

IBD 2009: Emerging Research Frontiers on the Path to a Cure

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The Crohn's and Colitis Foundation of Canada (CCFC) hosted a research symposium in April 2009. The current article presents short synopses of the presentations given at that symposium. Invitees included CCFC-funded clinician-scientists and researchers, research administrators and international leaders in inflammatory bowel disease research. Research challenges were outlined while acknowledging advances made in several domains relevant to informing the search for cures. Following the scientific presentations, discussions endorsed current activities of the CCFC and supported the creation of a new pediatric inflammatory bowel disease initiative.

Key Words: *Environmental exposure; Genetic risk; Inflammatory bowel disease; Microbiome; Mucosal immune responses; Pathogenesis*

The symposium titled 'IBD 2009: Emerging Research Frontiers on the Path to a Cure' provided an exciting showcase for new opportunities, ideas, research and investigators addressing the mission of the Crohn's and Colitis Foundation of Canada (CCFC) to find cures for inflammatory bowel disease (IBD). The title conjures an image of research frontiers arising from current knowledge and eventually converging on the final path to a cure. In reality, this image translates into identifying important and focused research goals and key initiatives in which to invest. However, when considered broadly, the most important scientific research challenges and themes in IBD include the following:

- Determining the functional significance of genetic associations in gastrointestinal diseases;
- Determining the interaction between environmental exposures and genetic risk in the pathogenesis of gastrointestinal disorders;
- Defining the neural-gut interactions in health and disease;
- Learning how to induce mucosal immune tolerance;
- Developing means of enhancing intestinal repair mechanisms;
- Assessing the role of the microbiome in gastrointestinal health and disease;
- Finding pathogenic microbes in gastrointestinal disorders;
- Developing safe and effective means of altering the mucosal immune response in disease states; and

Les MII 2009 : Les obstacles émergents de la recherche sur la voie d'un traitement curatif

La Fondation canadienne des maladies inflammatoires de l'intestin (FCMII) a organisé un symposium de recherche en avril 2009. Le présent article présente des résumés des présentations qui y ont été données. Les invités étaient des scientifiques-cliniciens et des chercheurs financés par la FCMII, des administrateurs de recherche et des chefs de file internationaux dans le secteur de la recherche sur les maladies inflammatoires de l'intestin. On a fait ressortir les défis de la recherche tout en reconnaissant les progrès réalisés dans plusieurs domaines afin de documenter la quête de traitements. Après les présentations scientifiques, des exposés ont sanctionné les activités en cours de la FCMII et appuyé la création d'un nouveau projet sur les maladies inflammatoires de l'intestin en pédiatrie.

- Translating advances in clinical research into health benefits for individuals with gastrointestinal disorders.

The present overview summarizes a selection of the symposium presentations that were designed to stimulate thought and discussion regarding some of the themes and challenges discussed above. As such, the current article provides a review of the existing state of knowledge and research in selected areas of IBD in which the largest advances toward cures are likely to arise.

IBD AND THE MICROBIOME

Understanding the microbiome

Advances in genome sequencing technologies and bioinformatics have created a new field of research – metagenomics – that enables comprehensive analysis of entire microbial communities, even those that cannot be cultured. The Human Microbiome Project (HMP) and the associated Canadian Microbiome Initiative are examining the composition of human microbial communities. Genetic and environmental factors such as geography, diet, age and lifestyle, influence an individual's microbiota. Although more than 500 bacterial species are represented in the human microbiome, most belong to only three phyla: *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. Associations have already been made between colonizing microbes and some chronic diseases (7). It is anticipated that this tremendous undertaking will clarify the relationship between innate microbial communities and human health and disease.

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The intestinal microbial population appears shortly after birth and influences the development and maturation of the host immune system (11). Only the thin intestinal barrier of enterocytes and mucus, and the active mucosal immune system prevent intestinal organisms from invading the body. If barrier permeability increases, organisms can penetrate the intestinal barrier and cause severe tissue damage and illnesses such as IBD. Illness may be accompanied by microbial population alterations. Significant differences are known to exist between microflora in healthy individuals and those with IBD (14).

The microbiome and colitis

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A variety of mouse models are available to study the intestinal microbiota in infectious colitis. *Citrobacter rodentium* causes an extracellular colitis in mouse models. After infection, the number of bacteria in the intestinal tract decrease and the microbial phyla distribution shifts from primarily *Firmicutes* and *Bacteroidetes* to *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. Resolution of infection is accompanied by a return to the normal population equilibrium (3).

Colitis induced by dextran sulphate sodium produces changes in the microbiota similar to those produced by *C rodentium* infection (3). Inducible nitric oxide synthase is important to the inflammatory response, and mice that do not produce it have a hypoinflammatory response during dextran sulphate sodium-induced colitis. This reduced inflammatory response is associated with a smaller shift in the intestinal microbial population. In contrast, interleukin (IL)-10 is an anti-inflammatory cytokine. Mice that do not produce IL-10 spontaneously develop colitis. The microbiota of these hyperinflammatory mice shifts the same way as it does in infection.

Treatment with streptomycin, tetracycline or vancomycin also shifts the colonic microbiota composition. The specific shifts produced vary with the antibiotic used. Streptomycin or vancomycin pretreatment promotes intestinal colonization with *Salmonella typhimurium* and development of infectious colitis. *S Typhimurium* infection reduces total intestinal bacteria, increases the proportion of proteobacteria and promotes intestinal inflammation, with an increase in the inflammatory cytokines tumour necrosis factor-alpha and monocyte chemotactic protein-1. The ability to manipulate intestinal microbial populations provides an important toolkit to study inflammation.

The environment, the microbiome and IBD

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The gastrointestinal tract can be regarded as a diverse collection of bacterial ecosystems that changes both longitudinally and across the gut. The oral cavity hosts approximately 200 species, while the colon hosts approximately 400 to 500 species. A cross-section of the gut would reveal nonadherent luminal populations and different populations in the mucus overlying the epithelium, in the deep mucus layer in the crypts and on the epithelium itself. The microbiota shifts over the first years of life until the relatively stable adult population develops.

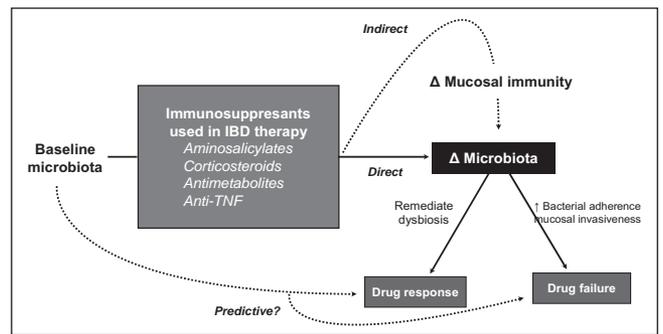


Figure 1) Potential interaction between anti-inflammatory and immunosuppressant drugs used in inflammatory bowel disease (IBD), change (Δ) in gut microbiota and treatment response. TNF- α Tumour necrosis factor-alpha

The gut microbiota occupies the interface between the host and the environment. Environmental stressors such as diet, smoking, infections, stress, antibiotics and appendectomy, may affect the stability of the gut microbiota. Of these factors, antibiotics, infection, smoking and appendectomy have been implicated in the development of IBD (8,48) (Figure 1).

Because intestinal bacteria may be important mediators of environmental influences, it is important to study the host, the function and composition of the host microbiota, and the influence of environmental factors in both health and disease conditions such as IBD. As such, it is important to determine whether commonly used IBD drugs affect the gut microbiota. Such bacteriological effects could play a role in modulating the therapeutic response to some of these agents. It is also possible that baseline microbiota composition predicts response to therapeutic interventions.

Probiotics, diet and the microbiome

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The intestinal microbiome has an important effect on metabolism. In germ-free mice, colonization of the gut produces weight gain. Microbial fermentation of dietary polysaccharides increases the amount of monosaccharides available for absorption, and microbial regulation of host genes promotes lipid deposition in adipocytes (9). In fact, major metabolic processes are linked to microbial activity in the gut. Prebiotics – nondigestible oligosaccharides that stimulate probiotic growth – and probiotics substantially modulate host lipid, carbohydrate and amino acid metabolism, which may affect glucose metabolism, insulin sensitivity, antioxidant function and steroidogenesis (10).

Probiotics and resident microflora interact in various ways that are likely strain specific and concentration dependent; however, most of these effects remain unknown. Microbe-microbe and microbe-host interactions significantly alter both microbe and host gene expression. The efficacy of probiotics may depend on the host genotype, interactions with other gut microflora and the use of immunomodulatory strains. The benefit of probiotics may be mediated through resident microflora through direct signalling to the host or through a combination of these effects. Evidence supporting the probiotic benefit in ulcerative colitis is accumulating, but the picture remains far less clear in Crohn's disease (12).

Characterizing the microbiome: Approaches and technology

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Studying the gut involves the microbiome, the human genome and the intimate interaction between the two. In Crohn's disease, the diversity of gut bacteria declines (13). Such declines are considered to be an indicator of disrupted homeostasis. *Firmicutes*, *Bacteroidetes*, bifidobacteria and lactobacilli numbers decrease, and *Faecalibacterium prausnitzii* – an anti-inflammatory commensal organism – disappears. Total and facultative bacteria counts increase, and various bacteria associated with granulomas invade the crypts and mucosa. *Proteobacteria* and adherent-invasive *Escherichia coli* increase. Similar changes are seen in ulcerative colitis in which the diversity of bacteria also decreases, with declines in *Firmicutes*, *Bacteroidetes* and lactobacilli (14). Total bacteria increase, with invasion of crypts. *Proteobacteria* – not necessarily *E coli* – increase.

The microbiologist attempts to determine the specific organisms present, their numbers, and their function and impact on the local environment. These tasks have proven to be challenging. Traditional methods of studying microorganisms rely primarily on direct microscopy and culture. Only 5% to 10% of the organisms in the gut can be cultured using standard techniques.

New microbial ecology techniques began developing during the second half of the 20th century. Acceleration of the development of sequencing technology (such as pyrosequencing) with the human genome project produced new techniques that could be used to study microorganisms. Fast and economical, pyrosequencing provides accuracy to the bacterial genus and good phylogenetic resolution, facilitating the study of bacterial genomes and entire bacterial ecosystems. Metagenomics can demonstrate changes in ecosystem and subsystem function over time, including indicators of pathogen invasiveness. These techniques, which can be used in conjunction with traditional methods, may help to elucidate the relationship between the microbiome changes in IBD and the disease process.

RODENT MODELS OF COLITIS

Understanding mouse models

Studies with experimental animal models enable the examination of potential pathophysiological mechanisms at a molecular level and the identification of specific functional defects. Human genetic studies and research with mouse models have contributed significantly to the understanding of gut immunoregulation and generated a hypothesis of IBD pathophysiology. This hypothesis states that loss of tolerance and an excessive immune response directed against the intestinal microbiota is an important trigger of chronic bowel inflammation and tissue destruction.

Many transgenic and gene-targeted mouse strains displaying altered intestinal immunological or functional properties have now been developed. These strains can be classified according to their mucosal immunity defects, which are related to epithelial integrity or permeability, the innate immune response and the adaptive immune response. These mouse strains can be developed by using gene targeting to modify chromosomes in embryonic stem cells. 'Knock-in' mice are created by replacing an endogenous DNA sequence with the desired sequence

whose gene products are to be studied. 'Knock-out' mice are created by deleting a specific DNA sequence from embryonic stem cells, allowing the impact of the deletion in a specific disease to be studied. The modified stem cells can then be used to generate mice that transmit the new trait to their offspring.

In the healthy gut, intestinal commensal organisms do not stimulate a strong inflammatory response due to the induction of immune tolerance. In IBD, however, defects in the intestinal barrier or local immunity produce chronic inflammation that is not self limiting. Evidence now indicates that a T-helper cell (Th) 2 immune response associated with epithelial barrier dysfunction may be characteristic of chronic inflammation in ulcerative colitis, whereas an interleukin-23-dependent, pro-inflammatory Th17 immune response may be involved in Crohn's disease. Advances in understanding the molecular basis of the gut immune system and the changes that occur in IBD make the development of novel biological therapies possible.

Animal models of IBD: Lessons learned

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More than 30 mouse models have now been developed to study IBD. In all of these models, mucosal CD4-positive (CD4⁺) T cells are the effector cells and the bacterial flora drive the disease process. Gene-targeted mice have provided major insights into the pathogenic mechanisms involved in IBD and the role of innate immunity. Innate immune cells direct and shape adaptive immune responses. Multiple IBD susceptibility genes affect innate immunity, and others affect adaptive and regulatory cells and mechanisms. For a given mutation, modifier genes affect IBD severity. Combinations of genes appear to be required for disease. Pathogenic CD4⁺ T cells are the major effector cells responsible for IBD. Among these, Th 17 cells seem to play an important role in progression.

However, many important questions remain. The contribution of genetic variants to IBD pathogenesis, the specific bacterial antigens or strains that stimulate pathogenic T cell responses, and the role of intracellular infection in Crohn's disease are all currently unknown. It is also important to determine whether immune regulation is defective in IBD, and whether fluctuation in regulation accounts for relapses and remission of disease. The immunological basis of segmental inflammation and the role of microbial localization need to be established. We also need to understand how defective innate immunity generates an excessive pathogenic CD4⁺ T cell response and whether the microbiota induce regulatory T cells. The pathogenesis of ulcerative colitis may involve different innate and adaptive immune mechanisms.

The types of animal models needed to help answer these questions may differ from those that proved useful in the past, and may include experimental models with an intact immune system, knock-in models with human disease genes inserted and knock-out models.

Exploring colitis mechanisms using infectious organisms

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Not only do bacteria contribute to inflammation in IBD, they are also associated with tissue repair. Commensal bacteria

activate mucosal protection and repair processes through toll-like receptors (TLRs), a series of microbial pattern recognition receptors found throughout the gastrointestinal tract. TLRs initiate innate immune responses via signal transduction by myeloid differentiation factor 88 (MyD88).

Innate signalling has both inflammatory and tissue protective roles. The ability to separate innate pathways causing inflammation from those promoting mucosal homeostasis and modulating their function could reduce inflammation and optimize tissue repair in IBD. Inadequate mucosal repair leads to crypt loss and ulceration, whereas exaggerated tissue repair produces fibrosis and strictures.

The *Citrobacter rodentium* infectious colitis model has been used to study innate signalling. Comparison of the effects of *C rodentium* infection in wild type and MyD88^{-/-} (knock-out) mice demonstrated that MyD88 signalling protected against mucosal injury in infectious colitis, with expression of tissue repair factors, such as interleukin-6, stimulation of epithelial cell proliferation and maintenance of the epithelial barrier. *C rodentium* infection in different TLR-deficient (TLR2^{-/-} and TLR4^{-/-}) mouse strains demonstrated that TLR2 promoted barrier function in an interleukin-6-dependent manner, and maintained mucosal homeostasis and repair. In contrast, TLR4 promoted inflammation and did not protect the host from infection. *Salmonella typhimurium* infection in MyD88^{-/-} mice demonstrated that fibrosis was also regulated by MyD88 signalling because these mice had reduced collagen deposition and smaller numbers of fibroblasts. These models enable investigators to distinguish innate pathways triggering inflammation from those promoting mucosal repair and those inducing fibrosis.

Creating opportunities with murine genetic engineering

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Complex interactions between the epithelium and different immune cell populations control the inflammatory response in the gut. These cells secrete a variety of mediators with multiple effects. Modification of one factor produces a cascade of molecular changes. Genetic engineering in mice provides an opportunity to understand the molecular pathology of IBD, especially when the focus is a specific cell type.

Constitutive knockout mice harbour the genetic change in all cells and throughout development. This classical gene knockout approach has important limitations including embryonic and neonatal lethality and nonspecific tissue or organ targeting. Site-specific recombination systems, such as the Cre/loxP system, can create tissue-specific gene knockouts that could not otherwise be produced. Cre recombinase (Cre) is an enzymic tool used to modify genes and chromosomes. Creation of conditional knockout mice uses other genetic strategies that allow genetic changes to be induced at different stages of development or in selected cell types. For example, the tamoxifen-activated CreER gene targeting system can be used to develop a range of transgenic mice in which tamoxifen injection induces the cell-specific change under investigation.

Hepatocyte nuclear factor 4-alpha is a transcription factor involved in many hepatic processes. It is also downregulated in ulcerative colitis. Genetic engineering has created mice

deficient in hepatocyte nuclear factor 4-alpha only in intestinal cells. These animals have a phenotype similar to those seen in patients with IBD, demonstrating chronic inflammation.

Genetic engineering of mice makes it possible to investigate specific cells involved in inflammation and the development of IBD, and the molecular mechanisms involved in the control of intestinal inflammation.

Using germ-free facilities: Science and opportunities

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Gnotobiotic research – in which only known and precisely controlled organisms are introduced – and germ-free research provide a unique opportunity to model components of the interaction between the microflora and host immunity.

It is possible to investigate the ways in which the intestinal microflora shape the development of the immune system to elucidate immune homeostatic mechanisms, and to study how the immune system shapes the intestinal microflora. Gene-targeted mouse models can be used to study various facets of IBD under controlled gnotobiotic conditions. Gnotobiotic research enables the selective manipulation of the intestinal flora, which may determine whether dysbiosis – an imbalance of bacteria – is a cause or a result of intestinal inflammation.

Intestinal commensal bacteria strongly influence mucosal and systemic immunity. Germ-free mice have fewer, less-developed Peyer's patches; smaller, less cellular mesenteric lymph nodes, germinal centres and lymphoid follicles; fewer lamina propria CD4⁺; fewer intestinal intraepithelial CD8⁺ lymphocytes; and reduced expression by Paneth cells and intestinal epithelial cells of factors involved in the maintenance of the epithelial barrier.

NEW INVESTIGATOR SHOWCASE

Adherens junctions in IBD

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The epithelial apical junctional complex comprises tight and adherens junctions, and plays a role in regulating cell structure and function. Adherens junctions are part of the intestinal barrier, and composed of E-cadherins, transmembrane adhesion proteins involved in epithelial cell adhesion and movement along the crypt-villous axis (Figure 2). Extracellular domains of E-cadherin molecules on adjacent cells form homodimers, whereas intracellular tails bind to anchor proteins, including alpha-, beta- and gamma-catenin, which bind actin filaments within the cells.

In ulcerative colitis, increasing evidence points to a primary barrier defence defect involving apical junctional complex proteins. The protein tyrosine phosphatase receptor S (PTPRS) gene encodes protein tyrosine phosphatase-sigma (PTPσ), which uses E-cadherin and beta-catenin as substrates. Studies of the human PTPRS gene have identified three single nucleotide polymorphisms (SNPs) located in an area of PTPσ regulated by splicing. SNPs associated with the pathogenesis of ulcerative colitis resulted in alternating splicing.

Crohn's disease is linked to intestinal epithelial permeability defects that are not explained by known IBD susceptibility

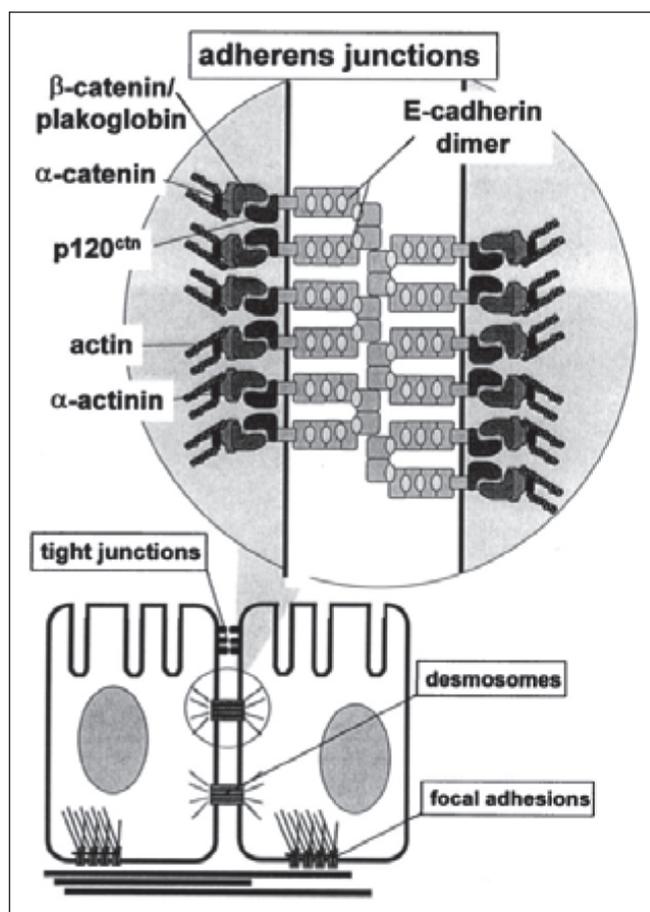


Figure 2) Structure of intestinal epithelial adherens junction. *ctn* Catenin

genes. E-cadherin plays a vital role in maintaining the integrity of the intestinal barrier. Investigation of SNPs in the *CDH1* gene, which codes for E-cadherin, demonstrated that individuals with Crohn's disease had increased cytoplasmic accumulation of E-cadherin and beta-catenin, which may explain the increased permeability seen in some patients with Crohn's disease.

Polymorphisms in barrier defence genes, in association with polymorphisms in other IBD susceptibility genes, may lead to the development of IBD. Genes grouped in biological pathways may be more important than isolated single genes.

IL-11: Anti-apoptotic signalling and healing in intestinal epithelium

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The treatment of IBD today focuses on reducing inflammation and promoting healing to arrest the disease process. The ideal management approach would be to intervene at a preclinical stage to prevent development of clinical disease.

IL-11 is a cytoprotective cytokine with epithelial-specific effects. Although these effects are largely undefined, IL-11 protects intestinal epithelial cells in models of IBD, acute graft-versus-host disease, intestinal ischemia, colitis caused by *Clostridium difficile* infection and during chemoradioablation. IL-11 is produced primarily by subepithelial myofibroblasts and

injury-activated macrophages. Clinically, the full potential of IL-11 has not been explored, although treatment with recombinant IL-11 induces remission in active Crohn's disease more effectively than placebo (30).

Nuclear factor- κ B (NF- κ B), a transcription factor regulating many aspects of cellular activity, is important in intestinal epithelial cell restitution, healing and intestinal barrier homeostasis. NF- κ B essential modulator activates NF- κ B, and the I κ B-kinase (IKK) complex appears to regulate NF- κ B-mediated innate immune responses. Because IL-11 is also involved in epithelial protection, IL-11/NF- κ B signalling in intestinal epithelial cells may modulate pathways promoting resistance to injury and facilitating healing.

A variety of experiments (31) delineated the effects of IL-11 on the NF- κ B pathway and determined that IL-11 stimulated NF- κ B in a nonclassical manner. IL-11 secreted by subepithelial myofibroblasts and recruited macrophages stimulates IKK and NF- κ B, inhibiting Fas ligand-mediated apoptosis in intestinal crypt cells and promoting epithelial healing (36). Intestinal epithelial apoptosis may contribute to the barrier dysfunction characteristic of IBD; IL-11 signalling appears to be a major factor in mucosal protection.

Macrophage phenotype in IBD

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Macrophages are critical in defending against pathogens, in healing and in scavenging debris. Gut macrophages are sequestered and adapted to be anergic or tolerant. Differentiation in the presence of transforming growth factor-beta enhances their ability to produce IL-10, an anti-inflammatory cytokine with an immunoregulatory role in the gut. Immunoregulatory mechanisms in the gut maintain homeostasis by balancing pathogen defence with environmental tolerance. Macrophages respond to environmental cues, producing populations active across the homeostatic continuum comprising host defence, wound healing and immune regulation.

Classically activated 'killer' macrophages are induced by microbial products and function in host defence. In contrast, alternatively activated 'healer' and 'regulatory' macrophages are activated by IL-4. Healer macrophages function in resolving inflammation and healing. Immunosuppressive regulatory macrophages act in immune regulation. Type II activated macrophages are anti-inflammatory. Macrophages can also be deactivated by IL-10 and transforming growth factor-beta. Except for classically activated macrophages, the different macrophage populations produce less IL-12, a proinflammatory cytokine, and more anti-inflammatory IL-10.

Macrophage depletion worsens disease outcome in IL10^{-/-} mice but improves disease outcome in wild-type mice, perhaps indicating the importance of alternatively activated macrophages (41). SH2 domain-containing inositol 5'-phosphatase (SHIP) suppresses the generation of alternatively activated macrophages. Different disease models in SHIP^{-/-} mice demonstrate pulmonary pathology, myelofibrosis, decreased colonic inflammation in IBD, and enhanced gut fibrosis in colitis induced by *Helicobacter hepaticus* infection. These observations highlight the range of macrophage immune functions.

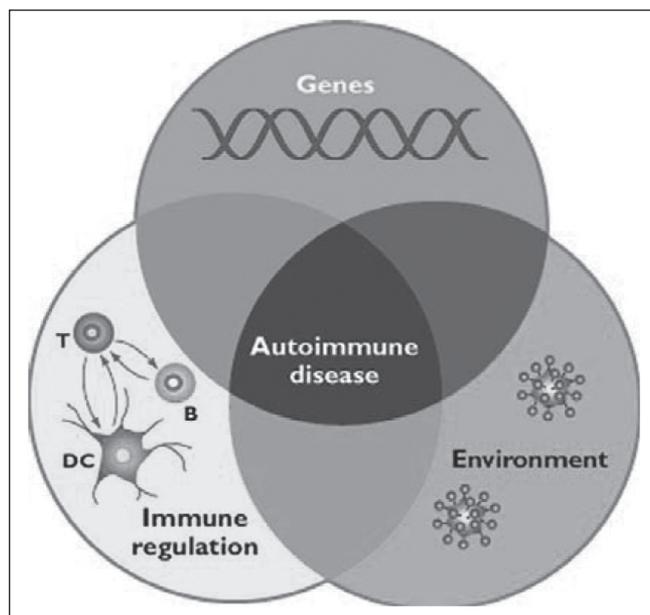


Figure 3) Pathogenesis of inflammatory bowel disease involves interaction between genetic, environmental and immune factors. B B cell; DC Dendritic cell; T T cell

OPPORTUNITIES IN IBD RESEARCH

Crohn's disease: Prospective evaluation of genetic, environmental and microbial factors of disease pathogenesis

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The complex nature of the pathogenesis of IBD makes it difficult to know how or where to look for disease triggers (Figure 3). The heterogeneity of environmental exposures, genetic factors and intestinal microbial flora, and the difficulty in determining whether observed associations are the cause of IBD or simply an effect of the disease or its treatment, complicate retrospective analysis of IBD risk factors. Only a large prospective study can generate an adequate body of data to identify disease triggers.

The Michael J Howarth Genetic, Environmental, and Microbial (GEM) Project is a multidisciplinary human study that will evaluate intestinal barrier function, bacterial flora, immune function, environmental exposures and genetic makeup. The objective of this prospective observational cohort study is to define the keys to triggering IBD.

The GEM Project will identify and enrol participants with an increased risk of developing Crohn's disease, defined as healthy siblings or offspring of individuals with Crohn's disease, and follow them for a minimum of five years. Subjects who develop Crohn's disease will be compared with subjects who do not develop Crohn's disease with respect to demographic data, environmental exposures, dietary patterns and intestinal permeability. Blood and stool samples will be collected and will be available for analysis of genetic, serological and microbial risk factors. Recruitment began in March 2008 and will involve multiple centres across Canada.

Pediatric versus adult IBD: Learning from the differences

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Approximately 25% to 30% of IBD cases are diagnosed in the pediatric population. Early-onset pediatric disease and late-onset adult IBD differ. In Crohn's disease, diffuse colonic involvement with disease extension during the first 10 years after diagnosis is commonly seen in children, whereas localized ileal involvement is more common among adults. Disease extension is less common in adults, but the time to the first surgery is shorter than in children (44). In ulcerative colitis, extensive disease is common in children, with a short time to colectomy, whereas the prevalence of left-sided disease is higher in adults. It is unknown whether these differences are due to the age at which disease manifests or the disease stage seen in patients of different ages.

Early-onset disease may have different or additional genetic determinants or markers, or different gene expression patterns than late-onset disease. Before 2008, no linkage studies or genome-wide association studies had focused on early-onset IBD. To determine differences in gut microbiology between early- and late-onset IBD, it is first necessary to define the gut microbiota in normal children, identify any differences in pediatric IBD and compare the intestinal flora with that seen in adult-onset IBD. Cytokine and immune cell profiles differ in early and late inflammation. Comparison of immune responses in pediatric and adult IBD patients would provide useful information with therapeutic implications.

Determining the overall degree of similarity or difference between early- and late-onset IBD may provide evidence to support or disprove the contention that these conditions are pathophysiologically distinct.

The environment and IBD: Progress and challenges

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Environment plays a fundamental role in the pathogenesis of IBD. Factors associated with IBD include industrialization, urbanization, economic growth, shifts in income and diet, and improved accessible health care. The emergence of ulcerative colitis in a society is followed by an increase in the incidence of Crohn's disease. In high-prevalence areas, the offspring of individuals emigrating from low-prevalence areas have an increased risk of IBD compared with the parental generation. These findings suggest genetics alone cannot explain the pathogenesis of IBD.

Smoking is the strongest environmental risk factor for IBD. There is an increased risk of Crohn's disease among smokers, and an increased risk of ulcerative colitis among nonsmokers and ex-smokers (46,47). However, the vast majority of smokers do not develop Crohn's disease and most Crohn's disease patients are nonsmokers.

Environmental exposures are difficult to study for many reasons. Multiple interactions appear to be involved in the pathogenesis of IBD, different phenotypes exist, and there are methodological challenges in identifying RR as a result of variations in the data collected and in gene distribution.

Without prospectively identified risk factors, disease-specific studies are inherently biased and underpowered.

Environmental exposures cannot be assessed in isolation. It is critical to perform an appropriately designed large trial to study a population-based inception cohort of IBD patients and controls. Patients and controls need to be comprehensively characterized by means of phenotyping, genotyping, serological profiling, microbial fingerprinting and assessment of environmental exposures. Computational systems biology can then assess the effect of gene-environment-microbe interactions and evaluate the mechanisms of these interactions using a variety of experimental methodologies.

Bench-to-bedside research in IBD

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Several bench-to-bedside research opportunities can be identified in IBD. A combination of genetic and environmental studies was used in the Walkerton Health Study, the follow-up investigation of water contamination in Walkerton, Ontario, and is being addressed in the GEM Project. Studying the intestinal microbiota, another opportunity for bench-to-bedside research, is part of the HMP.

Translational research may benefit from the use of the humanized mouse model. Humanized mice are immunodeficient mice that either express human transgenes or can be engrafted with human tissue or cells. These mice facilitate translational research in many areas, including immunity, infection, hematological malignancy and regenerative medicine. This model can be used to study human IBD, including possible use in preclinical drug testing.

The recurrence of Crohn's disease in previously uninvolved intestine following the surgical resection of the involved bowel is another area warranting investigation or representing a research opportunity because the inflammation and the occurrence of complications, such as strictures, develop *de novo* in previously unaffected intestine.

Neural regulation of IBD merits investigation. Emotion, inflammation and IBD morbidity are linked – a fact recognized by most patients. The inflammatory reflex is a neurophysiological mechanism that was described several years previously. Vagal stimulation produces a selective cytokine response that rapidly and predictably inhibits acute inflammation. Impairment of this reflex in depression can result in relapse or disease worsening. Antidepressant therapy can prevent relapse from occurring.

Bedside-to-bench research in IBD

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A variety of bedside-to-bench IBD research opportunities exist in diagnosis, prognosis and therapy. In the area of diagnosis, it is important to be able to measure disease accurately and to identify the different phenotypes. However, the Vienna classification of Crohn's disease yields 24 permutations, and the Montreal modification provides 72 permutations – far too many categories for practicality or clinical utility. Accurate prognostic information is vital to improve clinical management.

Currently available tools, such as calprotectin and serological markers, are inadequate. Reliable and accurate risk stratification models can help determine which patients need aggressive treatment. Generating and analyzing the necessary genetic, biochemical, microbiological and clinical data to develop these models requires a highly integrated bedside-to-bench approach. Despite some dramatic advances, the specific mechanisms of action of the drugs used for IBD therapy are unknown.

The most important research opportunities lie in answering the following questions:

- *How can disease be classified?* A systematic approach to the diagnosis of disease is greatly needed.
- *Why does disease recur?* Endoscopic recurrence following surgery for Crohn's disease is almost universal.
- *Why do tumour necrosis factor- α antagonists fail?* The curves for primary and secondary failure for the three available drugs are very similar.
- *How do drugs work?* The combination of an immunosuppressant and a biologic improves outcomes, but the mechanism is unknown.
- *How are antibodies formed and metabolized?* Decreased drug levels increase antibody formation. It is not known whether immune tolerance can be induced.

Focus, direction and collaboration will be critical in developing these research opportunities fully.

CONCLUSION

The CCFC IBD Research Institute was created in 2002 to address research needs in IBD and includes all those interested in participating in or monitoring IBD research in Canada. More than one-half of the total amount that the CCFC has spent on research since its inception in 1974 has been awarded since 2002. The recently completed CCFC strategic plan includes several priorities to guide the Foundation's activities over the next five years, and the newly established 'Research Report Card' will facilitate more informative tracking of research efforts and outcomes (www.cfc.ca/). Between 2004 and 2008, the CCFC provided \$21.7 million in project-directed research funding: 84% was dedicated to basic and biomedical research, 14% to clinical research and 2% to population research. It is anticipated that the funding provided to clinical and population research will increase, but it is important that this not be at the expense of basic and biomedical research.

Following the presentations at IBD 2009, facilitated discussions of research programs and directions were held. Enthusiastic support for the GEM project and the Grants-in-Aid of Research program was received. A clear research priority that emerged from IBD 2009 was the need for a pediatric IBD research initiative that would do more to bridge basic science, with outcomes-focused advances to benefit patients with IBD. Such an initiative has since been established and it is intended to complement the GEM project. Other areas of research activity that were discussed were not identified as clearly requiring specific emphasis or focus at this time. This lack of clear consensus regarding specific investigative topics suggests that a broad approach to the research enterprise is most likely to provide the significant advances that are still needed in the understanding of IBD.

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