Published in final edited form as:
Expert Rev Clin Immunol. 2010 July ; 6(4): 585–596. doi:10.1586/eci.10.32.

Crohn's disease as an immunodeficiency

Bu'Hussain Hayee[†,1], **Farooq Z Rahman**[1], **Gavin Sewell**[1], **Andrew M Smith**[1], and **Anthony W Segal**[1]

[1]Department of Molecular Medicine, University College London, London, WC1E 6JJ, UK

Abstract

The pathogenesis of Crohn's disease (CD) has widely been regarded as the consequence of a dysregulated T-cell-mediated response to intestinal microbes, and the majority of the worldwide research effort has focused on characterizing and treating the chronic inflammatory phase of the disease. However, recent molecular biological and clinical investigations indicate that CD is actually a primary immunodeficiency. At first counter-intuitive, the apparent paradox of a pathogenic innate immune defect can be linked mechanistically to the granulomatous chronic inflammation characteristic of the disease. Genome-wide association studies have corroborated the involvement of innate immune dysfunction in the pathogenesis of CD, but less than 20% of the heritable risk is accounted for. By contrast, *in vitro* and *in vivo* stimulation of the immune system has highlighted novel areas of interest that may lead to the development of targeted therapeutic and diagnostic tools.

Keywords

bacteria; Crohn's disease; immunodeficiency; innate immunity; macrophage; neutrophil

Established intestinal lesions in Crohn's disease (CD) contain a massive, mixed inflammatory cell infiltrate associated with a complex storm of cytokines in a self-perpetuating, chronic inflammatory response [1–3]. Most research to date has focused on the the immunological characteristics of established lesions and drawn inferences about their initiation. The predominance of a classically Th1-associated cytokine profile in these lesions led to the assumption that T lymphocytes were central to the initial pathogenesis. More recently, the role of regulatory (Treg) FoxP3$^+$ and IL-23-induced responsive Th17-type T lymphocytes has gained popularity [2,4,5]. Treg cell populations are diminished in patients with CD [6] and, although the hypothesis of CD as an immunodeficiency does not necessarily exclude defects in T-cell function, to date, no convincing intrinsic (primary) defect has been identified to implicate these cells in the pathogenesis of human CD.

The hypothesis that immunodeficiency underpins the development of CD seems, at first, to be in direct contrast with histological observations of established intestinal lesions and the

[†]Author for correspondence: Tel.: +44 207 679 6170 Fax: +44 207 679 0967 b.hayee@nhs.net.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Europe PMC Funders Author Manuscripts

use of immunosuppressive therapy (albeit with varying results [7]). However, several lines of evidence support the progression of innate immunodeficiency to granulomatous, chronic inflammation and recent research indicates that this process occurs in CD.

Association between immunodeficiency diseases & CD

There is a well-known and strong association between primary immunodeficiency disorders and non-infectious granulomatous intestinal inflammation. Chronic granulomatous disease (CGD), glycogen storage disease type 1b, leukocyte adhesion deficiency, Chediak–Higashi and Hermansky–Pudlak syndromes are all strongly associated with intestinal inflammation that is indistinguishable from CD [8,9]. These diseases are rare, with a ‘one in a million’ prevalence or less in most cases (and would therefore not be identified by population screening), but are important for a number of reasons. First, they are monogenic diseases in which the cellular dysfunction is well characterized. Second, they all involve – with varying emphasis – neutrophil dysfunction. Third, the final common result of these defects is an impairment in the ability of the host to deal with invading microorganisms [8]. Last, a granulomatous chronic inflammatory response ensues, in the absence of any intrinsic T-cell hyperreactivity, from the initial failure of bacterial clearance in these conditions.

A failure of bacterial clearance in CD

Neutrophils are phagocytic cells with a primary role in the defense against (and clearance of) invading microorganisms [10]. A profound defect of neutrophil influx to sites of acute inflammation has been demonstrated in abrasion-induced ‘skin windows’ in CD [11] and following experimental injury of the intestinal mucosa [12], indicating a systemic defect of neutrophil recruitment in CD. Importantly, in both studies, inflammatory controls (patients with rheumatoid arthritis and ulcerative colitis [UC]) displayed normal responses. More recently, this phenomenon was demonstrated in response to an experimental inoculation of killed *Escherichia coli* in CD using radiolabeled neutrophils [13].

The immunodeficiency hypothesis proposes that an inherently weak acute inflammatory response in CD is insufficient to remove bacteria and other organic material gaining access to the tissues of the bowel wall. The delayed clearance of such antigenic material, and its subsequent persistence, would then act as a trigger for granuloma formation and the T-cell-mediated chronic inflammation that is the hallmark of CD. Bacterial clearance depends on adequate neutrophil influx [14,15] and it was therefore hypothesized that the impaired neutrophil influx observed in CD would lead to a failure of bacterial clearance.

This hypothesis was tested directly by measuring the clearance of radiolabeled *E. coli* from a subcutaneous injection site. Using a single inoculum of 30 million bacteria, clearance was demonstrated to be markedly delayed in CD, with approximately 50% of the injected inoculum still present 72 h after injection (compared with <10% in healthy controls or patients with UC) [13]. These findings led us to propose a ‘three-stage hypothesis’ for the generation of inflammatory lesions in CD (Figure 1) [16]. The first stage: bacterial entry into, or invasion of, the intestinal mucosa would occur as the result of either an inherent weakness of the intestinal barrier or as the result of contact with an invasive pathogen. This would act as the stimulus for the second stage – a particular abnormality in CD: a relatively

weak innate immune response characterized by poor neutrophil accumulation. The third stage would be an expected response to bacterial persistence [17]: granulomatous chronic inflammation characteristic of CD, with adaptive immune responses to fecal material in the tissues of the bowel wall generating the clinically apparent systemic inflammatory response.

Predisposition to infection in CD: the first paradox

Given that such a profound defect of bacterial clearance was demonstrated in our studies in CD, the obvious question is whether this relates to a more general susceptibility to systemic bacterial infections. At first glance, this represents the first paradox of the immunodeficiency hypothesis. However, it is not clear whether patients with CD should actually be predisposed to systemic infection. Even in CGD, where the defect in innate immunity is monogenic and more profound, infections are intermittent and may, in some cases, present only in later life [9].

Notwithstanding this, there are both direct and indirect data documenting an increased incidence of urinary tract [18], gastrointestinal [19] and post-surgical wound infections [20] in patients with CD. However, confounding factors such as immunosuppressant medication, antibiotic therapy, fistula formation, malnutrition and hospitalization make it difficult to establish causality. Interestingly, a Swedish disease registry study reports higher rates of pre-morbid infections, particularly those associated with Gram-negative bacteria, in children who go on to develop intestinal CD (either pediatric or adult onset) [21]. It remains unclear, however, whether this represents underlying susceptibility or causation.

Initial microbial load is important

To further characterize the abnormal clearance of radiolabeled bacteria, we determined the effect of varying the initial size of the bacterial inoculum [12]. In healthy controls, the magnitude of the inflammatory response was exponentially related to the size of the bacterial inoculum, and this relationship was significantly attenuated in CD. However, most interesting was that the inflammatory response and subsequent clearance of smaller inocula in patients with CD containing 10^5 and 10^6 *E. coli* was comparable to healthy controls. This indicated a threshold of bacterial number beyond which the attenuated local inflammatory response in CD would be overwhelmed. This threshold would be even lower in individuals with inherited disorders of innate immunity (Figure 2).

In general, the required magnitude of stimulus could only readily occur in an area of high microbial and antigenic load: the intestinal tract, specifically the terminal ileum and colon [22]. The occurrence of oropharyngeal lesions in CD may also be attributed to high antigenic load [23], but this region is also uniquely susceptible to traumatic breach of the epithelial barrier. Invasive organisms, adherent-invasive *E. coli* (AIEC) in particular, have been associated with CD [24]. While there may be a number of mechanisms by which these organisms contribute to pathogenesis, it is interesting to speculate that their invasive capacity may rapidly overwhelm a weak innate immune response, as discussed previously. It should also be noted that AIEC also appear intrinsically capable of evading intracellular killing (discussed later).

Microbial species may influence clearance

Despite the difficulties in interpreting observational studies, infections that commonly involve Gram-negative organisms in other organs, for instance urinary tract infections [18], appear more frequently in CD than healthy individuals. By contrast, extrapolating from treatment registries and postoperative analyses, infections commonly caused by Gram-positive organisms, such as pneumonia, appear no more frequently in adults with CD compared with healthy or differentially treated populations [21,25–27] (although Gram-negative pneumonia has been reported in patients receiving anti-TNF therapy [28]). Responses to Gram-positive and Gram-negative bacteria are very different, most likely due to the differing antigenic stimuli presented by these organisms [29]. We have demonstrated deficient inflammatory responses to *E. coli* (a Gram-negative organism) in CD, which are not apparent when the Gram-positive organism *Staphylococcus aureus* is used as the stimulus [30]. These differences might also explain why defective innate immunity in CD appears to be most overtly apparent in the GI tract.

What is the underlying defect?

The similarity between CD and the primary inherited disorders of neutrophil function has been discussed. Neutrophil function has been extensively investigated and, while defects in various aspects of neutrophil function have been reported, the data are conflicting [12,31] and lead to the conclusion that intrinsic neutrophil defects do not appear to make a primary contribution to pathogenesis in the majority of patients.

Neutrophil migration to inflammatory sites requires a chemotactic gradient, but is also dependent on the upregulation of adhesion molecules and chemokine secretion by the activated vascular endothelium [32,33], which is induced classically by TNF-α. In the inflamed intestinal lamina propria, the source of TNF-α and other proinflammatory cytokines has been localized to macrophages [34–36], which are derived from (and continually replenished by) circulating monocytes [37].

Macrophages are the sentinels of the immune system and play a critical role in orchestrating inflammatory responses. Impaired macrophage-mediated neutrophil recruitment in CD would result in impaired clearance of antigenic material from the bowel wall, initiating a damaging, chronic adaptive response [38–40].

In vitro studies of the responses of peripheral blood monocyte-derived macrophages in response to *E. coli* demonstrated markedly diminished secretion of proinflammatory cytokines, such as TNF-α, in CD [12,13]. No abnormality was observed in macrophage anti-inflammatory cytokine and chemokine secretion.

Macrophage cytokine secretion is a complex process governed by an intricate network of regulatory steps. Impaired proinflammatory cytokine secretion in CD is not caused by abnormal transcription, processing or translation of cytokine mRNA. Rather, it appears to be due to abnormal secretion, with the defect lying in the stages between these two processes. Microarray data from stimulated macrophages revealed the majority of differentially expressed genes in CD to be associated with cytoskeletal rearrangement and vesicle trafficking. Molecular biological studies using inhibitors of vesicle trafficking confirmed a

defect in the complex exocytic pathways of CD macrophages, whereby normally-produced cytokines were targeted for degradation in the lysosomal compartment, rather than being secreted. The cause of this malfunction is yet to be determined.

The failure of a secretory system provides a generic mechanism for the impaired release of a number of functionally related cytokines and is particularly compelling given recent descriptions of the association between autophagy gene variants and CD susceptibility. Vesicle packaging and trafficking play a central role in this process. The common end result of these disparate genetic influences (impaired vesicle trafficking) is also reflected in the Chediak–Higashi and Hermansky–Pudlak syndromes. Both of these rare congenital immunodeficiencies arise from mutations in genes involved in vesicle transport and have a strong (>30%) association with CD-like intestinal inflammation [8].

If macrophages fail to secrete TNF-α in CD, why do anti-TNF agents work?

The observation that macrophages from patients with CD secrete less TNF-α in response to *E. coli* stimulation represents the second apparent paradox of the immunodeficiency hypothesis – given that anti-TNF agents are often efficacious in treating active CD.

It must first be understood that the processes being considered are temporally and biologically distinct. The acute inflammatory response orchestrated by the secretion of proinflammatory cytokines and neutrophil chemotaxis in healthy individuals is associated with bacterial clearance within a few hours. The attenuation of this process in CD leads to the failure of bacterial clearance and, as discussed, generates granulomatous chronic inflammation lasting much longer than the processes that initiated it. Furthermore, even when cytokine secretion by individual cells is sufficient, the overall effect of a massive, mixed chronic inflammatory cell infiltrate (as opposed to an early acute inflammatory response) would be sufficient to create a tissue-damaging environment.

Inflammatory responses also develop through distinct phases, with cytokines performing diverse roles at different stages. The effect of TNF-α blockade or deficiency in the dextran-sodium sulfate murine model of colitis is highly instructive. While TNF-α inhibition is effective in ameliorating established dextran-sodium sulfate-colitis in wild-type animals, TNF-α-deficient mice are actually more susceptible to the induction of colitis [41]. The dichotomous role of this pivotal cytokine supports the concept of the ‘phasic’ nature of CD pathogenesis and suggests that cytokines with a deleterious effect during chronic inflammation may confer protection during the preceding acute inflammatory response. There is some evidence to suggest that a duality of response in acutely activated, as opposed to chronically activated, macrophages may play a role in CD [38].

Finally, the major mechanism of action of anti-TNF agents is likely to be the induction of apoptosis in inflammatory cells rather than a direct effect on soluble TNF-α [42]. The anti-TNF agent etanercept does not induce apoptosis and was not effective in treating CD [43]. Of particular note in this regard is that an apparent resistance to apoptosis has been demonstrated in leukocytes in CD [44–46]. There is also some evidence to indicate that azathioprine metabolites, rather than merely suppressing adaptive inflammation, stimulate the release of proinflammatory cytokines by macrophages [38]. As a corollary, it is

interesting to note case reports where the development of CD in patients with other inflammatory disorders has been precipitated by the use of treatments that downregulate TNF [47,48].

Other factors influencing bacterial clearance *Intestinal barrier dysfunction*

The first stage of the three-stage hypothesis requires entry of luminal contents and bacteria through the mucosal layer. In healthy individuals, this is prevented by an intact intestinal mucosal barrier – achieved by the local secretion of antimicrobial peptides and maintenance of intercellular tight junctions. There is ample and elegant evidence to demonstrate that both mechanisms are defective in CD [49,50], are influenced by *NOD2* polymorphisms, and that bacterial stimuli via Toll-like receptors are required for their maintenance [51,52]. This is in keeping with early observations of increased intestinal permeability in CD [53].

Disruption of the mucus layer may also contribute to a failure of the intestinal barrier in CD, but may be more relevant to the initiation of inflammation in UC. In humans, polymorphisms in *MUC19* have been associated with an increased susceptibility to CD [54], and aberrant expression of mucin genes has been demonstrated in both inflamed and uninvolved intestinal epithelial biopsies [55], particularly at the edges of ulcers. The interruption of mucin secretion by *MUC1* deletion results in a worsening of colitis in *IL-10*-deficient mice [56], while *MUC2*-deficient mice develop spontaneous intestinal damage and more severe chemical colitis [57]. Mucin disruption may therefore have an early role to play in increasing susceptibility to mucosal damage and the impaired healing of established ulcers.

Components of the intestinal barrier are likely to play a more active role: an *in vitro* study has demonstrated the potential for cross-talk with, or recruitment of, inflammatory cells, particularly by dendritic cells [49,58]. Their role as antigen-presenting cells capable of influencing T-cell responses makes dendritic cell function in CD an intriguing avenue of study. However, present data regarding their pathogenic role are conflicting and do not support an intrinsic defect in these cells [57]. Impaired autophagy in epithelial cells may also have a role to play in the defense against invasive organisms [59]. This may only represent an extension of the ‘barrier’ function as, although ‘infected’ epithelial cells certainly have the capacity to produce proinflammatory cytokines *in vitro* [60,61], the weight of evidence from *in situ* hybridization and immunohistochemical examination of intestinal biopsies indicates that they do not appear to assume this role *in vivo* [34–36]. Furthermore, bacterial species that have been associated with CD [24] appear capable of invading even an intact epithelial barrier. The relative pathogenic contribution of these factors is unclear.

Intestinal microbes

The presence of luminal microbes is clearly important for the development of CD: active CD responds (albeit only partially) to broad-spectrum antibiotics and distal recurrence is prevented by diversion of the fecal stream.

A recent metagenomic study of bacterial diversity in the gut compared the profiles in a Spanish cohort (four patients with CD compared with 21 with UC and 14 healthy controls)

[62]. A highly significant difference was observed between patients with CD and healthy and disease controls. Although exact species were not identified, a previous study highlighted *Bacteroidetes* and *Clostridium* species as under-represented in patients with CD [63]. Using an alternative method in different populations, similar profiles were observed between CD and UC [64]. Although it is impossible to establish causality with this approach, focused metagenomic studies represent a powerful tool for further study of this aspect of the intestinal environment in health and disease and should be applied across populations. *Bifidobacterium* species and *Faecalibacterium prausnitzii* appear to have a clinically relevant association with CD, but it remains to be determined whether 'restoration' or alteration of the balance of microbial species in the bowel is of therapeutic benefit [65,66] (although again, this would not necessarily demonstrate causality).

A few CD-associated microbial species have been described (notably AIEC and *Mycobacterium* species) that appear able to either bypass an intact intestinal barrier or evade or suppress macrophage function [24,67–69]. These species might therefore be able to initiate a chronic inflammatory response even in 'innate immunocompetent' hosts. AIEC are of particular interest given the 'threshold effect' in the defective clearance of *E. coli* seen in patients with CD. As discussed, the invasive nature of these organisms would predispose to a rapid overwhelming of a defective innate immune response. Impaired host defense (defective autophagy) may also offer a selective advantage to AIEC [59], and provides evidence that interplay between host susceptibility and particular bacterial strains may be of primary pathogenic importance.

With other microbial species (i.e., *Yersinia*), CD-like intestinal inflammation tends to resolve without recurrence once the infectious organism is removed [70]. By contrast, CD recurs after cessation of antibiotics [71] and this approach is unsuitable and ineffective as a mainstay of therapy. Antimycobacterial therapy has failed to show any benefit in active disease [72]. The former does not necessarily preclude a nonspecific infectious etiology, but this cannot be applied to the latter. The requirement for antigenic material in the development of CD is not in question, but it remains unlikely that there is a single infectious etiology for this disease.

Lifestyle factors may influence intestinal barrier & innate immune dysfunction

Smokers have up to a fivefold increased risk of developing CD [73,74] and are more likely to have an aggressive clinical course [75] with fistulizing disease and more frequent surgical intervention. Cigarette smoking, and more specifically nicotine, is known to have detrimental effects on the generation of proinflammatory cytokines in patients with CD [76,77], in keeping with the predominantly immunosuppressive effects of smoking on the immune response [78]. Recently, differential gene expression profiles have also been demonstrated in the colonic mucosa of smokers compared with nonsmokers with CD [79], but the significance of these findings is unclear. With respect to macrophage function, a specific, suppressive effect of smoking may be achieved through the α7-nicotinic acetylcholine receptor. This receptor is expressed on macrophages (as well as other immune cells), where its predominant effect is anti-inflammatory [80]. The effect of nicotine acting

through this receptor to suppress lipopolysaccharide-induced cytokine secretion in particular [81] indicates a mechanism by which smoking may exacerbate macrophage hyporesponsiveness [12] and contribute further to bacterial persistence in CD.

There is some evidence to suggest that smoking is associated with disruption of the mucus layer [82]. Although this was initially studied (and is likely to be of more relevance) in patients with UC, a similar mechanism may be involved in CD. Increased local blood flow, which has been demonstrated to be related to the influx of inflammatory cells into sites of acute inflammation [83,84], is reduced by cigarette smoking in the intestinal mucosa, both acutely and in long-term smokers with or without inflammatory bowel disease [85,86]. This may exacerbate the diminished blood-flow responses that we have demonstrated in patients with CD in response to *E. coli* challenge [12,13] and contribute further to pathogenesis.

The increasing incidence of CD in Japan has been attributed to the adoption of a Western diet [87]. Dietary fat, in particular, has been shown to negatively influence elemental diet therapy [88,89]. Effects on arachidonic acid metabolism and prostaglandin synthesis have been postulated as mechanisms by which dietary fat may influence inflammation [89,90], but changes in the composition of bile may also be important. Although bile salts have important physiological properties, they may play a role in disease. This is particularly true for the more toxic secondary bile salts produced by the action of intestinal bacteria (notably those associated with CD [91,92]).

The composition of gallbladder bile in patients with CD favors toxicity [93,94], but it is difficult to determine whether these changes are merely due to disturbed enterohepatic recirculation. While gallbladder bile may not accurately reflect the composition of bile reaching the terminal ileum, its composition is similarly altered in patients with purely colonic CD [95], suggesting that alterations in bile composition may not be secondary to altered reabsorption in the terminal ileum. There is evidence from both animal and *in vitro* studies using human ileal fluid to suggest that bile salts increase intestinal permeability and membrane fragility [96,97], either by a direct toxic effect [98] or by modulating tight junction structure and function [99,100]. It is also conceivable that bile salts have detergent-like effects on cell membranes, resulting in cell lysis [101]. In addition, hydrophobic bile salts have been shown to induce apoptosis [102], which could result in barrier disruption and altered permeability in intestinal epithelial cells [103].

In a recent study, cyclo-oxygenase-2-knockout mice were fed with an atherogenic diet containing cholate (a primary bile acid) [104]. After 4 weeks, a high proportion of mice developed ileocaecal inflammation. This suggests that, in a model of disease where impaired acute inflammation exists, bile acids alone may be sufficient to result in intestinal mucosal damage that triggers inflammatory bowel disease. In another model, supplementation with the secondary bile acid deoxycholate alone was sufficient to induce colitis, even in wild-type mice [105].

The hygiene hypothesis suggests that early exposure to a wide variety of intestinal pathogens is protective against the development of CD in later life, while increasingly sanitized environments increase the risk of CD development [106]. These observations may

also explain (together with genetic variations and dietary differences) the relatively low incidence of CD in non-developed countries. For example, the carriage rates of helminths such as *Trichurus suis* are greater in areas with a low incidence of CD [107] and these worms have also been used successfully to treat active CD [108]. However, the mechanisms for this effect remain unclear and the hygiene hypothesis, while attractive, is unconfirmed. It may be that exposure to a wide range of microbes (including helminths) from an early age results in a continuous low-level stimulation [109] which may prime the immune response and make it more vigorous when infection is encountered.

Stress may also have a role to play in modulating barrier function and aspects of the innate immune system in the intestine [110]. Catecholamines attenuate lipopolysaccharide- and other ligand-mediated responses in monocyte/macrophage populations *in vitro* [111,112] by inhibiting NF-κB-mediated transcription [113]. and serotonin appears to inversely influence the development of macrophage-mediated adaptive responses [114].

Animal models & genome-wide association studies: evidence for innate immune dysfunction

In general, the polymorphisms identified by genome-wide association studies (GWAS) to date indicate a deficiency of innate immune function in CD, in keeping with the functional observations of impaired barrier function and defective bacterial clearance in patients with CD. Interestingly, using GWAS data, comparison of genetic variation profiles for classification has highlighted that CD does not cluster with the other autoimmune diseases studied [115].

Genome-wide association studies have revolutionized our understanding of the potential pathogenic mechanisms underlying CD as well as our general knowledge of innate immunity in the gut. However, the contribution of the susceptibility loci identified to date accounts for a relatively small proportion of the genetic risk for CD, perhaps up to 20% at most, leading to the concept of ‘missing heritability’ [54,116].

The contribution of each susceptibility locus to disease development, as opposed to the risk of disease, should also be considered. For example, homozygous polymorphisms in *NOD2* are associated with a 20- to 40-fold increase in the relative risk for CD [117,118]. The relative frequencies of these polymorphisms in the general population, however, mean that for every CD patient homozygous or compound heterozygous for a *NOD2* polymorphism, there would be approximately 20 individuals with the same genotype who do not develop disease. Despite conferring an odds ratio of 1.32 on disease risk, the influence of *ATG16L1* disease-associated polymorphisms would be weaker still, particularly considering the similar frequencies in CD and control populations [119,120].

There has been a wealth of research to elucidate, in particular, the contributions of *NOD2* and *ATG16L1* to innate immune function [121], and recent research has elegantly demonstrated the potential for their interdependence [122,123]. However, mice deficient in either gene do not develop spontaneous enterocolitis [124–127]. This is disappointing as *NOD2* polymorphisms have been shown to be mechanistically important in determining the

responsiveness of macrophages to muramyl dipeptide [12] and have been linked to intestinal barrier function as discussed. In addition, defects in the capture or killing of bacteria have been demonstrated in epithelial cell lines [128] and macrophages [129] from CD patients expressing the *ATG16L1* polymorphism. However these responses were studied with microbial species (such as *Salmonella typhimurium* or *E. coli*) that have been described to have an intrinsic capacity to evade killing [130–132]. It has been suggested, therefore, that the CD-associated *ATG16L1* polymorphism is more relevant to bacterial processing and antigen presentation than to primary antibacterial activity [133]. These observations do not rule out a primary pathogenic contribution of these loci to human disease, but the high frequency of these polymorphisms in healthy individuals must be kept in mind. They may therefore represent disease 'modifiers' that become relevant in the presence of causative 'triggers'. It may be that only a combination of such factors in an individual leads to disease, but the presence of these trigger components is likely to be of primary importance.

STAT3 polymorphism has also been linked to the development of CD in humans [54]. In mice, *STAT3* deletion has been targeted to affect myeloid cells exclusively (as completely *STAT3*-deficient mice were non-viable). Impaired neutrophil influx to bacterial stimuli, defective bacterial clearance and spontaneous 'Th1-type' granulomatous chronic intestinal inflammation mediated by Toll-like receptor- and IL-12-dependent responses have all been described in these animals [134–137]. This is in keeping with the hypothesis that innate immunodeficiency can generate chronic inflammation in the absence of primary T-cell defects.

Furthermore, neutrophil depletion or blockade of their adhesion by monoclonal antibodies has been examined in experimental colitis [138]. Chemically-induced and transfer colitis models were exacerbated by both of these interventions, again suggesting that neutrophils are required to prevent the transition to a chronic phase of inflammation.

Taking into account the negative animal studies, it must be concluded that the strongly-associated polymorphisms identified by GWAS are neither necessary nor sufficient for the development of CD. An alternative hypothesis suggests that they may represent compensatory mechanisms for a primary innate immunodeficiency, the causes of which have yet to be fully elucidated.

Expert commentary & five-year view

The last few years have seen an explosion of interest in the genetics and immunopathology of CD. Prevailing theories advocating a central role for T-cell dysfunction in the pathogenesis of CD have been challenged, with renewed focus on a primary defect of innate immune function. Novel studies have reorientated our understanding of the pathogenesis of CD, adding considerable credence to the concept of primary macrophage-mediated innate immune dysfunction. The next 5 years will hopefully extend this work by addressing missing heritability and elucidating the molecular mechanisms underlying innate immunodeficiency. It may be that improved or augmented genetic screening tools will be able to detect risk alleles of lower frequency than those currently described [116], and further study of epistasis (gene–gene interactions [139,140]) may be key to understanding

Europe PMC Funders Author Manuscripts

the pathogenesis of complex phenotypes. There is potentially a wealth of knowledge contained within the known IBD loci [121], and fine mapping of the genes within these regions will identify novel genetic influences. Pharmacogenomics may play an increasing role, and has been introduced by advances in the understanding of thiopurine metabolism [141]. Awareness of genetic influences such as *MDR1* polymorphisms [142] may help tailor therapy to individuals.

It is difficult to ascertain, however, whether the wealth of current genetic knowledge generated by GWAS will lead to the development of novel therapies [143], given the apparent disparate nature of the risk alleles identified and their relatively small contribution in real terms to pathogenesis in individuals. Functional phenotypic, rather than genetic, information may become more important in this regard. Our own studies have demonstrated a common phenotype of deficient cytokine secretion by macrophages in CD that can be linked mechanistically to pathogenesis and is not apparent in disease controls, particularly patients with UC [13]. This phenotype appears to be determined by genetic influences that are likely to fall well below the threshold of standard population genetic screens, but are clearly demonstrated by analysis of gene expression in specific cell types. In this circumstance, identification of individuals with a cellular phenotype may be more useful in determining what therapy they ultimately receive, as well as potentially highlighting missing heritability genes. Familial linkage studies may also achieve this goal.

Since the macrophage defects we described are not apparent in UC, and the underlying gene expression profiles appear distinct, this approach could also add to the armamentarium of diagnostic tools used to differentiate the two inflammatory bowel diseases. This type of gene-chip approach has met with some success [144,145], but the identification of novel genetic markers in specific cell types would strengthen the power of these diagnostic tools.

The recognition of immunodeficiency in CD has important implications for its clinical management. Suppression of chronic inflammation at the expense of innate immune function might predispose patients to relapse (even if therapy is continued) and potentially convert a sporadic disorder into a chronic relapsing–remitting condition. Providing a low level of immunostimulation in patients with quiescent disease may help to maintain remission and prevent intercurrent flares. The randomized trial of granulocyte macrophage colony-stimulating factor in active disease was affected by a high placebo response rate, but the treatment did appear efficacious [146]. If used in remission or in the post-operative setting to prevent lesions developing, these therapies may be more effective [147]. The identification of novel therapeutic targets may also improve results.

Concepts regarding current therapy, particularly for maintenance of remission in CD, may require re-evaluation. Defining the precise underlying molecular defects remains a significant challenge given patient heterogeneity, but should offer novel diagnostic tools and therapeutic targets in the future.

Acknowledgments

The authors wish to acknowledge funding from the Wellcome Trust, CGD Trust, UCL Charities and the Broad Medical Research Program.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Rogler G, Andus T. Cytokines in inflammatory bowel disease. World J. Surg. 1998; 22(4):382–389. [PubMed: 9523521]
2. Abraham C, Cho JH. Inflammatory bowel disease. N. Engl. J. Med. 2009; 361(21):2066–2078. [PubMed: 19923578]
3. Hanauer SB. Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. Inflamm. Bowel. Dis. 2006; 12(Suppl. 1):S3–S9. [PubMed: 16378007]
4. Brand S. Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. Gut. 2009; 58(8): 1152–1167. [PubMed: 19592695]
5. Neurath MF. IL-23: a master regulator in Crohn disease. Nat. Med. 2007; 13(1):26–28. [PubMed: 17206128]
6. Boden EK, Snapper SB. Regulatory T cells in inflammatory bowel disease. Curr. Opin. Gastroenterol. 2008:24733–24741.
7. Peyrin-Biroulet L, Deltenre P, Ardizzone S, et al. Azathioprine and 6-mercaptopurine for the prevention of postoperative recurrence in Crohn's disease: a meta-analysis. Am. J. Gastroenterol. 2009; 104(8):2089–2096. [PubMed: 19568226]
8. Rahman FZ, Marks DJ, Hayee BH, et al. Phagocyte dysfunction and inflammatory bowel disease. Inflamm. Bowel. Dis. 2008; 14(10):1443–1452. [PubMed: 18421761] • Review of the inherited disorders of neutrophil function and their relationship to the development of Crohn's disease (CD).
9. Marks DJ, Miyagi K, Rahman FZ, et al. Inflammatory bowel disease in CGD reproduces the clinicopathological features of Crohn's disease. Am. J. Gastroenterol. 2009; 104(1):117–124. [PubMed: 19098859]
10. Segal AW. How neutrophils kill microbes. Annu. Rev. Immunol. 2005:23197–23223.
11. Segal AW, Loewi G. Neutrophil dysfunction in Crohn's disease. Lancet. 1976; 2(7979):219–221. [PubMed: 59239] • First report of a failure of neutrophil recruitment in CD patients.
12. Marks DJ, Harbord MW, MacAllister R, et al. Defective acute inflammation in Crohn's disease: a clinical investigation. Lancet. 2006; 367(9511):668–678. [PubMed: 16503465]
13. Smith AM, Rahman FZ, Hayee B, et al. Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. J. Exp. Med. 2009; 206(9):1883–1897. [PubMed: 19652016] •• Presents data supporting the concept that a failure of macrophage-mediated acute inflammation is key to the pathogenesis of CD.
14. Li Y, Karlin A, Loike JD, et al. A critical concentration of neutrophils is required for effective bacterial killing in suspension. Proc. Natl Acad. Sci. USA. 2002; 99(12):8289–8294. [PubMed: 12060772]
15. Li Y, Karlin A, Loike JD, et al. Determination of the critical concentration of neutrophils required to block bacterial growth in tissues. J. Exp. Med. 2004; 200(5):613–622. [PubMed: 15353554]
16. Sewell GW, Marks DJ, Segal AW. The immunopathogenesis of Crohn's disease: a three-stage model. Curr. Opin. Immunol. 2009; 21(5):506–513. [PubMed: 19665880]
17. Adams DO. The granulomatous inflammatory response. A review. Am. J. Pathol. 1976; 84(1):164–192. [PubMed: 937513]
18. Kyle J. Urinary complications of Crohn's disease. World J. Surg. 1980; 4(2):153–160. [PubMed: 7405253]
19. Porter CK, Tribble DR, Aliaga PA, et al. Infectious gastroenteritis and risk of developing inflammatory bowel disease. Gastroenterology. 2008; 135(3):781–786. [PubMed: 18640117]

Europe PMC Funders Author Manuscripts

20. Myrelid P, Olaison G, Sjodahl R, et al. Thiopurine therapy is associated with postoperative intra-abdominal septic complications in abdominal surgery for Crohn's disease. Dis. Colon Rectum. 2009; 52(8):1387–1394. [PubMed: 19617749]
21. Hildebrand H, Malmborg P, Askling J, et al. Early-life exposures associated with antibiotic use and risk of subsequent Crohn's disease. Scand. J. Gastroenterol. 2008; 43(8):961–966. [PubMed: 19086166] • Discusses increased pre-morbid infections in children who go on to develop CD.
22. Farthing MJ. Bugs and the gut: an unstable marriage. Best. Pract. Res. Clin. Gastroenterol. 2004; 18(2):233–239. [PubMed: 15123066]
23. Diaz PI, Chalmers NI, Rickard AH, et al. Molecular characterization of subject-specific oral microflora during initial colonization of enamel. Appl. Environ. Microbiol. 2006; 72(4):2837–2848. [PubMed: 16597990]
24. Darfeuille-Michaud A, Boudeau J, Bulois P, et al. High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. Gastroenterology. 2004; 127(2): 412–421. [PubMed: 15300573]
25. Colombel JF, Loftus EV Jr, Tremaine WJ, et al. The safety profile of infliximab in patients with Crohn's disease: the Mayo clinic experience in 500 patients. Gastroenterology. 2004; 126(1):19–31. [PubMed: 14699483]
26. Lichtenstein GR, Feagan BG, Cohen RD, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. Clin. Gastroenterol. Hepatol. 2006; 4(5):621–630. [PubMed: 16678077]
27. Colombel JF, Loftus EV Jr, Tremaine WJ, et al. Early postoperative complications are not increased in patients with Crohn's disease treated perioperatively with infliximab or immunosuppressive therapy. Am. J. Gastroenterol. 2004; 99(5):878–883. [PubMed: 15128354]
28. Hofmann A, Beaulieu Y, Bernard F, et al. Fulminant legionellosis in two patients treated with infliximab for Crohn's disease: case series and literature review. Can. J. Gastroenterol. 2009; 23(12):829–833. [PubMed: 20011736]
29. Warner EA, Moldawer LL. Using innate immunity to characterize the host response to microbial invasion in severe sepsis. Future Microbiol. 2008:3177–3189.
30. Hayee BH, Sewell G, Rahman FZ, Bloom SL, Smith AM, Segal AW. Differential bacterial clearance and cytokine secretion by macrophages explains localisation of Crohn's disease to the gut. Gut. 2010; 59(4 Suppl. 1):A61.
31. Morain CO, Segal AA, Walker D, et al. Abnormalities of neutrophil function do not cause the migration defect in Crohn's disease. Gut. 1981; 22(10):817–822. [PubMed: 7028577]
32. Zarbock A, Ley K. Neutrophil adhesion and activation under flow. Microcirculation. 2009; 16(1): 31–42. [PubMed: 19037827]
33. Zarbock A, Ley K. Mechanisms and consequences of neutrophil interaction with the endothelium. Am. J. Pathol. 2008; 172(1):1–7. [PubMed: 18079440]
34. Arai F, Takahashi T, Furukawa K, et al. Mucosal expression of interleukin-6 and interleukin-8 messenger RNA in ulcerative colitis and in Crohn's disease. Dig. Dis. Sci. 1998; 43(9):2071–2079. [PubMed: 9753275]
35. Murch SH, Braegger CP, Walker-Smith JA, et al. Location of tumour necrosis factor α by immunohistochemistry in chronic inflammatory bowel disease. Gut. 1993; 34(12):1705–1709. [PubMed: 8031350]
36. Mazzucchelli L, Hauser C, Zgraggen K, et al. Expression of interleukin-8 gene in inflammatory bowel disease is related to the histological grade of active inflammation. Am. J. Pathol. 1994; 144(5):997–1007. [PubMed: 8178948]
37. Smythies LE, Maheshwari A, Clements R, et al. Mucosal IL-8 and TGF-β recruit blood monocytes: evidence for cross-talk between the lamina propria stroma and myeloid cells. J. Leukoc. Biol. 2006; 80(3):492–499. [PubMed: 16793909]
38. Zhou L, Braat H, Faber KN, et al. Monocytes and their pathophysiological role in Crohn's disease. Cell Mol. Life Sci. 2009; 66(2):192–202. [PubMed: 18791847]
39. Casanova JL, Abel L. Revisiting Crohn's disease as a primary immunodeficiency of macrophages. J. Exp. Med. 2009; 206(9):1839–1843. [PubMed: 19687225] • Good commentary for [12].

40. Adams DO, Hamilton TA. The activated macrophage and granulomatous inflammation. Curr. Top. Pathol. 1989; 79:151–167. [PubMed: 2644083]
41. Naito Y, Takagi T, Handa O, et al. Enhanced intestinal inflammation induced by dextran sulfate sodium in tumor necrosis factor-α deficient mice. J. Gastroenterol. Hepatol. 2003; 18(5):560–569. [PubMed: 12702049]
42. Wong M, Ziring D, Korin Y, et al. TNFα blockade in human diseases: mechanisms and future directions. Clin. Immunol. 2008; 126(2):121–136. [PubMed: 17916444]
43. Sandborn WJ, Hanauer SB, Katz S, et al. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. Gastroenterology. 2001; 121(5):1088–1094. [PubMed: 11677200]
44. Palmer CD, Rahman FZ, Sewell GW, et al. Diminished macrophage apoptosis and reactive oxygen species generation after phorbol ester stimulation in Crohn's disease. PLoS. One. 2009; 4(11):e7787. [PubMed: 19907654]
45. Brannigan AE, O'Connell PR, Hurley H, et al. Neutrophil apoptosis is delayed in patients with inflammatory bowel disease. Shock. 2000; 13(5):361–366. [PubMed: 10807010]
46. Boirivant M, Marini M, Di FG, et al. Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. Gastroenterology. 1999; 116(3):557–565. [PubMed: 10029614]
47. Yazisiz V, Avci AB, Erbasan F, et al. Development of Crohn's disease following anti-tumour necrosis factor therapy (etanercept). Colorectal Dis. 2008; 10(9):953–954. [PubMed: 18294265]
48. Charach G, Grosskopf I, Weintraub M. Development of Crohn's disease in a patient with multiple sclerosis treated with copaxone. Digestion. 2008; 77(3–4):198–200. [PubMed: 18607107]
49. Wehkamp J, Schmid M, Fellermann K, et al. Defensin deficiency, intestinal microbes, and the clinical phenotypes of Crohn's disease. J. Leukoc. Biol. 2005; 77(4):460–465. [PubMed: 15618294]
50. Zeissig S, Burgel N, Gunzel D, et al. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. Gut. 2007; 56(1):61–72. [PubMed: 16822808]
51. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function. Gastroenterology. 2007; 132(4):1359–1374. [PubMed: 17408640]
52. Froy O. Regulation of mammalian defensin expression by Toll-like receptor-dependent and independent signalling pathways. Cell Microbiol. 2005; 7(10):1387–1397. [PubMed: 16153239]
53. Hollander D, Vadheim CM, Brettholz E, et al. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. Ann. Intern. Med. 1986; 105(6): 883–885. [PubMed: 3777713]
54. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat. Genet. 2008; 40(8):955–962. [PubMed: 18587394]
55. Buisine MP, Desreumaux P, Leteurtre E, et al. Mucin gene expression in intestinal epithelial cells in Crohn's disease. Gut. 2001; 49(4):544–551. [PubMed: 11559653]
56. Beatty PL, Plevy SE, Sepulveda AR, et al. Cutting edge: transgenic expression of human MUC1 in IL-10$^{-/-}$ mice accelerates inflammatory bowel disease and progression to colon cancer. J. Immunol. 2007; 179(2):735–739. [PubMed: 17617560]
57. van der Sluis M, De Koning BA, De Bruijn AC, et al. *Muc2*-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology. 2006; 131(1): 117–129. [PubMed: 16831596]
58. Silva MA. Intestinal dendritic cells and epithelial barrier dysfunction in Crohn's disease. Inflamm. Bowel. Dis. 2009; 15(3):436–453. [PubMed: 18821596]
59. Lapaquette P, Glasser AL, Huett A, et al. Crohn's disease-associated adherent-invasive *E. coli* are selectively favoured by impaired autophagy to replicate intracellularly. Cell Microbiol. 2010; 12(1):99–113. [PubMed: 19747213]
60. Glasser AL, Boudeau J, Barnich N, et al. Adherent invasive *Escherichia coli* strains from patients with Crohn's disease survive and replicate within macrophages without inducing host cell death. Infect. Immun. 2001; 69(9):5529–5537. [PubMed: 11500426]

61. Stadnyk AW. Cytokine production by epithelial cells. FASEB J. 1994; 8(13):1041–1047. [PubMed: 7926369]
62. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010; 464(4):59–68. [PubMed: 20203603]
63. Manichanh C, Rigottier-Gois L, Bonnaud E, et al. Reduced diveristy of fecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut. 2006:55205–55211.
64. Ott SJ, Musfeldt M, Wenderoth DF, et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut. 2004; 53(5):685–693. [PubMed: 15082587]
65. Lindsay JO, Whelan K, Stagg AJ, et al. Clinical, microbiological, and immunological effects of fructooligosaccharide in patients with Crohn's disease. Gut. 2006; 55(3):348–355. [PubMed: 16162680]
66. Sokol H, Pigneur B, Watterlot L, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc. Natl Acad. Sci. USA. 2008; 105(43):16731–16736. [PubMed: 18936492]
67. Martin HM, Campbell BJ, Hart CA, et al. Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. Gastroenterology. 2004; 127(1):80–93. [PubMed: 15236175]
68. Mpofu CM, Campbell BJ, Subramanian S, et al. Microbial mannan inhibits bacterial killing by macrophages: a possible pathogenic mechanism for Crohn's disease. Gastroenterology. 2007; 133(5):1487–1498. [PubMed: 17919633]
69. Subramanian S, Roberts CL, Hart CA, et al. Replication of colonic Crohn's disease mucosal *Escherichia coli* isolates within macrophages and their susceptibility to antibiotics. Antimicrob. Agents Chemother. 2008; 52(2):427–434. [PubMed: 18070962]
70. Vantrappen G, Ponette E, Geboes K, et al. *Yersinia enteritis* and enterocolitis: gastroenterological aspects. Gastroenterology. 1977; 72(2):220–227. [PubMed: 830572]
71. Isaacs KL, Sartor RB. Treatment of inflammatory bowel disease with antibiotics. Gastroenterol. Clin. North Am. 2004; 33(2):335–345. [PubMed: 15177542]
72. Swift GL, Srivastava ED, Stone R, et al. Controlled trial of anti-tuberculous chemotherapy for two years in Crohn's disease. Gut. 1994; 35(3):363–368. [PubMed: 8150348]
73. Bridger S, Lee JC, Bjarnason I, et al. In siblings with similar genetic susceptibility for inflammatory bowel disease, smokers tend to develop Crohn's disease and non-smokers develop ulcerative colitis. Gut. 2002; 51(1):21–25. [PubMed: 12077086]
74. Lindberg E, Tysk C, Andersson K, et al. Smoking and inflammatory bowel disease. A case control study. Gut. 1988; 29(3):352–357. [PubMed: 3356367]
75. Lindberg E, Jarnerot G, Huitfeldt B. Smoking in Crohn's disease: effect on localisation and clinical course. Gut. 1992; 33(6):779–782. [PubMed: 1624159]
76. Sher ME, Bank S, Greenberg R, et al. The influence of cigarette smoking on cytokine levels in patients with inflammatory bowel disease. Inflamm. Bowel. Dis. 1999; 5(2):73–78. [PubMed: 10338374]
77. Aldhous MC, Prescott RJ, Roberts S, et al. Does nicotine influence cytokine profile and subsequent cell cycling/apoptotic responses in inflammatory bowel disease? Inflamm. Bowel. Dis. 2008; 14(11):1469–1482. [PubMed: 18618634]
78. Sopori M. Effects of cigarette smoke on the immune system. Nat. Rev. Immunol. 2002; 2(5):372–377. [PubMed: 12033743]
79. Nielsen OH, Bjerrum JT, Csillag C, et al. Influence of smoking on colonic gene expression profile in Crohn's disease. PLoS One. 2009; 4(7):e6210. [PubMed: 19603079]
80. de Jonge WJ, Ulloa L. The α7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. Br. J. Pharmacol. 2007; 151:915–929. [PubMed: 17502850]
81. Hamano R, Takahashi HK, Iwagaki H, et al. Stimulation of α7 nicotinic acetylcholine receptor inhibits CD14 and the Toll-like receptor 4 expression in human monocytes. Shock. 2006; 26:358–364. [PubMed: 16980882]
82. Cope GF, Heatley RV, Kelleher JK. Smoking and colonic mucus in ulcerative colitis. Br. Med. J. (Clin. Res. Ed). 1986; 293(6545):481. [PubMed: 3091168]

83. Issekutz AC. Vascular responses during acute neutrophilic inflammation. Their relationship to *in vivo* neutrophil emigration. Lab. Invest. 1981; 45(5):435–441. [PubMed: 7029133]
84. Issekutz AC. Effect of vasoactive agents on polymorphonuclear leukocyte emigration *in vivo*. Lab. Invest. 1981; 45(3):234–240. [PubMed: 6944561]
85. De Bruin AF, Schouten SB, de Kort PP, et al. The impact of chronic smoking on rectal mucosal blood flow. Tech. Coloproctol. 2009; 13(4):269–272. [PubMed: 19730785]
86. Srivastava ED, Russell MA, Feyerabend C, et al. Effect of ulcerative colitis and smoking on rectal blood flow. Gut. 1990; 31(9):1021–1024. [PubMed: 2210447]
87. Shoda R, Matsueda K, Yamato S, et al. Epidemiologic analysis of Crohn disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn disease in Japan. Am. J. Clin. Nutr. 1996; 63(5):741–745. [PubMed: 8615358]
88. Bamba T, Shimoyama T, Sasaki M, et al. Dietary fat attenuates the benefits of an elemental diet in active Crohn's disease: a randomized, controlled trial. Eur. J. Gastroenterol. Hepatol. 2003; 15(2): 151–157. [PubMed: 12560759]
89. Gassull MA, Fernandez-Banares F, Cabre E, et al. Fat composition may be a clue to explain the primary therapeutic effect of enteral nutrition in Crohn's disease: results of a double blind randomised multicentre European trial. Gut. 2002; 51(2):164–168. [PubMed: 12117873]
90. Shoda R, Matsueda K, Yamato S, et al. Therapeutic efficacy of N-3 polyunsaturated fatty acid in experimental Crohn's disease. J. Gastroenterol. 1995; 30(Suppl. 8):98–101. [PubMed: 8563904]
91. Lewis R, Gorbach S. Modification of bile acids by intestinal bacteria. Arch. Intern. Med. 1972; 130(4):545–549. [PubMed: 4562151]
92. Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J. Lipid Res. 2006; 47(2):241–259. [PubMed: 16299351]
93. Vantrappen G, Ghoos Y, Rutgeerts P, et al. Bile acid studies in uncomplicated Crohn's disease. Gut. 1977; 18(9):730–735. [PubMed: 604194]
94. Pereira SP, Bain IM, Kumar D, et al. Bilecomposition in inflammatory bowel disease: ileal disease and colectomy, but not colitis, induce lithogenic bile. Aliment. Pharmacol. Ther. 2003; 17(7):923–933. [PubMed: 12656695]
95. Lapidus A, Akerlund JE, Einarsson C. Gallbladder bile composition in patients with Crohn 's disease. World J. Gastroenterol. 2006; 12(1):70–74. [PubMed: 16440420]
96. Zhao DL, Hirst BH. Bile salt-induced increases in duodenal brush-border membrane proton permeability, fluidity, and fragility. Dig. Dis. Sci. 1990; 35(5):589–595. [PubMed: 2158881]
97. Zhao DL, Hirst BH. Comparison of bile salt perturbation of duodenal and jejunal isolated brush-border membranes. Digestion. 1990; 47(4):200–207. [PubMed: 2100270]
98. Merrett MN, Owen RW, Jewell DP. Ileal pouch dialysate is cytotoxic to epithelial cell lines, but not to CaCo-2 monolayers. Eur. J. Gastroenterol. Hepatol. 1997; 9(12):1219–1226. [PubMed: 9471029]
99. Fasano A, Verga MC, Raimondi F, et al. Effects of deconjugated bile acids on electrolyte and nutrient transport in the rabbit small intestine *in vitro*. J. Pediatr. Gastroenterol. Nutr. 1994; 18(3): 327–333. [PubMed: 8057216]
100. Raimondi F, Santoro P, Barone MV, et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. Am. J. Physiol. Gastrointest. Liver Physiol. 2008; 294(4):G906–G913. [PubMed: 18239063]
101. O'Connor CJ, Wallace RG, Iwamoto K, et al. Bile salt damage of egg phosphatidylcholine liposomes. Biochim. Biophys. Acta. 1985; 817(1):95–102. [PubMed: 4039949]
102. Powell AA, LaRue JM, Batta AK, et al. Bile acid hydrophobicity is correlated with induction of apoptosis and/or growth arrest in HCT116 cells. Biochem. J. 2001; 356(Pt 2):481–486. [PubMed: 11368775]
103. Gitter AH, Bendfeldt K, Schulzke JD, et al. Leaks in the epithelial barrier caused by spontaneous and TNF-α-induced single-cell apoptosis. FASEB J. 2000; 14(12):1749–1753. [PubMed: 10973924]

104. Lin JA, Watanabe J, Rozengurt N, et al. Atherogenic diet causes lethal ileo-cecocolitis in cyclooxygenase-2 deficient mice. Prostaglandins Other Lipid Mediat. 2007; 84(3–4):98–107. [PubMed: 17991612]
105. Bernstein H, Holubec H, Bernstein C, et al. Unique dietary-related mouse model of colitis. Inflamm. Bowel. Dis. 2006; 12(4):278–293. [PubMed: 16633050]
106. Lashner BA, Loftus EV Jr. True or false? The hygiene hypothesis for Crohn's disease. Am. J. Gastroenterol. 2006; 101(5):1003–1004. [PubMed: 16696784]
107. Elliott DE, Urban JF Jr, Argo CK, et al. Does the failure to acquire helminthic parasites predispose to Crohn's disease? FASEB J. 2000; 14(12):1848–1855. [PubMed: 10973934]
108. Summers RW, Elliott DE, Urban JF Jr, et al. *Trichuris suis* therapy in Crohn's disease. Gut. 2005; 54(1):87–90. [PubMed: 15591509]
109. Elliott DE, Urban JF Jr, Argo CK, et al. Does the failure to acquire helminthic parasites predispose to Crohn's disease? FASEB J. 2000; 14(12):1848–1855. [PubMed: 10973934]
110. Stasi C, Orlandelli E. Role of the brain–gut axis in the pathophysiology of Crohn's disease. Dig. Dis. 2008; 26(2):156–166. [PubMed: 18431066]
111. Zinyama RB, Bancroft GJ, Sigola LB. Adrenaline suppression of the macrophage nitric oxide response to lipopolysaccharide is associated with differential regulation of tumour necrosis factor-α and interleukin-10. Immunology. 2001; 104(4):439–446. [PubMed: 11899430]
112. Li CY, Chou TC, Lee CH, et al. Adrenaline inhibits lipopolysaccharide-induced macrophage inflammatory protein-1 α in human monocytes: the role of β-adrenergic receptors. Anesth. Analg. 2003; 96(2):518–523. [PubMed: 12538206]
113. Tracey KJ. Reflex control of immunity. Nat. Rev. Immunol. 2009; 9(6):418–428. [PubMed: 19461672]
114. Young MR, Matthews JP. Serotonin regulation of T-cell subpopulations and of macrophage accessory function. Immunology. 1995; 84(1):148–152. [PubMed: 7890297]
115. Sirota M, Schaub MA, Batzoglou S, et al. Autoimmune disease classification by inverse association with SNP alleles. PLoS Genet. 2009; 5(12):e1000792. [PubMed: 20041220]
116. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. Nature. 2009; 461(7265):747–753. [PubMed: 19812666]
117. Cuthbert AP, Fisher SA, Mirza MM, et al. The contribution of *NOD2* gene mutations to the risk and site of disease in inflammatory bowel disease. Gastroenterology. 2002; 122(4):867–874. [PubMed: 11910337]
118. Hampe J, Cuthbert A, Croucher PJ, et al. Association between insertion mutation in *NOD2* gene and Crohn's disease in German and British populations. Lancet. 2001; 357(9272):1925–1928. [PubMed: 11425413]
119. Hampe J, 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(2): 207–211. [PubMed: 17200669]
120. Van LJ, Russell RK, Nimmo ER, et al. Autophagy gene ATG16L1 influences susceptibility and disease location but not childhood-onset in Crohn's disease in Northern Europe. Inflamm. Bowel Dis. 2008; 14(3):338–346. [PubMed: 18088053]
121. Cooney R, Jewell D. The genetic basis of inflammatory bowel disease. Dig. Dis. 2009; 27(4): 428–442. [PubMed: 19897957]
122. Cooney R, Baker J, Brain O, et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. Nat. Med. 2010; 16(1):90–97. [PubMed: 19966812]
123. Travassos LH, Carneiro LA, Ramjeet M, et al. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat. Immunol. 2010; 11(1):55–62. [PubMed: 19898471]
124. Fujita N, Saitoh T, Kageyama S, et al. Differential involvement of Atg16L1 in Crohn disease and canonical autophagy: analysis of the organization of the Atg16L1 complex in fibroblasts. J. Biol. Chem. 2009; 284(47):32602–32609. [PubMed: 19783656]
125. Saitoh T, Fujita N, Jang MH, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production. Nature. 2008; 456(7219):264–268. [PubMed: 18849965]

126. Pauleau AL, Murray PJ. Role of Nod2 in the response of macrophages to Toll-like receptor agonists. Mol. Cell. Biol. 2003; 23(21):7531–7539. [PubMed: 14560001]

127. Kobayashi KS, Chamaillard M, Ogura Y, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science. 2005; 307(5710):731–734. [PubMed: 15692051]

128. Kuballa P, Huett A, Rioux JD, et al. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. PLoS. One. 2008; 3(10):e3391. [PubMed: 18852889]

129. Hudspith B, Rayment N, Prescott N, et al. *In vitro* responses of macrophages to *E. coli* challenge in Crohn's disease. Gastroenterology. 2009; 136(5 (Suppl. 1)):A250.

130. Buchmeier NA, Heffron F. Intracellular survival of wild-type *Salmonella typhimurium* and macrophage-sensitive mutants in diverse populations of macrophages. Infect. Immun. 1989; 57(1):1–7. [PubMed: 2642463]

131. Glasser AL, Boudeau J, Barnich N, et al. Adherent invasive *Escherichia coli* strains from patients with Crohn's disease survive and replicate within macrophages without inducing host cell death. Infect. Immun. 2001; 69(9):5529–5537. [PubMed: 11500426]

132. Lapaquette P, Glasser AL, Huett A, et al. Crohn's disease-associated adherent-invasive *E. coli* are selectively favoured by impaired autophagy to replicate intracellularly. Cell Microbiol. 2010; 12(1):99–113. [PubMed: 19747213]

133. Fujita N, Saitoh T, Kageyama S, et al. Differential involvement of Atg16L1 in Crohn disease and canonical autophagy: analysis of the organization of the Atg16L1 complex in fibroblasts. J. Biol. Chem. 2009; 284(47):32602–32609. [PubMed: 19783656]

134. Takeda K, Clausen BE, Kaisho T, et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of *Stat3* in macrophages and neutrophils. Immunity. 1999; 10(1):39–49. [PubMed: 10023769]

135. Takeda K, Noguchi K, Shi W, et al. Targeted disruption of the mouse *Stat3* gene leads to early embryonic lethality. Proc. Natl Acad. Sci. USA. 1997; 94(8):3801–3804. [PubMed: 9108058]

136. Kobayashi M, Kweon MN, Kuwata H, et al. Toll-like receptor-dependent production of IL-12p40 causes chronic enterocolitis in myeloid cell-specific Stat3-deficient mice. J. Clin. Invest. 2003; 111(9):1297–1308. [PubMed: 12727921] • *STAT3*-deleted mouse develops spontaneous enterocolitis as the sole consequence of impaired myeloid cell function.

137. Matsukawa A, Takeda K, Kudo S, et al. Aberrant inflammation and lethality to septic peritonitis in mice lacking STAT3 in macrophages and neutrophils. J. Immunol. 2003; 171(11):6198–6205. [PubMed: 14634136]

138. Kuhl AA, Kakirman H, Janotta M, et al. Aggravation of different types of experimental colitis by depletion or adhesion blockade of neutrophils. Gastroenterology. 2007; 133(6):1882–1892. [PubMed: 18054560]

139. van Heel DA, Udalova IA, De Silva AP, et al. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NFκB transcription factors. Hum. Mol. Genet. 2002; 11(11):1281–1289. [PubMed: 12019209]

140. Okazaki T, Wang MH, Rawsthorne P, et al. Contributions of IBD5, IL23R, ATG16L1, and NOD2 to Crohn's disease risk in a population-based case–control study: evidence of gene–gene interactions. Inflamm. Bowel Dis. 2008; 14(11):1528–1541. [PubMed: 18521914]

141. Ansari A, Hassan C, Duley J, et al. Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. Aliment. Pharmacol. Ther. 2002; 16(10):1743–1750. [PubMed: 12269967]

142. Farrell RJ, Murphy A, Long A, et al. High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy. Gastroenterology. 2000; 118(2):279–288. [PubMed: 10648456]

143. Lees CW, Satsangi J. Genetics of inflammatory bowel disease: implications for disease pathogenesis and natural history. Expert. Rev. Gastroenterol. Hepatol. 2009; 3(5):513–534. [PubMed: 19817673]

144. Csillag C, Nielsen OH, Borup R, et al. Clinical phenotype and gene expression profile in Crohn's disease. Am. J. Physiol Gastrointest. Liver Physiol. 2007; 292(1):G298–G304. [PubMed: 16959948]

145. Lawrance IC, Fiocchi C, Chakravarti S. Ulcerative colitis and Crohn's disease: distinctive gene expression profiles and novel susceptibility candidate genes. Hum. Mol. Genet. 2001; 10(5):445–456. [PubMed: 11181568]

146. Dieckgraefe BK, Korzenik JR, Anant S. Growth factors as treatment options for intestinal inflammation. Ann. NY Acad. Sci. 2006; 1072:300–306. [PubMed: 17057209]

147. Dejaco C, Lichtenberger C, Miehsler W, et al. An open-label pilot study of granulocyte colony-stimulating factor for the treatment of severe endoscopic postoperative recurrence in Crohn's disease. Digestion. 2003; 68(2–3):63–70. [PubMed: 14581762]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Key issues

- A dysregulated T-cell-mediated response to intestinal bacteria is thought to underlie the development of Crohn's disease (CD), but no intrinsic T-cell defects have been demonstrated.
- Inherited neutrophil disorders lead to non-infectious, granulomatous intestinal inflammation in the absence of T-cell defects and are characterized by a failure to clear invading microbes.
- Neutrophil influx to, and clearance of, a bacterial stimulus is defective in CD.
- Challenge with small numbers of bacteria (below the number likely to be encountered in the GI tract) results in normal responses in patients with CD.
- Increased intestinal permeability facilitates the entry of larger numbers of microbes into the bowel wall, overwhelming the weak acute inflammatory response in CD.
- Deficient secretion of proinflammatory cytokines by macrophages underlies the defect in neutrophil influx.
- The level of expression of genes involved in vesicle trafficking in macrophages appears to be responsible for the defect in cytokine secretion and distinguishes patients with CD from those with ulcerative colitis.
- A mouse model of impaired neutrophil and macrophage function (*STAT3*) leads to spontaneous granulomatous enterocolitis characterised by a Th1- and IL-12-predominant response.
- Lifestyle factors known to influence CD have defined effects on barrier and innate immune (particularly macrophage) function in the intestine.
- Genome-wide association studies highlight the role of innate immune dysfunction.

Europe PMC Funders Author Manuscripts

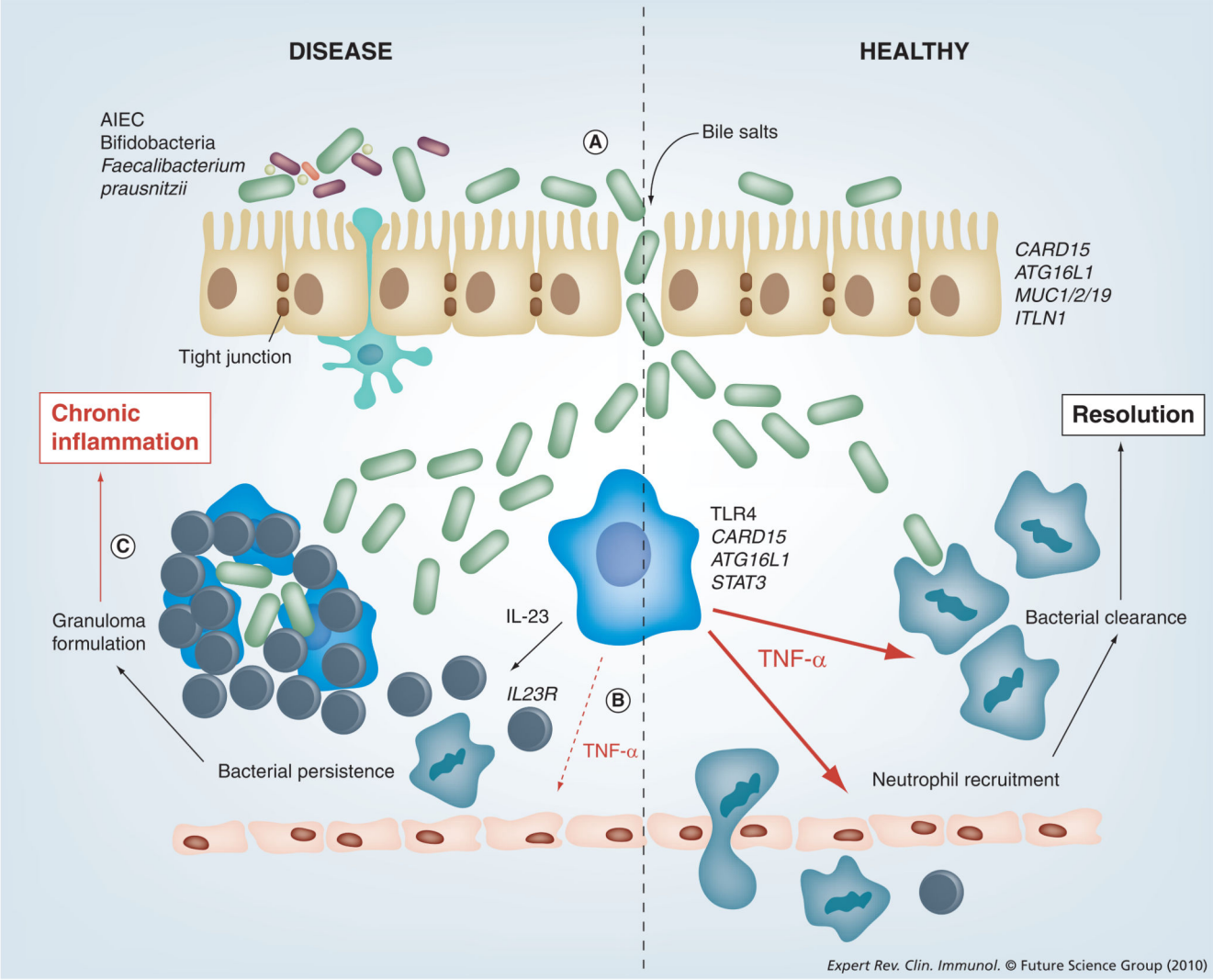

Figure 1. Three-stage hypothesis for the development of Crohn's disease

In a healthy individual (resistant to the development of Crohn's disease (CD); right-hand side of the image), bacterial entry/invasion occurring despite the action of antimicrobial peptides and maintenance of tight-junction (TJ) stability elicits an acute inflammatory response. Stimulated macrophages secrete TNF-α (and other proinflammatory cytokines) to facilitate neutrophil influx and bacterial clearance – promoting resolution of inflammation. In a patient susceptible to CD (left-hand side of the image), bacteria may invade as a consequence of a failure of barrier function or an intrinsic capability of the microorganisms themselves **(A)**. Macrophages encountering these bacteria produce less proinflammatory cytokines and neutrophil influx is impaired **(B)**. Bacterial persistence and chronic macrophage activation result in granulomatous chronic inflammation **(C)**. Known disease-associated polymorphisms are shown in italics with the relevant cell type.

AIEC: Adherent-invasive *Escherichia coli*; TLR: Toll-like receptor.

Redrawn with permission from original artwork by Bu'Hussain Hayee.

Europe PMC Funders Author Manuscripts

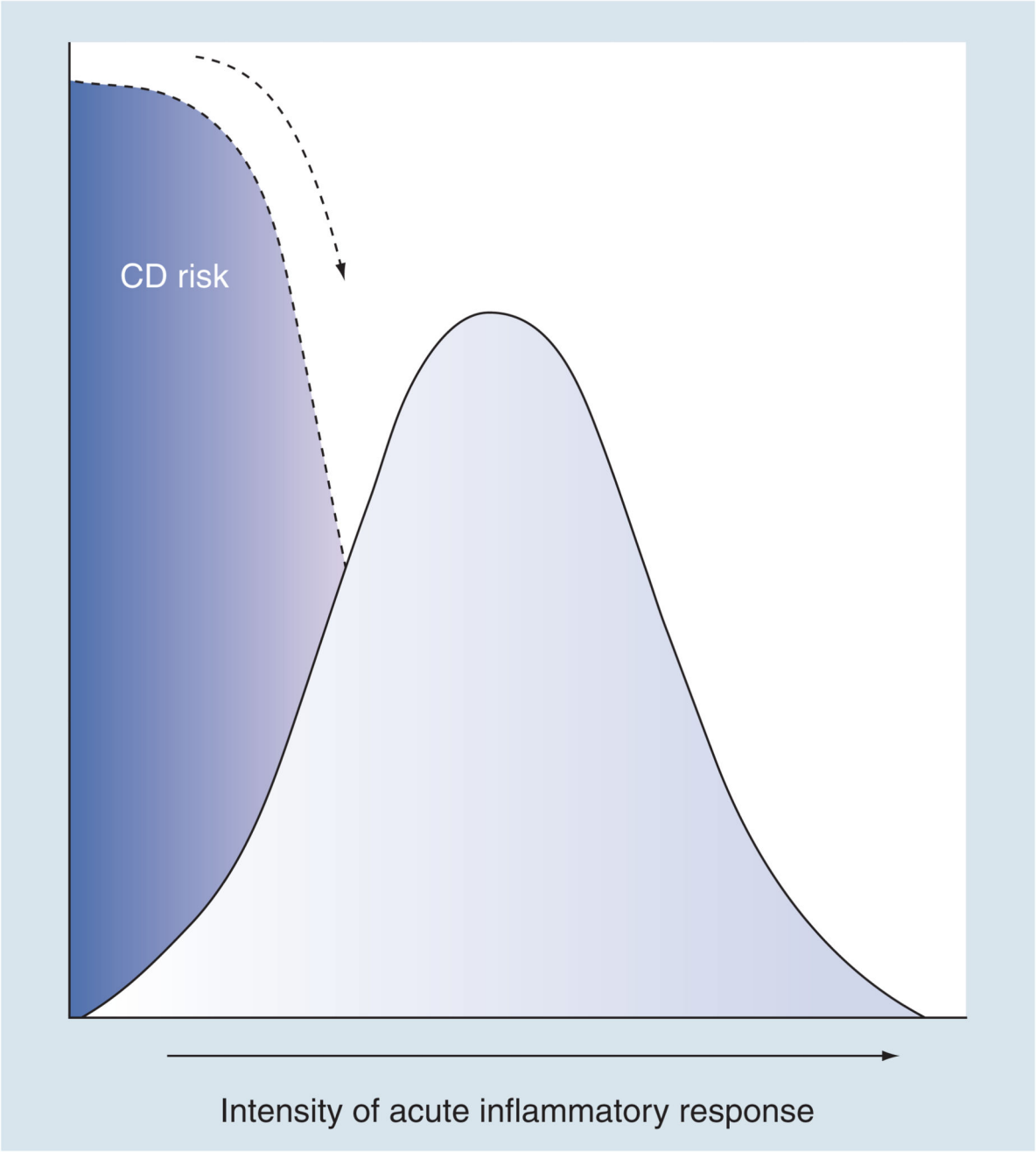

Figure 2. Relationship between the acute inflammatory response and disease development

The acute inflammatory response is considered to follow a normal distribution across a population. As the lower end of the spectrum is approached, individuals are increasingly likely to develop CD. At the lowest extreme, patients with inherited diseases of immune function are also predisposed to systemic microbial infection.

CD: Crohn's disease.