Review

Microbiota and immunity: from preclinical data to clinical practice

Eleonora Giannetti, Annamaria Staiano

Department of Translational Medical Science, University of Naples "Federico II", Naples, Italy

Proceedings

Abstract

The intestinal microbiota is composed of 10^{13}-10^{14} microorganisms, with at least 100 times as many genes as our genome, the microbiome. Its composition is specific for each individual, changes among individuals and also shows an intra-individual variability during life. Although the gastrointestinal microbial communities of adults are often believed to be stable, there is evidence that, even though at lower rates than in childhood, they change with time, and effects of this variability on health have not been determined yet. The interaction between microbiota and environment is close and widely demonstrated. Gut flora composition is deeply influenced by a number of factors, including diet, age, medications, illness, stress and lifestyle. Intestinal microflora has protective, metabolic and trophic functions. Commensal microbiota can deeply influence the development of the gut mucosal immune system, modulating the maturation of the gut-associated lymphoid tissue and preventing exogenous pathogen intrusion, by stimulation of the immune system and by direct interaction with pathogenic bacteria. The increasing amount of preclinical studies regarding the interaction between intestinal microbiota and immune system and the multiple observations of altered microbiota in human diseases have paved the way for a number of clinical trials aimed at verifying the potential benefits deriving from the manipulation of the microbial ensemble. Several probiotic bacteria have been assessed for their potential applicability in human diseases, albeit with different levels of success. In conclusion, the gut microbiota codevelops with the immune

system beginning at birth. The development of the microbiota and its interactions with the cellular populations of the bowel provide a substantial contribution to shaping the structure and dynamic operations of the innate and adaptive immune systems. Manipulation of the microbiota, particularly through the administration of specific probiotic strains, represents a unique opportunity for the development of new therapeutic tools in several human diseases.

Keywords

Microbiota, bowel, immune system, bacteria, probiotics, dendritic cells.

Corresponding author

Annamaria Staiano, MD, Translational Medical Sciences, Section of Pediatrics, University of Naples "Federico II", Via S. Pansini, 5, 80131 Naples, Italy; tel.: +39.081.7462679; fax: +39.081.5469811; email: staiano@unina.it

How to cite

Introduction

The intestinal microbiota is composed of 10^{13}-10^{14} microorganisms, with at least 100 times as many genes as our genome, the microbiome. Its composition is specific for each individual, changes among individuals and also shows an intra-individual variability during life. The human microbiota develops from an initial inoculum that is determined by mode of delivery, and subsequently moves towards its adult state through a dynamic process during the first 1-3 years of life.

Although the gastrointestinal microbial communities of adults are often believed to be stable, there is evidence that, even though at lower rates than in childhood, they change with time, and effects of this variability on health have not been determined yet [1].

It is often thought, incorrectly, that newborns are sterile and are colonized after birth by environmental microbes. However, placental mammals give birth through a birth canal that is heavily colonized by microbes and the hypothe-

sis that a prenatal mother-to-child efflux of commensal bacteria may exist has been confirmed by several studies.

Particularly, Jiménez and coworkers showed that bacteria, mostly belonging to the genuses *Enterococcus spp.* and *Staphylococcus spp.*, may be isolated from umbilical cord blood of healthy neonates and from murine amniotic fluid obtained by caesarean section [2]. The same authors also demonstrated that the inoculation of pregnant mice with a genetically labeled *E. faecium* strain resulted in the detection of the same labeled strain in the meconium of the term fetuses born from the inoculated animals. In contrast, it could not be isolated in samples obtained from a non-inoculated control group [3].

The interaction between microbiota and environment

The interaction between microbiota and environment is close and widely demonstrated. Gut flora composition is deeply influenced by a number of factors, including age, diet, medications, illness, stress and lifestyle.

Environmental interferences on human microbiota have been clearly shown by a 2.5-year case study aimed at correlating life events to microbiome composition and function. For example, functional genes involved in plant polysaccharide metabolism were shown to be present before the introduction of solid food, priming the infant gut for an adult diet. Similarly, ingestion of table food is associated to a sustained increase in the presence of *Bacteroidetes*, elevated fecal short chain fatty acid levels, enrichment of genes associated with carbohydrate utilization, and a more stable composition, all of which are typical of the adult microbiome [4].

Similarly, diet has a relevant impact on human intestinal microbiota, as suggested by the relevant microbial diversity highlighted in the comparison between gut microbiota from children on a modern western diet and from children on a rural diet. Particularly, children from a rural African village of Burkina Faso were showed to have a significant enrichment in bacteria from the genuses *Prevotella spp.* and *Xylanibacter spp.*, known to contain a set of genes for cellulose and xylan hydrolysis, completely lacking in children on a modern western diet. On the other hand, *Enterobacteriaceae* such as *Shigella spp.* and *Escherichia spp.* were significantly

underrepresented in African subjects when compared to European children [5].

Intestinal microflora has protective, metabolic and trophic functions, and its close relationship with immunity has been universally recognized. Indeed, together with digestive enzymes, mucins, peristalsis and epithelial barrier with tight junctions, microbiota belongs to the so called non-immune component of mucosal immunity and is able to establish a "cross-talk" with the mucosal immunity, including cellular and soluble elements [6]. Commensal microbiota can profoundly influence the development of the gut mucosal immune system, modulating the maturation of the gut-associated lymphoid tissue (GALT) and preventing exogenous pathogen intrusion, by stimulation of the immune system and by direct interaction with pathogenic bacteria [7].

Neonatal colonization

Furthermore, Olszak demonstrated that colonization of neonatal – but not adult – germ-free mice with a conventional microbiota protects the animals from mucosal invariant natural killer T cells accumulation in the colon and related pathology. Therefore, it is likely that age-sensitive contact with commensal microbes has a role in establishing mucosal tolerance to later environmental exposures and confirms the importance of microbiota in modulating the immune response [8].

During the adjustment of the newborn to the extrauterine environment, several factors influence initial intestinal colonization. These include the infant's genetic signature, the kind of delivery, the use of antibiotics during the perinatal period and the maternal expression of an inflammatory condition [9, 10]. Once a normal colonization has been achieved, the pool of microorganisms establishes a symbiotic relationship with the intestinal epithelial and lymphoid tissues. This interaction between gut microbiota and epithelial and lymphoid cells results in the expression of a number of molecules, which mediate host defense or metabolic activities within the intestine [11].

Although bacterial populations are segregated from the gut epithelium by a thick mucin layer produced by goblet cells that is embedded with anti-microbial factors such as immunoglobulin A (IgA), α and β defensins, the cross-talk between the gut microbiota and host is extensive, and involves both innate and adaptive immunity.

The immune system, in close proximity with a dense microbial population, needs to establish an appropriate balance between tolerance to the commensal microbiota and vigilance against infectious agents and opportunistic pathogens. This homeostasis is maintained as an inflammatory tone, allowing a rapid and self-limiting response appropriate to a stress or infectious agent [12].

Like immune cells, epithelial cells are a crucial component of the immune system of the gut. The expression of specific receptors by epithelial cells activate signalling cascades that finely tune the production of antimicrobial products and chemokines, strictly depending on the signals that are delivered by the microbiota. Thus, gut epithelial cells form a potent and inducible physico-chemical barrier, which limits bacterial growth and access to the intestinal mucosa. They can also recruit leukocytes to integrate their barrier function, participating in the activation of gut adaptive immune responses. In mammals, the development of GALTs is initiated before birth by a genetic programme. However, GALT maturation and the recruitment of IgA secreting plasma cells and activated T cells to mucosal sites only occur postnatally and is strictly dependent on microbiota-derived signals; these signals influence the cross-talk between epithelial cells and gut dendritic cells, thereby modulating the nature and intensity of intestinal B and T cell responses [13, 14]. In immunocompetent mice, gut colonization stimulates the production of secretory IgA, the differentiation of effector T helper (Th) 1, Th2 and Th17 cells, and the development of regulatory T cells [15].

Maturation of mucosal immune function and consequent immune homeostasis is not complete until the process of oral tolerance occurs. Oral tolerance is a systemic reduction in cellular and humoral immunity to commensal bacteria and antigens through exposure via the oral route. In the presence of colonizing bacteria, antigens or nonpathogenic bacteria interacting with submucosal dendritic cells determine a preferential production of T regulatory cells and stimulate the maturation of a specialized microenvironment that facilitates their development. These cells release Transforming growth factor beta (TGF-β), an oral tolerogenic cytokine, which reduces the Th1, Th2 and Th17 response to antigens/bacteria [16]. Therefore, it is likely that normal initial intestinal colonization is needed to establish oral tolerance and, as showed

by Walker, this can be broken with extensive use of broad-spectrum antibiotics [17].

The increasing amount of preclinical studies regarding the interaction between intestinal microbiota and immune system and the multiple observations of altered microbiota in human diseases have paved the way for a number of clinical trials aimed at verifying the potential benefits deriving from the manipulation of the microbial ensemble. Particularly, the increased understanding of molecular mechanisms of the gut microflora has resulted in a growing body of literature focused on the effects of probiotics on several human diseases, mainly affecting the gastrointestinal tract.

The beneficial effects of probiotics

The mechanisms behind the beneficial effects of probiotics include the enhancement of immune defenses and the antagonism with infectious agents, which have led to recommendations for several clinical applications of probiotics [18]. Several probiotic bacteria have been assessed for their potential applicability in human diseases, albeit with different levels of success.

Recently, applications of *Bifidobacteria* for the prevention and treatment of the most common enteric and non-enteric diseases in infants and children have been described. Moreover, a number of possible applications of these probiotic bacteria have been suggested for the treatment of diseases for which the probiotic approach appears promising on the basis of recent *in vitro* studies [19]. As demonstrated in a recent preclinical study, *Bifidobacteria* significantly improved the antigen uptake and processing by dendritic cells from patients with Crohn's disease, which are known to present an impaired autophagic functionality, whereas, in dendritic cells from ulcerative colitis and non inflammatory bowel disease controls, no prominent effect of probiotic mixture was observed. This improvement of antigen sampling and processing could partially solve the impairment of intestinal innate immunity and reduce uncontrolled microorganism growth in the intestine of children with inflammatory bowel disease [20].

Conclusions

In conclusion, the gut microbiota codevelops with the immune system beginning at birth. The development of the microbiota and its interactions with the cellular populations of the bowel provide a substantial contribution to shaping the structure and dynamic operations of the innate and adaptive immune systems. Manipulation of the microbiota, particularly through the administration of specific probiotic strains, represents a unique opportunity for the development of new therapeutic tools in several human diseases. Despite a number of well-designed clinical trials have already been carried out, larger, better controlled, and universally standardized studies are needed for the rigorous scientific evaluation of probiotic therapies and the comparison of clinical outcomes.

Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Dominguez-Bello MG, Blaser MJ, Ley RE, Knight R. Development of the human gastrointestinal microbiota and insights from high-throughput sequencing. Gastroenterology. 2011;140(6):1713-9.

2. Jiménez E, Fernández L, Marín ML, Martín R, Odriozola JM, Nueno-Palop C, Narbad A, Olivares M, Xaus J, Rodríguez JM. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol. 2005;51(4):270-4.

3. Jiménez E1, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, Fernández L, Rodríguez JM. Is meconium from healthy newborns actually sterile? Research in Microbiology. 2008;159(3):187-93.

4. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci USA. 2011;108(Suppl 1):4578-85.

5. De Filippo C1, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA. 2010;107(33):14691-6.

6. Liu Z, Li N, Neu J. Tight junctions, leaky intestines, and pediatric diseases. Acta Pediatr. 2005;94(4):386-393.

7. Purchiaroni F, Tortora A, Gabrielli M, Bertucci F, Gigante G, Ianiro G, Ojetti V, Scarpellini E, Gasbarrini A. The role of intestinal microbiota and the immune system. Eur Rev Med Pharmacol Sci. 2013;17(3):323-33.

8. Olszak T1, An D, Zeissig S, Vera MP, Richter J, Franke A, Glickman JN, Siebert R, Baron RM, Kasper DL, Blumberg RS. Microbial exposure during early life has persistent effects on natural killer T cell function. Science. 2012;336(6080):489-93.

9. Molloy MJ, Bouladoux N, Belkaid Y. Intestinal microbiota: shaping local and systemic immune responses. Semin Immunol. 2012;24(1):58-66.

10. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118(2):511-21.

11. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. Nat Rev Immunol. 2008;8(6):411-20.

12. Peterson CT, Sharma V, Elmén L, Peterson SN. Immune homeostasis, dysbiosis and therapeutic modulation of the gut microbiota. Clin Exp Immunol. 2015;179(3):363-77

13. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol. 2010;10(3):159-69.

14. Rescigno M, Di Sabatino A. Dendritic cells in intestinal homeostasis and disease. J Clin Invest. 2009;119(9):2441-50.

15. Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity. 2009;31(4):677-89.

16. Spiekermann G, Walker WA. Oral tolerance and its role in clinical disease. J Pediatr Gastroenterol Nutr. 2001;32(3): 237-55.

17. Walker WA. Initial intestinal colonization in the human infant and immune homeostasis. Ann Nutr Metab. 2013;63(Suppl 2):8-15.

18. Floch MH, Walker WA, Madsen K, Sanders ME, Macfarlane GT, Flint HJ, Dieleman LA, Ringel Y, Guandalini S, Kelly CP, Brandt LJ. Recommendations for probiotic use-2011 update. J Clin Gastroenterol. 2011;45(Suppl):S168-71.

19. Di Gioia D, Aloisio I, Mazzola G, Biavati B. Bifidobacteria: their impact on gut microbiota composition and their applications as probiotics in infants. Appl Microbiol Biotechnol. 2014;98(2):563-77.

20. Strisciuglio C, Miele E, Giugliano FP, Vitale S, Andreozzi M, Vitale A, Catania MR, Staiano A, Troncone R, Gianfrani C. Bifidobacteria Enhance Antigen Sampling and Processing by Dendritic Cells in Pediatric Inflammatory Bowel Disease. Inflam Bowel Dis. 2015;21(7):1491-8.