Good bug, bad bug: in the case of enteric inflammatory disease does the epithelium decide?

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Many studies demonstrate that intestinal inflammation is either initiated or exaggerated by a component of the normal microbiota, most likely commensal bacteria or products derived from these organisms. We review the nature of human inflammatory bowel disease, the evidence for the involvement of the normal bacterial flora in these disorders and the relevance of maintaining the integrity of the epithelial barrier. Moreover, we, and others, have shown abnormal mitochondria structure in tissue resections from patients with inflammatory bowel disease and tissues from rodents that demonstrated psychological stress-induced increases in epithelial permeability. Thus, we also consider the possibility that a defect in epithelial mitochondrial function would predispose an individual to respond to their commensal bacteria flora – no longer considering them as a beneficial passive inhabitant, but rather perceiving them as a threatening and pro-inflammatory stimulus. In support of this postulate, we discuss our recent findings from an in vitro model showing that the human colon-derived T84 cell line exposed to the metabolic stressor, dinitrophenol, and the non-pathogenic, non-invasive, Escherichia coli *(strain HB101) display a loss of barrier function, increased signal transduction and increased production of the chemokine, interleukin 8.*

Key words: intestine - metabolic stress - commensal bacteria - T84 cell

The interplay with microbiota is a pivotal determinant of human health and well-being. Bacteria have been implicated in a myriad of disorders ranging from autoimmune conditions to myocardial infarction to inflammatory and functional diseases. Nowhere is this better illustrated than in the gut, where bacteria have been implicated as initiating or exaggerating factors in idiopathic inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). In this commentary we review the central hypothesis relating to IBD, such that with the appropriate genetic background disease is manifest via an inappropriate immune response to an element of the commensal flora. We will introduce the reader to gut form and function, highlight our cell of interest – the epithelial cell – and then reflect upon the role of altered barrier function, stress and commensal bacteria in the induction of IBD. Finally, we present for the readers' consideration a new model that integrates many of the key finding in the analysis of enteric inflammation into a unifying schema.

Gut form and function

The mammalian gut is a tremendously complicated organ. It contains approximately the same number of neurones as the brain (Wood 1991), is the single largest repository of immune cells (Brandtzaeg et al. 1999) and houses a complex microbiota, which out-number host eukaryotic cells ten-fold (Shanahan 2002). The gastrointestinal tract is responsible for nutrient absorption and digestion, excretion of non-digestible material and, seemingly paradoxically, excluding the entry of lumen-derived unprocessed dietary or microbial antigen and microorganisms into the mucosa. The efficient functioning of the gut requires the coordinated activity of structural/stromal cells and regulatory elements (i.e. neuroendocrine and immune-derived factors) (Perdue & McKay 1994). Similarly, while it is instinctive to consider the microbiota as inherently bad and potentially pathogenic, the former is not true as it is quite clear that a normal intestinal flora is a requirement for normal intestinal function (Rook & Stanford 1998). For instance, intestinal morphology of gnotobiotic-raised animals is different from animals maintained under conventional housing, and bacteria-liberated short-chain fatty acids are an important energy source for colonocytes.

The single cell thick epithelial lining of the small and large intestines is composed of columnar transporting enterocytes, mucus-producing goblet cells, enteroendocrine cells, and defensin-producing Paneth cells (Madara 1994). These cells originate from a common stem cell in a lower-mid crypt position and migrate down to the base of the crypt (i.e. the Paneth cells) or up along the villus (or to the apex of the crypt in the colon). A specialized and enigmatic immune cell, the intra-epithelial lymphocyte, resides above the basement membrane that the enterocytes rest on and may migrate in and out of the epithelial compartment. This diversity of cell types indicates that the epithelial layer is a multifunctional tissue, and the dynamic nature of this tissue is aptly demonstrated by the 3-5 day lifespan of the average transporting enterocyte. The asymmetrical distribution of channels, transporters and ion pumps in the polarized epithelium is criti-

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cal for it's ability to vectorially move nutrients, electrolytes and water between the two compartments that it separates – namely, the gut lumen and the mucosa or body proper (Barrett & Dharmsathaphorn 1991).

Barrier function of the epithelium

In the absence of lesions in the epithelium, material can enter the mucosa via three routes. Specialized microfold "M" cells that overly Peyers' patches in the small bowel take up particulate antigen and pass it to the underlying immune aggregate; a portal of entry that can be exploited by microbial pathogens (Neutra et al. 1996). Given the significant increase in surface area of the transporting enterocytes compared to that of M cells, the majority of lumen-derived antigen should gain access to the mucosa via either transcytosis, negotiating the epitheliums' apical and baslolateral membranes, or passing between adjacent enterocytes, the paracellular permeation pathway. Enteric epithelial cells are joined at their apical (i.e. luminal) margins by tight junctions (TJ). The current model of the tight junction is one of membrane spanning, inter-digitating occuldin and claudin molecules that are integrated with the actin cytoskeleton via adaptor proteins such as zona occludens-1 (ZO-1) (Cereijido et al. 2000). The tight junction acts a "molecular fence" and maintains cell polarity by ensuring that proteins are inserted in the correct membrane – e.g. basolateral membrane components such as the N^+/K^+ ATPase pump do not locate to the apical membrane. The TJ is also the rate-limiting structure controlling paracellular permeability. Once considered static, fixed structures it is now clear that the TJ is a dynamic "organelle" that functions in an energy-dependent manner, and that paracellular permeability is controlled by extrinsic and intrinsic factors, such as bacterial toxins, nutrients, medications, cytokines and growth factors (McKay & Baird 1999). This control of the paracellular pathway can act via altering the epithelial cytoskeleton, the molecular composition of the TJ, or the number of TJ strands that comprise the junction. For example, enteropathogenic *Escherichia coli* infection disrupts the perijunction F-actin (Philpott et al. 1996), loss of claudin expression is associated with a leaky epithelial barrier (Mitic et al. 2000), and in tissues from patients with ulcerative colitis, where there is increased epithelial permeability, TJ density can be reduced (Schmitz et al. 1999).

Awareness of transcellular passage of antigen across the epithelium is gaining momentum. The perspective that all antigens entering the epithelium would be degraded (or processed) is being eroded by an increasing number of studies showing that intact protein antigen can cross the epithelium (Marcon-Genty et al. 1989, Berin et al. 1999, Schurmann et al. 1999). If the gut is sensitized to the antigen the antigen can cross surprisingly quickly, within minutes of exposure to the jejunal epithelium (Yu et al. 2001). The impact of this could be of paramount importance in mucosal immunity and the tenuous balance that exists between health and disease.

Inflammatory bowel disease

The IBD, Crohn's disease (CD) and ulcerative colitis (UC), have different clinical presentations and symptomatology but share the distinction of unknown aetiologies (Fiocchi 1998). Corticosteroids and broad spectrum immunosuppressive agents reduce the severity of the disease and can manage disease relapse, but there is no cure for either condition (with the exception of colectomy for UC). The development of safer, more effective therapies and ultimately a cure for IBD still requires extensive research to define the full significance of the major players in these multi-faceted conditions.

Is IBD a consequence of loss of epithelial barrier function? - There is no doubt that IBD is associated with increased epithelial permeability and over a decade ago Hollander (1992) proposed that reduced barrier function was a primary defect in CD. A postulate supported by assessment of the IL-10 deficient mouse, which showed increased epithelial permeability prior to the onset of the spontaneous overt inflammation (Madsen et al. 1999). However, it is feasible that low grade inflammation or immune activation that may go undetected by conventional assays preceded the barrier defect in these mice. Equally provocative has been the repeated demonstration of increased gut permeability in first-degree relatives of patients with CD and preliminary data showing that spouses of patients with CD can also have increased intestinal permeability (May et al. 1993, Söderholm et al. 1999, Breslin et al. 2001, Thjodleifsson et al. 2003). Whether such individuals go on to develop IBD or other enteropathies is unknown. An intriguing case report did show that a youth with increased intestinal permeability was diagnosed with CD a number of years later (Irvine & Marshall 2000). However, the debate continues as to whether the obviously increased gut permeability sets the stage for the development of inflammatory disease or if the barrier defect is a consequence of an existing immune or inflammatory response – certainly a number of studies have shown the potential for immune mediators to directly increase epithelial permeability (McKay & Baird 1999).

Increased gut permeability is a facet of IBD. Many insults such as alcohol consumption and the use of non-steroidal anti-inflammatory drugs (NSAIDs: we will return to this later (Zamora et al. 1999)) increase gut permeability, at least transiently, but not all users develop IBD. Clearly, in individuals that develop IBD other factors are in play.

Is IBD a consequence of reacting to bacteria and/or bacterial products? - An infectious cause of IBD has always found favour amongst a cadre of investigators (McKay 1999). A variety of potential pathogens have been proposed as the cause of CD (e.g. *Myobacterium paratuberculosis*), but despite extensive efforts identification of a single species responsible for CD or UC has not been forthcoming. The recognition of *Helicobacter pylori* as a cause of gastric ulceration suggests that abandoning the research for an infectious agent behind IBD could be premature, however, the pendulum has swung away from bacterial pathogens towards a consideration of ones own commensal flora as the driving force in IBD. Numerous studies can be cited in support of this postulate. Some of the most graphic are the almost universal descriptions of reduced disease severity in animal models of enterocolitis when the animals are maintained in germ-free conditions (Khun et al. 1993, Bouma & Strober 2003).

Patient studies reveal increased serum antibodies against gut microbes (MacPherson et al. 1996), that fecal diversion and bowel rest can improve CD (D'Haens et al. 1998), and that some patients with CD can generate immune responses against components of their own flora (Duchmann et al. 1996). The latter study led the authors to suggest that the tolerogenic mechanisms that prevent individuals from responding to their commensal flora had been lost in this cohort of patients.

As an adjunct to the concept that an exaggerated response against the commensal microflora is important in the pathogenesis of IBD, we, and others, have suggested that inflammation could be evoked or exacerbated by bacterial products such as peptidoglycan (PG-PS), lipopolysaccharide and superantigens (Sartor et al. 1985, Zareie et al. 2001, Lu et al. 2004). Additional credence is given to this hypothesis by the increasing awareness of the importance of the innate immune response and demonstrations of altered expression of, and mutations in, intracellular sensor/receptor proteins for bacterial products in some patients with CD and in cells exposed to inflammatory mediators (Hugot et al. 2001, Bonen et al. 2003, Girardin et al. 2003).

Is IBD a consequence of stressful life events in susceptible individuals? - Relapse in IBD is commonly coincident with periods of stress. Data from animal studies have convincingly shown that both acute and chronic stress episodes will increase small and large bowel permeability (Saunders et al. 1994, Castagliuolo et al. 1996, Santos et al. 1999). The barrier defect involves both the paracellular and the transcellular pathways, and cholinergic nerves, mast cells and corticotrophin releasing hormone (CRH) have been implicated in these stress responses. Söderholm et al. (2002b) showed that intestine from rats exposed to a chronic, but mild, stress paradigm not only had a significant increase in gut permeability, but also displayed increased numbers of bacteria attached to and penetrating the epithelium. Observations that were very reminiscent of those reported from studies with tissues obtained from patients with CD, were increased bacterial attachment to the epithelium was reported (Swidsinski et al. 2002). Söderholm et al. (2002b) also noted the presence of numerous swollen and irregular mitochondria in the epithelium of colonic segments from the stressed rats.

Human studies, although less numerous, are in general agreement that stress can increase epithelial permeability (Santos et al. 1998, Hart & Kamm 2002).

Epithelial energy balance and gut form and function - Regions of inflamed hamster intestine that are heavily infected with *Campylobacter jejuni* have swollen and irregular epithelial mitochondria (Humphrey et al. 1986). Similar ultrastructural mitochondrial abnormalities have been found in tissues from patients with UC and CD (Delpre et al. 1989, Söderholm et al. 2002a) and also following surgical stress (Ramachandran et al. 2001). Given that maintenance of the epithelial TJ seal is an energy-dependent process, it is not surprising that infective organisms or toxins that directly disrupt the epithelial barrier can do so by damaging the mitochondria (Dickman et al. 2000, He et al. 2000). Moreover, and presumably more of a concern for the physician, medications such as NSAIDs or formulations with sodium caprate (C10, a component of suppositories) can increase epithelial permeability and there is a concomitant disruption of mitochondrial structure and function (Somasundaram et al. 1997, 2000, Söderholm et al. 1999, Zamora et al. 1999, Basivireddy et al. 2002). These findings from a diverse series of studies can be interpreted to indicate that an epithelial mitochondria defect (i.e. perturbed epithelial energy balance) can be a predisposing factor for increases in epithelial permeability and subsequent enteric inflammation.

A new model

There is substantial evidence in support of IBD being a multi-factorial condition, in which the disease appears as a consequence of an uncontrolled immune response to the commensal microflora that may have unimpeded access to the mucosa by virtue of increased permeability. All of the components of this hypothesis have been examined, typically in a pair-wise manner, e.g. stress can increase epithelial permeability, patients with IBD can have altered gut permeability, some CD patients are reactive to their own microflora, etc. In an attempt to integrate these findings we hypothesised that epithelia under metabolic stress will become responsive to non-pathogenic commensal bacteria, leading to altered barrier function and the production of mediator molecules that could lead to inflammatory disease. In initial proof-of-concept experiments we adopted an in vitro cell culture model in which monolayers of the human T84 colonic epithelial cell line were exposed to low-dose dinitrophenol (DNP, 0.1 mM, uncouples oxidative phosphorylation) ± the non-pathogenic *E. coli* strain HB101 and epithelial function monitored for a 24 h period (Nazli et al. 2004). The exposure to DNP or *E. coli* HB101 individually had negligible effects on epithelial monolayers as gauged by barrier function and production of the chemokine, interleukin-8 (IL-8), when the epithelium was assessed 24 h post-treatment.

In contrast to this, simultaneous DNP+*E. coli* HB101 exposure resulted in dramatic changes in epithelial function: epithelial permeability was markedly increased, the non-pathogenic, non-invasive *E. coli* HB101 were internalized and translocated across the epithelium, and IL-8 release increased two-fold (although this was a relatively small increase compared to that evoked by tumour necrosis factor α). Thus, the metabolically stressed epithelium was now responsive to a commensal bacterium! Additional control experiments revealed that the epithelial response was not evoked by a bacterial product or killed bacteria, and was not the consequence of DNP converting the commensal organism into a "pathogen". The loss of epithelial barrier function and increased IL-8 were the net effect of a dynamic interaction between the viable bacteria and the enterocyte that was under the additional pressure of metabolic stress caused by disruption of mitochondrial structure and function (Nazli et al. 2004). This epithelial response is reminiscent of that elicited by enteropathogenic *E. coli* (EPEC), and while changes in the epithelial cytoskeleton induced by DNP + *E. coli* HB101 did

resemble those caused by EPEC infection, pharmacological assessment of the drop in transepithelial resistance (a marker of paracellular permeability) revealed significant differences between DNP + *E. coli* HB101 and EPEC infection (Nazli et al. 2004).

When the NSAID, indomethacin, was substituted for DNP and coupled to *E. coli* HB101 exposure similar changes were observed in the epithelial monolayers – notably there was a significant increase in paracellular permeability. The putative impact of these preliminary findings are underscored by the previous discussion and the widespread use of over-the-counter and prescribed NSAIDs.

Our in vitro data suggest that such events in vivo – loss of the epithelial barrier, bacterial translocation and IL-8 production – could initiate or exaggerate enteric inflammation. This conjecture is support by data showing that direct instillation of DNP (or NSAIDs) into rat ileum evoked the expected disruption of epithelial mitochondrial structure but also led to increased epithelial barrier function, bacterial translocation and a subtle, but detectable, immune cell infiltrate 6-24 h post-treatment (Somasundaram et al. 2000, Nazli et al. 2004).

Concluding remarks

The idiopathic IBD are insidious debilitating conditions. Concerted research efforts are producing a comprehensive picture of the cells and molecules involved in the inflammatory process in the gut and the potential trigger agents that initiate the process. Indeed this knowledge is being converted into new therapies involving biologicals (e.g. anti-TNFα antibody) and the need for scientific evaluation of some neutraceuticals and traditional herbal medicines. However, many of the basic questions relating to IBD remain unanswered – Is the barrier defect primary or secondary to the inflammation? Why do some individuals become reactive to their own microflora, if indeed this is the common denominator in IBD? What is the fundamental difference in aetiology between CD and UC? Is stress, of any kind, a predisposing factor for the development of IBD or more important in evoking disease relapse? Clearly we have much let to learn about IBD. In making a contribution to this field, our recent studies demonstrate that epithelia under metabolic stress now respond to non-pathogenic bacteria and elicit a programme of events that could result in inflammation – data that could be pertinent to the initiation of IBD, and certainly to disease relapse in a cohort of patients.

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