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Article type   : Review

The role of neutrophils in the pathogenesis of Crohn's disease

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Abstract

Crohn's disease (CD) is caused by a trigger, almost certainly enteric infection by one of a multitude of organisms that allows faeces access to the tissues, at which stage the response of individuals predisposed to CD is abnormal. In CD the failure of acute inflammation results in the failure to recruit neutrophils to the inflammatory site, as a consequence of which the clearance of bacteria from the tissues is defective. The retained faecal products result in the characteristic chronic granulomatous inflammation and adaptive immune response. Impaired of digestion of bacteria and fungi by CGD neutrophils can result in a similar pathological and clinical picture. The neutrophils in CD are normal and their inadequate accumulation at sites

of inflammation generally results from diminished secretion of pro-inflammatory cytokines by macrophages consequent upon disordered vesicle trafficking.

Keywords: Crohn's, Inflammatory Bowel Disease, CGD, Immunology, Infection, Bacteria, Gastroenteritis,

General introduction

The enigma that is the cause of Crohn's disease (CD) has puzzled clinicians and scientists from time immemorial. It[1] generally presents as chronic inflammation in the bowel where it usually involves the terminal ileum, as well as the caecum and colon to a variable extent. The lesions are patchy, known as "skip lesions", and associated with strictures, and fistulae between the bowel and other loops of bowel, the skin, and pelvic organs like the bladder and vagina. Anal disease affects about 40% of these patients[2] exemplified by abscess, fistulation and skin tags. The inflammation is described as transmural, extending deep into the wall of the bowel, and contains diagnostic granulomata, collections of macrophages which represent a characteristic tissue response to retained foreign material. "The basic etiological factor in the case of all granulomas is probably the presence of a nidus of insoluble material which, if small enough is ingested by phagocytic cells, or, if too large, remains extracellular"[3]. The central macrophages in these granulomata are surrounded by lymphocytes. The condition can also be associated with extra intestinal manifestations most commonly affecting the joints, skin, eyes and mouth[4].

Causation

It is generally accepted that CD results from an aberrant immune response to commensal microflora in genetically susceptible individuals[5]; however, the nature of the immune defects, the responsible microflora and the genetic susceptibility have only recently, and incompletely, been uncovered.

The three phases of Crohn's disease

A unifying model of CD pathogenesis has been proposed in which this condition develops in three temporally distinct phases[6]:

- The trigger - gastrointestinal infection;
- A defective response to the consequences of this infection;
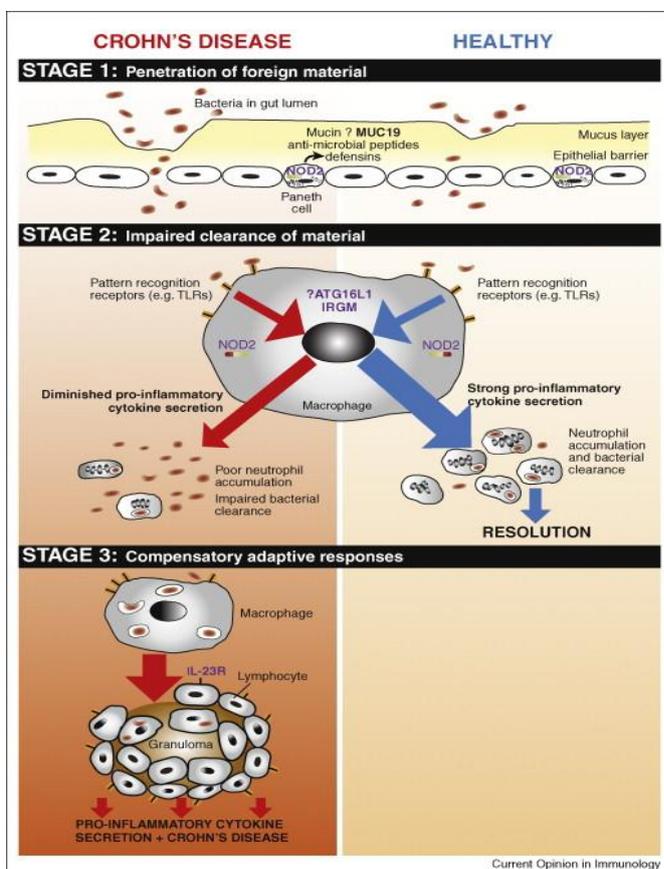- A subsequent prolonged chronic inflammatory adaptive immune response.

Figure 1. The immunopathogenesis of CD occurs in three temporally distinct stages.

Penetration of luminal contents into underlying tissues occurs in stage 1, which may be facilitated by environmental factors such as infection, or inherent defects in the mucosal barrier. In healthy individuals, resident macrophages secrete pro-inflammatory cytokines in response to this material, resulting in neutrophil accumulation, clearance of the material, and thereby resolution. In CD patients, defective secretion of pro-inflammatory cytokines by macrophages results in impaired neutrophil influx and impaired clearance of foreign material (stage 2). Subsequently, chronic inflammatory responses (stage 3) will be triggered, giving rise to the characteristic features of the CD lesion. From [6]Figure 1

Phase 1: The trigger - The infectious environmental factor

Infection has long been considered to cause CD. In the first description by Danziel in 1913 of what was later to be called Crohn's disease, the similarity between "chronic interstitial enteritis" and Johne's disease in cattle [7], which is caused by infection with *Mycobacterium avium paratuberculosis*, was commented upon[8].

There is strong epidemiological evidence for the role of an infectious environmental factor in the pathogenesis of CD. This is most obviously seen when populations or families emigrate from one country to another.

Several prospective studies have followed the course of patients after infections with enteric organisms and all have found an increased incidence of IBD as compared with uninfected control subjects[9-10]. In one of these[11] the risk was similar whether or not an infecting agent was identified, suggesting that it was the damage to the bowel rather than a specific infection that was important.

Most gastrointestinal infections do not generally produce homogeneous mucosal damage but lead to focal areas of ulceration [12,13], often in those regions of the bowel affected by CD. Because infection with invasive gastrointestinal pathogens is a stochastic process[14], the age at which this occurs is highly variable, as is the outcome after the infection. This outcome will

depend upon the severity of the infection, extent of ulceration, quantity of bowel contents gaining access to the tissues and on the effectiveness of the innate immune response. Individuals with a genetic predisposition to CD might not express this if their bowel mucosa is not sufficiently damaged to induce the disease. This lack of penetrance can cause confusion in genetic studies.

The age of onset peaks at between 20 and 30 years of age, which coincides with a stage in life accompanied by major lifestyle changes. These include the movement of individuals out of the family home, in which the ambient microbiome is likely to be relatively stable, into environments in which the risks of exposure to infection are much greater. The main two ways in which young adults are exposed to infectious enteric organisms is through the ingestion of contaminated food or fluids, or by person to person contact, the risk of both being increased by travel to places where exposure to novel organisms is more likely.

Although enteric infections are generally considered to be foodborne, only about one half are in fact transmitted in this way[15], most of the rest being transferred by person to person contact. Sexual transmission is worthy of consideration as a means of transmission of faecal organisms between individuals because, as might be expected, the peak age for the acquisition of sexually transmitted diseases is very similar to that of CD.

Diet has a major influence on the gut microbiota [16] which will influence the state of inflammation or "priming" of the innate response, the adaptive response, and the reception given by the microbial flora to invasion by novel organisms.

Phase 2: A defective inflammatory response to the damaged bowel

The submucosa of the bowel is particularly vulnerable to microbial invasion. If the mucosal barrier is breached large numbers of organisms can achieve rapid access, and the conditions are conducive to microbial proliferation. There is inadequate time for adaptive immunity to take effect, and reliance must be placed on the innate system to contain and eliminate potentially harmful stimuli. At its heart this means the rapid and florid release of

pro-inflammatory cytokines from lamina propria macrophages[17], recruited from blood monocytes[18], mast cells [19,20], eosinophils and innate lymphoid cells[21-38] when activated by bowel contents. Paneth cells reside in small clusters at the base of crypts of Lieberkühn in the small intestine, and they discharge antimicrobials, such as the alpha defensins, into the crypt lumen. These effector molecules also diffuse from the crypt and disseminate into the mucous layer that overlies the mucosal epithelium, where they contribute to the mucosal antimicrobial barrier[24].

Pro-inflammatory cytokines induce changes in the microvasculature[25,26] leading to the extravasation of plasma proteins and to the recruitment of neutrophils[27]. A critical concentration of neutrophils is required to eliminate invading bacteria[28], and immediately after bacterial penetration of the mucosa there is direct competition between bacterial replication and neutrophil recruitment and bacterial phagocytosis, followed by killing. In the absence of specific antibodies, uptake of the foreign material is enhanced by non-specific opsonins like pentraxins, collectins and complement[29]. The neutrophils then undergo apoptosis or necrosis, and the purulent collection is most probably discharged into the bowel lumen, with the residual debris being phagocytosed and cleared by macrophages[30].

Immunoparesis of the acute inflammatory response is the underlying Crohn's phenotype

The underlying pathology in Crohn's disease is the ineffective manner in which the faecal material entering the tissues through the damaged mucosa is dealt with. Infective damage to the mucosa followed by the entry of faecal material, with a bacterial count of greater than 10^{11} bacteria per ml, into the tissues, poses an existential threat that must be dealt with vigorously. This is accomplished by the acute inflammatory response, a non-specific local reaction to tissue damage that recruits the innate immune system. It includes the secretion of inflammatory mediators from mast cells and macrophages, complement activation, markedly increased blood flow, capillary dilatation and increased permeability, the deposition

of a fibrin network, and most importantly in the context of CD, a massive influx of neutrophilic leukocytes that ingest and kill invading bacteria and fungi and digest foreign organic material.

The underlying, and unifying, predisposition to the development of CD is a systemic incompetence of this acute inflammatory response. The evidence supporting this immunoparesis is derived from experiments performed on CD patients and healthy control subjects, and in some cases patients with ulcerative colitis (UC) and rheumatoid arthritis, and represent a unique set of data.
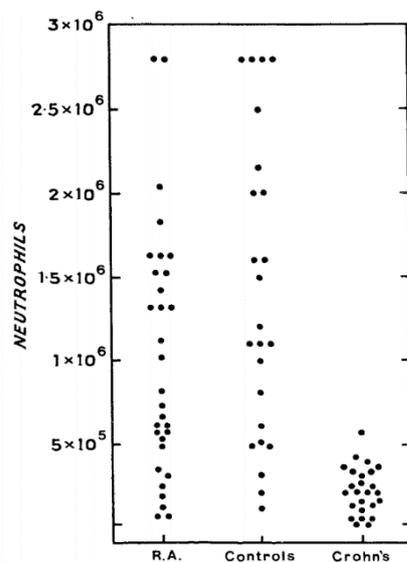

Figure 2, From [31]. Migration out of skin windows of neutrophils from patients with rheumatoid arthritis, controls and *patients with Crohn's disease.*

 The delay in the recruitment of neutrophils to sites of trauma to the body by the innate immune response has been demonstrated in patients with CD in several different but complimentary ways. In 1976 we demonstrated that the accumulation of neutrophils in

superficial abrasions on the arm, called "skin windows", was grossly deficient when compared with healthy subjects or patients with another chronic inflammatory condition, rheumatoid arthritis[31]. It was observed that *"This abnormality of neutrophil function in Crohn's disease appears to be secondary to a defective acute inflammatory response, as the neutrophils themselves were found to behave normally on in-vitro testing. A weak acute inflammatory response to particulate or antigenic material in the bowel wall could result in the chronic inflammation observed in this condition."*

The next in these series of experiments was conducted on the ileal and rectal mucosa, and again on the skin[32]. A small mucosal biopsy was taken from the ileum or rectum, and this was then followed 6 hours later by a further biopsy of the previous biopsy site, to determine the extent of the inflammatory response induced by the initial biopsy trauma. Once again there was a major delay in the recruitment of neutrophils in CD, and this was observed in both regions of the bowel. In addition to healthy subjects, control individuals with UC were studied, and their neutrophil recruitment was normal. Trauma to the skin reproduced the impaired neutrophil recruitment into skin windows, and the secretion of IL-8 and IL-1β from the windows was abnormally low.

The direct injection of heat killed *E. coli* into the subcutaneous tissues of the forearm of normal subjects is followed by profound rise in local blood flow. This was considerably impaired in CD, but not in UC. Blood flow is important in recruiting innate immune cells to sites of inflammation.

The third set of these experiments directly measured the accumulation of neutrophils at the site at which *E. coli* had been injected subcutaneously into human subjects, and then subsequently, the rate of clearance of these organisms. In this study peripheral blood neutrophils were purified from the individual under investigation, labelled with the gamma-ray emitting radioisotope Indium-111[33], and reinjected intravenously at the same time that unlabelled *E. coli* were injected subcutaneously into the forearms. The rate of accumulation

of the radioactive neutrophils over the site of the injected bacteria was determined[34]. A much smaller proportion of neutrophils were recruited to the injected bacteria in the CD subjects than in the human controls (HC) or UC individuals (Figure 3).

The next step was to radiolabel the *E. coli* with Phosphorus-32 and to then determine the rate of clearance of the bacteria from the tissues. This was a two-phase process in HC and UC subjects with a very rapid initial clearance lasting about 4 hours followed by a slower phase, with total clearance being achieved by 7 to 10 days. In the CD subjects, initial clearance was much less efficient and total clearance was markedly delayed and was predicted to last from several weeks to infinity. This study showed unequivocally that in CD coliform bacteria are cleared much less efficiently from the tissues than normal. It might be considered that this delayed recruitment of neutrophils to bacteria in the tissues should predispose these individuals to an increased incidence of clinically evident infections, which is not an obvious manifestation of CD. The reason for this apparent discrepancy is that the numbers of bacteria injected into the tissues were required to reach a certain critical load before the clearance defect was unmasked (Figure 3e). In this study 10^6 organism were cleared normally whereas 10^7 were not, indicating that a significant bacterial load must enter the tissues before the clearance systems are overwhelmed. The bowel is the only location in the body where such a burden of microbes is readily available to enter the tissues.
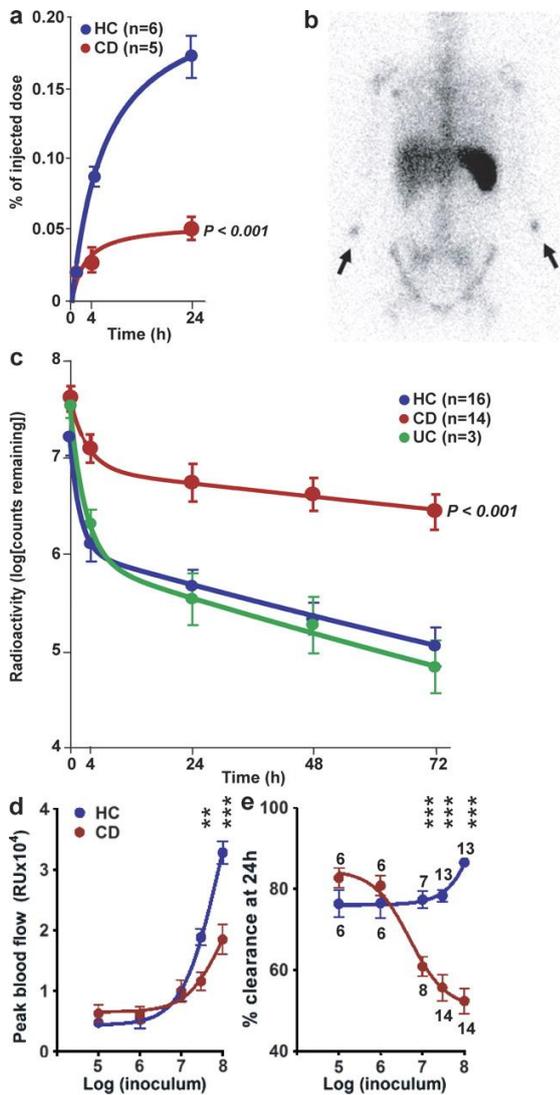
Figure 3. Neutrophil accumulation and subsequent clearance of *E. coli* from the tissues is markedly delayed in a dose-dependent manner in CD. From[34]. [111]Indium-labeled autologous neutrophils were injected intravenously at the same time as killed *E. coli* were injected subcutaneously into each forearm. (a) Radioactivity measured over the injection sites showed a much smaller proportion of labelled cells accumulating in CD subjects. (b) γ-Camera image of a CD patient at 24 h after injection, demonstrating focal accumulations of radioactivity at bacterial injection sites (arrows) and confirming lack of bowel inflammation, demonstrating that the CD was in an inactive phase. (c) ^{32}P-labeled,

killed *E. coli* were injected into the subcutaneous tissues of the forearm and radioactivity was measured at the skin surface. Clearance of radioactivity was much slower in CD than in HC or UC. Extrapolating these curves indicated that almost complete removal (99%) would take 10.2 and 7.1 d in HC and UC subjects, respectively, compared with 44.3 d in CD. Effect of increasing bacterial dose from 10^5 to 10^8 on blood flow (d) and bacterial clearance (e). The numbers of subjects studied in the dose response experiment are depicted in e. All results are expressed as mean ± SEM (**, P < 0.01; ***, P < 0.001).

Phase three: The consequences of the failure to clear intestinal contents from the bowel wall.

In the absence of an adequate acute inflammatory response and the complete clearance of the inciting agent by neutrophils, the retained foreign material produces a granulomatous inflammation[35-36] . *E. coli, Streptococci* and *Listeria* have been demonstrated immunochemically in macrophages, giant cells and lymph nodes of CD patients[37], and *E. coli* DNA has been identified in Crohn's granulomata isolated by laser capture microdissection[38]. The retention of this faecal material within the bowel leads to an intense adaptive immune response, and the tissues become infiltrated with large numbers of T-cells. It is not therefore surprising that when actively inflamed CD tissues are biopsied, any number and variety of adaptive immune cells can be identified and immune mechanisms evoked in the pathogenesis of the condition. The macrophages and adaptive immune cells, reacting to the foreign antigenic material, will produce cytokines such as IL-1β and TNFα[39,40] that lead to local inflammation and systemic symptoms[41].

The clinical picture of an inflamed bowel containing large numbers of macrophages and T-cells[42,43] has led to the erroneous belief that Crohn's was an autoimmune disease[44]. It is however clear that the cytokines produced by these inflammatory foci in their response to foreign faecal material contribute to the local and systemic inflammation, and failure of

mucosal healing, as evidenced by the, often dramatic, responses to anti-TNF drugs. However, only about half the patients respond to this treatment, and in those that do the response is often partial and temporary[45].

This failure to clear organic material from the tissues offers an explanation for the false-positive Kveim tests observed in CD [46]. The Kveim test[47] was designed to diagnose sarcoidosis, another chronic granulomatous disease. The intradermal injection of a crude homogenate of an extract of sarcoid tissue, usually from lymph node, produced epithelioid cell granulomas in subjects with sarcoidosis, reproducing those diagnostic of this disorder. Initially it was thought that the injected material contained some sarcoid specific factor, such as an infectious agent or antigen[48] but it has been recognised more recently that it relates to inefficient cellular immune processing, due most often to impaired myeloid dendritic cell function of unknown cause[49]. This is precisely the nature of the pathogenic mechanism in CD, and it is therefore not surprising that positive tests are found in both conditions[50] and that both diseases occasionally coexist in the same individual[51].

On the location of the CD lesions

Symptomatic lesions are largely confined to the terminal ileum, caecum and colon, probably due to the combination of mucosal damage by enteric infection coupled with the ready presence of massive numbers of bacteria to penetrate into the wall of the bowel when this happens. However, it is becoming apparent that the gastrointestinal tract is generally diffusely, sub-clinically, abnormal.

Oral manifestation of CD, particularly aphthous ulcers, are estimated to occur in 20-50% of patients[52]. A prospective endoscopic study identified upper gastrointestinal (GI) manifestations of CD in 55% of 108 untreated, newly diagnosed adult patients with CD, irrespective of symptoms. About a quarter of the patients had lesions in both the stomach and duodenum and in about 20% they were in one or other of these organs. In roughly 2% of patients the gastric outlet is obstructed by a granulomatous inflammation requiring

surgical intervention[53]. Aphthous ulcers in the oesophagus were present in 7% of these

subjects. Most of these lesions exhibited a granulomatous inflammation on histology.

In view of the systemic nature of the impairment of the innate immune system in CD, it is of

great interest, although not altogether surprising that patients with chronic granulomatous

disease (CGD)[54] exhibit very similar gastrointestinal pathology. CGD is a condition in which

there is a failure of microbial killing and digestion by neutrophils as a result of excessively

acid phagocytic vacuoles in which the pH is too low for the efficient activity of the neutral

protease digestive enzymes which fail to kill and digest the microbes[55,56] . Some of these

cases can present early and make up about 10% of a group of children with very early onset

bowel inflammation[57] .

Aphthous ulceration and other oral lesions are common in CGD. Oesophageal, gastric and

duodenal inflammation were detected in 21%,74% and 37% of 78 patients[58]. Large bowel

lesions were present in the majority[58] and are indistinguishable from those of CD[59,60].

Between 4%[61] and 15%[62] of these patients also develop gastric outflow obstruction.

Identifying the molecular cause/s of the CD phenotype

The abnormality of innate immunity in most cases of CD lies in the macrophages

Defective secretion of pro-inflammatory cytokines in CD may be the explanation for the

observed impairment in neutrophil recruitment [34,63-64]. In CD, the neutrophils themselves are

normal[65], exhibit normal migration *in vitro*[31,66,67] and will migrate out of skin windows if

chemoattractant substances are placed over them[32]. In the absence of a primary abnormality

of neutrophil function, CD macrophages showed defective secretion of pro-inflammatory

cytokines, but normal release of chemokines, in response to stimulation with *E. coli*[34] (Figure

4). The genes for these pro-inflammatory cytokines were transcribed and translated, but the

proteins were misdirected to lysosomal degradation rather than secretion, suggestive of
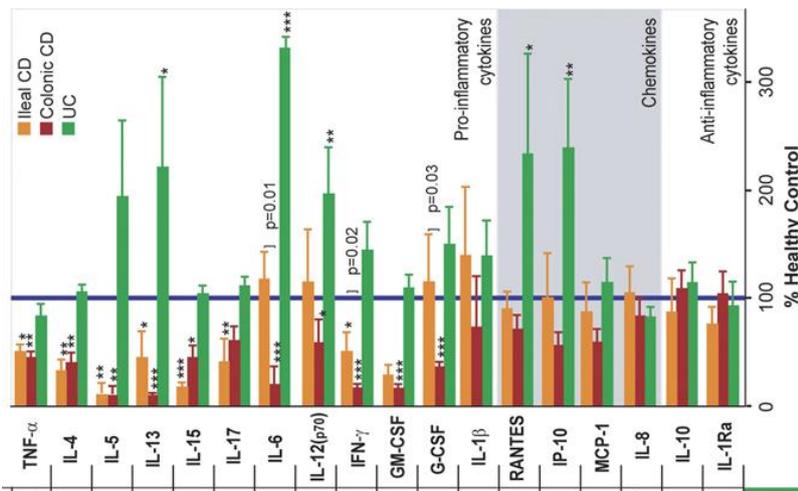
disordered vesicle trafficking.

Figure 4. Proinflammatory cytokine secretion by macrophages from CD patients is deficient in response to *E. coli*.

Cytokine and chemokine release expressed as a percentage of that secreted by HC cells (blue line) from ileal and colonic CD patients. From [34].

What is the molecular cause of the impairment of acute inflammation?

There is a strong genetic component to the aetiology of CD. The sibling recurrence risk (risk of developing the disease in the context of an affected sibling) is approximately 13-36%[68], and approximately 12%[69] of CD patients have at least one affected first degree relative. Furthermore, the study of over 300 twin pairs has demonstrated a higher concordance of disease phenotype in monozygotic (30%) compared with dizygotic twins (4%)[70].

Technological advances have provided the means of interrogating the genetic basis of CD.

1. Linkage and Genome Wide Association studies (GWAS)

GWAS

Increasingly large GWAS have been performed on CD and the results meta-analysed[71,72]. No single or small number of penetrant mutations have been found that independently cause the disease. The latest study of over 20,500 CD cases and 41,600 controls of European ancestry identified 145 loci associated with CD at $p<5\times10^{-8}$. The mean OR of the top SNPs representing these 145 loci was 1.16, and the mean control allele frequency was 0.48. Four SNPs had an OR exceeding 1.5 of which three were within *NOD2* and the fourth was in *IL23R*. The mean difference in allele frequency between cases and controls was only 0.02.[73]

NOD2

The nucleotide-binding oligomerization domain (NOD) protein, NOD2, belonging to the intracellular NOD-like receptor family, detects conserved motifs in bacterial peptidoglycan and promotes their clearance through activation of a pro-inflammatory transcriptional program and other innate immune pathways, including autophagy and endoplasmic reticulum stress[74].

ATG-16L1 (ATG16L1 (autophagy-related 16-like 1))

The process of autophagy influences several aspects of mucosal immune responses. Initially described as a "self-eating" survival pathway that enables nutrient recycling during starvation, autophagy has now been connected to multiple cellular responses, including several aspects of immunity. Initial links between autophagy and host immunity came from the observations that autophagy can target intracellular bacteria for degradation. However, subsequent studies indicated that autophagy plays a much broader role in immune responses, as it can impact antigen processing, thymic selection, lymphocyte homeostasis, and the regulation of immunoglobulin and cytokine secretion[75].

ATG16L1 interacts with NOD2. NOD2 has recently been identified as a potent autophagy inducer and a physical interaction of NOD2 and ATG16L1 appears to be required for autophagic clearance of intracellular pathogens[76].

IL-23R

This gene codes for a subunit of the receptor for IL-23. This protein pairs with the receptor molecule IL-12Rβ1 (IL12RB1), together forming the IL-23 receptor complex, and both are required for IL-23 signalling. This protein associates constitutively with Janus kinase 2 (JAK2), and also binds to transcription activator STAT3 in a ligand-dependent manner. IL-23 is pro-inflammatory[77] and the IL23R gene has been shown to protect against Crohn's disease[78].

GWAS have been performed for many different diseases, and IBD-associated loci have been shown to be shared with several other immunologically mediated diseases including Rheumatoid arthritis, Systemic lupus erythematosus (SLE), Ankylosing spondylitis, Coeliac disease and Sarcoidosis[79]. These associations are not surprising, as comorbidities of some of these conditions are well recognised in the context of IBD [80-81] and because almost all are associated with an increased incidence of similar pathologies, such as arthritis[82], uveitis[83,84] and bowel inflammation [85-86].

Other investigations to identify causal molecules

Macrophage expression profiling

In the knowledge that the release of pro-inflammatory cytokines by macrophages from CD subjects is depressed as a result of impaired vesicle trafficking[34], an attempt was made to identify genes contributing to this deficiency by looking for outlier levels of gene expression in these cells. The most commonly under-expressed genes identified were Optineurin and ADAMDEC1.

Optineurin (OPTN)[87,88] is a 67-kDa linker, or adaptor, molecule that is important in vesicle trafficking. It has several binding partners, including Rab8, Huntingtin, and Myosin VI, a multifunctional motor protein. OPTN plays a role in acute inflammation and neutrophil recruitment[89]. OPTN-deficient mice were more susceptible to *Citrobacter* colitis and *E. coli* peritonitis, and showed reduced levels of pro-inflammatory TNFα in their serum, diminished neutrophil recruitment to sites of acute inflammation and greater mortality, compared with wild-type mice. OPTN-knockdown zebrafish infected with *Salmonella* also had a higher mortality.

ADAMDEC1 (ADAM-like Decysin-1) is a member of the ADAM (A Disintegrin And Metalloproteinase) family, the expression of which is restricted to the macrophage/dendritic cell populations of the gastrointestinal tract. Its biological function is unknown, but it has been hypothesised to play a role in immunity. Adamdec1^{-/-} mice were more susceptible to the induction of bacterial and chemical induced colitis, and greater numbers of *Citrobacter rodentium* were found in the spleen, suggestive of a breakdown in mucosal immunity which resulted in bacteraemia[90].

DNA sequencing

Several studies have focussed on the analysis of Ashkenazi Jews (AJ) because they have a roughly fourfold increased incidence of CD. Two studies, one in a population of patients with sporadic disease[91] and the other, using a family-based approach[92], identified a frameshift mutation in *CSF2RB* as a strong causal candidate in a population of CD patients..

CSF2RB is the common or shared β subunit of the receptors for granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3, and IL-5[93]. The distinct α chains of these receptors provide cytokine specificity whilst the β chain is responsible for high-affinity binding, and is the major downstream signalling component of the receptor complexes. Incubation of cells with GM-CSF resulted in high levels of phosphorylation of signal transducer and activator of transcription (STAT5), which was defective in cells containing the mutant CSF2RB[92].

GM-CSF is produced by myeloid cells, dendritic cells (DCs), T cells, B cells, and several non-immunological cells including epithelial cells[94] following exposure to inflammatory stimuli to promote the production and function of myeloid haemopoietic cells including haemopoietic progenitor cells and differentiated cells such as basophils, neutrophils, eosinophils, macrophages and certain dendritic cells[95] to deal with the cause of the inflammation.

IL-3 is predominantly produced by activated T cells, natural killer (NK) cells and mast cells and IL-5 stimulates mainly the production and function of eosinophils. The major source of IL-5 is T-cells with relatively lower amounts produced by mast cells and eosinophils[96].

LRRK2 has also been associated with CD in this population of patients[97]. LRRK2 is found in immune cells, in lamina propria macrophages, B-lymphocytes, dendritic cells, and neutrophils, and levels are markedly increased in the bowel in CD[98] and in microglia in the nervous system[99]. It interacts with small GTPases including Rab32 and Rab38 with which it co-locates to transport vesicles and recycling endosomes[100] and it is important for the elimination or intracellular *Salmonellae*[101] and *Legionella*[102]. The association of variants in *LRRK2* with CD is consistent with the observation of abnormal vesicle trafficking in macrophages from patients with this condition[34]. Rab32 and Rab38 play an important role in the biogenesis and traffic of melanosomes and lysosomes and this system is disordered in Hermansky-Pudlak syndrome[103], accounting for the characteristic partial albinism. If LRRK2 and its associated proteins are important for immunological resistance to the development of CD then it might be expected that CD would be more common in conditions in which the LRRK2 system is disordered, which is in fact the case - a clear association exists between CD and Parkinson's disease,[104,105] and CD and Hermansky-Pudlak[106].

LRRK2 complexes with, phosphorylates and activates the NLRC4 inflammasome during host defence against Salmonella infection and LRRK2$^{-/-}$ mice exhibited impaired clearance of the bacteria[107], providing further evidence that impaired inflammation predisposes to CD.

The majority of genes identified as causal or associated with CD relate to the immune system, either directly as in the case of NOD2 and CSF2RB, or to molecules that are important for its function, like Optineurin that is involved in vesicle trafficking and cytokine secretion.

Future treatment options

Treatment of CD poses a conundrum. The logical approach to correcting the underlying problem would be to develop means of enhancing innate immunity, although no such range of drugs is currently available. It would be dangerous to attempt to do this in the presence of ongoing bowel inflammation, but it could be useful to maintain patients in remission after they had been cleared of disease by surgical resection, or through the use of non-immunosuppressant therapies such as elemental diets[108].

The use of GM-CSF to increase the numbers of circulating neutrophils was found to be ineffective[109], probably because the defect in CD relates to the attraction of neutrophils into injured tissues, rather than into the circulation.

The primary pathology in most case of CD appears to affect macrophages recruited from the blood as monocytes[110]. Advances in gene editing with the CRISPR-Cas[111,112] technology make the corrective treatment of CD a real possibility in the relatively near future. Once a primary causal mutation has been identified, and validated in animal models, bone marrow could be extracted, edited and reinfused into a conditioned patient in much the same way as is being applied to gene therapy for primary immunodeficiencies[113].

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