

NOD2 Signaling and Its Relation to Inflammation of the Mucosa

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Over the past several years we have conducted studies to determine the mechanisms underlying the fact that mutations of *CARD15* (encoding NOD2) is a susceptibility factor in Crohn's disease. In initial studies we showed with in vitro studies that activation of NOD2 by its ligand, muramyl dipeptide (MDP) causes negative regulation of peptidoglycan-mediated stimulation of Toll-like receptor (TLR) 2 and that in the absence of such activation (i.e., in cells from NOD2-deficient mice), TLR2 signaling leads to heightened Th1 responses. To support this concept in vivo, we then showed that mice deficient in NOD2 but not normal mice were susceptible to a transient Th1 colitis if they were exposed to recombinant *E.coli* organisms expressing ovalbumin peptide and were infused with T cells recognizing this peptide; in addition, this colitis was not seen in mice who were deficient in both NOD2 and TLR2.

In further studies we determined the susceptibility of mice to experimental colitis if they bore a transgene that expressed *CARD15* or were administered expression plasmids expressing wild-type NOD2 or NOD2 with a Crohns-like mutation. We found that mice expressing increasing amounts of NOD2 due to the presence of a transgene or an expression plasmid was less susceptible to colitis induction than wild-type mice; in addition mice administered an expression plasmid expressing NOD2 with a Crohns-like mutation did not confer reduced susceptibility. Then in our most recent studies we have shown that pre-stimulation of dendritic cells with MDP dramatically reduces subsequent cytokine responses to multiple TLR ligands through the induction of enhanced IFN-regulatory factor 4 (IRF4) activity. This is mirrored in the finding that pre-treatment with MDP administration protects mice from trinitrobenzene sulphonic acid or dextran sodium sulfate-induced colitis, again via induction of IRF4. Finally, tying these findings to Crohn's disease, we showed that repletion of NOD2 in NOD2-deficient mice with a NOD2 expression plasmid expressing wild-type NOD2 restores their ability to be protected from colitis by MDP administration, whereas, repletion of NOD2 in these mice with an expression plasmid expressing a NOD2 with a Crohns-like mutation did not bring about restoration.

Taken together, these studies provide solid evidence that mutations in *CARD15* are susceptibility factors in Crohn's disease because in the absence of normal NOD2 function one has reduced regulation of TLR signaling involving multiple TLR ligands.

References:

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