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REVIEW ARTICLE

Gut Microbiota and Human Health with Special Reference to Autoimmunity

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ABSTRACT

Human body is basically composed of human and microbial cells. The microbial cells outnumber the human cells by 10 fold. These microbes are an integral part of human body residing on mucosal and skin surfaces. In this way, we have got two genomes. The gastrointestinal tract is the most significant niche for majority of the microbiota. Gut is considered as a huge fermenter producing a variety of metabolites/products affecting human health. Such products may be beneficial or harmful. Apart from different metabolic products, the microbes are speculated to be the trainers of the immune cells. Therefore, a particular time-point of colonization by specific type of microbes decides the fate of immunity, whether protective or detrimental. Dysbiosis may lead to a variety of metabolic, autoimmune and infectious diseases. In this review, we have focused on the issue of gut microbiota and its possible role in causation of different types of diseases, e.g., autoimmunity, asthma, obesity, etc. Further, we have looked into what can be done to modify this genome in favor of good health with change in diet, antibiotics, probiotics, bacteriophages, exercise, Ayurvedic *Panch Karma*-like practices, etc.

Keywords: Autoimmunity, Dysbiosis, Gut microbiome, Inflammatory bowel disease, Obesity.

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INTRODUCTION

The entire multicellular organism exists as ametaorganism, comprising both the macroscopic host and its commensal microbiota which is usually symbiotic. The human body consists of assembly of 30 trillion own cells and about 40 trillion microbes. Interestingly, the human genome expresses 30,000 genes, while the commensal microbes express about 50,00,000 genes, 10 times more than the former.[1] The gastrointestinal tract, the longest mucosal absorptive surface area, is projected to be occupying about 250 m^2 (approximately the size of a tennis court).[2] Similarly, skin is the other big surface area which occupies about 2 m^2.[3] The number of microbes on the skin ranges between 10 and $10^5/cm^2$. The presence of microbes is overwhelming in the gut, from mouth to colon.[4] It is really difficult to guess whether human cells are parasitizing the microbial cells or *vice versa*. Despite this dogma, we must appreciate that human body has got two genomes, the one which is inherited is stable throughout the life and the other is extremely dynamic and acquired usually after birth. Obviously, the later genome may be easily influenced by a number of intrinsic and extrinsic factors, i.e., age, diet, hormonal cycles, travels, therapy, and different types of illnesses.

Humans are born sterile and microbial colonization starts with the advancement of labor.[5] The type of delivery and subsequent feeding decide the establishment of the variety of microbiota, especially during infancy.[6] However, by the age of 3 years, microbiota assumes almost like an adult gut.[7] Specific microbial signatures at different body sites and significant intraindividual variability over the time have been observed when a longitudinal microbiome analysis was carried out on different biological samples. It has been reported that more than 1,000 different species colonize the human gut, belonging to a small number of phyla.[8] It is important to note that despite intraindividual variability in the gut microbiota composition, a core gut microbiota shared by healthy adults has been identified. These shared species are considered to be playing an important role in the maintenance of health. The gut may be considered as a big fermenter and a number of following functions may be assigned to the core microbiota to know a few of the mystery of this magic box:

(i) Development of mucosal epithelium and musculature of the intestine; (ii) immune system development; (iii) defense against infections; (iv) polysaccharide digestion; (v) synthesis of vitamins; (vi) fat storage; (vii) angiogenesis regulation; (viii) behavioral development; (viii) providing ATP to colonic cells; and (ix) many other unknown functions yet to be explored.

Interestingly, genes encoded by the human core microbiome encode proteins essential for host survival, but not

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present in the human genome. Such type of observation led to the definition of the microbiome as "our forgotten organ." This organ is definitely affecting the state of health based on differential colonization of commensals, time point of colonization and subsequently immune response against different commensal and potential pathogens and also although part of the normal flora but colonizing at site other than natural sites. Colonization with different bacterial population may affect the state of obesity or starvation. We will be discussing on these three aspects of the gut microbiota and human health.

GUT MICROBIOTA AND METABOLIC SYNDROME: OBESITY/MALNOURISHMENT

Malnourishment and obesity have a substantial impact on human health worldwide. Globally, obesity has tripled since 1975. In 2016, approximately 650 million adults were obese and 41 million children under 5 years were obese, and over 340 million children and adolescents aged 5 to 19 years were obese. About 62 million children <5 years of age have been reported with severe malnutrition, while 155 million had stunted growth. Although nutrition is the most important factor causing either obesity or protein-energy malnutrition, other environmental factors may also contribute to the above-mentioned condition. Gorden et al[9] have proposed that early life dietary intake is a strong driver in deciding the composition of intestinal microbes.

Apart from these factors, delivery by cesarean section leading to early colonization of skin microbe,[10] children on formula feed,[11] and intake of antibiotics[12] have been observed to be associated with obesity. There is a study stating that *Akkermansia muciniphila* reduces the high-fat intake-induced metabolic disorders inhibiting fat mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance.[13] It has been seen that over colonization by *Firmicutes* rather than by *Bacteroidetes* results in the development of obesity, as they are more potent in extracting energy from the gut content. It has been proved that the fecal microbiota from obese person contaminating the feed of a group of mice and other with the feces from thin subject resulted in obesity in the previous group.[14] Thus, manipulation of gut microbiota may be applied for weight reduction in obese persons. It is quite likely that a diet prescribed in the treatment of obesity may be able to change the gut flora in favor of *Bacteroidetes* than the *Firmicutes*.

Similarly, Smith et al[15] showed that by transforming the fecal flora from Malawian twin, discordant for severe malnutrition in germ-free (GF) mice leads to malnutrition, indicating that microbiota does mediate the symptoms of malnutrition. Further, when these mice were put on a diet of therapeutic food, malnutrition could not be reversed fully. It implies that early microbiota has a persistent effect on metabolism and immunity. To elucidate the mechanism, Trehan et al[16] have shown that there was significant improvement in nutritional recovery and mortality rates postantibiotic treatment in severe acute malnutrition. This might have occurred as the antibiotics might have favored the over growth of the bacteria which have better potential for nutrient uptake. Alternatively, we may try to find out the bacteria in the gut which are more efficient in energy production and assimilation by the host. These bacteria may be selectively eradicated using specific bacteriophages. This might result in weight loss in due course of time. This philosophy might be used by the physician practicing Ayurveda, where *Panchkarma* is used to wash out the whole gut microbiota by different methods followed by a defined diet. This logic is further supported by the common practice of using antibiotics in poultry and piggery for weight gain.

GUT MICROBIOTA AND AUTOIMMUNE DISORDERS

Autoimmune diseases have registered an alarming increase worldwide since the end of the Second World War. This pandemic includes more than 80 autoimmune disorders and increase in both the incidence and prevalence of autoimmune disorders, such as Crohn's disease, rheumatoid arthritis, multiple sclerosis, and type I diabetes has been observed. It is far more commonly found in women and is one of the top 10 leading causes of death in female children and women of all age groups. Symptoms involve many medical specialties and can affect all body organs. Genetic predisposition, environmental factors (including infections), and gut dysbiosis play major roles in the development of autoimmune diseases. Autoimmunity develops in due course of time, and preclinical autoimmunity precedes clinical diseases by many years and can be detected in the peripheral blood in the form of circulating autoantibodies. Initially, symptoms of autoimmune disorders are vague and include fatigue, low-grade fever, muscle and joint aches, and malaise. They usually progress and become debilitating with significant morbidity. Factors, such as genetics, the environment, infections, and the gut microbiota all play a role in the mediation of autoimmune disorders. There have been tremendous recent advances in our better understanding of the interplay of these factors. It is clear that since birth, the gut microbiota has a profound and long-term effect on the host immune system. It is also evident that it plays a significant role in autoimmune diseases both inside and outside the gut. The mechanisms hypothesized to be involved in the breakdown of tolerance are: (i) Failure

to delete autoreactive lymphocytes, (ii) central tolerance failure, (iii) peripheral tolerance failure, (iv) molecular mimicry, (v) abnormal presentation of self-antigens, (vi) aberrant expression of major histocompatibility complex class II molecules, (vii) coupling of self- and non-self-antigens, (viii) overproduction of self-antigens, (ix) disclosure of cryptic T-cell epitopes, (x) release of sequestered self-antigens, (xi) epitope spreading, and (xii) polyclonal lymphocyte activation.

The exposure to microbial components just after birth is one of the most important triggers of immune response development. Since gut microbiota can be changed, there is an opportunity to manipulate it in favor of changing the host susceptibility to immune-mediated diseases. It is interesting to observe that when GF mice were inoculated either by lavage of cecal content or were co-housed with specific pathogen-free (SPF) mice at different points of time in postnatal period, the mice colonized at the age of 3 weeks permanently changed the gut flora, which was not achievable at 1 week. Interestingly, the experimental GF mice at the age of 3 weeks were found to have increased proinflammatory cytokines. In contrast to this, the GF mice kept with the colony of SPF mice had the composition of SPF mice.[17] Mucosal secretions have immunoglobulin A (IgA) antibody in the most abundant quantity.[18] Surface immunoglobulin IgA (SIgA) maintains the homeostasis of noninvasive commensal bacteria and inactivation of invasive microbes; SIgA prevents the adhesion of commensal bacteria with the apical surface of intestinal epithelial cells. The IgA also intercepts microbes and toxins inside the intestinal epithelial cells. However, these protective effects are initiated without activation of complement cascade resulting in minimal damage to the epithelial cells. There are two compartments where the evolution of SIgA takes place: Peyer's patches (PP), a highly organized follicular structure in the small intestine, which develops during fetal life, independent of colonization by the microbes. This is the most prominent IgA inductive site of microbial colonization, i.e., gut-associated lymphoid tissue. The other organ which is dependent on microbial colonization is called isolated lymphoid follicles (ILFs) which develop after birth, in both the small and large intestine. These SIgA restrict the access of microbes and other antigens and also control the quality and quantity of local immune response. Traditionally, IgA is known for neutralizing the toxins and some pathogens; it also maintains the diversified community of commensal flora. The precise contribution of PP and ILFs remains to be fully elucidated and the optimization of IgA responses is yet to be explored. Is there any role of commensal flora in the induction of SIgA response? The understanding of these aspects will facilitate the treatment of intestinal inflammatory disorders and food allergies.

In response to microbial and environmental encounters, the immune system has an extraordinary capacity to adapt and react to highly diverse challenges both through innate and adaptive immunity. The absence of commensal flora during infancy results in increase in the number of invariant natural killer T (i-NKT) cells which subsequently leads to higher susceptibility to asthma and colitis.[19] It is speculated that early colonization leads to epigenetic suppression of gene expressing CXCL16 chemokine, resulting in reduced i-NKT cells accumulation in lymphoid tissues.[19] Conventionally, it is assumed that T cells are primarily modified in thymus and those T/B cells are eliminated which are directed against available antigens. Strictly, the major lymphoid tissues are found in and around the gut, and the T lymphocytes which are coming in contact with newly colonized bacteria are modified in such a way that they do not react with the commensals. There are two types of T cells: T reg and T eff. The early exposure of microbes leads to induction of T reg cells and resulting in controlled immune response.[20] However, the absence of early colonization or antibiotic therapy results in enhanced IgE response.[21] This overexpression has been correlated with isotypic switching of B cells to IgE rather than IgA.[22,23] Interestingly, the induction of IgE is not suppressed by colonization with microbes later in life or even early microbiota colonization with limited complexity.[23]

The response of T cells and B cells to the gut microbiota is tightly regulated. The possible mechanism may be T-cell depletion and anergy. In this case, antigens derived from commensal microbes might induce apoptosis of antigen-specific T cells.[24] The other possible mechanism may be through induction of CD4+ T reg cells that express forehead box protein-3 (FoxP3) protein. Although T reg cells expressing CD4 and Fox P-3 are distributed throughout the body, a high proportion of such cells are present in the lamina propria of the intestine.[25-28] T reg cells play an important role in the maintenance of homeostasis, tolerance to gut commensals and dietary antigens. The parental T reg cells disappear under GF condition. It is quite likely that these T reg cells are induced by gut microbiota.[29-31] When experiments were carried out to trace the fate of immature T cells expressing transgenic T-cell receptor cloned from colonic T reg cells, it was demonstrated that differentiation of T cells to T reg cells occurred in colon in the presence of corresponding commensal bacteria and not in the thymus.[32] RORyt+ T reg cells are induced by oral antigens and microbiota-derived short-chain fatty acids. But this induction is absent in GF or antibiotic-treated animals.[29] It is worth mentioning

that T reg cells express the transcription factor RORγt. It is believed that cells from adaptive immune system that are primed in microbiota-sensing mucosa reside on the mucosal surfaces that protect them.

Initially, it was stated that $CD4^+$T cell differentiates into two subsets with reciprocal functions: T helper 1 (Th-1) cells characterized by the production of interferon leading to cell-mediated immunity, especially acting against intracellular pathogens, while the other subset called Th-2 produce interleukin-4 (IL-4) which is responsible for humoral immunity. Later, in 2005, a third type of CD^+4 was reported called Th-17.[33,34] The Th-17 produces IL-17; Th-17 subtype plays a special role in those extracellular bacteria and fungi for which Th-1 and Th-2 have got limited role.[33,34] Human Th-17 cells have been demonstrated to generate multiple inflammatory and hemopoetic effects on epithelial, endothelial, and fibroblast cells.[35-37] The IL-17 mediates powerful inflammatory immune response mediated by pro-inflammatory cytokines and recruitment of leukocytes and accordingly innate and adaptive immune responses. This is essentially needed for the host defense. However, overstimulation of Th-17 might be having significant role in the pathogenesis of several autoimmune and inflammatory diseases.

A local cytokine milieu plays an important role in naïve CD4+ cells during differentiation. On the contrary, IL-2 leads to Th-1 and IL-4 to Th-2 responses expressing the transcription factor GATA-3. Th-17 cells are basically modified Th-2 cells/response under the influence of IL-23, a heterodimeric cytokine that shares a subunit with IL-12.[33,34,38,39] It has recently been stated that Th-17 differentiation occurs because of combination of transforming growth factor-β (TGF-β) and IL-6.[40,41] The transformation is further enhanced by tumor necrosis factor-2 and IL-1β. However, TGF-β is well known to inhibit most of the T-cell responses and for induction of differentiation of FoxP3 expressing regulatory T cells (T reg cells). Segmented filamentous bacteria have been reported with immunomodulatory effect on T follicular and Th cells. T follicular helper and Th-17 cells both contribute to autoimmunity. It has been speculated that certain benign commensals may cause the young host to display an overexpression of the immune system. *Corynebcaterium mastitidis,* when cultured with immune cells from the conjunctivae, induce production of IL-17. It may be suggested that colonizers of immunomodulatory commensals may offer microbe-based therapy of autoimmune diseases. In future, elucidation of the mechanisms by which the commensal microbes deliver antigens in order to achieve immunogenic *vs* tolerogenic effect will be very interesting.[42] It may help in the development of the mucosal vaccines.

Genetic composition of the host, environmental factors, and gut microbiota has been proposed to be involved in the genesis of autoimmunity.[43,44] While it is difficult to manipulate genetic composition of an individual or the environmental factors, interestingly, the gut microbiota may be modified for the improvement of the clinical condition. Microbiome plays a significant role in the maintenance of a healthy state in adulthood. Anatomical site and quantitative and qualitative alterations in the composition of the gut microbiome could lead to pathological dysbiosis and have been related to an increasing number of intestinal and extraintestinal diseases. With the increasing knowledge about gut microbiome functions, it is becoming possible to develop novel diagnostic, prognostic, and most importantly, therapeutic strategies based on microbiome manipulation. Still, there are questions that remain to be answered: Does the immune system shape the gut microbiota or vice versa? This complex and dynamic symbiosis needs further elucidation that may help in determining the outcome of autoimmune diseases in patients. Of the above-mentioned mechanisms of autoimmunity, antigen mimicry may be one of the most important mechanisms leading to autoimmunity. However, other mechanisms like the abnormal presentation of self-antigens, coupling of self- and non-self-antigens, epitope spreading, and polyclonal lymphocyte activation deserve to be explored further in the pathogenesis of autoimmunity. If we could pinpoint the causative etiology in terms of infectious agent/s as has already been done in cases of rheumatic heart disease, it would help the clinicians to assist the patients by being aware of the triggers of autoimmune disorders. The role of microbiota can be emphasized because upon antimicrobial therapy, either improvement or exacerbation of immune-mediated diseases including ulcerative colitis, autoimmune uveitis, asthma, type I diabetes, rheumatoid arthritis, psoriasis, multiple sclerosis, etc., has been observed in humans as well as in animal models.

In this way, our traditional Indian medicine system of Ayurveda becomes extremely relevant where the emphasis is given on *Aahar* (food can change the composition of gut flora), *Vihar* (exercise affects the microbiome), and *Vichar* (thought process affects the immune response) to deal with the problem of autoimmunity. Moreover, if a single pathogen is proved to be the culprit, it can be eradicated by highly specific and targeted therapy with bacteriophage, leading to the cure of that autoimmune disease.

DYSBIOSIS AND INTESTINAL DISORDERS

There are several reasons for microbial dysbiosis, i.e., genetic predisposition, infections, diet, nutritional status,

use of antibiotics, pH-modifying drugs, etc.[45,46] In a murine model, consumption of the diet rich in milk fat resulted in an increase of taurocholic acid consuming *Bilophila wadsworthia*. This bacterium induces Th1 cells and accelerates the onset of colitis.[45] The gut microbiota leads to epigenetic suppression of the gene inducing chemokine CXCL16,[19] resulting in suppression of i-NKT cells in the gut and lungs. Early colonization of commensal bacteria results in the recruitment of T reg cells to mucosal sites.[47] If early colonization is prevented, then B cells undergo partial isotypic switching from IgA to IgE.[22,23]

In inflammatory bowel disease (IBD) animal models, adherent *Escherichia coli* are frequently observed. It causes an active response of Th-I7 mutation in certain genes of host, resulting in shift in the composition of gut microbiota, e.g., NOD 2 mutation in the subset of people with loss of bacterial recognition culminates in microbial dysbiosis.[48]

Although the ability of certain bacteria triggering autoimmune disease has yet to be elucidated, *Prevotella copri* density is increased in cases of new-onset rheumatoid arthritis[49] and similarly increased prevalence of Veillonellaceae and Fusobacteriaceae has been seen during the onset of Crohn's disease in children.[50]

Almost all these chronic IBD have been observed with dysbiosis where there is a strong association with T eff cells in response to potentially pathogenic bacteria. Loss of barrier function of epithelial cells and deregulated responses to commensal microbiota has also been noted. Such type of dysbiosis-induced immune response can be transformed also. Responses to flagellin antigens (known as CBir) that are expressed by commensal flora (*Clostridia* cluster X1va) have been detected in people with Crohn's disease.[42] It is noteworthy that transfer of such CD4-expressing T cells specific to CBir in immune-deficient mice colonized with commensal *Clostridia* causes severe colitis.[51] The disruption of the epithelial barrier due to the complex interplay of commensal and pathogenic bacteria might be responsible for dysregulated immune response to the gut microbiota.

Since the incidence of IBDs is far outpacing the genetic drift in the human population, epigenetics seem to play a major role in the etiopathogenesis. This statement is further supported by the fact that several genome-wide association studies have disclosed risk alleles associated with both ulcerative colitis and Crohn's disease. However, the culprit alleles have been detected in only a small proportion of cases.[52] Among the epigenetic factors, stress, diet, antibiotics use, prenatal exposure to infection, etc.,[53] have been reported to be associated with aberrant intestinal microbiota. Intriguingly, IBD does not occur in animal models living in GF conditions.[54,55] Association with aberrant microbial communities before and after the treatment has been reported.

Preterm infants face a lethal threat of necrotizing enterocolitis (NEC)[56,57] that may be occurring due to structural abnormalities in the intestine and ischemic injuries. In classical NEC, patients manifest with extensive tissue necrosis,[58] raised serum pro-inflammatory cytokines, bacteremia, and endotoxemia.[59-61] It is speculated that intestinal microbiota may be involved in the pathogenesis. The premature gut epithelial barrier is leakier than the mature one. Breast-feeding is favored in the conditions for the colonization of good microbe. On the contrary, antibiotics use and formula feeding may further add to the severity of the problem. The role of microbiota is, as further ascertained by the observation of the beneficial effect of probiotics, in the improvement in NEC incidence. Bergmann et al[62] have reported improvements in gut epithelial integrity and decrease in the incidence of NEC when rat pups were fed with *Bifidobacteria*. This commensal bacterium is known to thrive well in healthy breast-fed pups. It has been proposed that this bifidobacterium along with other probiotic bacteria may be used to modulate the NE dysbiosis.[63]

In conclusion, we may propose that it is the time now to explore the safe manipulation of gut microbiota of human being for the better health, cure of diseases, and prevention/cure of several of autoimmune diseases. It will be better to work on controlled colonization to be started just after the birth and onward. It will be beneficial to find out the microbe which is playing a significant role in an individual. Can we eradicate the culprit bacterium and see the effect? Can we correct the dysbiosis by diet? Should we study further regarding fecal transplantation? There are numerous questions to be addressed in future before manipulating this second unstable genome. The diet, *panch karma* followed by specific diet, elimination through bacteriophages, selective colonization with beneficial bacteria, etc., may be the tools to manipulate the gut flora, the magic box of the human body.

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