and prevent crucial activating interactions with the ST2 receptor.

But when is IL-33 secreted? Unlike IL-1β, which is readily secreted by macrophages and dendritic cells after stimulation with LPS and PMA, IL-33 remained intracellular (Lüthi et al., 2009). In apoptotic macrophages, caspase-mediated processing ensured inactivation of IL-33, but the processing fragments were nevertheless kept cell associated. In contrast, most IL-33 was released from macrophages induced to undergo necrotic cell death (Lüthi et al., 2009). As expected, IL-33 was not processed in necrotic endothelial cells (Cayrol and Girard, 2009) because caspases are not activated during this cell death process.

Together, these findings suggest that IL-33 is specifically released during necrotic cell death, which is thought to be associated with tissue damage during trauma or infection. Under these conditions, extracellular IL-33 may engage the ST2 receptor on mast cells and other immune cells in order to alert the immune system of tissue damage and infection and to promote the initiation of healing responses (Figure 1). In support of this hypothesis, IL-33 is highly expressed in endothelial cells of most organs and in the epidermal and gastrointestinal epithelium (Moussion et al., 2008). These tissues may become exposed to pathogens, allergens, and other environmental agents that can trigger tissue damage. In this respect, IL-33 appears highly reminiscent of IL-1α and HMGB1, two dual-function proteins that play important roles as both intracellular nuclear proteins and extracellular cytokines. Moreover, all three proteins lack classical secretion signals and display cytokine activity independently of processing. In addition, all three are released by necrotic cells, but kept intracellular during apoptosis. Because of these features, HMGB1 and IL-1α have been referred to as "endogenous danger signals" or "alarmins." The work by Lüthi et al. (2009) now also bestows IL-33 with this title.

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Integr-ating IL-1α in Antiviral Host Defenses

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Adenoviral vectors used in gene therapy induce inflammation, although the underlying mechanisms are currently unknown. In this issue of *Immunity*, Di Paolo et al. (2009) implicate interleukin-1α (IL-1α) in virus-induced inflammation and identify the β3 integrin as the key receptor regulating IL-1α activity.

To combat invading viruses and survive infection, eukaryotic hosts deploy an arsenal of defensive measures. The first of these is the innate immune system. Innate immunity controls virus infection and elicits the T and B cell responses of adaptive immunity, which are required to eliminate virus-infected cells. Several classes of germline-encoded pattern recognition receptors have been identified which recognize different components of viruses. In most cases, viruses are sensed via their genomes or their replicative or transcriptional activities (Pichlmair and Reis e Sousa, 2007). Toll-like receptors 3, 7–8, and 9 recognize dsRNA, ssRNA, and ssDNA respectively. The cytosolic RNA helicases RIG-I and MDA5 discriminate between different classes of RNA viruses. RIG-I senses the nascent 5′ triphosphate moiety of viral genomes or virus-derived transcripts of negative-sense ssRNA viruses, whereas MDA5 is activated by long dsRNA, a typical intermediate of the replication of plus-sense ssRNA viruses. The genome of DNA viruses are sensed by

DNA-dependent activator of IFN regulatory factors, (DAI; also called DLM-1 or ZBP1) or by absent in melanoma-2 (AIM2). TLRs, RNA helicases, and DAI control transcription of inflammatory cytokine and type I IFN genes, whereas AIM2 forms a caspase-1-activating inflammasome involved in the maturation of interleukin-1β and IL-18. Members of the NOD-like receptor family also form inflammasomes and regulate IL-1β and IL-18 in response to microbial products, endogenous danger signals, and environmental insults (Franchi et al., 2009).

Although the molecular sensors of virus induced-interferon responses are well defined, those regulating virus-induced inflammation are poorly characterized. This is particularly true in the case of adenoviruses that are used in gene therapy to deliver genes for the treatment of both genetic and nongenetic deseases (Muruve, 2004). An enduring problem with Ad vectors is the systemic inflammatory response, which contributes to significant morbidity and mortality in transduced hosts (Raper et al., 2002; Schnell et al., 2001). The earliest events in infection with Ad have been worked out and involve the binding of Ad fiber coat protein to the coxsackievirus and Ad receptor (CAR), the primary attachment receptor for cell infection for most Ad serotypes. Subsequently, RGD (Arg-Gly-Asp) motifs within the viral penton base-coat protein interact with integrins, allowing the internalization of attached virus particles and triggering of downstream signaling, the culmination of which is the transcription of proinflammatory cytokine and chemokine genes. One such gene is interleukin-1 (IL-1). There are three members of the IL-1 gene family: IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra) (Dinarello, 1996). IL-1α and IL-1β are agonists, whereas IL-1Ra is a specific receptor antagonist. All three bind the same receptor, IL-1R1, and control production of more proinflammatory cytokines and chemokines, which drive inflammation. Earlier work from Shayakhmetov and colleagues using IL-1R-deficient mice implicated IL-1 as a key factor driving inflammation in vivo to adenoviral vectors. Indeed, inhibition of IL-1 with IL-1ra reduced hepatotoxicity without compromising vector transduction into the liver (Shayakhmetov et al., 2005). Understanding how IL-1 is regulated therefore and its role in adenovirus-induced inflammation is critical for the future of Ad-based gene therapies to be realized. It is also likely that IL-1 plays a role more generally in the immune response to viruses, particularly those associated with an inflammatory response. Understanding how IL-1 is produced in virus-infected cells will be important not only for the development of therapeutic agents to limit IL-1 production or action in situations in which it is detrimental to host survival but also to enhance IL-1 activity in order to boost innate immune responses and host defenses.

In this issue of *Immunity*, Di Paolo et al. (2009) have followed up on their earlier studies and identified MARCO and CD169-positive macrophages in marginal zones of the spleen as IL-1α- and IL-1β-producing cells in vivo. IL-1 is produced very rapidly within 10 min after infection, and production of IL-1 is dependent on the interaction of the viral capsid with macrophage receptors. Surprisingly, macrophage-derived IL-1α and not IL-1β was the factor responsible for IL-1RI-dependent production of a cascade of downstream proinflammatory cytokines and chemokines in vivo. This is an intriguing finding because IL-1α has been implicated previously as a mediator of sterile inflammation rather than a central regulator of antimicrobial defenses (Chen et al., 2007). In an effort to define the receptors and downstream mechanisms responsible for the IL-1α-mediated response, the authors examined the role of candidate pattern recognition receptors previously implicated in adenoviral innate defenses. Mice lacking TLR9- and the NOD-like receptor family member NLRP3 did not show a defect in IL-1R1-driven cytokine production. This latter result is particularly surprising in light of Muruve et al.'s recent study, which identified NLRP3 as a critical sensor of adenovirus-induced inflammation (Muruve et al., 2008). In their study, Muruve et al. identified NLRP3 as a key regulator of IL-1β production in adenovirus-infected macrophages and the control of a cascade of cytokines and chemokines downstream of IL-1R1. Although both studies identified IL-1β as a downstream target of adenovirus infection, Di Paolo et al. (2009) suggest that in vivo it is IL-1α and not IL-1β that regulates the IL-1R1-dependent response. In their

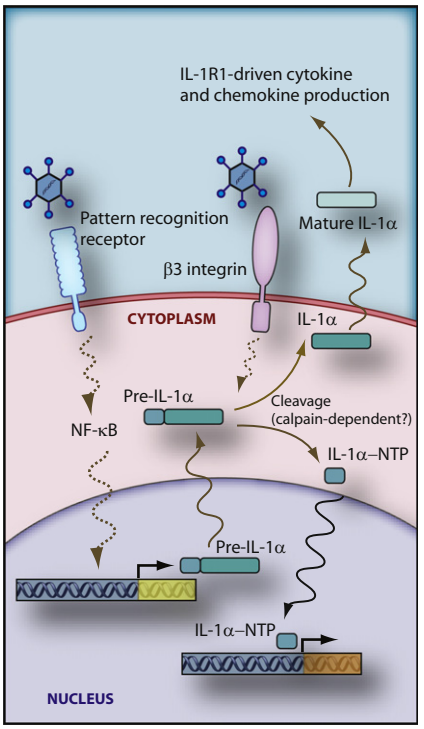

Figure 1. Model of Adenovirus-Induced Inflammation

The interaction of components of adenovirus with an unidentified pattern recognition receptor induces signaling leading to transcription of pre-IL-1α. NFκB activation is likely to mediate these events. The association of β3 integrins with virus RGD motifs then trigger intracellular signaling that promotes virus internalization and processing of pre-IL-1α. Pre-IL1α is processed into IL-1α-NTP and mature IL-1α. The mature cytokine is released from cells to trigger IL-1R1-dependent cytokine and chemokine production. IL-1α-NTP translocates to the nucleus and probably regulates additional target genes independently of IL-1R1. Viral RGD-β3 integrin associations also lead to the uptake of virus and rupture of endosomes, thereby amplifying pre-IL1α gene transcription, synthesis, and processing.

studies, the induction of IL-1R1 target genes was unaffected in mice lacking NLRP3, as well as other components of the NLRP3 inflammasome, apoptotic speck protein containing a C-terminal caspase recruitment domain (ASC), caspase-1, and IL-1β itself, consistent with this model. The discrepancy between these two studies are however difficult to reconcile on the basis of the present data. Differences in the route of delivery, the experimental readout, or the timing of the analyses could contribute.

The importance of virus RGD motifs and their interaction with macrophage β3 integrins was shown to be critical for the IL-1α-IL-1R1-dependent inflammatory

response. Several RGD motif-interacting integrins (v1, 31, v3, v5, v6, 51, M2, and L2) have previously been shown to serve as secondary receptors promoting Ad internalization into different cell types in vitro. This study highlights the role of β3 integrins not only for viral entry but also for IL-1α-driven inflammation. Like IL-1β, IL-1α is synthesized as a preprotein, pre-IL-1α (Dinarello, 1996); however, unlike IL-1β, IL-1α is not processed by caspase-1-containing inflammasomes. Rather, pre-IL1α is processed in the cytoplasm by neutral proteases, including calpains, thereby leading to the translocation of the N-terminal IL-1α propiece (IL-1α-NTP) to the nucleus, whereas mature IL-1α is released from the cell. Staining of spleen sections indicated that IL-1α was primarily localized to the nuclei of infected cells, suggesting that IL-1α was processed in adenovirus-infected cells. Adenovirus-induced *Il1a* gene transcription and synthesis occurred normally in β3-integrin-deficient mice; however, pre-IL1α was not detected in the nucleus. Therefore, the β3 integrin pathway appears to regulate pre-IL-1α processing rather than transcription of the IL-1α gene. The role of β3 integrin in regulating IL-1a activity is shown in Figure 1. Viral RGD-β3 integrin associations also lead to the uptake of virus and rupture of endosomes, thereby amplifying *Il1a* gene transcription, synthesis, and processing. How pre-IL-1α processing is regulated and the role of calpain in these events will require follow-up studies. It will be intriguing to examine IL-1α and cellular integrins further in innate immunity to other viruses and bacterial pathogens. Moreoever, defining the relative role of the N-terminal IL-1α propiece and the mature IL-1α released from cells will likely uncover novel roles for this cytokine in host-defense and pathogenesis.

Adenoviral vectors induce significant inflammation and overcoming this obstacle will be important for the full potential of this approach to be realized. The discovery of the β3-integrin-dependent IL-1α pathway as the primary mediator of inflammation in vivo identifies a pathway that could be targeted therapeutically to overcome this obstacle. A feasible strategy could be to modify the viral capsid to alleviate β3-integrin-driven inflammation. Additionally, the inclusion of immunomodulatory genes in gene therapy vectors that interfere with β3-integrin signaling, with IL-1α production and/or with IL-1α-IL1R1 function, could also be a worthwhile endeavor. Notably, inflammation driven by adenoviral vectors is not always a bad thing and could be beneficial and desirable in certain instances; for example, the ability of the viral vector to trigger IL-1α-driven inflammation could be useful in vaccine development or cancer gene therapy in which inflammation is a desirable adjuvant or bystander effect.

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How to be Naive

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The transcription factor KLF2 directs expression of receptors involved in trafficking of naive T cells. In this issue of *Immunity*, Weinreich et al. (2009) demonstrate that KLF2 additionally represses IL-4 production, which otherwise induces CXCR3 expression.

The adaptive immune system faces a formidable logistic challenge, which is to respond quickly to an invading pathogen while maintaining a large repertoire of different antigen (Ag)-specific lymphocytes. Thus, only few T cells are able to recognize and become activated by a given Ag derived from microbial intruders, whereas microbes may enter the body through any epithelial surface, such as skin, lungs, or gastrointestinal tract. To meet this challenge, a surveillance system has evolved in mammals, and such a system ensures continuous encounters of lymphocytes with Ag-presenting cells in strategically positioned secondary lymphoid organs (SLOs) (Junt et al., 2008). Blood-borne naive lymphocytes