INFLAMMATORY BOWEL DISEASES:

GENETICS, INFLAMMATORY MEDIATORS, NEW TREATMENTS

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The inflammatory bowel diseases (IBD), ulcerative colitis (UC) and Crohn's diseases (CD) are considered distinct disease entities although they share some common features. Ulcerative colitis is a continuous mucosal inflammation that almost invariably involves the rectum and may proceed proximally to involve part or the whole colon but only the colon. Crohn's disease is a discontinuous transmural inflammation of the intestine with a predilection for the ileocecal area but may involve any part of the gastrointestinal tract. Both diseases are chronic with an unpredictable relapsing course. A subset of patients with UC or CD develops extraintestinal manifestations with involvement of joints, eyes or skin (shared features).

The incidence of Crohn's disease in northern Europe and the United States has been increasing since 1960 but the increase appears to have leveled off since the 1980s, whereas incidence rates for UC have remained stable over the observed period from 1960 to 1994 (Figure 1).[1] The most recent estimates of incidence and prevalence of IBD from a Canadian study show an equal incidence of UC and CD at $14/10^5$/year with a prevalence of $198/10^5$ for CD and $170/10^5$ for UC.[2] If the prevalence of IBD is similar in the U.S., then there are about 900,000 patients with IBD in this country.

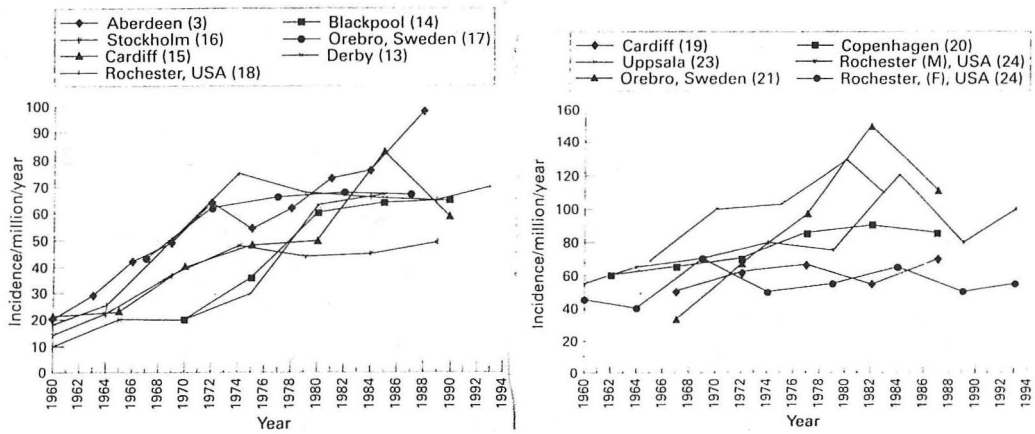

Fig. 1. Incidence rates of CD and UC

The etiology and pathogenesis of IBD are unknown. Most investigators in the IBD field support the hypothesis that IBD is due to an abnormal mucosal immune response to antigens of the normal enteric bacterial flora in a genetically susceptible host.[3] This review outlines the evidence for this hypothesis with special emphasis on the genetics of IBD and the inflammatory mediators of the

mucosal immune response, and it concludes with an overview of the new treatments of IBD based on new immunologic concepts of these inflammatory diseases.

Genetics

The concept that genetic susceptibility may play a role in the etiology of IBD has slowly gained recognition over the past several decades since Kirsner and Spencer first reported on the familial aggregation of IBD.[4] There are now many studies that have confirmed that the risk of developing IBD is increased in relatives of patients with IBD (Table 1).[5-10]

n	Disease	%	Country
400	CD	8	Holland
637	IBD	10	Denmark
1048	CD	13.4	Sweden
443	CD	18	UK
640	CD	20.6	Belgium
188*	IBD	23.4	US

* 82% Ashkenazi

Table 1. Risk estimates of familial IBD

It is estimated that 10 to 20% of patients with IBD have affected relatives. The risk of developing IBD is greater in first-degree than in second-degree relatives. In one study, it was found that first-degree relatives had a 10-fold increased risk of developing the same disease as the affected patients (UC or Crohn's). The risk of developing UC in a first-degree relative of a patient with Crohn's disease was also increased but to a lesser extent, whereas the risk of developing Crohn's disease in relatives of patients with UC was not increased in this study. The same trend has been observed in most studies of familial occurrence of IBD i.e. the highest risk to first-degree relatives is to develop the same type of IBD as in the index case. Another trend that has emerged from these family studies of IBD is that patients with familial CD have an earlier age of onset and more extensive disease.[11-12] In a study from Oxford, UK, involving 433 adult patients with Crohn's disease, it

was found that 78 families (18%) had at least one first- or second-degree relative who also had IBD.[13] Three relatives were affected in 14 families and four in two families. Both CD and UC occurred in the multiply affected families. Siblings were most commonly affected and Crohn's disease was more common than UC in the affected siblings. Based on the prevalence rates for CD and UC in the UK, the relative risks in siblings of patients with Crohn's disease were calculated to be 36.5 for CD and 16.6 for UC. The relative risk is defined as the recurrence risk of a relative of a proband divided by the risk for the general population and is commonly denoted γ_R or λ_S (sibling). The magnitude of λ_S is related to the degree of concordant inheritance of genetic determinants in affected siblings. Mapping of genetic traits with high λ_S (>10) is much more likely to be successful than in traits with low λ_S (<2). The high λ_S for CD and UC observed in the UK study may be compared with type I diabetes (λ_S=15) where genetic mapping has been applied successfully. The variability in incidence rates among the many family studies may be due to selection bias or due to ethnic variability within the study population. It should also be emphasized that all these studies were conducted in Europe or North America in patients with similar ethnic background. Ethnicity plays a definite role in the incidence of IBD as reflected by the high incidence of IBD in Ashkenazi Jews who have a 2- to 4-fold higher risk of developing IBD as compared to Caucasians living in the same environment.[14] Ashkenazi Jews also have a higher risk when compared to Sephardic or Oriental Jews. The incidence of IBD in Asia, Africa and South America appears, on the other hand, to be much lower than that observed in Caucasians as judged from the few series published from some of the countries on these continents.[15-17]

The most compelling evidence for a genetic component to IBD etiology comes from twin studies. In a study from Sweden comprising 25,000 pairs of twins, 80 pairs were identified where one or both suffered from IBD (UC:36 pairs; Crohn's:44 pairs) (Table 2).[18] In the UC group, there were 16 monozygotic and 20 dizygotic pairs, and only one pair of monozygotic twins was concordant for the disease and none of the dizygotic pairs were concordant. In the Crohn's disease group, there were 18 monozygotic and 26 dizygotic pairs of which eight monozygotic pairs and one dizygotic pair were concordant for the disease. All 80 twin pairs had been brought up together.

25,000 Swedish twin pairs;80 pairs with IBD

	UC		CD	
	MZ	DZ	MZ	DZ
	16	20	18	26
Concordant	1	0	8	1
Discordant	15	20	10	25
Concordance rate	6.3%		44.4%	

Table 2. Swedish twin pair study

A significantly higher concordance rate in monozygotic than in dizygotic twins argues for a genetic influence if the environment is shared. It was concluded from this study that Crohn's disease has a strong genetic component (~50%) whereas the evidence for genetic factors in ulcerative colitis is relatively weak. The data from the Swedish twin study were confirmed in a Danish twin study comprising 37 twin pairs with Crohn's disease (10 monozygotic; 27 dizygotic) and 62 twin pairs with UC (19 monozygotic; 43 dizygotic).[19] Four of the 10 monozygotic twin pairs with Crohn's disease were concordant whereas only one of the 19 monozygotic twin pairs with UC was concordant. Finally, some rare genetic diseases are associated with inflammatory bowel disease. These diseases include Turner syndrome, Hermansky-Pudlak syndrome (albinism and platelet dysfunction), and glycogen storage disease type 1b (neutrophil dysfunction); all of which have an increased incidence of a Crohn's disease-like enterocolitis.

The family and twin studies support the hypothesis that genetic susceptibility is an important component of the etiology of IBD. It should also be emphasized that spouses of IBD patients or adopted family members in an IBD family have the same risk of developing IBD as a control population despite the shared environment. The mode of inheritance of IBD is complex and does not fit a simple Mendelian pattern of a monogenic disorder. Two models of inheritance have been proposed to account for this complexity: a polygenic model and an oligogenic (multilocus) model. The polygenic model suggests that multiple genes, each with a small effect, cause the disease.

McConnell, a proponent of this hypothesis, suggested that 10 to 15 genes were involved in the IBD genotype.[20] If a person had only a few of these genes, he had increased susceptibility to UC, and if a person had most of these genes, he was at risk for Crohn's disease. This model would explain why a relative of a patient with Crohn's disease is more likely to develop IBD than relatives of patients with UC, as the relatives of Crohn's patients have inherited more risk genes. However, the polygenic model has been rejected by genetic analyses of large family studies of IBD patients. These analyses concluded that the risks to the relatives of IBD patients are increased more than would be explained by a polygenic model.

The oligogenic model proposes that only a few major genes interact to induce the disease phenotype. While there is little scientific evidence to support this model at this time, it is a theoretically attractive model. For example, this model may explain the occurrence of both UC and Crohn's disease in the same family if one postulates that one gene may be a susceptibility gene to both diseases and interaction with one or more specific genes is necessary to induce the specific disease phenotypes (UC or Crohn's). The correct genetic model should become evident once the genes involved in the etiology and pathogenesis of IBD have been identified.

Finally, it has been suggested that there may be genetic heterogeneity within IBD i.e. there may be several genetically distinct subtypes within the same phenotype.[21] There is convincing evidence that UC and Crohn's disease are related disorders. Some patients have clinical features of both diseases, so called indeterminate colitis, and there are families where some relatives have Crohn's disease and others UC. The interrelationship of CD and UC indicates that some genes confer susceptibility to both diseases while other susceptibility genes are distinct for either CD or UC. The concept of genetic heterogeneity is also supported by the fact that subsets of UC or CD patients may develop extraintestinal manifestations. Additionally, the presence of the serologic marker pANCA in a certain proportion of both UC and CD patients is evidence for the existence of subtypes within these two diseases.

The quest to identify the IBD susceptibility genes is hampered by the fact that it is a complex trait and therefore requires the inclusion of a large number of multiply affected families in the genetic analysis. Three types of genetic analysis have been used: complex segregation analysis, genome-wide scanning with microsatellite markers and candidate gene approach. Complex segregation analysis is based on the distribution of the disease in nuclear families (parents and their offspring)

and it involves fitting a general model to the inheritance patterns of a trait in pedigrees. Segregation analysis has been performed in three large family studies of IBD.[22-24] Two studies included patients with UC or Crohn's disease and one only Crohn's disease. All three studies suggested that Crohn's disease best fits a model with a recessive gene with incomplete penetrance but that only from 7 to 30% of cases are due to the presence of this gene. The two studies that included UC also agreed that a major dominant gene with low penetrance is present in about 10% of UC cases. It should be emphasized, however, that segregation analysis cannot distinguish between simple Mendelian models and complex traits.

The genetic analysis of IBD that has been most successful has been family-based linkage analysis with a systematic mapping of the whole genome with multiple microsatellite markers.[25] The linkage analysis basically asks the question whether a disease (i.e. CD or UC) is linked to a specific chromosomal segment identified with one or more genetic markers. Linkage analysis does not require any prior knowledge of the location of the genes of interest. Because large chromosomal segments are usually shared between parents and offspring and between siblings due to few recombinations in two generations, the chance that a genetic marker segregates with a susceptibility gene (linkage) is high if the marker and the gene are sufficiently close [10-20 cM (centiMorgans)]. Genome-wide scanning requires a large number of microsatellite markers (300-400) that are evenly spaced about 10 cM apart to properly conduct a linkage analysis. The first genome-wide scanning study was conducted in France and included a total of 78 nuclear families, each with at least two affected siblings with Crohn's disease.[26] A highly significant linkage (lod 3.17; $p<1.5x10^{-5}$) was observed to a locus on chromosome 16 which was called IBD1 (Table 3). Since then, an additional seven studies have been published comprising studies conducted in Europe, North America and Australia, either as genome-wide scans or replication studies.[27-33] Five of the seven studies confirmed the linkage of CD to chromosome 16. The most significant linkage was observed in the Australian study with a lod score of 6.3. Importantly, two studies failed to show linkage to chromosome 16. There has been debate among geneticists of which lod score constitutes a significant linkage in the analysis of complex traits. It has been proposed that a lod score above 3.6 is required to show statistical significant linkage in sib pair analysis ($p<3x10^{-5}$).[34] It is clear then that only the Australian study fulfills this criterion. The fact that five additional studies have shown linkage to this chromosome, albeit with less significant lod

Country	Design	Study size asp*	Disease	Lod
France	Genome	112	CD	3.17
UK	Genome	81	CD	2.6
USA	Replication	75	CD	2.41
Australia	Replication	54	CD	6.3
USA	Genome	175	CD	1.69
Canada	Replication	114	CD	Neg
Belgium	Replication	79	CD	Neg
Germany	Genome	162	CD	1.71

* affected sibling pair

Table 3. Linkage of CD to chromosome 16

scores, has been accepted as confirmatory evidence for a susceptibility gene(s) for CD on chromosome 16.

Seven groups have examined evidence for linkage of IBD with chromosome 12. The initial study from UK found a highly significant linkage (lod 5.47; $p<2.7x10^{-7}$) for IBD (both UC and CD) to a locus on this chromosome.[27] Three subsequent studies have confirmed a linkage to chromosome 12 (1 IBD, 2 CD only) although with lower lod scores.[28,35,36] Three groups from the U.S., Canada and Belgium, respectively, however, found no evidence for linkage of IBD to any locus on chromosome 12.[30-32] More recently, evidence for linkage of IBD or CD to other chromosomes has been observed.[37-40] These linkages include loci on chromosomes 1, 5, 6, 14 and 19 (Table 4). The lod scores for these five loci were 3.6 or greater which imply significant linkage. The studies of linkage to chromosomes 1 and 6 were replication studies i.e. confirmatory evidence of a previously identified locus, whereas the linkage to chromosomes 5, 14 and 19 are newly identified loci. The many groups in the U.S., Europe and Australia who study the genetics of IBD have now formed an 'International IBD Genetics Consortium' located in Canberra, Australia. The Consortium has just completed a large IBD family study (581 families: 382 CD, 91 UC, 108 mixed).[41] The families were genotyped with markers spanning the identified loci on chromosomes 12 and 16. The linkage of CD to chromosome 16 was again confirmed (lod 5.2). UC was not linked to the chromosome 16 locus. Neither IBD nor CD or UC were significantly linked to the

Country	Design	Study size asp	Disease	Chromo-some	Lod
USA	Genome	181	IBD	19	4.6
			CD	5	3.9
USA	Genome	127	CD	14	3.6
USA	Replication	239	CD	1	3.9
Germany	Replication	428	IBD	6	4.2

Table 4. Linkage of IBD to other chromosomes.

locus on chromosome 12. Currently, none of the susceptibility genes in the regions of interest on the chromosomes have been identified. The distance between the microsatellite markers used in most studies is about 10 cM which corresponds to about 10 million base pairs and contains hundreds of genes.

Another method that has been utilized in the genetic analysis of IBD is the candidate gene approach. This approach starts with the hypothesis that a candidate gene may contribute to the disease phenotype. Many of these studies have focused on the HLA region on chromosome 6 which contains a number of genes involved in the immune response. The studies involve genotyping of IBD families and using nonparametric analysis to identify sharing of specific alleles among affected sibling pairs. A large UK study of IBD families showed a positive association of UC with an HLA class II allele, DRB1*0103 which was found in 8.2% of UC patients vs. 3% in controls.[42] The HLA-DRB1*1502 allele is found in 49% of Japanese UC patients vs. 13.8% in controls. This allele is very rare in Caucasian populations. No positive associations have been observed between CD and specific HLA class II alleles. Other candidate genes that have been studied include T cell receptor genes, cytokine genes (IL-1, IL-2, IL-4 and IL-10) and mucin genes. Specific polymorphisms of these genes that are positively associated with IBD have so far not been identified. Thus, the candidate gene approach has until now been generally unrewarding in the genetic analysis of IBD.[43]

In summary, genome-wide scanning with multiple markers appears to be the most promising approach to the genetic analysis of IBD. The region identified on chromosome 16 is significantly linked to Crohn's disease. The loci identified on chromosomes 1,5, 6, 14 and 19 also appear to be promising targets for closer scrutiny. The regions of interest need to be further narrowed down before fine mapping and, ultimately, identification of the genes can be accomplished which still represents a formidable challenge. The completion of the Human Genome Project with a more detailed map of the chromosomes and the collaboration among the many individual groups that study the genetics of IBD, which is now in place by the formation of the International IBD Genetics Consortium, may aid in this endeavor.

Inflammatory Mediators of the Mucosal Immune System

The intestinal inflammation observed in IBD is thought to be a dysregulated immune response to antigens from the normal intestinal bacterial flora.

Intestinal Microflora

The normal bacterial flora of the human intestine is exceedingly complex and the bacterial composition changes both quantitatively and qualitatively from proximal small intestine to the colon.[44] The bacterial counts are low in the jejunum (10^2-10^3 cfu/g) and the dominant bacteria are gram-positive and aerobe. In the colon, the bacterial counts are as high as 10^{12} cfu/g and 99% of the viable bacteria are obligate anaerobe. So far, about 350 different bacterial species have been identified in the colonic microflora. There are no apparent qualitative or quantitative differences of the intestinal microflora between normal controls and IBD patients. There have been multiple attempts over the years to implicate specific bacteria as causative agents of IBD. For example, both mycobacterium paratuberculosis and Listeria have been proposed as causes of Crohn's disease but carefully controlled studies have been unable to substantiate these claims.[45] Thus, there is no evidence that CD or UC are infectious diseases. There is no doubt, however, that infections can either initiate IBD or, more typically, reactivate quiescent IBD. It is estimated that up to 50% of relapses of IBD are associated with respiratory infections. The huge load of bacteria in the distal intestine releases numerous antigens such as surface antigens as well as cell wall components (LPS, peptidoglycan-polysaccharide) and secreted oligopeptides (FMLP) which are capable of initiating an immune response in the intestinal mucosa.

Mucosal Immune System

The defense against antigens released by the intestinal microflora is mediated by the mucosa-associated lymphoid tissue (MALT) which consists of several compartments: lymphoid follicles (Peyer's patches), lamina propria lymphocytes, intraepithelial lymphocytes and the secretory IgA system.[3] The lymphoid follicles are located in the lamina propria throughout the intestine underneath a specialized epithelium, the follicle-associated epithelium, where the M cells are found (Fig. 2).

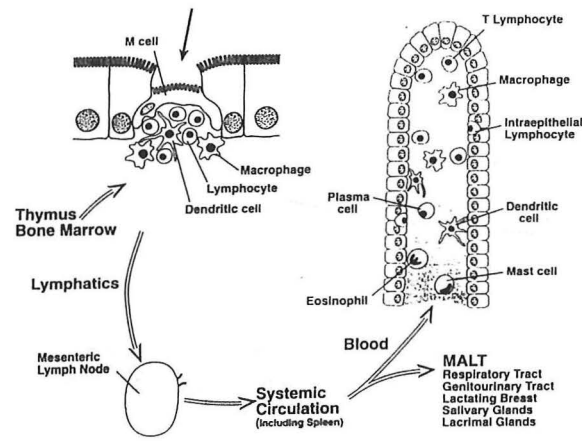

Fig. 2. Mucosal immune system

M cells are derived from the enterocyte population and have acquired a pinocytotic function. Bacterial antigens are actively taken up by these cells and transported into the lymphoid follicles. The follicles are composed of B cells, T cells, macrophages and dendritic cells i.e. all the necessary components for antigen processing and presentation to B and T cells to initiate an immune response. Activated lymphocytes leave the follicles via efferent lymphatics, reach the systemic circulation and eventually home back to the intestine where they take up residence as lamina propria or intraepithelial lymphocytes. The 'homing' back of lymphocytes to the lamina propria is governed by a sequential series of interactions between cell adhesion molecules (selectins, integrins, ICAM) on the lymphocytes and the endothelial cells. The majority of lamina propria lymphocytes are T cells (2/3 CD4 and 1/3 CD8). They have the phenotypic characteristics of memory cells and are activated i.e. they upregulate cytokine production upon stimulation. Current evidence suggests that lamina propria lymphocytes regulate the mucosal immune response to

luminal antigens. Animals raised under germ-free conditions do not develop lymphoid follicles and have very few lamina propria lymphocytes which suggests that the development of MALT depends on the exposure to luminal bacterial antigens.

The intraepithelial lymphocytes migrate up into the epithelial layer from the lamina propria and are found interspersed among the epithelial cells. These lymphocytes are primarily CD8 cells and have cytotoxic activity. They are situated as a frontline defense against luminal antigens and microorganisms but their precise role in the mucosal immune system is still debated. They may serve a protective role against parasitic and viral infections by secretion of IFNγ.

There are also a large number of plasma cells in the lamina propria of which the majority produces IgA. Dimeric IgA is endocytosed across the basolateral membrane of enterocytes by a specialized transport system, transported across the cells and released into the intestinal lumen as secretory IgA (sIgA). Secretory IgA binds to viruses, bacteria and toxins, thereby preventing mucosal uptake and activation of the mucosal immune system. In man, there is a daily production of 3-4 g of sIgA. Production of secretory IgA in germ-free animals is virtually absent which again illustrates that luminal antigens drive the mucosal immune response.

Regulation of the Mucosal Immune System

Bacterial antigens taken up by the M cells first encounter macrophages or dendritic cells of the innate immune system. These cells possess a specialized set of receptors (pattern recognition receptors) which transport bacterial antigens into the cells where they are degraded into peptide fragments. The peptide fragments are subsequently presented by the MHC class II molecules on the cell surface to the T cell receptor (TCR) on naive T cells (Th0) to initiate an immune response. The activated T cells may then develop into several subsets depending on the predominant cytokines secreted by macrophages or dendritic cells.[46] Secretion of IL-12 drives a Th1 response and this subset of CD4 T cells secretes IL-2, IFNγ and TNFα and mediates cellular immunity (Fig. 3). Secretion of IL-4 results in differentiation of Th0 cells into a Th2 subset. These cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13 and mediate humoral immunity. The two subsets, Th1 and Th2, mutually inhibit each other.

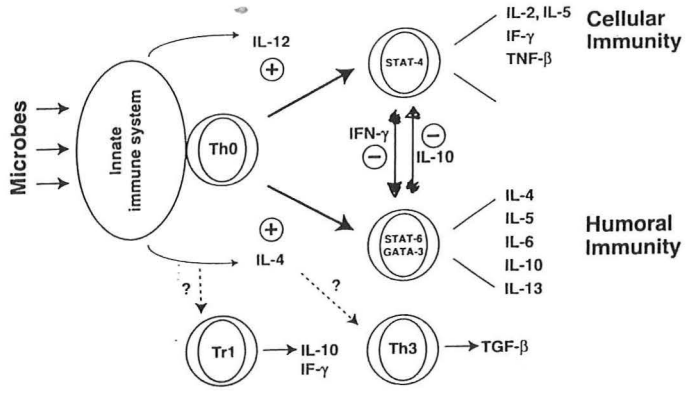

Fig. 3. CD4 T cell subsets

Secretion of IFNγ by Th1 cells inhibits a Th2 response and IL-10 secreted by Th2 cells suppresses a Th1 response. It is apparent that the decision to respond to a bacterial antigen with a Th1 or Th2 response is made by the innate immune system by selective secretion of either IL-12 or IL-4. The mechanisms determining these selective responses are largely unknown but genetic factors play a definite role. For example, most strains of mice infected with Leishmania major develop a Th1 response and clear the infection.[47] In contrast, BALB/C mice with a different genetic background mount a Th2 response and die from an overwhelming infection. The balance between a Th1 response and a Th2 response and their mutual regulation is thought to represent an important mechanism in the prevention of mucosal inflammation. There are now a number of animal models that illustrate this point.[48-52] Both knockout and transgenic models have been developed in mice or rats (Table 5) where either specific cytokines have been deleted or overexpressed or where T cell receptor function has been disrupted. All of these models are characterized by the development of colitis when mice or rats are raised under normal conditions but, importantly, absence of colitis when raised under germ-free conditions. Furthermore, the cytokine profile from stimulated lamina propria lymphocytes from inflamed colon is characteristic of a Th1 response in most models except for the TCRα negative mouse. These observations emphasize two points: intestinal bacteria are necessary for induction of colitis and a Th1 response

Gene	Species	Defect	Pathology	Germ-free
IL-2	Mouse	ko*	Colitis	Healthy
IL-10	Mouse	ko	Entero-colitis	Healthy
TCRα	Mouse	ko	Colitis	Healthy
TGFβ	Mouse	ko	Colitis	Healthy
TNFΔARE	Mouse	tg#	Entero-colitis	Healthy
HLA-B27	Rat	tg	Entero-colitis	Healthy

* knockout;#transgenic

Table 5. Animal models of colitis

is a common characteristic of intestinal inflammation. There is only limited inflammation in the intestine of normal persons, so-called physiologic inflammation, which has been interpreted as evidence for tolerance against the antigens from the normal intestinal microflora.[53] Naive T cells (Th0) can differentiate into two more recently defined subsets of CD4 T cells.[54] One subset is labeled Th3 and the predominant cytokine secreted by these cells is TGFβ. The other subset is Tr1 (r=regulatory) and the major product of these cells is IL-10. The cytokines that regulate these two subsets are still undefined. Both subsets play a major role in the development of tolerance by preventing an inflammatory response to antigens. Tolerance can be induced in experimental animals by feeding repeated low dose antigens which result in an increased production of IL-10 and TGFβ by lamina propria lymphocytes which, in turn, suppress a proinflammatory Th1 response.[55] Feeding a high dose of antigen leads to tolerance by clonal anergy and deletion by apoptosis of both Th1 and Th2 clones. The evidence that a state of tolerance exists in man was shown in experiments with isolated lamina propria lymphocytes obtained from intestinal biopsies.[56] These lymphocytes failed to respond to antigens from bacterial cultures of the same biopsies but did respond to antigens from bacterial cultures from other individuals. Furthermore, CD4 T cells isolated from the lamina propria of normal human intestine proliferate poorly in vitro when stimulated with bacterial antigens.[57] However, the T cells regain a proliferative response if

antibodies to IL-10 or TGFβ are included in the cultures. The lack of a proliferative response of normal lamina propria T cells is presumably due to secretion of the inhibitory cytokines IL-10 and TGFβ by the regulatory subsets Tr1 and Th3. This, in turn, results in a state of tolerance to bacterial antigens released from the resident intestinal microflora and limited intestinal inflammation. The induction of tolerance to common bacterial antigens is the major reason that normal man lives in a peaceful symbiosis with a multitude of intestinal bacteria.

Dysregulated Immune Response in IBD

The earliest intestinal lesions in Crohn's disease are the aphthous ulcers. These discrete ulcers develop in the epithelium overlying the lymphoid follicles. Thus, the local immune response is presumably directed towards bacterial antigens taken up by the M cells and processed in the lymphoid follicles. A number of studies have now been performed in an attempt to characterize cytokine production in the lamina propria of inflamed intestine using different approaches such as an RNA expression, organ culture, or stimulation of isolated lymphocytes from intestinal biopsies or resections. Recent studies of cytokine production by isolated lamina propria lymphocytes obtained from intestinal biopsies of patients with CD, UC or controls showed increased production of IFNγ and TNFα and decreased production of IL-4 upon antigen stimulation in CD compared to UC and controls.[58-60] In addition, there were spontaneous expression and production of IL-12 in patients with CD, but no expression was observed in UC. IL-12 is produced by macrophages in response to bacteria and plays a pivotal role in T cell differentiation into a Th1 subset.[61] For example, activation of lamina propria T cells with anti-CD3 antibody in fetal gut explants results in minimal IFNγ and TNFα secretion and no tissue injury. Addition of IL-12 with anti-CD3 antibody leads to a marked increase in IFNγ and TNFγ secretion and severe tissue injury due to secretion of metalloproteinases.[62] The central role of IL-12 in the Th1 response has also been demonstrated in a murine model of colitis where administration of IL-12 antibodies leads to normalization of IFNγ production and reversal of colitis.[55] IL-12 increases the production of IFNγ by CD4 cells and IFNγ, in turn, upregulates macrophage IL-12 production (Fig. 4). This positive feedback loop may serve to perpetuate an inflammatory response. Thus, the cytokine profile displayed by the T cells in the lamina propria in CD is most compatible with a Th1 response.

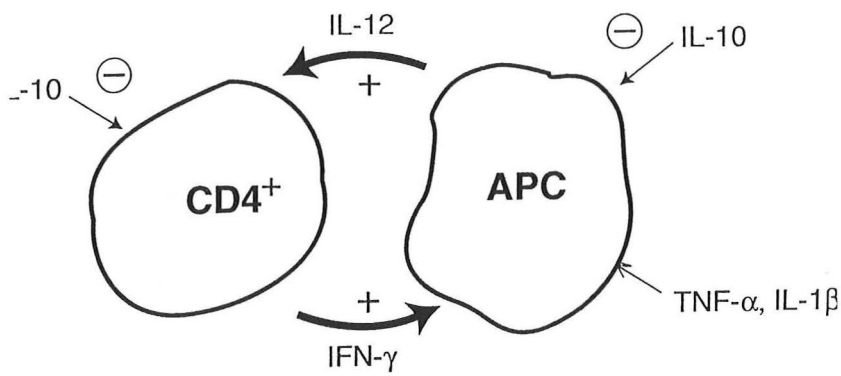

Fig. 4. Interrelationship between IFNγ and IL-12 secretion

It is currently not known whether the imbalance in the Th1/Th2 response in CD is due to an upregulation of IL-12 production and/or a downregulation of IL-4 production and which genetic or environmental factors determine this response. The cytokine profile in ulcerative colitis, on the other hand, conforms more to a Th2-like response. IL-4, an important cytokine of the Th2 response, is produced in normal or increased quantities by stimulated lamina propria T cells from UC patients.[60] IL-5 production is also elevated and IL-12 production is absent. It should be emphasized that the concept that UC is a Th2 mediated inflammation is not accepted by all investigators in the IBD field because some studies have shown low IL-4 production which is thought to be incompatible with a Th2 response.[58] The evidence that a Th2 response can indeed induce colitis is illustrated by two murine models. About 60% of TCRα negative mice develop a UC-like colitis. T cells isolated from lamina propria of inflamed colon produce IL-4, IL-5, IL-6 and IL-10 typical of a Th2 response.[63] Treatment of these mice with an antibody against IL-4 prevents colitis. Oxazolone colitis, another murine model of colitis induced by rectal installation of oxazolone, is characterized by mucosal inflammation of the distal one-half of the colon and increased production of IL-4 and IL-5 by lamina propria lymphocytes consistent with a Th2 response.[64] Treatment of oxazolone colitis with IL-4 antibodies results in rapid resolution of the inflammation.

The limited inflammation observed in normal intestine despite constant stimulation by bacterial antigens argues for a precise regulation of the mucosal immune system by suppression of inflammation and maintenance of the Th1/Th2 balance. The obvious question then is whether IBD is due to defects in immune regulation? The two T cell subsets, Tr1 and Th3, serve important roles in suppression of inflammation and induction of tolerance. An interesting model that illustrates the function of these two subsets has been developed. CD4 T cells can be divided into two subtypes based on their expression of the marker CD45RB.[65] $CD45RB^{hi}$ cells are naive T cells and $CD45RB^{lo}$ are memory cells. Transfer of $CD45RB^{hi}$ cells into SCID (severe combined immunodeficiency) mice results in severe colitis whereas transfer of $CD45RB^{lo}$ cells has no effect. Simultaneous transfer of both subtypes also has no systemic or local effect in SCID mice. Thus, normal mice have a T cell population that can induce colitis but normal mice do not develop colitis due to the presence of a second subtype which suppresses the other subtype. $CD45RB^{hi}$ cells produce IFNγ and $CD45RB^{lo}$ cells produce TGFβ and IL-10 typical for the regulatory subsets, Tr1 and Th3. As stated previously, the regulatory subsets are important for induction of tolerance. Emerging evidence suggests that tolerance may be abnormal in IBD patients. Isolated lamina propria lymphocytes from IBD patients react to both autologous and heterologous bacterial antigens with increased cytokine production whereas lamina propria lymphocytes from normal colon only react to heterologous bacterial antigens.[56] This observation suggests that T cell tolerance to luminal bacteria is broken in patients with IBD. Whether the loss of tolerance is an epiphenomenon or a primary defect is currently unknown. The unraveling of the mucosal immune response continues to evolve and the number of cytokines involved in the inflammatory response is ever expanding. Recently, the interleukins 7, 15 and 18 have been implicated as important proinflammatory mediators particularly in Crohn's disease.[66-68]

To summarize, then, the dissection of the immune responses in IBD has firmly established that CD is characterized by a Th1 response and that UC is more characteristic of a Th2 response. The dysregulated immune response in IBD may in part be due to loss of tolerance to bacterial antigens. Importantly, these studies have identified several new molecular targets for therapeutic intervention.

New Treatments of IBD

Upregulation of cytokine production plays a central role in the inflammatory response in IBD. The

proinflammatory cytokines TNFα, INFγ and IL-1 induce the production of cell adhesion molecules (selectins, integrins, ICAM) on local endothelial cells and on circulating neutrophils, lymphocytes and monocytes, which result in recruitment of these cells into the lamina propria and amplification of inflammation. Activated neutrophils secrete proteases and metalloproteinases which induce tissue injury and monocytes differentiate into activated macrophages and produce proinflammatory cytokines i.e. amplification of the inflammatory cascade. The inflammatory cells also generate a number of nonspecific inflammatory molecules such as prostaglandins, thromboxane, leukotrienes and nitric oxide which contribute to tissue injury.

Nuclear Factor κB (NFκB)

The goal of effective medical therapy of IBD is to ameliorate or preferentially heal the intestinal inflammation and, thus, provide symptomatic relief and clinical remission. The ideal target for a therapeutic agent would be a molecule that regulates the expression of the inflammatory mediators. The transcription factor NFκB occupies a central role in the inflammatory response (Fig. 5).

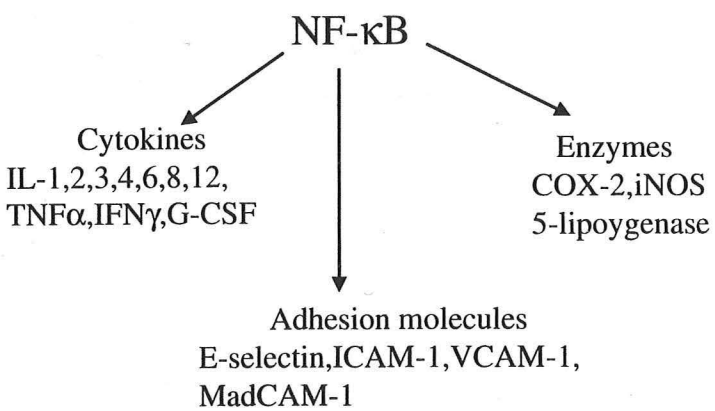

Fig. 5. Genes regulated by NFκB

NFκB is found in most cells and regulates the expression of a number of genes (> 100 genes) among others many cytokines (IL-1, IL-2, IL-4, IL-6, IL-8, IL-12, TNFα, IFNγ), cell adhesion molecules and enzymes such as iNOS, 5-lipoxygenase and COX-2, to name just a few.[69,70]

NFκB is a heterodimer composed of two subunits, typically p65L(relA) and p50, but other subunits exist (relB, C-rel, p52). NFκB is complexed with another protein IκB (inhibitor of κB) in the cytoplasm of unstimulated cells. IκB covers the nuclear localization signal of NFκB. Stimulation of cells by, for example, TNFα or LPS, activates a kinase complex (IKKα and IKKβ) in the cytoplasm which phosphorylates IκB, and IκB is then degraded by the ubiquitin-proteasome pathway. The nuclear localization signal of NFkB is uncovered and the transcription factor translocates to the nucleus and binds to specific promoter sequences of the target genes and initiates transcription. One of the target genes for NFκB is the IκB gene. Nuclear localization of NFκB increases the synthesis of IκBα which enters the nucleus, captures NFκB and transports it back to the cytoplasm as an inactive complex and, thus, terminates the response. Thus, agents that interfere with nuclear translocation of NFκB should downregulate the inflammatory response.

Sulfasalazine, the 5-aminosalicylates (5-ASA) and corticosteroids have been the mainstay of the medical treatment of IBD for years. It has now been found that one mechanism of action of these compounds is downregulation of NFκB activity. The salicylates inhibit the activity of IKKβ and corticosteroids induce IκB synthesis.[71-73] The functional result of these two actions is to retain NFκB as an inactive complex with IκB in the cytoplasm. The fact that 5-ASA and corticosteroids are often ineffective in inducing remission serves to emphasize that NFκB is not the only regulator of the inflammatory response.

Infliximab

TNFα plays an important role in the Th1 response in Crohn's disease and a chimeric monoclonal antibody against TNFα (cA2, infliximab) was approved by the FDA in 1998 as first an open-label study and, then, a placebo-controlled, randomized study showed promising results. Infliximab is administered as an intravenous infusion and in the first open-label study, eight of nine patients with Crohn's disease achieved clinical remission after a single infusion.[74] The placebo-controlled, randomized study included 108 patients with moderate to severe Crohn's disease that

was treatment resistant. The patients received a single infusion of placebo or infliximab at 5, 10 or 20 mg per kilogram and the response was evaluated at four weeks. The highest clinical response (81%) was seen in those given 5 mg/kg compared to 50% at 10 mg/kg and 64% at 20 mg/kg. The response in the placebo group was 17% (Figure 6). Overall, 65% of the patients obtained a clinical response after a single infusion of infliximab. A reevaluation at 12 weeks revealed that a clinical response was maintained in 43% of those treated with infliximab. Adverse effects were similar in the treated and placebo groups. There was initial concern about antibody development against infliximab which is a mouse-human antibody, but only six of the treated patients had detectable antibodies (anti-cA2) at completion of the study.

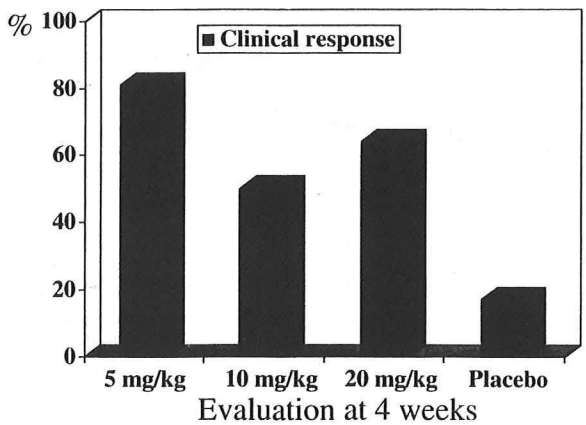

Fig. 6. First randomized, controlled study of infliximab

A recent randomized, placebo- controlled study has shown that multiple infusions of infliximab are an effective treatment for closure of fistulas in Crohn's disease.[76] Patients with draining fistulas were enrolled (n=94) and received three infusions of infliximab (5 mg/kg or 10 mg/kg) or placebo at 0, 2 and 6 weeks. The endpoint was a 50% reduction in the number of fistulas and was achieved by 68% (5 mg/kg) and 56% (10 mg/kg), respectively, compared to 26% in the placebo group. Complete closure of all fistula was observed in a significantly higher proportion of infliximab treated patients compared to controls. The median duration of response was about three months. Subsequent open-label studies have confirmed these results i.e. about 2/3 of patients with therapy-resistant Crohn's disease respond to infliximab and 1/3 have no response.[77] Examination of colonic or ileal biopsies from patients who responded to infliximab showed a significant reduction in inflammation with a disappearance of neutrophils and a decrease in mononuclear cells.[78] The

main effect of infliximab is presumably exerted by binding soluble TNF as well as neutralizing membrane-bound TNF in the areas of inflammation. The duration of a response would, therefore, be expected to be limited in time as infliximab is cleared from the tissue. It is now being recognized that the mean duration of a favorable response is 3 to 4 months after which disease activity slowly returns to pre-treatment levels. Interestingly, in a small endoscopic study of infliximab responders, it was observed that the pre-treatment intestinal lesions which had healed completely after treatment, reappeared at the same location with similar distribution.[79] Retreatment with infliximab of those who respond initially is a therapeutic option and has been examined in a small randomized, placebo-controlled study (n=73).[80] The actively treated group received infliximab at 10 mg/kg every eight weeks and the placebo group received an inactive infusion at similar intervals. Sixty-two percent of patients treated with infliximab maintained a clinical response throughout the treatment period compared to 37% of the placebo treated group. The difference failed to reach statistical significance (p=0.057). A larger study of similar design is currently being conducted to firmly establish the role of repeated infliximab infusions. Two patients in this study developed anti double-stranded DNA antibodies and seven patients had detectable human anti-chimeric antibodies in low titers. Thus, infliximab appears to be a valuable adjunct to our medical armamentarium in the treatment of Crohn's disease. Currently, it should be reserved for patients who are resistant to conventional medical treatment.

Thalidomide

Thalidomide was introduced as a sedative and antiemetic almost fifty years ago, but its use was discontinued after its potent teratogenic effects were recognized. Interest in thalidomide has been rekindled after it was found to be efficacious in inflammatory conditions such as lepromatous leprosy, pyoderma gangrenosum and graft- versus-host disease. Thalidomide has been shown to inhibit both TNFα and IL-12 production in in vitro experiments.[81] It also has an antiangiogenic effect.[82] A couple of encouraging case reports which demonstrated clinical efficacy of thalidomide in refractory Crohn's disease led to two open-label dose-finding studies.[83,84] Both studies were conducted in the U.S. The first study enrolled only men (n=12) and used either 50 or 100 mg thalidomide per day; the second study enrolled 22 patients (16 men, 6 women) and used either 200 or 300 mg thalidomide per day. Both studies lasted 12 weeks and all patients had refractory Crohn's disease. Ten patients completed the first study, 70% had a clinical response and 20% achieved clinical remission. Only 14 of the 22 patients completed the second study but all 14 had a clinical response and nine went into remission. The adverse effects of thalidomide were sedation

in most patients and peripheral neuropathy in 40% in the first study, and in 10% in the second study. Both studies concluded with a recommendation for a larger randomized, placebo-controlled study. Thalidomide analogs have now been developed which are more potent inhibitors of TNFα production in vitro and have shown no teratogenic effect in animal studies.[85]

Interleukin-10

IL-10 suppresses a Th1 response and would, therefore, be expected to induce a clinical response in Crohn's disease. Three studies of recombinant human IL-10 have been conducted in Crohn's disease. The first study enrolled 46 patients with steroid refractory disease and they received a daily i.v. infusion of IL-10 (0.5 to 25 μg/kg) or placebo on seven consecutive days.[86] The response was evaluated at 4 weeks. Complete remission was achieved in 50% of the IL-10 treated patients compared to 23% in the placebo group (Figure 7). The second study included only patients with mild to moderate Crohn's disease on no other medications (n=95).[87] These patients received a daily subcutaneous injection of IL-10 (1 to 20 μg/kg) or placebo for 28 days. Complete remission was seen in 29% of IL-10 treated patients and in none of the placebo controls. The third study was similar in design as the second study, but enrolled only patients with steroid refractory disease (n=329).[88] The result of this study was disappointing as IL-10 treatment failed to show any clinical benefit. Similar negative results were observed with IL-10 treatment of UC.[89] Thus, IL-10 appears to have lost its appeal and further studies of its use in IBD are not anticipated.

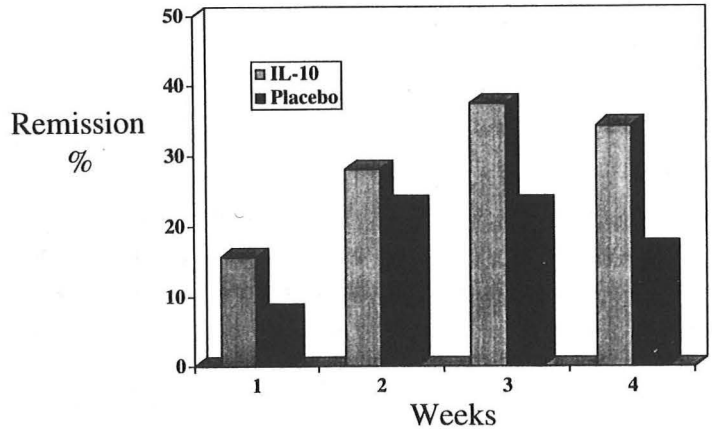

Fig. 7. Randomized, controlled study of IL-10 in CD

Interleukin-11

Interleukin-11 is related to IL-6 and has been shown to have anti-inflammatory activity in animal models of colitis. It also stimulates platelet production. So far, the effect of IL-11 has been examined in one randomized, placebo-controlled study which enrolled 76 patients with active Crohn's disease.[90] Patients received subcutaneous injections of IL-11 (5, 16 or 40 μg/kg/wk) or placebo for three weeks. The study was mainly designed as a safety and dose-finding study but a clinical response was observed in 42% compared to 7% in the placebo group. The highest dose of IL-11 caused thrombocytosis but otherwise there was no significant difference in adverse effects between the IL-11 treated and placebo group.

ISIS 2302

The adhesion molecule, ICAM-1, is upregulated in inflamed tissue and facilitates recruitment of neutrophils, lymphocytes and monocytes into the area of inflammation. ISIS 2302 is an antisense oligonucleotide which binds to ICAM-1 mRNA and induces its degradation. ISIS 2302 has been shown to inhibit cytokine-induced ICAM-1 expression in vivo. The safety and efficacy of ISIS 2302 has been examined in a small placebo-controlled, randomized study of patients with active, steroid-dependent CD (n=20).[91] The patients received intravenous infusions of ISIS 2302 (0.5, 1 or 2 mg/kg) or placebo every other day for 26 days and were followed for six months. At the end of the treatment 47% of the treated patients were in remission compared to 20% of the placebo group. The remission was maintained up to six months for the responders to ISIS 2302. The compound was well tolerated with no significant adverse effects. A larger, controlled study of ISIS 2302 is currently ongoing to establish efficacy and safety.

Summary

In summary, recent advances in the research of IBD support the evidence for genetic susceptibility and a dysregulated immune response to antigens from the normal intestinal microflora. Genetic mapping has identified significant linkage to loci on chromosomes 1, 5, 6, 14, 16 and 19. The immune response in CD is a Th1 response whereas the response in UC is Th2-like. The elucidation of the cytokines involved in these inflammatory responses has identified new targets for therapeutic intervention and spurred the development of new anti-inflammatory agents of which infliximab, so far, appears most promising.

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