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Influence of maternal microbiota during pregnancy on infant immunity

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Summary

Microbiota from various maternal sites, including the gut, vagina and breast milk, are known to influence colonization in infants. However, emerging evidence suggests that these sites may exert their influence prior to delivery, in turn influencing fetal immune development. The dogma of a sterile womb continues to be challenged. Regardless, there is convincing evidence that the composition of the maternal gut prior to delivery influences neonatal immunity. Therefore, while the presence and function of placental microbiome is not clear, there is consensus that the gut microbiota during pregnancy is a critical determinant of offspring health. Data supporting the notion of bacterial translocation from the maternal gut to extra-intestinal sites during pregnancy are emerging, and potentially explain the presence of bacteria in breast milk. Much evidence suggests that the maternal gut microbiota during pregnancy potentially determines the development of atopy and autoimmune phenotypes in offspring. Here, we highlight the role of the maternal microbiota prior to delivery on infant immunity and predisposition to diseases. Moreover, we discuss potential mechanisms that underlie this phenomenon.

Keywords: infant immunity, microbiota, pregnancy

Introduction

The human body is home to a range of microorganisms, including viruses, bacteria, fungi, archae and unicellular eukaryotes. The bacterial contingent of this community, the microbiota, are known to affect our health in profound ways, bringing to the forefront the symbiotic relationship that exists between these microbes and their human host. Vertical transmission of microbes from various maternal body sites contribute to the developing infant gut microbiota, including the gut, vagina, skin and breast milk [1,2]. Many of these exert their influences during delivery or postpartum, after exposure to maternal vagina, stool and skin [3], but emerging evidence suggests that their influence may begin *in utero*. Historically, it has been assumed that the uterine environment is sterile [4]. This dogma has since been challenged [5–8], with unique placental microbiome in some studies [5,9] but not others [7,10].

Regardless of whether there is a fetal or placental microbiome, there is evidence of a strong influence of maternal gut microbiota during pregnancy on infant microbiota. Maternal gut strains have been shown to be more persistent in the infant gut and ecologically better adapted compared to those from other sources [11]. In mice, genetically labeled bacteria were present in meconium samples that matched those that were orally administered to the mother [8]. In addition, germ-free pregnant dams transiently colonized with a modified *Escherichia coli* strain and returned to germ-free status prior to delivery [12] had pups with altered innate lymphoid and mononuclear cells, suggesting that transient changes in maternal microbiota during pregnancy drive fetal immune programming. Therefore, whether or not the fetus is indeed colonized *in-utero*, the period during pregnancy is the first point of maternal microbes influencing fetal immunity. The mechanisms through which maternal microbiota prior to delivery program neonatal immunity are yet to be elucidated. Here, we review current literature on the impact of maternal microbiota during pregnancy on infant immunity.

Prenatal gut microbiome and fetal immunity

Antibiotic-induced shifts in microbiota

A significant body of work implicates exposures and events during gestation as critical determinants of offspring disease predisposition later in life [13–16]. This concept has formed the basis of the developmental origins of health and disease (DOHaD) hypothesis [17,18]. These influences include maternal diet, toxins, stress and smoking and may act through changing metabolism, epigenetics and/or microbiota [19]. Support for microbial-mediated mechanisms are widespread. Tormo-Badia *et al.* [20] observed significantly increased proportions of $CD8^+$ T cells in the mesenteric lymph nodes (MLN) of pups born to non-obese diabetic (NOD) dams treated with antibiotics during pregnancy compared to control pups. Similarly, pups born to mothers treated with antibiotics orally during gestation and postpartum exhibited increased susceptibility to vaccinia virus infection and reduced interferon (IFN)-γ production by $CD8^+$ T cells when compared to controls [21]. Follow-up experiments revealed that the observed $CD8^+$ T cell impairment was driven by an altered activation and expression of T cell receptor (TCR) critical for sustained cytokine production [22]. We recently showed, using mouse models, that altering maternal intestinal microbiota during pregnancy alone impacted inherent immunity in pups at 14 days postpartum [1] (Fig. 1). We intended to only alter the microbiota in maternal gut and not at other body sites by administering oral vancomycin, which has poor oral bioavailability, during gestation. Indeed, we did not detect any vancomycin in maternal plasma 2 days after delivery, indicating that the antibiotic was not systemically absorbed. Although the intervention was targeted to maternal gut in our model, we observed perturbations in breast milk microbiota, and to a lesser degree vaginal

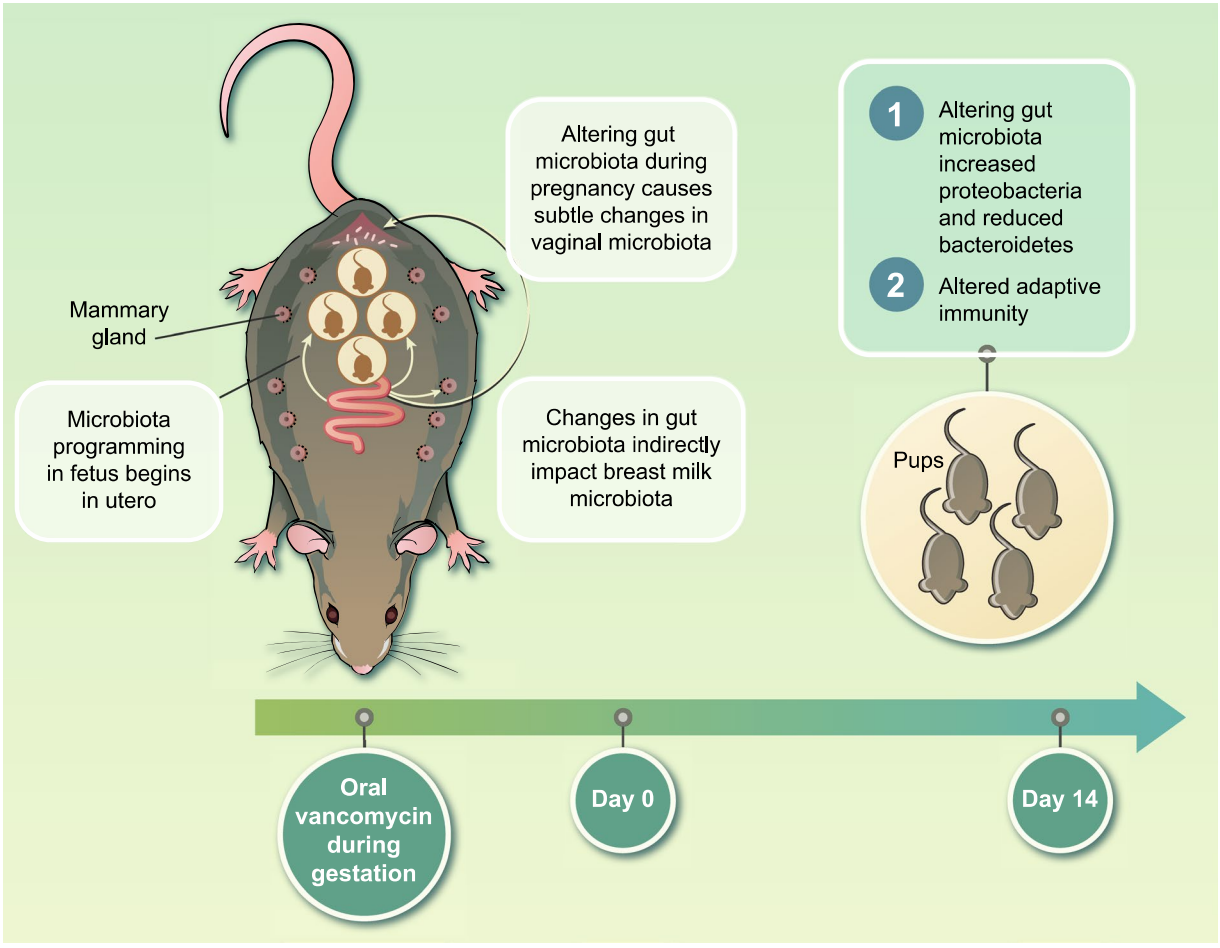

Fig. 1. Experimental model of the impact of maternal gut microbiota during gestation on offspring immunity. To test the role of maternal gut microbiota during gestation on offspring immunity, pregnant BALB/c dams were fed vancomycin in drinking water for 5 days prior to delivery. No antibiotics were administered to control dams. All mice in these groups received normal water after delivery. Pups born to dams treated with vancomycin during gestation only had altered intestinal microbiota compared to controls. In addition, pups born to vancomycin breeders had significantly higher splenic cell counts, higher numbers of total B cells as well as follicular B cells *versus* control pups [1].

microbiota postpartum, suggesting that gut microbiota during pregnancy may also impact that at distal sites [1] and this may, in part, be a mechanism through which maternal gut microbiota influence neonatal immunity.

Gut–breast milk bacterial axis

An early study by Martin *et al.* reported that select gut bacteria from the maternal gastrointestinal tract can access the mammary glands through an enteromammary pathway [23]. Although controversial, some studies have offered a scientific basis for such physiological translocation (reviewed in [24]). The mechanisms could involve dendritic cells (DCs) and $CD18^+$ cells [25,26], which take up non-pathogenic bacteria from the gut lumen and subsequently transport them to other locations, including lactating mammary glands. Bacterial translocation from the gut to the mammary glands and milk has been observed in mice late in gestation [27]. Two lactic acid bacteria strains, *Lactococcus lactis* and *L. salivarius*, were orally administered to pregnant dams and later detected in mammary tissue and milk [27]. While the breast milk microbiota influences immune development postpartum [28–30], the composition of these microbiota is partly shaped prior to delivery and is dependent on gut microbiota during pregnancy [1]. Therefore, gut composition prior to delivery indirectly drives early offspring immune development via the gut–breast milk axis. However, further research is needed to explore the existence of the bacterial enteromammary pathway. This novel form of maternal–neonatal communication could influence our present understanding of fetal immune development.

Vaginal microbiota during pregnancy and infant immunity

The vagina contains more than 170 species of bacteria, and these communities are less diverse and remarkably stable throughout pregnancy [31–33]. Previous work suggests that the vagina might be a source of microbes that reach the placenta, amniotic fluid and fetus, via translocation across the choriodecidual plate [34,35]). However, microbes in the vagina are probably themselves critical in programming neonatal immunity. A great deal of data exist regarding immunological differences between vaginal *versus* caesarean-delivered infants, suggesting a role of vaginal microbiota in immune education in offspring [36–38]. Maternal vaginal microbiota during labor and delivery is probably determined during pregnancy. There is also evidence that maternal vaginal microbiota during pregnancy impacts infant immunity even before passage through the vaginal canal during delivery *in utero*. Newborns whose mothers were vaginally colonized with *Lactobacillus* during pregnancy had higher proportions of $CD45RO^+$ cells and reduced IL-12 in cord blood, indicating that lactobacilli in the maternal vagina impact fetal immune development [39]. Benn *et al.* [40] found that the presence of certain maternal vaginal microbes was associated with risk for wheezing in children at 4–5 years of age. The mechanisms of this interaction remain unknown, but could be due to bacterial metabolites, ascending organisms or simply that women's vaginal microbiota is largely dependent on that of the gut microbiota.

Maternal microbiota during pregnancy and offspring immune-related disorders

Allergy and asthma. There is substantial epidemiological evidence that exposure to farming and pets in early life is associated with reduced incidence of asthma and allergies [41–43]. In addition, a large European study revealed that mothers exposed to farm animals during pregnancy were less likely to have children who developed allergies, and the immunological tolerance to allergens was already present in cord blood [44]. Similarly, a recent study in a Chinese cohort observed that maternal exposure to farming during pregnancy impacted the quantity and function of neonatal regulatory T cells (T_{regs}), partly contributing to reduction of incidences of allergies and asthma in offspring [45]. Maternal pet exposure has been associated with reduced cord blood levels of immunogloblulin (Ig)E [46] and increased numbers of T_{regs} which ameliorate the effect of allergy-mediated T helper type 2 (Th2) cytokines [47,48]. Douwes *et al.* [49] showed that maternal exposure during pregnancy to animals and/or grain and hay reduced the symptoms of asthma, hay fever and eczema in their children. Prenatal exposure was found to contribute to low prevalence of these atopic diseases, and continued exposure only contributed additional protection in some cases. Importantly, the timing of these exposures is crucial, with the strongest effects observed *in utero* and during the first year of life [50]. The interaction between farm-derived biological factors and the immune responses and disease susceptibility in the host has also been tested in mouse models. An interesting study by Conrad *et al.* [51] investigated the asthma-protective effect of prenatal exposure to farm-derived microorganisms. Intranasal exposure to *Acinetobacter lwoffii* F78 (cowshed-derived bacterium) protected against the development of experimental asthma in the progeny, and this protection was dependent on intact maternal Toll-like receptor (TLR) signaling [51]. While the exact mechanism of allergic protection is unclear in humans, as both prenatal and postnatal pet exposure alters infant gut microbial composition [52] it is likely that modulation of the microbiota plays a role.

Microbiome modulation by probiotics has also been shown to impact pediatric allergy development [53]. Combined pre- and postnatal probiotic supplementation

was shown to be crucial for the preventive effects of probiotics on infant eczema; prenatal or postnatal supplementation alone was ineffective [53,54]. However, others have found the prenatal component to be more influential [55]. While probiotic-induced changes in maternal gut microbiota during pregnancy and lactation may reduce incidence of pediatric allergies, further research is warranted to determine optimal timing and dosage.

More direct evidence for the influence of maternal microbiota during pregnancy on offspring atopic disease is antibiotic use during pregnancy, which can cause prolonged alterations to the microbiota and their metabolites [56]. In a Danish birth cohort, the use of prenatal antibiotics was associated with increased odds of atopic dermatitis at 18 months of age in infants of mothers with atopy [57]. In addition, in children aged 2–10 years, maternal use of any antibiotics during pregnancy was associated with a 1·3-fold increased risk of asthma in the offspring [95% confidence interval (CI) = 1·21–1·42] ([58]). However, in a Swedish study by Örtqvist *et al*. [59] antibiotic exposure in fetal life was associated with increased risk for asthma in a cohort analyses but not sibling analyses, suggesting that the link between antibiotic use and atopy may be confounded by shared familial risk factors. Furthermore, a recent study showed that maternal antibiotic exposure during pregnancy is associated with a dose-dependent increase in child asthma risk [60], but so was maternal antibiotic use before pregnancy and in the 9 months postpartum. However, in a well-designed sibling-control study, Mulder *et al.* [61] found prenatal antibiotic use to be predictive of childhood asthma even compared to sibling controls. Although some studies show influence regardless of trimester of maternal antibiotic use [57], this study found the influence to be more profound when antibiotic use occurs during the third trimester [61]. Although more research is needed to determine whether timing is crucial, multiple lines of evidence suggest the maternal microbiota during pregnancy plays a key role in preventing an allergy-prone immune phenotype in infants.

Type 1 diabetes. Apart from allergy and asthma, the prenatal microbiome has also been implicated in other immune disorders, including type 1 diabetes and inflammatory bowel disease. Studies in rodent models of spontaneous type 1 diabetes (T1D) have linked the gut microbiota to disease susceptibility [62,63]. Livanos *et al.* [64] demonstrated that administering a subtherapeutic low-dose penicillin (STAT) to mice during the late pregnancy period and postpartum accelerates the incidence of T1D in male offspring compared to untreated controls; however, whether these effects were mediated in the antepartum period is unclear. Either way, there were no differences in the frequencies of the lamina propria T_{reg} or Th17 cells between STAT and control pups. In an elegant murine study conducted by Hu *et al.*, oral administration of a combination of neomycin, polymyxin B and streptomycin to pregnant NOD dams led to a delay in the development of diabetes in these susceptible offspring. The incidence of diabetes was also significantly reduced relative to offspring born to untreated dams [65]. Authors observed shifts in microbiota of both dams and pups, suggesting a direct role of the microbiota in modulating the predisposition of T1D in offspring [65]. Furthermore, antibiotic treatment of NOD dams during pregnancy led to lower frequencies of both $CD4^+$ and $CD8^+$ T cells production of IFN-γ in the MLN in pups compared to those born to untreated dams. Interestingly, dams treated with vancomycin during pregnancy had different alterations in gut microbiota and increased susceptibility to T1D via opposing immunological effects, possibly mediated by alterations in antigen-presenting cells (APCs) [66].

Inflammatory bowel disease. Beyond T1D, the microbiota has also been implicated in inflammatory bowel diseases (IBD) [67–69]. The composition of the gut during pregnancy has been shown to influence IBD outcomes in offspring. For example, antibiotic exposure during pregnancy but not during infancy was associated with an increased risk of very early onset IBD, regardless of whether there was antecedent gastroenteritis [70]. In a murine study using an IL-10 knock-out colitis model, exposing dams to antibiotics from the third week of gestation until weaning led to increased susceptibility to chemically induced dextran sulfate sodium (DSS) colitis and inflammation in her offspring, lasting into adulthood [71]. This suggests that maternal microbiota during pregnancy is a critical determinant of IBD development. Taken together, the T1D and IBD studies discussed demonstrate that maternal dysbiosis during pregnancy has immunological consequences in the offspring and is a determinant of infant predisposition to autoimmune disorders.

Potential mechanism of cross-talk between maternal microbiota and offspring immunity

Although the exact mechanisms remain to be revealed, microbiota during pregnancy are thought to initiate offspring immune programming in various somewhat interrelated ways which are not necessarily mutually exclusive (Table 1, Fig. 2).

Maternal microbiota influence fetal microbiota. There are data supporting the possibility that the fetal microbiota may develop *in utero* via the placental barrier or through ingestion of amniotic fluids [72], and therefore may impact the developing fetal immune system. Various studies have

Table 1. Mechanisms of cross-talk between maternal microbiota and offspring immunity

Study theme	Outcome	Species	Refs
Maternal microbiota influence fetal microbiota	1. Maternal gut bacteria translocate to meconium in offspring	Mouse	[73]
	2. Fetal gut is colonized with bacteria during pregnancy	Human	[76–78]
	3. Placenta harbors a unique microbiome	Human	[5,9]
Maternal microbiota influence infant microbiota and immunity	1. Maternal gut strains are more persistent in infant gut compared to those from vagina and skin	Human;	[11]
	2. Maternal microbiota during gestation impacts offspring microbiota and immunity	Mouse	[1,12,20]
	3. Offspring born to mothers with an altered microbiota are susceptible to viral and bacterial infections	Mouse	[99,100]
	4. Maternal gut microbiota indirectly impacts extraintestinal microbiota	Mouse	[1]
Bacterial metabolites	1. SCFA impact intestinal immunity	Mouse;	[86,87,89,90,101]
	2. SCFA produced during pregnancy impact fetal immunity	Mouse	[12,91,93]
Maternal IgG	1. Maternal gut microbiota during pregnancy impacts maternal IgG effectively influencing passive immunity to offspring	Mouse	[1]
	2. Immunoglobulins are involved in microbial opsonization	Human;	[97]
	3. IgG mediates bacterial transfer *in utero*	Mouse	[12]

indicated that certain bacteria from the maternal gut may translocate to extradigestive sites in healthy hosts [73–75]. Murine and human studies have shown that meconium is colonized with bacteria during pregnancy [76–78]. Jimenez *et al.* [8] isolated a tagged *Enterococcus faecium* from the meconium of offspring after orally inoculating the strain to pregnant dams, indicating that maternal gut microbes potentially cross the placenta to offspring gut.

In theory, fetal intestines may be exposed to commensal microbes and their products in swallowed amniotic fluid, which may therefore be an important contributor to early immune development. For example, memory $CD4^+$ and $CD8^+$ T cells can be identified towards the end of the first trimester in human fetal gut [79]. Memory CD4 T cells in fetal intestines have been shown to co-localize with APCs and produce IFN-γ, IL-2 or tumor necrosis factor (TNF)-α, promoting intestinal development [80,81]. Altogether, these suggest that early fetal exposure to microbial antigens may impact immunity. While it is not clear what the relative contribution of maternal *versus* fetal microbiome is to offspring immunity, it is plausible that both these microbiota are critical in programming fetal immunity prior to delivery.

Maternal microbiota during pregnancy influence early-life infant microbiota and immunity. Although not a direct effect during pregnancy, maternal microbiota during pregnancy shape the vaginal and breast milk microbiota, which will alter the pioneer infant microbiota during a critical window in immune development. Indeed, we have recently demonstrated that altering maternal gut communities only during gestation indirectly impacts breast milk and, to a lesser degree, vaginal microbiota [1] (Fig. 1). In addition, vancomycin-induced shifts in maternal gut microbiota profoundly impacted infant gut microbiota 14 days postpartum. Pups born to dams treated with vancomycin during gestation had significantly higher numbers of $CD4^+$ T and B cells compared to controls [1]. Together, our findings reveal a multi-factorial link among maternal gut microbiota during pregnancy, breast milk microbiota, infant intestinal microbiota and postnatal immune development.

Bacterial metabolites. Maternal microbiota may impact infant immunity by the action of bacterial metabolites. Gut bacteria produce numerous metabolites that are critical mediators of various host physiological functions, immune modulation and energy production [82]. The immune system senses microbial products (including metabolites) and pathogen-associated molecular patterns, and the recognition of these molecules can influence host immunity [83–85]. Short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate are end-products of bacterial anaerobic fermentation and have been shown to impact intestinal immunity [86], T_{reg} development [87], DC biology [88,89] and epithelial integrity [90]. During pregnancy, SCFAs produced by maternal gut bacteria may indirectly impact the developing fetal immunity. For example,

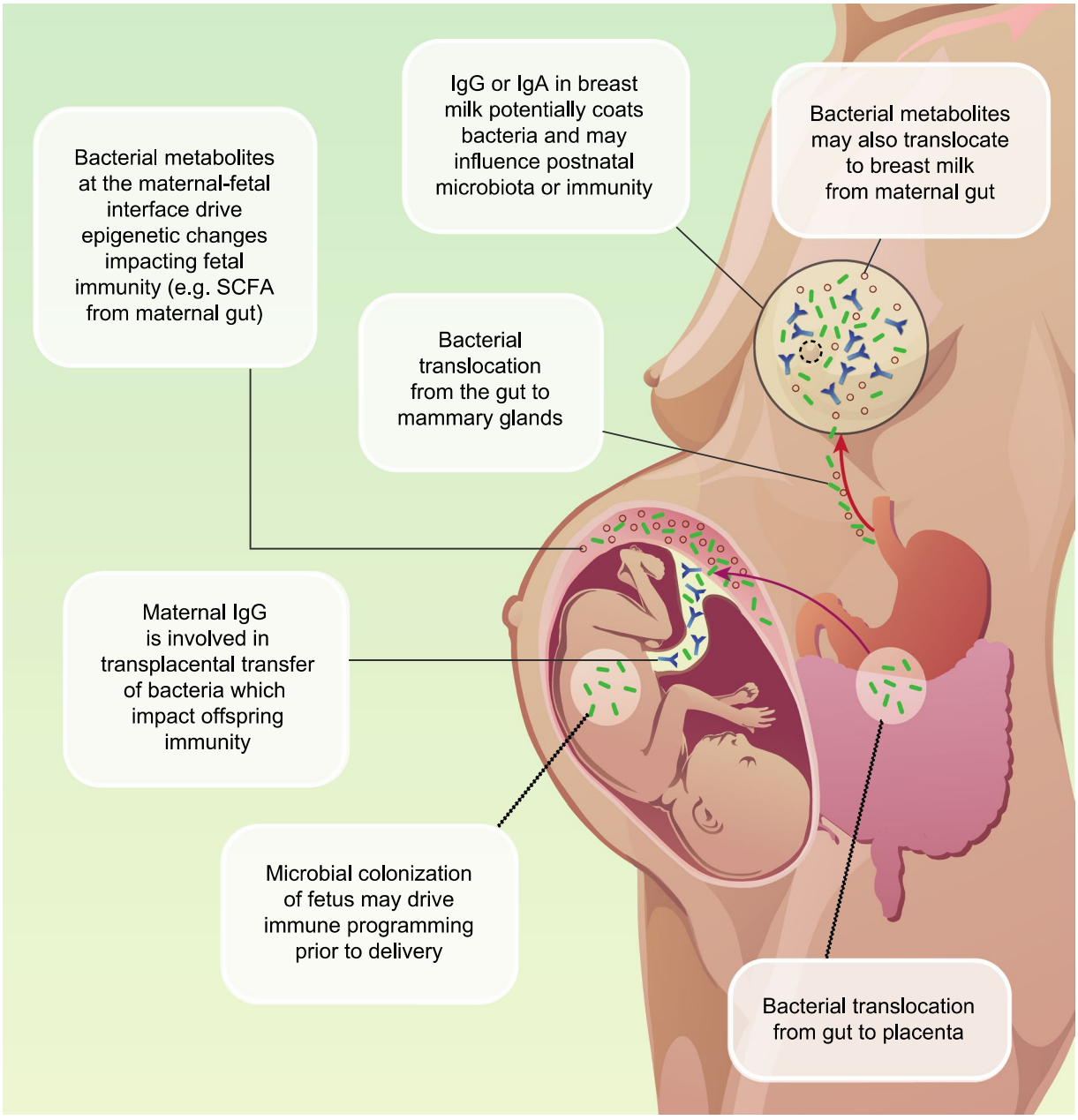

Fig. 2. Potential mechanisms of crosstalk between maternal microbiota and offspring immunity. Maternal gut microbiota during pregnancy translocate to the maternal-fetal interface. Commensals microbes translocate from the maternal gut to the placenta or fetal gut during pregnancy (maternal gut placenta axis) or to mammary glands. These microbes impact developing fetal immunity via various mechanisms including epigenetic changes, release of short chain fatty acids and alteration of the cytokine environment. Bacteria or bacterial metabolites transfer to the mammary glands (gut-breastmilk axis) impacting infant gut colonization and continued immune development after delivery.

bacterial metabolites potentially translocate from maternal gut during pregnancy to mammary glands, and could influence postnatal immune development during breastfeeding. Furthermore, a reversible maternal colonization model showed that microbial constituents such as aryl hydrocarbon ligands induce transcriptional changes in the fetal gut, enhancing cellularity of the innate immune system [12]. Similarly, maternal retinoic acid (RA) induces fetal type 3 innate lymphoid cells and therefore secondary lymphoid organ development [91]. *Clostridia* spp. in the gut can modulate RA concentration by suppressing the expression of retinol dehydrogenase 7 (Rdh7) in intestinal epithelial cells [92].

Epigenetics. In addition, metabolites or other molecules produced by the bacteria potentially impact the developing

fetal immune unit through epigenetic modulation. Thorburn *et al*. [93] observed that exposing mice to acetate in drinking water during pregnancy led to suppression of allergic airway disease in offspring by enhancing T_{reg} cell number and function. Acetate exerted its effects through increased acetylation at the forkhead box protein 3 (FoxP3) promoter, probably by histone deacetylases (HDAC)9 inhibition. In humans, maternal farm exposure has been shown to have an effect on epigenetic regulation of neonatal FoxP3 expression by impacting the T_{reg}-specific demethylated region (TSDR) conserved element [94]. The amount of demethylated TSDR was higher with any single exposure compared with that seen after no exposure, although significantly only for maternal intake of farm milk during pregnancy [95]. Michel *et al*. (2013) observed changes in DNA methylation patterns of asthma- and allergy-related genes in children. Regions in ORMDL sphingolipid biosynthesis regulator 1 (ORMDL1) and signal transducer and activator of transcription (STAT)-6 were hypomethylated in DNA from farmers' compared to non-farmers' children, while regions in RAD50 double-strand break repair protein (RAD50) and IL-13 were hypermethylated [96]. Farm exposure possibly mediates epigenetic effects through changes the gut microbiota which, in turn, alters bacterial metabolites [52,96]. Therefore, fetal exposure to maternal bacterial metabolites can impact the developing immune system via induction of epigenetic changes.

Maternal immunoglobulins. Maternal microbiota during pregnancy alters maternal immunity which ultimately impacts passive immunity to the offspring. We recently observed altered levels in breast milk IgG in mothers with vancomycin-induced alterations in intestinal microbiota during pregnancy [1]. While the transferred antibodies in milk are meant to provide immune protection in the neonate, they may also be transferring IgG-bound bacteria that could impact on the developing immune system. Indeed, the concept of IgA- or IgG-coated bacteria has previously been demonstrated in various body sites [97]. Moreover, there is evidence of transplacental IgG-mediated bacterial transfer. For example, when Gomez de Aguero *et al.* performed their aforementioned maternal transient colonization experiments [12], they tested the role of IgG in mediating the effects by transferring serum from the transiently colonized dams to unexposed pregnant mice and observed a similar effect on offspring immunity. However, when the serum was depleted of IgG prior to transfer, the impact on offspring immunity was lost. This suggest that fetal programming of immunity *in utero* is partially dependent on IgG-mediated transfer of bacterial components. Apart from IgG-mediated immunomodulation, transplacental immune regulation may also be mediated by cytokines and hormones [98], as well as bacterial components such as lipopolysaccharides [51].

Conclusions

Here, we highlight current literature on the role of maternal microbiota during pregnancy on the developing fetal and infant immunity, including the development of immune-mediated diseases such as autoimmunity and atopy. We describe potential mechanisms through which maternal gut microbes during pregnancy impact infant immunity. It is clear that immune development in the fetus begins prior to delivery and is probably driven by translocation of microbiota or their metabolites from the maternal gut to the maternal–fetal unit or other mucosal surfaces. While it is appreciated that the largest infusion of microbes occurs at delivery when the neonate comes into contact with external microbiota, data are limited on the role of vaginal microbiota during pregnancy on fetal immunity. Maternal immunoglobulins at these sites augment transfer of these components to the fetus, contributing to microbiota or immune reprogramming. Altogether, the gestational microbiota induce an immune imprint in the fetus that has lasting postnatal immunological consequences.

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