

The long-term health effects of neonatal microbial flora

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Purpose of review

Colonization of the newborn intestine is a complex process evolving over the first year of life. It is partly responsible for guiding immunologic development within the infant. Given the sharp escalation in immunologic diseases such as allergy and inflammatory bowel disease (IBD), this microbial–host interaction has become the focus of intense interest. DNA-based detection techniques have allowed increased identification of specific microbes involved in this symbiosis.

Recent findings

Epidemiologic studies have demonstrated a link between allergic diseases and alterations in the colonizing flora of infants. Concurrently, other work has demonstrated that interactions between gut flora and the intestinal epithelium seem to be central to the pathogenesis of IBD. In both allergy and IBD, certain bacteria seem to provide beneficial, protective effects via immune modulation.

Summary

There is a complex interaction between the bacteria within the developing gut and the immune system of the host. Colonization of the neonatal gut represents a critical window in this process. It appears clear that disruption within this flora has long-term health consequences as diverse as eczema, allergic rhinitis, and IBD. Guided establishment of specific species within the flora may reduce the incidence of these diseases.

Keywords

atopy, colonization, eczema, inflammatory bowel disease, probiotics

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Introduction

An infant's transition from the prenatal to postnatal existence results in many physiologic and anatomical changes that are critical to future development. Primary among these changes is complete dissolution of the protective sterile environment. The infant is bombarded by microbes through the mechanics of birth. Epidemiologic studies have evidenced steep increased incidence of immunologically based diseases such as allergy and inflammatory bowel disease (IBD) [1]. As allergic disease is often expressed in infancy, possible causative factors have been sought out during this time period. Colonization represents a massive immunologic stimulus and has earned a spotlight for research into the pathogenesis of allergy and IBD.

Establishment of gut colonization

The sterile amnion provides a barrier of protection throughout gestation, holding the microbial world at bay. During birth, the infant encounters these microbes at multiple surfaces, including the skin and mouth. This oral exposure leads to an exceedingly complex process of

bacterial niche establishment and resultant colonization of the neonatal intestine. Individual bacterial species establish their colonies sequentially on the basis of birth exposure and the local intestinal microenvironment [2]. There is wide variation of colonization across individuals and over time [3] and microenvironment. Species present at the time of initial inoculation are paramount to establishing the evolving gut flora. This initial inoculation can vary, particularly on the basis of mode of delivery. Vaginal delivery first exposes the infant to the vaginal and fecal microflora of their mothers. These bacteria include *Group B Streptococcus*, *Escherichia coli*, and *Listeria monocytogenes* [4], which are taken up by the oral route. These species readily establish a niche in the neonatal gut as they are facultative anaerobes and thrive in this oxygen-rich environment. Given the relative sterility of the intestine at this time, there is no competition among bacteria. In contrast, infants born by cesarean section are more likely to be colonized with *Klebsiella*, *Enterobacter*, and *Clostridia* [5,6] bacteria present in the medical environment. For all infants, as oxygen in the intestine is deprived via bacterial utilization, strict anaerobes begin to dominate [7]. *Bifidobacterium* and *Bacteriodes* are examples of anaerobic bacteria that gain hold in this milieu beginning at about

1 week of age [8]. There are other anaerobic bacteria present in smaller numbers. Among these is *Lactobacillus* [9]. However, a recent study [8] longitudinally examining the colonization patterns of more than 100 Swedish infants found *Staphylococcus aureus* to be present in all infants, regardless of delivery. This species represents a major change in species present in the neonatal intestine as compared with prior decades in the same country [8]. The same study also demonstrated delayed colonization time for *E. coli*. This may well result from a decreased initial bacterial load at birth, given the extensive hygienic practices employed at the time of delivery. Thus, alterations in exposures via changes in hygiene may alter this immunologic stimulus.

The next major event in inoculation of the gut occurs with initiation of feeding. Introduction of oligosaccharides and other milk components provides differential growth media to various bacterial species. With major efforts to create infant formula that increasingly resembles breast milk, differences in resident bacteria between breast-fed and formula-fed infants has not been documented consistently [2]. However, it is clear that bottle-fed infants support increased numbers of both *Clostridia* and *Bacteroides* in their gut [9]. In addition, oral antibiotics can have a marked impact on bacterial colonization. This can result in particular effect on both *Bifidobacteria* and *Bacteriodes* [10].

The epithelial cell as immune system arbiter

The single-cell layer of epithelium lining the lumen of the intestine negotiates interactions between copious luminal bacteria and the underlying mucosal immune system. These cells must distinguish between resident bacteria and those that represent potential harm. Epithelial cells serve as a physical barrier, separating immune cells from bacteria. It is through this bacterial–epithelial interaction that much of the beneficial effect of colonization occurs. Intestinal epithelium has evolved developmentally regulated expression of surface glycoconjugates [11]. This allows particular bacterial anchoring to the epithelial cell surface, thus creating more likelihood for approximation. In turn, certain bacteria can induce this glycosylation and reinforce their tether within the lumen. *Bacteroides* species colonized in the gut of germ-free mice will induce expression of these glycoconjugates [12]. The complete story behind tolerance to commensal bacteria has not been completely elucidated, although it is clear that the epithelial layer is a critical link to this lack of inflammatory response. The epithelial cell's location serves as a means of host surveillance. Bacterial recognition via Toll-like receptors and other pattern recognition receptors serves as a means of translating this epithelial lookout into useful information to nearby

immune cells. This information will be translated into very different immune responses, inflammation versus tolerance. Therefore, the epithelial cells have a powerful role in instructing the downstream immune response.

Specialized epithelial cells, referred to as M cells, transport luminal antigens across the epithelial barrier. These antigens then encounter dendritic cells, which process and present antigen to T cells. Dendritic cells serve as the link between antigens, epithelial cell input, and effector lymphocytes. Given this interaction, the dendritic cell–epithelial cell interface has received much warranted attention. Dendritic cells are diverse cells, which have been designated into ever increasing numbers of subsets. These subsets have enormous functional significance, as they will instruct T cells to differentiate variably. Dendritic cells can take up antigen directly by extension of processes through tight junctions between the epithelial cells. In turn, they can directly deliver these antigens to T cells. Recent work [13] has shown that gut-specific dendritic cells prefer steering local T cells toward a Th2 response, which is noninflammatory. This seems logical given the constant presence and recognition of bacteria just one cell layer away. If these dendritic cells were to continuously nudge T cells in an inflammatory direction, there would be local tissue damage that could not be controlled. This kind of dysregulation may be a main driving force in the pathogenesis of IBD. In IBD, there is accumulating evidence that the mucosal immune system responds to commensal bacteria with an aberrant inflammatory response. In fact, treatment with antibiotics, in both humans and experimental animals with IBD, leads to decreased intestinal inflammation [14]. Reinforcing the importance of the 'right' bacterial colonization, there are major disruptions in bacterial representation in IBD [15•]. As mentioned previously, *Bacteroides* is a common colonizing organism present in most infants, typically in the first few months of life. A very recent study implicates this species in protection against IBD. One of its surface polysaccharides (PSA) protects against colitis in an experimental model [16•]. This is accomplished by PSA-mediated expansion or generation of T cells that express IL-10. *Bacteroides* colonization then helps to create a tolerant immunologic milieu in which it resides in the gut. It was shown that although there were increased numbers of bacteria overall, *Bacteroides* was relatively lacking in stool from colonic biopsies of patients with bowel inflammation [17]. Other common colonizing organisms, *Lactobacillus* and *Bifidobacter*, helped attenuate colonic inflammation when given in a double-blind trial [18]. Patients with IBD flares have fewer numbers of both anaerobic bacteria and *Lactobacillus* species compared with patients with inactive disease [19]. Epithelial and bacterial interaction, dendritic cell modulation, and resultant T-cell differentiation elicit an aberrant and

harmful inflammatory response in the presence of alternative microbes at the gut mucosal surface. Large-scale studies aimed at identifying genetic markers of disease have identified nucleotide-binding oligomerization domain containing 2 (*NOD2*)/caspase recruitment domain family, member 15 (*CARD15*) on chromosome 16 as a candidate gene for Crohn's disease [20–22]. This intracellular pattern recognition receptor activates a signaling cascade, which activates the transcription factor nuclear factor kappa B (NFκB). This transcription factor is well known to direct cellular release of proinflammatory cytokines. Therefore, people with IBD may have genetic variations, which result in aberrant recognition of and responses to resident bacteria.

Allergy is related to a suboptimal gut niche

IBD has been rising in tandem with atopic diseases. Given that these conditions are of seemingly disparate causes, is there a causal connection? Perhaps aberrations in gut mucosal function and colonization variably give rise to these conditions based on host genetic influence and environmental factors. The gut flora has become a focus of intensive basic and clinical research in allergy. The 'hygiene hypothesis' coined by Strachan [23] implicates reduced microbial exposure in the cause of atopic disease. This swings the host immune system balance to allergic default. As allergic disease predominantly initiates in childhood, major clinical studies have been undertaken to elucidate the relationship of microbial exposure to allergic disease. A recent prospective birth cohort study [24**] examined the gut flora and relationship to allergy in nearly 1000 infants from the Netherlands. The authors noted that nearly all infants were colonized with *Bifidobacteria* and *Bacteroides*, as found previously. There was no relation between these groups of bacteria and allergic disease. However, infants colonized with *Clostridium difficile* had a higher risk of atopic dermatitis. It is known that colonization with *C. difficile* inhibits the ability of more symbiotic bacteria to develop niches within the gut. Although this study did not find a protective effect resulting from colonization with certain bacteria, the results nevertheless do evidence the role of proper colonization in immune balance. In general, intensive hygiene practices prior to and after delivery may affect these sorts of colonization changes. In Western countries, infants are no longer colonized with *E. coli* in the first days of life. This has been attributed in part to more sterile birthing practices and households in general. Instead, it now takes 6 months before 80% of infants are colonized with this species [25*]. This alteration may have major repercussions for the microbial flora long term, as *E. coli* has previously been one of the initial colonizing microbes, establishing a certain milieu for subsequent, specific bacteria to thrive. Thus, the entire pattern of microbial species may be altered throughout

the first year of life and beyond. In tandem with increasing hygiene surrounding vaginal births, the rate of cesarean deliveries has increased substantially. Birth by cesarean section leads to colonization by substantially different bacteria than those that colonize after vaginal delivery. A recent meta-analysis [26*] that included 20 published articles found a 20% increase in asthma risk for children born by cesarean section. Another study [8] also replicated previously mentioned findings that cesarean section leads to later colonization of important commensals such as *Bifidobacter*, *Bacteroides*, and *E. coli*. However, the first prospective study [27**] investigating the role of cesarean delivery and risk of atopic disease was recently published. In this study, over 400 children were followed for 9 years, with about half being formally assessed for allergic sensitization. The investigators found that cesarean section was associated with increased risk of allergic rhinitis and atopy in general [27**]. A parental history of atopic disease was also a clear risk factor for these children to develop allergy. The authors also replicated their previous finding in this cohort of the allergy-protective effects of early exposure to endotoxin. The relationship between endotoxin exposure and allergy has been studied extensively. A link has been demonstrated between children who live on farms, endotoxin exposure and later risk for allergic rhinitis, atopic asthma, and atopic wheeze [28]. Within this same population of children living on farms, a study [29] recently demonstrated that they are protected from development of IgE antibodies to grass.

As allergic disease occurs relatively early in childhood, usually within the first year or two, the focus of potential causative or protective exposures has been driven back toward early infancy. Atopic dermatitis in infancy heralds later food allergy, allergic rhinitis, and asthma. It is the most common allergic manifestation in childhood. Recently, in a study [30**] of about 100 infants, atopic dermatitis was found to be more prevalent in 18-month-old children who had less diversity within the intestinal microbial flora measured at 1 week of life. This study was replicated from the same population previously studied in which no clear differences existed in the presence of bacterial species as measured by culture-based techniques. In the present study, PCR-based techniques were used. These novel techniques allow increased detection of diverse species that may not survive culture-based techniques. Similar techniques are now being utilized more broadly, which will likely change some previous study results, similar to this work. In addition to an improved general ability to identify bacteria colonizing the gut lumen, these techniques have afforded a means by which variation across species within a given genus may also relate to host immune status. It is well known that *Bifidobacter* is one of the predominant bacteria present early in gut development. A recent study found that

a particular species, *Bifidobacterium pseudocatenulatum*, was found in almost 30% of patients with eczema compared with only 4% of controls. This finding was particularly compelling because, although the *Bifidobacter* genus is well represented in the gut in geographic populations of varied allergy incidence, *B. pseudocatenulatum* was found repeatedly and only in those populations with higher incidence of allergic disorders. One group demonstrated that this bacterial species stimulated IL-10 production by dendritic cells. They hypothesize that IL-10, in the absence of IL-12, can cause Th2 T-cell expansion. Downstream repercussions, at the macroscopic level, include allergy [31]. In contrast, another *Bifidobacter* species prevalent in Ghana, where allergic rates are low, did not alter dendritic cell IL-10 release dramatically. Findings such as these continue to elaborate on the incredible complexity of the intestinal microbiota and the resultant macroscopic effects on the host immune system.

The suggestion that variety within the microbial flora is protective against allergy reinforces the notion that exposure, in general, is beneficial to the host. Western civilizations have reduced microbial exposures through aseptic practices. It may be possible to introduce these bacteria in a controlled way that does not involve a reversal of current practices. Probiotics have become a source of investigation for their potential role in establishing a beneficial, protective gut flora. Much has been done *in vitro*, but many recent studies have involved clinical trials. A randomized, double-blind, placebo-controlled trial [32**] of probiotic administration to mother and infants demonstrated protection against atopic dermatitis. Findings such as these present the possibility that allergic disease could be modified by probiotic supplementation and manipulation of the host's intestinal flora.

Conclusion

Establishment of intestinal colonization in the newborn is a complex process influenced by countless variables. The role of the commensal bacteria in host immune development and function is currently being investigated. Many clues have been elucidated by considering certain diseases, which may be influenced by a host's intestinal colonization. Both allergy and IBD have been implicated as possible repercussions of the altered microbial exposures and resultant colonization of people in Western societies. Although IBD and allergic diseases have been the focus of much of this work, other disease states are also being considered such as overweight [33*]. With improved microbial identification techniques and a constantly evolving understanding of mucosal immunity, the functional significance of the neonatal microbial flora will no doubt be enhanced.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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