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THERAPEUTIC TARGETS FOR ANKYLOSING SPONDYLITIS

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ABSTRACT

Ankylosing spondylitis is one of a group of rheumatic diseases known as the spondyloarthritides and is characterized by a stiffening of the joints in the vertebrae due to inflammation. It is not known whether ankylosing spondylitis is an autoinflammatory or autoimmune condition and there is no cure. Current therapy focuses on slowing disease progression, controlling pain and preventing spinal cord and nerve root injury. To date, first-line therapy consists of a nonsteroidal anti-inflammatory drug (NSAID) in combination with physical exercise. In refractory cases, corticosteroids or immunomodulating agents are used. However, the search for effective treatment strategies for ankylosing spondylitis continues and this article provides insight into the search for new and improved treatment options by detailing the putative targets for drugs currently under active investigation for the disorder.

INTRODUCTION

Ankylosing spondylitis is a disease characterized by a stiffening of the joints in the vertebrae due to inflammation. It is a chronic, progressive and painful disease that affects, in particular, the sacroiliac joint and the vertebrae of the lower back, which may eventually become fused. The disease may also progress upwards causing the entire spine to become rigid, resulting in loss of normal curvature, movement and quality of life. Pain and stiffness can spread to the rib cage, causing difficulties with chest expansion, and other joints may be involved, including the hip, knee, elbows, hands and ankles. The accompanying inflammation may also affect the gastrointestinal tract, eyes and, occasionally, the lungs and heart (1-3).

Ankylosing spondylitis is one of a group of rheumatic diseases known as the spondyloarthritides. This group also includes reactive arthritis, enteropathic arthritis (i.e., with inflammatory bowel disease)/spondylitis, arthritis/spondylitis with psoriasis, juvenile-onset spondyloarthritis and a subgroup of undifferentiated forms of spondyloarthritides. These diseases share rheumatic symptoms and a high prevalence of the susceptibility gene *HLA-B27* in affected individuals. The HLA-B27 protein is a member of the class I major histocompatibility complex (MHC) and is normally involved in the presentation of self- and pathogen (e.g., microbial)-derived peptide fragments to T cells. However, the role of HLA-B27 in the pathogenesis of ankylosing spondylitis has not been elucidated (1-5). It is known that HLA-B27 contributes to only about 20-40% of the total risk for the disease, suggesting that other genetic and/or environmental factors are involved. Studies suggest that bacterial infection may play a role in individuals who are genetically predisposed to the disorder. Although direct evidence is lacking, it is thought that the disease may initiate with a compromise in intestinal defense mechanisms, allowing bacteria to pass into the bloodstream and to the sacroiliac joints, where they initiate an immune response (1, 6, 7).

In ankylosing spondylitis, the inflammation in the sacroiliac joint involves both innate and adaptive immune responses since infiltration of $CD3^+$, $CD4^+$ and $CD8^+$ T cells, $CD68^+$ macrophages, $CD20^+$ B cells, proliferating fibroblasts and neovascularization have been observed. The inflammatory cytokines interleukin-1 (IL-1), IL-8 and tumor necrosis factor α (TNF-α) have been detected in the synovial fluid of the joints of affected individuals, and this provides a rationale for the efficacy of TNF-α blockers in treating the disease. Erosion of the cartilage–bone interface (i.e., enthesis) accompanies the inflammation, and fibrosis and mineral deposition follow. This leads to the formation of bony syndesmophytes in the vertebrae, and eventually the discs may be completely replaced by bone and the vertebrae fuse. It is still unclear as to whether ankylosing spondylitis is an autoinflammatory or autoimmune condition. To date no autoimmune antibodies have been found (1-4, 8, 9).

The prevalence of ankylosing spondylitis is highly dependent on race, ranging from 0.007% in Japanese people to 0.5-1.2% in Caucasians and up to 10% in certain native tribes from the arctic. These figures largely reflect the prevalence of the *HLA-B27* gene in these populations (0.5%, 8% and up to 50%, respectively). The incidence of ankylosing spondylitis in white Europeans is estimated to be 0.5-8.2/100,000 population (1, 10, 11).

There is currently no cure for ankylosing spondylitis and therapy is aimed at slowing disease progression, controlling pain and preventing spinal cord and nerve root injury. To date, first-line therapy con-

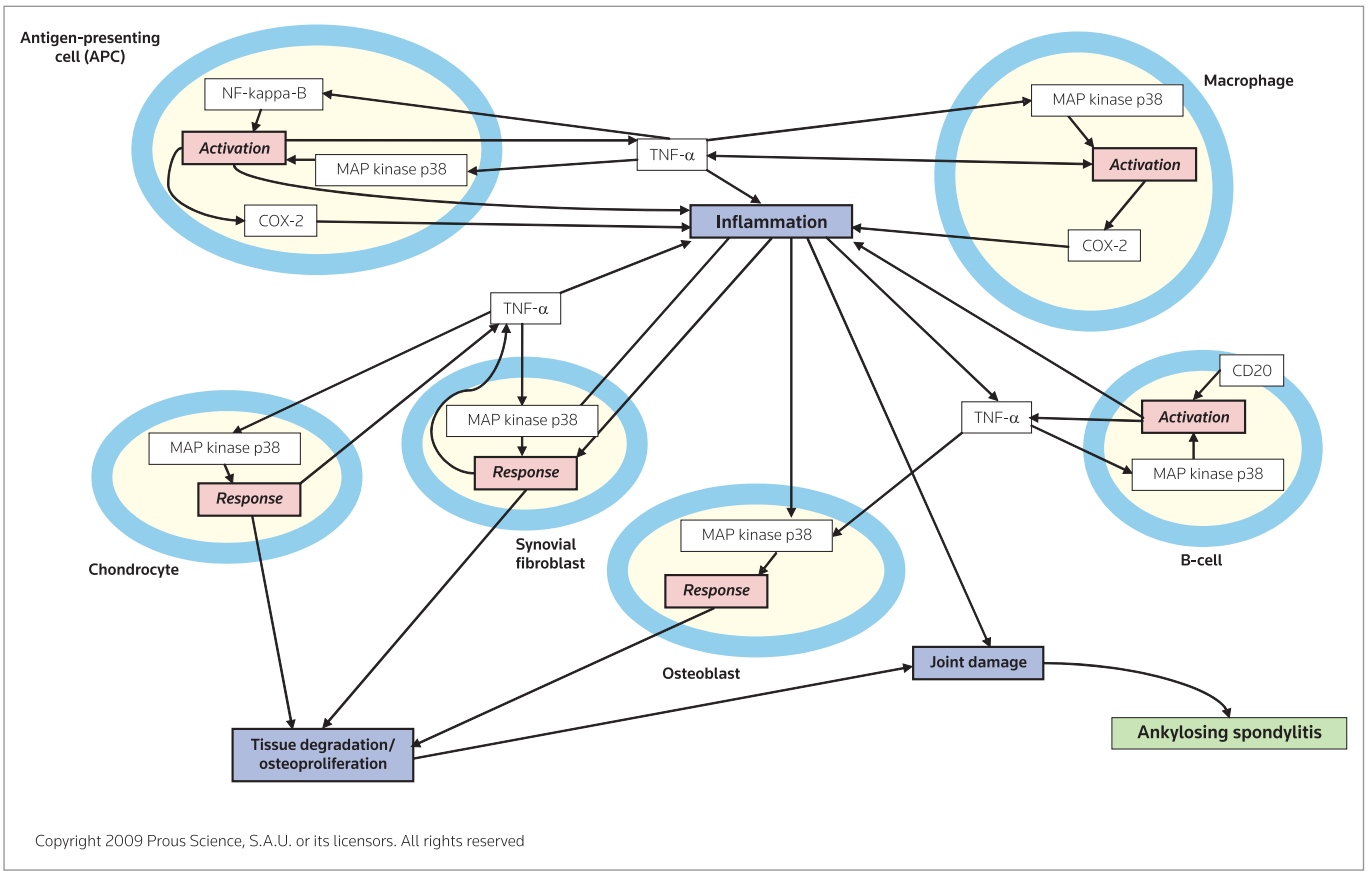

Figure 1. Ankylosing spondylitis targetscape. A diagram showing an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of ankylosing spondylitis and their biological actions. Arrow: positive effect. Abbreviations: COX, cyclooxygenase; MAP kinase p38, mitogen-activated protein kinase p38; NF-kappa-B, nuclear factor NF-kappa-B; TNF-α, tumor necrosis factor α.

sists of a nonsteroidal anti-inflammatory drug (NSAID) in combination with physical exercise under the guidance of a rheumatologist. In refractory cases, corticosteroids or immunomodulating agents are used. TNF-α blockers are increasingly used in refractory cases, with some patients exhibiting a rapid and dramatic response (1, 8, 9).

The search for effective treatment strategies for ankylosing spondylitis continues, with research focusing on the identification of novel targets for drug development. Those targets which are currently under active investigation are discussed below (see Figure 1). Table I provides a selection of products under active development for each target.

TARGETS

CD20

CD20 is a 33- to 37-kDa transmembrane glycoprotein of the immunoglobulin superfamily. It is expressed on the surface of normal and malignant B cells, residing within lipid rafts of the phospholipid membrane, where it functions as a store-operated calcium channel following the binding of the B-cell receptor with antigen. No natural ligands of CD20 have been identified. However, CD20 has been shown to participate in antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cellular cytotoxicity (CDCC) and cell growth. Immunohistological studies of specimens from patients with ankylosing spondylitis removed during spinal surgery have shown significantly increased numbers of $CD20^+$ B cells within those joints exhibiting persistent inflammatory lesions. Antibodies directed against CD20 may eliminate pathogenic B cells and therefore may be effective in the treatment of ankylosing spondylitis (12-15).

Cyclooxygenase (COX)

COX, also known as prostaglandin endoperoxide synthase or prostaglandin G/H synthase (EC 1.14.99.1), is an enzyme that catalyzes the two steps in prostaglandin (PG) synthesis, forming PG_2 and PGH_2 from arachidonic acid. The two major forms of the enzyme are COX-1 and COX-2. Recently, COX-3, a distinct COX-1 variant, and two smaller COX-1-derived proteins (partial COX-1 or PCOX-1 proteins) have been cloned and found to be expressed in canine cerebral cortex and other tissues; COX-3 is predominantly expressed in canine cerebral cortex and heart and has been shown to be selectively inhibited by NSAIDs, suggesting the involvement of this isoform in pain and fever. COX-1 is constitutive and present in endothelium, stomach and kidney. It is involved in the maintenance of platelet and kidney function and is considered a housekeeper enzyme, maintaining homeostasis. COX-2 is not present at baseline

Table I. *Selected targets and products launched or being actively investigated for ankylosing spondylitis (from Prous Science Integrity).*

Target	Product	Source	Phase
CD20	Rituximab	Roche	II/III
COX-2	Diclofenac	Novartis	L-1975
	Ibuprofen	Abbott	L-1985
	Meloxicam	Boehringer Ingelheim	L-1996
	Celecoxib	Pfizer	L-2005
	Etoricoxib	Merck & Co.	Reg. 2008
NF-kappa-B	Sulindac	Neopharmed	L-1976
	Ibuprofen	Abbott	L-1985
p38 MAP kinases	ARRY-797	Array Biopharma	II
TNF-α	Infliximab	Schering-Plough	L-2003
	Etanercept	Amgen/Wyeth Pharmaceuticals	L-2003
	Adalimumab	Abbott	L-2006
	Golimumab	Centocor/Schering-Plough	Prereg.

but is inducible during inflammation by cytokines and endotoxins. It has been shown to play a role in the propagation of inflammatory cascades leading to disorders such as Alzheimer's disease, rheumatoid arthritis and osteoarthritis, cancer, kidney disease, osteoporosis and ankylosing spondylitis. Clinical trials have established the efficacy of NSAIDs, including selective COX-2 inhibitors, in the pharmacological management of inflammation and pain in osteoarthritis, rheumatoid arthritis, acute gouty arthritis, low back pain, acute postoperative pain, primary dysmenorrhea and ankylosing spondylitis. Thus, inhibition of COX-2 may be effective in preventing the development and progression of these conditions and alleviating the associated symptomatic pain. It should be mentioned that concerns have been raised regarding the continuous use of NSAIDs in ankylosing spondylitis due to the possible increase in cardiovascular risk associated with these agents (16-19).

Nuclear factor NF-kappa-B

Nuclear factor NF-kappa-B is a protein transcription factor and intracellular mediator of the inflammatory cascade involved in the generation of adhesion molecules (ICAM-1, V-CAM 1), inducible nitric oxide synthase (iNOS), COX-2, cytokines (i.e., IL-1β, IL-2, TNF-α, IL-6, interferon gamma) and chemokines (IL-8). Other genes which are regulated by NF-kappa-B include those encoding the IL-2 receptor, the IL-12 p40 subunit and c-Myc. Recent findings suggest that NF-kappa-B provides a mechanistic link between inflammation and cancer, controlling the ability of preneoplastic and malignant cells to resist apoptosis-based tumor surveillance mechanisms and regulating tumor angiogenesis and invasiveness. NF-kappa-B activity is closely associated with the I-kappa-B kinase (IKK) complex, and aberrant or constitutive NF-kappa-B activation has been detected in many human malignancies, including solid tumors and hematological cancers such as acute myeloid leukemia (AML) and chronic myelogenous leukemia (CML). It has also been reported that constitutive activation of the tyrosine-protein kinase receptor FLT3 is responsible for IKK activation. In addition, TNF activation results in NF-kappa-B activation and plays a role in inflammation. Therapeutic inhibitors of NF-kappa-B activation may be a useful option for treating cancer including breast, lung, colorectal and prostate cancer, AML and CML, respiratory disorders including asthma, neurological disorders including multiple sclerosis, psychiatric, cardiovascular, renal, gastrointestinal, dermatological, endocrine, infectious, metabolic, hematological and immunological diseases. Special attention has been given to NF-kappa-B activation inhibitors as a possible therapeutic strategy for the treatment of musculoskeletal and connective tissue disorders such as rheumatoid arthritis and ankylosing spondylitis (20-22).

p38 MAP kinases

p38 MAP kinases are a class of mitogen-activated protein kinases (MAPKs; EC 2.7.11.24) composed of four isoforms: MAP kinase p38 alpha (CRK1), MAP kinase p38 beta, MAP kinase p38 gamma (ERK-6) and MAP kinase p38 delta. These are activated by a variety of cellular stresses, including osmotic shock, inflammatory cytokines, lipopolysaccharide (LPS), ultraviolet light and growth factors, and result in inflammatory, apoptotic, growth and differentiation responses. Activation of p38 MAP kinases occurs via mitogen-activated protein kinase kinase kinase (MAPK/ERK, MEKK) and dual-specificity mitogen-activated protein kinase kinase (MEK) by phosphorylation at Thr180 and Tyr182. Activated p38 MAP kinases have been shown to phosphorylate and activate MAPKAPK-2 (MAP kinase-activated protein kinase 2) and the transcription factors ATF-2, Mac and MEF2. Inhibition of p38 MAP kinase signaling and thus inflammatory responses may also be effective in the treatment of ankylosing spondylitis (8, 9, 23).

TNF-α

TNF-α, also known as cachectin, is a proinflammatory cytokine that belongs to the TNF family of cytokines. It is released by activated macrophages and lymphocytes, and acts via TNF-R1 and TNF-R2 receptors, triggering several signal transduction pathways resulting in the activation of transcription factors such as NF-kappa-B and c-fos/c-jun. TNF-R1 (also known as CD120a, p55/60) is expressed in most tissues and is fully activated by both the membrane-bound and soluble trimeric forms of TNF. TNF-R2 (also known as CD120b, p75/80), however, is found only in cells of the immune system and is activated by the membrane-bound form of the TNF homotrimer. Activated factors induce the transcription of antiapoptotic, prolifera-

tive, immunomodulatory and inflammatory genes. NF-kappa-B is the major survival factor in preventing TNF-α-induced apoptosis and inhibition of this transcription factor may improve the efficacy of apoptosis-inducing cancer therapies. TNF-α is also a crucial cytokine in the establishment and maintenance of inflammation in multiple autoimmune diseases, and inhibition of the TNF-α signaling pathway (e.g., TNF-α blockers, blockers of p38 MAP, JNK and/or ERK kinases, inhibitors of transcription factor NF-kappa-B activation) is a viable therapeutic strategy for the treatment of Crohn's disease, psoriasis, psoriatic arthritis, uveitis, sarcoidosis, Behçet's syndrome, graft versus host disease and ankylosing spondylitis (8, 23-26).

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