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The immune system and microbiome in pregnancy

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ABSTRACT

Hormonal changes during pregnancy instigate numerous physiological changes aimed at the growth and delivery of a healthy baby. A careful balance between immunological tolerance against fetal antigens and immunity against infectious agents needs to be maintained. A three-way interaction between pregnancy hormones, the immune system and our microbiota is now emerging. Recent evidence suggests that microbial alterations seen during pregnancy may help maintain homeostasis and aid the required physiological changes occurring in pregnancy. However, these same immunological and microbial alterations may also make women more vulnerable during pregnancy and the post-partum period, especially regarding immunological and infectious diseases. Thus, a further understanding of the host-microbial interactions taking place during pregnancy may improve identification of populations at risk for adverse pregnancy outcomes.

Introduction

During pregnancy, the female body undergoes numerous anatomical and physiological changes to allow the successful implantation of a fertilized egg, the growth of an MHC mismatched fetus and timely parturition. Most of these changes are transient, and will revert to 'normal' after delivery. The basis for all these physiological changes lies in rises in hormone levels that take place upon conception. Implantation of a fertilized egg results in the production of human chorionic gonadotrophin (hCG) by placental trophoblasts. Systemic levels of hCG steadily increase to reach peak levels around week 10 of gestation, and drop to baseline values shortly thereafter [1]. hCG initiates the production of progesterone by cells of the corpus luteum, a temporary endocrine structure that remains in the ovary after ovulation. The corpus luteum also produces estrogen, which in turn contributes to progesterone biosynthesis. Production of both progesterone and estrogen are taken over by the growing placenta after several weeks of pregnancy, and their levels thus rise progressively during the first trimester (T1) and reach their peak in the third trimester (T3) of gestation (Fig. 1A).

While the main purpose of hCG has long been considered to be the stimulation of progesterone production, numerous processes are now known to be dependent on hCG activity. In particular, promotion of placental growth and vascularisation appear to be a significant part of hCG functionality. Furthermore, hCG stimulates fetal organ growth and differentiation and prevents premature uterine muscle contraction [2]. Progesterone plays a role in early pregnancy by inducing differentiation of stromal cells into decidual cells, and helps reshape the cervix to adapt it to fetal implantation. Furthermore, progesterone sustains pregnancy by prohibiting uterine contractions through reduction of oxytocin and prostaglandin receptor production, and inhibition of contractibility of smooth muscle cells [3,4]. Estrogens, while not stimulating contraction, do appear to facilitate processes leading up to parturition [5]. Other important functions of estrogens include placental neovascularisation and preparation of breast tissue for lactation [6,7]. In addition to the gross anatomical and physiological alterations initiated during pregnancy, a pivotal role for pregnancy hormones in the modulation of immunological processes throughout pregnancy is emerging, which is essential for the allogenic fetus to grow and thrive.

How to deal with fetal alloantigens

Upon fertilization of an egg, half of the DNA of the ensuing fusion product is from paternal origin. Thus, the implanted embryo

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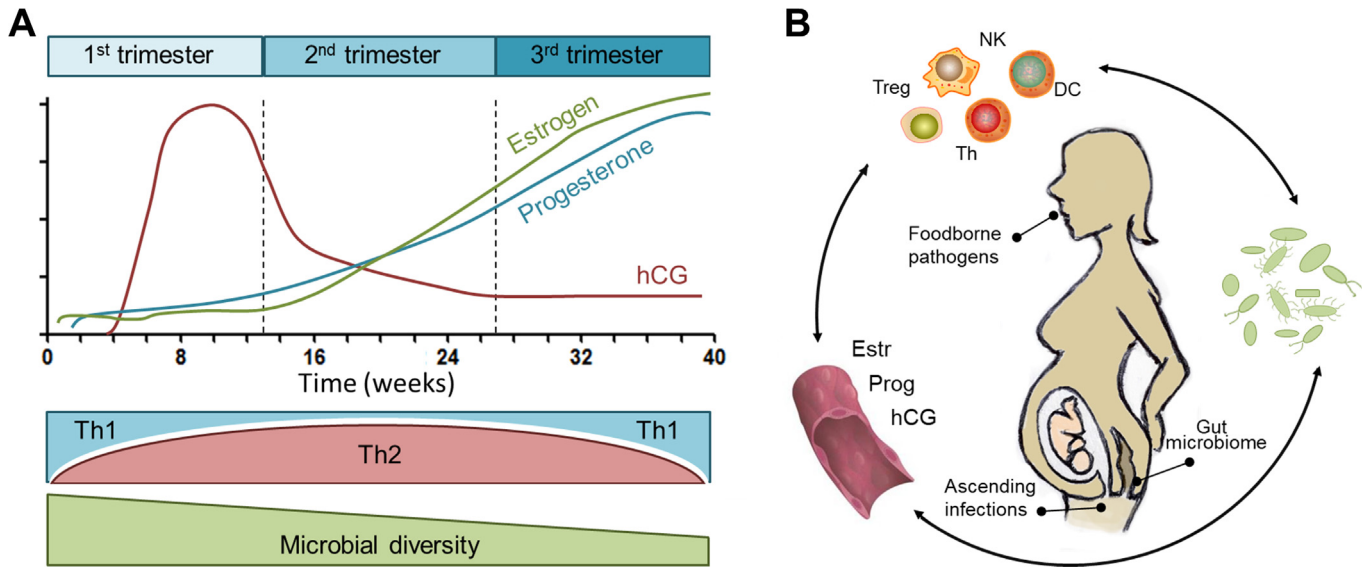

Fig. 1. Schematic representation of the hormonal, immunological and microbial changes during pregnancy. (A) Levels of human chorionic gonadotropin (hCG) rise early in pregnancy, with progesterone and estrogen levels following suit. Immunological changes occur at the placenta, and may extend to the periphery. While inflammatory responses are required during implantation and decidualization (characterized by mostly Th1 cytokine patterns), most of gestation is characterized by a reduced inflammation (shift towards Th2 patterns). Parturition again requires inflammatory signals. During pregnancy, fecal and vaginal microbiomes changes, with a reduced diversity at later trimesters one of the most consistent findings to date. (B) Pregnancy can be threatened by bacterial infections. The most common routes for these bacteria to reach the placenta are through ascending infections (vaginal microbiota) or hematogenous dissemination of food-borne or intestinal microbes. Pregnancy hormones play an important role in shaping immunity during pregnancy, which in turn can have an effect on microbial composition. In turn, microbiome may also be directly modulated by pregnancy hormones and diet, and *vice versa* may exert their effect on immunological parameters as well as hormone levels.

as well as the fetal placenta express paternal/fetal antigens which can be potentially recognized by the maternal immune system, but are nevertheless tolerated. While the fetal-maternal interface which develops during pregnancy separates the maternal and fetal blood streams, not only nutrients, oxygen and metabolic waste products cross the placental interface from fetus to mother and *vice versa*. Already in the early sixties it was noted that small numbers of leukocytes and platelets were able to cross the placenta from mother to fetus [8]. In the decade after, fetal cells were also observed in the blood of pregnant women, showing the bi-directionality of cellular transport [9]. This poses the question as to how the maternal immune system deals with such a challenge: fetal antigens and fetal MHC molecules on which they are presented will not be recognized as 'self' by the mother's immune system. This in turn means that maternal immunity against the fetus may cause harm to the developing infant. Case in point is rhesus alloreactivity, where a mother who does not possess the red blood cell-expressed rhesus D-antigen (Rh) may develop antibodies against Rh when pregnant with a Rh + child. This may have disastrous consequences in a subsequent Rh + pregnancy, due to destruction of fetal red blood cells by maternal antibodies crossing the placenta [10]. Nevertheless, in most cases, pregnancies are not ended by maternal immunological responses, and already in the early 50's Sir Peter Medawar proposed that pregnancy is accompanied by some form of immune-tolerance in order to protect the fetus against the maternal alloresponse [11].

Implantation vs transplantation

In the search for immunological parameters explaining fetal tolerance in pregnancy, implantation of an allogenic fetus during pregnancy has often been liked to organ transplantation. Several cell types are involved in immunological reactions towards foreign cells. Allogenic cells can be directly recognized by their non-self MHC molecules (either MHC-II on immune cells or MHC-I on most other cell types) by either $CD8^+$ cytotoxic T-cells or $CD4^+$ Thelper (Th) cells. This direct allorecognition causes a rapid expansion of T-cell clones and which can subsequently attack the foreign tissue [12]. Alternatively, foreign cells or their products are taken up by phagocytosing antigen presenting cells (APCs) such as dendritic cells, macrophages and activated B-cells. Intracellular breakdown and processing of foreign cells then takes place, resulting in antigen presentation of foreign MHC-derived antigenic peptides in the context of self MHC-II molecules. These can then be recognized by $CD4^+$ and $CD8^+$ T-cells, in what is known as the in-direct pathway of allorecognition [13]. In turn, activated $CD4^+$ Th cells will proliferate and differentiate into different Th lineages which can each produce their own array of (pro-)inflammatory cytokines. In addition, $CD4^+$ cells will activate antigen-specific B-cells to induce antibody production, whereas $CD8^+$ cytotoxic will kill nucleated foreign cells through self-MHC restricted pathways. The ensuing result is rejection of the tissues carrying the original antigens through antibody and T-cell mediated attack. While in transplantation biology immunosuppressive medication is required to prevent rejection of allografts, a natural immune-tolerant state has been proposed to occur during pregnancy to avoid immunological rejection of the fetus.

Immune tolerance in pregnancy – recognition of paternal antigens

Several maternal immune cell subsets are present at the maternal decidua. The majority of early infiltrating immune cells (>70%) are natural killer (NK) cells and macrophages recruited by endometrial hCG, progesterone and estrogen [14], but T-cells are also found. The physiological role of NK cells is to kill cells under stress, such as tumor and virally infected cells, and the recognition and lysis of cells lacking self MHC-I. Thus, the presence of large numbers of NK cells in the decidua might be expected to result in cytolysis of the fetal 'missing self' cells, as has been suggested in the

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organ transplantation setting [15]. However, uterine NK (uNK) cells express different cell-surface receptors from peripheral NK cells and are not cytotoxic [16]. This is partly due to the fact that fetal trophoblasts show an a-typical MHC expression, with and some of these (human histocompatibility leukocyte antigen [HLA]-E, G and F) actively suppressing uNK activity [17]. In addition, fetal trophoblasts are lacking in several other MHC-I and II molecules, which means they can to some extent escape recognition by the maternal immune system [18]. However, this MHC decrease does not appear to be the predominant factor in maternal-fetal tolerance, as tolerance is maintained even when paternal MHC is artificially re-expressed [19]. Studies on human third trimester decidual T-cells indicate that these cells do proliferate in response to fetal challenge *in vitro* [20], suggesting that recognition of paternal antigens does occur [21]. Mouse studies have indicated that T-cell responses to paternal antigens expressed by trophoblasts also occur at the placental interface, but that these are limited to indirect allogen recognition [22]. This means that maternal APCs are required in order to present fetal antigens to maternal T-cells, rather than direct recognition of intact fetal MHC molecules by maternal immune cells, which may severely limit the immune reactivity [23]. In addition, several mechanisms are in place to ensure that decidual APCs are less efficacious as compared to their peripheral counterparts. Decidualisation of the endometrium reduces the number of dendritic cells (DCs), one of the most efficient APC, and immobilizes remaining DC to prevent their T-cell activating properties [24]. *In vitro*, hCG is able to stimulate peripheral blood DC subsets to maintain a tolerant phenotype [25]. Together, these data suggest that limited recognition and response to fetal cells both contribute to fetal tolerance during pregnancy.

Immune tolerance in pregnancy – T-cell subsets

In addition to reduced recognition of alloantigens, the response of resident immune cells to activating triggers is also modulated via alternative mechanisms. It has been suggested that maternal T-cells themselves may acquire a state of tolerance against paternal alloantigens [26]. This may be in part be due to the presence of regulatory T-cells (Treg), a subset of T-cells that limit proliferation of $CD4^+$ and $CD8^+$ T-cells compartments via production of anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β [27]. Up to 20% of decidual T-cells are composed of Tregs, which have the capacity to suppress fetal alloresponses [28]. These cells may be actively recruited from the peripheral blood [29], or local expansion of Tregs can be induced by trophoblast-derived IL-10 [30]. Growth factors released by the placenta also induce a shift in differentiation of macrophages from an inflammatory phenotype (M1) to a wound-healing phenotype (M2), which further increases local IL-10 production [31]. M2 macrophages, as well as trophoblasts and DCs, are also an important source of the soluble enzyme indoleamine 2,3,-dioxygenase (IDO1) [16], which is required for the suppression of T-cell-induced local inflammatory reactions against fetal alloantigens [32]. This enzyme converts the essential amino acid tryptophan (Trp) into kynurenine, which has the dual effect of inducing apoptosis of $CD8^+$ T-cells by Trp depletion, and skewing $CD4^+$ T-cells to Treg differentiation [33]. Thus, local decidual responses are all geared towards the production of regulatory T-cells.

Tregs are not the only effector T-cells to be modulated during pregnancy, and a lot of attention has been directed towards the different Thelper (Th) cell subsets present during pregnancy. Traditionally, Th cells were divided into two subclasses, Th1 and Th2, based on their differential capacity to produce cytokines [34]. More recent evidence suggests that this distinction was somewhat crude, and additional Th populations (e.g. Th17, Th22, Th9) have now been identified. Th1 cells are mostly associated with IFNγ production and responses to intracellular pathogens and viral infections, whereas Th2 cells specifically produce IL-4, IL-13 and IL-5 and form the main response to helminths [35]. During pregnancy, IL-25, an IL-17 family member expressed by decidual T-cells, NK cells, Tregs and macrophages, stimulates the production of IL-4 and IL-10 in decidual T-cells, thereby contributing to a Th2 environment in first term placentas [36]. Furthermore, human pre-term placentas show increased levels of Th1 cytokines compared to term placentas, which express more Th2 cytokines [37]. Based on such data, it has been postulated that a shift from Th1 to Th2 cytokine profiles is required for a successful pregnancy [38], and that Th2 responses are more permissive for pregnancy.

However it is now becoming more and more evident that each trimester is associated with specific immunological needs. In particular, implantation and early pregnancy require a pro-inflammatory environment to allow for tissue remodeling that takes place during decidualisation. The blastocyst breaks through the epithelial lining of the uterus to implant, and placenta formation requires invasion of trophoblasts into the surrounding tissue [39]. Endometrial cells become specifically sensitive to IL1 family members, and produce pro-inflammatory and angiogenic factors [40]. Additionally, uNK and uDC cell activity in particular play a major role in vascularisation of the placenta, through their production of angiogenic factors such as TGFβ and vascular endothelial growth factor (VGEF) [41]. Furthermore, even though first trimester placentae are characterized by high IL-10 levels, IL-6, IL-8 and TNFα are also present or required for blastocyst implantation [42]. From first to second trimester, cellular populations in the uterus change, with total T-cell numbers increasing and macrophages and DC subsets decreasing as well as acquiring a tolerogenic phenotype [43]. Tolerance appears to be the predominant state throughout the main duration of pregnancy, but towards labor IL-10 levels decrease, and IL-6, TNFα and IL1β increase [44]. Thus, inflammatory processes are required for successful term labor and delivery, and immunological profiles may fluctuate during pregnancy (see Fig. 1).

Immunology of pregnancy outside the uterus

The general consensus thus far appears to be that implantation is associated low grade inflammation and a Th1 cytokine pattern, the main duration of pregnancy requires a shift towards tolerance-associated Th2 cytokines, while labor is initiated through Th1 cytokines at the uterus. To what extent these local immunological changes translate to peripheral effects is less clear. Case reports have suggested that pregnancy may reduce immunosuppressive therapy requirement in transplantation settings [45] and chronic inflammatory conditions such as inflammatory bowel disease [46]. In addition, disease course of several anti-immune diseases, including rheumatoid arthritis and multiple sclerosis were shown to be ameliorated during pregnancy [47–49], suggesting that this immune-tolerance goes beyond the placenta. It has indeed been proposed that pathological alterations in Th subsets occurring *in utero* during pregnancy failure are mirrored by peripheral alterations in these subsets [50]. However, studies investigating serum cytokine levels in healthy pregnancy over the three trimesters have been inconsistent. A recent study showed increased levels of both Treg cytokines (IL-10, sTNFRII) as well as Th1 cytokines (INFγ, IL-2, IL-12, IL-27) during the second trimester of pregnancy [51]. Two studies reported a decrease in TNFα from early to late pregnancy [52,53], although three other studies did not corroborate this finding [54–56] and one reported an increase of TNFα across the trimesters [57]. The most consisting finding to date appears to be increasing levels of IL6 (often considered a Th1 cytokine) from early to late pregnancy [53–55,58,59]. Similar controversy is seen for

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circulating immune cells. A recent study showed that peripheral blood subsets of NK cells and $CD4^+$ T-cells increased during pregnancy, while monocyte and $CD8^+$ T cell numbers remained stable over the trimesters, and peripheral Treg numbers decreased from upon conception [60]. However, others found no peripheral blood changes of $CD4^+$ cells, and increased peripheral Treg populations during healthy pregnancy [61]. Despite these controversies, it has been demonstrated that peripheral immune cells from pregnant women react differently to external stimuli as compared to heathy controls: less cytokines of either the Th1 or the Th2 variant are produced when isolated blood cells from women in their second trimester are challenged with phorbol 12-myristate 13-acetate and ionomycin (a general, non-receptor stimulant), suggesting that a peripheral immune dampening effects of pregnancy may be present.

Infectious diseases during pregnancy

In addition to providing fetal tolerance, immunological changes occurring during pregnancy might be also expected to affect the way pathogenic threats are handled during this time. Recognition of pathogens by the adaptive immune system depends on presentation of their antigens on MHC-I molecules of infected cells (mostly in the case of viruses) or presentation of ingested microbes by MHC-II on APCs (mostly for bacteria) [62]. Furthermore, as mentioned above, viral and intracellular pathogens are generally associated with the development of a Th1 immune response. Thus, with the immunological changes taking place during pregnancy, it might be expected that pregnant women are more susceptible to infection with bacteria and viruses. Clinical and epidemiological evidence suggests that while pregnant women are not completely defenseless against pathogens [63], an increased susceptibility to some infectious diseases is indeed present [64]. Already in the 50's it was noted that pregnant women may be more susceptible to malaria-causing parasites. Seroconversion to *toxoplasmosis gondii* (a parasite transferred in food and sometimes spread via cats) is increased during pregnancy [65]. Viral infections also appear to be more common or more severe in pregnancy, as has been demonstrated for measles virus, influenza A virus, Hepatitis E virus and herpes simplex virus [66]. Nevertheless, seroconversion rates in response to influenza vaccines appear to be similar for pregnant and non-pregnant women, and are not affected gestational time during vaccination [67,68]. With regards to bacterial agents, it has been suggested that pregnancy either increases susceptibility to, or allows re-activation of latent infections, of the gastric bacterium *Helicobacter pylori*, which is linked to gastric ulcers and gastric cancer [69]. Furthermore, pregnant women are 17 times more likely to be affected by the foodborne Gramm positive bacterium *Listeria monocytogenes*, which can cause premature delivery, miscarriage and stillbirth [44]. Thus, while for the most part, systemic immunity towards most pathogens appears to be functional in pregnancy, in particular severity of disease upon contraction of some infectious agents is increased in pregnant women.

Infections threatening the placenta/fetus

One of the most common threats to healthy pregnancy is infection of the placenta or fetus with microbial, viral, protozoal or fungal agents. It is still debated whether the healthy placenta contains a bacterial microbiota. While some studies have shown the presence of bacteria at the maternal side of the placenta [70,71], other studies were unable to find evidence of a unique placenta microbiome [72,73]. The presence of bacteria at the fetal side of the placental circulation has also been observed, with amniotic fluid described to contain Proteobacteria such as *Enterobacter* and *Escherichia/Shigella* [74], but again, others were unable to replicate these findings [75,76]. Thus, controversy surrounds the presence of bacteria in the healthy placenta and with low bacterial abundant tissues prone to contamination and sequencing artifacts, studies should be interpreted with care. It may be that bacterial presence in the placenta is relevant only in pathological situations. Microbial contaminants seen in infected membranes include the genera *Fusobacterium*, *Streptococcus*, *Mycoplasma*, *Aerococcus*, *Garderella* and *Ureaplasma* and the family Enterobacteriaceae [77], with *Ureaplasma urealyticum* and *Mycoplasma hominus* presenting as the most common causes of infection [78]. Several bacteria seen in chorioamnionitis, including *Bacteroides* spp, Group B *Streptococcus*, *Staphylococcus*, *E. coli* and *Klebsiella* are also seen at low abundance in the healthy vaginal microbiota, consistent with ascending infections [79]. Interestingly, pregnancy hormones may modulate these vaginal bacteria, as demonstrated by decreased rates of Group B *Streptococcus* in patients taking progesterone for the prevention of preterm birth [80]. Furthermore, a lower prevalence of *Ureaplasma* and *Mycoplasma* was seen during pregnancy [81]. Pregnancy is associated with a reduced vaginal bacterial richness and diversity and a higher abundance of *Lactobaccillus* spp [82], both signs of a healthy vaginal microbiome and associated with improved fertility rates. Thus, it appears that pregnancy hormones may modulate the vaginal microbiome to reduce pathogen-derived risk to the fetus.

Intestinal pathogenic threat to the fetus

In addition to ascending bacterial infections which are characterized by the spread of vaginal/cervical pathogens to the uterus, hematogenous spread of blood borne pathogens from maternal to fetal circulation poses a second threat [83] (see Fig. 1B). Several food contaminants are known to be able to infect the placenta to detrimental effects, including *Listeria*, *Salmonella*, *Brucella* and *Campylobacter* [84–87]. Generally, these bacteria are prevented from reaching the blood stream by the epithelial lining of the gastrointestinal tract and its antimicrobial products (e.g. defensins and mucins). However, some bacteria, like *Listeria*, are able to invade and spread via epithelial and immune cells and may thereby enter the circulation [44]. Additionally, microbial composition, medications, dietary peptides, infiltrating immune cells, and cellular stress may all modulate the strength of the epithelial barrier and affect bacterial translocation.

Several mechanisms are in place to ensure limited damage from these food borne pathogens. Pregnancy hormones such as progesterone and estrogen directly enhance barrier function in *in vitro* model systems [88], which is supported by data in animal models [89]. Pathogens and their products which do manage to cross the feto-maternal interface in the placenta are there recognized by specialized pattern recognition receptors. Uterine epithelial cells express several of these toll like receptors (TLR), microbial peptide recognizing transmembrane molecules which play a role in innate immunity. Ligation of these receptors on human uterine epithelial cells induces the release of the chemokine IL-8 as well as monocyte chemotactic protein-1 (MCP1), which ensures the recruitment of phagocytes to eliminate bacterial threats [90]. On the other hand, TLR3 ligation on endometrial cells increases the production of IDO1 in the uterus [91], which has several anti-inflammatory properties, including the stimulation of Treg differentiation. In mice the expression of several TLRs are increased during pregnancy [92], and their expression is further enhanced by maternal viral infection [93]. While some level of TLR is required to maintain a defense against biological agents, too much inflammation can also be harmful in pregnancy. A reduced presence of the lipopolysaccharide receptor TLR4 on villous trophoblasts and decidua is associated

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with spontaneous abortion [94], but enhanced syncytium TLR4 levels were shown in patients with preeclampsia [95]. Additionally, TLR expression and functionality may depend on gestational stage, as at least in peripheral blood, TLR4 stimulated cytokine production is only increased in the first and third trimester of pregnancy [96]. Thus, inflammatory and anti-inflammatory processes need to be carefully balanced in order to eliminate potential pathogenic threats while maintaining a healthy pregnancy.

Other interactions between the gut microbiome and pregnancy

While microbes may occasionally pose a pathogenic threat, the human body hosts over 10^{13} bacteria, in what is generally a mutually beneficial symbiosis [97]. Most of these microbes reside in the gastrointestinal tract, where they are now being recognized as a vital part of the human metaorganism [98]. Animal studies have shown the importance of the intestinal microbiome for shaping local and peripheral immunity [99,100]. Bacteria are essential for the efficient metabolisation of indigestible fibers and production of vitamins K and B. Metabolites produced by intestinal bacteria include short chain fatty acids (SCFA), of which butyrate and propionate are a nutrient source for colonocytes and perform functions in gluconeogenesis, while acetate is a growth factor for other bacteria and plays a role in cholesterol metabolism [101].

With the recognition of the intestinal microbiota as important player in homeostasis, its potential role in metabolic disease is now also emerging. Early studies studying the microbiota in obese mice and humans pointed towards decreased ratio of Bacteroidetes/Firmicutes and reduced microbial diversity [102–104]. Subsequent studies and re-analyses of these data have challenged the importance of this ratio in obesity, leaving the exact microbial changes open [105,106]. Nevertheless, while the exact signature of the microbiota for obese individuals is unclear, obese and lean individuals can be accurately classified by their microbiomes [107], consistent with the fact that transplantation of fecal microbiota from obese individuals to lean mice can cause metabolic changes and weight gain in these mice [108]. The microbiome in patients with metabolic syndromes shows reduced levels of *Bacteroides* species and butyrate producing *Faecalibacterium* [109]. Furthermore, the microbiome in patients with type 2 diabetes (T2D) is altered, and modulation of thereof by *Akkermansia muciniphila* administration, fecal transplants or the use of antibiotics can restore insulin sensitivity [110–112].

During pregnancy, insulin sensitivity fluctuates over time, to accommodate the changes in energy demand that accompany different phases of pregnancy. Early in pregnancy, insulin sensitivity is enhanced to allow establishment of fat stores for later energy demands, while at third trimester insulin sensitivity decreases and endogenous glucose production rises [113]. Interestingly, while the intestinal microbiome early in healthy pregnancy resembles that of non-pregnant women, late pregnancy is accompanied by reduced microbial diversity and reduced levels of *Faecalibacterium* [53,114,115]. Transfer of third trimester fecal bacteria to germ free mice resulted in increased weight gain, development of insulin resistance and inflammatory responses in these mice [116]. These data suggest that microbial alterations occurring during late pregnancy contribute to the physiological metabolic processes required in pregnancy. In up to 10% of women, insulin sensitivity drops to a point where they develop gestational diabetes mellitus (GDM) [117], which generally normalizes post-partum, but can nevertheless pose severe long-term health risks for mother and child. Obesity in pregnant women was also associated with specific microbial alterations, and weight gain in pregnancy has been correlated with increased *Bacteroides* levels [118] and reduced Firmicutes [119] dominance mid-pregnancy. GDM was associated with increased rather than decreased levels of alpha diversity from middle to late pregnancy, with increased Firmicutes and *Faecalibacterium* and reduced *Bacteroides* [120]. Thus, pregnancy induces microbial changes corresponding to metabolic syndrome, but metabolic syndrome development in pregnancy does not appear to be a simple exaggeration of these microbial alterations.

Microbial changes as cause or consequence of metabolic alterations in pregnancy

Although some studies did not observe any microbial changes over time during gestation [121], a consistent finding in most studies appears to be a reduced diversity in microbial composition [114,116,122,123]. This begs the question as to how this process is regulated. One obvious answer would be that dietary changes during pregnancy may affect the microbiome. Consciously or subconsciously, pregnant women take in less alcohol, meat and caffeine, while intake of milk products, fruits and sweets increases [124]. In mice, periconceptional diet was shown to influence microbial changes taking place during pregnancy [125]. It is also conceivable that changes in diet work in concert with molecular changes occurring during pregnancy. As described previously, pregnancy increases levels of the enzyme IDO1, which catalyses the degradation of Trp, an essential amino-acid derived from diet, to kynurenin. Trp is also degraded by the intestinal microbiome to form beneficial indole metabolites which drive IL22 production and increase insulin sensitivity. In mice, it has been demonstrated that high fat diet induces IDO1 expression, which in turn skews Trp metabolism from microbial-related indole production to IDO-1-mediated kynurenine production, and a decreased insulin sensitivity [126]. Although IDO1 levels in the gut and adipose tissues have not been directly studied in pregnancy, serum levels of kynurenine increase during pregnancy, suggesting increased systemic effects of IDO1 activity and increased IDO1 levels have been associated with metabolic disease [127]. Interestingly, the microbiome itself is also dependent on host IDO1 levels, demonstrating a reciprocal relationship between host and microbiome [126].

As mentioned earlier, there is also a reciprocal relationship between the microbiome and host immunity. Microbes play an important role in shaping the host immune response, and intestinal dysbiosis is a contributing factor to immunological disorders such as inflammatory bowel disease (IBD) and lupus erythematosus [89,128,129]. A loss of butyrate producing bacteria is associated with intestinal inflammation by increasing of IL17 levels and decreasing IL10 production and Treg generation [130]. Pregnancy improves inflammatory markers in patients with IBD, which is associated with microbial alterations and normalization of the microbiome in these patients [53]. While data on butyrate levels during human pregnancy is scarce, butyrate enhances embryo survival in rats when given during early pregnancy [131]. Conversely, immunological alterations also affect the microbial composition. For instance, overexpression of IL-15 in the intestinal epithelium drives microbiota changes associated with a decrease in butyrate producing bacteria and butyrate levels [132]. Enhanced (local) IL-15 levels have been associated with adverse pregnancy outcomes [133]. Thus, it is tempting to speculate that immunological alterations occurring during pregnancy also contribute to reshaping of the microbiome in pregnancy.

Pregnancy hormones may play a role in the reciprocal interaction between immunity and microbiome. Indeed, *in vitro* studies indicate that progesterone reduces bacterial richness but stimulates *Faecalibacterium*, *Bacteroides* and *Bifidobacterium* growth, amongst others [134]. Animal studies have shown that administration of exogenous estrogens at pregnancy levels alter the fecal

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microbiome in rats [135]. In this study, estrogen also protected against autoimmune encephalomyelitis-induced changes of microbiome, as well as development of EAE symptoms. These authors further showed that estrogen treatment was associated with mucosal expansion of regulatory B-cells and M2 macrophages, demonstrating a local protective effect and suggesting that estrogen may cause enrichment of bacteria with immune modulatory function. Furthermore, both progesterone and estrogen strengthen epithelial barrier function, which in turn may also affect bacterial composition [88]. *Vice versa*, the microbiome itself may adjust the levels of sexhormones produced [136], adding a further layer of complexity.

Conclusions

While it is by now well accepted that pregnancy is accompanied by substantial hormonal, immunological and microbial alterations, the exact interactions and processes governing these changes remain elusive. Several challenges contribute to our lack of understanding. For one thing, much of our knowledge regarding the local changes in immunological and microbial patterns is derived from either mouse experiments, or from pathological pregnancies. However, mouse placentae are considerably different from human placentae, in both morphology and the presence of uNK cell receptors [137]. Furthermore, most of what we have gleaned from human placentae comes from the study of different pathological situations, as otherwise healthy placental tissue at different gestational time points are generally unavailable. Secondly, it is becoming clear that like hormone levels, changes in immunological and microbial patterns fluctuate during pregnancy, and no longitudinal samples have ever been taken from healthy human placentae. Third, while local alterations associated with adverse pregnancy outcomes are to some extent correlated to peripheral immunological changes [50], there is remarkably little consensus as to the immunological changes taking place in the peripheral blood or other tissues during healthy pregnancy [89]. Similarly, studies investigating microbial changes occurring during pregnancy have not all been consistent. Technical differences between studies may account for the fact that not all studies show similar changes in immunological and (vaginal, intestinal, oral) microbial parameters, but that these parameters do change is not disputed [115]. The most consistent immunological and microbial findings include an increase in IL-6 cytokine levels and a decreased intestinal microbial diversity towards the third trimester. Cause and consequence of immunological and microbial alterations in pregnancy are difficult to distinguish. While pregnancy hormones can directly alter immunological responses as well as (intestinal) microbiomes [134], reciprocal interactions are present between the microbiome and immunity which may reinforce fluctuations in both. Thus, the concept of microbiome relating to our health as part of a meta-organism appears to extrapolate to the state of pregnancy.

Thus far, what is clear is that in general, tolerance against an MHC-mismatched fetus predominates in pregnancy, and that an altered susceptibility or response to certain viral or bacterial infections may exist. To what extent pregnancy improves of worsens (auto)inflammatory responses remains debated, and may depend on the disease and time point in pregnancy. Significant reduction of pro-inflammatory cytokine levels during pregnancy has been observed for patients with inflammatory bowel disease [53]. Similarly, pre-pregnancy differences in bacterial diversity normalized during pregnancy in these patients. A similar beneficial effect of pregnancy on disease activity was seen for rheumatoid arthritis, although increased flaring post-pregnancy may occur [49]. While a role for the intestinal microbiome was shown for lupus erythematosus, amelioration of disease by antibiotics treatment was seen for non-pregnant animals, but not for post-partum pregnant animals [138]. While these latter data also indicate that pregnancy alters the host-microbial interactions, in this case pregnancy was not beneficial. Similarly, microbiome alterations are seen in patients with multiple sclerosis [139], yet pregnancy worsens post-pregnancy disease course in these patients [140]. Thus, some immunological diseases may be affected favorably during pregnancy, but post-partum flaring of disease is an imminent risk. Thus, special care should be taken both during pregnancy and post-partum regarding infectious diseases and inflammatory disorders. Overall, expanding our knowledge regarding the interactions between host and microbiome and the time-sensitive modulation thereof by hormones during pregnancy would perhaps allow a better identification of patients at risk of complications of either their pregnancy or concomitant diseases.

Practice points

- Immunological changes are seen during pregnancy, which may extend beyond the placenta
- Tolerance against fetal antigens during pregnancy does not mean a reduced immunological reactivity *per se*.
- Microbial alterations during pregnancy can have both pathogenic and protective effects
- Hormonal, immunological and microbial alterations taking place during pregnancy may alter susceptibility of pregnant women to infection and inflammatory disease.
- Pregnant patients should be carefully monitored for infectious disease and inflammatory disorders, both during pregnancy and post-partum

Research agenda

- Longitudinal changes in peripheral immune responses and microbiome alterations should be better defined.
- Identifying women at risk for pregnancy complications including GDM or pre-term birth could be improved by studying the interaction between host and microbiome

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