Is there a long-term price to pay for infants not exposed to the stress of labor? How the microbiome and the immune system can affect our lives

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The transition between intrauterine and extrauterine life is one of the most dramatic and fundamental phenomena in biology. What evolution accomplished over millions of years (namely, the emergence of life from the sea into a terrestrial environment) must be accomplished in a matter of hours through spontaneous labor and delivery in viviparous species. Hugo Lagercrantz and Theodore Slotkin[1] emphasized the importance and adaptive value of intrapartum stress in their seminal article "The 'Stress' of Being Born." The authors described 4 main transitions that occur at birth: (1) emergence from an aquatic environment where oxygen is acquired through the placenta to a dry environment in which respiratory exchange occurs through the lungs, (2) change from a warm environment in which the fetus has a temperature that is 1 degree higher than the mother on average to a cooler environment at room temperature, (3) moving from a continuous supply of nutrients through the placenta to intermittent feeding in the neonatal period, and (4) going from a sterile bacterial environment to the establishment of the neonatal microbiome (eg, skin, respiratory tract, gut). Lagercrantz and Slotkin's views have gained relevance with time and are now buttressed by a considerable body of work that suggests that the microbiome plays an important role in the developing immune system.[2-5]

In this issue of the Journal, Cho and Norman[6] review the evidence of short- and long-term consequences of cesarean delivery on the immune system. The authors present a thoughtful review of the data, which suggests that infants who are born by cesarean delivery are at increased risk for type I diabetes mellitus, asthma, allergies, and gastrointestinal disorders, among other conditions. After assessing the strengths and weaknesses of epidemiologic evidence, Cho and Norman focus on the potential mechanisms that may underlie such a predisposition. Three major mechanisms are reviewed: (1) acquisition of an atypical microbiome at birth, (2) the effect of labor on the immune system, and (3) the development of memory of the first 2 events through epigenetic changes that modify the nature of the immune response and predispose to immune-related disorders (such as asthma or type I diabetes mellitus).

Acquisition of the first microbiota

Under normal circumstances, the fetus lives in an environment that is devoid of bacteria that is determined by the cultivation of amniotic fluid or the use of molecular techniques (the situation for viruses has not been adequately studied).[7,8] Birth constitutes a critical stage for the acquisition of the first microbiota. During the process of vaginal delivery, the conceptus is exposed to the vaginal microbiota,[9-15] and such bacteria become the pioneer microorganisms that invade the formerly sterile body of the infant and establish the first microbiota.[16] Using sequence-based techniques, Dominguez-Bello et al[17] demonstrated that infants who are born by cesarean delivery are colonized with bacteria that are similar to that found on maternal skin; those infants who are born by vaginal delivery had flora close to that of the vagina. One may think that this state of affairs would be a transitory phenomenon that could be altered rapidly by breastfeeding, ingestion of food, and other new activities. However, follow-up studies have shown that the number of bacteria in the stool of infants who are born by cesarean delivery is lower than that of those born by vaginal delivery; this difference persists long after the first days of life. Qualitative differences in bacterial composition in the stool have been documented 6 months after birth[18,19] and, in 1 study, 7 years later.[20]

Why would differences in the microbiota that are acquired at birth be important? There is now compelling evidence that microbial exposure shapes the nature of the innate and adaptive immune response.[21,22] Exposure to bacteria is critical to the education of the immune system.[22,23] This is consistent with the observation that neonates who are born by cesarean delivery have a higher number of immunoglobulin A– and G–secreting cells than those who are born vaginally.[24] When thinking about the importance of microbiota, it is worth reflecting on the fact that the human body harbors at least 100 trillion microbial cells[25] and a quadrillion viruses.[26] Therefore, numerically, each of us consists of more microbes than human cells—we are symbionts, and microbes contribute substantially to human life.[27,28]

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Changes in the intestinal microbiome have been implicated in physiologic and pathologic states. Earlier this year, the laboratory of Ruth Ley and Rob Knight reported that the gut microbiota of pregnant women changed drastically from the first to the third trimester.[29] When stool from women in the third trimester was administered to germ-free mice, they experienced greater adiposity and insulin resistance.[29] This is consistent with other observations that support a role for intestinal microbiota in the regulation of energy disposition. Indeed, the intestinal microbiota of obese patients is different from nonobese individuals.[30,31]

Moreover, feeding germ-free mice with the stool of obese individuals has been reported to result in weight gain, compared with feeding mice with the stool of nonobese individuals.[32] Supplementation with a methyl donor–rich diet has been reported to increase the frequency of experimental asthma in the offspring.[33] Perplexing as this may sound, a recent study suggests that the normal gut microbiota play a role in brain development and behavior, which has led investigators to consider the existence of a brain-microbiome axis.[34,35] So, if the phrase "you are what you eat" was born out of an ideologic belief in the importance of diet, scientific evidence is now coalescing to confer validity to this notion.

Disease states, such as autism,[36] type II diabetes mellitus,[31,37,38] inflammatory bowel disease,[39,40] and gastric cancer[41] have been associated with changes in the intestinal microbiota. A role for bacteria has also been implicated in susceptibility to influenza,[42] retrovirus transmission,[43] and/or colon cancer.[44] Data are emerging rapidly that support the idea that microbial colonization of the gastrointestinal and respiratory tract in the perinatal period have an important role in the development of mucosal homeostasis and in the predisposition to chronic inflammation.[22] The full scope of the effect of the microbial-host interaction during human development and its consequences later in life remain to be understood fully.[45] It is possible that allergic and autoimmune diseases may constitute only part of a broad spectrum of disorders that result from disturbances in the acquisition of the first microbiome, its disturbance over time (eg, with diet, antibiotics), and subsequent host-microbial interactions.

The effect of labor on the immune response

One may expect that an individual who lives in a sterile intraamniotic environment would need to prepare its immune system to adapt to the microbial world of postnatal life. How does this happen? For several decades, there have been hints in the literature that labor per se primes the immune response.

In 1981, Charles Dinarello et al[46] reported a study that described products of white blood cells that were capable of inducing a fever in rabbits (this was a standard bioassay to determine the presence of endogenous pyrogens). The investigators found that white blood cells from neonates who were born after a vaginal delivery (when incubated with heat-killed bacteria) were able to elicit a temperature elevation in rabbits. However, this change in temperature could not be elicited or was very weak when the experiment was repeated with white blood cells from neonates who were born by cesarean delivery before the onset of labor. Subsequently, it was found that endogenous pyrogens were cytokines that not only induced a fever but also enhanced the activity of the immune system.[46] We now know these cytokines to be interleukin-1, tumor necrosis factor–α, and others. Thirty years after the experiment of Dinarello et al, we know that umbilical cord white blood cells of fetuses who are born by cesarean delivery without labor produce less interleukin-1, tumor necrosis factor–α, and interleukin-6 than those of neonates who are born by vaginal delivery. This interpretation is consistent with evidence that fetal white blood cells of women in term[47] or preterm labor[48] are activated, which has been determined by flow cytometry. Thus, labor enhances the activity of the immune system, and we would argue that it does so to prepare for the transition from a sterile to a nonsterile environment.

How can information about exposure to the first microbiota and labor be stored by the immune system?

Even if the neonatal microbiota after a cesarean delivery is different from that of neonates born vaginally and if foregoing labor could lessen the activity of the immune system, this information would need to be remembered for an individual to be predisposed to an immune disorder later in life. Is this possible?

Memory is an important feature of the immune system; it accounts, among other things, for the success of vaccination. Exposure to microorganisms transforms naïve T cells into memory T cells (often called "pathogen-specific memory lymphocytes").[49] Epigenetic changes (mediated through gene methylation and chromatin modifications) are considered the molecular basis of immunologic memory. Schlinzig et al[50] first reported that umbilical cord leukocytes that are obtained at the time of cesarean delivery have a higher degree of global methylation than those that are obtained after vaginal delivery and proposed that vaginal delivery is associated with global demethylation. Because methylation "silences" gene expression, this is an attractive mechanistic explanation underpinning the priming of the immune response that is observed with the stress of labor. After the online publication of the review by Cho and Norman,[6] a study from the University of Michigan reported no difference in global methylation of leukocytes that were obtained from neonates who were born by cesarean or vaginal delivery.[51] However, this does not exclude the possibility that exposure to labor affects the epigenome in a gene-specific (rather than global) manner. The next step is to determine whether labor elicits epigenetic changes in genes that are involved in the immune response.

Does cesarean delivery before labor predispose to type I diabetes mellitus, allergies, and asthma?

What are we to make of the observation that cesarean delivery is associated with an increased risk of immune-related disorders? The establishment of a causal relationship between prela-

bor cesarean delivery and conditions diagnosed years or decades later presents a challenge for epidemiology. Specifically, it would be extremely difficult to control for the relevant factors that occur between exposure and the diagnosis of disease that could explain the observed association or to ensure that no meaningful antecedent factors have escaped consideration in estimating these relationships.

Cho and Norman[6] emphasize the limitations of studies in which these associations are based and highlight the complexities in controlling for confounding variables that are notoriously difficult to measure. Importantly, the authors also note that, because some studies did not distinguish between prelabor elective cesarean delivery and emergency cesarean delivery that was performed after the onset of labor, the magnitude of the positive associations may be underestimated.

Our assessment of the evidence is that there may be an association between prelabor cesarean delivery and the subsequent development of immune disorders that are diagnosed later in life; however, the magnitude of this association appears modest at this time. For example, 1 metaanalysis that report an association between cesarean delivery and asthma/allergic rhinitis estimated that only 1-4% of all cases can be attributed to cesarean delivery.[52] We believe that the potential effect of prelabor cesarean delivery in predisposing to later immune disease is worthy of further investigation, given the epidemic nature of cesarean delivery and the accumulating evidence that the microbiota play a critical role in shaping the innate and adaptive immune response.[2] Future investigation about the long-term effects of prelabor cesarean delivery will need to include a systematic survey of the different ecologic niches for bacteria/viruses with the use of sequence-based techniques, to characterize the nature of the immune response over time, and to prespecify the disorders of interest so that potentially confounding factors (genetic and environmental) can be measured appropriately and controlled for.

Who could have predicted that a surgical procedure introduced to save the lives of mothers because of obstructed labor would be performed so frequently in the 21st century[53] for such different indications[54] and that questions about the long-term effects on the microbiome, immune system, and the predisposition to allergic and autoimmune diseases would arise? Such are the unexpected turns of biology and medicine in the context of pregnancy and birth. ■

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REFERENCES

1. Lagercrantz H, Slotkin TA. The "stress" of being born. Sci Am 1986;254:100-7.
2. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 2010;330:1768-73.
3. Molloy MJ, Bouladoux N, Belkaid Y. Intestinal microbiota: shaping local and systemic immune responses. Semin Immunol 2012;24:58-66.
4. Atarashi K, Honda K. Microbiota in autoimmunity and tolerance. Curr Opin Immunol 2011;23:761-8.
5. Littman DR, Pamer EG. Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe 2011;10:311-23.
6. Cho CE, Norman M. Cesarean section and development of the immune system in the offspring. Am J Obstet Gynecol 2012. Epub ahead of print.
7. DiGiulio DB, Gervasi MT, Romero R, et al. Microbial invasion of the amniotic cavity in pregnancies with small-for-gestational-age fetuses. J Perinat Med 2010;38:495-502.
8. Gervasi MT, Romero R, Bracalente G, et al. Viral invasion of the amniotic cavity (VIAC) in the midtrimester of pregnancy. J Matern Fetal Neonatal Med 2012;25:2002-13.
9. Zhou X, Brotman RM, Gajer P, et al. Recent advances in understanding the microbiology of the female reproductive tract and the causes of premature birth. Infect Dis Obstet Gynecol 2010;2010:737425.
10. Gajer P, Brotman RM, Bai G, et al. Temporal dynamics of the human vaginal microbiota. Sci Transl Med 2012;4:132ra52.
11. Bai G, Gajer P, Nandy M, et al. Comparison of storage conditions for human vaginal microbiome studies. PLoS One 2012;7:e36934.
12. Hillier SL. The complexity of microbial diversity in bacterial vaginosis. N Engl J Med 2005;353:1886-7.
13. Fredricks DN, Marrazzo JM. Molecular methodology in determining vaginal flora in health and disease: its time has come. Curr Infect Dis Rep 2005;7:463-70.
14. Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. Annu Rev Microbiol 2012;66:371-89.
15. Fredricks DN. Molecular methods to describe the spectrum and dynamics of the vaginal microbiota. Anaerobe 2011;17:191-5.
16. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol 2007;5:e177.
17. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107:11971-5.
18. Adlerberth I, Lindberg E, Aberg N, et al. Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? Pediatr Res 2006;59:96-101.
19. Gronlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. J Pediatr Gastroenterol Nutr 1999;28:19-25.
20. Salminen S, Gibson GR, McCartney AL, Isolauri E. Influence of mode of delivery on gut microbiota composition in seven year old children. Gut 2004;53:1388-9.
21. Gill N, Finlay BB. The gut microbiota: challenging immunology. Nat Rev Immunol 2011;11:636-7.
22. Renz H, Brandtzaeg P, Hornef M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. Nat Rev Immunol 2012;12:9-23.
23. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009;9:313-23.
24. Huurre A, Kalliomaki M, Rautava S, Rinne M, Salminen S, Isolauri E. Mode of delivery: effects on gut microbiota and humoral immunity. Neonatology 2008;93:236-40.
25. Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. Proc Natl Acad Sci U S A 1998;95:6578-83.
26. Haynes M, Rohwer F. The human virome. In: Nelson KE, editor. Metagenomics of the human body. New York: Springer; 2011:63-78.
27. Gordon JI, Klaenhammer TR. A rendezvous with our microbes. Proc Natl Acad Sci U S A 2011;108(suppl 1):4513-5.
28. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449:804-10.
29. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012;150:470-80.
30. Ley RE. Obesity and the human microbiome. Curr Opin Gastroenterol 2010;26:5-11.

31. Kootte RS, Vrieze A, Holleman F, et al. The therapeutic potential of manipulating gut microbiota in obesity and type 2 diabetes mellitus. Diabetes Obes Metab 2012;14:112-20.
32. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027-31.
33. Hollingsworth JW, Maruoka S, Boon K, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest 2008;118:3462-9.
34. Diaz Heijtz R, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 2011;108:3047-52.
35. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 2012;13:701-12.
36. Williams BL, Hornig M, Buie T, et al. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. PLoS One 2011;6:e24585.
37. Larsen N, Vogensen FK, van den Berg FW, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 2010;5:e9085.
38. Wu X, Ma C, Han L, et al. Molecular characterisation of the faecal microbiota in patients with type II diabetes. Curr Microbiol 2010;61:69-78.
39. Dicksved J, Halfvarson J, Rosenquist M, et al. Molecular analysis of the gut microbiota of identical twins with Crohn's disease. ISME J 2008;2:716-27.
40. Rajilic-Stojanovic M, Biagi E, Heilig HG, et al. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology 2011;141:1792-801.
41. Atherton JC, Blaser MJ. Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications. J Clin Invest 2009;119:2475-87.
42. Ichinohe T, Pang IK, Kumamoto Y, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc Natl Acad Sci U S A 2011;108:5354-9.
43. Kane M, Case LK, Kopaskie K, et al. Successful transmission of a retrovirus depends on the commensal microbiota. Science 2011;334:245-9.
44. Kostic AD, Gevers D, Pedamallu CS, et al. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome Res 2012;22:292-8.
45. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell 2012;148:1258-70.
46. Dinarello CA, Shparber M, Kent EF Jr, Wolff SM. Production of leukocytic pyrogen from phagocytes of neonates. J Infect Dis 1981;144:337-43.
47. Thilaganathan B, Meher-Homji N, Nicolaides KH. Labor: an immunologically beneficial process for the neonate. Am J Obstet Gynecol 1994;171:1271-2.
48. Berry SM, Romero R, Gomez R, et al. Premature parturition is characterized by in utero activation of the fetal immune system. Am J Obstet Gynecol 1995;173:1315-20.
49. Weng NP, Araki Y, Subedi K. The molecular basis of the memory T cell response: differential gene expression and its epigenetic regulation. Nature Rev Immunol 2012;12:306-15.
50. Schlinzig T, Johansson S, Gunnar A, Ekstrom TJ, Norman M. Epigenetic modulation at birth: altered DNA-methylation in white blood cells after caesarean section. Acta Paediatr 2009;98:1096-9.
51. Virani S, Dolinoy DC, Halubai S, et al. Delivery type not associated with global methylation at birth. Clin Epigenetics 2012;4:8.
52. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. Clin Exp Allergy 2008;38:634-42.
53. Zhang J, Troendle J, Reddy UM, et al. Contemporary cesarean delivery practice in the United States. Am J Obstet Gynecol 2010;203:326.e1-10.
54. ●●●●. AQ: 3