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# The importance of gut microbiota in mediating the effect of NOD2 defects in inflammatory bowel disease

Gerhard Rogler

It is well accepted that genetic factors contribute to the pathogenesis of Crohn's disease. Susceptibility genes have been identified; however, the exact mechanisms of how those risk genes contribute to the pathogenesis of Crohn's disease still remain to be elucidated. Therefore, insights such as those provided by Barreau and co-workers from the group of Jean-Pierre Hugot in this issue of *Gut*<sup>1</sup> (see page 207) are important and helpful.

The proof of the 'genetic susceptibility concept' for Crohn's disease was achieved in 2001 by Hugot and others by the finding that single nucleotide polymorphisms (SNPs) in the NOD2 gene are significantly associated with the risk of developing Crohn's disease.<sup>2-4</sup> About 20–40% of all patients in Europe and North America—depending on the genetic background—carry variants of NOD2 in contrast to 10–15% in the healthy population. Three major NOD2 genetic variants have been associated with Crohn's disease in Caucasians in several independent studies. In contrast, NOD2 variants are irrelevant in the Asian population and do not play a role in the pathogenesis of Crohn's disease in Japan or China.<sup>5 6</sup>

The function of Nod2 has been intensively investigated since then. Muramyl dipeptide (MurNAc-L-Ala-D-isoGln, MDP), a component of the bacterial wall derived from peptidoglycan, was found to be the major ligand for NOD2.<sup>7 8</sup> Interestingly, MDP was long known to be the essential structure in Freund's adjuvans, important for vaccination. Nod2 protein variants associated with susceptibility to Crohn's disease seem to be deficient in their recognition of MDP.<sup>8</sup> Subsequently, they have been regarded as 'loss of function' mutations.

Nod2 protein expression was mainly found in mucosal macrophages which represent a central component of the innate immune system. They are capable of phagocytosis and destroy pathogens. They also react to bacteria by secreting cytokines and toxic oxygen radicals.<sup>9</sup> A role for the epithelial Paneth cells in NOD2-related Crohn's disease pathophysiology was supported by the finding that these cells were mostly prominent, expressing Nod2 in normal and Crohn's disease mucosa.<sup>10 11</sup>

In inflamed mucosa from patients with Crohn's disease increased Nod2 expression was found in intestinal epithelial cells (IECs) and macrophages<sup>9 12</sup> but not in T cells. This raised the question of how the NOD2 variants could finally lead to a 'T cell disease'.

NOD2 is a member of a superfamily of genes, the nucleotide-binding site and leucine-rich repeat (NBS-LRR) proteins, which are involved in intracellular recognition of microbes and their products.<sup>13</sup> NBS-LRR proteins play an important role in the innate immune system as pattern recognition receptors (PRRs). The microbial patterns recognised by PRRs are evolutionarily highly conserved. Already plants have an innate immune system of PRRs recognising the same patterns of microbes as compared to humans. As Nod2 is an important intracellular sensor of bacterial wall products it was assumed that it would not only play a role in Crohn's disease pathophysiology. Subsequently, a high impact of NOD2 variants on disease development, pathogenesis and even disease-associated mortality could also be demonstrated in graft versus host disease (GvHD) after allogeneic bone marrow transplantation.<sup>14 15</sup> GvHD is associated with increased intestinal permeability. Experimental models indicate the primacy of gastrointestinal damage; conditioning-related damage of the intestinal epithelium results in bacterial translocation followed by increased cytokine release by macrophages and finally T cell activation. The

incidence of severe GvHD (and associated gastrointestinal GvHD) increases from 18% in Stem Cell Transplantation (SCT) donor/recipient pairs without any NOD2 variant to 37% in pairs with either donor or recipient mutations with a subsequent increase of treatment related mortality (TRM) from 33% to 60%.<sup>14 15</sup>

Again the question arises from those data how a reduced recognition of invading bacteria at the intestinal barrier and subsequent reduced activation of the innate immune system may cause chronic mucosal inflammation. How can a reduced inflammatory response finally lead to an increased and uncontrolled inflammatory response? Do bacteria play an essential role for this? Barreau and co-workers<sup>1</sup> now provide some important answers. They show that both anti-CD4<sup>+</sup> and anti-interferon  $\gamma$  (anti-IFN $\gamma$ ) antibodies abrogate the increased bacterial translocation found in Peyer's patches (PPs) of Nod2<sup>-/-</sup> mice. This indicates that CD4<sup>+</sup> T cells are able to influence epithelial functions such as trans- (and para-cellular?) permeability. However, obviously this part of the cross-talk between epithelium and T cells must be secondary to the primary hit in which T cells are recruited and the T cell function is altered. Bacteria certainly are involved in this so far unknown primary event as antibiotic treatment in the Nod2<sup>-/-</sup> mouse could reverse the mucosal phenotype. Barreau and co-workers subsequently hypothesise that the ileal flora promotes recruitment and activation of CD4<sup>+</sup> cells inside the PPs. These cells secrete cytokines such as IFN $\gamma$  which causes upregulation of tumour necrosis factor (TNF) receptor2 expression in the epithelium. This could be followed by increased permeability of the epithelium (transcellular or paracellular: TNF has been shown to upregulate expression of the pore-forming tight junction protein claudin-2) and further uptake of bacteria into the mucosa. That again would lead to increased recruitment of CD4<sup>+</sup> cells and close a circle leading to chronification of inflammation.

While this concept is attractive, several questions remain open. Why is there an initially increased recruitment of CD4<sup>+</sup> cells into the PP region caused by bacteria? How can it be explained that in human GvHD the relative amount of CD4<sup>+</sup> cells in the colonic lamina propria is significantly reduced in GvHD patients with NOD2 variants as compared to patients with NOD2 WT.<sup>16</sup> The group of Jean-Pierre Hugot has started to characterise the Nod2<sup>snp13/snp13</sup> mouse model and found an increased number of PPs and increased

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## Commentaries

number of immune cells per PP as well as increased translocation of *Escherichia coli* in Ussing chamber experiments similar to the NOD2<sup>-/-</sup> mice. These first experiments are mentioned in the manuscript by Barreau *et al.*<sup>1</sup> We look forward to a more detailed characterisation of this mouse model with the assays the group has established to obtain more answers on this important aspect of Nod2 function and the initiation of inflammation in patients with Crohn's disease.

**Competing interests** None.

**Provenance and peer review** Commissioned; not externally peer reviewed.

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## The new 'connexin' to the pathogenesis of *E coli* diarrhoea

Alastair J M Watson

Infectious diarrhoea is a major cause of morbidity and mortality causing 1.5 million deaths worldwide in 2005.<sup>1</sup> Thus the pathobiology of diarrhoea is of considerable interest. However, despite considerable research over the last 50 years, significant gaps remain in our knowledge of the pathogenic diarrhoea mechanisms of specific microorganisms.

Diarrhoea can be regarded simply as an excess of water in faeces. The healthy adult intestine must absorb approximately 7 litres per 24 h to produce a normal stool. Failure of absorption or, alternatively, secretion of water into the intestine or a combination of the two will cause diarrhoea.

*Vibrio cholerae* or enterotoxigenic *Escherichia coli* (ETEC) secrete toxins which cause the accumulation of the second messenger

cyclic AMP or, in the case of ETEC, cyclic GMP in intestinal epithelial cells. These second messengers cause secretion of water into the intestinal lumen through a couple of mechanisms. Secretion occurs predominantly in the intestinal crypts. They can directly open an anion channel on the apical membrane of epithelial cells called cystic fibrosis transmembrane conductance regulator (CFTR) allowing the efflux of chloride ions resulting in osmotic gradients that pull water into the intestinal lumen; a process called electrogenic chloride secretion.<sup>2</sup> They also activate a neural reflex within the intestinal wall releasing the neurotransmitter vasoactive intestinal polypeptide (VIP) near epithelial cells, again causing accumulation of cAMP in epithelial cells and electrogenic chloride secretion.<sup>3 4</sup> On villi, cholera toxin and *E coli* STa toxin inhibit the process NaCl absorption, called electroneutral NaCl absorption, which is mediated by the coupled action of Na/H and anion exchangers. The mechanism of inhibition of these exchangers is not fully understood but involves at least nine regulatory proteins.<sup>4</sup>

The attaching and effacing (A/E) pathogens enterohemorrhagic *E coli* (EHEC), enteropathogenic *E coli* (EPEC) and mouse A/E pathogen *Citrobacter rodentium* do not cause secretion as described above but rather trigger changes in the structure of epithelial cells and tight junctions between epithelial cells which increases the permeability of intestinal epithelium to water. In these circumstances hydrostatic pressure in blood vessels and lymphatics will drive fluid, protein and cells into the intestinal lumen.<sup>5 6</sup> Induction of inflammation by A/E pathogens with the release of TNF will further exacerbate this exudation through an increase in vascular permeability.<sup>7</sup>

Work over the last decade has shown that A/E pathogens cause a profound remodelling of the actin cytoskeleton of intestinal epithelial cells causing the formation of a characteristic pedestal on their apical membrane. This is achieved by the bacteria injecting a number of effector proteins through a syringe-like structure called a Type III secretory system which flatten the microvilli of the apical membrane and raise the pedestal upon which individual bacteria reside.<sup>8 9</sup> The function of the pedestal may be to inhibit phagocytosis of bacteria but it also has the consequence of reducing the surface area available for absorption. The injected bacterial proteins EspF, EspG and Map disrupt the tight junctions by causing

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