Evolution of Human Microbiota

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Bacterial Colonization of the Newborn Gut, Immune Development, and Prevention of Disease

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Abstract

We now know that the fetus does not reside in a sterile intrauterine environment but is exposed to commensal bacteria from the maternal gut which cross the placenta and infiltrate the amniotic fluid. This exposure to colonizing bacteria continues at birth and during the first year of life, and it has a profound influence on lifelong health. Why is this important? Cross talk with colonizing bacteria in the developing neonatal intestine helps in the initial adaptation of the infant to extrauterine life, particularly in acquiring immune homeostasis, and provides protection against disease expression (e.g., allergy, autoimmune disease, and obesity) later in life. Colonizing intestinal bacteria are critical to the development of host defense during the neonatal period. Disrupted colonization (dysbiosis) due to cesarean section delivery, perinatal antibiotics, or premature delivery may adversely affect the development of host defense mechanisms in the gut and predispose to inflammation leading to increased susceptibility to disease later in life. Clinical evidence suggests that babies born by cesarean section have higher incidence rates of allergy, type 1 diabetes, and obesity. Infants given repeated antibiotic regimens are more likely to have asthma as adolescents. This observation helps to explain the disease paradigm shift in children from developed countries.

Introduction

In the last half century, the disease burden in developed countries has shifted from a predominately infectious to an immune basis [1]. For example, there has been a striking increase in allergic and autoimmune diseases. This shift and increase in disease has been attributed to the nature of initial bacterial colonization
of the newborn intestine, a revision of the so-called “hygiene hypothesis” [2].
During the first 2 years of life, the infant establishes a complete colonization of
the gastrointestinal tract that remains as a microbial signature throughout his
life. The initial colonization process occurs in stages and has its greatest fluctua-
tions in the early few months of life. During the same neonatal period, the new-
born infant develops appropriate intestinal host defenses to protect him from
infectious and immune-mediated diseases. Since intestinal bacteria influence
gut metabolic and immunologic function, the fluctuations in bacterial coloniza-
tion at the time when immune homeostasis is developing has a profound effect
on the infant’s general health and the prevention of disease expression later in
life.

An example of this process is the development of immune tolerance. Immune
tolerance to innocuous antigens and commensal bacteria is the absence of a sys-
temic immune response to their stimulus and thus an absence of autoimmune
disease states. Oral tolerance develops only with bacterial colonization [3] which
also defines appropriate immunologic responses to stimuli including the nature
of the T-helper cell subset response and other immunologic cellular subsets [4].
As new studies are reported regarding the association between intestinal coloni-
ization and host defense, we have come to appreciate the importance of appro-
priate initial colonization and immune homeostasis.

**Normal Intestinal Colonization – Symbiosis**

We now know that the human fetus does not reside in a sterile environment [5].
Experimental and clinical evidence exists to suggest that the human fetus is ex-
posed to bacteria in utero. Microbiota have been identified in the placenta, am-
niotic fluid, and meconium of full-term, vaginally delivered, healthy newborns
suggesting that exposure to microbes in utero occurs under normal gestational
conditions. Furthermore, the nature of the pregnant mother’s environment
(e.g., weight gain or exposure to infection) during gestation can impact on fetal
exposure to microorganisms. Details of these processes are covered by other au-
thors/speakers in this symposium.

Normal intestinal bacterial colonization leading to immune homeostasis and
the absence of disease occurs in full-term, vaginally delivered newborns not ex-
posed to perinatal antibiotics. These infants exhibit so-called “pioneer” bacteria
which have a specific effect on the normal development of intestinal host defense
including oral tolerance [6]. Normal colonization occurs in various phases over
the first 18 months to 2 years of life (Table 1). Phase 1, the most important phase,
occurs with the ingestion of a healthy bolus of maternal vaginal/colonic bacteria
as the vaginally delivered, partially intrauterine-colonized infant passes through
the birth canal. Phase 2 is the stimulus of oral feeding on bacterial proliferation.
The nature of initial oral feeding (breast milk vs. formula) is a major determi-
nant of normal colonization. Phase 3 occurs when the infant is weaned to com-
plementary solid foods. By 18 months to 2 years of life, a mature intestinal colo-
nization signature exists which is unique to that child throughout his life. Final
colonization consists of microbiota (1,013/mL intestinal content) residing prin-
cipally in the distal small intestine and colon as anaerobic bacteria. The nature
of colonizing bacteria is very diverse, consisting of over 2,000 different species.
Although genetics contributes to the nature of colonizing bacteria, environmen-
tal factors are very important and will be considered in a separate section.

**Dietary Influence on Colonization**

Although other environmental factors (antibiotics, vaccinations, and hygienic
conditions) are very important determinants in intestinal bacterial colonization,
the most important environmental factor is diet. Diet is particularly important
in infancy when the microbial colonizing signature is evolving and has its major
influence on gastrointestinal host defense. Breastfed infants have strikingly dif-
f erent microbiota than formula-fed infants. Components of breast milk, includ-
ing oligosaccharides, stimulate so-called “pioneer” bacteria which can influence
intestinal function such as an increase in polymeric IgA and a decrease in the
intestinal IL-6 inflammatory response [7]. A new area of research has developed
to determine what other protective factors in breast milk (e.g., lactoferrin, trans-
forming growth factor-β, or fatty acids) influence initial bacterial colonization.
In a recent experimental study, for example, the absence of breast milk-induced
polymeric IgA given to newborn rodent pups affects the intestinal microbiota
and predisposes to a greater access of gram-positive organisms to the mucosal
barrier [8]. In addition, intestinal bacteria induced by breastfeeding can stimulate enterocyte genes that both promote intestinal development and immune protection [9]. Furthermore, recent studies have suggested that breast milk has its own microbiome consisting in part of commensal bacteria found in mother’s intestinal tract [10]. To what extent the ingestion of breast milk bacteria influence the ultimate composition of the infant’s intestinal microbiota has not yet been determined.

Other studies have suggested that diet can influence gut colonization at various periods throughout life. An experimental study has shown that when common probiotic organisms (bifidobacteria or lactobacilli) are grown in either high-carbohydrate-, (saturated and unsaturated) fat-, or protein-containing media, a differential proliferation of the probiotic bacteria can occur as well as a differential stimulation of specific bacterial genes [11]. In addition, a recent study comparing the microbiome of children living in a primitive village in Africa with that of children in a city in a developed country (Florence, Italy) showed a striking difference in their microbiomes suggesting that diet (a complex carbohydrate diet with no animal fat or protein [Africa] versus a standard Western diet including processed foods [Italy]) had a strong influence on the composition of intestinal colonizing bacteria [12]. Although not specifically studied, it is known that the disease burden between these 2 patient populations differs dramatically suggesting that the influence of diet on microbiome content may in turn impact on long-term health. Finally, a recent large clinical study suggested that long-term modifications in diet (carbohydrate vs. fat) affect [5] the individual’s enterotype (functional clusters of bacteria) expression of intestinal bacteria [13].

Immunologic Consequences of Normal Colonization

Evidence exists that during a full-term pregnancy components of mucosal immune function in the fetuses can develop. However, for these intestinal defense components to be activated, the intestine must first be stimulated by initial bacterial colonization with appropriate commensal bacteria [14] (Fig. 1). In a like manner, other host defense functions that provide an intact mucosal barrier such as tight junctions, microfold cells, or glycocalyx to prevent the uptake of pathogens and noxious antigens are stimulated by the initial colonization process [15]. Accordingly, an appropriate initial intestinal colonization is necessary for immunologic adaptation of the neonate to the extrauterine environment (Table 2).

Initial colonization is necessary for both innate and adaptive immune function. Colonizing bacteria stimulate enterocytes and mucosal lymphoid cells
Initial Intestinal Colonization and Host Defense

(e.g., macrophages and lymphocytes) to react to pathogens attempting to cross the mucosal barrier by evoking an inflammatory response. These reactions are mediated by pattern recognition receptors such as toll-like receptors expressed on these cells to interact with the pathogens initiating an inflammatory (IL-6 or IL-8) response to prevent penetration. Colonizing bacteria stimulate intestinal cells to upregulate signaling molecules in order to react to potential penetration. However, over time with repeated stimulation, these intestinal cells become tolerant to exposure in order to prevent chronic

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**Table 2.** Microbial colonization and immunologic adaptation to the extrauterine environment

<table>
<thead>
<tr>
<th>Protective enterocyte barrier functions</th>
<th>Innate immune responses</th>
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<tr>
<td></td>
<td>Adaptive immune responses</td>
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<td></td>
<td>Polymeric IgA secretion</td>
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<td></td>
<td>Balanced T-helper cell responses</td>
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<td></td>
<td>Oral tolerance</td>
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**Fig. 1.** The influence of colonization on intestinal function (intra- versus extrauterine gut) is depicted as a schematic cross section of the small intestine of a human fetus in utero versus that of a newborn infant. The fetal intestine appears thin and exhibits a slow epithelial proliferation rate with a paucity of gut-associated lymphoid tissue (GALT), whereas the infant intestine manifests a robust, diverse epithelium with a fast turnover rate and abundant GALT elements. Reprinted from Walker [14] with permission.
inflammation of the intestine [16] leading to diseases such as inflammatory bowel diseases.

In a like manner, colonizing bacteria prime T-helper cells subsets (Th1, Th2, Th17, and T_{reg}) to activate a balanced immunologic response and to evoke on intestinal immune homeostasis rather than inflammation [17]. These stimulated T-helper cells produce a balanced humoral and cellular immune response including an appropriate T-regulatory reaction leading to immune tolerance.

A major component of intestinal protective function is the capacity to develop oral tolerance to commensal bacteria and innocuous antigens. Oral tolerance occurs when repeated exposures to oral antigens stimulate mucosal T-regulatory cells to release cytokines such as IL-10 and TGF-β that downregulate both humoral and cellular immune responses (Fig. 2). Oral tolerance must exist to prevent inappropriate inflammatory reactions to these benign antigens and thus prevent inflammatory disease. The increase in immune-mediated diseases in developed countries is attributed to a lack of oral tolerance development. A normal, balanced, initial colonization during the newborn period is necessary to

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**Fig. 2.** Physiologic immune response to intestinal antigens is shown as a schematic representation of oral tolerance induction by gut microbiota. In the intestinal lumen, gut microbiota activates dendritic cells via the TLR2/TLR4 signaling pathways. Activated dendritic cells cause maturation of Th0 to subsets (Th3, Tr1) of T_{reg} cells via the release of IL-10 to stimulate TGF-β release and thereby suppress humoral IgE production. Reprinted from Walker [14] with permission.

activate oral tolerance and to prevent the expression of allergic and other immune-mediated diseases later in life [18]. Thus, normal initial colonization of the neonatal gastrointestinal tract is necessary for the development of appropriate intestinal immunoprotective function and the prevention of disease.

**Dysbiosis**

Dysbiosis, the abnormal colonization of the intestine with less species diversity and altered phyla, can unfortunately commonly occur with the initial colonization of the newborn intestine [19]. Thus, dysbiosis provides colonizing bacteria which either fail to activate the needed immunoprotective function or actually stimulate a distinct immune response (an imbalance in lymphocytes or the up-regulation of immune cells which favor inflammation). As a result, the developing intestine established abnormal immunologic responses to stimuli leading to the expression of chronic disease later in life. Details of dysbiosis in the newborn period will be discussed in detail by other authors/speakers. As stated, dysbiotic colonization can disrupt the normal development of intestinal host defenses which may result in the expression of immune-mediated diseases (allergy, type 1 diabetes, or celiac disease) later in life. Dysbiosis is thought to be a major contributor to the observed shift in the disease paradigm within developed countries over the last several decades. Although there is a genetic component to dysbiosis in newborn colonization, environmental factors are thought to be a major contributing factor. Lifestyle factors such as diet and stress can affect the nature of colonizing bacteria. In addition, the nature of initial exposure to microbiota in the perinatal period (birth by cesarean section or prolonged hospitalization in the neonatal intensive care unit) can also contribute. Furthermore, medical practices such as the use of antibiotics, excessive vaccination, and excessive cleanliness can disrupt the initial colonizing process. All these circumstances disrupt the appropriate development of intestinal homeostasis and favor disease processes which occur with inflammation.

The process of abnormal colonization differs strikingly from the normal process (Table 3). As a result of premature delivery, birth by cesarean section, and the use of perinatal antibiotics, initial colonization results in a sparse, inadequate phase 1 of the colonization process. Despite the stimulus of oral feeding and weaning to solid foods, the complete colonization of the intestine under these conditions is delayed until 4–6 years of life. During that time period, the infant is more susceptible to both infectious and immune-mediated diseases. Microbial dysbiosis as a result of abnormal colonization has been associated with an increased incidence of chronic diseases later in life. A scientific
study done on newborn rat pups given low-dose antibiotics has been attributed to their increase in weight gain leading to obesity when placed on a high saturated fat diet later in life [20]. This association occurs despite a return to normal intestinal microbiota after the initial exposure to antibiotics underscoring the importance of disrupted dysbiotic colonization early in life on long-term expression of disease. This same observation has been made clinically when mothers gain excessive weight during pregnancy resulting in disruption of their intestinal microbiota, which in turn is passed to their infants at the time of birth, predisposing these infants to excessive weight gain and eventual obesity [21]. Infants born by cesarean section have an increased incidence of allergic diseases such as asthma during adolescence as well as other autoimmune diseases. The same observation has been made with newborns receiving perinatal antibiotics. Thus, early dysbiosis may have profound effects on health later in life.

We have studied the pathogenesis of necrotizing enterocolitis (NEC) for many years. Using ex vivo models of human fetal intestine and primary enterocytes from NEC patients, we have shown that initial commensal colonizing bacteria evoke an inflammatory response as opposed to oral tolerance in immature enterocytes as a result of underdeveloped innate inflammatory genetic pathways [22]. Others have shown that the composition of microbiota in NEC patients differs from that of age-related premature infants in the absence of NEC. A recent study from this laboratory [23] indicated that the more immature the intestine of prematures (e.g. infants <1,000 g) the more dysbiotic is the colonizing microbiome indicating that intestinal immaturity may influence intestinal colonization. This observation suggests that the basis for NEC in premature infants appears to be related to a dysbiotic colonization as well as an immature, proinflammatory response to commensal bacteria.

Table 3. Phases of abnormal initial bacterial colonization (dysbiosis\(^1\))

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
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<tr>
<td><strong>Phase 1</strong>&lt;br&gt;Sparse, inadequate colonization due to:</td>
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Premature delivery  
Cesarean section delivery  
Use of perinatal antibiotics |
| **Phases 2, 3**<br>Introduction of oral feeding and weaning results in slight modifications |
| **Phase 4**<br>Delayed, incomplete initial colonization until 4–6 years |

\(^1\) More susceptible to pathogens and immune-mediated diseases, e.g. allergic diseases.
The use of probiotics to correct dysbiotic colonization (Fig. 3) will be discussed by other authors/speakers. The intent of this section is to briefly suggest that probiotics have been effective in partially restoring symbiosis to a dysbiotic colonization process. In our studies with breast milk, we have shown that compared to formula-fed premature infants those fed mother’s expressed breast milk have a microbiota which favors anti-inflammation over inflammation. This may be an explanation for the protective effect against NEC seen in premature infants given expressed breast milk. Other studies suggest that specific probiotics (e.g., *Bifidobacterium infantis*) may preferentially inhibit inflammation in premature infants. We have just published an observation to suggest that unique to the immature enterocyte response of those exposed to *B. infantis* secretions to decrease inflammation is the use of TLR4 expressed on the surface of these enterocytes [24]. Probiotics have also been effectively used to reduce the expression of atopic dermatitis in allergy-prone infants when given in later stages of pregnancy and during lactation in mothers with a history of allergy [25] (to be discussed by other authors/speakers).

This area of investigation requires additional clinical and scientific studies before recommendations for the routine management of premature infants can be made.

*Fig. 3.* Intestinal microbiota restoration with probiotics. Under conditions of dysbiosis, an imbalance exists between potentially harmful and helpful bacteria. Probiotics may help to restore the dysbiosis to a symbiotic state.

**Use of Probiotics**

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Summary and Conclusions

In this review, a case has been made for the importance of normal initial colonization of the newborn intestine for an optimum adaptation of the infant to the extrauterine environment. Evidence is provided that colonizing bacteria activate the appropriate expression of intestinal defenses including components of the mucosal barrier as well as both innate and adaptive immune responses including oral tolerance. Normal colonization is dependent on both genetic and environmental factors, especially the influence of diet on the composition of intestinal microbiota. This observation is particularly true of breast feeding as the initial source of oral feeding. Factors in breast milk affect the composition of intestinal microbiota by stimulating “pioneer” bacteria which are essential in the activation of intestinal immune defenses such as polymeric IgA production. Disruption of normal colonization, dysbiosis, caused by prematurity, cesarean section, or the use of perinatal antibiotics can adversely affect the interaction of symbiotic commensals and host defense predisposing to disease expression, e.g., allergy, later in life. Thus, the dysbiotic effect on disease expression may be reversed by the use of probiotics. However, additional clinical studies are required.

Disclosure Statement

The author has no conflict of interest.

References
