*Editorial*

# NOD2/CARD15 mediates the inflammatory responses in inflammatory bowel diseases (IBDs)

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Inflammatory bowel diseases (IBDs), Crohn's disease (CD) and ulcerative colitis (UC), are common, chronically relapsing and remitting intestinal inflammatory disorders. The IBDs are thought to result from an inappropriate response of the mucosal immune system of the normal enteric flora in a genetically susceptible individual. Despite recent advances in our understanding of IBDs, important questions that concern the immunopathology and genetic basis of CD and UC, remain unanswered.

Epidemiological studies, familial clustering of disease, ethnic variability and twin studies provide conclusive evidence that genetic factors have a crucial role in determining susceptibility and progression to IBDs, as well as that susceptibility to IBDs, in particular to CD, is inherited.[1] However, it is also supported that IBDs are not inherited as a Mendelian trait, but have a complex genetic basis with many contributing genes.[2] The genetic heterogeneity, gene-gene and gene-environment interactions have, for a long time, prevented the identification of specific genetic abnormalities.[2] The human genome project was an important catalyst for the substantial progress that was made during the 1990s in studies of the molecular genetics of IBDs. The genome wide screening has led to the first gene of IBDs and has given new insight into the regulation of chronic inflammation.

In 1996, Jean-Pierre Hugot's group identified a susceptibility locus for CD adjacent to the centromere on chromosome 16, confirmed by a number of centres.[3] Further analysis of this region identified a strong association with a single gene, *NOD*2, also known as caspase-recruitment domain protein 15 (*CARD*15), which encodes an intracellular molecule of the NOD family that is thought to be involved in the recognition of bacteria. Sequencing of this gene, mainly in patients with CD, indicated a cytosine insertion at position 3020 in exon 11 that gives rise to a stop codon and a truncated NOD2 protein. Several other polymorphisms were identified in patients with CD, including two common missense mutations, Gly908Arg and Arg702Trp.[4,5]

Between 10 and 30% of CD patients heterozygotes in at least one of the three mutations and 3-15% homozygotes or compound heterozygotes were found, in a European study, to be heterozygotes have a slightly increased risk (1.5-3 fold) of developing CD, whereas homozygotes and compound heterozygotes have a 10-40 fold risk. Approximately 30% of the overall susceptibility to CD may result from the effect of *NOD*2/*CARD*15 mutations.[6] Normal individuals might occasionally have *NOD*2/*CARD*15 mutations in the absence of disease and none of the three mutations in the *NOD*2/*CARD*15 was found in Japanese patients with CD. The latter provides strong evidence for the presence of genetic heterogeneity among patients of different ethnic groups.[6]

The most important contribution to the identification of *NOD*2/*CARD*15 mutations associated with CD lies in a better understanding of the mechanisms responsible for the pathogenesis of this disease. *NOD*2/*CARD*15 is an intracellular protein implicated in the recognition of intracellular pathogen-associated molecular patterns such as lipopolysaccharides and peptidoglycans.[7] This protein plays a crucial role in detecting bacterial antigen and in transmitting several signalling cascades that induce a complex innate gene programme. Structurally, this gene consists of two amino-terminal effector domains, known as caspase-recruitment domains (CARDs), which induce the nuclear factor-kB (NF-kB) signalling

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cascade, a central nucleotide-binding oligomerization domain (NOD), and multiple carboxy-terminal leucine-rich repeat (LRR) domains that can function as an intracellular sensor of bacterial components. The three common *NOD*2/*CARD*15 variants have been identified within or near the LRR domain.[7]

Furthermore, the CARD domain is known to be implicated in signal transduction, leading to apoptosis via the caspases. Overexpression of *NOD*2/*CARD*15 enhances apoptosis induced by caspase-9 expression. The apoptotic pathway is needed to regulate the life and death of cells, particularly in the immune system, in which discontinuous cycles of expansion and contraction occur to combat infection. However, the apoptotic apparatus is also needed in the development of peripheral tolerance, reducing the deleterious pro-inflammatory effects. It is attractive to speculate that the mutation of one protein, such as *NOD*2/*CARD*15, implicated in the apoptotic pathway, may trigger an impaired pro-apoptotic response, causing reflexive/or constant activation of the innate/adaptive immune system. Nevertheless, the relationship between *NOD*2/*CARD*15 mutations and apoptotic pathway has not been investigated much, and further studies are needed to determine the role of *NOD*2/*CARD*15 protein in the apoptotic pathway, in particular, on CD.[8]

*NOD*2/*CARD*15 was first demonstrated to be expressed by monocyte/macrophage cells, but recent studies provide information concerning *NOD*2/*CARD*15 function and regulation also in intestinal epithelial cells, which are a primary element of the intestinal barrier. The expressed *NOD*2/*CARD*15 functions as a defensive factor against intracellular bacteria in intestinal epithelial cells, initiating a gene programme aimed at eliminating invasive intracellular bacteria. The mutated *NOD*2/*CARD*15 protein is incapable of sensing lipopolysaccharides or peptidoglycans, and of initiating NF-kB signalling and impairs its ability to eliminate invasive bacteria in intestinal epithelial cells. The bacterial clearance was accelerated in cells expressing a functional *NOD*2/*CARD*15 protein, whereas cells expressing mutant protein were unable to clear the pathogens.[9]

The findings that concern the activity of *NOD*2/*CARD*15 and its dysfunction in CD have led to several possible explanations as to the pathogenesis of CD due to *NOD*2/*CARD*15 mutations. The main hypothesis, at present, is that in the absence of *NOD*2/*CARD*15 activity there is defective activation of macrophages that leads to a persistent infection of macrophages owing to a marked *NOD*2/*CARD*15-independent effector-T-cell response. However, persistent intracellular infection of macrophages has not been detected in CD, and other possibilities need to be considered. Another hypothesis is that in the absence of *NOD*2/*CARD*15 expression by intestinal epithelial cells, microbial products that normally activate epithelial cells to secrete chemokines and defensins fail to do so. This would be lead to the proliferation of bacteria in the crypts and to loss of barrier function allowing marked stimulation of mucosal cells by mucosal antigens. The recent observation that *NOD*2/*CARD*15 is expressed by epithelial cells at the base of the villous crypts, known as the Paneth cells, supports this possibility. A third hypothesis is that recognition of microbial peptides by *NOD*2/*CARD*15 normally conditions APCs in a way that leads to their induction of regulatory and effector-T-cell-responses, and so failure of this mechanism disrupts mucosal homeostasis. A final hypothesis could be that mutated *NOD*2/*CARD*15 may have an impaired effect *NOD*2/*CARD*15 on caspase-9-induced apoptosis.[9,10]

Considering all the above, it may be speculated that an intracellular antigen resulting from the *NOD*2/*CARD*15 mutations persists in the epithelial cells (as shown by reduction of NF-kB activity) leading to activation of the innate system. Since the *NOD*2/*CARD*15 function is impaired, the innate immune system is no longer able to exert its role and, hence, hyperactivity of the adaptive immune system develops. It may also be hypothesized that the adaptive immune system cells do not undergo apoptosis, remaining constantly activated. Lack of tolerance may ensue with amplification of the inflammatory process.

Notably, given the role of NF-kB in activating pro-inflammatory pathways, the finding of decreased NF-kB activation with CD-associated mutations is contrary to what might be expected. NF-kB is activated by several pro-inflammatory agents promoting intestinal inflammation, and many anti-inflammatory pharmacologic agents commonly used in the treatment of CD exert their effect by inhibiting NF-kB activity. From recent studies, it appears that some genes, under the control of NF-kB, may play a role in the initiation of inflammation, whereas others maybe involved in the resolution of inflammation and/or tissue repair. Complete pharmacological disruption of NF-kB activity in the intestine could therefore have deleterious consequences for the host by impairing medications able to maintain tolerance versus normal flora in intestinal epithelial cells. This data changes the present concepts of IBD treatment and suggests that the pharmacological correction of the *NOD*2/*CARD*15 signal

pathway in intestinal epithelial cells, increasing the NF-kB activation, could contribute to maintaining intestinal host homeostasis.

The discovery of specific *NOD*2/*CARD*15 mutations in patients with CD provided clear evidence that CD may be caused by defective host innate responses to bacterial components.

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