JIM Review

doi: 10.1111/joim.12945

Studies on patients establish Crohn's disease as a manifestation of impaired innate immunity

A. W. Segal

From the Division of Medicine, University College London, London, UK

Abstract. Segal AW (University College London, London, UK). Studies on patients establish Crohn's disease as a manifestation of impaired innate immunity (Review). *J Intern Med* 2019; https://doi.org/10.1111/joim.12945

The fruitless search for the cause of Crohn's disease has been conducted for more than a century. Various theories, including autoimmunity, mycobacterial infection and aberrant response to food and other ingested materials, have been abandoned for lack of robust proof. This review will provide the evidence, obtained from patients with this condition, that the common predisposition to Crohn's is a failure of the acute inflammatory response to tissue damage. This acute inflammation normally attracts large numbers of neutrophil leucocytes which engulf and clear bacteria and autologous debris from the inflamed site. The underlying predisposition in Crohn's disease is unmasked by damage to the bowel mucosa, predominantly through infection, which allows faecal bowel contents access to the vulnerable tissues within. Consequent upon failure of the clearance of these infectious and antigenic intestinal contents, it becomes contained, leading to a chronic granulomatous inflammation, producing cytokine release, local tissue damage and systemic symptoms. Multiple molecular pathologies extending across the whole spectrum of the acute inflammatory and innate immune response lead to the common predisposition in which defective monocyte and macrophage function plays a central role. Family linkage and exome sequencing together with GWAS have identified some of the molecules involved, including receptors, molecules involved in vesicle trafficking, and effector cells. Current therapy is immunosuppressant, which controls the symptoms but accentuates the underlying problem, which can only logically be tackled by correcting the primary lesion/s by gene therapy or genome editing, or through the development of drugs that stimulate innate immunity.

Keywords: Crohn's, immunity, immunology, infection, inflammatory bowel disease, macrophage.

Introduction

The cause of Crohn's disease has remained an enigma for more than a century. However, the application of direct experimentation on patients together with the application of molecular biology, molecular genetics and gene sequencing has resulted in major advances in our understanding of the underlying mechanisms that predispose to the condition. The common theme of the results produced by these investigations is that the acute inflammatory response and innate immunity are defective, and that the specific lesions span the whole spectrum of the effector mechanisms of these responses from receptors and signalling pathways, through cytokine secretion and effector cell function.

The three-stage hypothesis

We have proposed that CD develops in three distinctive phases [1](Figure 1):

Stage 1 – The trigger – gastrointestinal infection

The peak age of onset of CD is in the third decade of life. The development of the disease is a stochastic event. Affected individuals are generally entirely normal until they develop the condition.

There is much evidence to implicate infection as the triggering factor. Several prospective studies have examined the consequences of gastrointestinal infections on the incidence of CD and all have found it to be increased especially during the first

year after the infective episode [2–4] as compared with uninfected subjects. In one of these studies [3], the risk of developing CD remained whether or not an infecting agent was identified, indicating that damage to the bowel was to blame rather than infection by a specific organism.

Another pointer to the role of infection is provided by examining the emergence of CD after families or populations migrate. Several studies have described the development of CD in an unusually large number of members of families moving to new countries [5–7], and similar effects are seen at a population level [8,9]. There is some evidence that imported infections can spread through families causing CD [5].

Most gastrointestinal infections lead to focal areas of bowel ulceration [10,11], often in the ileocaecal region, the most common site of lesions in CD. Gastrointestinal infection is a stochastic process [12], leading to a very variable age at which this occurs. The consequences of the infection will depend upon the extent of ulceration, the amount of intestinal contents that penetrate into the tissues and to the effectiveness of the innate immune response.

Specific infecting agents are generally not isolated from patients with CD. Most gastrointestinal infections are brief and the causal organism will be cleared in the weeks or months before the diagnosis of CD has been established.

The possible influence of infection on the increased incidence of CD in developed countries and those that are rapidly evolving, like China and the Middle-East, could be driven by social changes including altered sexual behaviour [13].

Resistance to infection in the bowel involves a panoply of interrelated defences. These include the secretion of antimicrobial lysozyme and defensins from Paneth cells, and lectins, mucins, and secretory immunoglobulin A, that have the capacity to bind microbes and contribute to barrier function in the human gut [14]. Impairments of these barriers will naturally lead to an increased predisposition to infection, but this in itself will not cause CD. It is an impairment of the response to the consequences of this infection that is the underlying causal mechanism.

Stage 2 An impaired acute inflammatory response to bacteria in the tissues

The infecting agent breeches the mucosal barrier, permitting faecal material to enter the interior of the bowel wall. If the load of bacteria exceeds the ability of the innate immune system to clear it, it will be retained in the tissues, resulting in a granulomatous inflammatory response.

The unifying feature central to the pathogenesis of CD is the impaired capacity of the tissues to clear the bacterial load as a result of compromised acute inflammation or innate immunity.

Valuable information regarding the inflammatory response to *Escherichia coli* in the tissues in CD has emerged by determining the consequences of injecting these bacteria into the subcutaneous tissues of the forearms of patients with CD, ulcerative colitis (UC) and in some cases rheumatoid arthritis (RA), another chronic inflammatory disease [15,16] (Fig. 1).

Blood flow is depressed in CD

The dramatic increase in blood flow that has been shown to normally follow the injection of *E. coli* is markedly blunted in CD, more dramatically in colonic disease, and this is accompanied by supranormal neutrophil counts and levels of acute phase proteins in the circulation (Fig. 2).

Neutrophil accumulation is defective

Neutrophils normally swarm into sites of infection or tissue damage [17] where they play a pivotal role in removing the invading organisms and in the clearance of damaged tissues. Neutrophil migration is markedly delayed into inflammatory sites in CD. This impairment has been demonstrated in superficial dermal abrasions [15,18,19] (Fig. 3), in cantharidin skin blisters [20], following biopsies of the rectum and ileum [15] and following the injection of bacteria into patients [16] (Fig. 4a and b).

The fact that blood flow and neutrophil accumulation are defective in the skin and subcutaneous tissues as well as in the bowel is indicative of a systemic, rather than local enteric, abnormality of inflammation.

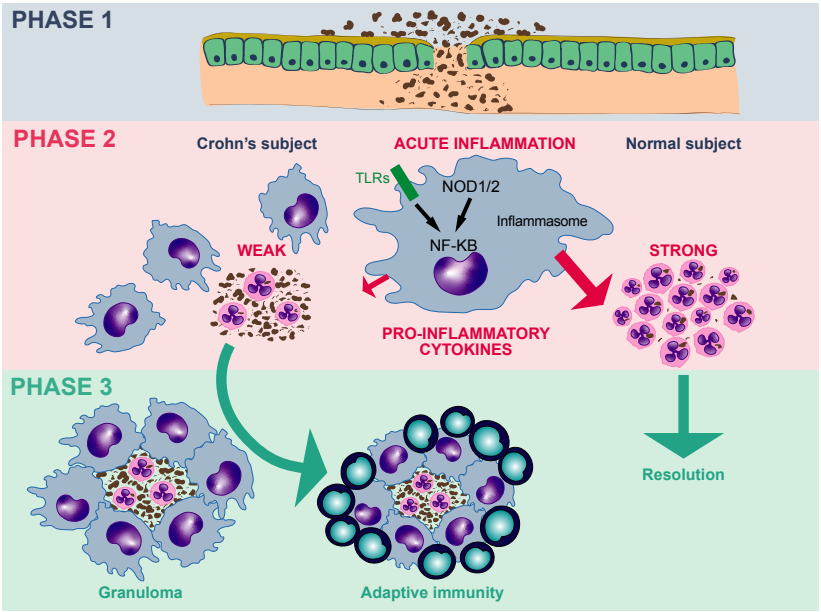

Fig. 1 *The Three-Stage Hypothesis as to the mechanisms leading to the granulomatous inflammation that characterizes Crohn's disease. Stage 1 Damage to the mucosa and penetration of faecal material into the bowel wall. Stage2 The stage at which the predisposition to CD is unmasked. This is the stage at which the effectiveness of the acute inflammatory response is critical. If this is adequate, leading to a florid recruitment of neutrophils, the faecal material is cleared and resolution ensues. If this does not occur it leads to: Stage 3 Inadequate removal of the bacteria and foreign material results in a granulomatous inflammation with an adaptive immune response.*

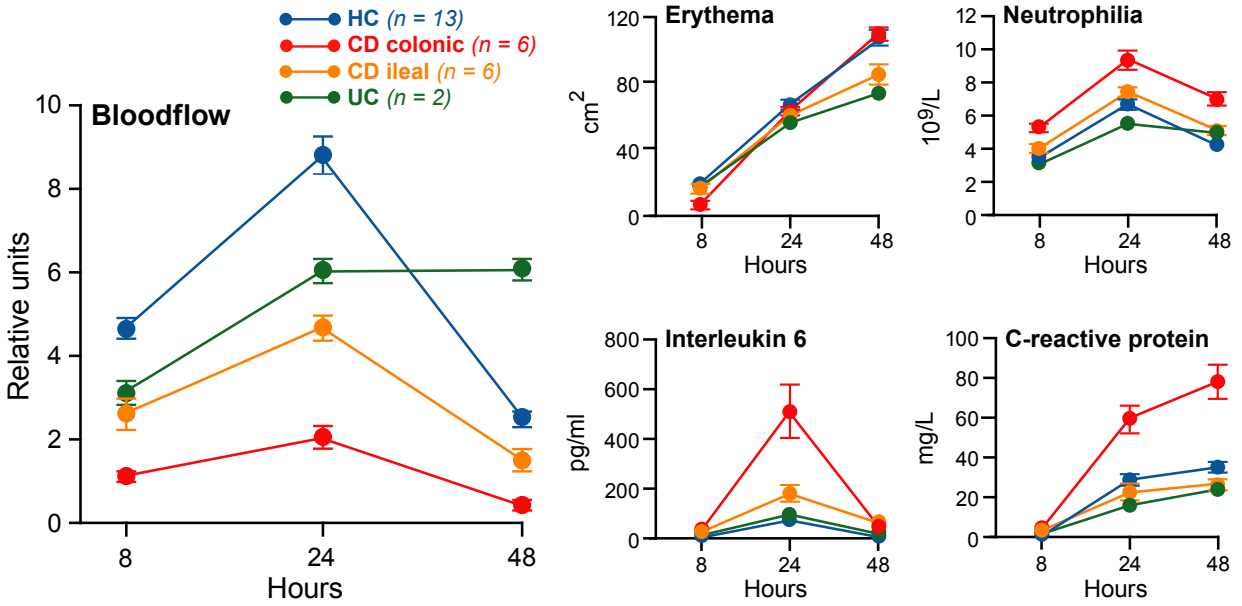

Fig. 2 *Blood flow in the forearm after the injection of* E. coli *subcutaneously into CD patients and control subjects, showing that this is severely compromised in Crohn's disease and protracted in ulcerative colitis [15] (Healthy control = HC). Interestingly, the erythematous response was fairly similar in all individuals tested. Peripheral blood neutrophilia, IL-6 and C-reactive protein levels were markedly elevated in the patients with colonic disease [15] (Healthy control = HC).*

Bacterial clearance from the tissues is delayed in CD

The demonstration that these impairments of acute inflammation result in failed or delayed clearance of bacteria from the tissues in CD was directly demonstrated by injecting Phosphorus-32-radiolabelled *E. coli* into patients [16] (Fig. 4). Whereas in normal subjects, the bacteria were cleared in about 10 days, clearance in CD took over four times longer. It was significant that this effect was dose dependent and only became apparent above a certain threshold, when $\geq 10^7$ of organisms were injected (Fig. 4d). This dose-dependent effect would explain why this particular disease presents largely in the bowel, and particularly in those regions prone to CD lesions, where very high concentrations of bacteria [21] are present in liquid, or semi-liquid faeces, that can more easily gain access the tissues through a damaged mucosa, and why systemic microbial infections are not unusually common in CD.

Stage 3 – a granulomatous inflammation and adaptive immune response

If the faecal material that enters the tissues attracts a robust neutrophil response, it will be phagocytosed and either digested by these cells or discharged into the lumen of the bowel. If on the other hand, inadequate numbers of neutrophils appear, or of those that do arrive are unable to digest normally, for example, due to abnormally

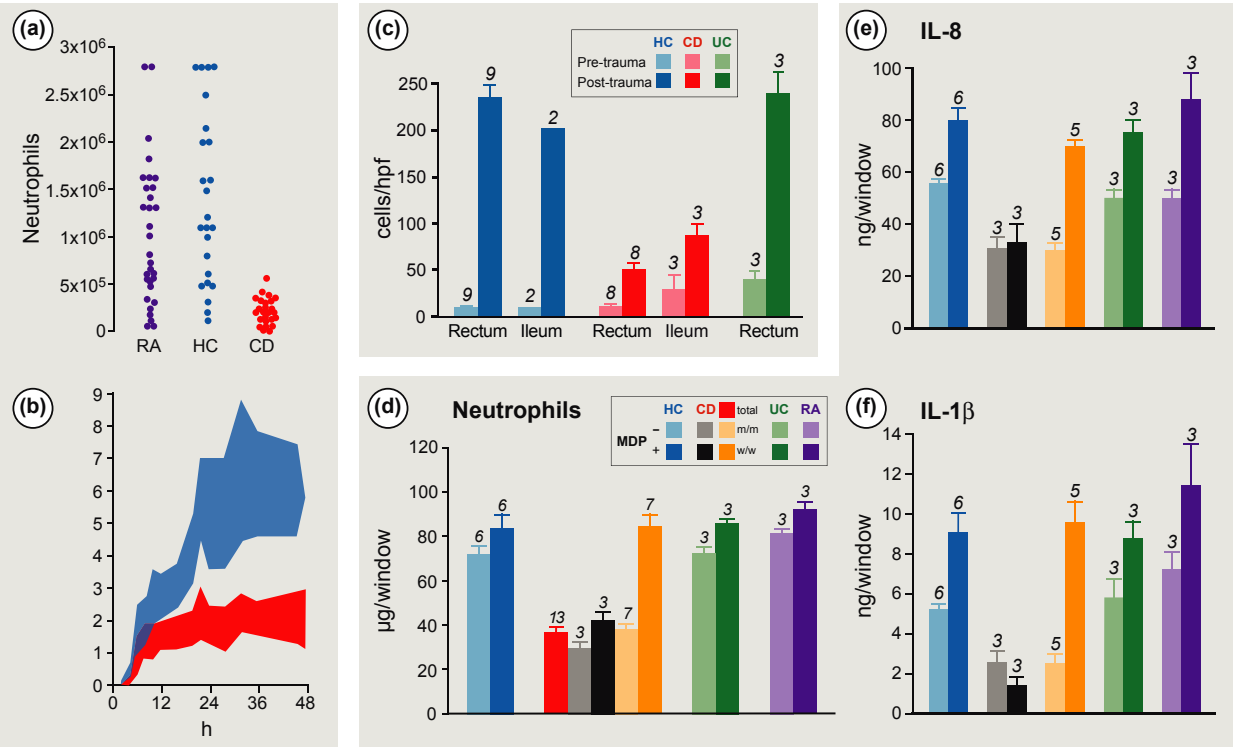

Fig. 3 *Neutrophil emigration into skin windows (a and d) after 5 h [18] and (b) over 48 h [19], and into rectum and ileum 6 h after trauma (c) [15] was markedly reduced in Crohn's disease. Of 42 inflammation-related cytokines, only IL-8 (e) and IL-1β (f) were grossly elevated in skin window effluent [15]. Healthy subjects and patients with Crohn's disease (CD), ulcerative colitis (UC) and rheumatoid arthritis (RA) were studied. The CD patients in (d-f) are presented as the total and those with CD-associated mutations in NOD2 (m/m) and those with wild-type NOD2 (w/w). In each case, the right hand column shows the results after the NOD2 agonist, muramyl dipeptide (MDP), has been placed on the skin window. (d-f) show that neutrophil accumulation and IL-8 and IL-1β secretion were depressed in CD, and that all these parameters responded to stimulation with MDP, but not in those CD patients with NOD2 mutations (m/m).*

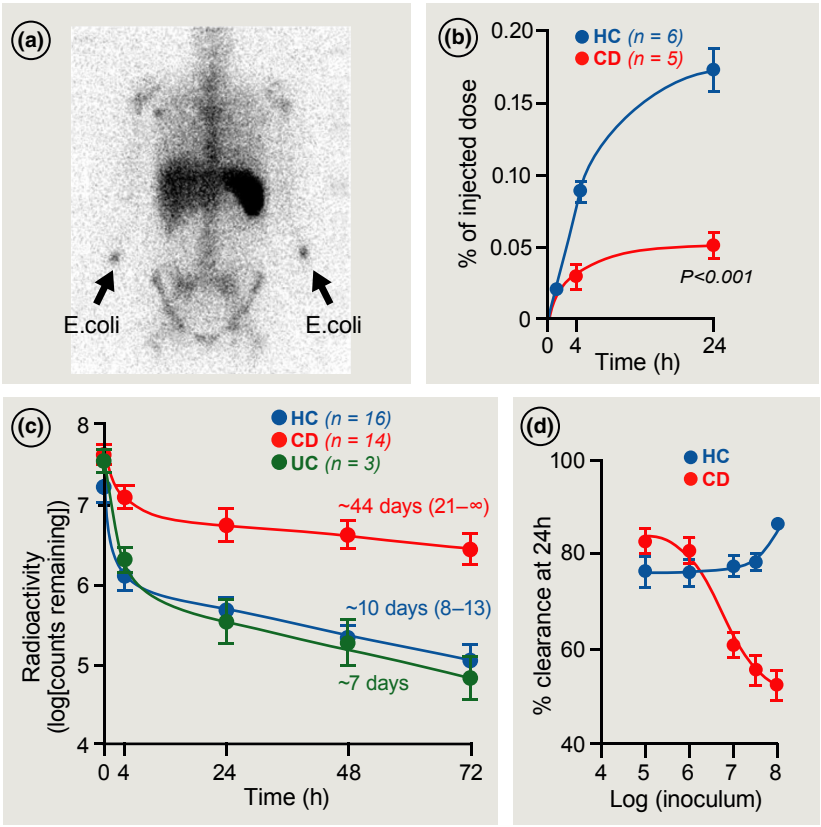

Fig. 4 *After* E. coli *are injected subcutaneously into the forearms, neutrophils accumulate at the sites of bacterial injection and the bacteria are cleared. Both the recruitment of neutrophils and clearance of bacteria are dramatically delayed in CD.* (a) The gamma-camera image of indium-111 labelled neutrophils injected intravenously at the same time as unlabelled E. coli were injected sub- cutaneously into the forearms (arrows). The rates of accumulation in healthy controls (HC) and CD patients of neutrophils at the sites of bacterial injection are shown in (b). In (c) and (d), Phosphorus-32 labelled E. coli were injected subcutaneously into healthy subjects and patients with CD and UC and the rates of clearance determined. Clearances in CD were grossly delayed. (d) Demonstrates that there is a threshold bacterial load (≥107) above which clearance is impaired.

low pH of the phagocytic vacuoles in chronic granulomatous disease (CGD) [22] or disordered granule biology in Wiscott–Aldrich syndrome, a granulomatous inflammation characteristic of CD develops. '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' [23], a granulomatous inflammation characteristic of CD develops.

The insoluble material that accumulates in CD granulomata is bacterial, at least in part. *E. coli, Streptococci* and *Listeria* have all been demonstrated immunochemically in macrophages, giant cells and lymph nodes of CD patients [24], and *E. coli* DNA has been identified in CD granulomata isolated by laser capture microdissection [25].

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. These cells, as well as macrophages, will react by producing cytokines [26,27] that cause local inflammation and systemic symptoms [28]. The bowel inflammation containing together with the infiltration by macrophages and T cells [29,30] has led to the misconception that CD is a T-cell-dependent autoimmune disease [31]. It is important to note that similar inflammation is produced by the largely mechanical condition of diverticulitis, where the trapped faeces produces an inflammatory response indistinguishable from that in CD [32] with an intense infiltration by lymphocytes [33]. Animal models and other work that has led to the erroneous belief in a causal role for lymphocytes in the development of CD have been reviewed separately [13].

Compromised release of inflammatory cytokines from macrophages forms the underlying pathogenesis in most cases of CD

Neutrophils from patients with CD are functionally normal [34]. They migrate normally *in vitro* [18,19,35] and will emigrate from skin windows in normal numbers in the presence of chemoattractants like IL-8 [15]. The failure to recruit neutrophils to regions of tissue damage in CD is most probably due to the failure of the secretion of inflammatory cytokines by their macrophages [16,36–39].

Damaged tissues produce a variety of molecules that stimulate cells through pattern recognition receptors. These include damage-associated molecular patterns (DAMPS) [40] receptors, and infecting microbes produce compounds, like MDP, that stimulate these cells through pathogen-associated molecular patterns (PAMPS) receptors. These receptors on resident macrophages and mast cells [41] activate the inflammasomes [42] and induce the secretion of pro-inflammatory cytokines and mediators to recruit neutrophils [43]. The neutrophils amplify the response by themselves producing pro-inflammatory cytokines [44].

The major PRRs include the toll-like receptor (TLR) family members, the nucleotide binding and oligomerization domain, leucine-rich repeat containing (NLR) family [45], the PYHIN (ALR) family, the RIG-1-like receptors (RLRs), C-type lectin receptors (CLRs) and the oligoadenylate synthase (OAS)-like receptors and the related protein cyclic GMP-AMP synthase (cGAS). The different PRRs activate specific signalling pathways to collectively elicit responses including the induction of cytokine expression, processing of pro-inflammatory cytokines and cell-death responses. These responses control pathogenic infection, initiate tissue repair and stimulate the adaptive immune system [46]. A central theme of many innate immune signalling pathways is the clustering of activated PRRs followed by sequential recruitment and oligomerization of adaptors and downstream effector enzymes, to form higher-order arrangements that amplify the response and provide a scaffold for proximity-induced activation of the effector enzymes. Underlying the formation of these complexes are co-operative assembly mechanisms, whereby association of preceding components increases the affinity for downstream components.

A consistent finding across several different investigations of macrophages derived from blood monocytes from hundreds of patients with CD has been that the secretion of pro-inflammatory cytokines is impaired in response to stimulation with a variety of agonists. These include *E. coli*, wound fluid (bearing mediators from damaged tissues), C5a (receptor C5aR1), and the toll-like receptor agonists PAM3, lipopolysaccharide and flagellin (Fig. 5) [15,16,36,39,47]. This defective secretion applies to a wide spectrum of pro-inflammatory cytokines, and the secretion is impaired to a greater extent from cells from patients with colonic rather than ileal disease [16].

In order to understand the cell biological basis underlying impaired secretion of TNF by macrophages, its synthetic machinery was investigated. It was demonstrated that mRNA expression was normal, as was protein translation, but the protein was then diverted to lysosomal degradation rather than to secretion [16], indicative of abnormal vesicle trafficking.

It is important to note that in almost all these studies on CD patients, patients with ulcerative colitis (UC) were included as a control group. This indicates that the abnormal test results in CD were not produced by chronic inflammatory mediators, or by bacteria or bacterial contents entering the circulation. In fact, quite the contrary was observed [48,49]. In UC, neutrophil emigration was normal, inflammatory responses to injected *E. coli* were exaggerated, sometimes to an alarming extent [49], with an abnormally high acute phase response, and the secretion of pro-inflammatory cytokines by monocyte-derived macrophages was exaggerated [16,48]. It is of interest that in a meta-analysis of GWAS described below, NOD2 and PTPN2, two of the strongest risk alleles for CD, showed significant protective effects in UC [50].

These results emphasize the fact the CD and UC are quite distinct diseases, and combining them in studies of 'inflammatory bowel disease' can be misleading.

The use of molecular biology and genetics to identify genes and molecules involved in the development of CD

The evolution of sophisticated techniques with which to explore pathological mechanisms has helped to reveal some of the molecular causes of CD.

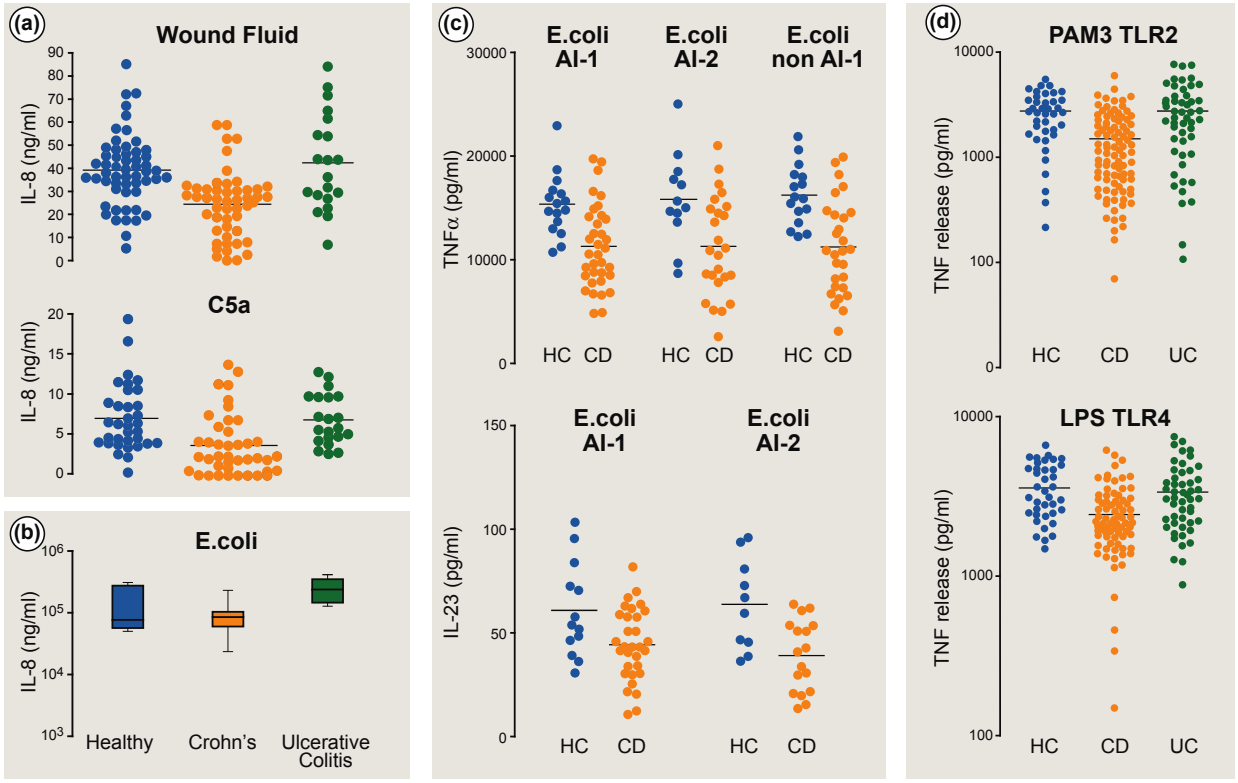

Fig. 5 *Results of four studies in which monocyte-derived macrophages from healthy subjects or patients with CD or UC were exposed to wound fluid or C5a (a, [15],* E. coli *(b, [39] and c, [36]), or the TLR ligands PAM3 or lipopolysaccharide (d, [16,47]) and the secretion of TNF, IL-8 or IL-23 measured.*

Macrophage mRNA expression

In an attempt to discover what it was about macrophages that caused them to produce subnormal amounts of pro-inflammatory cytokines, levels of expression of mRNAs were measured in these cells from 58 patients with CD and 48 healthy controls after stimulation with *E. coli*. The mRNA that was most obviously under expressed was that of Optineurin (Optn), which was abnormally low in 12% of these patients. Optn protein levels were also low in their macrophages, as was the secretion by them of TNF, Interferon-γ and IL-6 [47].

Genetics

Genetics plays an important role in the aetiology of CD. The risk of a sibling of a CD patient developing the disease is approximately 13–36 times that in the general population [51], and the risk is also significantly increased in first- (incident rate ratio 7.8), second- and third-degree relatives [52]. Furthermore, the study of over 300 twin pairs has demonstrated a higher concordance of disease phenotype in monozygotic (37%) compared with dizygotic twins (7%) [53].

Interestingly, about a third of first degree relatives do demonstrate some abnormal gastrointestinal function with modest increases in permeability [54–56] or indicators of inflammation like the neutrophil protein S100A12 [56].

However, despite this strong genetic influence, it has been difficult to discover causal genetic mutations. There are two main confounding factors. The first is the lack of complete penetrance, exemplified by the lack of concordance in monozygotic twins, which confirms the genetic predisposition whilst demonstrating the necessity for additional factors, probably environmental, for progression to overt

disease. The other factor that adds complexity is the presence of phenocopies, where the CD phenotype in a family or population has more than one genetic cause. Both these factors pose significant constraints to analysis, and greatly increase the numbers of subjects required to obtain statistically significance.

Greater precision might be conferred upon genetic studies if, in addition to overt disease, analysis was extended to subclinical parameters like increased permeability, or markers of bowel inflammation, across the families.

Linkage and Genome-Wide Association studies (GWAS)

Linkage

Linkage analysis is a technique for identifying a causal mutation within the genome by demonstrating that it is linked to expression of the disease. The association of mutations in *NOD2* with a predisposition to CD was identified by a positional-cloning strategy, based on linkage analysis followed by linkage disequilibrium mapping, of a known susceptibility region on chromosome 16 in 77 multiplex families [57]. Mutations in this gene remain the most strongly associated genetic risk factors for CD. No other causal genes for CD have been found by linkage analysis.

Genome-wide association studies

Increasingly, large GWAS have been performed on CD and the results analysed in meta-analyses [50,58–61]. No single, or small number, of penetrant mutations have been found that independently cause the disease.

With the exception of NOD2, the majority of the associated genes although highly significant have very small effect sizes, and they are thought to combine to produce a predisposition. However, the effect of their combined influence is small. The latest study of over 70 000 cases of IBD and controls found that the combined effect of these loci only explain about 14% of the disease [48].

Most of these GWAS studies have been related to IBD rather than CD and UC separately. These are very different diseases that are lumped together because they both involve the lower bowel and are associated with inflammation. It is difficult to obtain accurate phenotype data on the tens of thousands of patients studied in these GWAS, but by conflating these two different diseases the effects of the genes causing the one will be diluted by the lack of effect in the other. For example, NOD2 and PTPN2, two of the strongest risk alleles for CD, showed significant protective effects in UC [50].

Transcriptome-wide association studies (TWAS) are now being developed to integrate genome-wide association studies (GWAS) and gene expression datasets to identify gene-trait associations [62].

IBD loci are also markedly enriched in genes involved in primary immunodeficiencies, particularly those leading to Mendelian susceptibility to mycobacterial disease and leprosy [63,64]. The most significantly enriched gene ontology (GO) terms were cytokine production, lymphocyte activation and response to molecules of bacterial origin [50].

Apart from NOD2, the molecules most strongly associated with CD, are IRGM and ATG16L1, involved in membrane movements, and molecules of the IL-23/Il-17 axis that activates T cells of the adaptive immune system [65].

DNA sequencing

Genetic variation is determined by DNA sequencing, but this leads to the very considerable problem of determining whether or not variants are causally related to the disease process.

Whole genome sequencing at low coverage of 2513 adult patients with CD compared with 3652 population controls did not identify low-frequency risk variants, and little of the heritability was explained [66].

To improve the chances of identifying causally related variants, studies were conducted on Ashkenazi Jews (AJ) because this population has a restricted gene pool because it is thought to have arisen from a relatively small number of individuals about 30 generations ago [67]. CD is about four times as common in AJs as in the general population [68]. Two very large families [69] were investigated. Exome sequencing identified an inactivating missense mutation in DUOX2 in the smaller family, which impaired its function, and a truncating frameshift mutation in CSF2RB in the other. In an associated study, and in a further replication population, the same frameshift mutation in CSF2RB was statistically significantly associated with disease [70]. The mutant protein had a dominant negative effect on STAT5 signalling in

response to GM-CSF. Abnormalities in CSF2RB signalling pathways have also recently been associated with paediatric CD [71].

A further sequencing study on thousands of unrelated AJ patients found mutations in NOD2 and LRRK2 to show evidence of association to CD [72].

Expression databases (e.g. BioGPS) indicate that all three of the mutated molecules significantly associated with CD in AJs, NOD2, CSF2RB and LRRK2, are predominantly expressed in immune cells, most abundantly in neutrophils and monocytes, and not in the bowel. The evidence for the expression of NOD2 in mucosal cells, including Paneth cells is weak. It depends upon immunolocalization with antibodies. The original paper describing NOD2 in the Paneth cells [73], purported to demonstrate Paneth cell-specific staining in the ileum with their own antibody (2D9). The lamina propria macrophages and dendritic cells do not stain with this antibody although they express NOD2 and respond to MDP, and the 2D9 antibody shows positive staining in human foreskin fibroblasts. NOD2 expression was not found in the Paneth cells of mice [74].

The relationship between CD related molecules and inflammation

Crohn's disease is a misnomer. It is not one disease but a syndrome of regional enteritis with many different causes. In the majority of cases, there is a failure or abrogation of the response to bacteria and faecal material in the wall of the intestines, and this failure can occur anywhere along the inflammation pathway from initiation at the receptor systems and downstream signalling pathways, through secretion of pro-inflammatory cytokines, to defective function of the effector cells (Fig. 6). Thus, CD should be considered to be a group of 'hypo-inflammatory' disorders, caused not by an inherent susceptibility to infection in most cases, but as a result of an ineffective response to the faecal material entering the tissues as a consequence of such infections.

Receptor systems and signalling pathways

Pathogen-associated molecular pattern molecules (PAMPs) are derived from microorganisms and are recognized by pattern recognition receptor (PRR)-bearing cells of the innate immune system, as well as many epithelial cells. In contrast, damage-associated molecular pattern molecules (DAMPs) are cell-derived and initiate and perpetuate immunity in response to tissue damage, either in the absence or presence of pathogenic infection [46,75].

The bowel lesions in CD might activate either or both sets of receptors and signalling pathways. NOD-like receptors and inflammasomes provide mutual complementation, but also act in concert to produce an enhanced inflammatory response [76].

Inflammasome

The skin window experiments shown in Fig. 3d-f [15] provide important information as to the inflammatory response in CD. IL-1β and IL-8 were found in appreciable quantities and both were released in much lower concentrations in CD. Macrophages contain NALPs 1- 4 and 6, and AIM2[77] and are induced to secrete IL-1β by both pathogens [78] and cell damage [79]. When MDP was applied to the skin windows, it was without effect in those patients with CD-associated NOD2 mutations, however, in patients without such mutations it elevated the secretion of both cytokines and neutrophil emigration to normal levels. These results indicate that it is unlikely that the signals emanating from the skin windows come from stimulation by bacterial products and are likely to predominantly involve DAMPs, signalling through inflammasomes to secrete IL-1β. This suggests that inflammasome activity is depressed in CD.

It is of interest that NLRP2 was found high on the list of damaging variants in two separate studies of familial CD in AJs [80]. Very little is known about the biology of this NLRP. It does appear to activate MAP kinases [81] which is involved in the pro-inflammatory response of macrophages [82].

NOD (nucleotide-binding oligomerization domain) 2, the product of the CARD15 gene, is the most strongly of all molecules associated with the pathogenesis of CD. It is mainly expressed in peripheral blood monocytes [83] (and neutrophils), which are rapidly recruited to sites of acute inflammation [84] where they secrete pro-inflammatory cytokines [85,86] before transformation into macrophages.

Although it was initially thought that NOD2 mutations would be pro-inflammatory, in fact those associated with susceptibility to Crohn's disease in the LRR domain are inactivating mutations

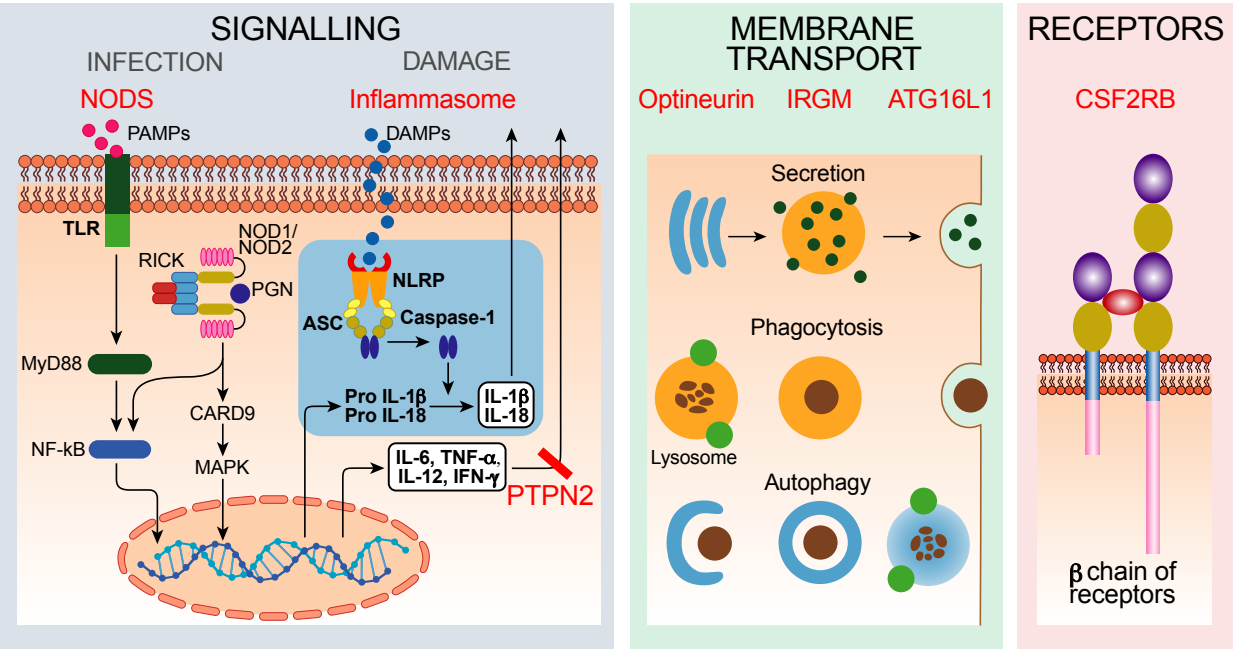

Fig. 6 *Crohn's disease-associated genes span the whole spectrum of the acute inflammatory response from receptors of pathogens PAMPs and tissue damage DAMPs, leading to the production of pro-inflammatory cytokines, their packaging, transport through, and secretion from, macrophages, and the activation of effector cells.*

because they prevent the recognition of muramyl dipeptides as shown in Fig. 3d-f [87]. Activating mutations are located in the nucleotide-binding domain and cause the autoinflammatory condition of Blau's syndrome [88] which does not manifest as bowel disease.

Membrane trafficking, lysosomal function and cytokine secretion

Many of the molecules that have been associated with CD are involved in the movement and organization of membranes in cells and play an important part in phagocytosis, lysosomal degranulation and the secretory apparatus.

The association of ATG16L1 (autophagy-related 16-like 1) and IRGM (immunity-related GTPase family M protein) with CD in GWAS have suggested a possible causal role of defective autophagy in the causation of the disease.

Autophagy is a process that takes place in the cytoplasm in which damaged or aged organelles and proteins are removed, or where cellular components are digested and redeployed under conditions of starvation [89].

ATG16L1 impairs signalling down the an unconventional pathway [90] in which it attaches to LC3 and the complex promotes degranulation of the cytoplasmic granules/lysosomes which kill and digest the engulfed organisms. IRGM interacts with NOD2, and ATG16L1, to form a molecular complex to modulate autophagy responses to microbial products [91].

The autophagy molecules have largely been identified in assays that measure autophagy in cultured cells, initially in mutant yeast [92]. Autophagy requires the movement of double membranous structures to enclose the intracellular target so that it can then be digested by lysosomal enzymes. Many of the proteins identified as being required for autophagy are engaged in the movement of membranes. ATG16L1 is an example of one of these autophagy-related molecules that has an effect unrelated to autophagy itself. The disease risk allele of ATG16L1 alters the lysosome and defensin containing vesicles in Paneth cells. These vesicles have an abnormal structure, are relatively deficient in lysozyme, and their secretion is disorganized [93]. Leucine-rich repeat kinase 2 (LRRK2) [94] and

Optineurin [95,96] also have roles not only in autophagy but also in vesicle trafficking and cytokine secretion.

An aspect of autophagy that might be of importance in the pathogenesis of CD is xenophagy, a process in which organisms that have escaped into the cytoplasm, such as *Shigella* or *Listeria,* or are sequestered in intracellular vacuoles, like *Salmonella* and *Mycobacteria* [97,98], are enclosed within autophagocytic vacuoles and digested. Xenophagy is abnormal in Niemann–Pick disease type C1 and in XIAP deficiency with NOD2 variants both of which predispose to CD [99].

By far the greatest numbers of bacteria that enter the body are phagocytosed, killed and digested by neutrophils, xenophagy only has to deal with a tiny minority escaping this process.

LRRK2 is predominantly found in myeloid cells [94] and in microglia in the nervous system [100] in which it is involved in vesicle transport and in recycling endosomes. LRRK2 had been extensively studied because mutations in it cause familial and sporadic Parkinson's disease (PD). Analysis of the extended LRRK2 locus in 24 570 CD cases, patients with Parkinson's disease (PD), and healthy controls revealed extensive pleiotropy, with shared genetic effects between CD and PD in both Ashkenazi Jewish and non-Jewish cohorts [101]. The LRRK2 N2081D CD risk allele is located in the same kinase domain as G2019S, a mutation that is the major genetic cause of familial and sporadic PD. Like the G2019S mutation, the N2081D variant was associated with increased kinase activity. The R1398H variant, which was protective of CD had increased GTPases activity, thereby deactivating LRRK2 [72]. An increased risk of PD was also seen in a Danish population with IBD [102].

Optineurin (Optn) [95,96]. Optineurin is a multifunctional adaptor protein intimately involved in various vesicular trafficking pathways. Through interactions with an array of proteins, such as myosin VI, Huntingtin, Rab8 and Tank-binding kinase 1, as well as via its oligomerization, optineurin has the ability to act as an adaptor, scaffold or signal regulator to coordinate many cellular processes associated with the trafficking of membrane-delivered cargo [103].

Effector cells

CSF2RB is the shared β subunit of the receptors for granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3 and IL-5[104]. These receptors are required for the normal function of myeloid cells, dendritic cells (DCs), T cells, B cells, natural killer (NK) cells, mast cells, basophils and eosinophils.

A large proportion of very early onset inflammatory bowel disease, or bowel inflammation, has been shown to be associated with Mendelian lesions in known immunodeficiency genes, about 50% of which are associated with neutrophil function defects (Uhlig undergoing re-review).

Roughly half the patients with chronic granulomatous disease (CGD), the preeminent primary immunodeficiency disease of neutrophil function has been shown to have bowel lesions indistinguishable from CD [105] and the other neutrophil defects, including Hermansky–Pudlak [106], glycogen storage disease 1b [107] and leucocyte adhesion deficiency all have a CD phenotype clinically, endoscopically and histopathologically, with granulomata evident on biopsy [105].

Adaptive immunity

Two other genes have been strongly linked to CD. IL-23 is produced by myeloid cells and attaches to its receptor IL23R. Variants that are strongly associated with CD in Caucasians are protective, and display loss of function due to impaired protein stability and intracellular trafficking [108]. This molecule is found on type 17 T helper cells (TH17 cells) which generate IL-17 and several other pro-inflammatory cytokines [109]. TNFSF15 variants contribute strongly to heritability in Asians [110]. It produces TL1A in several cell types and is important for T-cell differentiation, lymphocyte proliferation and cytokine production [111].

CD-associated variants in IL23R and in TNFSF15 are both deactivating. The protective effects of these inactivating mutations are understandable in that they would have a similar effect to immunosuppressants, reducing the adaptive immune response to the bacterial and other debris in the tissues.

The evidence presented above strongly supports the concept that it is the failure of the inflammatory

response and innate immunity, and not excessive inflammation that set the scene for the development of CD. There are two discordant pieces of evidence.

Interleukin-10 (IL-10) is well-recognized as an anti-inflammatory cytokine, potently inhibiting the production of numerous cytokines synthesized by T-lymphocytes, neutrophils and monocytes/macrophages [112], yet IL-10 deficiency, or receptor dysfunction, is associated with bowel inflammation. However, IL-10 can also be pro-inflammatory [112] and the presentation of patients with IL-10 or IL-10 receptor deficiency is similar to that of patients with primary neutrophil immunodeficiencies [113,114], with bacterial infections like folliculitis, and ear and chest infections. There was also a high prevalence of perianal disease with abscesses, fissures and fistulae that characterize neutrophil immunodeficiencies [105–107,115]. If, on the other hand, the IL-10 deficient phenotype was caused by the proposed excessive and unregulated macrophage response, then it might be expected that treatment with immunosuppressive therapy would be effective, which was found not to be the case [114].

The other argument is that CD patients respond to anti-inflammatory treatments like those with anti-TNF drugs, suggesting that it is caused by an excess of inflammation. The symptoms are indeed caused by too much inflammation in the third phase of the disease, which is the adaptive immune response to faecal material in the tissues. Current drug and biological treatments dampen down the secondary inflammation induced by the retained foreign material within the tissues. Anti-TNF treatments can be very helpful but do not provide a comprehensive answer and only one third of patients are in remission after one year on these treatments [116]. Immunosuppressant treatment further compromises the underlying innate immune deficit to mucosal damage, thereby increasing the likelihood of further infection and the influx of bowel contents into the tissues, and its impaired clearance. Thus, additional suppression of an already impaired inflammatory response could further impair the clearance of faecal material from the bowel wall, increasing the frequency of secondary inflammations and converting CD from a sporadic to a more chronic condition.

Treatment

Treatment of CD poses a problem. It would be logical to correct the underlying pathogenesis by enhancing innate immunity, however, no such drugs are currently available.

Immunostimulation might exaggerate ongoing bowel inflammation. However, if developed, such treatments, could be useful to maintain patients in remission after they had been cleared of disease by surgical resection, or through the use of nonimmunosuppressant therapies such as elemental diets [117], They might also be useful as a prophylactic measure in subjects like siblings of patients or members of families with multiply affected individuals at high risk of developing the disease.

Past attempts to stimulate immunity with Levamisole were unsuccessful [118], and GM-CSF was modestly effective but was never adopted as an FDA-approved treatment [119].

An alternative approach that is likely to be applied in the near future, given that most of the defective genes are in myeloid cells in the bone marrow, would be transplantation of autologous bone marrow transfected with the normal gene or altered by genome editing, into conditioned recipients.

There is evidence that allogeneic bone marrow transplantation can cure CD [120] but risk of death or major side effects precludes its routine use except in children with severe monogenic disease. It was hoped that autologous haematopoetic stem cell transplantation into conditioned patients might 'reset' the immune system without correcting the underlying genetic lesion, but a randomized trial did not result in a statistically significant improvement and was associated with significant toxicity [121].

Specific correction of the causal genetic lesions appears logical and is becoming increasingly feasible. Gene therapy using viral vectors to transfect haemopoietic stem cells with the wild-type gene now provides standard clinical practise for several primary immunodeficiency diseases and other conditions [122]. This approach should be currently applicable to treat CD where the causal gene defect is readily identifiable, for example, in subjects with homozygous truncating mutations in NOD2. In the near future, improvements in gene

editing technologies should lead to a personalized medicine approach with the correction of the genetic architecture of individual patients as the contributions to their disease by individual variants in genes regulating innate immunity become better defined and easier to identify.

Conflict of interest statement

No conflict of interest was declared.

Acknowledgements

I thank Adam Levine, Andrew Smith and Elly Schiff for critical examination of the manuscript and assistance and the Medical Research Council (MR/L 000261/1) for support. These studies were approved by the Joint University College London (UCL)/UCL Hospitals (UCLH) Committee for the Ethics of Human Research (project numbers 02/0324 and 04/Q0502/29) and the Administration of Radioactive Substances Advisory Committee (RPC597-790).

References

1 Sewell GW, Marks DJ, Segal AW. The immunopathogenesis of Crohn's disease: a three-stage model. *Curr Opin Immunol* 2009; **21:** 506–13.
2 García Rodríguez LA, Ruigómez A, Panés J. Acute gastroenteritis is followed by an increased risk of inflammatory bowel disease. *Gastroenterology* 2006; **130:** 1588–94.
3 Gradel KO, Nielsen HL, Schønheyder HC, Ejlertsen T, Kristensen B, Nielsen H. Increased short- and long-term risk of inflammatory bowel disease after salmonella or campylobacter gastroenteritis. *Gastroenterology* 2009; **137:** 495–501.
4 Jess T, Simonsen J, Nielsen NM, Jørgensen KT, Bager P, Ethelberg S, *et al.* Enteric Salmonella or Campylobacter infections and the risk of inflammatory bowel disease. *Gut* 2011; **60:** 318–24.
5 Joossens M, Simoens M, Vermeire S, Bossuyt X, Geboes K, Rutgeerts P. Contribution of genetic and environmental factors in the pathogenesis of Crohn's disease in a large family with multiple cases. *Inflamm Bowel Dis* 2007; **13:** 580–4.
6 Katsanos KH, Karetsos V, Tsianos EV. A family report of Crohn's disease in three children immigrating from Albania to Greece and review of the literature. *J Crohns Colitis* 2010; **4:** 582–5.
7 Freeman HJ, Hershfield NB. Anticipation in an Indo-Canadian family with Crohn's disease. *Can J Gastroenterol* 2001; **15:** 695–8.
8 Bar-Gil Shitrit A, Koslowsky B, Kori M *et al.* Inflammatory bowel disease: an emergent disease among Ethiopian Jews migrating to Israel. *Inflamm Bowel Dis* 2015; **21:** 631–5.
9 Li X, Sundquist J, Hemminki K, Sundquist K. Risk of inflammatory bowel disease in first- and second-generation immigrants in Sweden: a nationwide follow-up study. *Inflamm Bowel Dis* 2011; **17:** 1784–91.
10 Vantrappen G, Geboes K, Ponette E. Yersinia enteritis. *Med Clin North Am* 1982; **66:** 639–53.
11 Puylaert JB, Van der Zant FM, Mutsaers JA. Infectious ileocecitis caused by Yersinia, Campylobacter, and Salmonella: clinical, radiological and US findings. *Eur Radiol* 1997; **7:** 3–9.
12 Rock K, Brand S, Moir J, Keeling MJ. Dynamics of infectious diseases. *Rep Prog Phys* 2014; **77:** 026602.
13 Segal AW. Making sense of the cause of Crohn's – a new look at an old disease [version 2; referees: 2 approved]. *F1000Research* 2016; **5:** 2510.
14 Chairatana P, Nolan EM. Defensins, lectins, mucins, and secretory immunoglobulin A: microbe-binding biomolecules that contribute to mucosal immunity in the human gut. *Crit Rev Biochem Mol Biol* 2017; **52:** 45–56.
15 Marks DJB, Harbord MWN, MacAllister R *et al.* Defective acute inflammation in Crohn's disease: a clinical investigation. *Lancet (London, England)* 2006; **367:** 668–78.
16 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:** 1883–97.
17 Kienle K, Lämmermann T. Neutrophil swarming: an essential process of the neutrophil tissue response. *Immunol Rev* 2016; **273:** 76–93.
18 Segal AW, Loewi G. Neutrophil dysfunction in Crohn's disease. *Lancet* 1976; **2:** 219–21.
19 Wandall JH, Binder V. Leucocyte function in Crohn's disease. Studies on mobilisation using a quantitative skin window technique and on the function of circulating polymorphonuclear leucocytes in vitro. *Gut* 1982; **23:** 173–80.
20 Harbord MW, Marks DJ, Forbes A, Bloom SL, Day RM, Segal AW. Impaired neutrophil chemotaxis in Crohn's disease relates to reduced production of chemokines and can be augmented by granulocyte-colony stimulating factor. *Aliment Pharmacol Ther* 2006; **24:** 651–60.
21 Franks AH, Harmsen HJ, Raangs GC, Jansen GJ, Schut F, Welling GW. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol* 1998; **64:** 3336–45.
22 Levine AP, Duchen MR, de Villiers S, Rich PR, Segal AW. Alkalinity of neutrophil phagocytic vacuoles is modulated by HVCN1 and has consequences for myeloperoxidase activity. *PLoS ONE* 2015; **10:** e0125906.
23 Warren KS. A functional classification of granulomatous inflammation. *Ann N Y Acad Sci* 1976; **278:** 7–18.
24 Liu Y, van Kruiningen HJ, West AB, Cartun RW, Cortot A, Colombel JF. Immunocytochemical evidence of *Listeria*, *Escherichia coli*, and Streptococcus antigens in Crohn's disease. *Gastroenterology* 1995; **108:** 1396–404.
25 Ryan P, Kelly RG, Lee G *et al.* Bacterial DNA within granulomas of patients with Crohn's disease–detection by laser capture microdissection and PCR. *Am J Gastroenterol* 2004; **99:** 1539–43.
26 Mielke ME, Peters C, Hahn H. Cytokines in the induction and expression of T-cell-mediated granuloma formation and protection in the murine model of listeriosis. *Immunol Rev* 1997; **158:** 79–93.

27 Ozen S, Bilginer Y. A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin. *Nat Rev Rheumatol* 2014; **10:** 135–47.
28 Broderick L. Recurrent fevers for the pediatric immunologist: it's not all immunodeficiency. *Curr Allergy Asthma Rep* 2016; **16:** 2.
29 Elson CO, Alexander KL. Host-microbiota interactions in the intestine. *Dig Dis* 2015; **33:** 131–6.
30 Hansen JJ. Immune responses to intestinal microbes in inflammatory bowel diseases. *Curr Allergy Asthma Rep* 2015; **15:** 61.
31 Lord JD. Promises and paradoxes of regulatory T cells in inflammatory bowel disease. *World J Gastroenterol* 2015; **21:** 11236–45.
32 Gledhill A, Dixon MF. Crohn's-like reaction in diverticular disease. *Gut* 1998; **42:** 392–5.
33 Tursi A, Brandimarte G, Elisei W *et al.* Assessment and grading of mucosal inflammation in colonic diverticular disease. *J Clin Gastroenterol* 2008; **42:** 699–703.
34 Levine AP, Segal AW. What is wrong with granulocytes in inflammatory bowel diseases? *Dig Dis* 2013; **31:** 321–7.
35 Somasundaram R, Nuij VJAA, van der Woude CJ, Kuipers EJ, Peppelenbosch MP, Fuhler GM. Peripheral neutrophil functions and cell signalling in Crohn's disease. *PLoS ONE* 2013; **8:** e84521.
36 Elliott TR, Hudspith BN, Rayment NB *et al.* Defective macrophage handling of Escherichia coli in Crohn's disease. *J Gastroenterol Hepatol* 2015; **30:** 1265–74.
37 Campos N, Magro F, Castro AR *et al.* Macrophages from IBD patients exhibit defective tumour necrosis factor-α secretion but otherwise normal or augmented pro-inflammatory responses to infection. *Immunobiology* 2011; **216:** 961–70.
38 Sewell GW, Rahman FZ, Levine AP *et al.* Defective tumor necrosis factor release from Crohn's disease macrophages in response to Toll-like receptor activation: relationship to phenotype and genome-wide association susceptibility loci. *Inflamm Bowel Dis* 2012; **18:** 2120–7.
39 Vazeille E, Buisson A, Bringer M-A *et al.* Monocyte-derived macrophages from Crohn's disease patients are impaired in the ability to control intracellular adherent-invasive Escherichia coli and exhibit disordered cytokine secretion profile. *J Crohns Colitis* 2015; **9:** 410–20.
40 Areschoug T, Gordon S. Pattern recognition receptors and their role in innate immunity: focus on microbial protein ligands. In: *Trends in Innate Immunity*. In: Egesten A, Schmidt A, Herwald H, eds. Basel: KARGER; 2008: 45–60.
41 Cardamone C, Parente R, De Feo G, Triggiani M. Mast cells as effector cells of innate immunity and regulators of adaptive immunity. *Immunol Lett* 2016; **178:** 10–4.
42 Lamkanfi M. Emerging inflammasome effector mechanisms. *Nat Rev Immunol* 2011; **11:** 213–20.
43 Pittman K, Kubes P. Damage-associated molecular patterns control neutrophil recruitment. *J Innate Immun* 2013; **5:** 315–23.
44 Tamassia N, Bianchetto-Aguilera F, Arruda-Silva F *et al.* Cytokine production by human neutrophils: Revisiting the "dark side of the moon". *Eur J Clin Invest* 2018; **48:**e12952.
45 Pashenkov MV, Murugina NE, Budikhina AS, Pinegin BV. Synergistic interactions between NOD receptors and TLRs: mechanisms and clinical implications. *J Leukoc Biol* 2019; **105:** 669–80.
46 Vajjhala PR, Ve T, Bentham A, Stacey KJ, Kobe B. The molecular mechanisms of signaling by cooperative assembly formation in innate immunity pathways. *Mol Immunol* 2017; **86:** 23–37.
47 Smith AM, Sewell GW, Levine AP *et al.* Disruption of macrophage pro-inflammatory cytokine release in Crohn's disease is associated with reduced optineurin expression in a subset of patients. *Immunology* 2015; **144:** 45–55.
48 Rahman FZ, Smith AM, Hayee B, Marks DJB, Bloom SL, Segal AW. Delayed resolution of acute inflammation in ulcerative colitis is associated with elevated cytokine release downstream of TLR4. Bereswill S, editor. *PLoS ONE* 2010; **5:** e9891.
49 Marks DJB, Rahman FZ, Novelli M *et al.* An exuberant inflammatory response to *E. coli*: implications for the pathogenesis of ulcerative colitis and pyoderma gangrenosum. *Gut* 2006; **55:** 1662–3.
50 Jostins L, Ripke S, Weersma RK *et al.* Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012; **491:** 119–24.
51 Ahmad T, Satsangi J, McGovern D, Bunce M, Jewell DP. Review article: the genetics of inflammatory bowel disease. *Aliment Pharmacol Ther* 2001; **15:** 731–48.
52 Moller FT, Andersen V, Wohlfahrt J, Jess T. Familial risk of inflammatory bowel disease: a population-based cohort study 1977-2011. *Am J Gastroenterol* 2015; **110:** 564–71.
53 Brant SR. Update on the heritability of inflammatory bowel disease: the importance of twin studies. *Inflamm Bowel Dis* 2011; **17:** 1–5.
54 Teshima CW, Goodman KJ, El-Kalla M *et al.* Increased intestinal permeability in relatives of patients with Crohn's disease is not associated with small bowel ulcerations. *Clin Gastroenterol Hepatol* 2017; **15:** 1413–8.e1.
55 Fries W, Renda MC, Lo Presti MA *et al.* Intestinal permeability and genetic determinants in patients, first-degree relatives, and controls in a high-incidence area of Crohn's disease in southern Italy. *Am J Gastroenterol* 2005; **100:** 2730–6.
56 Pham M, Leach ST, Lemberg DA, Day AS. Subclinical intestinal inflammation in siblings of children with Crohn's disease. *Dig Dis Sci* 2010; **55:** 3502–7.
57 Hugot JP, Chamaillard M, Zouali H *et al.* Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411:** 599–603.
58 Ellinghaus D, Jostins L, Spain SL *et al.* Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. *Nat Genet* 2016; **48:** 510-8.
59 Huang H, Fang M, Jostins L *et al.* Fine-mapping inflammatory bowel disease loci to single-variant resolution. *Nature* 2017; **547:**173–8.
60 Mirkov MU, Verstockt B, Cleynen I. Genetics of inflammatory bowel disease: beyond NOD2. *Lancet Gastroenterol Hepatol* 2017; **2:** 224–34.
61 Verstockt B, Smith KG, Lee JC. Genome-wide association studies in Crohn's disease: past, present and future. *Clin Transl Immunol* 2018; **7:** e1001.
62 Wainberg M, Sinnott-Armstrong N, Mancuso N *et al.* Opportunities and challenges for transcriptome-wide association studies. *Nat Genet* 2019; **51:** 592–9.

63 Bach MA, Launois P. Mechanisms of mycobacterium leprae-specific T-cell deficiency in lepromatous leprosy. *Biochimie* 1988; **70:** 1013–8.
64 Casanova J-L, Abel L. Genetic dissection of immunity to mycobacteria: the human model. *Annu Rev Immunol* 2002; **20:** 581–620.
65 Teng MWL, Bowman EP, McElwee JJ *et al.* IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. *Nat Med* 2015; **21:** 719–29.
66 Luo Y, de Lange KM, Jostins L *et al.* Exploring the genetic architecture of inflammatory bowel disease by whole-genome sequencing identifies association at ADCY7. *Nat Genet* 2017; **49:** 186–92.
67 Carmi S, Hui KY, Kochav E *et al.* Sequencing an Ashkenazi reference panel supports population-targeted personal genomics and illuminates Jewish and European origins. *Nat Commun* 2014; **5:** 4835.
68 Rivas MA, Avila BE, Koskela J et al. Insights into the genetic epidemiology of Crohn's and rare diseases in the Ashkenazi Jewish population. *PLoS Genet* 2018; **14:** e1007329.
69 Levine AP, Pontikos N, Schiff ER *et al.* Genetic complexity of Crohn's disease in two large Ashkenazi Jewish families. *Gastroenterology* 2016; **151:** 698–709.
70 Chuang L-S, Villaverde N, Hui KY *et al.* A frameshift in CSF2RB predominant among Ashkenazi Jews increases risk for Crohn's disease and reduces monocyte signaling via GMCSF. *Gastroenterology* 2016;**151:**710–23.
71 Denson LA, Jurickova I, Karns R *et al.* Genetic and transcriptomic variation linked to neutrophil granulocyte-macrophage colony-stimulating factor signaling in pediatric Crohn's disease. *Inflamm Bowel Dis* 2019; **25:** 547-560.
72 Hui KY, Fernandez-Hernandez H, Hu J *et al.* Functional variants in the LRRK2 gene confer shared effects on risk for Crohn's disease and Parkinson's disease. *Sci Transl Med* 2018; **10:** eaai7795.
73 Ogura Y, Lala S, Xin W *et al.* Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut* 2003; **52:** 1591–7.
74 Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 2006; **313:** 1126–30.
75 Hayward JA, Mathur A, Ngo C, Man SM. Cytosolic recognition of microbes and pathogens: inflammasomes in action. *Microbiol Mol Biol Rev* 2018; **82:**e00015-18.
76 Kufer TA, Nigro G, Sansonetti PJ. Multifaceted functions of NOD-like receptor proteins in myeloid cells at the intersection of innate and adaptive immunity. *Microbiol Spectr* 2016; **4. https:**//doi.org/10.1128/microbiolspec.MCHD-0021-2015
77 Gicquel T, Victoni T, Fautrel A *et al.* Involvement of purinergic receptors and NOD-like receptor-family protein 3-inflammasome pathway in the adenosine triphosphate-induced cytokine release from macrophages. *Clin Exp Pharmacol Physiol* 2014; **41:** 279–86.
78 Lugrin J, Martinon F. The AIM2 inflammasome: sensor of pathogens and cellular perturbations. *Immunol Rev* 2018; **281:** 99–114.
79 Dubyak GR. P2X7 receptor regulation of non-classical secretion from immune effector cells. *Cell Microbiol* 2012; **14:** 1697–706.
80 Schiff ER, Frampton M, Ben-Yosef N *et al.* Rare coding variant analysis in a large cohort of Ashkenazi Jewish families with inflammatory bowel disease. *Hum Genet* 2018; **137:** 723–34.
81 Zhang X, Lu X, Yu L, Gu Y, Qu F. Downregulation of NLRP2 inhibits HUVEC viability by inhibiting the MAPK signaling pathway. *Mol Med Rep* 2018; **19:** 85–92.
82 Schorey JS, Cooper AM. Macrophage signalling upon mycobacterial infection: the MAP kinases lead the way. *Cell Microbiol* 2003; **5:** 133–42.
83 Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem* 2001; **276:** 4812–8.
84 Gerhardt T, Ley K. Monocyte trafficking across the vessel wall. *Cardiovasc Res* 2015; **107:** 321–30.
85 Yona S, Kim K-W, Wolf Y *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 2013; **38:** 79–91.
86 Auffray C, Fogg D, Garfa M *et al.* Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 2007; **317:** 666–70.
87 Inohara N, Ogura Y, Fontalba A *et al.* Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* 2003; **278:** 5509–12.
88 Manthiram K, Zhou Q, Aksentijevich I, Kastner DL. The monogenic autoinflammatory diseases define new pathways in human innate immunity and inflammation. *Nat Immunol* 2017; **18:** 832–42.
89 Clark SL. Cellular differentiation in the kidneys of newborn mice studies with the electron microscope. *J Biophys Biochem Cytol* 1957; **3:** 349–62.
90 Serramito-Gómez I, Boada-Romero E, Pimentel-Muiños FX. Unconventional autophagy mediated by the WD40 domain of ATG16L1 is derailed by the T300A Crohn disease risk polymorphism. *Autophagy* 2016; **12:** 2254–5.
91 Chauhan S, Mandell MA, Deretic V. IRGM governs the core autophagy machinery to conduct antimicrobial defense. *Mol Cell* 2015; **58:** 507–21.
92 Ohsumi Y. Historical landmarks of autophagy research. *Cell Res* 2014; **24:** 9–23.
93 Cadwell K, Liu JY, Brown SL *et al.* A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 2008; **456:** 259–63.
94 Gardet A, Benita Y, Li C *et al.* LRRK2 is involved in the IFN-gamma response and host response to pathogens. *J Immunol* 2010; **185:** 5577–85.
95 Ying H, Yue BYJT. Cellular and molecular biology of optineurin. *Int Rev Cell Mol Biol* 2012; **294:** 223–58.
96 Tumbarello DA, Kendrick-Jones J, Buss F. Myosin VI and its cargo adaptors – linking endocytosis and autophagy. *J Cell Sci* 2013; **126:** 2561–70.
97 Sorbara MT, Girardin SE. Emerging themes in bacterial autophagy. *Curr Opin Microbiol* 2015; **23:** 163–70.
98 Huang J, Brumell JH. Bacteria-autophagy interplay: a battle for survival. *Nat Rev Microbiol* 2014; **12:** 101–14.
99 Schwerd T, Pandey S, Yang H-T *et al.* Impaired antibacterial autophagy links granulomatous intestinal inflammation in Niemann-Pick disease type C1 and XIAP deficiency with NOD2 variants in Crohn's disease. *Gut* 2016; **66:** 1060-73.
100 Schapansky J, Nardozzi JD, LaVoie MJ. The complex relationships between microglia, alpha-synuclein, and LRRK2 in Parkinson's disease. *Neuroscience* 2015; **302:** 74–88.

101 Waschbüsch D, Michels H, Strassheim S *et al.* LRRK2 transport is regulated by its novel interacting partner Rab32. *PLoS ONE* 2014; **9:** e111632.
102 Villumsen M, Aznar S, Pakkenberg B, Jess T, Brudek T. Inflammatory bowel disease increases the risk of Parkinson's disease: a Danish nationwide cohort study 1977–2014. *Gut* 2019; **68:** 18–24.
103 Ryan TA, Tumbarello DA. Optineurin: a coordinator of membrane-associated cargo trafficking and autophagy. *Front Immunol* 2018; **9:** 1024.
104 Broughton SE, Dhagat U, Hercus TR *et al.* The GM-CSF/IL-3/IL-5 cytokine receptor family: from ligand recognition to initiation of signaling. *Immunol Rev* 2012; **250:** 277–302.
105 Marks DJB, Miyagi K, Rahman FZ, Novelli M, Bloom SL, Segal AW. Inflammatory bowel disease in CGD reproduces the clinicopathological features of Crohn's disease. *Am J Gastroenterol* 2009; **104:** 117–24.
106 Hazzan D, Seward S, Stock H *et al.* Crohn's-like colitis, enterocolitis and perianal disease in Hermansky-Pudlak syndrome. *Colorectal Dis* 2006; **8:** 539–43.
107 Dieckgraefe BK, Korzenik JR, Husain A, Dieruf L. Association of glycogen storage disease 1b and Crohn disease: results of a North American survey. *Eur J Pediatr* 2002; **161 (Suppl):** S88–92.
108 Sivanesan D, Beauchamp C, Quinou C *et al.* IL23R (Interleukin 23 Receptor) variants protective against inflammatory bowel diseases (IBD) display loss of function due to impaired protein stability and intracellular trafficking. *J Biol Chem* 2016; **291:** 8673–85.
109 Lubberts E. The IL-23–IL-17 axis in inflammatory arthritis. *Nat Rev Rheumatol* 2015; **11:** 415–29.
110 Yamazaki K, Umeno J, Takahashi A *et al.* A genome-wide association study identifies 2 susceptibility loci for Crohn's disease in a Japanese population. *Gastroenterology* 2013; **144:** 781–8.
111 Meylan F, Siegel RM. TNF superfamily cytokines in the promotion of Th9 differentiation and immunopathology. *Semin Immunopathol* 2017; **39:** 21–8.
112 Petitbertron A, Pedron T, Gross U *et al.* Adherence modifies the regulation of gene expression induced by interleukin-10. *Cytokine* 2004; **29:** 1–12.
113 Glocker E-O, Kotlarz D, Boztug K *et al.* Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N Engl J Med* 2009; **361:** 2033–45.
114 Engelhardt KR, Shah N, Faizura-Yeop I *et al.* Clinical outcome in IL-10- and IL-10 receptor-deficient patients with or without hematopoietic stem cell transplantation. *J Allergy Clin Immunol* 2013; **131:** 825–30.
115 Khangura SK, Kamal N, Ho N *et al.* Gastrointestinal features of Chronic granulomatous disease found during endoscopy. *Clin GastroenterolHepatol* 2015; 14: 395-402.e5
116 Ding NS, Hart A, De Cruz P. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn's disease - algorithm for practical management. *Aliment Pharmacol Ther* 2016; **43:** 30–51.
117 O'Sullivan M, O'Morain C. Liquid diets for Crohn's disease. *Gut* 2001; **48:** 757.
118 Sachar DB, Rubin KP, Gumaste V. Levamisole in Crohn's disease: a randomized, double-blind, placebo-controlled clinical trial. *Am J Gastroenterol* 1987; **82:** 536–9.
119 Korzenik JR, Dieckgraefe BK, Valentine JF, Hausman DF, Gilbert MJ. Sargramostim for active Crohn's disease. *N Engl J Med* 2005; **352:** 2193–201.
120 Lopez-Cubero SO, Sullivan KM, McDonald GB. Course of Crohn's disease after allogeneic marrow transplantation. *Gastroenterology* 1998; **114:** 433–40.
121 Hawkey CJ, Allez M, Clark MM *et al.* Autologous hematopoetic stem cell transplantation for refractory Crohn disease: a randomized clinical trial. *JAMA* 2015; **314:** 2524–34.
122 Milone MC, O'Doherty U. Clinical use of lentiviral vectors. *Leukemia* 2018; **32:** 1529–41.

Correspondence: A. W. Segal, Division of Medicine, University College London, 5 University Street, London WC1E6JF, UK.
(e-mail: t.segal@ucl.ac.uk)

