

REVIEW ARTICLE

Two faces of microbiota in inflammatory and autoimmune diseases: triggers and drugs

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The prevalence of chronic autoimmune and inflammatory diseases, such as inflammatory bowel disease, allergies, or rheumatic diseases, is steadily increasing in developed countries. This increase is probably accelerated by environmental factors, such as decrease in infectious burden or changes in food processing. These lifestyle changes then strongly influence the strongest stimulus for the immune system – commensal microbiota. Despite the differences in the affected organ, the immune-mediated diseases have one or more factors in common – microbe either as a trigger or as a protector, mucosal barrier dysfunction, and dysregulation of the immune system. The core questions, which microbes are involved and how these diseases can be cured or even prevented still remain unsolved. Powered by the recent progress in technology, by new insights into the function of immune system, by advances in microbiome research, and extended use of gnotobiological techniques, these mechanisms are now being unravelled and new therapeutic possibilities are emerging. To secure their niche, the microbes devised many ingenious ways, how to dampen the inflammation. Nonpathogenic microorganisms or microbial components isolated from probiotic, commensal or even pathogenic microbes could be, therefore, used to interfere with the pathogenetic mechanisms of immune-mediated diseases.

Key words: Inflammation; autoimmunity; probiotics; microbiota; intestinal barrier; inflammatory bowel disease; rheumatic diseases; celiac disease; diabetes; allergy; autism.

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There is a steady increase in prevalence of chronic autoimmune and inflammatory disorders, such as inflammatory bowel disease (IBD), rheumatic diseases and allergies, causing significant morbidity in developed countries (1). The crucial role of environment is supported by the fact that only 20–50% of the monozygotic twins are concordant for major autoimmune diseases and by the speed of this increase. The role of genetics is probably over-

estimated, because the twins often share the same environment, at least during the childhood (2).

Infections represent the most plausible modulators of autoimmune diseases. Microbes have been implicated as triggers of autoimmunity, via molecular mimicry and bystander damage, or polyclonal lymphocyte activation, as well as protective agents, via competition with potential pathogens and immunoregulation (2, 3). This knowledge led to intensive search for both the microbial triggers and

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potential protectors. So far, only few triggers and their mechanisms of action had been found, for example streptococci in rheumatic heart disease, *Campylobacter jejuni* in Guillain-Barré syndrome or Coxsackie B4 virus in diabetes (4–6).

The immune response to microbes starts by interaction of innate pattern recognition receptors (PRR) with specific conserved microbial components, microbe-associated molecular patterns (MAMPs) (7). Interestingly, PRRs could be also triggered by host's products of metabolism or by substances released from injured tissues, damage-associated molecular patterns (DAMPs), which supports the hypothesis that tissue damage have a potential to activate self-reactive cells and induce autoimmunity (8). After the PRR-MAMP interaction, activated innate immune cells start the adaptive immune response by presenting the antigen and by production of cytokines, which leads to antigen-specific, protective immune response. In inflammatory and autoimmune diseases this response goes adrift causing serious damage to host's tissues. Therefore, the immune response on the mucosal surfaces must be finely tuned to prevent the pathology in the constant flood of MAMPs from commensal microbiota.

Microbiota is a source of many regulatory signals, which are often necessary for host's physiological functions. These regulatory abilities may be suitable for therapy of diseases, where regulation of immune responses is needed; inflammatory and autoimmune diseases. This makes gut microbiota potential source of new drugs (9). In this review, we discuss how our current knowledge about pathogenesis of selected inflammatory diseases and about microbiota can drive the development of novel therapeutic strategies in the near future.

METHODOLOGICAL CHALLENGES

There are three approaches, currently used to analyze the participation of microorganisms in etiology of complex (polygenic) chronic diseases in humans. All of them are, however, only indirect. One of the most

frequently used is an epidemiological approach that is analysis of association of disease with the occurrence of infection in the particular population (10). Another approach is an immunological screening; where the titers of specific antibodies directed to a certain infectious agent are analyzed in sera of patients. The increased level of antibodies directed to specific microbial component is considered to be an indicator of specific immunological reaction developed during the contact with this agent (11). The last approach is to link infectious component with inflammatory or autoimmune disease by direct detection of an infectious agent in blood or biopsy samples of the affected tissue. For example, this approach linked *Chlamydia pneumoniae* in cerebrospinal fluid with multiple sclerosis (MS) (12), cardiotropic viruses (e.g. Parvovirus B19 or CMV) in endomyocardial samples with inflammatory myocardial diseases (13), or enterovirus in blood with type 1 diabetes (T1D) (14).

Owing to the considerable progress in molecular biology and microbiology, it is now possible to study the role of whole microbial populations in health by direct analysis of microbiota in the intestinal content, which truly revolutionized our understanding of gut microbial ecology. By using the 16S ribosomal RNA gene (rRNA) sequence-based methods, we are now able to analyze the gut microbiota in its full diversity that was often missed by the cultivation-based methods. The sequence-based methods also revealed that there is significant variability in the gut microbiota composition between individuals, yet vast majority of the microbes are members of the two bacterial divisions – Bacteroidetes and Firmicutes (15). As shown by metagenomic sequencing of human gut microbiota, many microbial genes are shared among individuals and the set of all gut microbial genomes of each individual contains set of genes involved in the homeostasis of the whole ecosystem – that is 'minimal gut metagenome' (16). This core metagenome is important for the proper function of the gut microbial community, and the analysis of the microbial metagenome could help elucidate the host-microbe interaction in the gut as well as the association

of the microbial genes with human disease or phenotypes. Another approach to study the gut microbiota is to analyze interactions between microbial and host metabolism. This approach, called metabonomics, uses advanced analytical techniques, such as high-resolution nuclear magnetic resonance or Gas chromatography coupled to mass spectrometry to analyze microbial products (17, 18). By understanding the intensive conversion between microbial and host metabolisms can uncover important physiological pathways or stimulate search for new drugs and therapies, but it could also reduce the adverse reactions of certain drugs (19). One particular challenge is then to link particular microbe, microbial component or even its metabolite, from vast metagenomic libraries with particular phenotype in a high throughput fashion. To overcome this challenge, cell-based screening assays have been developed to analyze either the anti-inflammatory or immuno-stimulatory effects (20).

Unfortunately, all these methods use the patients with already established diagnosis, which may be too late for the analysis of the early steps in disease pathogenesis, because the symptomatic phase of their disease can make the identification of the infectious components very difficult. For this purpose, it is necessary to use the animal models of human diseases. By keeping the susceptible animals in the conditions with controlled microbiota colonization by gnotobiological techniques, we can study the host-microbiota interaction in detail, as well as its physiological, immunological, and pathological consequences. Using these techniques, we showed that colonization of germ-free animals had profound effect on development of the immune system including the mechanisms that keep the intestinal homeostasis (21–25). Gnotobiology is now often used to show the importance of microbiota, or their defined components, in functioning of the immune system and/or in development of immunologically mediated diseases (26, 27). However, the differences in food/water composition and processing, and even in genetic stability of certain inbred strains among gnotobiological facilities could result in some incongruity in such experiments.

INTERACTIONS AMONG HOST'S MUCOSAL IMMUNE SYSTEM, GUT MICROBES, AND THE SPECIFIC GUT ENVIRONMENT

Vertebrates host a vast and complex ecosystem of microbes, which is formed by microbes belonging to approximately 500 species of Eubacteria kingdom and similar number of species derived from Archibacteria and Fungi, most of them located in the distal gut (28, 29). This ecosystem starts its formation after birth, and it represents major stimulus for the development of immune system and other physiological functions (22, 23, 30) and bestows the host with new functional features (16). Gut microbiota has evolved (or is regulated) to complement metabolic functions of the gut to reach higher efficiency of the metabolism (31).

Vast amount of food and beverages, consumed during our lives, extensively modulate the gut environment and the competition for specific substrates can influence the gut microbial community (32). Recently, the number of food additives and preservatives and substantial changes in food processing significantly changed the gut microbial community (33). Continuous pressure of food components changes the microbiota composition, metabolism, and gene expression, which can make the microbes more dangerous and aggressive than when they are grown in cultivation medium (34). There is also the possibility that microbes only catalyze formation of new substances from food, which then trigger the profound physiological or pathological changes in host. Therefore, microbes responsible for the disease triggering can be the product of specific gut environment, and they could behave this way only in the presence of certain environmental stimuli or substrates.

The tolerance to own microbiota is maintained by mechanisms of both innate and adaptive immunity. On the level of innate immunity, the undesirable activation of the complex pro-inflammatory networks by indigenous microbes is blocked either by stimulation of the negative regulators of PRR signaling, or by trigger PRR with opposing effect, or due to the compartmentalization of PRRs, as reviewed elsewhere (35, 36). At the same time, on the level of adaptive immune response, the

homeostasis on the mucosae is ensured by secretory antibodies and by various types of regulatory cells, as recently reviewed (37, 38).

COMMENSALS AS PATHOBIONTS OR PROBIOTICS

Human microbiota and mucosal barrier function are the key players in etiopathogenesis of many inflammatory and autoimmune diseases, as we have recently reviewed (39). Changes in mechanisms regulating mucosal immunity and tolerance, either inherited or induced by environmental triggers, can lead to impaired mucosal barrier function, increased penetration of microbial components from lumen into the mucosa and circulation, and consequently lead to exaggeration of aberrant immune responses and inflammation.

From the host point of view, the host-microbe interactions could be beneficial (probiotic), indifferent (commensals), or deleterious (pathogens). This classification by 'desirable/undesirable effect' for host is flawed, because microbes from different classes could share same interactions with host and certain effect could be both desirable and undesirable, depending on state of the particular host. These classes are also not fixed, because under certain conditions, commensals have the capacity to prevent or trigger disease, so they could act as probiotics or pathogens (pathobionts), respectively.

Probiotics are defined as live microorganisms, which when administered in adequate amounts confer a health benefit on the host (40). There are three main mechanisms, how probiotics contribute to human health and single probiotic bacterium could possess more than one of them. Probiotics shape the ecosystem, by competition for limited resources and adhesion sites, by decreasing the local pH via the production of organic acids, and by production of specific antibacterial substances (41), but they can also produce quorum sensing modifiers, molecules capable to signal to other members of the ecosystem to adjust their growth (42). Certain probiotics can influence the microbial ecology indirectly, by inducing the production of antimicrobial peptides in the host (43). Second, probiotics can improve the

barrier function, thus protecting from environmental threats. Both probiotic *Escherichia 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 (44, 45). It might be possible to convert adherent bacteria into nonadherent simply by administering certain microbial components orally (46). Third, probiotics have immunomodulatory properties on almost all levels of regulation, including downregulation of the PRRs expression, NF- κ B signaling and pro-inflammatory cytokine production (47–49), however, these abilities are strain dependent and may be still present in heat killed bacteria (50). There is increasing evidence, that similar beneficial effects can be achieved with microbial components isolated from probiotics or even from commensals (51–53). This fact may help to overcome the risks of severe infections, associated to the use of live microorganisms in severely ill patients (54, 55).

Interestingly, certain 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 (56–58). It would be therefore interesting to isolate the active component and to exploit it in the therapy of inflammatory and autoimmune diseases, without risking the infection. We will demonstrate some examples of this approach in subsequent paragraphs.

IBD

Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are severe chronic inflammatory illnesses of the gastrointestinal tract, that result from uncontrolled inflammatory response to indigenous intestinal microbiota in genetically predisposed individuals (59). The crucial role of gut microbiota in IBD pathogenesis is suggested by three facts. First, lesions in IBD predominate in areas of the highest bacterial exposure (60), and manipulation of luminal content using selective antibiotics, or fecal stream diversion, improves inflammation in IBD patients (61, 62). Second, in some animal models of IBD, intestinal inflammation is attenuated, or fails to develop

in germ-free conditions (63–67). Third, microbiological screening found dysbiosis, an imbalance in the intestinal bacterial ecosystem, and microbes with special ‘pathogenic’ abilities in IBD patients (e.g. Adherent-invasive *E. coli*, *Mycobacterium avium* s. paratuberculosis). These microbes can cause excessive immune response to microbiota in IBD patients, by disrupting the gut barrier and stimulating local and systemic immunity (68–71). However, dysbiosis or even increased virulence of gut commensals could either be a cause or consequence of the intestinal inflammation (69, 72).

Oral feeding with probiotics was successfully used in treatment of IBD, with acceptably high effectiveness and low incidence of adverse events (73–75). The clinical utility of probiotics in IBD therapy is, however, still controversial due to the lack of effect in some studies or because of the risk associated with live microbes. In animal models of IBD, certain beneficial effects of probiotics have been achieved even by their lysates or by isolated microbial components. Lysate of *Lactobacillus brevis* or *Streptococcus thermophilus* can induce apoptosis in lymphocytes, which could reverse the insensitivity to apoptosis of lamina propria lymphocytes in IBD patients (76). Oral treatment with 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 anti-inflammatory properties among commensals (77). Interestingly, some of these effects make animals less sensitive to the intestinal inflammation, so they might be used even in prevention of intestinal inflammation (78, 79).

The nature of the anti-inflammatory molecules and their mechanisms of action are now being unravelled using animal models of IBD. The anti-inflammatory effect of probiotic mixture VSL#3 can be mediated by its isolated DNA through toll-like receptor9 (TLR9)-dependent pathway (52). 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 (80). Another microbial

component, polysaccharide A isolated from gut commensal *Bacteroides fragilis*, protects mice from experimental colitis intestinal inflammation through changes in local immunomodulation (81). This immunomodulatory molecule has also ability to promote development of CD4⁺ T cells and could be therefore important for correct evolution of the oral tolerance (82). Another microbial surface molecule, lipoteichoic acid from *L. plantarum* was recently found to prevent the excessive immune cell activation via non-obese diabetic2 (NOD2)-related signaling pathways (83). All these data collectively show mechanisms, how microbes educate the mucosal immune system in early phylogeny, and suggest that microbial components can correct the defects in immune system reactivity that would normally result in IBD. These mechanisms are summarized in Fig. 1.

Celiac disease

Celiac disease (gluten sensitive enteropathy) is one of the few autoimmune diseases in which we know the triggering agent – dietary gluten. But environmental factor, such as certain infectious agent, for example Adenovirus 12, Hepatitis C virus, or *C. jejuni*, might be able to precipitate the disease in genetically predisposed individuals (84). Rod-shaped bacteria adhering to the epithelial layer of small intestine are a common finding in biopsies of patients with celiac disease. This typical colonization could be a result of unique carbohydrate structures in the glycocalyx or mucus layer of these patients (85). Interestingly, several papers recently described differences in microbiota composition between patients with active disease, patients on gluten free diet and healthy individuals (86–88).

So far, only few studies used animal models to study the host-microbiota interaction during early stages of celiac disease pathogenesis. Enteropathy, similar to celiac disease, can be induced during early postnatal period of germ-free rats, by oral administration of gluten (89). The presence of microbiota can significantly influence this inflammatory effect of gluten; Enterobacteriaceae promote the gluten-induced enteropathy, and the bifidobacteria suppress it (90). This disease-modulating ability of certain bacteria has been successfully used in another

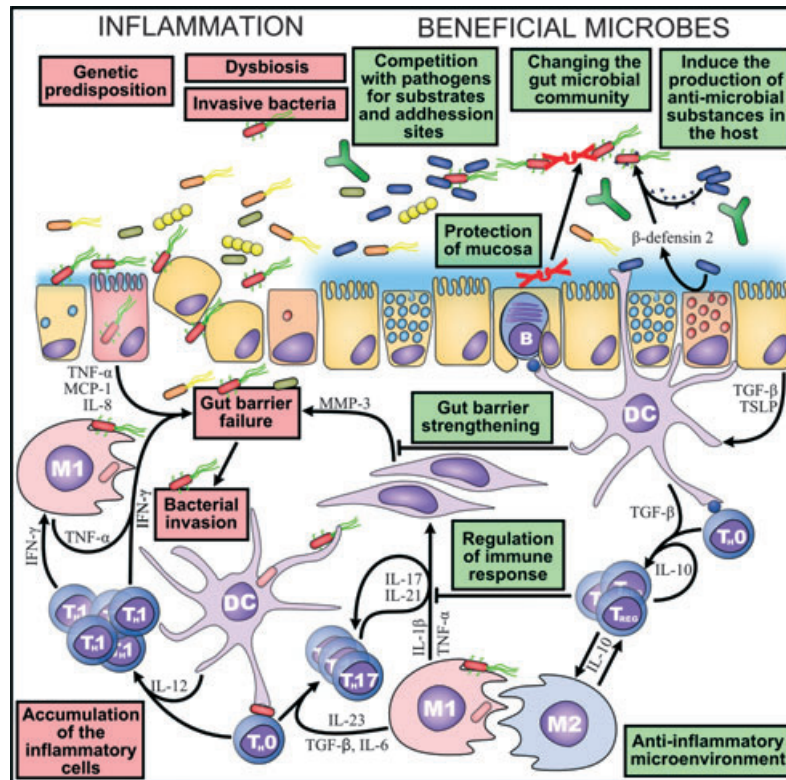


Fig. 1. Beneficial microbes in inflammatory bowel diseases pathogenesis. Beneficial microbes can interfere with the growth of certain pathogens, or correct the dysbiosis, by competition for limited resources and adhesion sites, by decreasing the local pH via the production of organic acids, and by production of specific anti-bacterial substance. But they are also able to induce the production of anti-microbial substances by the gut epithelium or produce molecules signaling to other members of the ecosystem to adjust their growth (quorum sensing modifiers). Certain beneficial microbes can also directly (by changing their gene expression) or indirectly (by induction of secretory antibodies by the host) interfere with adhesion capacities of other microbes or increasing the gut barrier integrity, thus maintaining the gut barrier function. Several microbes have capability to regulate the immune response, including downregulation of the pattern recognition receptors expression, NF- κ B signaling, and pro-inflammatory cytokine production. All these three pathogenic mechanisms are tightly connected by an intricate network of mucosal cytokines, suggesting that by addressing even one of them, we might be able to interrupt the vicious circle of inflammation.

animal model of celiac disease, where oral treatment with *L. casei* improved gut architecture (91). Oral treatment with probiotics still needs to be tested in humans. Certain microbes can improve celiac disease by enzymatic digestion of the immunotoxic gluten peptides (92, 93). Microbial proteolytic enzymes can promote beneficial effects even when used during food processing (94).

T1D

Type 1 diabetes mellitus is an organ-specific autoimmune disease, caused by the selective destruction of insulin-producing pancreatic β

cells by T lymphocytes (95). This reaction arises in genetically predisposed individuals, when the immune system is activated by environmental factors bearing epitopes similar to those in the β cell. Such epitopes were already described in enteroviruses or in cow's milk proteins, suggesting that either nutrition or infection could act as disease triggers (96, 97).

There are two commonly used animal models, BioBreeding (BB) rats and NOD mice, which spontaneously develop diabetes, and which are invaluable for elucidation of the T1D pathogenesis. The relationship between host and microbes in disease pathogenesis is very close. NOD mice reared in germ-free

conditions have substantially increased incidence of diabetes as compared to those reared in specific pathogen-free (SPF) conditions, which suggest the presence of protective factors in the normal microbiota (98). These results are not, however, found in all animal facilities, which suggest that protective microbes are not present in all animal facilities or their action is counteracted by deleterious ones (99). It is now possible to analyze the differences in microbiota composition between animal facilities by high throughput methods, to identify the microbe(s) with capacity to prevent or promote the autoimmunity (100).

The introduction of the protective microbes, probiotics, to the gut of children at genetic risk of T1D is now being tested as a measure of primary prevention (101). Microbes belonging to genus *Lactobacillus* seem like good candidates for T1D prevention, because their presence in feces is negatively correlated with diabetes development in the BB rats (102). Moreover, oral administration of *L. johnsonii* inhibits the disease development in the same disease model, not only by immunomodulation, changes in the metabolism of eicosanoids, but also by improvement of gut barrier function (103). Moreover, oral probiotic mixture VSL#3 prevents the development of diabetes in NOD mice, which is accompanied by increase in IL-10 production in Payer's patches and in pancreas (104). Oral administration of heat killed *L. casei* to NOD mice can reduce the incidence of diabetes, so the protective effect can be achieved also by lifeless microbial cells (105). Also other, often neglected, microbes could be responsible for T1D prevention, because either infection with the lymphocytic choriomeningitis virus (LCMV) or transfer of lymphocytes from LCMV-infected mice prevents diabetes in NOD mice (106). Similar protective effect as whole microbes could be achieved by systemic stimulation of TLR pathways *in vivo*. Systemic administration of TLR2 agonist P40 protein of *Klebsiella pneumoniae*, TLR3 agonist Poly(I:C) or TLR4 agonist lipopolysaccharide (LPS) all decreased the incidence of diabetes in NOD mice, if the treatment started early (i.e. between 4th and 10th week of life) (107). The immunomodulatory activities of gut microbiota were recently also ascribed to bacterial Heat Shock Protein

60 (HSP60), which is capable of inducing conversion of naïve T cells into regulatory T cells (108). Indeed, it is known for some time that immunization with complete Freund's adjuvant or with mycobacterial HSP65, protects NOD mice from spontaneous diabetes (109, 110). The stimulation of innate immunity by microbial TLR agonists could be used for new strategies of T1D therapy or prevention and could be also the mechanism underlying the hygiene hypothesis.

Neurological and psychiatric diseases

Recent studies showed that gut microbiota is an important modulator of brain development and function (111, 112), and changes in microbiota composition are associated with certain neurological and psychiatric diseases, such as MS or autism (113, 114). MS is a severe inflammatory disease caused by the destruction of myelin sheaths of the central nervous system by auto-reactive lymphocytes. There is a strong correlation between repeated and chronic infections during childhood and MS, and the exacerbation of the disease is associated with viral upper respiratory tract infections (115, 116). Patients with MS show also strong cross-reactivity in between sequences found in certain microbes and in myelin components, which strongly suggests that this disease is also triggered by infectious stimuli, by mechanisms of molecular mimicry (117). The finding of the peptidoglycan, a major component of the cell wall of Gram-positive bacteria in the antigen-presenting cells in the brain of MS patients also supports this theory (118). Based on epidemiological studies and the presence of Epstein-Barr virus (EBV)-specific antibodies in cerebrospinal fluid, EBV as a triggering agent was suggested (10).

Using animal model of MS, experimental autoimmune encephalomyelitis (EAE), group of L. Kasper convincingly demonstrated that microbiota is crucial for disease development (114, 119). Alteration of commensal bacterial populations in the gut could lead to development of autoimmune disease by induction of pro-inflammatory T-cell responses to microbiota, which then promote the EAE (120). On the other hand, certain components of commensal bacteria, such as polysaccharide from

Bacteroides fragilis or extracellular adherence protein of *Staphylococcus aureus*, could be used to prevent this condition (119, 121). Similar gut-brain axis in disease pathogenesis has been identified in neuropsychiatric disorders, such as anxiety, depression, eating and sleep disorders (122).

Several papers demonstrated the changes in microbiota composition and subtle inflammatory changes in the gut in autism (123, 124). Consistent with these changes, autistic children have unique metabolic phenotype that differs from healthy children, suggesting that not only gut microbiota but also its metabolic properties are abnormal in autistic individuals (125). This concept is supported by the findings that clinical onset of autism could be delayed by oral antibiotic with minimal absorption (126). Probiotics are therefore proposed to improve gut-related symptoms in childhood autism, and even alleviate behavior difficulties (113). Interestingly, certain probiotic bacteria can interact with the nervous system through production and recognition of neuro-active compounds (127). By interaction with the nervous system, either directly or indirectly via the immune system, and by decreasing the systemic exposure to gut antigens, through improvement of the intestinal barrier, probiotics can interfere with the pathogenesis of neurological and psychiatric diseases.

Rheumatic diseases

The relationship between the rheumatic diseases, microbial components, and elements of immune systems, is now being elucidated at the molecular level. Most human rheumatic diseases, and their animal models, involve participation of microbial components. In reactive arthritis, the microbes act as the disease triggers, and arthritis is the most common extra intestinal complication of IBD; condition, in which the immune response to gut commensal microbiota plays a crucial role (128). Moreover, patients with rheumatoid arthritis (RA) have microbial degradation products in their joints and the composition of their gut microbiota differ from this of healthy subjects (129, 130). Analysis of specific immune response and microbiota led recently to the suggestion that one particular microbe, *Proteus mirabilis*,

common cause of upper urinary tract infection, is the causative agent in RA (11).

The role of microbiota in pathogenesis of joint inflammation has been extensively studied in animal models. In animal models of human spondylarthropathy that is in HLA-B27 transgenic rats or in B10.BR mice, that joint inflammation did not develop in germ-free conditions (67, 131). Interestingly, while arthritis is strongly attenuated in germ-free K/BxN mice as compared to SPF K/BxN mice, their monocolonization with segmented filamentous bacteria is sufficient to reverse the disease dampening caused by germ-free state by induction of Th17 cells (132). Spontaneous T cell-dependent arthritis in IL-1Ra^{-/-} mice is absent under germ-free conditions, and it is markedly suppressed in TLR4-deficient mice, suggesting that TLR4-mediated signaling of resident microbiota is important for joint inflammation (133). All these experiments clearly show that microbiota, or at least certain microbial species, can trigger joint inflammation. Therefore, changes in microbiota or immunomodulation with microbial components and probiotics might be helpful in rheumatic diseases therapy.

The human studies using probiotics usually fail to prove efficiency on disease activity, but often report improvements in subjective symptoms or improve subjective well being (134, 135). Taking into account recent findings by Messaoudi et al., (136) this beneficial effect could be related more to their ability to change the disease experience through influencing central nervous processes, rather than change the disease activity through immunomodulation.

Oral probiotics have been successfully used in animal models of rheumatoid arthritis (137), and their effectivity is ascribed to induction of regulatory cells or potentiation of oral tolerance to collagen (138). The effect can be tracked down to the induction of tolerogenic dendritic cells in mesenteric lymph nodes and subsequent anti-inflammatory tuning of the immune system (139). Oral treatment with mycobacterial HSP65 during ongoing disease significantly reduced the activity of adjuvant-induced arthritis (140). Apart from this study, there are only few reports on anti-arthritis effects of bacterial lysates or isolated microbial

components, but these known to induce regulatory T cells in other inflammatory and autoimmune diseases might be hot candidates.

Allergy

The increase in allergy incidence is inversely correlated to family size and improvement in hygienic conditions in developed countries, which is now called 'hygienic hypothesis' (141). This hypothesis now states that decrease in the natural infectious stimuli in early life can disturb well-balanced development of T-cell subpopulations. At birth, the Th2-type of response dominates, but soon after delivery, it is balanced in favor of Th1-type and regulatory-type, due to the stimulation of the immune system by early colonization by microbes (142). The low diversity of gut microbiota in children born under strict hygienic condition in highly developed countries is now being linked to allergy pathogenesis (143).

The effect of early life colonization with probiotic *E. coli* O83 decreased incidence of infections and use of antibiotics early in life, but it also lowered incidence of allergies 10 and 20 years later (144, 145). Another study showed that even oral treatment of mothers with probiotic *L. rhamnosus* GG reduces incidence of atopic dermatitis (eczema) in their children (146), and that this beneficial effect is transmitted from mother to child through breast-feeding (147). Interestingly, although the decrease in eczema incidence is apparent even after several years, there is no decrease in allergic sensitization (146, 148), and although quite effective on eczema prevention, early intervention with this probiotic microbe does not affect airway inflammation later in childhood (149). This protective effect might require specific interaction between genes and environment, because a similarly designed study on different population did not find any beneficial effect of *L. rhamnosus* on eczema incidence (150). All these studies target the early period in life, aiming at the prevention of the disease. Treatment of existing disease with probiotics was successful for eczema (151, 152), but in case of birch pollen allergy, the results were quite inconsistent (153, 154). The variability in design and outcome of currently available

studies still does not allow general recommendation of probiotics in allergy prevention and therapy (155, 156).

Recently, two small studies successfully treated eczema patients either with oral lysate of *L. rhamnosus* or topical treatment with lysate of nonpathogenic gram-negative bacterium *Vitreoscilla filiformis* (157, 158). Interestingly, local application of *V. filiformis* lysate significantly decreased the numbers of *S. aureus* colonizing the affected skin (158). Because the products of *S. aureus* may contribute to eczema pathology, as recently reviewed (159), the local selective antimicrobial activity might be the mechanism responsible for the therapeutic effect. Recently published clinical trial has shown that the oral administration of lysate prepared from probiotic bacteria (*E. coli* Symbio and *Enterococcus faecalis* Symbio) in infancy, decreases the risk of atopic dermatitis in childhood. This effect is, however, apparent only if only one of the parents, especially father, was atopic (160).

Immunostimulatory oligonucleotides (CpG-ODN), TLR9 ligand resembling bacterial DNA have been successfully used in both treatment and prevention of allergic inflammation in animal models of atopic disorders (161). CpG-ODN is able to enhance effect of allergen immunotherapy, or even reverse the ongoing inflammation in animal models, which suggests that they could be useful component of allergen immunotherapy (162). Since the beneficial effect of probiotics, and their components, is now being linked to their ability to induce regulatory T cells, (163, 164), other lysates and components, known to induce regulatory cells in another models of autoimmune and inflammatory disorders, might be effective in allergies as well.

Periodontal disease

Periodontitis is a chronic inflammatory disease affecting the connective tissues and bone housing the teeth. The prevalence of the disease is high, about 50% of adults are affected by moderate form of this disease, and 5–15% of the population is affected by severe, progressive, and destructive form (165). Several species of periodontopathic bacteria present in oral cavity were implicated in etiology of the

disease: *Prevotella intermedia*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Actinobacillus actinomycetemcomitans*, and *Treponema denticola* (166, 167). However, some of these periodontal 'pathobionts', such as *P. gingivalis* and *T. forsythia*, are frequently isolated from healthy controls. Similarly to other chronic diseases occurring in mucosal surfaces, dysbiosis in the oral cavity can lead to periodontitis: oral microbiota shifts from gram-positive aerobes to gram-negative anaerobes were described. A succession of distinguishable microbial complexes develops (167). The complexity of the situation was demonstrated by using experimental models of the disease, including germ-free rats monoassociated with *P. gingivalis* (168), mice infected with *P. gingivalis*, ligated rat or hamster teeth and in some immunocompromised strains of mice.

Periodontitis with bone loss did not occur in animals reared in germ-free conditions. Experimental probiotic treatments *in vivo* and analysis *in vitro* characterized the effects of various probiotic strains on factors in periodontal health. Results of these studies suggest that probiotics may benefit periodontal health. However, more research is needed to define the optimal strain, therapeutic dosage, delivery mechanism, and patient profile for periodontal probiotics (169).

Metabolic diseases

The dramatic changes in lifestyle in the last few decades caused rapid increase in obesity and type 2 diabetes mellitus (T2D). These pathologies are responsible for high incidence of atherosclerosis and its complications, major causes of mortality worldwide (170). The relationship between inflammatory and metabolic pathways is very close, and inflammation is now recognized as a major player in obesity and metabolic diseases via insulin resistance and atherosclerosis, as recently reviewed by Caesar et al. (171). Several microbes implicated in their etiology, including intracellular bacterium *C. pneumoniae*, *Helicobacter pylori* and various oral bacteria suggested also as causative agents of periodontitis (*P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*). The association of periodontal disease with athero-

sclerosis is very close, because certain bacterial phylotypes are commonly found both in the atherosclerotic plaques and oral among microbiota, so the bacteria from the oral cavity may contribute to atherosclerosis pathogenesis (172). On the other hand, the atherosclerosis in atherosclerosis-prone experimental animals (Apo-E deficient mice) is accelerated in germ-free conditions, even if the mice are kept on standard low cholesterol diet (173). This suggests that atherosclerosis can be prevented by beneficial microbes in the commensal microbiota, similarly as suggested for T1D (98). Recently, this hypothesis was supported by pilot study, in which 'transplantation' of fecal microbiota from lean subjects to patients with metabolic syndrome increased their insulin sensitivity (174).

Moreover, chronic systemic stimulation of mice with LPS induces obesity and diabetes by CD14-dependent mechanism (175). This suggests that leak in mucosal barrier causes the excessive stimulation of systemic immune system with bacterial LPS or other microbial components, which could be the triggering factor of low-grade (metabolic) inflammation. Probiotics (as mentioned earlier) and prebiotics can improve the barrier thus decrease the inflammatory stimuli (176).

Lactobacillus acidophilus and *Bifidobacterium lactis* improve the lipid profile in individuals with T2D, which may contribute to the improvement of cardiovascular disease risk in these patients (177). Moreover, oral treatment of hypercholesterolemic patients with synbiotic (i.e. probiotic and prebiotic combination) *L. gasseri* CHO-220 and inulin for 12 weeks significantly reduced their plasma total- and low-density lipoprotein- cholesterol, as compared to placebo (178). Because these parameters are one of the main risk factors of atherosclerosis, similar therapeutic strategies could be used to decrease the risk atherosclerosis and its complications. The mechanism of the beneficial effect of probiotics on metabolic diseases is still poorly understood, but prevention of the low-grade inflammation by modulation of the immune system, changing the cholesterol metabolism by influencing the entero-hepatic cycle of bile acids, or changes in the energy recovery from dietary fiber might be one of the key mechanisms.

Other autoimmune and inflammatory diseases

Live or lysed probiotic bacteria have been also successfully used in treatment of irritable bowel syndrome, where the mild inflammation of the gut mucosa is a typical pathogenetic feature (179, 180). There are other autoimmune and inflammatory diseases with strong link to microbiota, such as psoriasis (181). The effect of probiotics or microbial components in their therapy is yet to be evaluated, but similarly as in those diseases mentioned above, tuning of mucosal immune response with similar 'immunomodulatory' microbial components can be suitable strategy for therapy, or even prevention, of these diseases.

CONCLUSIONS

The host-microbe interactions in the human gut influence many physiological and pathological processes. The new techniques of gut microbiota analysis and accumulation of our knowledge about their role in human health brings also new insight into the pathogenesis of immune-mediated diseases. In their pathogenesis, microbes could play a dual role, as disease triggers and as protectors. These effects are strain dependent, but could be shared by many different microbes, due to their molecular complexity. Once, we will learn the nature of the biologically active molecule present either in probiotics, commensals or pathogens, we might be able to correct the 'error in immune system function' present in autoimmune or inflammatory diseases. Since the mechanisms involved in prevention of one immune-mediated disease, for example improvement of gut barrier function, or induction of regulatory T cells, we can use knowledge in therapy of other inflammatory diseases. Thus, although the era, where 'vaccinations' against inflammatory and autoimmune diseases will be a routine method is still in the haze of the next century, the rapid progress in basic research of host-microbe interactions makes its vision clearer than ever.

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