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# MOUSE PEPTIDOGLYCAN RECOGNITION PROTEIN (PGRP)-S: THE ROLE IN BACTERIAL INFECTION

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**Abstract** Peptidoglycan recognition proteins (PGRPs) are pattern recognition receptors which are conserved from insects to humans. PGRPs can recognize bacteria and their cell wall components, peptidoglycans. Human PGRPs have bactericidal activities that can be accomplished by their amidase activities. Insect PGRPs consist of 16 subtypes and some have effector functions as the activation of Toll pathway (PGRP-SA), the activation of Imd pathway (PGRP-LC) and induction of autophagosome formation (PGRP-LE), thus contribute to the elimination of bacteria. However, the role of mammalian PGRPs in bacterial infection remains unclear. In this study, we report the function of mouse PGRP-S, the homologue of PGRP-SA, in bacterial infection. The recombinant protein was produced in *Escherichia coli* overexpression system and used for specific antibody production. We investigated the role of PGRP-S in infection with *Listeria monocytogenes*, a facultative Gram-positive bacterium that can grow intracellularly. The administration of recombinant PGRP-S before *L. monocytogenes* infection decreased the number of bacteria in the organs of infected mice. When endogenous PGRP-S was neutralized by antibodies specific to PGRP-S, the bacterial number increased. The levels of proinflammatory cytokines that are essential in the protection against *L. monocytogenes* infection were lower when the specific antibody was administered prior to infection. Together with these, it is suggested that PGRP-S plays a role in the protection against *L. monocytogenes* infection.

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**Key words:** PGRP-S; *Listeria monocytogenes*; cytokine; innate immunity

Innate immunity is an important process in host defense against microbial infections. It directs to components of microorganisms and recognizes them through a series of pattern recognition receptors, which are conserved through species from insects to mammals[1-3]. Peptidoglycan recognition proteins (PGRPs) are also a family of these pattern recognition proteins, conserved through species[4-9].

PGRP, first discovered in insects as a 19 kDa protein that could recognize peptidoglycans (PGNs), has been studied as a factor in innate immunity. The fruit fly, *Drosophila melanogaster* has 17 homologues of PGRPs[4,10]. In these PGRPs, seven secretable types and ten long membrane-bound types of PGRPs are contained. Each PGRP has specific functions. For example, PGRP-SA, -SD, -LC and -LE can activate two different pathways, that is, Toll and Imd pathways, that lead to the production of antibacterial peptides[11-17]. In addition, PGRP-LE is reported to be crucial for the induction of autophagy by *Listeria monocytogenes* in *D. melanogaster*[17]. Mammals have 4 homologues of PGRPs; PGRP-S, -L, -Iα and -Iβ in human[7,8], mice[18-20], rats[21] and cattles[22,23]. Mammalian PGRP-L is an *N*-acetyl-muramoyl-L-alanine amidase[5,24-27] and can cleave the peptide from the glycan chain of PGNs. Alternatively, human PGRP-S, -Iα and -Iβ show antibacterial activities[25]. Especially, mammalian PGLYRP-1 including human PGRP-S is localized in neutrophils and is likely to kill phagocytized bacteria[19,22,28]. Human PGRP-S is a secretory protein. Molecular and structural mechanisms

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of mammalian PGRPs for PGN binding and antibacterial activities have been studied[29-35] as well as insect PGRPs[36-39]. Although PGRP-S was initially considered as a pattern recognition receptor[4,8] and the immunomodulatory activity of PGRP-S in PGN-induced arthritis in mice have been recently reported[40], the mechanism of pattern recognition and following innate immunity in bacterial infection is still unclear.

*L. monocytogenes* is a Gram-positive intracellular bacterium that is an opportunistic pathogen in human. It is also an important pathogen for pregnant women and their fetuses. Host resistance to *L. monocytogenes* infection is controlled by cell-mediated immunity that consists of host innate immunity and following type I T helper cell responses. Tumor necrosis factor-alpha (TNF-α) and gamma interferon (IFN-γ) are known to be crucial in host resistance against *L. monocytogenes* infection[41-47]. The production of these cytokines is induced by *L. monocytogenes* infection via several pattern recognition receptors and the downstream components[1]. However, the role of PGRP-S in the protection against *L. monocytogenes* infection is not clear. In this study, we studied immunomodulatory activities of mouse PGRP-S in innate immune system as well as antibacterial activities.

Mouse PGLYRP-1 is known to be expressed in neutrophils at a high level[19]. To estimate whether PGLYRP-1 is regulated by bacterial infections, we investigated the expression of PGLYRP-1 in spleens and livers of mice infected with *L. monocytogenes*. C57BL/6 mice cared for under specific-pathogen-free conditions in the Institute for Animal Experimentation, Hirosaki University Graduate School of Medicine were used in all animal experiments in this study, following the Guidelines for Animal Experimentation of Hirosaki University. After infection with sublethal $5 \times 10^5$ CFU of *L. monocytogenes* 1b 1684, mice were sacrificed and RNA was obtained from spleens and livers for RT-PCR using TRIzol reagents (Invitrogen, Tokyo, Japan) according to manufacturer's instruction 0, 0.5, 1, 2, 4, 6 and 12 h after infection. Primers for amplification of *tag7* genes, coding PGRP-S[23], were; p-132 5'-CAT ATG TGC AGT TTC ATC GTG CCC CGC AG-3' and p-133 GGA TCC TCA CTC TCG GTA GTG TTC CCA GC. Primers for amplification of mouse glyceraldehydes-3-phosphate dehydrogenase (mGAPDH) genes were; mGAPDH-F 5'-TGA AGG TCG GTG TGA ACG GAT TTG G-3' and mGAPDH-R 5'-ACG ACA TAC TCA GCA CCG GCC TCA C-3'. PCR condition is; preheating at 94°C for 10 min, 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s and extra-elongation at 72°C for 10 min. PCR products were observed in the agarose gel after electrophoresis. The expression of PGLYRP-1 mRNA was up-regulated at 30 min to 6 h after infection and peaked at 1 h (data not shown). Quantitative real time RT-PCR was also performed and the consistent expression pattern was confirmed (data not shown). These results indicated that PGRP-S expression was induced at the early stage of *L. monocytogenes* infection in mice. From this result that PGRP-S was expressed after *L. monocytogenes* infection, we reached the idea that PGRP-S may be involved in the host protection against *L. monocytogenes* infection. Therefore, we assessed whether the endogenous PGLYRP-1 could be involved in host resistance against *L. monocytogenes* infection. Prior to this, we prepared recombinant mouse PGRP-S by *Escherechia coli* overexpression system. We also prepared polyclonal antibody against mouse PGRP-S from sera of rabbits immunized by recombinant mouse PGRP-S with Imject® alum (Pierce Biotechnology Inc., Rockford, IL, USA). Mice were injected with anti-PGRP-S antibody or normal rabbit globulin 24 h before infection with *L. monocytogenes*. On days 1, 2, 3 and 5 after infection, spleens and livers from infected mice were homogenized in PBS containing 1% (wt/vol) 3-[(cholamidopropyl)

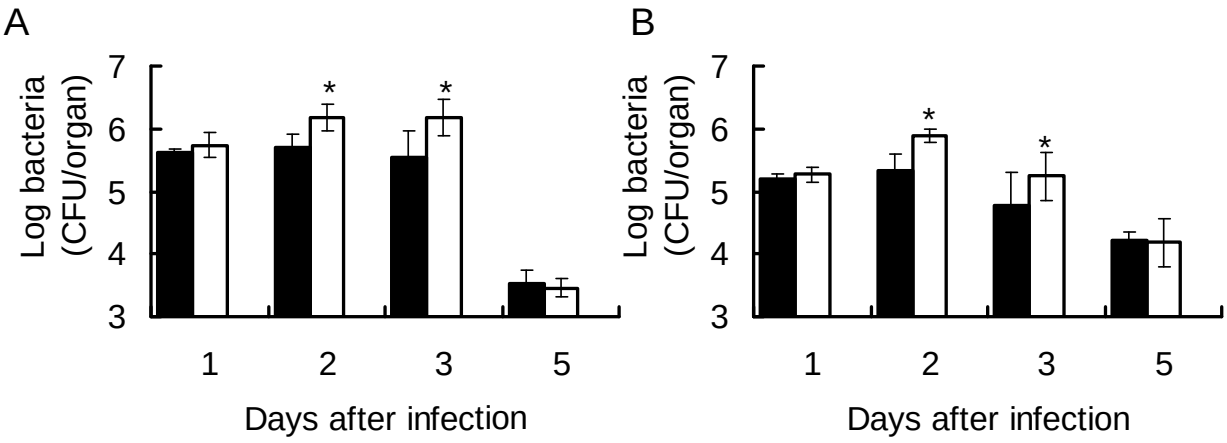


**Figure 1** Elimination of *L. monocytogenes* and endogenous cytokine production decreased by administration of anti-PGRP-S antibody in mice. C57BL/6 mice were injected with 1 mg of anti-PGRP-S antibody (open bar) or normal rabbit globulin (NRG) (closed bar) 24 h before infection with $5 \times 10^5$ CFU of *L. monocytogenes*, and viable bacterial numbers in the spleens (A) and livers (B) from mice 1, 2, 3 and 5 days after infection were determined. Each group consists of 6 mice. Data were expressed as means ± standard deviations, and Student's *t* test was used to determine the significance of the differences. An asterisk indicates the significant difference from NRG-treated group at $P<0.01$.

-dimethyl-ammonio]-1-propane sulfate (CHAPS, Wako, Osaka, Japan) to prepare 10% (wt/vol) homogenates. Ten percent homogenates were serially diluted in 10-fold and cultured on tryptic soy agar plates and colonies were counted 24 h later. The viable number of *L. monocytogenes* in the organs increased in anti-PGRP-S antibody-injected mice on days 2 and 3 after infection compared with that in NRG-injected animals (Fig. 1). This result suggests that endogenous PGRP-S is involved in the host protection against *L. monocytogenes* infection. Previous studies reported that TNF-α and IFN-γ are main cytokines in mammalian innate immunity against *L. monocytogenes* infection and following macrophage activation[41-47]. Therefore, we measured the levels of IFN-γ and TNF-α in the sera and spleens. TNF-α was determined by ELISA using cyto-set Elisa kit (Invitrogen, Tokyo, Japan) according to manufacturer's instrumentation. IFN-γ ELISA was performed as previously described[44]. The titers of IFN-γ and TNF-α in all organs tested significantly decreased in anti-PGRP-S antibody-treated group compared with NRG-treated group on day 1 after infection (data not shown). This result indicated that mice in which endogenous PGRP-S had been neutralized by the antibody showed the reduction of TNF-α and IFN-γ production and suggests that PGLYRP-1 induces TNF-α and IFN-γ production. The mechanism by which *L. monocytogenes* infection induces the expression of PGRP-S and by which PGRP-S induces the production of TNF-α and IFN-γ remains unclear. Recent studies demonstrated that the expression of PGRP-S was up-regulated by transcription factor NF-κB in brain ischemia[48] and that PGRP-S may regulate the inflammatory effect of PGLYRP-2[40]. It should be the next subject to elucidate the precise mechanism of the role of PGRP-S in *L. monocytogenes* infection. In summary, we demonstrated that the expression of PGLYRP-1 is induced by *L. monocytogenes* infection and that PGRP-S plays a role in the protection from *L. monocytogenes* infection through induction of TNF-α and IFN-γ.

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