Intestinal bacteria and inflammatory bowel disease

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Although the aetiology of idiopathic inflammatory bowel diseases (IBD) is incompletely understood, increasing evidence implicates the intestinal microorganisms in the initiation and maintenance of the inflammatory process of IBD. How much in the initiation and in the perpetuating of inflammation and how much in ulcerative colitis (UC) and in Crohn's disease remains as one of many unanswered questions. The contribution of microorganisms in the pathogenesis of both disorders are supported by the fact that IBD occurs preferentially in the area of relative stasis providing prolonged mucosal contact with luminal bacteria of the highest concentration (terminal ileum, rectosigmoideum) (23). Further, IBD can resemble enterocolonic infections, and decreasing luminal bacterial concentrations are often associated with Crohn's disease clinically improvement (36). IBD could represent a response to a specific pathogenic bacteria or an immunological response to microbial antigens. Recent advances in our understanding of the genetics of IBD, in particular the identification of NOD2/CARD15 have provided the opportunity to explore the genetic basis for the heterogeneity of IBD. The new data suggest that IBD comprises a heterogeneous family of oligogenic inflammatory disorders in which the characteristic clinical manifestations of disease in any patient are determined by the interaction of genetic and environmental factors (1).

Early last century Bacillus coli, B. proteus, B. pyocaneus, B. lactis aerogenes, streptococci and B. coli communis were suggested as possible causes of UC (26). Small haemorrhages, ulcerations, and a fibrinous exudate described in rabbits injected with dysentery bacilli or their toxins reminded the changes in UC (11). Although exceptionally some cases of primary acute shigellosis transform to UC, these cases are very rare. We had the opportunity to follow a patient with UC who came from a family where its five members went down with bacteriologically confirmed shigellosis, but only our patient became a typical UC. Many other bacteria were later implicated and discarded because of lack of conclusive evidence (12,14). Mařatka (24) stressed already 55 years ago in his monograph that no specific bacteria or parasites in association with UC have been confirmed as its aetiologic factor which is true of them up today. The aetiologic role of viruses, studied largely up till now by many gastroenterologists, has received little support. No one can fulfil Koch's postulates. Nevertheless, a large numbers of pathogens have been associated with clinical relapses of IBD or recovered from tissues of patients with active disease. Among viral pathogens are often quoted Cytomegalovirus (8), Epstein-Barr virus, Parainfluenza, Rubella, Respiratory syncytial virus, Influenza A and B, Adenovirus (19), Herpes simplex virus (35), Norwalk agent and Rotavirus (13), among bacteria Clostridium difficile (43), Shigella (21), Salmonella (41), Yersinia enterocolitica (32), Campylobacter jejuni (39), enteropathogenic E. coli (44), Fusobacterium varium (30), among parasites Entamoeba histolytica (21), Giardia lamblia (38) and Blastocystis hominis (28). Today we know that mucosally associated anaerobic and aerobic enteric bacteria are dramatically increased in Crohn's disease and UC with an intermediate level found in self-limited intestinal inflammation (40). Concentrations of adherent E. coli and enterococci are increased in

the neoterminal ileum of Crohn's disease after ileocolonic resection, increased numbers of enteroadherent/invasive E. coli strains were described in recurrent postoperative Crohn's disease (6). Recurrence of the early stage was associated with increased E. coli, enterococci, bacteroides, and fusobacteria (29). Clinical improvement after therapy with metronidazole, vancomycin and other specific anti-infection drugs supports a causative significance for Clostridium difficile, Cytomegalovirus infection etc. in relapse of IBD. However we must still consider also the well-known fact, that commensal enteric bacteria can induce protective as well as detrimental mucosal responses. A very important seems to be the observation that IBD patients exhibit loss of immunologic tolerance manifested by increased humoral and cellular immune responses to a number of commensal bacteria, such as E. coli, S. cerevisiae, C. elegans, etc. (31). It also enables better understanding why the patients with UC immunologically respond at a cellular level not only against colon antigen but also against E. coli antigens, eg. O:14 antigen (9).

The presence of non-pathogenic enteric microflora is required for the development of chronic enterocolitis, as determined by the lack of colitis in genetically engineered susceptible rats and mice raised in a germ-free environment (37). Experiments with germ-free animals also led to the clinically interesting results showing for example, that some animals develop rapid onset coecal inflammation when monoassociated with E. coli, but slow-onset distal colitis when monoassociated with E. faecalis (20).

As mentioned, for UC, there is no clear evidence for an infective agent, either bacterial or viral. For Crohn's disease, interest has been focused on many organisms, including RNA viruses and cell wall-deficient organisms. Mycobacterium avium subspecies paratuberculosis has been identified in some cases of Crohn's disease. M. paratuberculosis is an etiologic factor in Johne's disease in ruminants, which is characterized by chronic granulomatous lesions and clinical signs of enterocolitis. Isolation of M. paratuberculosis in Crohn's disease is a rare finding. This could be explained by a long-term and very difficult culture, by spheroplasts as a form of M. paratuberculosis, etc. Low prevalence of M. paratuberculosis in Crohn's disease does not exclude the possibility of its presence during the evaluation of the disease, because M. leprae is also not easily culturable in patients with leprosis, but its etiologic role in lepra is undoubted. M. paratuberculosis was also proven in the surgical tissue samples from the diseased intestine among some Czech patients with Crohn's disease (10). The same RFLP types of M. paratuberculosis in Crohn's disease and in paratuberculosis stress the possible relationship between Crohn's disease and paratuberculosis. The incidence of PPD responsiveness is greater in the Czech population (for healthy people as well as for patients with IBD), largely as a result of previous vaccination with BCG (42). The association of these findings with M. paratuberculosis is of little probability. The therapeutic response to "antimycobacteria" can sometimes be successful (17). The aetiology associated with M. paratuberculosis has not been disproven, but it is not generally accepted. It could be only a reasonably common environmental contaminant that preferentially invades ulcerated Crohn's disease tissue (37). It remains a question why ulcers in UC are not invaded, too.

Although the exact immune mechanisms may be unknown, there is little doubt that both humoral and cellular immune pathways are activated in the inflamed mucosa. Important role play intestinal microorganisms. Intestinal inflammation in patients with IBD is thought to result from an overwhelming uncontrolled activation of the mucosal immune system induced by antigens of the normal luminal flora in genetically susceptible individuals. IBD appears to be mediated by subsets of CD4 T cells or NK cells producing high levels of proinflammatory cytokines such as tumour necrosis factor alfa (TNF-alfa) (27). Only in the last years the great progress in understanding the mechanisms by which bacterial products activate immune responses in macrophages, dendritic cells, mesenchymal cells, and epithelial and endothelial cells started. The most important seems to be the discovery of a new class of surface and cytoplasmic receptors that bind bacterial adjuvans, which enable to reco-

gnize different bacterial products as peptidoglycans, lipolysaccharides, heat shock protein 60 (membrane bound TLR-2, 4, and 9, cytoplasmic NOD1, NOD2, etc) (2,18). Binding of bacterial adjuvans to the receptors is initiated through the activation of nuclear factor-kB (NFkB) transcription of many proinflammatory molecules found in active IBD. During active IBD NOD2 (nucleotide-binding oligomerization domain 2) and TLR-4 are upregulated by proinflammatory cytokines, especially TNF (37). Commensal gram-negative bacteria can activate NFkB through lipopolysaccharides binding to TLR-4 on colonic epithelial cells (16). As many years ago the main connection between E. coli O:14 and other enterobacteriaceae and UC was established, the determination of the role of different lipopolysaccharides from various gram-negative bacteria in activation of NFkB can now be assessed (9).

In this issue an interesting hypothesis concerning the importance of intestinal bacterial flora in the pathogenesis of IBD is published by Mařatka (25). The author puts his great and long-term experience with clinical, bacteriological and immunological problems of IBD and presents here the two-component hypothesis of UC. His hypothesis was put forward in 1948, but since this time he has assembled more of his own as well as the new literary data research and a more comprehensive version of his hypothesis was published in 1993. The primary lesion of UC consists of a haemorrhagico-catarrhal inflammation of idiopathic aetiology (the first component) and appears and disappears periodically. Secondarily, an ulcerative inflammation develops due to invasion of potential pathogenetic intestinal microflora as well as disturbance of immunity, sensitization, deficiencies, etc. (second component). In this stage the anti-infectious therapy can be more effective. This hypothesis has its rational grounds and can be applied to most cases of UC, and perhaps also to some cases of Crohn's colitis. As mentioned above, there also exist some uncommon cases where the primary attack from enteric bacteria can start UC. A genetic predisposition to abnormal immune responses to intestinal bacteria participating in the pathogenesis of IBD seems to be very probable here.

Importance to keep bacterial microflora in the intestine in a normal state is for UC-patients assumable. Prebiotics are dietary substances that stimulate the growth of beneficial native enteric bacteria, especially Lactobacillus and Bifidobacterium species. These bacteria act on the nonabsorbed carbohydrates. The produced short-chain fatty acids lead to a decreased luminal pH, which suppresses growth of detrimental bacteria and improves the state of epithelial cells including mucosal barrier function (3). These experiences are coming mainly from experimental studies.

More clinical experiences have been published with probiotics in IBD (15). Probiotics are commensal microorganisms with a beneficial effect on enteric bacteria and with a therapeutic effect in IBD, mainly in UC. Most are non-pathogenic bacteria, normally present in the human intestine such as lactobacilli, bifidobacteria and enterococci (5). Probiotic bacteria may modulate not only the intestinal microflora but also the mucosal immune responses. Unfortunately, controlled clinical studies with probiotics in IBD are still rare. Probiotics were used to maintain clinical remission either alone or together with usual pharmacological treatment. A cocktail containing high bacterial concentrations of four strains of Lactobacillus species, three strains of Bifidobacterium, and one strain of Streptococcus was superior to placebo in preventing acute pouchitis and in maintaining remission in chronic relapsing pouchitis (4). A non-pathogenic strain E. coli Nissle 1917 is proven to be as effective as mesalazine in preventing relapse of UC (22,33,34). Probiotics are harmless, well tolerated, and therefore represent a good alternative to pharmacological agents. As Mařatka stressed, the existing results seem to be an important support for the view that bacteria play a significant role in the pathogenesis of IBD. The role of enteric flora appears to be of greater significance than previously held. There exists the first experimental study in which antibiotics and probiotics display synergistic in vivo effects (7). Restoring the microbial balance between detrimental and protective luminal bacteria by combining antibiotic and probiotic approaches may be the most physiological approach to treat IBD and may alter the

course of these chronic relapsing diseases. In the future, manipulation of the colonic bacteria with antibiotics and probiotic agents may prove to be more effective and better tolerated than immunosuppressants.

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