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REVIEW

Current concept on the pathogenesis of inflammatory bowel disease-crosstalk between genetic and microbial factors: Pathogenic bacteria and altered bacterial sensing or changes in mucosal integrity take "toll" ?

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Abstract

The pathogenesis of inflammatory bowel disease (IBD) is only partially understood. Various environmental and host (e.g. genetic-, epithelial-, immune and non-immune) factors are involved. It is a multifactorial polygenic disease with probable genetic heterogeneity. Some genes are associated with IBD itself, while others increase the risk of ulcerative colitis (UC) or Crohn's disease (CD) or are associated with disease location and/or behaviour. This review addresses recent advances in the genetics of IBD. The article discusses the current information on the crosstalk between microbial and genetic factors (e.g. NOD2/CARD15, SLC22A46A5 and DLG5). The genetic data acquired in recent years help in understanding the pathogenesis of IBD and can identify a number of potential targets for therapeutic intervention. In the future, genetics may help more accurately diagnose and predict disease course in IBD.

Key words: Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Pathogenesis; Microbial factors; Genetics; NOD2/CARD15; SLC22A4/A5; DLG5

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INTRODUCTION

There has been a sharp increase in the incidence of inflammatory bowel disease (IBD) in the late 1900s in Western countries as well as in some Eastern parts of Europe and North America[1-3]. Both Crohn's disease (CD) and ulcerative colitis (UC) stem possibly from a common mechanism with an exact etiology that remains obscure[4,5]. Crohn's disease manifests itself as a chronic granulomatous inflammation of the gastrointestinal tract capable of affecting its entire length with the presence of "skip" lesions[6,7]. It preferentially affects the terminal ileum, as was originally described by Crohn *et al*[8]. Ulcerative colitis, on the contrary, presents as a continuous inflammatory lesion affecting the rectum and colon, lacking granulomatous characteristics.

IBD are multifactorial, polygenic diseases with probable genetic heterogeneity. According to this hypothesis, different genetic backgrounds may explain the various clinical patterns of the disease[4,5]. In addition to genetic predisposition, various environmental and host factors (e.g. genetic-, epithelial-, immune and non-immune) play a major role in the pathogenesis of IBD. Extensive heterogeneity is observed in terms of presentation, extraintestinal manifestations and location in CD, while behavior and response to treatment are heterogeneous in both CD and UC[9]. Furthermore, it is now undisputed that enteric bacterial flora play a key role in the pathogenesis of IBD, both in UC and in CD. The exact mechanism by which the intestinal mucosa loses tolerance to its bacterial neighbors remains elusive. The role of host genetic regulation of the innate immune response in the pathogenesis of CD has been brought to sharp focus by the identification of the NOD2 (CARD15) mutations.

The genetic aspect of research is quite focused on numerous chromosomal loci. The environmental contributors are diverse and an "infectious origin" of inflammatory bowel disease has not been confirmed. This review describes the various environmental, immunologic and genetic components leading to the manifestation of inflammatory bowel disease.

MICROBIAL FACTORS: ARE PATHOGENIC BACTERIA AND/OR ALTERED PERCEPTION OF NORMAL FLORA THE KEYS IN THE BREAKDOWN OF TOLERANCE?

The normal intestine encounters a high concentration of foreign antigens, bacteria and food. In the stomach and proximal small intestine, secretion of acid and bile, phasic 'housekeeping' motility patterns hinder colonization. However, the number of bacteria dramatically increases in the distal small intestine to an estimated 10^{10} - 10^{12} bacterial cells/g content in the colon, which contribute to 60% of the faecal mass[10]. More than 400-500 species of bacteria are represented, belonging to 30 genera. Although this antigenic load is separated from the largest complement of lymphocytes in the body (gut associated lymphoid tissue, GALT) by only a single layer of polarised intestinal epithelium, most people do not have an immune response to foreign antigens and interaction between the mucosal immune system and the fecal bacterial mass regulates physiologic bowel functions. The mucosal immune system has evolved to balance the need to respond to pathogens while maintaining active tolerance to commensal bacteria and food antigens. In IBD, this tolerance breaks down and inflammation supervenes driven by the intestinal microbial flora.

A large part of research has traditionally been devoted to finding a causative biological source of any disease. This has also been the case in IBD, but to date there is no compelling evidence of an etiological role for any single pathogenic microorganism.

PATHOGENIC MICROBES

The history of IBD is dotted by cyclic reports on the isolation of specific infectious agents responsible for CD or UC. Several microorganisms, such as *Mycobacterium paratuberculosis, Listeria monocytogenes, Chlamydia trachomatis, Escherichia coli, Cytomegalovirus, Saccharomyces cerevisiae*, have been proposed as having a potential etiologic role.

The suggested etiologic role of *Mycobacterium paratuberculosis* in CD is also controversial. This bacterium is the causative agent of Johne's disease, a chronic granulomatous ileitis in ruminants, which closely resembles CD. *M. paratuberculosis* was initially isolated from CD tissues some 20 years ago[11] and follow-up studies have tried to culture *M. paratuberculosis* for testing specific DNA sequences in intestinal tissues, or measuring serum antibodies against *M. paratuberculosis* with conflicting or inconclusive results[12-14]. Recently, *M. paratuberculosis* has been identified by *in situ* hybridization to the *M. paratuberculosis*-specific IS*900* gene in tissue specimens of Crohn's disease[15] and in 40% of Crohn's disease granulomas isolated from surgical specimens by laser capture microdissection[16]. Others have localized *M. paratuberculosis* by PCR to macrophages and myofibroblasts within the lamina propria[17]. However, the possibility of an association between *M. paratuberculosis* and CD remains inconclusive.

Additionally, *E. coli* bacteria possessing special adhesive properties have been associated with the development of ulcerative colitis.

A fascinating hypothesis was published in the *Lancet* in 2003[18]: an association between refrigeration and CD. Some so called psychrotrophic bacteria are capable of growing slowly at low temperature (known as "cold chain hypothesis"). Common pathogens are *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Clostridium botulinum*[19].

Several studies have demonstrated members of the *Yersinia* species in intestinal mucosal samples of Crohn's disease. The specific pathogens detected are either *Y. enterocolitica* or *Y. pseudotuberculosis*, and sometimes even both[20,21]. There are numerous aspects of yersiniosis which resembles the inflammatory reaction seen in Crohn's disease, making differential diagnoses of these two conditions, including ileitis or ileocolitis, mesenteric adenolymphitis, reactive arthritis and erythema nodosa. Additionally, granulomas may be observed in histological samples[18].

Recent data also demonstrate a role of mucosa-associated and intramucosal bacteria in the pathogenesis of IBD and colorectal cancer. In the study by Martin *et al*[22], mucosa-associated or intramucosal *E.coli* are present in 43% and 29% of CD, and 17% and 9% of controls, respectively, supporting a role of mucosa-adherent bacteria in the pathogenesis of Crohn's disease.

Finally, a viral etiology has also been proposed as the cause of IBD, particularly CD. An early measle infection during the perinatal period notably increases the risk of Crohn's disease[23]. The finding of paramixovirus-like particles in CD endothelial granulomas suggests an association between perinatal measles and predisposition to CD based on some epidemiological and serologic data[24]. However, these preliminary findings are not confirmed by later studies[25]. Importantly, the progressive decline of measle virus infection in the last decades with the concomitant rise of CD during the same period of time speaks against an etiologic role of measles in CD. The hypothesis that measle vaccination rather than measle infection, might be a risk factor for CD, has also been raised, but again results of additional studies fail to confirm this association[26]. In contrast, a role of cytomegalovirus infection is proposed in UC[27].

NON-PATHOGENIC INTESTINAL FLORA

In the last decade, the focus of interest in microbial etiology of IBD has shifted from infectious to commensal agents. Based on fairly solid data, there is a substantial body of evidence that the normal enteric flora plays a key role in the development of IBD[28]. This is even more evident after the discovery of the genetic factors (e.g. NOD2/CARD15, TLR4 and CD14) responsible for alterations in bacterial perception[29]. The beneficial effect of antibiotics in the treatment of CD, and to a lesser extent UC, has been appreciated for years. Diversion of the fecal stream from inflamed bowel loops has also been known to induce symptomatic improvement in CD patients, while re-emergence of inflammation often occurs upon restoration of intestinal continuity[30].

Changes are observed in the normal flora of IBD patients and dysbacteriosis is commonly reported. Based on the clinical picture of IBD, the intestinal flora

might play a role in the initiation and perpetuation of inflammatory reaction. *Fabia et al*[31] demonstrated that the concentration of anaerobic bacteria and *Lactobacillus* are radically decreased in active IBD patients. On the contrary, patients with inactive IBD show no such decrease. *Swidsinski et al*[32] compared over 300 IBD patients with 40 control individuals and found that abnormalities can be observed in the flora using various laboratory techniques. In addition, a much greater number and concentration of bacteria make up the biofilms covering the epithelium involved in IBD. Furthermore, a direct association has also been reported between bacterial concentration and disease severity.

More recently, probiotics (primarily lactic acid bacteria) defined as live microbial feeds that beneficially affects the host by modulating gut microbial balance, have been demonstrated to improve both human IBD and experimental colitis, primarily by preventing relapses, thus adding an important dimension to the role of gut flora in IBD[33,34].

Probably, the most convincing evidence comes from experimental data. In the majority of IBD animal models, intestinal inflammation fails to develop when they are kept in a germfree environment. This critical observation has led to the widely accepted paradigm "no bacteria, no colitis". However, once normal flora is introduced into their environment, the mutant-strain mice quickly develop colitis[35, 36].

It is worth mentioning that bacterial superinfection (most commonly *Clostridium difficile*, but also *Entamoeba histolytica, Campylobacter spp*, etc.) is also able to elicit relapse of IBD. In the study of Mylonaki *et al*[37], 10.5% of all relapses are associated with enteric infections. In another study[38], 20% of all relapses are correlated to *C. difficile* positivity.

The presence of bacterial antigens as the initiating factor in IBD has also been explored. The data support a role of flagellin proteins in activating the innate immune system. Elevated titers of serum anti-flagellin IgG against these proteins could be detected in CD patients[39]. Furthermore, elevated serum IgG2a titers could also be demonstrated in experimental IBD models, similar to results observed in humans. Recently, anti-CBir1 (anti-flagellin) expression has been independently associated with small-bowel, internal-penetrating and fibrostenosing disease features in CD[40].

Finally, the role of bacteria in the pathogenesis of IBD is further supported by the increased intestinal permeability in CD patients. Although it is not an environmental factor *per se*, it has been postulated as an early predisposing factor for the development of this condition. Increased intestinal permeability is also observed in asymptomatic first-degree relatives of CD patients, possibly encompassing another group of individuals who are at an increased risk of developing this form of IBD[41,42]. Documented observations of increased permeability preceding the onset of symptomatic disease are of further interest[43]. In relating this phenomenon to the UC form, electron microscopy has revealed deficiency of those elements comprising the tight junctions necessary for the wholeness of the intestinal epithelium[44]. Recent data reporting an association between genes important in mucosal transport and integrity (OCTN and DLG5) and IBD further support this assumption.

Table 1 Locations of nine major loci showing linkage to inflammatory bowel disease

IBD locus	Chromosome	Identified genes	Disease
IBD1	16q13	NOD2/CARD15	CD
IBD2	12q14	Not known (VDR, STAT6, MMP18, b2-integrin)	UC
IBD3	6p	Not known (HLA, TNF)	IBD
IBD4	14q11-12	Not known (TCR, LTB4 receptor)	CD
IBD5	5q31-33	SLC22A4/A5	CD
IBD6	19p13	Not known (ICAM1, C3, TBXA2)	IBD
IBD7	1p36	Not known (TNF-R family)	IBD
IBD8	16p12	Not known	CD
IBD9	3p26	Not known (CCR5, CCR9,hMLH1)	IBD
	10q23	DLG5	IBD

The phenomenon of decreased bacterial diversity of the intestinal microflora obtained from stool samples in IBD patients has also been suggested, based on culture-dependent microbiological techniques[45]. Recently, a German group has reported the relative lack of the *Bacteroides* group (usually accounting for 50%-90% of the anaerobic faecal microflora) by utilizing 16sDNA-based single strand conformation polymorphism (SSCP) fingerprint, cloning experiments and real time PCR. Moreover, the bacterial profiles are stable, at least for the observed period of six weeks. This is in concordance with previous findings, suggesting that the mucosa-associated microflora contains only a small number of bacteria of the *Bacteroides/Prevotella* group[52]. However, in a recent study comparing the faecal microflora of healthy controls and CD patients, no differences are noted between patients and healthy controls[46]. This lack of significant difference may be partially explained by the differences in mucosal and faecal microflora.

Why an abnormal response to normal endogenous gut bacteria exists in IBD is not clear, but recent data, especially on the genetic background of CD, suggest an association between gut inflammation and abnormal bacterial sensing.

GENETICS AND GENE FUNCTION

Possible genetic loci influencing the presentation of inflammatory bowel disease have already been identified on more than half of all chromosomes, including the X chromosome[47-49]. First, in 1996 Hugot *et al*[50] reported that the pericentromeric region of chromosome 16 (D16S408) is associated with CD renamed as IBD1[50]. This is confirmed by several studies. To this date, results of numerous studies have revealed a total of nine loci associated with specific linkage requirements, subsequently labeled as IBD1-9[51-55] (Table 1). Furthermore, it is clear that some loci correlate with either UC or CD while others are involved in the pathogenesis of both IBD forms[49].

FAMILIAL STUDIES

Evidence of genetic constituents in the induction of inflammatory bowel disease clearly lies within the results of familial studies. Several studies examining the relationship of IBD within families have reported positive findings in 10%-25% of families[56,57]. This finding goes back at least two decades when Farmer and Michener reported 40% concordance for IBD in first-degree relatives. They observed that sibling-sibling involvement occurs in addition to parent-offspring transmission.

Solid evidence of genetic predisposition showed that as many as 50% of monozygotic twins are affected by Crohn's disease whereas ulcerative colitis is seen in approximately 14%[58]. Monozygotic twins are not equally affected but still display greater concordance for IBD than dizygotic twins. The overall risk for IBD is increased by 5-20 times[59], being highest in twins and in siblings. The genetic component is more prominent in CD than in UC.

Finally, genetics certainly play a role in the observed phenotype of IBD, including disease behavior and location[60].

Genetic anticipation is suggested in early studies with a more severe and earlier presentation of the disease in subsequent generation[61], but this has not been replicated in more recent studies[62,63].

GENES INVOLVED IN THE RECOGNITION OF BACTERIA – NOD2/CARD15, NOD1/CARD4, TLR4 AND CD14

NOD2/CARD15

Three independent groups have identified NOD2 CARD15 on chromosome 16 in IBD1 as a candidate gene for CD[29,64,65].

NOD2/CARD15 is an intracellular element responsible for the indirect recognition of bacterial peptidoglycan through the binding of muramyl dipeptide[66,67]. It is a member of the Ced4-APAF1 protein superfamily and is expressed in various cells, including monocytes, dendritic cells, Paneth cells and intestinal epithelial cells[6]. Structurally, NOD2/CARD15 is composed of three segments: the first being composed of two CARD units, the central portion consisting of nucleotide-binding domain (NBD) and finally, a leucine-rich repeat (LRR) region as is found in TLRs[68]. The most intriguing question that remains to be answered concerns the mechanism whereby mutations in the NOD2/CARD15 gene predispose towards the chronic intestinal inflammation characteristics of CD.

At the molecular level, the binding of NOD2/CARD15 to a bacterial motif (muraryl dipeptide-MDP, a component of both Gram negative and positive bacterial cell walls) causes its binding to a second NOD2/CARD15 molecule, thus forming a dimer. Further interaction with other cytosolic proteins leads to the ultimate activation of nuclear factor κB (NF-κB), eliciting pro-inflammatory reactions[68] (Figure 1). Surprisingly, it is still unclear whether NF-κB expression is elevated or depressed in CD due to conflicting observations and studies. *In vitro* experiments demonstrated that the declined activity of this protein indicates a loss-of-function effect[69,70]. Hisamatsu *et al*[71] showed that

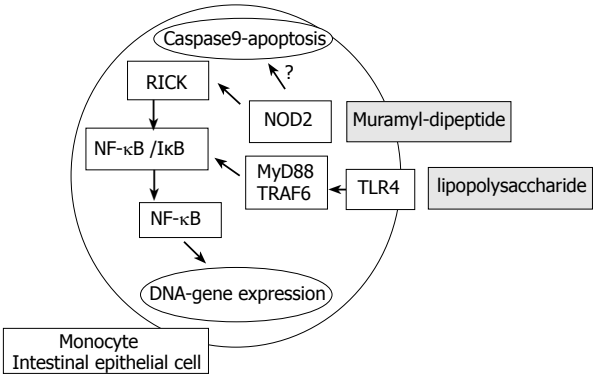

Figure 1 NOD2/TLR4 signaling pathway. NOD2 activation by CARD-CARD dimmer formation results in binding to RICK kinase (RIP-like interacting CLARP kinase). RICK then activates the nuclear factor kB inhibitor (IkB) kinase complex (IKK) via phosphorylation of IKKc. The IKK complex next phosphorylates IkB resulting in nuclear factor kB (NFkB) translocation to the nuclei and transcriptional activation of NFkB responsive genes, such as pro-inflammatory cytokines or defensins. TLR4 induces activation through other molecules (Myd88, IRAK and TRAF6).

CARD15/NOD2 may function as an antibacterial factor in CaCo2 intestinal epithelial cells. Cells stably transfected with a wild-type CARD15/NOD2 gene construct are able to prevent invasion of *Salmonella typhimurium*. This protective effect is lost in cells transfected with gene constructs of mutant CARD15/NOD2. It has also been demonstrated that NOD2/CARD15 expression in intestinal epithelial cells might be upregulated by the proinflammatory cytokine tumor necrosis factor α (TNF-α)[72]. The NF-κB activation is regulated by the LRR region and deletion within this region may theoretically result in its uncontrolled activation. In concordance with this, increased NF-κB activation is observed in the presence of 3020insC mutation by stimulation of MDP[73], which may explain the findings of higher NF-κB tissue levels in samples from IBD patients[74]. Interestingly, mice lacking NOD2 or possessing mutated NOD2 variants do not spontaneously develop Crohn's disease. These findings suggest that NOD2 mutations create an intestinal environment susceptible to IBD[73,75] rather than playing a direct causative role in disease development expression of α- and β-defensins.

Defensins are integral parts of the local intestinal immune system, and are secreted by intestinal epithelium as endogenous antimicrobial proteins. The consequential damage could finally lead to an intensified bacterial invasion and a chronic inflammation of the intestinal mucosa[76]. The possibility exists that NOD2/CARD15 is involved in the regulation of Paneth cell degranulation[77], as NOD2/CARD15 expression is noted in close proximity to the secretory granules of these cells. Kobayashi *et al*[75] and Wehkamp *et al*[78] have shown that carriage of NOD2/CARD15 variants may be associated with reduced α-defensin release from Paneth cells in response to bacterial cell wall components, and the defective defensin release by the Paneth cells could provide the missing link, whereby reduced NOD2/CARD15 activity impairs host defenses against bacteria and underlies persistent intestinal inflammation. Finally, NOD2/CARD15 is involved in the regulation of TLR2 stimuli by peptidoglycans. Defective

NOD2 function results in a pro-inflammatory cytokine bias after stimulation of mononuclear cells with TLR2 stimuli, which may contribute to the overwhelming inflammation in Crohn's disease[79].

Three major mutations have been identified within NOD2/CARD15: one frame shift (3020insC, SNP13) and two missense mutations (R702W-SNP8 and G908R-SNP12).

The presence of NOD2/CARD15 mutations increases the risk for CD by 1.4-4.3-fold in heterozygous patients and 17.6-44.0-fold in homozygous and compound heterozygous patients. Reports on homozygous individuals who are disease-free are available. One such family has been described by van der Linde *et al*[80]. It is estimated that any of these three common mutations involving NOD2/CARD15, is present in heterozygous form in 30-50% of CD patients and 7-20% of controls from North-America and Europe[81-85]. However, various geographical differences are noted. Furthermore, other races display a much lower prevalence of this mutation, sometimes lacking it altogether (e.g. African-American[86], Chinese[87] and Japanese[88]). The prevalence is also lower in other North European countries[89,90].

The association between NOD2/CARD15 and disease phenotype and behavior has also been investigated. The three common mutations are associated with ileal disease and fibrostenosing behavior, but they are relatively less frequent in colonic and fistulous disease[64,66,91,92]. The presence of mutations is not associated with extraintestinal manifestations or the response to infliximab therapy[93]. The mutations are also not associated with UC. In CD, the attributable risk for ileal disease is 40% determined by NOD2/CARD15 and 20% by HLA genes. The numbers are similar for ileo-colonic disease (NOD2/CARD15: 30%, HLA: 40%), while colonic disease is thought to be associated with HLA and other yet unknown genes[91].

It is worth mentioning that NOD2/CARD15 increases the risk for colorectal cancer (CRC). Kurzawski *et al*[94] have found that the presence of SNP13 mutation increases the risk of developing CRC by 2.23-fold in patients with an age >50 years at diagnosis of CRC. This has not been confirmed in a more recent study by Alhopuro *et al*[95].

NOD1/CARD4

A similar protein to NOD2/CARD15 by its structure and function, is located on chromosome 7p. Its particular location is in the midst of a region with strong IBD correlation, being translated into an intracellular bacterial pathogen-associated molecular pattern[96].

Zouali *et al*[97] denounced that NOD1/CARD4 plays a role in IBD following an investigation involving 63 IBD patients. The screening process was conducted on the eleven exons constituting the NOD1/CARD4 gene with the goal of identifying polymorphisms. Indeed, several variants were identified, none of which has proved any association with IBD[97]. On the contrary, McGovern *et al*[96] have located a complex insertion/deletion allele on NOD1, associating it with both an early onset of IBD and extraintestinal manifestations.

Toll-like receptors and CD14

Toll-like receptors (TLRs) expressed in myeloid cells play a major role both in detecting microbes (lipopolysaccharide) and in initiating innate immune responses. Accordingly, a disturbance in its function predisposes to infections with Gram negative bacterial pathogens, possibly influencing the advancement of IBD[98,99]. In contrast, little is known about the expression, distribution and function of TLRs in epithelial cells *per se*. TLR4 is also expressed in the Golgi apparatus of intestinal epithelial cells. Thus, LPS recognition in intestinal epithelial cells may occur in the Golgi apparatus and requires LPS internalization [100]. Recently, it has been suggested that the interaction of LPS with TLR4 / MD2 contributes to the perpetuation of the inflammatory epithelial cell injury via TNFα induced alterations of enterocyte turnover in an autoparacrine/paracrine manner[101]. TLRs may also influence the nature of immune response by skewing T cells toward a Th1 or Th2 profile. Myeloid cells are exquisitely sensitive to TLR ligands and produce significant IL-12p40. They appear to play a key role in the initiation and possibly the Th1/Th2 skewing of inflammatory responses. In this model the inflammation can be normally controlled by myeloid or lymphocyte-derived IL-10 acting through Stat3 in myeloid cells to block further production of IL-12/IL-23 and skewing the responses towards Th1 profile[102].

IBD is characterized by an altered expression pattern of TLRs on the surface of intestinal epithelium and TLR4 expression is up-regulated in patients with CD. In contrast, the expressions of TLR2 and TLR5 are unchanged, while TLR3 that recognizes viral replication is down-regulated[103]. The D299G (Asp299Gly) polymorphism of the TLR4 gene associated with LPS hyporesponsiveness[104] is associated with CD (OR:2.45-2.80) and UC (OR: 2.05)[98,105]. However, other studies have failed to replicate this association[85,106].

Another TLR which binds to and recognizes bacterial DNA, TLR9, may also play an important role in the pathogenesis of IBD. Rachmilewitz *et al*[107] reported that the anti-inflammatory effect of probiotics is transmitted through TLR9 in experimental colitis. More recently, an English group has reported a synergy between TLR9 and NOD2 that is lost in the CD patients carrying NOD2 mutation, indicating impairment in innate immunity[108]. Recently, *Torok et al*[109] reported that the -1237C promoter polymorphism of TLR9 is significantly associated with CD in German patients.

Contradictory data are available on the association between the bacterial receptor CD14 and IBD. Klein *et al*[110] found that the 159 TT genotype is associated with CD but not with UC. In a Japanese study[111], the same genotype is associated with UC (OR: 1.96) but not with CD and also a further study investigating Caucasian patients has failed to demonstrate any association[112].

MUCOSAL INTEGRITY AND TRANSPORT - SLC22A4/OCTN1, SLC22A5/OCTN2 AND DLG5

SLC22A4/OCTN1, SLC22A5/OCTN2

The association between 5q31 and CD is first noted in genome-wide screens (the genes for Th2 cytokines -e.g. IL-3, 4, 5, 9, 13 and IRF1- maps to this region). The

risk haplotype is associated only with a moderate risk for CD (OR: 1.4-1.5). The effect of NOD2/CARD15 is additive while the 5q31 haplotype is an independent risk factor[113-115]. A German study reported that the risk haplotype is also associated with UC while the IBD5 haplotype is not associated with the clinical presentation, nor is it correlated to IBD in Japanese[114]. Based on the available data, IBD5 increases mainly the overall risk for IBD, whereas NOD2/CARD15 mutations are primarily responsible for the determination of phenotype.

One of the most important findings in the genetics of IBD is the identification of OCTN1/SLC22A4 and OCTN2/SLC22A5 genes coding for integral membrane proteins. The function of these proteins is multispecific in bidirectional transmembrane transport of carnitine and organic cations. The SLC22A4 C1672T and SLC22A5 promoter G207C variants increase the risk for CD by 2-2.5-fold when present as a single copy and by 4-fold in homozygous carriers[55,116]. The elevated risk attributed to OCTN TC haplotype and NOD2/CARD15 mutations, is additive with an odds ratio of 7.3-10.5 in double carriers. A more recent Japanese study on SLC22A4/A5 and DLG5 polymorphisms and CD[117], has shown a weak association, concurring with the Ashkenazi population[118], where the frequency of the allele is lower, indicating racial differences. Variant alleles are associated with functional changes. The SLC22A4 variant encodes exchange of a leucine residue on the OCTN1 transmembrane domain for phenylalanine (L503F), a change which reduces carnitine but augments organic cation transporter activity. The SLC22A5 variant impairs heat shock protein-driven promoter transcriptional activation[116]. Carnitine is an essential mediator of fatty acid oxidation, a role subserved by promoting transport of long-chain fatty acids across the mitochondrial membrane. Inhibition of fatty acid oxidation can evoke clinical and pathologic signs of colitis[119], which may explain why impairments in carnitine transport may confer an increased risk for IBD. Another possibility is that the enhanced cation transporter activity of the OCTN1 503F variant may provoke disease by allowing aberrant uptake of toxic substrates.

A possible association between OCTN3 and CD has been reported[120,121]. In contrast, no association has been found between the proton-coupled bivalent metal antiporter located on 2q35 SLC11A1 and IBD[122].

DLG5

The association between DLG5 (Drosophilia Discs Large Homolog 5) at chromosome 10q22-23 is first reported by Stoll *et al*[123]. DLG5 is a member of the membrane-associated guanylate kinase (MAGUK) gene family which encodes cell scaffolding proteins and is also involved in the maintenance of epithelial integrity and regulation of cell growth[124], a role potentially impaired by expression of the CD-associated DLG5 variants causing increased permeability and disease. The impact on the overall risk of developing IBD is much smaller than NOD2/CARD15. The carriage of the 113A allele increases the risk for CD by 1.3-2.1-fold in German, Italian, Canadian patients and is associated with early onset of IBD in Scottish children[123,125,126]. Less common variants have also been identified (C4126A). Interaction between DLG5 and NOD2/CARD15 is also detectable. This however, has not been replicated by the same group in English and Scottish CD patients and by the German group from Munich[127,121].

HLA, CYTOKINE GENES AND OTHER GENETIC FACTORS

HLA genes

Reports on genetic trait of IBD are available, which investigated the genes associated with the immune response. The main region of interest is that of MHC (main histocompatibility complex) genes located on chromosome 6. The human leukocyte antigen (HLA) class II genes are candidates for a role in the pathogenesis of IBD because their products play a central role in the immune response. More than 100 known genes are located in the area, each is highly variable and several polymorphisms are known.

Several studies have addressed the possible association between certain HLA polymorphisms and the risk for IBD. Most of these studies have revealed contradictory results and their findings could not be replicated in different populations. Overall, genes in the HLA region may play a greater role in modifying IBD phenotype rather than in determining overall disease susceptibility[128-130].

The association of UC with HLA genes is stronger than CD[4,130]. In the white Caucasian population, the rare HLA-DRB1*0103 allele is positively associated with UC (OR: 3.42). Others have found that this allele predisposes to extensive disease and is additionally associated with the presence of extraintestinal manifestations[131]. The repeatedly observed association with HLA-DR2 has been confirmed in the cumulative odds ratio of 2.00, while the odds ratio for DRB1*1502 and DR9 is 3.74 and 1.54, respectively. In contrast, the presence of DR4 (OR: 0.54) and DRw6 is preventive for UC[132].

In CD, negative association has been found between DR2 (OR = 0.83) and DR3 (OR = 0.71), while HLA-DR7 and DQ4 seem to be associated with a moderately increased risk (OR = 1.42 and 1.88). The studies on allele DRB3*0301 showed that this allele is positively associated with Crohn's disease (OR = 2.18)[4,31]. Ahmad *et al*[91] investigated 340 polymorphisms in HLA genes and found that the 3 common NOD2/CARD15 alleles, the HLA DRB1*0701 (RR: 1.5) and Cw*802 (RR3.0) are associated with increased risk for CD, while the presence of HLA DRB1*1501 (RR: 0.6) is protective.

Investigating the genotype-phenotype relations, HLA DRB1*0103 allele in both UC and CD is associated with both extensive and severe disease as judged by the need for colectomy[131,133]. However, the low frequency of this allele even in UC patients suggests that this association is unlikely to be clinically useful in predicting disease course. The same allele is associated with late onset, colonic location (38.5% *vs* controls 3.2%) of CD[134], while the presence of HLA-DRB1*04 (OR:1.7) and HLA-DRB1*0701 (OR: 1.9) increased the risk for ileal location[135]. MICA*010 (MHC class I chain-related gene A) and HLA-DRB*0103 are associated with perianal

disease[91].

A number of genetic associations have also been described in extraintestinal manifestations of IBD. Studies comprising both UC and CD patients showed that migratory pauciarticular large-joint arthritis is associated with HLA-DRB1*0103 and B*27 and B*35 class I alleles in linkage disequilibrium. In contrast, chronic small-joint symmetric arthritis is associated with HLA-B*44[136]. Uveitis is correlated to HLA-B*27, B*58 and DRB1*0103, while erythema nodosum to B*15 and TNF-1031C[137]. However, due to the relatively small sample size of these studies, the evidence is lacking to draw a firm conclusion.

Cytokine, multidrug resistance and cell adhesion gene polymorphisms

Interaction between non-pathogenic, commensal bacteria and epithelial cells, M-cells and dendritic cells of the intestinal mucosa is characterized by a cytokine profile of mainly the Th2/Th3 (suppressor) type (IL-4, IL-5, IL-10, TGF-β), which incapacitates the development of an inadequate, progressive, proinflammatory cycle in healthy individuals[138]. Contrary to this, mucosal immune response against pathogenic species is largely mediated by Th1 cells and a specific cytokine milieu (IL-1, TNF-α, interferon-γ). Both ulcerative colitis and Crohn's disease are characterized by an increased expression of "general" proinflammatory cytokines (TNF-α, IL-1, IL-6), which suggests an abnormally intense inflammatory response against commensal bacteria. This loss of immune tolerance to non-pathogenic microbes results in pathologic immune reactivity and a self-supporting inflammatory cascade. In healthy individuals, the tight control of the inflammatory reaction between bowel mucosa and bacterial milieu involves antiinflammatory cytokines (IL-10, TGF-β)[139]. In case of cytokine imbalance, the inflammatory reaction intensifies. However, lack of antiinflammatory cytokines plays only a restricted role in the development and maintenance of IBD, which is suggested by their low therapeutic potential in IBD[140]. In contrast, the widely used chimera or humanized anti-TNF antibody represents one of the most fascinating new therapeutic options in severe and/or penetrating CD cases as well as UC[141-143]. Studies are also underway targeting IL-12[144].

Although the above mechanism is pivotal in the induction of local inflammatory changes from an immunological point of view, lack of reproducibility has challenged many of the other reported genotype-phenotype associations in IBD, such as association between interleukin polymorphisms and IBD.

The association between a low-producing allele, allele 2 of the interleukin (IL)-1RA gene and UC (OR: 1.3), extensive colitis, the need for colectomy[145,146] and development of pouchitis (OR: 3.1) in UC after ileal-pouch anastomosis[147] has been reported in Caucasian population. However, another study investigating 529 IBD patients has failed to confirm these results[148]. IL-10 polymorphisms also lack association with UC. However, the 627A allele is more frequent in patients with left sided colitis[149]. In contrast, the frequency of the high IL-10 producer allele (-1082*G) is decreased in IBD[150].

Bone loss and osteoporosis are well recognized in IBD, but the risk factors have not been clearly identified. Several studies investigated whether genetic markers may predict bone loss and found that variable number of tandem repeats adjacent to IL-6 or within IL-1RA and genes previously implicated in the paracrine stimulation of osteoclasts are associated with bone resorption[151]. A subsequent study from the same group has failed to identify an association with a putatively functional polymorphism in the IL-6 gene. Nemetz *et al*[152] reported that the 511*2 allele of IL-1b is associated with increased *in vitro* production of IL-1b by mononuclear cells in Hungarian CD patients[152].

Furthermore, microsatellite loci of TNF-α are associated with CD[153]. However, only data from a single study suggest that the TNF-308A polymorphism is associated with more intense inflammatory activity and an increased risk of arthritis in CD patients with fistulizing disease[154].

The A6 allele MICA is associated with UC and early onset of disease[155], while the MICA*011 allele increases the risk for UC by almost 2-fold. No association has been found between different polymorphisms of CTLA4, a receptor of activated T cells, which has an inhibitory function in regulating T-cell activation and IBD in different Caucasian and Asian populations[156,157].

Brant *et al*[158] have described a suggestive linkage on chromosome 7q containing the multidrug resistance (MDR)-1 gene, in association with the appearance of UC and CD. This particular gene is a membrane transport protein with several documented human polymorphisms having effects on intestinal absorption and drug pharmacokinetics. The significant mutation designated as Ala-893Ser/Thr is originally identified in knockout mice with spontaneous colitis[159,160]. An additional locus of mutation (C3435T) associated with 50% decreased protein secretion corresponding to UC (OR: 1.6-2.0) especially in extensive colitis (OR: 2.64), showed no manifestation in CD[161,162].

Adhesion molecules mediating cell-cell and cell-extracellular matrix interactions, are pivotal mediators of inflammation in IBD. Catenins and catherins are major contributors of integrity of the intestinal mucosa[163]. Dysregulation in E-cadherin-catenin complex formation leads to decomposition of the mucosal structure characterized by leaky epithelium, as observed in IBD.

Cell surface adhesion molecules conveying leukocyte-endothelial interactions, govern homing of activated inflammatory cells into the bowel mucosa. In order to slow down by rolling along the endothelium, circulating leukocytes in small vessels of the inflamed mucosa interact with adhesion molecules (e.g. CD44) expressed in the endothelium. This is followed by establishing a firm adhesion anchor. Extravasation and migration into the site of inflammation are mediated by integrins (mainly $\alpha_4\beta_2$ and $\alpha_4\beta_7$) and selectins (L-, E- and P-selectin). Vascular endothelium of the inflamed intestine, particularly in CD, is characterized by increased expression of adhesion molecules and integrin ligands, such as E-selectin, ICAM-1, MAdCAM-1, VCAM-1[164,165]. In UC, the soluble E-selectin levels show no preference between active or inactive forms. Also, the sVCAM concentration is far higher in the control group than in the inactive UC patients. Finally, unlike in CD, VEGF levels in UC cases are similar in active, inactive and control subjects[166]. Expression of

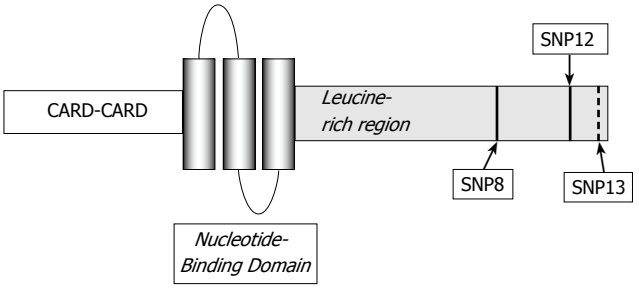

Figure 2 Structure of NOD2/CARD15 protein and locations of the three main mutations.

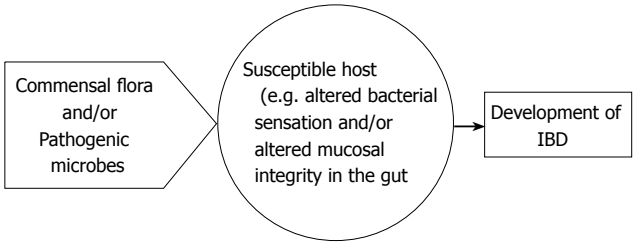

Figure 3 Role of microbial factors and genetics in the pathogenesis of IBD.

certain adhesion molecules (MAdCAM-1, CCL25) is also increased in tissues not primarily involved in the pathological process of IBD (e.g. liver), which might account for the extraintestinal manifestations of the disease[167].

Finally, in Japanese CD patients, the ICAM-1 K469 allele in IBD6 is associated with CD (OR: 2.6) and UC[168]. This association has also been confirmed in Caucasian population[169]. Targeted therapy against certain adhesion molecules (e.g. a4 and ICAM-1) is currently one of the major focuses of pharmaceutical trials in IBD[170,171].

In conclusion, various factors have been implicated in the pathogenesis of IBD, but its mechanism of action is still obscure. Recent data indicate that altered NOD2/CARD15 (or TLR4)-mediated bacterial sensing of normal commensal flora in the gut and mucosal permeability changes may be the key mechanisms (see Figure 2). At present, most efforts are devoted to a better understanding of the genetic changes underlying IBD and to further clarification of how the altered recognition of pathogenic and/or commensal microbial factors by the mucosal immune system leads to inflammation in IBD subjects but not in the general population (Figure 3).

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