The emerging role of the microbial-gastrointestinal-neural axis

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Abstract

The gastrointestinal tract and its associated mucosal immune system have been extensively studied in the context of their involvement in disease processes, both within the tract itself and in its associated organs. However, historically a number of aspects of both gastrointestinal physiology and pathophysiology have been to some extent overlooked. In particular, the relationship of the gastrointestinal tract with its indigenous microbiota, and also the influence of the tract on behavior and neural systems and vice versa. Here, we describe recent advances in our knowledge and understanding of these areas, and attempt to put these advances in perspective with regard to potential therapeutic strategies.

Introduction

The gastrointestinal (GI) tract is associated with a large range of diseases of significant morbidity and mortality, many of which are currently poorly treated or in some cases completely untreated.[1] This consequently places a large economic burden on society; the latest available statistics estimating that the direct annual cost of GI disease was over $85bn in 1998.[2] Within this disease set, conditions where the unmet medical need is high include the functional GI disorders (FGIDs) such as irritable bowel syndrome (IBS) and functional dyspepsia (FD), the inflammatory bowel diseases (IBD) particularly Crohn's disease and ulcerative colitis, colorectal cancer, and liver diseases such as liver cirrhosis and fibrosis.

From an anatomical perspective, the gut is uniquely exposed to the outside world and contains its own nervous and immune systems. The gut responds to the presence of luminal bacteria with controlled inflammation, mediated at least in part by receptors designed to recognize bacterial components (e.g. toll-like receptors (TLRs), nucleotide oligomerization domains (NOD)). IBD is perhaps the best documented example of a chronic disease that involves interactions between the luminal environment, the mucosa, and the enteric nervous system (ENS). The requirement for microbes in models of experimental colitis is supported by the significant improvement in tissue response in germ-free animals.[3] The NOD-2 mutations in Crohn's disease patients extend to humans the role that luminal bacteria have been shown to have in experimental models of IBD.[3]

The following review will provide a brief outline of some of the key recent findings which suggest that events in the GI tract may influence disease expression, not solely limited to the GI tract and its associated organs. In addition, it will focus on how these emerging ideas may change the therapeutic strategies that could be employed to treat them.

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Microbial-mucosal interactions

With an estimated population of 10^{14}, the gut commensal bacteria or microbiota outnumber their human cell counterparts by ten to one. The association of bacteria with the intestinal tract of animals appears older than human life itself, as it is already well established in non-vertebrates such as worms and flies.[4,5] These bacterial communities have co-evolved with their hosts, and contribute to a range of diverse physiological processes such as protective (pathogen displacement, production of anti-microbial factors), structural (immune system development, barrier fortification) and metabolic functions (synthesis of vitamins B7 and K, ion absorption, fermentation of dietary fiber) which are vital to the health and well-being of the host.[6]

Over the last few years, molecular geneticists and gastroenterologists alike have studied the types of bacteria that exist within the GI tract and the manner in which these organisms influence health and disease.[7,8] This attention, coupled with the increasing accessibility of animals reared in germ-free conditions, has led to a growing appreciation of the importance of microbial-host interactions and an understanding that disruption of this fine balance, or 'dysbiosis', can have dramatic consequences.

An example of dysbiosis-induced disease is antibiotic-associated diarrhea (AAD). *Clostridium difficile* are commensal bacteria that often exist in the normal human GI tract. However, in susceptible individuals treated with antibiotics, often in a hospital environment, the normal microbiota is altered and a subsequent overgrowth of *C.difficile* can result in colitis and diarrhea. Interestingly, replacement therapy with commensal bacteria (so called probiotics) has been used to treat this condition.[9] Another important aspect of the inflammation induced by *C. difficile* infection is the link that it provides between the lumen and the enteric nervous system. In rats, toxin A from *C. difficile* is pro-inflammatory, and this property is blocked by the anesthetic lidocane, implying that activation of sensory neurons and other enteric nerves is essential to this process.[10] This activation in turn leads to release of neutrophil chemoattractants (e.g. leukotriene B4 and TNF-α in rats, and IL-8 in human colon) and to activation of mast cells.

Patients with Crohn's disease can have

increased immune responses to certain antigens of the microbiota.[11] Serological expression cloning has identified a set of immunodominant antigens involved in murine colitis.[12] One-quarter of these antigens were previously unknown flagellins. Strong IgG immuno-reactivity to these flagellins was detected in sera from patients with CD, but not in sera from patients with UC or from normal controls. These data suggest that bacterial flagellin, possibly via its known ability to activate TLR5, may play a role in role in inducing/perpetuating colitis.

Clearly a complex and delicate balance exists between the microbiota and the host under normal conditions, and this can be most easily demonstrated by histological analysis of the gut mucosa, the interface between the two systems. The mucosa exists in a state of continuous and controlled or 'physiological' inflammation, containing numerous innate and adaptive immune cells in close apposition to the lumen of the gut and also, therefore, to the microbiota.[13] The complexity of host-microbe interactions is particularly evident in inflammatory bowel disease. For example, an increasing number of genetic polymorphisms of proteins which sense or otherwise interact with the microbial environment have been shown to predispose to Crohn's disease (see below). This finding is consistent with the proposal that the disease results, at least in some individuals, from an inappropriate immunological response to the commensal microbiota.[11] Molecular analysis of the gut microbiota has raised the possibility that a subset of patients with Crohn's disease or ulcerative colitis may have an abnormal or altered microbial composition in the gut.[14,15] While a pathogen waiting to be discovered cannot be excluded, work from several research groups has linked Crohn's disease with a virulent form of enteroadherent *Escherichia coli*.[16] Deficiency of the transcription factor T-bet in the murine innate immune system has been shown to lead to an alteration in the microbiota which becomes colitis-inducing and can transfer the disease to susceptible hosts.[17] These observations coupled with many predisposing genetic polymorphisms linked to this interplay (see below) have led to the hypothesis that IBD results from an inappropriate immunological response to the gut microbiota.[18] An apparent dysbiosis has been recently reported in IBS as well,[19] consistent with the finding that a subset of IBS patients can associate the onset of symptoms with an episode of acute gastroenteritis.[19] Inflammation due to gastroenteritis may act as a trigger to activate or make apparent underlying IBS, or it may be a true pathogenetic factor. More data are needed to answer this question.

It is becoming apparent, however, that inflammatory mediators such as substance P (SP) and calcitonin gene-related peptide (CGRP), along with increased tissue temperature can activate vanilloid receptors and mediate visceral pain that occurs both in Crohn's disease and in IBS. The transient receptor potential channel, vanilloid 4 (TRPV4) is highly enriched in colonic sensory neurons compared to other neurons in the mouse, but also in humans with Crohn's disease.[20] As in the example of *C. difficile* toxin A mediated activation of enteric neurons, these early findings suggest yet another link between the inflammatory process induced by luminal bacteria and a response of the enteric nervous system that could produce symptoms in intestinal disease.

In addition to diseases associated with the GI tract itself, the microbiota may play a role in the etiology of diseases in GI-associated organs. For example in mouse models of insulin resistance, the gut microbiota has been shown to have an influence on glucose tolerance[21] and development of fatty liver disease.[22] Also, in alcoholic cirrhosis, the erosive action of alcohol and its microbiota-oxidized product acetaldehyde can breach the intestinal epithelial barrier, causing aberrant exposure of the liver to gut bacteria and endotoxins.[23] Although data are only just emerging, evidence is accumulating for the influence of the gut microbiota on a number of extra-gastrointestinal disorders such as stress,[24] arthritis,[25] allergies,[26] and obesity.[27]

Some of the mechanisms by which commensal bacterial species modulate gut function include increased secretion of the anti-bacterial peptides bacteriocins and defensins from microbiota and epithelia, respectively.[28,29] Other effects on host immunity include enhanced regulatory function,[30] increased epithelial barrier function[31] and modulation of cytokine release.[32] Commensal bacteria have also been shown to modulate GI neuronal function by a variety of means including reduced visceral pain perception[33] and reduced excitability of colonic intrinsic primary afferent nerves.[34] It is clear from these studies, in combination with the dramatic phenotypic abnormalities observed in the GI tracts of germ-free animals[6] that the commensal bacterial 'virtual organ' is vital for the health and well-being of the host GI tract via direct, widespread, and specific effects on numerous physiological mechanisms. A thorough understanding of these mechanisms and the bacterial factors that mediate them will doubtless provide future opportunities for therapeutic intervention.

Mucosal immunological responses to microbiota

The immune system can be regarded as a sixth sense – the sense of microbial danger. It exhibits the attributes of all sensory systems: an afferent or uptake limb, central processing of information, an efferent or effector response limb, ability to learn, and memory. Like other sensory systems, the immunosensory system is present at birth but requires continual developmental education in early life. This is achieved through interaction with the environment, primarily by colonization with commensal organisms and, to a lesser degree, by episodic exposure to infection in childhood. In common with other forms of sensory development, disruption or deficit in the quality of sensory education during early life creates the risk of misperception or inappropriate (immune) responses to environmental stimuli in later life. Since many of the features of a modern lifestyle influence the gut microbiota directly or indirectly, the possibility arises that the increased frequency of immune-mediated disorders in developed countries might relate to disturbed microbial-immune education. A more optimistic prospect is the possibility of 'mining' the microbiota for the critical molecular signaling pathways required for microbial priming of host immune development. Indeed, the molecular basis of microbial-host signaling is beginning to emerge and provides the potential for novel treatments derived from microbial sources within the gut, including immunomodulatory and anti-inflammatory activities.[35-37]

A pivotal breakthrough linking GI disease to an abnormal mucosal immune response to gut bacteria came from identification of mutations in the pattern recognition receptor Nod2 in patients with Crohn's disease.[38] Nod2 (and Nod1) recognize peptidoglycan-derived products of bacterial cell walls, specifically muramyl dipeptides (MDP) in the case of Nod2.[39] As MDP is present in almost all Gram-negative and Gram-positive bacterial membranes, Nod2 acts as a general sensor of bacteria. It is not clear how the presumed loss-of-function NOD2 mutations lead to increased susceptibility in developing Crohn's disease. Possible mechanisms include regulation of α-defensins from Paneth cells, or downregulation of its own or other sensing mechanisms after initial activation. Either way, the luminal bacteria seem to play an important role in modulating the immune inflammatory response, and the NOD2 mutation in some patients modifies this effect and leads to inappropriate persistent activation, especially when the ileum is involved. Because some individuals who are homozygous for NOD2 mutations are healthy, other genetic or environmental factors must play a role.[3,39] Since these seminal findings, there has been an upsurge of interest in the field of mucosal immunology, leading to the identification of several other genetic associations pointing to a role of defective innate responses to bacteria in IBD.[40,41] Whilst the functional significance of many of these mutations remains to be deter-

mined, the NOD-2 mutant appears to regulate cytokine release (involving cross talk with TLRs)[42-44] and production of antimicrobial peptides.[45] Moreover, the seemingly simple question as to whether these mutations are loss or gain of function in CD remains to be conclusively demonstrated.[46] Whilst these genetic studies focused on IBD, recent data have extended these observations to indicate the importance of mucosal immunity and its relationship to the enteric microbiota in the pathogenesis of other diseases (see below).

Three broad and interrelated mucosal immune mechanisms have been identified as important in GI pathophysiology (adaptive-, innate- and neuro-immunology) and it is the nexus of these mechanisms on various cell types which is believed to be crucial for disease progression.

1. *Adaptive immunity:* mucosal T-cell responses are widely accepted to play a key role in mucosal inflammatory conditions. Historically, IL-12-driven Th1-derived proinflammatory cytokines were believed to be the primary mediators of Crohn's disease.[18] However, IL-23, a more recently described relative of IL-12 that shares the common subunit p40, has now been shown to be necessary for the development of T cell-mediated colitis in mice,[47] whereas this is not the case for IL-12. The paradigm has been altered by the discovery of a subunit, p19, that is unique to IL-23.[48] IL-23 seems more of a driving factor than IL-12 in models of other autoimmune/inflammatory diseases such as rheumatoid arthritis[49] and multiple sclerosis.[50] IL-23 is crucial in the activity and maintenance of the Th17 lineage, a T-cell subtype that is of rapidly gaining importance in the etiology of many autoimmune and inflammatory diseases, and indeed mutations in both the p40 subunit and in the IL-23 receptor have recently been genetically linked to Crohn's disease.[40] In addition to its recognised importance in the disease process of IBD, a role for low-grade mucosal inflammation and immune activation in the pathophysiology of IBS has also recently been proposed.[51] Another key factor in inflammatory disease progression in the GI tract is the role of regulatory T cells (Tregs) and endogenous innate immunosuppression or immunotolerance, as exemplified by the development of spontaneous colitis in the IL-10 knockout mouse. Indeed, the adoptive transfer of Tregs can control inflammation in animal models of IBD.[52,53] A recent study has suggested that the immunosuppressive effect of FTY720, a sphingosine-1-phosphate receptor agonist, in a model of IBD is due at least in part to the functional activation of Tregs.[54]

2. *Innate immunity:* epithelial tight junctions and secreted products (e.g. defensins) make up the epithelial barrier and play a critical role in maintaining intestinal homeostasis.[55] There are emerging clinical data suggesting that increased intestinal permeability correlates with GI diseases including IBD, celiac disease, and IBS,[56] and agents that normalize the epithelial barrier have been shown to be effective in the treatment of IBD.[57,58] There is an association between celiac disease and numerous other autoimmune conditions, such as Type 1 diabetes and dermatitis herpetiformis.[59] Crohn's disease patients have a reduced ability to clear bacteria and this has been suggested to be due to reduced innate immune function.[60] This defect is reversible by granulocyte-colony stimulating factor;[61] granulocyte macrophage-colony stimulating, factor may have utility in the treatment of IBD.[62] It is not clear whether this attenuation of inflammation is due to a true immune deficiency in GM-CSF, as is the case in chronic granulomatous disease, or whether it is related to another mechanism.

Recent data show that intestinal epithelial cells (IECs) and dendritic cells directly sample gut microbiota[63] and the decision to activate or suppress downstream effector pathways is dependent on the interaction between IECs and dendritic cells.[64,65] Moreover, the neuropeptide, vasoactive intestinal peptide (VIP) appears to play a role in the functional maturation of dendritic cells and in the production of regulatory T cells.[66,67] Emerging genetic data support the idea that defects in autophagy-mediated epithelial cell responses to intracellular microbes are involved in the pathogenesis of IBD,[41,68] and clinical data also suggest that modulating bacterial-sensing pathways, either via targeting cellular signaling pathways downstream of Toll-like receptors[69] or by the use of probiotic agents,[70] has utility in the treatment of IBS and IBD. Indeed, there is now good evidence that treatment with commensal bacteria is efficacious in conditions such as acute enteric infections[71,72] and necrotizing enterocolitis.[73] Probiotics are not uniform in their properties. Different strains should be selected on the basis of their desired function and intended disease indication. For example, protection from *Listeria* infection is a feature of some but not all probiotics, and in one instance, has been shown to be mediated exclusively by production of a bacteriocin.[28] However, the same probiotic protection against *salmonella* infection is not due to the activity of the bacteriocin. Other probiotics have been linked with a range of activities from immunomodulatory properties[32] to eliciting analgesic effects.[33]

3. *Neuroimmunology:* visceral pain is a cardinal feature of many GI disorders, suggesting that afferent pathways are intimately involved in these disease processes. Adaptive and innate immune cells can influence visceral hypersensitivity[74] and, importantly, that efferent pathways can modulate immune function.[75] Examples of the signaling molecules involved in these interactions include β-endorphin elaborated by lymphocytes and acting on μ-opioid receptors in the enteric nervous system to modulate pain perception.[74,76] Mild, mast cell hyperplasia is a feature common to a number of GI diseases including IBS;[77] data suggest a possible utility of mast cell stabilizers in murine post-operative ileus.[78] Evidence that gut commensal microbes influence pain perception comes from a study in which dysbiosis, induced by oral antibiotics, increased responses to colorectal distension in mice.[79]

ENS-CNS interactions: the gut brain axis

For more than a century, it has been widely accepted that there is a close interplay between emotions and gut physiology.[80] Despite this, the only treatments successfully developed to treat GI disease remain those which target the symptoms rather than the cause. Over the last twenty years, appreciation of the role performed by the gut-brain axis has improved immeasurably, both in relation to our understanding of normal physiological processes, such as the regulation of digestive processes and the gut immune system, but also with regard to GI diseases such as IBS, FD and IBD. The last ten years have also seen the documentation of multiple comorbidities associated with functional GI disorders, promoting the concept of a link between the functions of the central (CNS) and the enteric (ENS) nervous systems. This concept points the way to a common pathophysiology for some patients that could explain the involvement of multiple parts of the gut via the ENS (e.g. FD and IBS), as well as multiple somatic organs. For example, unknown luminal factors derived from indigenous microbiota can influence CNS development and the response to stress, at least in rodents.[24] Furthermore, signaling from the GI tract to the brain can occur not only via luminal interactions[81] but also via direct [77] and possibly indirect neuroimmune interactions.[82] This is a two-way process, and efferent pathways also have the capacity to modulate both immune as well as end organ function.[75] We postulate that behavioral change may influence gut physiology and susceptibility to inflammatory stimuli (Figure 1A), an observation supported by demonstrations of stress[83] or depressive-like behavior in rodents.[84,85] Stress may also influence the microbial content of the gut[86] and perturbation of gut bacteria results in low-grade inflammation and changes in gut physiology in mice.[79] In Figure 1B we postulate that dysbiosis not only induces low-grade inflammation and gut dysfunction[79] but also influences the brain. The latter notion is supported by observations that anxiety-like behavior in rodents is seen early during the course of enteric infections with *Campylobacter jejuni*[87] or *Citrobacter rodentium*.[88] The anxiety

behavior might be related either to luminal factors (e.g. toxins), activation of the ENS due to sensory or motor stimulation, or to systemic factors (e.g. fever). The relationship of these observations to the psychiatric comorbidity that occurs in patients with IBS following an acute bacterial infection is uncertain[89] although there are emerging human studies with certain probiotics suggesting an improvement in both anxiety/depression and improved mood states.

Both the ENS and CNS can alter the mucosal response to inflammation by neuropeptide secretion (e.g. substance P (SP), corticotrophin releasing factor (CRF), neurotensin (NT), vasoactive intestinal polypeptide (VIP), μ-opioid receptor agonists (MOR), and galanin).[90] Evidence from animal models shows both pro-inflammatory effects and augmentation of healing at later stages for many of these neuropeptides (Table 1). There is also emerging data to suggest that certain neuropeptides/neurotransmitters may also directly modulate microbiota via quorum sensing "interkingdom" signaling.[91,92] These factors provide the potential for similar common pathophysiological mechanisms to affect the GI tract as well as other organs and tissues.

Relevance of the microbial-gastrointestinal-neural axis

A unifying hypothesis with broad applicability to gastrointestinal disease?

A very large immune system (GALT, gastrointestinal associated lymphoid tissue) as well as a very large nervous system, the ENS, reside within the intestinal mucosa. Both of these systems are connected to their respective counterparts in the rest of the body, the systemic immune system and the CNS, respectively. However, despite the similarity of these gut-related organs and their systemic counterparts, there are distinct differences between them.

The intestine's uniqueness in being exposed to huge numbers of microbiota is important in maintaining a balance between tolerance and disease. It is not yet clear whether this balance is abnormal in disease, but it may turn out that subtle changes are an important trigger for disease.[93] Mucosal surfaces sense external stimuli and mount the appropriate response that involves components of both the innate and adaptive immune systems. A mediator of this sampling and sensing is the mucosal dendritic cell (DC).[94] These mucosal cells are CD11b$^+$ with only a small fraction also expressing CD8α$^+$ or B200$^+$, a marker phenotype like that of lung DCs but

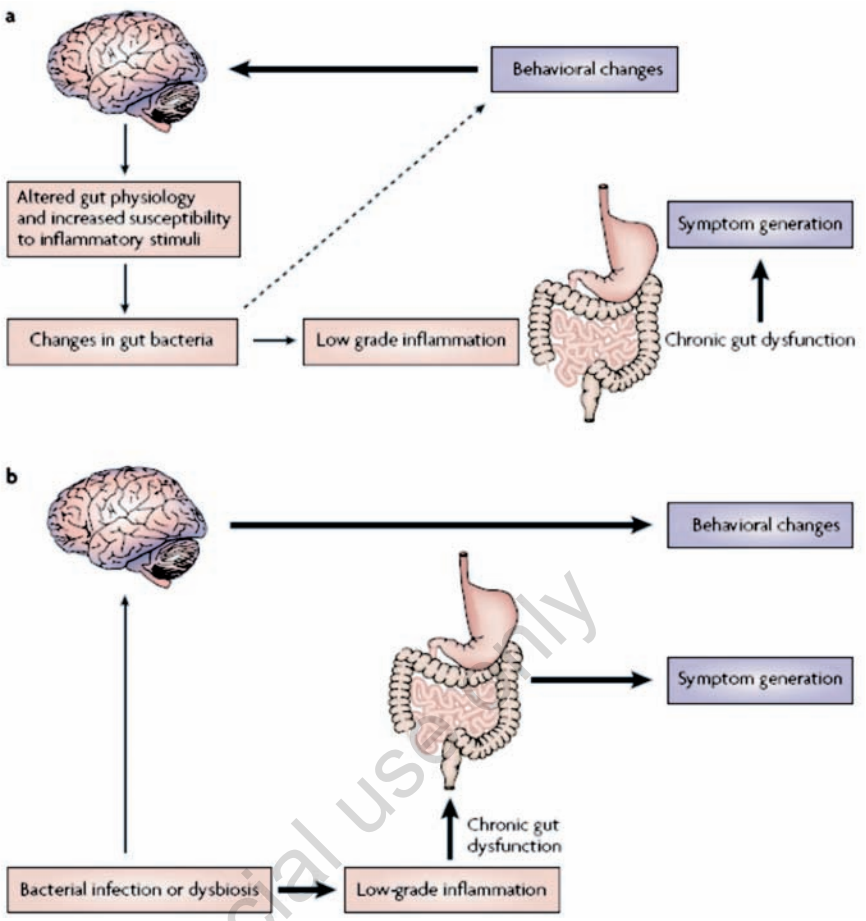

Figure 1. Microbes and inflammation: unifying concepts of functional GI disorders. It is now clear from multiple lines of evidence that microbial-gastrointestinal-neural communication is a two-way process involving both afferent and efferent exchanges of information. Theories of so-called 'top-down' (A) and 'bottom-up' (B) models of gut pathophysiology can no longer be thought of as being mutually exclusive, but rather that these are parallel and interrelated pathways that can result in both disease and behavioral changes. See text under "ENS-CNS interactions" for a further description of this model.

Table 1. Neuropeptide receptors, locations, and functions in the intestine.

Neuropeptide	Receptors	Major location	Examples of possible functions
Substance P (SP)*	NK-1, -2, -3	Myenteric/submucosal plexus, mast cells, neurons macrophages, dorsal root	NFkB activation, MMP activation TGF-α release, microbiota modulation
Calcitonin gene-gene product (CGRP)*	TRPV4	Colonic sensory neurons, dorsal root ganglia	Enhanced mechanosensory responses visceral pain, microbiota modulation
Corticotropin-releasing factor (CRF)*	CRF I, -II	Enterochromaffin cells, myofibroblasts, extrinsic nerve cells	Pro-inflammatory in colitis models, CRF-II responds to *C. difficile* toxin A
Neurotensin (NT)*	NT receptor I, -II	N cells in epithelium of jejunum & ileum	Degranulates mast cells, releases IL-1β from macrophages
Vasoactive intestinal peptide (VIP)^	VPAC1, -2	Myenteric plexus neurons, T cells, eosinophils	Decreased leukocyte migration, dendritic cell differentiation, Treg induction, microbiota modulation
Opioids^	μ-opioid receptor	Mesenteric & submucosal plexus in ileum & colon	Decreases inflammation via NO release
Galanin*	GalR 1, -2, -3	Enteric nerve termini	Increases NFkB activity

Modified from (90;91). *contributes to pro-inflammatory response, ^contributes to anti-inflammatory response.

unlike the plasmacytoid DCs in the spleen.[95] Moreover, the mucosal cells express fewer TLRs, but are still as responsive to oligonucleotides with the CpG motif. Similarly, the lymphatic tissue in the mucosa appears to have different characteristics from that in the regional lymph nodes.[96]

Each of these immune and neural components have been implicated in the mechanisms involved in the two major types of gastrointestinal diseases, those with minimal inflammation and a major component of the ENS and CNS (functional bowel disorders including IBS), and those diseases with a major inflammatory infiltrate (IBD).[97] In both extreme examples enteric nerves have been implicated, both sensory and efferent impulses going from neurons to immune cells. Cytokine and chemokine release are thought to play a role, and luminal triggers have also been implicated. Commensal bacteria (*Bifidobacterium infantis* and *Lactobacillus salivarium*) attenuate IL-8 secretion from an intestinal cell line and in addition stimulate IL-10 and TNF-α secretion from DCs, while modulating host responses to flagellin and enteric pathogens.[98] These commensal organisms are among those probiotic strains that have been shown to treat the symptoms of IBS, and to alter cytokine production. A different therapeutic agent, GM-CSF, has been reported to improve Crohn's disease[99] and in mice to expand the DC population and to increase type I interferon production, the effect blocked by a monoclonal antibody against a DC ligand.[100] Reverse translational studies such as these may provide explanations for drugs already in use to treat intestinal disorders.

Beyond gastrointestinal disease

These proposed links between environment, mucosal immune systems, and the nervous system are highly developed in this environment due to the juxtaposition of the microbiome, the mucosal immune system and the extensive ENS, but other mucosal surfaces have just as large a population of DCs to sample the environment, and have their own rich immune system.[101] These tissues include the lung, the nasal mucosa, the vagina, the urethra, and the skin. The ability to modulate the mucosal surface between tolerance and immunity is a function of all of these tissues, and these surfaces are also richly endowed with nerves, albeit from the somatic nervous system not the ENS. Nonetheless, all of the mucosal surfaces send signals to the spinal cord and the brain, as well as receiving input from the nervous system itself, and diseases in which afferent and efferent nervous input appear to modulate mucosal function include asthma (lung),[102] atopic dermatitis (skin),[103] and allergic rhinitis (nasal mucosa).[104] In conditions such as hepatic steatohepatitis, HIV infection, fibrosis, and cancer bacterially-driven inflammation has been linked with disease onset and progression.[105-108]

Over the last five years, molecular geneticists have started to map the human microbiome.[109] The majority of these bacteria are obligate anerobes and unculturable. With the advent of large scale microbiomics this biology is beginning to become unravelled,[7,8] and with this, a greater understanding of the role of the microbiota in health and disease is emerging.

These microbiomic studies are beginning to open new windows in our understanding of diseases as diverse as obesity,[27] allergy,[26] autoimmunity,[25] and even diseases of the CNS.[24,110] Furthermore, studies conducted with germ-free animals have added weight to the school of thought that human disease prevalence is changing as our environment is becoming increasingly sterile and our microbiota are presumably responding and evolving accordingly (the so-called 'hygiene hypothesis').[111]

Opportunities for novel therapies

Epithelial biology

One important aspect of epithelial biology research is currently focused on enhancing epithelial barrier function. Examples of such approaches include targets that increase tight junctions[112,113] or enhance defensin secretion.[114] Defects in autophagy may be associated with the lack of mucosal tolerance to luminal bacteria.[41]

Innate immune cells

Defects in macrophage and/or neutrophil function may predispose to colitis, and agents that boost innate immunity (e.g. GM-CSF and probiotics) may have clinical utility.[60] Disruption of tolerance towards the resident microbiota is thought to be a major mechanism involved in the development of GI disease. Dendritic cell (DC)-T cell interactions play a central role in regulating immune responses and in inducing tolerance. Examples of such programs include immune cell therapy (e.g. Tregs, DC priming) or turbo-probiotics aimed at IL-10 delivery to gut mucosa (see below). IL-35, a novel regulatory cytokine derived from innate immune cells and related to IL-12/IL-23, has been identified and can stimulate the production of Foxp3+ regulatory T cells and suppress Th17 cell development.[115]

Neuroimmune mechanisms

Vagal-immune interactions and mast cell-neuronal interactions feature prominently in current GI research efforts and may lead to novel therapeutic opportunities. For example, the vagus mediates important anti-inflammatory effects, and nicotinic receptors perform a critical role in nicotine-mediated reduction of NF-kB activity and cytokine production in macrophages.[116]

Microbial-host interactions

The Human Microbiome Project (HMP) is one of only four currently funded NIH roadmap 1.5 initiatives (http://nihroadmap.nih.gov/roadmap15update.asp). One of the key long-term goals of the HMP will be the isolation and characterization of molecules secreted by the gut microflora that have influence on both the microbial environment and host physiology. These molecules are now beginning to be identified[117] and have been shown to have profound effects on gut physiology. Future work to elucidate pathways crucial to the bacteria's ability to survive in and potentially influence the functionality of the human GI tract[118] will have a major influence on the therapeutic opportunities that present themselves.

Biopharmaceutical approaches

The concept of inducing oral tolerance (i.e. use of an orally-administered agent to induce immune tolerance) whilst having only limited success with administration of auto-antigens is now being viewed in a new light. The chronic inflammatory 'tolerant' state of the gut might be altered either from the lumen with probiotics or by altering the immune response directly. Two examples of the latter approach have utilized oral delivery of anti-CD3 antibodies in rodent models of multiple sclerosis and type-1 diabetes.[119,120] The efficacy observed with this treatment is postulated to occur via induction of tolerogenic T-cell subsets within the GI mucosa which can then result in widespread systemic effects and the suppression of autoimmune disease. If such studies translate to human disease, they may lead to treatment options for many immune-mediated conditions. With improvements in adjuvant and delivery technologies,[121] the possibility of developing mucosal vaccines capable of targeting dysbiosis generates a range of attractive opportunities. For example, a current high profile area of unmet medical need is nosocomial opportunistic infections, in particular antibiotic-associated diarrhea and colitis resulting from *C.difficile* overgrowth. Orally administered vaccines to *C.difficile* and other similar pathogens such as MRSA and *Shigella* would also constitute an area of high unmet medical need.

Another approach that is likely to gain increasing prominence in the treatment of a range of GI (and perhaps other) disease is the use of genetically-manipulated commensal bacteria – or "turbo-probiotics" – to deliver

biological therapeutics. This technique has now been carried out successfully by a number of groups, most notably the biotechnology company Actogenix (www.actogenix.com) who, in addition to a number of pre-clinical studies, have carried out a successful Phase I trial in Crohn's disease with a strain of *Lactococcus lactis* genetically engineered to secrete the anti-inflammatory cytokine IL-10[122] or trefoil factor. These technologies have wide-ranging potential for many GI indications and could be combined with techniques such as recombinant antibodies or RNAi. In the latter case, 'trans-kingdom siRNA' approaches have recently been used successfully to induce significant gene silencing both in the intestinal epithelium and in human colon cancer xenografts.[123] This has resulted in the formation of Cequent Pharma, an early-stage biopharmaceutical company with a therapeutic focus on IBD and colon cancer (www.cequentpharma.com).

Another approach worthy of mention is that of stem cells in the treatment of GI disease. Here, two main areas of interest can be identified: (1) bone-marrow derived stem cell therapy; (2) pharmacological manipulation of intestinal epithelial stem cell development and differentiation. Anecdotal evidence from a total of 33 IBD patients who have received bone marrow transplants suggests that such therapy is efficacious for at least a proportion of Crohn's disease patients.[124] Osiris Therapeutics (www.osiristx.com) recently completed a clinical efficacy trial with their proprietary preparation of donor-derived mesenchymal stem cells (termed Prochymal) for Crohn's disease amongst other indications. Despite the fact that the exact mechanism of action of such therapeutics is not yet known, it is possibly related to a reprogramming of the host's immune system so that it is less likely to respond to commensal bacteria. GI epithelial stem cells are generally accepted to be not amenable to transplantation. The mechanisms that drive these processes are now beginning to be elucidated, as are the factors that determine epithelial stem cell fate[125] and advances in this area will doubtless yield pharmacological targets for therapeutic intervention in epithelial barrier fortification.

Outlook

The studies reviewed above cover a wide range of mechanisms at the mucosal/luminal interface, involving multiple microorganisms and multiple sampling cells (epithelia, dendritic cells, neurons, enteroendocrine cells, Paneth cells). Surveillance at this interface is capable of generating signals that involve modulation of the innate immune system, and if organisms and/or their secreted products cross the mucosal barrier to enter the lamina propria or submucosa, elements of the adaptive immune system, neurones, vasculature, or other mucosal components might become involved. For example, surveillance might occur by recognition of specific microorganisms, either commensals or pathogens, by dendritic cells or by production of differential cytokine or defensin profiles. Moreover, the ability of the microbiome to produce unique but changing metabolic profiles represents a great opportunity for novel therapeutics, but much greater understanding of host-microbial interactions is needed to achieve rationale approaches. The optimistic side of this abundance of riches is that with so much activity moving forward on microbial-host interactions, new targets seem likely to emerge from understanding the effect of modulating the largest neuro-immune organ in the body.

Search strategy and selection criteria

To assess emerging areas of biology in the GI therapeutic area, we aimed to identify those areas that we believed will (1) change the way we think about disease pathogenesis and (2) have the widest possible application across the spectrum of GI disease. The first approach we used was to employ literature based searching which was applied across the entire PubMed database and then limited to the top three most cited biology or GI journals. The second approach identified the most heavily cited papers in the GI field over the last five years, and the third approach identified mechanisms relevant to GI disease understanding that have driven (or led to) an increase in publications over and above that which could have been predicted. These analyses clearly identified the areas of microbial-host interactions, mucosal immunology, gut-brain-axis as key areas of emerging GI biology.

Based on these initial analyses we undertook a detailed evaluation of peer-reviewed publications in the NCBI PubMed website for English language publications with key words: microbial-host interactions, mucosal immunology, gut-brain-axis. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. We also used publications that we were aware of due to our association with GI therapy and research over the past 20 years. Review articles are cited to provide readers with more details and more references than is within the scope of this review.

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