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MINI REVIEW

Adrenomedullin and endocrine control of immune cells during pregnancy

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The immunology of pregnancy is complex and incompletely understood. Aberrant immune activity in the decidua and in the placenta is believed to play a role in diseases of pregnancy, such as infertility, miscarriage, fetal growth restriction and preeclampsia. Here, we briefly review the endocrine control of uterine natural killer cell populations and their functions by the peptide hormone adrenomedullin. Studies in genetic animal models have revealed the critical importance of adrenomedullin dosage at the maternal–fetal interface, with cells from both the maternal and fetal compartments contributing to essential aspects underlying appropriate uterine receptivity, implantation and vascular remodeling of spiral arteries. These basic insights into the crosstalk between the endocrine and immune systems within the maternal–fetal interface may ultimately translate to a better understanding of the functions and consequences of dysregulated adrenomedullin levels in clinically complicated pregnancies.
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INTRODUCTION

Pregnancy presents a mysterious immunological paradox that is permitted by the complex, unique immunology of the maternal–fetal interface. We are only beginning to understand how this very specialized immune 'subsystem' differs from the systemic immune system and thus effectively protects the fetus from maternal rejection. For example, the sizable uterine natural killer (uNK) cell and macrophage populations found during early pregnancy are distinctive in their cell surface markers and functions compared to their peripheral counterparts.[1,2] Understanding the activity and control of these immune cell types will shed light on this immunological paradox and possibly inform the pathophysiology of complications of pregnancy.

During the past decade, numerous studies have characterized critical roles for the peptide hormone adrenomedullin (*Adm* gene; AM protein) in the establishment and maintenance of a healthy pregnancy. Here, we discuss the effects of AM on implantation and placentation, concentrating on the control of the uNK cell population and its subsequent involvement in the process of spiral artery remodeling—a necessary process for the maternal vascular adaptation to pregnancy. Importantly, studies addressing the link between AM and uNK cells exemplify an immunological basis for preeclampsia that can be strongly modulated by the maternal and fetal endocrine systems.

AM IS A VERSATILE PEPTIDE HORMONE EXPRESSED BY BOTH MATERNAL AND FETAL TISSUES

Originally isolated from pheochromocytoma extracts,[3] AM is a vasodilatory, angiogenic and anti-inflammatory protein with demonstrated roles in cardiac and lymphatic vascular development and tumor biology.[4,5] AM belongs to the calcitonin/calcitonin gene-related peptide family, which binds various combinations of G-coupled protein receptors and their associated receptor activity modifying proteins. The canonical receptor for AM is calcitonin receptor-like receptor when associated with either receptor activity modifying protein 2 or 3.[6] Estrogen, progesterone and hypoxia, which are all elevated within the placenta throughout pregnancy, are known to dramatically upregulate either *Adm* or AM receptor gene expression in several human and rodent female reproductive tissues including the uterus, ovary and placenta, thus underscoring the significance of AM signaling in female-specific reproductive physiology.[7–12]

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At the organismal level, plasma concentrations of AM are elevated two- to threefold above baseline levels in many disease states, such as cardiovascular, hepatic, renal and pulmonary disease, but interestingly, the largest increase in plasma AM levels occurs during a healthy pregnancy.[13] Whether this physiological elevation occurs during complications of pregnancy remains uncertain. However, polymorphisms in the human *Adm* gene are associated with preeclampsia,[14] and administration of an AM antagonist to pregnant rats caused placental and fetal pathologies.[15] A newly developed assay to detect a proteolytically cleaved precursor of active AM, mid-regional pro-adrenomedullin (MR-proADM), provides an alternative way to quantitate AM in humans and is currently being investigated as a biomarker of cardiovascular disease, pneumonia and sepsis.[16] While data on changes in AM levels in complications of pregnancy have been inconsistent, MR-proADM provides hope that consensus about changes in plasma AM levels during pregnancy complications can be achieved and may potentially be used as a surrogate for the prognostic determination of preeclampsia in early pregnancy.[17]

At the cellular level, many studies have described *Adm* expression in several tissues derived from both the mother and the fetus: ovary, uterus, placenta and fetal membranes.[18–24] For example, just prior to implantation in mice, *Adm* is highly expressed in the trophectoderm cells of the early blastocyst and the luminal epithelial cells of the uterine lining.[20,25,26] Shortly after implantation, and during the rapid expansion of the murine decidua, *Adm* expression is strongly centered within the primary decidual zone, a 3–5 cell-layer thick region surrounding the recently implanted embryo which serves as a temporary and physical barrier to immunological attack.[26] However, *Adm* expression is most enriched in mouse trophoblast giant cells (TGCs) throughout pregnancy, with approximately 30-fold higher levels in differentiated TGCs compared to undifferentiated precursors.[26] Because TGCs are active players in the processes of decidualization, implantation and placentation, this robust expression of *Adm* from these fetal cells implicates AM in many stages of pregnancy.[27] Moreover, TGCs of $Adm^{-/-}$ placentas undergo apoptosis, further suggesting that AM is critical for the survival of these cells that are central to the maintenance of a healthy pregnancy.[28]

MATERNAL-DERIVED AM IS NECESSARY FOR SUCCESSFUL IMPLANTATION AND PLACENTATION

During the generation of gene-targeted $Adm^{-/-}$ mice,[29] which are embryonic lethal by e14.5, it was observed that $Adm^{+/-}$ females had smaller litters than their wild-type counterparts, prompting questions about the fertility of $Adm^{+/-}$ dams. Subsequently, it was demonstrated that wild-type expression levels of *Adm* are important for uterine receptivity in mice during the peri-implantation period, specifically *via* the promotion of pinopode formation—a proxy for uterine receptivity—in the uterine luminal epithelium.[25] Healthy implantation, however, is likely determined by factors beyond uterine luminal epithelium, such as appropriate tempering of maternal immunity. Based on the interaction of AM with its anti-inflammatory binding partner, complement factor H, one could speculate that AM is also important for preventing an immune attack on the embryo during the peri-implantation period.[25]

Embryos that ably implant in $Adm^{+/-}$ uteri often do so unevenly both within and between uterine horns.[26] It is possible that this $Adm^{+/-}$ implantation phenotype is due in part to changes in ciliary beat frequency in the oviduct.[30,31] Embryos of $Adm^{+/-}$ dams are also more likely to die or demonstrate abnormalities symptomatic of poor placental perfusion during the development of the placenta between e9.5 and e12.5.[26] Furthermore, pathological placental morphologies observed in embryos developing within $Adm^{+/-}$ uteri exhibit aberrant invasion of *Adm*-expressing TGCs into the decidua.[26] Collectively, these studies in $Adm^{+/-}$ female mice demonstrate that the expression and dosage of maternal AM is a critical determinant for establishing normal uterine receptivity and enabling proper implantation.

LACK OF FETAL-DERIVED AM CONFERS PLACENTAL VASCULAR PATHOLOGIES AKIN TO PREECLAMPSIA POTENTIALLY *VIA* AN IMMUNE-BASED MECHANISM

Examination of $Adm^{-/-}$ mouse placentas and their vascular abnormalities revealed a direct link between fetal-derived AM and placental immunology. Notably, the placenta-perfusing spiral arteries of $Adm^{-/-}$ placentas are abnormally invested with a thick layer of vascular smooth muscle cells at e13.5.[28] By contrast, in wild-type placentas from neighboring littermates, these vascular smooth muscle cells have undergone apoptosis during the process of spiral artery remodeling, which transforms the spiral arteries into large, high-capacitance vessels associated with the migration of fetal trophoblast cells into the arteries.[32] Importantly, insufficient spiral artery remodeling has been implicated in the pathophysiology of preeclampsia. Therefore, the preeclampsia-like phenotypes of $Adm^{-/-}$ placentas recapitulate prior evidence of dysregulation of AM levels in complications of pregnancy.[33]

Because uNK cells are the largest population of decidua-specific immune cells and are established effectors of spiral artery remodeling, uNK cells were counted in $Adm^{-/-}$ mouse placentas and found to be fewer in number compared to wild-type placentas.[28] This reduction in uNK cell content was not associated with apopotosis but rather with under-recruitment of uNK cells to the decidua. Ovarian transplants ($Adm^{+/-}$ ovaries carrying *Adm*-null germ cells were placed in wild-type recipient females) further confirmed that the $Adm^{-/-}$ placental immune and vascular phenotypes were due to the loss of *fetal*-derived AM and independent of the genotype or dosage of *Adm* from the dam.[28] This conclusion highlights a critical function for fetal-derived AM as an essential signal to elicit changes to the maternal vasculature during pregnancy.[26]

AM RECRUITMENT OF UNK CELLS IS DOSAGE-DEPENDENT

Subsequent studies of a gene-targeted murine model of *Adm* overexpression (Adm^{hi} allele, which expresses *Adm* at levels three-fold higher than the wild-type allele) determined that uNK cell recruitment to the decidua by AM is dosage-dependent.[5,28] Specifically,

$Adm^{hi/hi}$ placentas demonstrated a 30% increase in uNK cells.[28] Because the uNK cells in these placentas were labeled with *Dolichos biflorus* agglutinin lectin, we can conclude that the uNK cell population that was quantified is, in fact, the one that expands during pregnancy.[34] Debate continues about whether this uNK cell population is derived from extra-uterine precursors that home to the uterus and differentiate *in situ* early in pregnancy or whether uNK cell precursors mature outside the uterus and then migrate to the uterus due to hormonal cues like AM.

Given the active participation of uNK cells in spiral artery remodeling as well as the uNK cell and vascular phenotypes of $Adm^{-/-}$ and $Adm^{hi/hi}$ placentas,[35] it stood to reason that AM could augment the effects of uNK cells on vascular smooth muscle cells. Indeed, treatment of primary mouse vascular smooth muscle cells with uNK cell-conditioned media caused changes in cell morphology and induced apoptosis;[28] these processes were enhanced when the uNK cell-conditioned media was supplemented with AM, suggesting that AM is important not only for the recruitment but also for the activation of uNK cells.[28]

AM DOSE-DEPENDENTLY STIMULATES THE EXPRESSION OF UNK CELL-SECRETED SIGNALING MOLECULES

uNK cells produce an array of cytokines and chemokines that engage these cells in a complex dialogue with trophoblast cells to execute spiral artery remodeling.[35,36] Therefore, it was expected that the dynamic fluctuations in uNK cell population size between $Adm^{-/-}$ and $Adm^{hi/hi}$ placentas would be mirrored by concomitant changes in the expression profile of these signaling molecules. Indeed, $Adm^{-/-}$ downregulated and $Adm^{hi/hi}$ upregulated Ccl7, Ccl17, Cxcl10, Xcl1 and tumor necrosis factor.[28] Concordantly, *in vitro* stimulation of isolated uNK cells by AM upregulated select signaling factors, including matrix metalloproteinase 9, which is involved in spiral artery smooth muscle cell apoptosis.[28] Given the diversity of angiogenic factors, growth factors, and other signaling molecules that are secreted by uNK cells, it is plausible that there are signaling cascades other than the ones already identified that are regulated by AM.

FUTURE DIRECTIONS

Collectively, these data support AM as a player in the pathophysiology of reproductive disorders *via* control of the uNK cell population size and of trophoblast invasion into the uterine luminal epithelium and into uterine spiral arteries. While perturbations in the size of the uNK cell population have been implicated in a variety of human reproductive disorders,[37,38] it has also been found that women with larger cytotoxic $CD56^{dim}CD16^{+}$ uNK cell populations are at a higher risk for infertility and recurrent pregnancy loss.[39] Therefore, not only the uNK cell population size is important, but also the delicate balance between the peripheral blood-like, cytotoxic $CD56^{dim}CD16^{+}$ uNK cells and the 'true' $CD56^{bright}CD16^{-}$ cytokine- and chemokine-producing uNK cells.[39] As argued by several groups, it is premature to base clinical decisions on information about size or type of uNK cell populations in patients.[38,40]

It will be interesting to determine whether AM exerts endocrine control over other immune cell populations at the maternal–fetal interface, such as decidual macrophages. There is evidence that AM can confer a semimature phenotype to dendritic cells, which provide instructions to T cells, but this may be of little consequence to placental immunology given the paucity of dendritic cells in the decidua.[41] Of course, it is also possible that other calcitonin/calcitonin gene-related peptide family peptides affect uNK cell recruitment. Adrenomedullin 2, also known as intermedin, has been shown to stimulate trophoblast invasion and therefore may participate in assemblage of the uNK cell population.[42,43]

CONCLUSIONS

Altogether, the aforementioned studies point to the complexity of the control of immune cells specific to the transient environment of the pregnant uterus. Here, we have emphasized that both maternal- and fetal-derived AM is important for establishing and maintaining a successful pregnancy. Specifically, haploinsufficiency for AM and lack of AM confer shallow trophoblast invasion into the uterine luminal epithelium during implantation and into spiral arteries, respectively. These observations have recently been recapitulated *in vitro*, whereby AM stimulates trophoblast invasion.[44] Future studies will aim to address the local control of AM dosage with the eventual goal of being able to exogenously control AM levels to promote a healthy pregnancy.

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