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Reply. I thank Danese et al. for their comments about our editorial on the induction of matrix metalloproteinase-9 (MMP-9) expression by *Helicobacter pylori* infection.[1] They report in their letter that MMP-9 expression has been reduced after *H. pylori* eradication both in epithelial cells and in mucosal fibroblasts. I acknowledge that their data confirm my findings and support my conclusions. I read with interest the data from this eradication study. They report no change of MMP-9 expression after eradication in macrophages. I observed no association between MMP-9 expression and *H. pylori* infection in macrophages in gastric ulcers.[1] These results suggest distinct mechanism of MMP-9 expression between epithelial cells and fibroblasts, and macrophages. MMP-9 expression in epithelial cells and fibroblasts is dependent on *H. pylori* infection, whereas in macrophages it is independent of *H. pylori* infection. The increase of MMP-9 in macrophages may be causally linked to the mucosal injury. The plasma levels of MMP-9 in patients with gastric cancer were significantly higher than those in healthy individuals.[2] It is possible that the plasma levels of MMP-9 are increased in patients with *H. pylori*-associated gastritis and gastric ulcer. I am under investigation the plasma levels of MMP-9 in these patients.

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The True Ligand of the NOD2 Receptor Is Peptidoglycan Instead of Lipopolysaccharide: A Schematic Representation of Ligand-Receptor Interactions and NF-κB Activation

Dear Sir:

It is with great interest that we read the article by Bonen et al. (Gastroenterology 2003;124:140–146) in which they assessed the functional activity of the major Crohn's disease (CD) associated NOD2 variants in response to lipopolysaccharide (LPS) and peptidoglycan (PGN).[1] Their findings showed a decreased nuclear factor κ-B (NF-κB) activation for all 3 major risk alleles (L1007fsinsC, G908R, and R702W), with a complete abolished NF-κB activation for the L1007fsinsC mutation, in response to LPS and PGN. This provides a strong support for the concept put forward by Gabriel Nuñez, that deficit NF-κB activation in the NOD2 signaling pathway is involved in susceptibility to CD. This concept is based on the fact, that activation of NF-κB signaling pathways in response to bacterial components mediates protection of the host against invading pathogens.

In this letter we summarize recent published findings, in part by the same authors,[1] concerning the contradictions in literature on the ligand of the NOD2 receptor. To provide a better understanding on

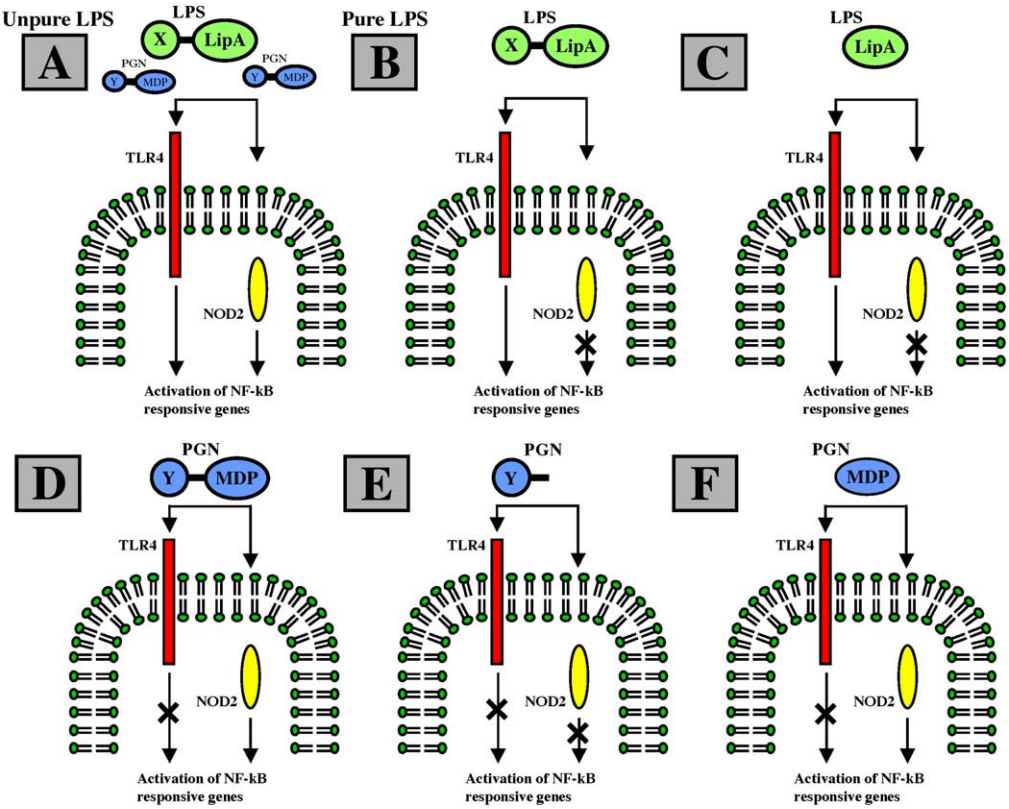

Figure 1. Schematic representation of the NF-κB activation pathways of the extracellular pathogen associated molecular pattern (PAMP) recognizer TLR4 and the intracellular NOD2 PAMP recognizer for LPS and PGN (based on experimental data[2–3]). (*A*) Poorly purified LPS (molecule in green) preparations contain PGN (molecule in blue) resulting in activation of NF-κB by both TLR4 and NOD2. (*B*) Pure LPS activates only TLR4. (*C*) LipA is the essential structure of LPS recognized by TLR4 but not by NOD2. (*D*) Highly purified PGN activates only NOD2. (*E*) PGN without the essential MDP structure does not activate NOD2 or TLR4. (*F*) The essential structure MDP of PGN activates NOD2. LPS, Lipopolysaccharide (molecule in green); PGN, peptidoglycan (molecule in blue); TLR4, Toll-like receptor 4: MDP, muramyl dipeptide; LipA, Lipid A; X, LPS without LipA; Y, PGN without MDP; NF-κB, nuclear factor κB.

this topic, a schematic representation is made with ligand-receptor interactions and subsequent NF-κB activation (Figure 1).

Recent biochemical and functional analyses identified only muramyl dipeptide (MDP), derived from PGN, as the essential structure in bacteria recognized by NOD2[2,3] (Figure 1*D–F*). Notably, Toll-like receptor 4 (TLR4), but not NOD2 was stimulated by highly purified LPS, prepared by gel-filtration chromatography, showing that previous results, identifying LPS as the ligand for NOD2, were based on the presence of PGN in poorly purified LPS preparations (Figure 1*A–B*). Further functional analyses identified MurNac-L-Ala-D-isoGln, derived from PGN, as the essential structure in bacteria recognized by NOD2 with a strong stereoselective recognition, as shown by replacement of L-Ala for D-Ala or D-isoGln for L-isoGln.[2] The lipidA fraction of LPS is the essential structure recognized by TLR4 and is, like complete LPS, not recognized by NOD2 (Figure 1*B–C*).

Although NOD2 can mediate the recognition of muropeptides, the mechanism involved is unclear and remains to be determined. Because the Leucine Rich Repeats (LRR), involved in the binding of the muropeptide part of PGN, are required for recognition, muropeptides could interact directly with NOD2 through its LRR's or via as yet to be identified cellular factor(s).

Since LPS is only present in Gram-negative bacteria, while PGN is present in both Gram-positive and Gram-negative bacteria, these data change our understanding of potential causative pathogens in relation to CD development.

A close analysis of Figure 2 (*A* and *B*) in the article of Bonen et al. suggests to us that even a very limited amount of PGN contamination in LPS results in a major NF-κB: pure PGN as compared to contaminated LPS resulted in only a factor 2–3 increase in the NF-κB activation.[1] This also raises doubts about the many ligands that have been described for other receptors, like the TLR4 receptor. It would be interesting to know whether the authors have investigated other ligands and receptors.

As shown by Bonen and colleagues, the distribution of the major risk alleles (L1007fsinsC, G908R and R702W) is not distributed evenly between different ethnic groups,[1,4] and extension of both genetic epidemiological and immunogenetic studies on this important CD gene are warranted to increase understanding of the molecular mechanisms whereby NOD2 mutations contribute to disease susceptibility. Indeed, recent work of Rosenstiel et al., investigating the role of IFN-γ and TNF-α in regulating expression of the CARD15 gene and the work of Hisamatsu et al. studying the expression of CARD15/NOD2 in intestinal epithelial cells, provide essential clues to a better understanding of the NOD2 gene in disease susceptibility and could potentially lead to new therapeutic approaches for CD.[5,6]

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Reply. We would like to thank the authors of the letter for summarizing our results and clarifying the findings to the readers of GASTROENTEROLOGY. In the initial studies describing the response of NOD proteins to microbial components, we reported that NOD2 mediates responsiveness to preparations of both lipopolysaccharide (LPS) and peptidoglycan (PGN). The results were unexpected in that PGN and LPS do not share any obvious structure. Because LPS and PGN preparations are often contaminated with additional bacterial components, we suggested that NOD2 may recognize LPS, PGN, or another bacterial molecule present in the bacterial preparations.[1] Subsequent work revealed that PGN and specifically MurNac-L-Ala-D-isoGln (MDP) present in PGN from practically all bacteria is the structure recognized by NOD2.[2,3] Notably, peripheral blood mononuclear cells from a CD patient homozygous for the main L1007fsinsC mutation are deficient in their response to MDP but retain their ability to response to LPS.[2] These results suggest that defective sensoring of bacteria by impaired recognition of PGN may be critical in the pathogenesis of Crohn's disease.

Morre et al. suggest that very limited amount of PGN contamination in LPS results in a major effect on NF-κB activation induced through NOD2 based on Figure 2 of our article.[4] However, this interpretation does not take into account the observation that the MDP and small structurally related muropeptides appear to be more active in stimulating NOD2 than undigested or muramidase-digested PGN. For example, monomeric MDP is more stimulatory than molecules with two copies or four copies of GlcNac-MurNAC attached to L-Ala-D-isoGln.[2] In addition, the major peak of NOD2-stimulatory activity in LPS preparations is consistent with muropeptides as determined by gel filtration (Inohara and Nunez, unpublished). Thus, small-sized muropeptides containing the MDP structure appear to be the most NOD2-stimulatory molecules in our assays.

Finally, we agree with Morre et al. that understanding the biological function of NOD2 should provide insight into the mechanism by which NOD2 mutations contribute to disease.

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