Genetics and epidemiology may contribute to understanding the pathogenesis of IBD – a new approach is now indicated

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AS PEÑA, JBA CRUSIUS, M OUDERK POOL, et al. **Genetics and epidemiology may contribute to understanding the pathogenesis of IBD – a new approach is now indicated.** *Can J Gastroenterol* 1993;7(2):71-75. The present article summarizes the genetic and epidemiological factors contributing to the pathogenesis of inflammatory bowel disease (IBD) in which a consensus seems to have been reached. The factors identified so far strongly support the involvement of the immune system in disease predisposition to IBD. The models that have resulted from segregation analysis of families with multiple cases of IBD have led to propose a multifactorial inheritance for Crohn's disease and ulcerative colitis – a recessive gene in Crohn's disease and a dominant gene for ulcerative colitis. At present, the lack of genetic markers does not allow the acceptance of any one of these models. Some environmental factors, such as perinatal infections, absence of breastfeeding, smoking in Crohn's disease, influence of transfusion in diminishing relapses in Crohn's disease and drugs that are active in controlling these diseases, may imply the existence of an abnormal immunoregulation in the control of inflammation. New technology is available to approach the problem.

Key Words: Crohn's disease, Epidemiology, Genetics, Human leucocyte antigens, Immunogenetics, Inflammatory bowel disease, Tumour necrosis factor, Ulcerative colitis

Considérations génétiques et épidémiologiques dans la compréhension de la pathogenèse de la maladie intestinale inflammatoire: indications d'une nouvelle approche

RÉSUMÉ: Le présent article résume les facteurs génétiques et épidémiologiques qui contribuent à la pathogenèse de la maladie intestinale inflammatoire au sujet desquels un consensus semble avoir été atteint. Les facteurs identifiés jusqu'à présent appuient nettement le rôle du système immunitaire dans la prédisposition

THERE IS OVERWHELMING EVIDENCE that genetic factors play a role in causing inflammatory bowel disease (IBD) (1-5). The observations supporting hereditary factors are increased familial incidence, higher prevalence of the disease in monozygotic versus dizygotic twins, higher rates in first degree relatives versus spouses and varied frequency found in different ethnic groups (Table 1). Since IBD has a variable age of onset, usually starting after 10 years of age, risks are dependent on the age-specific incidence; these have been calculated for patients in Baltimore, Maryland, USA (6) and for the Ashkenazi Jewish population from Los Angeles, California (7). This Jewish population has a three to four times higher risk of developing IBD than their non-Jewish neighbours. Ashkenazi Jews of middle European origin have an excess risk in relation to Jews of Polish/Russian origin (8,9).

The association of IBD with diseases of known genetic predisposition also supports the genetic predisposition. The diseases associated with IBD (such as ankylosing spondylitis, psoriasis, atopy and eczema, celiac disease, multiple sclerosis, autoimmune hemolytic anemia and primary sclerosing cholangitis) are linked to genes of the major histocompatibility complex (MHC).

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à cette maladie. Les modèles qui ont résulté de l'analyse des familles où se présentaient plusieurs cas de maladie intestinale inflammatoire ont permis de formuler une hypothèse multi-factorielle héréditaire de la maladie de Crohn et de la colite ulcéreuse, soit un gène récessif dans la maladie de Crohn et un gène dominant dans la colite ulcéreuse. À l'heure actuelle, le manque de marqueurs génétiques ne permet pas de confirmer l'un ou l'autre de ces modèles. Certains facteurs environnementaux, comme les infections péri-natales, l'absence d'allaitement maternel, le tabagisme dans la maladie de Crohn, l'influence des transfusions sur la diminution des rechutes de la maladie de Crohn et les médicaments actifs dans la maîtrise de ces maladies, peuvent permettre de supposer l'existence d'une immuno-régulation anormale dans le contrôle de l'inflammation. Une nouvelle technologie est disponible pour aborder le problème.

Some of these diseases are associated with class I antigens. For example, ankylosing spondylitis is associated with human lymphocyte antigen (HLA)-B27 and HLA-Bw60 (10). Recent studies from Germany showed a special type of genetic heterogeneity of patients with both ankylosing spondylitis and Crohn's disease. The phenotype HLA-B27, -B44 was markedly increased when both diseases coincided. Individuals with this phenotype have a relative risk of 68.8 for the concurrent manifestation of Crohn's disease and ankylosing spondylitis (11, 12). Other diseases found in association with IBD are linked to HLA class II antigens. Celiac disease primarily is associated with the HLA-DQA1*0501 and HLA-DQB1*0201 (13,14). These observations suggest that genes at the short arm of chromosome 6 are also of importance in the disease susceptibility to IBD.

Although in some families ulcerative colitis and Crohn's disease coexist, there is strong disease concordance among family members. In the majority of the studies reported so far (1-5), the familial prevalence of Crohn's disease and ulcerative colitis is higher when the proband has Crohn's disease. However, a recent study in Italy (15) found no significant differences; the prevalence of ulcerative colitis in first degree relatives of patients with this disease is seven per 1000 and the prevalence of Crohn's disease among first degree relatives of Crohn's disease patients is five per 1000 (15).

The observations of multiple cases in families of patients with IBD have led to the postulation of different forms

of segregation (3). Several models have resulted from these analyses. Some investigators propose multifactorial inheritance for Crohn's disease and ulcerative colitis, while others have put forward that segregation analysis of data in families of patients with Crohn's disease supports a recessive gene in Crohn's disease and a dominant gene for ulcerative colitis. No genetic markers exist to test the validity of these models. The numbers of pedigrees with multiple cases collected by any centre are not large enough to permit a valid statistical analysis. Drs G Hellers and L Iselius, from the Huddinge University Hospital, Huddinge, Sweden, are collecting pedigrees of families with at least two cases of Crohn's disease or ulcerative colitis to perform complex segregation analysis.

EPIDEMIOLOGICAL STUDIES

The different epidemiological factors which may be relevant in disease susceptibility to Crohn's disease and ulcerative colitis are shown in Table 2 (16-18). There are many studies showing that the relative risk of ulcerative colitis developing in nonsmokers definitely is higher compared with smokers (ex-smokers usually develop their disease after stopping smoking). Epidemiological studies (19-22) consistently support a different association of cigarette smoking with ulcerative colitis and Crohn's disease. It has been suggested that in genetically predisposed individuals, smoking exposure may determine the type of IBD that will develop. A study of identical twins in Sweden showed that the smoking pattern was similar in concordant and dis-

TABLE 1
Observations supporting a genetic predisposition to IBD

- Increased familial aggregations
- Higher concordance rate in identical twins
- If both parents are affected, higher prevalence in children
- Low prevalence in spouses
- Increased prevalence in some ethnic groups
- Association to diseases with known genetic predisposition

TABLE 2
Environmental factors in IBD

- Perinatal infections
- Lack of breast feeding or early weaning
- Higher urban prevalence
- Low socioeconomic status
- Smoking
- Use of oral contraceptives
- Viral and bacterial infections
- Increased incidence over too short a time span

cordant twins; after diagnosis two healthy monozygotic twins had given up smoking during a mean period of 6.5 years without developing ulcerative colitis, implying that the sharing of identical genes and smoking patterns are not enough to develop IBD and indicating that other environmental factors are important (23).

Heavy smoking produces a reduced ratio of T helper-inducer to T suppressor cells (24). There is also some evidence that smoking increases production of colonic mucus in vitro (25). Prytz *et al* (26) have shown that intestinal permeability is decreased as measured by the oral tracer ⁵¹Cr-EDTA in patients who smoke and have ulcerative colitis. This protective effect of smoking seems to be due to nicotine. Srivastava *et al* (27) have shown that 15 or 30 mg of nicotine administered transdermally for four weeks to patients suffering from mild ulcerative colitis produced a significant improvement (10 of 16 patients treated improved histologically). In Crohn's disease, vascular abnormalities are more severe

than in ulcerative colitis (28). The prothrombotic effects of smoking predominate since it is well known that smoking induces endothelial cell damage (29). Smoking could worsen the ischemic process. The thrombogenic vasoconstrictive effects would explain the higher susceptibility to tobacco in women using oral contraceptives (30,31). The risk of developing IBD in oral contraceptive users may be increased for Crohn's disease but not for ulcerative colitis (this issue, however, has not been settled).

The environmental factors identified so far, such as dietary factors, smoking patterns and the use of oral contraceptives, appear to be more important as risk factors for developing Crohn's disease than for ulcerative colitis.

Some of the environmental factors found in epidemiological studies, eg, increased perinatal infections in a cohort of patients with IBD, lack of breastfeeding and influence of blood transfusion (the number of relapses in Crohn's disease diminishes after blood transfusion [32]), may imply the existence of an abnormal immunoregulation in the control of inflammation. The kind of drugs active in controlling these diseases supports this idea.

THE IMMUNE SYSTEM, THE ENVIRONMENT AND IBD

In the interaction between genes and the environment, the ultimate defence mechanisms to eliminate pathogens, infected or malignant cells, is generation of a specific immune response. The immune system of the gastrointestinal tract consists of a complex network which has to distinguish between foreign proteins (the majority of which are beneficial to the organism) and the toxic proteins which have to be eliminated. To deal with these special environmental conditions, the gut has developed a specific way to recognize foreign proteins without mounting an immune response (tolerance) or producing secretory immunoglobulin A, which is not able to activate complement. T cells in the gut do not respond by proliferating but rather by producing a variety of

cytokines. Many genes are involved in the development of specific immune function. A set of closely linked genes at the short arm of chromosome 6 plays a unique role in regulating the immune system: HLA classes I and II are involved in immune recognition; CD4-positive T lymphocytes recognize antigens processed by the antigen presenting cells (eg, macrophages) and these are presented together with Class II molecules, resulting in proliferation and lymphokine production; CD8-positive T lymphocytes recognize peptides presented by class I molecules, resulting in killing of the target cell; and the region between class I and class II genes, the 'central region', encodes at least 20 genes whose macrophage products regulate B cell proliferation and antibody production (33,34). Several characteristics of the genes responsible for regulation of the immune response may explain the epidemiology of IBD. The polymorphism of the HLA system, ie, the occurrence of different alleles in each locus of the MHC, evolves through natural selection. It is believed that certain MHC alleles have provided protection of the species during evolution by protecting against fatal infections during childhood. These MHC alleles are transmitted from generation to generation. Evidence to support this suggestion comes from data obtained in Marek's disease of chickens (35), in Dutch survivors of typhoid and yellow fever epidemics in Surinam (36), and in malaria in west Africa. HLA-Bw53 and DRB1*1302 diminish the risk of severe malaria (37). In the latter, tumour necrosis factor (TNF) may be involved in selection of people with diminished risk to autoimmune disease (38). IBD is less frequent in areas where autoimmune disease is unusual. The immune system at birth is immature; immunity in the baby is partly obtained through breastfeeding, and perinatal infections may therefore play a role in the predisposition to IBD by exerting its effects when the immune system is not fully developed, probably by interfering with the normal mechanisms of tolerance. The immune system also is influenced by emotional factors, per-

TABLE 3

Working hypothesis to understand the interaction between genetic predisposition and environmental factors in IBD

- A specific combination of genes on chromosome 6
- Immune system under stress due to abnormal mucus composition, abnormal permeability
- Defect in control of inflammation, dysregulated cytokine production (other genes may be involved)
- In ulcerative colitis autoimmunity predominates, while in Crohn's disease defect in control of inflammation predominates

TABLE 4

Approach to the study of the genetic predisposition in IBD

Select patient subgroup

Immunological marker

- Neutrophil antibodies (pANCA) in a subset of patients with ulcerative colitis
- Antibodies to Mr 40,000 epithelial cell antigen in ulcerative colitis

Proposed gene markers to be studied

- HLA (class I and II), central region of the HLA system
- Complement haplotypes
- T cell receptor genes

Indirect markers

- Intestinal permeability
- Mucus glycoproteins

HLA Human leukocyte antigen; Mr Molecular weight

haps through neuropeptides and other hormones.

REGULATION OF GENE EXPRESSION

Gene transcription in the cells of the immune system can be induced by viral infections, heat shock and hormones. Many of these external and internal factors lead to the binding of positive regulatory molecules to specific sequences in the enhancer and promoter segments. For example, interferon (IFN)- γ and TNF induce class II expression. The regulatory molecules interact with DNA and activate the RNA polymerase, resulting in augmentation of RNA synthesis (34).

A MOLECULAR STUDY OF GENES FOR TNF IN IBD

TNF α (cachectin) and β (lymphotoxin) are cytokines secreted by activated macrophages, T cells, B cells and natural killer cells (TNF α), and mitogen-stimulated lymphocytes (TNF β) (39,40). TNF α plays a role in the acute-phase response to inflammation and induces an increase of MHC class II expression *in vitro* (41). Human T lymphocytes isolated from the lamina propria of colonic specimens triggered by anti-CD3 produce TNF α and IFN γ , which are able to kill cells from human colonic epithelial cell lines (42). These cytokines, therefore, may be important in the immune reactions observed in patients with IBD.

As mentioned above, the human MHC carries the genes for TNF α and β (43). Polymorphism in the TNF α gene has been detected in the New Zealand White mouse strain and appears to be implicated in the susceptibility of (New Zealand Black x New Zealand White) F1 hybrid mice to lupus nephritis. In humans, HLA-DR2 individuals produce less TNF α than individuals who are HLA-DR3 and HLA-DR4 (44,45).

TNF may contribute to the susceptibility of IBD. In a preliminary study of the bi-allelic TNF β NcoI polymor-

phism in 22 Dutch patients with ulcerative colitis, no major deviation of phenotypes was found when compared with the normal population (unpublished results). Further studies using microsatellite polymorphism will be carried out. TNF alleles in combination with high risk HLA-DR-DQ alleles may contribute to heighten the inflammatory reaction. On the other hand, an insufficient production of TNF may be a risk factor for the development of malignancy. The recent association found between HLA-DR2 and ulcerative colitis and HLA-DQB1 in Crohn's disease may suggest that the presence of different alleles in other genes of the Class II region or the central region at the short arm of chromosome 6 may determine whether a patient will develop ulcerative colitis (46-48).

The research on HLA antigens, complement system, TNF and T cell receptor genes is at present in an exciting phase. With rapidly developing new technology the search for genetic markers in IBD has reached a new dimension. The possibility to study with new technology two unlinked loci will enhance understanding of the way the immune system fails to modulate or wrongly directs the gastrointestinal tract defence system. A combination of

genes within the MHC may predispose the individual to suffer from IBD (Table 3). These are early days in this research and the possibility exists that other (clusters of) genes which regulate production of immunoglobulin, encode TNF-receptor(s), adhesion molecules, integrins and addressins in other parts of the human genome may turn out to be of interest in disease predisposition.

FUTURE STUDIES

A systematic study of the genes which regulate the immune response is indicated. The latest developments in genetics, like the possibility of typing sequence-specific oligonucleotides with the HLA and closely related genes, will undoubtedly help to discover the genes responsible for disease predisposition. However, since clinical features and location of disease at presentation together with the presence of specific complications and evolution of the disease give evidence for the existence of clinical subgroups in patients with IBD, a good integration should exist between the clinicians responsible for managing the patients and the basic scientists in order to select appropriate homogeneous groups (49) (Table 4).

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