and astrocytes, both of which suggest a state of exaggerated inflammation that at present is difficult to reconcile with a requirement for granulin in innate immune responses and particularly proinflammatory cytokine production. Survival during certain viral infections (notably infection by mouse cytomegalovirus and other herpesviruses) is strongly dependent on TLR9 signaling and may be taken as a sensitive indicator of TLR9 function. The conclusion that granulin is a molecule of central importance in signaling viral invasion thus awaits further testing.

Taken together, the available data suggest a hypothetical model in which secreted granulin encounters and binds to CpG-ODN, either extracellularly or (more probably) in phagosomes (Figure 1). Sortilin may function as the receptor for granulin, constitutively transporting it to endolysosomes. When bound to CpG-ODN or perhaps other TLR9 ligands, granulin may serve to concentrate ligand for optimal efficiency of receptor activation, as well as act as a cofactor for TLR9 binding and activation. Future studies will undoubtedly clarify the role of granulin in TLR9 signaling.

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Getting Closer to the Dirty Little Secret

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The molecular mechanism behind alum adjuvanticity is probably the oldest secret of immunology. In this issue of *Immunity*, Kuroda et al. (2011) and Kool et al. (2011) identify NLRP3 inflammasome-independent signaling to be crucial for the Th2 cell response induced by aluminum salts.

Edward Jenner's vaccination against smallpox in 1789 is the first and still most dramatic record of a successful manipulation of the immune system. Early trials of vaccine development revealed that the efficiency of vaccines depends on the presence of so-called adjuvants (Latin *adiuvare*, to help) in conjunction with the antigen. However, it took 200 years of research until Charles Janeway proposed the immunologic function of these little helpers—to stimulate the innate immune system. He suggested that adaptive immunity was not raised until the innate immune system provided clear evidence for the presence of pathogens—or for danger, as later extended by Polly Matzinger. The concept of PAMPs (pathogen-associated molecular patterns) and DAMPs (danger-associated molecular patterns) triggering innate PRRs (pattern recognition receptors) was born. The signals triggered by PAMPs and DAMPs strongly determine the type of adaptive immunity, ensuring an effective clearance of infection or appropriate inflammatory responses to sterile tissue damage. The most commonly used adjuvant in humans is alum. It induces so-called type 2 immune responses characterized by eosinophilia and production of IL-4, IgE, and IgG1. Although the discovery of alum's adjuvanticity dates back to 1926, the underlying molecular mechanism is still a matter of debate.

The discovery that the NLRP3 inflammasome senses particulates including monosodium urate (MSU) crystals (Martinon et al., 2006), silica, and asbestos, as well as alum (Dostert et al., 2008; Hornung et al., 2008) suggested a plausible mechanism for alum's effect as an adjuvant. Cellular uptake of particulates leads to reactive oxygen production and can inflict lysosomal damage. Both of these effects were suggested to act upstream in the activation of NLRP3 (Dostert et al., 2008; Hornung et al., 2008). Aluminum

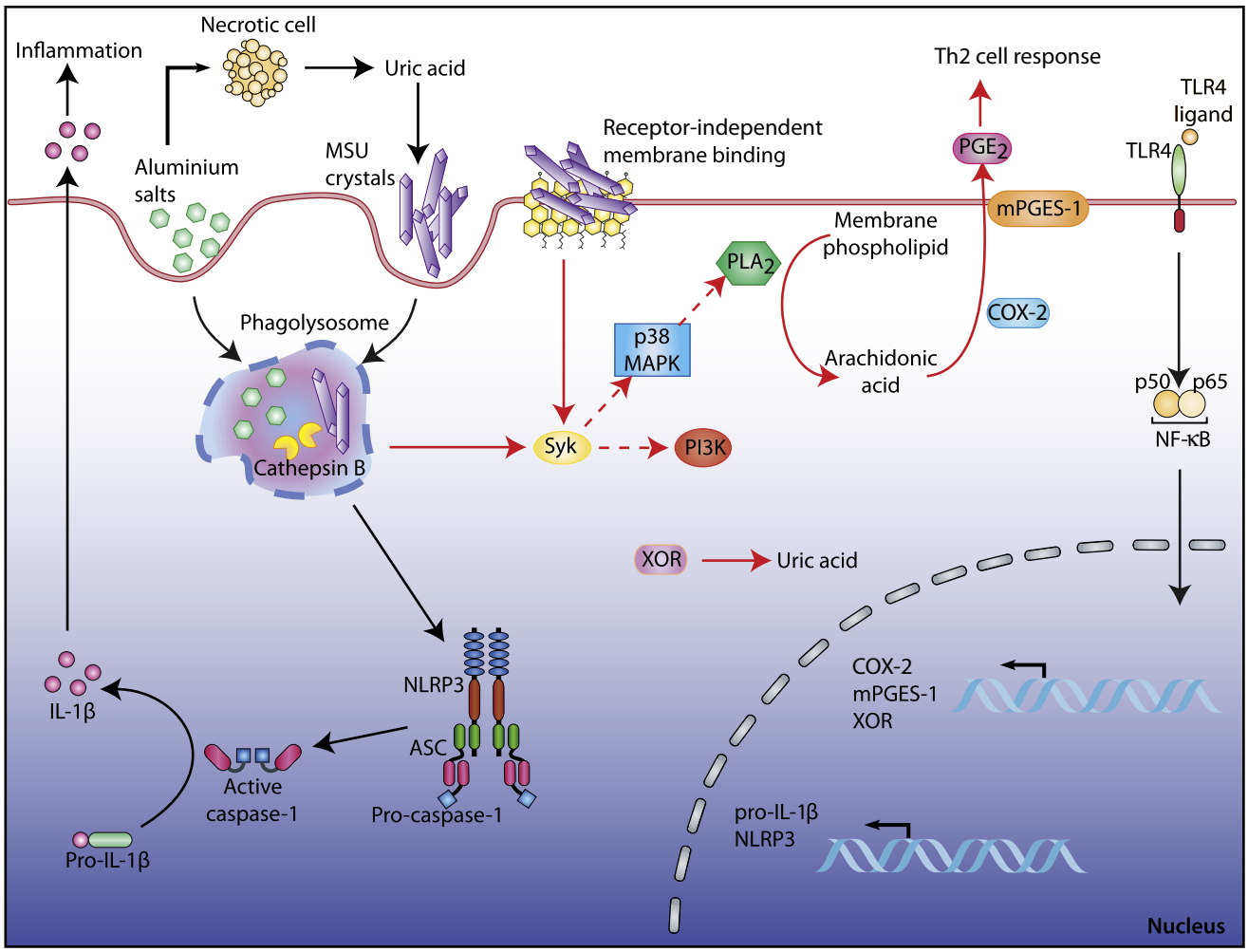

Figure 1. Mechanisms of Particulate-Induced Immunity: An Updated Model

Upon phagocytosis, particulates like aluminum salts or monosodium urate crystals (MSU) cause lysosomal damage. This initiates at least two separate signaling pathways. First is the activation of the NLRP3 inflammasome resulting from the release of enzymes like cathepsin B into the cytoplasm. Autocatalytically activated caspase-1 mediates the proteolytic cleavage of pro-IL-1β in biologically active IL-1β, accounting for the inflammatory response to particulates. Second is the activation of Syk, which in turn activates cytosolic phospholipase A2 (cPLA2), probably via p38 MAP kinase, resulting in the release of arachidonic acid from membrane lipids. Cyclooxygenase-2 (COX-2) and membrane-associated PGE synthase-1 (mPGES-1) convert arachidonic acid to prostaglandin E2 (PGE_2).

salt-mediated cytotoxicity can further induce cell death with subsequent release of uric acid (UA), which can also indirectly activate the NLRP3 inflammasome (Kool et al., 2008). Activated NLRP3 recruits the adaptor molecule ASC, which binds to and activates procaspase-1. Active caspase-1 catalyzes the cleavage of proforms of the IL-1β cytokine family into biologically active cytokines (Martinon et al., 2002). The crucial role of the NLRP3 inflammasome for the secretion of IL-1β cytokines is undoubted. However, results are controversial when it comes to the question of whether NLRP3 plays a role in alum-induced adjuvanticity and induction of type 2 immunity. Whereas some studies found NLRP3, ASC, and caspase-1 to be required for alum-induced adjuvanticity (Eisenbarth et al., 2008), others failed to observe a role of NLRP3 (Franchi and Núñez, 2008).

The studies from Kuroda et al. (2011) and Kool et al. (2011) aimed to clarify the molecular mechanisms by which immunogenic particulates initiate type 2 immune responses. Both studies argue for NLRP3-independent mechanisms. Kuroda et al. (2011) suggest a crucial involvement of prostaglandin PGE_2. Kool et al. (2011) identify UA as an essential initiator and amplifier of alum- and house dust mite (HDM) allergen-induced type 2 immunity (Figure 1). Kuroda et al. (2011) report the initiation of two separate pathways upon stimulation with particulates. As shown in previous studies, silica and alum induced the secretion of IL-1β cytokines in an NLRP3-dependent manner. In addition, the particulates induced the production of the proinflammatory arachidonic acid metabolite prostaglandin PGE_2. This second pathway, however, was independent of NLRP3, ASC, and caspase-1, but depended on cyclooxygenase-2 (COX-2) and membrane-associated PGE synthase-1 (mPGES-1), whereas neither COX-2 nor

mPGES-1 proved to be essential for IL-1β production. Furthermore, antigen-specific IgE amounts were reduced in mPGES-1-deficient mice after immunization with alum as an adjuvant. Conversely, immunization with NiO, which causes the secretion of PGE_2 but not of IL-1β, substantially enhanced IgE amounts. These studies support a role of PGE_2 rather than IL-1β in IgE production. Intriguingly, the mechanisms that lead to PGE_2 production are very similar to those that operate upstream of NLRP3 although the former process is NLRP3 independent. A priming step by a pro-inflammatory stimulus is required (to induce the expression of COX-2 and mPGES-1), and the induction of lysosomal damage by the particulate acts as a necessary second activation step. Based on studies with inhibitors, the authors further demonstrated that lysosomal damage leads to cPLA2 (cytosolic phospholipase A2) activation, probably via Syk and p38 MAP kinase, finally resulting in the release of arachidonic acid from membrane lipids and in the production of PGE_2.

The immunostimulatory effects of particulates not only enable their use as adjuvants, but they also account for the role of particulates in inflammatory diseases. In allergic asthma, for example, particulates like HDM trigger a Th2 cell response characterized by eosinophilic airway inflammation, mucus hypersecretion, and airway obstruction. In gout, MSU crystals activate NLRP3 leading to inflammation (Martinon et al., 2006). Here, Kool et al. (2011) report a major role for MSU in allergic asthma. Sensitization of mice with OVA-alum or HDM induced an allergic Th2 cell response upon antigen challenge. This response was characterized by eosinophilia, increased Th2 cell cytokines and antibody concentrations, as well as migration and activation of inflammatory monocytes and dendritic cells (DCs). Notably, uricase treatment of mice just before sensitization reduced Th2 cell immunity, suggesting that MSU played a critical role in alum- and HDM-induced asthma. Indeed, UA amounts increased upon treatment of mice with alum-OVA or house dust mite allergen. Consistently, asthmatic patients showed elevated UA concentrations after allergen challenge. Sensitization of mice with MSU-OVA revealed that MSU was not only necessary but also sufficient for the induction of an allergic Th2 cell response. Most notably, and in contrast to the well-known inflammatory pathway initiated by MSU in gout (Martinon et al., 2006), the induction of Th2 cell immunity by MSU did not require NLRP3, ASC, or IL-1R.

As previously reported, elevated UA amounts can reflect alum-induced cell damage leading to the release of endogenous DAMPs (Kool et al., 2008). In the case of HDM, Kool et al. (2011) propose yet another mechanism, which is the enhanced production of UA resulting from upregulation of xanthine oxidoreductase (XOR). The XOR gene promoter region contains an NF-κB control element. Strikingly, in contrast to UA production upon alum treatment, UA production upon HDM treatment was TLR4 dependent, suggesting that XOR upregulation might represent another important TLR priming effect.

Based on the finding that uricase treatment lost its profound effects if mice were sensitized by intratracheal instillation of OVA-pulsed DCs, the authors concluded that MSU had to act upstream of DC activation and recruitment. The molecular pathway for activation involved Syk and PI3K∂. Notably, previous studies proposed that MSU was able to directly engage cholesterol-rich cellular membranes of DCs in a receptor-independent manner and thereby triggered the activation of Syk (Ng et al., 2008).

Together, the work of Kuroda et al. (2011) and Kool et al. (2011) provide another jigsaw piece in the understanding of particulate-induced immunity. According to these authors, particulates not only activate the NLRP3 inflammasome to induce the secretion of IL-1β but also stimulate innate immunity in an NLRP3-independent manner (Figure 1). This latter pathway appears to be crucial for the initiation of Th2 cell immunity and could account for the controversy regarding whether NLRP3 is required for alum's adjuvant effect. Although representing two separate pathways, both pathways might go back to one common event induced by particulates, namely lysosomal damage.

Although the herein discussed studies represent a substantial step forward toward solving the mystery of alum's adjuvanticity, we are still struggling with depicting an exact molecular mechanism. It is likely that multiple pathways are involved and that triggering multiple pathways may actually be part of alum's secret of success. Four mechanisms have been proposed so far to explain the utility of alum as an adjuvant. First is the so-called depot effect, which refers to a slow release of antigen leading to prolonged stimulation of the immune system. Second, by converting soluble antigen into a particulate form, its uptake could be enhanced. Third, NLRP3 stimulation induces inflammation. And finally is the induction of type 2 immunity by NLRP3-independent signaling (Kool et al., 2011; Kuroda et al., 2011).

Our efforts to solve the oldest secret in immunology have taught us a great deal about our immune system and about what it takes to be a good adjuvant. These lessons have major implications for rational vaccine design. A good part of modern vaccines consist of purified antigens in combination with exogenous adjuvants. In order to achieve good efficiency, the formulation has to ensure efficient uptake of antigen in close conjunction with the adjuvant, and the choice of the adjuvant will strongly influence the type of the adaptive immune response. Finally, we are starting to recognize the great potential of effectively targeting the innate immune system by combining multiple stimuli. A current approach to dually target TLR4 and TLR7 with a nanoparticle-based vaccine provides an excellent example of how to gain effectiveness by making the secret a bit dirtier (Rhee et al., 2011).

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Resistance to Mousepox Virus: CD94 on a Special Mission

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NK cells play a key role in the control of ectromelia virus. In this issue of *Immunity*, Fang et al. (2011) demonstrate that the deletion of CD94 abolishes resistance to mousepox infection.

Natural killer (NK) cells are key actors in innate immunity. They protect the host from many types of viral infections by sensing proinflammatory cytokines in their environment as well as changes in the expression of major histocompatibility complex (MHC) class I and other molecules expressed on the cell surface during a viral insult. Effector functions of NK cells are regulated by integrating signals from their activating and inhibitory receptors. The engagement of inhibitory NK cell receptors by MHC class I molecules expressed by healthy cells prevents their activation. On the other hand, NK cells are activated either through the engagement of their activating receptors or a lack of engagement of their inhibitory receptors. Defusing NK cells is critical for their survival, so over time viruses have evolved a number of strategies to evade NK cell control (Lisnic et al., 2010). Virus-driven evolution of their natural hosts led to emergence of mechanisms able to oppose the viral immunoevasion. The best way to achieve this goal would be selection of activating NK receptors that specifically recognize virally infected cells. Indeed, unlike their inhibitory counterparts, many of the activating NK cell receptors bind to various molecular determinants of infection and demonstrate certain types of specificity to virally encoded molecules.

The most studied example of a virus-specific NK cell response is in C57BL/6 (B6) mice, which are constitutively resistant to murine cytomegalovirus (MCMV). These mice express Ly49H, an activating receptor on their NK cells that directly interacts with the MCMV m157 protein, leading to recognition and elimination of infected cells via cytolytic mechanisms (Arase et al., 2002). Another NK cell-dependent mechanism of MCMV resistance has been described in MA/My mice, whose activating Ly49P receptor recognizes the MHC class I allele H2-D^k bound to a viral protein encoded by the *m04* gene (Kielczewska et al., 2009). It has to be pointed out that the specificity of NK cell receptors to virally encoded molecules is not restricted to herpes viruses as shown by the fact that NKp46- and NKp44-activating NK cell receptors bind to influenza hemagglutinin (Arnon et al., 2006).

Mousepox or ectromelia virus is another virus whose pathogenesis is tightly controlled by NK cells. It belongs to *Orthopoxviruses*, a large family of DNA viruses that includes, in addition to ectromelia, the variola virus, a causative agent of smallpox, as well as vaccinia virus, cowpox virus, and monkeypox virus. Unlike several mouse strains that are highly sensitive to mousepox, B6 mice are able to successfully cope with mousepox infection without developing symptoms of the disease. The important role that NK cells play in the control of ectromelia infection is best illustrated by the fact that depletion of NK cells abolishes the resistance of B6 mice to this virus. In addition to reducing the viral burden during the early course of infection, functional NK cells are required for the generation of an optimal T cell response (Fang et al., 2008). A dramatic reduction of both $CD8^+$ and $CD4^+$ T cell responses is observed in ectromelia-infected mice depleted of NK cells, possibly as a consequence of virus-mediated eradication of dendritic cells. Although it is well established that NK cells play a key role in ectromelia virus infection in B6 mice, the molecular mechanism of the resistance and the receptors involved has remained elusive until now.

In this issue of *Immunity*, Fang et al. (2011) explain the mechanism of resistance of B6 mice to ectromelia virus through the involvement of the CD94 receptor (Figure 1). CD94-NKG2 receptors are composed of an invariant CD94 polypeptide, which forms a heterodimer with either NKG2A, NKG2C, or NKG2E