Journal of Digestive Diseases 2007; **8;** 171–178 doi: 10.1111/j.1443-9573.2007.00310.x

Inflammatory bowel disease: Progress and current concepts of etiopathogenesis

Franco SCALDAFERRI & Claudio FIOCCHI

Department of Gastroenterology and Hepatology, Department of Pathobiology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Over the past few years, none of the numerous conditions that are grouped under the broad designation of 'chronic inflammatory or autoimmune disorders' has undergone as much scientific and clinical progress as the two main forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC). Progress has occurred in all major areas relevant to IBD pathogenesis, which include the external environment, genetics, microbial factors, and the immune system. This review presents an update on the specific major advances that have occurred in each of these four areas, briefly discusses the therapeutic implications of the observed progress, and points out the additional work that needs to be accomplished in the next few years to reach a full understanding of IBD etiopathogenesis.

KEY WORDS: Crohn's disease, genetics, gut flora, inflammatory bowel disease, pathogenesis, ulcerative colitis.

INTRODUCTION

As short as a few decades ago, inflammatory bowel disease (IBD) was relegated to a vague group of chronic diseases whose etiology was unknown and whose pathogenic mechanisms were loosely related to autoimmunity or non-specific inflammatory reactions. When we judge that stage against our present knowledge of IBD etiopathogenesis, one remains astonished by the amazing progress that has been recently witnessed and the advanced knowledge that has been gained of the factors involved in predisposing, conditioning, and mediating both Crohn's disease (CD) and ulcerative colitis (UC). Even though progress has occurred in all those areas, it has not been equally strong in all of them, and these differences are emphasized in several recent publications.[1–5] Evidence as to which are the fundamental components of IBD has been consolidated mainly in the past decade, and there is general agreement among investigators that the external environment, the patient's genetic makeup, his or her intestinal microbial flora, and the immune system are all involved and functionally integrated in the generation of the chronic intestinal inflammatory reaction that characterizes IBD. Thus, the main goal of this article is to offer an update on the very latest knowledge that has recently been attained on each of the above four components of CD and UC, and further, to highlight the progress achieved since the last review published in this journal.[2]

Correspondence to: Dr Claudio FIOCCHI, Department of Gastroenterology and Hepatology, and Department of Pathobiology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA. Email: fiocchc@ccf.org

EXTERNAL ENVIRONMENT

It is indisputable that the emergence of IBD in various parts of the world is associated with social and economical progress, as initially observed in Northern Europe and North America, then the rest of Europe, South America and Japan, and further the Asian Pacific region, as we are now witnessing.[6] The 'hygiene hypothesis' still remains the main theory proposed to

explain the association between this rapid worldwide increase in the incidence and prevalence of IBD as well as several other autoimmune and chronic inflammatory conditions.[7] This hypothesis proposes that the increase is due to a drastic and brisk change from a 'dirty' lifestyle with high microbial exposure to a 'clean' lifestyle with low microbial exposure caused by safer food and water, widespread use of antibiotics, vaccines, lack of parasites, and fewer infections, which all together result in a great improvement in hygiene and sanitation of the affected populations. The theory further proposes that the progressive decline of infectious diseases early in life has led to a parallel surge in allergic, autoimmune, and chronic inflammatory diseases, including CD and UC, because of a less tested and therefore less prepared immune system; an immune system that is no longer equipped to handle strong immune challenges later on in life and would generate an ineffective and prolonged response because it is incapable of eliminating offending agents, or properly responding to common agents like the patient's own intestinal bacteria.[8]

A large number of unrelated environmental factors are considered risk factors for IBD, including smoking, diet, drugs (oral contraceptive and non-steroidal anti-inflammatory drugs, NSAIDs), geography and social status, stress, enteric flora, altered intestinal permeability, and appendectomy.[9] Among them, smoking still persists as the strongest modifier for risk of IBD. Over the past few years, no new significant risk factors have been added to this list, but various reports have confirmed previous observations and attempted to establish a more intimate and causal relationship between some of these factors and the development of IBD.

A multicenter case control study from Japan reiterates the potential importance of dietary factors in IBD pathogenesis.[10] In particular, this study claims that a higher consumption of sweets was positively associated with an increased risk for UC, whereas the consumption of sugars and sweeteners, fats, fish, and shellfish were positively associated with CD risk. Interestingly, the intake of vitamin C was negatively related to the risk of UC, while the intake of total fat, monounsaturated and polyunsaturated fatty acids, vitamin E, and n-3 and n-6 fatty acids was positively associated with CD.

In a recent population-based case control Canadian study, not living in a farm, lower consumption of unpasteurized milk, ever having smoked, having a relative with IBD, and living longer with a smoker were found to be risk factors for CD, while having pet cats before age 5 and living in large families protected against CD.[11] In the case of UC, patients were also less likely to consume unpasteurized milk, more likely to have a relative with IBD, and to ever have smoked. In another Canadian study of children with CD, day care attendance during the first six months of life and physician-diagnosed infections between the ages of 5–10 were associated with an increased risk of CD.[12] This pediatric study agrees with findings from a large adult British population showing that acute gastroenteritis is followed by an increased risk of IBD.[13] In contrast, in another population-based study from Manitoba, Canada, it was observed that high economic status, lower rates of enteric infections, and having multiple sclerosis were more common among CD patients, all findings consistent with the hygiene hypothesis theory.[14]

As stated above, smoking still represents the strongest environmental factor for IBD risk or modulation, being detrimental for CD and protective for UC. This observation has been recently tested and reconfirmed by a large meta-analysis evaluation.[15] Another recent study investigated whether cigarette smoking influences the phenotype of CD,[16] and concluded that this habit is associated with both age at diagnosis and disease location, as current smokers had less colonic disease than non-smokers or ex-smokers, while disease location (ileal) was associated with the rate of development of stricturing complications and the need for surgery.

Family studies, in addition to having been the primary reason for the subsequent and persistent interest in the environment and genetics, still offer interesting opportunities for investigating the influence of environmental factors on IBD. A recent report of a Moroccan family living in Belgium in which five family members developed IBD illustrates this point.[17] Serological markers of IBD, including ASCA, ASCAg, ALCA, ACCA, Omp, and ANCA, and genetic variants in *CARD15*, *TLR4*, *NOD1*, *CARD18*, and *DLG5* had no effect on whether family members were affected or unaffected, and the authors concluded that other major environmental factors unrelated to sanitation were responsible for the multiple occurrence of IBD in that family.

While the above reports overall support the conditioning influence of environmental factors in the development of IBD, it is also clear that various observations do not necessarily fit well into the hygiene hypothesis, as shown by the contrasting results on enteric infections and the either decreased or increased risk of CD. In addition, other factors, such as smoking, seem to work outside of this theory. Finally, the data generated from a study of a particular population, a particular country, or geographic region are not directly comparable due to intrinsic dissimilarities in population composition

and timing of the study. Therefore, the true relationship between the environment and development of gut inflammation is indisputably far more complex than previously anticipated, and must take into consideration the fundamental importance of the genetic background of the affected population.

GENETICS

Of the four components of IBD pathogenesis, none has undergone as rapid progress, generated more new information, and opened novel insights into possible pathways of gut inflammation as the field of genetics.[18] This is due in large part to technological advances in DNA analysis and sequencing, such as genome-wide associations (GWA), and the use of huge multicenter or multinational databases that allow not only the rapid screening for IBD-associated genetic mutations, but also their speedy confirmation by replication.[19]

The era of modern IBD genetics began in 2001 with the discovery of mutations in the *NOD2/CARD15* gene, the first susceptibility gene in CD (IBD1).[20,21] NOD2 is a cytosolic protein that recognizes bacterial muramyl dipeptide, a major component of bacterial cell wall.[22] Variations in *NOD2/CARD15* affect its ability to recognize bacterial components and set off NF-κB. Whether this results in a loss or gain of function in regard to immune reactivity is still being debated,[23] but this discovery launched a whole new way to investigate the relationship between genetic abnormalities and altered immune responses in IBD, particularly in regard to innate immunity.[24]

In 2004 genetic variations of the *DLG5* gene were reported to be associated with IBD,[25] and the *SLC22A4* and *SLC22A5* gene cluster (IBD5) with CD.[26] *DLG5* encodes a scaffolding protein involved in the maintenance of epithelial integrity, suggesting a pathogenic role of intestinal epithelial barrier dysfunction in IBD. *SLC22A4* and *SLC22A5* encode OCTN1 and OCTN2, two transmembrane organic cation transporters. Preliminary data suggest that an interaction may exist between the risk-associated haplotype of *DLG5* and *OCTNs*, respectively, and the risk-associated variants of *CARD15*.[25,26]

Over the past few years, a new wave of information on the genetics of IBD has surfaced with the widespread use of GWA, and novel genetic associations are widening the mechanistic horizons of IBD pathogenesis.[27] A significant association between IBD and the *IL23R* gene, which encodes a subunit of the receptor for the pro-inflammatory cytokine interleukin-23, has recently been described.[28] Interestingly, while variants of *IL23R* confer protection against CD, others apparently increase the risk for IBD. In this study, nine other markers in *IL23R* and in the intergenic region between *IL-23R* and the adjacent IL-12 receptor beta-2 gene (*IL12RB2*) were associated with ileal CD. However, unlike the *NOD2/CARD15* variants which are strictly linked to small bowel CD, these *IL23R* variants only seem to determine the susceptibility to, but not the phenotype of, IBD.[29] These findings have already been confirmed in a large European study.[27] The association of the *IL23R* gene with IBD is of great interest because IL-23 is a key cytokine involved in the generation of Th17 cells. These represent a novel type of effector T cells that produce IL-17, IL-6, and TNF-α, mediate inflammatory antibacterial responses,[30,31] and are detected in large numbers in IBD mucosa, as will be discussed later on.

GWA also revealed a novel and stimulating association of CD with genes involved in autophagosome formation and the autophagy pathway, which are critically involved in the processing of intracellular bacteria, an important function in innate and adaptive responses to pathogens. Two groups identified single nucleotide polymorphisms (SNPs) of the autophagy-related gene *ATG16L1*,[32,33] which apparently predisposes to ileal CD independently of *NOD2/CARD15* and *IBD5*.[34] Another group reported a contribution to CD susceptibility by sequence variants of the autophagy gene *IRGM*,[35] which belongs to the p47 immunity-related GTPase family. Besides the *ATG16L1* and *IRGM* genes, several other loci have been identified as heightening the risk for CD, including the gene for the prostaglandin receptor EP4, *PTGER4*, the gene *TBFSF15* encoding member 15 of the TNF superfamily, and several others.[33,35–37]

In addition to the above reports implicating the *ATG16L1* and *IRGM* genes as mediating defective recognition of bacteria in IBD pathogenesis, other reports support this notion by demonstrating polymorphisms of other gene products that are also involved in the host response to bacterial products. Polymorphisms of *TLR4* have been associated with an increased risk for CD as well as UC,[38,39] whereas a dominant-negative *TLR5* polymorphism is negatively associated with CD.[40] Polymorphisms related to the natural antimicrobial peptides defensins produced by Paneth cells have also been recently described. One recent study demonstrates that CD is associated with a reduced copy number of the beta-defensin 2 locus on chromosome 8, a finding primarily associated with colonic disease localization.[41] Also related to handling of bacterial products and xenobiotics are the multidrug resistance gene 1 (*MDR1*) and the pregnane X receptor (*PXR*), and he genetic variation in *MDR1* and *PXR* have been described as

being linked to UC and IBD, and CD and UC, respectively.[42,43]

This new wave of information on the genetics of IBD holds several key messages in regard to both disease susceptibility and the underlying mechanism of disease. First, the expanding number of gene variations being described in CD and UC leaves little room for doubting the increasingly accepted notion that genetic influences are critical determinants of disease development, phenotype, and clinical outcome. Second, if we consider the various functions ascribed to the products of the genetic alterations in IBD, they broadly fall into two main categories: immune reactivity and early response to bacteria. Together, this strongly suggests that genetics impact on how mutation-carrying individuals respond to microbial challenges mediated by innate immunity and predispose these subjects to the subsequent development of IBD, particularly CD. Third, if for every genetic variation there is a corresponding and unique pathophysiological consequence, then the spectrum of underlying mechanisms of disease may be disheartening large. This implies that a massive amount of work is ahead of us if we are to fully understand all the pathogenic aberrations that need to be corrected to provide IBD patients with rational approaches to their clinical management.

MICROBIAL FACTORS

Among the components of IBD pathogenesis, the investigation of role of infectious agents and the gut commensal flora is an area in which relatively less progress has occurred. There are two main reasons for this: (1) only isolated reports on new infectious agents with an etiologic potential for IBD have been published in several years; and (2) major methodological difficulties are encountered in the study of the flora in the human gut.

In regard to the previously proposed theory that the measles virus was somehow involved in causation of CD, no new studies have emerged, and this theory is now no longer being actively pursued. The status of *M. paratuberculosis* as another infectious cause of CD, a lingering and controversial topic, has very recently suffered a severe setback with the just published report from an Australian multicenter study showing that a 2-year administration of combined clarithromycin, rifabutin, and clofazimine antituberculous therapy offers no substantial or prolonged benefits to patients with CD.[44] This report would appear to contain the long sought-after proof that CD is not caused by *M. paratuberculosis*. Another microorganism that has been investigated as a potential IBD pathogen is a unique adherent-invasive *E. coli* that colonizes the ileal mucosa of CD patients, and has the ability to adhere to and invade intestinal epithelial cells through a CEACAM6-dependent mechanism.[45] This microbe is not found in UC, its presence being apparently restricted to CD.[46] However, whether this adherent-invasive *E. coli* directly or indirectly causes ileal CD, or is a secondary invader to a previously inflamed mucosa, is still uncertain.

In regard to the theory that IBD represents the consequence of the loss of immunological tolerance against the autologous gut flora, this is still held as being correct. Supporting this theory are a limited number of human studies and a large number of studies in animal models of IBD. The latter convincingly show that the presence of bacteria in the gut is essential to the development of experimental IBD in most models.[47] In some, like the ileitis occurring in SAMP1/YitFc mice, the commensal flora appears to exacerbate rather than directly cause disease,[48] and this concept may be also relevant to human IBD. It is widely accepted that in healthy subjects the normal commensal flora, especially in the colon, exhibits an extraordinary diversity,[49] but its detailed analysis is made quite problematic by the use of numerous culture- and nucleic acid-based analytical methods that yield different results and are not directly comparable.[50] Consequently, it has been impossible so far to reach a definitive answer to the central question of whether the intestinal flora in IBD is normal or abnormal.[51] The number of studies examining the gut flora in CD and UC in both inflamed and non-inflamed segments is fairly large, but results are inconclusive. Some of the most recent reports claim that the flora in CD and UC differs from that of healthy controls,[52] that biodiversity of the gut microbiota is stable in healthy and IBD subjects,[53] that there is reduced diversity of the flora in CD,[54] and that the bacteriology of mucosal biopsies differs among newly diagnosed and untreated CD and UC patients.[55] These studies may be technically correct, but the different source of the microbes (stools, lumen, mucosa) and the various analytical methods do not enable the gathering of conclusive evidence that specific or, at least, selective patterns or abnormalities of the gut flora are truly present in IBD, or that clear-cut differences exist between CD and UC.

IMMUNOLOGICAL FACTORS

The investigation of IBD pathogenesis has been dominated for a long time by studies of mucosal immunity and, in particular, studies of the function of local T cells in CD and UC tissues. This focus on the adaptive immune response has ultimately led to the notion that

the two main types of IBD represent clearly distinct forms of gut inflammation: CD represents a Th1-type condition dominated by the production of IFN-γ, while UC represents an atypical Th2 response characterized by an increased production of IL-13.[56,57] This separation has the important implication that the inflammatory mechanisms responsible for CD and UC are distinct, a proposition reinforced by recent proteomic analysis showing distinctive immune profiles in the inflamed mucosa of CD and UC patients.[58] Although this interpretation of the adaptive immune response in IBD is probably largely correct, there has been a surge of fresh information in regard to the role of innate immunity in IBD pathogenesis, and new data on adaptive immunity are emerging indicating that: (1) the mucosal Th1 and Th2 responses of CD and UC may be actually secondary to defects of the innate immune response, (2) the dysfunction of regulatory T cells may be contributing to mucosal immune abnormalities, and (3) the newly described Th17 cells are also prominently involved in the gut inflammatory response of both forms of IBD.[59]

Innate immunity

Innate immunity can be defined as a 'ready-to-go' defense system that jumps into action within minutes or hours, has limited specificity, and is mediated by a large variety of different cell types including epithelial cells, neutrophils, monocytes, macrophages, dendritic cells, and natural killer cells.[60] This form of immunity is directed primarily to the recognition of microbial antigens, a process mediated by pattern recognition receptors represented mainly by toll-like receptors (TLRs) on the cell surface and NOD proteins in the cytoplasm.[61,62] There is a growing body of evidence that the behavior of the cells mediating innate immunity and the expression and function of both TLRs and NOD proteins are altered in IBD.

A British study shows that mucosal neutrophil accumulation and production of IL-1β and IL-8 in response to trauma, and vasodilation in response to *E. coli* injections are selectively reduced in CD patients but not in UC patients.[63] This suggests the existence of a defective acute inflammatory response in CD, a phenomenon which appears to be independent of the presence of *NOD2/CARD15* mutations.[63] Transfection studies reveal that the NOD2 mutations most commonly associated with CD induce a defective ability to respond to LPS, and this defect may contribute to disease susceptibility.[64] Mucosal dendritic cells, the main antigen-presenting cell in the gut, display an activated phenotype in IBD tissues indicative of their involvement in the local chronic inflammatory reaction.[65] In intestinal epithelial cells, the expression of TLR3 is significantly downregulated in active CD but not UC, while TLR4 is strongly upregulated in both CD and UC.[66] In the mucosa of patients with postcolectomy active pouchitis, the expression of both TLR2 and TLR4 is also strongly upregulated.[67] Finally, patients with CD have a reduced content of α-defensins in the affected ileum, and this reduction is independent to the degree of mucosal inflammation.[68] These reports, combined with observations in animal models and the genetic associations previously discussed,[38,69] make an increasingly strong case for the role of defective innate immunity as being a key component or perhaps even the underlying trigger for IBD, and CD in particular.[70] Whether single defects of innate immunity are sufficient to trigger IBD, or a combination of them with or without the contribution of superimposed adaptive immunity alterations is needed to start and perpetuate gut inflammation, remains to be determined.

Adaptive immunity

In contrast to the innate immune response, adaptive immunity takes more time to develop (from a few to several days) and depends fundamentally on the type and number of T cells. Obviously, many different types of T cells exist but, for the purpose of this update, only T regulatory cells and the newly described Th17 cells will be discussed.

Regulatory cells, originally called suppressor cells, are cells capable of inhibiting or controlling the outgrowth of potentially pathogenic antigen-reactive T cells, and they exist in different varieties: (1) CD4+CD25– T cells (Tr1 cells) that exert their immune suppressor function primarily through the secretion of cytokines like IL-10, (2) CD4+CD25+FOXP3+ T cells (T regs) which require cell contact and cytokines for their function, (3) NKT cells that regulate through cytokine secretion and cytotoxicity, and (4) various types of CD8+ cells that utilize different mechanisms.[71] Information on regulatory T cells in IBD is based on a limited number of studies in human patients but a substantial amount of data from murine models of IBD. One study from Japan found that the relative proportion of CD4+CD25+ T regs was significantly increased in patients with active IBD,[72] whereas a German study found that the frequency of CD4+CD25+ T regs was variable with the activity of IBD.[73] In the latter study T regs were functional, but their number was reduced in the peripheral circulation and only moderately expanded in the inflamed mucosa.[73] Another report found that CD8+ regulatory cells were reduced in the gut of IBD patients.[74] Studies in animal

models are more abundant and convincing, showing that the administration of regulatory T cells can cure experimental colitis.[75] The therapeutic use of regulatory T cells in human subjects with IBD is theoretically feasible, but this approach will have to wait until a more definitive demonstration that an insufficient suppressor function is truly a key pathogenic event in IBD.[75]

The long established paradigm that CD4+ effector T helper cells are divided into two main separate subpopulations, e.g., Th1 and Th2, has been recently challenged by the discovery of a third Th lineage, Th17 cells, which are developmentally and functionally distinct from both Th1 and Th2 cells.[76] Th17 cells require IL-23, TGF-β1, and IL-6 for their differentiation and growth, and their main product is IL-17, in addition to IL-6 and TNF-α.[30,31] Th17 cells mediate immunity, inflammation, and tissue damage in infectious, autoimmune, and inflammatory conditions,[77] and are likely to be involved in IBD pathogenesis. In fact, IL-17+ cells have been detected by immunohistochemical staining in the inflamed mucosa of CD and UC patients.[78] A very recent report confirmed the presence of Th17 cells in CD mucosa but, interestingly, the authors also found a previously unreported subset of mucosal T cells sharing features of both Th1 and Th17 cells, i.e. concomitant producing IFN-γ and IL-17.[79] This raises the intriguing question of the functional relationship and the respective roles of Th1 and Th17 cells in mediating inflammation and gut tissue damage in this form of IBD. Of special interest to IBD is the fact that IL-23, a key cytokine needed for the production of IL-17, shares with IL-12 (the cytokine needed for production of IFN-γ) the common p40 unit, and the antip40 antibodies used for the treatment of CD block not only IL-12 but also IL-23 and the downstream molecule IL-17.[80] Therefore, the beneficial effects of these antibodies, originally ascribed to their capacity to block IL-12 and therefore IFN-γ (i.e. inhibiting a Th1 response) may actually be beneficial because of their effect in neutralizing IL-23 and Th17 cells (i.e. inhibiting a Th17 response). The true role of Th17 cells in IBD pathogenesis is currently undergoing intense scrutiny, and it is particularly fascinating that Th17 cells express on their surface the IL-23R, the product of the *IL23R* gene whose various mutations have been recently linked to both risk for and protection from IBD.[28]

ADDITIONAL FACTORS

In addition to the environment, genes, microbes, and the immune system, other factors also participate in IBD pathogenesis, and two in particular are worth mentioning: fibrosis and angiogenesis. Even though both the formation of scar tissue and new vessels are secondary to uncontrolled and long-lasting tissue inflammation (in IBD as well as in many other chronic inflammatory disorders), these two processes are intrinsic to CD and UC, and are responsible for important clinical symptoms and perpetuation of gut inflammation, respectively. New knowledge on these two components of IBD is fast emerging. The cellular and molecular mechanisms of fibrosis, and stricture and fistula formation, are being unveiled, and with that the possibility arises of preventing these serious complications.[81] Angiogenesis has recently been shown to be a novel and important component of IBD pathogenesis,[82] and one likely to contribute to the chronicity of the disease. Angiogenesis blockade is effective in decreasing inflammation in experimental colitis,[83] suggesting that preventing new vessel formation may open brand new therapeutic opportunities.[84]

CONCLUSIONS AND IMPLICATIONS FOR THERAPY

There is no question that the past few years have seen an unprecedented progress in our understanding of IBD pathogenesis. The key factors responsible for IBD are fairly well defined: the environment, genetic makeup, commensal flora, and immune response. More detailed information on their composition, function, and interaction is becoming increasingly accessible through the investigation of environmental changes, high throughput genomic approaches, molecular analysis of gut bacteria populations, and a more integrated understanding of the interaction between innate and adaptive immune responses. The growing number and diversity of genetic variations associated with IBD poses major challenges to the investigation of how they impact on immunity and inflammation in susceptible individuals, and a considerable amount of painstaking *in vitro* and *in vivo* work (in animal models) needs to be carried out to clarify structure-function-symptom relationships. However, this work is imperative to identify specific subgroups of IBD patients, because each group is likely to have unique mechanisms underlying their form of IBD. If we aim at truly specific, effective, and perhaps curative approaches to CD or UC, then custom-made therapies are indispensable. We are clearly not there yet, but if progress continues at the same pace that has been happening in the past decade (and it is likely to become even faster) we can envision that the molecular classification of patient phenotypes and the inflammatory pathway-specific interventions may become realities within a decade or two.

REFERENCES

1 Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417–29.
2 Fiocchi C. Inflammatory bowel disease pathogenesis: therapeutic implications. *Chinese J Dig Dis* 2005; **6**: 6–9.
3 Danese S, Fiocchi C. Etiopathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2006; **12**: 4807–12.
4 Kugathasan S, Fiocchi C. Progress in basic inflammatory bowel disease research. *Sem Ped Surg* 2007; **16**: 146–53.
5 Xavier RJ, Podolsky D. Unraveling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427–34.
6 Ouyang Q, Tandon R, Goh K-L, Ooi CJ, Ogata H, Fiocchi C. The emergence of inflammatory bowel disease in the Asian Pacific region. *Curr Opin Gastroenterol* 2005; **21**: 408–13.
7 Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; **296**: 490– 4.
8 Rook GAW, Brunet LR. Microbes, immunoregulation, and the gut. *Gut* 2005; **54**: 317–20.
9 Loftus EV. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology* 2005; **126**: 1504–17.
10 Sakamoto N, Kono S, Wakai K *et al*. Dietary risk factors for inflammatory bowel disease. A multicenter case-control study in Japan. *Inflamm Bowel Dis* 2005; **11**: 154–63.
11 Bernstein CN, Rawsthorne P, Cheang M, Math M, Blanchard JF. A population-based case control study of potential risk factors in IBD. *Am J Gastroenterol* 2006; **101**: 993–1002.
12 Amre DK, Lambrette P, Law L *et al*. Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn's disease: a case-control study. *Am J Gastroenterol* 2006; **101**: 1005–11.
13 Garcia-Rodriguez LA, Ruigomez A, Panes J. Acute gastroenteritis is followed by an increased risk of inflammatory bowel disease. *Gastroenterology* 2006; **130**: 1588–94.
14 Green C, Elliott L, Beaudoin C, Bernstein CN. A population-based ecological study of inflammatory bowel disease: searching for etiological clues. *Am J Clin Epidemiol* 2006.
15 Mahid SS, Minor KS, Soto RE, Hornung CA, Galandiuk S. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clinic Proc* 2006; **81**: 1462–71.
16 Aldhous MC, Drummond HE, Anderson N, Smith LA, Arnott IDR, Satsangi J. Does cigarette smoking influence the phenotype of Crohn's disease? Analysis using the Montreal classification. *Am J Gastroenterol* 2007; **102**: 577–88.
17 Joossens M, Simoens M, Vermeire S, Bossuyt X, Geboes K, Rugeerts P. Contribution of genetic and environmental factors in the pathogenesis of Crohn's disease in a large family with multiple cases. *Inflamm Bowel Dis* 2007; **13**: 580– 4.
18 Gaya DR, Russell RK, Nimmo ER, Satsangi J. New genes in inflammatory bowel disease: lessons for complex diseases? *Lancet* 2006; **367**: 1271– 84.
19 Duerr RH. Genome-wide association studies herald a new era of rapid discoveries in inflammatory bowel disease research. *Gastroenterology* 2007; **132**: 2045– 62.
20 Hugot J-P, Chamaiilard M, Zouali H *et al*. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599– 603.
21 Ogura Y, Bonen DK, Inohara N *et al*. A frameshift mutation in Nod2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603–6.
22 Inohara N, Ogura Y, Fontalba A *et al*. Host recognition of bacterial muramyl dipeptide mediated through NOD2. *J Biol Chem* 2003; **278**: 5509–12.
23 Eckmann L, Karin M. NOD2 and Crohn's disease: loss or gain of function? *Immunity* 2005; **22**: 661–7.
24 van Limbergen J, Russell RK, Nimmo ER *et al*. Genetics of the innate immune response in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 338–55.
25 Stoll M, Corneliussen B, Costello CM *et al*. Genetic variation in *DLG5* is associated with inflammatory bowel disease. *Nat Genet* 2004; **36**: 476–80.
26 Peltekova VD, Wintle RF, Rubin LA *et al*. Functional variants of *OCTN* cation transporter genes are associated with Crohn's disease. *Nat Genet* 2004; **36**: 471–5.
27 The Wellcome Trust Case Control Consortium. Genome-wide association of 14 000 cases of seven common diseases and 3000 shared controls. *Nature* 2007; **447**: 661–83.
28 Duerr RH, Taylor KD, Brant SR *et al*. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461–3.
29 Tremelling M, Cummings F, Fisher SA *et al*. IL23R varaiation determines susceptibility but not disease phenotype in inflammatory bowel disease. *Gastroenterology* 2007; **132**: 1657–64.
30 Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. *J Clin Invest* 2006; **116**: 1218–22.
31 McKenzie BS, Kastelein RA, Cua DJ. Understanding the IL-23/IL-17 immune pathway. *Trends Immunol* 2006; **27**: 17–23.
32 Hampe H, Franke A, Rosenstiel P *et al*. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007; **39**: 207–11.
33 Rioux JD, Xavier RJ, Taylor KD *et al*. Genome-wide association studi identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596–604.
34 Prescott NJ, Fisher SA, Franke A *et al*. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. *Gastroenterology* 2007; **132**: 1665–71.
35 Parkes M, Barrett JC, Prescott NJ *et al*. Sequence variants in the autophagy gene IRGM and multiple other replicatins loci contribute to Crohn's disease susceptibility. *Nat Genet* 2007: 39.
36 Libioulle C, Louis E, Hansoul S *et al*. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. *PLOS Genet* 2007; **3**: 538–43.
37 Yamazaki K, McGovern D, Ragoussis J *et al*. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. *Hum Mol Genet* 2007; **14**: 3499–506.
38 Franchimont D, Vermeire S, El Housni H *et al*. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 2004; **53**: 987–92.
39 Brand S, Staudinger T, Schnitzler F *et al*. The role of Toll-Like Receptor 4 Asp299Gly and Th399Ile polymorphisms and CARD15/NOD2 mutations in the susceptibility and phenotype of Crohn's disease. *Inflamm Bowel Dis* 2005; **11**: 645–52.
40 Gerwitz AT, Vijay-Kumar M, Brant SR, Duerr RH, Nicolae DL, Cho JH. Dominant-negative TLR5 polymosphism reduces adaptive immune response to flagellin and negatively associates with Crohn's disease. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G1157–63.
41 Fellermann K, Stange DE, Schaeffeler E *et al*. A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to Crohn disease of the colon. *Am J Hum Genet* 2006; **79**: 439–48.

42 Ho G-T, Nimmo ER, Tenesa A *et al*. Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. *Gastroenterology* 2005; **128**: 288–96.
43 Dring MM, Goulding CA, Trimble VI *et al*. The pregnane X receptor locus is associated with susceptibility to inflammatory bowel disease. *Gastroenterology* 2006; **130**: 341–8.
44 Selby W, Pavli P, Crotty B *et al*. Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Chron's disease. *Gastroenterology* 2007; **132**: 2313–9.
45 Barnich N, Carvalho FA, Glasser A-L *et al*. CEACAM6 acts as a receptor for adherent-invasive E. coli, supporting ileal mucosa colonization in Crohn disease. *J Clin Invest* 2007; **117**: 1566–74.
46 Rolhion N, Darfeuille-Michaud A. Adherent-invasive Escherichia coli in inflammatory bowel disease. *Inflamm Bowel Dis* 2007 [Epub ahead of print].
47 Elson CO, McCracken VJ, Dimmit RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive and regulatory mechanisms of host dialogue with the microbiota. *Immunol Rev* 2005; **206**: 260–76.
48 Bamias G, Okazawa A, Rivera-Nieves J *et al*. Commensal bacteria exacerbate intestinal inflammation but are not essential for the development of murine colitis. *J Immunol* 2007; **178**: 1809–18.
49 Eckburg PB, Bik EM, Bernstein CN *et al*. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635–8.
50 Tannock GW. New perceptions of the gut microbiota: implications for future research. *Gastroenterol Clin N Am* 2005; **34**: 261–382.
51 Guarner F. The intestinal flora in inflammatory bowel disease: normal or abnormal? *Curr Opin Gastroenterol* 2005; **21**: 414–8.
52 Sokol H, Seksik P, Rigottier-Gois L *et al*. Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 106–11.
53 Lepage P, Seksik P, Sutern M *et al*. Biodiversity of the mucosa-associated microbiota is stable along the distal digestive tract in healthy individuals and patients with IBD. *Inflamm Bowel Dis* 2005; **11**: 473– 80.
54 Manichanh C, Rigottier-Gois L, Bonnaud E *et al*. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic analysis. *Gut* 2006; **55**: 205–11.
55 Bibiloni R, Mangold M, Madsen KL, Fedorak RN, Tannock GW. The bacteriology of biopsies differs between newly diagnosed, untreated, Crohn's disease and ulcerative colitis patients. *J Med Microbiol* 2006; **55**: 1141–9.
56 Cobrin GM, Abreu MT. Defects of mucosal immunity leading to Crohn's disease. *Immunol Rev* 2005; **206**: 277–95.
57 Targan SR, Karp LC. Defects of mucosal immunity leading to ulcerative colitis. *Immunol Rev* 2005; **206**: 296–305.
58 Berndt U, Bartasch S, Philipsen L *et al*. Proteomic analysis of the inflamed intestinal mucosa reveals distinctive immune reponse profiles in Crohn's disease and ulcerative colitis. *J Immunol* 2007; **179**: 295–304.
59 Bamias G, Cominelli F. Immunopathogenesis of inflammatory bowel disease: current concepts. *Curr Opin Gastroenterol* 2007; **23**: 365–9.
60 Medzhitov R, Janeway C. Innate immunity. *N Engl J Med* 2000; **343**: 338–44.
61 Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. *J Immunol* 2005; **174**: 4453–60.
62 Inohara N, Nunez G. Nods: intracellular proteins involved in inflammation and apoptosis. *Nature Rev* 2003; **3**: 371–82.
63 Marks DJB, Hardbord MWN, MacAllister R *et al*. Defective acute inflammation in Crohn's disease: a clinical investigation. *Lancet* 2006; **367**: 668–78.
64 Bonen DK, Ogura Y, Nicolae DL *et al*. Crohn's disease-associated NOD2 variants share a signaling defect in response to lipolysaccharide and peptidoglycan. *Gastroenterology* 2003; **124**: 140–6.
65 Hart AL, Al-Hassi HO, Rigby RJ *et al*. Characteristics of intestinal dendritic cells in inflammatory bowel disease. *Gastroenterology* 2005; **129**: 50–65.
66 Cario E, Podolsky DK. Differential alterationin intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 2000; **68**: 7010–7.
67 Toyama Y, Araki T, Yoshiyama S, Hiro J-C, Miki C, Kusunori M. The expression patterns of toll-like receptors in the ileal pouch mucosa of postoperative ulcerative colitis patients. *Surg Today* 2006; **36**: 287–90.
68 Wehkamp J, Salzman NH, Porter E *et al*. Reduced Paneth cell α-defensins in ileal Crohn's disease. *Proc Natl Acad Sci USA* 2005; **102**: 18129–34.
69 Rakoff-Nahoum S, Hao L, Medzhitov R. Role of toll-like receptors in spontaneous commensal-dependent colitis. *Immunity* 2006; **25**: 319–29.
70 Korzenik JR. Is Crohn's disease due to defective immunity? *Gut* 2007; **56**: 2–5.
71 Jiang H, Chess L. An integrated view of suppressor T cell subsets in immunoregulation. *J Clin Invest* 2004; **114**: 1198–208.
72 Makita S, Kanai T, Oshima S *et al*. $CD4^{+}CD25^{bright}$ T cells in human intestinal lamina propria as regulatory cells. *J Immunol* 2004; **173**: 3119–30.
73 Maul J, Loddenkemper C, Mundt P *et al*. Peripheral and intestinal regulatory CD4+CD25+^{high} T cells in inflammatory bowel disease. *Gastroenterology* 2005; **128**: 1868–78.
74 Brimnes J, Allez M, Dotan I, Shao L, Nakazawa A, Mayer L. Defects in CD8+ regulatory T cells in the lamina propria of patients with inflammatory bowel disease. *J Immunol* 2005; **174**: 5814–22.
75 Huibregtse IL, van Lent AU, van Deventer SJH. Immunopathogenesis of IBD: insufficient suppressor function in the gut? *Gut* 2007; **56**: 584–92.
76 Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 2006; **24**: 677–88.
77 Steinman L. A brief history of Th17, the first major revision in the Th1/Th2 hypothesis of T cell-mediated tissue damage. *Nat Med* 2007; **13**: 139–45.
78 Fujino S, Andoh A, Bamba S *et al*. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 2003; **52**: 65–70.
79 Annunziato F, Cosmi L, Santarlasci V *et al*. Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007; **204**: 1849–61.
80 Fuss IJ, Becker C, Yang Z *et al*. Both IL-12p70 and IL-23 are synthesized during active Crohn's disease and are down-regulated by treatment with anti-IL-12 p40 monoclonal antibody. *Inflamm Bowel Dis* 2006; **12**: 9–15.
81 Rieder F, Brenmoehl J, Leeb S, Scholmerich J, Rogler G. Wound healing and fibrosis in intestinal disease. *Gut* 2007; **56**: 130–9.
82 Danese S, Sans M, de la Motte C *et al*. Angiogenesis as a novel components of inflammatory bowel disease pathogenesis. *Gastroenterology* 2006; **130**: 2060–73.
83 Danese S, Sans M, Spencer DM *et al*. Angiogenesis blockade as a new therapeutic approach to experimental colitis. *Gut* 2007; **56**: 855–62.
84 Childow JH, Shukla D, Grisham MB, Kevil CG. Pathogenic angiogenesis in IBD and experimental colitis: New ideas and therapeutic avenues. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G5–18.