Bacterial Peptidoglycan-Degrading Enzymes and Their Impact on Host Muropeptide Detection

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Abstract

Peptidoglycan (PGN) is a major component of the bacterial cell envelope in both Gram-positive and Gram-negative bacteria. These muropeptides can be produced or modified by the activity of bacterial glycolytic and peptidolytic enzymes referred to as PGN hydrolases and autolysins. Some of these bacterial enzymes are crucial for bacterial pathogenicity and have been shown to modulate muropeptide release and/or host innate immune responses. The ability of muropeptides to modulate host responses is due to the fact that eukaryotes do not produce PGN and have instead evolved numerous strategies to detect intact PGN and PGN fragments (muropeptides). Here we review the structure of PGN and introduce the various bacterial enzymes known to degrade or modify bacterial PGN. Host factors involved in PGN and muropeptide detection are also briefly discussed, as are examples of how specific bacterial pathogens use PGN degradation and modification to subvert host innate immunity.

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Basic Peptidoglycan Structure

The peptidoglycan (PGN) structure of both Gram-positive and Gram-negative bacteria comprises repeating disaccharide backbones of N-acetylglucosamine (NAG) and β -(1-4)-N-acetylmuramic acid (NAM) that are cross-linked by peptide stem chains attached to the NAM residues [1]. The first 2 residues of the stem peptide are generally L-alanine and D-glutamine or isoglutamine, while the last residue is typically D-alanine (fig. 1a). In contrast, the third residue of the stem peptide is a lysine in coccoid Gram-positive bacteria (such as *Staphylococcus* and *Streptococcus* species), but a meso-diaminopimelate (mDAP) residue in both Gram-negative bacteria and many rod-shaped Gram-positive bacteria such as *Listeria* and *Bacillus* species [1]. In the latter (DAP-type) PGN, mDAP residues from 2 adjacent stem peptides typically link directly to each other. Conversely, the stem peptides of Lys-type PGN are bridged by a variable peptide usually comprised of 2–5 glycine and serine residues [2, 3].

Common Modifications to the Basic PGN Structure

Modifications or variations to the basic PGN structure occur frequently amongst bacterial species. Many modifications are species specific, due to the expression of unique synthetic, modifying or degradative enzymes.

However, the PGN structure of a given bacterium may also be changed in response to changes in growth conditions. The benefits of such modifications include enhanced resistance to antibiotics and host degradative enzymes that target the cell wall, and possibly impairment of detection by host innate immune receptors for intact PGN or the peptide and glycopeptide fragments of PGN commonly referred to as muropeptides.

Substitutions and modifications to the basic PGN structure occur in both the peptide stem and bridge regions and in the disaccharide backbone. The disaccharide backbone can be modified by addition of glycolic acid, via N-acylation, to muramic acid residues – as occurs in *Mycobacterium tuberculosis* [4]. It is also common for bacteria to deacetylate N-acetyl glucosamine residues [5]. In some pathogenic bacteria, such as *Streptococcus pneumoniae* and *Listeria monocytogenes* [6, 7], the deacetylases responsible for removing these acetyl groups are crucial for lysozyme resistance and virulence in infected animals. *Staphylococcus aureus* employs O-acetylation as an alternative method for modification of PGN sugars to increase resistance to lysozyme [8].

Modifications to the peptide stem portion of PGN include substitutions of glycine or L-serine residues for the L-alanine in position 1 in *Mycobacterium leprae* and *Butyrivibrio rettgeri*, respectively [3]. Similarly, D-glutamine at position 2 in the stem peptide can be substituted with glycine, amidated glycine, amidated glutamine or an amidated alanine [5]. At position 3, L-ornithine, LL-DAP and hydroxy-lysine can take the place of the mDAP and L-lysine [5]. Finally, the length and proportion of glycine versus serine residues in the bridge peptides of staphylococcal Lys-type PGN can be altered by expression of FemAB and homologous nonribosomal peptidyltransferases [9–11].

Bacterial growth phase also affects PGN structure. For example, the PGN of stationary phase *Bacillus subtilis* is more highly cross-linked than that found in rapidly growing bacteria [12]. Likewise, *Escherichia coli* that is not actively growing has PGN with a higher degree of cross-linking, an increase in lipoprotein additions and shorter glycan strands compared to actively dividing *E. coli* [13]. Several other bacterial pathogens enter into more unique ‘nongrowth states’ associated with PGN modifications: *Bacillus* and *Clostridium* species substantially alter their PGN between vegetative and spore-forming stages. Pathogenic *Mycobacteria* do not form spores, but enter into dormant states with modified PGN structure during latent infection. When exiting nongrowth states, resuscitation-promoting factors promote cell wall

digestion and reactivation from dormancy in *Mycobacteria* [14, 15]. Such digestion modifies the PGN structure and promotes the shift from dormancy into active growth. In addition to possible direct effects on the reactivation of latent infections, the digestion of PGN by resuscitation-promoting factors and other PGN-digesting enzymes can also lead to the release of bioactive muropeptides. The release of muropeptides from bacterial pathogens can have profound effects on pathogenesis and the initiation and regulation of host immune responses. This is because eukaryotic host organisms as diverse as legumes, insects and man have evolved multiple innate immune receptors to detect intact PGN and several distinct muropeptides, including those illustrated in figure 1b.

Bacterial PGN Hydrolases

Bacteria employ several classes of PGN-hydrolyzing enzymes that participate in assembly and disassembly of the bacterial cell wall during the processes of bacterial growth and division. PGN-hydrolyzing enzymes that bind to and degrade intact bacterial cells or PGN of the producing organism are classified as autolysins. Several pathogenic bacterial species have hydrolases or autolysins that are required for bacterial pathogenicity in eukaryotic hosts.

PGN hydrolases are defined by their catalytic specificities. Two classes of these enzymes function to digest the PGN glycan backbone: N-acetylmuramidases cleave PGN between the NAG-NAM bond upstream of NAM and N-acetylglucosaminidases cleave the NAM-NAG bond (fig. 1a). In contrast, N-acetylmuramyl-L-alanine amidases cleave between NAM and the first alanine of the peptide chain (fig. 1a). Thus, catalysis by N-acetylmuramyl-L-alanine amidases separate the PGN sugar backbones from the stem peptide chain [16, 17]. Another common family of PGN hydrolase is the lytic transglycosylases (LTs). These enzymes cleave between the N-acetylmuramic acid and N-acetylglucosamine sugar chains (fig. 1a) to facilitate cell growth or, in some cases, to make space for secretion systems and flagella [18, 19] or to produce cytotoxic muropeptides [20].

Further digestion of the stem peptide requires cleavage between stem amino acids by several carboxy- and endopeptidases. Overall, a full complement of glycosidases, amidases and endopeptidases provides bacteria the capacity to fully degrade PGN and recycle cell wall glycans and amino acids. However, such complete degradation does not always occur, as several bacteria are

known to release cell wall fragments (muropeptides) that exert potent biological effects on the host [21]. Such bioactive muropeptides include a disaccharide-tetrapeptide fragment of PGN known as tracheal cytotoxin (TCT; fig. 1b). TCT is released by both *Bordetella pertussis* and *Neisseria gonorrhoeae* and causes death of tracheal and vaginal ciliated epithelial cells as well as induction of slow-wave sleep [22–26]. Muramyl dipeptide (MDP) and related muropeptides (for example, GMDP) influence the activation of host immune responses and have adjuvant activity in promoting antibody responses (fig 1b).

Domain Structures of PGN Hydrolases

Sequence motifs indicative of conserved catalytic and noncatalytic domains are both typically found in the primary amino acid sequences of PGN hydrolases. Thus, bioinformatics analyses can be used to estimate the distribution of hydrolases with a given predicted catalytic specificity within the genomes of different pathogen species and strains. Such analyses reveal that there are often multiple enzymes with the same predicted cleavage specificity within a given organism. Furthermore, the numbers of enzymes with a particular specificity varies between species or strains. For instance, *Bacillus anthracis* Sterne encodes 9 genes with type 2 or 3 N-acetylmuramoyl-L-alanine amidase domains (EC:3.5.1.28). In contrast, *M. tuberculosis* H37Rv has only 4 and *L. EGD-e* has only 3 genes with such domains (fig. 2a). The selective pressure for retaining such redundancy is not clear. However, variations in the assortments of putative noncatalytic do-

main, and in some case the absence of obvious secretion signal peptides, suggest that these enzymes differ in their regulation and/or localization within or outside the bacterium. Given that the expression patterns of these genes may also vary, it is possible that individual enzymes with apparently identical catalytic specificities may play distinct roles in the biology or pathogenicity of a given bacterium. Indeed, the greater number of N-acetylmuramyl-L-alanine amidases found in the genome of *B. anthracis* Sterne may reflect the need for unique or 'extra' amidase activity during sporulation or spore germination.

Redundancy is also seen in another class of PGN hydrolases, D-glutamate-mDAP endopeptidases, which cleave between the third and fourth amino acid residues of the stem peptide in DAP-type PGN. D-glutamate-mDAP endopeptidase activity is associated with the so-called NlpC/P60 domain based on experimental evidence from studies with the *B. subtilis* LytF protein [27]. NlpC/P60 domains are typically found at the C-terminus and contain a conserved C-terminal histidine residue. Multiple proteins with NlpC/P60 domains are found in individual Gram-positive bacteria. Deletion of the *B. subtilis* *lytF* gene impaired bacterial cell wall separation and caused filamentous growth (chaining) of bacteria [27]. Another *B. subtilis* protein containing an NlpC/P60 domain is CwlS, which cleaves PGN at the D-g-glutamyl-meso-diaminopimelate bond and is localized to the cell poles/separation sites of the bacteria [28]. These data suggest possible roles for CwlF and CwlS in *B. subtilis* septation or other processes that occur at these sites. *L. monocytogenes* codes for at least 3 proteins with NlpC/P60 domains: p60, p45 and Lmo0394 (fig. 2b). The C-terminus of p45 is highly homologous to the same region in p60 [29]. However, based on the inability to engineer deletion mutants of p45, this enzyme appears to be essential for viability of *L. monocytogenes*. Conversely, neither p60 nor Lmo0394 are required for *L. monocytogenes* viability [30].

Other conserved domains associated with PGN hydrolytic activity include the cysteine- and histidine-dependent amidohydrolase/peptidase (CHAP) domain [31, 32], which was first identified in the *S. aureus* LytA autolysin but is also present in *E. coli* glutathionylspermidine synthetase, the putative lysin protein from *Streptococcus pyogenes* and lytic enzymes from *Streptococcus agalactiae* bacteriophage B30 [33] and *S. aureus* phage phi MR11 [34]. Interestingly, the CHAP domain is often found in association with other putative catalytic PGN hydrolase domains within a single protein. Thus, CHAP domains may enable a single enzyme to have multiple or novel catalytic specificities.

Fig. 1. Structure of Lys-type and DAP-type PGN. **a** Lys-type PGN contains a lysine at the third position of the peptide stem and is predominantly seen in Gram-positive bacterial species. DAP-type PGN contains a mDAP at the third position of the peptide stem and is predominantly seen in Gram-negative bacterial species. Arrows indicate sites of PGN cleavage by PGN hydrolytic enzymes: 1 = N-acetylglucosaminidases; 2 = N-acetylmuramidases; 3 = N-acetylmuramyl-L-alanine amidases; 4 = glutamyl-mesodiaminopimelate endopeptidases of the p60/NLP family; 5 = other endopeptidases; 6 = carboxy- and trans-peptidases such as those of the penicillin-binding proteins (PBP) family. Lytic transglycosylases can also cleave between the sugar backbone moieties (1 and 2). **b** Biologically active PGN fragments (muropeptides) include tracheal cytotoxin (TCT), (glutamyl)-muramyl dipeptide [(G)MDP] and (glutamyl)-muramyl tetrapeptide [(G)MTP]. (G)MDP triggers host Nod2 and NALP3 activation, while murine Nod1 detects both TCT and MTP.

Fig. 2. Pathogenic bacteria encode variable repertoires of 'redundant' PGN-hydrolyzing enzymes that may permit unique biological functions. **a** Considerable variation is seen in the numbers of PGN-hydrolyzing amidases and domain assortments within these enzymes between *B. anthracis* Sterne, *M. tuberculosis* H37Rv and *L. monocytogenes* EGDe. Shown are SLH domains, LGFP domains and PG-binding-1 domains, all of which are distinct putative PGN cell wall-binding domains. Variability in these domains may facilitate distinct binding or cleavage specificities for the various amidases, perhaps to target specific PGN substructures or mucopeptides. Protein names listed are NCBI designations. **b** Domain structures of the 3 *L. monocytogenes* PGN hydrolases that contain a NlpC/p60 catalytic domain. SH3 is a proline-binding domain and LysM is a PGN-binding domain, while functions of the threonine-asparagine repeat region are unknown. These related enzymes appear to have distinct functions, since mutation of p60 in *L. monocytogenes* 10403S appears to uniquely reduce pathogenicity and host NK cell responses in infected animals.

PGN hydrolases and modifying enzymes also typically contain one or more conserved domains not associated with catalytic activity per se. Such noncatalytic domains may assist in localizing these enzymes to specific sites on the bacterial cell wall or permit their association with bacterial cofactors or perhaps host proteins. Examples of noncatalytic domains commonly found in bacterial PGN hydrolases include the GW domains that are associated with binding to lipoteichoic acid, SH3-like domains that may interact with carbohydrates and/or polyproline stretches, FtsN PGN-binding domains, choline-binding domains and LysM domains [35]. Interestingly, LysM-like domains are also found in receptor kinases important for recognition of bacterial nodulation factors during the establishment of symbiotic rhizobial infections in leguminous plants [36, 37].

Host Detection of PGN and Mucopeptides

The ability of PGN and mucopeptides to influence the biology of eukaryotes results from the triggering of eukaryotic receptor systems that have evolved to detect these molecules. On the eukaryotic host cell surface, Toll-like receptor (TLR) 2 recognizes PGN or molecules associated with PGN [38, 39]. PGN or mucopeptides also bind cell surface and soluble peptidoglycan recognition proteins (PGRPs). The 17 *Drosophila* PGRPs positively or negatively influence the activation of host innate immune responses to bacteria [40–43]. Mammals also produce 4 known PGRPs: PGRP-S, PGRP-Iα, PGRP-Iβ and PGRP-L [44]. However, unlike insect PGRPs, mammalian orthologs have no known signaling capabilities. Rather, some human PGRPs bind directly to the PGN on

invading pathogens and are thus thought to interfere with PGN synthesis [45]. PGRP-L also has N-acetylmuramoyl-L-alanine amidase activity [46], and thus digests PGN at position 3 in figure 1a. This enzymatic activity may digest intact bacteria and/or reduce host pro-inflammatory responses to PGN or muropeptides [47]. However, while PGRP-L contributes to antibacterial *Drosophila* immune responses [48–50], its influence on mammalian innate immunity is less clear [51].

Certain members of the Nod-like receptor (NLR) family also detect specific muropeptides. NLR proteins include NALPs, NODs and NAIPs, and are generally expressed constitutively by antigen-presenting cells and epithelia. They are also often induced by inflammation and inflammatory/immune regulatory cytokines such as TNF- α and IFN- γ [52]. The C-terminal leucine-rich repeat domains of NLRs are related to the extracellular (ligand-binding) regions of TLRs. Certain NLRs also contain a NACHT or NOD domain for oligomerization, as well as 1 or more protein-protein interaction domains at their N-termini [52, 53]. Signaling through NLR proteins profoundly influences the regulation of host immune responses. For instance, mutations in the *nod2* gene are risk factors for the development of Crohn's disease [54–56], an inflammatory disorder of the intestines. Nod2 contributes to host recognition of MDP and regulates the activity of host NF- κ B family transcription factors [57, 58]. Human Nod1 also influences NF- κ B activity, but senses distinct, mDAP-containing muropeptides derived from DAP-type PGN. Nod1 detects MTP and related structures (fig. 1b). The minimum structure sensed by Nod1 is a dipeptide comprised of L-glutamate-mDAP [59]. Like Nod2, the NALP3 NLR protein also appears to be activated by MDP [60, 61]. NALP3 forms a complex with Caspase 1 and other components to form an inflammasome that regulates the activation and secretion of pro-IL-1 β and other substrates. Also like *nod2*, mutations in *nod1* and *nalp3* genes predispose to inflammatory diseases, including asthma and Muckle-Wells syndrome [62, 63].

Subversion of Muropeptide Detection and Immune Responses

The activity of PGN hydrolases and autolysins can promote release of various muropeptides into an infected host or host cell or may, in some cases, destroy the ability of specific muropeptides to trigger PGRPs or NLRs. Hence, PGN hydrolytic activity and other mechanisms that skew muropeptide release can modify the host re-

sponse in a manner favorable for the pathogen. Indeed, evidence from studies with several bacterial pathogens support the concept that PGN digestion and modification are common pathogenic strategies to subvert muropeptide recognition and host innate immunity.

N. gonorrhoeae

AmiC is an *N. gonorrhoeae* N-acetylmuramyl-L-alanine amidase active during cell separation. Deletion of this autolysin results in growth inhibition and also reduced PGN fragment release. Specifically, metabolic labeling studies revealed that AmiC mutants fail to release PGN disaccharides [64]. Thus, AmiC may help reduce release of specific immune-stimulatory muropeptides. The putative *N. gonorrhoeae* LTs, LtgE and LtgD, also degrade PGN into monomers and free disaccharides. *N. gonorrhoeae* double mutants lacking both of these LTs released no PGN monomers or free disaccharide from the bacterial cell wall [20]. LtgE and LtgD are thought to degrade larger PGN fragments into smaller forms, as a way to break down the cell wall to components available for recycling into new PGN. Such activities may enable the bacterium to evade specific host muropeptide detection systems. In contrast, the *N. gonorrhoeae* LT LtgA appears to specifically enhance the production of PGN cytotoxin. This cytotoxic fragment of PGN is identical to the bioactive TCT muropeptide released from *B. pertussis* [65]. Bacteria deficient in LtgA show a decrease in PGN turnover during log phase growth, as well as decreases in PGN monomer and free disaccharide release. Thus, the major function of LtgA may be to induce the killing of ciliated cells via PGN cytotoxin/TCT release, which may in turn prohibit ciliary expulsion of invading bacteria. Enhancement of the inflammatory response and influx of inflammatory cells such as neutrophils are a by-product of the PGN release and may also contribute to dissemination of *N. gonorrhoeae* [20].

In addition to autolysins, several transporters and recycling machinery are required for efficient PGN turnover and muropeptide release. In *N. gonorrhoeae*, AmpG is a cytoplasmic PGN transporter and AmpD is an amidase that is specific for anhydro-muropeptides, which it separates into stem peptides and sugar residues. AmpD deficiency inhibits breakdown of larger PGN fragments and AmpG mutants release more PGN monomers [66]. Thus, changes in expression of AmpG and AmpD may further modulate release of TCT and other muropeptides.

B. pertussis

B. pertussis TCT is a DAP-containing muropeptide, GlcNAc-(anhydro)MurNAc-L-Ala-D-Glu-mesoDAP-D-Ala, present in DAP-type PGN and released during log phase growth. In humans, PGRPI α C binds to TCT, and in *Drosophila* TCT binds PGRP-LC and activates the Imd pathway. The result of TCT recognition is the destruction of lung and vaginal ciliated epithelial cells – as seen in whooping cough and gonorrhea [67]. TCT also induces production of TNF- α , IL-6, IL-1 β and KC secretion in wild-type mouse peritoneal macrophages, but not Nod1-deficient macrophages. The synthetic agonist FK156 also induces cytokine responses via Nod1, and can synergize with endotoxin to induce nitric oxide and cause epithelial damage.

In addition to releasing toxic PGN fragments, autolysins in *B. pertussis* also modify PGN to allow the release of other virulence factors into host cells. PtlE is a PGN hydrolase that has an active site similar to glycohydrolases [68]. PtlE deletion mutants or mutants with PtlE protein that lacks catalytic activity are both deficient in secretion of pertussis toxin, an ADP ribosyltransferase that interferes with signaling through G protein-coupled receptors. Thus, PGN hydrolysis by PtlE results helps *B. pertussis* to impair migration of host inflammatory cells.

Helicobacter pylori

H. pylori is a Gram-negative bacterial pathogen that infects the gastric mucosa. In the course of infection, *H. pylori* transfers PGN fragments directly into gastric epithelial cells via a type IV secretion system [69]. Bacterial mutants that lack the *cagPAI* genes that encode for this secretion system fail to induce NF- κ B signaling via Nod1. The Nod1 specificity of this interaction was also shown using dominant-negative CARD domain Nod1 mutants and siRNA to Nod1. Cells expressing such mutant Nod1 forms showed reduced activation of NF- κ B and production of IL-8 during *H. pylori* infection [69]. The LT activity of Slt enzymes appears to produce the muropeptides secreted by the *cag* secretion system [70]. Similarly, *Shigella flexneri* triggers Nod1-dependent responses and expresses a homolog of Slt, known as SltY.

H. pylori also undergoes shape changes during different phases of growth. In vitro, the bacterium is a curved rod, whereas the mature infectious form of the bacterium assumes a coccoid morphology. These coccoid *H. pylori* are abundant in severely damaged regions of infected stomachs [71]. The PGN of both curved and coccoid forms of *H. pylori* is rich in pentapeptide side chain

muropeptides. However, in the coccoid form there is an accumulation of dipeptide monomers and a decrease in tri- and tetrapeptide fragments. During stationary phase there is also an increase in the number of glycine-containing peptides in *H. pylori* PGN [71]. Since epithelial cells require Nod1 signaling to induce inflammatory responses, the morphological changes in *H. pylori* appear to help it dampen an inflammatory immune responses and escape detection [72]. Thus, PGN from the spiral form of *H. pylori* activates NF- κ B via Nod1 and leads to IL-8 production, but the coccoid form activates NF- κ B via Nod2 and fails to induce IL-8. AmiA is a PGN hydrolase important in the change from spiral to coccoid forms [72]. AmiA deletion mutants of *H. pylori* are impaired in their ability to change into coccoids and deficient in the release of the Nod2 agonist GMDP. These data suggest a contribution of increased Nod2 signaling to reduced host inflammatory responses against *H. pylori*. Furthermore, defective Nod2 signaling may predispose to inflammation-induced cancer, since the Nod2 R702W mutation has been associated with gastric lymphoma [73].

M. tuberculosis

Like *H. pylori*, *M. tuberculosis* employs autolysins that alter the PGN layer between different growth states. Instead of changing shapes, *M. tuberculosis* moves from active to dormant growth states. The resuscitation-promoting factors (Rpf) are PGN-hydrolyzing enzymes involved in this growth state shift from dormancy into reactivation of the bacterium. Some of these Rpf proteins, specifically RpfB and RpfE, associate with RipA and colocalize to the septae of the cells [14]. *M. tuberculosis* deficient in 4 (ABCD) or even 5 (ABCDE) of the 5 Rpf show delayed colony formation on agar plates, but are viable. However, such strains are avirulent in mice [14]. Conversely, deletion of any single Rpf is not sufficient to cause virulence or reactivation defects. Thus, some of the Rpf may be able to compensate for the loss of others. However, deletion of both RpfA and RpfB in *M. tuberculosis* results in a defect in reactivation from dormancy [15]. Furthermore, bone marrow-derived macrophages infected with RpfA and RpfB double mutants produced approximately 2-fold higher levels of IL-6 and TNF- α compared to wild-type *M. tuberculosis* infection [15]. Thus, Rpf proteins and their PGN products may also reduce pro-inflammatory responses during reactivation of *M. tuberculosis* from dormancy.

L. monocytogenes

L. monocytogenes uses both autolysins and deacetylases to influence PGN structure and release as well as host immune responses. The autolysins p60 and NamA are secreted by the SecA2 secretion system and cleave PGN at sites predicted to increase release of the Nod2 agonist muropeptide, GMDP [70]. The p60 autolysin may also alone disrupt Nod1-dependent responses by cleaving the D-glutamate-mDAP bond. Work from our laboratory has also shown that the p60 autolysin is required for *L. monocytogenes* virulence and also that expression of p60 enhances NK cell activation during the innate immune response to this pathogen [74]. Depletion of NK cells reduces *L. monocytogenes* growth in infected animals [75, 76], thus p60-dependent enhancement of the NK cell response may improve bacterial growth and dissemination within the host.

The cell surface autolysin IspC [77] has also been implicated in *L. monocytogenes* virulence [78]. While IspC deletion does not impact in vitro growth of the bacteria, it does show decreased bacterial burden and mortality in infected mice. Like the PtlE hydrolase in *B. pertussis*, IspC has function beyond PGN hydrolysis, as it appears to be required for surface display of other virulence factors, such as the *L. monocytogenes* ActA protein [78]. Finally, NAG fragments of PGN may be deacetylated into glucosamine by the *L. monocytogenes* N-deacetylase. Deletion of this gene renders *L. monocytogenes* more susceptible to lysozyme and impairs its access to the host cell cytosol [7]. Fully acylated PGN fragments are more readily detected by Nod proteins, so the deacylation of *L. monocytogenes* PGN may allow the pathogen to avoid signaling via Nod proteins during infection.

Conclusions

Recent years have seen the identification and study of several host pathways that detect intact bacterial PGN or muropeptides, including TLRs, PGRPs and NLR family proteins. Conservation of such pathways in mice, man and other eukaryotes highlights their importance but also provides selective pressure for bacterial virulence mechanisms that subvert PGN and muropeptide detection. Bacteria employ multiple enzymes that regulate PGN modifications and release of muropeptides. In some cases these enzymes promote generation of 'stealth' muropeptides that are not detected by the host, as seen with the Rpf and AmiA enzymes of the chronic bacterial pathogens *M. tuberculosis* and *H. pylori*. For other bacteria, including *B. pertussis*, *N. gonorrhoeae*, *S. pneumoniae* and *L. monocytogenes*, pathogenesis requires specific PGN hydrolases or other enzymes that permit release of bioactive muropeptides like TCT, (G)MTP, and (G)MDP. Although detection of (G)MPD by Nod2 appears to prevent chronic inflammation and tissue damage associated with Crohn's disease, the detection of such muropeptides by most PGRPs, Nod1 or NALP3 generally increases inflammation. It thus remains to be determined how the sum of the various host muropeptide detection systems influences immune activation and the context in which such activation can promote bacterial pathogenicity. Future studies in the field should more precisely delineate how bacterial PGN degradation and modification machinery influence host inflammatory and antibacterial immune responses. A better understanding of the effects of muropeptide detection should ultimately improve therapeutic strategies for directing host inflammatory responses in the context of colonization by commensal versus pathogenic bacteria.

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