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Controversy Over *NOD2*, Inflammation, and Defensins

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Abstract

Simms LA, Doecke JD, Walsh MD, et al. Reduced alpha-defensin expression is associated with inflammation and not NOD2 mutation status in ileal Crohn's disease. Gut. 2008;57:903–910.

Bevins CL, Stange EF, Wehkamp J. Decreased Paneth cell defensin expression in ileal Crohn's disease is independent of inflammation, but linked to the NOD2 1007fs genotype. *Gut*. 2009;58:882–883; discussion 883–884.

Several years ago, Bevins, Stange, Wehkamp and colleagues[1] reported decreased human α-defensin 5 and 6 expression in ileal Crohn's disease (CD) patients harboring the NOD2^{1007fs} variant (encoded by *NOD2*^{3020insC}, "snp13") and hypothesized that decreased Paneth cell antimicrobial activity might contribute to disease pathogenesis in ileal CD. Simms et al[2] recently challenged this model by reporting that decreased α-defensin expression is a consequence of inflammation per se and unrelated to the *NOD2* polymorphic state, implying that altered antimicrobial function might be secondary to active CD rather than a contributor to intestinal inflammation. This apparent contradiction has now been addressed by Bevins et al[3] in a letter published in *Gut*, which triggered a counter-response by Simms et al[4] and a consequent reply by Wehkamp et al.[5]

Specifically, Bevins et al[3] remark that Simms et al have not stratified for the NOD2^{1007fs} variant but pooled all 3 major *NOD2* variants. Moreover, they pointed to the fact that stratification with regard to inflammation in the ileum did not affect HD5 expression in the Wehkamp et al[1] study, nor was human defensin-5 (HD5) regulation observed in non-CD ileal inflammation as deduced from measurements in pouchitis mucosa. Bevins et al speculated that a mix of surgical and biopsy specimens in the CD group might have been a detrimental confounder for the analysis in the Simms et al study, since the control group solely consisted of biopsy specimens.

In their response, Simms et al[4] now provide a stratification of HD5 and HD6 mRNA levels by *NOD2* genotypes, which does not show significant differences including that associated with the NOD2^{1007fs} variant. Moreover, these authors claim varying results in the other group's previous articles with regard to defensin levels, and reject the idea that sample bias might have contributed to the divergent results since statistical analyses included adjustment for confounders including sample type.[4] Simms et al bring up another potential confounder

distinguishing the Wehkamp et al study from the Simms et al study, namely, medical treatment, as 5-aminosalicylates and immunosuppressants, which were used differentially in the 2 study cohorts, and might have vastly different effects on intestinal bacteria.[4]

COMMENT

Needless to say, this invited *Selected Summary* can certainly not be intended to resolve the apparent academic dispute between the 2 groups, and it would be pointless to discuss individual merits and limitations of either study beyond the points already raised in the respective letters discussed above. In fact, several major discoveries, especially over the last few years, have revealed a perplexingly complex picture of inflammatory bowel disease (IBD) that essentially excludes the possibility that any single, coherent mechanism will account for all, or even the majority, of patients developing any of the 2 forms of IBD or its subtypes.[6] This perception has arisen from many areas of research, in particular from genetic studies that have up to now discovered >40 individual genetic loci associated with CD and ulcerative colitis (UC), which altogether still may only account for up to 20% of heritability of the disease in CD.[7] More than one-third of genetic loci are shared between CD and UC, suggesting that genetics might determine susceptibility and environmental aspects might shape phenotypic presentation.[8] Another possibility might be that distinct particularly rare genetic polymorphisms that present in a simple Mendelian fashion might have profound effects as recently described for IL-10R variants,[9] and numerous distinct pathways could account for the familial forms of IBD, especially those associated with early-onset disease. Hence, while clinically classified subtypes of disease might not form pathophysiologically homogenous entities, quite the contrary, there might even be a substantial mechanistic overlap between both types of IBD. What we phenotypically observe in the clinic could represent the limited set of available responses of the intestine toward innumerable (genetic and environmentally determined) events, and hence disease presentation might not reflect a variety of distinct individual mechanistic pathways.

Undoubtedly, *NOD2* remains the individual genetic locus with the largest contribution to heritability to CD.[7] NOD2 acts as an intracellular pattern recognition receptor and binds muramyl dipeptide, a breakdown product of bacterially derived peptidoglycan, but might also bind virally derived molecules.[10] Its mechanistic association with CD is extremely important, and several mechanistic aspects may distinguish CD-associated *NOD2* variants from wildtype variants. These include decreased activation of NF-κB,[11] loss of regulatory function upon TLR2 stimulation,[12,13] absence of an adequate IL-10-mediated regulatory response,[14] and decreased defensin expression in Paneth cells.[1,15] However, a consistent model of how NOD2 and NOD2 variants lead to CD has still not yet emerged, likely due to the fact that neither *Nod2*$^{-/-}$ mice[15] nor mice that harbor the murine homolog of the human CD-associated *NOD*^{23020insC} variant[16] develop spontaneous disease. Remarkably, *Nod2*$^{-/-}$ mice express decreased levels of individual α-defensins (cryptdins)[15] similar to what has been proposed by Bevins et al[1] and NOD2 appears to control the commensal flora in the intestine in mouse models.[17] In turn, the presence of a commensal flora increases intestinal NOD2 expression.[17] Despite this regulation of commensal flora by NOD2 (presumably via defensins derived from Paneth cells), the commensals' contribution to disease pathogenesis is only speculative. Specifically, mice with a genetic inability to convert pro-cryptdins into

cryptdins,[18] or mice with a targeted depletion of Paneth cells,[19] do not develop intestinal inflammation.

However, changes in intestinal microbial composition and quantity might indeed be associated with CD and UC While overall changes in microbial composition as revealed through 16S rRNA sequencing are statistically significant, but rather subtle, a distinct “IBD subset” is discernable with profound alterations compared to healthy controls.[20] Remarkably, only a subset of CD and UC patients possess microbiotas that map to a so-called “IBD subset,” while the majority of CD and UC patients exhibit microbiotas that are indistinguishable from healthy controls.[20] It is currently unknown whether host genotype predicts mapping to the “IBD subset.” However, since the microbiotas of CD *and* UC patients were present in largely similar frequencies in this subset, it is unlikely that *NOD2* polymorphisms would account for this property since such genotypes are specific for CD. Moreover, the “IBD subset” contained decreased numbers of bacteria, which contrasts with the increased load of commensals in *Nod2*$^{-/-}$ mice[17] and predictions based on decreased defensin expression in human ileal CD. However, even this situation might be far more complex than appreciated until recently, as inflammation per se, no matter whether it is induced by a microbial pathogen or a host genetic defect, might dramatically alter the composition of the intestinal microbiota.[21] Based on the intriguing studies by Garrett et al,[22] altered host genes may render the intestinal microbiota “colitogenic,” i.e., creating a niche that changes the microbiota in a way that allows it to induce colitis on its own. However, with regard to the aspects specifically studied in the articles under discussion here, it is currently unknown whether inflammation or *NOD2* host genotype could possibly render the intestinal microbiota into a form that allows the microbiota to induce (or perpetuate) intestinal inflammation. In addition, data have not yet been published that show that altered defensin expression is sufficient to cause intestinal inflammation. However, the emerging close relationship between specific constituents of the intestinal microbiota and the organization of the intestinal and systemic immune system[23] render it plausible that future functional studies could indeed reveal “inflammatory” properties of the microbiota secondary to alterations in antibacterial functions of the host (no matter what the inflicting mechanism for altered antibacterial function is—be it genetic, or be it ongoing inflammation). Consistent with this, it has recently been observed that segmented filamentous bacteria (which adhere closely to epithelial cells) might induce Th17 cells in the lamina propria[24] and a single polysaccharide from *Bacteroides fragilis* may prevent colitis induced by a commensal with pathogenic potential.[25] These observations serve as illustrative and intriguing examples for how alterations in the microbial communities may exert effects on the mucosal immune system that may culminate in inflammation (or protection from inflammation).

In this context it is remarkable that several IBD genetic risk factors apart from *NOD2* also converge on Paneth cell function. Autophagy has recently been identified as a CD pathophysiologic mechanism via the discovery of polymorphisms in *ATG16L1*[26] and *IRGM*.[27] Paneth cells from mice harboring a hypomorphic ATG16L1 variant exhibit structural alterations in granules as well as changes in mRNA expression levels of inflammatory genes.[28] However, oral infection with *Listeria monocytogenes*, a model

pathogen whose translocation to liver and spleen is increased in *Nod2*$^{-/-}$ (and *Xbp1*$^{\Delta IEC}$ [see below]) mice, was not affected by hypomorphic ATG16L1 function, and these mice also did not exhibit spontaneous intestinal inflammation.[28] Of note is that myeloid deficiency of *Atg16l1* resulted in increased sensitivity to dextran sodium sulfate (DSS) colitis, and *Atg16l1*$^{-/-}$ macrophages secreted increased levels of IL-1β and IL-18 upon stimulation with bacterial lipopolysaccharide, a TLR4 ligand.[29] This is notable, as increased inflammatory responsiveness has also been reported secondary to *Nod2* deficiency,[12] although *Nod2*$^{-/-}$ mice do not develop spontaneous inflammation or increased susceptibility to DSS colitis.[15] Intestinal inflammation secondary to unresolved endoplasmic reticulum (ER) stress is another pathophysiologic mechanism that is relevant to both forms of IBD.[30,31], Specifically, genetic deletion of the ER stress associated transcription factor X-box binding protein 1 (XBP1) in the intestinal epithelium results in spontaneous enteritis closely resembling human IBD and increased sensitivity to DSS colitis, and *XBP1* variants have been associated with CD and UC.[32] Notably, XBP1 deficiency results in Paneth cell dysfunction and decreased cryptdin expression, and at the same time increases the inflammatory responsiveness of the intestinal epithelium to microbial-derived (e.g., TLR5 ligands) or mucosa-derived factors (e.g., TNF).[32] Altogether, these data may suggest a model whereby a proinflammatory responsiveness of the epithelial or myeloid compartment may be essential in addition to decreased Paneth cell antimicrobial function to elicit inflammation at the mucosal surface.

In summary, a bidirectional cross-regulation of host and microbes in the intestines that may profoundly contribute to intestinal inflammation as observed in IBD is emerging as an important paradigm. We are only at the very beginning of our understanding of these mechanisms and their relationships to IBD. Given the enormous genetic complexity of IBD, and even more so the still completely unresolved contribution of what we call “environmental factors,” it appears self-evident that numerous biological pathways may contribute to disease in IBD, and a unifying mechanism is currently unlikely to emerge for CD or UC.

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