Cesarean Versus Vaginal Delivery: Long-term Infant Outcomes and the Hygiene Hypothesis

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KEYWORDS

• Microbiota • Mode of delivery • Hygiene hypothesis

The journey of a thousand miles begins with one step.
Lao Tsu

In the United States, the rate of cesarean delivery (CD) has increased up to 48% since 1996, reaching a level of 31.8% in 2007.[1] This trend is reflected in many parts of the world, with the most populous country in the world, China, approaching 50%[2] and some private clinics in Brazil approaching 80%.[3] Although a significant number of CDs are preformed for obstetric indications, some are simply because of maternal request and may incur several risks for the child. Well known among these risks are neonatal depression due to general anesthesia, fetal injury during hysterotomy and/or delivery, increased likelihood of respiratory distress even at term, and breastfeeding complications. Concurrent with the trend of increasing CD numbers, there has been an epidemic of both autoimmune diseases, such as type 1 diabetes, Crohn disease, and multiple sclerosis, and allergic diseases, such as asthma, allergic rhinitis, and atopic dermatitis.[4,5] The occurrence of these diseases is higher in more affluent, Western, industrialized countries. Several theories have emerged suggesting that environmental influences are contributing to this phenomenon. Most notably, the hygiene hypothesis

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suggests that an overly clean environment, especially in early childhood, may contribute to the development of several childhood diseases. The hypothesis was first proposed by Strachan,[6] who observed an inverse correlation between hay fever and the number of older siblings. This report was subsequently extended by others, from the allergies to autoimmune diseases such as type 1 diabetes.[5] Whether the increase in CD incidence is also causally related is addressed in this review.

The interplay between the emerging microbial ecology of the gastrointestinal tract and the developing mucosal immune system serves as a backdrop for a relationship between CD and the emergence of some of these diseases. With the highly immunoreactive intestine serving as the largest surface area of the body that is exposed to the environment, especially a vast array of luminal microbes and antigens, it is intriguing to speculate that the intestinal environmental interaction during early development of the immune system may relate to these diseases. One intriguing component of this speculation relates to the early development of the intestinal microbiota, the developing immune system, and the early influence of cesarean versus vaginal delivery (VD) on these phenomena. The immune system undergoes major development during infancy, and the development is highly related to the microbes that colonize the intestinal tract.[7–9] It has been suggested that different initial exposures depend on mode of delivery (VD vs CD). The microbes that seed the intestine during either CD or VD may lead to changes in long-term colonization and subsequent altering of immune development (**Fig. 1**). This article provides background about the human microbiota and its relationship to the developing immune system as well as the relationship of mode of delivery on the colonization of the infant intestine, development of the immune system, and subsequent childhood allergies, asthma, and autoimmune diseases.

THE HUMAN MICROBIOTA

The human body, consisting of about 100 trillion cells, carries about 10 times as many microorganisms in the intestines.[10–12] It is estimated that the gut flora have around 100 times as many genes in aggregate as there are in the human genome.[13] The

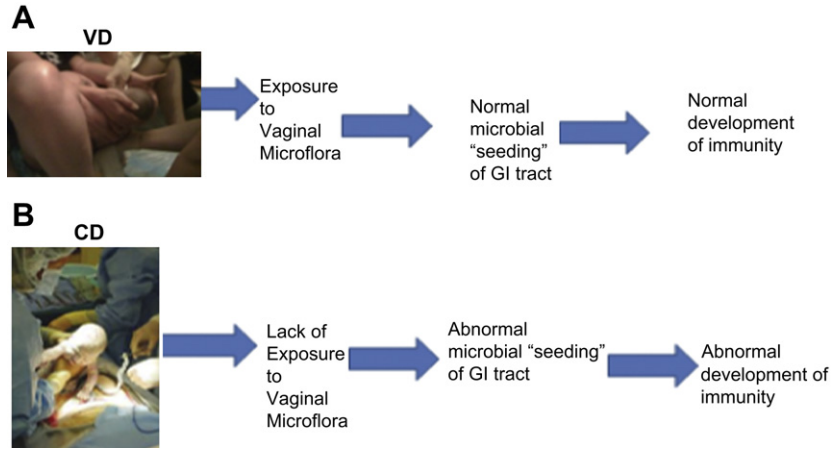

Fig. 1. (*A*) VD picture. (*Obtained from* website Available at: http://wisewomanchildbirth.blogspot.com/2009_01_01_archive.htm. Accessed February, 2011.) (*B*) CD picture. (*Obtained from* website Available at: http://makeupandbeauty.com/wp-content/uploads/2010/06/Cesarean-delivery.png. Accessed February, 2011.) GI, gastrointestinal.

metabolic activities performed by these bacteria resemble those of an organ, leading some to liken gut bacteria to a "forgotten" organ.[12] Microorganisms perform a host of useful functions, such as fermenting unused energy substrates, training the immune system, preventing growth of harmful pathogenic bacteria, regulating the development of the gut, and producing vitamins for the host (such as biotin and vitamin K).[14] Excitement about the potential of harnessing the intestinal microbiota for therapeutic purposes and health is reflected by the popularity of probiotics and prebiotics and by even such seemingly esoteric therapies as human fecal transplant.[15]

Not all the microbial species in the gut have been identified because most cannot be cultured,[10] and identification is difficult. An effort to better describe the microflora of the gut and other body locations using newly developed non–culture-based technologies[16] has been initiated and termed the Human Microbiome Project.[17] This project has a mission of generating resources enabling comprehensive characterization of the human microbiota and analysis of its role in human health and disease. Although the human intestine is the site where most studies are being focused, other sites such as the skin, bladder, mouth, and vagina harbor distinct microbial populations and are also likely to play major roles in health and disease.[16]

INTESTINAL MICROECOLOGY OF THE FETUS AND NEWBORN

Most current literature suggests that the gastrointestinal tract of a normal fetus is sterile. During birth and rapidly thereafter, bacteria from the mother and the surrounding environment colonize the infant's gut. It is obvious that exposure at birth would differ by mode of delivery. The long-term sequelae or impact of this difference in exposure on the child has yet to be determined.

Some recent research work suggests that colonization may begin even earlier. Although the paradigm has been that babies' intestines are sterile until birth, a recent work found a microbial community already dwelling in the meconium of some babies born prematurely.[18] It has also been shown that the amniotic fluid of mothers with preterm labor contains a large and diverse spectrum of bacterial ribosomal DNA.[19] While a baby is in utero, it typically swallows 400 to 500 mL of amniotic fluid per day at term, and the hypothesis that intra-amniotic infection is the driving force behind preterm labor is being widely studied in obstetrics.[20] Whether the microbes or microbial components swallowed in the amniotic fluid stimulate an inflammatory response resulting in preterm birth remains to be evaluated. The effect these organisms have on the developing immune system, aside from their role in preterm labor, also raises interesting questions.

Currently, very few studies have investigated the development of the human microbiota after birth using non–culture-based techniques. In a step toward greater systematic investigation of babies born at term, Palmer and colleagues[21] evaluated the developing microbiota of infants during the first year after birth using microarray techniques to detect and quantify the small subunit ribosomal RNA (rRNA) gene sequences of most currently recognized species and taxonomic groups of bacteria; this was performed along with sequencing of cloned libraries of polymerase chain reaction (PCR)-amplified small subunit ribosomal DNA to profile the microbial communities in 14 healthy full-term infants during the first year after birth. To investigate possible origins of the infant microbiota, the researchers also profiled vaginal and milk samples from most of the mothers as well as stool samples from all of the mothers, most of the fathers, and 2 siblings. The investigators found that the composition and temporal patterns of the microbial communities varied widely from baby to baby, but the distinct features of each baby's microbial community were recognizable for intervals of weeks to

months. The strikingly parallel temporal patterns from a set of dizygotic twins suggested that incidental environmental exposures play a major role in determining the distinctive characteristics of the microbial community in each baby. By the end of the first year of life, microbial ecosystems in each baby, although still distinct, had converged toward a profile characteristic of the adult gastrointestinal tract. Of interest, bifidobacteria were not found in the infants studied using the aforementioned techniques. This finding could be highly significant in that it may debunk the large amount of attention this microbe has received as a potentially important microbe that may be harnessed as a probiotic. On the other hand, the finding could be the result of a technical problem that still needs to be solved using newly developed methodologies.

Although a few studies have monitored the bacterial communities in preterm infants, the picture of the intestinal microbiota still remains limited. To determine whether non-cultured bacteria represent an important part of the community in premature babies' intestinal ecosystems, Magne and colleagues[22] used 16S rRNA genes and PCR-based electrophoretic profiling of 288 clones obtained from the fecal samples of 16 preterm infants. These clones were classified into 25 molecular species. The mean number of molecular species per infant was 3.25, ranging from 1 to 8. The researchers found high interindividual variability. The main bacterial groups encountered belonged to the Enterobacteriaceae family and the genera *Enterococcus*, *Streptococcus*, and *Staphylococcus*. Seven preterm infants were colonized by anaerobes and only four by bifidobacteria (again seeming to minimize these taxa during development). The researchers did not determine the relative effects of delivery mode, sex, gestational age, birth weight, age at sampling, feeding modes, and antibiotic therapies. They concluded that species diversity was low and interindividual variability was high in the feces of preterm infants, as revealed by sequences of 16S rRNA genes and PCR–temporal temperature gradient gel electrophoresis profiles. The intestinal ecosystem of these preterm infants had no typical characteristic.

In summary, whether the fetal intestinal ecosystem is sterile at the time of birth remains a question. This paradigm may be the case in some infants, but not necessarily in others, especially preterm infants, may in turn play a role in the initiation of preterm labor. Nevertheless, the species diversity does seem to be low in most infants shortly after birth, but this diversity increases with environmental exposure. At present, very little is known about the specific emergence of the microbial community of infants during the first year after birth and how this emergence specifically relates to development of immunity and subsequent health and disease.

FUNCTIONS OF THE INTESTINAL MICROBIOTA

A comprehensive review of the functions of the intestinal microbiota is beyond the scope of this review, but the article focuses on their immunologic functions because of their importance in development of the immune system and on the possible pathogenesis of several known allergic and autoimmune diseases. Intestinal bacteria are key to promoting the early development of the gut's mucosal immune system, both in terms of its physical components and function, and continue to play a role later in life in the system's operation. The bacteria stimulate the lymphoid tissue associated with the gut mucosa to produce antibodies to pathogens. The immune system recognizes and fights harmful bacteria but leaves the helpful species, a tolerance developed in infancy and sometimes termed the "old friends" hypothesis (**Fig. 2**).[23] This hypothesis seems to be a synthesis of the hygiene hypothesis, which proposes that these microorganisms that have evolved with humans play an essential role in the establishment of the immune system wherein the microorganisms and the host have evolved

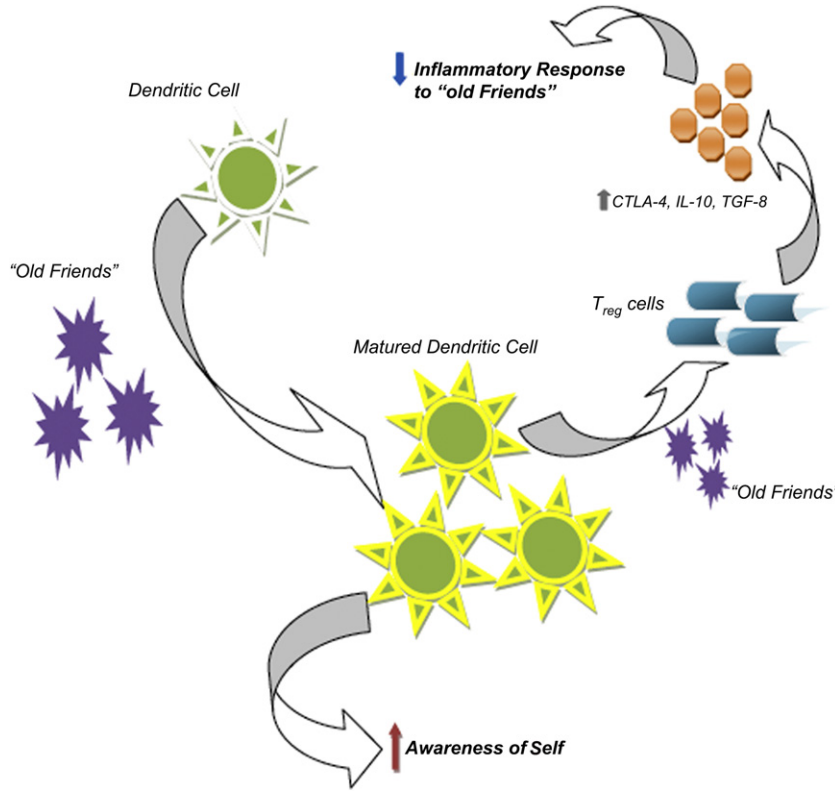

Fig. 2. CTLA-4, cytotoxic T-lymphocyte antigen 4; IL-10, interleukin 10; TGF-β, transforming growth factor β; T_{reg}, Regulatory T.

a codependence: the most relevant organisms are those that coevolved with mammals. These microorganisms interact with other modern lifestyle and environmental changes, such as inappropriate diet, obesity, psychological stress, vitamin D deficiency, pollution (dioxins), and perhaps even CD, leading to enhanced inflammatory responses. The range of chronic inflammatory disorders that can affect the child is potentially larger than usually assumed, including allergies, autoimmunity, inflammatory bowel disease, vascular disease, some cancers, depression/anxiety, as well as perhaps neurodegenerative disorders and type 2 diabetes.

Basic laboratory-based research is supplementing the epidemiologic studies. Recent findings have shown that gut bacteria play a role in the expression of toll-like receptors (TLRs) in the intestines. TLRs are 1 of the 2 classes of pattern recognition receptors (PRRs) that provide the intestine the ability to discriminate between pathogenic and commensal bacteria. These PRRs identify the pathogens that have crossed the mucosal barrier and trigger a set of responses that act against the pathogen, involving 3 main immunosensory cells: surface enterocytes, M cells, and dendritic cells.[24] The other class of PRRs is known as the nucleotide-binding oligomerization domain/caspase recruitment domain isoforms, which are cytoplasmic proteins that recognize endogenous or microbial molecules or stress responses and form oligomers that activate inflammatory caspases. This reaction results in the cleavage and activation of important inflammatory cytokines and/or activates the NF-κB signaling pathway to induce the production of inflammatory molecules.[24]

Bacteria can influence the phenomenon known as oral tolerance, in which the immune system is less sensitive to an antigen (including those produced by gut bacteria) once it has been ingested. This tolerance, mediated in part by the gastrointestinal immune system and liver, can reduce overreactive immune responses such as those found in allergies and autoimmune disease.[25]

There are several antenatal and perinatal events that might also affect the development of the intestinal microbiota. Therapy with broad-spectrum antibiotics is a common practice for mothers who go into premature labor or who have a CD. This treatment can reduce the biodiversity of the fecal microbiota and may be a factor in the cause of necrotizing enterocolitis.[26,27] Studies in mice show that intestinal commensal microbiota have an influence on early postnatal immune development via interactions with intestinal TLRs, which in turn are likely to influence the development of the mucosal immune system and mucosa-related diseases.[28] Other studies suggest that specific microbes may induce regulatory T (T_{reg}) cell development. For example, a prominent human commensal, *Bacteroides fragilis*, directs the development of Foxp3(+) T_{reg} cells with a unique inducible genetic signature.[29] Monocolonization of germ-free animals with *B fragilis* increases the suppressive capacity of T_{reg} cells and induces antiinflammatory cytokine production exclusively from Foxp3(+) T cells in the gut. This effect seems to be mediated by an immunomodulatory molecule, polysaccharide A (PSA), of *B fragilis*, which mediates the conversion of $CD4^+$ T cells into Foxp3(+) T_{reg} cells that produce interleukin 10 during commensal colonization. Functional Foxp3(+) T_{reg} cells are also produced by PSA during intestinal inflammation, and TLR2 signaling is required for both T_{reg} cell induction and interleukin 10 expression. These studies also show that PSA has the ability to not only prevent but also cure experimental colitis in animals and therefore, demonstrate that the *B fragilis* T_{reg} cell lineage differentiation pathway in the gut actively induces mucosal tolerance.[29]

VD VERSUS CD

During VD, the contact with the maternal vaginal and intestinal flora is an important source for the start of the infant's colonization. During CD, this direct contact is absent, and non–maternally derived environmental bacteria play an important role in the intestinal colonization of infants.[30] Some investigators have suggested that the composition of the very first human microbiota could have long-lasting effects on the intestine in breast-fed infants. For example, Grönlund and colleagues[31] showed that the primary gut flora in infants born by CD may be disturbed for up to 6 months after birth. Another study using culture-based techniques showed that the mode of delivery was associated with differences in intestinal microbes 7 years after delivery.[32] The clinical relevance of these changes is unknown, and even longer follow-up periods are needed to establish how long these alterations of the primary gut flora can last.

Nevertheless, there is accumulating evidence that intestinal bacteria play an important role in the postnatal development of the immune system.[33] Thus, if the intestinal flora develops differently depending on the mode of delivery, the postnatal development of the immune system might also be different. Available epidemiologic data show that atopic diseases occur more often in infants after CD than after VD.[34–37] The composition of enteric microbiota in early days of life seems, therefore, to be a very important factor for achieving and maintaining good health in the years to come. It is fundamental to identify more thoroughly the intestinal ecosystem of the newborn.

Although there is an increasing body of evidence that the intestinal microbiota play an essential role in the postnatal development of the immune system, the mechanisms remain poorly understood. Malamitsi-Puchner and colleagues[38] found that only VD

promotes the production of various cytokines implicated in neonatal immunity. Hallstrom and colleagues[39] found a link between CD, disturbed intestinal colonization, and, possibly, occurrence of necrotizing enterocolitis in preterm infants. Although the epidemiologic studies demonstrated that elective CD provides an increased risk for allergic diseases in later childhood, confounding factors could also play intermediate roles. Data available from several studies indicate a delayed onset of lactation with CD.[40,41] Thus, many infants born by CD also lacked the early support of breast milk as stimulator for a physiologic intestinal flora. Both the nonphysiologic start of colonization and the missing early dietary support by delayed start of lactation might result in these long-lasting effects.

Babies are born with immunologic tolerance that is instructed by the mother by preferential induction of T_{reg} lymphocytes,[42] which might allow the baby to become colonized by this first inoculum. The mechanism is via substantial numbers of maternal cells crossing the placenta to reside in fetal lymph nodes, inducing the development of $CD4^{+}CD25highFoxp3(+)$ T_{reg} lymphocytes that suppress fetal antimaternal immunity and persist at least until early adulthood. However, only a subset (if any) of the microbes to which the newborn is initially exposed will permanently colonize available niches and contribute to the distinctive microbiota harbored by the body habitats of adults.[21] As more and more deliveries bypass the vagina, babies may not be exposed to these microbes at birth. Differences in delivery mode have been linked with differences in the intestinal microbiota of babies.[30,31,43,44] Initial communities may serve as a direct source of protective or pathogenic bacteria very early in life.

Another recent study[45] offers a detailed look at the early stages of the body's colonization by microbes. Babies born vaginally were colonized predominantly by *Lactobacillus*, whereas babies born by CD were colonized by a mixture of potentially pathogenic bacteria typically found on the skin and in hospitals, such as *Staphylococcus* and *Acinetobacter*, suggesting babies born by CD were colonized with skin flora in lieu of traditionally vaginal type of bacterium.

The effect of delivery mode on the development of childhood disease has just recently begun to be explored (**Table 1**). The effect seems to be most robust in the

Table 1
CD-associated childhood diseases

Disease	Odds Ratio (95% Confidence Interval) vs VD
Allergic Rhinitis	
All CDs	1.37 (1.14–1.63)
Repeat CDs only	1.78 (1.34–2.37)
Asthma	
All CDs	1.24 (1.01–1.53)
Female	1.53 (1.10–2.10)
Female & repeat CD[a]	1.83 (1.13–2.97)
Celiac disease	1.80 (1.13–2.88)
Diabetes mellitus (type 1)	1.19 (1.04–1.36)
Gastroenteritis[b]	1.31 (1.24–1.38)
Gastroenteritis and asthma	1.74 (1.36–2.23)

[a] Increase not appreciated for male fetuses.
[b] Requiring hospitalization.
Data from Refs.[46,47,50]

area of immune-mediated diseases. CD has been associated with a significant increased rate of asthma, especially in women, and allergic rhinitis, but not atopic dermatitis.[46] This increase was even more apparent when accounting for the factors surrounding the CD. The risk of asthma was increased by 60% in women who underwent a repeat CD without ruptured membranes versus those women with ruptured membranes and/or labor before CD.[46]

Children born by CD are also significantly more likely to experience celiac disease and to be hospitalized for gastroenteritis.[47] No association has been found between CD and Crohn disease or ulcerative colitis. However, whereas preterm birth has been implicated in the development of inflammatory bowel disease, mode of delivery has not.[48]

Type 1 diabetes mellitus has been on the increase in the recent decades, mirroring the increase in CD.[49] Meta-analysis found a 19% increase in type 1 diabetes mellitus in children born by CD when controlling for confounders such as gestational age, maternal age, and birth weight.[50] A recent retrospective study of children in Scotland failed to show such an association.[51] However, the Scotland study had a very small number of subjects (n = 361) compared with the meta-analysis (n = 9938), and the rate of CD was only 14% in the Scottish study (much less than the US average).

SUMMARY

Although CD is necessary in modern obstetrics, the procedure seems to shift a baby's first bacterial community. A better understanding of this early colonization, which is also influenced by events such as breastfeeding, may lead to medical practices for establishing healthy bacterial colonization. The causal relationship between CD, the shift in microbiota, and many childhood diseases continues to be studied. However, there are several problems with the studies reviewed in this article.

It is impossible to lump CD into one category without delineating the indication for CD. A baby delivered after arrest at 8-cm dilation after a long labor would be exposed to a much different microbial environment than a baby born by CD for maternal request before rupture of membranes. It is naive to think that the fetus is only exposed to microbes as the head passes through the vaginal introitus onto the perineum and to ignore the constant exposure to vaginal flora after rupture of membranes. Sonntag and colleagues[48] failed to show a relationship between mode of delivery and inflammatory bowel disease. However, the average age of a subject in this study was 42 years. Indication for CD in the late 1960, before the common use of external fetal monitoring, is strikingly different than modern obstetric indications. The intrapartum exposures of these subjects are most likely vastly different than a more contemporary cohort. Future studies must be more meticulous in categorizing CD to fully understand the effect of CD on colonization and childhood disease.

The role of antepartum and intrapartum antibiotics must also be accounted for in future studies. What effect, if any, these antibiotics have on the microbiota of the fetus and/or subsequent development of disease is unknown. Nearly 20% of women in the United States are colonized with group B streptococci and subsequently receive intrapartum antibiotics. The standard of care also dictates that antibiotics be administered before CD and to mothers in preterm labor and/or with premature prolonged rupture of membranes. Given all these facts, the exposure to antenatal antibiotics is significant. Dominguez-Bello and colleagues[45] noted a difference in fetal colonization based on mode of delivery. However, none of their patients who underwent VD received antibiotics and the CD cases received cephalosporin several hours before incision, which is not the recommended course in the United States. Whether this exposure accounts for the difference, or if fetuses who receive antibiotics per standard guidelines in the

United States show a different colonization pattern, is an important research area to explore.

The link between mode of delivery and subsequent childhood pathology is important. This link becomes even more important as maternal desire for primary CD is increasing and rates of vaginal birth after CD are declining in the United States. This new information about colonization differences with differing modes of delivery seems to be taking the hygiene hypothesis to an entirely new level.

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