Review articles

Personalized vaccination? II. The role of natural microbiota in a vaccine-induced immunity[1]

Henryka Długońska, Marcin Grzybowski

Department of Immunoparasitology, Chair of Immunology and Infectious Biology, University of Lodz,
12/16 Banacha Street, 90-237 Lodz, Poland

Corresponding author: Henryka Długońska; E-mail: hdlugo@biol.uni.lodz.pl

ABSTRACT. Inter-individual variation in immune response to widely used prophylactic vaccines against infectious diseases is strongly influenced by sex, MHC (Major Histocompatibility Complex), age and current hormones status of vaccinated individuals. Numerous findings showed that microorganisms residing at different sites of human or animal body (natural microbiota), especially in the gastrointestinal tract, appear to contribute to nearly every element of the host's physiology. Recently, the microbiota is also supposed to be an underappreciated yet, but very important factor responsible for diverse vaccine efficacy observed in humans from developing vs. developed countries. In the article, selected aspects of the microbiota – host relation are presented: importance of the gut microbiota in the development of both the intestinal mucosal and systemic immune responses, bacteria of a predominant role for the immunity (e.g., SFB, Segmented Filamentous Bacteria), and several clinical observations on the varied immunogenicity of the same vaccines in different human populations. In the light of our current knowledge, manipulation of the microbiota by probiotics and/or prebiotics is becoming a realistic therapeutic and prophylactic strategy for many infectious, inflammatory and even neoplastic diseases within the gut but it may be also used for improving vaccine efficacy.

Key words: microbiota, vaccine, immunity

Introduction

The previous article [1] discussed selected aspects of inter-individual variations in artificially acquired active immunity, i.e. induced by vaccination. The most prominent factors determining the profile and intensity of immune response in humans and animals are sex, age, major histocompatibility antigen complex and current hormones levels. Immunoprophylaxis against infectious diseases performed with vaccines according to the up-to-date immunization calendar is based on mass vaccination and does not take into consideration individual characteristics of vaccinated subjects despite accumulating data which advocates personalized vaccination.

The present study highlights another factor underestimated to date in vaccine-induced immune responses, i.e. intrinsic natural body „microflora" currently called „microbiota" as a result of exclusion of bacteria and fungi from the kingdom Plantae.

Microorganisms (microbiota) inhabiting the human body

At birth, human body is composed of exclusively human cells. After delivery the sterile body of a neonate is instantly colonized by microorganisms, the process being dependent on the mode of delivery, hygiene, infant diet and administered medication [2]. Life-long intense colonization accounts for only 10% of human cells in the total of all cells in the human organism at the moment of death, with 90% of microbial cells. The genome of this microbial community (bacteria, archaeons, viruses, fungi and protozoa) residing inside and outside the body is called a microbiome. The total number of genes forming a microbiome approximately 100 times exceeds the number of human genes, i.e. only about 2×10^4 genes encoding proteins [3]. Microorganisms dwell on the human body unevenly – most of them inhabit the gut, with relatively few occurring in the respiratory system. Its lower segments were so far considered as sterile,
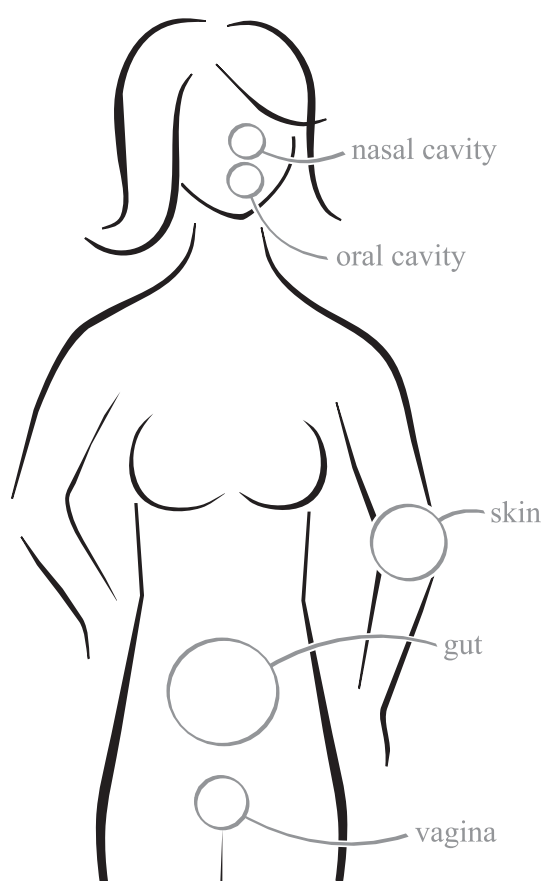
Fig. 1. Natural human microbiota – 5 sites from which specimens in „Human Microbiome Project" (HMP) are collected, http://www.hmpdacc.org/sampling.php.

but using molecular techniques characteristic local consortia of bacteria have recently been found also in the bronchial tree [4]. Intestinal microbiota dominant in humans (70% of the pool of microorganisms) consists of about 10^{14} bacteria belonging to 500–1000 species and over 7000 strains forming the biomass of as much as 1.5 kg. Intestinal population of bacteria reaches the thickest density in the large intestine amounting to 10^{11}–10^{12}/ml [5–6]. Classic culture-based methods revealed years ago the presence of almost solely anaerobes of species *Bacteroides*, *Clostridium*, *Lactobacillus*, *Fusobacterium*, *Bifidobacterium*, *Eubacterium*, *Peptococcus*, *Peptostreptococcus*, *Escherichia* and *Veilonella* [7]. New generation methods based on sequencing DNA directly from environmental samples (metagenomics) allow, however, detecting uncultivated microorganisms. The early analysis performed by Ekburg et al. [8] in 2005 revealed enormous diversity of gastrointestinal tract microorganisms. Up to 60% of the obtained 16S rDNA sequences were novel, and

up 80% of sequences were derived from uncultivated species. Further metagenomic research has shown that 99% gastrointestinal tract bacteria belong in 99% to four types (phylotypes): Firmicutes (79.4%), Bacteroidetes (16.9%), Actinobacteria (2.5%), and Proteobacteria (1%), and dominating species were *Faecalibacterium*, *Ruminococcus*, *Eubacterium*, *Dorea*, *Bacteroides*, *Alistipes* and *Bifidobacterium* [9]. Intestinal microbiota is a multifunctional and very important, though underestimated, organ which coexists in mutual relationship with host organism and coevolves with it [10]. The composition and metabolic activity of microbiota influences, among others, the state of host nutrition, effectiveness of taken medication, natural and acquired immunity and, therefore, it is no overstatement to say that it is of decisive significance for health and disease [3,11,12].

Is it possible in this complex and extremely abundant ecosystem as microbiota to find a distinct genus or species which selectively performs some specific function, for instance affects immune system reactivity? New and not numerous studies to date give positive answer.

Intestinal bacteria playing a special role in immunity

A study on gnotobiotic (germ free) mice showed that anaerobic Gram-negative rods commonly appearing in natural microbiota may neutralize numerous immunological defects in those animals concerning local and systemic immunity, stimulating development and maturation of lymphocytes T and setting the correct ratio of Th1/Th2 lymphocytes and proper cytokine balance. It is worth emphasizing that intestinal colonization with only one bacteria species *B. fragilis* (mono-colonization) is sufficient to restore appropriate immunological status, and some capsular polysaccharides of these bacteria are directly responsible for this phenomenon [13].

Unculturable bacteria SFB (Segmented Filamentous Bacteria), similar to anaerobic rods of genus *Clostridium* (provisionally classified as *Candidatus Arthomitus*), colonize the small intestine of numerous mammals just after weaning a baby and participate in the development of local immune system GALT (Gut-Associated Lymphoid System). Scanning microscope images show that, in the way of transcytosis through microfold (M) cells, SFB come into direct and close contact with mononuclear cells in intestinal mucosa [14]. It has

been demonstrated that the settlement of SFB in gut (after their oral administration into gnotobiotic mice) causes a rise in the total number of intraepithelial lymphocytes $T\alpha\beta$ (especially of subpopulation $CD8\alpha\beta+$), expression of MHC class II on epithelial cells and rise in the level of plasmocytes producing IgA-class antibodies in *lamina priopria* [15]. Recently it has been found that SFB are responsible for the increase in the intestinal CD4+ lymphocytes level and well-orchestrated development of the local immune response mediated by those lymphocytes, including both the expression of pro-inflammatory (IFN-γ) and regulatory (IL-17, IL-10) activities [16]. Ivanov et al. [17] showed that SFB selectively induce only the subpopulation of helper lymphocytes Th17 producing IL-17 and IL-22 without alteration in the level of helper lymphocytes Th1 producing IFN-γ and CD4+ regulatory lymphocytes (Foxp3+ Treg). Lymphocytes Th17 and their soluble mediators play a key role in the development of local anti-infectious immunity on the mucosa [18].

The aforementioned studies have explicitly shown that the immunological response of the organism with the aid of lymphocytes T depends on isolated intestinal commensals, which influence not only local immunity in gastrointestinal tract, but also systemic immunity.

Diverse immunological response to vaccines in humans

The range of prophylactic vaccines for humans against infectious diseases systematically increases. Many of them are used in large-scale all over the world. Numerous serological data show that immunogenicity of vaccines varies in developed versus developing countries. It concerns, among others, the vaccine used commonly since 1962, namely Albert Sabin's attenuated oral vaccine against Heine-Medin disease (poliomyelitis). Patriarca et al. [19] reported that trivalent oral poliovirus vaccine caused seroconversion in 100% of children from industrialized countries, but only in approximately 70% of children from developing countries. Similar observation concerns orally administered inactivated cholera vaccine including killed vibrios of *Vibrio cholerae* O139 strain. In-depth comparative study on the vaccine immunogenicity in children from Stockholm and Leon in Nicaragua showed that Swedish children responded by a higher production of serum antibodies IgA against the cholera toxin than Nicaraguan children [20]. Even in individuals living

in the same region diverse degree of post-vaccination immunity was reported depending on socioeconomic conditions. Studies performed in Peru revealed that seroconversion rates after oral administration of live cholera vaccine (CVD 103-HgR) were higher in students and physicians of medical faculty than in slums inhabitants and the difference in vibriocidal seroconversions depended on the vaccination dose (e.g., with lower dose seroconversion rates were respectively 78 and 49%) [21]. The proximal small intestine in healthy children and adults is practically free from rods of Enterobacteriaceae family ($<10^4$/ml of intestinal aspirate). It was noted that the presence of rich intestinal microbiota (correlated with intensive exhalation of H_2 as the product of sugars fermentation by microorganisms) was linked with impairment of antibodies production after an oral application of live cholera vaccine (CVD 103-HgR) in children in Santiago [22].

The foregoing observations on diversified post-vaccination immunogenicity in various human populations call for causal explanations. Both the genetic and environmental factors, including socioeconomic conditions, nutritional and immunological status, may be considered. It has been suggested that frequent and intensive contact of some individuals with microorganisms leads to the development of specific tolerance to their antigens and resulting lack of post-vaccination immunity. One of the factors modulating immunological response may also be microbiota, particularly in gastrointestinal tract. The question of taking it into account in formulation and administration of vaccine is therefore fully justified [23]. The potential influence of microorganisms is associated not only with their immunoregulatory activity but also, in case of vaccines introduced on the mucosa (oral, nasal, rectal and vaginal routes), with metabolic changes in vaccination material made by numerous microorganisms which live there.

Probiotics, prebiotics and the immune response to vaccines

Probiotics are defined as live non-pathogenic microorganisms (mainly lactic acid bacteria) which, when administered in adequate amounts, confer certain health benefits. Taken orally, they may alter the composition and activity of intestinal microbiota, whereas the profile of their immunoregulatory action concerning both the innate and acquired immunity is strictly dependent

on the probiotic strain [24]. It was demonstrated on the basis of varying cytokine production (IL-10 and IL-12) by human peripheral blood mononuclear cells (PBMC) stimulated by different *Lactobacillus plantarum* strains [25].

It was documented that probiotics may enhance immunogenic activity of vaccines. For instance, in the case of oral rotavirus vaccine, in a group of infants who received *Lactobacillus casei* GG just before the administration of the vaccine followed by 5 days of probiotic intake a statistically significant increase in the level of IgM secreting cells and a higher seroconversion rate in contrast to the placebo control group (IgA presence – from 74 to 93% of the vaccinated individuals) were found [26]. Similarly, in the case of oral live *poliomyelitis* vaccine given together with probiotics (*Lactobacillus rhamnosus* GG or *L. acidophilus* CRL431), significantly higher titres of poliovirus neutralizing antibodies, as well as specific IgG and IgA were noted [27]. On the other hand, Matsuda et al. [28] tested *Bifido-bacterium breve* strain Yakult (BBG-01) for the enhancement of immunogenicity of an oral inactivated cholera vaccine but no significant differences comparing BBG-01 and placebo group were found. An exceptionally good research model for the assessment of the influence of probiotics on the immune response to vaccines seems to be influenza vaccination – the flu vaccines are widely used and, importantly, a significant decline in their effectiveness in the elderly is commonly observed. It has been shown that daily consumption of Actimel® significantly enhances humoral response induced by trivalent seasonal vaccine, although a statistically significant difference in immune response between the probiotic and placebo group was noted only in relation to the type B influenza virus [29].

Other interesting observations were also made analyzing the immunomodulatory activity of prebiotics. These are non-digestible substances whose beneficial effect on health is attributed to selective growth stimulation of microorganisms inhabiting the human body. To prebiotics belong oligosaccharides (e.g., lactosucrose) and poly-saccharides (e.g., fiber and its split products) [30].

Promising results were achieved by Benyacoub et al. [31], who investigated the effect of fructooligosaccharides (FOS)/inulin mix on murine response to the live attenuated *Salmonella* vaccine. In the group of animals fed the diet containing prebiotics compared with control mice a statistically significant increase in the level of specific serum IgG and faecal IgA antibodies was found.

Moreover, after the challenge with a lethal dose of a virulent strain of *S. typhimurium*, a 33% increase in the protection rate was noted upon feeding FOS/inulin mix as compared with the control group. The murine model of vaccination response was also used to evaluate the immunomodulatory effect of a prebiotic product Immunofortis® (short-chain galactooligosaccharides and long-chain fructooligo-saccharides in combination with pectin-derived acidic oligosaccharides) on the immune response to influenza vaccine. Based on the vaccine-induced DTH responses and microbiota parameters, it was shown that supplementation with the prebiotic modulates the priming phase of immune response in relation to the shift in the Th1/Th2 balance to be mediated by microbiota. Concurrently, attention was drawn to the necessity of considering kinetics of prebiotic activity to understand better their interaction with natural intestinal microbiota and the immune system [32].

In summary, research up to date shows that using an appropriate genus and strain of a probiotic associated or not with a prebiotic preparation may increase immunogenic properties of the vaccine and trigger a proper profile of immunological response.

Final remarks

The composition, activity and biological significance of microbial consortia residing in organism of humans and other mammals are subjected to more and more numerous studies. For instance, the number of publications only on intestinal microbiota has increased five times in the last twenty years [12]. A proof of special interest in human microbiota and increasing recognition of its role is a five-year research effort „Human Microbiome Project" (HMP) launched in 2007 by National Institutes of Health in the USA aiming at characterization of ontocenoses (microbial communities at various sites of the human body), selection of their phylogenetic core, i.e., dominant and most frequent microorganisms, and determination of their role in health and disease [3]. This enormous enterprise raises hope, among others, to recognize new diagnostic health markers („microbiological" health) and enrich pharmaco-poeia in medication including microbiota represen-tatives, important for human health.

In the light of up-to-date knowledge, microbiota composition is individually diversified, evolves during the life of the host and changes under the influence of internal and external factors, e.g.,

antibiotics. Purposeful manipulation of microbiota by probiotics and/or prebiotics has recently become a realistic strategy for prevention and therapy in many infectious, inflammatory and neoplastic diseases and may also become the tool for efficacy improvement in prophylactic or therapeutic vaccination. It is possible that in the future each vaccination will be preceded by microbiological analysis and, if need be, experimentally determined composition of natural microbiota will be corrected to activate strong vaccine-induced immune response of the organism.

The signalled problem of prophylactic and therapeutic vaccines efficacy depending on composition and biochemical and immuno-modulatory activity of natural microbiota is so far poorly represented in literature. However, it deserves attention and calls for further intensive studies.

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Szczepienia spersonalizowane? II. Rola naturalnej mikrobioty w indukowanej odporności poszczepiennej

H. Długońska, M. Grzybowski

Różnice osobnicze w odpowiedzi immunologicznej na szeroko stosowane szczepionki profilaktyczne przeciw chorobom zakaźnym są determinowane przez płeć, MHC (główny układ zgodności tkankowej), wiek i aktualny status hormonalny szczepionych osobników. Liczne badania wykazały, że mikroorganizmy rezydujące w różnych miejscach organizmu człowieka lub zwierząt (naturalna mikrobiota), zwłaszcza w przewodzie pokarmowym, wpływają wieloraki na procesy fizjologiczne gospodarza. Sugeruje się ostatnio, że mikrobiota stanowi niedoceniany dotychczas, chociaż bardzo znaczący czynnik, odpowiedzialny za zróżnicowaną skuteczność szczepień w krajach rozwiniętych i rozwijających się.

W niniejszym artykule przedstawiono wybrane aspekty relacji mikrobiota–gospodarz: znaczenie mikrobioty jelitowej w rozwoju miejscowych i uogólnionych reakcji odpornościowych, bakterie o szczególnej roli w odporności (np. SFB–Segmen - ted Filamentous Bacteria) i dane kliniczne dotyczą - ce zróżnicowanej immunogenności szczepionek w różnych populacjach ludzkich. W świetle aktualnej wiedzy, manipulowanie mikrobiotą za pomocą probiotyków i/lub prebiotyków, staje się realistyczną strategią terapeutyczną i profilaktyczną w przypadku chorób infekcyjnych, zapalnych, a nawet no - wotworowych w obrębie przewodu pokarmowego, ale może również służyć zwiększaniu skuteczności szczepień.