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Danger signals, inflammasomes, and the intricate intracellular lives of chlamydiae

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ABSTRACT

Chlamydiae are obligate intracellular bacterial pathogens, and as such are sensitive to alterations in the cellular physiology of their hosts. Chlamydial infections often cause pathologic consequences due to prolonged localized inflammation. Considerable advances have been made in the last few years regarding our understanding of how two key inflammation-associated signaling pathways influence the biology of *Chlamydia* infections: inflammation regulating purinergic signaling pathways significantly impact intracellular chlamydial development, and inflammasome activation modulates both chlamydial growth and infection mediated pro-inflammatory cytokine production. We review here elements of both pathways, presenting the latest developments contributing to our understanding of how chlamydial infections are influenced by inflammasomes and purinergic signaling.

The bacterial family *Chlamydiaceae* includes several species which promiscuously or sporadically infect humans. *Chlamydia trachomatis* is the most common nationally notifiable infection in the USA [1], the most common bacterial cause of sexually transmitted infection worldwide [2], and also a prominent cause of preventable blindness following repeated conjunctival infections in the developing world [3]. *C. trachomatis* can also cause pneumonia in infants following exposure during birth [4]. *Chlamydophila pneumoniae* is one of the leading causes of pneumonia in the developed world [5] and may increase the risk of developing atherosclerotic lesions in coronary artery disease [6]. *Chlamydophila psittaci*, while primarily an avian pathogen, sporadically causes human pneumonia [7,8]. *Chlamydophila abortus* and *Chlamydophila caviae* zoonotic infections have been reported in humans but are rare [9,10]. Untreated *C. trachomatis* genital tract infection in women may

ascend the endometrial endothelium to reach the fallopian tubes, with the associated chronic inflammation leading to pelvic inflammatory disease (PID), which may also cause miscarriage [11], ectopic pregnancy [12], or tubal scarring and infertility [13]. Repeated and chronic infection of the conjunctiva with *C. trachomatis* leads to recruitment of lymphocytes and the formation of follicles and inflammation mediated conjunctival thickening, which subsequently causes the deformation of the eyelids and corneal damage via scraping of the cornea by in-turned eyelashes [14]. Pneumonia caused by *C. pneumoniae* develops slowly and leads to inflammation of the lungs but with limited production of purulent sputum. *C. pneumoniae* also causes infections of the upper respiratory tract including pharyngitis, sinusitis, and bronchitis. Inflammation due to repeated infections with *C. pneumoniae*, or unrecognized and untreated infections, may contribute to chronic obstructive pulmonary disorder (COPD) [15,16]. Human disease following infection with *Chlamydiaceae* species bears a consistent hallmark: chronic, localized inflammation.

Two major pathways relevant to the induction and regulation of localized inflammation have recently received considerable research emphasis and have been demonstrated to be particularly relevant during *Chlamydia* infection: purinergic signaling, and the formation of macro-molecular inflammasomes. Here we review the basic concepts of purinergic signaling in the context of immune function and inflammation regulation, and inflammasome mediated inflammatory cytokine production, followed by an examination of the recent literature evaluating the impact of host purinergic signaling and inflammasome activation on chlamydial infection.

Extracellular purines and purinergic receptors

A wide range of extracellular purine concentrations are physiologically relevant [17], and they are met by a similarly broad spectrum of sensory affinity in the purinergic receptor families [18]. ATP is released from cells under normal physiologic conditions reaching nanomolar to low micromolar concentrations in the immediately adjacent extracellular space [19–21]. Higher concentrations of ATP or ADP result from various forms of cell stress [22], platelet degranulation [23], or are present in tumor microenvironments [24]. The concentration of ATP in cells ranges from 3 to 10 mM, and thus in the context of cell damage or necrosis the neighboring cells are exposed to low millimolar levels of extracellular ATP and purine metabolites. ATP may be released via degranulation in cell types which produce ADP or ATP rich granules, via pannexin channels [25,26], or following cell damage. At colonized mucosal epithelial surfaces, ATP may also be directly released by bacteria [27]. ATP is also released from cervical epithelial cells *in vitro* during *C. trachomatis* infection, particularly during the late stages of inclusion development when there is likely more cellular stress [28].

Receptor mediated purine signaling is an evolutionarily conserved cellular function, and is involved in a wide variety of physiological processes in mammals including neurologic signaling, vascular function, and immune cell regulation.

Receptors which recognize purine nucleotides and nucleosides are termed purinergic receptors. Purinergic receptors are grouped into families based on functional similarity: P1 receptors are engaged by the purine nucleoside adenosine, while P2 receptors are activated by nucleotides and are further subdivided into gated ion channels (P2X) or G-protein coupled seven transmembrane receptors (P2Y).

Adenosine receptors couple via G-proteins to adenylyl cyclase to modulate cAMP generation in cells. A1 and A3 receptors associate with Gi proteins to inhibit adenylyl cyclase and prevent cAMP upregulation, whereas A2a and A2b receptors interact with Gs proteins to activate adenylyl cyclase, leading to elevated intracellular cAMP. Adenosine receptors are expressed in a wide variety of cell types, with particularly well described roles for A1 and A3 receptors in cardiac function, and for A2a receptors in immune cell function and A2b on epithelial and endothelial cells. A2b also mediates intracellular signaling via Gq and phospholipase C [29]. Recent excellent reviews have organized the great depth of studies related to adenosine receptor mediated regulation of immune cells [30,31].

P2X purinergic receptors are ligand gated ion channels which are activated by ATP, and possibly ADP in the case of P2X4, and are increasingly recognized to play a role in inflammation and immune cell function [32]. There are seven described P2X receptors, the best characterized being P2X7. P2X7 plays a critical role in the production of IL-1β and other inflammatory cytokines both in the context of infection and sterile inflammation [33,34]. Initial stimulation of P2X receptors leads to the opening of small-cation permeable pores (approximately 0.85 nm for P2X7), while prolonged ligation then leads to increased permeability to larger molecules (greater than 1 nm for P2X7) [35], either by P2X7 pore dilation or by P2X7 coupling to pannexin1 and the pannexin pore.

P2Y receptors respond to purine and pyrimidine nucleotides and mediate intracellular signaling via regulation of cAMP or activation of PLC. Eight P2Y receptors have been characterized ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$), and while their activity relevant to inflammation and immune cell function is less well characterized than for P2X receptors, some P2Y receptors are expressed on immune cells [18,36,37].

While there is nuance to the roles that purine signaling plays in local and systemic immune regulation, in general, ATP mediates pro-inflammatory responses, and adenosine receptor stimulation is anti-inflammatory [38]. Thus soluble or cell surface associated extracellular enzymes play a critical role in determining the response to extracellular purines. Adenine nucleotides released from cells are dephosphorylated by members of several families of purine enzymes to generate adenosine. Enzymes such as CD39 (ENTPD1) and alkaline phosphatase dephosphorylate ATP and ADP to generate AMP, and CD73 (ecto-5′-nucleotidase) or alkaline phosphatase dephosphorylates AMP to generate adenosine. Adenosine can be captured by adjacent cells via nucleoside transporters for purine salvage, or metabolized by adenosine deaminase to produce inosine. Enzymes regulating extracellular purine metabolism have been more completely described in recent reviews [39,40].