Multiple Interleukin-1β–Converting Enzymes Contribute to Inflammatory Arthritis

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Mouse models of inflammatory arthritis are strongly driven by excessive production of interleukin-1β (IL-1β). Much attention has been focused on the generation of IL-1β by inflammasomes and caspase 1 in monocytes and macrophages. However, a number of other proteases can also process proIL-1β into the mature, bioactive cytokine. Arthritis has complex etiologies based on a variety of genetic and environmental factors, and global blockade of IL-1β signaling strongly ameliorates arthritis in experimental models. Yet, the mechanisms behind the relative contributions of the different IL-1β–processing enzymes to disease initiation and perpetuation remain elusive. Two articles in this issue of *Arthritis & Rheumatism*, by Joosten et al and Guma et al (1,2), shed more light on the complex proteolytic mechanisms behind the excessive maturation and release of IL-1β that perpetuates inflammatory arthritis in experimental models. These findings have significant implications for the design of future treatment strategies.

Rheumatoid arthritis (RA), which occurs in ∼1% of the population, is a complex chronic inflammatory disease that presents as a symmetric polyarthritis, usually in the small joints of the hands and feet. RA is characterized by synovial inflammation as well as joint swelling, stiffness, and pain, which leads to pannus formation and joint destruction, and ∼25% of patients with RA will require joint replacement. However, RA can also display systemic manifestations, including pulmonary and cardiovascular implications as well as a higher risk of cancer. The synovium is primarily composed of synovial fibroblasts (synoviocytes) and macrophages, the latter being the main source for many inflammatory cytokines, including tumor necrosis factor α (TNFα), IL-1β, and IL-18, that are active in the joints of RA patients and are responsible for disease perpetuation, and which are therefore significant targets for therapeutic intervention.

The current standard treatment for RA is administration of disease-modifying antirheumatic drugs (DMARDs), such as methotrexate and sulfasalazine, which results in remission (as defined by manageable disease) in ∼20–30% of all patients, but these treatments also display significant toxicity. Those patients whose disease is nonresponsive to methotrexate are usually treated with combination therapy, involving a combination of DMARDs with one of the TNFα blockers (etanercept, adalimumab, or infliximab) or the IL-6 blocker (tocilizumab), which also results in remission in ∼20–30% of patients. However, the disease in 40–50% of all patients remains refractory to any of the current standard therapies. Therefore, the efficacy of blocking therapy against any of the other cytokines active in RA, including IL-15, lymphotoxin β, Light, B lymphocyte stimulator (BLyS; trademark of Human Genome Sciences, Rockville, MD), APRIL, and RANKL, is currently under investigation in phase II clinical trials (3). Anakinra, which is a nonglycosylated recombinant IL-1 receptor antagonist (IL-1Ra) that interferes with binding of IL-1β to its receptor, is the only other approved anticytokine biologic therapy. Although anakinra is inferior to TNFα blockers, it is moderately effective in RA but has been proven to be highly effective and safe for the treatment of adult-onset Still’s disease (4), systemic onset juvenile idiopathic arthritis (5), Schnitzler’s syndrome (6), gouty arthritis (7,8), pseudogout (9), and hereditary periodic fever syndromes (10).

The significant role of IL-1β in the development of RA is long known and has been directly demonstrated by the strong arthritogenic response in experimental mouse models, resulting from either intraarticular injec-
tion of recombinant IL-1β or articular expression of IL-1β by local gene transfer (11,12). Plasma levels of IL-1β are strongly correlated with RA disease severity and joint destruction, and in all experimental models, bone and cartilage erosion is highly dependent on IL-1β (13,14). This is consistent with the IL-1β–dependent potent activation of synoviocytes, chondrocytes, osteoblasts, and osteoclasts. TNFα induces IL-1β and vice versa, but TNFα–independent production of IL-1β is observed in experimental models of inflammatory arthritis induced by streptococcal cell wall (SCW) fragments. In addition, there is increasing evidence that TNFα mediates its arthritogenic effects via production of IL-1β, and therefore TNFα-deficient mice still develop type II collagen–induced arthritis (CIA), while anti–IL-1RI antibodies completely block joint inflammation in TNFα–transgenic mice (15,16). CIA and arthritis induced by human T lymphotrophic virus type I is ameliorated in IL-1β–deficient mice, and CIA is also prevented by prophylactic treatment and by treatment of established disease with the pharmacologic caspase 1 inhibitor VE-13,045 (17,18). Mice deficient in IL-1Ra spontaneously develop an inflammatory arthritis that resembles RA, and those with CIA develop more severe disease, whereas in IL-1Ra–transgenic mice, CIA is ameliorated (19–21).

IL-1β is a pleiotropic cytokine with immune- and inflammation-modulatory activities. To explain how IL-1β promotes inflammatory arthritis, one has to understand its principal mechanism of activation. It belongs to the IL-1 family, which also includes IL-1α, IL-1Ra, IL-18, and IL-33, in addition to others. Generation of biologically active IL-1β is highly regulated at several levels. Mediators of inflammation and infection, including IL-1β itself, induce NF-κB–dependent transcription of the IL1B gene. IL-1β is further regulated by control of RNA stability and translation, and requires posttranslational processing to be released. Once in circulation, its effects are further controlled by multiple IL-1 receptors. IL-1β only binds with low affinity to IL-1RI, but recruitment of the IL-1R accessory protein (AcP) results in formation of a trimeric high-affinity complex. IL-1RII, which lacks the intracellular Toll/IL-1R (TIR) signaling domain, acts as a decoy receptor, and IL-1Ra, which is constitutively released from cells, competes with IL-1β for receptor binding. In addition, there are soluble forms of IL-1RI, IL-1RII, and AcP, which further allow fine-tuning of the IL-1 response.

Once the IL-1RI is engaged, signal transduction is initiated by clustering of the intracellular TIR domain, recruitment of myeloid differentiation factor 88 and IL-1R–associated kinases, and activation of downstream transcription factors. Its potent proinflammatory effects are mediated by induction of additional inflammation mediators, including TNFα, IL-1α, IL-1β, IL-6, IL-8, cyclooxygenase 2, and prostaglandin E2, which account for the pain, swelling, and tenderness typically observed in RA joints. IL-1β mediates pannus formation, leading to destruction of cartilage and bone and impaired processes of repair, which thus directly enhances the loss of patient functionality and causes disability in RA patients. For example, IL-1β activation of synoviocytes induces secretion of matrix-degrading enzymes, such as matrix metalloproteinases (MMPs), which drive cartilage breakdown. In chondrocytes, IL-1β potently inhibits proteoglycan synthesis and induces collagen degradation, thus impairing the maintenance of cartilage, while long-term exposure to IL-1β causes apoptosis of chondrocytes via excessive production of nitric oxide. IL-1β is also responsible for bone resorption by increasing the expression of RANKL in osteoblasts, which promotes differentiation of osteoclasts and enhances their bone-resorptive activity, while at the same time causes apoptosis of osteoblasts to prevent bone formation (22).

The critical step in the release of IL-1β from macrophages is the requirement for proteolytic processing to convert the 31-kd precursor into the 17-kd mature, bioactive IL-1β, which is then released. The best-characterized protease that processes IL-1β is caspase 1. Caspase 1, which was initially identified as the only IL-1β–converting enzyme (ICE), is activated in inflammasomes and generates a 28-kd intermediate, inactive form of IL-1β and a 17-kd, bioactive form of IL-1β (23,24). Therefore, one would expect that preventing caspase 1 activity should impact disease severity to a level similar to that observed in IL-1β–deficient mice. However, in contrast to the effects of deficiency of IL-1β or IL-1RI, which completely ameliorates inflammatory arthritis in many experimental models, inhibition of caspase 1 itself, surprisingly, only partially inhibits inflammatory arthritis, to ~50%.

Caspase 1 is essential for the maturation of IL-1β by inflammasomes in macrophages, but perhaps the most interesting discovery has been that caspase 1 is not the sole ICE; a number of serine proteases can also proteolytically cleave the IL-1β precursor at distinct sites, some of which give rise to a mature peptide that closely matches caspase 1–processed IL-1β (Figure 1). Several of these enzymes are stored in azurophil granules in neutrophils (neutrophil elastase, proteinase 3, cathepsin G), mast cell granules (chymase, cathepsin G), cytotoxic T lymphocyte (CTL) granules (granzyme A),
and MMPs (stromelysin 1, gelatinase A, gelatinase B), which are produced by a number of cells, as well as chymotrypsin, trypsin, plasmin, keratinocyte stratum corneum chymotryptic enzyme (SCCE), and Staphylococcus aureus protease. Whether all of these proteases are relevant in vivo is not known, but several are found in inflammatory lesions.

Neutrophil elastase processing of proIL-1β occurs after I_{103}, which results in only a 10-fold increase in activity (25). In addition, neutrophil elastase can also cleave after Y_{113}, which is a hot spot for cleavage by several inflammatory fluid proteases, and this yields 17-kd and 18-kd mature peptides with significant activity (26). Proteinase 3 has been identified as one of the main neutrophil ICEs in a monocyte–neutrophil coculture system, in studies using selective inhibitors and purified proteins (27). Proteinase 3 produces mature, bioactive IL-1β as an intracellular protease, and not as a secreted extracellular protease, indicating that this mechanism parallels the caspase 1–mediated IL-1β processing, in which processing precedes release of IL-1β. A cell-permeable peptide blocked activity, whereas α₁-antitrypsin, which specifically inhibits extracellular serine proteases, did not affect IL-1β maturation by neutrophils (28).

Cathepsin G also cleaves proIL-1β after Y_{113}, resulting in a mature (17-kd) peptide that is identical to that produced by chymase, chymotrypsin, and neutrophil elastase (26). Mast cells store chymase in their secretory granules, and chymase also cleaves proIL-1β downstream of Y_{113}, generating a mature (17-kd) IL-1β, which displays activity similar to that of the caspase 1–derived IL-1β (29). CTL-produced granzyme A converts proIL-1β into the mature, bioactive cytokine (17 kd) by cleaving downstream of R_{120}, which is just 4 amino acids downstream of the major caspase 1 cleavage site at D_{116} (30). Granzyme A–produced IL-1β is biologically active, albeit to a lesser extent (~30% activity) compared with that of the caspase 1–derived IL-1β.

Localized sites of inflammation, such as inflamed joints, also contain MMPs, which are involved in tissue destruction, but stromelysin 1 (MMP-3), gelatinase A (MMP-2), and gelatinase B (MMP-9) can also function as an ICE (31,32). Although stromelysin 1 and gelatinase A are less potent, require higher enzyme concentrations, and require prolonged incubation times as compared with gelatinase B, all of these MMPs process proIL-1β into a number of distinct peptides in the 14–17-kd range, with larger intermediate products. Nevertheless, such MMP concentrations, even the high levels of gelatinase A, can be found in serum or at sites of inflammation. Furthermore, prolonged incubation of stromelysin 1 resulted in complete degradation of the mature peptide, suggesting that MMPs are capable of both positive and negative regulation of IL-1β (31). This is reminiscent of the observations in studies of SCCE, which was found to produce bioactive IL-1β in amounts slightly larger than those of caspase 1–derived IL-1β, whereas prolonged incubations with SCCE resulted in degradation and loss of IL-1β activity (33).

S aureus protease processing of proIL-1β causes a similar increase in activity (~300-fold), following cleavage after E_{111} (18 kd) (25). Significantly, S aureus infections can develop into septic arthritis and can also develop in mouse models of experimental arthritis. Chymotrypsin also converts IL-1β by cleavage after Y_{113} (17 kd) (25). Chymotrypsin-mediated maturation results

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**Figure 1.** Schematic overview of the interleukin-1β (IL-1β)–converting enzymes and their cleavage sites in the IL-1β precursor. The wide arrowhead indicates the caspase 1 cleavage site of the IL-1β precursor of 269 amino acids, while narrow arrowheads indicate the serine protease cleavage sites. The cleavage site after which the precursor is processed by each enzyme is indicated in parentheses. The cleavage sites for plasmin, keratinocyte stratum corneum chymotryptic enzyme (SCCE), proteinase 3 (PR3), and matrix metalloproteinases (MMPs) (stromelysin 1, gelatinase A, and gelatinase B) have not been precisely mapped and are estimated based on the published molecular weight of the processed IL-1β.
in a 500-fold increase in activity, as compared with the activity of proIL-1β. In contrast, trypsin processing occurs further upstream, after R25 or K36, which results in a poorly active cytokine of 25 kd, with only a 7-fold increase in bioactivity, and treatment with plasmin can also generate a mature peptide of 23 kd, with only slightly increased activity (25,32).

From these observations, it seems obvious that processing of proIL-1β has to occur close to the caspase 1–processing site in order to produce bioactive IL-1β, while conversion into larger peptides is generally correlated with severely reduced biologic activity. Recombinant IL-1β purified from Escherichia coli, containing an additional 46 amino acids of the pro domain (IL-1β71–269), shows some pyrogenic activity in rabbits, but removal of all but 5 amino acids of the pro domain (IL-1β112–269) increases the specific activity by 50-fold (34). In another study, trypsin-produced IL-1β77–269 is 10,000-fold less active than the elastase-produced IL-1β114–269, clearly emphasizing the significance of the specific processing site for bioactivity (26).

Whether it is essential to have the proper NH₂ region, or whether the correct size of the mature peptide is critical, is currently not known. Many inflammatory reactions are not systemic, but rather are localized, such as in RA joints, and the proteases present in these extracellular fluids could be highly significant for determining these localized inflammatory reactions. For example, incubation of recombinant proIL-1β with synovial fluid from arthritic joints results in the generation of several IL-1β peptides with distinct molecular weights and pI values, in addition to the caspase 1–derived mature IL-1β, and inflamed synovial fluid is known to contain a number of these proteolytic enzymes (26). However, many of the original experiments were performed in vitro, and thus not all of the ICEs might actually be relevant in vivo (35).

In this regard, the 2 present studies reported in this issue of Arthritis & Rheumatism (1,2) are highly significant, because they demonstrate this proteolytic activity in an experimental disease model in vivo, indicating that, indeed, different cell types with a distinct repertoire of ICEs producing distinct bioactive IL-1β can contribute to inflammatory arthritis. Considering that most of these proteases are actually released from different cells, it is noteworthy that several cell types, such as fibroblasts, smooth muscle cells, and endothelial cells, can produce proIL-1β but lack a caspase 1 maturation mechanism, although release of proIL-1β has been described even in activated monocytes.

In addition, sterile inflammatory conditions are frequently associated with infiltration of neutrophils, which are short-lived cells and might be a source of proIL-1β following cell death. These IL-1β precursors could be employed by the proteases present in the inflammatory fluid to produce bioactive IL-1β. Nevertheless, activated macrophages also rapidly release active caspase 1, together with other inflammasome components and IL-1β, and it is feasible that this extracellular caspase 1 is also involved in proteolytic processing of proIL-1β. The precise mechanism by which IL-1β is released is still controversial, and results from several models have suggested mechanisms of either cell rupture, ATP-induced blebbing of microvesicles, or exocytosis of secretory lysosomes (36).

The molecular mechanism by which ICEs are activated is distinct between the caspase 1 and serine protease activation pathways. Activation of caspase 1 depends on the formation of inflammasomes. The inflammasomes are protein platforms that link recognition of damage-associated molecular patterns (DAMPs) by members of the NOD-like receptor (NLR) family of cytosolic pattern-recognition receptors (PRRs) to the activation of caspase 1–dependent processing and release of IL-1β and IL-18 in macrophages (37). In response to the recognition of DAMPs from either pathogens (pathogen-associated molecular patterns) or cellular stress (stress-associated molecular patterns, or danger signals), NLRs undergo NTP-dependent oligomerization and recruit the adaptor protein ASC. Caspase 1 is then recruited by ASC into the NLR–ASC complex, which results in activation of caspase 1 by induced proximity.

Although little is known about the stimuli that activate inflammasomes, it appears that each NLR is selectively activated. For example, NLRP1, one of the 22 human NLRs, in concert with NOD-2, is involved in the recognition of peptidoglycan and Bacillus anthracis lethal toxin, while NLRC2 and neuronal apoptosis inhibitor protein are required for recognition of intracellular flagellin in conjunction with a bacterial type III or type IV secretion system. NLRP3 (cryopyrin), perhaps the best-studied NLR, is activated in response to a diverse set of infection- and stress-associated signals, including peptidoglycan, bacterial and viral RNA, reactive oxygen species, asbestos and silica particles, skin irritants, and, in concert with P2X7 receptors, extracellular ATP (38). Furthermore, NLRP3-containing inflammasomes are relevant in rheumatic diseases and are activated in response to uric acid and calcium pyrophosphate crystals that cause the painful symptoms in gout and pseudogout (39).
In contrast, activation of serine proteases is uncoupled from the PRR system, and thus is independent from direct recognition of infections and cellular stress. Serine proteases present in vesicles are activated by the lysosomal exocysteine peptidase dipeptidyl peptidase I (DPPI; cathepsin C), which removes the NH₂-terminal leader peptide in cathepsin G, neutrophil elastase, proteinase 3, granzyme A, MMP-9, and chymase, thereby promoting their maturation. DPPI itself also requires autocatalytic removal of its pro domain for activation, which is enhanced at low pH (40). Many MMPs, including MMP-3 and MMP-9, can be activated by plasmin and also by other MMPs. Plasmin itself is activated by conversion of plasminogen into plasmin by urokinase plasminogen activator, tissue plasminogen activator, and factor XII, and subsequently can autoactivate itself and chymotrypsin.

The reason that different cell types have acquired several alternative mechanisms that can convert IL-1β into the bioactive form is not fully understood. One simple explanation could be that having redundant mechanisms for releasing this potent cytokine essential for the inflammatory host response might ensure that the bioactive cytokine is available, even if one system fails. It is well established that besides macrophages, neutrophils and mast cells also significantly contribute to inflammatory arthritis (41–44). This might explain the observations that caspase 1-deficient mice are not fully protected from IL-1β-dependent inflammatory diseases, including arthritis. Because most of the potential IL-1β-converting serine proteases have the DPPI-activating process in common, it is not surprising that DPPI-deficient mice are largely resistant to the development of CIA (45). However, consistent with the activity of multiple ICEs, a number of mice still develop inflammation and bone erosion, likely resulting from inflamasome-dependent IL-1β generation. Although the existence of distinct ICEs has been known for some time, their relative contribution to the development of inflammatory arthritis has not been investigated, and the 2 studies reported herein are the first to address this.

As suggested by Joosten et al and Guma et al (1,2), serine proteases play a dominant role during the acute phase of arthritis, which is characterized by strong neutrophil infiltration, whereas caspase 1 could play a major role during the chronic phase of arthritis, which is highly dependent on macrophages (Figure 2). Joosten and colleagues tested an acute K/BxN serum transfer model as well as models of acute and chronic SCW-induced arthritis, and the results showed that arthritis progressed similarly in caspase 1-deficient and wild-type mice during the first 4 days, while caspase 1-deficient mice showed partial protection against joint inflammation and against chondrocyte proteoglycan synthesis inhibition. In contrast, IL-1β-deficient mice were fully protected. In a model of chronic arthritis induced by repeated SCW injections, caspase 1 deficiency ameliorated disease, concomitant with a significant reduction in neutrophil infiltration, although the effects were not as efficient as IL-1β deficiency. Dual blockade of caspase 1 and DPPI-dependent serine proteases using pharmacologic caspase 1 inhibition by pralnacasan in a DPPI-deficient background resulted in significantly ameliorated disease, comparable with the effects of IL-1β deficiency. Based on the observations in 2 additional models of arthritis in mice deficient in MMP-9 or lacking neutrophil elastase and cathepsin G (Beige/Beige mice), Joosten et al concluded that proteinase 3 is the main serine protease working in concert with caspase 1 in the development of inflammatory arthritis.

Guma and colleagues also tested the K/BxN model and the uric acid crystal-induced peritonitis model to demonstrate the significant contribution of serine proteases in inflammatory arthritis, but based on their findings, the authors concluded that mast cell chymase and neutrophil elastase might be the alternative ICEs in arthritis. These investigators also observed only partial protection in caspase 1-deficient mice, and based on the previously described contribution of mast cells
mast cells and neutrophils play a considerable role in the development of inflammatory arthritis. Moreover, these studies provide more insight into the disease mechanisms, which can be expected to have a significant impact on future therapies. Nonetheless, it should be noted that IL-1β is not the sole substrate of caspase 1, which also cleaves 2 other IL-1 family cytokines, IL-18 and IL-1F7, and at least IL-18 is also processed by protease 3 and contributes to arthritis (46,47). IL-33, yet another IL-1 family cytokine relevant in RA, has been proposed to require caspase 1 for maturation, but recent data suggest a predominantly intracellular function of IL-33 that is similar to that of IL-1α, which is released only during damage of cells. Recent findings also indicate that cleavage by caspase 1 in fact inactivates IL-33 (48). Thus, the role of these proteases in arthritis could be far more complex than being limited to the maturation of IL-1β.

One of the problems of anakinra is its very short half-life of only a few hours, and novel anti–IL-1β therapies are designed to improve the limitation of the required daily administration of anakinra. Therefore, much emphasis has been placed on the development of improved anti–IL-1β therapies, including caspase 1 inhibitors. Two other drugs that target IL-1β are currently approved or are in clinical trials for the treatment of the cryopyrinopathies, but could also benefit patients with other IL-1β–dependent diseases. Rilonacept is based on the Trap technology, which is a fusion of IL-1RI and IL-1AcP with the Fc region of human IgG1, and canakinumab is a fully humanized monoclonal anti–IL-1β antibody. For global inhibition of IL-1β by anakinra and other cytokine traps, potentially considerable side effects, similar to the risks associated with global TNFα inhibition, need to be considered, because patients may be severely immunocompromised. As such, partial inhibition of the IL-1β pathway by selectively targeting a particular IL-1β–converting protease could be a promising and superior approach. Such strategies would also allow for personalized therapy that is customized to the patient’s need. The viability of this approach was recently demonstrated by the identification of an NLRP3-specific inhibitor that abrogates caspase 1 activation only downstream of NLRP3 but has no effect on the other NLRs, which might have implications in the treatment of gouty arthritis without the side effects of global caspase 1 inhibition.

It is currently not understood whether there is a particular NLR defect in RA that causes excessive caspase 1 activation, but NLRP1 and NLRP3 have been linked to rheumatic diseases. Genetic variants of NLRP1 are associated with a number of autoimmune diseases, including RA, while NLRP3 has been linked to gout and pseudogout, and elevated expression of these NLRs is detected in the synovial fluid of patients with RA (49,50). Whether specific defects promote the increased activation of serine proteases in inflammatory arthritis or whether this is merely the result of the enhanced infiltration of neutrophils and mast cells will need further investigation. In any case, several specific serine protease–inhibitory drugs that target, among others, neutrophil elastase and MMPs are currently approved by the US Food and Drug Administration, and in light of the results from the 2 studies reported herein, these agents could also be evaluated for efficacy in inflammatory arthritis.

**REFERENCES**