Innate immunity includes defensins

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We would like to comment on the comprehensive review of Karantanos and Gazouli in *Annals of Gastroenterology* [1] about genetics and innate immunity in inflammatory bowel disease (IBD).

The authors appreciate the important role of innate immune sensing and the production of antimicrobial peptides in maintaining the integrity of the mucosal barrier. Pertinent studies on the subject are presented and the authors concur with the current understanding of Crohn's disease (CD) as the manifestation of an abnormal immune response to the intestinal microbiome in individuals with genetic susceptibility.

Yet, in our view, the body of literature justifies the proposition of an integrative model of pathogenesis for ileal CD (iCD), focusing on the role of the Paneth cell (PC) products, the α-defensins human defensin 5 (HD-5) and HD-6. Associations between PCs and defensins in small intestinal inflammation are plentiful, and we feel that in the interpretation by Karantanos' article, this aspect is not given the due attention.

PCs, a characteristic epithelial cell line of the small intestine localized at the bottom of the intestinal crypts, constitutively secrete considerable amounts of antimicrobial peptides (AMP), the expression levels of α-defensins thereby exceeding those of other PC antimicrobials like lysozyme and sPLA2 by a factor of up to 100 [2].

Activation of pattern recognition receptors (e.g. Toll-like receptors, nucleotide-binding oligomerization domain (NOD)-like receptors, RIG-I-like receptors) by pathogen-associated molecular patterns (PAMPs, derived from resident and pathogenic bacteria) leads to the release of PC secretions into the intestinal lumen [3]. Expression of intracellular receptors like NOD2 itself depends on the presence of commensal bacteria [4]. In turn, the composition of microbial species found in the small intestinal lumen can be regulated by the luminal antimicrobials [2,5].

A link between NOD2 and iCD has already been demonstrated by Cuthbert et al [6] in 2002, when the mutations R702W, G908R and 3020insC were identified as strong independent risk factors (in patients of Caucasian descent). NOD2 protein and mRNA were found to be most prominently expressed in the terminal ileum, with localization to crypt base cells and mononuclear cells of the lamina propria [7]. Subsequent work from our group reported decreased α-defensin mRNA levels in ileal biopsy specimens, which were even more pronounced in patients carrying NOD2 mutations [8]. On a functional level, Petnicki-Ocwieja et al showed that the bactericidal activity of crypt secretions of the terminal ileum was severely compromised by NOD2 deletion in a murine model [4]. Studies with other knock-out animals, lacking functional cryptidins (mouse-homologs to defensins are called cryptidins) due to deficiency of the cryptidin-processing enzyme matrilisin, revealed that intestinal crypt secretions had decreased antimicrobial activity [9], and that these mice are more susceptible to orally administered bacterial pathogens or to DSS-induced colitis.

NOD2 mutations are but one of the findings that underline the link between PC, defensins and iCD. In 2007, a genome-wide association study identified ATG16L1 as susceptibility locus for small intestinal CD [10]. ATG16L1 protein mediates degradation of phagocytosed or invasive bacteria, although it is commonly known for its involvement in autophagy, a process responsible for the degradation of intracellular structures. Cadwell et al provided evidence that in mice with conditional Agt16l1 knock-out, granule exocytosis is abnormal [11] and that in patients with the variant T300A, morphologic distortions in PC can be observed. As PC granules contain huge amounts of α-defensins, impaired exocytosis of these antimicrobials presumably weakens the mucosal barrier. This could explain part of the increased disease susceptibility found with mutations in ATG16L1, though other mechanisms for the phenotypic development of iCD with this genetic background have to be considered as well. ATG16L1 deficient macrophages for example exhibit proinflammatory properties [12], which points to an additional involvement of myeloid cells in disease pathogenesis.

A conditional deletion of X-Box binding protein (Xbp)-1 shed light on the association of Xbp-1 variants with IBD (CD and ulcerative colitis). Xbp-1 normally functions as transcription factor for the unfolded protein response under conditions of endoplasmic reticulum (ER) stress, maintaining proper folding, export and degradation of proteins by the ER. Knock-out of Xbp-1 in the intestinal epithelium led to spontaneous small bowel inflammation, altered PC morphology and a decreased antimicrobial activity of crypt secretions [13]. These findings furthermore support the concept that different

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genetic alterations lead to dysfunctional PCs, resulting in a weakened mucosal barrier and disease susceptibility.

Yet another association between iCD and defensins becomes apparent when alterations in the Wnt pathway, which governs PC differentiation [14], are considered. More specifically, the transcription factor TCF-4 has been linked to α-defensin expression, as heterozygous Tcf-4 knock-out mice with decreased levels of Tcf-4 exhibit compromised cryptdin expression and weakened antimicrobial activity of crypt extracts [15]. Furthermore, a reduced mRNA expression of Tcf-4 was observed in patients with iCD. Following investigations aimed at the regulatory regions of Tcf-4 revealed that in iCD, a SNP in the Tcf-4 promoter region (rs3814570) was significantly more frequent than in controls, solely colonic CD or ulcerative colitis [16]. Taken together, the genetic variant in the promoter of Tcf-4 gives a further rationale for the α-defensin deficiency in iCD on the basis of PC malfunction.

In a murine model, inhibition of the K⁺-pump/Ca²⁺-channel KCNN4, which regulates Ca²⁺-fluxes important for the secretion mechanisms for AMPs from PC, led to a reduced bactericidal activity and AMP secretion [17]. An association of the SNP r2306801 with CD in general, and with iCD the strongest, was recently observed in a combined cohort from Australia and New Zealand [18], though the reproduction of these results in a larger cohort is not yet available. KCNN4 mRNA expression in non-inflamed mucosal biopsies of individuals with NOD2 mutations was reduced, leading to the assumption that functional NOD2 is important for the expression of KCNN4.

All the mentioned different genetic variants and functional studies converge on the PC and its ability to provide the crypt lumen with functional antimicrobial peptides, which are, in the end, predominantly α-defensins. With this addendum, we depict our PC model of defensin deficiency in iCD (Fig.1), which respects the interplay between genetic variants, host factors like AMPs and the microbiome in the pathogenesis of small intestinal inflammation [19]. It should be noted that compromised defensin expression is also a feature of colonic CD [20], although the details are beyond the scope of this comment. We recommend that the important role of defensins in IBD pathogenesis should be given credit.

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Innate immunity includes defensins

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