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Gut microbiota biomodulators, when the stork comes by the scalpel

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ABSTRACT

The microbial communities that reside in the human gut (microbiota) and their impact on human health and disease are nowadays one of the most exciting new areas of research. A well-balanced microbial intestinal colonization in early postnatal life is necessary for the development of appropriate innate and adaptive immune responses and to establish immune homeostasis later in life. Although the composition and functional characteristics of a 'healthy' gut microbiota remain to be elucidated, perturbations in the microbial colonization of an infant's gastrointestinal tract have been associated with an increased risk of short- and long-term immunologically mediated diseases. Emerging evidence suggests that gut microbiota biomodulators, such as probiotics, prebiotics, synbiotics, and postbiotics may support disease prevention in infants who tend to have a delayed and/or aberrant initial colonization with reduced microbiota diversity (delivery by caesarean section, premature delivery, and excessive use of perinatal antibiotics). Under these dysbiosis conditions probiotics could act as 'surrogate' colonizers to prevent immune-mediated diseases. This review focuses on the influence of delivery mode on the colonization of the infant gastro-intestinal tract. In particular, it examines the manipulation of the gut microbiota composition through the use of gut microbiota biomodulators, in the management of aberrant initial gut colonization and subsequent consequences for the health of the offspring.

"Pray for us now and at the hour of our birth."
[Thomas Stearns Eliot]

1. Introduction

Caesarean section (CS) is the most common surgical procedure performed on women worldwide. According to the World Health Organization (WHO), CS rate (percentage of births managed by CS) exceeding 15% lacks medical justification and it could be linked with adverse maternal and child health consequences [1]. CS rate is elevated especially in tertiary center mostly because of risk pregnancy and preterm deliveries. Contrary to widespread belief, Caesarean delivery is not safer than vaginal delivery for premature and small for gestational age infants [2]. A recent research showed that small for gestational age babies delivered early by CS had higher rates of respiratory distress syndrome than similar preterm babies who were born vaginally [3].

Although since 1985 WHO has recommended that the rate is not to exceed 10–15%, there is no empirical evidence for an optimum range of percentages, despite of a growing body of research showing a negative effect of high rates. It should be noted that the proposed upper limit of 15% is not a target to be achieved but rather a threshold not to be exceeded. Nevertheless, the rates in most developed countries and in many urban areas of lesser-developed countries are above that threshold. Rates of CS have increased beyond the recommended level especially in high-income areas such as North America, Italy, France, Germany, and the United Kingdom of Great Britain. An estimated one-third of all births in the United States occur by CS, many of which are elective. Across Europe, there are differences between countries: Italy has the highest Caesarean rate of Europe (38% in 2008) while in the Nordic countries CS rate is 14% [4]. Furthermore, the cost of the global "excess" CS was estimated to amount approximately 2.32 billions $, while the cost of the global "needed" CS was approximately 432 million $ [5].

Abbreviations: B, *Bifidobacterium*; CS, Caesarean section; C, *Clostridium*; CAMPs, commensal associated molecular patterns; DCs, dendritic cells; EPS, exopolysaccharide; ESPACI, European Society for Paediatric Allergology and Clinical Immunology; ESPGHAN, European Society for Paediatric Gastroenterology Hepatology and Nutrition; FAO, Food and Agriculture Organization; FoxP3, forkhead box protein 3; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; GALT, gut-associated lymphoid tissue; GMB, gut microbiota biomodulators; HMOs, human milk oligosaccharides; sIgA, immunoglobulin A; iTreg, inducible Treg; IFN, interferon; IL, interleukin; IECs, intestinal epithelial cells; LPS, lipopolysaccharide; NEC, necrotizing enterocolitis; nTreg, natural Treg; NF-κB, nuclear transcription factor-κB; NOD, nucleotide-binding oligomerization domain; NLRs, NOD-like receptors; nDCs, normal dendritic cells; HMO, oligosaccharides; PAMPs, pathogen associated molecular patterns; PRRs, pattern recognition receptors; DCreg, regulatory dendritic cells; Treg, regulatory T; TJs, tight junctions; TLRs, toll-like receptors; TGF, transforming growth factor; TNF, tumor necrosis factor; Th1, type 1 helper T cells; Th2, type 2 helper T cells; WHO, World Health Organization.

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Vaginal delivery and exclusive breast-feeding during the first months of life have short- and long-term beneficial effects, such as protection against infectious diseases, reduced infant morbidity and mortality, and low incidence of immunological disorders. The human immune system undergoes major development during infancy and is highly related to the microbes that colonize the intestinal tract in early postnatal life [6]. Gut microbiota has protective, metabolic, trophic, and immunological functions. Neonates born by vaginal delivery are exposed to the mother's vaginal and intestinal flora as they pass through the birth canal and typically harbor communities of bacteria that resembled those of the mother's microbiota. On the other hand, infants delivered via CS are colonized by bacteria that are most similar to the skin communities of the mothers [7]. Such perturbed microbiota composition (dysbiosis) can alter immune regulatory networks that normally restrain intestinal inflammation, and may contribute to a number of intestinal and extraintestinal immune-mediated diseases. Neonatal dysbiosis has been proposed as one of the environmental factors that may play a role in the increasing incidence of both allergic and autoimmune diseases [8,9].

In other words, the potential disadvantages of CS include altered bacterial profile of the neonate/infant intestinal microbiota, which, in turn, leads to immune dysfunction and increased tendency for immune-mediated diseases. Rising CS rates in Western countries make such a potential relation an important public health concern.

2. "*Homo bacteriens*" [10]

Human beings are born into, and develop in, a microbial world. Some scientists believe that early in the history of the planet, different types of microbes joined together to form a new type of organism. These microbes were engulfed by larger bacteria, forming a microbial symbiosis and the host cell protected the smaller microbe inside, while benefiting from the skills of its new partner. In 1981 Lynn Margulis proposed that the main organelles of the eukaryotic cell were primitive prokaryotic cells that had been engulfed by a different, bigger prokaryotic cell. The "endosymbiotic theory of eukaryote evolution" claims that mitochondria were originally separate organisms that entered into a symbiotic relationship with eukaryotic cells through endosymbiosis.

Humans coexist with an enormous quantity of microbial organisms collectively termed microbiota. The microbiota represents an ensemble of microorganisms that resides at various sites on and inside the human body, including skin, nares, oral cavity, urogenital tract, and gut. The gut microbiota (formerly called gut microflora) represents the best studied microbial ecosystem coexisting with human subjects. The gut ecosystem plays a key role in the maturation of the immune system and in other physiological processes including mucosal barrier function. Under normal conditions, this immunologically and metabolically active 'foreign' body exists in a state of symbiotic tolerance with its host and remains relatively stable over time.

Because of more than 100 trillion microbes, ten-fold the number of human cells, including at least 1000 separate bacterial species which constantly interact with themselves and their host, the intestine has evolved to our main immune organ. It is estimated that the microbiota-encoded microbiome (the collective gene set of all colonizing microbes) contains 8 million genes, 150 times more unique genes than are encoded by the human genome. Consequently, our immune system is charged with the critical task of distinguishing between beneficial and pathogenic microbes [11]. Given this important role, while enabling the uptake of large amounts of nutritional products, it is not surprising that the intestine harbors over 70% immune-competent cells.

3. The multi-omics approach

The development of the neonatal fecal microbiota has been studied by culture-dependent techniques from the 1950s onwards. A considerable portion of the microbial cosmos inside our body is composed of bacteria that cannot be cultivated by current microbiological methods. However, recent technological advances as well as a growing interest in the human gut ecosystem have led to a surge of progress in this area. Over the last few years a number of crucial technological innovations have been introduced to shed more light on the composition and functionality of human gut microbiota, offering a complementary support to the classical microbiology. Thanks to their ability to identify a large number of species that cannot be cultivated, they allowed a more complete and rapid assessment of the gastrointestinal ecosystem and the approach to the study of human microbiota has become multidimensional. Although there have been over 50 large bacterial families (phyla) described to date, the human gut microbiota is dominated by only two of them: the *Bacteroidetes* and the *Firmicutes*, whereas *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Cyanobacteria* and *Verrucomicrobia* are present in minor proportions.

Integrative multi-omics approach, defined as the study of related sets of biological molecules in a comprehensive fashion, yields a global picture of the microbial community structure and the metabolic status of the gut ecosystem, which is paramount to establishing correlations with host physiology. The multi-omics approach includes data obtained from genomics (i.e., the large-scale genotyping of single nucleotide), transcriptomics (i.e., the measurement of all gene expression values), proteomics (i.e., the identification of all proteins present in a cell) and metabolomics (i.e., the identification and quantification of all metabolites) of bacteria, host cells and intestinal contents. By omics-technologies we can gain important new insight how early-life events like mode of delivery, type of feeding or genetic background may interfere with the colonization pattern.

The metabolomic approach, in which a large number of small molecule metabolites can be defined in a biological sample, offers a promising avenue to 'fingerprint' microbiota functional status and a powerful strategy to elucidate the molecular mechanisms involved in host-microbial interactions in the complex gut ecosystem [12]. Since fecal samples contain endogenous human metabolites, gut microbiota metabolites, and other compounds, the use of omics-technologies opens the possibility of applying fecal metabolomics to study the gut microbiota functions related to human health. Changes in the metabolomic profile of feces reflect, among others, quantitative and qualitative changes of intestinal microorganisms. A growing number of studies have demonstrated that fecal metabolome profiling, obtained by high-resolution nuclear magnetic resonance spectroscopy-based metabolomics, can reveal the significant metabolites differentiating comparative groups. Comparative analysis of individual human gut microbiomes has revealed the existence of a distinct infant and adult-type microbiota. The fecal microbiota is commonly used as a reflection of the intestinal bacterial composition. Profiling the fecal metabolome can produce information that may be used to point to the existence of a distinct neonatal colonization pattern, markedly influenced by several postnatal factors such as the mode of delivery (vaginal versus caesarean delivery) and infant feeding (breast milk versus infant formula). The performance of metagenomic studies have provided the basis for the use of dietary interventions aimed at counteracting microbiota aberrancies. Fecal metabolomics, in fact, may explore the effects of new therapeutic strategies to optimize the microbial ecosystem by gut microbiota biomodulators such as probiotics, prebiotics, synbiotics or postbiotics.

The potential clinical value of such biomarkers for the early assessment of perturbations in the infant microbial colonization remains, however, a complex task. Understanding the influence of microbial communities during colonization is complicated by multiple factors: the complexity to assess a 'healthy' gut microbiota, knowledge of its composition under eubiosis or dysbiosis conditions, and the difficulty in gaining a global view of microbe–microbe and microbe–host interactions.

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