NLRP3 inflammasome activation: CD36 serves double duty

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Studies have linked the NLRP3 inflammasome pathway to the elaboration of sterile inflammation. CD36 serves a dual role by priming transcription of the gene encoding interleukin 1β (IL-1β) and inducing assembly of the NLRP3 inflammasome complex, which leads to the release of active IL-1β.

Studies of innate immune responses in mammals have focused mainly on the roles of various pattern-recognition receptors (PRRs) in responding to microorganisms or their products. As has been postulated[1], the pressure that drove the evolution of modern innate immune systems was probably the need to prevent microbial colonization. PRRs such as the Toll-like receptors (TLRs), RIG-I–like receptors, Nod-like receptors (NLRs) and various C-type lectin receptors evolved to detect microbes in germ-free tissues of human bodies[2]. It is therefore logical that most studies of PRR function have focused on their role in infection. In addition to the evolutionary implications of PRRs in the context of infection, ample clinical evidence supports the idea that defects in TLR function predispose humans to microbial infections that 'wild-type' humans control. In contrast, a new study by Sheedy *et al.* shows that the scavenger receptor CD36, through the recognition and uptake of endogenous soluble ligands (including oxidized low-density lipoprotein (LDL), amyloid-β peptide and amylin peptide), delivers two signals that lead to activation of the NLRP3 inflammasome in germ-free settings of 'sterile' inflammation[3].

An interesting outcome of research on the role of PRRs in controlling infection has been the appreciation that PRRs also contribute to noninfectious maladies. Although they are not caused by microbial infections, such diseases are nevertheless associated with so-called 'sterile' inflammatory responses that contribute to disease. For example, PRR-induced inflammation seems to underlie the symptoms associated with atherosclerosis, Alzheimer's disease and type 2 diabetes. Although such studies have emphasized the clinical importance of PRRs in noninfectious disorders, very little is known about the underlying mechanisms by which PRRs operate in this context. Among the PRRs linked to sterile inflammation, the NLRs seem to have attracted the most attention[4,5]. Most members of the NLR family are unusual PRRs in that they do not function to upregulate the transcription of genes encoding inflammatory cytokines, chemokines or interferons. Rather than regulating the expression of cytokine-encoding genes, many NLRs trigger secretion of cytokines of the interleukin 1 (IL-1) family. The secretion of IL-1 is achieved via the ability of NLRs to assemble cytoplasmic protein complexes called 'inflammasomes'. The inflammasome is a protein-processing machine that uses proteases to cleave precursor ('pro-') forms of IL-1 in the cytosol, which are then somehow secreted to induce inflammation. Because NLRs cannot activate transcription, yet they promote the secretion of inducible cytokines, NLRs usually depend on other PRRs to induce cytokine expression. Thus, present models of infection-induced immune responses mediated by the IL-1 family propose that two signals are necessary. Signal 1 is provided by a transcription-inducing PRR (such as a TLR), which upregulates expression of members of the IL-1 family and some NLRs themselves. Signal 2 is then provided that activates the NLR to assemble an inflammasome and cause the secretion of proinflammatory members of the IL-1 family[6]. In an interesting turn of events, Sheedy *et al.* now report that the scavenger receptor CD36 can provide both signal 1 and signal 2 to promote inflammasome activation by sterile stimuli[3]. This finding helps explain the importance of CD36 in atherosclerosis[7] and identifies a previously unknown means by which the innate immune system can be activated differently by microbes or endogenous triggers.

Sheedy *et al.* focus their attention of CD36 and its role in atherosclerosis[3] because their previously published work demonstrated that this scavenger receptor promotes the activation of TLR-dependent innate immune responses to oxidized LDL (oxLDL), the critical inflammatory trigger in atherosclerotic plaques[8]. Interestingly, that study showed that oxLDL induces the assembly of an unusual dimer of TLR4 and TLR6 that is responsible for activating the expression of inflammatory cytokines. Those data are considered in the context of two additional pieces of evidence to justify their investigation of the role of CD36 in activation of the inflammasome. First, as stated above, TLR-dependent signals augment activation of the NLRP3 inflammasome by inducing expression of *Il1b* and *Nlrp3*. The finding that CD36 triggers the TLR4-TLR6–dependent expression of cytokines in response to oxLDL suggests that CD36 might facilitate inflammasome activation. Second, CD36 and other scavenger receptors bind to and mediate the internalization of a range of cargo of both microbial and endogenous origin[9,10], much of which has been shown to activate the NLRP3 inflammasome.

Through the use of a classic microscopic approach, the authors make the intriguing finding that CD36 captures soluble oxLDL and delivers this molecule to lysosomes, where it is converted into a crystalline substance[3]. LDL crystals then somehow destabilize lysosomes, which causes them to release their contents into the cytosol. These experiments also show that the oxLDL-capturing activity of CD36 is independent of its ability to induce TLR4-TLR6–dependent expression of cytokines. In these studies, CD36 is required for the formation of intracellular LDL crystals, but cells lacking TLR4 or TLR6 are not. Those findings are congruent with published work demonstrating that lysosomal rupture can be a cell-intrinsic trigger of NLRP3 activation[11]. Consistent with that idea, the authors find that oxLDL is able to induce the aggregation of ASC (a component of the NLRP3 inflammasome) in the cytosol of macrophages[3]. As ASC aggregation is a marker of activation of the NLRP3 inflammasome, these data provide compelling evidence that oxLDL on its own can induce formation of the NLRP3 inflammasome. Thus, it becomes clear that CD36 has distinct activities that could be considered as activators of signals 1 and 2 in mediating inflammasome-dependent cytokine secretion (**Fig. 1**). CD36 is able to recognize oxLDL and somehow activate

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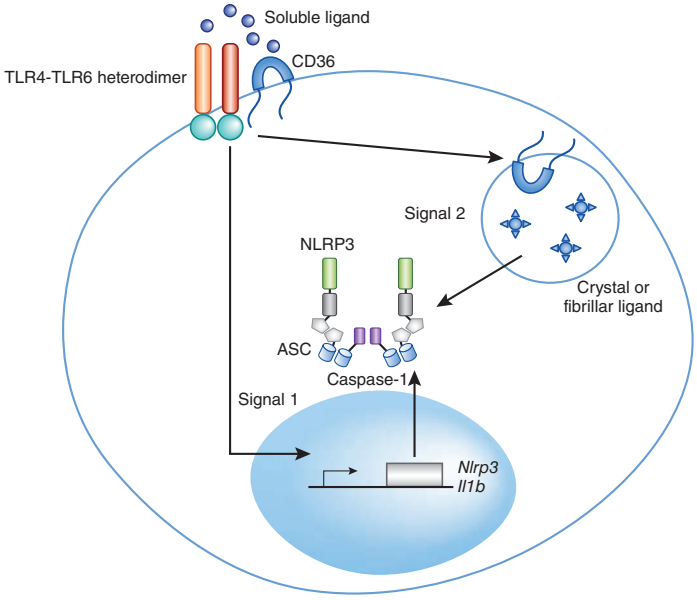

Figure 1 The role of CD36 in activating the NLRP3 inflammasome in the absence of infection. CD36 and the TLR4-TLR6 heterodimer recognize oxLDL, which accumulates in atherosclerotic lesions. That interaction initiates a signaling pathway that leads to transcriptional upregulation of NLRP3 and pro-IL-1β (signal 1). CD36 also mediates the internalization of oxLDL into the lysosomal compartment, where the ligands are converted into crystals that induce lysosomal rupture and activation of the NLRP3 inflammasome (signal 2).

cytokine expression mediated by TLR4 and TLR6 (signal 1). CD36 is also able to internalize oxLDL into lysosomes, where it becomes a crystal that ruptures the lysosomal membranes (signal 2). The authors provide convincing genetic evidence to support that idea by the demonstration that CD36, TLR4 and TLR6 are each essential for functions of the NLRP3 inflammasome in response to oxLDL[3]. Thus, CD36 can use its abilities as a TLR activator and an endocytosis receptor to serve as an 'all-purpose' activator of the NLRP3 inflammasome, in the absence of microbial stimuli (**Fig. 1**).

The findings of Sheedy *et al.*, substantiated by several complementary experiments with animal models[3], highlight a critical distinction between infectious and endogenous activators of the NLRP3 inflammasome. The ability of CD36 to induce both signal 1 and signal 2 probably explains its role in sterile inflammation that results from atherosclerosis and may also explain its role in other sterile inflammatory responses. Although the role of CD36 in such diseases may be shared, the ligands that activate CD36-dependent responses are not. For example, Sheedy *et al.* nicely demonstrate that CD36 serves a similar function in activation of the NLRP3 inflammasome by triggers of Alzheimer's disease (soluble amyloid-β peptides) and type 2 diabetes (amyloid-containing amylin–islet amyloid polypeptides)[3]. It is also noteworthy that CD36 is able to direct soluble cargo (such as oxLDL and prefibrillar amyloid-β) to the lysosomal compartment, where subsequent crystallization leads to lysosomal destabilization, as the soluble forms are thought to make critical contributions to disease etiology. Future work should focus on elucidating how CD36 is able to capture those seemingly diverse soluble ligands from the extracellular space, perhaps in concert with different coreceptors that can influence cargo trafficking and signaling and thus activation of the NLRP3 inflammasome. Another area of enquiry that is now appropriate revolves around the question of exactly how lysosomal rupture activates NLRP3. Various models for this have been proposed, including release of lysosomal cathepsins into the cytosol[11,12] and Ca^{2+} mobilization[13,14]. Interestingly, in the latter context, CD36 signaling has been linked to activation of Src kinases and phospholipase C proteins that may regulate such Ca^{2+} mobilization. Alternatively, CD36-mediated destabilization of lysosomes itself is sufficient for Ca^{2+} mobilization and the associated production of mitochondrial reactive oxygen species, which may promote inflammasome activation[13,14]. Finally, given the diverse ligand-binding specificity and phagocytic ability of other scavenger receptors[9,10], it would be interesting to determine if they can function similarly to CD36 in activating the NLRP3 inflammasome and thus extend the model proposed here whereby the activity of an intracellular PRR is contingent on a cell surface–resident, endocytic receptor. Conceivably, in some such cases, the critical activity of the scavenger receptor is to deliver the internalized ligand to the cytosolic compartment (rather than to enable crystallization and lysosomal destabilization), especially for those molecules that do not form crystals.

Finally, there is the question of evolutionary origin. Did CD36 evolve to activate TLR4-TLR6 dimers and induce NLRP3 activation after lysosomal rupture? Or is NLRP3 activation simply a byproduct of scavenger receptor activity that captures ligands that happen to form crystals in lysosomes? A scenario could be imagined in which CD36 evolved to link microbial products (such as bacterial lipoproteins) to TLRs as a means of promoting inflammation in response to infection. CD36 may also have evolved to capture self molecules that might indicate tissue damage or dysfunction (such as oxLDL). Both of those activities would serve the body well, helping to rid it of infections or restore tissue homeostasis. However, in the case of sterile tissue damage, CD36 seems to be doing more harm than good in that it promotes the inflammatory symptoms associated with several human diseases. In this context, it remains unresolved whether CD36 evolved to try to do a good thing (clear dangerous molecules from circulation) but ended up causing more trouble than it is worth (promoting inflammasome activation and causing pathology). The real question then becomes the following: does CD36 'know' that the ligands it internalizes will ultimately form crystals and activate NLRP3 inflammasomes? If CD36 does 'know' that the ligands it captures will form crystals in lysosomes, then the question remains of how the crystal-forming potential of soluble ligands for CD36 is determined. Regardless of the answer to that question, it is clear from this study that CD36 is a major participant in controlling NLRP3 activation in response to molecules involved in several human diseases. Sheedy *et al.* therefore not only have provided important insights into the diverse means by which inflammasomes can be activated but also have identified CD36 as an potential target for therapeutic intervention[3].

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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The IL-20 cytokine subfamily: bad guys in host defense?

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Resistance to infection of the skin with *Staphylococcus aureus* depends on early production of interleukin 1β (IL-1β) and IL-17A by skin-resident cells. However, several members of the IL-20 subfamily of cytokines (IL-19, IL-20 and IL-24) can inhibit the local generation of those two cytokines.

Staphylococcus aureus is a major human pathogen that is the most frequent cause of superficial and invasive skin infections. It also participates in the pathogenesis of skin diseases such as atopic dermatitis. Systemic *S. aureus* can cause life-threatening infection of the blood, lungs, bone, meninges and heart and is the leading cause of hospital-acquired infection. The high prevalence of *S. aureus* that has become resistant to treatment by most antibiotics (that is, methicillin-resistant *S. aureus*) has increased the need for alternative therapies such as vaccination. Most vaccination strategies against *S. aureus* have focused on generating humoral responses to surface proteins and have produced largely disappointing results in clinical trials[1]. Adaptive responses involving the T_H17 subset of helper T cells develop after exposure to *S. aureus*. Studies of mice have found that protective immunity to *S. aureus* is associated with the generation of T_H17 responses[1]. In addition, human patients with mutations in the gene encoding the transcription factor STAT3 that result in defective T_H17 differentiation develop autosomal dominant hyper-IgE syndrome (Job's syndrome, named after the biblical character Job, whose faith was tested with recurrent boils) and suffer recurrent infection of the skin with *S. aureus*[2]. Notably, it is now appreciated that resistance to primary infection of the skin with *S. aureus* critically depends on early production of interleukin 1β (IL-1β) and IL-17A by skin-resident cells[3,4]. Those cytokines initiate an inflammatory cascade that recruits neutrophils, the key effector cells responsible for abscess formation and the clearance of *S. aureus*. In this issue of *Nature Immunology*, Myles *et al.* demonstrate that several members of the IL-20 subfamily of cytokines—specifically, IL-19, IL-20 and IL-24—inhibit the local generation of IL-1β and IL-17A during infection with *S. aureus*[5]. This results in greater severity of infection and identifies an unexpected pathway that suppresses immune responses to *S. aureus*. Targeting this pathway may provide new therapies for the treatment of antibiotic-resistant *S. aureus*.

The IL-20 cytokine subfamily is part of the larger IL-10 cytokine family and includes IL-19, IL-20, IL-22, IL-24 and IL-26. IL-19, IL-20 and IL-24 are produced by myeloid and epithelial cells and signal mainly through a heterodimer of the α- and β-chains of the IL-20 receptor (IL-20R) expressed on epithelial cells. Engagement of that receptor activates STAT3 and promotes wound healing, epithelial proliferation and elaboration of antimicrobial peptides[6]. Those functions are similar to the better studied cytokine IL-22 that is required for resistance to infection with *Citrobacter rodentium* in the gut and participates in driving keratinocyte proliferation in skin lesions of psoriasis[7]. Although certain members of the IL-20 family have also been epidemiologically and mechanistically associated with atopic dermatitis and psoriasis in humans and mice, the present data by Myles *et al.* provide the first evidence that these relatively little studied cytokines also have an important role in the context of infection[5].

To explore whether IL-19, IL-20 and IL-24 participate in host defense, the authors introduce *S. aureus* into the skin of IL-20R-deficient mice by intradermal injection. Contrary to expectations, those mice develop smaller lesions with fewer colony-forming units than those of wild-type or *Il22*$^{-/-}$ mice. Consistent with those observations, production of IL-19 and IL-24 and expression of IL-20R is greater in the skin of wild-type mice shortly after infection. Coinjection of *S. aureus* and recombinant IL-19 or recombinant IL-20 results in greater infection severity associated with the recruitment of fewer neutrophils into the infection site. Because the production of IL-17A by dermal γδ T cells is important for the recruitment of neutrophils and resistance to infection with *S. aureus*[4], the authors determine whether this cell type could be affected by IL-20R signaling. Indeed, coinjection of recombinant IL-19 and recombinant IL-20 suppresses the production of IL-17A by dermal γδ T cells and the production of defensin-β4, an IL-17-dependent antimicrobial peptide.

IL-20R is not expressed by γδ T cells, which indicates that the effect must be indirect. The induction of IL-17 production in γδ T cells depends on IL-1β and IL-23 and, to a lesser extent, IL-6 (which is more important for T_H17 differentiation). The abundance of all three cytokines increases in the skin within hours of *S. aureus* infection, an effect suppressed by coinjection of recombinant IL-19 or recombinant IL-20. IL-20R-deficient mice have higher expression of pro-IL-1β and, to a lesser extent IL-23, than do wild-type mice. To investigate IL-20R-mediated regulation of IL-1β, the authors switch to an *in vitro* system. Mouse keratinocytes exposed to *S. aureus* have a greater abundance of mRNA encoding pro-IL-1β, as well as of the fully processed protein, and both effects are suppressed by the presence of recombinant IL-19 or recombinant IL-20. Expression of IL-1β-inducing proteins, such as TLR2 or IL-1 receptors, is not altered in

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