Review

Innate Immunity

16(3) (2010) 168–174

ISSN 1753-4259 (print)
10.1177/1753425910366059

Mammalian peptidoglycan recognition proteins (PGRPs) in innate immunity

Roman Dziarski, Dipika Gupta

Indiana University School of Medicine Northwest, Gary, Indiana, USA

Peptidoglycan recognition proteins (PGRPs or PGLYRPs) are innate immunity proteins that are conserved from insects to mammals, recognize bacterial peptidoglycan, and function in antibacterial immunity and inflammation. Mammals have four PGRPs – PGLYRP1, PGLYRP2, PGLYRP3, and PGLYRP4. They are secreted proteins expressed in polymorphonuclear leukocytes (PGLYRP1), liver (PGLYRP2), or on body surfaces, mucous membranes, and in secretions (saliva, sweat) (PGLYRP3 and PGLYRP4). All PGRPs recognize bacterial peptidoglycan. Three PGRPs, PGLYRP1, PGLYRP3, and PGLYRP4 are directly bactericidal for both Gram-positive and Gram-negative bacteria and have no enzymatic activity, whereas PGLYRP2 is an *N*-acetylmuramoyl-L-alanine amidase that hydrolyzes bacterial cell wall peptidoglycan. Peptidoglycan recognition proteins influence host–pathogen interactions not only through their antibacterial or peptidoglycan-hydrolytic properties, but also through their pro-inflammatory and anti-inflammatory properties that are independent of their hydrolytic and antibacterial activities. The PGRPs likely play a role both in antibacterial defenses and several inflammatory diseases. They modulate local inflammatory responses in tissues (such as arthritic joints) and there is evidence for association of PGRPs with inflammatory diseases, such as psoriasis.

Keywords: innate immunity, peptidoglycan, pattern recognition, bacteria, inflammation

INTRODUCTION

Peptidoglycan recognition proteins (PGRPs) were first discovered in the hemolymph of insects as proteins that bind bacterial peptidoglycan and activate the prophenoloxidase pathway, an antimicrobial host defense mechanism in insects.[1] Cloning of insect PGRP has led to the discovery of mouse and human PGRP orthologs, thus showing that PGRPs are highly conserved from insects to mammals.[2–4] All PGRPs function in antibacterial defenses and innate immunity. Insects have many PGRPs with diverse functions. Insect PGRPs either sense bacteria and trigger host defense pathways that generate antibacterial products, or they hydrolyze or non-enzymatically neutralize pro-inflammatory bacterial peptidoglycan and thus limit inflammation.[5,6]

Mammals have four PGRPs, three of which were initially identified as bactericidal proteins and one as a peptidoglycan-hydrolytic enzyme, amidase.[5] Recent results, however, indicate that mammalian PGRPs also modulate inflammation and immune responses independently of their bactericidal and enzymatic activities.[7]

Genes and protein structure

Mammals have four PGRPs that were initially named PGRP-S, PGRP-L, and PGRP-Iα and PGRP-Iβ (for 'short', 'long', or 'intermediate' transcripts, respectively), by analogy to insect PGRPs.[4] The names for human PGRPs were then changed by the Human Genome Organization Gene Nomenclature Committee to PGLYRP1, PGLYRP2, PGLYRP3, and PGLYRP4, respectively, and this nomenclature has been adopted for all mammalian PGRPs.

PGRPs have at least one C-terminal type 2 amidase domain, called a PGRP domain, that is ~165

Received 15 January 2010; Revised 12 February 2010; Accepted 16 February 2010

Correspondence to: Roman Dziarski, Indiana University School of Medicine Northwest, Gary, IN 46408, USA. Tel: +1 219 980 6535; Fax: +1 219 980-6566; E-mail: rdziar@iun.edu

amino-acids long and is structurally homologous to bacteriophage and bacterial type 2 amidases.[2–6] The PGRP domain is longer at its N-terminus than type 2 amidase domain, contains a PGRP-specific segment, not present in type 2 amidases, and, as discussed later, not all PGRP domains have amidase activity.[5,6,8] Mammalian PGLYRP1 is ~200 amino-acids long, has a signal peptide, one PGRP domain, and a molecular mass of ~18–20 kDa. PGLYRP2 has one C-terminal PGRP domain and an N-terminal sequence that is twice as long and has no homology to PGRP domain or any other proteins. PGLYRP3 and PGLYRP4 have two PGRP domains, which, however, are not identical and, for example, in human PGLYRP3 and PGLYRP4 have only 37–43% identity.[4–6]

All mammalian PGRPs have two closely spaced conserved Cys in the middle of their PGRP domain that form a disulfide bond, which is needed for the structural integrity and activity of PGRPs.[5,6] A mutation in one of these Cys in human PGLYRP2 (C419A) abolishes its amidase activity.[9] Mammalian PGRPs have two additional conserved Cys that form a second disulfide bond, and many mammalian PGRPs (PGLYRP1 and the C-terminal PGRP domain of PGLYRP3 and PGLYRP4) have another conserved pair of Cys that form a third disulfide.

The crystal structure of PGRPs reveals a general design similar to type 2 bacteriophage amidases: PGRP domain has three peripheral α-helices and several central β-sheet strands.[8,10,11] The front face of the molecule has a cleft that forms a peptidoglycan-binding groove, and the back of the molecule has a PGRP-specific segment (not present in bacteriophage amidases) that is often hydrophobic and is also more diverse among various PGRPs. PGLYRP2 and all non-mammalian amidase-active PGRPs have a conserved Zn^{2+}-binding site in the peptidoglycan-binding groove, which is also present in bacteriophage type 2 amidases and consists of two His, one Tyr, and one Cys (C530 in human PGLYRP2). In non-amidase PGRPs (PGLYRP1, PGLYRP3, and PGLYRP4), this Cys is substituted with Ser.[9,12] Thus, the general design of PGRP domains in all PGRPs is similar to accommodate binding of muramyl pentapeptide fragment of peptidoglycan, but the fine details of the structure of PGRP domains in various PGRPs differ depending on the function and fine specificity for various types of peptidoglycan.

All mammalian PGRPs are secreted and PGLYRP1, PGLYRP3, and PGLYRP4 form disulfide-linked homodimers.[13–15] Moreover, if PGLYRP3 and PGLYRP4 are expressed in the same cells, they almost exclusively form disulfide-linked heterodimers.[15] PGLYRP2 also forms dimers, but they are not disulfide-linked.[16] Schematic structures of PGRP dimers are shown in Table 1 modeled on available crystallographic data for PGLYRP1 and C-terminal PGRP domain of PGLYRP3 and PGLYRP4.

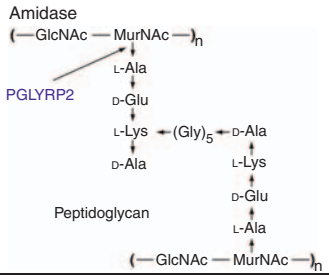
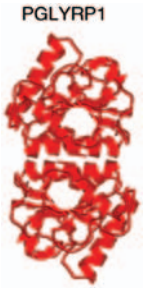
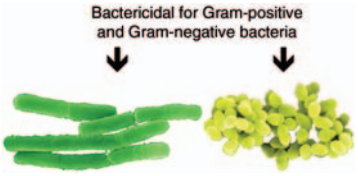
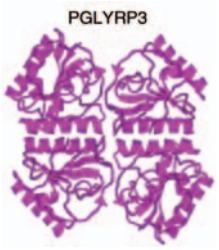
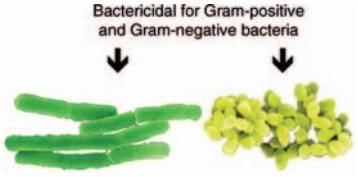
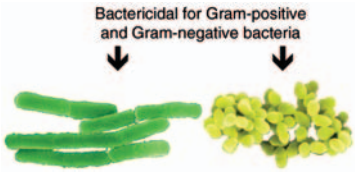
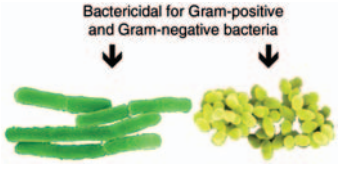
Expression

PGLYRP1 is highly expressed in the bone marrow in polymorphonuclear leukocytes and their precursors,[2,4] and the protein is almost exclusively present in the tertiary (secretory) granules,[17–21] from which it could be released by exocytosis during phagocytosis. PGLYRP1 is also expressed in the lactating mammary gland,[22] and bovine PGLYRP1 is expressed in eosinophils, in addition to neutrophils.[18] Lower expression of PGLYRP1 is found in intestinal M-cells[23] and also in many non-immune cells, such as epithelial cells and fibroblasts.[7,24,25]

PGLYRP2 is constitutively expressed in the liver, from where it is secreted into blood.[13,14] This liver PGLYRP2 and serum *N*-acetylmuramoyl-L-alanine amidase (that was earlier identified but not cloned) are the same protein encoded by the PGLYRP2 gene.[14] PGLYRP2 is also expressed at lower levels in oral and intestinal epithelial cells;[23,24] its expression is induced in keratinocytes and other epithelial cells and fibroblasts by exposure to bacteria and cytokines.[7,24–27] Peptidoglycan-induced expression of PGLYRP2 is nucleotide-binding oligomerization domain (NOD)2-dependent.[7] Some mammals express multiple splice forms of PGLYRP2 that may have different expression and possibly multiple functions. For example, pigs have two PGLYRP2 splice forms, short and long. They both have amidase activity, and the long form has a similar expression to human PGLYRP2, whereas the short form is constitutively expressed in several tissues, including bone marrow, intestine, liver, spleen, kidney, and skin.[28]

PGLYRP3 and PGLYRP4 proteins are selectively expressed in the skin epidermis, hair follicles, sebaceous glands and sweat glands; in the eye's ciliary body and corneal epithelium; in the mucus-secreting cells of the main salivary (submandibular) gland; and in mucus-secreting glands in the throat (both mucus-secreting glands selectively express PGLYRP4, but not PGLYRP3); in the tongue and esophagus in squamous epithelial cells; in the stomach in acid-secreting parietal cells (PGLYRP3) and glycoprotein-secreting neck mucous cells (PGLYRP4); and in the small and large intestine in the columnar absorptive cells, but not in mucus-secreting goblet cells and not in the crypts in Paneth cells, which produce antimicrobial peptides.[15,29] Bacteria and their products increase the expression of PGLYRP3 and PGLYRP4 in keratinocytes,[15] fibroblasts,[7] and oral epithelial cells,[24] likely through activation of Toll-like receptor (TLR)2, TLR4, NOD1, and NOD2.

Table 1. Structure, expression, and biological activities of mammalian PGRPs

Enzymatic	Expression	Activity[a]	Other effects
	Liver → serum Skin keratinocytes Oral and intestinal epithelium		Pro-inflammatory alarmin Peptidoglycan-induced inflammation and arthritis
Antibacterial	**Expression**	**Activity[a]**	**Other effects**
	Bone marrow polymorphonuclear neutrophils Intestinal M cells Epithelial cells Fibroblasts Eye corneal epithelium		Anti-inflammatory
	Skin Epidermis Hair follicles Sebaceous glands Sweat glands Oral and intestinal epithelium Stomach parietal cells Eye ciliary body and corneal epithelium		Anti-inflammatory
	Skin Epidermis Hair follicles Sebaceous glands Sweat glands Oral and intestinal epithelium Mucus-secreting cells in salivary glands and throat Stomach neck mucous cells Eye ciliary body and corneal epithelium		Anti-inflammatory
	Skin Epidermis Hair follicles Sebaceous glands Sweat glands Oral and intestinal epithelium Eye ciliary body and corneal epithelium		Anti-inflammatory

PGLYRP structures were rendered by RasMol and arranged as homodimers or heterodimers (the arrangements are arbitrary, because structures of dimers have not been yet solved). The structure of PGLYRP1 and C-terminal half of PGLYRP3 and PGLYRP4 are based on PDB entries 1yckA, 1SK3A, and 2EAX, respectively; the remaining structures were predicted by Swiss-Model; structure of the N-terminal portion of PGLYRP2 cannot be predicted. Thus, PGLYRP1 homodimer has two PGRP domains (one from each monomer), PGLYRP2 homodimer also has two PGRP domains (at the C-terminus of each monomer), and PGLYRP3 and PGLYRP4 homodimers, and PGLYRP3:4 heterodimer have four PGRP domains (two from each monomer).

[a]PGLYRP1, PGLYRP3, PGLYRP4, and PGLYRP3:4 do not have amidase activity. Images of bacteria provided by Dennis Kunkel Microscopy, Inc.

Peptidoglycan binding

Mammalian PGRPs recognize peptidoglycan, which is an essential cell wall component of all bacteria and which is not present in eukaryotic cells.[5,6] Peptidoglycan is a polymer of β-(1→4)-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc), cross-linked by short peptides containing alternating L- and D-amino acids.

Crystallographic analysis of human PGLYRP1 and C-terminal PGRP domain of PGLYRP3, as well as insect PGRPs, shows that PGRP domain has a ligand-binding groove that binds peptidoglycan and is specific for muramyl-tripeptide.[5,6,8,10,11,30] It binds GlcNAc–MurNAc–tetra- and –pentapeptide with higher affinity, but it does not bind muramyl-dipeptide or a peptide without MurNAc.[31,32] Binding of muramyl-pentapeptide to the C-terminal fragment of PGLYRP3 induces a conformational change in the PGRP domain that locks the ligand in the binding groove, which contributes to the high affinity of binding of PGRPs to peptidoglycan.[33]

Mammalian PGRPs bind to both Gram-positive and Gram-negative bacteria.[15,17] Although PGRPs can discriminate between amino acids present in the peptides of various peptidoglycans and although this discrimination imparts the specificity of PGRP-mediated responses in insects,[5] there is little evidence for similar functional discrimination by PGRPs in mammals. Moreover, some PGRPs may also bind to other polymers, such as lipoteichoic acid (LTA) and lipopolysaccharide (LPS) and also to some fungi.[15,17,19] However, human and mouse PGRPs have the highest affinity for peptidoglycan and much lower for LTA and LPS,[15,17] whereas bovine PGLYRP1 seems to have high affinity for LTA and LPS.[19] Binding results, however, should be always interpreted with caution when commercial ligand preparations are used, because they may have some contaminants. It is also not clear whether these other ligands bind to the peptidoglycan-binding groove or to another portion of the PGRP molecule, such as the hydrophobic region on the opposite side of the molecule. Binding of peptidoglycan outside the peptidoglycan-binding groove contributes to the formation of oligomers[34] or dimers of some insect PGRPs.[35]

Amidase activity

Mammalian PGLYRP2 is an *N*-acetylmuramoyl-L-alanine amidase that hydrolyzes the lactyl bond between the MurNAc and L-Ala in bacterial peptidoglycan (Table 1).[9,12] The preferred substrates are soluble peptidoglycan fragments, such as products of peptidoglycan digestion by the second host peptidoglycan-hydrolyzing enzyme, lysozyme, or by bacterial peptidoglycan hydrolases, whereas intact cross-linked peptidoglycan in the cell wall is a poor PGLYRP2 substrate. The minimum peptidoglycan fragment hydrolyzed by PGLYRP2 is muramyl tripeptide (similar to the minimal PGRP-binding fragment), whereas muramyl dipeptide is not hydrolyzed by PGLYRP2.[9] Serum PGLYRP2 may have a scavenger function similar to amidase-active insect PGRPs, and thus may neutralize pro-inflammatory peptidoglycan,[36] although, in tissues, PGLYRP2 has an opposite effect and participates in induction of inflammatory response.[7] The latter process, however, is independent of its amidase and peptidoglycan-binding activities.[7] PGLYRP1, PGLYRP3, and PGLYRP4 do not have amidase activity, because, as already mentioned, they have a Ser instead of Cys (which is critical for Zn^{2+} binding) in the enzyme's catalytic site.[9,12]

Bactericidal activity

Human PGLYRP1, PGLYRP3, PGLYRP4, and PGLYRP3:PGLYRP4 heterodimers, and bovine PGLYRP1 are bactericidal or bacteriostatic for many pathogenic and non-pathogenic Gram-positive and Gram-negative bacteria.[15,18,19,37] PGLYRP1, PGLYRP3, and PGLYRP4 from other mammalian species are also likely to have similar bactericidal activity. Bovine PGLYRP1 also has some microbicidal activity against a fungus, *Cryptococcus neoformans*.[18,19]

Mammalian PGLYRP1, PGLYRP3, and PGLYRP4 form a new class of bactericidal proteins that have a different structure, mechanism of action, and expression than currently known mammalian antimicrobial peptides.[15,37,38] The PGRPs are much larger than all currently known vertebrate antibacterial peptides: PGLYRP-1, PGLYRP-3, PGLYRP-3:4, and PGLYRP-4 proteins are disulfide-linked glycosylated 44 kDa, 89 kDa, 98 kDa, and 115 kDa dimers, respectively,[15] and vertebrate antimicrobial peptides are typically 3–15 kDa. PGRPs require divalent cations and *N*-glycosylation for bactericidal activity, which are not usually required by membrane-permeabilizing antibacterial peptides, such as defensins or magainin. Mammalian PGRPs also differ from antimicrobial peptides in their mechanism of bactericidal activity: the former kill bacteria by interacting with cell wall peptidoglycan and the latter by permeabilizing bacterial membranes. Furthermore, the expression patterns of mammalian PGRPs and antimicrobial peptides are different, and some cells that produce large amounts of these peptides (*e.g.* Paneth cells, which produce defensins, phospholipase A_2, and lysozyme) do not express PGRPs.[15] The PGRPs, however, are present in similar

sites in the body as antimicrobial peptides (phagocytic cells, skin, mucous membranes) and kill bacteria synergistically with antimicrobial peptides.[37] It has been proposed that PGRPs kill bacteria by inhibiting peptidoglycan synthesis,[38,39] but this hypothesis has not been directly proven.

Modulation of inflammation

One would expect that amidase-active mammalian PGLYRP2 should have an anti-inflammatory effect *in vivo*, because insect PGRPs with peptidoglycan-hydrolytic amidase activity have anti-inflammatory properties and protect insects from excessive inflammation by hydrolyzing pro-inflammatory peptidoglycan.[40–42] However, contrary to this prediction, recent results show that PGLYRP2 plays a pro-inflammatory role in a model of peptidoglycan-induced acute inflammation and arthritis in mice.[7] PGLYRP2 is required for the induction of peptidoglycan-induced arthritis and local inflammation, because PGLYRP2$^{-/-}$ mice are resistant to peptidoglycan-induced arthritis and inflammation. This property is not shared by other PGRPs and is dependent on NOD2-mediated induction of PGLYRP2 expression in local non-immune cells. Moreover, amidase activity of PGLYRP2 is not required for its pro-inflammatory effect.[7] By contrast, PGLYRP1 has an opposite anti-inflammatory effect in the same model of inflammation. Thus, in addition to its enzymatic activity, PGLYRP2 functions as an alarmin, similar to antimicrobial peptides, such as β-defensins, which in addition to their antimicrobial activity enhance immune responses and inflammation.[43]

Role in disease and future directions

The etiology of inflammatory diseases is often unknown or uncertain and these diseases are determined both by environmental factors and genetic predisposition, usually with multiple genes determining susceptibility. Examples of such complex inflammatory diseases are arthritis, systemic lupus erythematosus, inflammatory bowel disease, psoriasis, asthma, and atherosclerosis. There are some indications that PGRPs may be involved in the pathogenesis of several of these inflammatory diseases. For example, PGLYRP3 and PGLYRP4 are likely associated with psoriasis. Human PGLYRP3 and PGLYRP4 genes are located in the epidermal differentiation gene cluster in the psoriasis sensitivity *psors4* locus, and the expression of PGLYRP3 and PGLYRP4 is co-ordinated with the expression of other genes in this locus.[44] Recent genetic association studies indicate that mutations in PGLYRP3 and PGLYRP4 genes significantly contribute to the pathogenesis of psoriasis.[44,45] This association was significant in some, but not all, populations suggesting that other factors, such as environmental factors, may also play a role. An important environmental factor, so far unexplored with PGRPs, could be microbial flora,[46] which is influenced both by the genetic make-up of the host and by the life-style and diet. Recent data also indicate an association of PGLYRP1 with atherosclerosis.[47] Moreover, PGLYRP1 (also known as Tag7) also plays a role in lymphocyte cytotoxicity for tumor cells.[48]

PGLYRP2 plays a role in local tissue inflammation and acute arthritis.[7] Thus, PGLYRP2 may be one of the innate immunity molecules required for maintaining the proper balance between local inflammatory response (which is needed for efficient defense against infections) and exaggerated response (which induces pathology). It should be also noted that co-ordinated action of many molecules are needed to produce appropriately measured inflammatory responses. For example, peptidoglycan-induced local inflammation and arthritis are not only dependent on PGLYRP2, but also on NOD2 (which is needed for the induction of local expression of PGLYRP2), TLR4 and MyD88 (which are needed for the maturation of polymorphonuclear neutrophils (PMNs)), and several chemokines (which are needed to attract and activate PMNs).[7] PMNs are crucial effectors of inflammation in this model.[7]

One of the most frequent inflammatory diseases of unknown etiology in the intestinal tract is inflammatory bowel disease (IBD) which involves chronic relapsing inflammation of the gastrointestinal tract (likely due to dysregulated immune response to intestinal bacteria) and includes Crohn's disease and ulcerative colitis. Initial genetic analysis identified nine genetic loci that predispose patients to IBD. The first gene identified in one of these loci that predisposes patients to the Crohn's disease form of IBD is mutated NOD2, and recent large-scale genome-wide association screenings identified more than 30 other genes associated with IBD, including *IL23R*, *ATG16L1*, *IL12B*, and *IL10*.[49,50] These screenings also indicate that many more so far unidentified genes are also associated with IBD. One of the IBD susceptibility loci, 19p13 (originally named IBD6 locus), for which the susceptibility gene still has not been identified,[50] harbors human *PGLYRP2* at position 19p13.12. Thus, *PGLYRP2* could be one of the human IBD susceptibility genes in this locus. Further genotyping of IBD patients should verify this possibility. Parallel studies in animal models of inflammatory diseases should determine the *in vivo* role of PGRPs in host response to bacteria and inflammation.

Other aspects that still need to be resolved are the structures of the entire PGLYRP2, PGLYRP3, and PGLYRP4, and the structures and the role of PGRP dimers and possibly multimers. Solving the structure of PGLYRP2 would be especially important (in view of its

recently identified pro-inflammatory function), because PGLYRP2 in addition to the C-terminal PGRP domain (which harbors its amidase activity) has a long N-terminal segment that has no homology to any other PGRPs or to any other known proteins,[4,9] lacks any identifiable functional motifs, and is unique to PGLYRP2, and thus may have unique functional motifs.

The exact mechanism of antibacterial activity of mammalian PGRPs also needs to be determined. It has been proposed that PGRPs kill bacteria by inhibiting peptidoglycan synthesis, but this hypothesis needs to be experimentally tested. Some PGRPs also have different splice forms that may have different functions. For example, pig PGLYRP2 has two splice forms, both of which have amidase activity, but also seem to play a role in induction of β-defensin synthesis.[28] Thus, the presence and functions of PGRP splice forms need to be determined.

Acknowledgements

This work was supported by USPHS Grants AI028797 and AI073290 from NIH.

References

1. Yoshida H, Kinoshita K, Ashida M. Purification of peptidoglycan recognition protein from hemolymph of the silkworm, *Bombyx mori*. *J Biol Chem* 1996; **271**: 13854–13860.
2. Kang D, Liu G, Lundstrom A, Gelius E, Steiner H. A peptidoglycan recognition protein in innate immunity conserved from insects to mammals. *Proc Natl Acad Sci USA* 1998; **95**: 10078–10082.
3. Werner T, Liu G, Kang D, Ekengren S, Steiner H, Hultmark D. A family of peptidoglycan recognition proteins in the fruit fly *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 2000; **97**: 13772–13777.
4. Liu C, Xu Z, Gupta D, Dziarski R. Peptidoglycan recognition proteins: a novel family of four human innate immunity pattern recognition molecules. *J Biol Chem* 2001; **276**: 34686–34694.
5. Royet J, Dziarski R. Peptidoglycan recognition proteins: pleiotropic sensors and effectors of antimicrobial defenses. *Nat Microbiol Rev* 2007; **5**: 264–277.
6. Dziarski R, Gupta D. The peptidoglycan recognition proteins (PGRPs). *Genome Biol* 2006; **7**: 232.1–13.
7. Saha S, Qi J, Wang S *et al*. PGLYRP-2 and Nod2 are both required for peptidoglycan-induced arthritis and local inflammation. *Cell Host Microbe* 2009; **5**: 137–150.
8. Kim M-S, Byun M, Oh B-H. Crystal structure of peptidoglycan recognition protein LB from *Drosophila melanogaster*. *Nat Immunol* 2003; **4**: 787–793.
9. Wang ZM, Li X, Cocklin RR *et al*. Human peptidoglycan recognition protein-L is an *N*-acetylmuramoyl-L-alanine amidase. *J Biol Chem* 2003; **278**: 49044–49052.
10. Guan R, Malchiodi ML, Wang Q, Schuck P, Mariuzza RA. Crystal structure of the C-terminal peptidoglycan-binding domain of human peptidoglycan recognition protein Iα. *J Biol Chem* 2004; **279**: 31873–31882.
11. Guan R, Wang Q, Sundberg EJ, Mariuzza RA. Crystal structure of human peptidoglycan recognition protein S (PGRP-S) at 1.7 Å resolution. *J Mol Biol* 2005; **347**: 83–91.
12. Gelius E, Persson C, Karlsson J, Steiner H. A mammalian peptidoglycan recognition protein with *N*-acetylmuramoyl-L-alanine amidase activity. *Biochem Biophys Res Commun* 2003; **306**: 988–994.
13. Xu M, Wang Z, Locksley RM. Innate immune responses in peptidoglycan recognition protein L-deficient mice. *Mol Cell Biol* 2004; **24**: 7949–7957.
14. Zhang Y, van der Fits L, Voerman JS *et al*. Identification of serum *N*-acetylmuramoyl-L-alanine amidase as liver peptidoglycan recognition protein 2. *Biochim Biophys Acta* 2005; **1752**: 34–46.
15. Lu X, Wang M, Qi J *et al*. Peptidoglycan recognition proteins are a new class of human bactericidal proteins. *J Biol Chem* 2006; **281**: 5895–5907.
16. De Pauw P, Neyt C, Vanderwinkel E, Wattiez R, Falmagne P. Characterization of human serum *N*-acetylmuramyl-L-alanine amidase purified by affinity chromatography. *Protein Expr Purif* 1995; **6**: 371–378.
17. Liu C, Gelius E, Liu G, Steiner H, Dziarski R. Mammalian peptidoglycan recognition protein binds peptidoglycan with high affinity, is expressed in neutrophils, and inhibits bacterial growth. *J Biol Chem* 2000; **275**: 24490–24499.
18. Tydell CC, Yount N, Tran D, Yuan J, Selsted M. Isolation, characterization, and antimicrobial properties of bovine oligosaccharide-binding protein. *J Biol Chem* 2002; **277**: 19658–19664.
19. Tydell CC, Yuan J, Tran P, Selsted ME. Bovine peptidoglycan recognition protein-S: antimicrobial activity, localization, secretion, and binding properties. *J Immunol* 2006; **176**: 1154–1162.
20. Dziarski R, Platt KA, Gelius E, Steiner H, Gupta D. Defect in neutrophil killing and increased susceptibility to infection with non-pathogenic Gram-positive bacteria in peptidoglycan recognition protein-S (PGRP-S)-deficient mice. *Blood* 2003; **102**: 689–697.
21. Cho JH, Fraser IP, Fukase K *et al*. Human peptidoglycan recognition protein S is an effector of neutrophil-mediated innate immunity. *Blood* 2005; **106**: 2552–2558.
22. Kappeler SR, Heuberger C, Farah Z, Puhan Z. Expression of the peptidoglycan recognition protein, PGRP, in the lactating mammary gland. *J Dairy Sci* 2004; **87**: 2660–2668.
23. Lo D, Tynan W, Dickerson J *et al*. Peptidoglycan recognition protein expression in mouse Peyer's patch follicle associated epithelium suggests functional specialization. *Cell Immunol* 2003; **224**: 8–16.
24. Uehara A, Sugawara Y, Kurata S *et al*. Chemically synthesized pathogen-associated molecular patterns increase the expression of peptidoglycan recognition proteins via toll-like receptors, NOD1 and NOD2 in human oral epithelial cells. *Cell Microbiol* 2005; **7**: 675–686.
25. Ghosh A, Lee S, Dziarski R, Chakravarti S. A novel antimicrobial peptidoglycan recognition protein in the cornea. *Invest Ophthalmol Vis Sci* 2009; **50**: 4185–4891.
26. Wang H, Gupta D, Li X, Dziarski R. Peptidoglycan recognition protein 2 (*N*-acetylmuramoyl-L-ala amidase) is induced in keratinocytes by bacteria through the p38 kinase pathway. *Infect Immun* 2005; **73**: 7216–7225.
27. Li X, Wang S, Wang H, Gupta D. Differential expression of peptidoglycan recognition protein 2 in the skin and liver requires different transcription factors. *J Biol Chem* 2006; **281**: 20738–20748.
28. Sang Y, Ramanathan B, Ross CR, Blecha F. Gene silencing and overexpression of porcine peptidoglycan recognition protein long

isoform: involvement in β-defensin-1 expression. *Infect Immun* 2005; **73**: 7133–7141.
29. Mathur P, Murray B, Crowell T *et al*. Murine peptidoglycan recognition proteins PglyrpIα and PglyrpIβ are encoded in the epidermal differentiation complex and are expressed in epidermal and hematopoietic tissues. *Genomics* 2004; **83**: 1151–1163.
30. Guan R, Roychowdhury A, Ember B, Kumar S, Boons G-J, Mariuzza RA. Structural basis for peptidoglycan binding by peptidoglycan recognition proteins. *Proc Natl Acad Sci USA* 2004; **101**: 17168–17173.
31. Kumar S, Roychowdhury A, Ember B *et al*. Selective recognition of synthetic lysine and meso-diaminopimelic acid-type peptidoglycan fragments by human peptidoglycan recognition proteins Iα and S. *J Biol Chem* 2005; **280**: 37005–37012.
32. Swaminathan CP, Brown PH, Roychowdhury A *et al*. Dual strategies for peptidoglycan discrimination by peptidoglycan recognition proteins (PGRPs). *Proc Natl Acad Sci USA* 2006; **103**: 684–689.
33. Guan R, Brown PH, Swaminathan CP, Roychowdhury A, Boons GJ, Mariuzza RA. Crystal structure of human peptidoglycan recognition protein Iα bound to a muramyl pentapeptide from Gram-positive bacteria. *Protein Sci* 2006; **15**: 1199–1206.
34. Lim J-H, Kim M-S, Kim H-E *et al*. Structural basis for preferential recognition of diaminopimelic acid-type peptidoglycan by a subset of peptidoglycan-recognition proteins. *J Biol Chem* 2006; **281**: 8286–8295.
35. Chang CI, Chelliah Y, Borek D, Mengin-Lecreulx D, Deisenhofer J. Structure of tracheal cytotoxin in complex with a heterodimeric pattern-recognition receptor. *Science* 2006; **311**: 1761–1764.
36. Hoijer MA, Melief M-J, Debets R, Hazenberg MP. Inflammatory properties of peptidoglycan are decreased after degradation with human *N*-acetylmuramyl-L-alanine amidase. *Eur Cytokine Network* 1997; **8**: 375–382.
37. Wang M, Liu LH, Wang S *et al*. Human peptidoglycan recognition proteins require zinc to kill both Gram-positive and Gram-negative bacteria and are synergistic with antibacterial peptides. *J Immunol* 2007; **178**: 3116–3125.
38. Dziarski R, Gupta D. Mammalian PGRPs: novel antibacterial proteins. *Cell Microbiol* 2006; **8**: 1056–1069.
39. Cho S, Wang Q, Swaminathan CP *et al*. Structural insights into the bactericidal mechanism of human peptidoglycan recognition proteins. *Proc Natl Acad Sci USA* 2007; **104**: 8761–8766.
40. Mellroth P, Karlsson J, Steiner H. A scavenger function for a *Drosophila* peptidoglycan recognition protein. *J Biol Chem* 2003; **278**: 7059–7064.
41. Zaidman-Remy A, Herve M, Poidevin M *et al*. The *Drosophila* amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity* 2006; **24**: 463–473.
42. Bischoff V, Vignal C, Duvic B, Boneca IG, Hoffmann JA, Royet J. Down regulation of the *Drosophila* immune response by peptidoglycan-recognition proteins SC1 and SC2. *PLoS Pathog* 2006; **2**: e14. 139–147.
43. Biragyn A, Ruffini PA, Leifer CA *et al*. Toll-like receptor 4-dependent activation of dendritic cells by β-defensin 2. *Science* 2002; **298**: 1025–1029.
44. Sun C, Mathur P, Dupuis J *et al*. Peptidoglycan recognition proteins Pglyrp3 and Pglyrp4 are encoded from the epidermal differentiation complex and are candidate genes for the *Psors*4 locus on chromosome 1q21. *Hum Genet* 2006; **119**: 113–125.
45. Kainu K, Kivinen K, Zucchelli M *et al*. Association of psoriasis to *PGLYRP* and *SPRR* genes at PSORS4 locus on 1q shows heterogeneity between Finnish, Swedish and Irish families. *Exp Dermatol* 2009; **18**: 109–115.
46. Sansonetti PJ, Medzhitov R. Learning tolerance while fighting ignorance. *Cell* 2009; **138**: 416–420.
47. Rohatgi A, Ayers CR, Khera A *et al*. The association between peptidoglycan recognition protein-1 and coronary and peripheral atherosclerosis: observations from the Dallas Heart Study. *Atherosclerosis* 2009; **203**: 569–575.
48. Dukhanina EA, Kabanova OD, Lukyanova TI *et al*. Opposite roles of metastasin (S100A4) in two potentially tumoricidal mechanisms involving human lymphocyte protein Tag7 and Hsp70. *Proc Natl Acad Sci USA* 2009; **106**: 13963–13967.
49. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat Rev Immunol* 2008; **8**: 458–466.
50. Budarf ML, Labbé C, David G, Rioux JD. GWA studies: rewriting the story of IBD. *Trends Genet* 2009; **25**: 137–146.