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Inflammation in Reproductive Disorders

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Abstract

Inflammatory disorders account for a significant percentage of gynecologic disease, particularly in reproductive age women. Inflammation is a basic method by which we respond to infection, irritation, or injury. Inflammation is now recognized as a type of nonspecific immune response, either acute or chronic. In gynecology, inflammation leads to anatomic disorders primarily as a result of infectious disease; however inflammation can affect ovulation and hormone production as well as be associated with endometriosis. Similarly, immune cell trafficking is an important component of cyclic endometrial development in each menstrual cycle. These immune cells are required for endometrial function, producing a vast array of inflammatory cytokines. Inflammation alters endometrial receptivity, however it may also play a role in tissue repair and remodeling. Finally, inflammation affects the trophoblast and trophoblast—endometrial interaction. Some components of the immune response are required for optimal fertility and normal tissue remodeling. A better understanding of the necessary role of inflammation in reproduction will allow more rational and targeted treatment of inflammatory disorders in reproductive medicine.

Keywords

Reproduction; inflammation; ovulation; infertility; implantation

INTRODUCTION

This article is a compilation of talks on gynecology and inflammation at a Society for Gynecologic Investigation satellite meeting hosted by the Dr Felice Petraglia at the University of Siena. As demonstrated in the following sections, inflammation has a significant role in gynecology and infertility, affecting the ovary, uterus as well as the embryo and implantation. This manuscript reflects the presentations given at this meeting and is intended to solidify recognition of the need for further study in this area. The role of inflammation is apparent. Future challenges include dissection of this complex phenomenon; inflammation has both beneficial and harmful attributes. A more nuanced approach will likely lead to a better understanding of this process, with the potential for treatment.

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INFERTILITY AND INFLAMMATION

There are three major categories of problems that account for most causes of infertility. These are anatomic abnormalities, ovulatory problems, and male factor. Male factors are beyond the scope of this discussion. Suffice to say that male infections are clearly related to infertility. Certainly, adult mumps is a significant cause of infertility in men.

Anatomic Abnormalities

Most anatomic abnormalities that cause infertility are acquired. The causes of acquired anatomic abnormalities include infection, inflammation, ischemia, and surgical injury, which may result in pelvic pain, pelvic adhesions, and infertility. The most significant cause of inflammatory infertility is *Chlamydia trachomatis* infection that may result in infertility in 10% to 30% of infertile couples in developed countries. The proportion of infertility related to pelvic inflammatory disease (PID) in developing countries is up to 85%. The overall prevalence of chlamydia infection in the United States in adults aged 18 to 26 is 4.19%. Women are more likely to be infected than men, 4.79% versus 3.67%. The prevalence of chlamydia infection varies dramatically in different racial groups. By way of example, the highest male incidence in the United States is black men with an incidence of 11.12%. The lowest in the United States is Asian men, 1.14%.¹

In all, 70% to 80% of infected women and 50% of infected men are asymptomatic. Half of asymptomatic women will clear spontaneously. In the remaining women, cervical infection will persist for years; 10% will ascend and produce PID, which is a danger to future fertility.²

The cervix is a reservoir of infection which may ascend to the tube, attached to sperm. Only a fraction of women with chlamydial cervicitis get PID.

A major issue is why there is such variable expression of adverse effects of infection amongst individuals and races. Based on the evidence to be presented I would suggest that the reasons for variable expression and racial differences are genotypic. Different genotypes cause variable expression of inflammatory cytokines, and different genotypes are either protective or facilitatory to the development of PID from chlamydia infections.

A total of 277 couples were screened for chlamydia. The presence of chlamydia was not associated with either semen characteristics of in vitro fertilization (IVF) or success. Infertility is associated with inflammation rather than the presence of the organism.³

Heat shock proteins (HSPs) are ubiquitous, highly structurally conserved proteins. In the presence of increased temperature, most organisms switch off most protein synthesis and synthesize HSPs. Heat shock proteins have many roles including that of molecular chaperones, intracellular transport, and are protective of protein degradation. Heat shock proteins are induced by many stressors. Antibodies to cHSP60 are found in 70% of women with occluded fallopian tubes and in <20% of infertile women with patent tubes. Untreated chlamydia infection produced high levels of cHSP60 and huHSP60. Because of their structural similarities, antibodies to cHSP60 may cross-react with huHSP60 to produce increased tissue damage. It is possible that tissue damage from chlamydia infection may have an autoimmune component. cHSP60 may have a specific role in regulating the immune system during chlamydial infections. cHSP60 treatment of peripheral mononuclear cells from women with tubal occlusion produces increased interleukin 10 (IL-10) production.⁴ One third of T-lymphocyte clones that respond to chlamydia antigen recognized cHSP60 as a target antigen. Most produced IL-10. Thus, cHSP60 may be a major T-lymphocyte antigen involved in tubal damage.⁵

In a Kenyan population of infertile couples, normal except for tubal occlusion, 50% were chlamydia positive by microimmunofluorescence (MIF), a less sensitive test than polymerase chain reaction (PCR), the current standard. Two class II alleles, HLA DRI*1503 and DRB5*0101, were less common in chlamydia-positive women. These alleles may lead to an immunologically mediated mechanism of protection against chlamydia. An alternative hypothesis, however, is that increased risk of tubal disease due to other causes is related to these alleles.⁶

Using an ex vivo model from human fallopian tubes viewed by scanning electron microscopy and immunohistochemistry, tissue destruction was noted after chlamydia infections, especially ciliated cells. Interleukin 1 was detected after infection. Interleukin 1 receptor antagonist limited the destruction. Interleukin 10, an anti-inflammatory cytokine, reduced damage. Interleukin 1 induced IL-8, a neutrophil attractant, so IL-1 can generate a cellular infiltrate. Thus, IL-1 can imitate the tissue destruction seen in fallopian tubes produced by chlamydia.⁷

Enhanced IL-10 secretion and reduced antigen-specific lymphocyte proliferation and interferon γ (IFN- γ) response were found in chlamydia-infected subjects with IL-10–1082GG genotype when compared to IL-10–1082AA genotype. Thus impaired cell-mediated response to chlamydia is associated with IL-10 genotype.⁸

Among women with PID, carrying DQA*0301, DQA*0501 and DQB*0402 alleles (all HLA class II DQ variants) altered the occurrence of lower genital tract infection, upper genital tract infection, and fertility. This may help explain why only few infected women get upper tract disease with tubal occlusion. It can be seen from the above information that genotype can determine susceptibility to chlamydia infection.⁹

Ovulatory and Endocrine Abnormalities

While some endocrine illnesses effecting reproduction have autoimmune causes, such as diabetes and Hashimoto's thyroiditis, probably the best example of the relationship between ovulatory infertility and inflammation is premature ovarian failure (POF). The incidence of POF is 1% of women under the age of 40. The current therapy is egg donation. Women with POF occasionally (~25%) spontaneously ovulate. About 10% of women become pregnant without medical assistance. Waxing and waning of symptoms spontaneously is a hallmark of autoimmune disease. Premature ovarian failure is associated with several alleles of the estrogen receptor as well as a variety and growing list of other variant genes. There are many causes of POF including extrinsic causes such as ovarian surgery, irradiation, chemotherapy, smoking, and extrinsic toxins. Intrinsic causes of POF include karyotypic abnormalities and genetic diseases such as galactosemia. About 50% of POF cases have been ascribed to autoimmune disease. Antiovarian antibodies have been noted in some of these cases.

Interferon- γ and tumor necrosis factor alpha (TNF- α) can induce major histocompatibility complex (MHC) Class II antigen expression in ectopic sites and are implicated in the cause of autoimmune disorders. In the study of Class II MHC antigens in ovaries of normal and POF women, normal ovaries only occasionally had antigen expression and granulosa cells were negative. However, in POF patients extensive Class II antigen expression could be induced and Class I MHC antigen expression was enhanced in granulosa cell cultures after addition of IFN- γ .¹⁰

Accumulation of monocytes and dendritic cells and the clustering of dendritic cells in endocrine organs is one of the first phenomena of an autoimmune endocrinopathy. In a prospective study, peripheral monocytes of 46% of POF patients had decreased

chemoattractant-induced monocyte polarization compared with none in the controls. Peripheral blood dendritic cells of 36% of the POF patients showed decreased cluster capability. Correction of hormonal alteration did not affect the outcome. It has been suggested that redistribution of active monocytes and dendritic cells from periphery to ovary may cause POF.¹¹ Thus, ovulatory abnormalities may have a significant basis in inflammation.

Endometriosis

Endometriosis can cause infertility by producing adhesions that may result in tubal occlusion. Endometriosis may distort pelvic anatomy by producing large ovarian masses. Endometriosis may also be destructive to germinal epithelium, decreasing the number of available oocytes. It is not clear whether the products of endometriosis are a cause of infertility or a paraphenomenon. Endometriosis is related to retrograde menstruation; however, retrograde menstruation occurs in >90% of women. Endometriosis has a 10% incidence.

Autoimmune responses may contribute to the onset of endometriosis. Endometriosis results in tissue damage, production of antibodies against endometrium, ovary, phospholipids, histones, and is associated with other autoimmune diseases such as thyroiditis or systemic lupus erythematosus (SLE), all of which suggest an autoimmune cause.

Peritoneal fluid (PF) is present in women with endometriosis of increased volume. Increased white blood cells (WBCs) and macrophages and increased macrophage activation also occur. Expression of IL-1, IL-6, and TNF- α is increased in PF of women with endometriosis.

Both IL-6 and TNF- α promote endometrial cell proliferation, adhesion, and angiogenesis. Endometriotic lesions may secrete TNF- α and IL-1.¹² There is decreased endometrial receptivity due to aberrant expression of proteins such as adhesion factors during the window of receptivity in women with endometriosis.

Aromatase expression is increased in eutopic endometrium from endometriosis patients compared to controls. Endometriosis implants have markedly increased aromatase compared to eutopic endometrium. Cyclooxygenase-2 (COX-2) expression is increased similarly. Both were highest in red implants, the most active form of endometriosis.¹³

Thus, women with endometriosis differ in many ways from normal women including different secretion of cytokines. This may help explain the decreased fecundity with patients with endometriosis. In summary, inflammation is a major cause of infertility affecting essentially all components necessary for reproduction.

INFLAMMATION IN OVULATION AND CORPUS LUTEUM FUNCTION

Inflammation is a basic way in which the body reacts to infection, irritation, or other injury. Inflammation is a complex biological response of vascular tissue to harmful stimuli. Inflammation is now recognized as a type of nonspecific immune response that is categorized as acute or chronic.

Since antiquity, the defining clinical features of inflammation have been known in Latin as rubor (redness), calor (warmth), tumor (swelling), and dolor (pain). These hallmarks of inflammation were first described by Celsus—Aulus (Aurelius) Cornelius, the Roman physician and medical writer, who lived from about 30 BC to 45 AD.

That the physical appearance of the ovary showed these characteristics first led to the suggestion that various aspects of ovarian function, including ovulation, can be likened to an inflammatory process.

Certain recent advances in our understanding of ovarian function have provided additional data that various processes which occur in the ovary are reminiscent of inflammatory processes. Various mediators of acute inflammatory reactions appear to be important components of ovulation, luteal formation, and luteal demise, that is using the same cell types and/or biochemical mediators.

The inflammatory response directs immune system components to the site of injury or infection and is manifested by increased blood supply and vascular permeability. This allows chemotactic peptides, neutrophils, and mononuclear cells to leave the intravascular compartment. In the case of infection, microorganisms are engulfed by phagocytic cells (eg, neutrophils and macrophages) in an attempt to contain the infection in a small tissue space. The response includes attraction of phagocytes in a chemotactic gradient of microbial products, followed by movement of the phagocyte to the inflammatory site and contact with the organism. Phagocytosis (ingestion) of the organism, development of an oxidative burst directed toward the organism, fusion of the phagosome and lysosome with degranulation of lysosomal contents, and death and degradation of the organism then occurs. When quantitative or qualitative defects in neutrophil function result in infection, the infection usually is prolonged and recurrent, and responds slowly to antimicrobial agents.

The process of acute inflammation is initiated by local blood vessels with exudation of plasma proteins and leukocytes into tissue. Increased blood flow causes characteristic swelling, reddened color, and heat. Blood vessels permit extravasation of leukocytes through endothelium and basement membranes. Vasodilation and increased permeability cause slowing of blood flow. With slowing of blood flow, leukocytes marginate along endothelial cells. Normal blood flow prevents this because shearing force along the periphery of vessels moves cells through the middle of the vessels. Extravasation mediated by recruitment of leukocytes is receptor mediated. Inflammation promotes expression of P-selectin on endothelial cells. P-selectin binds to carbohydrate ligands on the leukocyte surface. Cytokines secreted from injured endothelial cells induce expression of E-selectin and integrins which further slow leukocytes. A subgroup of locally produced cytokines, the chemokines, act as leukocyte chemoattractants, which act in concert with other specific chemotactic factors to accumulate and activate subsets of leukocytes appropriate for each specific tissue and condition.⁸²

The temporal changes in the ovary which appear to resemble inflammation first led to the concept that ovulation is reminiscent of an inflammatory response. Early electron microscopy studies that monitored the morphological changes in the ovary during follicular development, ovulation, and luteal formation demonstrated significant rearrangement of connective tissue and fibroblast activation in the theca externa and tunica albuginea.¹⁴ Maintenance of connective tissue architecture requires a balance between the actions of matrix metalloproteinases (MMPs), the enzymes that degrade connective tissue components, and the action of the endogenous inhibitors of MMP action, the tissue inhibitors of metalloproteinases (TIMPS). Inflammatory features of ovulation in addition to extracellular matrix (ECM) degradation include vascular changes; expression of chemokines, cell adhesion molecules and integrins; and recruitment of leukocytes.

Positive feedback of estradiol results in the luteinizing hormone (LH) surge, which is essential for ovulation. That LH binding to its receptor is required is demonstrated in LH receptor null mice who do not ovulate. LH stimulates granulosa cells to secrete

prostaglandins (PGs), progesterone (P), and cytokines (C). In turn, PG, P, and C act on granulosa and theca cells to induce secretion of MMPs that degrade the ECM. In addition, both granulosa and theca cells secrete TIMPs, which protect the follicle from excessive MMP action and also stimulate cellular proliferation of endothelial cells.¹⁵

Remodeling of the ECM in many tissues appears to involve two families of zinc-binding metalloproteinases, the MMP family and the members of a more recently described group of metalloproteases, the ADAM (a disintegrin and metalloproteinase) family. An ADAM subgroup, the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family of proteases are upregulated in inflammation and show antiangiogenic activity.⁸³ ADAMTS-1, the first member of ADAMTS family described, cleaves aggrecan and versican. ADAMTS-1 null mice show female infertility, specifically decreased ovulated oocytes in superovulated null mice, compared to wild type.

In addition to follicular rupture, the process of ovulation requires expansion of the cumulus oocyte complex, a hyaluronan-rich ECM formed by cumulus (granulosa) cells. LH induction of hyaluronic acid synthase-2 expression and prostaglandin E2 (PGE2) induction of hyaluronic acid—binding protein tumor necrosis factor alpha stimulated gene 6 (TSG-6), in concert with the covalent coupling of plasma inter-alpha-trypsin inhibitor to hyaluronic acid, results in increased synthesis of hyaluronic acid. It is thought that ADAMTS-1 by cleaving versican plays a role in cumulus expansion because versican is an LH-induced, hyaluronic acid—binding factor.

Although many gene products have been implicated in ovulation, PGs and P are required for ovulation. The LH surge induces COX-2. That PG are obligatory for ovulation is demonstrated by inhibition of ovulation by PGs synthesis inhibitors. In addition, COX-2 deficient mice fail to exhibit cumulus expansion. Ovulation is inhibited by P synthesis inhibitors and P receptor antagonists.

Although both follicle rupture and luteinization are critically dependent upon the LH surge, follicle rupture appears to be independent of luteinization. This is demonstrated by the fact that blocking rupture of the ovarian follicle does not interfere with P synthesis. Luteal formation requires proper neovascularization, which is regulated by vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Macrophages and leukocytes accumulate in the corpus luteum during the late luteal phase and may be actively involved in luteolysis in primate species.

THE ENDOMETRIAL CYCLE: INFLAMMATORY CHANGES

Dynamic cellular remodeling of the cyclic endometrium is a prominent feature in the uterine biology of women. The evolution of menstruation is a relatively recent phylogenetic development, occurring in human and simian females as well as in some species of bats, and tends to correlate with the length of the reproductive cycle and degree of placental invasion. By contrast, rather than desquamating and shedding their uterine lining, in most mammals changes in the endometrial lining occur without bleeding. Several hypotheses have been proposed to explain the evolutionary advantages of menstruation. Profet¹⁶ suggested that endometrial shedding, which occurs about 2 weeks following peak copulatory activity, might protect the uterus from sperm-borne pathogens; Strassmann¹⁷ argued that menstruation was energetically less costly than metabolic maintenance of the endometrium in a receptive state; Finn¹⁸ proposed that menstruation is a nonadaptive consequence, or epiphenomenon, of endocrine and inflammatory responses within the uterus. The latter is my preferred hypothesis, and I will attempt to frame this brief review within the context of Finn's postulate.¹⁸

Immune cell trafficking into the human endometrium has been recognized for decades and was highlighted in the classical description of histological changes in this tissue by Noyes et al.¹⁹ A dramatic influx of innate immune cells into the *functionalis* layer of the endometrium occurs during the secretory phase of the uterine cycle. Macrophages and natural killer (NK) cells accumulate in the mid-late secretory phase, whereas neutrophils and eosinophils appear during the premenstrual phase only.²⁰ My colleagues and I proposed that endocrine signals, via the induction of specific endometrial chemokines and immunomodulatory proteins, orchestrate the cyclical recruitment and turnover of uterine immune cells.²¹ In turn, these leukocytes influence physiological changes and disease susceptibility within the female genital tract. For the sake of brevity, this review will focus only on the trafficking of endometrial macrophages, as these cells play a critical role in the establishment of pregnancy.²²

Chemokines and Macrophages Regulate Cyclic Endometrial Turnover

Among several monocyte-selective chemokines (eg, monocyte chemoattractant protein MCP-1, MCP-3, and macrophage inflammatory protein MIP-1 α) my laboratory has extensively characterized the expression of RANTES (regulated upon activation, normal t-cell expressed and secreted) within human endometrial and endometriosis tissues. RANTES is an 8-kd “C-C” or “ β ” chemokine, expressed in endometrial stromal but not epithelial cells.²³ Endometrial RANTES is bioactive as a monocyte chemokine in Boyden chamber assays²⁴ and its mRNA and protein concentrations are highest in the mid-late secretory phase.²⁵ These findings support a functional role for RANTES as a secretory phase chemoattractant, which we postulate establishes a concentration gradient within the endometrial stroma and serves to recruit monocyte precursors from the bone marrow and circulation into the uterus, where they differentiate into tissue macrophages, secrete MMPs and PGs and participate in menstrual breakdown (Fig. 1). Macrophage activation products, such as IL-1 β , significantly upregulate endometrial stromal cell production of RANTES mRNA and protein, creating a proinflammatory, feed-forward loop²⁶ in the premenstrual tissue.

An Immunomodulatory Protein Plays a Role in Maintenance of Early Pregnancy

Given that the above mentioned immune system is primed to effect an inflammatory and degradative environment leading to menstruation, what happens in conception cycles to preserve integrity of the implantation site? Based on the pioneering studies of Seppälä and colleagues,²⁷ among others, we have investigated the role of glycodelin-A (GdA) in this context. Endometrial GdA is a 28-kd glycoprotein synthesized and secreted by secretory phase endometrial epithelial cells.²⁸ The gene encoding GdA is regulated by a promoter that contains 4 canonical P responsive elements,²¹ however its transcriptional regulation is complex.²⁹ Glycodelin-A transcription and translation can be stimulated by progestins³⁰ and possibly relaxin³¹ in vitro and by human chorionic gonadotropin infusion into the baboon uterus in vivo.³² We postulate that hormones secreted in early pregnancy from the corpus luteum and growing trophoblast stimulate the local production of GdA from endometrial glands.

The precise physiological effects of GdA have not been fully characterized, but this glycoprotein can inhibit the function and/or proliferation of NK cells,³³ T cells,³⁴ and monocytes.³⁵ We have shown that GdA dose dependently inhibits monocyte chemotaxis in Boyden chamber assays.³⁵ The mechanism appears to be via induction of apoptosis, evidenced by DNA laddering, *TUNEL* (Terminal deoxynucleotidyl transferase biotin-dUTP Nick End Labeling) and through the activation of caspases-2, -3, and -8 in primary and transformed human cells of this lineage.³⁶ Induction of leukocyte apoptosis by GdA in the vicinity of the implanting gestation thereby would create a local anti-inflammatory

environment without significantly compromising systemic maternal immunocompetence (Fig. 2). Such a mechanism provides an example of “the immunological indolence or inertness of the mother” as initially proposed by Sir Peter Medawar to explain maternal tolerance of the fetal allograft in eutherian species.³⁷

Although much remains to be learned about the exact functions of endometrial macrophages in menstrual breakdown, fetal allograft tolerance, and rescue of early pregnancy, the hypotheses put forth in this review are readily testable using in vitro models that we and others have established and continue to develop. Moreover, despite the economic and ethical challenges of subhuman primate research, some of these species, and the baboon in particular,³⁸ provide opportunities for direct in vivo observations that are likely to be highly relevant to clinical physiology in women (Figures 1 and 2).

REGULATION OF IMPLANTATION BY HOX GENES: THE ROLE OF INFLAMMATION

Implantation is the rate limiting step in achieving pregnancy; this is true for both spontaneous pregnancies and those achieved through assisted reproductive technologies. Implantation is relatively inefficient in the human. When performing IVF, we frequently transfer multiple embryos to achieve an acceptable pregnancy rate due to the low implantation efficiency of any individual embryo. This has led to an increase in multiple gestations and the related complications. Although the major determinant of implantation and successful pregnancy is embryo quality, endometrial defects can also prevent or reduce implantation. The effect of the endometrium has been most recently clearly demonstrated with conditions such as hydrosalpinx, which decreases IVF implantation rates by nearly 50%.^{39,40} Other common medical conditions also decrease implantation, including endometriosis, polycystic ovary syndrome as well as uterine polyps and fibroids.

Endometrial defects are still poorly characterized. What causes endometrial defects and how can we detect them? A reproductive medicine network trial evaluated endometrial biopsies from fertile and infertile women using histology and the classic Noyes criteria.^{41,42} They showed that this method of evaluating the endometrium has poor predictive value. An “out-of-phase” biopsy was found more commonly in fertile women than infertile women. These data suggest that endometrial defects are not typically observable by histologic evaluation but may be detected at the molecular level.

Numerous studies have analyzed the expression of genes normally found in a particular period of the menstrual cycle, including the window of implantation. Microarray studies have shown the ability to classify normal versus abnormal endometrium using expression patterns of multiple genes.^{43,44} However, very few genes have a well-characterized endometrial receptivity phenotype. Although mutation of many genes cause infertility for multiple reasons, very few such mutations lead to specific endometrial defects while not interfering with follicular maturation or subsequent embryogenesis.

In our laboratory, we have studied *HOX/Hox* (human/murine) genes.^{45–48} *Hoxa10* and *Hoxa11* have a well-characterized endometrial receptivity phenotype.^{49,50} These highly evolutionary conserved transcription factors play an essential role in embryonic development.⁵¹ In addition, they are necessary for endometrial receptivity. *Hoxa10*- or *Hoxa11*-deficient mice are sterile, however the endometrial histology is normal.⁴⁹ *Hoxa10*- or *Hoxa11*-deficient mice may be a good model for human endometrial defects, which similarly display normal histology but deficient implantation. *Hoxa10*- and *Hoxa11*-deficient mice ovulate normally, however, the uterus will not allow implantation. These *Hox*-deficient embryos are viable when transferred into a wild-type pseudo-pregnant mouse,

however even wild-type embryos when transferred into the uterus of the *Hoxa10*- or *Hoxa11*-deficient mouse fail to implant. Hence, these mice have specific endometrial defects.

Similarly, *HOXA10* is dynamically expressed in the human uterine endometrium during the menstrual cycle and in pregnancy.^{52–54} There is increase in expression in the midsecretory phase at the time of implantation. This increase is predominantly due to the increased expression in the glands. Glandular expression is very low in the proliferative phase and dramatically increases at the window of implantation. *HOXA10* and *HOXA11* are stimulated by estrogen and P; both appear to have a similar role but nonredundant roles in humans. In addition to regulations by sex steroids, *HOX* genes are also regulated by vitamin D and negatively regulated by testosterone.^{55,56}

In addition to sex steroid regulation, *HOX* genes in the uterus are also dramatically regulated by inflammatory cytokines.⁵⁷ Treatment with IL-1 beta leads to a 90% reduction in *HOXA10* expression, suggesting inflammation may play an important role in regulating the genes necessary for human endometrial receptivity. Interleukin 1 β treatment of human endometrial cells leads to a nearly complete suppression of *HOXA1* and approximately 90% reduction in *HOXA9*, *HOXA10*, and *HOXA11* expression. This reduction in expression persists even in the presence of P that normally stimulates *HOXA10* expression. It appears to be very similar to the suppression of *HOX* gene expression seen in the inflammatory milieu of hydrosalpinx fluid that dramatically decreases *HOX* expression, or the inflammatory environment of polycystic ovary syndrome (PCOS), or endometriosis.^{58–60} All of these conditions we have previously shown decrease *HOXA10* expression, and we believe an inflammatory component may play a role in these alterations.

In endometriosis, several models have been devised in animals that allow us to determine cause and affect. Normal human endometrium can be used in an immunodeficient mouse or endometrium can be autotransplanted into the baboon to develop experimental endometriosis.^{61–63} In the baboon model, the inflammatory phase initially resulting from the surgery required to establish the disease, does little to alter *Hox* expression. Long-term establishment of endometriosis and the creation of an inflammatory environment downregulates *HOXA10* and *HOXA11* as well as insulin-like growth factor binding protein 1 (IGFBP1) in the baboon and in mouse models.^{63,64}

Although the inflammatory changes associated with hydrosalpinx can be corrected by resection of the hydrosalpinx, surgical removal of endometriosis is only minimally effective in improving pregnancy rates. Could there be long-term changes in gene expression after the inflammatory insult is gone? We have recently identified epigenetic alterations leading to changes in *Hox* gene expression in animal models of endometriosis.^{63,64} The Guo laboratory was the first to show that humans with endometriosis have increased methylation of the promoter and intronic regions of the *HOXA10* gene.⁶⁵ Similarly, in our mouse model of endometriosis, we have demonstrated that even the transplantation of normal endometrium into the peritoneal cavity of a mouse can induce altered DNA methylation in the *Hoxa10* gene, suggesting that the endometriosis is not due to inherently abnormal endometrium but due to the transplantation of normal endometrium.⁶³ This normal endometrium in ectopic location leads to altered methylation through many mechanisms, likely including an inflammation-mediated pathway.

We have also recently described the contribution of bone marrow-derived stem cells to endometrium.^{66–68} We have previously shown that bone marrow-derived cells can trans-differentiate into endometrial cells. This likely occurs with greater incidence after injury. Stem cells may be recruited to the site of injury to help repair the endometrium. In a murine

model of endometriosis, we also demonstrated that stem cells from outside the uterus contribute to the endometriosis.⁶⁷ The molecules responsible for this recruitment are still unknown, however, preliminary data suggests that IL-1 β may be an important mediator of stem cell recruitment both to the normal endometrium and to endometriosis. Hence, an inflammatory cytokine may be both helpful and harmful. Inflammatory cytokines may be an important signal that recruits stem cells to the endometrium to repair damage. It may also be an important marker of endometriosis that causes further progression through contribution of stem cells to the endometriosis.

In summary, inflammation plays an important role in endometrial pathology and likely in endometriosis. However, inflammation may also be useful in recruiting stem cells to injured tissue, including the uterus to assist with repair and regeneration. Understanding the pathology associated with inflammation as well as the benefits of inflammation may allow us to harness the good affects while blocking those that are abnormal. This knowledge may have potential for improving both embryo implantation and endometrial repair in response to injury.

THE TROPHOBLAST, THE IMMUNE SYSTEM, AND THEIR INTERPLAY IN THE INFLAMMATORY STATE OF PREGNANCY

There is a fundamental paradox in human reproduction. Although it is critical to the survival of the species, the process is relatively inefficient. Of the pregnancies that are lost, 75% represent a failure of implantation.⁶⁹ Implantation failure and placental pathologies including recurrent miscarriage and preeclampsia are believed in many cases to originate in immune disturbances.⁷⁰ Indeed, effector cells and molecules of the immune system are key participants in the development and function of the placenta, which by virtue of its paternally encoded genes is semiallogeneic to the mother. A better understanding of interactions between trophoblast and immune system during implantation and placentation may improve clinicians' ability to treat disorders related to these processes.

The Interplay of the Trophoblast and the Immune System in the Inflammatory State Of Pregnancy

Effector cells and molecules of the immune system, leukocytes, cytokines, and immunoregulatory factors, can act both to promote and to limit placental development through influencing trophoblast cell survival, proliferation, and differentiation (Figure 3). The juxtaposition of trophoblast cells, stromal cells, and immune cells within the placenta greatly facilitates cell cooperation. There are two distinct immunological interfaces in pregnancy. The first (interface 1) is between maternal immune cells and the fetal trophoblast in the deciduas, which dominates during early pregnancy (Figure 4). The second immune interface of pregnancy (interface 2) is anatomically distinct from the first and involves interactions between circulating maternal immune cells and the syncytiotrophoblast (Figure 5). These interactions expand with the growth of the placenta to become the dominant interface toward the end of pregnancy and involve systemic, instead of local, immune responses.⁷¹

Intuitively it seems that pregnancy should be accompanied by suppression of maternal immune activity, and indeed, T-lymphocyte responses of the type 1 phenotype associated with acute allograft rejection are suppressed during pregnancy, with skewing toward potentially less damaging type 2 responses.² Indeed, previous investigations of the Th1/Th2 immune responses during pregnancy had shown that a distinct shift toward Th2 type reactions occurs at the feto-maternal interface and at systemic sites (the Th1/Th2 hypothesis).⁷² Although there is undoubtedly a bias toward Th2 cytokine production, which

has been demonstrated consistently in humans, there is no evidence that this is essential to the success of pregnancy and it is now emerging that other aspects of immune function are heightened.⁷³

During the first trimester characterized by a predominance of the local immune response, NK cells and macrophages normally accumulate around cytotrophoblasts. In the human placenta, the syncytiotrophoblast surface fails to express MHC class I or class II molecules and is, therefore, unable to induce the antigenic stimulation of maternal T cells. The decidual (extravillous) cytotrophoblast does not express the classic MHC class I genes encoding human leukocyte antigens HLA-A, HLA-B, or HLA-D, the principle stimulators of T-cell—dependent graft-rejection responses. However, it does express the nonclassic MHC genes encoding HLA-C, HLA-E, and HLA-G. Human leukocyte antigen HLA-C is now recognized to interact with killer cell immunoglobulin-like receptors (KIRs) on decidual NK (dNK) cells and, consequently, has the potential to control their repertoire of cytokine production. HLA-E is believed to interact with dNK cells and to be important in preventing NK cytotoxicity. HLA-G is immunosuppressive to CD8+ and CD4+ T cells, but its primary role is to interact with decidual macrophages and specialized CD56 bright dNK cells. This stimulates the production of cytokines and angiogenic factors that are beneficial to trophoblast invasion, implantation, and placentation.⁷⁴ Decidual NK cells in early pregnancy mediate angiogenesis and trophoblast chemoattraction, two key functions of early pregnancy, and it was shown that dNK cells, but not NK cells derived from the peripheral blood, control trophoblast invasion; this control occurs through the release of IL-8 and IFN-inducible protein-10 (IP-10), chemokines that bind to receptors expressed on invasive trophoblast cells.⁷⁵ These findings add to evidence that maternal NK cells in the uterus of pregnant healthy women do not use their cytotoxic functions. Instead, the cells have a regulatory function, cooperating with trophoblast and stromal cells to assure proper placental development.^{76,77} In this context, it is noteworthy that dNK and trophoblast cells produce cytokines that play major roles in the inflammatory process, including IL-12 and IFN- γ .^{71,78} Thus, implantation and placentation can be considered proinflammatory states, contrary to the Th1/Th2 hypothesis.

Toward the end of pregnancy characterized by the predominance of systemic, instead of local, immune response, there is a systemic activation of the innate immune compartment. Peripheral blood-neutrophils and -monocytes in the circulation express molecules indicative of a systemic inflammatory response. These leukocytes have been shown to contain increased intracellular reactive oxygen species (iROS) and express more markers of activation in pregnancy than in nonpregnant women.⁷⁰ In quality and magnitude, some of these changes mimic the response to acute infectious insults such as septicemia. Normal gestational inflammation has been demonstrated not only in terms of phagocytic activity but also in terms of the production of proinflammatory cytokines, including interleukin IL-6, IL-12, and TNF- α . These changes represent a sterile inflammatory response brought about by the pregnancy itself instead of infection.⁷¹ In contrast, the adaptive immune system, including T cells and B cells, does not express activation markers during normal pregnancy. Thus, maternal–fetal systemic immune interactions in humans might be predominantly NK cell instead of T-cell-mediated, in the same way as they are locally in the uterus. Although the interaction between HLA-G and HLA-C and uterine NK cells can explain the inflammatory response in the decidua, a similar mechanism cannot operate in the periphery because the syncytiotrophoblast surface lacks MHC antigens.⁷¹ This raised the question as to what constitutes the inflammatory stimulus in all pregnancies and also the more severe systemic inflammation resulting in the clinical symptoms of preeclampsia. Recent studies have focused on the potential role of syncytiotrophoblast debris. Syncytiotrophoblast apoptotic debris, which is shed into the maternal circulation in normal pregnancy, is a candidate activator of the innate immune system. In the third trimester of normal pregnancy,

the mother tolerates daily shedding of several grams of dying placental trophoblast into the maternal circulation. It is considered to be released into the maternal circulation as a result of the normal turnover and renewal of the placental surface and, thus, extends interactions between circulating maternal immune cells and the syncytiotrophoblast throughout the body of the mother. These subcellular microparticles shed from the placenta are part of a wider range of other syncytiotrophoblast debris, including whole cells and soluble syncytiotrophoblast cytokeratin, in addition to trophoblast DNA and RNA. All these are present during normal pregnancy and in significantly increased amounts in preeclampsia. During normal pregnancy, syncytiotrophoblast microparticles are taken up and cleared by monocytes and dendritic cells, resulting in the production of TNF- α and IL-12 but low levels of IL-18 and IFN- γ . Syncytiotrophoblast apoptotic debris, which is shed into the maternal circulation in normal pregnancy may also contribute to the suppression of Th1 responses seen in pregnancy.

In summary, the major responses at the local interface as in the periphery are primarily inflammatory, involving NK cells instead of T cells.⁷¹ Indeed, normal pregnant women exhibit many characteristics of a systemic inflammatory response, which, in some aspects, can be as strong as those seen in patients with sepsis but do not seem to harm the mother in any way.

Their Malfunction in Preeclampsia

Preeclampsia occurs in an estimated 3% to 5% of births and is a leading cause of both fetal and maternal morbidity and mortality worldwide. These disorders are characterized by hypertension and proteinuria in previously normotensive subjects, which can also progress to eclampsia characterized by convulsions.

Examination of the placenta from normal and preeclamptic pregnancies has revealed that preeclampsia is associated with an apparent failure of trophoblast cells to invade and remodel the maternal environment. A failure to remodel the maternal spiral arteries, for example, is thought to restrict the blood flow to the developing foetus and may be a contributing factor to the onset of preeclampsia.

The pathophysiology of the disease involves impaired trophoblast invasion, abnormal genetic polymorphism, vascular endothelial cell activation, immune intolerance by the maternal immune system but also an exaggeration of a systemic inflammatory process.⁷⁹ Immunocompetent cells such as neutrophils, monocytes, NK cells, T cells, and B cells are activated in preeclampsia. In preeclampsia, hypoxic conditions, excessive inflammation, or oxidative stress induce necrosis or aponecrosis of trophoblasts. Syncytiotrophoblast apoptotic debris, which is shed into the maternal circulation in increased amounts in preeclampsia, may be the stimulus for this response. Excessive immunostimulation by necrotic trophoblasts, a hypoxic condition and poor angiogenesis, may overcome this immunoregulation system resulting in a systemic inflammatory condition and systemic endothelial dysfunction in preeclampsia. This activation enhances the production of cytokines, and these cytokines must play some roles at the materno-fetal interface or in the whole body. In preeclampsia, the excessive microparticle load stimulates higher levels of IL-18, which in turn stimulates IFN- γ by NK cells, resulting in the intense systemic inflammatory response.⁷⁹

Another important aspect of preeclampsia is that the balance of Th1 type cytokines to Th2 type cytokines, which occurs in normal pregnancy shifts to Th1 type immunity in preeclampsia. When macrophages or dendritic cells phagocyte necrotic or apo-necrotic trophoblasts, they produce type 1 cytokines such as TNF- α , IL-12, and IFN- γ , and augment

inflammation. These conditions may induce apoptosis of extravillous cells (EVT), resulting in poor placentation in preeclampsia.⁸¹

Take Home Messages

While some aspects of the immune response are reduced in pregnancy, effectors from both the innate and adaptive immune compartments are important. Indeed, it appears that innate immunity, in addition to adaptive immunity, plays a central role during pregnancy and preeclampsia and that NK cells are central to this process. Moreover, throughout its entire life span, the placenta produces as well as responds to a great diversity of inflammatory stimuli. Many signalling molecules and concurrent pathways responsible for the propagation of an inflammatory response have been identified in placental cells. Thus, as an integrative organ, the placenta relays or enhances the contribution made by the cells of the immune system in nonpregnant individuals.⁸⁰ Despite recent advances, considerably more research is needed to understand how immune system integrates to positively influence development of the placenta, and how subtle perturbations in immune status can affect pregnancy.

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Figure 1.

Secretory phase RANTES production by endometrial stromal cells induces the recruitment of monocytes into the uterus. Macrophage activation results in the production of cytokines (eg, IL-1 β) that stimulate NF- κ B to augment biosynthesis of RANTES, other cytokines, MMPs, and PGs, which contribute to endometrial breakdown and menstruation. IL-1 β , interleukin 1 β ; MMPs, matrix metalloproteinases; RANTES, regulated upon activation, normal t-cell expressed and secreted.

Figure 2.

Early pregnancy rescues the corpus luteum, resulting in the enhanced production of progesterone (P4) and relaxin. These hormones, in addition to direct effects of human chorionic gonadotropin (hCG), stimulate endometrial epithelial cells to secrete glycodelin (GdA), which in turn can induce apoptosis in local uterine inflammatory cells including invading macrophages.

Figure 3.

Schematic representation of immune cells in the deciduas. When the trophoblast invades the decidua, immune cells constitute 40% of the total decidual cell population and are dominated by mediators of innate immunity, including decidual natural killer (dNK) cells (70%–75%), Hofbauer cells considered the placental resident macrophages (20%–25%) and dendritic cells ([DCs] 1%–2%) while T-lymphocytes are more sparsely distributed.¹³

Figure 4. Schematic representation of the first interface (interface 1) between maternal immune cells and the fetal trophoblast in the deciduas that is dominating during early pregnancy.³

Figure 5. Schematic representation of the second interface (interface 2) that involves interactions between circulating maternal immune cells and the syncytiotrophoblast that forms the villous surface of the hemochorial placenta. This interface expands with the growth of the placenta to become the dominant interface toward the end of pregnancy, engaging systemic, instead of local, immune responses.³