Effect of genetical, environmental and clinical factors on the aetiology and natural course of inflammatory bowel disease

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Results of our clinical studies concerning the aetiology and the management of inflammatory bowel disease

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Chapter 1. Introduction

I. Aetiology.

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder with unknown aetiology affecting varied segments of the gastrointestinal tract. The two common forms of IBD are ulcerative colitis (UC) and Crohn's disease (CD). The importance of IBD arises from the fact that its frequency is on the increase and it disables a lot of patients while generating a significant burden on the health care system. IBD is the disease of the twentieth century, the aetiology, the pathogenesis, the factors, which influence the prognosis and the natural course of IBD are intensively researched. These studies emphasize the importance of three key aetiologic factors. It seems that genetic susceptibility is the major risk factor in developing IBD. Environmental factors are the second most important and most frequently studied elements, which play a trigger role. These external influences initiate a pathologic host immune response. The target of these processes is the mucosa of the gastrointestinal tract leading to the ulceration of the mucosa and the inflammation of the bowel.

A. Genetic susceptibility.

Evidence of familiar aggregation is largely documented in several studies (1). Over 15% of patients with IBD have first-degree relatives who also have the disease. The lifetime risk of developing IBD in first-degree relatives of individuals with IBD is 8.9% for offspring, 8.8% for siblings, and 3.5% for parents (2). The incidence of IBD among first-degree relatives of IBD patients is 30 to 100 times higher than that of the general population (3). Dizygotic (nonidentical) twins have the same rate of concordance for IBD as what would be expected for siblings, whereas monozygotic twins (identical) have higher rates of concordance for both diseases (i.e., UC and CD). The concordance rate for identical twins with CD ranges from 29% to 67% in various studies (4). There is not a reported case of monozygotic twins in which one twin had CD and the other had UC. Also, there is no increased risk for IBD among spouses of individuals with IBD. Ethnic differences in disease frequency have also been described (5), thus the prevalence is the highest among Whites, while lower in Blacks and even smaller in Asians (6).

a. Candidate genes in inflammatory bowel disease. Since there are several genes potentially involved in the pathogenesis of IBD, first it is advantageous to study genes of known function. These are the so-called "candidate genes" (Table 1.). One of the first events in immune response is the interaction between antigen-presenting cells and T cells. This antigen recognition process is restricted to molecules encoded by genes of the major histocompatibility complex (MHC), which is called the human leukocyte antigen (HLA) complex in man. The genetic features and the biology of the MHC system have been reviewed several times and their role in the autoimmune disease is a current topic. The MHC complex is located on the short arm of chromosome 6. Class I genes are named A, B, C, and class II genes HLA-DP, -DQ and DR. Each gene is highly polymorph, several combinations of allels can occur as well as showing a typical pattern in distinct races. Some of the autoimmune disorders are associated with certain allelic combination.

b. HLA and ulcerative colitis. The genetic background of the different populations is diverse, therefore examination of the specific allelic combination revealed discrepant results in the distinct populations. Some studies from Japan, USA and Western Europe suggested a positive association between UC and HLA-DR2, especially DRB1*15 (7,8,9). A few other European publications found that the allelic combination of DRB1*15 associates frequently with extensive disease localisation, namely pancolitis (10). Masuda and co-workers found that in Japanese

population DRB1*1502 was significantly more frequent in therapy resistant UC patients, whereas DRB1*1501 often occurred in cases with a more benign disease course (11). These results have been confirmed in Dutch patients (12) as well as in an English patient's population among those who underwent colectomy for extensive disease (13). Furthermore, association have also been detected with IBD subgroups with distinct arthritic manifestations (14). According to a Spanish publication a negative correlation may exist between HLA-DR3 subtype and UC (15). These studies suggested an association between the structure of HLA molecule and the extension, severity and therapeutic suggestibility of UC. Although the HLA genes do not seem to have a major effect on the evolution of IBD, the above mentioned data suggest that there is a relationship between a specific HLA structure and a different subgroup of IBD, which can influence the natural course of the disease. Therefore genetic factors may have an influence on the severity of the disease in a peculiar population.

c. HLA and Crohn's disease. The clinical manifestation of CD is more heterogeneous than UC. Studies on HLA class II genes in CD have revealed positive or negative association with a large number of alleles. These results are often controversial. It is likely that the different clinical phenotypes, e.g. behavioural subgroups of CD, have a distinct genetical background. Some authors have found a positive association between DR1 and DQw5 allels and CD (16). A strong negative correlation have been published between the DRB1*03 allel and CD with perianal involvement (penetrating subtype) (17). This allel often associates with the polymorphism of the tumour necrosis factor (TNF) promoter haplotype –308 (18). Another study revealed that there is a significant difference in the DRB1 allel frequency between the penetrating and the non-penetrating CD subgroup (18). Bouma et al. 1998). This important publication gives evidence about the distinct genetic background of the different CD subgroups.

d. Cytokine genes. Cytokines play a central role in each inflammatory process, so these proteins are basically important in acute and chronic bowel inflammation. The balance of the proinflammatory and anti-inflammatory (suppressor) cytokines warrants the natural course of the inflammation (19). The mutation and polymorphism of cytokine encode genes can cause genetically determined imbalance, which can affect the inflammatory process. A mucosal imbalance of interleukin (IL)-1 and IL-1 receptor antagonist (IL-1ra) has been reported in patients with IBD in comparison with healthy controls (20). Generating suppressor predominance with synthetic molecules, which is the base of immunomodulatory therapy, is a current and hopeful therapeutic strategy of IBD, especially in CD.

Considering the pathophysiology of IBD, interleukin-1 is a central cytokine in the regulation of inflammation both systemically and at the mucosal level of the gastrointestinal tract. The source of this cytokine is the activated endothelial cells, lymphocytes, macrophages and fibroblasts. IL-1 acts on receptors of various inflammatory target cells leading to an overproduction of prostanoids and platelet activating factor. It also has a systemic effect characterised by fever and an increased level of acute-phase proteins.

IL-1 plays a central role in the inflammatory mechanism, as well as in the regulation of the connective tissue differentiation and in distinct hormonal and neurological functions. Its effects, such as the induction of fibroblast activity, can affect the course and the severity of IBD (21). Enhanced level of IL-1ß has been reported both in gut tissue of animal colitis models and in the mucosa of patients with active IBD (22,23). IL-1ß level in gut lavage of IBD patients was increased, showed a good correlation with the activity of the disease, and it was predictive for relapse. In addition, it has been reported that the non-perforating form of CD was associated with a higher expression of IL-1 mRNA in intestinal tissue compared with the penetrating subtype of CD (24) Gilberts et al. 1994).

A large inter-individual variation was found in the production of IL-1 in healthy populations, and these variations were associated with different genetic polymorphism in the IL1 gene (25). The IL1 genes, IL1ß and IL1ra, are located in close proximity to each other within 430 kb on chromosome 2q12-21. Recent studies suggest that polymorphism of the IL1 gene complex may be a significant severity factor in a number of multifactorial, often chronic, inflammatory disorders (26). Four polymorphisms are described in the IL1 gene: two in the promoter region, one in the intron four and one in the exon five (27,28,29). Two of their bi-allelic base exchange polymorphisms were suggested to have an influence on the in vitro production of the IL-1ß cytokine. One, completing an AvaI site, is located in the promoter region at the position –511 (IL1ß-511), while the other one (TaqI) is in the exon 5 at the position +3953 (IL1ß+3953). Although these two polymorphisms play a role in the pathogenesis of IBD, the clinical significance of IL-1ß polymorphism in IBD was not established earlier (30,31) Bioque et al. 1995; Heresbach et al. 1997; Mansfield et al. 1994, Roussomoustakaki et al. 1997).

e. NOD2 gene family. Scientists from the United States and Europe reported important findings from two independent studies that demonstrated a mutation in a gene known as NOD2, located on chromosome 16, that appears to be associated with Crohn's disease (30). The NOD2 gene family was initially identified as a class of plant disease-resistant gene products that function as receptors within the cell for bacterial lipopolysaccharide (LPS) (31). Subsequently, NOD2 was characterised as being highly restricted to monocytes, with the ability to induce nuclear factor kappa-B (NF-kappa-B) activation. NF-kappa-B activation by LPS should result in monocyte activation and a protective immune response(32). Using positional-cloning strategy, Hugot and colleagues (33) identified 3 NOD2 gene mutations that confer susceptibility to Crohn's disease by altering recognition of LPS and interfering with normal NF-kappa-B activation in monocytes. At the same time, in an independent study, Ogura and associates (34) demonstrated a truncated NOD2 protein associated with Crohn's disease. These investigators showed that the disease-associated NOD2 variant was functionally less active in conferring responsiveness to bacterial LPS. The NOD2 gene mutations that have been described to date are found in a subset of Crohn's disease patients. Therefore, Crohn's disease in patients with the NOD2 gene mutations may, in fact, be due to a genetically determined inability to mount an appropriate immune response against bacterial LPS.

B. Environmental factors

Several findings show that environmental factors play a "trigger" role in the expression of IBD. This is supported by the fact that incidence of IBD is higher in urban than in rural areas (35).

a. Smoking. Smoking is the most frequently investigated and the most important environmental factor that may influence the clinical course in IBD. The effects of smoking are contrary in IBD: favourable in UC and disadvantageous in CD. The harmful effect of smoking is dose-dependent in CD. It increases the use of steroid and immunosuppressive drugs as well as the risks of relapse and surgical intervention (36). The cessation of smoking influences the clinical course of CD favourably. Interestingly, a recent study even suggests that CD patients who quit smoking for more than one year have a more benign disease course than as if they had never smoked (37). Smoking may activate the procoagulations' factors and damage the microcirculation of the intestine which finally leads to the development of several microthrombi in the small vessels of the intestine. In contrast, UC is the disease of non-smokers: the rate of smokers is lower among UC patients than in the general population (38). Furthermore, former smokers have a greater risk to develop UC (39). There are some observations about the favourable effect of smoking on the clinical course of UC. Lower rates of hospitalisation and surgical intervention as well as later age

of disease onset have been observed among smoking UC patients (40). There are not enough data on how the onset of smoking influences the activity of UC. It is likely that nicotine is the key to the favourable effect. Transdermal nicotine patches and nicotine enemas have proved to be useful in mild to moderate active ulcerative colitis, and nicotine has a steroid sparing effect as well (41). There are some data based on a study (42) that smoking significantly affects cytokine levels in the colon. Patients who smoke have an elevated level of IL-8 in the colonic mucosa. On the other hand, patients with CD have a significant reduction in IL-8, and smoking patients with CD have a significant reduction in both IL-8 and IL-1 (43). The reduced level of these cytokines may provide protection against UC, but the significance of isolated reduction of IL-8 in CD patients is still unknown.

b. Passive smoking. There are not many studies about passive smoking in IBD and the results of these studies are controversial. Children are usually non-smokers and the risk of their developing inflammatory bowel disease (IBD) may be related to passive smoking. A study, which evaluates passive smoking exposure in 72 non-smoking children with recently diagnosed IBD (39 with UC and 33 with CD) and in an equal number of peer-nominated controls revealed, that passive smoking exposure at birth was significantly associated with the development of IBD. The effect was greater in CD (odds ratio 5.32) than in UC (odds ratio 2.19). Maternal smoking at birth was also significantly associated with the development of IBD (odds ratio 2.09), an effect that was also greater in CD than in UC. There was a dose-response relationship between packs smoked per day and IBD, and packs smoked at home per day and IBD. At symptom onset, the risk of developing IBD from passive smoking exposure was increased but was not significant (odds ratio 1.88, 95% confidence interval 0.84-4.18). The authors conclude that passive smoking exposure as well as maternal smoking at birth and, to a lesser extent, passive smoking exposure at symptom onset are associated with an increased risk of developing IBD in children. The association is stronger in CD than in UC, and there is a dose-response effect. The specific toxic exposure is more likely to be inhaled rather than passed through the placenta or in breast milk (44). Another study from Israel and two studies from New York hospitals found no evidence of association of IBD with maternal age at birth, birth order, maternal smoking, passive smoking or season of birth (45.46).

c. Oral contraceptives. Several studies described a weak association between the use of oral contraceptives and the risk of CD and UC (47). Another observation was that the elevated risk of CD decreases simultaneously with the cessation of oral contraceptives (48). The effect mechanism how of oral contraceptives is similar to that of smoking, but while smoking damages microcirculation, oral contraceptive use is rather associated with the thromboembolic disorder of the larger vessels. However, data are conflicting and recent studies and meta-analyses could not confirm the role of oral contraceptives either in the development or in the clinical course of UC or CD.

d. Caffeine and alcohol intake. There is no exact evidence that caffeine and/or alcohol consumption plays any role in the pathogenesis or affects the clinical course of IBD.

C. Immunology and inflammatory bowel diseases

In a genetically susceptible individual, IBD appears to be the result of defective regulation of mucosal immune interactions with enteric microflora triggered by an environmental factor. Although the aetiology of IBD is unclear, the pathogenesis of these disorders is better understood, and it is increasingly clear that these diseases represent the outcome of two essential interactive co-factors: enteric microflora and mucosal immunity.

a. Mucosal immune system. The mucosal immune system is required to sense and interpret the local microenvironment, recognise and avoid reacting to commensal flora (tolerance), yet retain the capacity to respond to episodic challenge from pathogens. One of the theoretic pathogenesis of IBD is a breakdown in the regulatory constraints on mucosal immune responses to enteric bacteria. The nature of the immune response and profile of cytokines generated are under genetic control and determine the features of the inflammatory process. Although there is some evidence that the profile of mucosal cytokines in early lesions may differ from that of chronic lesions, CD is associated with type 1 helper T cells (TH1) cytokines, such as interferon, TNF alpha and IL-12 (49). In UC cytokine patterns are less clear. It is not a TH1 response, rather it appears, at least in established diseases, to be a modified TH2 response associated with cytokines, such as IL-5 and IL-10 (50).

The lamina propria is the major compartment of the mucosal immune system, and contains numerous cell types (51). B lymphocytes constitute 15-40%, with a predominance of IgA-producing cells. T lymphocytes constitute 40-90% and are predominantly CD4+ (helper) cells. In IBD the total number of lymphoid cells within the intestinal mucosa is increased two to fourfold. Both T and B lymphocyte numbers are increased. The ratio of T to B lymphocytes, and the T cell CD4/CD8 (helper/suppressor) ratio of lamina propria lymphocytes, is similar in both diseased and normal bowel, and approximately 6:1 and 2:1, respectively. Intestinal intraepithelial lymphocytes (IELs) are located at the basolateral aspect of adjacent epithelial cells, and lie on the basement membrane of the epithelial layer. The frequency of IELs in relation to epithelial cell numbers varies throughout the gastrointestinal tract, ranging from 1:6 in the ileum to 1:20 in the colon. Intestinal IELs are heterogeneous population (52). The majority of IELs have pan-T-cell marker CD3. In contrast to lamina propria lymphocyte, the majority (75-80%) of IELs are CD8+ cells. This heterogenity probably reflects a diverse range of functional properties. IELs have a variety of effector functions, including cytotoxicity, modulation of epithelial cell function and proliferation, and immunoregulatory roles, including tolerance to dietary antigens and suppressor functions (53).

In IBD the antigenic trigger is probably the presence of common, non-pathogenic microbial agents within the intestine, against which the patient mounts an activated immune response. In healthy individuals, there is a finely tuned, low-grade chronic inflammation in the intestinal lamina propria caused by intestinal bacteria. Failure to suppress these inflammatory response results in an uncontrolled immune response within the intestine of IBD patients.

b. Cytokines. The role of cytokines in IBD has received the most attention in the recent past. Cytokines are glycosylated proteins that are synthesised by a variety of cell types in response to tissue injury. Certain cytokines, such as TNF α and IL-1, -6, -8, and -12 are categorised as "pro-inflammatory," due to their ability to induce inflammation; others, such as IL-4, -10, -11 and -13, are categorised as "anti-inflammatory" on account of their ability to reduce inflammation (54). There is increasing evidence that a disturbance in the balance between pro-inflammatory and anti-inflammatory cytokines occurs in IBD (55). For example, tissue levels of pro-inflammatory cytokines are elevated in active IBD and correlate with the severity of the inflammation (56). This has led to the development of treatments directed at blocking the proinflammatory actions of these cytokines. Among these treatments the administration of monoclonal antibody infliximab against TNF has received the most attention, and it is the only biologic agent approved for the treatment of IBD to date.

TNF serves as a pivotal inflammatory mediator, and treatments based on downregulating and/or eliminating the effector cells that produce TNF and other inflammatory cytokines have proved effective in Crohn's disease. Downregulation of activated effector cells may be achieved using

suppressor cytokines or stimulating suppressor-T-cell (T-helper 2) function. Elimination of activated effector cells may be achieved by inducing the cells to undergo apoptosis. TNF has numerous biologic properties including inflammation, proliferation and differentiation. TNF production leads to the activation of macrophages, the priming of neutrophils, and an increase in intestinal epithelial permeability (57).
TNF is primarily a product of activated macrophages, although lymphocytes and natural killer cells also produce it. Immune-mediated tissue injury results in the development of TNF from the induction of proteases, prostaglandins, leukotrienes, eicosanoids, and other products. However, some of these products (such as prostaglandin E2) may also directly cause diarrhoea by prompting mucosal secretion of chloride and potassium (58).

D. Neuropeptides and inflammatory bowel diseases
The significance of the "gut-brain" axis in gastrointestinal secretory and motor function supposes that the central nervous system might also have a modulatory effect on gastrointestinal immune function. Not only can the gut be referred to as the largest endocrine organ in the body, but also it can say to being the largest immune organ as well as having an extensive, relatively independent nervous system with roughly the same numbers of neurons as the spinal cord. Evidence for bidirectional communication between the nervous and immune systems is well established. Peptide and nonpeptide messengers effect communication in both systems. Interestingly, as the messengers are often structurally identical, it may be the differential expression of receptors and timing of release that predicts varied responses. The two forms of this potential are illustrated with the variably proinflammatory and healing effects of substance P (59). The bi-directional nature of the neuroimmune communication is an important feature. This is as true in the gut mucosa as elsewhere (60). Increasingly, cytokines, the soluble mediators of immune function, have been shown to regulate several neural and endocrine systems (61). As if to emphasize interdependence of the nervous and immune systems, many of the secretory products of the immune system are actually produced by the neuroendocrine system, and similarly mediators traditionally considered to be exclusive products of neuroendocrine tissues are produced by immunocytes (62). Although maintaining distinction between cytokines, neuropeptides, growth factors, and local and systemic hormones can be limiting, the role of these mediators in potent neuroimmune cross-talk is clear. Evidence of the biologic significance of the close relationship between nerves and intestinal immune cells is evident in examples of neurogenic inflammation. As a model, sensory neuropeptides in the skin are not only afferent transmitters but also participate in efferent regulation of tissue inflammation (63). There is evidence that this phenomenon occurs in the gut as well (64). Locally released neuropeptides act directly on contiguous vasculature as well as mast cells, prompting their release of histamine and other mediators, fanning the inflammatory response. In the process, local sensory neurons are stimulated with a potentially amplifying result. Recent studies have underlined the importance of the neuropeptide substance P in the activation of neurogenic inflammation with the promising suggestion that decreased release of substance P, with agents such as lidocaine, or blockade of the substance P receptor with specific antagonists, may be an important intervention to prevent inflammation (65).
In health, the gastrointestinal mucosal immune response is an intricately balanced weave of local, regulatory communication systems. These comprise a diverse peptide network, the enteric nervous system, the heterogeneous interactive mucosal immune populations, and other cells within the mucosa. The complexity of this microenvironment makes it difficult to clarify the heirarchy or relative importance of individual regulatory factors during inflammation. Basic

research in this area promises much but is only slowly having a decisive impact on clinical medicine.

IBD is a chronic idiopathic inflammation, a condition in which environmental factors and genetic predisposition converge to express the disease. Stress may be one of the most interesting environmental factor (smoking is the other one), which can induce IBD in a genetically susceptible individual. Initially, colitis was considered to have a major psychological component and was listed among psychosomatic conditions. As our awareness of the role of the immune system in IBD has increased, less attention has been paid to the role of the enteric nervous system and stress in the expression of these conditions, despite some florid demonstration of a modulatory role of the nervous system in precipitating relapse in humans (66). Another hypothesis was that early life stresses may predispose a person to the development of IBD. The placebo effect in clinical trials of IBD is substantial and has promoted the impression that IBD is a condition extremely receptive to influence of neuropsychic factors (67).

The stress response in a healthy organism is generally viewed as a warning and thus protective reaction to a threat. However, the response may be deleterious if it is linked to an inflammatory stimulus or if it proceeds an inflammatory event. Prior stress enhances the response to an inflammatory stimulus by a mechanism that is independent of the release of hypothalamic corticotropin-releasing factor or arginine vasopressin (68). Putative mechanism include an increase of intestinal permeability as well as the release of neuropeptides (mainly substance P). Within the gastrointestinal tract, neuropeptides are involved in the physiological control of several digestive functions, including motility, fluid and electrolyte secretion, blood flow and homeostasis. In addition, there is a mounting evidence that neuropeptides play a pivotal role in the regulation of immunoinflammatory responses, and that bi-directional communication exists between the enteric nervous and mucosal immune system.

Substance P and tachykinins. Substance P (SP) and neurokinin A (NKA), the two most thoroughly characterized members of tachykinin family of neuropeptides, are putative neurotransmitters that exert important physiological functions in both the central nervous system and peripherial tissues. In the very first study on SP published in 1931, it was reported that this factor was present both in intestine and brain (69), clearly showing that focus was on the nervous system and, in a way, on the brain-gut complex. The actions of tachykinins on the digestive effector systems are mediated by three different types of tachykinin receptor, termed NK1, NK2 and NK3. Most prominent amongst the gastrointestinal effects of tachykinins is their action on motility, and they can not only stimulate but also inhibit motility, the net response depending on the type and site of tachykinin receptors that are activated. Nerve-independent facilitation of gastrointestinal motor activity is brought about by NK1 receptors on interstitial cells of Cajal and NK2 receptors on muscle cells. NK3 receptors are largely confined to enteric neurones and mediate predominantly cholinergic contraction of the intestinal musculature within the enteric nervous system. SP and NKA can also depress motor activity through release of inhibitory transmitters such as nitric oxide (an effect exterted via NK3 receptors and in particular NK1 receptors on inhibitory motor pathways). Tachykinins also participate in the neural control of secretory activity in the intestine. Thus, tachykinins can act directly on NK1 or NK2 receptors on enterocytes to stimulate chloride and bicarbonate secretion. In addition, SP and NKA, acting via NK1 and NK3 receptors on enteric neurones, participate in the transmission to secretomotor neurones, which cause ion secretion through release of acetylcholine and/or vasoactive intestinal polypeptide. On the other hand, tachykinins can be released from axon collaterals of intrinsic sensory neurones close to the epithelial effector cells and elicit chloride secretion via an axon reflex type mechanism (70).

Inflammatory disorders of various aetiologies involve changes in the peptidergic innervation of the gut, and are associated with NK1 receptor upregulation in intestinal blood vessels and lymphoid structures. Thus, in the diseased gut the contribution of tachykininergic neurones seems out of balance and there is a shift away from cholinergic towards tachykininergic regulation. Similarly, in animal experiments, tachykinin receptor antagonists are little active in the normal gut but are able to correct disturbed motility, hypersecretion, tissue homeostasis and pain associated with certain forms of intestinal anaphylaxis, infection and inflammation. Therefore tachykinin receptors are potential therapeutic targets in gastroenterology (71).
VIP. Vasoactive intestinal peptide (VIP) is a neuropeptide with a broad distribution in the body that exerts very important pleiotropic functions in several systems. This neuropeptide could be included in the group of cytokines since it is produced and secreted by different immunocompetent cells in response to various immune signals, plays a broad spectrum of immunological functions, and exert them, in a paracrine and/or autocrine way, through three different specific receptors. Although VIP has been classically considered as an immunodepressant agent, and its main described role has been an anti-inflammatory effect, several evidences suggest that this peptide is also a modulator of the homeostasis of the immune system. In the last decade, the pharmacology of VIP has spectacularly grown, and VIP itself as well as more stable VIP-derived agents have been used or proposed as efficient therapeutic treatments of several disorders, specially inflammatory and autoimmune diseases, such as CD (72).

II. Extracts in clinical features of inflammatory bowel diseases

Remission and relapse - these are the two controversial conditions which are characteristic of the clinical course of both forms of IBD. The goal of the therapy and the management of patients is a long-term symptom-free status. However, there are a significant number of patients who have a chronic permanently active disease with a poor quality of life. They are the candidates of the immunosuppressive and the immunomodulatory – biological – therapy. Although these therapeutic modalities offer a better prognosis, the possibility of the different side effects is also high. The acute phase therapy of a relapse is henceforward steroid treatment. During this therapy the risk of a drug-induced problem is also high and a recent Cochran analysis showed no reduction in relapse with any of the oral steroids at 6, 12 or 24 months and the same applies to Budesonide in controlled trials. So, only 25% of IBD patients treated with steroids are going to be well in remission at the end of the year (73). The others are either going to become steroid dependent or still symptomatic on steroids. Increased expression of glucocorticoid receptor beta and increased expression of the multidrug resistance drug pump P-glycoprotein 170 have been proposed as markers of drug resistance to glucocorticoids (74). Therefore, the provision of appropriate therapy for IBD poses a serious problem to the therapist because of the great variability of the extent and the association with possibly severe complications. A correct diagnostic method should determine the maximum spread of the inflammatory process and should thus reveal fistulas and abscesses without imposing more than minimal strain on these severely ill patients. The severity of IBD and the individual response to the therapy show a great variability as well. Until now there is no such prognostic factor which gives us a significant help in the evaluation of the disease course at the time of the first symptoms. Management of patients with IBD is not only treating the relapse. It is a complex challenge providing medical, psychological and social guidance. The medical part of management is also complex, because there are some special conditions, which can occur during the years after the diagnosis. The most

relevant special problems are extraintestinal manifestations, pregnancy and a risk of malignancies.

A. Subgroups of Crohn's disease

Evidence is accumulating that CD consists of different subgroups, based on the localisation of macroscopic disease, clinicopathological features and the heterogeneous course of the disease. Division of patients into subgroups was carried out according to criteria of the Vienna classification, shortly summarised below:

Age (A): In case the diagnosis is established before the age of 40, the patient is defined to belong to group A1, while when it is established later than the age of 40, to group A2.

Localisation (L): if the maximum extent of the disease is restricted to the terminal ileum, the patient belongs to subgroup L1. If she/he presents with colonic localisation only to L2, if, additionally, the terminal ileum is also involved, then L3, while patients with disease localisation proximal to the terminal ileum regardless to additional involvement are regarded as L4.

Disease behaviour (B): patients with inflammatory disease which presents neither stricturing nor penetrating symptoms are defined as group B1. Patients who have stricturising disease are defined as B2, while patients with penetrating disease are defined as B3 (75).

B. Prognostic significance of epitheloid granuloma in Crohn's disease

Granuloma formation is a host response by the localized accumulation of epitheloid cells, macrophages and lymphocytes. Granulomas can be found in many infective, allergic and neoplastic disorders (76). Although the first description of granulomas in Crohn's disease was published in 1913 by Dalziel (77), the exact role and significance of this "specific" histological lesion remains a puzzle. The probability is that the granuloma is the site where the aetiological agent resides, the antigen specification occurs and the T cell differentiation into Th1 cell starts (78), yet there is no evidence to confirm this theory. Pathological host response is one of the important basic concepts in the development of Crohn's disease besides genetic predisposition and environmental factors. The different individual host responses may explain the presence or absence of granuloms.

The first publication which tried to examine whether the presence of granulomas is associated with good or poor prognosis came out more than forty years ago (79). Only a few studies have been published since then which examine the prognostic role of granulomas, and their results are controversial. The most studied topic was the postsurgical recurrence of CD. Anseline et al. examined several factors that might predict recurrence after operation in 130 patients over a 24-year period. A highly significant positive association was revealed between the presence of granulomas and the likelihood of recurrence ($p=0.003$) on the basis of the multivariate regression analysis (80). Trnka et al reported a similar tendency. They found that there was an increasing chance of recurrence in a subgroup of patients with ileocolonic disease (81). On the other hand, some studies suggest just the opposite (82,83,84). These publications suggest that patients with granulomas have less frequent postoperative recurrence and/or have a better prognosis. These studies were the pioneers in this topic, therefore the selection of patients, the diagnostic and the statistical methods might be less accurate than the ones in the latest publications.

There has been only one study so far in which the presence of granulomas was determined by endoscopic biopsies (85). Markowitz et al examined a paediatric population, and rectosigmoid biopsies were taken from 58 subjects. All biopsies were obtained from newly diagnosed patients before any therapy was started and the children were followed for at least one year. The frequency of granulomas was 32.7%. More severe perianal complications, a higher frequency of

surgery and more extensive inflammatory involvement were observed in patients with granulomas. Medical treatment and the need for hospitalisation were similar in both groups.

C. Clinical value of different diagnostic procedures in active Crohn's disease

a. Computed tomography and leukocyte scintigraphy. CD is a chronic transmural granulomatous inflammatory bowel disease of unknown aetiology, with alternating periods of symptomatic exacerbations and clinical remissions. There are not any specific characteristics of an acute attack because of the different clinical courses and the heterogeneous localisation of the disease. Appropriate therapy of an exacerbation therefore requires the knowledge of the maximum extent of involvement, the behaviour type (inflammatory, stricturing or penetrating), the current clinical activity and the presence of complications. Although endoscopic and radiological examinations are the gold standard procedures to determine the localisation and the severity of inflammation, these methods are not so valuable for the detection of the complications of CD, such as abdominal abscesses and/or suspected fistulas. Furthermore, bowel preparations are relatively contraindicated in patients with severe active disease. Imaging techniques, which do not impose a great load on patients, are therefore of high clinical relevance in the follow-up of CD patients, and especially in those who undergo a severe active relapse. Non-invasive imaging methods, such as isotope-labelled leukocyte or granulocyte scintigraphy and spiral computed tomography are useful in cases involving a suspected complication (86,87), but the accuracy of these techniques in different types of active CD has not yet been well established. Only one comparative prospective study is to be found in the literature, in which the two methods were performed simultaneously and the results were compared with endoscopic and operative findings in patients with inflammatory bowel disease (88).

b. Jejunoscopy. Since the first description by Gottlieb and Alpert in 1937 (89), CD of the upper gastrointestinal tract has been recognised with changing, but increasing frequency. The diagnosis of CD of the upper GI tract was based on clinical, radiological, endoscopic and histological features. Nugent at al. analysing 89 patients with duodenal CD, established the diagnostic criteria of CD involving the upper GI tract. These criteria are the following: 1. Histological evidence of non-caseating granulomatous inflammation of upper GI tract exists with or without obvious CD elsewhere in the intestinal tract, and without evidence of a systemic granulomatous disorder. 2. Documented CD elsewhere in the intestinal tract, and radiological and/or endoscopic findings of diffuse inflammatory changes in the upper GI tract consistent with CD (90). The involvement of the upper gastrointestinal tract has been considered to be a rare manifestation of CD. Retrospective studies have reported prevalence figures of 0.5-13% (91,92). In contrast to these retrospective studies, during the last decade several studies have been performed to evaluate the degree of involvement of upper GI tract in CD. These prospective studies, in which patients with CD underwent routine endoscopic evaluation with biopsies, revealed a much higher frequency of endoscopic and histological abnormalities. These studies have found endoscopic alterations in 22-60% of cases and granulomas in 7-16% of biopsies (93,94).

The diagnosis and the management of an upper gastrointestinal CD is a difficult problem. Therapy for CD of the upper GI tract consists of drug therapy and endoscopic or surgical interventions and is, in fact, similar to that for distal CD. Corticosteroids are still the most important drugs in the treatment of CD of the upper GI tract. Sometimes adjunctive therapy, e.g. gastric antisecretory drugs, mucosa protective agents and, in severe cases, proton pump inhibitors, is beneficial. Endoscopic evaluation of the upper GI tract with biopsies is an important part of the work-up of CD patients (95).

D. Special problems in the management of patients with inflammatory bowel diseases

a. Extraintestinal manifestations. Extraintestinal manifestations are relatively common complications of IBD. Predominantly inflammatory reactions may affect different organs in some 25-50% of IBD patients (96). Inflammation of the joints (musculosceletal manifestations), skin and eyes are the most frequent associations. Identified pathogenic autoimmune mechanisms of the development of extraintestinal manifestations include genetic susceptibility, cytokine imbalances, antigenic display of autoantigen, aberrant self-recognition and immunopathogenetic autoantibodies against organ-specific cellular antigen(s) shared by the colon and the extracolonic organs (97). Microbes may play an important role, probably by molecular mimicry (98).

Usual extraintestinal manifestations of IBD fall into one of the following groups: 1) Reactive conditions, such as acute arthropathy and skin eruptions. These manifestations are more frequent in patients with colonic involvement. 2) Associated conditions, like ankylosing spondylitis and chronic liver disease that may precede or follow the activity of IBD, but not as an immediate response to the presence of an inflamed gut. 3) Consequences of long standing IBD that include metabolic disorders, malabsorption, and renal and genitourinary problems. 4) Consequences of drug therapy, such as neutropenia, interstitial nephritis and folate deficiency anaemia. 5) Other unusual extraintestinal manifestations include pulmonary fibrosis, alveolitis, haematological autoimmune disease, urogenital involvement, carditis, pericarditis and vasculitis (99).

Musculoskeletal manifestations are the most common extraintestinal complications of inflammatory bowel disease. The spectrum of musculoskeletal manifestations in inflammatory bowel disease patients includes all of the clinical features of spondylarthropathies: peripheral arthritis, inflammatory spinal pain, dactylitis, enthesitis (Achilles tendinitis and plantar fasciitis), buttock pain and anterior chest wall pain. Radiological evidence of sacroileitis is common but not obligatory. Joint manifestations begin either concomitantly or subsequent to the bowel disease; however, the onset of spinal involvement often precedes the diagnosis of inflammatory bowel disease. Symptoms usually disappear after proctocolectomy. The pathogenetic mechanisms that produce musculoskeletal manifestations in inflammatory bowel disease are unclear (100). Several arguments favour an important role of the intestinal mucosa in the development of spondylarthropathy. The natural history is characterised by periods of flares and remissions, therefore the efficacy of treatment is difficult to establish. Most patients respond to rest, physical therapy and nonsteroidal anti-inflammatory drugs, but these drugs may activate bowel disease and their use is not recommended in IBD. Sulphasalazine may have a favourable effect in some patients. There is no indication for systemic use of steroids (101).

The term metastatic CD is used when microscopically Crohn-like granulomatous inflammation is observed in an extraintestinal organ simultaneously with the typical involvement of the gastrointestinal tract (102). Skin and genital associations are the most frequent of these uncommon associations (103).

Disorders of the urinary tract are rare but well-known complications of CD, especially with ileal involvement (104). Stricture of the right ureter due to periileal inflammation and nephrolithiasis caused by the defect of oxalate metabolism are the most common associations; fistula formation between the bladder and some part of the ileo-colonic tract are less frequent, but severe complications of Crohn's disease, which often require surgical intervention (105).

Cardiac disorders are among the least common extraintestinal associated reactive conditions. Up to 2001 about 150 IBD patients were reported with associated cardiac diseases as extraintestinal manifestations of IBD or the consequence of drug-induced side effects (Medline Express, SilverPlatter International N.V.). Pericarditis was found to be the most frequent symptom (70% of the cases), followed by myocarditis (10%), cardiac hypersensitivity to 5-aminosalicylic acid or

other drugs (9%), bacterial endocarditis (6%) and AV blockage I/III (5%) (106,107,108). The majority of these cases were interpreted as true extraintestinal manifestations of IBD; only 9 patients were considered to develop cardiac complications as a side effect of treatment (sulfasalazine in 3, mesalamine in 5 and azathioprine in 1 case) (109,110,111,112). Prognosis varies among reported cases, including complete recovery, remission with recurrence and fatal disease.

Osteoporosis is a common problem for individuals with IBD. Corticosteroids play an important role in the development of osteoporosis in these patients; however, active disease and longer disease duration also appear to increase the risk of bone loss. The development of osteoporosis in IBD seems to be a phenomenon related to the disease process and/or the treatment modalities in IBD. By using markers such as interleukin 6, osteocalcin, parathyroid hormone, and N-telopeptide cross-linked type 1 collagen, Pollak et al. showed that chronic IBD is associated with increased bone loss. Some inflammatory factors, like interleukin-6, play a significant role in this process (113).

b. Pregnancy. The management of both male and female patients with IBD who wish to have a baby is challenging. Fertility in women with UC is usually normal, however, the situation for CD is different: fertility is reduced. Although women with UC, who undergo proctocolectomy with ileal pouch-anal anastomosis, are at risk for less fertility after the procedure than before (114). For women the most important factor to bear in mind is that the outcome of pregnancy is largely influenced by disease activity at the time of conception (115). Women with quiescent disease are likely to have an uncomplicated pregnancy with the delivery of a healthy baby, whereas women with active disease are more likely to have complications, such as spontaneous abortion, miscarriage, stillbirth and exacerbation of the disease. This especially stands for patients with CD rather than patients with UC (116). In view of the risk of low birth weight, all women with CD who become pregnant should be followed carefully during pregnancy, paricularly those who have ileal disease or who have previously undergone bowel resection (117). Furthermore, smoking cessation needs to be aggressively pursued in these patients. Although the safety of medications used during pregnancy is an important issue, the impact of the medications used to treat IBD is less important in comparison to disease activity itself. 5-Aminosalicylic acid (5-ASA) products appear to be safe during pregnancy; corticosteroids are probably safe; 6-mercaptopurine and azathioprine should be used with caution (118); and methotrexate is contraindicated (119). There are inadequate data on the use of infliximab during pregnancy. In regard to men with IBD, the disease itself does not seem to have any negative impact on fertility. However, the effect of 6-mercaptopurine and azathioprine use prior to and during fertilisation is still controversial. In view of possible adverse pregnancy outcomes, it would be wise to withhold 6-mercaptopurine and azathioprine therapy in men with IBD for 3 months prior
to conception, when feasible (120). Most IBD medications should be continued before, during, and after pregnancy, with careful attention to the known cautions and exceptions. If IBD in a pregnant patient is in remission, the prognosis for pregnancy is the same as if she did not have IBD. Active disease should therefore be treated aggressively and remission accomplished before pregnancy is attempted. Similarly, a woman who unexpectedly becomes pregnant while her IBD is active should be treated aggressively, as remission remains the greatest investment for a favourable pregnancy outcome. However, there are not enough data how on pregnancy itself affects the activity signs of IBD or what the risks of a relapse during pregnancy and breast-feeding are.

c. Risk of carcinoma in IBD. Although Crohn is known for his description of regional ileitis, the first case of cancer in IBD reported and described was actually one of ulcerative colitis-

associated rectal cancer by Crohn and Rosenberg in 1925 (121). It was 66 years after the classic description of UC by Sir Samuel Wilks in 1859 (122). The colon cancer complicating "regional enteritis" was first described more than 50 years ago (123). Since then there have been many reports on colorectal cancers in series of patients with CD (124). On the evidence of these publications it turned out that there is an analogy in the incidence of colorectal cancer in ulcerative and Crohn's colitis of similar duration and extent (125). IBD cancers are preceded by dysplasia, and the relative risk increases with duration of the IBD. CD cancers are more proximally distributed than UC cancers. Both tend to occur at the site of the overt disease and both develop at earlier ages (47 UC, 50 CD) than in the de novo colorectal cancer (70 years). The absolute cummulative colon cancer frequencies (8% UC, 7% CD) are identical after 20 years, emphasising the importance of regular surveillance in both types of IBD (126). The duration and the extension of the inflammation are the two most significant risk factors of the development of colorectal cancer in IBD patients. Some of the data indicate that patients with CD older than 45 years and/or those with a recent change in symptoms may be at particular risk (127). Moreover, the increased risk of colon cancer exists in patients with CD even when CD is confined to the small bowel, and patients with IBD have increased risks of developing extraintestinal and reticuloendothelial tumours in both CD and UC, as well as ano-vulval and malignant melanoma in CD. Cholangiocarcinoma is a usual tumour association in UC patients suffering from PSC, and there is a tendency of an increased risk of developing lymphoma among males with CD (128). Colorectal cancers associated with IBD are often diffuse, extensive, multiple and right-sided with insidious presentation. The prognosis is not worse after operation than that of de novo colon cancer. Most small bowel cancers in CD are adenocarcinomas, rather than sarcomas, and present at a younger age, more diffusely and more distally than de novo cancers. Because of this feature adenocarcinomas are usually invisible for the diagnostic procedures at a curable early stage; indeed, two-thirds of the tumours present themselves with intestinal obstruction (129).

Strictures of the colon are common in patients with IBD and they have a 10-fold risk of colon cancer. The risk increases with disease duration. Indications for surgery are absolute, relative and incidental. Procedures include segmental resection, total proctocolectomy, subtotal colectomy and palliative surgery (130).

Therefore it is recognised that IBD predisposes to the development of colorectal adenocarcinoma, and the molecular pathway of this process differs from that of sporadic colorectal cancer. However, several important details regarding the risk factors of and the molecular changes underlying IBD-related colorectal carcinogenesis have only come to light lately. First, recent data suggest that environmental factors related to long-standing inflammation contribute more to this increased cancer risk than inherited susceptibility (131) Second, molecular changes that may represent the first steps in the development of neoplasia are being increasingly identified in non-dysplastic, colitic mucosa (132). Third, there is now good evidence suggesting that IBD-related colorectal cancer may develop along more than one molecular pathway (133).

It is strongly recommended that all patients with extensive Crohn's and ulcerative colitis be kept in a registry and that the same surveillance programs already used in UC be strongly considered for patients with extensive Crohn's colitis. Only future prospective studies will determine the appropriate time intervals for surveillance (134). Dysplasia associated lesion/mass (DALM) and sporadic adenoma can be diagnosed during the colonoscopy. As origin of a lesion from within colitic bowel is not always obvious endoscopically, there has been recent interest in ways of distinguishing between DALMs and sporadic adenomas occurring in UC (135). This interest has been particularly stimulated by suggestions that sporadic adenomas can be dealt with by polypectomy alone, whereas a diagnosis of a DALM indicates proctocolectomy (136). Several

clinicopathological features may help distinguish between the two lesions. Compared with sporadic adenomas in UC, DALMs are more likely to show or associate with the following: younger patient; active colitis; longer duration of UC; tubulovillous or villous change; inflammation of the polyp stroma and epithelium; and a mixture of normal and dysplastic superficial epithelium (137). As, however, there is not one feature that absolutely distinguishes these two types of lesions (138), a variety of analyses have been tested to facilitate this procedure. While again no absolute distinguishing marker has emerged, the presence of immunostaining for p53 and lack of bcl2 and nuclear-catenin immunostaining appear to favour a diagnosis of DALM over sporadic adenoma. Molecular studies have shown further differences between the two lesions, with LOH at the von Hippel-Lindau (vHL) (3p) and the p16ink4a (9p) gene loci occurring in 30-70% of DALMs compared with < 15% of adenomas (139).

III. Immunosuppressive treatment of IBD

Immunosuppressive drugs have proved their efficacy in the management of patients with a complicated form of IBD. Immunosuppressor agents are primarily of use to maintain remission and to achieve steroid sparing in patients with chronic active disease. They are likely to be increasingly used due to the recognition that corticosteroids, including budesonide, do not provide effective maintenance therapy for either froms of IBD. The most commonly used immunosuppressive agents are azathioprine, 6-mercaptopurine (this drug is not available in Hungary), cyclosporine and methotrexate. Cyclosporine is mainly effective in acute cases, it can induce remission mainly in UC, while the other three drugs are optimal for maintaining remission in both forms of IBD.

A. Azathioprine

Azathioprine/6-mercaptopurine should be tried first in both chronic active or steroid dependent CD and UC at a dose of 1.5-2 mg/kg/24 h, respectively. The thioguanine derivative, azathioprine, is a prodrug of 6-mercaptopurine that is further metabolised by various enzymes present in the liver and gut. During azathioprine – 6-mercaptopurine metabolism imidazole derivates are formed and they can cause fever, rash and nausea in some individuals. These symptoms can occur within a few hours after the drug intake. This mechanism is called early azathioprine intolerance (140). Azathioprine and 6-mercaptopurine have been used in the treatment of IBD, i.e. UC and CD, for more than 30 years. However, widespread use of azathioprine or 6-mercaptopurine in inflammatory bowel disease is of more recent origin, the primary reason being a long-standing debate on the efficacy of these agents in IBD. Both drugs are slow acting, which is why clinical efficacy cannot be expected until several weeks or even months of treatment have elapsed (141).

In CD, a large number of controlled trials recently meta-analysed (142) showed a 60-70% response rate (possibility to stop steroids or reduce their dose to <10 mg/24 h, while maintaining clinical remission), a 20-25% failure rate and ~10% of drug interruption because of side effects. The effectiveness of these drugs is not well documented in UC. When remission is achieved, azathioprine/6-mercaptopurine discontinuation leads to a high relapse rate (~40% within the first year) (143). The useful duration of therapy is not known yet, most of the authors recommend at least a four-year-long therapy. Preliminary evidence suggests that discontinuation of the drug after this long period is followed by a low relapse rate, but a controlled trial should be performed (144). Today, azathioprine and 6-mercaptopurine are the most commonly used immunomodulatory drugs in the treatment of IBD. Their clinical effects are probably identical,

although their exact mode of action is still unknown. The azathioprine's mode of action is thought to be multifactorial. The first step is a conversion to 6-mercaptopurine (which acts as a purine antimetabolite), a possible blockade of thiol groups by alkylation. The next effects are the inhibition of several pathways in nucleic acid biosynthesis (preventing proliferation of cells involved in the determination and amplification of the immune response) and damage to DNA through the incorporation of thiopurine analogues. However, 6-thioguanine nucleotides may accumulate in toxic doses in myeloid precursor cells, resulting in life-threatening myelosuppression. Azathioprine and 6-mercaptopurine are further known to alter lymphocyte function, reduce the number of lamina propria plasma cells and affect natural killer cell function (145). Measurement of 6-thioguanine nucleotide concentrations may be useful for optimising treatment with azathioprine and 6-mercaptopurine. Polymorphisms at three loci in the thiopurine methyltransferase gene are known to be responsible for azathioprine and 6-mercaptopurine toxicity. A baseline determination of thiopurine methyltransferase phenotype or genotype may predict early leukopenia in patients treated with azathioprine or 6- mercaptopurine (146). A loading dose of intravenous azathioprine does not accelerate the time of response in patients with steroid-treated CD (147).

B. Methotrexate

When purin analogues are discontinued for failure or adverse effects, methotrexate may be used as a second-line drug in chronic, active steroid-dependent CD. It acts through both immunomodulatory and anti-inflammatory effects. A 40% success rate (defined as steroid discontinuation and clinical remission) versus 20% in the placebo group was shown by the main controlled trial (148). It should be given at the dose of 25 mg once a week, intramuscularly (im.) or subcutaneously. Im. administration is not easily recommended in clinical practice. It is associated with high medical cost and compromised patient quality of life because of the need for increased clinic visits. Frequent use of im. injections have also been reported to cause peripheral nerve injury, local irritation, pain, bleeding, fibrosis, abscesses (sterile or non-sterile), gangrene, and contractures (149,150). As an alternative method of parenteral administration, subcutaneous injection has been
shown to have similar pharmacokinetics to im. injection, yet it is easier to administer and leads to reduced local toxicity at the injection site and greater patient comfort than im. injection. There are some narrative data about the beneficial effect of methotrexate given orally, although two randomised controlled trials, using orally administered methotrexate at 12.5 mg weekly and 15 mg weekly, respectively, failed to show the efficacy of methotrexate in chronic active CD (151)

Methotrexate inhibits the enzyme dihydrofolate reductase, and its metabolites strongly inhibit folate-dependent enzymes distal to dihydrofolate reductase (152,153). The net result of methotrexate therapy consists of impaired thymidylate and purine production, impaired folic acid metabolism, inhibition of methionine production, and local accumulation of adenosine. Although cancer therapy with high-dose methotrexate takes advantage of its antiproliferative effect (154), the cellular and physiological mechanism of low-dose methotrexate in inflammatory conditions is still unclear. Proposed theories include: - immunomodulation through inhibition of leukocyte proliferation, - induction of apoptosis of activated T cells, - inhibition of proinflammatory cytokine production (e.g. IL-1, IL-2, IL-6, and IL-8), - decreased arachidonic acid metabolism, - especially leukotriene B4 production, - impaired immunoglobulin synthesis, and local accumulation of adenosine (155, 156, 157, 158, 159). Methotrexate acts rapidly and may be stopped at 1-2 months of treatment if ineffective. The optimal duration of methotrexate therapy is

unknown, although the cumulative dosage of 1.5 g is certain to cause hepatic damage. A pre-treatment liver biopsy is indicated in patients who have abnormal liver function tests and in those at potentially increased risk of hepatic toxicity. Follow-up liver function tests are not a good predictor of toxicity. Therefore, it is prudent to perform a liver biopsy after a patient has received a cumulative methotrexate dosage of 1.5 g and periodically thereafter (160).

C. Anti-TNFα therapy

Tumour necrosis factor plays an important role in mediating the inflammation of CD. Strategies aimed at reducing TNF level in patients with CD include the mouse/human chimeric monoclonal antibody infliximab, the humanised monoclonal antibody CDP571, the human recombinant TNF receptor fusion protein etanercept, and the small molecule thalidomide. CDP571 is effective in treating active CD, steroid sparing, and, possibly, in closing fistulas and maintaining remission. Side effects occurring in patients treated with CDP571 include anti-idiotype antibodies, infusion reactions, and formation of autoantibodies. Pilot studies have suggested that etanercept and thalidomide may also be beneficial (161, 162,163,164).

Infliximab (Remicade; initially known as cA2) is a chimeric IgG1 monoclonal antibody composed of 75% human and 25% murine sequences. This antibody has been demonstrated to have high specificity and affinity for TNF. When given as a single intravenous infusion of 5 mg/kg of body weight, infliximab has a half-life of about 10 days. Pharmacokinetic data have demonstrated that this agent does not accumulate when given in 3 doses at 2- to 4-week intervals or when given in repeated doses at 8-week intervals (165).

In the initial multicenter study, Targan and colleagues (166) reported the first phase of a randomised, double-blind, placebo-controlled efficacy trial. The dose-ranging portion of the study identified the most effective dose (5 mg/kg given as a single dose), and the re-treatment phase reported the efficacy of repeat infusions at a dose of 10 mg/kg given every 8 weeks for maintenance of remission. The following studies gave evidence that infliximab is effective in treating active CD, maintaining remission and closing fistulas. Side effects occurring in patients treated with infliximab include human anti-chimeric antibodies, infusion reactions, formation of autoantibodies and rarely drug induced lupus (167,168,169).

D. Mycophenolate mofetil

Mycophenolate mofetil is of proven efficacy and safety in transplantation and in some autoimmune disorders. It is an inhibitor of purine synthesis and could constitute an alternative therapy of CD. The preliminary data are promising, it is possible that this novel immunosuppressor is effective in the treatment of chronic active and perianal CD in patients who failed or were intolerant of 6-mercaptopurine or azathioprine (170,171).

Chapter 2. Aims

I.A Genetic examinations

a. The Interleukin 1ß and Interleukin-1 Receptor Antagonist gene polymorphism in Hungarian population with inflammatory bowel disease.
To our knowledge, no investigations have been performed on genetic polymorphism in IBD in the region of Eastern Europe. We have studied a selected and mixed Hungarian population, which is unique in the sense that in Europe it is ethnically related only to the Finns. Our aim was to test the hypothesis that the association of certain IL1ß and IL1RA genotypes is significantly different in IBD patients compared with an ethnically matched Hungarian population.
b. Interleukin 1ß gene polymorphism and the course and the severity of inflammatory bowel disease.
Genetic predisposition to suffer from chronic IBD is well recognised (172,173,174,175). Both CD and UC patients can be subdivided into a number of subgroups based on the course of the disease, clinical markers, and pathology.
Two biallelic base exchange polymorphisms are described in the IL1ß genes which influence IL-1ß production. So far no association has been found between the IL1ß-511 or the IL1ß-c3953 gene polymorphisms and patients with CD or UC (176,177,178,179). Recently CD patients with perianal fistulas have been found to have significantly different DRB1 allelic frequencies compared with healthy controls (HC). The IL-1RA allele 2 were predominantly found in patients with UC, who developed extensive colitis (180). This suggests a different genetic background for subgroups of patients with CD. Part of the above mentioned data raise the possibility that allelic variations IL1ß are important in the manifestation of different phenotypes in IBD. Therefore, our aim was to analyse the frequency of IL1ß allelic combinations in IBD,especially in relation to severity factors, such as fistula formation and operation in CD, and the extension and severity of UC.

IB. Studies concerning environmental factors

a. The role of environmental factors in the pathogenesis of inflammatory bowel disease.
Environmental factors have an important role in the initiation of IBD. A lot of studies examined these factors with smoking habits being the most frequently researched element (181,182). The protective role of smoking in UC, and its harmful effects in CD are well-known thanks to epidemiological studies. The effect of passive smoking and oral contraceptives on the pathogenesis of IBD are more confusing. There are not enough data about the impact of coffee and alcohol consumption before the onset of IBD. The majority of the previous studies examined IBD patients compared to a similar group of healthy population. Not enough data are available concerning the fact that environmental factors, which may have a role in the etiopathogenesis of IBD, are also involved in other autoimmune diseases. Therefore the aim of the epidemiological examinations was to investigate the etiologic factors possibly involved in the pathogenesis of inflammatory bowel diseases as compared with a group of patients with autoimmune diseases and with a healthy control group.
b. The effect of smoking on the activity of ulcerative colitis.
Smoking is a protective factor against UC, so this bad habit can decrease the incidence of UC. There is not clear evidence regarding the connection between smoking started after the onset of disease and the activity symptoms. We have been making allowances for our IBD patients to

change their smoking habits during the follow-up visits. Therefore we were able to analyse how the activity of IBD changes under smoking. Our goal was to investigate the effect of the initiation of smoking on the activity symptoms of UC.

IC. Neuropeptides and inflammatory bowel diseases

It has been hypothesized that an unbalanced function of the enteric nervous system and the regulatory neuropeptides may profoundly influence the pathophysiology of acute and chronic intestinal inflammation, contributing to the motor, secretory and immunological disturbances which characterize human IBD, but their significance in the pathogenesis of CD is controversial. Our aim was to study the amount and distribution of enteric ganglion cells (GCs) as well as different neuropeptide positive nerve fibres in surgically resected bowel segments involved by active CD.

II. Clinical studies

A.Prognostic value of granulomas in Crohn's disease
Granulomas are considered to be the hallmarks of microscopic diagnosis in Crohn's disease, but granulomas can be detected in only 40-50% of surgically removed bowel segments of CD patients (183). The frequency of granulomas in bioptic samples varies according to different authors (184,185). The severity of the disease shows great variability as well. Several factors (e.g., localisation of the disease, maximum extent of involvement, behaviour type: inflammatory, stricturing or penetrating) influence the clinical course of the disease. There are inconsistent data in the literature about the prognostic significance of granulomas in CD. Our aim was to divide our newly diagnosed CD patients into two subgroups on the basis of the presence or absence of granulomas at the time of diagnosis. We have been accomplishing follow-up studies for at least two years in order to get information about these two different groups (patients with or without granuloma), whether they show any difference in the clinical course.

B. Clinical value of different diagnostic procedures in Crohn's disease
a. Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease.
Provision of appropriate therapy for acute relapse of CD poses a serious problem to the therapist because of the great variability of the extent and the association with possibly severe complications. A suitable diagnostic method should determine the maximum spread of the inflammatory process and should thereby reveal fistulas and abscesses without improsing more than minimal strain on these severely ill patients. Both computed tomography (CT) and leukocyte scintigraphy (LS) are able to meet these requirements (186,187), but the radiation dose of CT is about fourfold higher. The aim of the present study examinations was to evaluate and compare the diagnostic accuracy of technetium-99m-HMPAO-labeled leukocyte scintigraphy (LS) and spiral computed tomography (CT) on the basis of clinical parameters and endoscopic, radiological and surgical findings in patients with clinically active CD. The comparison of these techniques was necessary because only 17 CD patients had been reported previously, in whom these two methods were performed simultaneously without the awareness of the behaviour type of the disease. Therefore, the other important goal was to determine the diagnostic efficacy of these two commonly used non-invasive methods in the different subgroups of CD.
b. Clinical significance of jejunoscopy in Crohn's disease. CD is rarely limited to the upper gastrointestinal (GI) tract alone; the association of gastroduodenal lesions with ileal and/or

colonic involvement occurs more frequently. The frequency of the oesophageal, gastric and duodenal involvements is more or less known. Not enough data are available about the frequency of jejunal involvement and, since these lesions frequently remain undiagnosed due to the lack of the endoscopic studies for the evaluation of jejunum, the endoscopic characteristics and clinical signs of jejunal CD are also almost completely unknown. Furthermore, histology and immunohistochemistry of oesophageal, gastric and duodenal mucus is also still ill-defined in CD patients. Epitheloid granuloma and focally enhanced gastritis are the most common characteristic features of gastric histology in CD, but the typical endoscopic and microscopic pictures of the lower part of the duodenum and the jejunum have not been thoroughly studied. The majority of histological analyses of the stomach in CD were performed without the awareness of Helicobacter pylori. However, since Helicobacter pylori is the most frequent cause of gastritis and the most important etiologic factor in peptic ulcer disease, it is important to assess the contribution of H. pylori in the interpretation of the abnormalities observed in the upper GI tract in patients with CD. On the basis of the above mentioned data, our goal was to perform a prospective complex analysis of the clinical, endoscopic, pathologic and immunohistochemical features of the upper GI tract (from the oesophagus to the proximal part of the jejunum) in patients with diagnosed small and/or large bowel CD. We wanted to be informed about the Helicobacter pylori status of the examined patients and the frequency of granulomas in different parts of the upper gastrointestinal tract. We performed an analysis of the inflammatory cells with immunohistochemical methods to find out what type of immunological alteration is characteristic of CD of the upper gastrointestinal tract.

C. Special problems associated with inflammatory bowel diseases

a. Extraintestinal manifestations. Careful clinical observation showed that almost all organs could be affected in IBD. Some of the extraintestinal manifestations may precede IBD, although the majority accompanies the underlying disease and are influenced by its activity. Prompt recognition of extracolonic organ involvement in IBD is important because of the relative refractoriness for the therapy and a possible increase in morbidity and mortality. Therefore during the follow-up of patients particular attention should be paid to identify extraintestinal symptoms in time. Our aim was to diagnose the rare extraintestinal complications of IBD. We were also interested in cardiac and urogenital manifestations. Despite a relatively high number of reported CD cases with bladder fistula, we have been unable to find any previous cases with proven metastatic granulomatous lesion of the urinary bladder.

b. Effect of pregnancy on the activity of inflammatory bowel disease. IBD is predominantly the disease of young people therefore it often coincides with the childbearing age. There are some publications about the pregnancy and delivery of female patients with IBD. However, previously only a few groups had examined the reverse of the medal, namely the impact of pregnancy on the activity of IBD. The results of these publications are controversial. The aim of our study was to retrospectively examine the effect of pregnancy on the course of IBD. We analysed the data of each pregnancy which had occurred up to the time of the analysis. The other important part of our work was to investigate the change of clinical symptoms during breast-feeding. We would have liked to know when treatment should be restarted if it was stopped before conception.

c. Carcinomas and inflammatory bowel diseases. Association of a malignant tumour is a possible risk of long-standing, extended IBD. Aggregation of carcinomas in a patient with IBD is an uncommon clinical problem, no such case reports have been published yet. Although some cases of gastric malignancy have been reported in patients with CD, the association remains

controversial, particularly because most patients have not had gastric CD involvement. We observed the case of a 59-year-old female patient with CD and associated gastrointestinal malignancies (rectal and gastric cancer with metastasis in the skin). Our aim was to determine the immunophenotype of these carcinomas. Using this method, we wanted to adjudge whether the microscopical and immunohistochemical structures of the tumours were similar or different, if these were synchronic or metachronic carcinomas, that is if they were different or not in their origins according to examinations. We tried to collect data about the pathogenesis of these tumours retrospectively.

III. Our experiences with the use of immunosuppressive and immunomodulator therapy

A significant part of our patients with IBD are on immunosuppressive therapy. The indication, the effect and the risk of this therapy become more and more obvious due to studies published recently mainly in USA. In Hungary, only unpublished data have been announced at some congresses, therefore it seems that the relation between IBD and immunosuppressive therapy is not clear enough.
During the past years, several multicenter studies examined the safety and the effectiveness of Remicade. This chimeric IgG1 monoclonal antibody proved to be effective in the management of chronic active, therapy resistant and fistulizing CD. Our department was one of the Hungarian centres, where we treated ten CD patients.
We would have liked to collect the clinical data of each patient on immunosuppressive and biological therapy with a retrospective analysis of the last two years. Our aim was to review our clinical experience of the use of immune modulator therapy in both forms of IBD. We examined the indication of therapy, the safety of the different drugs and their effect on remission.

Chapter 3. Patients and methods

The ethical committee of our department approved all study. Informed oral consent was obtained from the patients before the participation in the studies.

IA. Genetic examinations

Patients. Ninety-six UC, 97 CD patients and 132 randomly selected ethnically matched healthy individuals were included in the study. All were unrelated white Caucasian subjects from three IBD centres in Hungary. Half of the IBD patients and all controls were randomised from our centres. The diagnosis of UC and CD was determined on the basis of conventional radiological, endoscopic and histological criteria as described by Lennard-Jones (188). Radiology and/or endoscopy determined the localisation of the disease. Table 2 shows the demographic and clinical features of the patients. Classification of patients' subgroups (fistulizing and non-fistulizing) was determined on the criteria set by Sachar and co-workers.

Methods of DNA isolation. Genomic DNA was extracted from ethylenediamine-tetraacetate – anticoagulated peripheral blood according to the standard proteinase-K digestion and phenol-chloroform extraction method.

a. Significant differences in the Interleukin 1ß and Interleukin-1 receptor antagonist gene polymorphism in a Hungarian population with inflammatory bowel disease

IL1ß TaqI restriction fragment length polymorphism. The region containing the TaqI polymorphic site within exon 5 of the IL1ß was amplified by the polymerase chain reaction (PCR). The oligonucleotides 5' GTTGTCATCAGACTTTGACC 3' and 5' TTCAGTTCATATGGACCAGA 3' flanking this region were used as primers. Amplification was performed by using a thermal cycler, Perkin-Elmer 9600 (Perkin-Elmer, Norwalk, Conn., USA): first, 97°C for 90 sec, 55°C for 90 sec, and 74°C for 60 sec (three cycles), and second, 32 cycles: 97°C for 30 sec, 55°C for 30 sec, and 74°C for 30 sec. A final incubation at 72°C for 10 min and cooling to 4°C was then performed. The PCR products were analysed by electrophoresis on 2% agarose gel stained with 0.1% ethidium bromide. TaqI digestion of the 249-bp fragments resulted in fragments that either remained intact (allele 2) or were cut in two segments of 135 bp and 114 bp (allele 1). The resulting fragments were analysed by electrophoresis on 3% agarose gel containing 0.1% ethidium bromide.

IL1RA gene polymorphism. The region in the second intron of the IL1RA gene, containing variable numbers of an identical tandem repeat (VNTR) of 86 base pairs, was amplified by PCR. The oligonucleotides 5' CTCAGCAACACTCCTAT 3' and 5' TCCTGGTCTGCAGGTAA 3' flanking this region were used as primers. The program was initial denaturation at 94°C for 1 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, elongation at 72°C for 5 min, and cooling at 4°C. The PCR products of 410 bp (allele 1, four repeats of the 86-bp region), 240 bp (allele 2, two repeats), 500 bp (allele 3, five repeats), 325 bp (allele 4, three repeats), and 595 bp (allele 5, six repeats) were analysed by electrophoresis on 2% agarose gel containing 0.1% ethidium bromide.

Statistical analysis. Allele frequencies and genotypes of IL1ß and IL1RA were compared in the study group by means of chi-square and, when appropriate, Fisher's exact test (2x2 contingency tables) was used. In order to study whether the IL1ß polymorphism acts synergically with the IL1RA gene polymorphism in susceptibility to IBD, individuals were at first divided into carriers and non-carriers of allele 2 at each polymorphic locus. The strength of the association between these pairs of genes was estimated with the odds ratio (OR).

b. Interleukin 1ß gene polymorphism and the course and severity of inflammatory bowel disease
IL1ß polymorphism typing. Polymerase chain reaction (PCR) was used to identify the genotype status of the two polymorphic regions. Primers used for the region containing a single base pair (bp) polymorphism at position –511 in the promoter region (189) were: 5' TGGCATTTGATCTGGTTCATC 3' and 5' GTTTAGGAATCTTCCCACTT 3'. PCR amplification (Perkin-Elmer, Norwalk, Conn.) was performed according to the following parameters: 94 7C for 1 min followed by 30 cycles at 94 7C for 1 min, 55 7C for 1 min, and 72 7C for 1 min. The PCR amplification followed by an Ava (Gibco BRL, Life Technologies, Rockville, Md.) digestion resulted in fragments that either remained intact (allele 2) or were cut in two fragments (allele 1). Fragments were analysed by electrophoresis on a 2% agarose gel containing 0.1% ethidium bromide. The region that containing the Taq I polymorphic site within the exon 5 of the IL1B gene was analysed as described earlier in our laboratory.
Statistical analysis. The Mantel-Hanszel method was applied to compare allele frequencies and carriage rates of the two IL1B gene polymorphisms between the study groups, and when the appropriate Fisher's exact test was used (SAS/STAT 6.12.; Sas Institute Inc., Cary, N.C.).
In order to determine whether the IL1B polymorphisms act synergistically with each other in susceptibility to IBD, individuals were at first divided into carriers and non-carriers for allele 2 at each polymorphic locus. The strength of the association between these pairs of genes was estimated by odds ratio (OR) and 95% confidence intervals (CI). The probability values for comparisons between subgroups were corrected for multiple comparisons, taking into account the number of parameters considered.

B. Studies concerning environmental factors

a. Environmental factors predisposing to inflammatory bowel disease
Methods. Patients with UC and CD were studied retrospectively by using a questionnaire to assess the factors that may play a role in the development of the diseases. Patients with different autoimmune diseases were selected as controls. The results were analysed using the chi-square test.
Patients. 112 patients with inflammatory bowel disease were studied. There were 63 patients with UC (34 female, 29 male; at start of the disease: 30.25 years old on average) and 49 patients with CD (31 female, 18 male; at start of the disease: 29.40 years old on average). The third group of patients was 64 patients with different autoimmune diseases (59 female, 5 male; at start of the disease: 38.04 years old on average). This latest group consisted of 27 patients with systemic lupus erythematousus, 20 patients with primary Sjögren's syndrome, 8 patients with rheumatoid arthritis, 8 patients with ankylosing spondylitis, and 1 patient with polymyositis.
b. Effect of smoking on the activity of ulcerative colitis.
Based on our computerised patient registration system a retrospective analysis was performed about the smoking habits of our patients with UC. During the period between November 1987 and June 1994, out of 260 regularly controlled patients with UC 221 were non-smoker, 19 were smoker, while 20 patients changed his/her smoking habit. Out of them, 13 patients started to smoke. Their UC was diagnosed earlier on the basis of standard endoscopic and histological criteria. There were eight male and five female patients; their median age was 37.4 years (range: 25-53). The median duration of UC was six years. The extension of the inflammatory process was the following: six proctitis, six procto-sigmoiditis and one pancolitis.
We examined the effect of starting smoking by comparison of the activity signs of UC during a half-year period before and after the initiation of smoking. Data of two controls of each patient

from both periods were available. The general well-being, number of stools, number of stools containing blood, number of mucus and bloody mucus, number of all defecation have been registered by the patients for two weeks up to the controls. The median value of these activity signs of both twenty-six visits (from the period preceding and following the onset of smoking) was calculated and compared. The changes in laboratory values characteristic of the activity of UC, sedimentation rate (mm/h), serum fibrinogen level (mg/dl), haematocrit (%), platelet count (G/l), serum iron level ((mol/l), as well as serum sodium level (mmol/l), were also studied in six out of thirteen patient.
The medication of each patient was also recorded, therefore we were able to compare to median 5-ASA, sulphasalazine as well as steroid dose.
Statistics. Data are expressed as means / standard deviation. Correlation coefficients were calculated by using Student's T- test.

C. Neuropeptides and inflammatory bowel diseases

Patients with CD. The diagnosis of CD was based on case-history and clinical examinations as well as endocopic and histopathological finding. Specimens of small and/or large bowel was taken from the resected bowel. The cause of the surgical intervention was severe complication or failure of the conservative treatment in all cases. None of the patients were operated on for dysplasia or cancer. Surgically removed bowels of twenty patients with CD (9 females and 11 males; mean age: 34.47 years; disease localisation: 16 ileo-colonic, 3 isolated colon and one duodenal; disease behaviour: 9 penetrating, 11 stricturing; histology: 12 granuloma positive and 8 negative) were examined using immunohistochemical techniques.
Controls. Eight patients (all males, mean age: 63.28 years) operated on for colon carcinoma and without signs of inflammatory bowel disease were used as controls. No patient had bowel obstruction or other additional colonic disease. Several whole-wall specimens were taken from a region at the farthest possible distance from the tumuor. The specimens were stained for routine histological examinations.
Immunohistochemistry. Immediatly after resection, the specimens were immersed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.2, for 24 hours and transferred to 20% sucrose in PBS. Then 10-μm cryostat sections were cut on chrome-alum-gelatin coated glass slides. The sections were immunostained using standard methods (190). Immunsera were made by Dako, Copenhagen. Peripherin, an intermediate filament which is a specific marker for peripheral neurons was used to label enteric ganglion cells. Neuropeptide positive cells were immunostained for chromogranin-A (CgA) (secretory protein highly sensitive for each type of neuroendocine cells), serotonin, substance P and vasoactive intestinal polypeptide (VIP). The positive cells were scored on a scale of 0 to 3+ in each layer of the bowel wall.
Statistical analysis. Mann-Whitney and Kruskal-Wallis tests were used as statistical methods.

II. Clinical studies

A. Prognostic significance of epitheloid granulomas in Crohn's disease
Patients. Fifty-six patients with recently diagnosed CD were included in the study during the period from January 1996 to September 1999. Their CD was diagnosed on the basis of standard clinical, radiological, endoscopic and histological criteria. The female/male ratio was 31/25, the median age of patients was 36.29 years (range:18-65). The localisation of CD was as follows: in 33 patients both the small and large bowels were involved (among them, in 6 patients upper GT

involvement, oesophageal, gastric and duodenal, was also observed), in 18 patients only the large bowel, while in 5 the terminal ileum alone was inflamed.
Clinical parameters. Blood samples were taken from all patients on each visit to determine the haemoglobin and hematocrit, as well as the C reactive protein (CRP) values. The Crohn's disease activity index (CDAI) (Best) (191) was calculated to measure the clinical disease activity.
Localisation of CD, endoscopic activity index (EAI). To determine the extent and the severity of the involved bowel segments physical examination, abdominal ultrasound, jejunoscopy, small bowel enteroclysis and colonoscopy were performed in all patients at the time of the first admission, when the study started. Four large bowel segments (the caecum and ascending colon, the transverse colon, the descending colon, and the sigmoid colon and rectum) were examined by colonoscopy and scored according to the findings. The proximal small bowel segment alterations (jejunum together with the duodenum) were detected by jejunoscopy, and scored by using the protocol of Mary and Modigliani with some modification (0 = normal mucosa, 1 = oedema, erythema, granularity of the mucosa, and aphthous lesions, 2 = sporadic or superficial ulcerations, and 3 = extensive deep ulcerations, and stenosis) (192). The severity of the inflammation in the small bowel was established by enteroclysis in 43 patients, while in 13 patients the terminal ileum was also inspected during colonoscopy. The radiological findings were classified by the same radiologist as normal (0), oedema and granularity of the mucosa (1), ulcerations (2), and stenosis (3). (193) The total of the proximal (duodeno-jejunal) and distal small bowel (ileal) scores were applied to show the small bowel activity. This way these diagnostic methods could produce quantifiable data about the severity of the inflammation in each part of the gastrointestinal tract.
Disease behaviour. According to the behaviour of the inflammation (Vienna Classification) (194) patients were divided into three subgroups: 23 patients were in the inflammatory (non-stricturing, non-penetrating) type, 15 patients in the stricturing and 18 patients in the penetrating-fistulizing type of CD. Two groups of patients were created according to the presence (group I) or absence (group II) of granuloma in any bioptic samples.
Follow up. The schedule of follow up visits depended on the actual severity of CD. The completely symptom-free patients were controlled every three months, and naturally, patients with active disease were observed more frequently. Two gastroenterologists controlled all the patients.
Statistics. Data are expressed as means ± standard deviation. Correlation coefficients were calculated by using student's T and Chi-Square tests.

B. Clinical value of different diagnostic procedures in Crohn's disease

a. Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease

Patients. 28 patients who had suffered an acute relapse or a severe first attack of CD were eligible for inclusion in the study during the period from January 1998 to March 1999. Their CD was diagnosed on the basis of standard clinical, radiological, endoscopic and histological criteria. Jejunoscopy, small bowel enteroclysis, colonoscopy, LS and CT were performed in all patients within 7 days of admission. The median age of the patients was 32.5 years (range: 18-59). The median duration of CD was 7.47 years (range: 0.1-25).
Clinical parameters. Following admission, a blood sample was taken to determine the sedimentation rate, haemoglobin content, thrombocyte and leukocyte counts, C reactive protein (CRP), and serum iron, albumin and fibrinogen levels. Crohn's disease activity index (CDAI) and van Hees index (195) were also calculated as disease activity parameters. The above mentioned investigations demonstrated the extent of involvement of the inflammatory process in all patients.

The maximum extents of CD were as follows: 7 patients with isolated small bowel, mainly ileal, localisation (5 ileal, 1 jejunal + ileal, and 1 duodenal + ileal), 10 with a colonic localisation only, and 11 patients with an ileo-colonic localisation. The clinical characteristics of patients are summarised in Table 3.

Endoscopy, radiology and surgery operation. One endoscopist performed all jejunoscopies and colonoscopies, in order to assess the extent of CD and to classify the endoscopic mucosal inflammatory changes. Four bowel segments (the caecum and ascending colon, the transverse colon, the descending colon, and the sigmoid colon and rectum) and the duodenum-upper part of the jejunum were scored by protocol of Mary and Modigliani with some modification (see above) (0 = normal mucosa, 1 = oedema, erythema, granularity of the mucosa, and aphthous lesions, 2 = sporadic or superficial ulcerations, and 3 = extensive deep ulcerations, and stenosis). The severity of the inflammation in the small bowel was established by enteroclysis in 23 patients, while in 5 patients the terminal ileum was also inspected during colonoscopy. The radiological findings were classified by the same radiologist as normal (0), oedema and granularity of the mucosa (1), ulcerations (2), and stenosis (3). The total of the duodeno-jejunal and ileal scores was used to measure the small bowel activity. With these diagnostic methods, we were able to quantify the severity of inflammation in each part of the gastrointestinal tract. Laparotomy was performed on 9 patients during their hospitalisation, because of suspected abscess in 5 cases, and on account of severe stenosis in 4. All patients with a suspected intraabdominal abscess were operated on, and the intraoperative findings served as gold standard.

Involved segments were divided into three subgroups according to the behaviour of the inflammation: inflammatory (non-stricturing, non-penetrating) type, stricturing type and penetrating-fistulizing type.

Technetium-99m-HMPAO-labelled leukocyte scintigraphy. In vitro leukocyte labelling was carried out by a routine method (196), with some modification. 60ml of venous blood was collected in a syringe containing 6 ml of 3.8% sodium citrate and 12 ml of 6% hydroxyethyl starch solution. After spontaneous sedimentation of erythrocytes, the supernatant was centrifuged at 100- x g for 5 min, and the mixed leukocyte pellet was collected. 99mTc-HMPAO was formed by adding 99mTc in 1-1.5 ml to HMPAO (Leuco-Scint OSSKI, Budapest, Hungary). Mixed leukocytes were resuspended in 1 ml of 99mTc-HMPAO. After incubation with careful shaking for 10 min at room temperature, the unbound 99mTc-HMPAO was removed by centrifugation (450 x g, 5 min). Following slow reinjection into patients (mean activity being 345 MBq; range: 208-614 MBq), images were taken in the anterior view after 30 min and 2 hours. The maximum radiation dose was 2.2 mSv. The inflammatory process indicated by LS was localised in 5 segments: the small intestine, the ascending, transverse and descending colon, and the rectosigmoideum. The leukocyte uptake of each segment was scored relative to the normal bone marrow uptake (0= no uptake, 1= less than normal bone marrow uptake, 2= normal bone marrow uptake, and 3= bone marrow uptake). The LS activity index was calculated by summing the segment scores.

Spiral computed tomography. Every CT examination was performed with a helical CT scanner (Somatom Plus 4; Siemens, Erlangen, Germany) after the administration of oral contrast material. Patients received 2 litre of diluted sodium amidotrizoate (meglumin amidotrizoate, Gastrografin; Schering AG, Germany) 2 hours before the scanning, and drank it slowly up to the beginning of the examination. After an unenhanced study, 100 ml of Iopromide (Ultravist Schering AG, Germany) was administered. The slice thickness was 10 mm, the pitch was 1, and the reconstruction interval was also 10 mm. The maximum radiation dose was 8 mSV. The images were reviewed by two radiologists who were unaware of the clinical symptoms. The thickness,

appearance and enhancement of the bowel wall, and the mesenteric changes, lymph nodes, fistulas and abscesses, were assessed as indicators of the inflammatory activity, and were scored for each of the above mentioned bowel segments on a 4 point scale, adopted from the study of Kolkman et al. (197). (0= no thickening of the bowel wall, and normal mesentery; 1= a thickened bowel wall, a homogenous aspect, no enhancement with intravenous contrast, and no double-halo sign; 2= a thickened bowel wall, enhancement with intravenous contrast or a double-halo sign, ulceration and mesenteric fibrofatty proliferation; and 3= a thickened bowel wall, enhancement with intravenous contrast, ulceration and mesenteric fibrovascular strands).
No clinical information was provided to the observers; only the gastroenterologists knew exactly the clinical histories of the patients.
Statistics. Data are expressed as means (standard deviation or means and range). Correlation coefficients were calculated using the Spearman rank test.
b. Clinical significance of jejunoscopy in Crohn's disease
Patients. Patients with CD. 57 CD patients with known involvement of the small and/or large bowel were involved in the study after signing the declaration of agreement. The median age of the patients was 37.66 years, (range: 18-78). The sexual distribution was 22 male, 35 female. CD involved the small and the large bowel simultaneously in 24 patients, while 24 patients had isolated colon, and 9 isolated small bowel CD. At the time of the examination 40 patients had a clinically active disease (CDAI>150), while 17 patients were in remission according to the Best index (<150).
Patients with UC. 17 patients with earlier diagnosed UC were enrolled in the study. The median age of these patients was 43.8 years, (range 18-77).
IBD-free patients. 36 patients with different abdominal symptoms indicating endoscopic examinations of the upper gastrointestinal tract were also involved in the study as IBD-free controls. Their median age was 57.2 years (range 16-80).
Methods. Jejunoscopy. Olympus JF type jejunoscope was used for all examinations. One gastroenterologist performed each examination. Six parts of the upper GTgastrointestinal tract were individually graded: oesophagus, body region of the stomach, antral region of the stomach, bulbar part of the duodenum, distal part of the duodenum and the proximal part of the jejunum. Two bioptic samples were obtained from each segment mentioned above. Endoscopic scores. The endoscopic pictures of each part of the upper GT were scored by protocol of Mary and Modigliani with some modification (0 = normal mucosa, 1 = oedema, erythema, granularity of the mucosa and aphthous lesions, 2 = sporadic or superficial ulcerations, and 3 = extensive deep ulcerations, and stenosis).
Tissue samples, histological evaluation. The tissue was routinely processed and stained with Hematoxylin-Eosin. The Helicobacter pylori bacterial status was visualised with Giemsa stain. An experienced histopathologist, who was not imformed about the clinical condition, evaluated each biopsy specimen.
Antibodies. The antibodies used are listed below. Anti-CD3, anti-CD4, anti-CD8, and KPI were all from Draco (Lustrum, Denmark). The target cells were CD+ T lymphocytes, CD4+ helper T cells, CD8+ suppressor T cells, and CD68+ macrophages. Frozen sections were processed by the immunohistochemistry service in the Department of Pathology. Subsequently, slides were stained using an automated staining process in which they were labelled with the antibodies mentioned above.

C. Special problems associated with inflammatory bowel diseases

a. Extraintestinal manifestations. Metastatic Crohn's disease of the bladder. Case report. A 21-year-old romani male patient with previously proven CD was hospitalised in January 1998 because of severe abdominal pain associated with diarrhoea (3-4 watery stool daily), fever, nausea and vomiting. The diagnosis was established three years before by colonoscopy and histologyic examination, which disclosed an ileo-colonic localisation. He responded well to mesalazine and steroid therapy and became symptom free following a three-month therapy. He did not return thereafter and we did not see him until his recent admission. He was not taking any drug during the last two years. Physical examination showed moderate malnutrition (weight: 48 kg, height: 176 cm), mild anaemia, diffuse abdominal tenderness, systolic epigastrial murmur and painful resistance on the right side of the lower abdomen. He did not have fever (temperature was 37.2 0C), but his pulse rate was elevated, 102/min. Blood pressure was low, 105/70 mm Hg. On admission, the significant laboratory data were as follows: sedimentation rate 26 mm/h, haemoglobin 11.2 g/l, white cell count 8.5 x 109/l, platelet count 452 x 109/l, C reactive protein 91 mg/l, fibrinogen 7.24 g/l, serum iron 2.1 (mol/l. The modified Crohn's Disease Activity Index [Best index] was 410. The urine was sterile, and no symptoms of urinary tract involvement were observed. The abdominal ultrasound examination and the computed tomography revealed severe inflammation with a thickened wall of the terminal ileum and the right part of the large bowel. An abscess with a diameter of 4 cm was detected beside the ileum. The wall of the urinary bladder was also moderately thickened. An exploratory laparotomy was performed on the fourth day of hospitalisation. During the operation an inflammatory mass involving the terminal ileum, the caecum and the ascending colon close to the right side of the bladder was found. An abscess in close adhesion to the bladder wall was seen. Ileocolic resection, ileocolic anastomosis,
partial bladder excision, and drainage of the abscess were performed in one-stage procedure. The histo-pathological examination revealed no fistula in the bladder wall but an epitheloid granulomatous inflammation was present. The patient recovered completely. Twelve months after the operation, he is still in remission on maintenance therapy with mesalazine (3 g daily). A recent abdominal ultrasound and cystoscopy did not reveal any pathological findings.

Pericarditis associated with indeterminate colitis. Case report. A 29-year-old female patient was referred to our clinic in 1996 because of a severe relapse of IBD, with a 6-month history of bloody diarrhoea. She was observed in another hospital for 3 months before her present admission. At that time, her disease was diagnosed on the basis of endoscopic and histological findings as UC, and sulfasalazine was started at a daily dose of 3 g. Five weeks later sulfasalazine administration was discontinued because of upper gastrointestinal symptoms and was replaced by mesalamine (500 mg twice daily). On admission, the woman had 4-7 episodes of bloody diarrhoea daily, fever, nausea, cough, dyspnoe and chest pain. Auscultation of the heart sounds and of the lungs was normal (no pericardial rub was audible) and examination of the abdomen was also negative. Her temperature was 38.6 °C, blood pressure 120/80 Hgmm and pulse rate was 105/min. Laboratory data with the exception of erythrocyte sedimentation rate (ESR: 72 mm/h), haemoglobin (11.9 g/dl), white cell count (12.2x109/l), platelets (533x109/l) and fibrinogen (6.34 g/l) were within the normal ranges. Viral serology showed no evidence of recent viral infection, repeated blood cultures were negative, and the pANCA was positive. Results of other immunoserology (antinuclear antibody, anti-DNA, Latex test, Lupus erythematosus preparation and complement measurement) were normal. The chest X-ray and electrocardiogram were both negative. Endoscopy, histology and technetium-99m HMPAO labelled leukocyte scintigraphy demonstrated active left-sided (indeterminate) colitis without a clear distinction between UC and CD. The echocardiogram revealed pericarditis with moderate-sized

circumferential pericardial fluid 1.5 cm posteriorly. 125 mg methyl-prednisolone was started, and mesalamine therapy was continued without a break. By discharge her fever and bloody diarrhoea had disappeared. The steroid was tapered off and one year later she is completely well. The last echocardiogram confirmed the disappearance of the pericardial effusion. She is currently on a maintenance dose of 3 g of mesalamine daily, with 4 mg of methyl-prednisolone orally on alternate days. Repeated colonoscopy revealed a typical cobble stone appearance of the mucosa, suggesting CD with the narrowing of the proximal sigmoid colon. Histology did not differentiate between the two types of IBD.

b. Pregnancy. 320 (159 female) patients with UC and 110 (66 female) patients with CD were registered in our out-patient clinicambulance in December of 1996. Out of them 95 UC and 53 CD female patients were in childbearing age. We analysed the data of ten years (from April 1987 till October 1996). Twenty-six pregnancies occurred during this ten-year period.

Out of these 26 pregnancies, 19 pregnancies of 18 patients were suitable for analysis. During the outpatient visits, the activity signs of the fourteen days up to the controls were recorded. General well-being, number of stools, number of stools containing blood, number of mucus and bloody mucus, number of all defecation, severity of the abdominal pain, treatment and severity of joint pain, arthralgia, as the most frequent extraintestinal manifestation, have been registered by the patients for two weeks up to the control. All data of each patient were collected and analysed according to three aspects: 1. During the 19 pregnancies 57 controls were registered, while these patients had 430 controls from their non-pregnant period. All controls' data of the pregnant and non-pregnant period were suitable for analysis. 2. For statistical analysis, we compared the data of altogether four visits of one pregnancy of each patient, two visits before and two after the conception. These visits were randomly selected from two one-and-a-half-year periods (duration one and a half years), before and after the conception. Therefore, 36 visits before and the same number of visits after the conception were selected, and the activity signs were compared. Correlation coefficients were calculated by using student's T test.

3. We tried to collect data about the change of the disease activity during pregnancy and breast-feeding. Ten pregnancies of 7 UC and 3 CD patients were followed retrospectively, and data of 5 visits of each pregnancy were compared. The dates of these visits were the following: first: 3-6 month before conception; second: at the time around conception; third: first half of pregnancy; fourth: second half of pregnancy; fifth: 3-6 month after delivery, so during the breast-feeding. Scheffe test was used for statistical analysis. Naturally, the data of the newborns were also recorded and compared according to the mother's disease (namely UC or CD).

c. Carcinomas and IBD. Case report. A case of a 59 years old female patient with CD and associated gastrointestinal malignancies is presented. After a ten year period of diffuse abdominal pain and recurrent diarrhoea, symptoms of bowel obstruction evolved in 1991. The patient was operated on urgently, and the diagnosis of CD was established during the operation. Ileo-sigmoidostomy was performed because of the stenosis of the transverse and descending colon due to CD associated with tubulovillous adenoma in the caecum. A rectal cancer was removed (pT3N0) four years later together with benign serosus ovarian cystadenoma. A gastric cancer with metastasis in the skin of the chest was diagnosed (pT2N2M1) in 1998. Two weeks after the operation of this latest cancer, the patient died.

Immunophenotypisation. Determination of immunophenotypes of the cancers was performed by using routine immunohistochemical method. The removed malignant tissues were studied by using immunohistochemical staining with bioclonal anticytokeratin antibodies 7, 10/13, 19, 20, AE1/AE3, MNF IIG, HMW (high molecular weight), monoclonal anti p53 protein antibody, and antioestrogen receptor antibody. The result of immunohistochemistry was graded as follows: 0 =

no staining, 1+ = <50% of cells positive, and 2+ = > or =50% of cells positive. We compared the immunophenotype of rectal and gastric cancer.

III. Our experiences with the use of immunosuppressive and immunomodulator therapy

We revised records of all patients who were controlled because of UC or CD during between January 1997 and December 2000. Data of the indication, frequency and efficacy of immunosuppressive treatment, as well as the number and severity of side effects were examined. During this period 144 CD patients and 228 UC patients were controlled regularly (at least twice in a year) at our out-patient department.
At least a one-year steroid free interwall, under immunosuppressive treatment was considered as effective therapy. The goal of the immunosuppressive therapy was the maintenance of remission in the majority of cases. Therefore the at least a one-year steroid-free remission was used to measure the effectiveness of immunosuppressive therapy.
Case report. The history of a patient with a specific problem due to immunosuppressive treatment is briefly reported. A 65-year-old male patient with left-sided ulcerative colitis, which had been known for seven years, was admitted to our department in 1999. Azathioprine therapy had been started three months earlier because of recurrent relapses, which required intravenous steroid loading therapy twice a year. Prior to the immunosuppressive treatment a chest X-ray showed signs of former tuberculosis without the evidence of inflammation activity. On admission, a repeated chest X-ray revealed postprimary tuberculosis. Mycobacterial staining and the culture of the sputum confirmed the diagnosis. Successful tuberculostatic treatment was started with the interruption of immunosuppressive therapy.

Chapter 4. Results

IA. Genetic studies

a. Significant differences in the Interleukin 1ß and Interleukin-1 receptor antagonist gene polymorphism in a Hungarian population with inflammatory bowel disease.

We found no significant differences in the IL1ß allele frequencies between patient groups and control subjects, or between CD and UC patients. Similarly, there were not any statistically significant differences in allele frequencies or in genotype distribution of IL1RA in the three groups of subjects. From the point of view of disease localisation, we found no association of any genotypes or alleles of IL1RA in UC patients.

To investigate the possible association between certain alleles of IL1ß and IL1RA gene polymorphism, individuals were divided into carriers and non-carriers of allele 2 of these two polymorphic genes. The strength of this association was compared in each of the patient groups and controls. In the CD group homozygotes for IL1ß allele 1 were more often present (72% versus 28%; p=0.01, OR=2.85) in the subgroup of patients carrying at least one copy of the IL1RA allele 2 (IL1RA 2+). No association was detected in healthy controls or in the UC group; moreover, we found a similar distribution of genotype combinations of the two polymorphisms in the two groups (Table 4). The distribution of these genotypes combinations differed significantly between CD and healthy controls (chi-square = 8-83, p=0.03). However, when we divided the population on the basis of being homozygous carriers for both IL1ß and IL1RA allele 1 and not being homozygous carrier for this allele, we observed a significant negative association in CD (p=0.006, OR=0.31). This association was again not present in UC or in the controls. To investigate the hypothesis that, besides the distribution of patients into carriers and non-carriers of allele 2 of IL1ß and IL1RA genes, certain other genotype combinations are more dominant and thus probably more important in developing IBD, we considered "rare" genotype combinations present in less 10 individuals as one group. These genotype combinations were 1.1-1.3; 1.1-1.4; 1.1-2.3; 1.2-1.3; 1.2-1.4; 1.2-2.2; 2.2-1.2; 2.2-1.3; and 2.2-2.2. We found that these otherwise rare combinations were more frequent both in UC (p<0.01) and CD (p<0.03). Moreover, cluster analysis has shown that these allelic combinations statistically behave as one group, supporting the hypothesis that they might form one important group in the determination of IBD.

Several IL1ß - IL1RA genotype combinations characterised by the presence of at least one copy of the IL1RA allele 2 (1.1-1.2; 1.1-2.2; 1.1-2.3; 1.2-2.2; 2.2-1,2; 2.2-2.2) were more common in CD (chi-square = 10.45, p = 0.001) and UC (chi-square = 3.905, p = 0.049) than in healthy controls.

b. Interleukin 1ß gene polymorphism and the course and severity of inflammatory bowel disease.

We did not find any significant differences in allele and genotype frequencies of the IL1ßc3953 gene polymorphism comparing the groups of patients and control subjects (Table 5). However, in CD patients, homozygotes for allele 1 at IL1ßc3953 were more often represented in the subgroup of patients carrying at least one copy of allele 2 at IL1ß-511 (p = 0.009, OR = 3.007 Cip1.3–6.95). The significance proved to be stronger in patients with non-fistulizing disease (p = 0.002 OR = 8.00 CI = 2.53–25.31), while no association was found in patients with perforating-fistulizing disease. We found a similar association (p = 0.024 OR = 4.15 CI = 1.34–12.84) only in unoperated CD patients., but not in the operated subgroup, in HC, or in UC (Table 5). The distribution of allelic pairs in the non-fistulizing group was significantly different from HC (P~0.05), UC (P~0.03), and the fistulizing group (P~0.05); however, after correction for multiple comparisons this difference was no longer significant.

Concerning the IL1ß-511 allelic polymorphism we found an association between carriership of allele 2 and the severity of spontaneous bleeding in patients with UC. Patients who presented with bloody diarrhoea without spontaneous rectal bleeding were more likely to be carriers of allele 2 at position –511 (IL1ß-511*2), than patients with spontaneous bleeding (p = 0.006). This difference was also present in comparison with HC (p = 0.02) (Table 3). The frequency of IL1ß-511*2 was somewhat higher in patients with proctitis only in comparison with extensive UC, HC, or patients with CD. The difference was larger in proctitis cases that had not progressed in at least five years of follow-up (50% vs. 37% , 35%, and 35% respectively). However, this difference was not significant, probably due to the small number of patients in this subgroup (proctitis vs. HC p = 0.18). Perianal fistulas are genetically different both from the HC and from the unselected CD population (198). The present study provides further evidence for the hypothesis that fistulizing and non-fistulizing CD are distinct entities. A recent in vitro study reported that mononuclear cells from healthy individuals, who were carriers of IL1ß-511*2 and non-carriers of allele 2 at position c3953 (IL1ßc3953*2), had a slightly elevated capacity to produce IL-1 ß protein (199). Our results therefore are probably consistent with earlier studies by Gilberts and co-workers (200), who found that patients with non-perforating Crohn's disease had a significantly higher IL-1 ß mRNA expression in intestinal tissue than healthy controls or patients with the perforating form of the disease. The importance of this allelic association in influencing the severity of the disease is further supported by our results concerning unoperated CD patients. Between the two severity factors (fistulizing disease and having operations) there was not a large overlap: more than half of the operated patients were exempt from fistulizing symptoms.
Among our UC patients the carriership of the "high-producer" allele IL1ß-511*2 seemed to influence severity; this allele was over-represented in patients with less severe bleeding symptoms compared with UC patients with more severe bleeding symptoms, and compared with HC patients. In addition, the frequency of this allele was higher among patients with proctitis, especially when considering that the diagnosis was established at least five years previously. This finding is underlined by the fact that in a number of patients the extension of the disease progresses after initial proctitis. Concerning the IL1ß-511 gene polymorphism, a similar trend in genotype distribution could be observed in the French population (201). There, the carriership of IL1ß-511*2 was greater in unoperated patients compared with operated patients (78% vs. 49%), and in patients with a less extensive disease than in those with pancolitis (60% vs. 54%). Unfortunately, in the French study no data is given on the frequency of IL1ß-511*2 carriers in proctitis. The importance of this IL1ß gene polymorphism is understandable, since it is located in the promoter region of the gene.

B. Studies concerning environmental factors

a. Environmental factors predisposing to inflammatory bowel disease.
Cigarette smoking was more prevalent among CD patients than among patients in the control groups –e.g. UC patients and patients with autimmune disease- (45.7% vs. 15.9% and 21.9%, p<0.001). Passive smoking exposure in childhood (in the questionnaire: maternal smoking) was also significantly more frequent in the CD group (37.1% vs. 6.4% and 6.3%, p<0.01). Use of oral contraceptives before disease onset was also more common in female patients belonging to the CD group (41.7% vs. 11.7% and 18.6% p<0.01%). Proportion of former smokers was significantly higher in the UC group than in the other ones (14.3% vs. 2.9 and 4.7% p<0.01). According to the questionnaires, stress is more common at the onset of inflammatory bowel diseases than in control patients with other autoimmune diseases (41.9% and 39.8% vs. 17.2%

p<0.01). Caffee intake in ulcerative colitis and alcohol consumption in autoimmune diseases could be traced less frequently before disease onset (Table 6).

b. Effect of smoking on the activity of ulcerative colitis.

Each examined activity sign showed a significant improvement according to the controls performed within a half-year period after the initiation of smoking. Analysing the data of the thirteen patients, the median number of fortnightly stools decreased from 49.80 to 36.68; the median number of stools containing blood from 18.08 to 4.24; mucus from 10.92 to 3.52; bloody mucus from 5.08 to 0.64; while the median number of defecation during fourteen days decreased almost by fifty percent: from 83.88 to 45.08. The amelioration of general well-being did not reach the level of significance (Table 7). The laboratory parameters did not change significantly during the controls before and after the start of smoking (Table 8).

C. Neuropeptides and inflammatory bowel diseases

The number of peripherin positive GCs was significantly higher in CD (3.90 vs. 1.71; p=0.009). The total count of neuroendocrine cells labelled by CgA also increased (5.42 vs. 2.57; p=0.03) in CD, mainly due to the significantly higher count of VIP positive cells (6.95 vs. 4.28; p=0.004). The number of each type of positive cells increased significantly in the myenteric and submucosal plexus in CD (2.23 vs. 0.71; p=0.011 and 2.42 vs. 1.57; p=0.008); there was no significant difference in the other layers of bowel wall between CD and controls. The count of GCs was significantly higher in the penetrating group of CD (4.66 vs. 3.08; p=0.01), than in the stricturing group. The number of VIP positive cells was significantly higher in the granuloma positive group (7.83 vs. 6.00; p=0.023).

II. Clinical studies

A. Prognostic significance of epitheloid granulomas in Crohn's disease

Granulomas were found in at least one tissue sample in 44.6% of the cases (25 patients). The average age of patients in groups I and II was 29.6 and 36.4, respectively. The proportion of female patients was moderately higher in the group with granuloma (60 vs. 51.3%) Involvement of the upper GI tract was about 2,5 times more frequent (16.0 vs. 6.4%) in the granuloma group. Penetrating disease behaviour was slightly more often associated with granulomas (44% vs. 23%). The clinical characteristics of the two groups of patients are summarised in Table 9.

Patients with granulomas had more pronounced activity parameters at the time of the biopsies (CDAI: 218.9±108.2 vs. 144.1±92.5, p=0.01; CRP level: 62.7±84.9 vs. 48.5±87.8 p<0.05). Overall endoscopic scores tended to be higher in group I than in group II (6.5±3.5 vs. 4.7±3.0, p=0.08), but it did not reach the level of significance.

The average follow-up times were similar in the two groups: 27.5±7.34 and 23.8±3.83 months. The appearance of extraintestinal manifestations was more often observed in the granuloma group, but there was no significant difference between the two groups. Arthritis was equallyfrequent in both groups, with sacroileitis and sero-negative peripherial arthritis being the two most common disorders. Aphthous stomatitis was associated with CD in about 7% of all patients. Noticeably every aphthous stomatitis started simultaneously with the first symptoms of the CD. Iridocyclitis, cutan vasculitis and metastatic CD were other extraintestinal manifestations (Table 10).

Surgical interventions were performed more often in the granuloma group (48% vs. 22.5%, p<0.05). Stenosis, abscess, fistula and perforation were the indications for surgery.

The use of immunosuppressive drugs was significantly more frequent in patients with granulomas (60% vs. 32.2%, $p<0.05$).

B. Clinical value of different diagnostic procedures in Crohn's disease

a. Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease. All investigations were well tolerated and there were not any complications. Endoscopy and/or enteroclysis gave a positive result in at least one segment of the gastrointestinal tract in all patients. Both LS and CT gave positive results in 25 (but different) cases (25/28: 89.3%). Endoscopy and/or enteroclysis revealed positive results in 71 segments. LS gave positive results in 54 of these 71 segments, while CT did so in 51 segments. The distribution of positive segments is illustrated in Figure 1. Endoscopy and enteroclysis did not indicate any abnormalities in 67 segments. LS gave negative results in 60 of these segments, while CT did so in 56 segments. Table 11 presents the correlations between LS and CT scores and endoscopic (and radiological) activity scores.

Analysis of the data above demonstrated that the sensitivity and specificity of LS were 76.1% and 91.0%, whereas those of CT were 71.8% and 83.5%. The accuracy of LS was 82.6%, and that of CT was 77.5%. The sensitivity and specificity of LS and CT differed in the various anatomic localisations of the involved segments: LS was more effective in colonic inflammation, whereas CT was better in small bowel processes (Table 12).

The total of the endoscopic and radiological activity scores (EAS) was 159, that of the LS scores (LSAS) was 121, and that of the CT scores (CTAS) was 120. EAS correlated significantly with LSAS ($r=0.61$, $p<0.005$) and CTAS ($r=0.58$, $p<005$), and also with the number of involved segments detected by LS ($r=0.61$, $p(0.005)$ or by CT ($r=0.59$, $p<0.005$). On the basis of the endoscopic, radiological and operative findings, the 72 involved segments were divided into three groups according to disease behaviour. There were 44 inflammatory, 15 stricturing and 13 penetrating-fistulizing segments. As regards the disease behaviour type, the sensitivity of LS and CT was 77% and 100% in the penetrating-fistulizing, 80% and 73% in the stricturing, and 68% and 64% in the inflammatory form of CD, respectively. LS detected the inflammatory and stricturing processes more correctly, while CT exhibited an excellent sensitivity in the penetrating-fistulizing form of inflammation.

Intraoperatively, 5 abscesses and 4 fistulas were found. The rate of abscess detection with CT was excellent: all of them were detected without false-positive results, while 3 out of 4 fistulas were diagnosed preoperatively, again without false-positive results (sensitivity 100% and 75%, specificity 100%). LS indicated 3 out of 5 abscesses and 2 out of 4 fistulas, without false-positive finding (sensitivity 60% and 50%, specificity 100%).

Correlations were calculated and analysed between LSAS and CDAI and both clinical activity indices and each laboratory parameter. Inflammatory activity scores measured by LS displayed a closer correlation with the Best index ($r=0.71$, $p<0.0005$ vs. $r=0.63$, $p<0.001$), the van Hees index ($r=0.61$, $p<0.005$ vs. $R=0.59$, $p<0.005$), the serum fibrinogen level ($r=0.67$, $p<0.005$, vs. $r=0.59$, $p<0.005$) or the CRP level ($r=0.64$, $p<0.005$, vs. $r=0.51$, $p<0.01$) than CT. The significant correlations are presented in Table 13.

b. Clinical significance of jejunoscopy in Crohn's disease

Symptomatology. Ulcer –, or dyspepsia-like symptoms occurred less frequently in CD groups than in UC (18% in CD groups vs. 42% in UC and 65% in control groups)

Endoscopy. The frequency of different detected mucosal lesions in at least one part of the upper gastrointestinal tract was similarly high in each group. An abnormal endoscopic result was found in 85% of CD patients, as well as in 88% of UC patients, while 81% of control patients exhibited

different mucosal lesions. Antral hyperaemia with or without erosions was the most frequently observed endoscopic finding in all groups (57% in CD vs. 70% in UC and 68% in control group). Hyperaemia, granulation and oedema of the lower duodenal tract occurred more frequently in CD than in control groups (38% in CD vs. 17 in UC and 14 % in control group, $p<0.05$). The averages of endoscopic scores showed a significant difference in the distal part (duodenum and jejunum) of the upper gastrointestinal tract, the most severe disorders were noted in CD patients (Figure 2).

Helicobacter pylori. The H. pylori prevalence was significantly lower in IBD patients than in the control group (21% in CD group, 28% in UC group whereas 60% among the controls, $p<0.001$). This significant difference in the H. pylori status was not manifested in the endoscopic scores of the antro-bulbar region in the three groups (0.52 in CD, 0.35 in UC and 0.49 in control groups). (Figure 3).

Histology. Granulomas were exclusively observed in CD patients. The frequency of granuloma in the CD group was 10.5% (in 6 out of 57 patients). Granulomas were found in each part of the upper gastrointestinal tract, except for the corpus of the stomach (granulomas were observed in one oesophageal, three antral, one duodenal and one jejunal biopsies). Focal enhancement of inflammatory cells occurred characteristically in CD patients, and this lesion was observed mainly in the stomach; 65% of H. pylori negative CD patients had such a lesion. The other typical histological abnormality characteristic of CD was intraepithelial lymphocytosis (in 63.1% of CD patients). More than half (55%) of the jejunal biopsies in CD patients also showed intraepithelial lymphocytosis (Figure 4).

Immunohistochemistry. The hallmark of focally enhanced gastro/duodeno/jejunitis with H. pylori negative CD was the perifoveolar and periglandular accumulation of CD 68+ hystiocytes and CD3+ T lymphocytes. An increased number of macrophages were seen in all parts of the upper gastrointestinal tract in CD. These cells were also dominant in garnulomas. CD3+ T lymphocytes were the dominant cells in jejunal biopsies, whereas the same types of lymphocytes with CD68+ macrophages in the antral biopsies. The CD4/CD8 ratio of lamina propria lymphocyte was 2:1, whereas the proportion of CD4/CD8 IELs was 1:3.

C. Special problems in the management of patients with inflammatory bowel diseases

a. Pregnancy. I. First of all, we analysed the data of each controls. During the 19 pregnancies 57 controls were registered, while these patients had 430 controls from their non-pregnant periods. The activity of IBD was lower during the controls of the pregnant period than in the non-pregnant period. The most frequent extraintestinal manifestation, arthritis, was also less frequently observed during pregnancy (Table 14). II. We examined the data of 36 visits before and the same number of visits after conception, thus we obtained data suitable for statistical analysis. General well-being, number of stools, number of stools containing blood, number of mucus and bloody mucus, number of all defecation, as well as the severity of the abdominal pain also showed a mild, non-significant improvement during the one-and-a-half year period including pregnancy and breast-feeding (Table 15). 7 patients discontinued the drug therapy after the conception, while the other 11 patients had a fixed therapy during pregnancy and breast-feeding. 6 patients received corticosteroid (with an average dose of 6 mg prednisolone daily), while 7 patients received only sulphasalazine (with an average dose of 2.7 g daily). (Table 16). The dose of steroid and sulphasalazine was lower during pregnancy than before conception. 3 patients received 5-aminosalicylate with a constant dose before and after conception (Table 17). III. Although the clinical course of the disease did not change significantly during pregnancy and

breast-feeding, some tendencies could be observed. Each activity parameter showed moderate improvement in the first half of pregnancy. The number of stools, stools containing blood as well as number of bloody mucus has increased during the second half of pregnancy and after delivery (Table 18).
Data of the infants are presented in the Table 19. One child of an UC mother was born with a total transposition of great vessels, which could be completely corrected through surgical intervention. The mother did not receive any drug, and her UC was continuously inactive during pregnancy. It was her first delivery, since that time she has had three other healthy children.
The average term of delivery, the mean height, as well as the mean weight of the newborns were calculated and the two groups according to the mother's disease (UC and CD) were compared. Each mother with CD gave birth to a baby weighing less than 3000 grams (average: 2500 gram), in contrast with the normal mean birth weight (3311 grams) of infants born to mothers with UC (Table 20).
b. Carcinomas and inflammatory bowel diseases
Rectal tumour. Histology was adenocarcinoma gelatinous, the stage was pT3N0, Dukes B. The immunophenotype of this cancer was: CK7 (-), CK 10/13 (-), CK 19 (+++), CK 20 (+), CK AE1/AE3 (+++), CK MNF IIG (++), CK HMW (+), p53 (-), ER (-).
Gastric tumour. The microscopic diagnosis adenocarcinoma at stage pT3N2M1. There were not any macroscopic or microscopic signs of CD in the stomach. An alcian blue positive intracytoplasmatic mucus production was detected in the tumour. The immunophenotype of the cancer was CK7 (-), CK 10/13 (-), CK 17 (-), CK 18 (++), CK 19 (+++), CK 20 (++), CK AE1/AE3 (+++), CK MNF IIG (+++), CK HMW (-), p53 (-), ER (-). The infiltrative dermal process of the chest was the metastasis of the gastric cancer with the same immunophenotype.
The microscopic structure, the way of mucus production, as well as the immunophenotype were also different in both types of cancer (e.g. rectal and gastric). Therefore, it seems that gastric cancer is a second metachron primary malignant tumour.

III. Our experiences with the use of immunosuppressive and immunomodulator therapy

Frequency. 44% of CD patients (63 of 144) and 21% of UC patients (48 of 228) received immunosuppressive therapy in this period. Azathioprine was the first choice for each patient. If it did not work or the patient could not tolerate it, another drug was selected. Methotrexate was given for 6.3% of CD and 4.1% of UC patients. 10 CD patients were selected in a clinical study of Infliximab, so 7.6% of our CD patients received anti-TNFα therapy. 5 UC patients (2.1%) received cyclosporine due to severe, steroid refractor relapse. Mycophenolat-mofetil (CellCept) was tried in 2 CD patients (1.4%).
Indication. Chronic activity was the most frequent indication of immunosuppressive therapy. 62 out of 111 (63 CD + 48 UC), that is 56% of immunosuppressed patients received the drug because of this indication. Steroid dependency indicated imunosuppression in 26% of the patients (29 of 111). Immunosuppressive treatment due to a perianal or enterocutan fistula was started in 14 CD patients (13% of all immunosuppressed, and 22% of immunosuppressed CD patients). Associated autoimmune condition (vasculitis or AHA) was the indication in 5% of the patients (Figure 5). Half of the patients treated with anti-TNFα (5 patients) had fistulas, while the other 5 patients had chronic active disease.
48.4% of CD patients with penetrating behaviour and 51.5% of CD patients with multiple segmental involvement were on immunosuppressive therapy. Similarly, in 66.6% of UC patients with pancolitis immunosuppressive therapy was indicated.

Effectiveness. At least a one-year steroid-free remission was achieved in 67.3% of CD and 60% of UC patients. Fistulas healed in 58.3% of CD patients with manifested fistulas.
Side -effects. All side -effects due to azathioprine therapy were observed. Mild symptoms of intolerance (headache, nausea, vertigo, pruritis) appeared in a quarter of patients, but the therapy could be continued in all of them. Azathioprine intake was discontinued in the 14.6% of UC and 6.2% of CD patients. Symptomps of early intolerance (effect of imidazole derivates) lead cessation in all, but two patients. These two patients developed severe pancytopenia as a sign of thiopurine-methyl transferase deficiency. Reactivation of pulmonary tuberculosis resulted in discontinuation of azathioprine therapy in one UC patient. We did not observe any side effects during Infliximabe therapy.

Chapter 5. Discussion and conclusions

IA. Genetic susceptibility

a. Ulcerative colitis. Our data are consistent with prior studies in other European regions describing no significant differences in the genotype and allele frequencies of IL1ß TtaqI polymorphism between healthy control, UC and CD groups (202). On the other hand, although several reports described a higher frequency of allele 2 of the IL1RA RFLP polymorphism in UC patients, especially in the subgroup of patients with a more severe course, we did not observed a significant difference. In the English population a significant overrepresentation of allele 2 of the IL1RA was described in UC patients, this association being the strongest in patients with pancolitis (203). This observation could not be confirmed by the Oxford Group at first (204); however, when they examined pancolitic patients who underwent proctocolectomy, the association with this allele was confirmed (205). In Amsterdam a similar increase of allele 2 of IL1RA gene was found in pancolitis (206), while Hacker et al. (207) found no association in spite of having 54% of patients with total colitis. These conflicting results may be due to the heterogeneity of patient populations, since in Lille a higher frequency of allele 2 of IL1RA polymorphism was observed only in surgically treated patients (208). Thus the lack of association in Hungarian patients with UC may be due to the fact that only 25% of these patients had pancolitis. This can not be compared with the 78% of a previous study showing association with this allele. Also interestingly only a small number of patients in Hungary (4.2% in an eight years follow up) underwent surgery, indicating a less severe disease. There is another possible explanation, since IL1RA allele 2 frequency is influenced by the frequency of alleles of the TaqI IL1ß polymorphism. Therefore it seems that in this population the presence of allele 1 of the IL1RA is protective against developing a severe form of UC. This would explain why rare allelic combinations with allele 3 and 4 in the IL1RA gene polymorphism were also more frequently found among Hungarian UC patients. In conclusion, the presented data support the assumption that in severe UC there is an imbalance in the genetic control of IL1ß and IL1RA gene polymorphisms, which stresses the importance of disease heterogenity in assessing the significance and strength of the observed associations.

Among our UC patients the carriership of the "highproducer" allele IL1ß-511*2 seemed to influence severity; this allele was over-represented in patients with less severe bleeding symptoms compared with UC patients with more severe bleeding symptoms, and compared with HC patients. In addition, the frequency of this allele was higher among patients with proctitis, especially when considering that the diagnosis was established at least five years previously. This finding is underlined by the fact that in a number of patients the extension of the disease progressed after initial proctitis. Concerning the IL1ß-511 gene polymorphism, a similar trend in genotype distribution could be observed in the French population. There, the carriership of IL1ß-511*2 was greater in non-operated patients compared with operated patients (78% vs. 49%), and in patients with less extensive disease than in those with pancolitis (60% vs. 54%). Unfortunately, in the French study no data is given on the frequency of IL1ß-511*2 carriers in proctitis patients in the French population. The importance of this IL1ß gene polymorphism is understandable, since it is located in the promoter region of the gene.

b. Crohn's disease. The importance of genetic association studies using candidate genes may increase when disease heterogeneity is taken into account. It has been shown in several studies

that patients with perforating/fistulizing CD undergo a more aggressive disease course compared with patients with non-perforating disease (209). Moreover, recent results have suggested that patients with peri-anal fistulas are genetically different both from healthy controls and an unselected CD population. The present study provides further evidence for the hypothesis that fistulizing and non-fistulizing CD are distinct entities. A recent in vitro study reported that mononuclear cells from healthy individuals, who were carriers of IL1ß-511*2 and non-carriers of allele 2 at position c3953 (IL1ß-c3953*2), had a slightly elevated capacity to produce IL-1ß protein. Our results therefore are probably consistent with earlier studies by Gilberts and co-workers, who found that patients with non-perforating Crohn's disease had a significantly higher IL-1ß mRNA expression in intestinal tissue than healthy controls or patients with the perforating form of the disease. The importance of this allelic association in influencing the severity of the disease is further supported by our results concerning non-operated CD patients. There was not large overlap between the two severity factors (fistulizing disease and having operations): more than half of the operated patients did not develop fistulizing symptoms.

The established difference between UC and CD patients in IL1ß genotype distribution also supports the hypothesis that these conditions are polygenic disorders, sharing some but not all susceptibility genes. The association between these polymorphisms may be interpreted as suggestive of another gene in linking imbalance with the IL1 gene family. However, given the fact that the two polymorphisms of IL1ß gene influence the course and severity of the disease, it is possibile that the gene which controls IL-1ß production is involved in the pathogenesis of the disease itself.

B. Studies concerning environmental factors

a. Environmental factors predisposing to inflammatory bowel disease.

Our study confirmed the contradiction of smoking habits in the two major types of IBD: UC is the disease of non-smokers, while the ratio of smokers among CD patients is significantly higher than in healthy controls. The prevalence of smokers was also low in the autoimmune disease group; there was not a statistically significant difference between the UC and the autoimmune group. It may be worthwhile doing further investigations, as presumably, smoking may have a protective effect not only against UC, but also against other autoimmune diseases. Although there are some other observations, former smokers are likely to have a greater risk of developing UC. Our results confirm this elevated risk. Furthermore, Hanauer suggested that ex-smokers form a different group in UC: their disease course is worse and they produce a different therapeutic response than never-smokers (210). Our most interesting observation was the increased passive smoking exposure in childhood (in the questionnaire: maternal smoking) in the CD group (37.1% vs. 6.4% and 6.3%, $p<0.01$). These data suggest that passive smoking exposure in childhood may influence the development of CD at a later age (211). Our data suggest some addictive role of oral contraceptive use before disease onset in the development of CD. The results of publications in this topic are conflicting; there is not enough evidence to forbid CD patients taking contraceptive pills, moreover, according to the majority of studies concerning CD a safe method of contraception is even required. According to the questionnaires, a significant part of patients with IBD think that stress is an important factor in the development of their disease. Caffee consumption in ulcerative colitis and alcohol consumption in autoimmune diseases were seen less frequently before disease onset, but the importance of these observations are less clear than the others mentioned above.

b. Effect of smoking on the activity of ulcerative colitis.

Since the first reports by Harries (212), a lot of publications suggested a strong relationship between UC and non-smoking (213). On the basis of several publications it is clear that the smoking habits of patients with UC and CD are just the opposite. Furthermore, smoking influences the clinical course of these diseases in a contradictory manner. While smoking decreases the chance of developing UC and has a favourable effect on the course of disease as well, smoking patients with CD require significantly more surgical interventions and the relapses are also more frequent (214). There are significantly more smokers among CD patients. There are not enough data about how smoking influences the activity and the development of IBD. It is likely that nicotine is the key to the favourable impact. Nicotine is a potent vasoconstrictor agent, which causes a transient diminution of rectal blood flow. The peak of this effect can be observeed 30-60 seconds after smoking. The highest plasma concentration of nicotine can also be measured at this time (215). Vasoconstriction may be harmful in CD, but probably it is not the basis of the favourable effect in UC. However, there are some data about the connection between smoking and the cytokine levels in the colon and in the serum. A decrease in proinflammatory cytokines rovide protection against the development and relapse of UC. Some data suggested that the glycoprotein IV level of the mucus synthesised in the colon decreased in UV patients (216). According to some observations smoking could normalise the glycoprotein IV level in the mucus (217).

Several reports suggest that smoking can decrease the activity signs of UC, but no one has yet performed a self-controlled study, because there is a limited number of patients who change their smoking habits after the diagnosis of UC. Our results indicated that the initiation of smoking moderates the activity of UC irrespectively of drug therapy. However, this favourable effect was not reflected in the laboratory results. About half of our patients participating in this study had proctitis only and their laboratory results rarely changed even if the inflammation involved only a short segment of the bowel.

Although our study was self-controlled, the relatively little number of patients limited its efficacy. Further studies should be carried out in order to prove "therapeutic effect" of smoking in active UC and the exact pathomechanism should also be revealed before endorsing smoking as a treatment of UC. It would be important to determine the risks and benefits, and the dose response of smoking, and comparative trials, using medications such as aminosalicylates and immunomodulators, should be done (218). However, the number of possible harmful effects of smoking will limit its therapeutic use.

C. Neuropeptides and inflammatory bowel diseases

The number of GCs and neuroendocrine cells increases in myenteric and submucosal plexus in the resected bowel segments of CD patients. There is a little doubt that neuropeptides play an important role in intestinal inflammation as well as their pro- and antiinflammatory effect has been clearly demonstrated. There are some biologic evidence for neuroimmunomodulation in IBD. Direct interconnection of the nervous and immune system has been proved by the demonstration of intimate cellular contact between substance P and CGRP peptidergic enteric neurons and mucosal mast cells (219). Reports identifying specific receptors and functional effect of various neuropeptides on both systemic and mucosal components of the immune system are increasing (220). Most of the receptor study suggest potential physiologic significance. Therefore, this observed higher concentration of peptiderg neurons and neuroendocine cells in the place of severe CD might be an important part of the inflammatory process. Since earlier studies of mucosal peptide concentration in CD have yielded conflicting results, with concentration

stated to be either greater, lower or no different from control tissue (221,222,223), our results require further similar observations to make consequences. However, the different results may be explained by many factors that determine peptide concentrations in mucosal biopsies. These include, for example, the depth of biopsy, because peptides are concentrated at certain layer within the gut wall. Another important factor is the speed of processing, which may alter the degree of peptides loss through by hydrolysis by tissue enzimes (224). There is a general aggreement that peptide-containing nerves are thickened and coarse in CD, but the relationship of these changes to the disease process remains to be established.

Bishop at el reported increased concentrations of VIP in surgically resected colonic mucosa affected by CD (225) and O'Morain et al reported increased concentration of VIP in rectal biopsies obtained from patients with CD but normal concentration in biopsies obtained from patients with UC (226). Our similar observation, namely the raised number of GCs as well as VIP positive neuroendocrine cells suggests a pathogenetic role in the altered motility, secretory and immune response observed in CD, but the exact mechanism of these events require further investigation.

Sustance P, as well as acethylcholine, is known to be an excitatory transmitter of enteric neurons to the circular muscle of the guinea pig ileum (227). On the other hand, VIP generally causes relaxation of the intestinal muscle, including the circular muscle of the guinea pig small intestine (228). This information leads to the conclusion that the excitatory nerve fibers to the muscle contain SP, and that the inhibitory fibers contain VIP. The available evidence from human material also indicates that SP contracts gastrointestinal external muscle (229), whereas VIP relaxes it (230). Furthermore, VIP is a potent stimulant of mucosal water and electrolyte secretion, hence, VIP may cause increased local secretion in the ileal reservoir resulting in the loose stools, and also, as its name suggests affects blood flow (231).

Although the physiological and pathological implications of these data remains speculative, most of the result (as well as ours) suggest an important role for neuropeptides in the pathophysiology of IBD. In a therapeutic perspective, these results suppose a role of the variety of high-affinity and selective antagonist are currently available for the treatment of CD.

II. Extracts in clinical features of IBD

A. Prognostic significance of epitheloid granuloma in Crohn's disease

Granuloma formation is a host response by the localised accumulation of epitheloid cells, macrophages and lymphocytes. Granulomas can be found in many infective, allergic and neoplastic disorders (232). Although granulomas in CD were first described by Dalziel in 1913 (233), the exact role and significance of this "specific" histological lesion remains a puzzle. The likelihood is that granuloma is the site where the etiological agent resides, the antigen specification occurs and the T cells differentiation into Th1 cells starts (234), but there is not any evidence to confirm this theory. Pathological host response is one of the important basic concepts in the development of CD besides genetic predisposition and environmental factors. The differencies in individual host responses may give an explanation to the presence or absence of granulomas.

The first publication that tried to examine whether the presence of granulomas is associated with good or poor prognosis came out more than forty years ago (235). Since then there has only been a few studies which have examined the prognostic role of granulomas, and their results are controversial. The most widely studied topic was postsurgical recurrence of CD. Anseline et al.

examined several factors that might predict recurrence after operation in 130 patients over a 24-year period. A highly significant positive association was revealed between the presence of granulomas and the likelihood of recurrence (p=0.003) on the basis of the multivariate regression analysis (236). Trnka et al published a similar tendency; they found that there was an increased chance of recurrence in a subgroup of patients with ileocolonic disease (237). On the other hand, some studies suggest just the opposite (238,239,240). These publications suggest that patients with granulomas have less frequent postoperative recurrence and/or have a better prognosis. They were pioneer studies in this topic, therefore the selection of patients, the diagnostic and statistical methods were probably less accurate than the ones in the latest publication.

There has only been one study so far in which the presence of granulomas was determined by endoscopic biopsies (241). Markowitz et al examined a paediatric population, and rectosigmoid biopsies were taken from 58 subjects. All biopsies had been obtained from newly diagnosed patients before any therapy was started, and the children were followed for at least one year. The frequency of granulomas was 32.7%. More severe perianal complications, a higher frequency of surgery and more extensive inflammatory involvement were observed in patients with granulomas. Medical treatment and the need of hospitalisation were similar in both groups.

We found a higher frequency of granulomas in the biopsies of our patients than Markowitz et al, however, we took biopsies from more (even intact) segments of the entire gastrointestinal tract. We examined newly diagnosed patients as well, and we also found a more severe clinical course in the first two years of the disease in patients with granulomas in any part of the gastrointestinal tract. Considering that these were the first symptoms and the first examinations of the patients, we can conclude that the onset of CD was also more severe in the granuloma group.

Naturally, our results with a small number of cases are not capable to solve the controversy about the prognostic significance of epitheloid granulomas in CD, but suggest that the presence or absence of granulomas is a useful prognostic marker. We propose that all gastrointestinal segments obtained by different endoscopic methods should be checked histologically for granulomas. The presence of granulomas may suggest a more aggressive form of IBD and this should influence our therapeutic strategy and the frequency of follow-up visits.

B. Clinical value of different diagnostic procedures in active Crohn's disease

a. Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease.

The comparison of CT and LS was necessary because only 17 CD patients had been previously reported, in whom the two methods were performed simultaneously. The sensitivity we observed in LS is similar to the range reported in previous studies: 79-92% (242,243). However, the sensitivity of CT concerning the segmental inflammatory activity was earlier assessed only by Kolkman et al. They reported 68% sensitivity, but the disease behaviour type of their patients was not stated. However, this is a very important factor: in our work the sensitivity of CT in the different disease types ranged from 64% in the inflammatory form to 100% in the penetrating form of CD. It seems that the different types of CD (e.g. inflammatory and stricturing) may occur simultaneously in a single patient. The management of the patient is determined by the complications (stenosis or fistula), so that classification of each segment is usually not necessary in clinical practice. On the other hand, a correct measurement of the value of different diagnostic methods does necessitate the biological classification of each segment.

In our prospective comparative study LS detected the extent of gastrointestinal involvement caused by CD more accurately. CT gave a better result only when there was small bowel involvement and in segments with penetrating behaviour. LS presented a relatively low sensitivity to detect abscesses (3 out of 5). Technetium 99m has a relatively short half-life of 6 h,

but at neutral pH Tc-99m and HMPAO form a lipophilic complex which is rapidly incorporated into the leukocytes in vitro. Tc-HMPAO labelled leukocytes, therefore, migrate to the site of the inflammation within 30 min, but they disappear from there rapidly. In a number of studies involving this isotope technique, scintigrams were reported to be prepared in 1 and in 2-4 hours. If indium 111 is used, scanning can be performed later on, because this isotope has a half-life of 65 hours. This method might well lead to superior results concerning the detection of abscesses. However, indium is not only more expensive and not too easily available, but also a large degree of self-radiation has been encountered.

Although both methods slightly underestimated the severity of the inflammation and the length of the involvement, their accuracy was sufficient enough for adequate therapy. CT displayed excellent suitability for the recognition of extraintestinal complications.

The CT and LS scores and the number of involved segments detected by CT and LS in our study correlated well not only with the endoscopic results and laboratory parameters, but also with the CDAI and the van Hees index. Although several authors claim that the most frequently used indices only correlate poorly with the endoscopic findings (244) and have little clinical relevance, our results suggest just the opposite.

In conclusion, our results indicate that, in a severe first attack of CD, when the radiological and endoscopic procedures are relatively contraindicated, both LS and CT are convenient for the patient without any complications. Although both methods have a significant diagnostic value in active CD, the accuracy of these investigations is not the same in the different disease behaviour types.

We suggest that in a severe active CD patient with unknown disease behaviour type and localisation, LS and CT should be performed simultaneously. In a patient with an acute flare-up and a known disease behaviour type, one of these procedures is sufficient: preferably LS in the inflammatory and stricturing form, and CT in the penetrating form of CD.

b. Clinical significance of jejunoscopy in Crohn's disease. Several earlier studies suggested that characteristic microscopic alterations could be present in the majority of stomach biopsies in patients with CD. Focally enhanced gastritis in H. pylori negative CD patients seemed to have high diagnostic efficacy in CD. The histological picture of this type of gastritis is characterised by focal, perifoveolar accumulation of CD3+ lymphocytes, CD68+ hystiocytes and, in 80% of cases, granulocytes.

In IBD the total number of lymphoid cells within the intestinal mucosa is increased two to fourfold. The ratio of T to B lymphocytes is similar in both diseased and normal bowel, and approximately 6:1, respectively (245). The T cell CD4/CD8 (helper/suppressor) ratio of lamina propria and intestinal intraepithelial lymphocytes (IELs) is varied: helper cells are characteristically present on the surface, while suppressor cells are mainly found among IELs. This heterogenity probably reflects a diverse range of functional properties. IELs have a variety of effector functions, including cytotoxicity, modulation of epithelial cell function and proliferation, and immunoregulatory roles, including tolerance to dietary antigens and suppressor functions (246). Our study also confirmed an increased number of T lymphocytes in the inflamed and non-inflamed mucosa of the upper gastrointestinal tract in patients exclusively with CD. But this increase in T cells could be detected not only in the stomach, but, in more than half of the patients, in the jejunum too. Immunohistochemical examinations showed a diverse ratio of helper and suppressor T cells mentioned above.

Granuloma is thought to be a diagnostic histological lesion of CD. The frequency of granulomas in the biopsies taken from the upper gastrointestinal tract of CD patients varied among 5-20%. Our percentage (10.5%) is similar to those observed by others (247,248). The characteristic cells

of granulomas were the macrophages, although an increased number of macrophages were observed in the mucosa without granuloma and without macroscopic signs of inflammation; therefore they seem to be characteristic of CD.

Our method also covered a symptomatic and an endoscopic score. These examinations were rarely performed earlier; however, they confirmed some of our former assumptions. CD patients had less dyspepsia and ulcer-like symptoms than IBD-free patients, although the severity of endoscopic lesions was similar in both groups. The symptoms, the endoscopic picture and the histological appearances are three different things; each one has a significant importance without dependence on each other.

The jejunum was a rarely examined part of the gastrointestinal tract in CD. Jejunoscopy takes a little bit longer than gastroscopy, it is a little bit more uncomfortable for the patients, and it requires special endoscopic forceps. In our case, all of our investigations were well tolerated, there were no complications, and in the majority of cases, no sedation was needed. The endoscopic and histologic appearance of the jejunum in CD is very similar to the lesions of the stomach or duodenum as well as those of the terminal ileum, however, in a few cases, the examination of the jejunum can support the diagnosis.

Since our prospective study revealed a high frequency of endoscopic and microscopic alterations in CD patients without relevant clinical symptoms, we think that the endoscopic evaluation of the upper gastrointestinal tract with biopsies of the inflamed and non-uninflamed mucosa should be an important part of the routine procedures in CD. These specific findings may help to establish the correct diagnosis in indeterminate colitis and should suspect CD of the distal part of the gastrointestinal tract in patients with unknown gastrointestinal disease.

C. Special problems in the management of patients with inflammatory bowel diseases

a. Extraintestinal manifestations. Metastatic CD in a CD patient was first described as a cutaneous granulomatous involvement at a site separated from the gastrointestinal tract by normal skin in 1976. Up to date about 40 similar metastatic cases have been reported (Ovid Netscape Database). Necrobiosis and granulomatous perivasculitis are the histopathologic characteristics of metastatic skin CD (249). The second most common extraintestinal granulomatosus association is the genital involvement. CD of the penis (250), vulva and cervix (251) have all been observed. However, other organs can also be infiltrated by specific granulomas, such as the gall bladder (252), the bone (253), the muscles (254) and the lung (255). Metastatic inflammatory lesions in the abdominal cavity are probably caused by an infiltrative process, but the mechanism of distant metastases is unknown and necessitates further investigations.

The reported frequency of urologic complications associated with CD is about 15% percent (256), the incidence of nephrolithiasis ranges from 3% to 19% and urethral obstruction unrelated to nephrolithiasis from 1% to 25 % (257).

CD characterised by fistula formation developed in 32% to 35% of patients (258). One such fistula is the enterovesical one, which was reported to affect 2% to 6% of patients suffering from CD (259). About 90% of the patients with enterovesical fistula ultimately required surgery, but since in most of the cases the urinary bladder was preserved during the operation (260), therefore, microscopic analyses of the bladder wall is extremely rare in CD patients. This may explain why granuloma formation in the bladder wall has not been reported yet. Patients suffering from CD associated urological complications may be completely asymptomatic, or the associated urinary symptoms are non-specific (261). For example, our patient did not have any symptoms of urinary involvement that should have required the examination of the bladder. In our case computed tomography, as well as abdominal ultrasound showed a moderate thickening of the bladder. In

another study, computed tomography revealed focal bladder wall thickening in 4 out of 275 urologically symptom free CD patients (262). Our case report suggests that histological examinations could also reveal granulomas in these asymptomatic cases, which underlines the importance of computed tomography and ultrasound examination that may call the attention to bladder involvement in CD. CD can mimic primary bladder tumour (263), even transitional cell carcinoma of the bladder has been reported in association with CD, (264) and CD can also develop in an ileal conduit (265). These cases also confirm that ileo-colonic CD may be associated with different types of urological involvement. Granulomatous inflammation in an extraintestinal site in patients with proven CD suggests a widespread systemic involvement. Whether these cases represent a particular subgroup of patients with CD remains to be investigated. Urinary symptoms in CD patients should alert the clinician and urological consultation is indicated.

Pericarditis was described to be the most frequently observed cardiac association with IBD. This extraintestinal manifestation usually starts together with a relapse of the bowel symptom (266), or may be the initial manifestation of IBD (267). Most cases of pericarditis are associated with colonic involvement in both common types of IBD (268), nevertheless the incidence of pericarditis is slightly higher in UC. The drugs used to treat IBD may also cause pericarditis, as well as myocarditis or pleuritis. The sulfapyridine moiety of sulfasalazine can induce a lupus-like syndrome, which can also present with pleural or pericardial effusion (269). Similar reactions have rarely been reported in connection with mesalamine products (270,271) and azathioprine (272). The pathogenesis of drug-induced cardiac damage (like the etiology of IBD) remains unknown. In our case pericardial effusion and relapse of IBD presented together more than one month after mesalamine therapy had been started without any complications. Possible infections were excluded; immunoserological findings were negative. 5-ASA treatment was never stopped and pericarditis did not relapse. This case shows that serious cardiac complications may be associated with IBD as an unusual extraintestinal manifestation. During the follow-up of patients with IBD, extraintestinal cardiac manifestations should be carefully attended to.

b. Pregnancy. Fertility in women with IBD seems a little bit reduced when compared with that of the general population. According to recent reports this difference is not significant regarding female UC patients. The situation for CD is different: fertility is reduced (273). Some studies showed that fertility is lower when the colon is also involved than when there is only an ileal involvement, perhaps because of the higher incidence of perianal, perineal and rectovaginal fistulas and abscesses (274). Therefore, it seems that it is not the localisation of the inflammatory process that is the most important, but the behaviour of the diseases, and that patients with a penetrating disease have hygiene difficulties and dyspareunia. Although far more difficult to quantify, fear of pregnancy is probably also a major factor reducing fertility in young women with CD. Either the obstetrician or the gastroenterologist often introduces this fear by overemphasising the potentially negative effect of drug therapy itself on the outcome of the foetus (275). The risk of preterm birth is higher in CD than in UC, and it seems that women with the latter disease do not have an elevated risk (276). Comprehensive reports showed a similar risk of congenital abnormality in IBD (0 to 3%) and in a normal population (277). On the basis of our data and the literature, it seems that the influence of pregnancy on the course of the disease could usually be correlated with the activity of IBD at the time of conception. If the inflammatory process of the patient is active at the time of conception, the disease could become more active during pregnancy (278). However, when drug therapy results in remission, the disease is usually in the quiescent phase during pregnancy (279).

Young women at childbearing age should be informed about the influence of pregnancy on the course of IBD and about the management of IBD during pregnancy and breast-feeding. It is very important that conception should occur in an inactive phase of the disease. Exacerbation during pregnancy should be treated as aggressively as necessary to induce remission as soon as possible. In our cases, the disease was in remission or showed only a mild activity in the first phase of pregnancy and the activity of IBD did not increased until delivery. These results suggest that if conception occurs in a relatively inactive period of the disease, pregnancy does not enhance the activity of IBD.

Most drugs used in the treatment of pregnant IBD patients are the same as those used in non-pregnant patients. These drugs include sulfasalazine and its derivate 5-aminosalicylic acid, and even corticosteroids. It is not recommended to start immunosuppressive therapy during pregnancy, however, if the severity of IBD requires the administration of azathioprine (or 6-mercaptopurine) before conception, the therapy can be continued safely during pregnancy (280). The use of methotrexate is contraindicated during pregnancy because of its teratogen effect. Metronidazole use was associated with at least two infants born with craniofacial abnormalities to mothers treated for amebiasis in the first trimester of pregnancy, but causal relation was not established (281). There was not any evidence of foetal complication in a large report of more than 800 pregnancies in more than 300 patients who received metronidazole during the first trimester. However, we do not recommend this drug during pregnancy. Table 16 shows our pregnant patients' drug therapy. No immunosuppressive therapy was used, but sulphasalazine, 5-aminosalicylic acid, as well as a little dose of corticosteroid did not influence the pregnancy in an unfavourable manner.

Our results suggest that the activity of IBD can increase during breast-feeding. Therefore, if the patient has not received any therapy during pregnancy, it should be restarted after delivery as soon as possible.

The pathomechanism of pregnancy influencig the course of IBD is not known yet. Beside the role of hormones produced by the placenta, the change in the maternal immune system might be another important factor. Some authors call this mechanism "natural immunosuppression" (282).

Pregnancy does not influence the course of IBD unfavourably. On the contrary, it even seems that IBD patients, who have already been pregnant, have a better disease course than those who have never been pregnant (283). It is important to note that after episiotomy, perineal involvement may become more frequent in CD patients. Therefore, in CD patients with known earlier perianal involvement, section should be the method of choice of delivery.

Babies born to mothers with CD have a lower birth weight than babies of mothers with UC and of healthy population (284). This tendency could be detected in our work too, but we did not perform a statistical analysis due to the low number of patients.

IBD patients should be optimistic about a potential pregnancy. Inactive IBD is not associated with increased preterm delivery. Drugs usually used to treat IBD do not have teratogen effect. Pregnancy can result in a more favourable disease course later on and a good change in the mother's psyche, whichs help IBD patients to tolerate life with IBD more easily.

c. Carcinomas and inflammatory bowel diseases.

The carcinogenesis, especially the molecular background of cancer development on the basis of IBD has been extensively studied for the last decades. It is well known that UC patients have an increased risk of developing colorectal carcinomas. Much less is known about the risk of colonic dysplasia and cancer in chronic Crohn's colitis. The first description of a colon cancer complicating "regional enteritis" was published more than 50 years ago (285). Since then there have been many reports on colorectal cancers in a series of patients with CD (286) accumulating

evidence for similarity in the incidence of colorectal cancer in ulcerative and Crohn's colitis of similar duration and extent. These two factors, namely the duration and the extension of the inflammation, seem to be the two most significant risk factors of the development of colorectal cancer in IBD patients. Some of the data indicate that patients with CD older than 45 years of age and/or those with a recent change in symptoms may be at a particular risk (287). Strictures of the colon are common in patients with IBD, and they have a 10-fold risk of developing colon cancer (288).

Goldman reported that the occurrence of colon carcinoma in CD was approximately 3% of all cases (289). This value is less than that of ulcerative colitis because of earlier surgical intervention due to developing complications and because lesions frequently remain undetected. The risk of developing colorectal carcinoma in patients with CD is 2.5-20 times greater than that of the general population (290). Carcinomas associated with CD are characterised by a long clinical history of IBD (15-24 years), a relatively young age of onset (mean 48-63 years), (291) equal incidences of carcinomas on the left and right sides of the large intestine (292), multiplicity (10%) (293), frequent occurrence of mucinous carcinomas and signet ring cell carcinomas (294), a close relationship to active inflammation, dysplasia, stenosis and fistula (295), and a 5-year survival rate of 44-46% (296). The localisations of tumours associated with CD are the following: tumours of small bowel in 25% of cases, CRC in 70% of cases, while tumours of other organs occur in only 5% of all cases (297).

As for our case, CD was diagnosed in a relatively old age (52 years old). The patient was 56 years old at the time of the diagnosis of rectal cancer and was 59 years old when gastric cancer developed.

Environmental factors and molecular process together formed the special characteristic of "IBD-cancer" which is not different from either sporadic nor genetically determinant CRC. The likelihood that the increased risk of CRC in IBD is primarily environmental in nature and related directly to colorectal inflammation is supported by several observations. The risk of CRC increases with duration and extent of disease (more common in pancolitis) (298) and appears to be reduced with anti-inflammatory therapies. It has long been assumed that noxious compounds released during inflammation could damage DNA and/or alter cell proliferation or death, hence promoting oncogenesis. Only recently, however, have possible candidates and pathways for this role been identified. Reactive oxygen species (ROS) are known to damage DNA (299), having been linked, for example, to increased p53 mutation (300). Increased levels of ROS and increased expression of inducible nitric oxide synthase, which can react with superoxide anions to produce further toxic oxidizing agents, have both been demonstrated in UC (301). Cyclooxygenase 2 (COX2) expression is increased in active UC and UC-related neoplasm (302), and is also known to induce the expression of regulators of apoptosis, e.g. bcl2 protein (303). Finally, the interferon-inducible gene 1-8U has recently been shown to be up-regulated both in active colitic mucosa and in UC-related CRC (304). While the function of 1-8U is as yet unknown, the persisting expression of a gene induced by an inflammatory mediator may provide further clues as to how inflammation may promote carcinogenesis in IBD.

Several studies examined the possible molecular abnormalities which lead to the development of CRC in IBD. Initial studies in this area searched for molecular aberrations that were already known to be characteristic of sporadic CRCs, namely mutations of p53, APC, DCC and k-ras and up-regulation of bcl2 protein expression (305). The results of these publications suggest that IBD-related CRCs develop along a different molecular pathway than sporadic CRCs. This has led to a search for other molecular aberrations that may be specifically important to IBD-related colorectal carcinogenesis. As opposed to other CRCs, APC and DCC deletion, as well as

mutation of TGF, ß-RII and the RER seem to be missing in CRC associated with IBD (306). Traditionally, IBD-related dysplasia has been defined as any form of non-invasive epithelial neoplasia occurring within the large bowel affected by IBD. While IBD-related dysplasia has thus been referred to as a single entity, these lesions may be subdivided into flat dysplasia and dysplasia associated lesions/masses (DALMs), defined as elevated tumours occurring within colitic bowel. Regarding the frequency of dysplasia in CD, several studies (307) have shown the presence of severe dysplasia in 27-100% of patients with colorectal carcinomas in association with CD. After a rectal biopsy, dysplasia was found in 5% of the patients with CD, and a subsequent 11.1% of these patients developed carcinomas (308).
Adenocarcinoma of the stomach is extremely rare in CD (309) and if it does occur, the granulomatous involvement of the stomach could be excluded. The pathogenesis of the association between these two diseases is unknown, coincidence is most likely (310).
The time of the onset of CD is unknown in our case, but it is likely to have developed several years before the first examination. The stenotic process of the rectum was recognised first, but an inflammatory process was suspected. Surgical intervention was indicated due to multiple stenosis diagnosed by radiological examination of the bowel. Microscopic examination of the removed bowel showed colonic CD and a villous adenoma. Eight months after the operation, a postoperative recurrence was diagnosed in the neoterminal ileum and in the rectum. Endoscopic recurrence very often develops in the neoterminal ileum in 3-6 month after operation. To decrease the frequency of postoperative relapse rate is a very difficult problem during the management of patients with IBD. Recent data suggested that azathioprine/mercaptopurine is the most cost-effective therapy to prevent postoperative relapse (311). After 3 years without any signs of active CD, the symptoms of rectal stenosis reappeared. It was two months before the second operation. Neither palpation nor endoscopic examination could differentiate between inflammatory versus malignant process. Gastric cancer, which developed two and a half years after the iloestomy, was localised on the place of a little hyperplasiogen polyp which had been observed 6 years before. As other premalignant conditions (ulcer, adenomatous polyp, Helicobacter pylori infection) were not seen, it is possible that this benign disorder was the basis of the malignant transformation.
On the basis of our patient we conclude that CD patients have a higher risk of developing malignancy of any part of the entire gastrointestinal tract. As in our patient, these tumours may be metachron cancers and can also involve non-inflamed mucosa. Therefore, cancer surveillance is one of the most important aspects of the management of patients with IBD, even if the disease initiates at a relatively old age, involves the whole colon and/or a long part of the small bowel, and if there are fixed stenosis and fistulas. When years have passed after the diagnosis of IBD, the risk of cancer becomes more and more relevant.

III. Immunosupressive and immunomodulator therapy of inflammatory bowel disease

The indications of immunosuppressive therapy became more and more obvious on the basis of recently published reviews (312). Chronic continuous activity and steroid dependency are the two most common resons for commencing therapy. These two clinical conditions indicated immunosuppression in 82% of our cases. Immunosuppressive and immunomodulator therapy are becoming more and more frequent mainly in the treatment of CD, although the frequency of use is increasing in ulcerative colitis as well. Almost half of our CD patients included in the study were on immunosuppressive therapy and this rate has been increasing continuously. It seems that if a UC patient has pancolitis or more than one segment is involved in CD, the possibility of the

need of immunosuppression is high. Therefore the extension of bowel involvement is a predictive factor of immunosuppression in both diseases. Our results suggest that the effectiveness of immunosuppressive therapy is almost the same in the two diseases. Side -effects were more frequently observed and were more severe in the UC group in our study. Immunosuppressive drugs can increase the frequency of underlying infection, especially pulmonary tuberculosis (313). Not only imidazole purine analogues, but also the increasing use of cytokine-based therapy enhances the danger of tuberculosis. This is proved by the fact that the first CD patient with activated pulmonary tuberculosis during anti-TNFα treatment has been published recently (314). We suggest that chest X-rays should be routine examinations before the onset of immunosuppressive therapy. Finally, metastatic CD should be taken into consideration in the differential diagnosis of a granulomatous tissue inflammation in a patient with known CD mainly if microbiological examinations do not reveal mycobacterial infection.

Conclusions

Genetic susceptibility. The difference found between UC and CD patients in the IL1ß genotype distribution also supports the hypothesis that these conditions are polygenic disorders. The severity of UC seem to depend on the genetic control of the IL1ß and IL1RA gene polymorphism. The two examined polymorphisms of the IL1ß gene influence the course and severity not only of the UC, but also of the CD. Therefore, it is possible that the gene itself which controls IL-1ß production is involved in the pathogenesis of the disease.
Environmental factors predisposing to inflammatory bowel disease. UC is the disease of non-smokers, while the ratio of smokers among CD patients is higher than in the controls. Former smokers have a greater risk to develop UC. Passive smoking exposure in childhood may influence the development of CD at a later age.
Effect of smoking on the activity of UC. The initiation of smoking moderates the activity of UC irrespectively of drug therapy.
Prognostic significance of granulomas in CD. The fact that patients might or might not develop granulomas is a useful prognostic marker to be noted. All endoscopically available parts of the gatrointestinal tract should be examined histologically as well at the time of the first examination of the patient to search for granulomas. The presence of granulomas may suggest a more aggressive form of inflammatory bowel disease and this fact should influence our therapeutic strategy and the frequency of follow-up visits.
Efficacy of CT and LS in CD. In a severe first attack or relapse of CD, when the radiological and endoscopic procedures are relatively contraindicated, both LS and CT are convenient for the patient without any complications. Although both methods have a significant diagnostic value in active CD, the accuracy of these investigations is not the same in the different disease behaviour types. In a patient with unknown disease behaviour type and localisation, LS and CT should be performed simultaneously. In a patient with an acute flare-up and a known disease behaviour type, one of these procedures is sufficient: preferably LS in the inflammatory and stricturing form, and CT in the penetrating form of CD
Jejunoscopy in CD. The endoscopical and histological appearance of the jejunum in CD is very similar to that of the lesions of the stomach, however, in a few cases the examination of the jejunum can establish the diagnosis. Endoscopic evaluation of the upper gastrointestinal tract with biopsies of the inflamed and non-inflamed mucosa should be an important part of routine procedures in CD because of the high frequency of relevant asymptomatic endoscopic and microscopic alterations in CD patients. The examination of the upper gastrointestinal tract may help to establish the correct diagnosis in indeterminate colitis and should suspect CD of the distal part of the gastrointestinal tract in patients with unknown gastrointestinal disease.
Extraintestinal manifestations. Metastatic granulomatous inflammatory processes can involve the urinary bladder. Granulomatous inflammation in extra-intestinal sites in patients with proven CD suggests a widespread systemic involvement. During the management of patients, the urological, cardial and hematological status should also be controlled regularly.
Pregnancy and IBD. The influence of pregnancy on the course of the disease could usually be correlated with the activity of IBD at the time of conception. It seems that the activity of IBD can increase during breast-feeding. Therefore, if the patient has not received any therapy during pregnancy, it should be restarted after delivery as soon as possible. Babies born to mothers with CD have a lower birth weight than babies of mothers with UC and of healthy population.
Cancer and IBD. Carcinoma of the gastrointestinal tract is a possible risk factor not only in UC, but also in CD. The tumours in CD may be metachron cancers and can also involve non-inflamed

mucosa. Cancer surveillance is one of the most important aspects of the management of patients with IBD, even if the disease presents at a relatively old age, involves the whole colon and/or a long part of the small bowel, and if there are fixed stenosis and fistulas.
Immunosuppressive therapy. The extension of bowel involvement is an early predictive factor of the latest immunosuppression in IBD. Azathioprine seems as effective in UC as in CD, but the frequency and the severity of side- effectsside effects may limit its use in UC. Underlying tuberculosis is a real danger during immunosuppressive therapy. The degree of the danger can be evaluated at the start of the therapy.

Chapter 6. Figures and legends

Table 1. **Potential "candidate genes" in IBD**

HLA complex class I and class II (DR, DP, DQ)
Immunoglobulin
T-cell receptor
Complement system
Cytokines

Adapted from Volk BA. Immunogenetics in inflammatory bowel disease. In Inflammatory Bowel Diseases. Progress in basic research and clinical implications. Kluwer Academic Publishers 1991.

Genetic studies

Table 2. **Demographic and clinical features of the patients**

	UC	CD
Number of patients	96	97
Sex ratio (M/F)	39/57	42/55
Mean age	40.6	3 7.9
Range	18-79	19-96
Median	37	36
Age at onset	32.6	30.2
Range	14-78	15-56
Median	28	27
Disease duration	7.7	7.5
Median	6	6
Previous operation (n (%))	4 (4.2)	40 (41.2)
Localisation (%)		
In UC patients		
Rectum/proctitis	21.8	
Left-sided colitis	52.1	
Pancolitis	25.3	
In CD patients		
Ileum		20.6
Colon		28.6
Ileum + colon		46.4
Upper gastrointestinal tract		4.1

Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease

Table 3. **Clinical characteristics of the patients**

No. Of patients (F/M)	28 (15/13)
CDAI (mean±SD)	230,26 ± 125,65
Van Hees index (mean±SD)	164.81 ± 67.09
ESR (mean±SD), mm/h	43.28 ± 30.50
Hb (mean±SD), g/l	11.98 ± 2.31
Thrombocyte count (mean±SD), $x10^9$/liter	411.28 ± 158.58
Leukocyte count (mean±SD), $x10^9$/liter	10.46 ± 4.10
Serum iron (mean±SD), μg/l	7.93 ± 6.54
Serum albumin (mean±SD), g/l	40.39 ± 7.42
CRP (mean±SD), mg/l	63.64 ± 81.01
Fibrinogen (mean±SD), g/l	5.38 ± 1.64

CDAI = Crohn's disease activity index, ESR = erythrocyte sedimentation rate, CRP = C reactive protein

Significant differences in the Interleukin 1β and Interleukin-1 receptor antagonist gene polymorphism in a Hungarian population with inflammatory bowel disease

Table 4. **Frequencies of associations of interleukin-1 receptor antagonist (IL-1ra) allele 2 and IL-1β alleles in healthy control (HC) individuals and in patients with ulcerative colitis (UC) and Crohn's disease (CD)**

		Healthy controls (n=132)		UC patients (n=96)		CD patients (n=97)	
Carriers of IL-1ra allele 2	Carriers of IL-1β allele 2	number	%	number	%	number	%
Pos	Neg	24	18.2	22	22.9	33	34.0
Pos	Pos	24	18.2	21	21.8	12	12.4
Neg	Pos	36	27.3	24	25.0	27	27.8
Neg	Neg	48	36.4	29	30.2	25	25.8

Interleukin 1β gene polymorphism and the course and severity of inflammatory bowel disease

Table 5. **Carriers of allele 2 in IL1β+3953 and in IL1β-511 in healthy controls and different IBD patients**

Study group (n)	Carrier	Non-carrier	Significance
	of IL1β33953*2 n (%)		
Healthy controls (132)			
Carriers of IL1β-511*2 (74)	32 (43)	42 (57)	NS
Non-carriers of IL1β-511*2 (58)	28 (48)	30 (52)	
Ulcerative colitis (96)			
Carriers of IL1β-511*2 (60)	28 (47)	32 (53)	NS
Non-carriers of IL1β-511*2 (36)	16 (44)	20 (56)	
Crohn's disease (98)			
Carriers of IL1β-511*2 (58)	18 (31)	40 (96)	p=0.009
Non-carriers of IL1β-511*2 (40)	23 (57)	17 (43)	
Crohn's disease fistulizing group (36)			
Carriers of IL1β-511*2 (21)	9 (43)	12 (57)	NS
Non-carriers of IL1β-511*2 (15)	5 (33)	10 (67)	
Crohn's disease non-fistulizing group (62)			
Carriers of IL1β-511*2 (37)	9 (24)	28 (76)	p=0.002
Carriers of IL1β-511*2 (25)	18 (72)	7 (28)	
Crohn's disease operated patients (43)			
Carriers of IL1β-511*2 (28)	9 (30)	19 (70)	NS
Carriers of IL1β-511*2 (15)	7 (47)	8 (53)	
Crohn's disease non-operated patients (55)			
Carriers of IL1β-511*2 (30)	9 (32)	21 (68)	p=0.024
Carriers of IL1β-511*2 (25)	16 (64)	9 (36)	

Environmental factors predisposing to inflammatory bowel disease

Table 6. **Frequency of use of different environmental factors at the onset of inflammatory bowel diseases**

Exposure	Frequency of occurrence			
	Ulcerative colitis	Crohn's disease	Autoimmune disease	Significance
Current smoker	15.9%	**45.7%**	21.9%	p<0.001
Former smoker	**14.3%**	2.9%	4.7%	p<0.01
Maternal smoking	6.4%	**37.1%**	6.3%	p<0.01
Contraceptive pill	11.7%	**41.7%**	18.6%	p<0.01
Psychical stress	**41.9%**	**39.8%**	17.2%	p<0.01
Coffee consumption	49.2%	65.7%	76.6%	NS
Alcohol use	31.8%	25.7%	**11.2%**	p<0.01

The frequencies signed with bold characters differ significantly with the others; NS = non-significant

Effect of smoking on the activity of ulcerative colitis

Table 7. **Changing of activity parameters due to the onset of smoking**

Activity parameters	Before smoking	After smoking	Significance
Number of stools / 14 day	49.8 ± 41.38	36.68 ± 24.34	p=0.02
Stools containing blood /14 day	18.08 ± 25.63	4.24 ±11.82	p=0.01
Number of mucus / 14 day	10.92 ± 15.70	3.52 ± 7.93	p=0.01
Bloody mucus /14 day	5.08 ± 11.23	0.64 ± 2.29	p=0.04
General well-being	0.39 ± 0.55	0.20 ± 0.43	NS

NS = non-significant

Effect of smoking on the activity of ulcerative colitis

Table 8. . **Changing of average of laboratory parameters due to the onset of smoking**

Laboratory parameters	Before smoking	After smoking
Sedimentation (mm/h)	3.66	4.83
Serum fibrinogen (mg/dl)	230.66	206.58
Haematocrit (%)	44.50	45.51
Platelet count (G/l)	239.08	245.91
Serum iron (umol/l)	16.00	15.82
Serum sodium (mmol/l)	142.41	144.50

Prognostic significance of epitheloid granuloma in Crohn's disease

Table 9. **Clinical characteristics of patients in the two different groups**

	Patients with granuloma Group I	Patients without granuloma Group II
Number of patients	25	31
Female/male ratio (N°)	15/10	16/15
Median age (years)	29.60±11.32	36.46±12.94
Localisation		
Small bowel only	1 (4%)	4 (12.9%)
Large bowel only	10 (40%)	8 (25.8%)
Small and large bowel	10 (40%)	17 (54.8%)
UGT involvement	4 (16%)	2 (6.4%)
Disease behaviour type		
Inflammatory	8 (32%)	15 (48.4%)
Stricturing	6 (24%)	9 (29.0%)
Penetrating	11 (44%)	7 (22.6%)

UGT = upper gastrointestinal tract

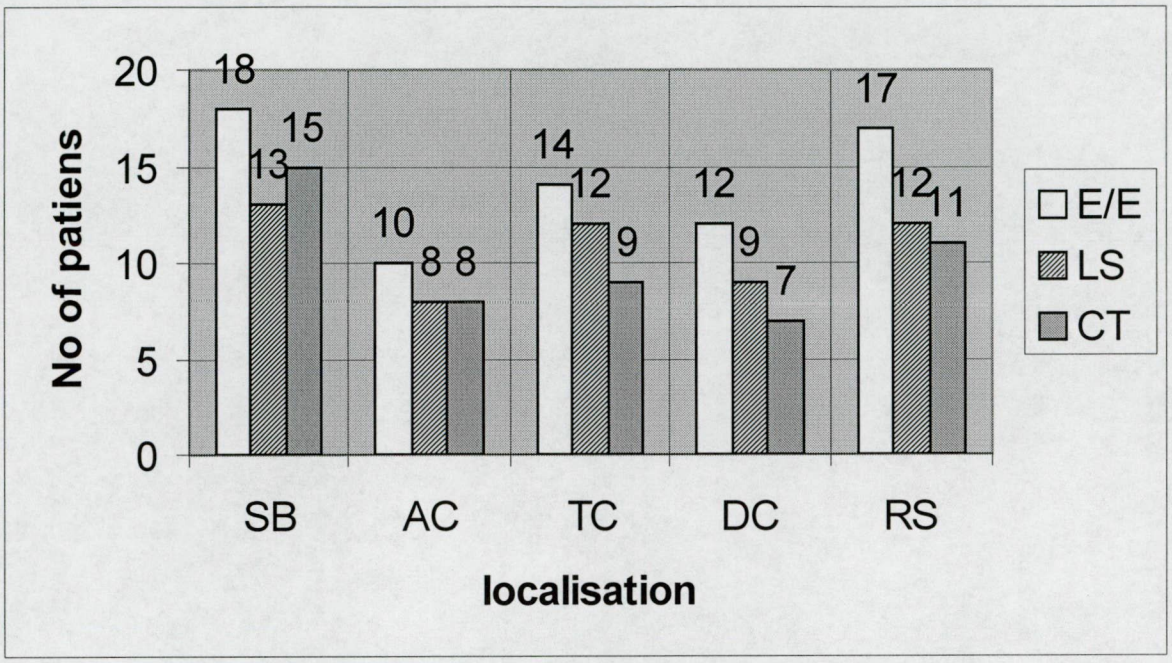
Prognostic significance of epitheloid granuloma in Crohn's disease

Table 10. **Frequency of different extraintestinal manifestations in patients with or without granuloma**

	Patients with granuloma		Patients without granuloma	
N° of extr. manifest.	10/25	40%	7/31	22.6%
Arthritis	6/25	24%	4/31	12.9%
Eye manifestation	0/25		1/31	3.2%
Cutan manifestation	2/25	8%	0/31	
Aphthous stomatitis	2/25	8%	2/31	6.4%

Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease

Figure 1. **Anatomic distribution of the positive segments**



SB = small bowel, AC = ascending colon, TC = transverse colon, DC = descending colon, RS = rectum and sigmoid colon, E/E = endoscopy and/or enteroclysis, LS = leukocyte scintigraphy, CT = computed tomography

Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease

Table 11. **Segmental inflammatory activity as determined with LS and CT compared to the endoscopic and histologic findings**

Endoscopy		0	1	2	3
		67	11	32	28
LS	0	61	6	7	4
	1	1	3	6	5
	2	4	1	11	13
	3	1	1	8	6
CT	0	56	7	8	5
	1	5	3	8	5
	2	4	1	12	7
	3	2	0	4	11

LS = leukocyte scintigraphy, CT= computed tomography

Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease

Table 12. **Sensitivity and specificity of LS and CT in the different anatomic localisation**

		SB	AC	TC	DC	RS
Sensitivity (%)	LS	72	80	85	75	70
	CT	83	80	71	58	65
Specificity (%)	LS	90	100	100	81	73
	CT	90	75	86	94	82

LS = leukocyte scintigraphy, CT = computed tomography, SB = small bowel, AC = ascending colon, TC = transverse colon, DC = descending colon, RS = sigmoid colon and rectum

Clinical value of computed tomography and leukocyte scintigraphy in active Crohn's disease

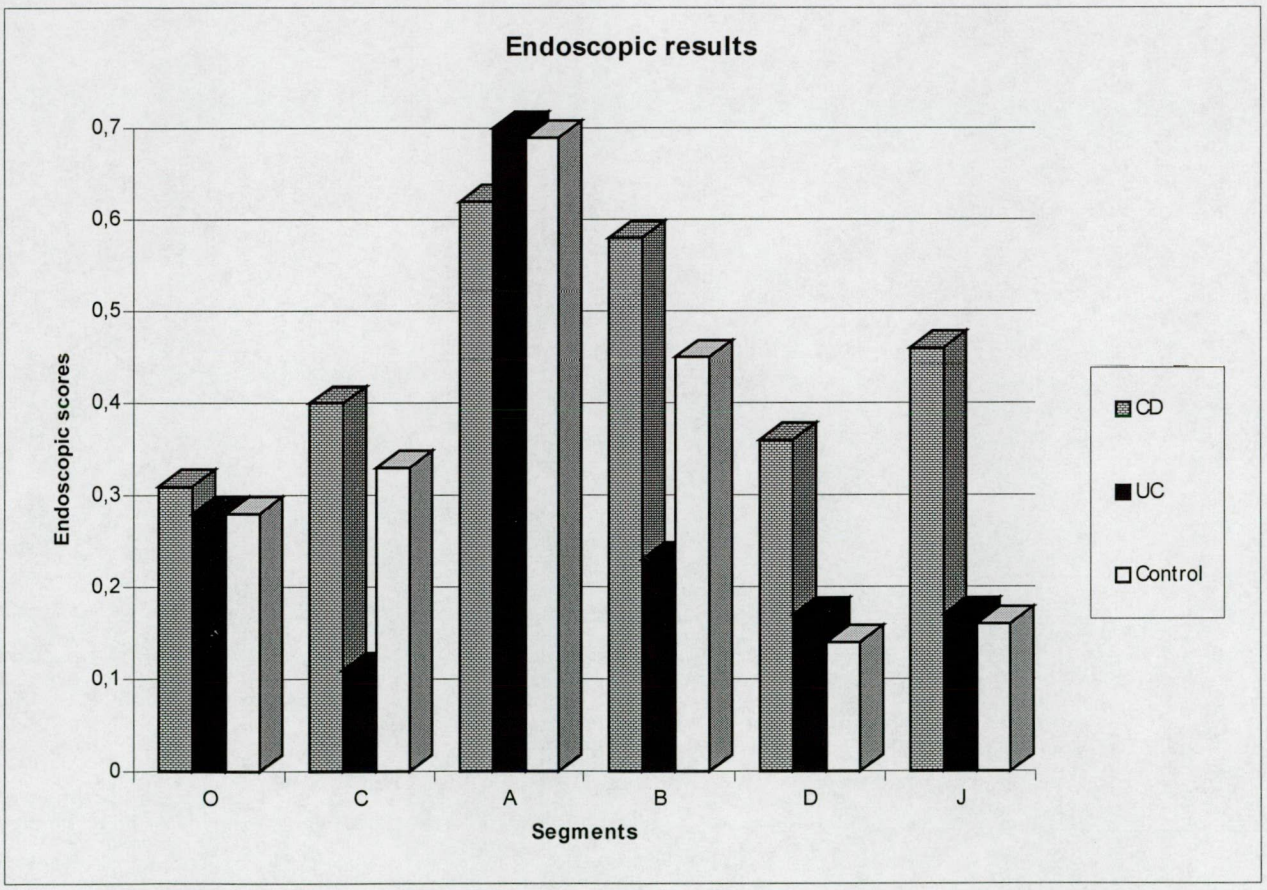
Table 13. **Correlation between activity indices, laboratory parameters and endoscopic, LS and CT scores**

	Van Hees	CDAI	Fibrinogen	CRP	Sediment.	Se albumin	Se iron	Thr. count
EAS	r=0.87 p<0.00001	r=0.78 p<0.0001	r=0.78 p<0.0001	r=0.71 p<0.0005	R=0.80 p<0.00001	r=-0.68 p<0.0005	r=-0.78 p<0.00001	r=0.73 p<0.0005
LSAS	r=0.61 p<0.005	r=0.71 p<0.0005	r=0.67 p<0.0005	r=0.64 p<0.001	R=0.59 p<0.005	r=-0.57 p<0.005	r=-0.50 p<0.01	r=0.59 p<0.005
LSEX	r=0.56 p<0.005	r=0.66 p<0.001	r=0.59 p<0.005	r=0.56 p<0.005	R=0.60 p<0.005	r=-0.46 p<0.05	r=-0.52 p<0.01	r=0.53 p<0.01
CTAS	r=0.57 P<0.005	r=0.63 p<0.001	r=0.59 p<0.005	r=0.51 P<0.01	R=0.51 p<0.01	r=-0.59 p<0.005	r=-0.52 p<0.01	NS
CTEX	r=0.52 p<0.01	r=0.63 p<0.001	r=0.58 p<0.005	r=0.49 p<0.05	R=0.56 p<0.005	r=-0.49 p<0.05	r=-0.56 p<0.005	NS

EAS = endoscopic activity score, LSAS = leukocyte scintigraphy activity score, LSEX = leukocyte scintigraphy extension (number of involved segments detected by leukocyte scintigraphy), CTAS = computed tomography activity score, CTEX = computed tomography extension (number of involved segments detected by leukocyte scintigraphy), CDAI = Crohn's disease activity index, CRP = C reactive protein, Sediment = sedimentation, Thr = thrombocyte

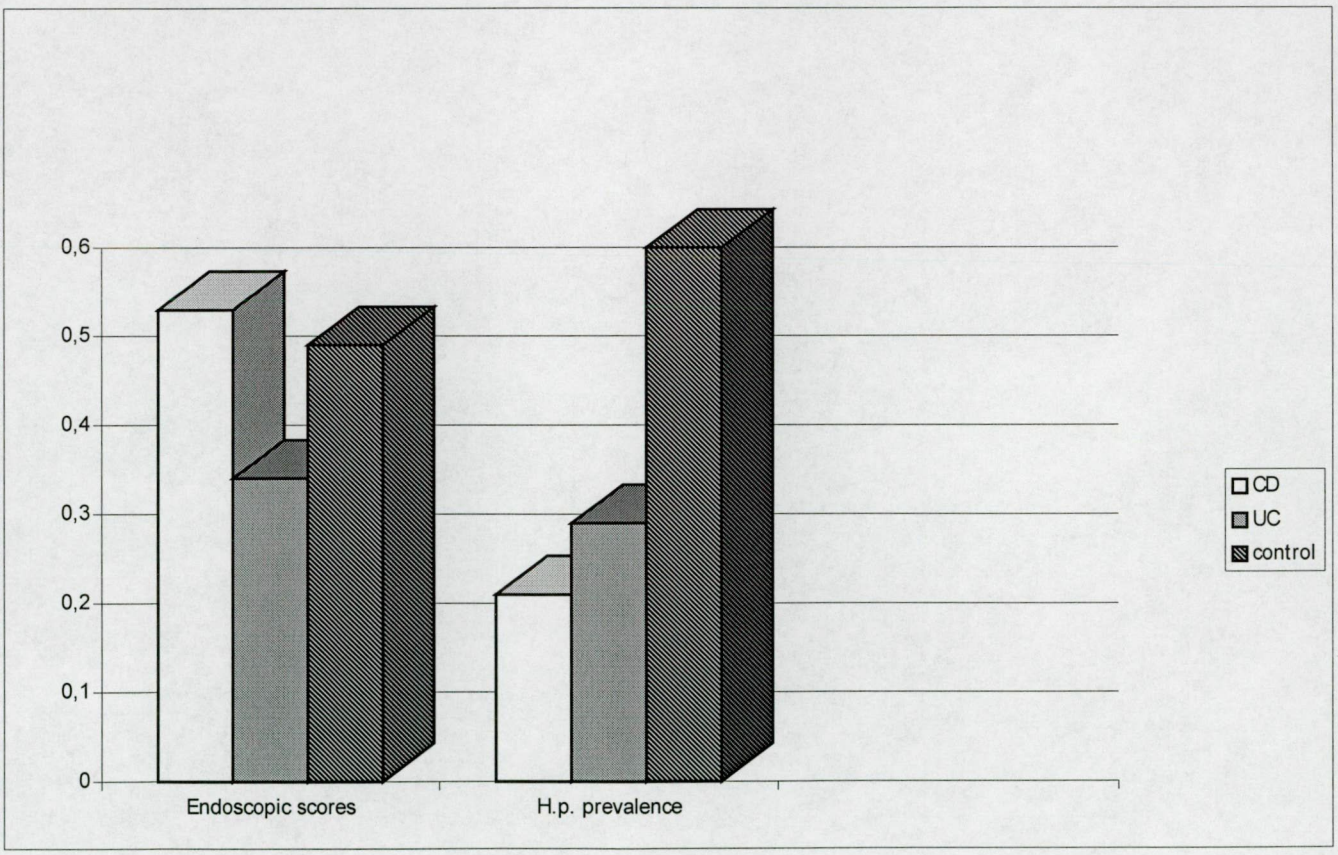
Clinical significance of jejunoscopy in Crohn's disease

Figure 2. **Endoscopic scores of the different segments**



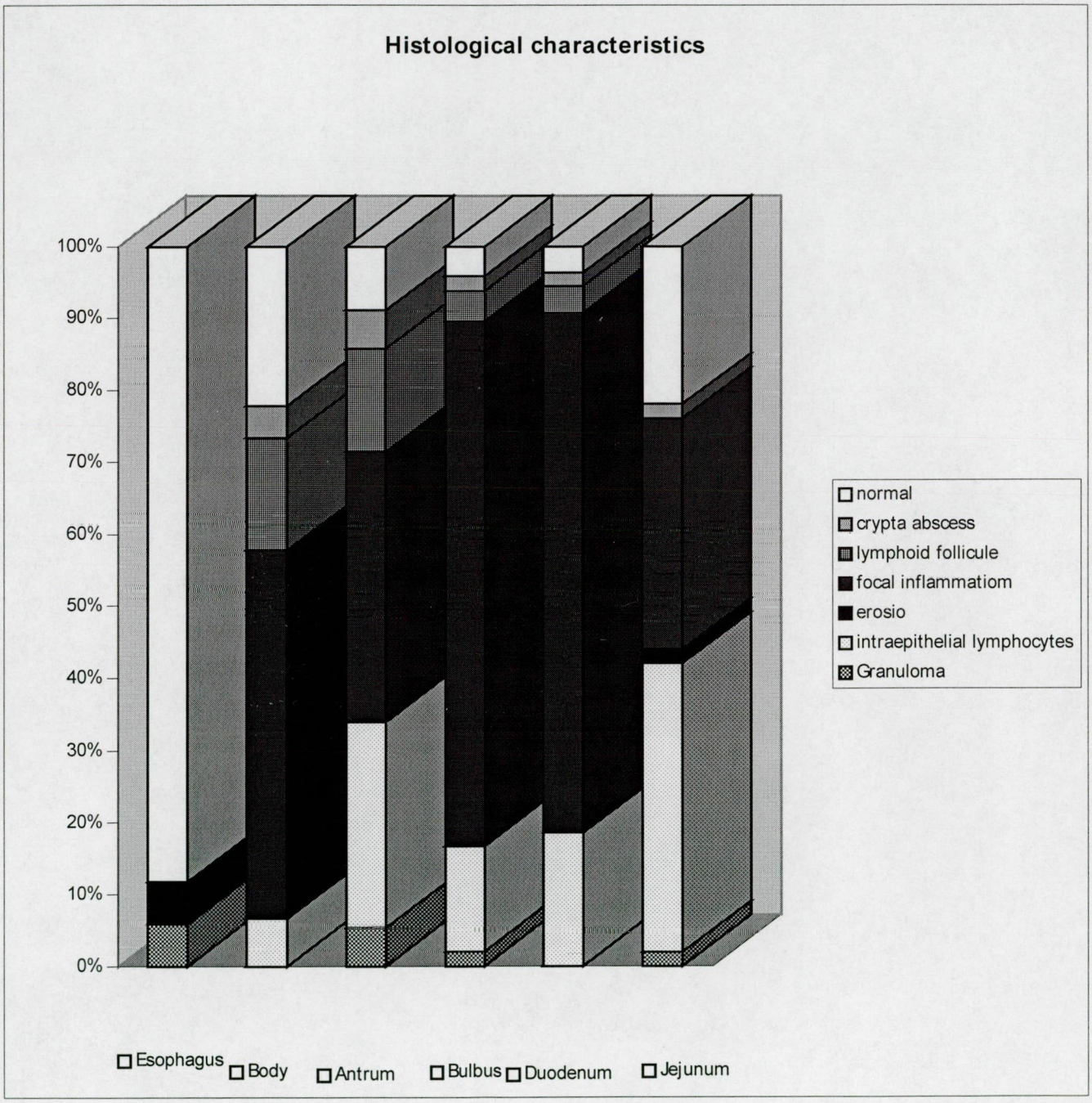
Clinical significance of jejunoscopy in Crohn's disease

Figure 3. **Endoscopic scores of the antro-duodenal region and Helicobacter pylori prevalence**



Clinical significance of jejunoscopy in Crohn's disease

Figure 4. **Results of microscopic examinations of the different segments**



Effect on pregnancy on the activity of inflammatory bowel disease

Table 14. **Comparison of the clinical data of pregnant and non-pregnant periods**

Clinical parameters	Non-pregnant period (430 controls)	Pregnant period (57 controls)
General well-being	0.38 ± 0.49	0.24 ± 0.48
Number of stools / 14 days	30.60 ± 19.37	24.26 ± 13.15
Stools containing blood / 14 days	7.03 ± 14.79	4.90 ± 9.35
Number of bloody mucus / 14 days	5.00 ± 14.70	2.65 ± 5.68
Intensity of joint pain	0.22 ± 0.41	0.11 ± 0.31

Effect on pregnancy on the activity of inflammatory bowel disease

Table 15. **Comparison of the activity of IBD on the basis of controls' data before and after the conception**

Clinical parameters	36 controls before conception	36 controls after conception	Significance
General well-being	0.38 ± 0.50	0.28 ± 0.44	NS
Number of stools / 14 days	26.0 ± 19.10	23.90 ± 14.60	NS
Stools containing blood / 14 days	4.97 ± 9.20	3.02 ± 6.80	NS
Number of bloody mucus / 14 days	8.79 ± 27.00	1.23 ± 3.58	NS
Number of / 14 days	34.8 ± 29.8	25.10 ± 15.40	NS
Intensity of abdominal pain	0.34 ± 0.50	0.18 ± 0.40	NS

NS = non-significant

Effect on pregnancy on the activity of inflammatory bowel disease

Table 16. **Therapy during pregnancy**

Patients	Disease type	Sulphasalazine (g/day)	5-ASA (g/day)	Prednisolone (mg/day)
1	UC	0	0	0
2	UC	0	2.5	10
3	UC	0	0	0
4	UC	0	0	0
5	UC	2.0	0	0
6	UC	2.0	0	0
7	UC	3.0	0	0
8	UC	0	2.0	0
9	UC	2.0	2.0	10
10	UC	0	0	0
11	UC	0	0	0
12	UC	3.0	0	0
13	UC	1.0	0	0
14	CD	1.5	0	5
15	CD	4.0	0	0
16	CD	0	0	0
17	CD	1.0	0	5
18	CD	0	0	0

UC = ulcerative colitis, CD = Crohn's disease, 5-ASA = 5-aminosalicilate

Effect on pregnancy on the activity of inflammatory bowel disease

Table 17. **Dosage of the drugs before and after the conception**

Drugs	36 controls before conception	36 controls after conception
Sulphasalazine (g/day)	1.94 ± 1.62	1.08 ± 1.28
5-aminosalicilate (g/day)	0.36 ± 0.83	0.36 ± 0.83
Prednisolone (mg/day)	3.05 ± 3.88	2.22 ± 3.40

Effect on pregnancy on the activity of inflammatory bowel disease

Table 18. **Clinical course of IBD during pregnancy and breast-feeding**

Controls	General well-being	Number of stools / 14 days	Bloody stools / 14 days	Bloody mucus / 14 days
Before conception	0.33 ± 0.44	27.8 ± 21.5	3.4 ± 6.3	8.1 ± 24.3
At the time of conception	0.17 ± 0.31	27.1 ± 14.7	2.2 ± 4.7	4.6 ± 13.1
First part of pregnancy	0.26 ± 0.47	25.3 ± 12.1	3.2 ± 5.2	3.4 ± 4.7
Second part of pregnancy	0.28 ± 0.60	27.3 ± 13.9	5.0 ± 6.7	2.4 ± 3.3
Breast-feeding	0.25 ± 0.38	31.1 ± 21.0	2.4 ± 4.8	8.4 ± 25.2
Significance	NS	NS	NS	NS

NS = non-significant

Effect on pregnancy on the activity of inflammatory bowel disease

Table 19. **Characteristic data of newborns**

Newborns New-borns	Mother's disease	Gestation time	Height of newbornnew-born	Weight of newbornnew-born
1	UC	42	52 cm	4000 gram*
2	UC	37	49 cm	2700 gram
3	UC	40	49 cm	3110 gram
4	UC	40	52 cm	3730 gram
5	UC	36	50 cm	3500 gram
6	UC	36	48 cm	2600 gram
7	UC	40	54 cm	4360 gram
8	UC	40	50 cm	3400 gram
9	UC	39	51 cm	3500 gram
10	UC	39	52 cm	3005 gram
11	UC	39	52 cm	3300 gram
12	UC	?	?	2900 gram
13	UC	39	53	3100 gram
14	UC	?	?	3150 gram
15	CD	?	?	2050 gram
16	CD	32	42 cm	2000 gram
17	CD	?	?	2950 gram
18	CD	41	51 cm	2900 gram
19	CD	40	52 cm	2600 gram

UC = ulcerative colitis, CD = Crohn's disease, * = newborn with big vessels transposition

Effect on pregnancy on the activity of inflammatory bowel disease

Table 20. **Comparison of newborns' data according to mother's disease**

Mother's disease	Gestation time (mean)	Height of newborns (mean)	Weight of newborns(mean)
Ulcerative colitis	38.9	51.0	3311
Crohn's disease	37.6	48,3	2500

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Chapter 8. Publications

Full papers

1. Molnár T., F. Nagy: Autoimmun hemolitikus anaemia colitis ulcerosában. Orvosi Hetilap 1993,134:2263-69.
2. Molnár T., F. Nagy: A dohányzás hatása a colitis ulcerosa aktivitási tüneteire. Magyar Belorvosi Archívum 1996,49:207-11.
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12. T. Molnár, Cs. Gyulai, F. Nagy, J. Lonovics: Mycobacteria and Inflammatory Bowel Diseases: A Cumulative Association Due to Immunosuppressive Therapy? Scandinavian Journal of Gastroenterology (in press)
13. T. Molnár, L. Tiszlavicz, Cs. Gyulai, F. Nagy, J. Lonovics: Prognostic value of granuloma in Crohn`s Disease (submitted)

B. Published abstracts related to the thesis

1. T. Molnár, F. Nagy: Autoimmune hemolytic anemia in ulcerative colitis. Z. Gastroenterol 1992;30: A
2. T. Molnár, F. Nagy: Effect of smoking on the activity of ulcerative colitis. Z Gastroenterol 1995;33:155A.
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18. T. Molnár T, F. Nagy: Efficacy and safety of immunosuppressive treatment for inflammatory bowel disease. Z Gastroenterol 39;A408,2001.
19. F. Nagy, T. Molnár: Colorectal malignancies associated with ulcerative colitis. Z Gastroenterol 39;A432,2001.
20. T. Molnár, L. Tiszlavicz, T. Krenács, F. Nagy, J. Lonovics. Increased number of enteric ganglion and neuroendocrine cells in the bowel of patients with Crohn's disease. Z Gastroenterol (in press).