Inflammatory bowel disease: definition, epidemiologic, etiologic aspects, and immunogenetic studies

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**DISEASE DEFINITION**

Several causes exist for inflammation of the gut such as infection, toxin, autoimmune reaction, radiation and ischemia. When none of these causes are identified, a group of diseases with unknown etiology remains which are called chronic inflammatory bowel diseases (IBD). The chronic inflammatory bowel diseases include two distinct entities, ulcerative colitis (UC) and Crohn’s disease (CD), although there is a small group of patients with an intermediate form.

UC was first described in 1859 by Walks\(^3\). It is an inflammatory process confined to the colon in all instances, which presents itself clinically with bloody diarrhea, mucus in the stools, abdominal pain, and weight loss. The colonic inflammation is usually superficial, continues diffusely and often begins in the rectum. Microscopic changes show edema and congestion of mucosa, infiltration of lymphocytes, plasma cells and polymorphonuclear granulocytes, crypt abscesses, crypt architecture distortion, and ulceration. When the inflammation is confined to the rectum, the condition is called proctitis. When the inflammation does not extend beyond the descending colon, it is called left-sided colitis. If the inflammation extends proximal from the rectum to at least the hepatic flexure (sometimes defined as beyond the splenic flexure), it is termed pancolitis or total colitis. It is called reverse ileitis or backwash ileitis, when inflammation of the entire colon extends to the terminal ileum as well.

Crohn, Ginsberg and Oppenheimer described ileitis as a pathological and clinical entity different from intestinal tuberculosis in 1932\(^2\). It was named Crohn’s disease after the name of the first author. However, in 1913, Dalziel gave a remarkable accurate description of the disease\(^3\). CD can affect any part of the digestive tract from the mouth to the anus. The terminal ileum is the commonest site for the disease. Lockhart-Mummery and Morson first described the colon localization of CD in 1960\(^4\). CD is clinically presented with abdominal pain, diarrhea and weight loss. Occasionally with an abdominal mass, intestinal obstruction, or fistula. The inflammation is focal, segmental and transmural, often complicated by fissures, fistulas, abscesses, and intestinal obstruction. The macroscopic changes of CD reveal aphthous ulcers and the microscopic features are lymphoid aggregates, chronic inflammatory cell infiltration and epitheloid granulomas.

Terminal ileitis refers to CD limited to the last part of the ileum. It is called regional or segmental enteritis, when the disease involves several segments of the small intestine. Granulomatous colitis refers to the colonic involvement of the disease.

About 10% of the patients with colonic inflammation cannot be classified as either CD or UC. These patients are categorized as “undeterminate colitis”\(^5,6\). Follow-up of these patients has shown that the majority of these patients developed UC\(^6\).

**EPIDEMIOLOGY**

The incidence of IBD varies greatly in different geographic areas of the world\(^7-31\). A high incidence is seen in North-West Europe and North America. It is uncommon in Asia and Africa. The incidence of IBD in western countries is about 2 to 15 per 100000/year for UC and 0.9 to 11.6 per 100000/year for CD. The peak age for UC is 30 years and for CD is 20 years. Women generally have a 20%-30% higher risk than men in developing CD. The reports on sex differences in UC are variable, but there seems to be a tendency to a male preponderance.

Data from Copenhagen from 1962-1987, show that the incidence of UC remained stable at a mean of 8.1/100000, whereas the incidence of CD increased by 6 times, from less than 1/100000 to
A recent epidemiological study of the European collaborative study group on IBD gave 10.4/100000 (95% CI 7.6-13.1) as the incidence for UC and 5.6/100000 (95% CI 2.8-8.3) for CD from the South to the North of Europe[29]. In this study, higher overall incidence rates were found in Northern Europe than in Southern Europe, but the magnitude of the difference was less than expected on the basis of previous studies[7-24]. One explanation is that the results may reflect a recent increase in incidence of IBD in Southern Europe and a stable incidence in the North.

The prevalence of IBD varies widely from 30-200/100000 for UC and 1.2 - 106/100000 for CD[30]. Determining accurate prevalence rates, referring to the number of cases at one moment divided by the total population of the area, creates certain problems. Incidence figures, especially of prospective studies, are therefore more likely to be correct than prevalence figures[31].

In areas with a high incidence of UC, the incidence of CD also appears to be high. The ratio of UC to CD is approximately 2:1. No data are available about the exact incidence of IBD in developing countries. IBD was thought to be a rare disease in Asian countries in the past. However, the number of UC and CD cases has surprisingly increased over the past 10 years[32]. There is a need for epidemiological studies in these areas as to judge the strength of genetic and environmental factors in the pathogenesis of IBD.

**ETIOLOGY**

The etiology of IBD is not yet known but is likely to be multifactorial. The pathogenesis of the disease is largely determined by environmental and immunological factors on a genetically predisposed host.

**Environmental factors**

Since UC and CD were first described, much effect has been made to search for infectious agents. Although several bacteria can induce identical symptoms and pathologic changes reminiscent to those found in UC and CD, bacterial infection has different features with a self-limited course, effectiveness of antibiotic treatment and returning to normal histology after treatment[33]. *Campylobacter jejuni*, *Shigella*, *Salmonella* and pathogenic coli are the most common causes for self-limited colitis[33,34]. Parasites (Amoebae, schistosomiasis) and viruses (e.g. herpes, cytomegalovirus, rotavirus, Norwalk agent and influenza) are also explored as possible causative agents in IBD or as inducing relapses of IBD, but no definite evidence has been provided[35]. Most recently, measles virus has been proposed as a causative agent for CD[36-40]. The connection remains disputed[41-44]. Mycobacterium paratuberculosis has been isolated from the bowel of some patients with CD[45-47] and identified in more than 50% of biopsy specimens, in less than 20% in the healthy control group and in UC[48,49]. But recent studies do not support the hypothesis that Mycobacterium paratuberculosis is involved in the etiology of CD[50,51]. Most antimycobacterial treatment in CD patients has been unsuccessful[52-55].

Other factors, including smoking, use of oral contraceptives, socio-economic status, nutrition and dietary habit, blood transfusion and perinatal infections, have been suggested as risk factors in IBD[56]. However, all factors should be reconfirmed except smoking. Smoking has been considered as the strongest exogenous risk factor for IBD. In this context UC and CD seem to be opposites. Non-smokers are associated with UC and ex-smokers are at even higher risk of developing UC than never-smokers[56,61]. The protective effect of smoking in UC seems to be related to nicotine, but clinical trials have shown variable results[62-64]. In contrast to UC, smoking is associated with the development of CD by thrombogenic and vasculitic effects[65-67], and apparently influences the clinical course and quality of life of the patient[66,67].

**Immunological factors**

Immunology is one of the most actively studied aspects in IBD. Disturbance of immune reaction often has been observed in the patients, such as increased numbers of immune cells in the lamina propria, the demonstration of humoral and cellular immune activation, association with other immune-related diseases, and effective treatment with steroids and immunosuppressive agents. Thus, abnormalities of the intestinal mucosal immune function have been postulated to explain the clinical and histopathological nature of IBD.

The immunological hypothesis proposes that IBD represents an abnormal immune response to a normal stimulus in a genetically susceptible host[68]. This hypothesis was strongly supported by recent experiments on knockout mice in which deletion of immune-related genes has resulted in chronic colitis[69-71]. Alternatively the infections hypothesis proposes that some unidentified pathogens cause IBD and that the immune system is just responding appropriately to these pathogenic stimuli. Even though no such pathogens have been found, this hypothesis is still possible since chronic gastritis has recently been confirmed to be caused by *Helicobacter pylori* infection[72,73]. Moreover, intestinal inflammation develops in mice as a consequence of an abnormal immune response in the presence of a single pathogen, such as *Helicobacter*...
hepaticus or Helicobacter bilis[74,75]. However, in these two hypotheses the immune system will respond to ubiquitous antigens. In CD, the response may be elicited by luminal constituents, whereas in UC, an autoimmune response may be the causative factor[76].

The antigenic challenge to the gastrointestinal immune system is enormous, including pathogenic bacteria, normal resident intestinal flora, bacterial products, toxin, viruses, ingested chemicals, even food and drinks. Thus, the immune system of the gut has evolved at least two different directions. On one hand, it has to provide the host with protective mechanisms against invasion of pathogens across the surface of the mucosa, and on the other hand it has to take up large nutrient substances and tolerate the normal intestinal flora, all of which may potentially be immunogenic. For this purpose, the gut mucosal immune system has developed specialized structures, such as Peyer’s patches and isolated lymphoid follicles, which is termed the gut-associated lymphoid tissue (GALT), lamina propria, intra-epithelial lymphocytes and mesenteric lymph nodes. Intestinal antigens pass through membrane cells (M-cells) and are presented by antigen-presenting cells (APC) in conjunction with the MHC class I or class II antigens in Peyer’s patches. The interaction between the MHC-peptide complex on the APC and T cell receptor (TCR) determines the nature of the T cell responses. Because of an enormous antigen challenge in the intestine, it is apparent that the mucosal immune system is in a constant state of response, mainly down regulating mechanisms of inflammation.

Recently, a defective epithelial barrier in the intestinal mucosa has been postulated as one of the etiologic factors in IBD[76]. The evidence of enhanced mucosal permeability in CD[77-79] and colonic mucin and sulfur compound alteration in UC[80-82] support this hypothesis. However, other[83-86] can not confirm the increased intestinal permeability in CD. A defective epithelial barrier may allow large uptake of antigens and pro-inflammatory molecules, such as luminal bacteria and bacterial products, N-formyl-methionyl-leucyl-phenylalanine (FMLP), and peptidoglycan-polysaccharide polymers, and lipopolysaccharide (LPS), and give more opportunities for antigens to initiate immunological and inflammatory responses.

One of the important immunoregulatory abnormalities in IBD is related to the T cell response[6,87,88]. The intestinal lamina propria contains approximately two thirds of CD4+ T cells and one-third of CD8+ T cells in a proportion similar to the peripheral blood lymphocytes[68]. Activation of T cells by different stimuli shows a rapid increase of the expression of several surface antigens. CD3 is present on all T cells and is used as a marker for the T cell lineage. CD4 is present on those α/β T cells that interact with the human leukocyte antigen (HLA) class II molecules and CD8 is present on α/β T cells that interact with HLA class I molecules[89,90]. Most of the lamina propria T cells have the CD45RO + CD45RA- phenotype characteristics of memory T cells. Functionally, lamina propria lymphocytes are characterized by their reduced proliferative and cytokine responses through the TCR, but increased reactivity through the alternative CD2 activation pathway. When T cells are activated, they express interleukin-2 (IL-2) receptor alpha chains (IL-2Rα or CD25), HLA-DR, CD98 (an activation molecule recognized by the monoclonal antibody 4F2) and the transferrin receptor. They also produce many cytokines, and thus take part in the regulation of B and T cell responses and other immune activities[68,87,91-93].

Histopathology of IBD has shown an increased number of lymphocytes in the lamina propria. Immunohistochemical studies on frozen sections of intestinal tissue or cytofluorometric analysis of isolated intestinal lamina propria mononuclear cells from IBD patients shows no significant changes in the CD4+ and CD8+ T cell subpopulations[94]. There are, however, no obvious defects in these helper and suppressor T cell functions in isolated lamina propria lymphocytes from CD patients on poleweed mitogen stimulation[95]. However, memory T cells expressing CD45RO+ are increased in the intestinal mucosa of IBD patients[68,91,96]. The T-cell population from the peripheral blood or lamina propria of intestinal mucosa in CD patients showed early activation markers: IL-2R, CD98 and the transferrin receptor T9[97,1], and HLA-DR antigens. Some studies have shown that HLA-DR is not increased in lamina propria T cells[69]. One study from Korea describes an increase of IL-2R, the transferrin receptor, CD3 and HLA-DR in lamina propria lymphocytes in UC, whereas CD3, TCRα/β, and TCRα/β was decreased in CD[98]. In another study, higher numbers of T cells expressing IL-2R are found in CD patients, whereas macrophages express IL-2R predominantly in UC patients[99]. These phenomena may be related to different activation patients observed in UC and CD. High concentrations of circulating soluble IL-2R in serum or high expression of IL-2R in mucosa is strongly indicative for an active stage of CD and UC[99-102].

CD4 positive T cells can be divided into two subpopulations, Th1 cells and Th2 cells according to their secreted cytokines. Cytokines are small soluble peptides with molecular weight ranging from 5 to 50kDa, which regulate the function of target cells through binding to specific cell surface receptors. A large number of different cytokines have been
identified and each cytokine has special functions and cell origin. Cytokines act in conjunction and form complex interactions in a cytokine network. It is interesting that CD has selectively activated Th1 cells which produce pro-inflammatory mediators, such as IL-2 and IFN-γ, and UC has selectively activated Th2 lymphocytes which produce anti-inflammatory cytokines, such as IL-4 and IL-10 [76,103]. These observations also suggest that CD and UC have different patterns of abnormal immunity. Recently a Th3 subset of T cells has been reported to exist in GALT which plays a key role in the production of transforming growth factor beta (TGFβ). This cytokine is an active suppression component of oral tolerance, a state of unresponsiveness to immunity, and has downregulatory effects on Th1 cells [104,105]. Activation of human mucosal T cells has been shown to cause tissue injury in organ cultures [106], indicative of the importance of T cell activation in the mucosal lesions for IBD.

Another important immunoregulator abnormality in IBD is related to the imbalance of cytokine regulation [76]. Although cytokines consist of a wide range of structurally distinct peptides, the majority are categorized into three major groups by their overall dominant effects [107]:

1) Pro-inflammatory cytokines, so called Th1 type: TNFα, IL-1, IL-2, IL-6, IL-12 and interferon γ (IFN-γ), lymphotoxin alpha (LTα) or tumor necrosis factor beta (TNFβ) are responsible for cell mediated immune responses. IL-1, IL-6, IL-8, and TNFα are predominantly monocyte or macrophage derived and have preferential pro-inflammatory activities [108].

2) Whereas the Th2 type subset secretes immunomodulatory cytokines IL-1ra, IL-4, IL-5 and IL-10, and IL-13 predominantly synthesized by T cells and responsible for humoral or B cell immunity [108].

3) Growth factor and regulatory cytokines, including the colony-stimulating factors, transforming growth factor beta (TGFβ), epidermal growth factor (EGF) family, insulin like growth factors, fibroblast growth factors, have growth regulation and proliferative effects on different cell types.

An imbalance between positive and negative factors in favor of a proinflammatory response can initiate an inflammatory cascade. One of the most interesting immunoregulatory balances in the IL-1/IL-1ra ratio, the former is a pro-inflammatory cytokine, whereas the latter is an anti-inflammatory cytokine which inhibits the actions of IL-1 by binding to IL-1 receptors without agonistic effects. A decreased IL-1ra/IL-1 ratio has been observed in the mucosa of IBD patients when compared with the ratio in the mucosa from healthy individuals and from other inflammatory disorders [109]. The same study group had reported blockade of endogenous IL-1ra to exacerbate and to prolong inflammation in rabbit immune colitis [100] and deletion of the IL-1ra gone to increase susceptibility to experimental colitis in mice [111]. Holt and colleagues think that a decreased ratio of IL-1ra/IL-1 mRNA is the result of inflammation rather than a unique IBD-related abnormality [112]. However, Kojouharoff et al showed that treatment with anti-IL-1 reagents, anti-TNF monoclonal antibodies, and dexamethasone leads to aggravation of acute colitis in mice induced by 5% sodium dextran sulphate, but with IL-1 activity-inhibiting reagents for chronic colitis failed to show any significant effect, whereas the other two significantly reduced the colitis [113]. This study suggests that TNF but not IL-1 plays a major role in perpetuation of chronic inflammation. Recently, several cytokines, such as IL-4, IL-10, and IL-13 were reported to be capable of inhibiting the pro-inflammatory cytokines [114-116]. Kucharzik et al did show that combinations of IL-10 plus IL-4 and IL-10 puls IL-13, respectively, inhibited IL-1β, and TNFα response of peripheral monocytes stimulated by pokeweed mitogen much more than IL-4, IL-10, or IL-13 alone [117].

The classical anti-colon antibodies are found more common in UC patients than in CD patients, but are of minor diagnostic value [118,119].

Anti-neutrophil cytoplasmic antibodies (ANCA) have been indentified [120]. Perinuclear staining (pANCA) occurs in up to 70% of patients with UC, but in less than 20% of CD patients and in an even lower percentage of healthy controls.

Yang et al showed that pANCA-positive patients have a significantly increased frequency of HLA-DR2 as compared with pANCA negative patients, whereas pANCA-negative patients have a high frequency of HLA-DR4. These authors therefore proposed at least two genetically subclinical markers an HLA-DR2 associated pANCA positive group and an HLA-DR4 associated pANCA negative group [121]. These results could not be confirmed by other studies [122,123] (Bouma, personal communication). Recently, Abad et al reported the IBD associated ANCA is different from vasculitis-associated ANCA [124].

Tropomyosin is an actin-binding cytoskeletal protein localized in the apical cytoplasm and brush border of colonic enterocytes and may possess epitopes cross-reacting with bacterial products [125]. Anti-tropomyosin antibody, a 40kD protein present in epithelial cells of the colon, skin and biliary tract was found in the circulation of more than 55% of UC patients and the cross-reaction may be able to induce complement activation mediated by IgG1 [125-127]. These data suggest that UC is an
autoimmune disorder, but CD is not. However, the notion that UC and CD are autoimmune disease has been supported by very little documentation[76].

The final common pathway of immune activation in IBD is the local influx of lymphocytes, macrophages, and polymorphonuclear neutrophils (PMN) which induce tissue damage, and subsequently results in clinical manifestations of IBD. These cells produce many immune and inflammatory mediators that amplify the inflammatory reaction. Among these are cytokines, complement components, eicosanoids, platelet-activating factor (PAF), leukotrienes, adhesion molecules, neuropeptides, reactive oxygen metabolites (ROMs), and nitric oxide (NO).

ROMs are one of the key mediators in the inflammatory final common pathway[128]. Therefore, removal of large amounts of free oxygen radicals can relieve inflammation[129-131] and probably one of the major working mechanisms of 5-aminosalicyclic acid and sulphasalazine used in the treatment of IBD[132].

The unrestrained activation of the immune response and an imbalance in the immune regulation are characteristics of IBD. This process appears to be a secondary response following the initial unknown stimulation or primary trigger. From the recent development of experimental colitis in animal models with application of targeted deletion of genes (knockout mice) involved in immune processes and transgenic approaches[133-138] we have learned that:

1) The intestinal epithelial and mucosal immune system are necessary for maintaining normal intestinal homeostasis, and damage of the intestinal epithelial barrier and imbalance of mucosal immune regulation will contribute to the development of IBD;

2) In some of these models without normal intestinal flora or even without some challenge by an exogenous external agent, inflammation does not occur. This means that activation of the immune response in the intestinal mucosa needs persistent antigen stimulation. The antigens can be pathogenic bacteria, bacterial products, the normal intestinal flora, and even dietary antigens;

3) Genetic susceptibility of the host is also necessary to develop IBD as an important interaction between environmental factors and genetic predisposition.

As for the immune mechanism of IBD, Sartor proposes that the pathogenesis of IBD progresses through a series of steps: initiating events, perpetuating events, immunoregulatory abnormalities, tissue damage and clinical symptoms[76]. The initiating factors may be infections, toxins, and NSAIDs. These factors can break the intestinal epithelial barrier. Then the inflammatory process may be continued by exposure to large amounts of resident luminal antigens and thereby induce a mucosal immune response and immunoregulatory abnormalities in genetically susceptible host, and finally tissue damage and clinical symptoms of IBD may occur. Although no specific initial pathogen has been found, and large and often conflicting results exist, it is now clear that IBD is a disease of or at least involving the mucosal immune system.

Genetic factors

There is overwhelming evidence that genetic susceptibility plays a role in the development of IBD[139-143]. This is supported by several studies.

Firstly, there is an increased risk for the relatives of IBD patients to develop the disease. The frequency of a positive family history in first degree relatives of IBD is increasing in comparison with the normal population. Familial aggregation is more frequent in CD than in UC, suggesting that CD has a stronger genetic predisposition than UC. However, the data on familial aggregation do not fit a simple Mendelian pattern of inheritance[144,145].

There is a higher concordance rate (which means that both twins suffer from the disease) in monozygotic twins than in dizygotic twins in both CD and UC, but this rate is higher in CD than in UC[146,147]. These twin studies also suggest the importance of genetic factors in the predisposition to IBD and can explain the phenomenon of familial aggregation. Since monozygotic twins share 100% of their genes, the observation that not all monozygotic twins are concordance for the disease, suggests that the penetrance of the IBD genotype is reduced and environmental factors also play a role in the development of IBD. Interestingly, one recent report first documented a case of UC and CD occurring in a monozygotic twin pair[148]. This case confirmed the important genetic contribution to the development of IBD, but also highlighted the role of environmental factors in dictating the IBD phenotype.

Using complex segregation analysis of a large population of IBD patients, two studies have concluded that up to 30% of IBD cases may result from a major gene defect. In CD, a major recessive gene has been suggested and in UC the results are consistent with the involvement of a major dominant gene[144,149].

There is a limited number of reports on the risk of developing IBD in spouses of patients with IBD. These studies suggest that the incidence of IBD in spouses in not increased over the risk of disease in the general population and is dramatically less than in siblings[150-152]. The high rates of IBD in the first-degree relative versus the lack of high rates in spouses[153] also support the importance of the
genetic susceptibility to IBD.

Secondly, a large difference in the incidence and prevalence of IBD is found at different geographic locations and in various ethnic groups. Western countries, especially in North and West Europe and North America, have a higher incidence and prevalence of both UC and CD than the rest of the world\[^{31,143}\]. As for certain ethnic groups, a high incidence of IBD was found in Caucasians and a low incidence in black and Asian people\[^{31,154}\].

The most interesting observation is that the Jewish population has a 2-9 times higher risk of developing IBD than other ethnic groups living in the same geographic area and during different time periods\[^{155-158}\]. Furthermore, Ashkenazi Jews of European origin and American origin have a greater risk of coming down with IBD than the Jews of North African and Asian origin\[^{158-160}\]. The prevalence of IBD is higher in Ashkenazi Jews of middle European origin than in those of Polish or Russian origin\[^{161,162}\]. These ethnic aggregation phenomena can be explained by both genetic and environmental factors, but with such a consistently increased incidence and prevalence of IBD in Jewish people, it is hard to exclude genetic factors.

Thirdly, IBD is often associated with specific genetically determined syndromes, such as Turner’s syndrome, Hermansky-Pudlak syndrome, and glycogen-storage disease type 1b. UC or CD are also often associated with several other immune-related disorders with a clear genetic component but otherwise unknown etiology, such as ankylosing spondylitis, primary sclerosing cholangitis, psoriasis, multiple sclerosis, coeliac disease, and autoimmune thyroid disease\[^{143}\]. Ankylosing spondylitis, primary sclerosing cholangitis, multiple sclerosis and coeliac disease are linked to the genes of the MHC\[^{163}\].

Finally, recent whole genome screening studies using microsatellite markers which survey the entire genome for IBD-related loci in families with multiple IBD patients, have stressed the importance of genetic factors in the development of IBD. These are ongoing studies that have demonstrated a susceptibility locus for CD on chromosome 16\[^{164,165}\]. The locus has been called IBD1. Later, Ohmen et al from the US confirmed this finding and described linkage with this locus only in non-Jewish CD sibpairs and not in Ashkenazi Jewish CD sibpairs\[^{165}\]. Satsangi et al have detected the susceptibility loci for both CD and UC on chromosomes 3, 7 and 12\[^{166}\]. Identification of susceptibility loci for IBD by these powerful technic is just beginning and progress will lead to a substantial understanding of the genetic contribution to the pathogenesis of IBD.

The experiments in transgenic mice and knockout mice to induce the phenotypically similar intestinal and colonic inflammation also prove the contribution of genetic susceptibility and heterogeneity of IBD. Since the pathogenesis of IBD involves immune responses, it is not surprising that a large number of candidate gene studies are focusing on the associations between IBD and immunerelated genes, including MHC genes, cytokine genes, T cell receptor genes, complement factor genes, and genes encoding adhesion molecules, e.g. intercellular adhesion molecule-1 (ICAM-1).

**IMMUNOGENETIC FACTORS**

To identify the genes that predispose to IBD, two methods are now widely used. These include population-associated studies and family-linkage studies. The population-associated studies are based on epidemiologic investigations and designed to compare the frequencies of genetic markers or polymorphic candidate genes between unrelated affected cases and unaffected controls. An association between a disease and a distinct genetic marker may be suggestive for a causal relationship of an associated gene (i.e., susceptibility gene), or may result for linkage disequilibrium, which may indicate the nearby location of the actual disorder gene. Linkage analysis tests the chromosomal location of disease susceptibility genes by identifying polymorphic markers of known location transmitted in families with multiple affected members. Linkage studies examine whether a certain allele of a genetic marker locus is transmitted within a family with the disease of interest. Existence of linkage between a given marker and the disease indicates that this marker is located in close physical proximity to that gene. Both the methods have been used to detect genetic markers in IBD.

**HLA genes**

The major Histocompatibility Complex (MHC) in humans called the Human Leukocyte Antigen (HLA) complex, is located on the short arm of chromosome 6, which occupies a large segment of DNA extending about 3500 kilobases or 4 centimorgan. More than 100 different MHC genes have been identified in the HLA complex. The major HLA class I genes encode HLA-A, HLA-B and HLA-C molecules, and HLA class II genes encode HLA-DR, HLA-DQ and HLA-DP molecules. Class I molecules present peptides derived from proteins that are endogenously synthesized in the cytosol to CD8+ T cells. Class II molecules present peptides derived from exogenous or membrane-bound proteins to CD4+ T cells. Each HLA gene encodes a cell surface molecule and most HLA genes are highly polymorphic. Since HLA molecules play an important role in antigen recognition and in the immune response, the genes are intensively studied in immunerelated diseases,
clinical heterogeneity in UC and CD may explain negative association that had been found\[184-188\]. Studies did not show this association or even a positive correlation of UC with HLA-DR2 in Japanese\[169,171,176-178\]. By the DNA-typing methods, HLA-DRB1-1502 has been found as the allele responsible for this association. HLA-DRB1-1502 is the most frequent subset of HLA-DR2 in Japanese. Several studies in European and North American populations confirm these results and revealed that HLA-DRB1-1501 is the most frequent subset of HLA-DR2 in Caucasians\[179-183\]. However, other studies did not show this association or even a negative association had been found\[184-188\]. Recently in some studies a positive association between HLA-DRB1-0103 and extensive UC has been observed\[186,189\]. It is interesting to know that the frequency of HLA-DR4 is significantly decreased in UC patients, suggesting that HLA-DR4 may have a protective effect against UC\[170,179,186,190\].

In CD, results of studies are not uniform. Several studies have shown an increased frequency of HLA-B44\[191-195\], HLA-B18\[196\], whereas other studies do not find any association with HLA class I alleles\[197-200\]. As for HLA class II genes, HLA-DR1\[201\], DR4\[176,202-205\], DR7\[206\], DRB3-0301\[187\] are found more often in CD, whereas HLA-DR3\[201,206\], DQA1 *0102\[204\], and DPB1-0401\[207\] are found less frequent in CD. Of these genes, only HLA-DR4 was frequently related with CD in studies in the Japanese population. Ethnic variation as well as clinical heterogeneity in UC and CD may explain these confusing results.

HLA linkage studies have been conducted to evaluate co-segregation of HLA haplotypes in IBD families with multiple affected members. Increased haplotype sharing among affected siblings has been found in some studies\[186,208-210\] but not in others\[185,190,211,212\].

**Cytokine genes**

**TNFα genes and LTα** Tumor necrosis factor alpha (TNFα) and tumor necrosis factor beta (TNFβ or lymphotoxin alpha, LTα) are potent cytokines with numerous biological functions such as the hemorraghic necrosis of tumors, cytotoxicity and immunoregulation. The genes encoding TNFα and LTα are tandemly arranged in the class III or central region of the MHC at the short arm of chromosome 6\[213\]. This region, encoding at least 30 different proteins that regulate macrophage and T cell function, B cell proliferation, and antibody production, plays a unique role in the regulation of the immune system\[214\].

Several polymorphisms in TNF region have been described. Partanen and Koskimies reported a rare EcoRI restriction fragment length polymorphism (RFLP) in the LTα gene in 1988\[215\]. Later, Messer et al showed a NcoI RFLP in the first intron of the LTα gene in 1991\[216\] and thereafter Ferencik et al described an AspHI RFLP in the first intron of the LTα gene\[217\]. Of five polymorphisms in the TNFα gene, four were found to be G to A transition polymorphisms at positions 376\[218\], -308\[219\], -238\[220\], and 163\[218\] in the promoter region of the TNFα gene, and one was a C insertion polymorphism in a C-stretch at position +7 in the first exon of the TNFα gene\[221\]. As for microsatellites in the TNF- region, five polymorphic loci have been found\[222\]. Two are located 3.5kbp upstream of the LTα gene, one is present in the first intron of the LTα gene, and the positions of the other two are downstream of the TNFα gene. Recently, a compound tetra, dinucleotide microsatellite polymorphism was found in TNF/LT α locus\[223\].

A limited number of studies on associations between the TNFα 308 gene polymorphism and UC and CD have been carried out. Mansfield et al found no evidence for the involvement of this polymorphism in either UC or CD\[224\]. Allele 2 of the TNFα -308 polymorphism was slightly decreased in CD\[225\]. A specific allelic combination of five microsatellite alleles, linked with the HLA-DR1-DQ5 haplotype, was increased in CD patients\[226\]. Studies from our group have shown that in the Dutch population there are only five haplotypic combinations at four polymorphic sites in the TNFα and LTα genes\[227\].

**IL-1 and IL-1ra genes** In man, the genes encoding interleukin-1α (IL-1α) and IL-1β, and their antagonists, interleukin-1 receptor antagonist (IL-1ra) are located in each others vicinity on the long arm of chromosome 2 (2q13 - 14)\[228\]. Polymorphisms of IL-1α\[229-231\], IL-1β\[232,233\], and IL-1ra\[234\] genes have been described in recent years. The IL-1ra gene polymorphism in intron 2 is characterized by an 86bp tandem repeat (VNTR), with five different alleles encountered in the Caucasian population. Mansfield et al from England studied the association between IBD and IL-1α, IL-1β and IL-1ra genes. Allele 2 of the IL-1ra gene was significantly over-represented in UC patients,
especially in patients with total colitis, a similar situation was not seen in CD\textsuperscript{[224]}. Our group did confirm that IL-1ra allele 2 is related with severity in UC\textsuperscript{[235]}, and found non-carriers of IL-1β allele 2 to be more often present in carriers of IL-1ra allele 2 in UC and CD than in healthy controls\textsuperscript{[236]}. This suggests that the imbalance of the IL-1/IL-1-ra ration observed in IBD\textsuperscript{[109,237]} has a biological basis. Brett \textit{et al} found an increased frequency of IL-1ra allele 2 in UC, with the majority of the association arising from the pouchitis group, suggesting that the presence of allele 2 in patients with UC affects the disease outcome\textsuperscript{[238]}. However, three other reports show no association between the allele 2 and UC\textsuperscript{[225,239,240]}.

Other candidate genes From the United States, Yang \textit{et al} have reported studies on single base polymorphisms in exons 4 and 6 of the ICAM-1 gene in IBD patients. The results show that these two polymorphisms are not associated with CD or UC. Only after stratifying patients by pANCA status, differences are demonstrated between subgroups of UC and CD\textsuperscript{[241]}. The significance of this unconfirmed observation is uncertain.

Elmgreen \textit{et al} have reported a study on a polymorphism in the complement C3 gene in IBD. A single nucleotide substitution in the C3 gene did result in a fast moving product on electrophoresis (C3F), that is more often present in CD patients, but not in UC patients\textsuperscript{[242]}. As for T cell receptor (TCR) gene, two studies show no associations of IBD with TCRα and β-chain polymorphisms\textsuperscript{[243,244]}. One study found that the TCR constant beta gene was associated with UC\textsuperscript{[190]} and two other studies found that TCR-V-β 8 was associated with CD\textsuperscript{[145,146]}.

With regard to transporter associated with antigen processing (TAP) genes, which are located between HLA-DP and HLA-DQ, Heresbach \textit{et al} found no difference between overall UC or CD and unrelated healthy controls\textsuperscript{[123,247]}. Above all, the results from these candidate gene studies are often conflicting and require further confirmation.

SUMMARY

The etiology of IBD remains unclear. Environmental factors, a genetic predisposition and a disturbed immunological response all contribute to the development of the disease.

Even though pathogens have not been identified as a cause of IBD, persistent infections are highly suspected, including Mycobacterium paratuberculosis and measles virus in CD, and an unknown bacterial infection and/or bacterial products in UC. Smoking has been suggested to be a strong risk factor in CD, and in determining the clinical course of UC and CD.

Disturbances of the mucosal immune system and an imbalance of immune regulation presented by cellular and/or humoral abnormalities as well as functions of pro-inflammatory cytokines and anti-inflammatory cytokines, and other mediators were observed in IBD patients and are considered to be fundamental to the pathogenesis of IBD. Because of the multiplicity and complexity of the interactions of all these elements, it is difficult to distinguish between primary and secondary, and pathogenic and non-pathogenic phenomena\textsuperscript{[248,249]}. With the development of transgenic and knockout rodent models, it is possible to define the critical immune elements necessary to develop IBD. Recent studies demonstrated that mucosal homeostasis is important and a defect in mucosal barrier may allow unrestrained uptake of luminal antigens and pro-inflammatory molecules and thus initiate immune responses. Some evidence for an imbalance of pro-and anti-inflammatory cytokines and of Th1/Th2 lymphocytes have been found in chronic intestinal inflammation. pANCA are present in more than 70% of UC patients, but their role as a subclinical marker of UC needs to be confirmed.

As to genetic factors, epidemiological studies have demonstrated the influence of genetic factors on the development of IBD by phenomena such as ethnic variations, familial aggregation, monozygotic twins versus dizygotic twins, and first degree relatives versus spouses. A genetic influence weights more for CD than for UC. Much interest has been focused on association studies of HLA-genes, cytokine genes and other immunemediating genes. These studies do not explain the over all disease susceptibility to either UC or CD, but may in future studies shed more light on the relevance of these markers for subgroups of patients. The definition of clinical subgroups in IBD, relevant to these studies forms a challenge to the clinicians. Recent studies using systematic whole genome screening in families with multiple affected members found the first susceptibility locus for CD on chromosome 16, and susceptibility loci for both CD and UC on chromosomes 3, 7 and 12 have been reported. Further studies are needed to clarify the complex genetic mechanisms in IBD.

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