A critical role for peptidoglycan N-deacetylation in Listeria evasion from the host innate immune system.

Ivo G Boneca, Olivier Dussurget, Didier Cabanes, Marie-Anne Nahori, Sandra Sousa, Marc Lecuit, Emmanuel Psylinakis, Vassilis Bouriotis, Jean-Pierre Hugot, Marco Giovannini, et al.

SUPPLEMENTARY FIGURES LEGEND

Supplementary Figure 1. Growth of the *pgdA* mutant in serum. Despite the in vitro sensitivity of the *pgdA* mutant to lysozyme, either strain (EGDe and the *pgdA* mutant) grew normally in 25% fetal calf serum independently of prior decomplementation (A). Growth in 25% serum correlated with an increase in CFU/ml (B) indicating that the *pgdA* mutant was not sensitive to the complement or the normal amounts of lysozyme found in the serum.

Supplementary Figure 2. Morphology of *L. monocytogenes* EGDe (A and B) and the *pgdA* mutant (C and D) in the absence of presence of lysozyme (10 μg/ml). Both strain had normal bacillary morphology (A and C) in BHI broth as observed by optical microscopy. Addition of lysozyme in stationary phase had no effect on the wild type EGDe strain (B) but lead to cell rounding of the *pgdA* mutant (D).

Supplementary Figure 3. (A) *Escherichia coli* MC1061 and *L. monocytogenes* EGDe PG were compared for their ability to activate NF-κB in a Nod1 and Nod2-dependent manner. Transiently transfected HEK293 cells were stimulated either with native, amidase digested or muramidase digested PG. *Listeria* PG induced NF-κB in a Nod1 and Nod2-dependent manner only after digestion into soluble muropeptides by the muramidase. (B) Native PG from strain EGDe and its isogenic *pgdA* mutant were used to activate NF-κB in a Nod1 and Nod2-dependent manner. Native PG from the *pgdA* mutant was more efficient in inducing a Nod1 and Nod2-dependent response. Taking together, our results suggested that *L. monocytogenes* contains in its native PG the Nod agonists but that these are not readily available to the host. (C) To further establish a possible link between *N*-deacetylation of PG and escape from Nod proteins, we decided to *N*-deacetylate a PG known to be fully acetylated using a recombinant

PG *N*-deacetylase BC1960 from *Bacillus cereus* (*1*) and highly purified PG from *Helicobacter pylori.* This PG was used to activate NF-κB in a Nod1 and Nod2-dependent manner. While native PG induced both Nod1 and Nod2, *in vitro* fully *N*-deacetylated native *H. pylori* PG failed to activate NF-κB in a Nod1 and Nod2-dependent manner. (D) Analysis of native and *in vitro N*-deacetylated *H. pylori* peptidoglycan by HPLC and MALDI-PSD. *H. pylori* has a classical *meso*DAP-type PG. Treatment of native *H. pylori* PG with recombinant BC1960 PG deacetylase from *Bacillus cereus* resulted in a complete N-deacetylation of *H. pylori* PG as assessed by HPLC and MALDI-PSD of soluble muropeptides. Peaks 1 to 5, and, 1* to 9* represent native monomeric and *in vitro N*-deactylated monomeric muropeptides of *H. pylori*. The nomenclature of each peak correspond to previously described nomenclature (*1*).

Supplementary Figure 4. Enhanced inflammatory response of the *pgdA* mutant. TNF-α (A) and IL-1ß (B) production by RAW264.7 macrophages was enhanced by the *pgdA* mutant compared to the parental strain EGDe. Cytokine production was assayed by ELISA method. The inflammatory response was also enhanced as measured by the amount of IL-6 (see Figure 5A and C) and, particularly, IFN-ß (see Figure 5B and D) production.

Supplementary Figure 5. Model of host recognition of the *pgdA* mutant. In the intestinal lumen, the *pgdA* mutant is rapidly eliminated as a result of the bacteriolytic activity of lysozyme (thick arrow) that is massively produced by Paneth cells. The few bacteria that escaped from the intestinal lumen (dashed arrow), disseminate by spreading either from cell-to-cell or by entry into phagocytic cells. The *pgdA* mutant sensitivity to lysozyme results in its enhanced destruction in phagosomes and release of cell wall components such as muropeptides and LTA. LTA is readily available to target membrane bound TLR2 while

muropeptides can be delivered to the cytosol to target Nod1 through either a listeriolysin O-dependent mechanism or by endogenous transporters. Activation of the TLR2 and Nod1 pathway results in an induction of pro-inflammatory cytokines. Type I interferons are also induced in a TLR2-dependent fashion. Production of IFN-ß induced by the *pgdA* mutant is only partially dependent on MyD88 indicating that TLR2 might activate Type I interferons by a yet unknown pathway. Type I interferons and cytokines enhanced production by the *pgdA* mutant results in a stronger inflammatory response contributing to its impaired virulence.

REFERENCES

1. E. Psylinakis *et al.*, *J Biol Chem* **280**, 30856 (Sep 2, 2005).