

Future of IBD Pathogenesis: How Much Work Is Left to Do?

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About one-half century ago inflammatory bowel disease (IBD) was a true “Cinderella” of the autoimmune/chronic inflammatory disease cluster, with probably the most inadequate and least dependable amount of information on what might be causing Crohn’s disease (CD) or ulcerative colitis (UC), what could be leading to them, and what the mechanisms underlying inflammatory tissue damage might be.¹ This situation is dramatically reversed at the beginning of the 21st century, and it is fair to state that more progress has been achieved in IBD than in any other condition characterized by chronic inflammation of a specific target organ. At present, the IBD research community is satisfied, if not convinced, with the general notion that both CD and UC are closely related to 4 fundamental components that explain their pathogenesis: broad *environmental changes*, *genetic predisposition*, the *enteric commensal flora*, and the *immune system*.^{2,3} Progress in each of these areas has not been necessarily even, which is primarily due to logistic difficulties intrinsic to large-scale studies, limits on financial means, variable technological progress, and the availability of enough “hands and brains” to perform basic studies or implement laborious and time-consuming experiments at the bench. Let us briefly analyze each of the above 4 pathogenic components.

To further expand our understanding of the contribution of environmental changes to IBD pathogenesis obviously cannot be done at the bench. To generate strong solid evidence in support of the “hygiene hypothesis,”^{4,5} which links environmental changes to an inappropriate immune reactivity at the systemic or intestinal levels, massive, expensive, long-term prospective studies of diverse populations in various parts of the world would be needed. No single investigator or even groups of investigators alone will ever be able to gather the financial and logistic resources needed to properly carry out these studies, which should be relegated to international governmental agencies. Since this is highly improbable to occur, the lack of adequate IBD epidemiologic studies will continue to hinder efforts to better understand the impact of the environment on the emergence of IBD worldwide.

Quite an opposite situation has taken place in the field

of IBD genetics. The recent development of technologies that can generate a comprehensive portrait of the human genome, methods that allow rapid sequencing of DNA from preselected and reasonably well-defined patient and control populations, coupled with the creation of genetic consortia in the USA, Europe, and elsewhere have allowed the performance of massive genome-wide association (GWA) studies.⁶ These are not necessarily “bench-based” activities, but combined they have yielded 2 critical pieces of information: the first is the discovery of novel, numerous, strong links between genetic variations and IBD, particularly in CD; the second is the essentially irrefutable proof that genetic predisposition is required to develop IBD.⁷ This is an excellent example of how long-held intuitions about disease mechanisms based only on clinical and laboratory experience are transformed into reality because of technological advances. While the progress in the field of IBD genetics has been impressive, as is often the case, new challenges are created by new knowledge. At the moment >30 genetic variations have been reported in IBD, but they explain <30% of all IBD cases. How many more variations are to be expected? If the ones described so far represent the most frequent because they are the easiest to detect by GWA, will we uncover an even greater number of variations in unyielding numbers of small subsets of CD and UC patients?⁸ If so, this is a disheartening and daunting perspective, and one that may keep investigators very busy for quite some time unless additional screening tools become available.⁹

The long-lasting emphasis put on immunological studies during several decades has caused the field of microbiology to be relatively neglected. Scientists are now suffering from this gap in knowledge, which has become far more acute after the discovery that the human genome contains “only” 20,000 genes—instead of the predicted 100,000—and the realization that essential functions needed for health depend on complementary functions codified by genes of the human microbiota.¹⁰ This is particularly true in the gastrointestinal tract, whose function is almost entirely dependent on the presence of the enteric commensal flora. Lack of adequate knowledge of the enteric microbiota has become a pressing issue for IBD pathogenesis after the realization that gut flora appears to be the target of the aberrant immune response driving inflammation and tissue damage in the gut of CD and UC patients.^{11,12} To alleviate our ignorance, the recently established *Human Microbiome Project* and the increasingly widespread use of DNA-based rather than culture-based tech-

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niques will be essential to classify bacterial populations in the human ecosystem.¹³ Such classification is essential to provide an answer to the vital question of whether or not the gut flora in IBD patients is qualitatively or quantitatively different from that of healthy subjects and, if so, how. Unfortunately, we are nowhere near to answering this question. The different molecular approaches used to analyze bacteria yield different types of data, are not directly comparable, and not only is the source of patients dissimilar, but even the source of bacterial DNA, which can derive from the fecal stream, the mucosa, endoscopic washes, or expelled stools.¹⁴ We are not even sure whether these sources yield the same microbial components. Moreover, the composition of the flora turned out to be far more complex than previously anticipated, and flora changes from birth to adult life, and varies among populations in different regions of the world.

The increasing attention that genetics and enteric microbiology is receiving is eroding the supremacy of the immune system as the predominant area of IBD pathogenesis on which to concentrate investigational resources. Moreover, immunological studies of IBD are taking newly uncovered directions. From a “T-cell centric” or *adaptive immunity* view of IBD immunopathogenesis, investigators are quickly switching their allegiance to *innate immunity*.¹⁵ This switch has resulted in the more and more commonly accepted view that IBD may be caused by defects in innate immunity, and that the involvement of T cells is a secondary event. The bases of such change are multiple: a vastly increased knowledge of the cells mediating innate immunity (like macrophages and dendritic cells), the identification of specific molecular moieties that allow recognition of microbial molecular patterns (like Toll-like receptors [TLRs] and NOD-like receptors [NLRs]), the identification of several mutations in genes whose products mediate innate immunity pathways, and the apparent clinical efficacy of some therapies using cytokines that boost rather than suppress the immune system. Even some of the fixed-in-stone paradigms of IBD adaptive immunity appear to be crumbling: the belief that CD is a Th1-like disease dependent on the IL-12/IFN- γ axis has been badly shaken by the recent identification of a new subset of T helper cells, the Th17 cells, that produce IL-17, IL-6, and TNF- α ,¹⁶ and the identification of abundant Th17 cells in inflamed IBD tissues.¹⁷ Even Th cells that simultaneously produce both IFN- γ and IL-17 have been recently detected in the mucosa of CD patients.¹⁸ Thus, the IL-23/IL-17 axis appears on the verge of replacing or at the least joining the IL-12/IFN- γ axis on the pedestal of “fundamentally important” mechanisms of inflammation.¹⁹ Will the capacity to quickly uncover new genes and analyze bacterial populations extend to immunology? Will we be soon witnessing the discovery of additional and unsuspected subsets of T helper cells, T regulatory cells, B cells, and dendritic cells? Will nonimmune cells, like epithelial, endothelial, mesenchymal,

neural, and fat cells reach the level of “IBD pathogenicity” comparable to that of classical immune cells?²⁰ Will this expand our IBD immunological universe, making it more complicated although more relevant? Continuous investigation, this time truly at the old-fashioned bench, should provide answers to these key questions.

How much work is left to do to gain a fuller understanding of IBD pathogenesis? We still need a long series of continuing combined studies to grasp the functional implications of the various IBD genetic variations at the molecular, cellular, and clinical levels. This will entail: 1) detailed DNA sequencing of highly selected IBD population, 2) large genotype–phenotype association studies, 3) testing of the gene products of interest in functional assays, 4) the development of conditional cell-specific “knock out/in” and transgenic animals, 5) to study of the effect(s) of gene mutations in IBD animal models, and 6) the modulation and therapeutic screening of IBD-associated mutations in animal models. These directions will be successful and fruitful, and will help to advance our overall understanding of IBD. However, they alone will not be enough, as long as each area is explored within itself. To reach a true understanding of IBD on a global scale, we will need integration of new knowledge emerging from the investigation of environmental changes, genetic make-up, the enteric commensal flora, and the immune system. For instance, very recent evidence shows how environmental toxins can directly skew the immune response toward Th17 or T regulatory cell dominance through the same receptor (aryl hydrocarbon receptor [AHR]),²¹ and how environmental pollutants like silica and asbestos can directly activate the inflammasome.²² Along these lines, we should relatively soon understand how IL-23R mutations impact the immune response of IBD patients, and how *NOD2*, *IRGM*, and *ATG16L1* mutations alter the handling of bacteria in CD patients. This integration will represent the next phase of IBD investigation, and will require an even greater coordination of knowledge among investigators’ groups and an open mindset, where no single component is more important than the other.

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