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EDITORIAL

The Hidden Effect of Nod2 in the Host/Microbiota Relationship

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Anderson et al[1] present new insights on the role played by nucleotide-binding oligomerization domain-containing protein 2 (NOD2) receptor in shaping and regulating the intestinal microbiota. NOD2 is a pattern recognition receptor expressed by specialized cells of the epithelium, such as Paneth cells, and some immune cells, and which has been involved in inflammatory disorders (first susceptibility gene identified in Crohn's disease).[2–4] NOD2 is a receptor for muramyl dipeptide derived from peptidoglycan degradation of both gram-positive and -negative bacteria.[5] Acting as a gatekeeper, NOD2 activates nuclear factor-κB and MAPK pathways following muramyl dipeptide recognition, contributing to intestinal defense and integrity.[6]

In mice, genetic deletion of NOD2 seems to associate with abnormal immune responses and exacerbated susceptibility to experimental colitis.[7–9] Moreover, based on its role in sensing bacterial compounds, loss of NOD2 function has regularly been associated with alteration in microbiota composition in both human and mice.[10] For example, the same team previously described a bacterial overload, especially from the Bacteroidetes phylum, in feces and ileal biopsies of patients with Crohn's disease with *Nod2* SNP13 mutation and *Nod2*-deficient mice.[11] Such data suggest a key role played by NOD2 in shaping the intestinal microbiota, which can play a role in driving susceptibility to chronic intestinal inflammation.[12] However, other studies failed to observe significant bacterial taxonomic differences between *Nod2*-KO mice and wild-type (WT) true littermates[13,14] at steady state, suggesting that NOD2-deficiency associated dysbiosis could require other perturbations of the host/microbiota relationship to arise.

In this new study, Anderson et al[1] informed the debate by showing an absence of genotype effect on fecal microbiota composition at the homeostatic state. The authors next examined to which extent NOD2 deficiency impacts microbiota composition recovery following antibiotic exposure, known to dramatically disrupt gut bacterial population, in young adult (20 weeks old) and middle-age (52 weeks old) mice. They importantly revealed a delayed weight loss recovery following antibiotics exposure in *Nod2*-KO mice compared with WT animals, in an age-independent manner. Regarding microbiota composition, neither WT nor *Nod2*-KO mice recovered their initial microbiota composition following antibiotics exposure, as assessed by 16S rRNA gene sequencing. However, both Bray-Curtis and Jaccard distance analyses revealed a greater resilience of WT mice microbiota to shift back toward its initial preantibiotic state compared with *Nod2*-KO mice. Such phenotype was driven by specific operational taxonomic units and demonstrate the role played by NOD2 in shaping microbiota resilience to antibiotic exposure.

In addition to the bacteriome, the authors also investigated the mycobiome, still largely understudied. Indeed, fungi are part of the gut "dark matter," even if recent findings demonstrate the important role played by this community in the gastrointestinal environment.[15] Here, the authors demonstrate that mycobiome displayed an enhanced diversity after antibiotic treatment. Moreover, contrary to the bacteriome, mycobiome configuration did not shift back toward its initial state, with no differences observed between WT and *Nod2*-KO mice.

Interestingly, fecal transplantation of *Nod2*-KO microbiota collected 7 weeks postantibiotic withdrawal into germ-free WT animals demonstrated no impact on metabolic parameters or small intestine inflammation, whereas an enhanced colonic inflammation was observed, with an increased histologic score and elevated inflammatory markers, compared with germ-free mice receiving similar WT-derived microbiota. These results demonstrate functional impacts of postantibiotic microbiota in *Nod2*-KO mice compared with WT littermates, in line with a recent study demonstrating that NOD2 deficiency is leading to decreased microbiota resilience after antibiotic exposure.[14] Based on previous studies, the authors suggested that underlying mechanisms by which NOD2 deletion impacts the post-antibiotic microbiota resilience may involve altered expression of antimicrobial peptides/proteins, inflammatory markers, or autophagic/endoplasmic reticulum stress signals. However, how this altered microbiota in *Nod2*-KO mice after antibiotic exposure is able to promote inflammation when transplanted to WT recipients remains elusive. Did the lack of some specific species foster expansion of more proinflammatory microorganisms and/or detrimentally impacted the intestinal immune system? Another mechanism could be linked to microbiota encroachment, known to play a central role in chronic intestinal inflammation, which can be favored in *Nod2*-KO-derived post-antibiotic microbiota.[16,17] Hence, further studies are required to elucidate the functional role of *Nod2*-KO-associated microbiota.

In conclusion, the work by Anderson et al[1] provides novel insights regarding the role played by NOD2 in shaping the gut microbiota, especially regarding its resilience following environmental perturbation. Although microbiota of *Nod2*-KO and WT animals are similar at steady state, composition alterations were observed in the postantibiotic phase, further highlighting the multifactorial etiology of inflammatory bowel diseases in which multiple hits and/or susceptibility genes need to be associated for the disease to occur. Moreover, this study importantly suggests that investigation of microbiota composition and function in true littermate genetic model, at steady state, in specific pathogen-free environment, and in the absence of any

additional hint/perturbator (eg, antibiotic treatment, pathobiont infection) might mask important relevant differences.

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Conflicts of interest
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