

***NOD2* in Crohn's disease – loss or gain of function mutations?**

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The recognition of disease-associated alleles in the *NOD2/CARD15* gene has boosted research on the pathogenesis of Crohn's disease. Among the three risk alleles harbouring single nucleotide polymorphisms (SNPs), the C-insertion in the leucine-rich region of the gene has been most extensively studied. Although many questions about the role of *NOD2/CARD15* in the immune response have been elucidated, one seemingly simple question remains unanswered: do the risk alleles confer a gain or a loss of the protein function? The paper by Netea *et al.* in this issue of the *Netherlands Journal of Medicine*¹ adds to the discussion in favour of the last option, but the argument has not been settled yet.

NOD2 is a receptor for a pathogen-associated molecular pattern (PAMP) called muramyl dipeptide (MDP). MDP is a component of bacterial peptidoglycans and is bound by the leucine-rich region of the protein. As with the Toll-like receptors, a family of membrane bound molecules that bind all sorts of bacterial and viral compounds, *NOD2* is thought to constitute an important link between innate immunity and the adaptive immune response. Upon binding of MDP, NF- κ B is activated through a cascade of cytoplasmatic events and a proinflammatory immune response will occur.

The C-insertion at position 3020 of the gene leads to a truncated protein that lacks a lot of the MDP binding part of the protein.² Therefore, presumably, this defect in an *NOD2* protein structure will lead to an impaired NF- κ B activation and decreased production of proinflammatory

cytokines. This is indeed supported by *in vitro* studies using cell lines transfected with the Crohn's disease-associated *NOD2* variants,³ giving arguments for the hypothesis that these variants result in a loss of function of the protein.

However, Crohn's disease is characterised by an increased NF- κ B activity. This apparent discrepancy led to the studies addressing this question *in vivo*, using different mice models.

Two studies based on experiments with *NOD2*-deficient mice provided two different hypotheses explaining the possible mechanism of a loss-of-function allele in the pathogenesis of Crohn's disease. Watanabe *et al.* observed reduced response of splenic macrophages to MDP in *NOD2*-deficient mice, yet found that PGN stimulation led to elevated levels of IL-12 in these mice.⁴ Thus, an *NOD2*-mediated negative regulation of TLR2 signalling would be lost in an *NOD2*-deficient condition, leading to an enhanced cytokine response by macrophages to commensal bacteria and resulting in inflammation.

Another hypothesis was proposed by Kobayashi *et al.* who suggested that a loss-of-function *NOD2* allele might affect epithelial cells rather than macrophages.⁵ In this study, *NOD2*-deficient mice developed more severe infection to *Listeria monocytogenes* when the pathogen was given orally compared with systemic administration. This, together with the finding of specific *NOD2* expression in intestinal crypt epithelial cells in wild-type, but not in *NOD2*-deficient mice, supported the hypothesis of epithelium-mediated impaired local control of pathogenic bacteria thus resulting in an inflammatory condition. Interestingly, in contrast to the findings of Watanabe *et al.*, in the study by Kobayashi *et al.* TLR2 stimulation of bone-marrow derived macrophages did not result in enhanced proinflammatory cytokine produc-

tion in *NOD2*-deficient mice, underlying the importance of the studied cell type for this kind of experiments. On the other hand, Maeda *et al.* used a mice model with insertion of one of the mutations associated with Crohn's disease. In this model, enhanced production of IL-1 β has been observed upon MDP stimulation of bone marrow-derived macrophages in mutated mice.⁶ Based on these findings, a hypothesis of gain-of-function *NOD2*-associated mutations has been postulated, suggesting that in the presence of this mutation, the stimulation of antigen-presenting cells (i.e. macrophages or dendritic cells) with bacterial components binding *NOD2* would directly lead to the production of proinflammatory cytokines. The mice studies mentioned have definitely provided interesting insights into the pathogenetic mechanisms of Crohn's disease-associated *NOD2* mutations. However, the question of the relevance of these models for human pathology remains. Moreover, these studies have lead to strikingly different hypotheses depending on the particular genetic modification approach used. Therefore, it is clear that for a better understanding of the *NOD2*-mediated pathogenesis of Crohn's disease, data from studies with human material are crucial.

Therefore, the report by Netea *et al.* published in this issue is of particular importance.¹ The authors used peripheral blood mononuclear cells to demonstrate decreased IL-1 β production in homozygous *NOD2*-mutant Crohn's disease patients upon stimulation of these cells with MDP and TLR2 ligands. These results providing possible arguments for a loss-of-function hypothesis are in relative discrepancy with the study by Maeda *et al.* in mice with inserted Crohn's disease-related *NOD2* mutation. Besides the general differences between mice and human models, the contradictory findings can be explained by the different cell types used in these two studies. It has been shown that *NOD2* expression differs in monocytes and macrophages,⁷ and may be enhanced by stimulating the cells with different TLR ligands and TNF- α .⁸ Therefore, trying to make conclusions based on the results of these two studies using different cell types, might lead to mistaken interpretations.

On the other hand, it is not clear whether results obtained with stimulation of peripheral blood-derived monocytes may be translated into general statements on pathogenetic mechanisms of *NOD2* mutations in humans. Antigen-presenting cells, macrophages and dendritic cells seem to be particularly involved in the development of mucosal inflammation. Furthermore, the specific local inflammatory milieu in which these cells interact with luminal bacterial flora is not fully reflected in an *in vitro* experimental set-up using peripheral blood monocytes. Therefore, more human studies are needed, using different cell types, especially antigen-presenting cells, to resolve the paradigm of loss-of-function or gain-of-function mechanism of action of Crohn's disease-related *NOD2* allelic variants.

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