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NOD2 prevents emergence of disease-predisposing microbiota.

Thomas Secher¹, Sylvain Normand^{2,3,4,5}, Mathias Chamaillard^{2,3,4,5}.

¹ INRA, Institut National de la Santé et de la Recherche Médicale, UMR 1043, F-31024 Toulouse, France. ²University Lille Nord de France, F-59000 Lille, France. ³ Institut Pasteur de Lille, Center for Infection and Immunity of Lille, F-59019 Lille, France. ⁴ Centre National de la Recherche Scientifique, Unité Mixte de Recherche 8204, F-59021 Lille, France. ⁵ Institut National de la Santé et de la Recherche Médicale, U1019, F-59019 Lille, France.

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Correspondence should be addressed to: mathias.chamaillard@inserm.fr

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ABSTRACT

The gut flora is composed of a huge number of diverse, well-adapted symbionts that interact with epithelial lining throughout the host's entire life. Not all commensals have the same ability to maintain quiescent, protective inflammation. Importantly, instability in the composition of gut microbial communities (referred to as dysbiosis) has been linked to loss of gut barrier in the context of common human illnesses with increasing socio-economic impacts, such as Crohn's disease and colorectal cancer. Our recent findings suggest that disease-predisposing dysbiosis can now be intentionally manipulated by targeting the major Crohn's disease-predisposing *NOD2* gene. That knowledge will not only add a new dimension to the often overlooked microbiology of Crohn's disease and colorectal cancer, but will also have a broad impact on biomedical sciences worldwide.

A foetus is sterile *in utero* but is progressively colonized by a huge number of diverse microbial communities (also referred as the microbiota) [1]. One can consider the intestinal microbiota as our “extended self”, in as much as this multicellular organ benefits to the host by consuming, storing and redistributing energy harvested from nutrients. With this reductionist organ centered-view in mind, it is interesting to contemplate the dynamics of symbiosis (defined as an association between organisms of two different species in which each is benefited). Symbiosis is well exemplified by the co-evolutionary, beneficial interactions between the microbiota and the intestinal barrier, which has widely differing living requirements. Over the past ten years, several initiatives (including the MetaHIT program, the Canadian Microbiome Initiative and the Human Microbiome Project) have already provided critical insights into our understanding of the composition of the human microbiota which primarily consists of three enterotypes among the human population [2]. Noteworthy, over 80% of phylotypes of the fecal microbiota belongs to either Bacteroidetes, Firmicutes and Actinobacteria in adults [3]. Besides antibiotics, the composition of the intestinal microbiota is also influenced across age and geography [4, 5] and by the mode of delivery [6, 7]. It is also noteworthy that long-term protein/fat and carbohydrate diets respectively influence not only the abundance of *Bacteroidetes* and *Firmicutes* species in humans [8], but also the functionality of the microbiota [9]. Whereas profiles of dominant phylotypes appears specific to each individuals, high-throughput sequencing approach revealed the existence of a phylogenetic core composed of Firmicutes, Bacteroidetes and Actinobacteria [10] and of several core pathways (including the ribosome and translational machinery, nucleotide charging and ATP synthesis, and glycolysis) [11]. The physiological impact of the microbiota is indeed intrinsically determined by the microbiome - a term introduced by the Nobel laureate Joshua Lederberg to describe the collective genomic content of the gut microbiota which consists of hundred time more genes than the human genome *per se* [12]. It remains now to be determined whether the afore-mentioned core microbiome may act as a rheostat for maintenance of gut functionalities. More recently, whole-transcriptome shotgun sequencing unveiled a panel of xenobiotic-responsive genes in the active human gut microbiome [13], which may be determined by inter-individual genetic variations in the microbiota [14]. However, despite an extensive research in this field, it is still not yet known how successive waves of colonization may comprehensively shape the overall health status of the host and (ii) which selective genetic forces may ensure stability and resilience of symbionts from the first days of life onwards.

One can also hypothesize that not all commensal lineages (of which *Bacteroides* is the most prominent), have the same ability to act as a failed pathogen for the benefit of the immune response [15]. It is worth mentioning *Bacteroides thetaiotaomicron* was found to behave as a more versatile commensal, in that genetically predisposed hosts may perceived it to be an opportunistic microorganism [16]. Those that do are referred to herein as pathobionts. By contrast, secretion of polysaccharide A by *Bacteroides fragilis* confers its ability to function as a true symbiotic microorganism by exploiting innate immunity [17]. In the absence of any commensal lineages, antibody production is reduced and the colonic mucosa is defective in goblet cell differentiation and lymphoid tissue architecture (e.g., isolated lymphoid follicles) [18]. Gut microbiota plays a key role against enteric bacterial pathogen infection by out-competing towards specific nutritional requirements [19]. Throughout the host's entire life, the coexistence of certain resident commensals and a single layer of intestinal epithelial cells therefore has a beneficial impact on post-natal maturation of the gut innate and adaptive immune barrier, as illustrated by the key observation on acid-producing lactic bacilli by the 1908 Nobel laureate Elie Metchnikoff. Notably, the absence of gut microbiota impacts intestinal epithelial cell function with decreased production of antimicrobial peptides [20]. However, it remains challenging for immunologists to understand how the host coordinates the biologic dynamics of the microbiota.

Understanding this paradigm is of importance because instability in the composition of gut microbial communities (referred to as dysbiosis) has been linked to ageing [21] and loss of gut barrier function in common human illnesses with increasing socio-economic impacts, such as malnutrition [22, 23], type I diabetes [24], type II diabetes [25] and Crohn's disease [26]. Crohn's disease is an alarming public health care problem that mostly affects young adults in industrialized countries. In Europe, the combined prevalence is about 250-300 cases per 100,000 inhabitants that result in as many as 2.2 million sufferers [27]. Whereas Crohn's disease can affect work and social interactions in diverse ways, the lifetime therapeutic management of Crohn's disease is still indeed far from optimal. Notably, 25-30% of patients fail to respond to current biologics and/or to immunosuppressive drugs, resulting in accumulation of adverse events. Consequently, the estimated annual economic burden of health care of Crohn's disease patients ranges from €2.1 billion to 16.7 billion in Europe. Resection surgery is also required in about two-third of Crohn's disease patients, but does not cure the disease. Colorectal carcinoma still represents the most common cause of morbidity in Crohn's disease. It affects around 10% of Crohn's disease patients after 30 years of disease progression [28]. Similarly, prominent inflammation enhances tumour incidence and progression in experimental models of colitis-

associated colorectal cancer. On the clinical level, it is noteworthy that Crohn's disease lesions have a tendency to develop where the bacterial load is greatest. Hence, short-term antibiotic treatment and surgical diversion of the fecal stream are used as a non-specific therapy in some Crohn's disease patients [29, 30]. In particular, Crohn's disease is linked to a reduced diversity of the microbiota, suggesting that disappearance of certain ancestral microbial commensal indigenous species might be deleterious for the host [31]. Both fecal- and mucosal-associated of Crohn's disease patients is characterized by a lower abundance of *Firmicutes* and a greater preponderance of certain *Bacteroidetes* [32, 33, 34]. More importantly, subclinical dysbiosis has been observed in healthy, first-degree relatives of Crohn's disease patients [35]. However, it remained to be seen whether dysbiosis may act as the premise or a consequence of disease risk.

Our knowledge of bacterial symbiosis now suggests that the major Crohn's disease predisposing *NOD2* gene functions as a key remote control mechanism for the gut microbial communities. *NOD2* is an intracellular receptor for bacterial peptidoglycan that share similarities with the superfamily of plant disease-resistance proteins. In both mice and humans, impaired functionality of *NOD2* is associated with a profound dysbiosis that is characterized by lower abundance of certain *Firmicutes* and by preponderance of intestinal *Bacteroidaceae* [36, 37, 38, 39]. More recently, Couturier-Maillard and coworkers described an unexpected role of the major Crohn's disease predisposing *NOD2* gene in the protection against intestinal inflammation and tumorigenesis afforded by maternally-transmissible gut bacterial communities in mice (Figure 1). Reciprocal fecal microbiota transplantation led to long-term changes in intestinal microbial communities and decreased the vulnerability of *Nod2*-deficient mice to colonic injury [40]. Consistently, treatment with a cocktail of broad spectrum antibiotics efficiently improved disease risk in *Nod2*-deficient animals. Conversely, disease risk was enhanced in wild-type hosts that were recolonized with dysbiotic fecal microbiota from *Nod2*-deficient mice [40]. Most recent high-throughput, culture-independent sequencing of bacterial communities revealed an expansion of members within the *Bacteroidetes* phylum, including *Bacteroides* spp. in mice deficient for *Nod2*. Unlike "conventional" pathobionts, the afore-mentioned commensal lineage was associated with a communicable risk for colitis in a non-genetically predisposed host, suggesting the urgent need to refine dogmas on symbionts. Furthermore, a decreased abundance of microorganisms related to *Butyrivibrio* and *Lachnobacterium* has been correlated with enhanced disease risk, but further metagenomic investigations should be considered in order to determine *Butyrivibrio*'s and/or *Lachnobacterium*'s specific ability to enhance the epithelial barrier. Collectively, *NOD2* was identified as a genomic force that shape evolutionary transitions in

bacterial symbiosis. However, one roadblock to cataloguing a disease-predisposing microbiota is that it does not provide any functional indications on the key “outside-in” features of the causative microbe. It is therefore eagerly awaited to clarify how NOD2 act as key remote control mechanisms for the gut microbial communities.

On this basis, licensing of the microbiota is a key remote control mechanism for disease risk in the gut. The mechanism may result from the differential ability of disease-predisposing commensal lineages to out-compete true symbionts for nutrients. As is observed for classical enteropathogens [41], one may consider that access to reactive oxygen species may also assist growth and colonization by a subset of disease-predisposing commensals within inflamed tissues. To understand how potentially distinct mechanisms lead to the genesis of dysbiosis, we shall seek to determine the potentially common innate and adaptive cellular firewall(s) through which NOD2 prevents growth and colonization of disease-predisposing commensal lineages since birth. From a bench-to-bedside perspective, establishing whether a particular dysbiotic state is sufficient to instigate Crohn’s disease and/or colitis-associated colorectal cancer may open up the way for new therapeutic strategies, including the potential for preventive manipulation of the gut microbiota by either nutrition, probiotics and immunomodulatory drugs in humans (Figure 1). Notably, anti-interleukin-6 receptor treatment was associated with less severe disease and changes on gut microbial communities [40], suggesting that protective assembly of the microbiota may result from a specific microbiome-plus-treatment interaction. Inflammation has indeed been recently identified as a key remote control mechanism of gut microbial ecology [42]. Understanding microbiome-plus-treatment interactions may not only add a new dimension to the often overlooked microbiology of Crohn’s disease, but may also provide us unique opportunities for improving drug design and for selecting more efficient therapies [43].

Figure 1 : Schematic of regulation of bacterial symbiosis by NOD2. The regulation of the composition of the microbiota by NOD2 (1) controls epithelial barrier function (2) and immune homeostasis upon injury (3). Complete mucosal healing may subsequently influence growth and colonization of commensals (4). On this basis, a direct clinical perspective relies in the development and selection of novel therapies (including nutrition, probiotics and host-targeted drugs) aiming at restoring dysbiosis in the absence of NOD2.

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