Crohn's and Colitis Foundation of Canada

Fondation canadienne des maladies inflammatoires de l'intestin

2003

RESEARCH REPORT

Recent progress towards the cure

"The CCFC is the largest financial supporter of intestinal disease research in Canada and as a result is a key component in finding the cure for inflammatory bowel disease."

Dr. A. Hillary Steinhart
Vice-Chair, Executive Council
CCFC IBD Research Institute

Inflammatory bowel disease (IBD) affects more than 150,000 Canadian men, women and children. There is no known cause or cure.

The Crohn's and Colitis Foundation of Canada

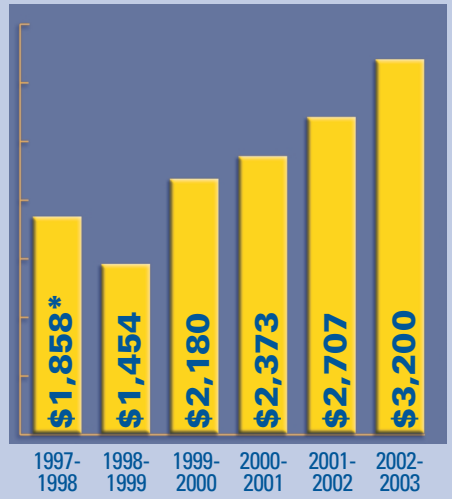
(CCFC) believes medical research is the key to finding a cure for these chronic digestive disorders. We have come to learn more about the fundamental biology of the intestine and IBD, thanks to intensive world-class research being conducted in Canada, much of which is sponsored by the CCFC.

As the leading sponsor of IBD research in Canada, the CCFC has invested close to $32 million in:

- More than 170 **Grants in Aid of Research** in university centres;
- The **Canadian IBD Network Tissue Bank** to facilitate the collection of IBD tissue and clinical data for collaborative research studies;
- The **Canadian IBD Research Institute** to link patients, clinicians and researchers across Canada;
- The **CCFC Group Grant** program to support collaboration among IBD researchers in various universities;
- The **"Innovations in IBD Research"** program to fund novel, less traditional research studies;
- The **CCFC IBD Research Scientist Award** to assist Canadian university-based investigators to conduct IBD research on an ongoing basis;
- **Laboratory equipment grants** to support young investigators establishing a career in IBD research;
- **Scholarships** to 158 summer students to receive training in IBD research;
- The development of the Canadian IBD research community, including the establishment of the first **Canadian Chair in Intestinal Disease Research** and the funding of two world-class **intestinal disease research units** at McMaster University and the University of Calgary.

CCFC Research Investments 1997 - 2003

(Number in thousands)



**Includes $755,000 CCFC IBD Network start-up grant from AstraZeneca.*

The Search for a Cure

This is a very exciting time for IBD research in Canada. Investigations into genetics, microbiology and intestinal physiology are telling us more and more about these diseases. New treatment avenues, such as the use of probiotics, are being explored.

The CCFC is at the forefront of several remarkable initiatives. Projects funded by the Foundation are adding significantly to our knowledge of these diseases and the Canadian scientists we fund are worldwide leaders in IBD research.

In the past year, the CCFC invested over $3 million in various IBD studies. And we're not stopping there! The CCFC is continually expanding its research initiatives, searching for new opportunities to intensify the search for a cure. To that end, the Foundation plans to fund an additional $25 million in research initiatives and training over the next five years.

This significant investment in the future of IBD research marks a new phase for the CCFC's research program, thanks to the generous support of our donors, supporters and volunteers.

The Future of IBD Research

In 2003, the Foundation embarked on an extensive new initiative that will significantly change the face of IBD research in Canada. The newly established **CCFC IBD Research Institute** held its first series of meetings and elected its Executive Council in the summer of 2003.

This "virtual" Institute is a major expansion of our research efforts to find the cure for IBD. Traditionally, the Foundation has invested the majority of its research dollars in project grants – studies of one aspect or another of the diseases' causes and development. The CCFC IBD Research Institute will address the major challenges in IBD research by:

- Attracting the most talented young researchers to the field of IBD research, investing in their research careers, and hosting scientific meetings where they can train with world-class scientists and clinicians.
- Conducting a national study to establish the incidence of IBD throughout Canada and determine the real cost of the diseases in terms of their impact on patients, families and our health care systems. Funding for this study was awarded to leading Canadian epidemiologist Dr. Charles Bernstein of the University of Manitoba in the summer of 2003.
- Bringing IBD to the attention of government and other funding bodies to encourage significant increases in their support of IBD research, which has often been overshadowed by more common and high profile diseases.
- Investing in Canada's first Clinical Trials Consortium, a network of the best clinical trial specialists in Canada who will work together to test new approaches to understanding the causes and treatments of IBD.
- Funding national and international collaborations between multiple research centres to study various approaches to finding the cure for IBD.

Research Grants

As we strive for new and innovative avenues in pursuit of the cure, the Foundation recognizes the continued importance of its traditional funding targets. The Grants in Aid of Research have been the cornerstone of the CCFC's Research Program for close to 30 years and will continue to be so. They provide funding to the most promising Canadian investigations into the causes, development, function and complications of Crohn's disease and ulcerative colitis. Each year, the CCFC carefully invests in a number of projects, the majority of which are conducted and funded over a three-year period. This year, in addition to funding eight new Grants in Aid of Research, the Foundation is increasing the dollar value of its Grants in Aid of Research from $75,000 to $125,000 per year, in recognition of the increasing costs of research and in order to attract researchers who have not previously worked in this field.

We also continued our emphasis on the training of future IBD researchers, funding two new CCFC Finkelstein Clinical Research Fellowships, a CCFC Post Doctoral Fellowship and four Summer Student Scholarships.

The Canadian IBD Network Tissue Bank

Tissue and clinical data from the Canadian IBD Network Tissue Bank continue to be a vital resource for IBD investigators. Several research projects, including some listed below and three large studies of microbes and bacteria in the development of IBD are being conducted with materials collected through the Tissue Bank.

Recent Research Findings

Each year, the CCFC carefully invests in a number of promising research projects. The majority of these projects are conducted and funded over a three-year period. Here is what we have learned from some of the research projects completed in 2003:

Mycobacterium Paratuberculosis and Other Microbes in Crohn's Disease

Mycobacterium paratuberculosis (Mpara) is a bacteria associated with Crohn's disease. *Drs. Charles Bernstein, James Blanchard, Gopi Nayar and David Relman* of the *University of Manitoba* examined whether this and other bacteria are linked to disease development.

Data and serum from IBD patients, siblings and healthy control subjects were studied for antibody responses to the bacterium. An antibody reaction, which suggested a previous infection with one of the bacteria, was considered seropositive. The rate of seropositive results for Mpara was similar for all four groups. A study of serological response to measles, mumps and rubella showed that people with Crohn's disease were less likely to have had previous rubella infection, but there was no significant difference in the seropositive rates for measles or mumps.

A tissue study done showed no relationship between Mpara and Crohn's disease. The investigation however, has led to more questions, which will be the basis of further studies that will advance the understanding of Crohn's and may ultimately lead to a cure.

Role of a Campylobacter jejuni Lipoprotein (JlpA) in Infection of Human Intestine

IBD may be caused by an uncontrolled immune response to microbes or bacteria in the intestine. Campylobacter jejuni (Cj) is a bacteria which can lead to inflammation through the production of a gene product called JlpA. *Dr. V.L. Chan* of the *University of Toronto* investigated the roles of Cj and JlpA and whether Cj infection is associated with IBD.

Surgical tissues from IBD and control patients were examined to determine if it contained the genetic sequence for JlpA or Cj. Six (12.5 per cent) of the 48 IBD samples had the Cj sequence, compared to two (5.1 per cent) of the 39 healthy control samples. While not statistically significant, the results indicate an association between IBD and Cj. The study also showed that JlpA is required for adhesion of Cj to intestinal epithelial cells, and that JlpA and Cj can induce factors which activate genes involved in inflammatory and immune responses.

Understanding the role of Cj could lead to the development of therapies to control not only Cj, but potentially the development and exacerbation of IBD.

Gastrointestinal Water Transport in Health and Disease

Regulating water balance in the body is an important role of the intestinal tract. Alterations in intestinal water transport may play a role in the diarrhea and resulting complications seen in IBD. *Dr. James A. Hardin* of the *University of Calgary* investigated the role of aquaporins (AQPs), a class of membrane protein channels involved in the movement of water.

At least 10 different AQP proteins have been identified and studies suggest these proteins play a role in intestinal fluid transport. Dr. Hardin identified a variety of AQP proteins on the surface of intestinal epithelial cells, suggesting these proteins may form a pathway for the movement of water across the intestine. Further investigation indicated that colonic AQP protein expression was decreased in both an animal model of colitis and human patients

diagnosed with ulcerative colitis, Crohn's colitis or infectious colitis. In human patients, the decrease in AQP expression appeared to correlate with disease activity. In animals, the decrease in colonic AQP expression was associated with a significant decrease in colonic fluid absorption.

The results suggest that AQPs may be involved in the pathophysiology associated with IBD and could lead to the development of therapies to control the diarrhea and water loss associated with IBD.

Reactivation of Gut Inflammation by Bacterial Products

Infection and bacteria may be involved in the relapse or flare up of IBD, but there is little solid evidence to confirm this. Some intestinal bacteria produce superantigens (SAgs) that are potent activators of immune cells called T-lymphocytes. Using a colitis model in which the disease had been chemically induced, *Dr. Derek McKay* of *McMaster University* showed that SAgs could reactivate and exacerbate inflammation.

Delivering the superantigens in a certain way (intra colonic) resulted in low-grade inflammation, suggesting that infection with a species of bacteria that produce superantigens can potentially predispose an individual to further inflammation. During the course of the study, Dr. McKay noted abnormal control mechanisms relating to ion transport and gained evidence suggesting that nitric oxide (derived from nerves) is pro-absorptive (i.e. anti-diarrheal), and therefore beneficial, in the chemically induced model of colitis.

Epigenetic Regulation of the Tumour Necrosis Factor Alpha Gene in Crohn's Disease

Overproduction of the immune molecule tumour necrosis factor alpha (TNFα) seems to play an important role in Crohn's disease. *Drs. Arturas Petronis and Jeffrey P. Baker* of the *University of Toronto* conducted an epigenetic analysis of the gene encoding TNFα. Epigenetics refers to the regulation of the expression of gene activity without alteration of the DNA structure.

Using DNA samples from Crohn's patients and healthy control subjects, researchers used a mapping process to identify specific chemical elements, which encode epigenetic information for TNFα, and evaluate which of such elements are different in Crohn's disease when compared to the normal controls. The results showed very complex epigenetic patterns of TNFα gene. Although the differences between the groups did not achieve statistical significance, the researchers said it would be premature to exclude epigenetic factors in TNFα misregulation due to challenges involved in the specific mapping process used. The traditional epigenetic techniques used allowed for the analysis of only small sections of DNA. Using new microarray–based technology, specifically adapted to epigenetic studies, the researchers are now preparing a large scale epigenetic analysis of genes thought to be involved in Crohn's. If the epigenetic factors of TNFα gene regulation are associated with the disease, development of DNA-modifying therapies will be considered.

Immunopathogenesis of IBD: Role of cytokines

Cytokines are regulatory proteins that control the immune response. *Drs. Ernest Seidman and José Menezes* of the *University of Montreal*, examined whether infectious microbes in the bowel induce the production of the pro-inflammatory cytokines that cause increased inflammation in IBD patients. Using colonic biopsies from IBD and control patients, they studied the cytokines produced in response to bacterial products and discovered the release of pro-inflammatory cytokines (TNAα and IL-1) was much increased in IBD tissue. They then investigated the possible role of viral infections in initiating relapses of Crohn's disease, and found that other key pro-inflammatory cytokines (Il-2Rα and Il-15) are increased in Crohn's disease tissue.

When exposing colonic tissue from Crohn's patients to Herpes virus, they discovered that only dendritic cells were infected - not any of the other immune cells or the surface lining epithelial cells. Dendritic cells are able to recognise and act against invading antigens, which are substances capable of inducing an immune response. When compared to control tissue, alterations in the number, state of activation and distribution of mature dendritic cells were found in Crohn's tissue.

This new information on the modulation of cytokine dysregulation in IBD and of the potential role of dendritic cells – something that has remained largely unknown – provide the opportunity to develop new strategies to discover the cause of IBD and thus new treatments for it.

Mechanisms of Leukocyte Migration into the Inflamed Intestine

The movement of leukocytes (white blood cells which exacerbate inflammation) is characteristic of IBD. These cells, and polymorphonuclear leukocytes (PMN) in particular, are found in patients' stool. To get into the stool, PMN must penetrate blood vessels, cross the underlying tissues and break through the epithelial lining into the intestine.

Dr. Andrew Stadnyk of *Dalhousie University* examined ways of preventing leukocyte movement in order to control inflammation. He has determined that preventing the cells from penetrating the blood vessels is not the optimal way to stop leukocyte migration because there is no way to specifically select PMN for blockage. As a result, all cells are blocked, creating a risk of general immune suppression. A better approach is to block the final stage of leukocyte migration, the movement of cells through the epithelial lining.

The study has increased understanding of leukocyte migration and the ways in which leukocytes attach themselves to other cells in order to cross through the epithelial barrier. This and further research to identify the adhesion molecules could lead to the development of blocking agents.

Role of Prostaglandin D2 in the Progression from Colitis to Colon Cancer

Colitis increases the risk of developing intestinal cancer. Drugs known as non-steroidal anti-inflammatory drugs (NSAIDs) seem to decrease the risk of intestinal cancers in the general population, however they exacerbate intestinal inflammation. *Dr. John L. Wallace* of the *University of Calgary* examined whether prostaglandin molecules, which are inhibited by NSAIDs, play a role in colitis and in increasing the risk of colon cancer.

The tests, conducted in a laboratory model of colitis, indicate a much higher production of prostaglandin D2 in ulcerative colitis which continued long after the disease activity had subsided. Further study showed prostaglandin D2 was an important signal for promoting colon cancer. Finally, reducing the severity of colitis with conventional anti-inflammatory drugs reduced the production of prostaglandin D2 and the incidence and severity of colon cancer.

Future studies will include the testing of experimental drugs that may someday be useful for treating colitis in humans.

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