

Anti-inflammatory Agents: Present and Future

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Inflammation involving the innate and adaptive immune systems is a normal response to infection. However, when allowed to continue unchecked, inflammation may result in autoimmune or autoinflammatory disorders, neurodegenerative disease, or cancer. A variety of safe and effective anti-inflammatory agents are available, including aspirin and other nonsteroidal anti-inflammatories, with many more drugs under development. In particular, the new era of anti-inflammatory agents includes “biologicals” such as anticytokine therapies and small molecules that block the activity of kinases. Other anti-inflammatories currently in use or under development include statins, histone deacetylase inhibitors, PPAR agonists, and small RNAs. This Review discusses the current status of anti-inflammatory drug research and the development of new anti-inflammatory therapeutics.

Introduction

Reducing pain, inflammation, and fever with salicylate-containing plant extracts can be traced throughout written human history. One hundred and fifty years ago, Felix Hoffman acetylated salicylic acid and created aspirin. Aspirin inhibits the cyclooxygenase (COX) enzymes COX-1 and COX-2, which synthesize inflammatory mediators called prostaglandins and thromboxanes. The ability to block production of prostaglandins and thromboxanes accounts for aspirin being the world’s most used therapeutic agent. Second to aspirin are nonsteroidal anti-inflammatory drugs (NSAIDs), which target COX-2 and hence the synthesis of prostaglandins, particularly PGE₂. Synthetic forms of natural cortisol (termed glucocorticoids) are also widely used to treat many inflammatory diseases, and despite their side effects, glucocorticoids remain a mainstay for reducing inflammation. Yet, it is still the challenge of the pharmaceutical chemist to develop more effective and less toxic agents to treat the signs and symptoms of acute inflammation as well as the long-term consequences of chronic inflammatory diseases.

Inflammation is a dynamic process with proinflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and vascular endothelial growth factor (VEGF) playing central roles. A number of “biologicals” have been developed to treat inflammation (Table 1), including agents that reduce the activity of specific cytokines or their receptors (anticytokine therapies), block lymphocyte trafficking into tissues, prevent the binding of monocyte-lymphocyte costimulatory molecules, or deplete B lymphocytes (Figure 1). Current anticytokine therapies have found a place in the treatment of autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, multiple sclerosis, and others. Without question, neutralization of specific proinflammatory cytokines has “canonized” their causative role in inflammation and has changed the lives of millions of patients with these diseases. One drawback of anticy-

tokine therapies is decreased host immune defense against infection and possibly cancer. Nevertheless, the benefits of anticytokine therapies outweigh the risks, and the risks can be reduced. Compared to the consequences of long-term glucocorticoid treatment to stem inflammation, anticytokine therapies are a major improvement. Indeed, organ toxicities are rarely, if ever, observed with anticytokine therapies as they operate almost exclusively in extracellular rather than intracellular compartments.

Kinases that act downstream of cytokine receptors have become new targets to tame inflammation, and orally active small-molecule inhibitors of intracellular signaling kinases will likely be the new frontier of anti-inflammatory drug development. However, because many intracellular signaling molecules are involved in normal cellular functions, the effective concentration that does not elicit organ toxicity will need to be carefully determined. Statins, a safe class of drugs used for lowering serum cholesterol, also have anti-inflammatory properties. Orally active inhibitors of histone deacetylases, which are also safe and used clinically, are effective drugs with anti-inflammatory properties that also block cell proliferation. Naturally occurring resolvins are also being developed as anti-inflammatory agents. This Review discusses current anti-inflammatory drugs as well as the development of new orally active, safe, and effective drugs for treating acute or chronic inflammation.

Inhibiting Prostaglandins: Targeting COX-2

The inflammatory molecule PGE₂ lowers pain thresholds, and the primary goal of oral inhibitors of PGE₂ is to reduce pain. There are two pathways for synthesizing the inflammatory molecule PGE₂: the constitutive COX-1 pathway and the inducible COX-2 pathway. Whereas COX-1 accounts for low levels of PGE₂ and regulates homeostatic mechanisms in health, COX-2 induces at least two orders of magnitude more PGE₂ compared to COX-1 and is primarily associated with inflammatory disease.

Table 1. Biologicals in the Treatment of Chronic Autoimmune and Inflammatory Diseases

Drugs	Function	Diseases Treated
Anti-CD3 (eplizumab); anti-IL-2 receptor MoAb (daclizumab)	Targeting T cells	Transplant rejection; Type 1 diabetes
Anti-CD20 (rituximab, crelizumab, ofatumumab); anti-CD22 (epratuzumab); anti-Blys MoAb IgG1 (belimumab)	Targeting B cells	Type 1 diabetes; rheumatoid arthritis; multiple sclerosis
Anti-TNF- α MoAb (infliximab, adalimumab, golimumab); anti-TNF- α pegylated Fab' (certolizumab); soluble TNF p75 receptor Fc fusion (etanercept)	Reducing TNF- α activities	Rheumatoid arthritis; Crohn's disease; psoriasis
Anti-IL-6 MoAb (MEDI5117); Anti-IL-6 receptor (tocilizumab)	Reducing IL-6 activities	Rheumatoid arthritis; juvenile arthritis
Anti-IL-12/23 (ustekinumab); Anti-IL-17 MoAb (AIN457/LY24398)	Neutralization of IL-12, IL-23, and IL-17	Rheumatoid arthritis; Crohn's Disease; psoriasis
IL-1 receptor antagonist (anakinra); soluble IL-1 receptor (rilonacept); anti-IL-1 β (IgG1) (canakinumab); anti-IL-1 β (IgG2) (Xoma 052); anti-IL-1R MoAb IgG1 (AMG 108)	Reducing IL-1 β activities	Autoinflammatory diseases (see Table S1)
Anti- α 4 integrins MoAb (natalizumab); anti-LFA-1 MoAb (efalizumab)	Blocking cell adhesion and migration	Multiple sclerosis; Crohn's Disease; psoriasis
CTLA-4 Ig fusion protein (abatacept)	Blocking T cell coreceptors	Type 1 diabetes; rheumatoid arthritis

Synthesis of COX-2 is absent or low in healthy individuals but is upregulated by proinflammatory cytokines such as IL-1 and TNF- α in response to infection or in inflammatory disease. Specific inhibitors of COX-2 have provided a major advance in the treatment of pain, particularly in patients with osteoarthritis or rheumatoid arthritis. For the most part, COX-2 inhibitors have significantly reduced gastrointestinal side effects compared to COX-1 inhibitors. However, the chronic use of some COX-2-specific inhibitors has been associated with an increase in cardiovascular as well as cerebrovascular events particularly in patients with an elevated risk of thrombosis. This increased risk may be due to the COX-2-mediated reduction in synthesis of prostacyclin, which is a natural inhibitor of platelet activation. In addition to their widespread benefit in arthritis, COX-2-specific inhibitors are used to reduce the development of colon cancer in high-risk patients as adenocarcinoma cells in the colon overexpress COX-2. There is still a need to develop safer, more effective COX-2 inhibitors.

Resolvins

There are several steps in the initial inflammatory cascade triggered by cytokines, including recruitment of myeloid cells (monocytes and neutrophils) into affected tissues (Figure 1). Inflammatory products of arachidonic acid oxidation (omega-6) including inflammatory prostaglandins (PGE2) and lipoxins (LTB4) are released from infiltrating myeloid cells. In contrast, products of eicosapentanoic acid (omega-3) oxidation, PGE3, and LTB5, have anti-inflammatory activities. Products of omega-3 fatty acid oxidation include resolvins of the E series (RvE1 and RvE2) (Serhan and Chiang, 2008), which are found naturally in nearly all inflammatory sites in mammals. The D series of resolvins are derived from docosahexaenoic acid. Specific receptors for resolvins have been identified and when activated are functional in reducing inflammation (Krishnamoorthy et al., 2010). In general, resolvins are part of the anti-inflammatory portfolio that coexists with inflammation. Synthetic forms of RvE1 are currently in clinical trials for treating ocular diseases and other local inflammatory conditions. In animal models of

sterile inflammation, RvE1 suppresses the number of infiltrating neutrophils and macrophages as well as decreasing expression of the genes encoding TNF- α , IL-1 β , and VEGF (Jin et al., 2009). The anti-inflammatory properties of omega-3 fatty acids include suppression of IL-1 β and TNF- α production (Endres et al., 1989), and the mechanism of action of omega-3 fatty acids may include boosting production of resolvins of the D and E series.

Glucocorticoids: Suppressing Cytokine-Driven Inflammation

Glucocorticoids are used widely on a chronic basis to treat most autoimmune diseases. Short-term glucocorticoid treatment is used in gout, and intra-articular injections of glucocorticoids are commonly used to treat painful osteoarthritic joints and tendonitis. Although there are several mechanisms by which glucocorticoids reduce inflammation, a major one may be to reduce expression of cytokine-induced genes. Glucocorticoids enter all cells and bind to the cytoplasmic steroid receptor, and then this complex translocates to the nucleus where it is recognized by specific DNA sequences. The major effect of binding to DNA is the suppression of transcription by opposing the activation of the transcription factors AP-1 and NF- κ B. AP-1 and NF- κ B induce expression of genes encoding nearly all proinflammatory cytokines. Glucocorticoids also suppress expression of inflammatory genes encoding T cell growth factors such as IL-2, IL-4, IL-15, and IL-17 as well as interferon- γ (IFN- γ). In addition, glucocorticoids reduce expression of genes encoding COX-2, inducible nitric oxide synthase, and intracellular adhesion molecule-1 (ICAM-1), which are normally induced by the cytokines IL-1 β and TNF- α . Glucocorticoids increase expression of genes encoding anti-inflammatory molecules, such as the cytokine IL-10 and the IL-1 type 2 decoy receptor.

Biologicals as Anti-inflammatory Agents Anticytokine Therapies

Although cytokines are studied in nearly every biological discipline, cytokine-mediated effects often dominate the fields of inflammation, immunology, atherosclerosis, and degenerative

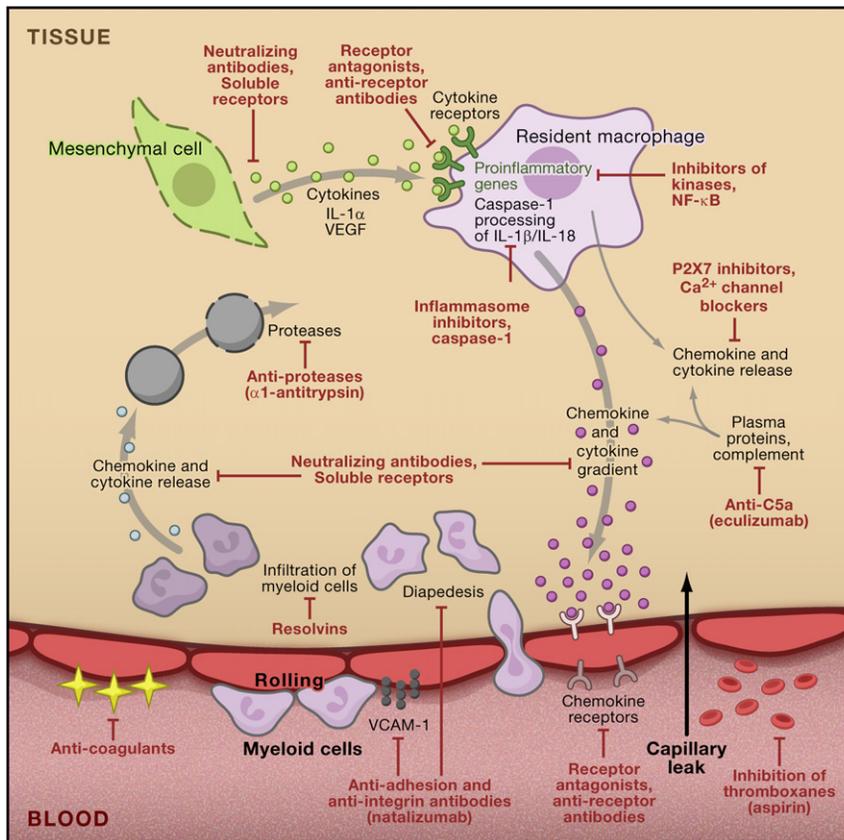


Figure 1. Inflammation and Points of Inhibition by Anti-inflammatory Agents

Shown is the inflammatory pathway associated with ischemia-induced tissue damage and the anti-inflammatory drugs that can be used to block inflammation at various points in the pathway. During ischemia, mesenchymal-derived cells undergo hypoxic damage, lose membrane integrity, and release their cytoplasmic contents. This is followed by the release of biologically active proinflammatory cytokines such as IL-1 and VEGF. These cytokines bind to and activate their cognate receptors on nearby macrophages. The activation of the cytokine receptors triggers tissue-resident macrophages to express an extensive portfolio of proinflammatory genes. Some gene products such as the IL-1 β and IL-18 precursor proteins require processing by caspase-1 and secretion as active cytokines. There is also chemokine production by activated tissue macrophages. A concentration gradient of secreted cytokines and chemokines forms between the macrophage and the endothelium of the microvasculature. Activation of cytokine and chemokine receptors on the endothelium takes place resulting in the opening of endothelial junctions. This leads to a capillary leak and the passage of plasma proteins into the ischemic area. During the ischemic event, there is activation of complement with induction of cytokines and chemokines, as well as a direct effect of C5a on chemoattraction. As part of the cytokine activation of the endothelium, there is expression of the adhesion molecule VCAM-1, which facilitates the rolling of innate immune cells such as monocytes

and neutrophils (myeloid cells) and their adherence to the endothelium. These inflammatory cells then leave the circulation and enter the tissue space by diapedesis. With increasing numbers of infiltrating myeloid cells, the healthy tissue surrounding the ischemic area (called the penumbra) is subjected to damage from the cellular infiltrate due to the release of cytokines and chemokines into the penumbral tissue. Release of damaging proteases from neutrophils contributes to the loss of tissue in the penumbra. Adherence of platelets to the endothelium causes further endothelial activation by increasing expression of receptors for platelets. During the development of an inflammatory infiltrate in the ischemic tissue, a cascade of clotting factors is initiated by cytokine-induced endothelial tissue factor. In addition, the cytokine-activated endothelium releases inhibitors of fibrinolysis. With increased coagulation in the microvasculature and decreased fibrinolysis, tissue perfusion slows and hypoxia and tissue damage worsen.

processes of aging. In addition, cytokine-driven chronic inflammation has been implicated in cancer formation as well as metastasis (see Review by S.I. Grivennikov et al. on page 883 of this issue). Cytokines are secreted by one cell and act on another cell in order to bring about a change in the function of the target cell. In a way, one can consider cytokines as the “hormones” of inflammatory responses, but whereas a hormone is the primary product of a specialized cell, cytokines can be produced by many different cell types including those of the immune system and epithelia. On a molar basis, cytokines are far more potent than hormones. For example, the concentration of IL-1 that induces COX-2 is 10 pM and the concentration of IL-12 that induces IFN- γ is 20 pM. In terms of anticytokine therapies, the amount of a neutralizing antibody or soluble receptor that blocks the activity of a cytokine can be relatively low compared to the amount of antibody needed to kill a microbe.

Blocking the activity of the proinflammatory cytokines IL-1, TNF, IL-6, IL-12, IL-17, IL-18, or IL-23 reduces inflammation and suppresses specific pathways that activate T cells. Blocking IL-32 and IL-33 may also be useful for treating inflammation as

well as allergy. Chemokines are also cytokines, and small-molecule chemokine receptor antagonists have been used to treat Crohn’s disease, an autoimmune inflammatory bowel disease (Proudfoot et al., 2010). Chemokines drive the migration of immune cells but they also affect angiogenesis and the activity of myeloid cells.

Anticytokine Therapies and Host Defense

The conundrum in considering anticytokine therapies for treating chronic diseases is that cytokines evolved many millions of years ago and provided a survival benefit for the host through what is now termed the “innate immune response.” The innate immune response is less specific than the adaptive immune response and is the first line of defense against infection or injury. It is characterized by an inflammatory response involving infiltration of neutrophils in response to cytokines resulting in phagocytosis and intracellular killing of the pathogens and the control of infection. In most cases, even without antibiotics, the inflammation subsides once the infection is eliminated, and there is little or no damage to the host. In fact, cytokines produced during inflammation also assist in the repair process after injury.

However, the same cytokines that orchestrate the infiltration of neutrophils acutely in order to fight infection are responsible for tissue remodeling and organ damage when produced chronically. Chronic inflammation destroys the joints, the ability of lung tissue to exchange gases, the patency of blood vessels, the intestinal barrier, and the myelin sheath that insulates nerve fibers in the brain and spinal cord. Hence, even though their primary function is to protect the host when challenged and to repair tissue when injured, these cytokines are mediators of disease and thus are targets for anticytokine therapy. Another paradox is provided by IFN- γ , which is essential for defense against intracellular microorganisms such as *Mycobacterium tuberculosis*, which causes tuberculosis. IFN- γ is also a major cytokine in the pathogenesis of several autoimmune diseases including multiple sclerosis, psoriasis, and lupus.

Blocking cytokines may reduce inflammation but also renders the host susceptible to infection and maybe even cancer. Anti-TNF- α treatment increases opportunistic infections (reviewed in Dinarello, 2005). On the other hand, anticytokine therapy has little or no organ toxicity or gastrointestinal disturbances and so is well tolerated.

Biologicals for Treating Autoimmune Diseases Autoimmune versus Autoinflammatory Diseases

Some chronic inflammatory diseases are autoimmune, whereas others are “autoinflammatory.” In autoimmune diseases, the T cell dominates as the primary dysfunctional cell or initiator of the disease process. A cluster of cytokines such as TNF- α , IFN- γ , IL-2, IL-12, IL-23, and IL-17 participate in maintaining autoreactive T cells. Rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, psoriasis, lupus, and multiple sclerosis are examples of autoimmune diseases in which the inflammation is secondary to a disease process that is driven by autoreactive T cells (See Essay by L.A. Zenewicz et al., in this issue). In contrast, autoinflammatory diseases are not mediated by the adaptive immune system and do not involve T cells but rather are caused by dysfunctional macrophages (see Essay by D.L. Kastner et al. in this issue). The mechanism of autoinflammatory disease appears to be due to increased secretion of IL-1 β , and treatment is uniquely based on reducing the activity of IL-1 β .

Most autoimmune diseases can be treated with any one of a number of “biologicals” (Table 1). The best example is rheumatoid arthritis where neutralizing TNF- α , blocking IL-6 receptors, depleting B lymphocytes, or preventing T cell costimulation are all effective therapeutic approaches. Even blocking IL-1 receptors or neutralizing IL-1 β is effective for treating rheumatoid arthritis, but this effect is due to protection of bone and cartilage rather than reduction of T cell activation. Thus, preventing the activity of only one cytokine or reducing the effect of one pathway can be effective in treating autoimmune diseases. Other examples include psoriasis and Crohn’s disease, which can be effectively treated by blocking IL-12, IL-23, or TNF- α or by using a humanized monoclonal antibody to block $\alpha 4$ integrins. For example, a monoclonal antibody directed against $\alpha 4$ integrins (natalizumab) prevents the migration of immune cells into tissues. One goal in treating autoimmune disease is to reduce the infiltration of autoreactive T cells into the affected tissue.

Another is the depletion of B cells using anti-CD20 monoclonal antibodies such as rituximab, ocrelizumab, or ofatumumab. Biologicals offer several options for treating autoimmune diseases. Although the field of biologicals began with the use of anti-TNF- α monoclonal antibodies to treat Crohn’s Disease, it has expanded considerably. Antibodies to IL-12, IL-17, IL-23, IL-1 β , and the IL-6 receptor have been approved, and antibodies to IL-23 and IL-17 are in clinical trials. Each “biological” can be effective in the treatment of more than one autoimmune disease, suggesting that there is considerable overlap in cytokine functions in inflammation. The success of a “biological” is best observed in patients with poorly controlled disease who are receiving the standard of therapy for that disease, for example rheumatoid arthritis. Adding a biological to standard therapy often decreases disease severity. The physician has an increasing list of biologicals from which to choose: anti-TNF- α antibodies or soluble TNF receptors to neutralize TNF- α , anti-IL-6 receptor antibodies to block IL-6, CTLA-4-Ig to block the binding of costimulatory molecules, rituximab (an anti-CD20 monoclonal antibody) to deplete B cells, neutralizing antibodies to block IL-12 and IL-23 activity, or natalizumab to reduce lymphocyte trafficking. Although the degree of benefit will differ depending on the patient, most experience improved control of their disease.

Why Just One?

Despite the multiple cellular and cytokine-mediated mechanisms for sustaining autoimmune diseases, blocking just one cytokine can be sufficient to bring the disease under control. Why is this? There is evidence that cytokines exist in “cascades” and that interrupting one cytokine interrupts the cascade. For example, blocking TNF- α reduces the activity of IL-6 and IL-1 β (Dinarello et al., 1986; Fong et al., 1989), blocking IL-1 β reduces IL-6 (Fitzgerald et al., 2005; Goldbach-Mansky et al., 2006; Hoffman et al., 2004; Pascual et al., 2005), and blocking IL-12 and IL-23 reduces IFN- γ . B lymphocytes make TNF- α and IL-1 β , and this may account for the beneficial effects of depleting B cells with monoclonal antibodies. IL-12 and IL-23 sustain production of IL-17, which may explain the success of using IL-12 and IL-23 inhibitors to treat autoimmune diseases.

There is also ample evidence that cytokines act in a synergistic rather than additive fashion. For example, synergistic cytokine pairs include IL-1 and TNF- α , TNF- α and IFN- γ , and IL-1 β and IL-6 (reviewed in Dinarello, 1996, 2009a). Thus, blocking one cytokine interrupts this synergy and reduces disease severity. Part of the success of TNF- α inhibitors for treating rheumatoid arthritis, Crohn’s disease, and psoriasis is the loss of TNF- α -bearing T cells. TNF- α is located on the T cell surface and anti-TNF- α monoclonal antibodies of the IgG1 class crosslink membrane TNF- α and induce death of T cells (reviewed in Dinarello, 2005).

Autoimmune Disease and B Cell Depletion

Rituximab and other monoclonal antibodies that target and deplete CD20-positive B cells have an unexpected benefit in treating rheumatoid arthritis, psoriasis, Crohn’s disease, multiple sclerosis, and type 1 diabetes (Table 1). Although depletion of B cells reduces immunoglobulin levels, this is unlikely to explain the efficacy of B cell depletion for treating these autoimmune diseases. B cells contribute to antigen presentation and thus

help in T cell activation. Also, increasing evidence links the pathogenesis of most autoimmune diseases with T cells producing IL-17. IL-17 is a proinflammatory cytokine that induces chemokine production and promotes infiltration of neutrophils and macrophages. There was a marked reduction in IL-17 produced by synovial cells from the joints of rheumatoid arthritis patients treated with rituximab (van de Veerdonk et al., 2009b). In peripheral blood mononuclear cells, the presence of rituximab reduced the levels of IL-17 as well as the number of T cells producing the cytokine.

Side Effects of Biologicals

The major side effect of biologicals is a reduction in host defense against infections. When detected early, these infections can be effectively treated with antibiotics. However, three biologicals used to treat autoimmune diseases have resulted in cases of progressive multifocal leukoencephalopathy (PML). PML is a rapidly demyelinating and potentially fatal disease that is caused by a virus and is often observed in patients treated with immunosuppressive drugs or in patients with AIDS. PML has been associated with patients with multiple sclerosis or Crohn's disease treated with the monoclonal antibody natalizumab (Major, 2009). PML also develops in patients treated with the B cell-depleting antibody rituximab and in psoriasis patients treated with the monoclonal antibody efalizumab. Natalizumab and efalizumab prevent the migration of T cells into tissues, whereas rituximab lyses CD20-bearing B cells and does not affect T cell migration. It is not clear why natalizumab, rituximab, or efalizumab cause PML.

Biologicals for Treating Autoinflammatory Diseases

Autoinflammatory diseases are chronic inflammatory conditions characterized by macrophage dysfunction and local as well as systemic inflammation (see Essay by D.L. Kastner et al. in this issue; Table S1 available online). Autoinflammatory diseases are noninfectious but can be exacerbated by infection. Recurrent fevers are common, with painful joints and muscles and elevated blood neutrophil counts. By inhibiting only IL-1 β , these diseases rapidly are brought under control; neutralization of TNF- α has little or no effect. Some of these debilitating diseases are due to gain-of-function mutations that affect the activation of caspase-1, which leads to increased processing of the inactive IL-1 β precursor and release of active IL-1 β (reviewed in Masters et al., 2009). Caspase-1 activation is initiated by the "inflammasome," a multiprotein intracellular complex (see Review by K. Schroder and J. Tschopp on page 821 of this issue). The first mutation affecting the processing of IL-1 β was discovered in the nucleotide-binding domain and leucine-rich repeat containing protein 3 (NLRP3), a component of the inflammasome (Hoffman et al., 2001). Mutations associated with the classic autoinflammatory disease familial Mediterranean fever also increase the secretion of IL-1 β (Masters et al., 2009). Another classic autoinflammatory disease is hyper-IgD (Drenth et al., 1999). Mutations in the gene encoding mevalonate kinase cause hyper-IgD and also periodic fever syndrome (International Hyper-IgD Study Group). In each of these diseases, IL-1 β can stimulate its own production, which plays a pivotal role in autoinflammatory disease pathogenesis (Dinarello et al., 1987; Gattorno et al., 2007).

Is Type 2 Diabetes an Autoinflammatory Disease?

Type 1 diabetes is a classic autoimmune disease in which autoreactive T cells attack the insulin-producing β cells of the pancreas. On the other hand, in type 2 diabetes, there is no T cell involvement but rather a chronic state of inflammation in which IL-1 β is produced and kills the β cells. Thus, type 2 diabetes falls into the autoinflammatory class of diseases, and the beneficial effects of IL-1 β blockade in patients with type 2 diabetes supports that concept. IL-1 β is also cytotoxic for β islet cells in autoimmune type 1 diabetes (Mandrup-Poulsen et al., 1986), but in type 2 diabetes high levels of glucose can stimulate IL-1 β production by the β islet cell itself (Maedler et al., 2002). IL-1 β is also produced by fat cells, an additional source of IL-1 β in type 2 diabetes. In general, the loss of β cell mass in the pancreas progresses over several years during which time patients are in a "pre-diabetic" state. It is therefore possible to "salvage" β islet cells by reducing IL-1 β -mediated inflammation.

Proof of a specific role for IL-1 β in the pathogenesis of type 2 diabetes was demonstrated in patients in a 13 week randomized, placebo-controlled study using the IL-1 receptor antagonist (IL-1Ra), also called anakinra, to block IL-1 β . There was a statistically and clinically significant improvement in insulin production and glycemic control (Larsen et al., 2007a). The clinical response to anakinra was linked to variants in the gene encoding IL-1Ra that result in low circulating levels of IL-1Ra (Larsen et al., 2009), raising the possibility that insufficient IL-1Ra contributes to inflammation in type 2 diabetes. In the 39 weeks after anakinra treatment, patients who responded to anakinra used 66% less insulin to obtain the same level of glycemic control. This observation suggests that blocking IL-1 β even for a short period restores the function of pancreatic β cells or possibly allows for some regeneration of β cells. Several trials blocking TNF- α in type 2 diabetes have succeeded in reducing C-reactive protein but did not improve glycemic control. The anakinra trial observations have been confirmed using a specific neutralizing monoclonal antibody to IL-1 β (Donath et al., 2009). These studies provide clinical evidence that type 2 diabetes is a chronic autoinflammatory disease in which IL-1 β -mediated inflammation progressively destroys the insulin-producing pancreatic β cells.

In diabetic mice, administration of a caspase-1 inhibitor reduces insulin resistance; in mice deficient in caspase-1, there is improved sensitivity to insulin (Stienstra et al., 2009). In addition, the thioredoxin-interacting protein (TXNIP) binds to NLRP3, which activates caspase-1 and the subsequent processing and secretion of active IL-1 β (Zhou et al., 2010). TXNIP-deficient mice exhibit improved glucose tolerance and reduced insulin resistance (Zhou et al., 2010). There are several studies linking TXNIP expression to type 2 diabetes and glucose regulation. Thus, it appears that in insulin-secreting pancreatic β cells, glucose-stimulated IL-1 β activity is caspase-1 dependent; production of IL-1 β by adipocytes is also caspase-1 dependent (Stienstra et al., 2009).

IL-1 Blockade in Stroke and Heart Disease

IL-1 receptor blockade in stroke patients results in a decrease in circulating neutrophils and IL-6 levels, which is often associated with a reduction in IL-1 β activity. Moreover, the severity of neurological impairment appears to decrease in stroke patients

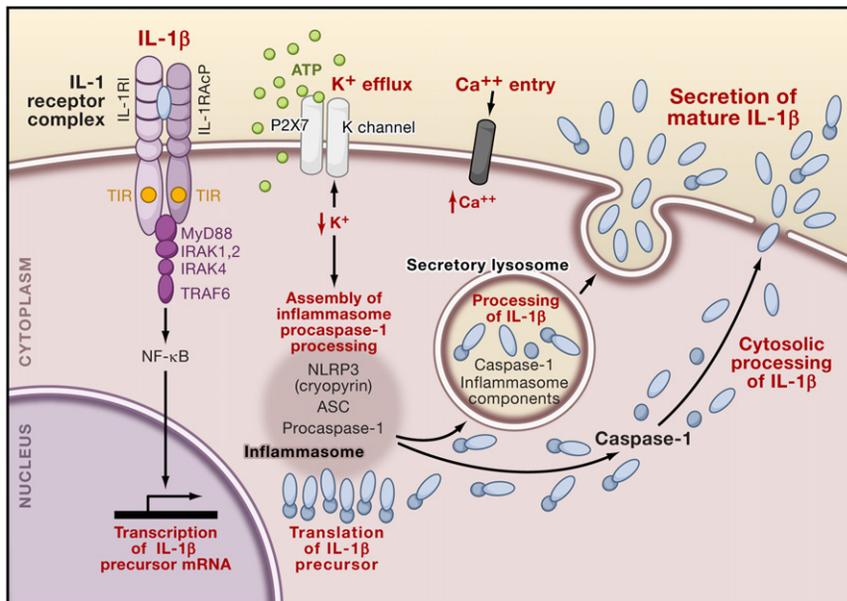


Figure 2. Production and Release of IL-1 β

(Top left) Primary blood monocytes, tissue macrophages, or dendritic cells are activated by the cytokine IL-1 β (blue oval) resulting in the formation of the IL-1 receptor complex (composed of IL-1RI and IL-1RAcP). The intracellular TIR domains of the IL-1 receptor complex recruit MyD88 and other signaling components leading to translocation of the transcription factor NF- κ B into the nucleus. This results in transcription of the gene encoding the precursor of IL-1 β and its translation into protein (blue dumb-bell) (Bocker et al., 2001). ATP released from activated monocytes (or from dying cells) accumulates outside the cell. As the extracellular levels of ATP increase, the P2X7 receptor is activated, triggering the efflux of potassium ions from the cell. Low intracellular potassium ion levels enable the components of the caspase-1 inflammasome to assemble. Assembly results in the conversion of procaspase-1 to active caspase-1. There is evidence that the components of the inflammasome localize to secretory lysosomes together with the IL-1 β precursor protein and lysosomal enzymes (Andrei et al., 2004). In the secretory lysosome, active caspase-1 cleaves the IL-1 β precursor, generating active mature IL-1 β . Mature IL-1 β is released along with the IL-1 β precursor and the contents of the secretory lysosomes (Andrei et al., 2004). There is evidence that secretion of active IL-1 β requires an increase in intracellular calcium ion levels (Kahlenberg and Dubyak, 2004). Processing of the IL-1 β precursor can also take place in the cytosol independently of caspase-1 and the inflammasome (Brough and Rothwell, 2007). Studies have also shown that Rab39a, a member of the GTPase family, contributes to the secretion of mature IL-1 β by helping IL-1 β traffic from the cytosol into the vesicular compartment (Becker et al., 2009) from where it is secreted by exocytosis (Qu et al., 2007) (not shown). Mature IL-1 β can also exit the cell through a loss in membrane integrity (Laliberte et al., 1999) or exocytosis via small vesicles (MacKenzie et al., 2001).

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treated with IL-1 receptor blockade compared to placebo-treated controls (Emsley et al., 2005). Does IL-1 contribute to heart failure after myocardial infarction? Animal studies show that reducing IL-1 activity prevents post-infarction myocardial remodeling (Abbate et al., 2008). In a placebo-controlled trial of anakinra in patients with myocardial infarction, daily anakinra treatment was added to standard therapy the day after angioplasty and continued for 14 days. Three months later, there was a statistically significant difference in the left ventricular end-systolic volume index (a surrogate marker of damage severity in myocardial infarction) in patients treated with anakinra compared to patients given a placebo (Abbate et al., 2010). The mechanism for the beneficial effect of the 14 day course of therapy is likely to be due to a reduction in the myocardial remodeling that takes place after loss of viable heart muscle, and that results in heart failure. The heart seems to be exquisitely sensitive to IL-1 blockade as the amount of anakinra used to treat the myocardial infarction patients was the same as that used in rheumatoid arthritis, that is, 100 mg subcutaneously each day. This dose results in a brief (4 hr) rise in blood levels not more than 1.0 μ g/ml.

Agents for Blocking IL-1 Activity

Specific monoclonal antibodies, such as canakinumab and Xoma 052, are the best agents for blocking IL-1 β activity. Canakinumab is approved for the treatment of a group of autoinflammatory diseases now termed cryopyrin-associated periodic syndromes (CAPS) that includes familial cold autoinflammatory syndrome, Muckle-Wells syndrome, and Neonatal Onset Multi-inflammatory Disease (see Essay by D.L. Kastner et al. in this issue; Lachmann et al., 2009). Both canakinumab and Xoma

052 are in clinical trials for treating type 2 (and type 1) diabetes, gout, Behcet's disease, and other autoinflammatory diseases. Riloncept is a construct of the two extracellular domains of the IL-1 receptor complex and binds to both IL-1 α and IL-1 β . Riloncept has been approved for the treatment of familial cold autoinflammatory syndrome (Hoffman et al., 2008) and has been used to treat gout. The extracellular domains of IL-1 receptor type II preferentially bind to IL-1 β . However, for optimal neutralization, the extracellular domain of IL-1 receptor accessory protein is required and, in clinical testing, the type II receptor was not effective. The extracellular domains of IL-1 receptor type I, which preferentially bind to IL-1Ra and IL-1 α , were not effective in patients.

Is it possible to specifically inhibit IL-1 activity with orally active agents? Currently, there are no orally active agents that prevent the binding of IL-1 to its receptor. Nevertheless, orally active agents that target the processing and secretion of IL-1 β have been developed. For IL-1 β to be released from the cell as an active cytokine, several steps are necessary (Figure 2). The purinergic receptor P2X7 is activated by extracellular ATP and appears to be a critical early step in the release of active IL-1 β (Netea et al., 2009; Perregaux et al., 2000). Once the purinergic receptor P2X7 is activated, there is a rapid efflux of potassium ions from the cell, subsequent oligomerization of the inflammasome, activation of caspase-1, cleavage of the IL-1 β precursor, and release of active IL-1 β via secretory lysosomes. Orally active, specific inhibitors of the P2X7 receptor reduce disease in several models of inflammation and are being tested in humans with rheumatoid arthritis. However, the P2X7 receptor serves other cellular functions, and it is unclear to what extent

inhibition of this receptor contributes to the amelioration of inflammation. Moreover, sites for caspase-1 activity are present in four members of the IL-1 family: IL-1 β , IL-18, IL-33, and IL-1F7. In the case of IL-33, inhibition of caspase-1 decreases the intracellular destruction of the IL-33 precursor. Orally active caspase-1 inhibitors also reduce inflammation in several animal models of disease and are effective in humans with autoinflammatory diseases (Stack et al., 2005). However, similar to inhibition of the P2X7 receptor, specific inhibition of caspase-1 will also impact processing of other IL-1 family members. Moreover, there are animal models of IL-1 β -mediated disease that are independent of caspase-1 processing of the IL-1 β precursor.

Although the oligomerization of inflammasome components initiates the conversion of pro-caspase-1 into the active enzyme for processing the IL-1 β precursor, there are ample examples of caspase-1-independent activities of IL-1 β . For example, the inflammation induced by muscle injury results in a large influx of neutrophils, tissue damage, and high IL-6 levels. This response is absent in mice deficient in IL-1 β but fully present in mice deficient in caspase-1 (Fantuzzi et al., 1997). Intra-articular injection of cell wall fragments derived from *Streptococcus pyogenes* into mice resulted in joint inflammation, cartilage destruction, and a robust infiltration of neutrophils. However, caspase-1-deficient mice exhibited the same response (Joosten et al., 2009). Similarly, injection of urate crystals intraperitoneally into mice induced a brisk peritonitis in mice with or without caspase-1 (Guma et al., 2009). These findings are important for the pathogenesis of autoinflammatory diseases as non-caspase-1 processing of IL-1 β in the extracellular compartment accounts for IL-1 β -mediated inflammation under conditions involving elevated neutrophil activation. Neutrophil enzymes that can process the IL-1 β precursor include elastase, proteinase-3, chymases, granzyme A, and cathepsin G (Fantuzzi et al., 1997; Guma et al., 2009; Joosten et al., 2009). Proteinase-3 cleaves the IL-1 β precursor into an active molecule (Coeshott et al., 1999; Joosten et al., 2009) (see Figure 1). Alpha-1 antitrypsin is an endogenous inhibitor of elastase and proteinase-3 and exhibits a broad range of anti-inflammatory properties (Lewis et al., 2008).

Natural Inhibition of Cytokines

Some cytokines function as natural inhibitors of inflammation, but are they useful therapeutically? Rabbits passively immunized against their own endogenous IL-1 receptor antagonist (IL-1Ra) experience severe colitis (Ferretti et al., 1994), and mice deficient in IL-1Ra develop diseases such as destructive arthritis (Horai et al., 2000), arteritis (Nicklin et al., 2000), and a psoriasis-like skin disorder. Furthermore, mice deficient in endogenous IL-1Ra develop aggressive tumors after exposure to carcinogens (Krelin et al., 2007). These data support the concept that relative amounts of IL-1 versus IL-1Ra affect the severity of some diseases. A single-nucleotide polymorphism (SNP) is associated with lower circulating levels of IL-1Ra (Larsen et al., 2009; Rafiq et al., 2007) and a concomitant increase in type 2 diabetes (Larsen et al., 2009). The same polymorphism is associated with reduced survival in patients with colon carcinoma compared to those with the wild-type allele (Graziano et al., 2009).

The notion of an imbalance between IL-1 β and IL-1Ra has gained considerable legitimacy with reports of infants born

with a genetic abnormality that results in nonfunctional IL-1Ra (Aksentijevich et al., 2009; Reddy et al., 2009). Soon after birth, the affected infants exhibit impressive local and systemic inflammation. Unless treated with exogenous IL-1Ra, the infants die. The infants suffer from multiple neutrophil-laden skin eruptions, vasculitis, bone abnormalities with large numbers of osteoclasts, osteolytic lesions, and sterile osteomyelitis. The inflammation resembles an infection with sepsis-like multiorgan failure, but all cultures were sterile for microbes. When treated with daily IL-1Ra (anakinra), the inflammation abated and the bone lesions were reversed. The infants are an extreme example of what happens without functional IL-1Ra: the activity of endogenous IL-1 is “unopposed” and IL-1-driven inflammation runs rampant. From these studies, one can propose that the outcome of inflammation is the balance of proinflammatory versus anti-inflammatory cytokines (Dinarello, 2009b).

Systemic Inflammation

Sepsis is an example of severe systemic inflammation and falls into the autoinflammatory class of diseases as macrophages but not T cells play a dominant role. The first studies that blocked cytokine activity in humans were in patients with sepsis. The clinical trials of IL-1 or TNF- α blockade were based on animal models of systemic inflammation caused by lethal endotoxin or live infection. The data from the animal studies were impressive: blocking either IL-1 or TNF- α reduced mortality. Hence, it seemed plausible that patients with sepsis could be treated by reducing IL-1 or TNF- α activity. Despite sophisticated intensive care, there are more than 500,000 deaths from sepsis annually in the US. Consequently, millions of dollars have been invested in the development of IL-1 and TNF- α blocking agents, and these have been tested in placebo-controlled clinical trials in over 12,000 patients. Only marginal reductions in 28 day mortality were achieved, insufficient to gain regulatory approval. A meta-analysis of the clinical trial data concluded that a survival benefit of blocking IL-1 or TNF- α was only observed in those patients most likely to die (Eichacker et al., 2002).

Other anticytokine therapies such as blocking IL-4 or IL-5 in the treatment of asthma were based on well-established animal models of airway antigen challenge, yet the data in several placebo-controlled trials did not show sufficient efficacy to warrant moving forward with these cytokine blockers. However, in autoimmune diseases and autoinflammatory diseases, blocking cytokines has proven consistently beneficial. The same IL-1 receptor antagonist and the same monoclonal antibodies to TNF- α or soluble TNF receptors that failed in clinical trials for sepsis have been approved for the treatment of rheumatoid arthritis, Crohn's disease, plaque psoriasis, and a spectrum of autoinflammatory diseases.

Protease Inhibitors

Proteases play an important role in initiating as well as sustaining inflammation. Many studies have focused on blocking matrix metalloproteases in order to stabilize fragile plaques in arteries or reduce the loss of cartilage in arthritic conditions. Posttranslational cleavage of the N-terminal amino acids of certain chemokines (CCL3, CCL5, and CCL23, for example) by proteases increases their potency (some by over 100-fold). Inhibition of proteinase-activated receptor 2 (PAR-2) reduces inflammation

in mouse models of rheumatoid arthritis. Inhibition of PAR-2 also reduces the destruction of chondrocytes in cartilage by IL-1 β . Antibodies to complement factor 5 (C5) prevent its protease-mediated conversion to C5a, the terminal component in complement-mediated inflammation. Thus, inhibition of proteases is a valid approach to controlling inflammation.

There are several serum protease inhibitors to combat protease-mediated inflammation. The serum protein α -1 antitrypsin inhibits serine proteases including neutrophil elastase and also proteinase-3. Activation of complement is also inhibited by α -1 antitrypsin. Humans born with a genetic deficiency in α -1 antitrypsin develop pulmonary emphysema early in life, possibly due to progressive destruction of lung alveoli due to insufficient inhibition of neutrophil-derived elastase by α -1 antitrypsin. Although α -1 antitrypsin inhibits neutrophil-derived elastase *in vitro*, it also blocks endotoxin-stimulated IL-1 β and TNF- α production by human blood monocytes and reduces translocation of the transcription factor NF- κ B to the nucleus in response to cytokine stimulation. Whole-blood cytokine production in patients lacking α -1 antitrypsin is elevated but decreases following an infusion of exogenous α -1 antitrypsin (Pott et al., 2009). Administration of exogenous α -1 antitrypsin to mice protects against allograft rejection (Lewis et al., 2008) and blocks IL-1 β -induced death of insulin-producing pancreatic β cells (Lewis et al., 2008); α -1 antitrypsin also prevents HIV-1 entry into host cells *in vitro* (Shapiro et al., 2001; Munch et al., 2007).

Orally Active Anti-inflammatories

Several orally active, disease-modifying drugs used to treat autoimmune diseases—methotrexate, cyclosporine, tacrolimus, and rapamycin—also possess anti-inflammatory properties. The most widely used of these is methotrexate, which is primarily an antiproliferative agent due to its ability to inhibit dihydrofolate reductase. However, methotrexate also decreases production of TNF- α and chemokines *in vivo*. The antiangiogenic properties of methotrexate may also contribute to its anti-inflammatory profile. Cyclosporine, tacrolimus, and rapamycin are immunosuppressive agents, which are used broadly in many clinical situations from preventing allograft rejection to treating psoriasis and controlling vasculitis. Their primary mechanism of action is as inhibitors of IL-2 and IFN- γ production, which results in lymphocytes producing less TNF- α . Reducing inflammation is part of their effectiveness but is likely to be an indirect effect.

Colchicine, which binds to tubulin and blocks microtubule formation, remains the standard of therapy for preventing attacks of urate crystal arthritis (gout) and for treating the autoimmune disease familial Mediterranean fever (see Essay by D.L. Kastner et al. in this issue). Colchicine is also used to treat a variety of chronic inflammatory diseases including autoimmune biliary cirrhosis. The mechanism of action of colchicine is to prevent the infiltration of neutrophils into inflamed tissues. Colchicine also inhibits the mobility of monocytes and macrophages, and colchicine therapy in patients with familial Mediterranean fever has dramatically reduced the development of life-threatening amyloidosis. Both familial Mediterranean fever and gouty arthritis are IL-1 β -mediated diseases. For example, the IL-1 receptor antagonist (anakinra) as well as monoclonal antibodies to IL-1 β (canakinumab) will reduce the pain and inflam-

mation of gouty arthritis. Similarly, in familial Mediterranean fever and Behcet's disease, blocking IL-1 β results in a rapid and sustained remission of inflammation.

In 1991, Gilla Kaplan reported that clinically achievable levels of thalidomide inhibited endotoxin-induced TNF- α in human blood monocytes (Sampaio et al., 1991). Thalidomide, orally active and structurally related to glutamic acid, had caused limb malformations in children born of mothers who used the drug to combat nausea during pregnancy. Thalidomide was approved to treat patients with leprosy and later, due to its antiangiogenic properties, was used widely to treat patients with multiple myeloma. Its use in treating various autoimmune diseases is based on its immunosuppressive effects and ability to affect the production as well as the activities of several cytokines including IL-1, IL-2, IL-6, IL-12, IL-23, TNF- α , and IFN- γ . For example, a beneficial effect of thalidomide in Crohn's disease patients is thought to be due to reduced TNF- α and IL-23 production. However, peripheral neuropathy and other adverse effects are a major problem with thalidomide. New analogs of this drug such as α -fluoro-4-aminothalidomide exhibit increased potency in blocking cytokine production. Analogs increase cAMP levels in macrophages by inhibiting phosphodiesterase-4, which results in reduced TNF- α production. The anti-inflammatory cytokine IL-10 is elevated in response to thalidomide analogs (Thiele et al., 2002). The thalidomide analog lenalidomide has been approved in the US and Europe in combination with dexamethasone as maintenance therapy for multiple myeloma (Falco et al., 2008). Although lenalidomide has reduced neurotoxicity, a hypercoagulation state has been observed in clinical trials. In controlled studies, lenalidomide failed to induce remission or be effective for treating Crohn's disease. Nevertheless, lenalidomide's clinical benefit in multiple myeloma is due, in part, to its anticytokine and antiangiogenic properties, and it continues to be evaluated in the treatment of several chronic autoimmune and autoinflammatory diseases.

Small-Molecule Inhibitors of Signal Transduction

It has been estimated that there are over 300 kinases that phosphorylate intracellular proteins, including autophosphorylation of kinases themselves. A key kinase of interest is the p38 mitogen-activated protein kinase (MAPK), which regulates several fundamental mediators of inflammation including cytokines, chemokines, COX-2, and nitric oxide synthase. The discovery of p38 MAPK began with a screen for small molecules that inhibited endotoxin-induced IL-1 β production as part of a program to develop orally active drugs for treating rheumatoid arthritis. Scientists at SmithKlineBeecham found that one of the compounds (SB20358) reduced IL-1 β production and also reduced arthritis in mice. In order to ascertain the mechanism of action of SB20358, they radiolabeled the compound, added it to cell lysates, and isolated an intracellular protein that was closely related to HOG-1 (high osmotic glucose-1), which is found in yeast and enables growth in high sugar concentrations (Kumar et al., 1995). The induction of cytokines and chemokines by immune cells treated with hyperosmotic concentrations of sodium chloride is dependent on p38 MAPK (Shapiro and Dinarello, 1995).

After the discovery of p38 MAPK in the drug screen, the next step was to develop small-molecule inhibitors against the

several isoforms of p38 MAPK. In general, specific inhibitors of p38 MAPK do not affect the downstream signaling component c-Jun NH2-terminal kinase (JNK). Inhibitors of the p38 MAPK α isoform reduce the production of IL-1 β and TNF- α in endotoxin-stimulated human monocytes, whereas the β , γ , and δ isoform inhibitors are less effective. Most animal models for sepsis, type 1 diabetes, rheumatoid arthritis, lupus, multiple sclerosis, and inflammatory bowel disease exhibit marked improvement when treated with pan-p38 MAPK inhibitors. The most studied inhibitor of p38 MAPK is SB20358, which inhibits phosphorylation of its substrate ATF-2.

Orally active p38 MAPK inhibitors are well tolerated in phase I clinical trials in healthy subjects, but hepatic toxicity is observed with increasing doses. Clinical targets for orally active inhibitors of p38 MAPK have focused on rheumatoid arthritis with limited success. Although there is improvement in clinical scores in rheumatoid arthritis patients treated with most p38 MAPK inhibitors, the window of efficacy and unwanted toxicity appears to be narrow and some clinical trials have been halted. The development of new p38 MAPK inhibitors for clinical testing continues because the kinase plays such a central role in many inflammatory processes (Marin et al., 2001).

Tyrosine kinases such as the Janus kinases (Jak) play a key role in many proliferative diseases including leukemias and are part of the angiogenic process in solid tumors. In rheumatoid arthritis, the synovial membrane, usually a single-cell layer in healthy subjects, proliferates and becomes vascularized. Like a tumor, the proliferating synovium (pannus) invades and destroys the surrounding joint. Jak3 is a tyrosine kinase that drives the proliferation and differentiation of lymphocytes. From *in vitro* studies, inhibition of the Jak signaling pathway appears to be a strategy in treating rheumatoid arthritis and possibly other chronic inflammatory conditions. Clinical trials of the orally active Jak inhibitor CP-690550 in patients with rheumatoid arthritis, renal allografts, or psoriasis showed a reduction in inflammation markers and improved quality of life for these patients. Mechanisms of action include a reduction in dendritic cell activation and proinflammatory cytokine production as well as increased production of the anti-inflammatory cytokine IL-10. A related Jak inhibitor reduced IL-17 and IFN- γ production by peripheral blood mononuclear cells. At higher doses, Jak inhibitors are associated with increased serum creatinine levels, indicating possible renal toxicity. An increase in infections, nausea, abdominal pain, diarrhea, and dyspepsia have been reported as the dose increases.

Spleen tyrosine kinase (Syk) participates in intracellular signaling in B cells as well as in cells expressing Fc- γ receptors. Fc- γ receptors are activated by IgG1 and are expressed by monocytes, macrophages, neutrophils, and mast cells. A specific inhibitor of Syk reduced production of IL-1 β , TNF- α , IL-6, and IL-18. There are controversial data on whether Syk inhibits IL-1 β via the inflammasome (Gross et al., 2009; van de Veerdonk et al., 2009a). In a mouse model of rheumatoid arthritis (collagen-induced arthritis), Syk inhibition reduced bone erosion, pannus formation, and synovitis. In a blinded, placebo-controlled clinical study in patients with rheumatoid arthritis, who were unresponsive to methotrexate, there was a rapid and significant improvement in all parameters of disease severity. Matrix metalloprotei-

nase 3 and serum IL-6 levels decreased within the first week. However, side effects included gastrointestinal disturbances and neutropenia (Weinblatt et al., 2008). Inhibition of Syk may be a valid strategy for reducing inflammation in autoimmune diseases.

Imatinib (Gleevec) is a tyrosine kinase inhibitor that is used clinically to block the kinase activity of the BCR-ABL oncoprotein in patients with chronic myeloid leukemia. Imatinib is also effective in ameliorating disease in a mouse model of rheumatoid arthritis and in reducing TNF- α production by synovial fluid mononuclear cells from patients with rheumatoid arthritis (Paniagua et al., 2006). This kinase inhibitor reduced TNF- α production by human blood monocytes in response to lipopolysaccharide but did not suppress production of the anti-inflammatory cytokine IL-10. In murine models of acute hepatitis, imatinib prevented TNF- α -dependent inflammatory damage to the liver induced by injecting concanavalin A (Wolf et al., 2005). These findings suggest that imatinib is able to act as an anti-inflammatory because it reduces TNF- α production. It remains unclear whether these observations will be of therapeutic value for treating TNF- α -mediated diseases.

Statins as Anti-inflammatory Agents

Mevalonate is the substrate for the synthesis of cholesterol as well as several biologically active sterols. The generation of mevalonate requires the reduction of the enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) by HMG-CoA reductase. Synthetic small-molecule inhibitors of the reductase are called "statins." Statins were developed to lower the endogenous synthesis of cholesterol in patients at high risk for myocardial infarction in whom elevated levels of low-density lipoproteins (LDL) were difficult to control. Initial epidemiological studies showed a highly significant reduction in cardiovascular events with statin treatment, and the number of high-risk patients being treated with statins increased rapidly. Several analogs of statins that also inhibit HMG-CoA reductase entered clinical trials; it became clear that all statins reduced LDL levels to the same extent but that the decrease in cardiovascular events was greater for some statins than for others. An analysis of the Framingham cohort (a long-term prospective study of the health of residents of Framingham, MA, USA) for serum levels of C-reactive protein (CRP) provided a value for predicting cardiovascular risk, although the association of elevated CRP with inflammation in coronary artery disease was not new (Liuzzo et al., 1994).

A large, placebo-controlled trial of nearly 18,000 patients evaluated the effect of rosuvastatin for cardiovascular events in patients with normal LDL-cholesterol levels (less than 130 mg/dL). After 1.9 years, CRP decreased by 37% in the treatment group and there were significantly fewer (range of 48%–55%) cardiovascular events including stroke. Death from any cause was 20% lower compared to the placebo group (Ridker et al., 2008). The study known as the JUPITER trial raised the epidemiological issue of the benefit of statins in apparently healthy subjects. CRP levels above 2.0 mg/l, a biomarker for occult inflammation including coronary artery inflammation, were used as an entry criterion for the JUPITER trial (~4% of the US population have CRP levels above 2.0 mg/l). The overall

conclusion of the study was that statins had anti-inflammatory properties independent of their ability to lower cholesterol and that reducing occult inflammation with statins could reduce degenerative processes of aging.

The statin-mediated reduction in mevalonate also reduces the synthesis of cholesterol via the isoprenoid pathway. The function of several intracellular signaling molecules is also affected by statins through a reduction in products of the isoprenoid pathway that are important for the formation of signaling complexes. There is no dearth of studies on the anti-inflammatory properties of statins *in vitro* and in animal models of autoimmune and inflammatory diseases. *In vitro*, statins reduce cytokine production and expression of endothelial adhesion molecules. Statins decrease expression of major histocompatibility complex (MHC) class II molecules but do not affect MHC class I. They also downregulate the maturation of dendritic cells. Statins bind to a unique allosteric site in $\beta 2$ integrin known as lymphocyte function-associated antigen-1 (LFA-1), which participates in many inflammatory processes. However, the anti-inflammatory effect of binding to LFA-1 is independent of the inhibition of HMG-CoA reductase. Fluvastatin induced apoptosis of synovial cells from patients with rheumatoid arthritis through a caspase-3 mechanism. Both atorvastatin and simvastatin suppressed expression of the RANK ligand by fibroblast-like synovial cells, and atorvastatin inhibited osteoclast formation in a coculture of peripheral blood mononuclear cells, suggesting that statins may inhibit osteoclast formation and bone destruction. However, studies on the anti-inflammatory effects of statins *in vitro* have also yielded inconsistent data: some studies report a reduction in cytokine production by human blood monocytes, whereas others report an increase in cytokine production (Kuijk *et al.*, 2008). It seems that animal models reveal consistent findings for the anti-inflammatory effects of statins. It is important to note that although the anti-inflammatory properties of statins are highly consistent in animal models, statins do not lower cholesterol in mice. Many studies demonstrate reduced disease severity in animals treated with clinically achievable levels of statins and suffering from a variety of inflammatory conditions including colitis, uveitis, myocarditis experimental allergic encephalitis, lethal sepsis, allograft rejection, and asthma (Abeles and Pillinger, 2006).

Statins have been used to reduce inflammation, tame immune cell activation, or arrest degenerative processes. Because of their widespread use and long-term safety record, some physicians prescribe statin therapy for nonapproved indications. A number of case reports describe the dramatic effects of statins added to standard therapies, but beneficial effects need to be confirmed in controlled studies. For example, in three patients with lupus glomerulonephritis who were not responding to the standard of therapy (cyclophosphamide and prednisone), adding high doses of simvastatin brought about a dramatic reduction in disease activity. There are randomized, placebo-controlled studies, but two issues cloud the results: the particular statin used and the dose. Clearly the anti-inflammatory properties of statins vary, whereas the cholesterol-lowering properties are similar. Six clinically used statins were examined *in vitro* for their ability to affect NF- κ B, phosphorylation of I κ B, and activation of tissue factor, the first step in coagulation. There was

a distinct difference between the statins: cerivastatin, atorvastatin, and simvastatin were more effective in reducing these parameters than fluvastatin, lovastatin, or pravastatin (Hilgen-dorff *et al.*, 2003).

Nevertheless, there seems to be no dearth of case reports and small uncontrolled trials that show the benefits of statins for reducing inflammation. In the case of rheumatoid arthritis where the anti-inflammatory effects of blocking cytokines have been shown repeatedly, there have been more than ten trials of statins resulting in a moderate reduction in joint inflammation associated with a fall in CRP and red cell sedimentation rate and decreased cytokine production by circulating monocytes. As many patients with long-standing rheumatoid arthritis are also at high risk for cardiovascular disease, adding statin therapy to the standard of care (methotrexate) is highly cost effective. Adding statins to the regimen of cyclosporine and sirolimus for kidney transplant patients lowered the rate of organ rejection. In patients with relapsing-remitting multiple sclerosis, statin therapy for 6 months significantly lowered the number of brain lesions detected by gadolinium, an established marker of disease activity. Examination of peripheral blood cells revealed no suppression in T cell responses but did reveal an increase in production of IL-10, an anti-inflammatory cytokine (Paul *et al.*, 2008).

Histone Deacetylase Inhibitors

In order to compact chromatin, DNA is tightly wrapped around nuclear histones, which are maintained in a state of deacetylation by histone deacetylases (HDACs). Histone acetylases, on the other hand, hyperacetylate histones, unraveling DNA and thus permitting transcription factors to bind to gene promoters and initiate gene expression. In humans, there are 18 HDACs divided into classes based on their dependence on zinc for enzymatic activity (Khan *et al.*, 2008). In addition to their ability to deacetylate the highly conserved N-terminal lysines of nuclear histones, HDACs also target many non-histone-containing cytoplasmic proteins affecting ribosomal function, cytoskeletal polymerization, and signaling pathways.

Synthetic inhibitors of HDACs are orally active and used widely in medicine. For example, valproic acid, the drug of choice for treating epilepsy as well as obsessive disorders (Gerstner *et al.*, 2008), is an HDAC inhibitor (Ren *et al.*, 2004). Valproic acid has also been used in patients with HIV-1 to purge the latently infected pool of memory T cells (Archin *et al.*, 2008). The HDAC inhibitor sodium butyrate is used to treat patients with sickle cell anemia and β -thalassemia (Atweh and Schechter, 2001). The development of newer, small-molecule synthetic inhibitors of HDACs began with the work of Paul Marks, a cancer researcher (Marks *et al.*, 2001). The goal was to inhibit HDACs in order to increase the expression of several proapoptotic genes that are often silenced in malignant cells. The first of this class was the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA). At micromolar plasma concentrations, (O'Connor *et al.*, 2006) SAHA has been approved for treating patients with cutaneous T cell leukemia (Santini *et al.*, 2007) and has shown benefit in the treatment of patients with acute myeloid leukemia (Garcia-Manero *et al.*, 2008). Many hematopoietic malignancies appear to be responsive to HDAC inhibitors, possibly related

to the ability of these compounds to reduce the production of cytokines, many of which are growth factors for leukemias and myeloproliferative neoplasms (Guerini et al., 2008). Indeed, HDAC inhibitors such as SAHA exhibit immunosuppressive and anti-inflammatory properties by reducing cytokine production (Leoni et al., 2002, 2005). Importantly, the anti-inflammatory properties of HDAC inhibitors are observed in the low nanomolar range compared to the micromolar range needed to treat cancer where the mechanism of action is to increase apoptosis by upregulating expression of proapoptotic genes. The recent expanding interest in HDAC inhibitors as orally active, safe, anti-inflammatory agents has been fueled by the ability of these inhibitors to reduce disease severity in several animal models of inflammatory and autoimmune diseases (Table S2).

ITF2357 is a hydroxamic acid-containing, orally active inhibitor targeting class I and II HDACs. In a phase II clinical trial in children with active systemic onset juvenile idiopathic arthritis, a daily oral dose of ITF2357 at 1.5 mg/kg for 12 weeks resulted in no organ toxicity and a significant ($p < 0.01$) reduction in parameters of systemic disease and the number of painful joints in over 75% of patients (Vojinovic et al., 2008). At a dose of 1.5 mg/kg, the peak blood levels of ITF2357 were 125–200 nM, which are the levels that inhibit cytokine production in vitro and by cultured blood cells from subjects receiving the drug in vivo (Oldoni et al., 2009). Given that targeting of IL-1-mediated inflammation to protect pancreatic islets has been tested in human trials (Donath et al., 2009; Larsen et al., 2007a), HDAC inhibitors may be useful for preventing inflammation of pancreatic islet tissue. Indeed, the HDAC inhibitors SAHA and ITF2357 reduced cytokine-mediated nitric oxide formation and reversed the decline in insulin secretion by isolated islets (Larsen et al., 2007b; Susick et al., 2007). In animal models of Alzheimer's disease, the most common neurodegenerative disease, HDAC inhibitors (Larsen et al., 2007a) decreased β -amyloid production, and effective neuroprotection was associated with increased acetylation of histones (Kozikowski et al., 2009) (see Review by C.K. Glass et al. in this issue). In a model of head trauma, the anti-inflammatory properties of HDAC inhibitors helped to prevent neurological impairment even when administered 24 hr after the injury (Shein et al., 2009). Although the role of inflammation in Huntington's disease is presently unclear, inhibition of caspase-1 delays the onset of motor abnormalities in a mouse model of this neurodegenerative disease (Charles et al., 1999) and treatment with oral SAHA reduces disease severity (Hockly et al., 2003) (Table S2).

Anticoagulants and Thrombolytics as Anti-inflammatory Agents

Despite several randomized, placebo-controlled trials of anti-inflammatory agents and anticytokine therapies, sepsis remains a leading cause of death. The only approved agent for reducing death in sepsis is activated protein C (aPC). Protein C is a natural component of human serum and upon activation inhibits the formation of thrombin and breaks down thrombi in blood vessels. aPC drives fibrinolysis through inhibition of plasminogen-activator inhibitor-1. As a therapeutic in septic patients, aPC is administered intravenously and restores circulation to hypoxic organs. Perhaps more importantly, however, aPC exerts a number of

anti-inflammatory effects. For example, aPC blocks the production of the proinflammatory cytokines IL-1 β and TNF- α by blocking the transcription factor NF- κ B (Esmon, 2006) and p38 MAPK (Nold et al., 2007), decreasing apoptosis of endothelial cells and inhibiting leukocyte adhesion and chemotaxis.

Similarly, other anticoagulants, which prevent the formation of microthrombi on the endothelium thereby maintaining a blood supply to organs, may also be useful as anti-inflammatory agents (Figure 1). Low molecular weight heparins and antithrombin exhibit anti-inflammatory effects in vivo, mostly by reducing cytokine production and endothelial activation by thrombin. However, in a controlled trial in septic patients, anti-thrombin did not reach statistical significance for reducing all cause 28 day mortality. Small-molecule antiplatelet agents used in patients at risk for myocardial infarction also act as anti-inflammatory agents. Although these small-molecule anticoagulants are used primarily to prevent clot formation, it is likely that many patients benefit from the anti-inflammatory properties of this drug class.

Inhibition of Activated Complement

Activation of complement results in the generation of the terminal component, complement 5a (C5a). C5a binds to its receptor C5aR and triggers the synthesis of cytokines, chemokines, and adhesion molecules with the subsequent infiltration of myeloid cells, mostly neutrophils, into the area of injury (Figure 1). As such, C5a is a major cause of acute inflammation, and activated complement appears to contribute to nearly all inflammatory processes. Not unexpectedly, there are natural inhibitors of complement activation such as C1 inhibitor and soluble Crry, which inhibit all complement activation pathways and target activation of C3 (Huang et al., 2008). Rheumatoid arthritis, immune complex diseases, psoriasis, lupus nephritis, acute lung injury, myocardial and cerebral infarction, and renal ischemia are considered diseases in which the activity of C5a could be targeted. Complement activation takes place in acute allograft rejection. Eculizumab, a monoclonal antibody directed against C5, has been approved for the treatment of paroxysmal nocturnal hemoglobinuria. A single chain of this antibody, pexelizumab, has been extensively studied in clinical trials in models of ischemia. Pexelizumab, which is directed against C5 and blocks the generation of C5a, has been used in acute ST-elevation myocardial infarction (STEMI) and the coronary artery bypass graft (CABG) procedure. Meta-analysis of over 15,000 patients from seven clinical trials showed no overall benefit of the antibody when added to the standard of care (Testa et al., 2008), but the antibody did reduce the risk of death by 26% in patients undergoing CABG.

Another approach to preventing the inflammatory sequelae of complement activation is direct inhibition of serine proteases. Nafamostat mesilate is a small-molecule serine protease inhibitor that targets the formation of the C3/C5 convertases. Several studies indicate that the interaction of macrophages or dendritic cells with T cells results in activation of complement. In mice with experimental autoimmune encephalomyelitis (a model of multiple sclerosis), nafamostat attenuated central nervous system inflammation, reduced demyelination, and decreased the numbers of immune cells producing IFN- γ and IL-17 (Li et al., 2009). Orally active convertase inhibitors or small-molecule antagonists of C5aR may become broadly useful for treating

chronic inflammatory conditions. Although there was no benefit in patients with active rheumatoid arthritis after 28 days of treatment with an orally active antagonist of C5aR, targeting inflammation by preventing the generation of C5a or blocking C5aR with orally active small molecules holds considerable promise.

The Anti-inflammatory Effects of PPARs

Endogenous, intracellular mechanisms exist to tame inflammation by targeting gene expression of proinflammatory cytokines, cell adhesion molecules, and genes such as COX-2 or inducible nitric oxide synthase. The peroxisome proliferator-activator receptors (PPARs) are members of the nuclear receptor superfamily. There are three separate gene products with highly conserved DNA-binding domains: PPAR α , PPAR β/δ , and PPAR γ . Functionally, PPARs form heterodimers with retinoic acid receptors. Although this interaction has downstream effects in the regulation of glucose metabolism, PPAR agonists are anti-inflammatory agents because they reduce expression of several proinflammatory cytokines, most chemokines, and cell adhesion molecules. There are several endogenous agonists of PPARs such as prostacyclin. However, there are orally active synthetic ligands for PPAR γ —rosiglitazone, pioglitazone, troglitazone, and fenofibrate—that are used to treat insulin resistance in patients with type 2 diabetes. These drugs belong to the chemical class called thiazolidinediones (or glitazones).

In vitro, the PPAR γ agonist pioglitazone reduces synthesis and gene expression of TLR2, TLR4, IL-1 β , TNF- α , IL-6, and monocyte chemoattractant protein-1 in human blood monocytes (Dasu et al., 2009). In vivo, pioglitazone reduces lipopolysaccharide-induced TLR2 and TLR4 expression on peritoneal macrophages (Dasu et al., 2009). In humans with type 2 diabetes, pioglitazone reduces serum CRP and TNF- α but increases VEGF levels. Rosiglitazone improves insulin resistance by increasing the production of the anti-inflammatory molecule adiponectin. In a mouse model of lupus nephritis, oral treatment with pioglitazone decreases immune complex deposition, renal inflammation, and intrarenal synthesis of TNF- α , IL-1 β , and VCAM-1 (Zhao et al., 2009).

Despite the anti-inflammatory portfolio of PPAR agonists, some clinical studies indicate that treatment of type 2 diabetic patients with thiazolidinediones, particularly rosiglitazone, increases cardiovascular events and death (Stafylas et al., 2009). A meta-analysis of data on the cardiovascular safety of glitazones concluded that clinical trials consistently indicate an increased risk of heart failure. Some studies have shown that pioglitazone may be beneficial for reducing major cardiovascular outcomes. Whether using PPAR γ agonists as anti-inflammatory agents will entail the same risks remains unknown. Nevertheless, PPAR γ agonists as a class of orally active drugs with anti-inflammatory properties will certainly be developed.

Prostaglandin Agonists and Phosphodiesterase-4 Inhibitors

Although PGE2 is inflammatory and lowers pain thresholds, it is also one of the most immunosuppressive natural products of inflammation. One of the four receptors for PGE2 is EP4, and selective EP4 agonists are anti-inflammatory. For example, EP4 agonists reduced levels of proinflammatory cytokines, che-

mokines, and adhesion molecules. PGE2 is a potent inhibitor of IFN- γ and IL-2 production but increases IL-17 production. In general, the immunosuppressive properties of PGE2 are due to an increase in the intracellular levels of cyclic AMP (cAMP), which suppresses production of TNF- α , IL-2, and IFN- γ . In fact, inhibitors of PGE2 synthesis result in lower levels of cAMP and higher levels of TNF- α . Analogs of prostaglandin-I2 (iloprost, cicaprost, treprostinil) inhibit the proinflammatory cytokines IL-12, TNF- α , IL-1 α , and IL-6 as well as the chemokines MIP-1 α and MCP-1 by boosting cAMP levels (Zhou et al., 2007).

Inhibitors of phosphodiesterase-4 (PDE-4) are anti-inflammatory and immunosuppressive because they prevent the breakdown of cAMP. Rolipram, roflumilast, piclamilast, and pentoxifylline are selective PDE-4 inhibitors that are in clinical use. A general property of PDE-4 inhibitors is the suppression of cytokine production as well as degranulation by neutrophils and TNF- α -induced neutrophil adherence to endothelial cells, which is a fundamental step in inflammation. Some studies indicate that rolipram and newer PDE-4 inhibitors are comparable or superior to methylprednisolone in suppressing inflammation in models of inflammatory bowel disease, atopic dermatitis, spinal cord injury, septic shock, cerebral ischemia, and hyperoxia-induced lung inflammation. PDE-4 inhibitors also decrease the binding of NF- κ B to DNA and the production of VEGF.

Conclusions

In this Review, I have made a case that the future of anti-inflammatory agents lies in the development of orally active drugs that reduce production or activities of proinflammatory cytokines. In addition, particularly with respect to treating the inflammatory component of degenerative diseases of aging, newer drugs need to have minimal risks of organ toxicity while ensuring that host immune defense against infection and cancer is not impaired. Some drugs currently used to treat a variety of diseases exhibit distinct anticytokine effects, for example, statins, histone deacetylase inhibitors, and PPAR agonists. Based on their long-term safety, increasing the anti-inflammatory and anticytokine properties of these classes of drugs is warranted. New agents are in a rapid stage of development, for example, orally active chemokine receptor antagonists. Based on animal studies, using small RNAs to downregulate gene expression and targeting microRNAs that regulate gene expression holds therapeutic promise. In addition, agents that increase the expression and activities of endogenous anticytokines such as the IL-1 receptor antagonist or IL-10 should be considered. The understanding of the pivotal role of inflammation in seemingly unrelated diseases has resulted in the use and development of new anti-inflammatory agents such as the biologicals. These have improved the quality as well as the duration of life for millions of patients. In addition, it appears that combating inflammation will slow the degenerative consequences of aging such as atherosclerosis, joint disease, and neurodegeneration.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two tables and can be found with this article online at doi:10.1016/j.cell.2010.02.043.

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