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Review

Is the inflammasome relevant for epithelial cell function?

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Abstract

Inflammasomes are intracellular protein complexes that sense microbial components and damage of infected cells. Following activation by molecules released by pathogens or injured cells, inflammasomes activate caspase-1, allowing secretion of the pro-inflammatory cytokines IL-1β and IL-18 from innate immune cells. Inflammasomes are also expressed in epithelial cells, where their function has attracted less attention. Nonetheless, depending on the tissue, epithelial inflammasomes can mediate inflammation, wound healing, and pain sensitivity. We review here recent findings on inflammasomes found in epithelial tissues, highlighting the importance of these protein complexes in the response of epithelial tissues to microbial infections.

Keywords: Caspase-1; Epithelium; Inflammation; NLRP3; Cell repair

1. Introduction

Inflammasomes are intracellular multi-protein complexes that sense the presence of pathogens and injured cells. In innate immune cells, inflammasomes induce the activation of caspase-1, which results in cleavage of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 into their active forms [34,48]. These cytokines stimulate fever, vasodilatation and recruitment of innate immune cells to the site of infection, ultimately leading to resolution of the infection and tissue repair.

Inflammasomes are typically composed of an immune sensor protein (a NOD-like receptor (NLR) such as NLRP1, NLRP3, NLRC4 or NLRP6), caspase-1, and, in many cases, the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD domain) [34]. Some inflammasomes are activated by direct interaction with specific pathogen-associated molecular patterns (PAMPs). For instance, the NLRP1 inflammasome is activated by the peptidoglycan motif, muramyl dipeptide, whereas NLRC4

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recognizes microbial proteinaceous ligands, such as flagellin [29]. An inflammasome formed with the immune sensor protein AIM2 (absent in melanoma 2) is activated by double-strand DNA (either from the host, bacteria or viruses). In contrast, the NLRP3 inflammasome is activated by a wide range of compounds, including bacterial and viral nucleic acids, lipopolysaccharide (LPS), and pore-forming toxins, as well as danger-associated molecular patterns (DAMPs) released from damaged cells, including extracellular adenosine triphosphate (ATP), uric acid crystals, and amyloid-β peptides. NLRP3 inflammasome activation also occurs in response to environmental irritants such as aluminum salts, asbestos, silica, and ultraviolet (UV) light [12].

Secretion of IL-1β or IL-18 typically requires two signals. The first signal may consist of a PAMP (e.g., LPS) which is recognized by pattern recognition receptors (PRRs; e.g., Toll-like receptors, TLRs) and whose activation leads to NF-κB nuclear translocation and gene transcription of the immature cytokines (i.e., pro-IL-1β, pro-IL-18) [29]. A second signal consisting of a DAMP released from damaged cells (e.g., ATP) and recognized by PRRs or purinergic receptors (e.g., P_2X7R) is needed for inflammasome complex formation and activation of caspase-1, resulting in secretion of mature IL-1β and IL-18 [40]. The observation that diverse molecules activate the NLRP3 inflammasome suggests that the activators might not directly interact with the NLRP3 protein, but may instead activate signaling pathways leading to inflammasome activation. Potassium efflux, reactive oxygen species (ROS), lysosomal damage and oxidized mitochondrial DNA represent upstream signals that activate the NLRP3 inflammasome [48,46].

As inflammasomes mediate the inflammatory response induced by a wide range of PAMPs and DAMPs, these protein complexes play a role in several pro-inflammatory conditions that include Alzheimer's disease, arthritis, atherosclerosis, asbestosis, cancer, gout, inflammatory bowel disease (IBD), microbial infections, silicosis, and type 2 diabetes [48,18]. Inflammasomes are also involved in mediating the inflammatory response induced by tissue damage resulting from injuries and trauma. Given the importance of cytokines and innate immune cells in the healing process, the inflammasomes are important for clearing the body of infectious pathogens and detecting tissue damage [48].

While the role of inflammasomes has been studied mainly in innate immune cells, recent studies indicate that epithelial cells of the eye, mouth, lung, blood vessel, kidney, intestine, cervix, and skin express active inflammasome complexes (Table 1). These epithelial inflammasomes have been shown to participate in inflammation, infection, wound healing, and pain sensitivity.

2. Role of epithelial inflammasomes in response to microbial pathogens

Epithelial tissues are the first tissues to respond to injuries and pathogens. Accordingly, epithelial cells secrete pro-inflammatory and anti-viral cytokines such as IL-6, IL-8 and interferon-β (IFN-β) [4,17] (Fig. 1) which can act as a first wave of signals to recruit innate immune cells to the site of injury and activate cell repair. Several epithelial tissues also contain an inflammasome and produce the pro-inflammatory cytokines IL-1β and IL-18, which may contribute to the inflammatory response (Table 1). For instance, *Staphylococcus aureus* induces the expression of NLRP3, ASC and caspase-1 proteins in conjunctival goblet cells [35]. In the presence of both *S. aureus* and extracellular ATP, the inflammasome complex is activated in the epithelial cells, leading to caspase-1 activation and IL-1β secretion. The NLRP3 inflammasome expressed in epithelial cells of the eye may thus help to protect the host against bacterial infection.

Viruses can also activate inflammasomes in epithelial cells. Human airway epithelial cells challenged with the influenza A virus secrete IL-1β in culture [2]. $NLRP3^{(-/-)}$, $ASC^{(-/-)}$ and caspase-$1^{(-/-)}$ mice that are infected intranasally with the influenza A virus show lower levels of pro-inflammatory cytokines and reduced numbers of monocytes and neutrophils in the lungs compared with wild-type (WT) animals [2,52]. Notably, the knockout mice show reduced survival in response to the viral infection, indicating that the NLRP3 inflammasome protects the host against viral infection.

The NLRP3 and NLRC4 inflammasomes are also upregulated in the oral mucosa in response to the fungal pathogen *Candida albicans* [53]. In mice, the NLRC4 inflammasome mediates the recruitment of innate immune cells to the oral mucosa and prevents systemic dissemination of *C. albicans*. Bone marrow chimeric mouse experiments show that the NLRC4 inflammasome, when expressed in the oral epithelium and stroma, limits mucosal candidiasis, whereas the NLRP3 inflammasome limits the extent of both mucosal and systemic infection when expressed in innate immune cells or stromal compartments. These observations suggest that inflammasomes expressed in epithelial cells and innate immune cells may play different roles depending on their expression profile.

Using mouse monoclonal antibodies against human NLRP1 and NLRP3, Kummer et al. [28] have shown that these inflammasomes have distinct expression profiles in human epithelial tissues. NLRP1 is expressed in epithelial cells lining the stomach, small intestine, colon, and lung, whereas NLRP3 is found in keratinocytes and non-keratinizing epithelia of the oropharynx, esophagus, and ectocervix. Epithelial inflammasomes may thus have distinct, site-specific functions in the inflammatory response.

The inflammasome has been studied in the intestinal epithelium which, along with mucus and innate immune cells located in the intestinal mucosa, forms a barrier against food antigens, pathogens, and commensal microbes. Notably, $NLRP3^{(-/-)}$, $NLRC4^{(-/-)}$, caspase-$1^{(-/-)}$ and $ASC^{(-/-)}$ mice show increased bacterial colonization, inflammation and weight loss following infection with the pathogen *Citrobacter rodentium* compared with WT mice [33,47,38]. Analysis of irradiated bone marrow chimeras show that protection from infection is due to NLRP3 and NLRC4 activation in non-hematopoietic cells (which include epithelial cells), as opposed to innate immune cells [47,38]. The effects of these