

Review articles

Natural microbiota in viral and helminth infections. Addendum to: Personalized vaccination. II. The role of natural microbiota in a vaccine-induced immunity.

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ABSTRACT. Numerous original and review papers have emerged over recent years concerning the natural microbiota and its interaction with the mammal host's body. This addendum supplements in short our previous review article on the role of microbiota in the host immunity [1] paying particular attention to such essential aspects as the composition and role of gut microbiota in viral infections as well as the interplay between the microbiota and the macrofauna inhabiting the mammalian gastrointestinal tract. The host immune system, commensal microbiota and macrofauna are elements of an integrated system in which the relationships are bidirectional. As demonstrated in the article, virus or helminth infection alters the composition of commensal gut microbiota but, in turn, commensal microbiota influences the fate of a virus or helminth infection. Natural microbiota located on external and internal surfaces of the host body is a prominent element of its health and condition, including the functioning of the immune system [1,2,3]. The gastrointestinal tract harbors the highest number and the greatest diversity of microbial organisms, so the studies presented in the article regard gut microbiota.

Key words: microbiota, viruses, helminths, host immunity

Microbiota and viral infections

Viruses are dominating etiological agents of mammals infections. They penetrate the host body using the sites abundant with natural microbiota (e.g. gut mucosa, skin etc.), which can potentially alter the fate and finally the outcome of the infection.

Hitherto a prevailing number of studies revealed that natural microbiota can play an ambivalent role in host anti-viral immunity. In some cases it helps the host effectively fight the infection, whereas in others it promotes the infection. Wilks and Golovkina [4] described and presented graphically three possible mechanisms of the microbiota action. The first scenario concerns influenza A virus infection in which certain commensal bacteria display a protective role. In the presence of intact natural microbiota, the respiratory infection of influenza A virus in mice leads to the induction of the NLRP3 inflammasome [5]. Inflammasomes are large multiprotein complexes, activated upon cell infection or stress stimulus, and play a key role in the innate immunity by participating in the production of the pro-inflammatory cytokines. Inflammasomes allow activation of caspase-1, able to process and activate proIL-1beta and proIL-18 molecules [6]. Inflammasome activation in influenza infection results in the migration of lung dendritic cells to the draining lymph nodes and the stimulation of virus-specific T-cell response. By using various antibiotic treatments, Ichinobe et al. [7] found that neomycin-sensitive commensal bacteria are exclusively associated with the induction of protective immune responses in the lung. The results imply that the very common treatment of respiratory diseases with antibiotics may make it impossible to initiate protective immune responses to the influenza virus. Besides, stimulants of inflammasomes seem ideal candidates

for an adjuvant in flu vaccines. Two other scenarios of the role of commensal microbiota are associated with microbiota-dependent promotion of viral infection. The study of Kuss et al. [8] revealed that natural microbiota directly enhances replication of such common enteric viruses as polioviruses (Picornaviridae) and reoviruses (Reoviridae) in experimentally infected mice. Futher detailed experiments performed on both mouse (embrionic fibroblasts) and human (HeLa) cell lines clearly showed that *N*-acetylglucosamine-containing surface polysacccharides of both Gram-positive bacteria (peptidoglycan) and Gram-negative bacteria (LPS) increased virus infectivity. These bacterial products, bound to virus particles, enhance the virus attachment to gut epithelial cells or, as shown by Kane et al. [9] for another unrelated enteric virus – MMTV (Mouse Mammary Tumor Virus, Retroviridae), virus-bound LPS triggers the TLR4 receptor on dendritic cells, leading to a subsequent IL-6-dependent induction of immuno-suppressive IL-10 in B lymphocytes. As it was found earlier, this cytokine directly inhibits effector and memory CD4+ T cell responses [10]. Establishment of MMTV chronic infection requires natural microbiota because antibiotic-treated or germ-free mice do not transmit MMTV infection to their offspring [9]. Indirect promotion of the virus replication by natural microbiota seems to be also possible and could result from stimulation or activation of host cells by commensal bacteria. This mechanism is suggested for retroviruses, which infect only proliferating target cells although the results of few studies on the murine leukemia virus are unequivocal [4]. Apart from natural infections, several live attenuated virus preparations (e.g. polioviruses, rotaviruses) are commonly used as a vaccine material (Polio Sabin – oral, Rotarix, RotaTeq etc.) inoculated in humans orally, so the composition of gastrointestinal tract microbiota is one of the crucial determinants of the development of a vaccine-induced immunity [1].

The studies concerning the influence of commensal bacteria on anti-viral immune response are usually conducted on either antibiotic-treated or germ-free (gnotobiotic, axenic) animals, particularly experimental rodents. The latter tool seems very valuable because it enables analyzing the relationship between microbiota and the immune system by comparising the effects of microbial colonization in both germ-free and wild-type inbred isogenic mouse strains [3,11]. Because both antibiotic-treated and gnotobiotic animal models are not perfect, it is recommended to carry out the planned experiments paralelly using both experimental systems.

Viral infections, regardless of the location, strongly affect natural intestinal microbiota composition. Bacteria of phylum Firmicutes (genera: *Enterococcus, Peptostreptococcaceae incertae sedi, Streptococcus, Weisella, Clostridium* and opportunistic genus *Shigella*) dominated in patients with diarhea caused by different viruses (adenoviruses, noroviruses, rotaviruses, and astroviruses), whereas phylum Bacteroidetes was dominant in healthly humans [12]. Additionally, the copy number of three genera: *Bacteroides vulgatus, Bifidobacterium,* and *Lactobacillus* was significantly reduced in virus-infected patients as compared to healthy controls. The beneficial effect of *Bifidobacterium* on the health is widely accepted. It has been recently demonstrated that the species profile of this genus was deeply changed in chronic hepatitis B disease by a shift from beneficial species (e.g. *Bifidobacterium longum*) to the opportunistic ones (e.g. *Bifidobacterium dentium*) [13].

Microbiota and macrofauna interaction

An experimental model for studying the microbiota and macrofauna relationship is genus *Trichuris* consisting of very common and succesful nematodes inhabiting the gastroitestinal tract of numerous mammalian hosts. Using this model, Hayes et al. [14] found that intestinal microflora mediated the establishment of chronic *Trichuris muris* infection within the epithelium of Lieberkühn crypts in the large intestine (cecum and colon). Microflora initiates hatching of *T. muris* ova: it attaches to the terminal plugs, which are removed enabling larvae to exist. A thick mucus layer keeps most bacteria away from the host epithelia but early larval stages of *Trichuris* burrow into the mucosa and allow the intestinal microbiota to reach the epithelium and stimulate the immune system. *In vitro* incubation of eggs with explants of mouse cecum containing substantial numbers of bacteria or *E. coli* culture induced the hatching. Direct contact between the bacteria and the eggs, and not secreted molecules, was required for the process. Further analysis confirmed that not only *E. coli* but also other bacteria (*S. aureus, P. aeruginosa*, and *S. typhimurium*) and yeast *S. cerevisiae* could induce efficient hatching. Reducing the number of bacteria

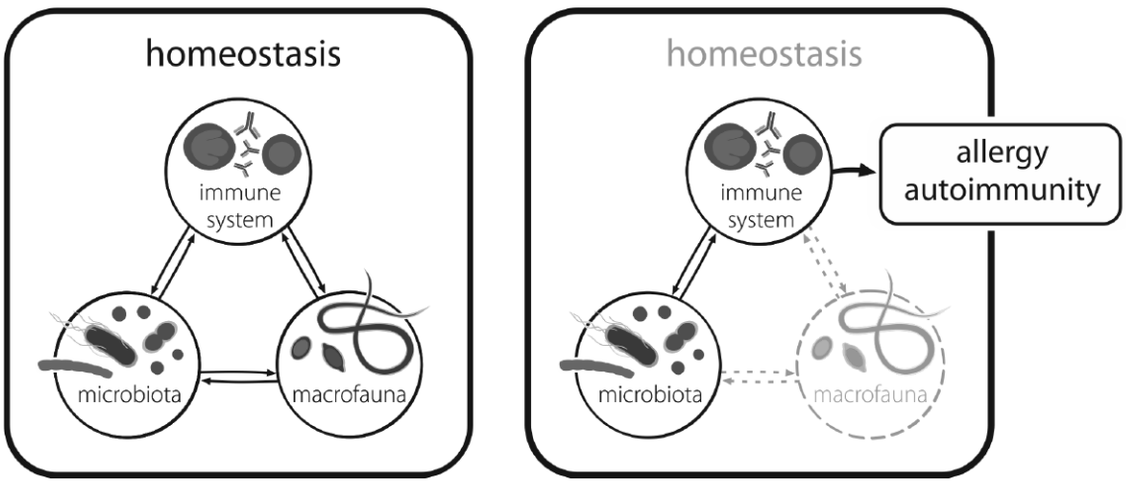

Fig. 1. Interactions between the mammalian immune system, microbiota and macrofauna

by enrofloxacin was accompanied by immunomodulation of mouse immune response and a decrease in the hatched parasite egg number.

As recently signalled in the review by Bancroft et al. [15], chronic *T. muris* infection is associated with the changes in cecum microflora (A. Houlden, I. Roberts, and R. Grencis, unpublished). A few of studies revealed also that helminth infections alter significantly composition of gut microbiota in mammals. Using the clone library analysis of the bacterial 16S rRNA encoding gene, Walk et al. [16] studied the effect of the *Heligmosomoides polygyrus* treatment on the composition of gastrointestinal tract microbiota in the distal small intestine (ileum) and the tip of the cecum. The infection of wild-type C57Bl/6 mice with *H. polygyrus* caused changes of both the bacteria number and composition. The total bacterial load was greater in infected mice versus controls but the most characteristic was the increase in the number of Lactobacillaceae, suggesting that some species find the conditions in the ileum of *H. polygyrus*-infected mice favorable and a microbial-helminth mutualistic relation is formed, where both bacteria and the worm benefit from symbiosis. The results may also imply that Lactobacillaceae (with dominant *Lactobacillus* species) play a role in the anti-inflammatory effects of *H. polygyrus* infection. Similarly, Li et al. [17] observed significant alterations in the proximal colon microbiota composition in *Trichuris suis* infected pigs as compared to the parasite naive control animals. The changes regarded approximately 13% of bacterial genera and induced deep shift in the metabolome of microbiota dwelling the proximal colon (for instance reducing carbohydrate metabolism and lysine biosynthesis). Further studies performed at 53 day postinfection (at that time some pigs were worm-positive and others were worm negative) showed that the percentage of the genera significantly affected was similar between the 21 (13%) and 53 (≈13%) day postinfection, regardless of the worm status of animals, although the composition profiles were a little distinct [18]. *Trichuris suis* infection was associated with a 3-fold increase in relative abundance of *Campylobacter* in worm-positive pigs but with a 3-fold decrease in worm-free, as compared to naive controls. These results revealed dynamic changes – *T. suis* infection promotes the growth of *Campylobacter* and adult nematode clearance had a quick therapeutic effect.

Concluding remarks

The relation „mammal body-microbiota-macrofauna" is very complex and dynamic. To date, the attention has been focused on commensal bacteria in the gastrointestinal tract and their interactions with the human body including the immune system. Other components of microbiota (e.g. archeons, viruses, fungi, and protozoa) and macrofauna as well as their mutual interactions and influence on the host immunity remain to be explained. For instance, viral diversity in the human gut as well as in other body habitats is poorly known as compared to bacteria. Particular attention should be paid to bacteriophages, which in many environments outnumber bacteria ten to one, being an essential regulator of bacteria density. Microbiota, macrofauna and the host immune

system must be viewed as an integrated and balanced system that has developed by a co-evolution for many millions of years (Fig.1). As stressed by Bancroft et al. [15], the recently observed elimination of macrofauna in humans living in highly developed countries disrupted the immune homeostasis and this disruption is suggested to be responsible for the increasing immunopathologic phenomena like allergic and autoimmune diseases. Strong hygiene regime as well as widely used antibiotics, probotics, prebiotics, and symbiotics influence significantly the relationship between the main co-players: immune system, microbiota and macrofauna. The described examples of a dual role of microbiota in selected viral infections suggest that a future therapy or prevention of infectious diseases should take into consideration the current composition of microbiota and macrofauna of an individual (a „personal identity card") and their possible interaction with the given infectious agent.

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