How nutrition and the maternal microbiota shape the neonatal immune system

Andrew J. Macpherson, Mercedes Gomez de Agüero and Stephanie C. Ganal-Vonarburg

Abstract | The mucosal surfaces of mammals are densely colonized with microorganisms that are commonly referred to as the commensal microbiota. It is believed that the fetus *in utero* is sterile and that colonization with microorganisms starts only after birth. Nevertheless, the unborn fetus is exposed to a multitude of metabolites that originate from the commensal microbiota of the mother that reach systemic sites of the maternal body. The intestinal microbiota is strongly personalized and influenced by environmental factors, including nutrition. Members of the maternal microbiota can metabolize dietary components, pharmaceuticals and toxins, which can subsequently be passed to the developing fetus or the breast-feeding neonate. In this Review, we discuss the complex interplay between nutrition, the maternal microbiota and ingested chemicals, and summarize their effects on immunity in the offspring.

Thalidomide
A drug that was prescribed primarily as a sedative or hypnotic. It was used to treat nausea and to cure morning sickness in pregnant women until it was discovered that it caused an absence of limbs in the offspring at birth.

From the point of view of immunologists who study mammalian systems, it is easy to forget that viviparity (that is, the birth of live offspring) has appeared independently many times in the animal kingdom during evolution[1]. This indicates the clear advantages of viviparity in protecting the developing animal in terms of guarding its nutrition, nitrogen balance, gaseous exchange and very existence. Mammals have evolved to provide protection in early postnatal life by means of nutritional and immune support through lactation. The zenith of maternal succour has been reached in the eutherian mammals. These are characterized by a placenta, which is as a highly adapted organ in which molecular exchange between mother and fetus occurs. Interestingly, placentation co-evolved with epigenetic imprinting[2], which is the DNA modification system that marks certain loci for selective expression in the offspring, depending on whether the loci have been derived from the male or female parent. The fact that this special genetic control mechanism has evolved to regulate placental function and resource allocation between a fetus and its mother indicates the delicate intimacy of having two individuals in such close contact. Despite its inevitable biological problems, placentation has permitted the rich molecular exchange between mother and fetus that has enabled the evolution of a human nervous system with advanced cognitive functions.

The mammalian fetus is supported during development by the umbilical cord[3], through which almost all nutrients, respiratory gases, excretion products and xenobiotics pass. It has been realized recently that molecules from the intestinal microbiota are also an important part of placental molecular exchange. After birth, the defining function of lactation is to support the nutrition of the newborn. However, to an extent that varies depending on the mammalian species concerned, lactation also serves as a route of immunoglobulin, macromolecular and xenobiotic uptake, and it has strong modulatory effects on the incoming microbiota of the offspring[4]. Although different toxicological, nutritional and microbial influences have been generally investigated by different disciplines[5], there is good reason to think that there are common chemical mechanisms that drive their developmental effects. Understanding intrauterine and neonatal development inevitably requires an interdisciplinary approach.

In other words, as the placental mammal develops from one cell into an organism of about 10^{13} cells and undergoes the challenges of postnatal adaptation, it is exquisitely dependent on molecular exchange of different types with its mother. The requirement for a diet sufficient in calories and balanced in micronutrients (vitamins and minerals) was established mainly from experiments with animals in the first half of the 20th century[3]. The importance of maternofetal molecular exchange is generally understood by the lay public to be crucial for healthy fetal development: pregnant women optimize their nutrition, and avoid alcohol and (after the occurrence of thalidomide-induced phocomelia) all non-essential medications.

Maurice Müller Laboratories, Departement Klinische Forschung, Universitätsklinik für Viszerale Chirurgie und Medizin Inselspital, University of Bern, Murtenstrasse 35, 3008 Bern, Switzerland.

Correspondence to A.J.M. and S.C.G.-V.
andrew.macpherson@insel.ch; stephanie.ganal@dkf.unibe.ch

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The intestinal microbiota modulates essentially all aspects of these processes, and contributes its own molecular signature to the tissues and the fetus of the pregnant mother. Indeed, the inherent microbial metagenomic diversity greatly expands the metabolic capacity of the host. Classical organic chemistry studies of selected molecules, modern wider-ranging metabolomics techniques and isotope-transfer studies all show that a diverse range of microorganism-derived molecules pervasively penetrate host tissues. In this Review, we examine the relative roles of nutrition and microbial metabolites in maternofetal molecular exchange, and discuss how these shape healthy immune system development. The evidence is mainly from mouse models because the data largely depend on our ability to experimentally manipulate diet and environmental factors, including colonization status. We also describe the processes of placentation, fetal development, postnatal lactation and immunoglobulin transfer, and we hypothesize how these processes might affect human host–microbial interactions in early life.

The fetal basis of adult disease

To emphasize the extent to which events in fetal life shape the long-term health of the offspring, we need to start with the effects of maternal nutrition on development and systemic disease[6]. Nutrition was originally shown to be a vital determinant of fetal growth and development from farm animal experiments that date from the first half of the last century[7] and from studies on humans living in the tragic conditions of malnutrition in Europe at the end of the Second World War[6].

There is abundant evidence that nutritional and other molecular events in fetal and neonatal life lay a foundation for future health that includes effects on immune system function. For example, adult abdominal adiposity (which itself predisposes to cardiovascular disease and type 2 diabetes) is associated with prior poor intrauterine fetal growth[8,9]. Lower birth weight has been linked to increased susceptibility in later life to osteoporosis, depression and prostate tumours[10–14]. This epidemiological evidence implies that the adaptation to reduced nutrition in early life has marked long-term consequences. One mechanism for this — which is discussed in more detail below — is that the fetal epigenetic reprogramming that attempts to salvage fetal growth in the presence of an inadequate macronutrient supply from the mother persists into adult life when nutritional conditions are better. Malnutrition also decreases the levels of maternal antibody that are transferred to the fetus via the placental neonatal Fc receptor (FcRn), and this has long-term effects on B cell repertoire development in the infants and their susceptibility to diseases, including allergic and autoimmune diseases[15,16].

The concept of a fetal basis of adult disease also extends to the consumption of xenobiotics. The consequences of exposure to the synthetic non-steroidal oestrogen diethylstilbestrol, which was prescribed to pregnant women with the intention of avoiding miscarriage, are a telling example of this. The effects of diethylstilbestrol on male and female infertility and reproductive tract tumours only became apparent in adult life, or even in the next-generation offspring of those who were exposed *in utero*[17]. This is an extreme example, although it should be noted that the intestinal microbiota has powerful modulatory effects on the toxicity of many environmental and pharmaceutical xenobiotics and heavy metals[18,19].

Maternal support of fetal immune development

Molecular transfer of nutrients and microbial molecules during early life immune development. The developing immune system of the fetus is shaped markedly by the *in utero* environment. Intrauterine growth retardation is strongly associated with susceptibility to postnatal infectious disease, and the effects are seen both in children and in young adults. *In utero* malnutrition impairs immune function through both direct and indirect mechanisms (FIG. 1). For example, a loss of macronutrients and micronutrients directly affects leukocyte development in the fetus. In addition, maternal malnutrition leads to stress responses in the mother and fetus that directly affect placental function and fetal immune development. Malnutrition also leads to maternal immunosuppression, which decreases the availability of maternal immunoglobulins for uptake into the fetus, and renders the mother susceptible to opportunistic or manifest infections. Any increase in systemic exposure to microorganisms will further increase the maternal stress responses that are mediated by the hypothalamic–pituitary–adrenal axis (HPA axis)[20] (FIG. 1).

There are several examples that illustrate how the direct limitation of *in utero* nutrient levels can affect immunity in neonates. An insufficient supply of vitamin A results in impaired fetal differentiation of B lineage cells, including the B1a and B1b innate-like B cell subsets, which express antibodies that are responsible for the early phase of protection against pathogens[21]. In addition, maternal retinoids influence the development of fetal lymphoid tissue inducer (LTi) cells and subsequently the size of secondary lymphoid organs in the offspring[22]. Retinoic acid is also required for thymic development and myeloid cell differentiation. As a final example, a deficient zinc supply limits the size of the thymus and spleen, and is associated with deficient B cell and T cell function, partly because of stress-induced corticosteroids[23].

Stress responses that are mediated by the HPA axis can be generated either directly because of protein calorie malnutrition or indirectly, as malnutrition leads to immunosuppression, poor intestinal barrier function and increased susceptibility to infection (FIG. 1). In a healthy pregnancy, the fetus is protected from maternal glucocorticoid exposure because of the activity of placental 11β-dehydroxylase, which converts glucocorticoids into non-active metabolites[24], but malnutrition results in reduced placental dehydroxylase levels. Despite this, the effects of maternally delivered glucocorticoids (which suppress the fetal HPA) are much less than are the effects of direct stimulation and activation of the fetal HPA by corticosteroids present within the fetus. This has

Hypothalamic–pituitary–adrenal axis
(HPA axis). One of the major neuroendocrine systems that controls, among other things, reactions to stress, the immune system, digestion and emotions. It consists of a complex set of feedforward and feedback mechanisms between the hypothalamus, the pituitary gland and the adrenal cortex. Neuroendocrine neurons in the hypothalamus produce corticotropin-releasing factor, which acts on the anterior pituitary gland to induce the production of adrenocorticotropic hormone (ACTH). ACTH induces the adrenal gland to release glucocorticoids, such as cortisol.

been shown experimentally using the administration of lipopolysaccharide (LPS) or CpG oligodeoxynucleotides to mimic the effects of bacterial or viral infections, respectively[16]. Such manipulations have clear lasting effects on many aspects of immunity in the offspring, including reduced levels of lymphocyte proliferation, lower antibody responsiveness and reduced natural killer cell activity. There is also a long-lasting effect on HPA activity, with the offspring showing increased stress responses in later life (reviewed in REF. 20). These effects are probably due to epigenetic programming, which is discussed further below.

Therefore, it is clear that maternal nutrition is vital for the healthy development of the infant immune system. This prompts the following question: to what extent does the microbiota (which is well known to increase the efficiency of energy extraction from food[25], provide vitamins and metabolize xenobiotics[26]) contribute to the general nutritional state in pregnancy? Of course, it should in addition be pointed out that the uptake of foods, vitamins and xenobiotics will also affect the maternal microbiota[27–29] and secondarily the microbiota of the offspring[30].

Effects of intestinal microorganisms on maternal nutrition. Intestinal microorganisms can increase the energy harvest from the diet by digesting complex carbohydrates (such as the constituents of plant cell walls), which are resistant to mammalian enzymes[25]. Clearly, the microbiota cannot itself serve as a substitute for a healthy diet, as there is insufficient energy to salvage when the diet is inadequate. However, microbial metabolic capability is required for vitamin synthesis, and to generate the short-chain fatty acids that sustain epithelial integrity[31] and regulatory T cell development in the mother[32–34]. The evidence for this comes from specific supplementation studies and from studying germ-free animals, which require fortified diets. The requirement for microbial metabolic capacity is particularly true for vitamin K: if animals that are raised in germ-free conditions are not supplemented with this vitamin, the synthesis of host clotting factors is impaired, which leads to a bleeding diathesis. B-group vitamins, including folate (also known as vitamin B_9) and vitamin B_{12}, are also synthesized by the microbiota but not by the host. The synthesis of these vitamins occurs mostly in the large intestine, in which uptake into the host is more restricted than in the small intestine[35].

As the maintenance of the germ-free status depends on rigorous sterilization of food, normally through prolonged autoclaving of chow, the diets administered to germ-free mice are generally heavily fortified with vitamins to avoid potential micronutrient deficiencies in the animals. Therefore, determining exactly where the boundaries of the microbial contribution to the maternal nutritional state lie requires chemically defined, rigorously sterilized diets. This is not as straightforward as it sounds because the doses of irradiation that are used to sterilize chemically defined diets also reduce the vitamin availability of the diet through radiochemical effects[36].

Coprophagia
The eating of faeces, which is a normal behaviour in many animals.

The proportion of micronutrients that is derived from the microbiota in mice depends on the extent of coprophagia, which is seen only in psychiatric cases in humans. Nevertheless, although most of the vitamins that are newly synthesized will be shed in the faeces, there is evidence, even in humans, that some vitamin B_{12} and folate are derived from microbial sources[37,38]. Pregnant mothers are widely supplemented with folate in developed countries to avoid facial clefting, and neural tube and cardiac defects. Folate and vitamin B_{12} are important as the catalysts for methylation reactions in epigenetic marking, the relevance of which to immune system development is discussed further below.

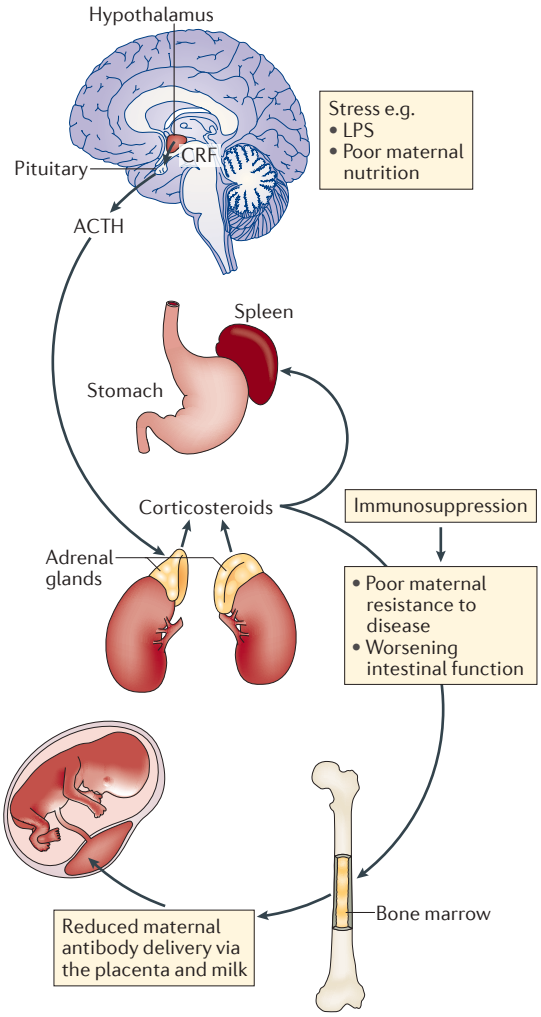


Figure 1 | **Maternal stress weakens maternal and offspring immunity.** Maternal stressors, such as exposure to lipopolysaccharide (LPS) or malnutrition, induce the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH triggers the release of corticosteroids from the adrenal glands, leading to suppression of the maternal immune system. In addition to increasing the susceptibility of a pregnant woman to infections, such stress-induced immunosuppression lowers the rate of immunoglobulin production in the bone marrow, resulting in the less efficient transfer of immunoglobulins to the offspring via the placenta and breast milk. CRF, corticotropin-releasing factor.

Haemochorial placenta
A type of placenta present in humans and some rodents, in which maternal blood is in direct contact with the chorion.

Allantois
A bag-like structure that forms part of the developing conceptus and that has a role in nutrition and excretion.

Given that intestinal motility is reduced in pregnancy owing to the secretion of progestogens, one cannot safely extrapolate physiological studies in non-pregnant individuals: nevertheless, it is likely that the microbial biomass of the lower small intestine increases in pregnancy[39,40], and the rate of molecular exchange of micronutrients between the microbiota and the host probably also increases.

Development of the maternofetal interface

The basis of maternofetal contact and the organ for molecular exchange is the placenta. The timelines of the development of this interface and of the different phases of embryogenesis are considered here in terms of maternofetal molecular transfer and fetal immune development.

Both mice and humans have a haemochorial placenta because, during the process of decidualization, the fetal trophoblast invades both the uterine epithelium and the endothelium of the maternal vessels, and so the syncytial cellular outer trophoblast layer is directly bathed in maternal blood (FIG. 2a). The outer trophoblast structure develops as a result of the trophectoderm becoming vascularized via the mesoderm of the allantois. The allantois emerges from the posterior end of the embryo, forming a vascular labyrinth as the trophoblast invades the mouse maternal decidua at embryonic day 12.5 (E12.5)[41] (FIG. 2a).

As the placenta is formed, the embryonic immune system is in a phase of stem cell formation in the splanchnopleuric mesenchyme that surrounds the developing heart, an area known as the aorta–gonad–mesonephros (AGM). In the mouse at around E10.5, the phase of tissue migration and lineage progenitor expansion starts with lymphopoiesis in the fetal liver and the thymus (FIG. 2b). This is aligned with the vascularization of the trophoblast and decidualization, yet the effects of maternally derived molecules on the developing fetus can occur even without this intimate contact when present in sufficiently high concentrations, as is evident from the teratogenic effects of a short-lived high dose of alcohol at E7 in mice[42]. Nevertheless, pharmacological studies of placental drug transfer indicate that there is greater fetal exposure to a standardized dose at later points in gestation[43]. This occurrence is inextricably linked to the fetal

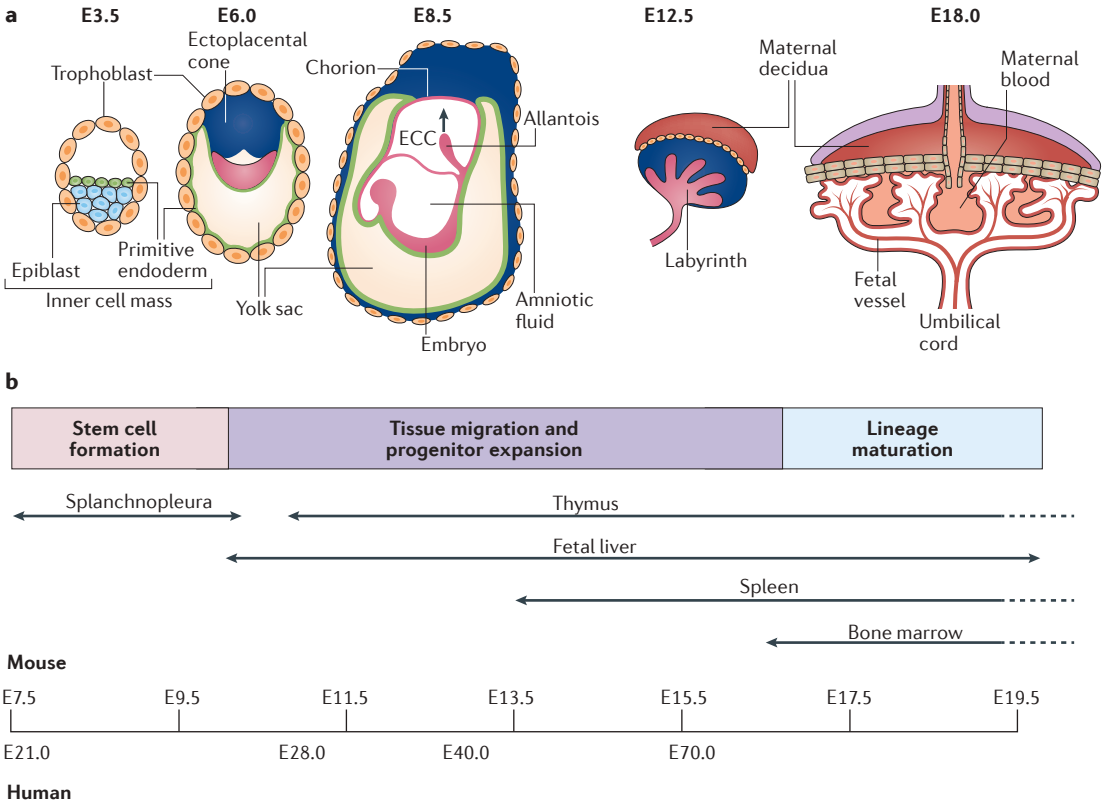


Figure 2 | **Placental development and fetal haematopoiesis. a** | A schematic view of mouse placental development showing the main stages, which correspond to embryonic day 3.5 (E3.5), E6.0, E8.5, E12.5 and E18.0. The development of the placenta starts upon implantation of the blastocyst into the maternal endometrium. The outer layer of the blastocyst becomes the trophoblast, which later forms the outer layer of the placenta. The maternal endometrium contributes the maternal decidua of the placenta, which is accommodated by maternal spiral arteries that ensure the blood supply of the placenta. Fetal umbilical arteries form a labyrinth while invading the placenta to form an arterio–capillary–venous system. **b** | A timeline of mouse and human haematopoiesis during embryonic and fetal development. Dashed lines indicate processes that are ongoing. ECC, extracoelomic cavity.

Box 1 | **Epigenetic environmental influences on the developing fetus**

As has been shown for imprinting, epigenetics has a marked effect on the developing fetus. Upon fertilization, the sperm-specific and egg-specific gene expression patterns and therefore epigenetic marks need to be erased in order to generate pluripotent progenitor cells. However, some maternal and paternal epigenetic marks (at imprinted genes) survive the genome-wide demethylation that occurs in embryonic somatic cells at the blastocyst stage. This leaves imprint control centres that organize appropriate differential gene expression patterns in the developing embryo (see FIG. 4). It also leaves non-epigenetically modified genetic territory in which epigenetic controls can be established to determine the proliferative and differentiation fate of pluripotent cells. By contrast, the parental imprints in primordial germ-line cells are erased and re-established in a gender-specific manner during gamete development.

The epigenetic processes are subject to external influences. The classical example of this is the brown Agouti (*A*/*A*) mouse, in which the coat colour of A^{vy}/a pups born to an *a*/*a* (black) mother can be determined by methylation status of the retrotransposon regulatory region in the A^{vy} allele (which encodes viable yellow agouti). This methylation can be manipulated by supplementation with the vitamins B_{12} and folate[70]; as described in the main text, *de novo* synthesis by members of the microbiota contributes to body stores of these dietary micronutrients, even without coprophagia. Other known epigenetic modifiers are cigarette smoke (which, in addition to other effects, results in the hypomethylation of the gene that encodes aryl hydrocarbon receptor repressor[71]) and alcohol (which, in addition to critical effects on the development of the central nervous system, also causes deficient lung development and defects in pulmonary innate immunity[72]).

The developing immune system requires phases of progenitor cell expansion and tissue migration, and later, lineage establishment and maturation. Epigenetic controls that are established through the different processes of methylation, histone modification and non-coding RNA expression are best described for the lineage differentiation of different T cell subsets in the adult animal[73]. The processes that guide the differentiation of haematopoietic stem cells and that characterize the lineage progenitors are emerging[74–76]. It is reasonable to assume that the pervasive maternal gestational effect from the microbiota will also have an epigenetic basis because of its durability and the environmental precedents cited above. The details of these mechanisms are still under investigation.

demands for oxygen and nutrients for growth: measurements with radioactive compounds have shown that both facilitated and diffusive exchange of metabolites increase between E16 and E19 (REF. 44), which is when the trophoblast area expands and the interhaemal distance is reduced.

The relationship between the fetus and the mother cannot be entirely mutualistic. There must be resource transfer from the mother to the fetus for it to develop. Even in times of famine, precious resources that are diverted from the mother to the fetus may also be consumed by the trophoblast. This equilibrium is highly regulated by epigenetic imprinting, which co-evolved with placentation. Epigenetic imprinting results in placental development and increased placental size being driven by paternally expressed alleles in the fetus (for example, an allele of the gene that encodes insulin-like growth factor 2 (IGF2)) and counterbalanced by genes expressed from maternal alleles (for example, an allele of the gene that encodes IGF type 2 receptor that targets the protein for degradation)[45]. Remarkably, most sex-specific epigenetic imprinting affects genes that are expressed in the placenta or the brain; imprinting therefore not only determines resource allocation between the fetus and mother, but also influences the maternal endocrine responses and behaviour that are necessary for successful nursing of the offspring[46]. The potential susceptibility of imprinting to external influences in humans was shown in a remarkable study of babies that were born from pregnancies in Holland during the famine of the 1944–1945 winter[47]. Even six decades later, individuals born during the famine showed reduced DNA methylation at the *IGF2* locus, possibly reflecting their demand for nutrition in fetal life, compared with unexposed siblings of the same gender[47] (also see BOX 1).

Although epigenetic imprinting is not known to have major direct effects on immune system development in vertebrates[48], it has important indirect effects, as it regulates the passage of maternal antibodies through the placental interface. Such antibodies transmit maternal immune memory of pathogenic[15] and non-pathogenic members of the microbiota to the neonate[49]. The timing of antibody transmission from the mother to her offspring is variable between different species, but in general it is a transgenerational effect that is very sensitive to adequate nutrition because the physiological expense of the immune response competes with the requirement for nutrients for maternal metabolism and fetal growth[50]. Maternal antibodies can be taken up into the circulation and tissues of the offspring to transfer immune memory, which may neutralize pathogens or components of the microbiota[15]. These antibodies are capable of limiting infectious disease during the period of neonatal immune immaturity and can shape the development of endogenous antibody repertoires in the offspring during early life[16].

Exposure of the infant to the maternal microbiota

Direct* in utero *exposure to live organisms of the maternal microbiota. Although the biomass of the host and of its microbiota are rather well separated, this separation is not absolute, and very small numbers of live microbial organisms can be detected in the systemic organ systems of the host, in both animal models and in humans[51,52]. During late pregnancy and in the immediate postnatal period, the translocation

Box 2 | **The effects of the maternal microbiota**

Exposure of the trophoblast and placenta to live microorganisms
Although the placenta is generally believed to be a sterile site, recent literature indicates a possible low-grade colonization of placental tissue with commensal bacteria[56,77]. However, colonization was estimated to be at low levels, and sampling of the human placenta without contamination is still a technical challenge.

Transfer of microbial endobiotics
Metabolites or fragments of the maternal endogenous microbiota can reach the maternal serum and systemic sites, and can thus be transferred to the unborn fetus via the placenta or to the newborn child through maternal milk[60].

Nutrition
Dietary components in the maternal intestine can be further metabolized by members of the intestinal microbiota, and the resulting molecular products can be absorbed into the mother's body and subsequently transferred to her offspring. In addition, the maternal diet can influence the composition or transcriptional state of the maternal intestinal microbiota itself[25,28–30,33,34].

Xenobiotics
As with dietary components, several members of the human intestinal microbiota have the potential to metabolize xenobiotic chemicals that originate from plant or pharmaceutical sources in the maternal gut, and thereby alter the chemical exposure of the fetus[26].

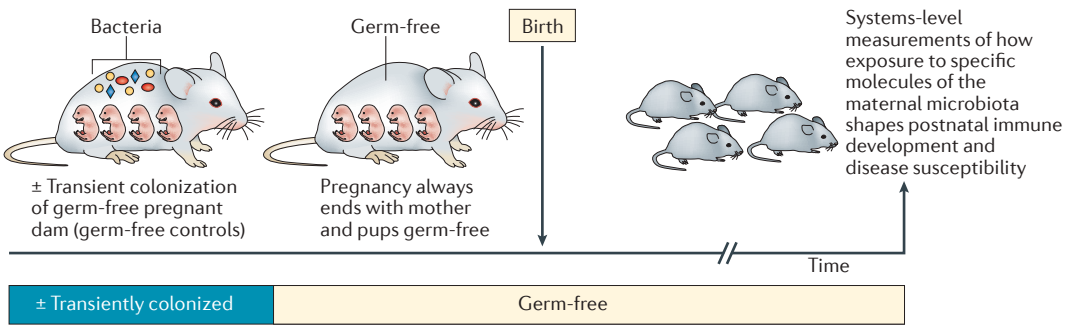
of intestinal and oral microorganisms is increased in both laboratory rodents and in humans[53–55]. This results in microorganisms being present in the placenta and in the milk[53,56]. At least for placental and fetal tissues, microbial numbers must be maintained at extremely low levels, otherwise the pre-term birth or stillbirth complications of intrauterine infection will ensue[57]. Nevertheless, there is evidence that these organisms contribute to the early colonization of the postnatal infant[53]; they may also have a role in supporting the developing fetal immune system *in utero*, although the extent of this is uncertain (BOX 2).

Exposure of the fetus and neonate to penetrant maternal microbial molecules. In experimental mice, one can show that the development of the immune system in the fetus is driven by maternal microbial molecules independently of the actual penetration of live microorganisms into maternal or conceptus tissues. For the most part, the effects of the microbiota on immune system development (as well as its effects on other organ systems) have been determined by comparing colonized and germ-free animals[58].

As germ-free animals are born to a germ-free dam and colonized animals are born to a colonized dam, comparing these two hygiene statuses does not provide information on the effects of the maternal microbiota on the development of the immune system of her pups. However, the effect of the maternal microbiota alone can be addressed by treating germ-free pregnant female mice with live *Escherichia coli* that lacks the synthetic pathways for the essential bacterial amino acids D-alanine and meso-diaminopimelic acid[59]. This mutant strain of *E. coli* can be grown in culture when D-alanine and meso-diaminopimelic acid are provided as supplements, but it does not permanently colonize the mouse intestine, as D-alanine and meso-diaminopimelic acid are not synthesized or available in the host to support bacterial replication in the intestinal luminal environment. The major advantage of this system is that the pregnant dam returns to a germ-free status before the delivery of her pups, and so the pups themselves are germ-free.

Box 3 | **An experimental model of transient gestational colonization in germ-free mice**

In this model, timed-pregnant germ-free mice are gavaged with 10^{10} colony-forming units of *Escherichia coli* HA107 between embryonic day 5 (E5) and E16, or are kept germ-free throughout pregnancy (see the figure). *E. coli* HA107 is an auxotrophic strain that harbours mutations in molecules that are involved in the pathways that synthesize meso-diaminopimelic acid and D-alanine. It can thus only survive in supplemented culture and colonizes a germ-free mouse intestine transiently for 24–72 hours. The pregnant dams return to a germ-free status before delivering their germ-free pups, which can subsequently be analysed for the effect of maternal gestational colonization on offspring immunity and disease susceptibility. To date, all experiments exploring the effects of gestational colonization on the development of the neonatal immune system have used the reversibly colonizing commensal bacterium *E. coli* HA107. Although *E. coli* is only a rare member of the intestinal microbiota in mice, it is present within the human intestine and is particularly abundant in early life[78,79]. By comparison, in pups born to diversely colonized, specific pathogen-free (SPF) mice or mice colonized with the altered Schaedler flora (a model microbiota comprising eight bacterial species), the immune system was very similar to that of germ-free pups that were born to mothers that had been gestationally colonized with *E. coli* HA107. This further indicates that colonization with *E. coli* HA107 during pregnancy can recapitulate most phenotypes that are observed in mice that were colonized with a diverse microbiota.



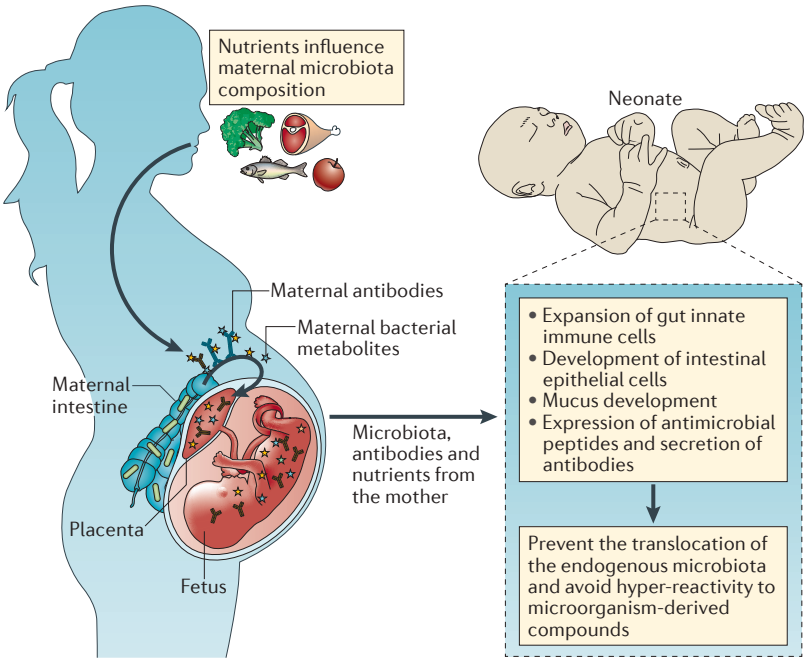


Figure 3 | **A schematic of the effects of the maternal microbiota and maternal nutrition on immune development in the offspring.** Molecular signals originating from the maternal microbiota during pregnancy can reach the offspring during *in utero* development via the placenta, and after birth through maternal milk. These signals contribute to immune development in the offspring, and have effects on processes including the expansion of innate immune populations in the intestine, the development of intestinal epithelial cells, mucus development, the expression of antimicrobial peptides and the secretion of antibodies into the intestinal lumen. Many of these effects are dependent on maternal antibodies. In addition, metabolites that originate directly from the maternal diet, or that originate from the maternal diet and are further processed by the microbiota, can be transferred to the offspring and may potentially alter its immunity.

Thus, one can compare the immune responses in the pups born to the transiently colonized mothers with those of the pups born to a control germ-free mother (BOX 3).

The results from experiments using this approach show that, compared with control germ-free pups, germ-free pups born to transiently colonized dams harbour increased numbers of class 3 innate lymphoid cells (ILC3s) in the small intestine and have increased numbers of intestinal mononuclear cells that express CD11c and F4/80, in both the small and large intestine[50]. These effects are durable, lasting from approximately postnatal day 5 until at least day 60. The effects are not limited to leukocyte populations, as intestinal mucosal transcriptional signatures (predominantly those of epithelial cells) are substantially reshaped in pups born to gestationally colonized dams[60]. In particular, intestinal tissues from pups show increased expression of antibacterial peptides (including C-type lectins of the REG family and defensins); increased cell division and differentiation of epithelial cells; increased production of mucus and expression of ion channels; increased metabolism of dietary xenobiotics, bile acids and complex lipids; and changes in sugar metabolism[60]. There is also increased expression of polymeric immunoglobulin receptor (pIgR), which is responsible for transporting IgA through the epithelial layer into the intestinal lumen[60]. Further experiments showed that many of these effects are dependent on the expression of antibodies by the transiently colonized mother, which implies that antibody transfer to the fetus and neonate augments the gestational colonization effect[60] (FIG. 3).

As the transiently colonizing *E. coli* strain is unable to replicate *in vivo*, it is possible to carry out isotope flux experiments using metabolically labelled bacteria. These experiments have shown that there is substantial transfer of metabolites from the intestinal bacteria into the mother, across the placenta and into the fetus[50]. As bacterial metabolites that are transferred into maternal tissues have a long dwell time, the uptake of maternally derived bacterial metabolites by the offspring continues during breastfeeding. Examples of maternal microbiota-derived metabolites that reach the offspring include aryl hydrocarbon receptor (AHR) ligands, fatty acids and retinoid compounds. These metabolites have been shown previously to have an impact on the development of the immune system[22,60]. There are therefore likely to be multiple molecules that are driving different effects of maternal gestational colonization on the offspring, but one group that is transferred without secondary metabolism comprises ligands for AHR. The administration of an authentic AHR ligand (indole-3-carbinol) to pregnant female mice is sufficient to recapitulate the effect of maternal intestinal bacteria on fetal ILC3 populations[60].

Such effects are likely to be important in preparing the neonatal mammal for its own microbial colonization. Experiments have shown that gestational colonization effects prepare pups to have a more intact intestinal barrier and blunt the responsiveness of their splenocytes to systemic challenge with LPS[60]. Clearly, such experiments would be unethical and technically unfeasible in humans, but they provide insights into possible ways in which the maternal microbiota may shape the fetal immune system in humans. Although mice continue to take up IgG from the maternal milk through the neonatal intestine until about postnatal day 12 (REF. 61), human IgG uptake is essentially completed via placental transfer at birth. However, most human transplacental IgG uptake occurs in the last month of pregnancy[62]. One may speculate that the immaturity of intestinal function that is seen in babies that are born pre-term (for example, their increased susceptibility to inflammatory necrotizing enterocolitis as a result of enteral feeding[63], and the relative protection afforded by feeding pre-term infants human colostrum[64], which contains IgG) can be explained, at least in part, by altered exposure of the neonate to maternal microbial components.

Postnatal lactation and the neonatal microbiota

Earlier in this Review, we described the transgenerational transfer of immune memory from mother to offspring that results from the transmission of systemic antibodies (mainly IgG) that can limit systemic infections. Whereas this is largely transplacental and antenatal in humans, in some species, such as ungulates, this happens postnatally through the colostrum.

Mice are in an intermediate position, and have both prenatal and postnatal phases of maternal antibody transfer.

In addition to containing antibodies that are specific for potential pathogens, milk contains antibodies that are induced by the maternal microbiota. These antibodies mainly comprise secretory IgA (sIgA) and sIgM, but also include IgG isotypes in mice, and they can protect the immature mucosal surfaces of the offspring. The evidence for this originates from heterozygous breeding studies using *scid*/+ mice, which are heterozygous for a severe combined immune deficiency (SCID)-associated mutation in *Prkdc* (which encodes DNA-dependent protein kinase catalytic subunit). When these mice were born to and nursed by *scid/scid* dams (mated with wild-type males), the heterozygous offspring induced endogenous mucosal sIgA early, at approximately postnatal day 15, when the maternal milk contained no antibody. By contrast, when *scid*/+ pups were born to and nursed by wild-type dams (mated with *scid/scid* sires), endogenous mucosal sIgA induction was delayed until after weaning[65]. This work was later developed in the $J_H^{-/-}$ strain, which is selectively deficient for antibodies owing to a lack of the gene that encodes the joining region of the immunoglobulin heavy chain (J_H), to confirm that the milk-borne antibodies protected the offspring against the intestinal microbiota. This was shown to be a mucosal protection effect because parenteral replacement of the early phase of IgG did not affect the early induction of endogenous IgA when the maternal milk lacked antibodies, and maternal milk antibodies prevented microorganisms from translocating to the mesenteric lymph nodes in the pups[66].

This heterozygous breeding approach has been taken further in experiments using pIgR-deficient mice, in which pIgR-knockout males were crossed with pIgR-heterozygous females, or pIgR-heterozygous males were crossed with pIgR-knockout females. In both cases, heterozygous and homozygous pups were obtained from each cross. In the cases in which the dam was pIgR-deficient, there was no IgA or IgM antibody secretion into the milk[67]. Regardless of whether the pup was able to secrete endogenous mucosal antibodies or not, maternal antibody was shown to protect the pup from developing an inflammatory transcriptional signature in the intestinal mucosa[67]. Furthermore, maternal antibodies differentially shaped the composition of the endogenous microbiota in the pup, with one notable effect being a long-term reduction in the proportion of Proteobacteria[67]. In mice, IgG is transferred from the mother to her offspring via FcRn, both across the placenta and via the neonatal duodenum. Experiments combining various strains of mice that are deficient for FcRn or selective antibody isotypes showed that the postnatal uptake of maternal T-independent IgG2b and IgG3 antibodies limits the development of neonatal T follicular helper cell and germinal centre responses against intestinal microorganisms[49]. This suggests that maternal antibodies are important for promoting mutualism with the incoming intestinal microbiota in the neonate.

Therefore, although other components of maternal milk (including lactalbumin, lysozyme, lactoferrin and lactoperoxidase) have antimicrobial effects, secreted milk antibodies protect the neonatal intestinal mucosal surface, as they limit the penetration of incoming intestinal microorganisms into systemic tissues, and restrict B cell and inflammatory responses in the neonatal mucosa itself. This results in a less-inflammatory composition of the microbial consortium that stably colonizes the mouse intestine in early life. This protective effect of secreted milk antibodies is independent of transgenerational immunoglobulin uptake in the neonatal intestine. In developing countries, severe protein-energy malnutrition, which is characterized by dysbiosis, small intestinal enteropathy and growth stunting, can supervene at the time of weaning[68,69]. A mammalian mother is preparing her offspring through the direct effects of nutrition and of the passage of immune memory, as well as the indirect effects of antibody-augmented molecular exchange, to stabilize the earliest stages of host–microbial mutualism.

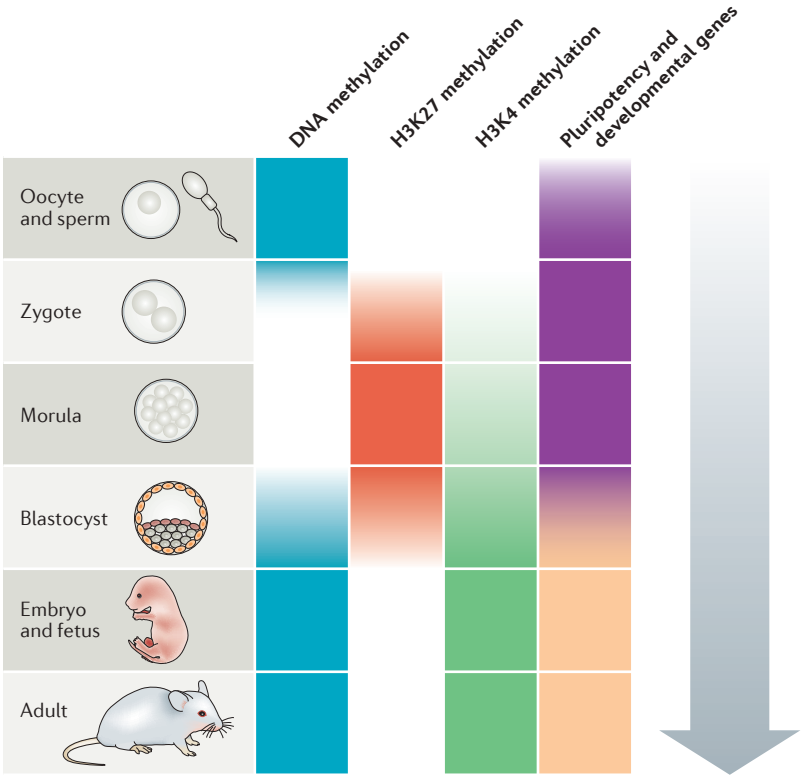


Figure 4 | **Epigenetic reprogramming during mammalian development.** Key developmental steps are shown in relation to epigenetic modifications and gene expression patterns (the intensity of the coloured shading indicates the level of methylation or gene expression). Soon after the fertilization of the oocyte, DNA methylation is erased, enabling the expression of genes that are associated with pluripotency. Developmental genes that are required for the differentiation of specific cell types are still repressed by methylation of Lys27 of histone H3 (H3K27). As development proceeds, the genes associated with pluripotency, and paternal or maternal imprinted genes, need to be silenced permanently by DNA methylation. At the same time, an increase in H3K4 methylation and a reduction in H3K27 methylation enable the expression of developmental genes.

Epilogue

In this Review, we have described how the intestinal microbiota and nutritional status of the mother interact

in a number of distinct ways during pregnancy and in the early life of the offspring. In addition to increasing the all-important energy yield, the maternal microbiota assists with micronutrient provision and xenobiotic metabolism. Furthermore, components of the maternal microbiota can reach the fetus via the placenta and the milk, and the transfer of these components to the fetus is augmented by maternal antibody. Intricate experimental studies have shown that maternal microbial components have a wide range of both immune and non-immune effects on the fetus; the durability of these effects and the established effects of known environmental xenobiotics suggest that underlying epigenetic alterations are likely to be involved.

Both gestational colonization-driven reprogramming of the neonate, as well as direct secretory antibody-mediated protection, prepare the immunologically immature intestinal mucosa for its own 'tsunami' of colonization by endogenous intestinal microorganisms and help to ensure that a healthy consortium — that will protect the offspring from diseases throughout life — is formed.

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The authors declare no competing interests.