these genes until later stages of differentiation. In either scenario, the complement of transcription factors, rather than lineage priming itself, may play a direct role in HSC cell fate determination. However, the two models may be distinguished by whether lineage priming occurs concomitant with or subsequent to cell fate decisions.

In this regard, the role of lineage priming in segregation of the LMPP and MkE fates remains to be resolved. Because Ikaros is not required for development of LMPPs but rather for lymphoid differentiation from LMPPs, a possible role for lymphoid-lineage priming appears to be restricted to cell fate decisions after segregation of the MkE fate (Ng et al., 2009; Yoshida et al., 2006). However, it remains possible that a critical Ikaros-independent lymphoid gene(s) may function in repression of the MkE fate. It is also unclear whether HSCs that coprime lymphoid- and myeloid- along with erythroid-lineage genes resolve these conflicting gene expression programs or whether they simply fail to undergo further differentiation (Figure 1). Although the degree of multilineage copriming appears to be low in this study, this may be due to the small number of genes examined. Therefore, analysis of a larger set of lineage-associated genes may reveal a higher degree of copriming and a need for resolution of conflicting gene expression programs at this stage. Acquisition of the LMPP fate could be the consequence of HSC differentiation concomitant with a failure of MkE-lineage priming (i.e., those cells that fail to activate MkE genes become LMPPs). In this respect, it is interesting that one of the primed E lineage genes examined is Gata1, a transcription factor that is essential for E development whereas none of the essential lymphoid transcription factors are a component of the lymphoid-lineage-primed set (Crispino, 2005). The ability to analyze chromatin and global gene expression patterns in single cells is a challenging future goal that will be required to understand how genome regulation influences cell fate choices. The identification of s-myly or other multilineage gene programs in progenitors with defined developmental potential is an important step in understanding how multiple lineages arise from HSCs.

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Fighting the Flu with Inflammasome Signaling

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A wide variety of stimuli induce the inflammasome, but little is known about its role in immune protection against viruses. In this issue of *Immunity*, Allen et al. (2009) and Thomas et al. (2009) describe a critical role for NLRP3 induction of the inflammasome and protection against influenza virus infection.

Influenza A virus is an important human pathogen that infects millions of people worldwide in seasonal epidemics and leads to more than 30,000 deaths annually in the United States alone (Taubenberger and Morens, 2008). The character of the immune response, and in particular the innate immune response, is a key determinant of influenza outcome wherein innate immunity mediates our essential first-line defense against infection. Pathogen-associated molecular patterns (PAMPs) present within influenza A virus that are generated during infection are recognized by three major classes of pattern-recognition receptors (PRRs), which form the basis for innate immune detection of viruses and other microbes. These PRRs include the Toll-like receptors (TLRs), retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs), and the nucleotide-binding domain-leucine-rich repeat-containing molecules (NLRs). Detection of influenza A virus by TLRs or RLRs lead to the production of type 1 interferons in bronchial epithelial cells (via RIG-I) and plasmacytoid dendritic cells (via TLR7) leading to tissue-specific and systemic antiviral states (Wang et al., 2007). In general, little is known

about the role of NLRs in the detection of virus infection and the induction of antiviral immune defenses.

The NLR family contains 23 members, of which only a handful have been characterized in terms of their role as PRRs of immune signaling (Franchi et al., 2009). The first NLRs identified, NOD1 and NOD2, are intracellular PRRs that detect bacterial peptidoglycan leading to the induction of NF-kB and MAP kinase signaling pathways. Three other family members NLRC4 (also known as IPAF), NLRP1, and NLRP3 (cryopyrin and NALP3) are intracellular proteins that form distinct caspase-1-activating complexes termed inflammasomes. NLRC4 responds to bacterial flagellin and oligomerizes to recruit caspase 1 via interactions between the caspase activation and recruitment domain (CARD) present on each molecule. Caspase 1 is then autocatalytically cleaved to its active form and is able to process proIL-1β and proIL-18 into their respective mature secreted forms, IL-1β and IL-18, which operate to induce inflammation to sites of infection and tissue damage. This processing is the hallmark of inflammasome activity. NLRP1 responds to muramyl dipeptide but does not contain a CARD domain, so it relies on the adaptor protein apoptotic speck-like protein containing a CARD (ASC) to confer downstream signaling. NLRP3 also signals through ASC and is reported to respond to numerous stimuli including bacterial DNA and RNA, pore-forming toxins, silica, asbestos, uric acid, and ion flux. A role for the NLRP3 inflammasome in response to virus infection is also emerging. For example, adenovirus DNA can stimulate inflammasome signaling in a NLRP3-dependent manner (Muruve et al., 2008). Moreover, myxoma virus (a poxvirus) encodes a protein that interferes with ASC-mediated inflammasome signaling, thus highlighting the importance of the inflammasome as a mediator of antiviral activity (Johnston et al., 2005). In a previous study, Kanneganti et al. (2006) demonstrated a role for NLRP3 in caspase 1 activation during virus infection of cultured macrophages, and recent studies have implicated a critical role for inflammasome signaling in the adaptive immune response against influenza A virus infection (Ichinohe et al., 2009). Now Allen et al. (2009) and Thomas et al. (2009) show that NLRP3 plays an essential role to direct a protective inflammatory response that limits lung damage and overall pathogenesis from influenza A virus infection.

Allen et al. (2009) and Thomas et al. (2009) both examined influenza A virus infection in a mouse model wherein wild-type mice and mice deficient in caspase 1 (*Casp1*$^{-/-}$), ASC (*Pycard*$^{-/-}$), NLRP3 (*Nlrp3*$^{-/-}$), or NLRC4 (*Nlrc4*$^{-/-}$) were infected with a high but sublethal dose of H1N1 influenza A virus strain A/PR/8/34. Allen et al. (2009) found that although ~70% of wild-type or *Nlrc4*$^{-/-}$ mice survived the virus challenge, only 40% of *Casp1*$^{-/-}$ or *Pycard*$^{-/-}$ and only 20% of *Nlrp3*$^{-/-}$ mice survived the challenge. This outcome supports a specific role for the NLRP3-ASC-caspase 1 inflammasome in protection from influenza A virus pathogenesis. Interestingly, histological analysis of lung tissue from the infected *Nlrp3*$^{-/-}$ mice showed less infiltration of inflammatory cells in the airway compared to wild-type mice. This observation was confirmed by counting cells present in bronchoalveolar lavage fluid (BALF), a marker of acute inflammation, which demonstrated an ~50% reduction in monocytes and neutrophils in the *Nlrp3*$^{-/-}$ animals compared to wild-type or *Nlrc4*$^{-/-}$ mice. Similarly, Thomas et al. (2009) observed that infection under similar conditions reduced survival from ~70% in wild-type animals to 40% in *Nlrp3*$^{-/-}$ or *Casp1*$^{-/-}$ animals. Likewise, the number of neutrophils, monocytic, and dendritic cells in the BALF was markedly reduced in *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ animals compared to the wild-type. However, Thomas et al. (2009) observed a different pattern of lung histology in *Nlrp3*$^{-/-}$ animals than that observed by Allen et al. (2009), in which there was an increase in pulmonary necrosis and collagen deposition in the lungs of infected mice when compared to wild-type animals. The reasons for the histologic discrepancies between these studies are unclear but could be caused by specific differences between mouse lines, given that the two groups used independently constructed lines of *Nlrp3*$^{-/-}$ mice. Although both groups agree that NLRP3 protects mice from influenza mortality, they also concluded that disruption of inflammasome signaling upon NLRP3 deletion did not impact the generation of adaptive immunity against influenza A virus. These results seemingly differ from the observations of Ichinohe et al. (2009), who showed that inflammasome-deficient mice had defective adaptive immune responses against influenza A virus A/PR8 infection that were attributed to ASC, caspase 1, or overall IL-1 receptor signaling but not to NLRP3 because *Nlrp3*$^{-/-}$ mice were not observed to display increased susceptibility to infection. The influenza A virus infection, however, differed among the studies and were conducted at a much lower dose (ten plaque-forming units [Ichinohe et al., 2009] versus 6000–8000 plaque forming units [Allen et al., 2009; Thomas et al., 2009]), suggesting that the protective effects of NLRP3 vary with initial viral challenge dose and acute viral load.

To define the role of NLRP3 in virus-induced inflammasome activity, Allen et al. (2009) and Thomas et al. (2009) each assessed the release of IL-1β, the characteristic marker of inflammasome activity. Similar to the findings of Ichinohe et al. (2009), both groups demonstrate that *Nlrp3*$^{-/-}$ mice secrete substantially less IL-1β into BALF upon influenza A virus infection than wild-type mice. To determine the effect of NLRP3 mediated inflammasome defects in the adaptive immune response against influenza A virus, Thomas et al. (2009) examined antigen-specific IgG or CD8^{+} T cell response at 11 days after infection. In agreement with the results of Ichinohe et al. (2009), no marked defects in adaptive immunity were observed in *Nlrp3*$^{-/-}$ animals. The work of Ichinohe et al. (2009) suggested that macrophages and dendritic cells, but not lung fibroblasts, were the cells responsible for NLRP3-dependent IL-1B production. Moreover, Allen et al. (2009) showed that primary human airway epithelial cultures could also induce increased NLRP3 expression and produce IL-1β in response to influenza A virus infection. Thus, both epithelial cells and macrophages may contribute to NLRP3 signaling during infection. Prior work has suggested that the common signal leading to NLRP3 activation could be deregulated lysosomal function within infected cells (Hornung et al., 2008), and such a function leads to the leakage of lysosome contents into the cytoplasm and/or the production of reactive oxygen species (ROS) that trigger inflammasome signaling. In agreement with this hypothesis, Allen et al. (2009)

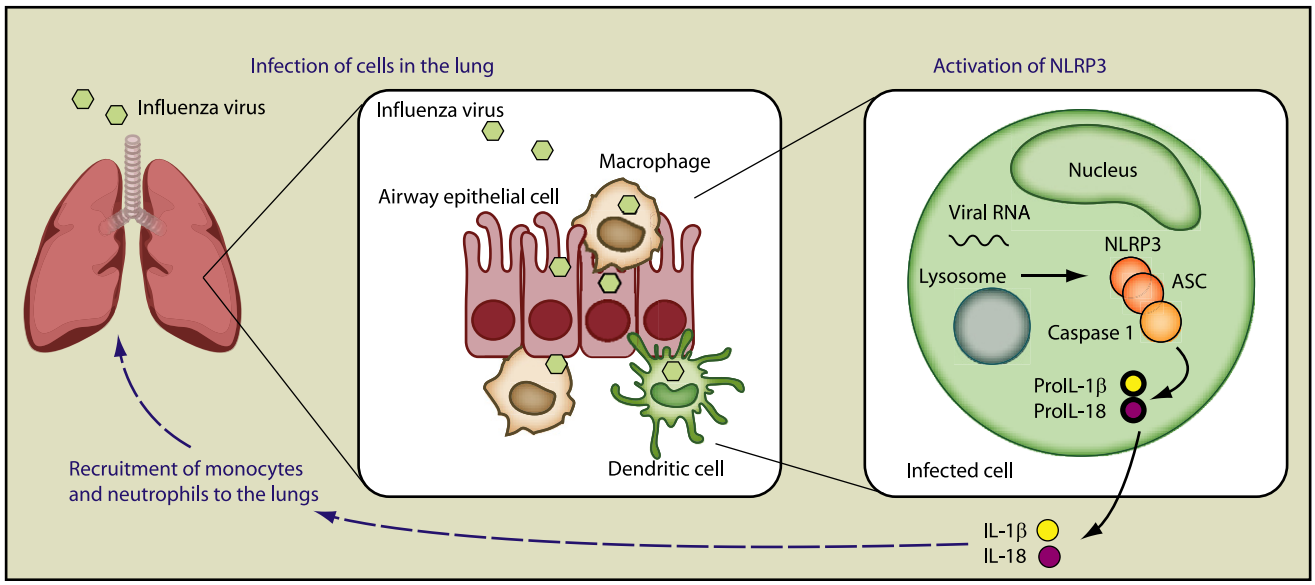

Figure 1. Influenza Activation of NLRP3 Inflammasome

Influenza A virus infects airway epithelial cells, macrophages, and dendritic cells in the lung (left). Viral RNA within an infected cell and/or virus debris taken up by phagocytic cells leads to NLRP3 activation and inflammasome formation in a manner dependent on viral RNA-PRR interaction and/or lysosome function. Caspase 1 in the inflammasome complex processes pro-IL-1β and pro-IL-18 into their mature, active forms (right). Increased IL-1β and IL-18 proinflammatory cytokine secretion recruits monocytes and neutrophils into the lung to control infection and tissue pathogenesis (center).

showed that influenza A virus-induced IL-1β production in human monocytes could be abrogated by blocking endosome acidification, thereby inhibiting the lysosomal protease cathepsin B, or by treatment with ROS inhibitors. Importantly, Thomas et al. (2009) and Allen et al. (2009) also showed that double stranded (ds) RNA could serve as the signal for NLRP3 activation. However, it is not known whether NLRP3 functions as a PRR for dsRNA and influenza A virus RNA.

These results lead to a model in which influenza A virus is sensed either in respiratory epithelial cells or macrophages at early time points of infection leading to NLRP3 signaling activation, IL-1β production, and the recruitment of neutrophils and monocytes to the site of infection where they further secrete inflammatory cytokines, engulf virus-infected cells, and serve to control virus spread, contain tissue pathology, and support respiratory function (Figure 1). By this model, NLRP3 is directly or indirectly activated in response to PRR engagement of viral RNA. However, neither study has shown that NLRP3 is an actual PRR in this case, and the actual PRR that recognizes dsRNA to activate NLRP3 remains to be defined. This model also implies that NLRP3 signaling is tightly controlled because a high amount of systemic proinflammatory cytokines can enhance viral pathogenesis by triggering a "cytokine storm" that drives a massive and toxic inflammatory response. Viral RNA is also an important PAMP for type 1 interferon induction, suggesting the potential for signaling crosstalk between TLR, RLR, and NLRP3 pathways. Indeed one member of the NLR family, NLRX1, has been identified as a key regulator the RIG-I signaling pathway and also facilitates ROS production, suggesting a regulatory connection of RLR and NLR pathways (Meylan and Tschopp, 2008). Further studies are required to understand the relative contributions of TLR, RLR, and NLR signaling in inflammation and the inflammatory response to virus infection.

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