Seminars in Fetal & Neonatal Medicine xxx (2016) 1–7

Contents lists available at ScienceDirect

Seminars in Fetal & Neonatal Medicine

journal homepage: www.elsevier.com/locate/siny

Review

The microbiome during pregnancy and early postnatal life

Josef Neu*

Division of Neonatology, Department of Pediatrics, University of Florida, Gainsville, FL, USA

Keywords:
Microbiome
Metabolome
Fetus
Neonate
Preterm birth
Mode of delivery

SUMMARY

We are changing our concept that the newborn infant emerges from a sterile environment. In-utero colonization may have major impacts on the developing mammal in terms of development of immunity and metabolism that, with epigenetic modifications, will lead to diseases in later life. In addition, the microbial profile that develops during and after birth depends on mode of delivery, type of feeding (human milk versus formula) and various other environmental factors to which the newborn is exposed. The goal of this review is to clarify that the microbiome in the maternal fetal unit as well as the immediate changes that occur as new microbes are acquired postnatally play major roles in subsequent health and disease. Rapidly developing technologies for multi-omic analyses and systems biology are shifting paradigms in both scientific knowledge and clinical care.

1. Introduction

Central paradigms to reproductive science have been that the fetus resides in a sterile environment and that the newborn attains its microbiota only after birth. Numerous studies in humans as well as other species contradict this dogma [1]. Research done more than 30 years ago demonstrated that, even without ruptured membranes, bacteria are often found in amniotic fluid [2]. Several studies since have provided evidence for a significant presence of microbes in the fetal maternal unit, which includes the placenta, amniotic fluid, and meconium [3–9]. This has brought forth the concept of a maternal–fetal–microbial triad comprising a "holobiont", somewhat analogous to a coral reef, that is highly interdependent upon its various components, which does poorly when its commensal microbiota are perturbed [10,11]. Similar to this analogy, an inflammatory response to altered intrauterine microbial communities has been linked to preterm delivery [12], as well as brain, lung, and eye disease in these infants [13,14].

We are thus changing our concept that the newborn infant emerges from a sterile environment. In-utero colonization may have major impacts on the developing mammalian host in terms of development of immunity and metabolism that, with epigenetic modifications, will lead to diseases in later life. However, the microbial profile that develops during and after birth depends on mode of delivery, type of feeding (human milk versus formula), and various other environmental factors to which the newborn is exposed. These will be further discussed in this review.

2. Proximal colonization sites: placenta, amniotic fluid and meconium as niches for microbes and preterm birth

Microbial taxonomy in placenta, amniotic fluid and meconium differs depending on the stage of gestation [3,5,15]. Whether prematurity is related to a true "dysbiosis" at these sites with microbes that have a pathogenic potential remains unclear. Even though these differences in taxonomy associate with preterm delivery, considerable work is needed to better determine causality between the microbes found in maternal distal sites (oral cavity, vagina, and intestine) or proximal sites (amniotic fluid, placenta, fetal gastrointestinal tract) and preterm birth. Here we will discuss these niches separately, then attempt to amalgamate them into a more cohesive pattern.

2.1. Placenta

Recent studies using DNA sequencing to interrogate the placental microbiota from infants born at various gestational ages have been performed [3,16–18]. It is now known that the placenta frequently harbors microbes, and that these microbes are associated with prematurity. An association between different taxa of microbes and level of prematurity as well as infections early in pregnancy has been found [3]. Intriguingly a similarity between

* Address: 1600 S.W. Archer Road, Human Development Building Room 112, Gainesville, FL 32610, USA. Tel.: +1 352 273 8985; fax: +1 352 273 9054.
E-mail address: neuj@peds.ufl.edu.

http://dx.doi.org/10.1016/j.siny.2016.05.001
1744-165X/

placental microbes and those found in the mouth derived from a database of non-pregnant women was discovered. The fact that *E. coli* was one of the largest taxa represented in the placental samples [3] is of interest in that this is a frequent resident of the intestine rather than of the oral cavity, thus suggesting a potential maternal intestinal origin. This requires additional studies wherein samples from different sites in the same woman are analyzed.

2.2. Amniotic fluid

Recent studies estimate the prevalence of microbial invasion of the amniotic cavity to be ≥30–50% higher than that detected by cultivation-based methods [4]. Highly intriguing is the fact that the presence and quantity of microbial DNA in amniotic fluid directly correlates with increased levels of white blood cells and inflammatory mediators, and indirectly correlates to gestational age at the time of delivery [7,19]. This suggests a sequence in which higher microbial loads lead to inflammation, subsequently triggering the neuroendocrine mechanisms of preterm birth [7,20,21].

2.3. The fetal gastrointestinal tract and meconium microbiome

Pediatricians are taught that the baby's first stool, meconium, is sterile. Studies from our group and others have demonstrated that meconium from both preterm and term neonates frequently contains both cultured and as-yet-uncultured microbes [5,22,23]. Our group was among the first to propose that the fetus develops in a non-sterile environment in utero and that the fetal intestinal microbiome may contribute to preterm birth [22]. Specifically, our investigation of the neonatal intestinal meconium revealed that microbial DNA was present in infant meconium samples and that the diversity of these microbes was linked to gestational age (GA) [22]. Importantly, these results were in contrast to the generally accepted dogma that meconium and the intrauterine environment of the fetus are sterile, and subsequent investigations by our group and others have replicated and expanded on these findings [5,6,24,25]. More recently, we characterized the neonatal microbiome using meconium samples from babies born between 23 and 40 weeks' GA and we identified specific microbial taxa linked to GA that have been previously detected in amniotic fluid [5]. Published data from our own laboratories [5] and others [6] demonstrate that the microbial taxa present in meconium are uniquely distinct from those found in subsequent fecal samples from the same infant, independent of gestational age. More specifically, meconium samples collected near the time of delivery from infants with various gestational ages still exhibited differences in the taxonomic composition from subsequent non-meconium fecal samples [6,22] underscoring the fact that microbial variation in meconium samples reflect the intrauterine environment and differ according to GA. To make an analogy, using meconium to evaluate intrauterine microbial status exhibits similarity to testing meconium for in-utero drug exposure, a well-known and established diagnostic test [26].

Existing data from animal [23,27] and human [28] studies that have characterized the fetal microbiome using meconium samples suggest that the fetal intestinal microbiome is likely seeded by microbes present in the maternal gut. How these microbes traverse the maternal gut and travel to the fetal GI tract remains a topic for future studies. In addition, studies are needed to verify whether these microbes originate from one of the other niches previously mentioned – the maternal mouth or vagina. It is highly likely that since the fetus swallows large quantities of amniotic fluid during the last trimester of pregnancy, meconium microbes are derived from swallowed amniotic fluid. This is supported by a high degree of similarity between meconium microbes found in our studies and those reported in the literature to be found in amniotic fluid [5].

3. Distal sites: vaginal, oral, and maternal intestinal dysbioses trigger preterm labor

Alternatives to the fetus obtaining vaginally derived microbes via ascension and translocation through the choriodecidual barriers include hematogenously derived sources such as the maternal mouth via inflamed periodontal tissue and the maternal intestinal tract due to higher intercellular junctional permeability and/or dendritic cell transport [29,30]. Here we will discuss these in greater detail.

3.1. Maternal intestine

Microbes in the pregnant mother's intestinal tract differ depending on stage of pregnancy. One study characterized fecal bacteria of 91 pregnant women [31]. Gut microbiota changed markedly from first to third trimesters, with an overall increase in Proteobacteria and Actinobacteria, and reduced richness (lower species count).

It has been hypothesized that intestinal dysbiosis in the pregnant woman is an emerging cause of pregnancy complications including fetal rejection (causing preterm delivery) [32]. The possibility of the maternal intestine as the source of in-utero/fetal microbes is derived from several studies: in one, a group of pregnant mice were orally inoculated with a genetically labeled *E. faecium* strain previously isolated from breast milk of a healthy woman. The labeled strain was isolated and polymerase chain reaction detected from the amniotic fluid of the inoculated animals. By contrast, it was not detected in the samples obtained from a non-inoculated control group [27]. In a follow-up study, they detected this labeled microbe in meconium of the offspring after feeding to pregnant mice [23]. In another study, it was found that a probiotic administered to pregnant women altered both placental and fetal intestinal inflammatory markers [28]. The concept of a dormant blood microbiome that distributes microbes between organs has also been raised [33]. It is highly intriguing that if the maternal intestine is the origin of the microbes found in the uterine/fetal unit, and if these microbes cause metabolic and inflammatory responses that may induce preterm labor, interventions might be developed that include maternal dietary manipulation and/or microbial therapeutic alterations by introducing certain microbes into the mother's diet. Recent studies pointing to this phenomenon suggest that the maternal intestine is a primary site of microbes associated with preterm labor [34].

3.2. Vagina

Microbes in the vagina differ depending on the stage of pregnancy and fetal maturity [9,35]. Early studies suggested that the vagina might be the origin of these microbes that ultimately reach the placenta, amniotic fluid, and fetus via translocation across the choriodecidual plate [29,36]. Of major interest is the fact that recovery of particular organisms from the placenta is associated with systemic perinatal inflammation [37], which relates to intrauterine growth restriction and central nervous system damage in the preterm [38,39]. This has also been linked to learning disabilities, attention deficit disorders, and developmental delays in those newborns who survive [40].

Please cite this article in press as: Neu J, The microbiome during pregnancy and early postnatal life, Seminars in Fetal & Neonatal Medicine (2016), http://dx.doi.org/10.1016/j.siny.2016.05.001