A Scavenger Function for a *Drosophila* Peptidoglycan Recognition Protein*

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Recent studies of peptidoglycan recognition protein (PGRP) have shown that 2 of the 13 *Drosophila* PGRP genes encode proteins that function as receptors mediating immune responses to bacteria. We show here that another member, PGRP-SC1B, has a totally different function because it has enzymatic activity and thereby can degrade peptidoglycan. A mass spectrometric analysis of the cleavage products demonstrates that the enzyme hydrolyzes the lactylamide bond between the glycan strand and the cross-linking peptides. This result assigns the protein as an N-acetylmuramoyl-l-alanine amidase (EC 3.5.1.28), and the corresponding gene is thus the first of this class to be described from a eukaryotic organism. Mutant forms of PGRP-SC1B lacking a potential zinc ligand are enzymatically inactive but retain their peptidoglycan affinity. The immunostimulatory properties of PGRP-SC1B-degraded peptidoglycan are much reduced. This is in striking contrast to lysozyme-digested peptidoglycan, which retains most of its elicitor activity. This points toward a scavenger function for PGRP-SC1B. Furthermore, a sequence homology comparison with phage T7 lysozyme, also an N-acetylmuramyl-l-alanine amidase, shows that as many as six of the *Drosophila* PGRPs could belong to this class of proteins.

A prominent feature of the innate immune system is the rapid and massive response to intruding microorganisms. Signal transduction pathways are immediately activated to induce genes for antimicrobial peptides or signaling molecules such as tumor necrosis factor-α (1). Pre-formed humoral protein cascades can also be triggered by a single microbe through strong binding to cell wall fragments (2). It is therefore important that organisms have efficient mechanisms to remove such immunogenic substances. This is necessary to be able to respond to a second infection and to minimize overreaction to foreign material and the risk of septic shock. Usually microbes are internalized for further processing after being bound to cellular scavenger receptors (3, 4).

In the late 1980s, Janeway (5) formulated the concept of pattern recognition. This concept implies that the innate immune system is set to recognize molecular patterns that are common to most microbes and that are essential structural parts of the microbial cell. Such pathogen-associated molecular patterns (PAMPs), e.g. lipopolysaccharide, peptidoglycan (PGN), and β-glucan, were postulated to be recognized by pattern recognition receptors (PRRs). In mammals, the Toll-like receptors (TLR) are involved in the response to a variety of PAMP molecules, and in at least one case, a TLR has been shown to be a true PRR (6, 7).

The differential recognition of different pathogens enables insects to respond with a somewhat adapted response, depending on the nature of the infecting pathogen (8). In *Drosophila melanogaster* there are 13 genes for peptidoglycan recognition proteins (PGRP) (9). A gene knock out of one of these, PGRP-SA, was shown to be defective in the response to *Micrococcus luteus* in adult flies via the Toll/Dif pathway (10). In similar genetic screens, one of the membrane-bound forms, PGRP-LCx, was shown to be required for activation of the Relish pathway (11–13). It had been demonstrated earlier (14) that PGRP is needed to trigger the prophenol oxidase cascade with PGN in *Bombbyx mori*. Because these three PGRP proteins have been shown to have affinity for peptidoglycan, they are all true PRRs (9, 14).

A homology comparison of PGRP and bacteriophage T7 lysozyme identified PGRP as part of the N-acetylmuramoyl-l-alanine amidase superfamily of proteins (15). This enzyme hydrolyzes the bond between the N-acetylmuramyl group in the glycan strand and the l-alanine in the stem peptide in peptidoglycan. None of the two receptor PGRPs (SA and LCx) has such amidase activity. However, N-acetylmuramyl-l-alanine amidase activity has been found in mammalian sera, and the enzymatic properties of a human enzyme have been well characterized (16, 17), although the gene for this enzyme is not known. This is in contrast to the many known genes of bacterial *N-acetylmuramoyl-l-alanine amidases*, which take part in the degradation of the bacterial cell wall (18).

In the present study we report that one member of the PGRP family, the *Drosophila* SC1B, is an N-acetylmuramoyl-l-alanine amidase, and we examine the immunogenicity of peptidoglycan being digested with this enzyme.

**EXPERIMENTAL PROCEDURES**

*Protein Expression and Purification—*The inserts of the cDNA clones pbacPAK9/PGRP-SC1B-His and pbacPAK9/PGRP-SA-His (9) were excised from the baculovirus vector pbacPAK9 with EcoRI and XhoI and ligated into the pMVT5-His expression vector (Invitrogen). Because the insert contains six histidine codons followed by a stop codon, neither the V5 epitope nor the vector His tag was utilized. The vector contains a copper-inducible metallothionein promoter.

The *Drosophila* Expression System (Invitrogen) was employed to produce Schneider 2 (S2) cell lines expressing the following His-tagged proteins.

1. The abbreviations used are: PAMP, pathogen-associated molecular pattern; PGRP, peptidoglycan recognition protein; PGN, peptidoglycan; TLR, Toll-like receptor; PRR, pattern-recognition receptor; PBS, phosphate-buffered saline; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; HPLC, high pressure liquid chromatography.
2. P. Mellroth and H. Steiner, unpublished results.
proteins: PGRP-SA(−His), PGRP-SC1B(−His), and the mutants PGRP-SC1B[C168S](−His) and PGRP-SC1B[C168A](−His). The vector pCoHYGRO encoding a hygromycin-B resistance gene was used for selection. Transfection was performed using the calcium phosphate method (19). Stable clones were selected in Schneider’s Drosophila medium (PAN Biotech) supplemented with 10% fetal calf serum and hygromycin-B (300 μg/ml). Transformed cells were adapted to serum-free medium (HyClone Hy-Q-CCM) and grown in 500-ml suspension cultures at 21 °C. At a concentration of 3 × 10⁶ cells/ml, the cells were induced with CuSO₄ (500 μM) and grown for 5 days. Cells were centrifuged (3,000 × g for 20 min at 4 °C), and the medium was assayed for protein. Cells in the medium were precipitated with 70% saturated ammonium sulfate overnight at 4 °C. The precipitate was spun down (8,000 × g, 40 min, 4 °C), and the pellet was dissolved in 30 ml of water. The suspension was dialyzed against 2 liters of binding buffer (5 mM imidazole, 600 mM NaCl, 20 mM Tris, pH 7.9) with two changes of buffer over an 18-h period. Proteins were applied to the superloop in a fast protein liquid chromatography system (Amersham Biosciences AB) and passed through a nickel-charged HiTrap chelating HP column (Amersham Biosciences) at 1 ml/min. The column was washed with 80 mM imidazole, 600 mM NaCl, 20 mM Tris, pH 7.9, and elution was performed with a linear gradient to 600 mM imidazole. The proteins eluted at ~20% elution buffer and were essentially free from other proteins, which was confirmed with a Coomassie Brilliant Blue-stained 15% SDS-polyacrylamide gel.

Peptidoglycan Purification—Insoluble peptidoglycan from Staphylococcus aureus Cowan 1, M. luteus MI 11, and Bacillus megaterium Bm 11 was prepared as described (20). In short, bacterial lawns were grown overnight at 37 °C on nutrient agar plates. Cells were collected and suspended in saline and boiled for 20 min. Bacteria were pelleted, washed with saline, water, and acetone, and then dried at 37 °C. Bacteria were resuspended in cold water with an equal volume of 0.1-MM glass beads and homogenized using a bead beater (Biospec Products) 10 times for 2 min and filtered through a glass filter. Unbroken cells were sedimented by centrifugation at 1,500 × g for 10 min at 4 °C, and cell walls were collected at 5,000 × g for 30 min at 4 °C. Cell walls were then treated with 100 μg/ml RNase A (Sigma), 50 μg/ml DNase I (Sigma), and 0.25% toluene. The suspension was incubated with slow shaking for 18 h at 37 °C. Trypsin (200 μg/ml) (Sigma) was added and incubated for another 18 h at 37 °C. Cell walls were collected, washed, and lyophilized. For teichoic acid removal, S. aureus and B. megaterium PGN were incubated with 5% trichloroacetic acid at 22 °C for 18 h. The insoluble walls were heated at 90 °C for 15 min, washed three times with water and three times with acetone, and dried.

Enzymatic Digestion of Peptidoglycan—Insoluble trichloroacetic acid-treated PGN from S. aureus was suspended in PBS (pH 7.2 at 4 mg/ml) and briefly sonicated using an XL2020 Sonicator (Misonix). The suspension was incubated in PBS under the same conditions. Digested or control PGN was added to the mbn-2 cells at a concentration of 5 mg/ml. As additional control, mbn-2 cells were treated with PGRP-SC1B (20 μg/ml), hen egg white lysozyme (20 μg/ml), and PBS. Total RNA was isolated from the mbn-2 cells at 2, 6, and 24 h after induction.

Isolation of RNA and Northern Analysis—Total RNA was isolated using Trizol (Invitrogen), essentially following the manufacturer's instructions. RNA (15 μg per lane) was separated on a denaturing 1% agarose gel and subsequently capillary-blotted onto a Hybond-XL nylon filter (Amersham Biosciences). The filter was probed with [32P]dCTP random prime-labeled (Amersham Biosciences Rediprime II kit) cDNAs for cecropin A1, attacin, diptericin, and ribosomal protein 49. High stringency hybridization at 42 °C was performed and a PhosphorImag-
Peptidoglycan (0.33 mg/ml) was incubated with PGRP-SC1B (10 μg/ml) in 60 mM Tris buffer, pH 8.0, 100 mM NaCl. The decrease in absorbance at 540 nm was recorded every 5 min.

| Peptidoglycan source | Teichoic acid* | Initial rate | Relative activity (ml/min-1μg⁻¹) % |
|----------------------|---------------|--------------|----------------------------------|
| Staphylococcus aureus | -             | 0.175        | 100                              |
| Bacillus megaterium   | +             | 0.025        | 14.3                             |
| Micrococcus luteus    | +             | 0.080        | 34.3                             |
| -                    | -             | 0.040        | 22.9                             |
| -                    | -             | 0.065        | 37.1                             |
*Teichoic acid was removed by treatment with 5% trichloroacetic acid at 22 °C for 18 h.

FIG. 2. HPLC separation of S. aureus peptidoglycan fragments. Samples (360 μg) of insoluble peptidoglycan were incubated in ammonium bicarbonate buffer (A), with PGRP-SC1B (B), and with PGRP-SC1B plus lysozyme (C). The samples were centrifuged, and the supernatants were fractionated using a Brownlee Spheri-5 column and an acetonitrile gradient (shown on the right-hand side).

A Drosophila Peptidoglycan Scavenger

RESULTS

PGRP-SC1B Degradates Bacterial Cell Walls—A recombinant PGRP-SC1B protein was synthesized using the Schneider expression system and S2 insect cells. Codons coding for 6 histidine residues were added to the C terminus by PCR to facilitate purification of recombinant protein. After metal chelate affinity column purification, the protein was essentially pure (Fig. 1C).

The enzyme is an N-Acetylmuramoyl-l-alanine Amidase—To find out which bond is cleaved by PGRP-SC1B, we compared the HPLC profiles of undigested S. aureus peptidoglycan and peptidoglycan cleaved with PGRP-SC1B (Fig. 2). The profile of undigested control peptidoglycan shows no peaks. In contrast, there is a complex pattern of peaks in the PGRP-SC1B-cleaved sample. Because S. aureus peptidoglycan has a high degree of cross-linking, the size and structure of the PGRP-SC1B-cleaved peptides vary greatly, which explains the profile.

To simplify the pattern, PGRP-SC1B-treated peptidoglycan was digested with the endopeptidase lysozyme, which hydrolysis screen was exposed to the filter. Scanning was done using a FLA-3000 scanner (Fuji film), and data were analyzed with Image Gauge 3.4.5 software (Fuji film).

MALDI-TOF Mass Spectrometry—The masses of the samples were measured by MALDI-TOF MS using a Voyager DE STR (Applied Biosystems) operating in the reflector, positive ion mode. The accelerating voltage was set to 20 kV with an extraction delay time of 125 ns. For each spectrum, 250 laser shots were averaged. Mass spectra were calibrated externally by Calibration Mixtures 1 (SequazymeTM Peptide Mass Standard Kit, PerSeptive Biosystems). The sample (0.5 μl) was applied and mixed with 0.5 μl of 2.5-dihydroxybenzoic acid in 50% acetonitrile on the sample plate.

Mutagenesis—Site-directed mutagenesis was performed to produce two mutants of PGRP-SC1B-His (C168S and C168A) using a two-step PCR strategy. First, two PCRs were done with pMT/PGRP-SC1B-His as template. One reaction used 5'CATCTCATGCAACTAA-3', a complementary sequence to the pMT/V5-His expression vector upstream of PGRP-SC1B, as the sense primer plus a mutagenic primer complementary to bases 483–519 in the coding sequence of PGRP-SC1B. The other reaction was with the complementary mutagenic primer plus 5'TAGAAAGCCACAGTCGAGG-3, a sequence downstream of PGRP-SC1B in the vector. The mutagenic primers substitute the Cys-168 TGC codon with a TCC serine codon or a GCC alanine codon. After separation on a 15% SDS-polyacrylamide gel, the fractions were collected manually and subsequently subjected to MALDI-TOF mass spectral analysis.

PCR and Plasmid DNA Sequencing—PCRs were run using a PC-9600 Gradient Thermal Cycler (Corbett Research) and Deep Vent polymerase (New England Biolabs). Oligonucleotide primers were purified with restriction enzymes from the first PCR step were used as templates with the same flanking vector primers as above.

Peptidoglycan Binding Assay—Insoluble trichloroacetic acid-treated peptidoglycan (1 mg/ml) from S. aureus was incubated with PGRP-SC1B, PGRP-SC1B(C168A), PGRP-SC1B(C168S), or PGRP-SA in Tris buffer (60 mM Tris, pH 8.0, 100 mM NaCl) for 30 min at 4 °C. The samples were centrifuged at 13,000 × g for 10 min, and the supernatants were collected. The PGN pellet fraction was washed with Tris buffer, centrifuged, and dissolved in SDS loading buffer. Supernatant and pellet fractions were analyzed on a 15% SDS-polyacrylamide gel followed by staining with Coomassie Brilliant Blue.

| Peptidoglycan source | Teichoic acid* | Initial rate | Relative activity (ml/min-1μg⁻¹) % |
|----------------------|---------------|--------------|----------------------------------|
| Staphylococcus aureus | -             | 0.175        | 100                              |
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| Micrococcus luteus    | +             | 0.080        | 34.3                             |
| -                    | -             | 0.040        | 22.9                             |
| -                    | -             | 0.065        | 37.1                             |
*Teichoic acid was removed by treatment with 5% trichloroacetic acid at 22 °C for 18 h.
lyzes the pentaglycine bridges that cross-link the stem peptides (1). The number of peaks in the chromatogram is dramatically reduced to one major and one minor peak (Fig. 2C). To determine the masses of the peptidoglycan fragments, the material in the major peak was analyzed using MALDI-TOF mass spectrometry.

The spectrum (Fig. 3) is dominated by peaks with masses corresponding to those of the fragments that can be derived from the known structure of S. aureus peptidoglycan (Fig. 3, inset). The pattern is compatible with PGRP-SC1B cleaving between N-acetylglucosamine and l-alanine in peptidoglycan. The multitude of peaks in the spectrum is fully explained by (i) lysostaphin cleavage at different positions in the penta-glycine bridges and by (ii) two parallel series of peaks generated by sodium replacement of a hydrogen ion. For example, the peak at 702.4 Da is in good agreement with a protonated stem peptide plus five glycine residues with a calculated mass of 703.3.

**Analysis of PGRP-SC1B Mutants**—In bacteriophage T7 lysozyme, Cys-130 is a zinc ligand essential for N-acetylglucosamyl-l-alanine amidase activity. To examine the impact of the homologous Cys-168 in PGRP-SC1B on amidase activity and PGN-binding properties, we constructed two mutants using site-directed mutagenesis. In the receptor type PGRPs and PGN-binding properties, we constructed two mutants using the homologous Cys-168 in PGRP-SC1B on amidase activity. The indicated masses at the peaks correspond to those of an S. aureus peptidoglycan stem peptide plus two to six glycine residues. A schematic structure of S. aureus peptidoglycan with PGRP-SC1B cleavage sites indicated is shown in the inset. NAG, N-acetylmuramyl-l-alanine; NAM, N-acetylmuramyl-l-alanine.

**Summary of peptidoglycan fragment mass mapping analysis**

| Peptide | Type of ion | Theoretical | Observed |
|---------|-------------|-------------|----------|
| AEKA    | [M + H]+    | 532.27      | 531.27   |
| AEKA + G2 | [M + H]+    | 589.29      | 589.30   |
| AEKA + G3 | [M + H]+    | 646.31      | 645.33   |
| AEKA + G4 | [M + H]+    | 667.31      | 667.32   |
| AEKA + G5 | [M + H]+    | 703.33      | 702.36   |
| AEKA + G6 | [M + H]+    | 724.33      | 724.35   |
| AEKA + G6 | [M + Na]+   | 759.35      | 759.39   |
| AEKA + G6 | [M + Na]+   | 781.35      | 781.38   |

The Enzyme Does Not Show Antibacterial Activity—The finding that PGRP-SC1B degrades peptidoglycan prompted us to test if PGRP-SC1B is bacteriocytic. PGRP-SC1B did not exhibit any antibacterial activity against any of the five Gram-positive and three Gram-negative bacterial strains employed in the plate assay (data not shown). Thus the PGN layer of living bacteria is not susceptible to degradation by PGPR-SC1B. As indicated in Table I, this inertness is partly due to the presence of teichoic acid. We cannot, however, exclude the possibility that PGRP-SC1B has antibacterial effects in synergy with other immune proteins.

**The Digested Peptidoglycan Has Lost Immunogenicity**—As PGN is a strong elicitor of immune responses in insects, we assayed the influence of PGRP-SC1B on this elicitor activity. Challenge of Drosophila mbn-2 cells with intact peptidoglycan (Fig. 5) results in a typical immune induction pattern, namely an early response of cecropin A1 that persists over time and a more delayed response of attacin and diptericin. Interestingly, the inducibility of the antibacterial peptide genes was drasti-
challenge with PGN (times are indicated above after 6 h and subjected to Northern analysis. The filter was probed with were stimulated with PBS, PGRP-SC1B, or lysozyme. RNA was isolated for activity.

T7 lysozyme and a mutational analysis, five amino acid residues have been shown to be 

due to make the peptidoglycan structure more accessible for PGRP engagement effect abolishing immunogenic PGN concentrations in the fly in which both lysozymes and PGRP-SC1B are constitutively expressed. The cells were practically unable to respond to the double-digested peptidoglycan, implying an efficient scavenging effect abolishing immunogenic PGN concentrations in the insect.

**DISCUSSION**

What is the physiological role for PGRP-SC1B? We have shown clearly that it degrades peptidoglycan and that the degradation products are less immunostimulatory, but not all peptidoglycans are equally well degraded. Those from *S. aureus* and *M. luteus* contain L-Lys and a peptide cross-linking bridge of a slightly different structure. *B. megaterium* and most Gram-negative bacteria have PGN with *meso*-diaminopimelic acid and direct cross-linking between stem peptides. This PGN is the most resistant of those tested, but the differences are small. We observed the largest difference between PGN with and without teichoic acid. In nature other enzymes may initiate removal of teichoic acid from the cell wall. In the physiological situation, PGRP-SC1B also works in concert with lysozyme to make the peptidoglycan structure more accessible for PGRP amidases to abolish PGN immunogenicity. With lysozyme it was shown that a threonine can be substituted for the lysine residue with retained but reduced activity. If one allows a threonine to replace a threonine in PGRP-SC1B; however, in T7 lysozyme it was shown that a threonine can be substituted for the lysine residue with retained but reduced activity. If one allows for a threonine in this position one observes that five other lysine residues are conserved from T7 lysozyme plus a threonine in high inducibility in the fat body

**Fig. 6.** Multiple alignment of active-site residues of PGRP family members. Protein sequences were aligned using the multiple alignment function in the GeneJockey II program (Biosoft, UK). Proteins with four active-site residues conserved from T7 lysozyme plus a threonine residue in position 128 are shown in bold face type. The GenBank or Swissprot accession numbers for the sequences used are as follows: bacteriophage T7 lysozyme (P00806); PGRP from *Trichoplusia ni* (O76537), *B. mori* (Q0XTN0), *Calpodes ethlius* (AF035445), *D. melanogaster* (SA, Q9VYX7; SB1, Q9V9V7; SB2, Q9V9V6; SC1A, Q9VB7; SC1B, Q8SSQ9; SC2, Q9V4X; SD, Q9VS97; LA, Q9V585; LB, Q9VG93; LCw, AAL89928; LCx, Q8TSQ2; LD, Q9GN97; LE, Q9VXX9), *Mus musculus* (S, AF076482; L, AF149837), *Bos taurus* (SA, Q0XTN0), *Calpodes ethlius* (AF035445), *D. melanogaster* (SA, Q9VYX7; SB1, Q9V9V7; SB2, Q9V9V6; SC1A, Q9VB7; SC1B, Q8SSQ9; SC2, Q9V4X; SD, Q9VS97; LA, Q9V585; LB, Q9VG93; LCw, AAL89928; LCx, Q8TSQ2; LD, Q9GN97; LE, Q9VXX9), *Mus musculus* (S, AF076482; L, AF149837), *Bos taurus* (AAL87002), and *Homo sapiens* (S, AF076483; Ia, AY035376; Ib, AY035377; L, AF384856).

**Fig. 5.** PGRP-SC1B-degraded peptidoglycan shows low elicitor activity. *Drosophila* mbn-2 cells were challenged with staphylococcal peptidoglycan (5 μg/ml). PGN (1 mg/ml) had been preincubated at 25 °C for 10 h in PBS with PGRP-SC1B (20 μg/ml), hen egg white lysozyme (20 μg/ml), or with a mixture of PGRP-SC1B (20 μg/ml) and lysozyme (20 μg/ml). Total RNA was isolated at 2, 6, or 24 h after challenge with PGN (times are indicated above the lanes). Control cells were stimulated with PBS, PGRP-SC1B, or lysozyme. RNA was isolated after 6 h and subjected to Northern analysis. The filter was probed with 32P-labeled cDNA for cecropin A1, attacin, and diptericin. The ribosomal protein 49 (rp49) was used as a loading control showing that similar amounts of RNA were loaded in each lane.
Interestingly, the two PGRPs with receptor functions, PGRP-SA and PGRP-LCx, both have a serine substitution in the position corresponding to the Cys-130 zinc ligand in T7 lysozyme. This modification removes one of the three potential zinc ligands and would make these proteins inactive enzymes. Furthermore, our analysis of PGRP-SC1B mutants shows Cys-130 to be essential for amidas activity but not for binding PGN. A cysteine residue in this position can thus serve as a marker for an effector type PGRP, and a non-cysteine residue could be an indication of a PGRP receptor function.

The concept of receptors and scavengers belonging to the same family of proteins contains an apparent logic. If PGRP-SC1B binds to the same motif in PGN as PGRP-SA and LCx, an obvious explanation for the scavenger effect of PGRP-SC1B is that it cleaves in the middle of the binding motif for the receptor PGRPs. This effect is not obtained with lysozyme because a different bond is cleaved. It has been suggested that the minimal PGN structure required to elicit an immune response in insects contains the two sugar moieties N-acetylglucosamine and N-acetylmuramic acid bound to the stem peptide Lys-Glu-Dap-Ala (25). This is consistent with our results, as such an active product is obtained by lysozyme cleaving the glycan strand but not by PGRP-SC1B, which instead hydrolyzes the bond between the peptide and the glycan strand. PGRP-SC1B is in this way efficiently destroying the PAMP properties of peptidoglycan.

In the mammalian system, recognition of peptidoglycan is thought to be mediated by TLR2 directly binding to PGN (6, 26). A receptor function for PGRP as in insects has not yet been demonstrated in mammals, but the PGN structure recognized seems to be similar if not identical in the two systems. Drosophila PGRP-SC1B is the first eukaryotic N-acetylmuramoyl-L-alanine amidase gene to be described. However, a human N-acetylmuramoyl-L-alanine amidase has been purified from serum and shown to reduce the immunostimulatory effect of peptidoglycan (27). It will be interesting to find out if the amidase activity in vertebrate sera also can be ascribed to PGRP proteins.

Studies of scavenger functions in Drosophila have concentrated on cell-mediated responses performed by macrophage-like cells, expressing scavenger receptors that engulf bacteria and bacterial fragments (28). One of these cellular receptors is a member of the PGRP family (13). Our study now shows that a secreted PGRP protein also can accomplish a scavenging task in innate immunity.

REFERENCES

1. Silverman, N., and Maniatis, T. (2001) Genes Dev. 15, 2321–2342
2. Takaki, Y., Seki, N., Kawai, H., Sawa, S., Iwana, T., and Muta, T. (2002) J. Biol. Chem. 277, 14291–14297
3. Gough, P. J., and Gordon, S. (2000) Microbes Infect. 2, 305–311
4. Aderem, A., and Underhill, D. M. (1999) Annu. Rev. Immunol. 17, 593–623
5. Janeway, C. A. J. (1989) Cold Spring Harbor Symp. Quant. Biol. 54, 1–13
6. Iwaki, D., Mitsuhashi, H., Murakami, S., Sano, H., Kojishi, M., Akino, T., and Kuraki, Y. (2002) J. Biol. Chem. 277, 24315–24320
7. Sieling, P. A., and Medlin, R. L. (2002) Curr. Opin. Microbiol. 5, 70–75
8. Lemaitre, B., Reichhart, J. M., and Hoffmann, J. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 14614–14619
9. Werner, T., Liu, G., Kang, D., Ekengren, S., Steiner, H., and Hultmark, D. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 13772–13777
10. Michel, T., Reichhart, J. M., Hoffmann, J. A., and Royet, J. (2001) Nature 414, 758–759
11. Choe, K. M., Werner, T., Stiven, S., Hultmark, D., and Anderson, K. V. (2002) Science 296, 359–362
12. Gottar, M., Gobert, V., Michel, T., Belvin, M., Duyk, G., Hoffmann, J. A., Ferrandon, D., and Royet, J. (2002) Nature 416, 644–644
13. Yoshida, H., Kinoshita, R., and Ashida, M. (1996) J. Biol. Chem. 271, 13854–13860
14. Zheng, W., Liu, D., and Zheng, J. (1996) Biochim. Biophys. Acta 1270, 63–71
15. Vanderwinkel, E., de Pauw, P., Philipp, D., Ten Have, J. P., and Baintner, K. (1996) Biochim. Biophys. Acta 701, 63–71
16. Shockey, G. D., Danese-Moore, L., Kariyama, R., and Massidda, O. (1996) Microb. Drug Resist. 2, 95–98
17. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, pp. 16–33. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. Rosenthal, R. S., and Dzierski, R. (1994) Methods Enzymol. 235, 253–285
19. Hultmark, D., Engstroem, A., Bennett, H., Kapur, R., and Boman, H. G. (1982) Eur. J. Biochem. 127, 207–217
20. Ghysen, J.-M., Strominger, J. L., and Tipper, D. J. (1968) Compr. Biochem. 26, 56–104
21. Ohita, K., Komatsu, M., and Sugimoto, H. (1998) Microb. Immunol. 42, 231–235
22. Cheng, X., Zhang, X., Peng, R., J., and Studier, F. W. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4034–4038
23. Ikemoto, M., Ishimura, K., Aoyama, K., Yamano, Y., and Morishima, J. (1999) Infect. Immun. 67, 4038–4043
24. Schwander, R., Dzierski, R., Wesehe, H., Rothe, M., and Kirschning, C. J. (1999) J. Biol. Chem. 274, 17404–17409
25. Heijer, M. A., Mellef, J. M., Debes, R., and Hazenberg, M. P. (1997) Eur. J. Biochem. 243, 375–381
26. Rasmussen, M., Pearson, R. C., Li, X. H., Koziel, H., Gehle, V., Chung, E., Krueger, M., and Enkowitz, R. A. B. (2001) Immunity 15, 1027–1038