INSIGHTS

Placental inflammasome signaling: Protection for mother and baby

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The second trimester of pregnancy is traditionally viewed as an immunosuppressive state. Megli et al. (https://doi.org/10.1084/jem.20200649) change this paradigm, showing that midgestation induces inflammasome signaling in placental trophoblasts to promote fetal and maternal antimicrobial defense. The placenta is thus a dynamic immunological organ.

The maternal–fetal interface is an active immunological site

Infections are a major concern during pregnancy. Infection can cause premature death, growth abnormalities, or severe congenital defects in the developing fetus. The maternal–fetal interface serves as a barrier that prevents the vertical transmission of pathogens and is composed of the maternally derived decidua and the fertilally derived placenta developed from the blastocyst trophectoderm.

Apart from established placental functions as a physical barrier and in delivering maternal antibodies to the fetus, the mechanisms by which the placenta prevents fetal infection are unclear. The placenta has a unique structure, containing a single layer of contiguous multinucleated syncytiotrophoblasts (SYNs) on its outermost surface, followed by an underlying layer of undifferentiated mononucleated cytotrophoblasts (CTBs). Together, these layers form a tight cellular barrier between the fetal compartment and maternal blood flow. During pregnancy, the underlying CTB layer thins and the progenitor CTBs differentiate into extravillous trophoblasts located at the tips of chorionic villi (Malassine et al., 2003). These developmental processes result in a single hemomonochorial layer of SYNs during the second and third trimesters of pregnancy (Malassine et al., 2003).

Megli and co-workers sought to determine whether trophoblasts protect the fetus during pregnancy (Megli et al., 2020). The authors used multianalyte Luminex methods to profile secreted factors from chorionic villi isolated from pregnant women. At midgestation, chorionic villi showed marked production of cytokines, chemokines, and antimicrobial proteins. Strikingly, uninfected trophoblasts showed strong secretion of IL-1β and IL-18 in the second trimester, and this declined with advancing gestation. These observations suggest a molecular basis for placental defense against fetal infection and demonstrate that the placenta is a dynamic immunological organ.

The placental NLRP3 inflammasome generates cytokines during midgestation

The finding that chorionic villi produced pro-inflammatory cytokines such as IL-1β and IL-18 at midgestation was surprising, given that this symbiosis between mother and fetus is generally considered to be an anti-inflammatory state. IL-1β/18 maturation and secretion are controlled by an immune signaling complex called the inflammasome, which assembles when an inflammasome-nucleating protein senses cell infection or damage (Chan and Schroder, 2019). SYNs are reported to recognize pathogens via Toll-like receptors and RIG-I-like receptors (Koga and Mor, 2008), but their capacity to sense microbes or cell stress via the inflammasome was unknown. To investigate the mechanism underpinning cytokine production by chorionic villi, Megli et al. (2020) performed detailed RNA sequencing analyses of trophoblasts isolated from chorionic villi. Indeed, midgestation trophoblasts expressed high levels of inflammasome-associated molecules such as caspase-1, NLRP3, IL-1β, and IL-18. In confirming the mRNA expression data at the protein level, the authors further showed that the inflammasome substrates, IL-1β and gasdermin D, are cleaved to their active forms in placental trophoblasts, suggestive of an active inflammasome in these cells. The specific NLRP3 inflammasome inhibitor MCC950 (Coll et al., 2019) blocked the release of IL-1β and IL-18 from midgestation chorionic villi. These results suggest the fascinating scenario that in healthy, uninfected pregnant women, placental trophoblasts secrete...
immune-modulating factors in a dynamic and timely manner without affecting immune tolerance. These data further indicate that midgestation somehow serves as a molecular trigger for inflammasome assembly and signaling, although the nature of this signal is unresolved. It is possible that pregnancy-induced tissue remodeling, such as the rapid expansion, growth, and differentiation of progenitor CTBs into extravillous trophoblasts, generates a sterile (i.e., nonmicrobial) inflammasome trigger for trophoblast cytokine secretion.

**Trophoblast IL-1β primes maternal monocytes**

The concept that a healthy pregnancy triggers inflammasome signaling in placental trophoblasts is new and exciting and raises several questions. What is the function of placental IL-1β and IL-18? What is the impact of these cytokines on fetal and maternal health, including immunity to microbes?

One well-established function of IL-1β in other settings is its capacity to “prime” cells for heightened responses to other stimuli, such as inflammasome-activating factors. Megli and colleagues showed that placental IL-1β production during midgestation is associated with increased IL-1β levels in serum and up-regulated expression of caspase-1 and IL-1β in circulating monocytes (Megli et al., 2020). Conditioned media from chorionic villous explants from pregnant women similarly primed the monocytes of nonpregnant women for up-regulated IL-1β, caspase-1, and NLRP3 expression. This suggests that placental IL-1β targets circulating maternal monocytes to prime them for inflammasome signaling by an inflammasome-activating factor (e.g., nigericin). This indeed was the case, as conditioned media from chorionic villous explants facilitated nigericin-induced inflammasome signaling in monocytic THP-1 cells in a manner blocked by IL-1 receptor neutralization or genetic deficiency. These data reveal a novel mechanism by which the NLRP3 inflammasome in the placenta drives systemic priming of circulating maternal monocytes to poise these cells for further inflammasome signaling in the periphery in a previously unappreciated interaction between fetus and mother (see panel A in figure).

**Pregnancy-induced inflammasome signaling enables maternal and fetal protection against Listeria monocytogenes**

Inflammasome signaling is crucial for host defense against a variety of pathogens, including *L. monocytogenes*. *L. monocytogenes* infection is a major concern during pregnancy, as this bacterium is vertically transmitted along the maternal-fetal barrier, where it can cause fetal infection and premature death. With the hypothesis that placental IL-1β poises circulating maternal monocytes for heightened antimicrobial responses to microbes that may endanger the fetus, Megli et al. (2020) assessed the impact of priming on monocyte defense against *L. monocytogenes*. THP-1 monocytic cells primed with chorionic villous-derived conditioned media were indeed less susceptible to *L. monocytogenes*. The protective effect conferred by conditioned media required NLRP3 inflammasome signaling in chorionic villi, as it was blocked when media were conditioned in the presence of MCC950 (see panel B in figure). The protective factor in conditioned media was identified as IL-1β, as IL-1 receptor deficiency rendered THP-1 cells susceptible to *L. monocytogenes* infection. These data indicate that during midgestation pregnancy, NLRP3 inflammasome–derived IL-1β from the placenta primes maternal circulating monocytes to promote their capacity to clear *L. monocytogenes* before this bacterium poses a threat to the fetus.

Megli et al. (2020) next investigated whether trophoblasts are themselves capable of defending the fetus in the scenario that maternal monocyte defenses fail to clear *L. monocytogenes*. SYNs form a protective layer around the placental epithelia, and these cellular barriers are well established to prevent microbial invasion of the fetus (Robbins et al., 2010). With the hypothesis...
that trophoblast inflammasome signaling contributes to antimicrobial defense, Megli et al. (2020) isolated chorionic villous explants from women in their second trimester of pregnancy. Explant infection with L. monocytogenes triggered strong IL-1β and IL-18 production. Chorionic villous explants showed strong resistance to L. monocytogenes infection, but this was compromised in the presence of MCC950 (see panel B in figure). In all, this suggests that NLRP3 inflammasome signaling in placental trophoblasts serves to protect the villi and fetus from potentially fatal infection with L. monocytogenes.

Pregnancy is associated with a dynamic immunological state

Megli and co-workers’ newly described pathway of immune protection during pregnancy adds to an emerging literature suggesting that pregnancy is associated with a dynamic immunological state. Pregnancy can be divided into three distinct immune phases based on pregnancy trimester: (1) the first trimester involves implantation, placentation, and ongoing wound healing, which requires a strong immune response, including the infiltration of maternal immune cells (Co et al., 2013; Dekel et al., 2010). This immune reaction is often associated with morning sickness. (2) The second trimester was traditionally viewed as a state of symbiosis between mother and fetus, reflecting an anti-inflammatory state (Ferreira et al., 2017). Megli et al. (2020) shift this paradigm, showing that the placenta forms an immunological barrier that also promotes maternal peripheral host defense. (3) The third trimester is associated with an influx of immune cells into the myometrium, which eventually promotes uterine contraction, placentation rejection, and parturition in a sterile pro-inflammatory state (Romero et al., 2006). During late-stage pregnancy, these infiltrating immune cells exhibit a pro-inflammatory phenotype with high expression of several inflammasome pathway members such as NLRP3, NLRC4, ASC, caspase-1, and GSDMD (Gomez-Lopez et al., 2019). Accordingly, cytokines (e.g., IL-1β) and chemokines are abundant in the amniotic fluid, maternal circulation, and placenta (Keelan et al., 2003).

A healthy pregnancy requires the activities of diverse maternally derived immune cells plus the fetal trophoblasts to orchestrate critical processes such as implantation, placentation, and parturition in their correct sequence and timing. The study by Megli et al. additionally reveals that an inflammasome-mediated immunological barrier is important for preventing maternal and fetal infection (Megli et al., 2020). This begs the question, can a pro-inflammatory state during pregnancy be beneficial?

Inflammasome inhibitors during pregnancy—safe or detrimental?

The natural pro-inflammatory state of late pregnancy, which is associated with contractility of the myometrium, cervical dilation, and rupture of the chorionic membrane, is also seen in spontaneous preterm labor. Premature labor is associated with microbial or sterile intra-amniotic inflammation. In some settings, microbes can breach the physical and immunological placental barrier, inducing a fetal systemic immune response leading to premature labor, which can be associated with fetal neurological and respiratory inflammatory diseases (Romero et al., 2014).

The benefits of inflammation during pregnancy thus come at a cost, which is the possibility of preterm labor. Several factors are implicated in driving an elevated fetal and maternal immune response that can induce preterm labor. One of these is a shortage of progesterone. Throughout gestation, progesterone suppresses IL-1β and TNF-driven apoptosis. A drop in progesterone levels may thus favor increased cytokine secretion, cell death, and maternal rejection (Tan et al., 2012). Another factor implicated in preterm birth is cell-free fetal DNA circulating in maternal blood, which is detected by TLR9 and disrupts immune tolerance (Scharfe-Nugent et al., 2012). With new anti-inflammatory drugs such as NLRP3 inflammasome inhibitors entering clinical trials for several diseases, longitudinal studies are required to determine whether such anti-inflammatory agents can prevent preterm labor or, alternatively, whether they risk compromising maternal and fetal defense against infection. The precise mechanisms underpinning maternal-fetal immune activation and antimicrobial defense require further clarification. A better understanding of fetal immune defense, the maternal-fetal immunological dialogue, and the maternal response is urgently required to address potential complications of pregnancy and assess the benefits and risks of prescribing new anti-inflammatory drugs to pregnant women.

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