

OPINION

Breaching the great wall:
peptidoglycan and microbial
interactions

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Abstract | Once thought to be a process that occurred only in a few human pathogens, release of biologically active peptidoglycan fragments during growth by Gram-negative bacteria controls many types of bacterial interaction, including symbioses and interactions between microorganisms. This Perspective explores the role of peptidoglycan fragments in mediating a range of microbial–host interactions, and discusses the many systems in which peptidoglycan fragments released during bacterial growth might be active.

Most bacteria are stabilized by peptidoglycan (polymeric glycopeptide murein, PG), which forms a layer of the cell wall in both Gram-negative and Gram-positive bacteria, although the PG layer in Gram-positive bacteria is thicker and more crosslinked. PG is composed of a network of glycan strands that are interlinked by short peptides (reviewed in REF. 1). The glycan chains are formed by alternating *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) linked by β -1,4-glycosidic bonds. A variable peptide is attached by an amide linkage to the carboxyl group of each muramic acid. In Gram-negative bacteria, the peptide is usually made up of four amino acids: L-alanine, D-glutamic acid, meso-diaminopimelic acid and D-alanine (FIG. 1a).

The PG sacculus regulates bacterial size, shape, internal pressure and diffusion of molecules into the cell^{1,2}. The mechanisms that control PG expansion during bacterial elongation and septation are not completely understood; however, it is known that the PG sacculus is remodelled during growth. Goodell showed that *Escherichia coli* breaks down nearly 50% of its PG every generation³. This breakdown is mediated by lytic transglycosylases, which cleave PG monomers from the PG sacculus, and by a carboxypeptidase, which removes the terminal alanine. In *E. coli*, the tripeptide monomers are transported into the cytoplasm by a membrane protein AmpG and then the tripeptide is cleaved from the sugar by the PG amidase, AmpD⁴ (FIG. 1b). It is thought that the sugars and peptides are then reincorporated into the PG layer,

but the complete mechanism is unknown (reviewed in REF. 5).

Until recently, it was believed that almost all Gram-negative bacteria recycled PG similar to *E. coli*, but the release of biologically active PG fragments during growth by Gram-negative bacteria was thought to be limited to two human pathogens, *Bordetella pertussis* and *Neisseria gonorrhoeae*. PG monomers released by *B. pertussis* and *N. gonorrhoeae* cultures have been shown to cause damage to ciliated cells in organ culture, similar to the pathology caused by *B. pertussis* and *N. gonorrhoeae*^{6–9}. Lysis of bacteria produces PG fragments that stimulate the innate immune system. However, this process is distinct from the release of PG fragments by growing Gram-negative bacteria in that cell death is required and the PG fragments lack a 1,6-anhydrobond.

Although it was once believed that the role of PG was confined to its involvement in cell-wall structure and septation, it is becoming increasingly evident that PG fragments have a much broader role in mediating interactions between bacteria and other organisms. PG fragments have been shown recently to be secreted into host cells by *Helicobacter pylori*¹⁰, thereby expanding the range of pathogens for which released PG fragments function as virulence factors. Intriguingly, the same PG fragment that functions in virulence also coordinates colonization of the squid *Euprymna scolopes* by its bacterial symbiont *Vibrio fischeri*¹¹. These data, and several additional studies, indicate that signalling by PG fragments is not merely a pathway used by the host

for detection of a few specific pathogens, but represents a mechanism of microbial interaction conserved among many types of bacterial relationships, including symbiotic associations, microbial interactions and pathogenesis in animals and possibly plants.

This Perspective discusses recent advances in our understanding of the role of PG fragments in microbial interactions and proposes that PG fragments have many diverse roles, including involvement in symbioses and microbial–plant interactions. Although PG fragments from Gram-positive bacteria also induce host responses (reviewed in REF. 12), here we focus on PG fragments released by Gram-negative bacteria — tracheal cytotoxin (TCT) and related PG fragments containing diaminopimelic acid.

PG fragments in pathogenesis

TCT is a single monomeric unit of PG, consisting of GlcNAc, MurNAc, glutamic acid, diaminopimelic acid, and two alanines¹³ (FIG. 1a). Goldman and co-workers established that TCT is responsible for the destruction of ciliated respiratory epithelial cells during *B. pertussis* infection⁶. TCT is released by *B. pertussis* during log-phase growth and functions synergistically with endotoxin to inhibit DNA synthesis in cultured tracheal epithelial cells, induce the production of interleukin-1 α and nitric oxide, and cause the selective epithelial damage that corresponds to the airway pathology of whooping cough (caused by *B. pertussis*)¹⁴.

Since the discovery of the role of TCT in *B. pertussis* pathogenesis, the function of TCT or related muropeptides in other diseases has been established through compound purification and testing, receptorspecificity studies and mutant screens. Sinha and Rosenthal showed that *N. gonorrhoeae* releases multiple PG fragments⁹, including TCT, which was shown by Melly *et al.* to induce sloughing of ciliated fallopian tube cells in a manner identical to that of whole gonococci⁸. PG fragments extracted from *Haemophilus influenzae* produce brain oedema, leukocytosis and protein accumulation in the cerebrospinal fluid in a rabbit model of meningitis¹⁵, and tympanic membrane inflammation, abnormal middle ear pressure and localized bleeding in the middle ear mucosa in a chinchilla model of otitis media¹⁶.

Viala *et al.* have shown that *H. pylori* releases several muropeptides, which are transferred into epithelial cells through a type IV secretion system¹⁰. Once translocated to the host cell, the PG monomers stimulate an intracellular receptor, NOD1

