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Human Fetal immune cells fight back

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It has long been postulated that the fetal immune system develops in a sterile environment, without antigen exposure, and therefore that it is essentially immature. Immune immaturity was also considered the main reason why newborns and infants are generally more susceptible to infections. However, more recent work has started to challenge these assumptions, not only the sterility of the placenta environment, but also the lack of functionality of the human fetal immune system. Furthermore, the growing realization that *in utero* exposures of the immune system may have a life-long impact, notably influencing many non-communicable diseases involving immune dysfunction (asthma, autoimmunity), is generating a push towards gaining a better understanding of the ontogeny and the functionality of the fetal immune system, not only under homeostatic conditions, but also in the context of infection and inflammation. However, as development of the immune system, particularly of the adaptive immune system, is markedly different in humans and small rodents, there is still a paucity of data on this topic. In this issue, Frascoli et al. bring us a step closer to identifying and understanding the functional capabilities of human fetal T cells (1).

Functional innate-like lymphocytes, natural killer cells, $\gamma\delta$ T cells, and mucosa-associated invariant T cells are all found in healthy human fetuses. Maturation of human fetal T cells also occurs *in utero*, with T cell zones appearing in the spleen as early as 18 weeks of gestation. This is in marked contrast from murine models, in which T cells emigrate from the thymus after birth. Although most of these cells are naive, CD4⁺ T cells with a memory phenotype are found in the human fetal intestine, spleen and skin (rev. in (2)). Further demonstrating that fetal T cells can be fully activated, it has long been known that infants exposed *in utero* to viral infections, such as HIV or CMV, are born with antigen-specific CD4⁺ T cells and CD8⁺ T cells, even when the infants are not infected themselves. Similarly, antigen-specific T cells are detectable at birth in many infants born to mothers harboring a variety of parasitic infections.

It has been proposed that one of the major influences on fetal T cells is maternal micro-chimerism. Substantial numbers of maternal cells cross the placenta by incompletely defined passive or active mechanisms and are found in fetal lymph nodes. In seminal studies, Mold et al. showed that human fetal CD4⁺ T cells preferentially differentiate into regulatory T cells (Tregs) when stimulated *in vitro*, and that this bias develops in response to cells bearing non-inherited maternal allo-antigens (NIMA) (3). These fetal Tregs are thought to prevent maternal–fetal conflict, and this control persists after birth, as Tregs in adolescents continue to be more efficient at dampening responses to maternal allo-antigens than to paternal antigens (3).

Another factor that probably shapes the fetal immune system is its exposure to microbial products and/or inflammatory mediators. Indeed, recent studies detected bacterial DNA sequences in the amniotic cavity more frequently than presumed historically, even in healthy pregnancies, although detection of background DNA could have been a confounding factor in some of these studies. Molecular evidence of microbial invasion of the amniotic cavity was strongly associated with spontaneous preterm birth, and intracellular bacteria were detected in the placental basal plate in about half of the women who deliver spontaneously before 28 weeks (rev. in (4)). This microbial signature correlated with signs of placental inflammation, or chorioamnionitis. Notably, the 16S rDNA copy number in the amniotic fluid was found to be significantly higher in the women with severe chorioamnionitis than in those with mild or no chorioamnionitis (5). These data thus corroborate previous studies where chorioamnionitis was diagnosed histologically in ~25% of late preterm and >50% of early preterm births, with or without rupture of membranes (rev. in (6)). Importantly, chorioamnionitis, particularly in very preterm births, is associated with neonatal diseases such as cerebral palsy, necrotizing enterocolitis, and bronchopulmonary dysplasia, as well as longer-term deleterious outcomes such as wheezing and asthma (rev. in (6)). Pro-inflammatory cytokines are high, not only in the amniotic fluid, but also in the cord blood of infants exposed to severe chorioamnionitis, demonstrating that the fetal immune system has been exposed, and has reacted, to either inflammatory mediators or bacterial products, or most likely both.

The paper by Frascoli et al. suggests that we should consider how the two influences of micro-chimerism and infection/inflammation might intersect in the context of inflammation-induced preterm birth. In this study, the authors analyzed immune profiles in the cord blood of term and preterm newborns, and their mothers. Signs of immune activation were obvious in the cord blood of many preterm babies, many of whom were exposed to chorioamnionitis. A notable finding of this paper is that maternal micro-chimerism was more profound in these preterm babies, thus providing additional evidence of inflammation-driven increase in maternal micro-chimerism. The same authors reported earlier that fetuses with severe congenital diaphragmatic hernia, who exhibit high inflammatory cytokines in cord blood, also have increased maternal micro-chimerism (7); a similar increase was also described in babies exposed to placental malaria (8). Interestingly, the latter study showed that both malaria infection and inflammation contributed to this enhanced transfer of maternal cells.

Even more strikingly, Frascoli et al. describe the presence of a subset of central memory CD4⁺ T cells with the characteristics of Th1 cells in the blood of preterm babies, whereas this population was almost undetectable in term babies. Importantly, they show that fetal CD4⁺ T cells from these preterm babies proliferate more vigorously in response to maternal antigens than those from term babies. Such activation appeared specific, as they did not exhibit a stronger reactivity to third-party allo-antigens. This study thus indicates that the human fetal immune system is influenced by maternal antigens, but the outcome is not always tolerogenic, in contrast to the model suggested by Mold et al (3). The mechanisms underlying these differences were not investigated in this paper, but the fact that preterm babies' dendritic cells exhibited an activated phenotype may give us a clue. Indeed, murine fetal/neonatal T cells were shown long ago to be fully competent to respond to mature adult dendritic cells (9). Thus, the model indicated by these findings is that, in the context of *in*

utero inflammation, the fetal immune system is exposed to more maternal allo-antigens in an inflammatory context, and this double hit results in the activation of effector cells, not classic Tregs (Figure 1). It will be important to directly test this hypothesis, showing that inflammation-triggered maturation of fetal dendritic cells leads to allo-specific fetal T cell activation. It would also be important to determine whether it induces NIMA-specific inflammatory Tregs, that express not only FoxP3 and other molecules associated with a Treg phenotype but also “effector” transcription factors such as T-bet or RORC and can produce effector cytokines upon stimulation. Indeed, from investigating the effect of severe chorioamnionitis on fetal T cells, we have shown the emergence of these inflammatory Tregs in the fetal spleen, and less abundantly in the blood (10). Although the authors did not find differences in the overall size of the Treg pool, they did not investigate in detail the functional characteristics of these cells.

Another intriguing finding of this study is that *in vitro* contact of T cells from preterm infants with a myometrial cell line stimulated uterine myometrial contractility, through enhanced IFN- γ and TNF- α production. In a murine model, adoptive transfer of IFN- γ - and TNF- α -competent T cells provoked fetal resorption. The authors thus postulate that activated fetal T cells could play an active role in pre-term labor, through cytokine-induced induction of uterine contractions, although whether fetal T cells produce enough cytokines to act on a distal site such as the uterus remains to be proven.

This study has limitations, one being that only peripheral immune cells, both maternal and cord blood cells, were analyzed, thus providing only a partial view of the activation processes. This is a caveat inherent to the study of the human neonatal immune system, as collecting biological samples other than the blood is even less feasible in this population than it is in other human subjects. Additionally, it is not known how long this activation persists after birth. These important points will likely be more easily addressed in animal models with an ontogeny of the immune system close to that of humans, such as non-human primates or pigs. Another limitation is that women who go into preterm labor are a heterogeneous group, as demonstrated by the large variability in the concentration of inflammatory cytokines measured in the cord blood in this study and others. Mechanisms explaining why more robust maternal micro-chimerism occurs in the context of inflammation need also to be elucidated, notably because the reverse traffic (fetal cells into the mother) did not appear to be affected. This suggests that at least some of the signals that control cellular movements across the placental barrier are selective and can be affected by inflammation. Overall, this study supports the growing concept that immune dysregulation can begin *in utero*, and it further emphasizes how important it is to precisely understand the interplay between inflammation, maternal micro-chimerism, and the activation of fetal responses.

Acknowledgments

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Figure 1. Inflammation induces activated α-NIMA effector cells that produce pro-labor effector cytokines

Left: typically, in healthy pregnancy, a combination of mechanisms, including a TGF- β rich environment, and immunosuppressive cells, such as CD71+ erythroid cells and Treg, combined with intrinsic DC immaturity, keep fetal dendritic cells (DCs) in a tolerogenic state. Exposed to maternal microchimerism, these DCs induce a predominantly Treg response against non-inherited maternal allo-antigens (NIMA). Right: In contrast, in the context of inflammation-induced prematurity, maternal microchimerism is more pronounced. In addition, fetal DCs are activated by microbial products and/or pro-inflammatory cytokines, present in the amniotic fluid and the fetal circulation. These DCs direct the activation of NIMA-specific effector cells, which produce pro-labor effector cytokines, such as IFN- γ and TNF- α . Although not studied in Frascoli et al, exposure to inflammation may also alter Treg function, further lifting the control of fetal APC function.