Linkage between genotype and immunological phenotype in Crohn's disease

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Abstract: Understanding the mechanisms that drive uncontrolled inflammation in Crohn's disease (CD) remains one of the most pressing challenges in contemporary experimental medicine. Recently, a three-phased view on the pathogenesis of CD was proposed in which following the breakdown of intestinal epithelial barrier function, CD patients fail to clear the resulting infectious debris, provoking subsequent immune responses. This view on CD is attractive in that it is testable and allows better diagnosis of disease if proven correct, apart from opening a window on new therapeutic horizons. Here we shall argue, however, that this scheme may be an oversimplification in that it ignores the genetic diversity of CD and thus does not fully take into account the nature of the intestinal epithelium, which appears a non-passive actor in this disease.

Keywords: Crohn's disease (CD); risk gene; innate immunity; epithelial barrier

Submitted Sep 07, 2015. Accepted for publication Sep 09, 2015.
doi: 10.3978/j.issn.2305-5839.2015.09.28
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.09.28

Crohn's disease (CD) is a major manifestation of inflammatory bowel disease (IBD) which presents itself with a variable and wide range of clinical phenotypes (1). Among its major characteristics are recurrent diarrhea, bloody stool (2), abdominal pain (3), the presence of aphthous ulcers anywhere in the gastrointestinal tract (4) and more generally substantial chronic fatigue (5), feverous attacks (6), malnutrition and associated weight loss (7) as well as a range of extra-intestinal manifestations, including skin rashes (8), bone loss (9) and uveitis (10). Treatment remains symptomatic and despite the widespread use of immunomodifying biologicals many patients require repetitive surgery (11). In combination with the ever increasing incidence of CD (12), the often chronic poor quality of life (13) and substantial economic costs (14) imposed by an incurable disease that usually manifests itself for the first time in adolescence make CD a substantial burden on society. Thus, further understanding of the pathological mechanisms underlying this disease so as to guide development of rational novel avenues for improved treatment of CD is warranted.

Pathophysiologically, the root mechanism underlying the clinical problem in this disease appears to be a chronic inflammatory response towards the microbiological constituents of the gastrointestinal tract (15). In support of this notion is the observation that although disease might manifest itself anywhere in the gastrointestinal tract, areas with increased microbiological load, like the ileum or the colon (16), are more often affected by CD as compared to other parts of the digestive system (17). Complicating this interpretation, however, is the observation that in patients with CD, there are areas of apparently healthy tissue right next to damaged intestine (18). In combination with familial clustering of disease (19), but a relatively poor concordance of disease incidence even in monozygotic twins (20), most researchers active in this area feel that only a combination of genetic, environmental, immunological and microbiological factors can explain why this disease develops in affected individuals.

Dai and colleagues have recently contributed an excellent editorial to the *Annals of Translational Medicine* with their vision on the future of translational research in

IBD (21). This editorial was a reaction to an earlier study which we published in *Science Translational Medicine* (22) in which we performed comparative kinome profiling of the intestinal mucosa of healthy controls as well as inflamed and neighboring non-inflamed tissues from CD patients. We observed that p21Rac1 GTPase signaling is suppressed in non-inflamed tissue and speculated that blocking p21Rac1 correlates with clinical improvement of IBD by boosting innate immune responses. Our data suggest that blocking p21Rac1 may be protective in IBD and further highlight the potential of kinome profiling techniques to uncover the inner mechanistics of disease processes (23,24). In their editorial Dai *et al*. employ our study as a scaffold to share their view on the field, in particular highlighting the need for further studies on the mechanisms mediating the primary immunodeficient aspect of CD. In this school of thought, CD involves inadequate innate immunity (25), allowing small-time infections to fester and provoking strong intestinal inflammation through secondary lines of defense and thus granulomatous disease. This view is strongly supported by high-quality studies showing diminished innate immunity in CD patients (26) and our own observations, as reported by Parikh *et al*., that remission of CD disease is associated with improved innate immunity led further credence to this model. In this sense we fully agree with Dai and colleagues that further understanding these innate defects, especially at the mucosal level is now a major frontier in experimental IBD research.

In their editorial Dai and colleagues further explore the possible nature of immune responses in the mucosa of patients with CD by making the distinction between an abnormal acute reaction and the subsequent chronic inflammation. The authors sketch a convincing and experimentally testable hypothetical three-phased outline as to how such chronic inflammation develops and leads to granulomatous disease. They envision that in the absence of adequate neutrophil mobilization to sites where barrier function has been compromised, residual luminal material will be phagocytized by macrophages to form the disease-characteristic granulomata, with secondary macrophage activation subsequently provoking deleterious Th1 cell-mediated chronic inflammation. However, we feel that these authors overlook the possible diagnostic implications of their vision of immunity in CD. Diagnosis of CD remains highly problematic (27) and for now can be only be made by the combination of clinical presentation, pathological investigation of biopsied material and medical imaging (28). Distinction of CD from other pathological entities, especially infectious colitis and ulcerative colitis, is often not straightforward. The molecular markers associated with the chronic process proposed by Dai *et al*. may however enable future pathologists to come up with immunohistochemical and/or molecular biological markers (think of genes specifically expressed during secondary macrophage activation or those present in the plasma cells that surround granulomata in CD) that will enable an unequivocal diagnosis of CD.

A further conclusion reached by these authors is that the environmental factors influencing CD should be further investigated and that in conjunction with increased insight into the nature of the immunological response this might lead to novel insights into potential new modes of therapy of CD. While this is no doubt true and worthy of further exploration, we feel that this notion overlooks the heterogeneous nature of the disease and especially does not take sufficiently into account the genetic differences between alternative patients with CD, which clearly influences the nature of the immune response (29). For instance, our own research has shown a genomic ATG16L1 risk allele-restricted ileal Paneth cell endoplasmic reticulum stress in quiescent CD, which had functional consequences for local microbiota composition in these patients (30). Furthermore, we demonstrated that in neutrophils of patients with CD an increased reactive oxygen species production was observed following fMLP stimulation, which was mirrored by an increased fMLP-triggered ERK and AKT signal activation (31). However, in patients bearing the NCF4 risk allele for CD, priming of this fMLP-mediated reactive oxygen production by pro-inflammatory cytokines was reduced (32). Other studies point to an association of NOD2 mutants and ileal disease (33), whereas evidence has been presented that pediatric CD fueled by the congenital absence of the interleukin 10 receptor, are refractory to most immunomodulators, including infliximab (34). Apparently, depending on the genetic predisposing context, CD and the results of its treatment can have different aspects.

This may be especially true for the intestinal epithelium that forms the first layer of defense against bacterial invasion of the mucosa (35). While Dai *et al*. acknowledge the importance of the epithelial layer as a barrier against bacterial passage; we view the epithelium as much more than solely a passive structure. The epithelial layer contains goblet cells that through mucus production influences luminal bacterial composition and also actively react to bacterial challenge with further NF-κB-driven mucus

secretion thus adjusting mucosal barrier function to local need (36). Paneth cells in particular, but also other epithelial cell types secrete antibiotics to combat local bacterial colonization (37). The epithelial layer contains antigen-presenting M cells (38) and dendrites of submucosal dendritic cells (39) that present mucosal antigens to the immune system. The epithelial cell itself can produce cytokines, chemokines and anti-bacterial products to combat bacteria. Importantly many of these functions can be influenced by IBD risk genes (40). In this context it is especially important to note that the intestinal $Lgr5^+$ stem cells constitutively express the canonical CD risk gene Nod2 which apparently mediates survival of this cell following increased bacterial load (41). One can imagine that NOD2 deficiency thus specifically influences the integrity of the intestinal layer. We thus envision that differences in IBD phenotype can partly derive from genetic differences in the CD-predisposing genomic context and the resulting altered epithelial function thereof.

In conclusion, we find the three-tiered hypothesis of Dai *et al*. very attractive in that it offers a testable framework as to how intestinal Crohn's can develop and provides a clear view as to how improved diagnosis of CD could be obtained. We do, however, argue for amendment of the proposed view on the immunopathogenesis of CD in that it does not take into account as to how differences into genotype with respect to IBD-predisposing phenotype may provoke different presentation of the disease. In addition, we find that the proposed scheme does not take fully into account the dynamics in the intestinal epithelial compartment and we would like to propose inclusion of these factors in the view of Dai *et al*. on the pathogenesis of CD as well.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Fuhler GM, Parikh K, van der Woude CJ, Peppelenbosch MP. Linkage between genotype and immunological phenotype in Crohn's disease. Ann Transl Med 2015;3(16):237. doi: 10.3978/j.issn.2305-5839.2015.09.28

