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PROBIOTICS AND BACTERIAL COMPONENTS IN INTESTINAL INFLAMMATION THERAPY AND PREVENTION

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SUMMARY

Crohn's disease (CD) and ulcerative Colitis (UC), the two major forms of inflammatory bowel disease (IBD), are both severe chronic inflammatory disorders. Although the exact etiology and pathogenesis of both forms of IBD have yet to be completely understood, it is widely accepted that they result from a continuous microbial antigenic stimulation of pathogenic immune response in genetically predisposed individuals. Genome-wide association studies identified several defects in genes responsible for mucosal barrier function, bacterial sensing and killing and for the regulation of the inflammatory response. The changes in microbiota composition or abilities (epithelial adhesion and invasion) are supposed to be the trigger of the inflammation. These findings are further supported by conclusions of studies in both humans and experimental animals. Those studies showed that impaired host reaction to commensal microbiota, or their abundant presence in the subepithelial layer, leads to the pathological stimulation of the mucosal immune system.

Several clinical studies as well as some interventional studies on animal models demonstrated that antibiotics, probiotics and bacterial components are useful in maintaining disease remission and in disease prevention. This effect is partially due to the changes in microbiota composition and partially due to imunomodulation.

During many years of co-evolution with humans, the microbiota, indigenous as well as pathogenic, have acquired immunomodulatory mechanisms to bypass our mechanisms of protective immunity. Our aim is to isolate the immunomodulatory components from bacteria and use them in IBD therapy and prevention. Compared to use of live bacteria, this approach seems to be safer and easily applicable in practice. The differences in immunomodulatory properties of these components also suggest the need of individualized therapy. This review will focus on IBD pathogenesis and the possibility of influencing it by therapy with bacterial components.

INTRODUCTION

The two major forms of inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis, are both severe relapsing inflammatory diseases of the intestine, each associated with a typical phenotype. Ulcerative colitis is characterized by diffuse continuous mucosal inflammation that extends proximally from the rectum to a varying degree. Although it is located only in the large intestine, in some patients, the terminal ileum is also affected (so-called backwash ileitis). Patients typically suffer from bloody diarrhea, abdominal pain, rectal bleeding and malnutrition. Crohn's disease is characterized by segmental and transmural inflammation, fistulas and granulomas in any part of the gastrointestinal tract, most commonly in terminal ileum. Crohn's disease leads to strictures, abscesses and fistulas and the clinical manifestation depends mainly on the disease localization. The IBD usually starts to manifest in the second and third decades of life and the majority of affected individuals progress to chronic relapsing disease (*Baumgart* and *Sandborn*, 2007).

The IBD is a systemic disorder and in almost half of the patients other organs are affected by the disease as the extraintestinal disease manifestation or disease (therapy) complications.

These manifestations and complications typically affect the musculoskeletal system (peripheral arthritis, ankylosing spondylitis and osteoporosis), skin (erythema nodosum, pyoderma gangrenosum and fistulas), eye (iritis and uveitis) and biliary system (primary sclerotising cholangiopathy and gall stones) (*Rothfuss* et al., 2006).

The highest prevalence of IBD is traditionally in North America, northern Europe and the United Kingdom, with averages ranging from 100 to 300 cases per 100 000 (*Ehlin* et al., 2003; *Loftus* et al, 2004). Recent epidemiological studies suggest that the increase in disease incidence has probably reached its plateau in these countries, however, there is a strong increase in IBD incidence in regions with traditionally low IBD prevalence (e.g. Asia and Latin America) (*Linares de la Cal* et al., 1999; *Yang* et al, 2000; *Lee* et al., 2000; *Yang* et al., 2008).

Although the etiopathogenesis of the IBD remains obscure, IBD is thought to be a result of uncontrolled inflammatory response to indigenous intestinal microbiota in genetically predisposed individuals. The genetic predisposition is associated with genes related to host bacteria interaction, suggesting the crucial importance of intestinal microbiota in IBD pathogenesis. General intestinal dysbiosis, presence of some pathogenic bacteria or even enhanced virulence of certain commensal bacteria (*Chiodini et al.*, 1989; *Darfeuille-Michaud* et al., 1998; *Swidsinski* et al., 2002; *Seksik* et al., 2003; *Frank* et al., 2007; *Rabizadeh* et al., 2007) were all proposed to be the trigger of the IBD. The importance of intestinal microecology is further supported by the finding that manipulating intestinal microbiota using probiotics and antibiotics is effective in IBD therapy. (*Guslandi* et al., 2000; *Kruis* et al., 2004; *Rahimi* et al., 2007)

Some strains of probiotic bacteria also possess immunomodulatory properties, so their effect in IBD therapy could be mediated by modulation of the mucosal immune system or intestinal barrier function, rather than by intestinal ecology changes. (*Damaskos* and *Kolios*, 2008) Therefore, we could use bacterial lysates or isolated bacterial components to mimic the therapeutic

effect that probiotics have. Use of sterile bacterial components with immunomodulatory properties seems to be a safer and more practical approach than the use of live bacteria.

IBD PATHOGENESIS

The luminal antigens can initiate the pathogenetic inflammatory cascade in the gut only after four conditions are met. First, the host's mucosal immune system must be genetically susceptible to recognize the antigens from indigenous microbiota and misinterpret them as potentially harmful. Second, the antigen must reach the gastrointestinal tract. Third, the antigen must pass through the intestinal barrier to reach the immunocompetent cells in the mucosa. And finally, the regulatory mechanisms of mucosal immune system must fail to control the inflammation.

Genetics

Family aggregation of IBD is a well known phenomenon; the life-time risk of developing IBD for first-degree relatives of a CD proband is 5 % and that of an UC proband 1.6% in white non-Jewish Euro-American population (8 % and 5.2% for Jews). The concordance in monozygotic twins is 28% and 16% for CD and UC respectively, and 4% for dizygotic twins for each disease (*Halfvarson* et al., 2003; *Halme* et al., 2006). On one hand, these data clearly indicate that genetic factors definitely contribute to IBD, but also that the environmental and developmental factors are more important in disease pathogenesis.

Genome-wide association studies for IBD susceptibility genes performed in the last few years have reported many genes that contribute to disease susceptibility. Interestingly, most of these newly identified genes are related to the epithelial barrier permeability, bacteria sensing and killing, and regulation of the immune system. (*Duerr* et al., 2006; *Rioux* et al., 2007) All these findings fit well into the current concept of IBD pathogenesis and stress the importance of host-microbiota interaction.

Microbiota

Humans are colonized by huge number of microbes; microbial cells form 90% (10^{14}) of all cells in adult humans (*Savage*, 1977). This colonization starts during the birth and continue during the first few years of life. After this period, the composition of the individual microbiota seems to be relatively stable. Although there are some representatives from archaea and eukarya, as well as viruses and bacteriophages, most members of gut microorganisms belong to the domain bacteria. (*Eckburg* et al., 2003; *Breitbart* et al., 2003; *Curtis* and *Sloan*, 2004). Over 98% of all gut bacteria in mammals belong to the two divisions of Bacteria - Firmicutes and Bacteroidetes. (*Eckburg* et al., 2005). Although this uniformity probably reflects the specific conditions in the gut, the differences on a species level is striking, as it resembles an individual fingerprint. The diversity of individual microbiota is caused by many variables such as dietary habits, use of pharmaceuticals, health status and by microbial exposure of both mother and child during the child's perinatal and infant period. Introduction of probiotic bacteria during this period could result in long term colonisation and could have impact on health later in adult life (*Kalliomäki* et al., 2001; *Lodinová-Zádníková* et al., 2003).

Although the microbiota composition has a distinctive pattern in every individual, under physiological conditions it serves important biological functions for all hosts. The microbiota prevent colonization with pathogens, providing important nutrients (vitamins and short-chain fatty acids), and modulate intestinal barrier maturation and development of the immune system (*Chapman* 2001; *Hooper* et al., 2002; *Tlaskalová-Hogenová* et al., 2004). Luminal microbiota also positively influence the development and preservation of oral tolerance in a strain-dependent manner (*Gaboriau-Routhiau and Moreau*, 1996; *Moreau and Gaboriau-Routhiau*, 1996; *Prioult* et al., 2003).

The close link of intestinal inflammation to microbiota was proposed many years ago and later proven with animal models of IBD, which showed that intestinal inflammation is much milder or even fails to develop, if animals are reared under germ-free conditions (*Sellon* et al., 1998; *Hudcovic* et al., 2001; *Stepankova* et al., 2007). These findings led to closer investigation of microbiota composition in IBD patients. Many studies initially focused on searching an individual pathogen responsible for IBD. The pathological similarity of Johne's disease in cattle to Crohn's disease in humans led to a proposition that *Mycobacterium avium* subsp. paratuberculosis (MAP) is the causative agent of IBD. This hypothesis was supported by finding of MAP in inflamed tissue of CD patients (*Chiodini*, 1984; *Sanderson* et al., 1992; *Fidler* et al., 1994). Although there were reservations about these studies, recent meta-analysis confirmed specific association of MAP with CD (*Feller* et al., 2007). The controlled trials for the therapy of CD with antimycobacterial drugs failed, so the question how this bacterium could influence the IBD pathogenesis remains unclear (*Goodgame* et al., 2001; *Selby* et al., 2007).

Several ecological studies of gut microbiota showed that there is a difference in microbiota composition in IBD patients compared to healthy individuals. This dysbiosis or increase in some bacterial group was proposed to cause or at least perpetuate the intestinal inflammation in IBD. These studies also identified some candidate bacteria that could be responsible for the IBD development in susceptible individuals (*Seksik* et al., 2003; *Sokol* et al., 2006). All these studies must be interpreted with caution, because the real trigger might be only transient and changes we are detecting are just secondary.

There are also changes in mucosa-associated bacteria, suggesting the increased ability of bacteria to adhere to mucosa or some other changes in the bacterial metabolism could be responsible for IBD triggering (*Darfeuille-Michaud* et al., 1998; *Swidsinski* et al., 2002) To date, the question remains open whether these bacteria are introduced into the gut from the outside environment, or whether this new ability is introduced to intestinal microbial society by horizontal gene transfer or by an other, yet unknown, signal.

Although we do not completely understand the natural relations between intestinal microbiota, the success of antibiotics and probiotics in IBD therapy clearly shows, that manipulation with intestinal microecology might be the future treatment of at least some forms of IBD.

Intestinal barrier function

Intestinal barrier prevents viable enteric bacteria from excessive interaction with lamina propria immune cells. This barrier is formed by several components including mucus layer, epithelial cells, tight junction, mucosa associ-

ated lymphoid tissue, SIgA and it is an incredibly dynamic and actively regulated apparatus.

Several studies reported that the intestinal permeability is increased in inflamed as well as in noninflamed IBD mucosa of patients and even in first degree relatives of CD patients (*Jenkins* et al., 1988, *Katz* et al., 1989). These findings clearly demonstrate the importance of the intestinal barrier function, and its genetic control, in IBD pathogenesis. Increased intestinal permeability also has been shown useful in the prediction of relapse in asymptomatic CD patients (*Wyatt* et al., 1993; *D'Incà* et al., 1999).

Several mechanisms might be involved in increased gut permeability. First, there is a defect in mucous production in IBD patients (*Buisine* et al., 2001). Mucus forms a rather thick layer (approximately 100 μm in jejunum and over 800 μm in colon) on the gut epithelium (*Atuma* et al., 2001). This layer acts as a mechanical and antimicrobial barrier protecting the underlying epithelium. In healthy individuals, this layer contains high concentrations of secreted IgA, lysozyme and other antimicrobial components keeping the epithelial surface free of bacteria.

Defensins, antimicrobial peptides produced by the Paneth cells in the base of the Lieberkühn's crypts, are concentrated in the mucus layer protecting vulnerable epithelium from invasive bacteria, yet allowing the presence of harmless enteric microbiota (*Meyer-Hoffert et al.*, 2008). The production of defensins is, however, significantly decreased in terminal ileum of CD patients, which may result in aberrant ileum colonisation causing the inflammation (*Wehkamp* et al., 2004; *Wehkamp* et al., 2007).

Another mechanism, capable to increase the intestinal permeability is a downregulation of tight (ZO-1 and occludin) and addherens (E-cadherin and α-catenin) junctions' proteins in the epithelium of IBD patients. The degree of this downregulation positively correlates with degree of inflammation, showing the importance of these proteins for intestinal epithelium integrity. (*Gassler* et al., 2001).

Another explanation for the increased intestinal permeability in IBD is increase of extracellular matrix degrading endopeptidases - matrix metalloproteinases (MMP). These enzymes were found upregulated in the inflamed gut tissue of IBD patients causing mucosal degradation and ulceration (*Heuschkel* et al., 2000). They are produced by activated gut myofibroblasts, macrophages and resident plasma cells in the presence of proinflammatory cytokines IL-1β and TNF-α (*Okuno* et al., 2002; *Gordon* et al., 2008).

Although the defect in intestinal barrier function could be the initial defect in IBD pathogenesis, the production of TNF-α and IFN-γ secondary to the inflammation perpetuates the increased intestinal permeability by reorganizing the tight junction, causing further leakage of luminal content to submucosa (*Ma* et al., 2005; *Wang* et al., 2005). This way, the vicious circle of inflammation is created.

Mucosal immune system and its regulation

In healthy subjects, there is an immunological tolerance to intestinal flora from autologous but not heterologous intestine. This tolerance is, however, broken during intestinal inflammation (*Duchmann* et al., 1995).

There is an increase of activated macrophages and dendritic cells (DCs) and changes in cytokine production in the intestinal mucosa of IBD patients. The cytokine profiles are, however, unique for each form of IBD. While proinflammatory cytokines TNF-α, IL-

1β and IL-6 are increased in both forms, the increase of IFN-γ, IL-12, IL-17, IL-23 and IL-27 are specific for CD and increase of IL-5 and IL-13 for UC (*Fujino* et al., 2003; *Fuss* et al., 2004; *Schmidt* et al., 2005). On the other hand, the deficiency in IL-10 and TGF-β signaling in the intestine might contribute to the development of IBD, because both cytokines are important in directing naïve T cell maturation to a regulatory pathway (*Hahm* et al., 2001). Moreover, local delivery of IL-10 with genetically engeneered bacteria, *Lactococus lactis* producing IL-10, shown good results in experimental colitis therapy (*Steidler* et al., 2000).

Even under normal physiological condition, the microbes in the intestine make contact with the host's immune system without inducing inflammation. It has been shown, that DCs in the lamina propria actively sample the gut lumen, but also there is active intake of IgA coated bacteria to the Peyer's patches through the M cells (*Rescigno* et al., 2001; *Mantis* et al., 2002).

There is also evidence that live commensal bacteria are shuttled by DC through the Payer's patches to mesenterial lymph nodes causing specific mucosal, but not systemic IgA response (*Macpherson* and *Uhr*, 2004).

All these observations shows that at least some intestinal microbes make contact with the mucosal immune system, but also raise the question of why this contact does not cause the inflammation as the loss of the intestinal barrier does. The answer is not known but it is thought, that the amount of antigen is too small to trigger the inflammation or it is well "guarded" by the unresponsive innate immunity cells. Once there is a big load of bacteria in the mucosa due to the barrier failure or the cells are more susceptible to the inflammatory response due to the defect in bacteria sensing, the mucosal immune cells overcome their unresponsivenes and the inflammation starts. Three immune mechanisms are involved in this process: defect in microbe sensing by the resident mucosal cells, the accumulation of the effector cell in the mucosa and a loss of the local tolerogenic signals.

Microbe sensing by the resident mucosal cells

Luminal antigens are continuously sampled by intestinal epithelium as well as by cells of innate immunity such as DCs using several evolutionarily conserved and structurally related receptors, pattern recognition receptors (PRR). These receptors could be membrane-bound (e.g. Toll like receptor (TLR) 1, 2, 4, 5, 6, 10 or membrane bound CD14), residing in the cytoplasm (e.g. nucleotide-binding oligomerization domain containing (NOD) 2 protein, TLR 3, 7, 8 and 9) or even released from the cell (e.g. mannan-binding lectin or soluble CD14). These receptors are recognizing conserved structural motives on microbiota or microbe-associated molecular patterns such as lipopolysaccharide, peptidoglycan, lipoteichoic acide, single- and double-stranded RNA and methylated DNA (CpG-motives).

Interestingly, epithelial cells are also expressing PRRs and are able to be activated in response to microbes and produce cytokines. This way, the epithelium could deliver the inflammatory signals to underlying cells in lamina propria. In normal gut, the epithelial cells are activated by invading pathogens yet maintain the tolerance to resident bacteria (*Duchmann* et al., 1995).

Antigen-presenting cells (APCs; e.g. DCs and macrophages) are found in a resting (inactive) state in lamina propria and Peyer's patches of normal gut. In this state the cells do not respond to

bacterial stimuli and pro-inflammatory cytokines (*Smythies* et al., 2005). They became activated with conserved structural motives, they migrate to the local lymph nodes and activate naive T cells. They also contribute to the T cell activation with production of IL-6, IL-12, IL-23 and TGF-β (*Drakes* et al., 2005). Interestingly, the expression of some of some PRR is dysregulated in the intestines of CD and UC patients. This may result in easier triggering of the inflammatory cascade in these individuals (*Frolova* et al., 2008). This is further stressed by the fact, that the IL-$10^{-/-}$ mice are resistant to spontaneous colitis, if they are lacking MyD88 - important adaptor protein in TLR signaling. (*Rakoff-Nahoum* et al., 2006).

Accumulation of the effector cell in the mucosa

Pro-inflammatory molecules, responsible for tissue damage during the inflammation, are produced by effector cells that have accumulated to high numbers in the inflamed mucosa. There are two main mechanisms responsible for the accumulation of effector cells in the inflamed intestine: enhanced recruitment due to the upregulation of adhesion molecules or chemokines, and by increased cell recruitment and prolonged survival caused by decreased cellular apoptosis.

High levels of inflammatory cytokines (e.g. TNF-α, IL-1β and IL-6) in the mucosa of IBD patients increase the expression of chemokines and adhesion molecules (on endothelium as well as on circulating CD^+ cells) causing circulating leucocytes to adhere to endothelium and enter to the inflamed mucosa (*Burgio* et al., 1995; *García de Tena* et al., 2006). The presence of TNF-α and IL-6 in the inflamed mucosa also mediates T-cell resistance against apoptosis causing their accumulation and further tissue damage (*Atreya* et al., 2000; reviewed in *Neurath* et al., 2001).

Loss of the local tolerogenic signals

While innate immunity is activated in both forms of IBD, the T cell response differs. In CD, there is upregulation of T_H1 (characterized by IFN-γ) and T_H17 (characterized by IL-17) pathways. The T_H1 response is inicated by IL-12 and T_H17 response is enhanced by the presence of IL-6, TGF-β and IL-23. These cytokines are produced by activated local APCs and other innate immunity cells upon bacterial colonization (*Becker* et al., 2003; *Kamada* et al., 2005). On the other hand, the UC is characterised by atypical T_H2 response, with increase IL-5 and IL-13 produced by natural killer T (NK-T) cells. These NK-T cells are stimulated by APCs bearing nonclassical MHC class molecule CD1d, which is specialised in presenting the lipids (*Fuss* et al., 2004).

In normal gut, the inflammatory response of effector T cells is regulated by T regulatory cells. To date, there are three types of T regulatory cells identified in humans. Naturally occurring T regulatory cells (T_{REG}) originate in thymus and typically express transcription factor forkhead box P3 (FoxP3) and high levels of CD25. These cells regulate the effector cells with cell-cell contact. The other two types, regulatory T cell type 1 (T_R1) and T helper type 3 (T_H3), originates in intestine and react to luminal antigens with production of IL-10 and TGF-β on bacteria or food proteins respectively. The function of T regulatory cells is under close control of APCs and local cytokine milieu as recently reviewed by *Belkaid* and *Oldenhove* (2008). Interestingly, the anti-inflammatory T_{REG} as well as pro-inflammatory T_H17 cells could be induced locally in the intestinal mucosa

from naïve T cells by TGF-β. The balance between these two functionally opposite subsets of T cells is kept with IL-6 and IL-21, because these cytokines promote the differentiation to T_H17 by blocking the expression of FoxP3 in the naïve T cells (*Fantini* et al., 2007).

PROBIOTICS AND BACTERIAL COMPONENTS IN INTESTINAL INFLAMMATION THERAPY

Recent advances in our understanding of IBD pathogenesis and mucosal immune response regulation led to a proposal of novel strategies in intestinal inflammation therapy.

As mentioned before, the indigenous microbiota is crucial for induction and perpetuation of intestinal inflammation and manipulation with microbiota composition with probiotics and antibiotics is possible approach in IBD therapy. However, each probiotic strain have unique effect on immune system, therefore deeper insight into the particular microbe-host interaction and careful choice of particular strain for particular function might be needed for successful therapy (*Maassen* et al., 2000).

Each strain could also have several mechanisms of action, interfering with one or more steps of IBD pathogenesis. Live probiotic bacteria could change composition of intestinal microbiota resulting in changes in host's sensitivity to inflammation. These changes could be mediated by simple competition with other microbes for limited resources and limited number of receptors or they could produce antimicrobial peptides or even increase the host antibody production against other microbes (*Kaila* et al., 1992; *Liévin* et al., 2000; *Hütt* et al., 2006). Moreover, probiotic *E. coli* Nissle 1917 and *Lactobacillus casei* DN-114 001 have inhibitory effect on adhesion and invasion of adherent invasive *E. coli* isolated from patients with Crohn's disease (*Boudeau* et al., 2003; *Ingrassia* et al., 2005). The mechanism of this action could also be explained by recent findings, that *L. acidophilus* secrete molecule(s) capable of downregulating expression of genes involved in attachment of enterohemorrhagic *E. coli* to gut mucosa (*Medellin-Peña* et al., 2007). This indicates that we might be able to convert adherent bacteria into non-adherent simply by administering this bacterial component to the gut. Furthermore, treatment with *L. casei* or mixture of probiotic bacteria VSL#3 results in an improvement of intestinal barrier integrity and thus prevents enteric antigens from excessive stimulation of lamina propria immune cells (*Madsen* et al., 2001; *Llopis* et al., 2005).

Several probiotics have shown immunomodulatory properties on basically all levels of regulation, including downregulation of the PRRs expression, NF-κB signaling and pro-inflammatory cytokine production (*Sturm* et al., 2005; *Matsumoto* et al., 2005; *Grabig* et al., 2006; *Sougioultzis* et al., 2006). Interestingly, some of these effects could be achieved by soluble factor produced by these bacteria or their lysates. Lysate of *Lactobacillus brevis* and *Streptococcus thermophilus* could induce apoptosis in immune cells, which could reverse the insensitivity to apoptosis of lamina propria immune cells in IBD patients (*Di Marzio* et al., 2001). Oral administration of lysate prepared from normal intestinal flora containing anaerobes reduces the severity of acute experimental colitis in mice which suggests that there might be some bacterial components with special

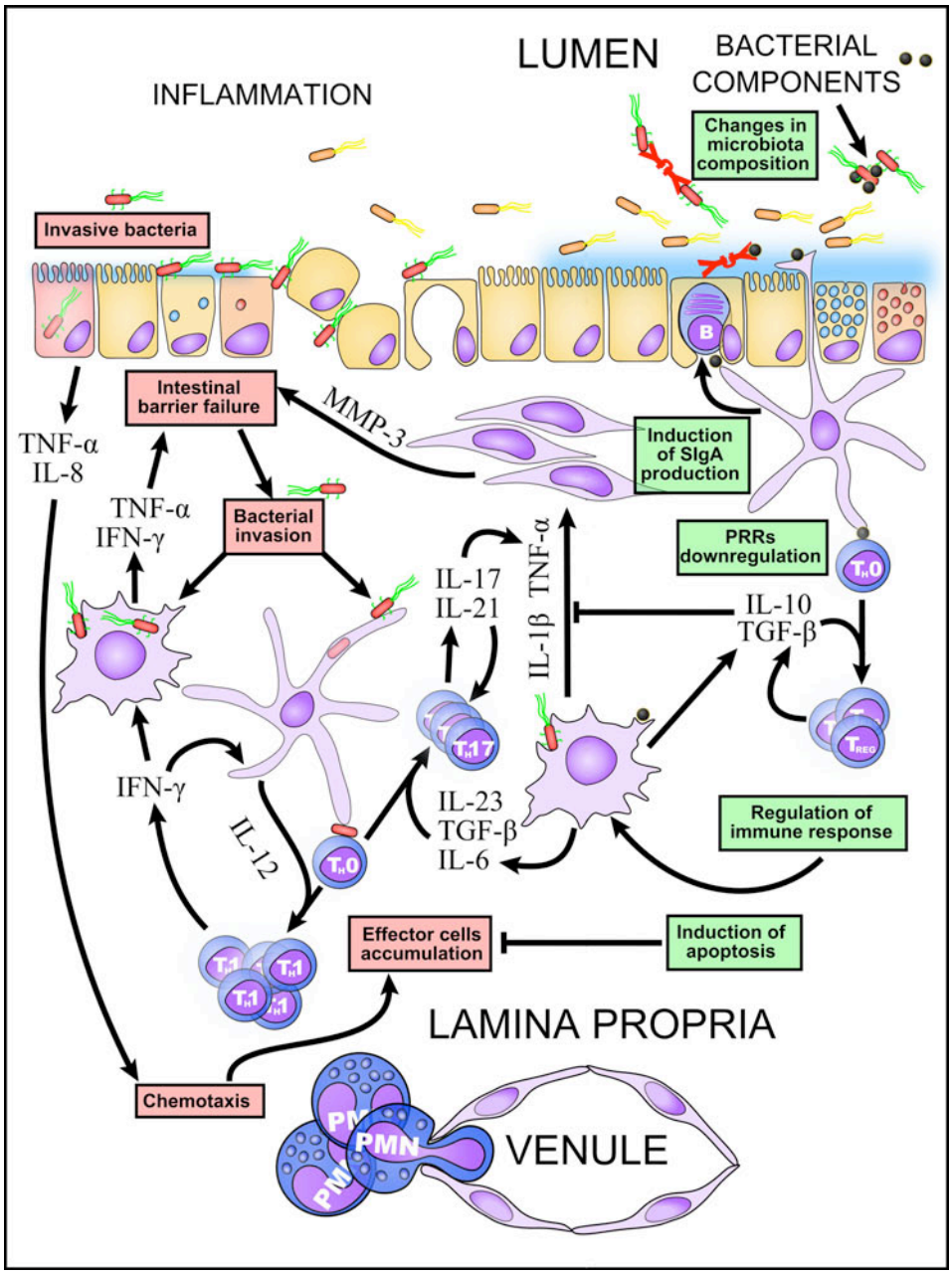

Figure 1: Simplified diagram of IBD pathogenesis summarizing the possible mechanisms by which bacterial components could beneficially influence the natural course of intestinal inflammation.

anti-inflammatory properties among commensals (*Verdù* et al., 2000).

Interestingly, also some well known pathogens could decrease the immune response by dampening inflammatory signals or inactivating immune cells which help them to evade host's immune system and establish infection or persistence (*Ruckdeschel* et al., 1997; *van der Velden* et al., 2008;

reviewed in *Sansonetti* and *Di Santo*, 2007). It would be therefore interesting to isolate the active component and to exploit it in the therapy of IBD with these components.

Several recent studies showed that TLR-9 ligand, an immunostimulatory bacterial oligonucleotide (CpG-ODN), ameliorates experimental colitis and decreases the production of proinflammatory cytokines by human colonic mucosa, but this effect is present only in certain CpG-ODN (*Rachmilewitz* et al., 2002; *Rachmilewitz* et al., 2006). Experiments with isolated bacterial DNA showed that intestinal epithelial cells respond to pathogenic bacterial DNA by increasing surface localization of TLR9 and production of IL-8, but remain unresponsive to DNA isolated from commensal or probiotic bacteria (*Ewaschuk* et al., 2007) In dextran-sulfate sodium (DSS) induced colitis, exposure to CpG-ODN during acute inflammation was found to exacerbate the disease, whereas preexposition proved to be protective (*Obermeier* et al., 2003). These results could explain the observed positive correlation between IBD and domestic hygiene in infancy, and they also suggest that CpG-ODN is a promising candidate for the maintenance therapy, but not for the therapy of active disease.

Another interesting approach is to think about bacteria that have been proposed as the IBD triggers as targets for vaccination. If we will identify the causative bacteria, than the protective immune response against this bacteria could prevent the IBD in otherwise susceptible individuals. Although *B distasonis* and mycobacteria were both proposed to be involveded in induction and perpetuation of intestinal inflammation, we found that introduction of *B. distasonis* lysate, its DNA or mycobacterial heat shock proteins by gavage led to decreased sensitivity of mouse to DSS colitis (*Kverka* et al., unpublished). We still do not know whether we induce the oral tolerance to indigenous microbiota or protective immunity against some closely related potential pathogen causing the inflammation, but this therapy promotes changes in cytokine production in the intestine. Recent findings on how could bacterial components beneficially influence the natural course of intestinal inflammation are summarized in figure 1.

It is important to mention that the reaction of the immune system to the addition of the bacterial component depends on the actual tuning of mucosal immune system. As shown on animal models the introduction of the bacterial component into the inflamed condition could lead to opposite effect.

Although there are still mysteries about the role of microbe-host interactions in IBD pathogenesis, our deeper understanding of these underlying mechanisms is important for new strategies in IBD therapy. It seems that the use of immunomodulatory properties of microbiota could be fundament for that purpose.

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