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Author

Beacham, Ifor, Headrick, John

Published

2013

Journal Title

Advances in Infectious Diseases

DOI

[10.4236/aid.2013.32016](https://doi.org/10.4236/aid.2013.32016)

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Bacterial UDP-Glucose Hydrolases and P2 Receptor-Mediated Responses to Infection: A Commentary

Ifor R. Beacham^{1*}, John P. Headrick²

¹Institute for Glycomics, Griffith University, Gold Coast Campus, Gold Coast, Australia; ²School of Medical Science, Griffith University, Gold Coast Campus, Gold Coast, Australia.
Email: *i.beacham@griffith.edu.au

Received March 7th, 2013; revised April 9th, 2013; accepted May 9th, 2013

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ABSTRACT

UDP-glucose hydrolases are a group of relatively little known membrane-bound or periplasmic enzymes found in *Salmonella enterica* and *E. coli*. UDP-glucose is an agonist for a specific P2 receptor (P2Y₁₄) found on epithelial cells and cells associated with innate immunity. It is also recognised as a ‘danger signal’. Cells respond to mechanical damage by releasing UDP-glucose which activates P2Y₁₄ to trigger an innate immune response; it is postulated that a similar response to bacterial infection may be protective against infection. However, the UDP-glucose hydrolases may constitute virulence factors able to abrogate this response by degradation of the released UDP-glucose.

Keywords: UDP-Glucose Hydrolase; P2Y₁₄ Receptor; Virulence Factor; *Salmonella enterica*; *E. coli*

1. Introduction

Surface localised enzymes in bacteria are well known to be involved in mediating the utilisation of molecules, which otherwise cannot access the cytoplasm, as sources of carbon and energy. Classical examples include alkaline phosphatase, 5'-nucleotidase, lipases and proteases. In Gram-negative bacteria, such enzymes are located either in the periplasmic space, on the outer membrane, or are exported to the external milieu.

In *Salmonella enterica* and *Escherichia coli*, UDP-glucose (UDPG) hydrolases were discovered as enzymes which catalyse the hydrolysis of the pyrophosphate bond of UDPG leading to the formation of UMP and glucose-1-phosphate [1,2]. In *S. enterica*, the enzyme (UshB) is located primarily in the inner membrane by virtue of a hydrophobic N-terminal topogenic sequence; it is in an N-terminus inside/C-terminus outside orientation with enzyme activity accessible from the outside [1,3,4]. In *E. coli* and *S. enterica*, the enzyme (UshA) is periplasmic and also possesses 5'-nucleotidase activity [1,2]. As well as differing in their cellular location, these enzymes display no sequence or antigenic similarities. Interestingly, whilst most *S. enterica* serotypes contain both activities, most serotype Typhimurium isolates contain an inactive

(cryptic) *ushA* allele [5-7]. Likewise, *E. coli* K-12 and natural isolates of *E. coli* contain no detectable membrane-associated UDP-glucose hydrolase activity [1] due to an inactive allele of *ushB* [5,8].

The role of the UDPG hydrolase (5'-nucleotidase) in *E. coli*, which has quite broad specificity for 5'- and other nucleotides, can be considered to be the degradation of external nucleotides enabling the uptake and use of nucleosides and phosphate [9]. A scavenging role for the *Salmonella* enzyme, with its narrower specificity for UDP-sugars, however, is not so clear. With the recent discovery of a class of P2 receptors for UDPG on the surface of eukaryotic cells, the role of UDPG hydrolases can be cast in a new light, relating to the cellular response to bacterial infection.

2. P2Y Receptors and Nucleotide/UDPG Signalling

Nucleotides in the form of adenine and uracil tri- and diphosphates have long been known to constitute signalling molecules in eukaryotic tissues, playing a role as endogenous “danger signals” following release from cells during injury, stress, infection or death [10]; concentrations of extracellular nucleotides can increase from 0.5 - 10 nM to >100 nM following such events [11].

*Corresponding author.

Their receptors, the P2 (purinergic) receptor family, are found on the surface of most animal tissues and fall into two families: The P2X family are ion channel receptors and the P2Y family comprise G-protein-coupled receptors. The P2Y family in mammals can be subdivided pharmacologically into adenine (P2Y₁, P2Y₁₂ and P2Y₁₃) or uracil-preferring (P2Y₄, P2Y₆ and P2Y₁₄) receptors. The P2Y₁₄ receptor, a relatively recent addition to the P2Y family, is notable since it is a specific receptor for UDPG and related sugar nucleotides [12,13], though UDP is also reported to be an agonist of the human receptor [14,15]. The intracellular concentration of UDPG is about 100 mM [16] and mild mechanical stimulation of a number of cell lines, by a change of medium, increases extracellular levels of UDPG to 1 - 20 nM [17]. P2Y₁₄ has the highest expression in placenta, adipose tissue, stomach and intestine [12,13], and is highly expressed in the surface epithelial cells of other tissues such as lower respiratory tract and in enterocytes in mice [18]. The receptor is also expressed in rodent and human brain, localised to glial cells (specifically astrocytes), and is particularly apparent in immune cell types including neutrophils, lymphocytes and megakaryocytic cells [19]. Activation of P2Y receptors by nucleotides involves a wide variety of responses [20,21], but of specific interest here is the regulation of the immune responses in response to UDPG, UDP and bacterial infection.

3. P2Y Receptors and the Innate Immune Response

Arase *et al.* [18] have shown that UDPG elicits innate mucosal immunity in the mouse female reproductive tract where its cognate receptor, P2Y₁₄, is expressed in epithelial cells. UDPG up-regulates expression of P2Y₁₄ and stimulates IL-8 production, in human endometrial epithelial cells, leading to enhanced neutrophil chemotaxis. These results were shown to be P2Y₁₄-dependent, with similar findings in mouse uterus. The P2Y₁₄ receptor may also regulate chemotaxis of select bone-marrow derived hematopoietic stem cell populations [22], and mast cell function, a key to defence against Gram-negative bacteria [23], is also P2Y₁₄ responsive [24]. Given that P2Y₁₄ is expressed on the surface of a variety of tissues, Arase *et al.* propose a model for induction of innate immunity by microbial infection, or other insults, that extends to the lumen of other organs in addition to the FRT: damaged cells release UDPG into the lumen, up-regulating P2Y₁₄ in undamaged cells, with P2Y₁₄ activation triggering IL-8 release and neutrophil recruitment. This response, in isolated human neutrophils, involves Rho-mediated signalling [25]. Thus UDPG may act as an endogenous "danger signal" (or one of a number of damage-associated molecular patterns or DAMPS) in an analogous fashion to bacterial pathogen-associated molecular

patterns (PAMPs) which act via TLR receptors to provoke production of IL-8 and other cytokines. Maturation of dendritic cells (DCs) sensitive to PAMPs is P2Y₁₄-dependent: the findings of Skelton *et al.* [26] suggest P2Y₁₄ activation by UDP-glucose may initiate immune responses by promoting DC maturation. Curiously, alternate effects may arise in terms of viral responses: extracellular UDPG, UDP and UTP inhibit type I interferon production by virus-challenged plasmacytoid DCs, implicating involvement of P2Y₁₄ (and/or P2Y₄, P2Y₆) receptors in down-regulating immune surveillance against viral infection [27].

Support for the above concept of UDP-glucose as an immune danger signal derives from work on P2Y₆, whose agonist is UDP [20]. P2Y₆ (and P2Y₁₄) is expressed in a murine macrophage-like cell line (RAW 264.7 cells), as well as peritoneal and bone marrow-derived macrophages. Activation of P2Y₆ by UDP results in increased chemotaxis to splenocytes, peritoneal macrophages and RAW264.7 cells. In a peritonitis mouse model, intraperitoneal injection with UDP promoted clearance of *E. coli*, in contrast to mice treated with PBS or UDP plus a P2Y₆ antagonist. Similarly, and remarkably, mice treated with UDP showed greatly increased survival when challenged with *E. coli*.

4. UDP-Glucose Hydrolases as Bacterial Virulence Factors

Rather than a purely catabolic role, these observations suggest a new role for UDPG hydrolases and 5'-nucleotidase: Clearly abrogation of the innate immune response to bacterial infection would advantage growth of invading bacteria, and could be effected by degradation of released UDPG to limit P2Y₁₄ signalling. Likewise, UDPG-hydrolase-5'-nucleotidase could perform a similar role with respect to P2Y₆ signalling since UDP is also a good substrate for the nucleotidase function of this enzyme [2], in addition to 5'-nucleotides and UDP-sugars. Significantly, UDP-sugars, in contrast to nucleotides, are resistant to degradation by eukaryotic ecto-nucleotidases and therefore constitute a stable endogenous signal [25], albeit susceptible to degradation by bacterial UDPG-hydrolases. Thus, these latter enzymes may be postulated to constitute virulence factors. Infection experiments, *in vitro* or *in vivo*, with isogenic mutant and wild-type strains of *S. enterica* or *E. coli*, either lacking, containing or over-expressing UDPG hydrolase (*S. enterica*) or UDPG hydrolase-5'-nucleotidase (*E. coli*), would answer this question. Accumulation of released nucleotides should be greater, and the innate immune response enhanced, in enzyme deficient strains, and *vice versa* for enzyme over-expressing strains. Differences between innate immune responses to infection with isogenic bacterial strains should be at least of similar magnitude to those obtained with or

without P2 receptor antagonists; if there is receptor (and signalling) cross-talk, then the isogenic strain comparison might be particularly marked as compared to the use of receptor specific antagonists.

P2Y₁₄ is expressed in the brain in humans and mice [12,19,28]. The rat homologue, VTR15-20, is also expressed in astrocytoma and neuronal cell lines and in rat primary astrocytes and microglia; in the latter, it is up-regulated following challenge with zymosan, a stimulator of macrophage phagocytosis [29]. Up-regulation was also seen in discrete brain regions following *in vivo* challenge with LPS [19,29]. Rat primary astrocytes respond to UDPG with increases in intracellular Ca²⁺ [30]. In view of the importance of glial cells in an immunological response to infection [31], such observations suggest that P2Y₁₄, and possibly other related receptors, such as P2Y₆, may be important in an innate immune response to infection within the brain [19]. Kinoshita *et al.* also recently reported constitutive P2Y₁₄ activity in glial cells (astrocytes), regulating TNF- α release and subsequent MMP-9 activity [32]. Gram-negative bacteria are amongst those that can infect brain *via* either the blood-brain barrier or the olfactory route of infection [33-36]. It is possible that they possess UDPG-hydrolases and/or nucleotidases, unrelated to those in enteric bacteria, that may counter this intrinsic defence pathway.

Should further studies substantiate the roles of bacterial UDPG-hydrolases and/or 5'-nucleotidases as virulence factors in pathogenic *S. enterica* and *E. coli*, the question arises as to whether selection is "coincidental" or "direct" [37]. *S. enterica* and *E. coli* can exist in other growth environments, both environmental [38] and animal hosts other than human. As zoonotic or environmental opportunists [37], and in view of the capacity of UDP-sugar hydrolases for using substrates as a carbon and energy source, it is therefore very plausible that, as postulated virulence factors, they are pre-adapted for survival in the external environment (e.g. water, food), or possibly during carriage and growth in animal hosts. Thus they could be considered as coincidental virulence factors [37]. This argument is more likely for UDPG-hydrolase (5'-nucleotidase), which can recycle nucleotides, than for the unrelated UshB, of *S. enterica* which is a specific UDP-sugar hydrolase. The latter could quite possibly be directly selected as a virulence factor in the human host. Their dual use in different environments may be a factor in the silencing by missense mutation of *ushA* in certain natural isolates of *S. enterica* Typhimurium if use is required more consistently in a human or animal host over time, and not required (selected) in the external environment [6]. This may also be the case for other coincidental virulence factors since silencing by missense mutation is not revealed by sequence analysis alone.

5. Concluding Remarks

The role of P2Y receptors in immune modulation is now well established. It is inherently likely that certain bacterial pathogens will have some capacity to counter this defence mechanism. Whilst catabolic UDPG-hydrolases and 5'-nucleotidases may at first seem unlikely candidates for such a role, we suggest that they are indeed likely virulence factors since their substrates are involved in immune modulation. The known existence of virulence factors that are pre-adapted in alternate growth environments, in opportunistic pathogens, is also consistent with such a role, at least in the case of a UDPG-hydrolase with 5'-nucleotidase activity.

6. Outstanding Questions

Can the release of nucleotides including UDPG, and the innate immune response be demonstrated in response to infection by *E. coli* or *S. enterica* infection *in vitro*?

Are the levels of released nucleotides and the magnitude of the innate immune response in the presence of infection by *S. enterica* or *E. coli* response increased in isogenic enzyme deficient strains?

Are the levels of released nucleotides and the magnitude of the innate immune response in the presence of infection response decreased in isogenic enzyme over-producing strains?

Is the survival of mice in response to infection by *E. coli* and *S. enterica* modulated by *ushA/B* deficiency or over-expression, hence replicating results with *E. coli* and UDP administration?

What is the relationship between the presence of active or inactive alleles of *ushA* or *ushB* and the pathogenicity of *E. coli* and *S. enterica* strains? Will active membrane-associated UDPG-hydrolase (UshB) be found in clinical isolates of *E. coli*?

Is the P2Y mechanism of up-regulation of innate immunity of particular relevance in the response to bacterial (or viral) infection in the brain?

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