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Reuiew

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

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DISEASE DEFINITION

Several cause 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 deseases (IBD). The chronic inflammatory bowel diseases include two distinet 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[1]. 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 abscresses, 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 splernic 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 infolvement of the disease.

About 10% of the patients with colonic inflammation can not 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 Compenhagen 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

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4.1/100000[13,14]. 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 suprisingly increased over the past 10 years[32]. There is a need for epdemiological 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, measle virus has been proposed as a causative agent for CD[36-40]. The connection remains disputed[41-44]. Mycobacterium paratuberculosis has been insolated 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 antimycobaterial 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[31]. However, all factors should be reconfirmed except smoking. Smoking has been considened as the strongest exogenous risk factor for IBD. In this contxt UC and CD seem to be opposites. Non-smokers are associated with UC and ex-smokers are at even higher risk of developing of UC than never-smokers[56-61]. The portective 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[58-61,65], 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-reiated 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 histonathological 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 elicted 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 chomicals, 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 ceallenge 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[76,87,88]. 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 suppresser 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], and HLA-DR antigens. Some studies have shown that HLA-DR is not increased in lamina propria T cells[68]. 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 impalance 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 I-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 rabit immune clitis[110] 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* show 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 prtients and the cross-reaction may be able to induce complement activation mediated by IgGI[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, neuropetides, 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 majoy working mechanisms of 5-aminosalicylate 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 appear to be a secondary response following the initial unknow 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 measns 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 sympotoms[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 facdtors also play a role in the development of IBD. Interestingly, one recent report frist 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 I b. UC or CD are also often associated with several other immune-related disorders with a clear genetic component but otherwise unknown etilogy, 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 lici 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 lici 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-DP, HLA-DQ and HLA-DR molecules. Class I molecules present peptides derived from proteins that are endogeneously synthesized in the cytosol to CD8+ T cells. Class ¢ò 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,

including IBD.

HL-A class I studies in Japan showed an increased prevalence of HLA-B5 and its subtype B52 in UC and this allele is in linkage disequilibrium with the class II HLA-DR2[167-171]. Studies from other countries have revealed the inconclusive results that HLA-A11 has increased the frequency in UC[172,173]. Two reports from China show that HLA-A31 was significantly more often detected in UC than in normal controls in the Chinese population[174,175].

The studies of HLA-class II genes show 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 happloype sharing among affected siblings has been found in some studies[186,208-210] but not in toher[145,199,211,212].

Cytokine genes

TNFα genes and LTα Tumor necrosis factor alpha (TNFα) and tumor necrosis factor beta (TNFα or lymphotoxin alpha, LTα) are potent cytokines with numerous biological functions such as the heemorrhagic 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 3.5kb 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 compoundtetra, dinucleotide microsatellite polymorphism was found in TNF/LT 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 sightly 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 antagonist, 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[224]. Our group did confirm that IL-1ra allele 2 is related with severity in UC[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[236]. This suggests that the imbalance of the IL-1/IL-1ra- ration observed in IBD[109,237] has a biological basis. Brett *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[238]. However, three other reports show no association between the allele 2 and UC[225,239,240].

Other candidate genes From the United States, Yang *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[241]. The significance of this unconfirmed observation is uncertain.

Elmgreen *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[242].

As for T cell receptor (TCR) gene, two studies show no associations of IBD with TCRα and β-chain polymorphisma[243,244]. One study found that the TCR constant beta gene was associated with UC[190] and two other studies found that TCR-V-β 8 was associated with CD[145,146].

With regard to transporter associated with antigen processing (TAP) genes, which are located between HLA-DP and HLA-DQ, Heresbach *et al* found no difference between overall UC or CD and unrelated healthy controls[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£Û248£Ý. With the development of transgenic and knockout rodent models, it is pollible to define the critical immune elements necessary to develop IBD. Recent studies demonstrated that mucosal homeostasis is important and a defection 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 clinicans. 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.

REFERENCES

1 Wilks S. The morbid appearance of the intestine of miss Banks. *Med Times Gazette*, 1985;2:264-269
2 Crohn BB, Ginsberg L, Oppenheimer GD. Regional enteritis. A pathological and clinical entity. *JAMA*, 1932;99:1323-1329
3 Dalziel TK. Chronic interstitial enteritis. *Br Med J*, 1913; 2: 1068-1070
4 Lockhart-Mummery HE, Morson BC. Crohn's disease (regional enteritis) of the large intestine and its distinction from ulcerative colitis. *Gut*, 1960;1:87-105
5 Price AB. Overlap in the specturm of non-specific inflammatory bowel disease: 'colitis indeterminate'. *J Clin Pathol*, 1978;31:567-577
6 Wells AD, McMillan I, Price AB, Ritchie JK, Nicholls RJ. Natural history of indeterminate colitis. *Br J Surg*, 1991;78:179-181

7 Moum B, Vatn MH, Ekbom A, Aadland E, Fausa O, Lygren I *et al.* Incidence of ulcerative colitis and indeterminate colitis in four counties of southeastern Horway, 1990-93. A prospective population-based study. The Inflammatory Bowel South-Eastern Norway (IBSEN) Study Group of Gastroenterologists. *Scand J Gastroenterol*, 1996; 31:362-366

8 Moum B, Vatn MH, Ekbom A, Andland E, Fausa O, Lygren I *et al*. Incidence of Crohn's disease in four counties in southeastern Norway, 1990-93. A pro spective population-based study. The Inflammatory Bowel South-Eastern Norway (IBSEN) Study Group of Gastroenterologists. *Scand J Gastroenterol*, 1996;31:355-361

9 Kildebo S, Breckan R, Nordgaard K, Burhol PG, Jorde R. The incidence of Crohn's disease in northern Norway from 1983 to 1986. *Scand J Gastroenterol*, 1989;24:1265-1270

10 Kidebo S, Nordgaard K, Aronsen O, Breckan R, Burhol PG, Jorde R. The incidence of ulcerative colitis in Northern Norway from 1983 to 1986. The northern Norewegian study group. *Scand J Gastroenterol*, 1990; 25:890-896

11 Haug K, Schrump E, Halvorsen JF, Fluge J, Hamre E, Hamre T. Epidemiology of Crohn's disease in western Norway. Study group of inflammatory bowel disease in western Norway. *Scand J Gastroenterol*, 1989;24:1271-1275

12 Ekbom A, Helmick C, Zack M, Adami HO. The epidemiology of inflammatory bowel disease: a large, population-based study in Sweden. *Gastroenterology*, 1991;100:350-358

13 Langholz E, Munkholm P, Nielsen OH, Kreiner S, Binder V. Incidence and prevalence of ulcerative colitis in Copenhagen county from 1962-1987. *Scand J Gastroenterol*,1991;26:1247-1256

14 Munkholm P, Langholz E, Nielsen OH, Kreiner S, Binder V. Incidence and prevalence of Crohn' s disease in the County of Compenhagen, 1962-87: A six fold increase in incidence. *Scand J Gastroenterol*, 1992;27:609-614

15 Martinez Salmeron JF, Rodrigo M, de Teresa J, Nogueras F, Garcia-Montero M, de Sola C *et al*. Epidemiology of inflammatory bowel disease in the province of Grandada, Spain: a retrospective study from1979 to 1988. *Gut*, 1993;34:1207-1209

16 Russel MGVM, Dorant E, Volvics A, Brummer RJM, Pop P, Muris JWM *et al*. High incidence of inflammatory bowel disease in the Netherlands: results of a prospective study. *Dis Colon Rectum*, 1998;41:33-40

17 Shivananda S, Pena AS, Mayberry JF, Ruitenberg EJ, Hoedemaeker PJ. Epidemiology of proctocolitis in the region of Leiden, The Netherlands. A population study from 1979 to 1983. *Scand J Gastroenterol*, 1987;22:993-1002

18 Shivananda S, Pena AS, Nap M, Weterman IT, Mayberry JF, Ruitenberg EJ *et al* . Epidemiology of Crohn's disease in region Leiden, the Netherlands. A population study from 1979 to 1983. *Gastroenterology*, 1987;93:955-974

19 Trallori G, Palli D, Saieva C, Bardazzi G, Bonanomi AG, d'Albasio G *et al*. A population-based study of inflammatory bowel disease in Florence over 15 years (1978-92). *Scand J Gastroenterol*, 1996; 31: 892-899

20 Tragnone A, Corrao G, Miglio F, Caprilli R, Lanfranchi GA. Incidence of inflammatory bowel disease in Italy: a nationwide population-based study. Gruppo Italiano per lo Studio del Colone del Retto (GISC). *Int J Epidemiol*, 1996;25:1044-1052

21 Vucelic B, Korac B, Sentic M, Milicic D, Hadzic N, Juresa V *et al*. Epidemiology of Crohn¡äs disease in Zagreb, Yugoslavia: a ten years prospective study. *Int J Epidemiol*, 1991;20:216-220

22 Vucelic B, Korac B, Sentic M, Milicic D, Hadzic N, Juresa V *et al*. Ulcerative colitis in Zagreb, Yugoslavia: Incidence and prevalence 1980-1989. *Int J Epidemiol*, 1991;20:1043-1047

23 Manousos ON, Koutroubakis I, Potamianos S, Roussomoustakaki M, Gourtsoyiannis N, Vlachonikolis IG. A prospective epidemiologic study of Crohn's disease in Heraklion, Crete.Incidence over a 5-year period. *Scand J Gastroenterol*, 1996;31:599-603

24 Kyle J. Crohn's disease in the northeastern and northern Isles of Scotland: an epidemiological review. Gastroenterology, 1992; 103:392-399

25 Stonnington CM, Phillips SF, Melton LI, Zinsmeister AR. Chronic ulcerative colitis: Incidence and prevalence in a community. *Gut*, 1987;28:402-409

26 Stowe SP, Redmond SR, Stormont JM, Shan AN, Chessin LN, Segal HL *et al*. An epidemiologic study of inflammatory bowel disease in Rochester, New York. Hospital incidence. *Gastroenterology*, 1990;98:104-110

27 Kawai K, Higashi A, Watanabe Y, Tada M. Epidemiological study of inflammatory bowel disease in Japan. International Falk Workshop of Inflammatory Bowel Diseases in Asia. Hong Kong; 1996:27-29

28 Wright JP, Froggatt J, O'Kewefe EA, Ackerman S, Watermeyer S, Louw J *et al* . The epidemiology of inflammatory bowel disease in Cape Town 1980-1984. *S Afr Med J*, 1986;70:10-15

29 Shivananda S, Lennard-Jones J, Logan R, Fear N, Price A, Carpenter L *et al* . Incidence of inflammatory bowel disease: is there a difference between north and south Results of the European collaborative study on inflmmatory bowel disease. *Gut*, 1996;39:690-697

30 Calkins BM, Mendeloff AI. The epidemiology of idiopathic inflamma tory bowel disease. In: Kirsner JB, Shorter RG, eds. Inflammatory bowel disease. *Baltimore: Williams & Wilkins*, 1995:31-68

31 Russel MGVM, Stockbrugger RW. Epidemiology of inflammatory bowel disease: an update. *Scand J Gastroenterol*,1996;31:417-427

32 Shivananda S, Sutherland L. Epidemiology of bowel inflammation. International Falk Workshop on inflammatory bowel disease in Asia. Hong Kong: 1996:3-38

33 Miskovitz P, Rochwarger A. The evaluation and treatment of the patient with diarrhea. In: Miskovitz P, Rochwarger A, eds. Infectious causes of diarrhea. *Boston: Andover Medical Publishers*, 1993:45-96

34 Rubin PH, Present DH. Differential diagnosis of chronic ulcerative colitis and Crohn's disease of the colon: one, two or many diseases In: Kirsner JB, Shorter RG, eds. Inflammatory bowel disease. 4 ed. *Baltimore: Williams & Wilkins*, 1995:355-379

35 Geboes K, Ectors N. The infectious track in inflamatory bowel disease: a controversial area. Research and Clinical Forums. *Wells Medical Ltd*,1995;17:41-48

36 Wakefield AJ, Pittilo RM, Sim R, Cosby SL, Stephenson JR, Dhillon AP *et al*. Evidence of persistent measles virus infection in Crohn's disease. *J Med Virol*, 1993;39:345-353

37 Wakefield AJ, Ekbom A, Dhillon AP, Pittilo RM, Pounder RE. Crohn's disease: pathogenesis and persistent measles virus infection. *Gastroenterology*, 1995;108:911-916

38 Lewin J, Dhillon AP, Sim R, Mazure G, Pounder RE, Wakefield AJ. Persistent measles virus infection of the intestine: confirmation by immunogold electron microscopy. *Gut*, 1995;36:564-569

39 Ekbom A, Daszak P, Kraaz W, Wakefield AJ. Crohn's disease after in-utero measles virus exposure. *Lancet*, 1996;348:515-517

40 Thompson NP, Montogomery SM, Pounder RE, Wakefield AJ. Is measles vaccination a risk factor for inflammatory bowel disease. *Lancet*, 1995;345:1071-1074

41 Iizuka M, Nakagomi O, Chiba M, Ueda S, Masamune O. Absence of measles virus in Crohn's disease. *Lancet*, 1995;345:199

42 Nakagomi O, Iizuka M. Measles virus in Crohn's disease. *Lancet*, 1995;345:660

43 Fisher NC, Yee L, Nightingale P, McEwan R, Gibson JA. Measles virus serology in Crohn's disease. *Gut*, 1997;41:66-69

44 Miyamoto H, Tanaka T, Kitamoto N, Fukuda Y, Shimoyama T. Detection of immunoreactive antigen, with a monoclonal antibody to measles virus, in tissue from a patient with Crohn's disease. *J Gastroenterol*, 1995;30:28-33

45 Chiodini RJ, van Kruiningen HJ, Merkal RS, Thayer WR, Jr., Coutu JA. Characteristics of an unclassified Mycobacterium species isolated from patients with Crohn's disease. *J Clin Microbiol*, 1984;20: 966-971

46 Chiodini RJ, v Kruiningen HJ, Thayer WR, Merkal RS, Coutu JA. Possible role of Mycobacteria in inflammatory bowel disease. I. An unclassified Mycobacterium species isolated from patients with Crohn's disease. *DigDisSci*,1984;29:1073-1079

47 Moss MT, Sanderson JD, Tizard MLV, Hermon-Taylor J, El-Zaatari FAK, Markesich DC *et al* . Polymerase chain reaction detection of Mycobacterium paratuberculosis and Mycobacterium avium subsp silvaticum in long term cultures from Crohn's disease and control tissues. *Gut*, 1992;33:1209-1213

48 Lisby G, Andersen J, Engbaek K, Binder V. Mycobacterium paratuberculosis in intestinal tissue from patients with Crohn's disease demonstrated by a nested primer polymerase chain reaction. *Scand J Gastroenterol*, 1994;29:923-929

49 Thayer WR, Jr., Coutu JA, Chiodini RJ, van Kruiningen HJ, Merkal RS. Possible role of mycobacteria in inflammatory bowel disease. II. Mycobacterial antibodies in Crohn's disease. *Dig Dis Sci*, 1984;29:1080-1085

50 Suenaga K, Yokoyama Y, Okazaki K, Yamamoto Y. Mycobacteria in the intestine of Japanese patients with inflammatory bowel disease. *Am J Gastroenterol*, 1995;90:76-80

51 Al-Shamali M, Khan I, Al-Nakib B, Al-Hassan F, Mustafa AS. A multiplex polymerase chain reaction assay for the detection of Mycobacterium paratuberculosis DNA in Crohn's disease tissue. *Scand J Gastroenterol*, 1997;32:819-823

52 Afdhal NH, Long A, Lennon J, Crowe J, O'Donoghue DP. Controlled trial of antimycobacterial therapy in Crohn's disease. Clofazimine versus

placebo. *Dig Dis Sci*, 1991;36:449-453
53 Thayer WR. The use of antimycobacterial agents in Crohn's disease. *J Clin Gastroenterol*, 1992;15:5-7
54 Prantera C, Kohn A, Mangiarotti R, Andreoli A, Luzi C. Antimycobacterial therapy in Crohn's disease: results of a controlled, double-blind trial with a multiple antibiotic regimen. *Am J Gastroenterol*, 1994;89:513-518
55 Swift GL, Srivastava ED, Stone R, Pullan RD, Rhodes J, Wilkinson S. Controlled trial of anti-tuberculous chemotherapy for two years in Crohn's disease. *Gut*, 1994;35:363-368
56 Nakamura Y, Labarthe DR. A case-control study of ulcerative colitis witrh relation to smoking habits and alcohol consumption in Japan. *Am J Epidemiol*, 1994;140:902-911
57 Boyko EJ, Koepsell TD, Perera DR, Inui TS. Risk of ulcerative colitis among former and current cigarette smokers. *N Engl J Med*, 1987;316:707-710
58 Lindberg E, Tysk C, Andersson K, Jarnerot G. Smoking and inflammatory bowel disease. A case control study. *Gut*, 1988;29:352-357
59 Franchesi S, Panza E, La Vecchia C, Parazzini F, Decarli A, Bianchi Porro G. Nonspecific inflammatory bowel disease and smoking. *Am J Epidemiol*, 1987;125:445-452
60 Calkins BM. A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig Dis Sci*, 1989;34:1841-1854
61 Persson PG, Ahlbom A, Hellers G. Inflammatory bowel disease and tobacco smoke: a case-control study. *Gut*, 1990;31:1377-1381
62 Lashner BA, Hanauer SB, Silverstein MD. Testing nicotine gum for ulcerative colitis patients. Experience with single-patient trials. *Dig Dis Sci*, 1990;35:827-832
63 Pullan RD,Rhodes J,Ganesh S,Mani V, Morris JS, Wiolliams GT *et al*. Transdermal nicotine for active ulcerative colitis. *N Engl J Med*, 1994;330:811-815
64 Thomas GAO, Rhodes J, Mani V, Williams GT, Newcombe RG, Russell MAH *et al* . Transdermal nicotine as maintenance therapy for ulcerative colitis. *N Engl J Med*, 1995;332:988-992
65 Silverstein MD, Lashner BA, Hanauer SB, Evans AA, Kirsner JB. Cigarette smoking in Crohn's disease. *Am J Gastroenterol*, 1989;84:31-33
66 Russel MGVM, Volovics A, Schoon EJ, v Wijlick EHJ, Logan RF, Shivananda S *et al*. Inflammatory bowel disease: is there any relationship between smoking status and disease presentation Results of the European Collaborative Study on inflammatory bowel disease. In: Russel MGVM Ph.D thesis. Incidence, risk factors and quality of life in IBD: Inflammatory bowel disease registry South Limburg. Maastricht: *University Maastricht*, 1997:117-129
67 Russel MG, Nieman FH, Bergers JM, Stockbrugger RW. Cigarette smoking and quality of life in patients with inflammatory bowel disease. South Limburg IBD Study Group. *Eur J Gastroenterol Hepatol*, 1996;8: 1075-1081
68 Elson CO, MCCabe RP. The immunology of inflammatory bowel disease. In: Kirsner JB, Shorter RG, eds. Inflammatory bowel disease. *Baltimore: Williams & Wilkins*,1995:203-251
69 Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, Tonegawa S. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell*,1993;75:274-282
70 Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell*, 1993;75:253-261
71 Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell*, 1993;75:263-274
72 Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*, 1983;1:1273-1275
73 Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*, 1983;1:1273-1275
74 Cahill RJ, Foltz CJ, Fox JG, Dangler CA, Powrie F, Schauer DB. Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection with Helicobacter hepaticus. *Infect Immun*, 1997;65:3126-3131
75 Shomer NH, Dangler CA, Schrenzel MD, Fox JG. Helicobacter bilis-induced inflammatory bowel disease in scid mice with defined flora. *Infect Immun*, 1997;65:4858-4864
76 Sartor RB. Pathogenesis and immune mechanisms of chronic inflammatory bowel diseases. *Am J Gastroenterol*, 1997;92(suppl 12):5S-11S
77 May GR, Sutherland LR, Meddings JB. Is small intestinal permeability really increased in relatives of patients with Crohn' s disease *Gastroenterology*, 1993;104:1627-1632
78 Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohon's disease and their relatives. A possible etiologic factor. *Ann Intern Med*, 1986;105:883-885
79 Hilsden RJ, Meddings JB, Sutherland LR. Intestinal permeability changes in response to acetylsalicylic acid in relatives of patients with Crohn's disease. *Gastroenterology*, 1996;110:1395-1403
80 Podolsky DK, Isselbacher KJ. Glycoprotein composition of colonic mucosa. Specific alterations in ulcerative colitis. *Gastroenterology*, 1984;87:881-998
81 Rhodes JM. Colonic mucus and mucosal glycoproteins: the key to colitis and cancer. *Gut*, 1989;30:1660-1666
82 Clamp JR, Fraser G, Read AE. Study of the carbohydrate content of mucus glycoproteins from normal and diseased colons. *Clin Sci (Colch)*, 1981;61:229-234
83 Teahon K, Smethurst P, Levi AJ, Menzies IS, Bjarnason I. Intestinal permeability in patients with Crohn's disease and their first degree relatives. *Gut*,1992;33:320-323
84 Ainsworth M, Eriksen J, Rasmussen JW, Schaffalitzky de Muckadell OB. Intestinal permeability of 51Cr-labelledethylenediaminetetraacetic acid in patients with Crohn;s disease and their healthy relatives. *Scand J Gastroenterol*, 1989;24:993-998
85 Ruttenberg D, Young GO, Wright JP, Isaacs S. PEG-400 excretion in patients with Crohn's disease, their first-degree relatives, and healthy volunteers. *Dig Dis Sci*,1992;37:705-708
86 Munkholm P, Langholz E, Hollander D, Thornberg K, Orholm M, Katz KD, Binder V. Intestinal permeability in patients with Crohn's disease and ulcerative colitis and their first degree relatives. *Gut*, 1994;35:68-72
87 Abreu-Martin MT, Targan SR. Regulation of T cells in the intestinal mucosa. *Curr Opin Gastroenterol*, 1996;12:569-576
88 Greenwald BD, James SP. Immunology of inflammatory bowel disease. *Curr Opin Gastroenterol*, 1997;13:293-301
89 Engleman EG, Benike CJ, Grumet FC, Evans RL. Activation of human T lymphocyte subsets: helper and suppressor / cytotoxic T cells recognize and respond to distinct histocompatibility antigens. *J Immunol*, 1981;127:2124-2129
90 Meuer SC, Schlossman SF, ReinherZ EL. Clonal analysis of human cytotoxic T lymphocytes: T4 + and T8 + effector T cells recognize products of different major histocompatibility complex regions. *Proc Natil Acad Sci USA*, 1982;79:4395-4399
91 Schieferdecker HL, Ullrich R, Hirseland H, Zeitz M. T cell differentiation antigens on lymphocytes in the human intestinal lamina propria. *J Immunol*, 1992;149:2816-2822
92 Qiao L, Schurmann G, Betzler M, Meuer SC. Activation and signaling status of human lamina propria T-lymphocytes. *Gastroenterology*, 1991;101:1529-1536
93 Targan SR, Deem RL, Liu M, Wang S, Nel A. Definition of a lamina propria T cell responsive state. Enhanced cytokine responsiveness of T cells stimulated through the CD2 pathway. *J Immunol*, 1995;154:664-675
94 Selby WS, Janossy G, Bofill M, Jewell DP. Intestinal lymphocyte subpopulations in inflammatory bowel disease: an analysis by immunohistological and cell isolation techniques. *Gut*, 1984;25:32-40
95 James SP, Fiocchi C, Graeff AS, Strober W. Immunoregulatory function of lamina propria T cells in Crohn's disease. *Gastroenterology*, 1985;88:1143-1150
96 Moore K, Walters MT, Jones DB, Garvey E, Harvey J, Cawley MI *et al*. An immunohistological study of CD4+ lymphocyte subsets within inflammatory lesions with special reference to rheumatoid arthritis and inflammatory bowel disease. *Immunology*, 1988;65:457-463
97 Pallone F, Fais S, Squarcia O, Biancone L, Pozzilli P, Boirivant M. Activation of peripheral blood and intestinal lamina propria lymphocytes in Crohn's disease. In vivo state of activation and in vitro response to stimulation as defined by the expression of early activation antigens. *Gut*, 1987;28:745-753
98 Lee HB, Kim JH, Yim CY, Kim DG, Ahn DS. Differences in immunophenotyping of mucosal lymphocytes between ulcerative colitis and Crohn's disease. *Korean J Intern Med*, 1997;12:7-15
99 Choy MY, Walker-Smith JA, Williams CB, MacDonald TT. Differential expression of CD25 (interleukin-2 receptor) on lamina propria T cells and macrophages in the intestinal lesions in Crohn's disease and ulcerative colitis. *Gut*, 1990;31:1365-1370
100 Brynskov J, Tvede N. Plasma interleukin-2 and a soluble / shed interleukin-2 receptor in serum of patients with Crohn' s disease. Effect of cyclosporin. *Gut*, 1990;31:795-799
101 Mahida YR, Gallagher A, Kurlak L, Hawkey CJ. Plasma and tissue interleukin-2 receptor levels in inflammatory bowel disease. *Clin Exp immunol*, 1990;82:75-80
102 Mueller C, Knoflach P, Zielinski CC. T-cell activation in Crohn's disease. Increased levels of soluble interleukin-2 receptor in serum and

in supernatants of stimulated peripheral blood mononuclear cells. *Gastroenterology*,1990;98:639-646
103 Mullin GE, Maycon ZR, Braun-Elwert LEA. Inflammatory bowel disease mucosal biopsies have specialized lymphokine mRNA profiles. *Inflammat Bowel Dis*, 1996;2:16-26
104 Weiner HL. Oral tolerance: immune mechanisms and treatment of autoimmune disease. *Immunol Today*, 1997;18:335-343
105 Strober W, Kelsall B, Fuss I, Marth T, Ludviksson B, Ehrhardt R *et al*. Reciprocal IFN-g and TGB-b responses regulate the occurrence of mucosal inflammation. *Immunol Today*, 1997;18:61-64
106 MacDonal TT, Spencer J. Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. *J Exp Med*, 1988;167:1341-1349
107 Fiocchi C, Podolsky DK. Cytokines and growth factors in inflammatory bowel disease. In: Kirsner JB, Shorter RG, eds. Inflammatory bowel disease. *Baltimore: Williams & Wilkins*, 1995:252-280
108 Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today*, 1996;17:138-146
109 Casini-Raggi V, Kam L, Chong YJT, Fiocchi C, Pizarro TT, Cominelli F.Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. *J Immunol*, 1995;154:2434-2440
110 Ferretti M, Casini-Raggi V, Pizarro TT, Eisenberg SP, Nast CC, Cominelli F. Neutralization of endogenous IL-1 receport antagonist exacerbates and prolongs inflammation in rabbit immune colitis. *J Clin Invest*, 1994;94:449-453
111 Melani L, Hirsch E, Guanzon M, Pizarro TT, Hirsh D, Cominelli F. Deletion of the IL-1 receptor antagonist (IL-1ra) gene increases susceptibility to experimental colitis in mice [Abstract]. *Gastroenterology*, 1997;112:A1040
112 Holt LC, Bocker U, Damiao A, Murphy M, Haskill JS, Sartor RB. Decreased ratio of IL-1ra / IL-1 mRNA is the result of inflammation rather than a unique IBD-related abnormality [Abstract]. *Gastroenterology*, 1997;112:A998
113 Kojouharoff G, Hans W, Obermeier F, Mannel DN, Andus T, Scholmerich J *et al* . Neutralization of tumor necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. *Clin Exp Immunol*, 1997;107:353-358
114 Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA. Potential antiinflammatory effecxt of interleukin 4: suppression of human monocyte tumor necrosis factor alpha, interleukin 1, and prostaglandin E2. *Proc Natil Acad Sci USA*, 1989;86:3803-3807
115 Kucharzik T, Stoll R, Lugering N, Domschke W. Circulating antiinflammatory cytokine IL-10 in patients with inflammatory bowel disease (IBD). *Clin Exp Immunol*, 1995;100:452-456
116 Kucharzik T, Lugering N, Adolf M, Domschke W, Stoll R. Synergistic effect of immunoregulatory cytokines on peripheral blood monocytes from patients with inflammatory bowel disease. *Dig Dis Sci*, 1997;42:805-812
117 Kucharzik T, Lugering N, Weigelt H, Adolf M, Domschke W, Stoll R. Immunoregulatory properties of IL-13 in patients with inflammatory bowel disease; comparison with IL-4 and IL-10. *Clin Exp Immunol*, 1996;104:483-490
118 Broberger O, Perlman P. Autoantibodies in human ulcerative colitis. *J Exp Med*, 1959;110:657-674
119 Broberger O, Perlman P. In vitro studies of ulcerative colitis. I. Reactions of patients serum with human fetal colon cells in tissue cultures. *J Exp Med*, 1963;117:705-715
120 Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. *J Allergy Clin Immunol*, 1990;86:202-210
121 Yang H, Rotter JI, Toyoda H, Landers C, Tyran D, McElree CK *et al*. Ulcerative colitis: a genetically heterogeneous disorder defined by genetic (HLA Class II) and subclinical (Antineutrophil cytoplasmic antibodies) markers. *J Clin Invest*, 1993;92:1080-1084
122 Duerr RH, Neigut DA. Molecularly defined HLA-DR2 alleles in ulcerative colitis and an anti-neutrophil cytoplasmic antibody-positive subgroup. *Gastroenterology*, 1995;108:423-427
123 Heresbach D, Alizadeh M, Reumaux D, Colombel JF, Delamaire M, Danze PM *et al*. Are HLA-DR or TAP genes genetic markers of severity in ulcerative colitis. *J Autoimmun*, 1996;9:777-784
124 Abad E, Tural C, Mirapeix E, Cuxart A. Relationship between ANCA and clinical activity in inflammatory bowel disease: variation in prevalence of ANCA and evidence of heterogeneity. *J Autoimmun*, 1997;10:175-180
125 Das KM, Dasgupta A, Mandal A, Geng X. Autoimmunity to cytoskeletal protein, a clue to the pathogenetic mechanism for ulcerative colitis. *J Immunol*, 1993;150:2487-2493
126 Takahasi F, Shan Hs, Wise LS, Das KM. irculating antibodies against human colonic extract enriched with a 40 kDa protein in patients with ulcerative colitis. *Gut*,1990;31:1016-1020
127 Halstensen TS, Das KM, Brandtzaeg P. Epithelial deposits of immunoglobulin G1 and activated complement colocalise with the M(r) 40 kD putative autoantigen in ulacerative colitis. *Gut*, 1993;34: 650-657
128 Grisham B. Oxidants and free radicals in inflammatory bowel disease. *Lancet*, 1994;344:859-861
129 Emerit J, Pelletier S, Tosoni-Verlignue D, Mollet M. Phase II trial of copper zinc superoxide dismutase (CuZnSOD) in treatment of Crohn's disease. *Free Radic Biol Med*, 1989;7:145-149
130 Keshavarzian A, Morgan G, Sedghi S, Gordon JH, Doria M. Role ofreactive oxygen metabolites in experimental colitis. *Gut*, 1990;31: 786-790
131 Keshavarzian A, Haydek, Zabihi R, Doria M, MDA, Sorenson JR. Agents capable of eliminating reactive oxygen species. Catalase, ER-2721, or Cu(II)2(3,5-DIPS)4 decrease experimental colitis. *Dig Dis*, 1992;37:1866-1873
132 Yamada T, Volkmer C, Crisham B. Antioxiant properties of 5-ASA: potential mechanism for its anti-inflammatory activity. *Can J Gastroenterol*,1990;4:295-302
133 Morales VM, Snapper SB, Blumberg RS. Probing the gastrointestinal immune function using transgenic and knockout technology. *Crr Opin Gastroenterol*, 1996;12:577-583
134 Podolsky DK. Lessons from genetic models of inflammatory bowel disease. *Acta Gastroenterol Belg*, 1997;60:163-165
135 MacDeemott RP. Etiology and pathogenesis of inflammatory bowel disease. *Curr Opin Gastroenterol*, 1997;13:303-306
136 Bregenholt S, Delbro D, Claesson MH. T-cell transfer and cytokine / TCR gene deletion models in the study of inflammatory bowel disease. *APMIS*,1997;105:655-662
137 Dieleman LA, Pena AS, Meuwissen SGM, van Rees EP. Role of animal models for the pathogenesis and tretment of inflammatory bowel disease. *Scan J Gastroenterol*, 1997;223(Suppl): 99-104
138 Sartor RB, ath HC, Sellon RK. Microbial factors in chronic intestinal inflammation. *Curr Opin Gastroenterol*, 1996;12:337-333
139 Pena As, Crusius JBA, Oudkerk Pool M, Casanova MG, Pals G, meuwissen SGM *et al* Genetics and epidemiology may contribute to understanding the pathogenesis of IBD-a new approach is now indicated. *Can J Gastroenterol*, 1993;7:71-75
140 atsangi J, Jewell DP, Rosenberg WMC, Bell JI. Genetics of inflammatory bowel disease. *Gut*, 1994;35:696-700
141 Ekbom A. Epidemiology of inflammatoy bowel disease. *Curr Opin Gastroenterol*, 1997;13:289-292
142 Satsangi J, Jewll DP. Genetic markers in inflammatory bowel disease. *Curr Opinion Gastroenterol*, 1996;12:322-326
143 Yang HY, Rotter JI. Genetic aspects of idiopathic inflammatory bowel disease. In: Kirsner JB, Shorter G, eds. Inflammatory bowel disease. *Baltimore: Williams & Wilkins*,1995;301-331
144 Orholm M, Iselius L, Sorensen TLA, Munkholm P, Langholz E, Binder V. Investigation of inheritance of chronic inflammatory bowel diseases by somplex segregation analysis. *BMJ*,1993;306:20-24
145 Hugot JP, Laurent-Puig p, Gower-Rousseau C, Caillat-Zucman S, Beaugerie, Dupas JL et al . Linkage analyses of chromosome 61oci, including HLA, in familial aggregations of rhn's disease. *Am J Med Genet*,1994;52:207-213
146 Tysk C, Lindberg E, Jamerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn¡äs disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut*, 1988;29:990-996
147 Thompson Np, Driscoll R, Pounder RE, Wakefield AJ. Genetics versus environment in inflammatory bowel disease: results of a British twin study. *BMJ*, 1996;312:95-96
148 Breslin NP, Todd A, Kilgallen C, O'Morain . Monozygotic twins with Crohn's disease and ulcerative colitis: a unique case report. *Gut*, 1997;41:557-560
149 Kuster W, Pascoe L, Purmann J, Funk S, Majewski F. The genetics of Crohn disease: complex segregation analysis of a family study with 265 patients with Crohn disease and, 187 relatives. *Am J Med Genet*, 1989;32:105-108
150 Mayberri JF, Rhodes J, Newcombe RG. Familial prevalence of inflammatory bowel disease in relatives of patients with Crohn's disease. *B Med J*,1980;280:84
151 Weterman IT, Pena AS. Familial incidence of Crohn's disease in The Netherlands and a review of the literature. *Gastroenterology*, 1984; 86:449-452
152 Bennett RA, Rubin PH, Present DH. Frequency of inflammatory bowel disease in offspring of couples both presenting with

inflammatory bowel disease. *Gastroenterology*, 1991; 100: 1638-1643
153 Pena AS. Genetic aspects In: Jamerot G,ennard-Jones J, Truelove S, eds. Inflammatory bowel diseases. *Lancaster: MTP Press*, 1992: 37-49
154 Samuels AD, Weese JL, Beman PM, Kirshner JB. An epidemiologic and demographic study of inflammatory bowel disease in black patients. *Dig Dis Sci*, 1974;19:156-160
155 Brahme F, Lindstom C, Wenckert A. Crohn's disease in a defined population. *Gastroenterology*, 1975;69:342-351
156 Novis BH, Marks IN, Bank S, Louw JH. Incidence of Crohn's disease at Groote Schuur Gospital during 1970.1974. *S Afr Med J*, 1975;49: 693-697
157 Pinchbeck BR, Kirdeikis J, Thomson ABR. Effects of religious affiliation and education status on the prevalence of inflammatory bowel disease in northem Alberta. *Can J Gastroenterol*, 19888;2(suppl. A):95-100
158 Odes Hs, Fraser D, Krugliak P, Fenyves D, Fraser GM, Sperber AD. Inflammatory bowel disease in the Bedouin Arabs of southem Israel: rarity of diagnosis and clinical features. *Gut*, 1991;32:1024-1026
159 Odes Hs, Fraser D, Krawiec J. Ulcerative colitis in the Jewish population of Southem Israel 1961-1985:epidemiological and clinical study. *Gut*, 1987;28:1630-1636
160 Gilat T, Grossman A, Fireman Z, Rozen P. Inflammatory bowel disease in Jews. In: McConnell R, Rozen P, Langman M, Gilat T, eds. The genetics and epidemiology of inflammatory bowel disease. *Basel: Karger*, 1986:135-140
161 Roth M-P, petersen GM, McElree C, Feldman E, Rotter JI. Geographic origins of Jewish patients with inflammatory bowel disease. *Gastroenterology*, 1989;97:900-904
162 Zlotogora J, Zimmerman J, Rachmilewitz D. Crohn's disease in Ashkenazi Jews. *Gastroenterology*, 1990;99:286-290
163 Pena As Genetics of inflammatory bowel disease. Research and Clinical Forums. *Wells Medical Ltd*, 1993;15:13-18
164 Hugot JP, Laurent-Puig P, Gower-Rousseau C, Olson JM,Lee JC, Beaugerie Let al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature*, 1996;379:821-823
165 Ohmen JD, Yang HY, Yamamoto KK, Zhao HY, Ma YH, Bentley LG *et al*. Susceptibility locus for inflammatory bowel disease on chromosome 16 has a role in Crohn's disease, but not in ulcerative colitis. *Hum Mol Gen*,1996;5:1679-1683
166 Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K *et al*. Two stage genome wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nature Genetics*, 1996;14:199-202
167 Tsuchiya M, Yoshida T, Asakura H, Hibi T, Ono A. HLA antigens and ulcerative colitis in Japan. *Digestion*, 1977;15:286-294
168 Hiwatashi N,Kikuchi T, Masamume O, Ouchi E, Watanabe H, Goto Y. HLA antigens in inflammatory bowel disease. *Tohoku J Exp Med*, 1980;131:381-385
169 Asakura H, Tsuchiya M, Aiso S, Watanabe M, Kobayashi K, Hibi T *et al*. Association of the human lympocyte DRz antigen with Japanese ulcerative colitis. *Gastroenterology*, 1982;82:413-418
170 Sugimura K, Asakura H, Mizuki M, Hibi T, Yagita A et al . Analysis of genes within the HLA region affecting susceptibility to ulcerative colitis. *Hum Immunol*, 1993;36:112-118
171 Futami S, Aoyama N, Honsako Y, Tamura T, Morimoto S, Nakashima T *et al* . HLA-DRB1-1502 allele, subtype of R15, is associated with susceptibility to ulcerative colitis and its progression. *Dig Dis Dci*, 1995;40:814-818
172 Asquith P, Mackintosh P, Stokes PL, Holmes GKT, Cooke WT. Histocompatibility antigens in patients with inflammatory bowel disease. *Lancet*, 1974;1:113-115
173 Papasteriades C, Spiliadis C, Emmanouilidis A, Papadimitriou C, conomidou J, Manousos O. Histocompatibility antigens (HLA-A,B) and ulcerative colitis in a Greek population. *Digestion*, 1986;33:229-232
174 Guo Z, Zhang PY, Wei K, Wang ZR, Ge KL, uo Q *et al*. Association of HLA with ulcerative colitis. *Genetics Dis* (*Chi*), 1988; 5:38-39
175 Wu L, Huang XQ, Wang XL, Huang NX, Zhang XL, Liu QY. Relation of HLA-A, B to ulcerative colitis. *Chin J Digest* (*Chi*), 1994;14:244-245
176 Kobayashi K, Atoh M, Yagita A, Konoeda Y,Inoko H, Ando A *et al*. rohn' s disease in the Japanese is associated with the HLA-DRw53. *Exp Clin Immunogenet*, 1990;7:101-108
177 Masuda H, Nakamura Y, Yanaka T, Hayakawa S. Distinct relationship between HLA-DR genes and intractability of ulcerative colitis. *Am J Gastroenterol*, 1994;89:1957-1962
178 Sugimura K, Assakura H, Hibi T, Ysuij T, Inoko H. Molecular analysis of genes responsible for the susceptibility to ulcerative colitis within the HLA region [Abstract]. *Gastroenterology*, 1991; 100: A619
179 Toyoda H, Wang SJ, Yang HY, Redford A, Magalong D, Tyan D *et al*. Distinct associations of HLA class II genes with inflammatory bowel disease. *Gastroenterology*,1993;104:741-748
180 Farrant JM, Bunce M, Artlett C *et al* . HLA DR2 is a susceptibility marker for UC in British patients irrespective of ANCA positivity [Abstract]. *Gastroenterology*, 1994;106:A679
181 Andus T, Caesar I, Vogl D, Scholmerich J, Gross V. Association of HLA-DR15, pANCA and IL-1 receptor antagonist allele 2 with ulcerative colitis [Abstract]. *Gastroenterology*, 1995;108:A770
182 De la Concha EG, Femandez-Arquero M, Santa-Cruz S *et al.* Positive and negative associations of distinct HLA-DR2 subtypes with ulcerative colitis (UC). *Clin Exp Immunol*, 1997;108392-395
183 Bouma G, Oudkerk Pool M, rusius JBA, Schreuder GMTh, Hellemans HPR, Meijer UGA *et al*. Evidence for genetic heterogeneity in IBD. HLA genes in the predisposition to suffer from ulcerative colitis and Crohn's disease. *Clin Exp Immunol*, 1997;109:175-179
184 Zetterquist H, Broome U, Einarsson K, Olerup O. HLA class II genes in primary sclerosing cholangitis and chronic inflammatory bowel disease: no HLA-Rw52a association in Swedish patients with sclerosing cholangitis. *Gut*, 1992;33:942-946
185 Mehal WZ, Lo YMD, Wordsworth BP, Neuberger JM, Hubscher SC, Fleming KA *et al.* HLA DR4 is a marker for rapid disease progression in primary sclerosing cholangitis. *Gastroenterology*, 1994;106: 160-167
186 Satsangi J, Welsh KI, Bunce M, Julier, Farrant JM, Bell JI *et al*. ontribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. *Lancet*, 1996;347:1212-1217
187 Forcione DG, Sands B, Isselbacher KJ, Rustgi A, Podolsky K, Pillai S. An increased risk of Crohn's disease in individuals who inherit the HLA class II DRB3* 0301 allele. *Proc Natl Acad Sci USA*, 1996;93: 5094-5098
188 Cottone M, Bunce M, Taylor CJ, Ting A, Jewell DP. Ulcerative colitis and HLA-phenotype. *Gut*, 1985;26:952-954
189 Roussomoustakaki M, Satsangi J, Welsh K, Louis E, Fanning G, Targan S *et al* . Genetic markers may predict disease behavior in patients with ulcerative colitis. *Gastroenterology*, 1997;112:1845-1863
190 Kobayashi, Atoh M, Konoeda Y, Yaghita A, noko H, Sekiguchi S. HLA-DR, DQ and T cell antigen receptor constant beta genes in Japanese patients with ulcerative colitis. *Clin Exp Immunol*, 1990;80: 400-403
191 Purrmann J, Bertrams J, napp M, leveland S, Hengels KJ, Gemsa R *et al*. Gene and haplotype frequencies of HLA antigens in 269 patients with Crohn' s disease. *Scand J Gastroenterol*, 1990; 25:981-985
192 Jacoby RK, Jayson MIV. HL-A 27 in rohn's disease. *Ann Rheum Dis* 1974;33:422-424
193 Thorsby E, Lie SO. Relationship between HL-A system and susceptibility to diseases. *Transplant Proc*, 1971;3:1305-1307
194 Kuhnl P, Sibrowski W, Bohm BO, Bender SW, Kalmar G, Loliger C. HLA antigen frequencies in familial Crohn's disease. *Beitr Infusionsther*, 1990;26:283-286
195 Purrmann J, Zeidler H, Bertrams J, Juli E, Cleveland S, Berges W *et al* . HLA antigens in ankylosing spondylitis associated with Crohn's disease. Increased frequency of the HLA phenotype B27,B44. *J Rheumatol*, 1988;15:1658-1661
196 can den Berg-Loonen EM, Dekker-Saeys BJ, Meuwissen SGM, Nijenhuis LE, Engelfriet CP. Histocompatibility antigens and other genetic markers in ankylosing spondylitis and inflammatory bowel diseases. *J mmunogenet*, 1977;4:167-175
197 Lewkonia RM, Woodrow JC, McConnell RB, Price Evans DA. HL-A antigens in inflammatory bowel disease. *Lancet*, 1974;4:167-175
198 Bergman L, Lindblom JB, Safwenberg J, Krause U. HL-A frequencies in Crohn's disease and ulcerative colitis. Tissue Antigens, 1976; 7:145-150
199 Pena As, Biemond I, Kuiper G, Weterman IT, van Leeuwen A, Schreuder I *et al* . HLA antigen distribution and HLA haplotype segregation in Crohn's disease. *Tissue Antigens*, 1980;16:56-61
200 Woodrow JC, Lewkonia RM, McConnell RB, berg-Loonen EM, Meuwissen SG, Dekker-Saeys J *et al*. HLA antigens in inflammatory bowel disease. *Tissue Antigens*, 1978;11:147-152
201 Danze PM, Colombel JF, Jacquot S, Loste, MN, Heresbach D, Ategbo S *et al* . Association of HLA class II genes with susceptibility to Crohn's disease. *Gut*, 1996;39:69-72

202 Fujita K, Naito S, Okabe N, Yao T. Immunological studies in rohn's disease. I. Associations with HLA syatems in the Japanese. *J Clin Lab Immunol*, 1984;14:99-102

203 Matake H, Okabe N, Naito S, Yao T. An HLA study on 149 Japanese patients with Crohn's disease. *Gastroenterol Jpn*, 1992;27: 496-501

204 Nakajima A, Matsuhashi N, Kodama T, Yazaki Y, Takazoe M, Kimura A. HLA-linked susceptibility and resistance genes in Crohn's disease. *Gastroenterology*,1995;109:1462-1467

205 Caruso C, Oliva L, Palmenri P, Cottone M. B cell alloantigens in Sicilian patients with Crohn's disease. *Tissue Antigens*, 1983;21:170-172

206 Reinshagen M, Loeliger C, Kuehnl P, Weiss U, Manfras BJ, Adler G *et al* . HLA class II gene frequencies in Crohn's disease: a population based analysis in Germany. *Gut*, 1996;38:538-542

207 Wassmuth R, Keller Y, Thomson G *et al*. HLA DPBI alleles provide protection against Crohn's disease in Caucasians. *Eur J Gastroenterol Hepato*, 1994;6:405-411

208 chwartz SE, Siegelbaum Sp, Fazio TL, Hubbell, Henry B. Regional enteritis: evidence for genetic transmission by HLA typing. *Ann Intern Med*, 1980;93:424-427

209 Achord JL, Gumm CH, Jackson JF. Regional enteritis and HLA concordance in multiple siblings. *Dig Dis Sci*, 1982;27:330-332

210 Shohat T, Cantor RM, Tyan D, McElree C, Rotter JI. Evidence for linkage of familial occurrence of inflammatory bowel disease [Abstract]. *Am J Med Genet*, 1989;45:248

211 Colombel JF, Guillemot F, van Gossum AV, Dufosse F, Cortot A, Dupont E *et al* . Familial Crohn's disease in multiple siblings: no linkages to the HLA system. *Gastroenterol Clin Biol*, 1989; 13:676 -678

212 Naom I, Lee J, Ford D, Bowman SJ, Lanchbury JS, Harris I *et al* . Analysis of the contribution of HLA genes to genetic predisposition in inflammatory bowel disease. *Am J Hum Genet*, 1996;59:226-233

213 Spies T, Morton CC, Nedospasov SA, Fiers W, Pious D, Strominger JL. Genes for the tumor necrosis factors alpha and beta are linked to the human major histocopatibility complex. *Proc Natl Acad Sci USA*, 1986;83:8699-8702

214 French MAH, Dawkins RL. Central MHC genes, IgA deficiency and autoimmune disease. *Immunol Today*, 1990;11:271-274

215 Partanen J, Koskimies S. Low degree of DNA polymorphism in the HLA-linked lymphotoxin (tumor necrosis factor beta) gene. *Scand J Immunol*, 1988;28:313

216 Messer G, Spengler U, Jung MC, Honold G, Blomer K, Pape GR *et al*. Polymorphic structure of the tumor necrosis factor (TNF) locus: a NcoI polymorphism in the first intron of the human TNF-beta gene correlates with a variant amino acid in position 26 and a reduced level of TNF-beta production. *J Exp Med*, 1991;173:209-219

217 Ferencik S, Lindemann M, Horsthemke B, Grosse-Wilde H. A new restriction fragment length polymorphism of the human TNF-beta gene detected by AspHI digest. *Eur J Immunogenet*, 1992;19:425-430

218 Hamann A, Mantzoros C, Vidal-Puig A, Flier JS. Genetic variability in the TNF-a promoter is not associated with type II diabetes mellitus (NIDDM). *Biochem Biophys Res Com*, 1995;211:833-836

219 *Wilson AG, di Giovine FS, Blakemore AIF, Duff GW. Single base polymorphism in the human tumor necrosis factor alpha gene detectable by NcoI restriction of PCR product. Hum Mol Gen*, 1992;1:353

220 D'Alfonso S, Momigliano Richiardi P. A polymorphic variation in a putative regulation box of the TNFA promoter region. *Immunogenetics*, 1994;39:150-154

221 Brinkman BMN, Kaijzel EL, Huizinga TWJ, Giphart MJ, Breedveld FC, Verweij CL. Detection of a C-insertion polymorphism in the human tumor necrosis factor alpha (TNFA) gene. *Hum Genet*, 1995; 96:493

222 Udalova IA, Nedospasov SA, Webb GC, Chaplin DD, Turetskaya RL. Highly informative typing of the human TNF locus using six adjacent polymorphic markers. *Genomics*,1993;16:180-186

223 Greenberg SJ, Fujihara K, Selkirk SM, Yu F, Du TL, Glenister N *et al*. Novel compound tetra, dinucleotide microsatellite polymorphism in the tumor necrosis factor/lymphotoxin locus. *Clin Diagn Lab Immunol*, 1997;4:79-84

224 Mansfield JC, Holden H, Tarlow JK, Di-Giovine FS, McDowell TL, Wilson AG *et al*. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology*, 1994;106:637-642

225 Louis E, Satsangi J, Roussomoustakaki M, Parkes M, Fanning G, Welsk K *et al*. Cytokine gene polymorphisms in inflammatory bowel disease. *Gut*, 1996;39:705-710

226 Plevy SE, Targan SR, Yang H, Fernandez D, Rotter JI, Toyoda H. Tumor necrosis factor microsatellites define a Crohn's disease-associated haplotype on chromosome 6. *Gastroenterology*, 1996; 110:1053-1060

227 Crusius JBA, Bing X, Mulder CJJ, Mearin ML, Pena AS. Relevance of haplotypes in the TNF region in celiac disease. *Eur Cytok Network*, 1994;2:168

228 Nicklin MJH, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1-a, interleukin-1-b, and interleukin-1 receptor antagonist genes. *Genomics*, 1994;19:382-384

229 McDowell TL, Symons JA, Ploski R, Forre O, Duff GW. A polymorphism in the 5' region of the interleukin-1 alpha gene is associated with juvenile chronic arthritis (JCA). *Br J Rheumatol*, 1993;32(Suppl. 1):162

230 Bailly S, di Giovine FS, Duff GW. Polymorphic tandem repeat region in interleukin1a intron 6. *Hum Genet*, 1993;91:85-86

231 Bailly S, di Giovine FS, Blakemore AI, Duff GW. Genetic polymorphism of human interleukin-1a. *Eur J Immunol*, 1993;23:1240-1245

232 di Giovine FS, Takhsh E, Blakemore AIF, Duff GW. Single base polymorphism at 511 in human interleukin-1b gene (IL1b). *Hum Mol Genet*, 1992;1:450

233 Pociot F, Melvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1b (IL-1b) gene correlates with IL-1b secretion in vitro. *Eur J Clin Invest*, 1992;22:396-402

234 Tarlow JK, Blakemore AIF, Lennard A, Solari R, Hughes HN, Steinkasserer A *et al* . Polymorphism in human IL-1 receptor antagonist gene intron 2 is cause by variable numbers of an 86 bp tandem repeat. *Hum Fener*,1993;91:403-404

235 Bioque G, Bouna G, Crusius JBA, Kostense PJ, Meuwissen SGM, Pena AS. Evidence for genetic heterogeneity in IBD. 1. The interleukin-1 receptor antagonist in the predisposition to suffer from ulcerative colitis. *Eur J Gastroenterol Hepatol*, 1996;8:105-110

236 Bioque G, Crusius JBA, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SGM *et al* . Allelic polymorphism in IL-1b and IL-1 receptor antagonist (IL-1RA) genes in inflammatory bowel disease. *Clin Exp Immunol*, 1995;102:379-383

237 Nishiyama T, Mitsuyama K, Toyonaga A, Sasaki E, Tanikawa K. Colonic mucosal interleukin 1 receptor antagonist in inflammatory bowel disease. *Digestion*, 1994;55:368-373

238 Brett PM, Yasuda N, Yiannakou JY, Herbst F, Ellis HJ, Vaughan R *et al* . Genetic and immunological markers in pouchilis. *Eur J Gastroenterol Hepatol*, 1996;8:951-955

239 Hacker UT, Gomolka M, Keller E, Eigler A, Folwaczny C, Fricke H *et al*. Lack of association between an interleukin-1 receptor antagonist gene polymorphism and ulcerative colitis. *Gut*, 1997;41:651-657

240 Andus T, Daig R, Vogl D *et al* . Imbalance of the interleukin 1 system in colonic mucosa-association with intestinal inflammation and interleukin 1 receptor agonist genotype 2. *Gut*, 1997;41:651-657

241 Yang H, Vora DK, Targan SR, Toyoda H, Beaudet AL, Rotter JI. Intercellular adhesion molecule 1 gene associations with immunologic subsets of inflammatory bowel disease. *Gastroenterology*, 1995; 109:440-448

242 Elmgreen J, Sorensen H, Berkowicz A. Polymorphism of complement C3 in chronic inflammatory bowel disease. *Acta Med Scand*, 1984;215:375-378

243 Randolph LM, Toyoda H, McElree CK, Shanahan F, Targan SR, Rotter JI. Lack of an association between polymorphisms of the T-cell receptor alpha-chain and ulcerative colitis. *Gastroenterology*, 1989; 98:1115-1120

244 gth polymorphism analysis of T-cell receptor genes in inflammatory bowel disease. *Scand J Gastroenterol*, 1989;24:381-384

245 Posnett DN, Schmelkin I, Burton DA, August A, McGrath H, Mayer LF. T cell antigen receptor V gene usage. Increases in V beta 8+ T cells in Crohn's disease. *J Clin Invest*, 1990;85:1770-1776

246 Lowes JR, Chahal H, Zewde M, Allan RN, Ibbotson JP. T cell receptor V beta gene usage in mesenteric lymph node mononuclear cell populations from patients with Crohn's disease. *Gut*, 1993;34(Suppl. 1):S23

247 Heresbach D, Alizadeh M, Bretagne JF, Dabadie A, Colombel JF, Pagenault M *et al*. TAP genes transporter polymorphism in inflammatory bowel disease. *Scand J Gastroenterol*, 1997;32:1022-1027

248 Fiocchi C. The immune system in inflammatory bowel disease. Acta *Gastroenterol Belg*, 1997;60:156-162