Wandering womb, water and worms: the history, myths and messy immunologic etiology of preeclampsia/eclampsia

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Introduction:

"When the womb remains in the upper abdomen, the suffocation is similar to that caused by the purgative hellebore, with stiff breathing and sharp pains in the heart. Some women spit up acid saliva, and their mouths are full of fluid, and their legs become cold. In such cases, if the womb does not leave the upper abdomen directly, the women lose their voices, and their head and tongue are overcome by drowsiness. If you find such women unable to speak and with their teeth chattering, insert a pessary of wool, twisting it round the shaft of a feather in order to get it in as far as possible-dip it either in white Egyptian perfume or myrtle or bacchar or marjoram. Use a spatula to apply black medicine (the kind you use for the head) to her nostrils. If this is not available, wipe the inside of her nostrils with silphium, or insert a feather that you have dipped in vinegar, or induce sneezing. If her mouth is closed tight and she is unable to speak, make her drink castoreum in wine. Dip your finger in seal oil and wipe inside her nostrils. Insert a wool pessary, until the womb returns, and remove it when the symptoms disappear. But if, when you take the pessary out, the womb returns to the upper abdomen, insert the pessary as you did before, and apply beneath her nostrils fumigations of ground-up goat or deer horn, to which you have added hot ashes, so that they make as much smoke as possible, and have her inhale the vapour up through her nose as long as she can stand it. It is best to use a fumigation of seal oil: put the coals in a pot and wrap the woman up-except for her head. So that as much vapour as possible is emitted, drip a little fat on it, and have her inhale the vapour. She should keep her mouth shut. This is the procedure if the womb has fallen upward out of place ..."

This description of a woman having an intrapartum seizure was state of the art during the time of Hippocrates in the late 5th and early 4th centuries BCE. In this passage from his treatise Diseases of Women, Hippocrates illustrates a woman in the throes of a seizure. According to Hippocrates, the treatment of such a clinical presentation would include applying black medicine, a feather, or a pessary with ground up goat or deer horn to her nostrils. Although such a treatment may seem absurd based on today's understanding of preeclampsia/eclampsia, it was based on the scientific background of the time. Many discoveries have fine-tuned our

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understanding of the complex pathogenesis of preeclampsia/eclampsia. Yet, much like the ancient Greeks, our understanding of this heterogeneous disease is still very incomplete. This paper will examine the development of the modern conception of preeclampsia/eclampsia, its surrounding myths, and the current understanding of its etiology.

A Brief History and Myths of Preeclampsia/Eclampsia:

During the time of Hippocrates, health was considered a result of the balance of the four major humors: blood, phlegm, yellow bile, and black bile. A balance of wet and dry was also important to specifically female physiology. It was believed that due to the soft, "wetter" nature of female skin, women were at higher risk of too much moisture leading to an increase in humors and consequent sickness. In addition to this fluid imbalance, it was also proposed that women got sick due to a wandering womb. In disease states, this wandering womb would leave the pelvis to seek what it was missing. In the case described above, Hippocrates held that the dry uterus wandered the body in search of moisture. This gave rise to the treatment of fumigations of ground goat or deer so the fumigations could provide the much needed moisture. Central to this idea of the wandering womb, is that in its movement around the female body it would cause damage to surrounding organs such as the liver, spleen and lungs. Further treatment of female maladies was aimed at balancing the fluids or humors through blood letting and diets. To maintain the balance, Hippocrates held that women needed to be pregnant, lactate, and menstruate.[1]

Essentially, versions of the humor/ fluid balance theory of female disease and specifically preeclampsia were maintained until the seventeenth century. The term eclampsia first appeared in the medical lexicon in 1619 in Varandaeus' work on gynecology. During the late 1600s and early 1700s, Francois Mauriceau was the first to describe eclampsia in its modern conception. He held that puerperal convulsions were due to decreased lochial flow or intrauterine fetal death. Even at this time, the Mauriceau pathophysiology of convulsions due to intrauterine fetal death was a result of the foul fluid from the dead fetus. Consequently, prevention of preeclampsia and eclampsia was still through a fluid correction via phlebotomy.[1]

Despite the continued treatment by phlebotomy during the 1800s, the 18th and 19th centuries are when the modern phenotype of preeclampsia/eclampsia began to truly take shape. In 1849, Dr. Williams Tyler Smith published Parturition and the Principles and Practice of Obstetrics. In this work, Smith held that pregnancy related convulsions were due to many causes such as mechanical or emotional neurologic stimuli, bleeding, environmental changes, irritation of the gastrointestinal or genitourinary tracts, and toxic substances in the body. These toxic substances irritate the central nervous system causing *toxemia*, a term some still use to describe preeclampsia. As depicted in Table 1, the late 18th and the 19th

centuries ushered in discoveries that truly have shaped the modern definition of preeclampsia.[1]

Table 1: The Development of the Modern Definition of Preeclampsia/Eclampsia in the 18th and 19th Centuries

Year	Author	Discovery
1797	Demanet	Connection between edema and eclampsia
1843	Lever	Proteinuria in eclamptics
1843	Johns	Preeclamptic symptoms in late pregnancy described such as headache, blurry vision, abdominal pain and edema
1897	Vaquez and Nobecourt	Ecclamptic hypertension

Although the phenotype of preeclampsia/eclampsia was taking shape during this time, it was not until the mid-20th century when the placental pathophysiology of preeclampsia began to take shape. It was during the 1960s that multiple groups demonstrated that placental trophoblast cells were unable to invade and convert high resistance spiral arteries into high capacity conduit vessels thereby causing hypoxia and decreased blood flow to the fetal-placental unit. As will be discussed later in this paper, there are many theories as to the modern molecular pathogenesis of preeclampsia. Yet, this placental dysfunction lies at the crossroads of all these theories.

Despite modern advances in the 20th century, medical science has not fully avoided foibles in the investigation of the cause of this disease. In 1983, 2 reports were published in the *American Journal of Obstetrics and Gynecology* suggesting a possible worm-based cause for the development of preeclampsia. Aladjem et al. reproduced a preeclampsia phenotype in pregnant beagles including hypertension, hypertensive retinopathy, proteinuria, liver dysfunction, glomerular endotheliosis, disseminated intravascular coagulation, fetal growth restriction and fetal death. They achieved this phenotype by intraperitoneally inoculating these pregnant dogs with placental extracts from human placentas that were affected by preeclampsia/eclampsia or gestational trophoblastic disease. A key finding in these placentas was the finding of a worm-like structure termed "*Hydatoxi lualba*".[2] This was observed in the first paper from the same group by Lueck et al demonstrating these worm-like forms in these diseased placentas. In the Lueck paper they describe 150 micrometer long larva forms with premordial eggs measuring 7-43 micrometers all of which are found in peripheral blood and placental tissue of preeclamptics and those with gestational trophoblastic disease by toluidine blue O staining. This description of these worm-like structures were held in comparison to other helminths such as hookworms, tapeworms, and roundworms. They purported the discovery of a biologically active, novel worm species associated with the development of these placentally-based diseases.[3]

Although an inflammatory, immunologic basis for the pathogenesis of preeclampsia is at the forefront of all the causes of preeclampsia, this apparent worm infestation was not a cause of this devastating disease. Subsequent articles investigate the origins of these worm-like structures. After centrifugation of peripheral blood and cord blood and passage through small filters, these "worms" could not be isolated through usual techniques. It was concluded that these worm-like structures were an artifact of the toluidine blue O staining process.[4,5] Therefore, worms are not the cause of preeclampsia.

The story of worms and preeclampsia highlights the importance of the rigor that must be taken in demonstrating a cause for preeclampsia. As research techniques have advanced beyond basic histology, the complexity of the multiple molecular processes involved in the development of preeclampsia make its investigation challenging in determining the causative from the correlative. Yet, breakthroughs in the molecular etiology of preeclampsia have expanded our knowledge of its cause and are pathways to future predictive and therapeutic modalities.

The Immunologic Etiology of Preeclampsia:

In 1966, T.N. Jeffcoate in the *Proceedings of the Royal Society of Medicine,* coined the term "the disease of theories" when describing the multiple causes of preeclampsia.[6] This description of preeclampsia still rings true as there are multiple molecular mechanisms that have been linked to the development of preeclampsia without a singular unifying, initial molecular cause. Endothelial cell dysfunction occur through alterations in the usually peripheral vascular resistance lowering milieu of pregnancy. This altered milieu includes changes in relaxin, the endothelin B- nitric oxide pathway, and matrix metalloproteinases which all have been associated with preeclampsia. Increases in reactive oxygen species from multiple sources and a dysfunction of normal antioxidant enzymes help contribute to the endothelial cell dysfunction in preeclampsia. Furthermore, the recent elaboration of dysregulated angiogenesis and the role of soluble vascular endothelial growth factor receptor in preeclampsia has placed a new emphasis on the vascular etiology of preeclampsia. Despite these multiple mechanisms, placental dysfunction remains at the core of preeclampsia.[7] Yet, how does the abnormal differentiation and invasion of placental trophoblasts and decreased flow in the placenta initiate?

There is much data to support that preeclampsia may be initiated by an immune, rejection-like response to the feto-placental unit. Among these observations is the finding that multiparous women who conceive by the same partner are at lower risk for developing preeclampsia.[8-10] Because different paternity is associated with an increased risk to that of primiparity,[11] this finding suggests that a tolerance to the antigen develops as a consequence of a prior pregnancy. This antigen is the paternal contribution to the fetus. In cases of in vitro fertilization in which the male was functionally aspermic, and therefore the mothers have never had

exposure to the "antigen," a three-fold increase in the incidence of preeclampsia was observed when women in this cohort were implanted with embryos fertilized with the surgically removed sperm of their partners.[12] In cases in which donor sperm or oocytes are used and there is no protection from self-antigens, there is also a significantly increased rate of preeclampsia.[13,14] Although somewhat controversial, lower rates of preeclampsia have been found by some groups in immunocompromised HIV+ women.[15] These rates approach the expected rate when women are treated with triple antiretroviral therapy.[16]

Unfortunately, defining the role of immune system mechanisms responsible in preeclampsia has proven elusive because of the many components involved. One common-held theory is that an immune system response results in damage to the endothelial cells of the microvascular system resulting in the multiorgan phenotype of preeclampsia.[17] This endothelial cell damage may also originate from endothelial cells themselves. Recent cell culture data have demonstrated that endothelial cells from nonplacental origins can release IL-6, secondary to the phagocytosis of necrotic trophoblasts, which can cause further endothelial cell activation. These data suggest how endothelial cell phagocytosis of necrotic trophoblasts at a distant site, such as pulmonary capillaries, may cause downstream activation of endothelial cells at the local and placental level.[18] This damage may occur as a consequence of exposure of the vessels to other cytokines causing an inflammatory response or triggering apoptosis.

What triggers the immune system response? What factors damage the vessels? Are multiple pathways involved? These questions are the research interests of many labs. The answers will be critical to designing drugs to specifically target the immune triggers of preeclampsia without disabling the entire immune system.

Among these studies are animal models that mimic preeclampsia. In a mouse model established by Zenclussen *et al.*,[19] Zenclussen and colleagues transferred activated Th1-like cells into pregnant mice causing the development of preeclamptic symptoms such as elevated blood pressure and proteinuria. Notably, these findings only developed when cells were transferred into pregnant mice. There was no hypertension and elevated proteinuria in non-pregnant mice.

One immune system factor that appears to be involved in the etiology of preeclampsia is indoleamine 2, 3-dioxygenase (IDO). IDO is an enzyme involved in the degradation of L-tryptophan, an essential amino acid. Depleting T cells of tryptophan inhibits their expansion due to an arrest in the cell cycle at the G1 phase.[20-22] In mice, a maternal T-cell reaction against paternal antigens was induced when IDO was pharmacologically inhibited[23] and fetal rejection occurred [24] indicating that IDO may be critical for tolerance of the fetus. The mean number and the developmental status of the syngeneic embryos were not affected. In contrast, allogeneic concepti in IDO inhibitor treated dams showed increased

hemorrhage, inflammation, and degeneration with the death of all allogenic concepti by 9.5 days post conception. They hypothesize that with the interruption of tryptophan catabolism by the addition of the IDO inhibitor, T-cell mediated destruction of paternal antigen possibly leads to the rejection of the allogeneic fetuses. Using semi-quantitative immunohistochemistry, Santoso et al.,[25] demonstrated that significantly less IDO was expressed in placentae of term preeclamptic women than in control placentae. IDO staining was present in the endothelial cells of most capillaries and some arteries of the villi in all placentae. In addition to its role in suppressing CD8+ T cells, IDO also reduces oxygen free radicals in the placenta by utilizing it to cleave L-tryptophan to form formylkynurenine. The reduced levels of IDO in preeclamptics may prove to be one of the key links between the immune response and oxidative damage of preeclampsia.

Other studies have examined more global indicators of imbalances in the immune system during preeclampsia. Jonsson et al.[26] found that women with preeclampsia had decreased levels of spontaneous interleukin (IL)-5 secretion. Interestingly, IL-10 secretion in response to paternal antigens was significantly decreased in women with preeclampsia. IL-10 has potent anti-inflammatory effects. In addition, decreased numbers of basophil granulocytes were found in preeclamptics. The decreased secretion of IL-5 and basophils indicate a decrease in systemic T helper type 2 (Th2)-like responses. Some have hypothesized that there is a Th2 shift in normal pregnancies because of the strong humoral response.[27] A Th1 response, including the presence of cytokines such as IL-2, interferon-gamma (IFN-gamma), and tumor necrosis factor-alpha (TNF-alpha) leads to the destruction of trophoblast *in vitro* and the loss of pups in pregnant mice.[19] In normotensive pregnant women, stimulated peripheral blood mononuclear cells (PBMC) produce predominantly Th2 cytokines (IL-4, IL-10); whereas, Th1 cytokines (IFN-gamma) are predominantly produced from women experiencing preeclampsia.[28] The presence of a Th1 phenotype may be important in the development of preeclampsia.

Preeclampsia has also been associated with changes in the levels of other inflammatory cytokines in serum. Luppi et al., investigated plasma levels of IL-6 as a marker of leukocyte activation.[29] They found significantly increased levels of IL-6 in preeclamptic women. Suggestive of a role in the endothelial dysfunction of preeclampsia, chronic infusion of IL-6 was found to affect the contraction and relaxation of systemic vessels in pregnant rats.[30,31] Consistent with the increase in IL-6, they found increased nuclear translocation of nuclear factor kappa-B (NF-κB). The strongly conserved IL-6 promoter sequence contains consensus binding sites for NF-κB.[32] Identification of immune system signals and their pathways will provide targets for therapeutic intervention in preeclampsia. The evidence of increased immune system signaling in response to the allogenic fetus begs the question: what serves as the initial antigen? Fetal trophoblast cells are a likely

immunogen. These cells lack HLA expression preventing them from being recognized by maternal CD8+ T cells; however, they are susceptible to lysis by natural killer (NK) cells.[33] The replenishment of the syncytiotrophoblast also causes the release of fetal debris into the maternal circulation.[34] *In vitro* studies have found that interactions between vesicles from syncytiotrophoblasts and human umbilical endothelial cells results in neutrophil activation.[35,36] Furthermore, these microparticles are able to stimulate primed peripheral blood mononuclear cells to produce cytokines including IFN-gamma and TNF-alpha, which are part of Th1 response.[37] Functional differences between the syncytiotrophoblasts microparticles released in normotensive and preeclamptic pregnancies have not been delineated.[38] Other fetal material, such as RNA, DNA, or other cell types can also be found in the maternal circulation.[39-42] The number of fetal erythrocytes in the maternal circulation has been found to be elevated in preeclamptic women versus normotensive pregnant women.[43,44] The concentration of fetal material may be significant to whether the maternal immune system tolerates the semi-allogeneic fetus or initiates a strong response against the fetus.

Groups have also investigated an increase of antibodies in preeclamptic women that is not found in women with normal pregnancies. Autoantibodies against angiotensin receptor-1 (AT_1) in the IgG fraction of sera from preeclamptic women will induce preeclamptic symptoms in pregnant mice. These effects were not seen using samples from normotensive women. The AT_1 autoantibodies induce excess activation of the AT_1 receptor which can cause hypertension through the interaction of the receptor with its ligand angiotensin II, a known mediator of elevated blood pressure. Blocking the AT_1 receptor reduced the effects in mice. The maternal antibodies that activate AT_1 may mediate some of the cellular changes observed during preeclampsia.[45] Thway *et al*. examined the ability of IgG from 16 severely preeclamptic patients to activate AT_1 receptors and to increase free intracellular calcium.[46] All of these samples activated the receptors and increased intracellular calcium in more than 50% of Chinese hamster ovary (CHO) cells. Immunoglobulin G (IgG) from normotensive pregnant women caused an activation of the AT_1 receptor in less than 20% of CHO cells. When intracellular calcium mobilization occurred in the CHO cells exposed to IgG from normotensive women (in 4 of 14 samples), it was not mediated through AT_1 receptor activation. Increased calcium can affect gene expression, platelet activation, and vasoconstriction. However, it is unlikely that these antibodies are the initial causative agent for preeclampsia as they have also been found in normotensive pregnant women without preeclampsia.[47] Nevertheless, these antibodies affect vasoconstriction and pharmacologic inhibition of AT_1 antibodies may aid in the treatment of preeclampsia.

It has also been hypothesized that the immune mediated preeclamptic pathophysiology is triggered by an extra-fetal source. Some groups have

suggested that infection may play a role in the development of pre-eclampsia. A recent systematic review and meta-analysis found an increased risk of preeclampsia in pregnant women with a urinary tract infection and periodontal disease.[48] Our own data demonstrate no significant difference between the overall intrapartum infection rates of control and preeclamptic parturients. However, we did identify significant differences when patients were stratified by soluble Heat Shock Protein 60 (s-HSP60) protein and antibody status. Heat Shock Protein 60 is a chaperone protein that is known to stimulate inflammatory and cytokine pathways in response to infectious stimuli. A higher intrapartum infection rate in preeclamptics versus controls who were positive for s-HSP60 protein and s-anti-HSP60 antibody. In addition, preeclamptic mothers who have sera positive for s-HSP60 have a significantly higher rate of intrapartum infection in comparison to those in their preeclamptic cohort who are sera negative. Further, preeclamptic mothers who have sera positive for *both* s-HSP60 and s-anti-HSP60 antibody also have a significantly higher rate of intrapartum infection in comparison to other preeclamptics. This relationship is not observed in preeclamptic mothers who are sera positive for s-anti-HSP60 antibody alone.[49] These types of differences in inflammatory and cytokine immunology underscore the importance of immunology in the pathogenesis of preeclampsia.

In order to better understand how the immune system affects pregnancy outcomes, it is important to understand the interface between mother and fetus. During implantation and placentation, the decidua is comprised of almost 40% immune cells. Of these, NK cells are most abundant; they represent approximately 70% of the immune cells.[50] These NK cells vary from the majority of peripheral blood cells in that their phenotype is cytokine producing and not cytotoxic.[51] Hanna *et al*. demonstrate that decidual NK cells, but not peripheral blood-derived NK cells can regulate trophoblast invasion through the production of IL-8 and interferon-inducible protein 10 (IP-10) chemokines. In addition, the decidual NK cells secrete several angiogenic factors, such as vascular endothelial growth factor and placental growth factor, and induce vascular growth in the decidua. Both the activating and inhibitory receptors of the NK cells and their ligands are important in establishing proper invasion and vascularization of the placenta. The ligands to the NK receptors may be provided by maternal stromal cells and trophoblast cells. In normal, healthy pregnancies decidual NK cells may be a key immune system factor in regulating placental development. Further studies of NK cells in preeclamptic women will be necessary to elucidate how these cells are different and to develop potential therapies to restore their beneficial function in pregnancy.

Conclusion:

The understanding of the underpinnings of preeclampsia has undergone steady change throughout history. From a wandering uterus, the unbalance of humors, worms to the current molecular theories on the cause of preeclampsia, each step in understanding must be

made with careful consideration of what is associated versus what is truly causative. It is increasingly evident that preeclampsia/eclampsia is a disease process that is heterogenous in nature. Moving forward, the challenge to researchers is to find novel predictive, therapeutic, and possibly curative targets that will address either the root cause of preeclampsia or the multitude of complex molecular processes involved in its development. Without this approach, clinicians will be left with a limited treatment arsenal in battling this "disease of theories."

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