The Autoinflammatory Side of Systemic Sclerosis

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The recent history of autoimmunity in general and rheumatological disease in particular has brought a significant number of major paradigm shifts, as represented by the wave of genome-wide association studies and new biotechnological treatments. The study of Martìnez-Godinez and colleagues [1] in this issue of *IMAJ* follows this direction and confirms the role of innate immunity and the inflammasome in systemic sclerosis (SSc). Indeed, the data presented support the NLRP3 over-expression in the SSc skin, as previously reported by others [2]. The present article, nevertheless, also identified a consequent increase in caspase-1, interleukin (IL)-1-β and IL-18, all downstream of the inflammasome activation. Interestingly, the inflammasome over-expression together with endothelin 1 (ET-1) over-expression has been found in both limited and diffuse SSc, suggesting a role in the disease-associated fibrosis of both the inflammasome and the vascular mediator ET-1.

From a clinical standpoint, SSc remains a challenging disease because available treatments are largely unsatisfactory, particularly once fibrosis of the skin or the lungs is established. Similarly, there is an unmet need for molecules capable of counteracting the progression of inflammation and vascular damage that also characterize the disease [3].

Inflammasomes are large cytoplasmic macromolecular signaling complexes that control the proteolytic activation of two highly pro-inflammatory interleukin (IL)-1 family cytokines, IL-1β and IL-18, under different danger signals with the purpose of host defense [4]. Several NOD-like receptor (NLR) family members assemble into the inflammasome complexes, among which NLRP3 remains the best studied and fully characterized inflammasome [5]. It consists of a basic NLR scaffold with a central nucleotide-binding and oligomerization (NACHT) domain, which is flanked by C-terminal leucine-rich repeats (LRRs) and N-terminal caspase recruitment and activation (CARD) or pyrin (PYD) domains, the adaptor molecule apoptosis-associated speck-like protein (ASC) and caspase-1. LRRs are believed to mediate the recognition of diverse ligands, whereas CARD and PYD domains are involved in the interaction with downstream signaling proteins [5]. Several pathogens and endogenous damage-associated molecular patterns (DAMPs) activate the NLRP3 inflammasome. The activation of the NLRP3 inflammasome requires two separate steps. The first signal, which can derive from Toll-like receptors (TLRs), tumor necrosis factor receptor (TNFR) or interleukin 1 receptor (IL-1R) signaling, activates NF-κB for the transcription and translation of the immature proforms of IL-1β and IL-18. As a second step, enzymatic cleavage is needed to secrete these pro-inflammatory interleukins into the extracellular space. This requires the oligomerization of NLRP3 with ASC, which facilitates proteolytic cleavage of pro-caspase-1 to its active caspase-1 (also called IL-1β converting enzyme, ICE). In addition, once activated, caspase-1 induces its own secretion. Caspase-1 then cleaves the pro-cytokines to mature IL-1β and IL-18 that can be secreted. Although the exact mechanism of second-signal NLRP3 activation is unknown, three distinct models have been proposed to account for NLRP3 activation: potassium efflux, the generation of reactive oxygen species, and phagolysosomal destabilization leading to cathepsin leakage into the cytosol [6].

Although NLRP3 inflammasome activation is critical to drive acute inflammation in the clearance of viral or bacterial infections, persistent activation of NLRP3 may be involved in the progression of several chronic fibrotic diseases, including pulmonary, kidney, heart and liver fibrotic diseases. Moreover, agents blocking inflammasomes, such as pirfenidone [7] and naclynamide [8], may be effective in counteracting fibrogenesis.

As previously mentioned, the most studied products of the activated inflammasome are IL-1β and IL-18, which are structurally similar and are involved in autocrine and paracrine signaling targeting parasitic, bacterial or viral infections, or in systemic inflammation as a result of chronic disease. IL-1β is produced by many cell types, e.g., epithelial cells, fibroblasts, T cells, etc., in response to pathogens or damaged tissues. IL-1β can modulate the expression of its own mRNA via signaling mediated by the IL-1 receptor, and the signaling pathways involved induce increased p38 MAPK phosphorylation and NF-κB activity. IL-1β requires two signals for the protein secretion; the first signal is usually obtained via TLR or IL-1R signaling and up-regulates IL-1β gene transcription, and the second signal is obtained via inflammasome activation, cleaving caspase-1 leading to IL-1β maturation and secretion. The acute release of IL-1β into the peripheral blood in response to cellular stimuli up-regulates

the expression of other pro-inflammatory cytokines such as IL-6 [9].

The effects of IL-18 (previously called interferon-gamma inducing factor) remain unclear, but, unlike IL-1β, IL-18 is constitutively expressed in its precursor form in many cells and only requires caspase-1 activation for its cleavage and secretion. Similar to IL-1β, on the other hand, IL-18 can modulate the expression of its own RNA, and its signal transduction is similar to that of IL-1β via NF-κB activity. IL-18 can activate T cells, resulting in the increased synthesis of tumor necrosis factor-alpha (TNFα), IL-2, and granulocyte macrophage-colony stimulating factor (GM-CSF), and polarizes the Th1 response by suppressing IL-4 [9].

The mechanisms by which the activated inflammasome may in some cases induce fibrosis instead of a self-limiting acute response remains enigmatic. Nevertheless, both IL-1β and IL-8 are involved in fibrogenesis. Indeed, it has been demonstrated that a short stimulation with IL-1β and transforming growth factor-beta 1 (TGFβ1) results in the inhibition of Smad3 phosphorylation and further inhibits downstream TGFβ1 signaling pathways. However, a longer exposure (24 hours) increases Smad3 phosphorylation and induces TGFβ1 expression [10]. In addition, prolonged exposure of microvascular endothelial cells to IL-1β can induce the permanent transformation of these cells to myofibroblasts [11]; and once differentiated, myofibroblasts synthesize large amounts of collagen, rendering a functional inflammasome. Since IL-1β can induce its own gene expression, chronic activation of the inflammasome, resulting in the continual cleavage of IL-1β in a positive feedback mechanism, could conceivably maintain an elevated level of active TGFβ1 protein, resulting in fibrosis. IL-18 has also been shown to be profibrotic. In vitro studies demonstrate a dose-dependent increase in type I and III collagen proteins and α2β1 integrin, one of the major collagen receptors with recombinant IL-18 [12]. Moreover, IL-18 was also shown to induce fibroblast proliferation [12]. Contrasting data have also been reported [13]. Interestingly, the direct targeting of IL-18 by the compound felodipine reduced perivascular fibrosis [14]. Calcium channel signaling has been shown to be important for inflammasome activation; therefore, the reduced secretion of IL-18 may be directly due to the inhibition of the inflammasome by this calcium channel blocker, thus suggesting an anti-fibrotic role of these compounds commonly used in the treatment of Raynaud's phenomenon.

The first report linking inflammasome to SSc-associated fibrosis was obtained in an animal model with the inflammasome and IL-1β found to be linked to bleomycin-induced lung fibrosis, an animal model of SSc lung disease [15]. In this setting, mice that are IL-1R1, ASC or MyD88 deficient manifest an abrogated fibrotic response to bleomycin or a benefit from IL-1R antagonist [15]. More recently, the up-regulation of several genes of the inflammasome platforms, e.g., AIM2 and NLRP3 [2], was observed in the skin and lung fibroblasts of patients with scleroderma. Furthermore, caspase-1 activity was found to be up-regulated in SSc fibroblasts, and lesional fibroblasts secreted more IL-1β and IL-18. This secretion could be abrogated with chemical or siRNA inhibition of caspase-1. These data suggest that the release of IL-1β and IL-18 is mediated by an inflammasome activation mechanism that drives fibrosis. The present report by Martìnez-Godinez et al. confirms the over-expression of NLRP3 inflammasome, IL-1β and IL-18 in scleroderma skin. Moreover, they found a similar over-expression of inflammasome and related cytokines in both subtypes of the disease, the limited and diffuse forms. These further demonstrate a correlation with the severity of skin involvement and with TGFβ and ET-1. Although data regarding the possible anti-fibrotic effect of endothelin inhibitors in clinical trial are negative or modest, ET-1 is believed to be pivotal not only in scleroderma vasculopathy, but also in scleroderma fibrosis, as plasma levels and skin protein expression of ET-1 are elevated in SSc [16,17]. Moreover, constitutive autocrine over-production of ET-1 in SSc lung fibroblasts occurs [18]. Several lines of evidence indicate that ET-1, either alone or together with TGFβ, induces a fibrotic phenotype in cultured fibroblasts [19]: i.e., fibroblasts exposed to recombinant ET-1 exhibit elevated production of extracellular matrix and CTGF while a transgenic mouse in which ET-1 is over-expressed in lungs results in the development of a progressive pulmonary fibrosis. Further, TGFβ induces ET-1, and blockade of the ET receptors impairs the ability of TGFβ to induce the expression of mRNAs encoding fibrogenic proteins, and blocking the ETA and ETB receptors with bosentan also impairs TGFβ-induced skin fibrogenesis in vivo. Ultimately, TGFβ and ET-1 appear to have a multiplying effect on their fibrogenic effects.

In view of the available evidence, we are convinced that a chronically activated inflammasome mediates the continuous release of IL-1β and IL-18 in SSc, thus inducing a specific autocrine signaling in fibroblasts and sustaining the profibrotic phenotype. Moreover, IL-1β, ET-1 and TGFβ exert a synergistic effect, promoting further secretion of collagen. Understanding the role of the NLRP3 inflammasome in SSc has a crucial role in the development of novel targets for treating this currently untreatable disease [20].

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