REVIEW

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Abnormalities in the handling of intracellular bacteria in Crohn's disease: a link between infectious etiology and host genetic susceptibility

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Abstract

The etiology of Crohn's disease (CD) is still poorly understood, but recent advances have highlighted the importance of the innate immune system and the critical relationship between the gut flora and the intestinal mucosa. Several combinations of genetic factors predisposing to CD have been described, with the most significant replicable associations including genes for intracellular receptors of bacterial cell walls (*NOD2*/*CARD15*) and for bacterial clearance and antigen processing via autophagy (*ATG16L1* and *IRGM*). One theoretical link between susceptibility genes *NOD2*/*CARD15*, *ATG16L1*, and *IRGM* is that CD is primarily induced by the presence of a dysfunctional immunological response to persistent infection by intracellular bacterial pathogens such as *Mycobacterium avium* subspecies *paratuberculosis* or adherent-invasive *Escherichia coli*, both first-rank candidates on the basis of host genetic susceptibility, which concerns impaired functions in the defense against intracellular bacteria.

Key words: Crohn's disease, *Mycobacterium avium* subspecies *paratuberculosis*, adherent-invasive *E. coli*, NOD2/CARD15, ATG16L1, IRGM, autophagy.

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INTRODUCTION

Idiopathic inflammatory bowel diseases (IBDs), which include Crohn's disease (CD) and ulcerative colitis (UC), are chronic disorders of the gastrointestinal tract with a combined prevalence of about 150–200 cases per 100,000 in western countries [5, 66]. Several lines of evidence suggest that bacteria play a role in the onset and perpetuation of IBD [53, 60]. Intestinal bacteria are essential for the development of intestinal inflammation since they are required for the onset of inflammation in numerous knockout models of IBD [19, 54, 65]. The pathogenesis of CD is complex and consists of three interacting elements: genetic susceptibility factors, priming by the enteric microflora, and immune-mediated tissue injury. In patients with CD, exposure of the terminal ileum post-surgically to luminal content is associated with increased inflammation, and diversion of the fecal stream is associated with improvement [58]. Patients with IBD have higher concentrations of mucosa-associated bacteria than controls without IBD [69]. Generalized or localized dysbiosis is observed in IBD, evidenced by the presence of low numbers of usual bacteria, high numbers of unusual bacteria, and sometimes a reduction in biodiversity. This upsets the balance between putative species of "protective" versus "harmful" intestinal bacteria and may promote inflammation [63, 70]. In addition, CD has features that might be the result of a microbial process in the gut. Some characteristic pathological elements of CD, including aphthous ulcers of the mucosa, mural abscesses, and macrophage and epithelioid cell granulomas, occur in well-recognized infectious diseases such as shigellosis, salmonellosis, and yersinial enterocolitis, in which invasiveness is an essential virulence factor of the bacteria involved (for a review see reference [75]). Interestingly, the earliest lesions in CD are aphthous ulcers in the intestine [26], which also occur in some viral and bacterial infections.

CD SUSCEPTIBILITY ALLELES RESPONSIBLE FOR DYSREGULATED HOST RESPONSE TO INTRACELLULAR BACTERIA

The theory of dysregulated host responses to intracellular microorganisms is emerging as a contributing factor in CD pathogenesis. Over the last decade, many IBD susceptibility loci have been identified. The most well-replicated IBD genetic association is the *NOD2* gene association to CD [35, 50]. Fine mapping of this locus led to the identification of the *NOD2/CARD15* gene (nucleotide-binding oligomerization domain 2)/(caspase activation recruitment domain 15). NOD2, a mediator of innate immunity, is a key cytoplasmic sensor of intracellular bacterial peptidoglycan. It can be activated by the minimal bioactive component muramyl dipeptide (MDP), which is present in both Gram-positive and -negative bacteria. NOD2 stimulation by MDP activates NF-κB and mitogen-activated protein (MAP) kinase pathways and contributes to antibacterial effects via various mechanisms: cytokine responses, upregulation of defensins, killing of intracellular microbes, and maintenance of epithelial barrier integrity [25, 27, 33, 42, 73]. Three uncommon polymorphisms (Arg702Trp, Gly908Arg, and Leu1007fsinsC) of *NOD2* are each highly associated with CD and located within or near the leucine-rich repeat corresponding to the bacterial sensing domain of this receptor [35, 50]. *NOD2* mutations are mostly associated with ileal involvement, structuring complications, and a slightly earlier age at onset [22].

Recent genome-wide association (GWA) studies, in addition to highlighting new susceptibility genes, have firmly established that some of them contribute to IBD, and in particular to CD [20, 31, 44, 56, 74]. A highly significant and replicated association between CD and variants in two separate autophagy genes, *ATG16L1* and *IRGM*, has been shown. In the GWA study of nearly 20,000 non-synonymous SNPs, an amino-acid polymorphism, Thr300Ala, within the *ATG16L1* (autophagy-related like 1) gene located on chromosome 2q37.1 was found to be highly associated with ileal CD [31, 56]. A second autophagic gene has been identified in CD susceptibility: *IRGM* (immunity-related GTPase family M) located on chromosome 5q33.1 [52, 74]. Autophagy is a process by which eukaryotic cells sequester and degrade cytoplasm and organelles via the lysosomal pathway, but it also plays a key role in the defense against intracellular microorganisms [6, 30, 46]. Interestingly, as already reported for *NOD2* mutations, the *ATG16L1* variant is found only in CD and has not been observed in UC [13].

ABNORMALITIES IN THE HANDLING OF INTRACELLULAR BACTERIA IN CD

Among the several combinations of genetic factors predisposing to CD that have been described, the most significant replicable associations include genes for intracellular receptors of bacterial cell walls (*NOD2/CARD15*) and for bacterial clearance and antigen processing via autophagy (*ATG16L1* and *IRGM*).

The ability of NOD2 to mediate antibacterial effects has been shown with invasive *Streptococcus pneumoniae*, *Salmonella* spp., *Mycobacterium* spp., and *Listeria monocytogenes* [2, 24, 33, 37, 51]. The frameshift mutation, Leu1007fsinsC, demonstrates a deficiency in the capacity to signal in response to MDP stimulation, since this mutant has impaired function as a defensive factor against intracellular *Salmonella* in intestinal epithelial cells [33]. This result suggests that patients carrying *NOD2* mutations may be unable to control infection with invasive bacteria. Likewise, NOD2-deficient mice show loss of protective immunity in response to bacterial MDP and the mice are susceptible to *Listeria* infection via the oral route [38]. However, NOD2 contribution to CD remains complex, and the functional consequences of *NOD2* mutations could be related to synergic effects of NOD2/Toll-like receptor (TLR) signaling and antimicrobial mechanisms [72]. In CD patients a loss of surveillance activity by NOD2 may result in the inability of local responses in the intestinal mucosa to control bacterial infection.

The *ATG16L1* gene product is part of a multimeric protein complex (ATG5, ATG12, and ATG16) required for autophagosome formation and thereby is essential for autophagy [46]. The ATG16L1 protein has been implicated in the clearance of intracellular pathogens such as *Mycobacterium tuberculosis* and *Salmonella* spp. [6, 67]. A functional knockdown of ATG16L1 by siRNA abrogates autophagy of the intracellular pathogen *Salmonella thyphimurium* in epithelial cells [56]. Parallel results have been obtained with IRGM protein, which is highly expressed in response to infection and seems to be critical for resistance to intracellular bacteria such as *Salmonella* spp. by a newly recognized form of pathogen clearance [71]. Moreover, mice rendered deficient in the homologue of *IRGM* gene have impaired ability to eliminate the intracellular pathogens *Toxoplasma gondii*, *L. monocytogenes*, and *S. thyphimurium* [14, 32]. Experiments in human macrophages showed that knockdown of *IRGM* leads to markedly prolonged survival of *M. tuberculosis* and that the murine *IRGM1* (*LRG-47*) induced autophagy and generated large autolysosomal organelles as a mechanism for the elimination of intracellular *M. tuberculosis* [67]. Defects in autophagy could predispose patients to CD by promoting prolonged survival of intracellular microorganisms within host cells.

The intracellular function of the NOD2 receptor and the defective autophagic pathway associated with genetic susceptibility to CD suggest that a primary causal organism would require (1) initial invasive ability and also (2) an inherent mechanism for chronic intracellular persistence. Thus the normal enteric flora is not sufficiently invasive or lacks virulence factors that are able to initiate disease progression. Bacterial candidates that exhibited these properties and can be detected in CD patients include *M. avium* subspecies *paratuberculosis*, the psy-

chotropic bacteria *Yersinia* spp. and *L. monocytogenes*, and adherent-invasive *Escherichia coli*.

M. AVIUM SUBSPECIES *PARATURBERCULOSIS* AS AN INTRACELLULAR PERSISTENT BACTERIA ASSOCIATED WITH CD

M. avium subspecies *paratuberculosis* causes spontaneous granulomatous enterocolitis in cattle with clinical manifestations of diarrhea and wasting, making this obligate intracellular pathogen a credible etiologic agent of CD [11]. In the past few decades, several dozen published studies have provided evidence either to claim or to dispute its association with CD. On the basis of 28 case-control studies, a meta-analysis demonstrated that the prevalence of (MAP) DNA detected by PCR in intestinal tissue samples was higher in patients with CD than in controls and UC patients [23]. Viable MAP was detected in peripheral blood in a higher proportion of CD patients than in controls [48]. The prevalence of antibodies against MAP antigens tested by ELISA was also higher in patients with CD than in controls. The most irrefutable evidence that MAP causes CD lies in the long--term remission of clinical manifestations and altered natural history of the disease following clearance of the infection by antibiotics. A recent Australian antibiotic trial, performed to evaluate the efficacy of a two-year combined antibiotic therapy, reported, however, that the proportion of patients who relapsed after two and three years was not significantly different between groups with or without antibiotic treatment [64]. Thus these results do not support a role of MAP in CD. However, the identification of mutations in genes encoding NOD2 receptor in CD patients [35, 50] and the GWA studies that identified new susceptibility loci for CD and implicated autophagy [31, 56], warrant additional research to confirm or disprove the role of MAP as an etiological agent in CD. This obligate intracellular pathogen that evades killing within phagolysosomes can selectively infect hosts with defective innate immune killing, such as NOD2 polymorphisms or defects in autophagy [57]. The mechanism of autophagy can eliminate intracellular mycobacteria [30, 67] and the NOD2 pathway has been implicated in the innate immune response to *Mycobacterium* infection, NOD2--deficient macrophages being impaired in their cytokine response to infection with *M. tuberculosis* [24]. However, no relation was found between *NOD2* mutations and positive MAP serology [4] and MAP was not preferentially recovered from ileal CD [12]

LISTERIA AND *YERSINIA* AS INVASIVE BACTERIA PUTATIVELY ASSOCIATED WITH CD

Epidemiologic assessment of familial environmental risk factors related to Western lifestyle, diet, bacteria, and domestic hygiene all pointed to refrigeration as a potential risk factor for CD. The pathogens incriminated are psychotropic bacteria that can exist and grow at temperatures between –1°C and 10°C. Referred to as the "cold chain hypothesis", this suggests that pathogenic bacteria such as *Yersinia* spp. and *Listeria* spp. contribute to CD [34]. Several studies have demonstrated the presence of *Y. enterocolitica* or *Y. pseudotuberculosis* in intestinal samples of CD [60, 61] and there are numerous aspects of yersiniosis, which resembles the inflammatory reaction seen in CD, including the presence of granulomas, that make differential diagnosis difficult [57]. *L. monocytogenes* has been identified in CD lesions by immunohistochemical analysis, but conflicting results are reported with PCR [23, 63–65]. However, *Listeria* could be a good candidate to drive inflammation since Kobayashi et al. [38] showed that NOD2-deficient mice manifested increased susceptibility to *L. monocytogenes* infection. This finding was associated with decreased levels of two specific types of mouse cryptin mRNA (defensin homologues) in Paneth cells. Moreover, mice deficient in LRG-47, the homologue of human *IRGM* gene, are unable to eliminate *L. monocytogenes* [14].

ADHERENT-INVASIVE *E. COLI* ASSOCIATED WITH ILEAL CD

E. coli is the predominant aero-anaerobic Gram--negative species of the normal intestinal flora, where it plays an important role in promoting the stability of the luminal microbial flora and maintaining normal intestinal homeostasis. As a commensal, *E. coli* and its mammalian host coexist in good harmony and rarely cause disease except when the normal gastrointestinal barrier is breached. However, some *E. coli* strains have acquired specific virulence factors that increase the ability of the bacteria to adapt to new niches and allow them to cause a broad spectrum of diseases [36]. Pathogenic *E. coli* strains are also potent inflammatory response inducers. As a general rule, the interaction between adherent and/or invasive *E. coli* and host cells induces inflammatory responses by activating several pathways which lead to NF-κB activation, upregulated IL-8 expression, and transmigration of polymorphonuclear leukocytes [16, 62]. In addition, for all motile *E. coli* strains the interaction of flagellin with TLR-5 induces a pro-inflammatory response [28, 68].

Modifications of luminal bacteria concentrations have been observed in IBD patients. Increased numbers of mucosa-associated *E. coli*, forming a biofilm on the surface of the gut mucosa, are observed in patients with CD and UC [15, 18, 39, 40, 43, 47, 49, 69]. A correlation between intestinal colonization by *E. coli* and bacterial adhesion of CD-associated *E. coli* strains to intestinal epithelial cells has been observed [18, 43]. Five independent studies have reported the presence of intramucosal *E. coli* or mucosa-associated *E. coli* with invasive properties in IBD patients [3, 17, 21, 43, 61]. On the

basis of the pathogenic traits of CD-associated *E. coli*, a new potentially pathogenic group of *E. coli* was designated AIEC for adherent-invasive *E. coli* [7]. AIEC bacteria are able to adhere to and to invade intestinal epithelial cells with a macropinocytosis-like process of entry dependent on the recruitment of actin microfilaments and microtubules. The interaction between adherent-invasive *E. coli* and intestinal epithelial cultured cells induces inflammatory responses with an upregulated expression of IL-8 and CCL20, leading to transmigration of polymorphonuclear leukocytes and dendritic cells in coculture models [21]. AIEC infection significantly decreases transepithelial resistance and induces disorganization of F-actin and displacement of ZO-1 and E-cadherin from the apical junctional complex, indicating that AIEC bacteria can reduce barrier intestinal functions [61]. In addition, AIEC bacteria are able to survive and to replicate extensively in large vacuoles within macrophages without triggering host cell death and can also induce the release of large amounts of TNF-α by infected macrophages [29]. Such behavior with macrophages indicates that these pathogens, which have evolved to resist phagocytosis and to persist within macrophages, may be involved in chronic antigenic stimulation and T-cell and macrophage activation. Moreover, in an *in vitro* model of human granulomas, AIEC bacteria cause infected macrophages to aggregate and some of them to fuse and form multinucleated giant cells, leading to recruitment of lymphocytes to form cell aggregates very similar to early stages of epithelioid granulomas [45]. This observation should be considered in conjunction with the fact that *E. coli* antigens are present in CD granulomas [8, 41] and that *E. coli* DNA is detected in 80% of micro-dissected granulomas from CD patients [59]. *In vivo*, certainly due to the lack of CEACAM6 expression in mice, the reference AIEC strain LF82 does not induce intestinal inflammation. However, in a dextran sulfate sodium-injured mouse colon model, AIEC strain LF82 significantly worsens colitis [9]. The inflammatory mucosal immune response to AIEC bacteria is dependent on the expression of bacterial flagella, and upregulated expression of TLR-5 and IPAF flagellin receptors is observed.

Adherent-invasive *E. coli* strains were found to be highly associated with ileal mucosa in CD patients [17]. The high prevalence of AIEC strains associated with the ileal mucosa observed in CD patients is related to abnormal expression of a specific host receptor recognized by bacterial lectin-like surface adhesins in a genetically predisposed host gut segment. CD-associated AIEC strains adhere to the brush border of primary ileal enterocytes isolated from CD patients but not from controls without inflammatory bowel disease [1]. Most AIEC strains associated with CD ileal mucosa express type 1 pili, which are responsible for bacterial adhesion to the carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) expressed on the apical side of ileal epithelial cells. CEACAM6, acting as a receptor for AIEC adhesion, is abnormally expressed by ileal epithelial cells in CD patients in both uninvolved and inflamed ileal mucosa. Increased CEACAM6 expression results from interferon (IFN)-γ or TNF-α stimulation and infection with AIEC bacteria, indicating that AIEC can promote their own colonization in CD patients. The presence of AIEC bacteria and their ability to induce the secretion of pro-inflammatory cytokines by infected macrophages lead to an amplification loop of colonization and inflammation (Fig. 1). However, this could be due to sustained immune stimulation with increased levels of inflammatory cytokines even in the absence of

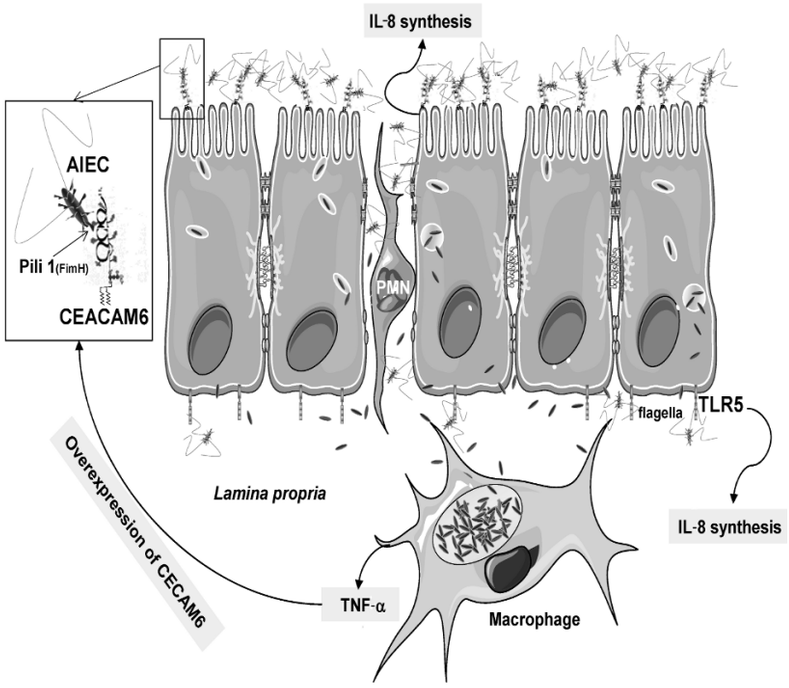

Fig. 1. Colonization of the ileal mucosa by AIEC bacteria. Adherent-invasive *E. coli* adhere via type 1 pili to CEACAM6, which is abnormally expressed by intestinal epithelial cells in Crohn's disease patients. AIEC bacteria adhering to intestinal epithelial cells induce the secretion of IL-8, through signaling via flagellin and TLR-5, and the transmigration of polymorphonuclear leukocytes. The invasive phenotype of AIEC allows the bacteria to cross the mucosal barrier. AIEC bacteria replicate within infected macrophages lying beneath the epithelial barrier and induce TNF-α secretion. Both colonization of the ileal mucosa by AIEC and stimulation of ileal epithelial cells by TNF-α released by AIEC-infected macrophages induce the overexpression of CEACAM6 at the lumen-faced surface of the mucosa, leading to an amplification loop of colonization and inflammation. Abbreviations: AIEC – adherent-invasive *E. coli*; CEACAM6 – carcinoembryonic antigen-related cell adhesion molecule 6; PMN – polymorphonuclear leukocyte; TNF-α – tumor necrosis factor-α; IL-8 – interleukin-8.

patent inflammation, as already reported in the intestinal mucosa of CD patients [55]. Alternatively, inducible expression of CEACAM6 in the ileal mucosa of CD patients could be genetically linked. We observed that cytokine stimulation of intestinal epithelial cells that did not express CEACAM6 did not induce CEACAM6 expression. The significantly increased ileal CEACAM6 expression found in uninvolved ileal mucosa of CD patients compared with controls without IBD suggests that patients expressing a basal level of CEACAM6 are genetically predisposed to overexpress that molecule. Of note, the gene encoding CEACAM6 is located on the long arm of human chromosome 19 (q13.2 between CYP2A and D19S15), close to a suggested IBD susceptibility locus [10]. In addition to *NOD2*/*CARD15*, *ATG16L1*, and *IRGM*, abnormal ileal CEACAM6 expression could be another susceptibility factor for CD. Accordingly, patients expressing a basal level of CEACAM6 would be genetically predisposed to develop ileal CD, and the presence of AIEC bacteria and the secretion of IFN-γ and TNF-α would lead to an amplification loop of colonization and inflammation.

No studies have yet investigated the link between expression of NOD2, ATG16L1, or IRGM variants and AIEC infection. Much of the mucosal inflammation observed with entero-invasive *E. coli,* a bacteria very similar to *Shigella* spp., is initiated by intracellular sensing of peptidoglycan by cytosolic leucine-rich receptors of the NOD family in epithelial cells and the invasive phenotype can account for the potent pro-inflammatory ability of the bacteria [28]. We can speculate that AIEC bacteria might take advantage of defects in intracellular sensing of bacteria or in autophagy.

CONCLUSION

One theoretical link between host susceptibility genes (*NOD2*/*CARD15*, *ATG16L1*, and *IRGM*) is that CD is primarily induced by the presence of a dysfunctional immunological response to persistent infection by intracellular bacterial pathogens. *M. avium* subspecies *paratuberculosis* and adherent-invasive *E. coli* are good candidates for an infectious etiology of CD. These microorganisms can invade, survive, and replicate within host cells and have been detected in many CD patients. In patients with impaired functions such as a defensive factor against intracellular bacteria, the multiplication of any persistent intracellular bacteria such as *M. avium* subspecies *paratuberculosis* and adherent-invasive *E. coli* is no longer regulated and could trigger chronic uncontrolled intestinal inflammation.

REFERENCES

1. Barnich N., Carvalho F. A., Glasser A. L., Darcha C., Jantscheff P., Allez M., Peeters H., Bommelaer G., Desreumaux P., Colombel J. F. and Darfeuille-Michaud A. (2007): CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. J. Clin. Invest., **117**, 1566–574.
2. Barnich N., Hisamatsu T., Aguirre J. E., Xavier R., Reinecker H. C. and Podolsky D. K. (2005): GRIM-19 interacts with nucleotide oligomerization domain 2 and serves as downstream effector of anti-bacterial function in intestinal epithelial cells. J. Biol. Chem., **280**, 19021–19026.
3. Baumgart M., Dogan B., Rishniw M., Weitzman G., Bosworth B., Yantiss R., Orsi R. H., Wiedmann M., McDonough P., Kim S. G., Berg D., Schukken Y., Scherl E. and Simpson K. W. (2007): Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. ISME J., **1**, 403–418.
4. Bernstein C. N., Wang M. H., Sargent M., Brant S. R. and Collins M. T. (2007): Testing the interaction between NOD-2 status and serological response to *Mycobacterium paratuberculosis* in cases of inflammatory bowel disease. J. Clin. Microbiol., **45**, 968–971.
5. Binder V. (2004): Epidemiology of IBD during the twentieth century: an integrated view. Best Pract. Res. Clin. Gastroenterol., **18**, 463–479.
6. Birmingham C. L., Smith A. C., Bakowski M. A., Yoshimori T. and Brumell J. H. (2006): Autophagy controls *Salmonella* infection in response to damage to the *Salmonella*-containing vacuole. J. Biol. Chem., **281**, 11374–11383.
7. Boudeau J., Glasser A. L., Masseret E., Joly B. and Darfeuille-Michaud A. (1999): Invasive ability of an *Escherichia coli* strain isolated from the ileal mucosa of a patient with Crohn's disease. Infect. Immun., **67**, 4499–4509.
8. Cartun R. W., Van Kruiningen H. J., Pedersen C. A. and Berman M. M. (1993): An immunocytochemical search for infectious agents in Crohn's disease. Mod. Pathol., **6**, 212–219.
9. Carvalho F. A., Barnich N., Sauvanet P., Darcha C., Gelot A. and Darfeuille-Michaud A. (2008): Crohn's disease-associated *Escherichia coli* LF82 aggravates colitis in injured mouse colon via signaling by flagellin. Inflamm. Bowel Dis., **14**, 1051–1060.
10. Chamaillard M., Iacob R., Desreumaux P. and Colombel J. F. (2006): Advances and perspectives in the genetics of inflammatory bowel diseases. Clin. Gastroenterol. Hepatol., **4**, 143–151.
11. Chiodini R. J. (1989): Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. Clin. Microbiol. Rev., **2**, 90–117.
12. Chiodini R. J., Van Kruiningen H. J., Thayer W. R., Merkal R. S. and Coutu J. A. (1984): Possible role of mycobacteria in inflammatory bowel disease. I. An unclassified Mycobacterium species isolated from patients with Crohn's disease. Dig. Dis. Sci., **29**, 1073–1079.
13. Cho J. H. and Weaver C. T. (2007): The genetics of inflammatory bowel disease. Gastroenterology, **133**, 1327–1339.
14. Collazo C. M., Yap G. S., Sempowski G. D., Lusby K. C., Tessarollo L., Woude G. F., Sher A. and Taylor G. A. (2001): Inactivation of LRG-47 and IRG-47 reveals a family of interferon gamma-inducible genes with essential, pathogen-specific roles in resistance to infection. J. Exp. Med., **194**, 181–188.
15. Conte M. P., Schippa S., Zamboni I., Penta M., Chiarini F.,

Seganti L., Osborn J., Falconieri P., Borrelli O. and Cucchiara S. (2006): Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease. Gut, **55**, 1760–1767.
16. Dahan S., Busuttil V., Imbert V., Peyron J. F., Rampal P. and Czerucka D. (2002): Enterohemorrhagic *Escherichia coli* infection induces interleukin-8 production via activation of mitogen-activated protein kinases and the transcription factors NF-kappaB and AP-1 in T84 cells. Infect. Immun., **70**, 2304–2310.
17. Darfeuille-Michaud A., Boudeau J., Bulois P., Neut C., Glasser A. L., Barnich N., Bringer M. A., Swidsinski A., Beaugerie L. and Colombel J. F. (2004): High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. Gastroenterology, **127**, 412–421.
18. Darfeuille-Michaud A., Neut C., Barnich N., Lederman E., Di Martino P., Desreumaux P., Gambiez L., Joly B., Cortot A. and Colombel J. F. (1998): Presence of adherent *Escherichia coli* strains in ileal mucosa of patients with Crohn's disease. Gastroenterology, **115**, 1405–1413.
19. Dianda L., Hanby A. M., Wright N. A., Sebesteny A., Hayday A. C. and Owen M. J. (1997): T cell receptor-alpha beta-deficient mice fail to develop colitis in the absence of a microbial environment. Am. J. Pathol., **150**, 91–97.
20. Duerr R. H., Taylor K. D., Brant S. R., Rioux J. D., Silverberg M. S., Daly M. J., Steinhart A. H., Abraham C., Regueiro M., Griffiths A., Dassopoulos T., Bitton A., Yang H., Targan S., Datta L. W., Kistner E. O., Schumm L. P., Lee A. T., Gregersen P. K., Barmada M. M., Rotter J. I., Nicolae D. L. and Cho J. H. (2006): A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science, **314**, 1461–1463.
21. Eaves-Pyles T., Allen C. A., Taormina J., Swidsinski A., Tutt C. B., Jezek G. E., Islas-Islas M. and Torres A. G. (2008): *Escherichia coli* isolated from a Crohn's disease patient adheres, invades, and induces inflammatory responses in polarized intestinal epithelial cells. Int. J. Med. Microbiol., **298**, 397–409.
22. Economou M., Trikalinos T. A., Loizou K. T., Tsianos E. V. and Ioannidis J. P. (2004): Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. Am. J. Gastroenterol., **99**, 2393–2404.
23. Feller M., Huwiler K., Stephan R., Altpeter E., Shang A., Furrer H., Pfyffer G. E., Jemmi T., Baumgartner A. and Egger M. (2007): *Mycobacterium avium* subspecies paratuberculosis and Crohn's disease: a systematic review and meta-analysis. Lancet Infect. Dis., **7**, 607–613.
24. Ferwerda G., Girardin S. E., Kullberg B. J., Le Bourhis L., de Jong D. J., Langenberg D. M., van Crevel R., Adema G. J., Ottenhoff T. H., Van der Meer J. W. and Netea M. G. (2005): NOD2 and toll-like receptors are nonredundant recognition systems of *Mycobacterium tuberculosis*. PLoS Pathog., **1**, 279–285.
25. Fritz J. H., Ferrero R. L., Philpott D. J. and Girardin S. E. (2006): Nod-like proteins in immunity, inflammation and disease. Nat. Immunol., **7**, 1250–1257.
26. Fujimura Y., Kamoi R. and Iida M. (1996): Pathogenesis of aphthoid ulcers in Crohn's disease: correlative findings by magnifying colonoscopy, electron microscopy, and immunohistochemistry. Gut, **38**, 724–732.
27. Girardin S. E., Boneca I. G., Carneiro L. A., Antignac A., Jehanno M., Viala J., Tedin K., Taha M. K., Labigne A., Zahringer U., Coyle A. J., DiStefano P. S., Bertin J., Sansonetti P. J. and Philpott D. J. (2003): Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science, **300**, 1584–1587.
28. Girardin S. E., Tournebize R., Mavris M., Page A. L., Li X., Stark G. R., Bertin J., DiStefano P. S., Yaniv M., Sansonetti P. J. and Philpott D. J. (2001): CARD4/Nod1 mediates NF-kappaB and JNK activation by invasive *Shigella flexneri*. EMBO Rep., **2**, 736–742.
29. Glasser A. L., Boudeau J., Barnich N., Perruchot M. H., Colombel J. F. and Darfeuille-Michaud A. (2001): Adherent invasive *Escherichia coli* strains from patients with Crohn's disease survive and replicate within macrophages without inducing host cell death. Infect. Immun., **69**, 5529–5537.
30. Gutierrez M. G., Master S. S., Singh S. B., Taylor G. A., Colombo M. I. and Deretic V. (2004): Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. Cell, **119**, 753–766.
31. Hampe J., Franke A., Rosenstiel P., Till A., Teuber M., Huse K., Albrecht M., Mayr G., De La Vega F. M., Briggs J., Gunther S., Prescott N. J., Onnie C. M., Hasler R., Sipos B., Folsch U. R., Lengauer T., Platzer M., Mathew C. G., Krawczak M. and Schreiber S. (2007): A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat. Genet., **39**, 207–211.
32. Henry S. C., Daniell X., Indaram M., Whitesides J. F., Sempowski G. D., Howell D., Oliver T. and Taylor G. A. (2007): Impaired macrophage function underscores susceptibility to *Salmonella* in mice lacking Irgm1 (LRG-47). J. Immunol., **179**, 6963–6972.
33. Hisamatsu T., Suzuki M., Reinecker H. C., Nadeau W. J., McCormick B. A. and Podolsky D. K. (2003): CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology, **124**, 993–1000.
34. Hugot J. P., Alberti C., Berrebi D., Bingen E. and Cezard J. P. (2003): Crohn's disease: the cold chain hypothesis. Lancet, **362**, 2012–2015.
35. Hugot J. P., Chamaillard M., Zouali H., Lesage S., Cezard J. P., Belaiche J., Almer S., Tysk C., O'Morain C. A., Gassull M., Binder V., Finkel Y., Cortot A., Modigliani R., Laurent-Puig P., Gower-Rousseau C., Macry J., Colombel J. F., Sahbatou M. and Thomas G. (2001): Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature, **411**, 599–603.
36. Kaper J. B., Nataro J. P. and Mobley H. L. (2004): Pathogenic *Escherichia coli*. Nat. Rev. Microbiol., **2**, 123–140.
37. Kobayashi K., Inohara N., Hernandez L. D., Galan J. E., Nunez G., Janeway C. A., Medzhitov R. and Flavell R. A. (2002): RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. Nature, **416**, 194–199.
38. Kobayashi K. S., Chamaillard M., Ogura Y., Henegariu O., Inohara N., Nunez G. and Flavell R. A. (2005): Nod2--dependent regulation of innate and adaptive immunity in the intestinal tract. Science, **307**, 731–734.
39. Kotlowski R., Bernstein C. N., Sepehri S. and Krause D. O. (2007): High prevalence of *Escherichia coli* belonging to the B2+D phylogenetic group in inflammatory bowel disease. Gut, **56**, 669–675.
40. Lederman E. N., Desrumeaux P. et al. (1997): Bacterial overgrowth in the neoterminal ileum after ileocolonic resection for Crohn's disease. Gastroenterology, **112**, A1023.
41. Liu Y., van Kruiningen H. J., West A. B., Cartun R. W.,

Cortot A. and Colombel J. F. (1995): Immunocytochemical evidence of *Listeria*, *Escherichia coli*, and *Streptococcus* antigens in Crohn's disease. Gastroenterology, **108**, 1396–1404.
42. Maeda S., Hsu L. C., Liu H., Bankston L. A., Iimura M., Kagnoff M. F., Eckmann L. and Karin M. (2005): Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. Science, **307**, 734–738.
43. Martin H. M., Campbell B. J., Hart C. A., Mpofu C., Nayar M., Singh R., Englyst H., Williams H. F. and Rhodes J. M. (2004): Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. Gastroenterology, **127**, 80–93.
44. Massey D. C. and Parkes M. (2007): Genome-wide association scanning highlights two autophagy genes, ATG16L1 and IRGM, as being significantly associated with Crohn's disease. Autophagy, **3**, 649–651.
45. Meconi S., Vercellone A., Levillain F., Payre B., Al Saati T., Capilla F., Desreumaux P., Darfeuille-Michaud A. and Altare F. (2007): Adherent-invasive *Escherichia coli* isolated from Crohn's disease patients induce granulomas *in vitro*. Cell Microbiol., **9**, 1252–1261.
46. Mizushima N. (2007): Autophagy: process and function. Genes Dev., **21**, 2861–2873.
47. Mylonaki M., Rayment N. B., Rampton D. S., Hudspith B. N. and Brostoff J. (2005): Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease. Inflamm. Bowel Dis., **11**, 481–487.
48. Naser S. A., Ghobrial G., Romero C. and Valentine J. F. (2004): Culture of *Mycobacterium avium* subspecies paratuberculosis from the blood of patients with Crohn's disease. Lancet, **364**, 1039–1044.
49. Neut C., Bulois P., Desreumaux P., Membre J. M., Lederman E., Gambiez L., Cortot A., Quandalle P., van Kruiningen H. and Colombel J. F. (2002): Changes in the bacterial flora of the neoterminal ileum after ileocolonic resection for Crohn's disease. Am. J. Gastroenterol., **97**, 939–946.
50. Ogura Y., Bonen D. K., Inohara N., Nicolae D. L., Chen F. F., Ramos R., Britton H., Moran T., Karaliuskas R., Duerr R. H., Achkar J. P., Brant S. R., Bayless T. M., Kirschner B. S., Hanauer S. B., Nunez G. and Cho J. H. (2001): A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature, **411**, 603–606.
51. Opitz B., Puschel A., Schmeck B., Hocke A. C., Rosseau S., Hammerschmidt S., Schumann R. R., Suttorp N. and Hippenstiel S. (2004): Nucleotide-binding oligomerization domain proteins are innate immune receptors for internalized *Streptococcus pneumoniae*. J. Biol. Chem., **279**, 36426–36432.
52. Parkes M., Barrett J. C., Prescott N. J., Tremelling M., Anderson C. A., Fisher S. A., Roberts R. G., Nimmo E. R., Cummings F. R., Soars D., Drummond H., Lees C. W., Khawaja S. A., Bagnall R., Burke D. A., Todhunter C. E., Ahmad T., Onnie C. M., McArdle W., Strachan D., Bethel G., Bryan C., Lewis C. M., Deloukas P., Forbes A., Sanderson J., Jewell D. P., Satsangi J., Mansfield J. C., Cardon L. and Mathew C. G. (2007): Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat. Genet., **39**, 830–832.
53. Podolsky D. K. (2002): Inflammatory bowel disease. N. Engl. J. Med., **347**, 417–429.
54. Rath H. C., Herfarth H. H., Ikeda J. S., Grenther W. B., Hamm T. E. Jr., Balish E., Taurog J. D., Hammer R. E., Wilson K. H. and Sartor R. B. (1996): Normal luminal bacteria, especially *Bacteroides* species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. J. Clin. Invest., **98**, 945–953.
55. Reimund J. M., Wittersheim C., Dumont S., Muller C. D., Kenney J. S., Baumann R., Poindron P. and Duclos B. (1996): Increased production of tumour necrosis factor-alpha interleukin-1 beta, and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. Gut, **39**, 684–689.
56. Rioux J. D., Xavier R. J., Taylor K. D., Silverberg M. S., Goyette P., Huett A., Green T., Kuballa P., Barmada M. M., Datta L. W., Shugart Y. Y., Griffiths A. M., Targan S. R., Ippoliti A. F., Bernard E. J., Mei L., Nicolae D. L.,. Regueiro M, Schumm L. P., Steinhart A. H., Rotter J. I., Duerr R. H., Cho J. H., Daly M. J. and Brant S. R. (2007): Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat. Genet., **39**, 596–604.
57. Rumsey J., Valentine J. F. and Naser S. A. (2006): Inhibition of phagosome maturation and survival of *Mycobacterium avium* subspecies paratuberculosis in polymorphonuclear leukocytes from Crohn's disease patients. Med. Sci. Monit., **12**, BR130–139.
58. Rutgeerts P., Goboes K., Peeters M., Hiele M., Penninckx F., Aerts R., Kerremans R. and Vantrappen G. (1991): Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet, **338**, 771–774.
59. Ryan P., Kelly R. G., Lee G., Collins J. K., O'Sullivan G. C., O'Connell J. and Shanahan F. (2004): Bacterial DNA within granulomas of patients with Crohn's disease: detection by laser capture microdissection and PCR. Am. J. Gastroenterol., **99**, 1539–1543.
60. Sartor R. B. (2008): Microbial influences in inflammatory bowel diseases. Gastroenterology, **134**, 577–594.
61. Sasaki M., Sitaraman S. V., Babbin B. A., Gerner-Smidt P., Ribot E. M., Garrett N., Alpern J. A., Akyildiz A., Theiss A. L., Nusrat A. and Klapproth J. M. (2007): Invasive *Escherichia coli* are a feature of Crohn's disease. Lab. Invest., **87**, 1042–1054.
62. Savkovic S. D., Koutsouris A. and Hecht G. (2003): PKC zeta participates in activation of inflammatory response induced by enteropathogenic *E. coli*. Am. J. Physiol. Cell Physiol., **285**, C512–521.
63. Seksik P., Sokol H., Lepage P., Vasquez N., Manichanh C., Mangin I., Pochart P., Dore J. and Marteau P. (2006): Review article: the role of bacteria in onset and perpetuation of inflammatory bowel disease. Aliment Pharmacol. Ther., **24** (suppl. 3), 11–18.
64. Selby W., Pavli P., Crotty B., Florin T., Radford-Smith G., Gibson P., Mitchell B., Connell W., Read R., Merrett M., Ee H. and Hetzel D. (2007): Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease. Gastroenterology, **132**, 2313–2319.
65. Sellon R. K., Tonkonogy S., Schultz M., Dieleman L. A., Grenther W., Balish E., Rennick D. M. and Sartor R. B. (1998): Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect. Immun., **66**, 5224–5231.
66. Shanahan F. (2002): Crohn's disease. Lancet, **359**, 62–69.
67. Singh S. B., Davis A. S., Taylor G. A. and Deretic V. (2006): Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science, **313,** 1438–1441.
68. Steiner T. S., Nataro J. P., Poteet-Smith C. E., Smith J. A.

and Guerrant R. L. (2000): Enteroaggregative *Escherichia coli* expresses a novel flagellin that causes IL-8 release from intestinal epithelial cells. J. Clin. Invest., **105**, 1769–1777.
69. Swidsinski A., Ladhoff A., Pernthaler A., Swidsinski S., Loening-Baucke V., Ortner M., Weber J., Hoffmann U., Schreiber S., Dietel M. and Lochs H. (2002): Mucosal flora in inflammatory bowel disease. Gastroenterology, **122**, 44–54.
70. Tamboli C. P., Neut C., Desreumaux P. and Colombel J. F. (2004): Dysbiosis in inflammatory bowel disease. Gut, **53**, 1–4.
71. Taylor G. A. (2007): IRG proteins: key mediators of interferon-regulated host resistance to intracellular pathogens. Cell Microbiol., **9**, 1099–1107.
72. Watanabe T., Kitani A., Murray P. J. and Strober W. (2004): NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat. Immunol., **5**, 800–808.
73. Wehkamp J., Salzman N. H., Porter E., Nuding S., Weichenthal M., Petras R. E., Shen B., Schaeffeler E., Schwab M., Linzmeier R., Feathers R. W., Chu H., Lima H. Jr., Fellermann K., Ganz T., Stange E. F. and Bevins C. L. (2005): Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc. Natl. Acad. Sci. USA, **102**, 18129–18134.
74. Wellcome Trust Case Control Consortium (2007): Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature, **447**, 661–678.
75. Zumla A. and James D. G. (1996): Granulomatous infections: etiology and classification. Clin. Infect. Dis., **23**, 146–158.