapy of Etarnecept combined with Methotrexate results in greater improvement than monotherapy with either drug [57]. Other TNF inhibitors common in clinical use include infliximab, adalimumab, certolizumab, and golimumab. A concern with TNF inhibitors is the increased susceptibility to infection, as TNF is an important signaling protein in the normal immune response [46]. The rate of infections in patients taking these medications is difficult to compare against all RA patients, however, as this population already exhibits a high background rate of infection [58].

Janus Kinase Inhibitors

Recent advances in RA treatment target the Janus kinase (JAK) pathway. Janus kinases are tyrosine kinases that mediate cytokine receptor signaling. These receptors do not have intrinsic kinase activity but instead associate with JAK kinases. Upon hormone or cytokine binding, a conformational change in the receptor brings JAK pairs together and causes their activation by transphosphorylation. JAKs phosphorylate tyrosine residues on the cytoplasmic domains of the receptor, which recruit downstream transcription factors called STAT (signal transducers and activators of transcription) proteins. JAK-mediated phosphorylation of STATs causes their dissociation from the receptor and subsequent dimerization and translocation into the nucleus, where they regulate gene expression [59]. Among the members of the Janus kinase family, JAK3 is specifically expressed on hematopoietic cells and is a critical component of the signaling pathway of interleukins 2, 4, 7, 9, 15, and 21, which are important in the development and survival of lymphocytes. Loss of function of JAK3 results in severe combined immunodeficiency due to the absence of lymphocytes. Furthermore, JAK3 is the only member of the JAK family expressed in RA synovium, macrophages, monocytes, and lymphocytes [60].

Because of its involvement in cytokine signal mediation, the JAK pathway is an important target for drugs designed to ameliorate the effects of immune overstimulation. Tofacitinib was the first JAK inhibitor (Jakinib) approved for clinical use to treat RA. It is an inhibitor primarily of JAK3 that has minor inhibitory effects on JAK1 and JAK2, which has shown to improve treatment outcomes in RA patients [61]. Other drugs with similar mechanisms of action, such as Baricitinib or Peficitinib are in various stages of clinical trials.

Krüppel-Like Factors in Regulation of Activation and Inflammation

The Krüppel-like factors (KLF) are a family of zinc-finger transcription factors that play a significant role in physiological processes such as cell growth, proliferation, and differentiation, as well as in responses to stress such as apoptosis. They function in many organ systems, including respiratory, immune, and hematological systems. Changes in their function are associated with diverse pathologies, from cardiovascular disease to metabolic aberrations to cancer [62]. Their name comes from their shared homology with the *Drosophila* Krüppel protein, which functions in embryonic development to regulate body segmentation [63]. In addition, they share homology with the transcription factor Sp1, which binds CG-rich regions of DNA with a zinc-finger structure; Sp1 and KLF are often classified as part of the same family. The Sp1/KLF family regulates the expression of housekeeping genes as well as tissue development and homeostasis [64]. We focus on a member of KLF family (KLF2), which plays an important regulatory

role in immune cell activation, inflammation, and function.

KLF2 is also known as lung KLF (LKLF) because it was first isolated from the lung of the adult mouse [65]. It is expressed during embryonic development in vascular endothelial cells and is highly upregulated in endothelial cells subjected to prolonged laminar flow shear stress [66]. Homozygous knockout mice die in utero from hemorrhaging due to compromised vessel integrity [67]. Because of its responsiveness to fluid shear stress, KLF2 is a regulator of hemodynamics of critical importance to cardiovascular development. Its atheroprotective quality is further demonstrated by its induction in response to statin drugs, which inhibit HMG-CoA reductase, the enzyme that catalyzes the rate limiting and committed step of cholesterol synthesis [68]. Furthermore, KLF2 expression in endothelial cells is suppressed by pro-inflammatory cytokines IL-1β and TNF-α, both of which contribute to the pathogenesis of atherosclerosis. TNF-α inhibits KLF2 via NF-κB and histone deacetylase (HDAC) 3 and 4 [69]. Moreover, overexpression of KLF2 inhibits pathways that depend on IL-1β signaling [70].

KLF2 in T Cells

Mature CD4+ and CD8+ T cells circulate in the bloodstream in a quiescent state until they encounter their cognate antigen bound to an MHC molecule on the surface of

a respective antigen-presenting cell. This event activates T cells, a process which involves the expression of hundreds of genes as well as cell cycle progression and cell proliferation. Transcription factors which include activation protein 1 (AP-1), nuclear factor of activated T cells (NFAT), cAMP response element binding protein (CREB), and NF-κB are known positive regulators of gene expression pertinent to T cell activation. On the other hand, KLF2 is an important regulator of T cell quiescence and survival [71]. This claim is bolstered by the observation that deficiencies of KLF2 result in spontaneous activation of the cell surface phenotype as well as a great loss in the peripheral T cell pool. It is suggested that KLF2 plays a central role in thymocyte and T cell trafficking, as it is induced during the maturation of single-positive T cells and is extinguished following their activation [72]. Furthermore, the T cell specific expression of KLF2 has been shown to repress the expression of chemokine receptors, and its absence has an inverse effect [73].

KLF2 in Endothelial Cells

Statin-induced expression of endothelial nitric oxide synthase (eNOS) is mediated by KLF2. As its name implies, eNOS is an enzyme expressed in endothelial cells that produces nitric oxide (NO) from arginine. NO then diffuses to the vascular smooth muscle surrounding blood vessels and causes vasodilation; in addition, NO has significant anti-inflammatory and antithrombotic properties

[74]. KLF2 also mediates the statin-dependent induction of thrombomodulin, an endothelial cell surface factor that increases the rate of activation of thrombin-catalyzed protein C, an anticoagulant [75]. Statins upregulate KLF2 via the inhibition of cholesterol synthesis and the Rho pathway. Statins bring about the depletion of mevalonate, a cholesterol precursor, in cells. In addition to cholesterol, mevalonate is a precursor for isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, both of which are important post-translational lipid modifications for Ras and Rho proteins [76]. Furthermore, siRNA-mediated knockdown of KLF2 attenuates the ability of statins to induce eNOS and thrombomodulin, reducing NO production and ultimately increasing inflammation [77].

KLF2 in Monocytes

KLF2 is expressed in monocytes, where it is postulated that it inhibits pro-inflammatory gene expression. This anti-inflammatory effect is mediated through inhibition of the NF-κB and AP-1 pathways [78]. The activation of monocytes is critical in the pathophysiology of atherosclerosis, a chronic inflammatory condition [79]. *In vitro*, KLF2 is highly expressed in primary monocytes and attenuated significantly upon cellular differentiation into macrophages; it is expressed at lower levels in the human monocytic cell line THP-1. *In vivo* studies reveal that expression of KLF2 is significantly reduced in atherosclerosis patients, however [78].

The function of monocytes in inflammation is to express increased levels of cytokines, chemokines, and other pro-inflammatory factors in response, enhancing phagocytic activity. In both primary monocytes and THP-1 cells infected with control empty virus (EV) and treated with LPS, significant induction of COX-2 and monocyte chemoattractant protein (MCP)-1 mRNA was noted. In cells infected with Ad-KLF2 and similarly treated with LPS, induction of these factors was appreciably attenuated. Furthermore, other pro-inflammatory factors including IL-1β, TNF-α, and macrophage inflammatory proteins, were likewise attenuated. KLF2 can inhibit secretion of pro-inflammatory factors, suppressing monocyte function and phagocytic capacity [78]. *In vivo*, KLF2 augments rather than inhibits recruitment of monocytes to the site of inflammation. It reduces inflammation significantly, however, by attenuating monocytic secretion of cytokines, chemokines, and growth factors, as was demonstrated in a carrageenan-induced inflammatory model.

NF-κB regulates the induction of MCP-1, COX-2, and tissue factor (TF) [80-82]. It is thought, therefore, that KLF2 reduces their expression by inhibiting the NF-κB pathway. Overexpression of KLF2, it turns out, does not alter nuclear levels of p65 or IKK, nor does it affect IκB

phosphorylation, degradation, or cytoplasmic levels of IKK. Moreover, it does not affect NF-κB binding to DNA; rather, KLF2 inhibits NF-κB transcriptional activity and in this way reduces the production of pro-inflammatory factors. The transcriptional activity of NF-κB depends on the recruitment of coactivators; KLF2 may interact with these, preventing their association with NF-κB. For instance, KLF2 has been shown to recruit the coactivator CBP-associated factor (PCAF), a protein with histone acetyl transferase activity that plays a role in transcriptional regulation.

KLF2 plays an important role in regulating host response to infection by modulation of myeloid cell activation. The site of a bacterial infection is characteristically hypoxic and filled with bacterial debris [83]. This microenvironment induces myeloid cell activation by way of the transcription factors hypoxia-inducible factor (HIF)-1α and NF-κB, molecules whose functions affect the other in myeloid development [84, 85]. KLF2 negatively modulates inflammation and prevents shock. Its experimental deficiency results in improved survival in mice with polymicrobial infection due to increased myeloid cell activation; however, it also makes animals more susceptible to spontaneous pro-inflammatory activation and endotoxic shock. Myeloid KLF2 is downregulated by hypoxia and inflammatory stimuli. Moreover, KLF2 regulates the transcription of HIF-1α mRNA via regulation of NF-κB. These findings identify KLF2 as a molecule of importance to the

innate immune response to bacteria and their products [86].

Taken as a whole, these findings support the view that the anti-inflammatory effects of KLF2 are due not to reduced monocyte recruitment to the site of inflammation but rather to the inhibition of pro-inflammatory gene expression. KLF2 modulates gene expression by altering NF-κB and AP-1 pathways at the transcriptional level, and does not affect the protein expression, nuclear accumulation, or DNA binding of these molecules.

KLF2 in Rheumatoid Arthritis

The protective nature of KLF2 against arthritis was demonstrated in experiments comparing inflammation in wild type $KLF2^{+/+}$ mice to hemizygous $KLF^{+/-}$ mice, all of which had methylated bovine serum albumin (mBSA) and IL-1β-induced arthritis [87]. This study found that arthritis was more severe in the hemizygous animals, which inherited increased numbers of inflammatory monocytes. More severe arthritis in KLF2 hemizygous mice was associated with higher levels of stress-related and pro-inflammatory molecules such as HSP60, HSP90, and MMP13, as well as attenuated levels of phosphorylated phosphatase and tensin homolog (pPTEN), p21, p38, and HSP25/27 in the bone marrow. Increased severity in hemizygous mice is due in part to the differentiation of monocytes towards osteoclasts, which significantly damage the bone and cartilage under arthritic conditions. The osteoclasts

from hemizygous mice, moreover, were found to be more functionally aggressive than those isolated from wild type mice. It is speculated that increased damage of arthritic joints and bones in hemizygous mice is due in this case to a greater recruitment of inflammatory cells to these tissues. Immunohistochemical analysis revealed that, while there was no difference between groups in the recruitment of lymphocytes, recruitment of monocytes and macrophages was higher in the $KLF2^{+/-}$ animals than in the wild type group.

KLF2 as a Potential Target Molecule for RA Therapy

Given its anti-inflammatory effects in varied contexts and the specific implications of its role in arthritic models, KLF2 is proposed as a potential target for the treatment of RA. KLF2 is important in the maintenance of T cell quiescence, and its upregulation may prevent the activation of T cells and attenuate their expression of cytokines. Though its influence on the immigration of monocytes into the synovium is incompletely understood, there is evidence that it does reduce their local production of pro-inflammatory factors by means of transcriptional regulation. In diverse inflammatory contexts, KLF2 has demonstrated a negative regulatory role in monocyte-mediated inflammation. It reduces monocyte differentiation towards the osteoclastic lineage. KLF2 inhibits NF-κB transcriptional

activity, thereby reducing inflammation by preventing the transcription of diverse pro-inflammatory molecules. Its significant anti-inflammatory properties affect many of the molecules and cells implicated in the pathogenesis of RA, which lends it promise as a targeted molecule in the treatment of RA.

Based on what is known about the effects of KLF2, a therapy that induced its expression in RA conditions may reasonably be expected to lower levels of stress-related proteins and reduce the recruitment and activation of immune cells to the synovium. Upregulation of KLF2 would have significant inhibitory effects on the NF-κB pathway, and the transcription of number of inflammatory molecules, such as COX-2, inflammatory interleukins, and TNF-α would be consequently attenuated. This would have significant effects in reducing the inflammation characteristic of RA, which would protect joints and slow the course of disease. Furthermore, KLF2 has a protective effect against joint inflammation and the erosion of bone and cartilage, as compared to hemizygous models. From this, it is postulated that its induction in RA conditions would lessen the severity of the disease.

There is a necessity, however, for future research to be carried out in order to confirm these effects in animal subjects. Specifically, experiments must be performed that observe the effects of conditional knockout and conditional overexpression of KLF2 in murine arthritis mod-

els. Assuming the results of such experiments are consistent with previous findings, KLF2 targeted therapies show great promise of translation into human patients.

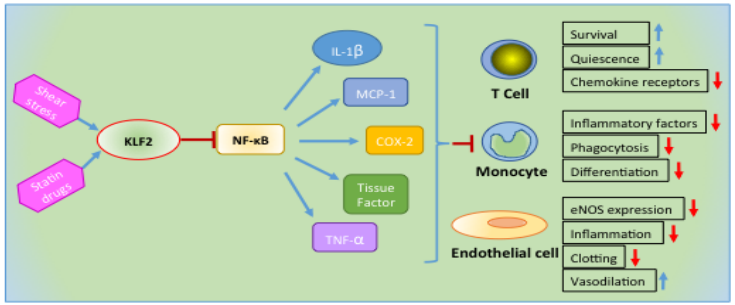

Figure 1: KLF2 stimulation and subsequent inhibition of NF-κB transcription has downstream effects on immune cell activation and function.

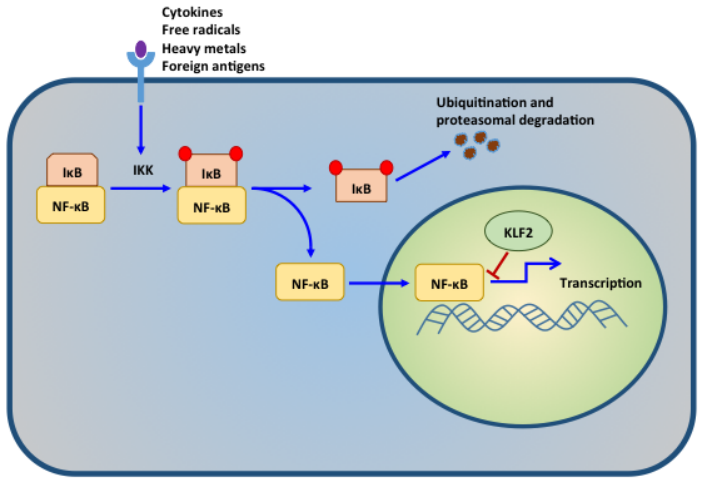

Figure 2: NF-κB activation and nuclear translocation are depicted. KLF2 exerts inhibitory effects resulting in attenuation of NF-κB mediated transcription.

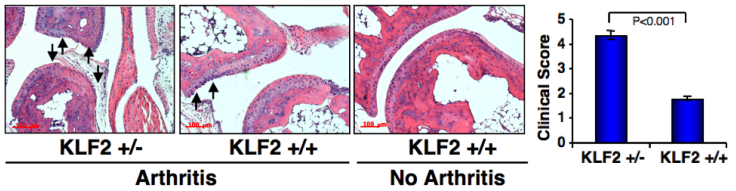

Figure 3: Severe arthritic pathogenesis in KLF2 hemizygous (KLF2+/-) mice. Arthritis was induced in wild type (KLF2+/+) and KLF2 hemizygous (KLF2+/−) mice via an intra-articular injection of mBSA and footpad injection of rIL-1β. Hematoxylin and eosin (H&E) staining was performed on the harvested tissue sections (left panels). A cumulative graphical presentation of clinical scores of arthritic damage for scoring is depicted in the right panel. Adapted from Das M. et al., Current Molecular Medicine (2012); 12: 113-125.

Conclusion

RA is characterized by severe irreversible destruction of the joints. It is associated as well with extra-articular manifestations that contribute to a heightened risk of early mortality in patients. For this reason, it is imperative that improved treatments be sought which reduce the severity of disease and associated symptoms and which halt the destruction of tissues. KLF2 is a molecular target with great therapeutic promise, as it has been demonstrated to mediate the transcription of a number of proteins with important roles in the inflammatory processes that characterize RA pathogenesis. Its presence at normal physiological levels has numerous anti-inflammatory applications and, more specifically, has shown to be protec-

tive in arthritic models. It exerts a regulatory influence on myriad signaling pathways central to the inflammatory response to stress. Because of this, it is suggested that therapies that aim to upregulate its expression would prove to be potent anti-inflammatory agents in the management of RA. Though existing data strongly predict its effectiveness against this disease, much work must be done to confirm its effects and elucidate the underlying mechanisms. Furthermore, much remains to be discovered about the etiology and pathogenesis of RA itself. Indeed, KLF2 holds great potential as a novel therapeutic target in RA treatment; its benefits are only beginning to be apprehended.

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