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Common Mechanisms in Immune-Mediated Inflammatory Disease

GARY S. FIRESTEIN and MARIPAT CORR

**ABSTRACT.** Characterization of the K/BxN mouse model of spontaneous arthritis contributed to the rediscovery of immune complex-mediated inflammation in rheumatoid arthritis (RA). Serum from these animals can transfer joint-specific inflammation to normal mice. Fc receptors, interleukin 1, mast cells, and complement are all essential for the development of arthritis after serum transfer. In RA, additional amplifying factors have been identified, including cytokines and intracellular signaling molecules, such as mitogen-activated protein kinases and nuclear factor kappa B, that perpetuate inflammation. Understanding the autoimmune and inflammatory pathways implicated in disease has led to targeted drug development and improved clinical outcomes. (J Rheumatol 2005;32 Suppl 73:8-13)

**Key Indexing Terms:**
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DISEASE MODELS, ANIMAL
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**THE NATURE OF THE IMMUNE RESPONSE**

Activation of the innate immune system is a common pathway that initiates normal inflammatory responses. Innate immunity, which plays a fundamental role in host defense, developed prior to the evolutionary appearance of highly specific adaptive immune functions. These rapid immune responses are mediated by germ line-encoded receptors that recognize highly conserved structures present in prokaryotes and other pathogens. These structures are referred to as pathogen-associated molecular patterns (PAMP), and include bacterial lipopolysaccharide (LPS) (endotoxin in the outer membrane of Gram-negative bacteria) and peptidoglycan (a component of the cell wall of Gram-positive bacteria), as well as bacterial and viral DNA motifs.

The receptors of the innate immune system that recognize PAMP are termed pattern-recognition receptors (PRR). They are expressed by many effector cells, including macrophages, dendritic cells, and mast cells. When PRR bind to their ligand, effector cells are activated and initiate a rapid response marked by release of inflammatory mediators and cytokines. Three classes of PRR have been characterized: secreted, endocytic, and signaling. One key PRR family is composed of Toll-like receptors (TLR), which are evolutionarily conserved and play a key role in the induction of immune and inflammatory responses in mammals. At least 11 mammalian TLR have been identified, including TLR2, which binds to peptidoglycan, and TLR4, which binds to LPS. When a TLR binds its ligand, the nuclear factor kappa B (NF-κB) signaling pathway is rapidly activated. NF-κB proteins in the cytoplasm translocate to the nucleus and enhance transcription of numerous inflammatory- and immune-response genes, including tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), and chemokines. The signals induced after recognition by the innate immune system, in turn, participate in the activation of adaptive immune responses. Adaptive immunity provides a second line of defense, and is characterized by its exquisite antigen specificity. Receptors on the specialized cells of the adaptive immune system (T cells and B cells) are generated somatically. The range and diversity of the receptor repertoire increase the probability that a particular antigen will be recognized, triggering amplification through the release of additional cytokines, and lymphocyte clonal expansion.

Immune responses are designed to be self-limited, and processes that initiate resolution are also triggered. For instance, a subset of regulatory T cells promotes tolerance, the resolution of inflammation, and tissue repair. As part of the repair process, fibroblasts and other mesenchymal cells can produce collagen and other components of the extracellular matrix. Ultimately, these counter-regulatory systems reestablish homeostasis in the host.
Additional experiments implicated mast cells, which are another component of the innate immune system in this model. When mast cell-deficient mice receive anti-G6PI, arthritis is markedly attenuated. Further, mast cell-deficient mice that are reconstituted with bone marrow-derived mast cells developed an intermediate course of arthritis. The role of cytokines has also been investigated in this immune complex model. IL-1 receptor-deficient mice do not develop the disease, indicating that IL-1 signaling is absolutely necessary for the development of arthritis following transfer of arthritogenic serum. Another requirement for the development of serum-transferred arthritis is the complement factor C5. Mice lacking the C5a receptor gene, or mice treated with an anti-C5 monoclonal antibody, were resistant to disease.

**THE ROLE OF TOLL-LIKE RECEPTORS**

As noted above, TLR are an evolutionarily conserved family of transmembrane receptors present on many cell types. TLR4 and TLR2 have been characterized in most detail. The former recognizes LPS from most Gram-negative bacteria. It also binds to several eukaryotic endogenous proteins that can be released at sites of damage or infection, such as heat shock protein 60 (HSP60) and fibronectin. TLR2 ligaes a wide variety of Gram-positive bacterial cell wall components including peptidoglycan, as well as lipoproteins found in both Gram-positive and Gram-negative bacteria and mycoplasma. TLR9 is an interesting member of the family that binds to bacterial CpG DNA motifs.

Although the extracellular domain of TLR differs from the IL-1 receptor family, similar cytoplasmic...
especially TNF-α, is an effective treatment strategy. Synovial macrophages and fibroblasts produce cytokines, including TNF-α, IL-1, IL-6, IL-8, and IL-18, that can activate cells in the local articular environment. Superimposed on this paracrine system is input from T cells, which produce interferon-γ and IL-17 (Figure 3). The innate immune system can also be activated by engagement of Fc receptors by rheumatoid factors and other autoantibodies. Proteoglycan in the rheumatoid synovium could bind to TLR, possibly representing another pathway to innate immune activation. This complex process contributes to a wave of cytokine production and enhanced adaptive immune response. In addition to therapeutic approaches targeting antibody formation, regulatory intracellular pathways that control cytokine production could be targeted in inflammation. The MAP kinases and NF-KB are key regulators of cytokine production and represent particularly attractive pathways. The MAP kinases include 3 main families: extracellular signal-related kinase, c-Jun N-terminal kinase (JNK), and p38. All 3 are expressed in rheumatoid synovial tissue, and the upstream kinases that activate MAP kinases (MAP kinase kinases including MKK3 and MKK6) are also activated in RA synovium.

p38 MAP kinase plays a key role in the regulation of IL-1 and TNF-α. It has 4 isoforms (α, β, γ, δ), of which the α isoform appears to be most important for cytokine production. p38 inhibitors are effective in animal models of arthritis and are now being investigated in humans. For instance, the adjuvant-induced arthritis model in the Lewis rat has been used to evaluate selective p38 inhibitors. SB 203580 has dose-dependent antiarthritic activity in this model, and SB 242235 inhibits paw swelling and protects bone and cartilage in rodent arthritis models.

Figure 2. The Toll-like receptor (TLR) pathway in RA. Initiation of inflammation by a variety of stimuli can indirectly lead to TLR activation by endogenous proteins. This can, in turn, lead to increased cytokine production and a positive feedback loop that enhances inflammation. HSP: heat shock proteins.
A second interesting target is JNK, a key regulator of matrix metalloproteinases (MMP) such as collagenase and stromelysin. Several JNK isoforms phosphorylate c-Jun after cells are exposed to cytokines, thereby enhancing the transcriptional activity of activator protein-1 (AP-1), a key regulator of MMP production. JNK blockade suppresses MMP and bone destruction in adjuvant arthritis\textsuperscript{15}. Further, mice lacking JNK2 function show less joint damage than wild-type mice in a passive collagen-induced arthritis model\textsuperscript{16}.

NF-κB participates in inflammatory disease through its ability to induce transcription of proinflammatory genes. It is highly activated at sites of inflammation in many diseases and enhances recruitment of inflammatory cells and proinflammatory mediators. NF-κB resides in the cytoplasm in an inactive form associated with the regulatory protein IκB. Phosphorylation of IκB leads to its proteolytic degradation, thereby releasing NF-κB for nuclear translocation. Two IκB kinases (IκB kinase: IKK1 and IKK2) mediate IκB phosphorylation in inflammatory arthritis\textsuperscript{17}. IKK2 is expressed in fibroblast synoviocytes and plays a central role in IL-1 and TNF-α-mediated NF-κB activation and expression of proinflammatory genes\textsuperscript{18}. Intraarticular gene transfer with a...
dominant negative IKK2 gene significantly ameliorates adjuvant arthritis. The selective IKK blocker SPC 839 inhibits paw swelling in an adjuvant arthritis model and appears to prevent joint damage (Figure 4). Therefore, NF-κB-targeted therapies may be effective in diseases such as RA, and blockade of IKK2 is a potential therapeutic approach.

RESOLUTION OF INFLAMMATION
Inflammation cannot continue unabated without serious hazard to the host. Hence, complex processes have evolved to suppress these responses, repair local damage, and reestablish homeostasis. Transforming growth factor-β (TGF-β) can broadly attenuate inflammation and immune responses while enhancing production of matrix proteins. Soluble receptors to TNF and IL-1 can also absorb excess cytokines in the local milieu. Natural antagonists, such as IL-1 receptor antagonist (IL-1Ra) and antiinflammatory cytokines, including IL-10 and IL-4, assist with this process. Protease inhibitors, including the tissue inhibitors of metalloproteinases and serine protease inhibitors that protect the extracellular matrix, are released by mesenchymal cells. Antioxidants, such as superoxide dismutase, are also produced as a component of the counter-regulatory mechanisms, as well as antiinflammatory prostanooids that suppress inflammation through peroxisome proliferator-activated receptor-gamma. Deletion of cells that participate in the innate and adaptive immune responses occurs through apoptosis and is mediated by proteins such as Fas ligand. This process removes effector cells from the local environment.

DEVELOPING TARGETED THERAPIES
Understanding the regulation of cytokines in inflammatory diseases can potentially lead to new therapeutic interventions. Inhibitors of TNF-α have demonstrated efficacy in many inflammatory diseases, including RA, Crohn’s disease, ankylosing spondylitis, psoriasis, and psoriatic arthritis. IL-1Ra, a natural antagonist of IL-1, has also been approved for use. Numerous additional cytokine-directed therapies, such as anti-IL-6 receptor antibody, are also in clinical development. Other attractive approaches include the targeting of IL-18, adhesion molecules, and chemokine receptors. Blocking intracellular kinases, such as MAP kinases, or regulators of NF-κB, has shown promise in animal models, and clinical development programs are under way.

Recently, there has been renewed interest in the therapeutic targeting of T cells and B cells in RA. For example, the monoclonal antibody rituximab binds to CD20-positive B cells. In addition to producing antibodies, B cells can act as antigen-presenting cells and serve as the source of cytokines that support the activity of autoreactive T cells. Thus, rituximab is an appealing candidate for treating autoimmune disorders and has already shown activity in RA and systemic lupus erythematosus. Promising data using the biologic agent CTLA4-Ig, which interferes with T cell costimulation, suggest that modulation of T cell function will also be an effective therapeutic strategy in RA. Other approaches that might have utility include the inhibition of proteases to preserve the extracellular matrix and induction of apoptosis to remove inflammatory cells.

CONCLUSIONS
Many cells and mediators are involved in the pathogenesis of autoimmune and inflammatory diseases such as RA. Interplay between B cells, T cells, macrophages, dendritic cells, fibroblasts, and mast cells upon a background of genetic susceptibility likely contributes to the development and progression of RA. Although RA is a uniquely human disease, animal models can increase our understanding of normal inflammatory and immune responses and can be used to evaluate therapeutic agents. By dissecting the various mechanisms of immune activation and resolution, novel targets might lead to more effective therapy.

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REFERENCES
13. Badger AM, Bradbeer JN, Votta B, Lee JC, Adams JL, Griswold DE. Pharmacological profile of SB 203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in


