REVIEW ARTICLES

CROHN'S DISEASE: A ROLE OF GUT MICROBIOTA AND NOD2 GENE POLYMORPHISMS IN DISEASE PATHOGENESIS

Lucia Hrnčířová^{1,2}, Jan Krejsek², Igor Šplíchal¹, Tomáš Hrnčíř¹

Institute of Microbiology, Academy of Sciences of the Czech Republic, Doly 183, 549 22 Nový Hrádek, Czech Republic: Department of Immunology and Gnotobiology¹; Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Králové, Czech Republic: Department of Clinical Immunology and Allergology²

Summary: Crohn's disease is a chronic immune-mediated intestinal inflammation targeted against a yet incompletely defined subset of commensal gut microbiota and occurs on the background of a genetic predisposition under the influence of environmental factors. Genome-wide association studies have identified about 70 genetic risk loci associated with Crohn's disease. The greatest risk for Crohn's disease represent polymorphisms affecting the CARD15 gene encoding nucleotide-binding oligomerization domain 2 (NOD2) which is an intracellular sensor for muramyl dipeptide, a peptidoglycan constituent of bacterial cell wall. The accumulated evidence suggests that gut microbiota represent an essential, perhaps a central factor in the induction and maintaining of Crohn's disease where dysregulation of normal co-evolved homeostatic relationships between intestinal microbiota and host mucosal immune system leads to intestinal inflammation. Taken together, these findings identify Crohn's disease as a syndrome of overlapping phenotypes that involves variable influences of genetic and environmental factors. A deeper understanding of different genetic abnormalities underlying Crohn's disease together with the identification of beneficial and harmful components of gut microbiota and their interactions are essential conditions for the categorization of Crohn's disease patients, which enable us to design more effective, preferably causative, individually tailored therapy.

Key words: Autoimmune diseases; Commensal microflora; Hygiene hypothesis; Inflammatory bowel diseases; Nod2

Crohn's disease

Crohn's disease (CD), ulcerative colitis, and intermediate colitis are immune-mediated chronic intestinal disorders named together as inflammatory bowel diseases (IBD). IBD is thought to be the result of an overaggressive immune response to a subset of commensal gut microbiota in a genetically susceptible host, with disease initiated by environmental triggers (1). CD is a segmental, transmural inflammation of intestinal wall that can affect any part of gastrointestinal tract from the mouth to the anus with skip areas interspersed between one or more involved areas. Most commonly CD affects terminal ileum, cecum, perianal area, and colon. Histologically, CD manifests itself by a transmural, dense infiltration of lymphocytes and macrophages, the presence of granulomas (in up to 60% of patients), fissuring ulceration and submucosal fibrosis. CD can cause significant morbidity such as diarrhea, pain, narrowing of the gut lumen leading to strictures and bowel obstruction, abscess formation, and fistulization to skin and internal organs. CD can also be associated with other medical conditions, including

ACTA MEDICA (Hradec Králové) 2014; 57(3): 89–96 http://dx.doi.org/10.14712/18059694.2014.46 arthritis, osteoporosis, eye inflammation, blood clots, liver disease, and skin rashes.

Incidence

IBD are a public health problem in industrialized countries, where two in 1,000 people are affected. Most patients are young adults. The incidence of IBD has increased greatly in western countries since the Second World War but is beginning to level off. However, the incidence is still rising in low-incidence areas such as Eastern Europe, Asia, and developing countries (2). The highest incidence of CD has been reported in northern Europe, the United Kingdom, North America (8–14/100,000) and New Zealand; the most affected is Canterbury County, New Zealand with incidence 16.5/100,000 people (3). The prevalence of CD in the West is 120-200/100,000 persons (3-5). The incidence is about 1-3 per 100,000 in southern Europe, South Africa, and Australia, and is even lower, less than 1 per 100,000, in Asia and South America. CD is more prevalent in whites than in African Americans and Asians. In the United States, Europe, and South Africa, Crohn's disease is 2–4 times more common among Jewish people of Ashkenazi origin than among other ethnic or social groups (6).

Etiology

The etiology of IBD has been extensively studied. However, causative factors in disease pathology are not yet fully understood. IBD is thought to result from the interaction between genetic and environmental factors that influence the composition of normal commensal gut microbiota to trigger an inappropriate mucosal immune response (7).

The mucosa of gastrointestinal tract with surface of approximately 200 m² forms a platform where large quantities of antigenic, mitogenic and toxic stimuli present in food together with resident commensal microbiota interact with cells of the human body. These two compartments are separated by physical barriers formed by epithelial layer single layer of interconnected, polarized epithelial cells, reinforced by tight junctions, and a basement membrane, which separates it from the connective and supporting tissue, by chemical barriers – mucus and humoral factors, i.e. secretory immunoglobulin A and antimicrobial peptides, and biological barriers, which ensure microbiota as a compact ecologic community. But these two worlds are not ultimately divided, in fact in the gut there is a complex network of sensing and regulatory signaling cascades that is essential for proper activation together with a timely inactivation of the pathway (8, 99).

A characteristic feature of the immune system in mucosa is mucosal tolerance, the ability of discriminating between potentially pathogenic microorganisms and harmless antigens, it has developed a great redundancy of various mechanisms that ensure essential defense functions and simultaneously the prevention of immune system stimulation to food antigens, environmental allergens and components of microbiota. The immune system has two, broadly cooperative components. The first line of defense, the innate nonspecific immune system, which comprises a large number of cell populations present in mucosa and mucosa-associated lymphoid tissue and relies on humoral factors and germline-encoded pattern recognition receptors, promotes the immediate detection and rapid destruction of microorganisms. The cells of innate immunity also produce factors essential for subsequent initiation of specific immunity. Tight control of innate immunity is critical to mucosal homeostasis in the intestine. An adaptive, antigen-specific immune system arises as a consequence of antigen exposure. Adaptive immunity is initiated when antigen-presenting cells, primarily dendritic cells, present antigen to lymphocytes in inductive immune compartments, such as lymph nodes and Peyer's patches. Naive T cells mature to effector T lymphocyte: T_C , T_H1 , T_H2 , T_H17 or T_{reg} lineages depending on additional signals and cytokine milieu. Intraepithelial lymphocytes serve to regulate intestinal homeostasis, maintain epithelial barrier function, respond to infection and regulate adaptive and innate immune responses (9). B cells mature in T cell dependent and T cell independent routes into plasma cells and commit predominantly to IgA production by class-switch recombination (8, 10, 11, 99).

The microenvironment of the gut is mainly tolerogenic, the dominant mucosal immune response mechanisms are those that dampen the immune and inflammatory responses and limit inflammation that could injure the mucosal layer. Mucosal tolerance to microbiota is a fundamental mechanism of maintaining intestinal homeostasis. The major mechanisms underlying immunologic tolerance generally include deletion of antigen-reactive T cells, clonal anergy of antigen-reactive T cells, and induction of antigen-specific regulatory T cells. Diminished T-cell-mediated responses efficiently suppress the otherwise unavoidable overstimulation of the immune system. The concomitant induction of humoral immune responses in the mucosal compartment and T-cell unresponsiveness in the systemic compartment are two mutually complementary mechanisms which in concert achieve fundamental defense principles (8).

During the last decade new metagenomic approaches were used to analyze the composition of microbiota and its metagenome. The interactions between the gut microbiota and the host are analyzed in fuctional studies. To investigate the role of microbiota in the development of immune system (12), the pathogenesis of inflammatory diseases (13, 14), autoimmune diseases and cancer (15) gnotobiological approaches are exploited (11).

Environmental factors

Environmental risk factors involved in IBD include factors that have an influence on the composition of gut microbiota (i.e. maternal exposure, breastfeeding, diet, antibiotics, infections), and factors that affect the mucosal immune system (i.e. smoking, non-steroidal anti-inflammatory drugs, oral contraceptive pills, stress, vaccination, intestinal permeability, and appendectomy) (1, 16, 17). Improved sanitation and hygiene along with decreased exposure to enteric organisms during early childhood, may lead to a greater susceptibility of developing an inappropriate immunological response upon exposure to new antigens later in life (18, 19). The finding is supported by epidemiological data and forms the basis of the so-called "hygiene hypothesis".

Genetic factors

An important role of genetic factors in IBD was first suggested by epidemiological studies showing familial aggregation of IBD and by twin studies. Monozygotic twin studies show the concordance rate for disease being 40-60% (20).

Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNP) in 71 genes that are associated with CD (21). Functionally, these genetic risks can be characterized broadly as abnormalities in innate immune responses, immunoregulation, or mucosal barrier function, namely, Nod2-dependent innate immunity, barrier function, epithelial restitution, microbial defense, innate immune regulation, reactive oxygen species (ROS) generation, autophagy, the inflammasome pathway, regulation of adaptive immunity, interleukin-23 (IL-23)-IL-17 circuitry, endoplasmic reticulum stress, and metabolic pathways associated with cellular homeostasis (22).

In particular, Nod2 on chromosome 16 was the first gene associated with susceptibility to CD (23). Among the three main SNPs identified in Nod2 (R702W, G908R, L1007fs), the L1007fs (frame shift stop-codon mutation that leads to a partial deletion of the terminal LRR of the protein) displays the strongest association with CD (23-25) and 1007fs homozygous patients demonstrate much severe disease phenotype than other patients with CD with an early disease onset and long-segment ileal stenosis and entero-enteral fistulas. They frequently need a surgical intervention and have a high risk of restenosis. This mutation is also associated with gastroduodenal involvement (26-28). In addition to CD, three missense mutations (R334W, R334QQ an L469F) in the nucleotide-binding domain of Nod2 confer susceptibility to another granulomatous disorder affecting the eyes, skin, and joints known as Blau syndrome (29) and early onset sarcoidosis (30).

Between 30% and 50% of CD patients in the Western hemisphere carry at least one mutated Nod2 allele. Individuals who carry two of these mutated Nod2 alleles have a 20-40-fold increased risk of developing CD (23, 24). The patients with double-dose mutations are characterized by a younger age at onset, a more frequent stricturing phenotype, and a less frequent colonic involvement than are seen in those patients who had no mutation (31). However, normal individuals might have Nod2 mutations on both chromosomes in the absence of disease (32) and also mice deficient in Nod2 do not develop CD spontaneously (33). In some populations the carrier rate in healthy controls of CARD15 mutations associated with Crohn's disease is 20% (34). None of the three mutations in the Nod2 gene was found in 483 Japanese patients with CD (35) and also were not to be associated with CD in Chinese (36) and Korean (37) patients. It is of great interest and relevance to note that the three LRR domain mutations are also associated with graft-versus-host disease (38).

Nod2 structure and function

Nod2 (nucleotide-binding oligomerization domain 2) is an important host defense and regulatory factor. It is a germ line-encoded pattern-recognition receptor, which belongs to the NLR (nucleotide-binding domain, leucine rich-repeat containing) family. Pattern recognition receptors of the innate immune system cells and epithelial cells, including Toll-like receptors (TLRs), NLRs, C-type lectins, and retinoic acid-inducible gene I-like receptors, sense microbial and danger motifs and are required for the activation and shaping of the adaptive and regulatory immune responses, mucosal regeneration and tissue repair (22).

Nod2 contains two tandem caspase recruitment domains (CARD1 and 2) on its N-terminal side, a central nucleotide-binding domain (NBD), and leucine-rich repeat (LRR) domain on its C-terminus. The P-loop containing the ATP-binding motif is located in the NBD domain and allows conformational change of the molecule. The LRR domain has receptor function and is also responsible for cell membrane association of Nod2 (39). The three main CD- and GvHD-associated SNPs (R702W, G908R and 1007fs) are in LRR domain and the Blau syndrome-associated mutations (R334W, R334Q and L469F) are in the NBD domain.

Nod2 recognizes muramyl dipeptide (MDP), a component of bacterial cell wall peptidoglycan, and viral ssRNA (40). Nod2 is found both in the cytoplasm and in association with the cell membrane through two amino acids in LRR domain, membrane association is necessary for Nod2 stimulation (39). Nod2 is expressed in myeloid cells such as monocytes (41), macrophages, dendritic cells, Paneth cells, and non-Paneth intestinal epithelial cells (42–45) and in T lymphocytes (46) and is recognized as an important mediator of inflammatory response largely dependent on NF- κ B activation. Nod2 stimulation can lead to Th2 driven adaptive response (47, 48) or Th1 and Th17 responses (47, 49) depending on cytokine milieu.

Binding of MDP to Nod2 causes conformational change of Nod2 structure, which results in an unfolding of the molecule, followed by oligomerization, and exposure of the CARD domain. The CARD domain of Nod2 binds to the CARD domain of RIP2, a threonine-serine kinase, and enables RIP2 to undergo polyubiquitination (50, 51). The pattern of polyubiquitination leads to mitogen-activated protein (MAP) kinase pathway (ERK 1/2, p38 and JNK) and/or NF-KB activation and the bifurcation in signaling between the NF-kB and ERK versus p38 and JNK pathways might involve differential recruitment of adaptor proteins that direct signaling towards specific inflammatory responses (52). Bid, a BCL2 family member, is required for downstream Nod2 activation of NF-KB, which confirms Nod2 cross talk with apoptosis proteins (53). Finally, Nod2 may also bind to one or more inflammasome proteins that contain CARD domains such as NLRP-1 or NLRP-3 and thereby participate in IL-1 β secretion (54).

Binding of viral ssRNA to Nod2 leads to translocation of Nod2 to mitochondria and interaction with mitochondrial antiviral-signaling protein (MAVS) and activation of NF- κ B in the RIP2-independent manner, and interferon regulatory factor-3, and production of interferon β (40).

Nod2 signaling is necessary for bacterial autophagy, the breakdown of invading bacterium by forming double-membrane vacuoles that ultimately fuse with lysosomes to eliminate proteins arising from cellular stress responses (55). Nod2 directly interacts with autophagy related 16-like 1 protein (ATG16L1) to recruit it to the plasma membrane at the

entry foci of bacteria and thus facilitates the formation of an autophagosome around the invading bacterium (56). Nod2 stimulation by MDP leads to upregulation of MHC class II surface expression and fusion of autophagosomes with specific MHC class II compartments (57). Autophagy has been reported to inhibit the generation of reactive oxygen species, which have been shown to trigger the activation of NLRP-3 inflammasomes (58). Autophagy may also inhibit pyroptosis, a highly inflammatory form of caspase-1-dependent cell death that has been observed in myeloid cells infected with intracellular pathogens (59).

Nod2 directly influences the composition of gut microbiota by regulating the production of a subgroup of intestinal antimicrobial peptides, known as cryptdins produced by Paneth cells in intestinal crypts (33). Impairment of Nod2 function leads to deficient production of α -defensins by intestinal crypt cells and thereby defective killing of bacteria and increased burden of commensal and pathogenic bacteria in the terminal ileum of Nod2-deficient mice that increases their invasion and contributes to the deeper, often transmural inflammation observed with ileal CD (60, 61).

Nod2 mediates an immune tolerance to bacterial products in human and mouse. In vitro, Nod2 stimulation by MDP lead to release of pro-inflammatory NF-kB-dependent cytokines (such as IL-1 β , TNF- α and IL-6), as well as secretion of IL-23 (which promotes Th17 differentiation) after costimulation with MDP and TLR-2 ligands and deletion of Nod2 in murine cells or loss of Nod2 function mutations in the LRR ligand recognition domains of Nod2 from human donors lead to a decrease in NF-kB activation, MAPK signaling and pro-inflammatory cytokine secretion (33, 41). Whereas in *in vivo* animal models of inflammation the role of NOD2 deficiency has varied, with some models showing deficient mice having increased inflammation (60, 62) and other models showing deficient mice having decreased inflammation (33, 63-65). These differences are related to the "chronicity" of the infection: during the early stages of infection Nod2 is a positive regulator of inflammation, however, after a period of sustained stimulation, the role of Nod2 switches and it becomes a negative regulator of inflammation, potentially via the induction of tolerance to further microbial stimulation through either Nod2 itself or other microbial receptors, such as TLR2 and TLR4 (65-67). This is dependent on IRF4 in mice and humans and downregulation of the IRAK-1 kinase and perhaps upregulation of IL-1R-associated kinase M (IRAK-M) in humans and also on early secretion of IL-10, TGF-β, IL-1Ra (10). Nod2-dependent release of IL-10 after MDP stimulation has been demonstrated to be specific to humans and is impaired in L1007fs cells (68). Resistance, which clears the invading organisms, and tolerance, which diminishes the negative effects of the host immune response, have been recognized as separate defense strategies in microbial defense.

During allogeneic bone marrow transplantation lack of Nod2 regulatory function in dendritic cells leads to enhanced proliferation and activation of allogeneic donor T cells presumably under the influence of endogenous TLR ligands, which results in target organ damage in graft versus host disease (GvHD) (69).

Nod2 is intrinsically required for T cell function. TCR and CD28 signaling triggers the activation of Nod2, which then interacts with NF- κ B inducing kinase (NIK) and c-Rel to form a complex that promotes c-Rel nuclear accumulation (46). C-Rel mediated IL-2 production positively regulates T cell activation and differentiation and plays a crucial role in T cell priming for IFN- γ production (70). Studies have demonstrated that c-Rel that is activated in response to TCR and CD28 triggering binds to regulatory element present in the forkhead box P3 (Foxp3) promoter region to facilitate Foxp3 expression and to promote T-regulatory (Treg) cell differentiation an thus may contribute to dysregulated immune response in Nod2-deficient host (69).

Nod2-deficient mice exhibit a hyperplasia and a hypertrophy of the Peyer's patches. The Th1 immune activation and increased levels of mucosal IFN- γ and TNF- α play a key role in the disruption of the epithelial barrier integrity of Peyer's patches of Nod2-deficient mice and lead to increased transcellular permeability and bacterial translocation in Peyer's patches (71).

Mutations in the Nod2 gene are strong genetic risk factors for ileal CD; however, the mechanism by which these mutations predispose to increased intestinal inflammation remains a subject of controversy. They include a role of Nod2 in the induction of defensins production, impaired autophagy and antigen presentation, mediating tolerance by attenuating inflammatory responses initiated by other receptors and secretion of anti-inflammatory cytokines, inhibiting processing and secretion of IL-1 β and pyroptosis and impaired T cell differentiation and proliferation.

Gut microbiota

Analysis of mouse models has revealed at least two major courses of disease: dysbiosis characterized by the depletion or alteration of commensal microbiota and chronic pathogen infection. Inflammation might arise from a lack of tolerance to antigens present in autologous microflora or from transient infection by traditional enteric pathogens which might break the mucosal barrier and activate pathogenic immune responses that are subsequently perpetuated by commensal enteric antigens in genetically susceptible host who is unable to repair epithelial breaches or downregulate the inflammatory response (72).

The essential role for the commensal microbiota as antigenic stimuli of effector immune responses in chronic intestinal inflammation is broadly accepted. Although it is not clear whether dysbiosis can cause IBD in humans or is a consequence of acute infection or host inflammatory response, several lines of evidence indicate that dysbiosis consisting of a decrease in beneficial bacteria and their metabolic end-products together with an increase of detrimental bacterial populations and their toxic metabolites drives activated inflammatory cascades leading to CD in genetically susceptible host (1, 73). Dysbiosis in CD is characterized by decreased microbial diversity in major phyla, such as Firmicutes and Bacteroidetes, altered ratio of beneficial and aggressive bacterial species and higher numbers of mucosa-associated bacteria than have healthy individuals (72).

Patients with ileal and colonic CD have significantly reduced concentrations of core commensals belonging to the Clostridiales order, such as *Faecalibacterium prausnitzii* and *Roseburia* (74). These genera are potent sources of short-chain fatty acids (75) and clostridial groups IV and XIVa promote the accumulation of Foxp3⁺ Treg cells in the mouse colon (76). Decreased number of *F. prausnitzii* in resected ileal CD mucosa is a predictive marker of postoperative ileal CD (77).

Bacterial species that are consistently increased in CD patients include *Escherichia coli*, specifically the B2 and D phylotypic groups and adherent/invasive strains (AIEC) associated with ileal CD (78, 79). AIEC adhere to the ileal mucosa through binding to molecules, which are overexpressed during CD (80, 81). AIEC can also disrupt the integrity of polarized cell monolayer, breach the intestinal barrier and penetrate into the gastrointestinal epithelium (82). By expressing long polar fimbriae, the bacteria interact with mouse and human Peyer's patches and translocate across microfold cells monolayers (83). AIEC are also able to survive and replicate extensively within a large, phagolysosome-like vacuole in macrophages (84) and AIEC-infected macrophages aggregate and fuse to form multinucleated giant cells *in vitro* (85).

An etiological role for other intracellular opportunistic pathogen, *Mycobacterium avium* subspecies *paratuberculosis* or measles virus is not widely supported. The higher prevalence of intracellular pathogens in the tissue of CD patients might arise from an inability of the dysfunctional innate immune system to control persistent infection by intracellular bacteria – possibly opportunistic pathogens in intestinal mucosa. However, CD-associated microbes could promote disease in genetically susceptible hosts with defects in innate immune system-mediated killing of microbes, mucosal barrier functions, or immunoregulation.

Pathogenesis of Crohn's disease

The consensus view of the cause of CD include genetic predisposition, an abnormal immune response to components of the normal gut microflora, and an environmental trigger and define CD as the genetically supported inappropriately aggressive Th1 and/or Th17 immune response to a subset of commensal intestinal bacteria, initiated and reactivated by transient infectious or environmental triggers (72). This excessive response can arise from an abnormal reactivity of the mucosal immune system to mucosal antigens or from increased exposure of luminal bacteria to normal mucosal immune system (54). Initial nonspecific immune response becomes chronic because of the constant drive of

commensal microbial antigens caused by genetic defects of epithelial barrier integrity, bacterial handling and immunoregulation (72).

Th1-mediated immune responses are typically triggered by an intracellular pathogen antigen presentation on MHC class II by antigen-presenting cell (APC) with costimulatory signals in the presence of IL-12 and is aimed to localize the infectious agent, promote intracellular killing or induce the differentiation of cytotoxic T lymphocytes. The hallmark of a Th1 response is a granuloma. Th1 cells under the control of a master transcriptional regulator Tbet produce pro-inflammatory cytokines IFNy, TNFa, and IL-2 (86). These cytokines act on local cell populations to promote intracellular killing, enhance recruitment of other inflammatory cells, enhance secretion of chemoattractant cytokines, and promote local tissue destruction. TNF- α is a central mediator of the intestinal inflammation and is produced by macrophages and Th17 cells (87). TNF- α in turn induces expression of IL-1 β and IL-6, both of which are also upregulated in serum of patients with IBD (88). IL-6 is an important factor for the synthesis of acute phase proteins, controls proliferation and resistance of resting T cells against apoptosis, activates Th2 cytokine production in CD4+ T lymphocytes and together with TGF- β induces the generation of Th17 cells while it inhibits differentiation of regulatory T cells. In particular, IL-6 trans-signaling appears to promote the maintenance of IL-17-secreting T lymphocytes in inflamed tissue (89).

Development of Th17 cells requires TGF-β and IL-6 (or IL-21) and is independent of the Th1 pathway. Th17 cells demonstrate substantial developmental plasticity after their commitment to the Th17 program: antigen activated naïve CD4+ T cells respond to TGFB to transiently co-express RORyt and Foxp3, but differentiate into either Th17 cells or induced regulatory T cells (iTreg) depending on the dominance of IL-6 or all-trans retinoic acid, respectively. Depending on the balance of TGF-B, IL-23, and IL-12, Th17 precursor diverges into progeny that express high levels of IL-17A and IL-17F (TGFβ dominance), IL-17A alone, IL-22 and IFNy (IL-23 dominance) or suppress IL-17A and IL-17F to express a Th1 pattern of cytokines dominated by IFNy (IL-12 dominance) (90). IL-23 is induced by PRR stimulation and is constitutively expressed in a small population of ileal dendritic cells. During CD, CD14+ intestinal macrophages secrete large amounts of IL-23 (91). IL-23 promotes a wide range of pathological responses in the intestine, mediated either by excessive innate immune activation or by enhancement of Th1 and Th17 responses, including enhanced proliferation of effector T cells, reduced differentiation of Foxp3+ Treg cells and the emergence of IL-17+IFN-y+CD4+ T cells (92). Th17 cells produce several cytokines, including IL-17A, IL-17F, IL-21 and IL-22 (93). IL-17A an IL17F have pro-inflammatory effects in the gut (94). IL-22 mediates either tissue-protective or pathogenic functions, depending on the absence or presence of IL-17A, respectively (95). Depending on the cytokine milieu and tissue in which it is expressed, IL-22 can regulate

the expression of genes encoding molecules associated with inflammation, repair or chemotaxis or the expression of antimicrobial peptides, which can orchestrate host-protective immunity, tissue inflammation, repair or homeostasis (96). CD patients have increased levels of IL-22 which correlate with increased disease activity and susceptibility-associated IL23R polymorphisms (97).

Th17 response is both permissive and inhibitory of the Th1 response, probably at different phases of the inflammatory cycle, so it is hypothesized that in Crohn's disease Th17 response may be more important in the regulation of the inflammation than in its induction: IL-23 inhibits Treg cell generation and counteracts the inhibitory effect of Treg cells on both Th1 and Th17 proinflammatory responses and IL-17 inhibits generation of IFNy-producing cells (98). In the proposed model of pathogenesis of Crohn's disease the intestinal inflammation consists of innumerable microenvironments, each exhibiting a progression of inflammatory patterns. In the initial and most intense phase of the inflammation Th1 responses predominate, at this point, production of IL-23 in a nascent Th17 response inhibits regulatory T-cells generation and feeds the inflammation. In a later phase, a mixed T-cell response prevails in which the Th1 response is still predominant but is now moderated by a Th17 response producing both IL-17 (which inhibits IFN- γ T cells) and IL-22 (98).

Conclusion

Since the first description of CD in 1932, the understanding of the etiology and pathogenesis of disease underwent a substantial progress. GWAS have identified numerous genomic regions containing CD-risk factors, revealing several features of the genetic architecture of CD. Furthermore, the importance of multiple environmental factors in the induction and maintaining of CD was established. The accumulated evidence suggests that gut microbiota represent an essential, perhaps a central factor in the development of CD where dysregulation of normal co-evolved homeostatic relationships between intestinal microbiota and host mucosal immune system leads to intestinal inflammation. Taken together, these findings identify CD as a syndrome of overlapping phenotypes that involves variable influences of genetic and environmental factors. A deeper understanding of different genetic abnormalities underlying CD together with the identification of beneficial and harmful components of gut microbiota and their interactions are essential conditions for the categorization of CD patients, which enable us to design more effective, preferably causative, individually tailored therapy.

Acknowledgements

This work was supported by Charles University in Prague, Faculty of Medicine in Hradec Králové, Czech Republic, project "PRVOUK" P37/10, by grant from the Grant Agency of Charles University in Prague (No. 906613) and by grants from the Academy of Sciences of the Czech Republic (No. M200201207), IGA Ministry of Health of the Czech Republic (No. NT14017), the Grant Agency of Czech Republic (P303/12/0535) and Institutional Research Concept (RVO: 61388971).

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Received: 30/04/2014 Accepted in revised form: 08/09/2014

Corresponding author:

Tomáš Hrnčíř, M.D., Ph.D., Department of Immunology and Gnotobiology, Institute of Microbiology, Academy of Sciences of the Czech Republic, 549 22 Nový Hrádek, Czech Republic; e-mail: tomas@gnotolab.com