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Another Reason to Thank Mom: Gestational Effects of Microbiota Metabolites

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Microbial colonization after birth profoundly affects development of the host. In a recent paper, Gomez de Agüero et al. (2016) reveal a new aspect of ontogeny influenced by the microbiota: the impact of gestational gut bacterial metabolites on early immune maturation of the neonatal intestine.

In many ways, newborn mammals are both immunological and microbiological blank slates. Microorganisms, including the consortium known as the microbiota, drive development of the immune system over the course of postnatal life. This includes, but is not limited to, maturation of secondary lymphoid organs and priming of innate immune barrier functions, such as the expression of antimicrobial factors by intestinal epithelial cells (Fulde and Hornef, 2014; Rakoff-Nahoum et al., 2015). Indeed, the microbiota is a major factor that confers protection from pathogens. However, postnatal immune development takes some time, leaving newborns vulnerable to infection. Infectious diseases are the leading cause of death worldwide in this demographic.

One strategy to overcome this ontogenetic "problem" is the transfer of protective factors from mother to offspring. In utero, antibodies such as of the immunoglobulin G (IgG) isotype are transported across the placenta and remain with the offspring through early infancy. After birth, mother's milk contains numerous factors including IgG and IgA, leukocytes, antimicrobial proteins, oligosaccharides, and potentially many others, which confer protection from infection.

Publishing in *Science*, Gomez de Agüero, Ganal-Vonarburg, et al. (Gomez de Agüero et al., 2016) report a significant advance in our understanding of how the gut microbiota impacts postnatal immune development. They also discovered a novel mechanism of vertically transmitted protection of the newborn. They find that bacteria in the mother's intestine during gestation can drive later innate maturation of the neonatal gut in the absence of colonization, through the transfer of specific bacterial metabolites to the fetus and via mother's milk.

We know that the microbiota strongly impacts the host and that much of these effects chronologically associate with colonization after birth. Does this mean that the effects of the microbiota are solely due to colonization of the newborn? To address this, one would need a system to reliably eliminate all microbes in the parental generation before the birth of the offspring. Previously, the Macpherson lab engineered an auxotrophic mutant of *E. coli* with a deletion of three genes required for synthesis of peptidoglycan, a component of the cell wall (Hapfelmeier et al., 2010). Introduction of 10^{10} CFU/gram feces of these bacteria to germ-free mice results in transient and reversible colonization whereby no bacteria are detectable in the gut 72 hr after administration (Hapfelmeier et al., 2010).

With these tools in hand, Gomez de Agüero et al. (2016) were able to colonize pregnant mothers while leaving newborns effectively germ-free. They then compared the immunological phenotype of newborn mice from germ-free mothers transiently colonized with these *E. coli* from days 4 to 15 of gestation, referred to as "gestational colonized," (the murine gestational period is roughly 21 days) to those born to "germ-never" moms, germ-free animals that were not gestationally colonized. Analysis of the pups from these two different groups of moms demonstrated three main differences (Figure 1). First, the intestines of pups from gestationally colonized mothers had 10-fold more of a particular type of immune cell known as type 3 innate lymphoid cells (ILC3; specifically NKp46$^+$RORγt$^+$ ILC3) compared to germ-never pups. These cells have not undergone T cell receptor rearrangement but share many properties of T cells such as lineage differentiation factors and cytokine production, including IL-22 (van de Pavert and Vivier, 2016). ILC3 have been best studied in lymphocyte-deficient mice where they have been shown to provide local protection against bacterial gut pathogens. Second, pups from gestationally colonized moms had significantly more intestinal monocytes belonging to the CD11c$^+$, F4/80$^+$ subset. It is unclear as to the function of these cells, although they may have immunoregulatory roles. Third, focusing on suckling mice at postnatal day 14, the authors performed transcriptional analysis of intestinal mucosa from each group and found higher expression of antimicrobial factors such as the Reg3 family of C-type lectins and also defensin-related proteins in pups from gestationally colonized moms.

Importantly, the authors found no evidence of colonization of either the placenta or pup intestine and no differences in other known aspects of microbiota-induced immune development such as local or lymphoid organ expansion of T and B cells. Together, these data demonstrate that specific aspects of early innate immune development in neonatal mice, particularly the ILC3-IL-22-antimicrobial protein axis, are induced by intestinal colonization of the pregnant mother and occur independently of live microbes in the offspring. Notably, these observations may also point to a non-redundant role of ILC3 in intestinal host defense specifically in

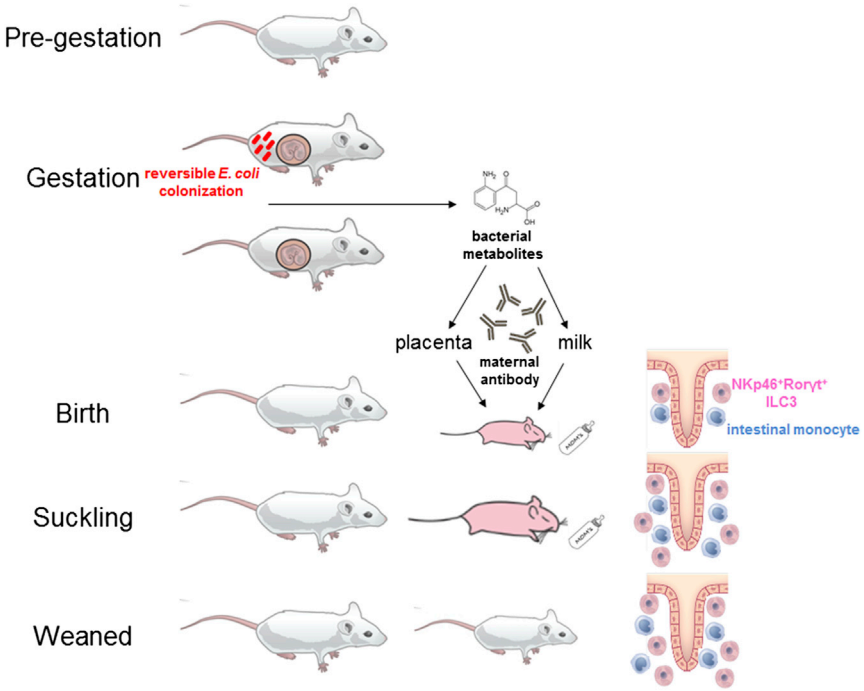

Figure 1. Metabolites Derived from Bacteria during Gestation Drive Early Postnatal Gut Innate Immune Development Independently of Neonatal Colonization

An auxotrophic mutant of *E. coli* was used to transiently colonize previously germ-free pregnant mice. Bacterial metabolites, such as AhR ligands, cross the placenta to the fetus and into milk through a mechanism partially dependent on maternal antibodies. This process led to expansion of type 3 innate lymphoid cells, specifically NKp46^{+}RORγt^{+} ILC3, and monocyte subsets in the intestine. Gestational colonization also regulated intestinal gene expression, such as for antimicrobial proteins (not depicted).

the neonate when adaptive lymphocytes have yet to expand.

The authors next investigated how this "information" of gestational colonization may be relayed to the neonate. Transfer of serum from gestationally colonized mothers to germ-never mothers increased intestinal ILC3 (but not the monocyte) populations in pups, but not when the IgG component was removed. Furthermore, pups of gestationally colonized mice deficient in the J chain gene, which have defective transport of di- and polymeric antibodies (such as IgA and IgM) into the intestinal lumen, had intestinal ILC3 levels similar to pups of germ-never mice. Cross-fostering experiments (where pups born to gestationally colonized or germ-never mothers are switched shortly after birth) demonstrated that transfer of this microbial information across the placenta and into mother's milk were both necessary for neonatal ILC3 expansion.

What form of information was transferred to the neonate to confer the early immune developmental program? Gomez de Agüero et al. (2016) used auxotrophic, carbon radiolabelled *E. coli* for gestational colonization and were able to detect fully labeled bacterial products across nearly all macromolecular classes including sugars, fatty acids, amino acids, and derivatives and in multiple organ compartments, including placenta, fetus, mother's milk, as well as the liver and intestine of newborn pups. Focusing on metabolites present in milk and those requiring maternal antibodies for transport, the authors identified enrichment of tryptophan metabolites, such as kynurenine. Such small molecules are ligands for the aryl hydrocarbon receptor (AhR), which is emerging as an important developmental regulator of the immune system. Notably, the AhR pathway has been implicated in the postnatal ontogeny of ILC3 (Kiss et al., 2011). By administering AhR ligands to pregnant germ-never mothers, the authors showed that this pathway is sufficient to drive the ILC3 phenotype in offspring.

Like any truly novel discovery, the work of Gomez de Agüero et al. (2016) stimulates compelling questions. How are the metabolites derived from the bacteria in the pregnant mother's intestine transferred to the fetus and milk? The requirement of antibodies for transport of a subset of bacterial-derived metabolites suggests that the neonatal Fc receptor (FcRn), which transcytoses IgG out of the intestinal lumen and also from maternal circulation across the placenta (Pyzik et al., 2015), is a good candidate. Are some small molecules bound to bacterial antigenic targets of maternal antibodies? Are others bound to carrier proteins such as albumin, which is known to both bind AhR ligands and cross the placenta? There is a range of consequences of systemic microbial products and toxins at the maternal-fetal interface during gestation. These include teratogenesis, preterm labor, impairment of neuro-cognitive development (Humann et al., 2016), effective defense against placental infection, and now the newly discovered benefits of transplacental AhR ligands on postnatal immune development. Mechanistic understanding of such transport may provide insights into how bacterial products are recognized and interpreted at the placenta.

Gomez de Agüero et al. (2016) clearly demonstrate that the effects of the gut microbiota on postnatal immune maturation are not simply due to colonization of the newborn after birth. They show this in a simplified model of gestational monocolonization with *E. coli* whereby AhR ligands derived from this bacterium drive a distinct early postnatal intestinal developmental program. Given the complexity of microbes present in the gestational gut, it will be exciting to learn whether there are other modules of priming induced by distinct microbes and their products. Along these lines, it is tempting to speculate that this transgenerational effect represents a predictive adaptive response (West-Eberhard, 2003) whereby mothers prepare the neonate for specific challenges that they are likely to encounter based on gestational environmental cues.

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Breathless in the Gut: Implications of Luminal O_2 for Microbial Pathogenicity

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Salmonella **employs a variety of strategies to survive and colonize the colon. In this issue of** ***Cell Host & Microbe*****, Rivera-Chávez et al. (2016) identify a new mechanism whereby antibiotic-mediated depletion of anaerobes (e.g.,** ***Clostridia*****) and associated decreases in butyrate result in increased tissue oxygen and increased aerobic expansion of** ***Salmonella*****.**

The healthy mammalian gastrointestinal tract harbors trillions of bacteria. A finely tuned mutualism exists within the intestinal mucosa, where microbes that are essential for host health can also contribute to the development and maintenance of multiple mucosal diseases (Lozupone et al., 2012). Nutrient provision by microbes is one benefit enjoyed by the host. Microbial metabolism in the gut liberates energy in the form of short-chain fatty acids (SCFAs) such as butyrate, which is the primary energy source for colonic epithelial cells.

The low-O_2 conditions that enable SCFA production also establish a unique niche for the adjacent colonic epithelium. A steep O_2 gradient exists between the lamina propria and the gut lumen (Albenberg et al., 2014; Kelly et al., 2015), which is dominated by anaerobic organisms. Situated at this interface, the colonic epithelium functions at a partial pressure of oxygen (pO_2) well below that of other tissues. Recent studies have demonstrated that active inflammation results in a further decrease in tissue pO_2, to localized levels that approach anoxia (Campbell et al., 2014). Such decreases in local O_2 during inflammation are driven largely by the activation of enzymes that generate reactive oxygen species (ROS), such as leukocyte NADPH oxidase (Campbell et al., 2014). In addition to ROS, other byproducts of inflammation (e.g., reactive nitrogen species) can be used as respiratory electron acceptors by some pathogens under anaerobic conditions (Lopez et al., 2015). From this perspective, surprisingly little is known about the influence of the tissue microenvironment on the expansion or retraction of specific microflora under inflammatory conditions.

A study by Rivera-Chavez et al. in this issue of *Cell Host & Microbe* (Rivera-Chávez et al., 2016) addresses this question head on. They proposed that aerobic respiration enzymes encoded within the bacterial genome might prove beneficial for the expansion of *Salmonella* in conditions where anaerobes become depleted (e.g., following antibiotic use). For these purposes, they utilized *Salmonella enterica* subsp. Typhimurium (*S.* Typhimurium) mutants lacking functional cytochrome *bd* subunits and examined competitive fitness advantages in vitro and in vivo. Results from these studies revealed that mice depleted of major anaerobic microorganisms (especially *Clostridia*) generate a more aerobic environment, which then allows for the luminal expansion of aerobic *S.* Typhimurium (Figure 1). These experiments revealed that the lack of cytochrome *bd* oxidase resulted in a growth disadvantage for *S.* Typhimurium that manifested only in antibiotic-treated animals. Further analysis revealed that cytochrome *bd* oxidase synergizes with nitrate reductases to drive postantibiotic *S.* Typhimurium expansion. Indeed, recovery of targeted *S.* Typhimurium nitrate respiration mutants (*nar*G, *nap*A *nar*Z mutant) was nearly 8-fold less than wild-type *S.* Typhimurium following antibiotic-mediated depletion of anaerobes. To document increases in tissue oxygenation associated with antibiotic treatment, these investigators employed the use of pimonidazole dyes, which adduct with