SUPPLEMENTARY INFORMATION

Bacterial strains and growth conditions. *Streptococcus pneumoniae* strain R36A was grown in a casein-based semisynthetic medium (C+Y) supplemented with yeast extract (1 mg/ml of growth medium). *Staphylococcus aureus* strain COL and RN4220 were grown in tryptic soy broth (TSB, Difco) with aeration at 37ºC in the presence (strain COL) or absence of sub-inhibitory concentrations of oxacillin (50 μg/ml). The presence of oxacillin in the medium induced strain COL to have a simpler peptidoglycan composition and made possible the purification of polymerized muropeptides. Micrococcus luteus strain DSM20030 was grown in Luria broth medium with aeration at 37ºC.

Cell wall preparation. Bacterial cell walls were prepared essentially as previously described (Severin and Tomasz, 1996). After the cells were harvested by centrifugation, they were suspended in ice-cold 50 mM Tris-HCl pH 7.0 buffer and quickly dropped into boiling sodium dodecyl sulfate (final concentration, 4%). The solution was boiled for 15 (pneumococcus) or 45 minutes (staphylococcus and micrococcus) to inactivate any wall-modifying enzymes. The cells were washed several times with water to remove the SDS and ressuspended in water (1/1000 of culture initial volume). Cell walls were mechanically broken by shaking with an equal volume of acid-washed glass beads with a FastPrep FP120 (Bio101 Savant) apparatus. After removal of unbroken cells and glass debris by low speed centrifugation (2,000 x *g*, 5 min), the purification of cell walls was done according to previously described procedures (Severin and Tomasz, 1996). Cell walls were digested with DNAse and RNAse for 3 h at 37°C and then overnight with trypsin (at 37°C). The enzymes were inactivated by boiling in 1% (final concentration) SDS. Cell walls were collected by centrifugation and washed twice with water, once with

8 M LiCl, once with 100 mM EDTA, and then two times with water before being washed with acetone. Finally, the broken cell walls were resuspended in water and lyophilized.

Peptidoglycan purification. For purification of peptidoglycan the cell walls (5 mg) were treated with 2 ml of 49% hydrofluoric acid (HF) for 48h at 4°C. The peptidoglycan was recovered and washed as previously described (Severin *et al*, 1997).

Enzymatic digestion. For liberation of muropeptides the peptidoglycan was digested with Mutanolysin (Sigma) as previously described (de Jonge *et al*, 1992).

HPLC. Analyses of muropeptides were done as in de Jonge *et al*, 1992 with the following modifications. The muropeptides were reduced with $NaBH_4$ in sodium borate buffer pH 9.0 and were separated in an 80 min gradient. The collected peaks were lyophilized, desalted by HPLC and the aminoacid composition was determined. The purification of the muropeptides for the inflammatory activity assay was carried out in the same HPLC gradient except they were not reduced. Fractions from the gradient were collected, lyophilized and desalted by HPLC. A small fraction of the purified samples were then reduced with sodium borohydride and re-injected in the same HPLC conditions in order to identify the muropeptide present in the sample and to assay its purity (Figure 1). Mass spectrometric measurements of the pneumococcal monomers were carried out, confirming the composition of the purified compound. The absence of contaminating endotoxin was confirmed by a negative result for the production of IL1 and IL6 in murine RAW 264.7 cells to a concentration of 10 g/ml of monomeric muropeptide. It was estimated there was less than 1pg LPS per g of monomeric muropeptide.

Peptidoglycan degradation experiments. We isolated hemolymph from wild type and GNBP1 mutant adults using a Nanoject II microinjector (Drummond Scientific). PG from

M. luteus or *Staph. aureus* was suspended in 25 mM sodium phosphate pH 5.5 to a final concentration of 5 mg/ml ($O.D._{600\ nm}$ around 0.85). Digestion was initiated with the addition of 12.5 µl of hemolymph enzymatic extract (6.5 µg of total protein) to 600 µl of peptidoglycan suspension and incubated at 37ºC with agitation.

REFERENCES

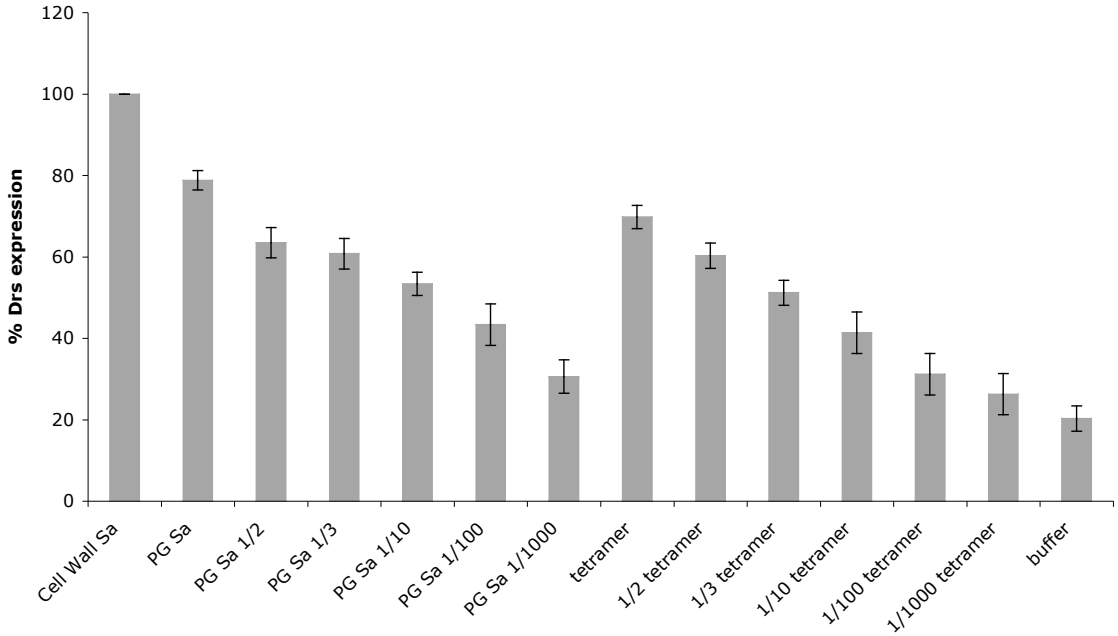
de Jonge BL, Chang YS, Gage D, Tomasz A. (1992) Peptidoglycan composition of a highly methicillin-resistant *Staphylococcus aureus* strain. The role of penicillin binding protein 2A. *J Biol Chem* **267:** 11248-11254

Severin A, Tomasz A (1996) Naturally occurring peptidoglycan variants of *Streptococcus pneumoniae*. *J Bacteriol* **178:** 168-174

Severin A, Horne D, Tomasz A (1997) Autolysis and cell wall degradation in a choline-independent strain of *Streptococcus pneumoniae*. *Microb Drug Resist* **3:** 391-400

FIGURE S1

A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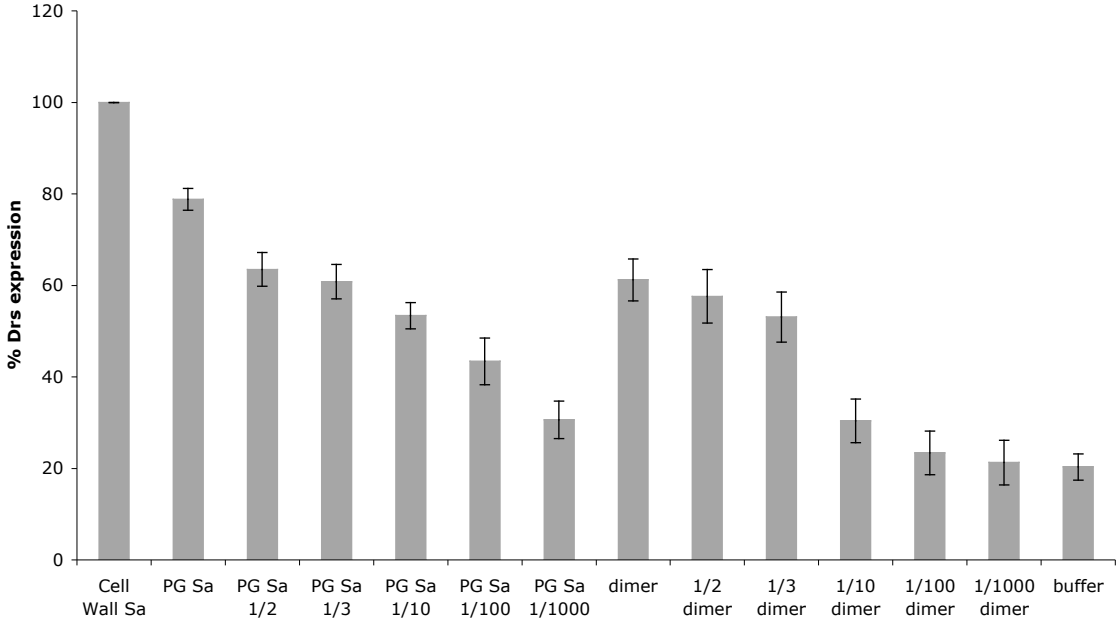
buffer

B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PG Sa 1/1000
dimer
1/2 dimer
1/3 dimer
1/10 dimer
1/100 dimer
1/1000 dimer
buffer

FIGURE LEGEND FOR SUPPLEMENTARY FIGURE S1

The dimeric and tetrameric muropeptides of *Staph aureus* follow the induction response curve of intact PG. Dose response curves of *drs* induction for the tetrameric (a) and dimeric (b) muropeptides of *Staph aureus* in respect to intact PG from the same bacterium. Muropeptides induce *drs* at levels comparable to respective PG dilutions, indicating their potency as activators of the host defence. Of note is the fact that the tetramer induces *drs* at a higher level that the dimer in 1/10 and 1/100 dilutions. At a 1/1000 dilution, induction for both muropeptide polymers is comparable to buffer injection. Values shown are a mean value of five independent experiments with the 100% set for the infection by the cell wall of *Staph aureus*. Error bars represent standard deviation estimates. The initial concentration was 5mg/ml (for PG) and 0.15mg/ml (for muropeptides).