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Review: hCG, Preeclampsia and Regulatory T cells

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Abstract

Human chorionic gonadotropin (hCG) is crucial for successful pregnancy. Its many functions include angiogenesis and immune regulation. Despite years of research, the etiology of preeclampsia remains unknown. Marked by insufficient trophoblast invasion and poor spiral artery remodeling, preeclampsia has also been linked to immune dysregulation. Here we discuss the roles of hCG in the context of endovascular cross-talk between trophoblasts and endothelial cells and immune tolerance. We propose that functional and glycosylation modifications of hCG may contribute to the pathogenesis of preeclampsia.

Keywords

Uterine natural killer cells; human chorionic gonadotropin; preeclampsia; T regulatory cells; angiogenesis

1. Introduction

Pregnancy is a dynamic process characterized by immune tolerance, angiogenesis and hormonal regulation. Human chorionic gonadotropin (hCG) can be detected on the first day of implantation; its levels peak around gestational week 12 and diminish to low levels during the remainder of pregnancy [1]. hCG has many important functions in pregnancy, including the promotion of progesterone production, implantation and decidualization, angiogenesis, cytotrophoblast differentiation, and immune cell regulation (reviewed in [2]). With these myriad functions in mind, hCG dysregulation could lead to adverse pregnancy outcomes. Preeclampsia is a condition marked by insufficient trophoblast invasion and maternal spiral artery remodeling [3]. Recent studies have established a link between preeclampsia and immune cell dysregulation, including reduced numbers of uterine and circulating regulatory T cells (Tregs) and natural killer (uNK) cells [4,5]. It is thus possible that alterations in hCG production or function could contribute to the development of preeclampsia.

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2. hCG Variants

hCG is composed of α- and β-subunits each consisting of a protein backbone with N-linked and O-linked oligosaccharides. It is now believed that there are four distinct variants: hCG, hyperglycosylated hCG (HhCG), the free β-subunit, and pituitary hCG [2]. Pituitary hCG is an intriguing phenomenon and is a subject of extensive debate in menopausal women. Low levels of hCG are detected during the preovulatory surge of luteinizing hormone (LH) [6]. These hCG forms can be further modified by partial degradation of the hCG molecule, nicking of the intact β-subunit, or variation of the attached oligosaccharides [7]. Variations in the number and type of these sugar branches result in significant differences in the molecule with important clinical implications. These variants play different roles in both normal and abnormal pregnancy [8]. HhCG, which has complex β-subunit N- and O-linked oligosaccharides structural alterations, is produced early during pregnancy; it does not have high affinity to LH/hCG receptors, yet promotes invasion and growth of cytotrophoblasts by interacting with transforming growth factor (TGF) β receptors [2,9–13]. After the first three to four weeks of pregnancy, the levels of HhCG become very low and hCG is the major form [2]. Recent studies have reported additional variants with distinct sialylated oligosaccharides of the Lewis type pattern on hCG isolated from serum of pregnant women or choriocarcinoma cell lines. Differential expression of such carbohydrates is associated with inhibition of E-selectin-mediated homing of leukocytes and may contribute to early pregnancy loss through poor placental-immune interactions [14–16]. It is apparent that in the time between placentation and parturition, a dynamic structural conversion of one form of hCG to alternate forms of hCG is choreographed. This suggests that impairment or alterations in hCG glycosylation patterns may affect its signaling and biological activities.

3. hCG, angiogenesis and immune tolerance

The maternal-fetal interface is replete with immune cells which cross-talk with hormonal, endocrine, and angiogenic regulators to program a normal pregnancy outcome. Among immune cell types, regulatory T cells (Tregs), a specialized CD4 T cell subset phenotyped as $CD4^+/CD25^+/Foxp3^+$, play an important role in protecting the fetus by dampening harmful inflammatory immune responses at the maternal-fetal interface. It has been shown in humans [17] that Treg numbers increase very early in pregnancy, peak during the early second trimester and then begin to decline until they reach pre-pregnancy levels. Tregs have also been shown to be crucial in immune tolerance of the fetus in the mouse pregnancy model [18] and also follow a gestational age-dependent presence in the uterus. Animal studies further indicate that tolerance to paternal antigens may be initiated during mating when seminal fluid and components of semen have been shown to trigger expansion of the Treg cell population [19]. Further, it has been shown that Tregs migrate toward areas of hCG production [20], indicating that in normal pregnancy, these cells may be attracted to hCG produced by trophoblasts at the maternal-fetal interface ensuring immune tolerance of the fetus. However, if hCG undergoes dysregulation during pregnancy, its control over immune tolerance pathways may be impaired.

Interleukin-10 (IL-10) and the tryptophan-metabolizing enzyme indoleamine 2,3-dioxygenase (IDO) are two important immune regulators. Levels of IL-10, a key immunosuppressant, increase in early pregnancy and remain elevated until the onset of labor [21], possibly regulating maternal immunity and allowing acceptance of the fetal allograft. As shown by our studies, IL-10 can regulate uNK cell maintenance and control their cytotoxic functions in response to pro-inflammatory challenges during pregnancy [22,23]. Further, decidual Tregs can inhibit immune stimulation of T cells through IL-10 production [24]. The temporal expression of IDO regulates the Tregs and prevents them from being converted to pro-inflammatory Th17 (T helper 17) cells [25]. hCG is able to stimulate IL-10

production in bone marrow derived dendritic cells (BMDC) from mice [26]. This same study found that treatment of BMDC with hCG and interferon gamma (IFN-γ) increased IDO mRNA production and enzyme activity, raising the question of whether hCG can stimulate IL-10 production and IDO activity in trophoblasts as well. Our unpublished results suggest that hCG can rescue pregnancy in IL-$10^{-/-}$ mice by subverting production of anti-angiogenic factors and by replenishing uterine immune cells.

It is noteworthy that hCG is now considered as an angiogenic factor [27,28] and thus may regulate an endovascular cross-talk between trophoblasts, endothelial cells, and immune cells represented by uNK cells. These specialized cells have been shown to play an important role in spiral artery remodeling and trophoblast invasion at least in animal studies [29,30]. We have recently demonstrated that vascular endothelial growth factor C (VEGF C) production by uNK cells is responsible for their non-cytotoxic activity, and that VEGF C producing uNK cells support endovascular processes *in vitro* [31]. It is possible that the tolerogenic phenotype of uterine NK cells during early decidualization is be influenced by hCG through stimulation of the quiescent angiogenic machinery. Recent studies indicate that the uNK cells are indeed influenced by hCG. Kane *et al* showed that hCG induces proliferation of human uNK cells, by interacting through the mannose receptor rather than the LH/hCG receptor [32]. Importantly, deglycosylated hCG was not able to bind to mannose receptors on uNK cells, again emphasizing the importance of carbohydrate patterns in the function of hCG.

4. hCG and preeclampsia

Preeclampsia, diagnosed by hypertension and proteinuria after 20 weeks of gestation, affects 5–10% of all pregnancies and remains a leading cause of maternal and fetal morbidity and mortality. Although based on clinical presentation, preeclampsia is considered as a late pregnancy disorder, but the molecular events leading to its onset seem to occur early in pregnancy. Portrayed as a two stage disorder, maternal symptoms of preeclampsia are considered to be consequences of pre-clinical placental pathology associated with poor placental perfusion, inflammation, ischemia/hypoxia, and trophoblast damage [33]. Despite the pro-angiogenic role of hCG, little is known about the endovascular interactions of trophoblasts and endothelial cells and its subsequent effects on spiral arteries especially in the presence of different forms of hCG. Recently we showed that injection of preeclampsia serum in pregnant IL-$10^{-/-}$ mice results in hypertension and proteinuria [34]. Importantly, the treatment also led to a perturbed immune cell population at the maternal-fetal interface. Interestingly, we found higher hCG levels in preeclampsia serum at term as compared to normal pregnancy serum [35]. It is possible that altered glycosylation patterns and/or presence of sialyl Lewis antigens on hCG in preeclampsia influences the recruitment and/or expansion of tolerance-imparting immune cell populations. Several studies have reported a decrease in Treg cell population both in the circulation and in placental bed sections in preeclamptic women as compared to those with normal pregnancy [3,36–38]. Since IL-10 and hCG are central to normal pregnancy outcome, it is tempting to speculate that deficiency in these molecules may predispose to severe preeclampsia pathology. Animal studies from our lab suggest that IL-10 deficient mice are more sensitive to serum- and hypoxia-induced onset of preeclampsia-like features, implying that IL-10 is likely to play a protective role against preeclampsia [34,39,40]. Preeclampsia serum [35] and 9.5% oxygen also affect uterine Treg numbers (unpublished observations). Thus given the functional associations co-regulated by hCG, IL-10 and Treg migration, it is possible that dysregulated hCG can have similar effects on uterine Tregs and contribute to preeclampsia (Fig. 1).

5. Conclusions

Preeclampsia is a disease of poorly understood etiology. Research continues to identify factors that contribute to its onset as a heterogeneous disease. hCG appears to be involved in many aspects of angiogenesis and immune tolerance (Fig. 2), prompting us to suggest that dysregulation of hCG could lead to pregnancy complications such as preeclampsia. This dysregulation could be either in the form of altered levels of hCG or modifications of the oligosaccharide side chains which have been proven to be vital in the function of hCG. Nevertheless, ramification of dysfunctional hCG that may occur in preeclampsia is likely to affect immune tolerance and angiogenesis, two vital features of successful pregnancy outcomes and needs further research. If it is indeed demonstrated that hCG plays a role in preeclampsia, it could prove to be a useful target for therapeutic intervention.

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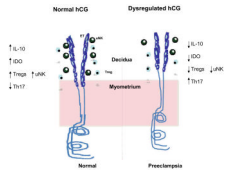

Fig. 1.

Dysregulation of hCG may lead to preeclampsia. In normal pregnancy, functional hCG, optimum expression of IL-10, and the temporal appearance of uNK and Tregs contribute to immune tolerance and angiogenesis. Dysregulated hCG due to altered structure/ glycosylation may influence expression of IL-10, IDO and appearance of Tregs and uNK cells resulting in pregnancy complications such as preeclampsia. hCG: human chorionic gonadotropin, IL-10: interleukin-10, IDO: indoleamine 2,3-dioxygenase, uNK : uterine natural killer cells, Treg: regulatory T cells, ET: endovascular trophoblasts.

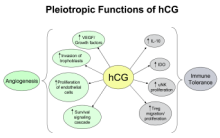

Fig. 2.

Angiogenic and immune tolerance function of hCG. hCG has many angiogenic functions such as stimulation of VEGF production, trophoblast invasion and proliferation of endothelial cells. By stimulating IL-10 and IDO expression, promoting uNK cell proliferation and Treg cell migration, hCG may play a role in immune tolerance at the maternal fetal interface. VEGF: vascular endothelial growth factor, hCG: human chorionic gonadotropin, IL-10: interleukin-10, IDO: indoleamine 2,3-dioxygenase, uNK: uterine natural killer cells, Treg: regulatory T cells.