RESEARCH HIGHLIGHT

The NOD2 receptor modulates cytokine response but does not alter the clinical outcome of Group B *Streptococcus*-infected mice

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Received: January 09, 2014
Published online: February 17, 2014

The nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is an intracellular receptor capable of sensing bacteria-derived muramyl dipeptide. We investigated the role of NOD2 in the pathogenesis of Group B *Streptococcus* (GBS) capsular type III, a crucial agent of life-threatening invasive infections, by using an adult NOD2$^{-/-}$ mouse model of infection. We demonstrated that NOD2 is not a key receptor to fight GBS infection and only partially contributes to the inflammatory response. This Research Highlight discusses the findings of this recent study and the investigators' active research on the involvement of receptors in the interaction between encapsulated bacteria and dendritic cells.

To cite this article: Lemire P, Segura M. The NOD2 receptor modulates cytokine response but does not alter the clinical outcome of Group B Streptococcus-infected mice. Receptor Clin Invest 2014; 1: e55. doi: 10.14800/rci.55.

The innate immune system has developed an arsenal of mechanisms to detect and eliminate pathogens [1]. Innate immune responses rely on pattern recognition receptors (PRRs) for detection of pathogen-associated molecular patterns (PAMPs). These receptors include Toll-like receptors (TLRs), RIG-I-like receptors and NOD-like receptors (NLRs) family of proteins [2]. NLRs recognize PAMPs in the cytosolic compartment and comprise more than 20 family members, including nucleotide-binding oligomerization domain-containing protein 1 or 2 (NOD1, NOD2) and NLR family pyrin domain-containing protein 3 (NLRP3) receptors [2]. NOD1 and NOD2 recognize bacterial components derived from peptidoglycan (PGN) synthesis and/or degradation, resulting in the activation of transcription factor nuclear factor κB (NF-κB) and the mitogen-activated protein kinases (MAPKs) [3]. NOD2 is found especially in myeloid cells such as dendritic cells (DCs) and macrophages [4, 5] and senses muramyl dipeptide (MDP) [4], a PGN component of both Gram-positive and Gram-negative bacteria. Many *in vitro* or *in vivo* studies established that NOD2 is a relevant mediator of host defense against a wide range of pathogens, including intracellular bacteria like *Listeria monocytogenes* [6, 7], *Mycobacterium tuberculosis* [8-10], *Neisseria gonorrhoeae* [11], *Helicobacter pylori* [12] and *Legionella pneumophila* [13]. NOD2 has also been shown to regulate the immune response to extracellular bacteria, such as *Streptococcus pneumoniae* [14-16], *Streptococcus pyogenes* [17], *Streptococcus suis* [18, 19] and *Staphylococcus aureus* [20-25].

Streptococcus agalactiae or Group B *Streptococcus* (GBS) is an important agent of severe invasive infections in pregnant women and newborns worldwide [26]. Clinical manifestations of GBS infection are principally related with pneumonia, septicemia, and meningitis. GBS is also

associated to invasive disease in nonpregnant adults, particularly among the elderly and individuals with underlying persistent illnesses [27]. Similarly to other bacterial pathogens, clinical isolates of GBS are coated with a capsular polysaccharide (CPS), known as the major factor for bacterial survival within the host. Among described GBS capsular types [26, 27], capsular type III is the principal type isolated from GBS meningitis [26]. Mancuso *et al.* [28] reported that GBS antigens can be found either in DC early and late phagosomes (containing intact bacteria) or in degradative vacuoles bearing lysosomal markers and containing partially digested GBS material co-localized with TLR7. Thus, it could be accepted that intracellular recognition of GBS is important in the immune response against this pathogen. Recently, Costa *et al.* [32] reported that activation of the inflammasome, an inflammatory signaling complex, by GBS is implicated in host defense against this pathogen. On the other hand, *in vitro* studies performed to date were unable to prove a clear role of NOD in GBS interactions with macrophages [29, 30].

In our recent study entitled "The NOD2 receptor does not play a major role in the pathogenesis of *Group B Streptococcus* in mice" [31], we used a mouse model of infection to better understand and give a first indication of the importance of NOD2 during GBS infection. We focused on the implication of NOD2 in the innate immune response against GBS during acute infection. Independently of the bacterial dose, similar survival and bacteremia levels were observed in infected $NOD2^{-/-}$ mice compared to control mice. Interestingly, *ex vivo* analysis of total spleen cells or sera from infected animals demonstrated that the absence of NOD2 results in diminished production of inflammatory cytokines. Nevertheless, this reduced inflammatory response does not seem to favor mouse survival. This study demonstrated that NOD2 is not an important receptor to sense GBS during infection and only weakly contributes to the inflammatory response. Further studies are necessary to measure the effect of NOD2 in the development of adaptive immunity, and more especially on the generation of anti-GBS specific antibodies. In this regard, in our recent study we observed that the expression levels of the T and B cell activation marker CD69, known as one of the earliest available indicators of leukocyte activation, were unaltered in $NOD2^{-/-}$ cells during GBS type III infection [31]. Compared to the results reported in the study of Costa *et al* [32], we concluded that NOD2 is not as relevant as NLRP3, at least in adult mice. These findings represent an important step in understanding how GBS interacts with immune cells and confirm the hypothesis that GBS use complex TLR-dependent [33], NLRP3-dependent [32], NOD2-dependent in addition to TLR/NOD2-independent pathways to modulate host immune responses.

Our research group use GBS and *S. suis* as models for the study of encapsulated bacteria. *S. suis* is a major swine pathogen and an emerging zoonotic threat in humans able to induce septicemia with sudden death, meningitis, endocarditis, pneumonia, and arthritis [34, 35]. GBS and *S. suis* share the feature of being the sole Gram-positive bacteria expressing terminal sialic-acid in their CPSs. Our work mainly focuses on the characterization of the interactions between these pathogens and DCs, with a particular interest on the receptors involved in the recognition of GBS or *S. suis* by these cells. Recently, we showed that encapsulated GBS is efficiently internalized by mouse DCs, yet the presence of CPS confers to GBS and intracellular survival advantage. Likewise, GBS internalization by DCs is largely required for modulation of IL-10, IL-12p70 and CXCL10 pathways [36]. The CPS seems to be important for the recognition of GBS via lipid rafts [37]. Receptors implicated in encapsulated GBS recognition within DC lipid raft domains are unknown. Sialic acid-binding immunoglobin superfamily lectins (Siglecs) or integrin CD11b/CD18 may be some of potential receptors used by GBS within lipid microdomains [37]. Encapsulated GBS interaction with lipid rafts might also facilitate bacterial contact with caveolin-1, leading to modulation of caveolin-related signaling pathways and activation of specific immune mediators, for instance CCL2 [37]. We also observed that highly purified GBS CPSs induced significant production of CCL3 by DCs, via partially TLR2- and myeloid differentiation factor 88 (MyD88)-dependent pathways, and CCL2, via TLR-independent mechanisms [38]. A similar pattern was observed with highly purified *S. suis* CPSs. In addition, DCs also recognize whole *S. suis* and become activated mostly through TLR signaling. Particularly, TLR2 is involved in the release of several cytokines and the expression of co-stimulatory molecules by *S. suis*-infected DCs [18]. Besides this major pathway, a multimodal recognition involving a combination of different receptors (NOD2 and TLR9 for example) seems essential for DC effective response to *S. suis* [18]. Like *S. suis*, production of cytokines by DCs in contact with GBS (at the extracellular interface) was shown to strongly rely on MyD88-dependent signaling pathways, suggesting that DCs recognize GBS and become activated mostly through TLR signaling (Lemire *et al*, unpublished results). Overall, these results demonstrate the implication of various receptors and the complexity of DC adaptive fitness in contact with GBS or *S. suis*.

We are interested in the future to further dissect the impact of certain families of receptors in recognition of GBS or *S. suis*, but also in characterizing the importance of receptors in the interaction of DCs with other immune

cells, including T cells and Natural Killer cells.

Conflicting interests

The authors have declared that no competing interests exist.

Acknowledgement

This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) through grant to MS (#342150-07). PL is the recipient of a PhD Award granted by the Fonds de recherche du Québec - Nature et technologies.

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