Blocking IL-1 in systemic inflammation

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A growing number of systemic inflammatory diseases characterized in part by recurrent fevers, leukocytosis, anemia, and elevated acute phase proteins are linked to interleukin (IL)-1 activity since rapid and sustained resolution is observed upon specific blockade of IL-1 receptors. Rapid resolution of systemic and local inflammation is now also reported in systemic onset juvenile idiopathic arthritis (SoJIA). Loss of control of the secretion of IL-1β might be a common mechanism explaining the aberrant activity of IL-1 in these diseases.

IL-1 synthesis and regulation

The production of IL-1β is via nonclassical pathways of protein secretion. Toll-like receptor (TLR) agonists such as endotoxins initiate the synthesis of the inactive IL-1β precursor (Fig. 1 A). Although most of the IL-1β precursor is in the cytosol, a fraction moves into specialized secretory lysosomes (1). There the IL-1β precursor colocalizes with procaspase-1 (1) (Fig. 1 B). The next step is the conversion of the inactive procaspase-1 to active caspase-1 by a complex of proteins termed the "IL-1β inflammasome" (2). Current thinking is that in resting cells procaspase-1 is bound to a large inhibitor molecule, which prevents its activation. During initiation of IL-1β synthesis, there is activation of caspase-1, which then processes the IL-1β precursor into a mature form ready for secretion. Processing and release are closely linked (Fig. 1, C and D). Activation of the nucleotide P2X7 receptor triggers the efflux of potassium ions out of the cell , and within minutes the secretory lysosomes begin releasing their contents of processed IL-1β into the extracellular milieu. In support of the role of P2X7, overexpression of the receptor increases the secretion of IL-1β (3) and its absence prevents the secretion of IL-1β (4). The small peptide LL37 released from activated neutrophils and epithelial cells also stimulates the release of processed IL-1β via the P2X7 receptor (5). The efflux of potassium ions signals the influx of calcium ions (3), which in turn activate phospholipases (6). It appears that calcium-independent phospholipase A2 is required for caspase-1 processing in the specialized lysosomes, whereas phosphatidylcholine-specific phospholipase C is required for lysosomal exocytosis and release (6). Dysregulation in any of these steps might account for increased secretion of IL-1β and for IL-1–mediated diseases.

IL-1 dysregulation in SoJIA

SoJIA (also known as systemic juvenile rheumatoid arthritis) is a devastating, systemic inflammatory disease that affects growing children for which there are few treatment options other than high dose corticosteroid treatment. In this issue, Pascual and colleagues show that blocking IL-1 activity with an IL-1 receptor antagonist (IL-1Ra, anakinra) resulted in convincing clinical and hematological responses in nine patients with SoJIA (7). Resolution of clinical symptoms including fever, marked leukocytosis, thrombocytosis, elevated erythrocyte sedimentation, anemia, and arthritis were rapid and sustained. The efficacy of IL-1Ra in these children contrasts sharply to that of blocking TNF in SoJIA. Neutralization of TNF, a successful treatment in some patients with rheumatoid arthritis, is now discredited in SoJIA since the TNF inhibitors etanercept and infliximab are associated with treatment failures, worsening of disease and/or exacerbations of other autoimmune diseases in these patients. Based on the present study and a similar observation (8), blocking IL-1 may become the standard of therapy for SoJIA. At present, only IL-1Ra is approved for use in humans, but other agents such as anti–IL-1β monoclonal antibodies or the IL-1 Trap molecule (9) reduce IL-1 activity and are likely to be effective. The rapid resolution of clinical, hematological, and biochemical manifestations of SoJIA after a few days of IL-1Ra treatment is reminiscent of the treatment of refractory adult onset Still's disease, a systemic inflammatory disease of adults characterized by similar manifestations of disease seen in SoJIA. (10). In SoJIA patients, reduction or complete withdrawal of long-term steroid treatment was achieved without a rebound in disease activity, as is also the case in adult onset Still's disease patients treated with IL-1Ra. For growing children, IL-1Ra therapy is safe and may eliminate the stunted growth associated with steroid therapy. In addition, IL-1Ra therapy in rheumatoid arthritis patients does not interfere with tetanus immunization, suggesting that this treatment will not interfere with childhood immunizations.

Pascual and coworkers took their investigation of disease mechanisms a step further than most studies. They added 20% serum from four SoJIA patients or from healthy controls to peripheral blood mononuclear cells (PBMCs) of healthy donors, and changes in gene expression were assessed by microarray analysis (7). Why these four sera of the 16 patients available to the authors were selected is not specified, nor is the reason for using 20% serum. Nevertheless, increases in gene expression of cytokines, cytokine receptors, cell adhesion molecules, and other markers of inflammation were observed in PBMCs incubated with patient sera but not control sera. The gene most highly induced by SoJIA sera was fibronectin (17-fold). The authors found that incubating PBMCs with 20% serum from SoJIA patients increased secretion of IL-1β compared

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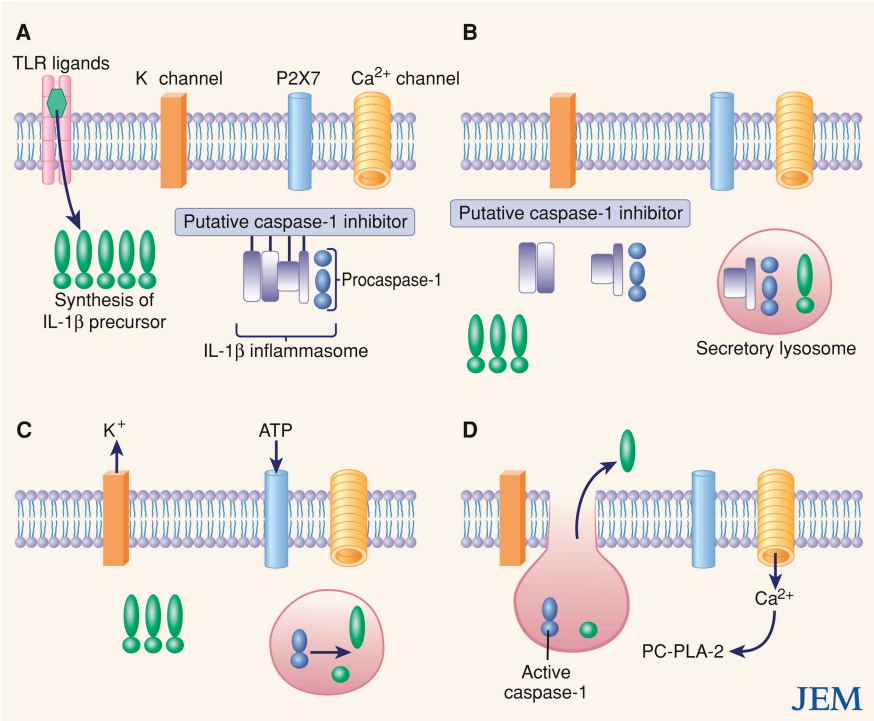

Figure 1. Steps in the processing and secretion of IL-1β. (A) TLR ligands such as endotoxin trigger gene expression and synthesis of the IL-1β precursor, which remains diffusely in the cytosol. In the same cell, inactive procaspase-1 is bound to components of the IL-1β inflammasome, which contains the products of the *NALP-3* gene. The IL-1β inflammasome is kept in an inactive state by binding to a large molecular weight putative inhibitor. (B) After TLR signals, there is a transient uncoupling of the inhibitor and *NALP-3* gene products from procaspase-1, which then colocalizes with the IL-1β in secretory lysosomes. (C) Activation of the nucleotide receptor P2X7 by ATP or LL37 initiates the efflux of potassium from the cell via a potassium channel. The efflux of potassium activates the autocatalytic processing of procaspase-1. Active caspase-1 cleaves the IL-1β precursor in an active cytokine. (D) The efflux of potassium ions results in the influx of calcium ions, which in turn activate phospholipases. Phosphatidylcholine-specific phospholipase C (PC-PLA-2) facilitates lysosomal exocytosis and secretion of IL-1.

with autologous sera. Furthermore, it appeared that the sera from patients with systemic disease induced more IL-1β secretion compared with patients with only active arthritis. It may be concluded that 20% serum from patients with SoJIA contains enough stimulant(s) to increase a portfolio of proinflammatory genes as well as induce secretion of IL-1β from healthy PBMCs, and that autologous sera does not contain such stimulants. In my opinion, however, such methods do not support the concept that disease is caused by a circulating factor(s); rather, the effects of sera on cultured cells may be an epiphenomenon. Presence of TLR ligands, such as bacterial lipopolysaccharide, in SoJIA sera would yield the same results, and a combination of particular serum acute phase reactants could also contribute to the observation. If the authors had added IL-1Ra to the cultures, they could have at least observed whether the serum stimulant(s) was IL-1 itself.

The authors also examined steady-state gene expression in PBMCs from 16 SoJIA patients with active disease and compared the results to PBMCs from 12 healthy children. Many of the same genes induced by SoJIA sera were spontaneously increased in the PBMCs of these 16 patients. Most notable and significant were the genes encoding IL-1β, the IL-1 decoy receptor, cyclooxygenase-2, TLR-2, and the complement receptor C1q. Pentraxin-3, an IL-1β–inducible gene (11), was also highly overexpressed, and being an acute phase protein, it likely contributes to the high sedimentation rate of red blook cells in SoJIA. Some genes that were up-regulated by the SoJIA sera were not increased in steady-state mRNA from PBMCs of the 16 patients, including several chemokines and fibronectin, casting doubt on the relevance of the high expression level these genes have in serum studies. Another potentially relevant gene overexpressed in SoJIA PBMCs is a potassium channel gene *KCNJ15*. As discussed above, influx of potassium ions is a trigger for activation of caspase-1 and secretion of IL-1β in response to activation of the nucleotide receptor P2X7.

Measurement of circulating IL-1β is not a reliable indicator of a role for this cytokine in disease, nor does it provide rationale for selection of a therapeutic intervention such as IL-1Ra. IL-1β is a highly active cytokine in humans; injecting a few nanograms per kilogram results in fever, neutrophilia, thrombocytosis, acute phase proteins, and circulating IL-6 (for review see reference 12). Thus, circulating levels of IL-1β in the picomolar range may easily escape detection by routine ELISAs or similar methods. Although there are numerous reports that circulating cytokine levels correlate with severity in a variety of diseases, it is only specific blockade or neutralization of a cytokine that provides a convincing case for causation. For this reason, the study by Pascual et al. is compelling (7). It is a general concept that IL-1–mediated disease severity is regulated at the level of ligand production and activity, and not at the receptor level. For example, IL-1 type I receptors are expressed on all cells in healthy individuals and increases of only two- or threefold occur in disease. On the other hand, in circulating monocytes and bone marrow macrophages from healthy individuals, IL-1β gene expression is absent but increases at least 100-fold when stimulated

with microbial products or inflammatory molecules, including products of activated T cells. The total amount of IL-1β precursor that is synthesized, however, does not necessarily equate to the amount of active IL-1β that is produced, as the caspase-1–dependent conversion of IL-1β precursor to an active secreted cytokine is a tightly controlled event, despite the presence of constitutive procaspase-1 in the same cell. Hence, the increase in IL-1β secretion from PBMCs of SoJIA patients is a highly relevant observation.

The study by Pascual and coworkers provides evidence for increased secretion of IL-1β by freshly cultured PBMCs from these patients compared with PBMCs from healthy subjects (7). In the absence of exogenous stimulation, cultured PBMCs from healthy subjects do not release IL-1β, but upon stimulation synthesize the IL-1β precursor and release the processed form of IL-1β into the supernatant. However, more than 50% of the total IL-1β precursor synthesized remains inside monocytes from healthy donors. The amount of IL-1β released from PBMCs of five SoJIA patients was more than 10-fold greater than five healthy controls (7). In the same cultures, the release of TNF and IL-6 was similar for healthy and affected subjects, suggesting that the elevated release of IL-1β was not due to increased monocyte numbers or increased activation of SoJIA PBMCs. Unfortunately, IL-1β secretion was induced using the unorthodox combination of PMA plus ionomycin, whereas most studies of dysfunctional IL-1β release use TLR agonists. Nevertheless, the disease-related increase in IL-1β secretion may explain the role for IL-1 and the responses to IL-1Ra in these patients who failed to respond to conventional treatments.

Secretion of IL-1β in other inflammatory diseases: a unifying mechanism?

Increased secretion of IL-1β from cultured PBMCs has also been reported for a growing number of inherited, chronic autoinflammatory syndromes, each of which responds to IL-1Ra (13–16). In these syndromes, increased secretion of IL-1β is due to a single amino acid mutation in the *NALP-3* gene, which controls the activation of caspase-1 found in the IL-1β inflammasome (17). Similar to the situation in SoJIA, circulating levels of IL-1β are not detected in these patients (13) but increased secretion of IL-1β is observed in vitro. The single point mutation in the *NALP-3* gene causes the loss of tight control of IL-1β processing. As a result, relatively minor stresses such as exposure to cold results in increased secretion of IL-1β with consequent systemic disease (13). It appears that the *NALP-3* gene provides an important roadblock to control the secretion of IL-1β and raises the issue of a defective roadblock in SoJIA. Although the reason for the increased secretion of IL-1β in the SoJIA patients remains unclear, both SoJIA patients and patients with *NALP-3* mutations share the phenotype of systemic disease and increased secretion of processed IL-1β in vitro. There are chronic inflammatory syndromes without mutations in the *NALP-3* gene but nevertheless elevated IL-1β release in vitro and also respond to IL-1Ra therapy; presently, these are mutation-negative neonatal onset multisystem inflammatory disease (NOMID; reference 18), pyogenic arthritis, pyoderma gangrenosum, acne syndrome (PAPA; reference 19), and familial Mediterranean fever (FMF; reference 20). Both PAPA and FMF are genetic diseases associated with the intracellular protein pyrin,

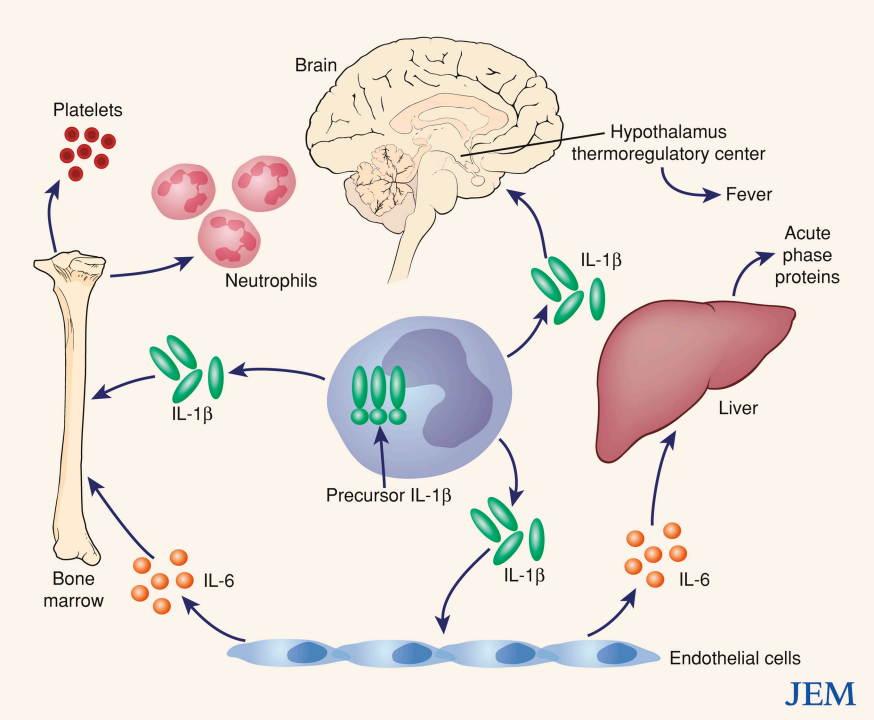

Figure 2. Systemic manifestations of IL-1β. Active IL-1β is secreted by many cell types including monocytes and macrophages (center). IL-1β enters the circulation and triggers IL-1 receptors on the hypothalamic vascular network resulting in synthesis of cyclooxygenase-2, which causes brain levels of prostaglandin E2 to rise, thus activating the thermoregulatory center for fever production (reference 27). In the periphery, IL-1β activates IL-1 receptors on the endothelium resulting in rashes and the production of IL-6. Circulating IL-6 stimulates liver hepatocytes to synthesize several acute phase proteins, which accounts for the increase in erythrocyte sedimentation rate in SoJIA. IL-1 also acts on the bone marrow to increase mobilization of granulocyte progenitors and mature neutrophils, resulting in peripheral neutrophilia. IL-1–induced IL-6 increases platelet production, which results in thrombocytosis. IL-1 also causes decreased response to erythropoietin, which causes anemia.

which participates in maintaining procaspase-1 as an inactive enzyme. Mutations of the *Pyrin* gene in mice, similar to those found in humans with FMF, result in increased caspase-1 activity and increased secretion of IL-1β (21).

Although these systemic, multisystem syndromes are not common diseases, they reveal a fundamental role for IL-1 in systemic inflammation regardless of the cause. As shown in Fig. 2, IL-1 affects several targets that account for the manifestations of systemic disease. These are recurrent fevers, neutrophilia, thrombocytosis, elevated serum amyloid A and C–reactive protein, and anemia. Skin rashes and urticaria are also common. Hearing loss, developmental delay, and aseptic meningitis can also be observed in childhood. The endothelium is a prime target for IL-1–mediated inflammation as IL-1 receptors on the endothelium trigger prostaglandin E production, cause bone marrow release of neutrophils, and induce the production of IL-6. In fact, IL-1 induction of IL-6 accounts for hepatic acute phase protein synthesis and the thrombocytosis. The finetuning of IL-1–mediated inflammation is revealed upon stopping and restarting IL-1Ra. Upon cessation of IL-1Ra therapy, clinical signs and symptoms of disease as well as biochemical and hematological abnormalities rebound within days and resolve upon resumption of IL-1 receptor blockade (10, 14–16). In the Pascual study, IL-1Ra therapy was temporarily withdrawn in two patients due to viral-like illness, during which time the erythrocyte sedimentation rate increased, only to fall upon restarting therapy (7).

Although SoJIA clearly fits with the clinical, hematological, and biochemical manifestations of the autosomal dominant autoinflammatory syndromes, and although SoJIA shares with these inherited diseases increased secretion of IL-1β, no genetic mutations have yet been described in SoJIA patients that relate to IL-1β secretion or any other genetic marker. However, to our knowledge no systematic examination of gene defects in the components of the IL-1β inflammasome has been made in SoJIA. There is no clear pattern of inheritance in SoJIA, and like other autoimmune diseases, the incidence is greater in families but without identification of a specific gene or mutation.

It is likely that other agents that prevent IL-1β–mediated disease will be effective in SoJIA. These are the IL-1 Trap, IL-1β–specific monoclonal antibodies, IL-1 receptor type I–specific monoclonal antibodies, and the caspase-1 inhibitor (22). It is also possible that agents that inhibit the nucleotide receptor P2X7 will reduce IL-1β–mediated disease (23). If SoJIA is due to dysfunctional control of caspase-1 activity or the P2X7 receptor, treatment options may be targeted to the mechanism of IL-1β secretion. It is a general principal in therapeutics to target the most distal mechanism of a disease process. Several levels of control of the synthesis, processing, and secretion of IL-1β have evolved, and one may assume that these function to limit inflammation. Moreover, once released, IL-1β must contend with competition for receptor occupancy with the naturally occurring IL-1Ra, the binding and neutralization by the IL-1 type II decoy receptor (24), and the formation of inactive complexes with constitutively secreted soluble IL-1 receptor accessory protein (25, 26), each of which also limit IL-1β responses. Although increased secretion of IL-1β may account for IL-1 activity in SoJIA, loss of control of these additional mechanisms may also be disrupted in SoJIA.

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