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Immunoregulatory effects of necroptosis in bacterial infections

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Necroptosis is an increasingly appreciated pathway of regulated cell death, which has a role in the pathogenesis of bacterial infection distinct from that of apoptosis or pyroptosis. It has been shown to have pathological consequences in some models of infection and ischemic injury [1], but can be beneficial in specific types of bacterial infection [2]. The hallmark of necroptotic cell death is the involvement of the necrosome kinases, receptor interacting protein kinases 1 (RIPK1) and 3 (RIPK3) and the mixed lineage kinase like (MLKL) protein [1, 3, 4]. Phosphorylation of RIPK1 leads to recruitment and activation of RIPK3, recruitment to the plasma membrane, MLKL oligomerization, followed by membrane permeabilization [1, 4]. Like the other cell death pathways the kinases that activate necroptosis are highly regulated and linked to the expression of the caspases that function in apoptosis [3]. Necroptosis occurs in the absence of caspase-8 that acts to counter-balance necroptosis by cleaving RIPK1 and RIPK3. Caspase-8 also targets the deubquitinase CYLD preventing RIPK1 initiation of necroptosis [3, 5]. Necroptosis is classically activated by TNFR signaling but can also be induced by a variety of mechanisms including: cell death receptors, toxins, toll-like receptors, DNA and RNA sensors and interferons [3]. Necroptosis triggers inflammation through the release of intracellular contents, damage associated molecular patterns (DAMPS) during cell death. Inflammation is an important aspect of the host response and is required for control of infection. However, regulation of inflammation is critical as the local pathology associated with excessive inflammation limits pathogen clearance and leads to the development of inflammatory diseases. It is this balance that we observe to be important in determining outcomes at different sites of infection with *Staphylococcus aureus*.

In the airway *S. aureus* trigger a robust neutrophil dominated inflammatory response often associated with lung pathology. The regulatory effects of alveolar macrophages are important in balancing pro and anti-inflammatory signaling. Our initial studies demonstrated that *S. aureus* activates necroptosis in macrophages [6]. The necroptotic cell death was dependent upon toxin-induced pore formation associated with; α-toxin, phenol soluble modulins and leukocidin AB, but not PVL. Interfering with necroptosis, either through the use of chemical inhibitors (necrostatin-1s, an inhibitor of RIPK1) or using gene deleted mice (RIP3), resulted in increased survival of alveolar macrophages that regulate proinflammatory signaling. Alveolar macrophages from *Ripk3*$^{-/-}$ mice expressed increased levels of CD206 and CD200R, major anti-inflammatory receptors [6]. *Ripk3*$^{-/-}$ mice had significantly

reduced production of proinflammatory cytokines and improved clearance of *S. aureus* from the airway [6].

Our current study demonstrates the importance of necroptosis in limiting inflammation in skin and systemic infections caused by *S. aureus* [2]. In these studies we used *Mlkl*$^{-/-}$ mice that lack the pore-forming effector of necroptosis; in contrast to the *Ripk3*$^{-/-}$ mice, a deletion which also affects the activation of caspase-1 and the NLRP3 inflammasome as well as necroptosis [6]. Deletion of *Mlkl* in models of skin or sepsis led to increased bacterial burden and mortality with an associated increase in proinflammatory cytokine levels. In a model of cutaneous infection, *Mlkl*$^{-/-}$ mice had increased bacterial loads in local skin lesions, accompanied by increased proinflammatory cytokines, more tissue damage and greater numbers of recruited neutrophils, macrophages and γδ T cells. Confirming the *Mlkl*$^{-/-}$ results, pharmacological inhibition of RIPK1 with necrostatin-1s also increased inflammation and impaired staphylococcal clearance in skin infection [2]. A *S. aureus* sepsis model similarly demonstrated increased bacterial burden when necroptosis was pharmacologically inhibited and *Mlkl*$^{-/-}$ mice also exhibited increased mortality. This current study contrasts with our work in the airway whereby necroptosis contributed to inflammation [6], an observation observed with other pathogens of the airway [7], as well as in bacterial sepsis with *Salmonella* [8]. This new study indicates that in the setting of a highly proinflammatory bacterial infection, necroptosis participates in limiting inflammation and facilitating bacterial clearance.

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