MICROBIOME

Initial exploration of in-utero microbial colonization

Analysis of the intestinal content of fetuses suggests very limited bacterial colonization with potential immunological roles opening new research questions.

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The establishment of the gut microbiome in newborns and infants is one of the key steps toward priming the developing immune system and promoting a healthy microbiome-host symbiosis. While several studies are unravelling the microbial and immunological aspects of this colonization during and after delivery [1,2], it is still highly debated whether there is a functional relationship between the fetus and the bacteria at this time. In this issue of *Nature Medicine*, Rackaityte *et al.,* analyze the fetal intestinal content collected from terminated pregnancies for bacteria and find some evidence for early bacterial colonization [3].

Rackaityte *et al.,* looked for bacteria in fetal intestine using a combination of methodologies ranging from microbial culture to visual bacteria-like morphology identification with fluorescence and SEM microscopy, as well as molecular approaches including Sanger sequencing and 16S rRNA gene amplicon sequencing. The same set of tools have been used in the past to survey the presence of bacterial organisms on tissues in contact with the fetus such as the placenta [4,5] and the amniotic fluid [6] or for analyzing the after-birth meconium content [7]. These previous studies raised an intense debate around whether the bacteria identified were in fact the result of contamination [8] but most importantly they were limited by the intrinsically indirect approach at testing the non-sterility of the fetal gut. Rackaityte and colleagues pioneer the direct exploration of the human intestinal fetal content by sampling what would be called meconium when collected after birth.

The authors found bacterial DNA in 40 out of 50 of the fetal intestinal contents studied. Among the samples in which they identified bacteria, three fetal bacterial profiles were found: one dominated by Micrococcus (n=9), another represented by *Lactobacillus (n=6)* and a third which included *Lactobacillus* and *Micrococcaceae*, as well as *Bacteroides, Bifidobacterium*, and *Prevotella* (n=25) although this last profile resembled that of the negative biological controls and the first two were found only in a minority of the samples. Interestingly, the fetal bacterial profiles were linked to distinct fetal epithelial transcriptomes and to distinct patterns of T-cell

composition suggesting varying immune imprinting. The authors were able to isolate, grow and identify a specific bacterium from the fetal intestine, *Micrococcus luteus*. This microbe was found in other meconium samples collected just after birth but was lowly prevalent in fecal samples collected later on during the first months of life. They carried out *in vitro* experiments with the bacterial isolate under conditions that mimic the pregnancy hormonal environment (progesterone and estradiol) and found that under these conditions it was able to proliferate as well as to persist inside phagocytes. Furthermore, when the authors exposed the cultivated *M. luteus* strain to primary human fetal intestinal epithelial cells *in vitro* the cells had a similar transcriptome profile to that observed in the *ex vivo* fetal intestine.

While the study provides evidence that bacteria can be present in the fetal intestine and that these bacteria may have a role in shaping the immune system, there are a number of intriguing questions that are left for future investigations. But before targeting such questions, it is crucial that the findings are replicated in different studies and independent cohorts. Previous investigations of the bacterial presence in the healthy placenta, amniotic fluid and meconium indeed demonstrated the need for such validation. These previous studies reported the isolation of specific bacterial strains with *Cutinobacterium*, *Staphylococcus* and *Enterobacteria* being the most common ones [6] although *Enterococcus*, *Ralstonia* and *Candida* have been also identified. Other studies only partially validated the presence of these bacterial groups in the same tissues [9,10]. Still, unexplained inconsistencies on the detected bacterial genera between studies are worrisome and demand caution in the interpretation of the data. Cultivation of low biomass samples in a rich culture media would also promote the growth of bacteria from the environment, and it is thus not easy to assign specific origin to the isolates. The authors of the new study are indeed well aware that these unavoidable limitations apply also to the analysis of the fetal gut, so the ultimate confirmation for the presence of viable bacteria in the fetal intestine would then need to come from other studies in other populations reporting similar colonization rates by a taxonomically consistent set of bacteria.

One of the next questions to ask would be about the type and extent of the effect of in utero viable bacteria on the fetus. In contrast with the study here, a recent study showed that *Ralstonia insidiosa,* a bacterial strain isolated from human placenta, did not induce an *in vitro* pro-inflammatory immune response [11]. The key challenge is thus to understand whether viable and immunologically active bacteria have a biological role for the health conditions of the infant and can influence disease risks later on in infancy and childhood. Because the viable bacterial presence is found to be “limited” in the study, the key related question is whether infants that experienced different levels of in utero colonization (from no colonization to colonization by multiple bacteria) have or not detectable difference in short-to-long-term health outcomes. That is: what is the size of the effect on the developing infant microbiome of in-utero colonization compared to the massive diversity of bacteria the newborn experiences during and after delivery? It is well-known the effect of potential pathogenic bacteria during pregnancy for fetal and neonatal health but very little data is available for non-pathogenic bacteria in healthy pregnancies.

Furthermore, bacterial viability may not even be strictly needed to promote host stimulation of the immune system. Different studies are showing that the bacterial cell-walls and other

bacterial components as well as bacterial metabolites would interact with the immune system even in absence of active colonization [12]. Multiple routes can thus be available for fetal microbiome-host crosstalk and understanding whether and which are specifically relevant in the fetal environment should be the focus of future studies.

Altogether, this study provides new evidence on the host-microbial interactions during life in utero, however, how and when the fetal intestine control the bacterial presence, and which diversity of taxa populate the fetal intestine and how they persists remains underexplored. And even more importantly, the biological implications of in-utero colonization for human development are currently yet to be understood.

Figure 1. Assessing bacterial presence and effect in the fetal intestine.
Distinct bacterial profiles are present in the fetal intestine and linked to distinct fetal epithelial transcriptomes and distinct patterns of T-cell composition. Rackaityte *et al.,* isolate viable *M. luteus* strainsand find its genome differs from other *M. luteus* strains. *In vitro* experiments under pregnancy hormonal environment reported its ability to proliferate and to persist inside phagocytes. *M. luteus* strain in vitro exposition to primary human fetal intestinal epithelial cells showed similar transcriptome profile to that observed in the ex vivo fetal epithelial tissue.

The authors declare no conflict of interest

References:

1. Ferretti, P. *et al.* Mother-to-Infant Microbial Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. *Cell Host Microbe* **24**, 133–145.e5 (2018).
2. Shao, Y. *et al.* Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature* **574**, 117–121 (2019).
3. Rackaityte, E. *et al.* Viable bacterial colonization is highly limited in the human intestine in utero. *Nat. Med.* (2020) doi:10.1038/s41591-020-0761-3.
4. Aagaard, K. *et al.* The placenta harbors a unique microbiome. *Sci. Transl. Med.* **6**, 237ra65 (2014).
5. Seferovic, M. D. *et al.* Visualization of microbes by 16S in situ hybridization in term and preterm placentas without intraamniotic infection. *Am. J. Obstet. Gynecol.* **221**, 146.e1–146.e23 (2019).
6. Collado, M. C., Rautava, S., Aakko, J., Isolauri, E. & Salminen, S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci. Rep.* **6**, 23129 (2016).
7. Stinson, L. F., Keelan, J. A. & Payne, M. S. Comparison of Meconium DNA Extraction Methods for Use in Microbiome Studies. *Front. Microbiol.* **9**, 270 (2018).
8. de Goffau, M. C. *et al.* Human placenta has no microbiome but can contain potential pathogens. *Nature* **572**, 329–334 (2019).
9. Zhu, L. *et al.* Bacterial Communities in the Womb During Healthy Pregnancy. *Front. Microbiol.* **9**, 2163 (2018).
10. Younge, N. *et al.* Fetal exposure to the maternal microbiota in humans and mice. *JCI Insight* **4**, (2019).
11. Parnell, L. A. *et al.* Functional characterization of Ralstonia insidiosa, a bona fide resident at the maternal-fetal interface. *bioRxiv* 721977 (2019) doi:10.1101/721977.
12. Laforest-Lapointe, I. & Arrieta, M.-C. Patterns of Early-Life Gut Microbial Colonization during Human Immune Development: An Ecological Perspective. *Front. Immunol.* **8**, 788 (2017).