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Influenza, Immune System, and Pregnancy

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Abstract

Influenza is a major health problem worldwide. Both seasonal influenza and pandemics take a major toll on the health and economy of our country. The present review focuses on the virology and complex immunology of this RNA virus in general and in relation to pregnancy. The goal is to attempt to explain the increased morbidity and mortality seen in infection during pregnancy. We discuss elements of innate and adaptive immunity as well as placental cellular responses to infection. In addition, we delineate findings in animal models as well as human disease. Increased knowledge of maternal and fetal immunologic responses to influenza is needed. However, enhanced understanding of nonimmune, pregnancy-specific factors influencing direct interaction of the virus with host cells is also important for the development of more effective prevention and treatment options in the future.

Keywords

influenza, pregnancy, innate immunity, adaptive immunity

Introduction

Influenza epidemics and pandemics pose a serious health problem worldwide. Understanding the illness and its effects on the immune system will aid in better disease management, including effective prevention and improved treatment strategies. The fact that certain segments of the population are more susceptible to severe infections and experience increased morbidity and mortality adds to the complexity of the disease. The goal of this review is to explore literature on the influenza virus and its immunology in humans and animals in an attempt to understand the increased morbidity and mortality rates associated with influenza during pregnancy.

Epidemiology

Viral Characteristics

Influenza virus is a single-stranded RNA (ssRNA) virus belonging to the Orthomyxoviridae family.[1] There are 3 major types of influenza virus—types A, B, and C—of which types A and B are responsible for most of the infections globally observed in humans. The genome for the influenza A virus (IAV) is composed of 8 negative-sense RNA strands that encode at least 13 viral genes. Of these, 2 major surface glycoproteins, hemagglutinin (HA; with 16 different subtypes) and neuraminidase (NA; with 9 different subtypes), are the major antigenic sites for the influenza virus.[2,3] The main natural reservoir for influenza viruses is the intestinal tract of aquatic birds and possibly the source of all human pandemic IAV strains.[4] Before the 2009 pandemic, there were 2 IAV subtypes prevalent (eg, the seasonal H1N1 and the H3N2 viruses) and 1 influenza B virus responsible for annual epidemics. In contrast, the influenza type C is antigenically stable and causes only mild illness in immunocompetent individuals.

Pandemics occur when an influenza virus strain enters the population and is antigenically different from seasonal virus strains. The generation of antigenically novel viruses occurs through point mutations in genes encoding HA and NA (antigenic drift) or through viral genome reassortment of subtypes (antigenic shift). The reassortment events occur during interspecies transmission followed by the introduction of the novel influenza strains into the human population.[3,5] The H1N1 strain of IAV, which caused the pandemic in 2009, contained genetic elements from human, avian, and swine viruses.[6] In contrast, the influenza pandemic of 1918 (Spanish flu) was attributed to a highly pathogenic strain of IAV. This H1N1 strain is thought to have spread from birds to humans after undergoing point mutations resulting in genomic adaptation.[7] The other 20th century pandemics occurred because of viral reassortment events: the 1957 pandemic (Asian flu) was caused by the generation of an H2N2 viral strain and the 1967 pandemic (Hong Kong flu) was caused by the generation of an H3N2 strain.[5,8]

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During the last decade, observed outbreaks of localized influenza infections mainly in Southeast Asia involved novel influenza viral strains. A highly pathogenic H5N1 influenza virus, first identified in 1997, is typically transmitted directly from birds (especially chickens) to humans; however, there is concern that with further adaptation, infection with this virus could evolve into a major pandemic.[9] More recently, the emergence of an H7N9 virus in 2013 raised public health concerns around the pandemic potential of this highly virulent strain of influenza virus.[10]

Influenza Infection and Pregnancy

Although the 2009 H1N1 IAV has generally been characterized as a self-limited, uncomplicated infection, severe illnesses and deaths have been reported among several high-risk patient groups. Groups at risk of seasonal influenza complications are age specific (individuals less than 2 and more than 65 years of age) and characterized by individuals with chronic illnesses (eg, chronic lung disease, neurological disorders, and diabetes).[11,12] Reports indicate that pregnant women with severe influenza infection are also at increased risk of having pregnancy-related complications.[13-16] An analysis of the pandemic 2009 IAV epidemic among pregnant women in the United States showed a disproportionately high risk of mortality in this group. There were a total of 280 intensive care unit admissions and 56 deaths among the 788 reported cases of pregnant women in the first 8 months of the pandemic. Pregnant women accounted for 5% of the deaths in the United States, although they only represented approximately 1% of the population. Pregnant women in all 3 trimesters were at increased risk of influenza-associated complications, especially when early antiviral treatment was not instituted within the first 48 hours after symptom onset. Among the deaths, 7.1% occurred in the first trimester, 26.8% in the second, and a remarkable 64.3% in the third.[13] Medical reports from the 1918 influenza pandemic also noted high mortality rates in pregnant women with influenza infection.[17,18] Besides adverse maternal outcomes, influenza virus infection during pregnancy is associated with preterm delivery, stillbirth, and abortion.[19-21]

Pathogenesis

Virus–Host Interaction

Research on the interaction between virus and immune system in the host provides insight into the pathogenesis and immunogenicity of influenza. Both human and animal studies have been fruitful, suggesting that the pathogenesis of a complex disease like influenza likely involves a combination of the direct effects of the virus and an imbalance between the beneficial and the harmful effects of the mediators released by immune cells.[22]

The influenza virus attaches to respiratory epithelial cell surface sialic acid residues linked to surface glycoproteins through binding to the HA molecules on the viral surface. The HA protein is critical for the binding of virus and cellular receptor, fusion of the viral and cellular membranes, and subsequent endocytosis. Viral RNA replication and transcription are carried out by 3 polymerase subunits (PB2, PB1m, and PA) and nucleoprotein. Newly synthesized viral ribonucleoprotein complexes are exported from the nucleus to the cytoplasm by nuclear export protein (formerly called "NS2") and matrix protein M1 and are assembled into virions at the plasma membrane. The NA protein facilitates the release of mature viral particles from infected cells by cleaving sialic acid bonds between cell surface glycoproteins and viral HA proteins.[2,4] The resulting progeny virions then spread to adjacent cells, where the replicative cycle is repeated. Viral NA also decreases the viscosity of mucous film in the respiratory tract, exposing cell surface glycoprotein receptors and promoting the spread of virus-containing fluid to the lower respiratory tract. Through this process, within a short time, many cells in the respiratory tract become infected and are eventually killed directly by the virus. Reportedly, the most significant and life-threatening pathology after IAV infection occurs in the lower respiratory tract.[23]

Influenza viral infection is also known to enhance host susceptibility to secondary bacterial infections.[12,24-26] Viral damage to the epithelium of the respiratory tract lowers epithelial resistance to secondary bacterial invaders, especially staphylococci, streptococci, and *Haemophilus influenzae*. Influenza infection has also been demonstrated to enhance the production of the anti-inflammatory cytokine interleukin (IL) 10 (IL-10), thereby suppressing the overall immune response and increasing susceptibility to secondary infections.[26] Pneumonia complicating influenza infections can be viral, secondary bacterial, or both[12] and is attributed to loss of ciliary clearance, dysfunction of phagocytic cells, and provision of a rich bacterial growth medium by alveolar exudate. Edema and mononuclear infiltrations in response to cell death and desquamation as a result of viral replication likely account for local symptoms (cough and sore throat), whereas prominent systemic symptoms associated with influenza (eg, headache, feverishness, chills, myalgia, and malaise) probably reflect the production of cytokines. An infectious virus is very rarely recovered from blood; however, viremia may occur in severe infections or with highly pathogenic IAV strains (eg, H5N1).[27]

Viral Interaction With the Fetoplacental Unit

During pregnancy, there are several systems protecting the fetus. These systems include mechanical defenses (amniotic fluid, fetal membranes, the placenta, and maternal decidua) and immunological defenses (including humoral and complement systems, and cell-mediated immunity).[28] The IAV has been isolated from the placenta and amniotic fluid in both fatal[29,30] and nonfatal cases,[31] although direct infection of the fetus has also been uncommonly reported.[32] Placentitis induced by influenza virus infection has been characterized by hyperplasia and degeneration of amniotic cells, placental trophoblasts, decidual cells, and vascular endothelial cells. Viral antigens have been

detected in affected cells and in lymphoid cell infiltrates.[33,34] Human decidua seems to provide a more favorable environment for viral replication than placental tissues.[35] These studies suggest that the influenza virus is capable of spreading from the maternal blood stream to the maternal decidua in order to replicate within the tissue and then infect the fetal chorion and amnion. Other studies have suggested that viral infection and replication have a direct cytopathic effect by inducing apoptosis in chorion cells, a process that may contribute to influenza-associated pregnancy loss.[36-39]

Immune Responses to Viral Infection

Innate and adaptive immune responses form the mainstay of the host defense against microbes. The innate immune system is genetically programmed to detect the invariant features of invading microbes, conferring rapid and early protection against an infection. Neutrophils, macrophages, and dendritic cells (DCs), among others, play a primary role in the innate immune response to influenza.[40] Host microbial sensors called ''pattern-recognition receptors'' (PRRs) recognize viral components, such as IAV ssRNA, and induce antiviral innate immune responses. The PRRs are expressed in multiple cell types, especially in innate immune cells such as DCs.[41-43] The DCs represent the most effective antigen-presenting cells capable of inducing robust CD4+ and CD8+ T-cell immunity in vivo, thereby regulating subsequent adaptive immune responses.[40,44,45]

Function of Toll-Like Receptors

Toll-like receptors function as PRRs that principally sense conserved molecular motifs called ''pathogen-associated molecular patterns'' (PAMPs), which are found in a variety of pathogens.[46,47] In mice, 13 TLRs have been described, among which only TLRs 1 to 9 and 11 to 13 are functional but TLR10 (thought to arise from a retrovirus insertion) is not.[48] Interestingly, in humans only 10 functional TLR molecules have been identified, whereas TLRs 11 to 13 appear to have been lost from the human genome (Kawai and Akira[48]). The TLRs can be divided into 2 groups depending on their cellular localization and respective PAMP ligand. One group includes TLR1, 2, 4 to 6, and 11, which are expressed on cell surfaces and mainly recognize microbial membrane components such as lipids, lipoproteins, and proteins; the other group comprises TLR3, 7, and 9, which are expressed within intracellular structures—such as the endoplasmic reticulum (ER), endosomes, lysosomes, and endolysosomes—where they respond to microbial nucleic acids.[48] These intracellular TLRs play an important role in antiviral immunity.

Toll-like receptor 3 exclusively signals through a myeloid differentiation factor 88 (MyD88)-independent pathway that uses toll–IL-1 receptor [TIR] domain-containing adaptor-inducing interferon [IFN] β (TRIF) as an intracellular adaptor protein and uses alternate pathways that lead to the activation of transcription factors (IFN-regulatory factor [IRF] 3 [IRF3], nuclear factor kappa B (NFκ-B), and mitogen-activated protein [MAP] kinases) to induce inflammatory cytokines.[48,49] The TLR3 and its signaling-associated adaptor molecule TRIF play a key role in the immune response of respiratory epithelial cells to IAV in humans.[50] Moreover, the importance of TLR3 signaling in antiviral immunity has been demonstrated in a study using TLR3 knockout mice,[22] which compared the time course of several parameters, including animal survival, respiratory distress, viral clearance, and inflammation in infected control wild-type versus TLR3-deficient mice. The TLR3-deficient mice displayed significantly reduced inflammatory mediators as well as fewer CD8+ T lymphocytes in the bronchoalveolar airspace.[22] Polyriboinosinic–polyribocytidylic acid (poly[I: C]) is a synthetic ligand for TLR3 that plays an immunomodulating role in prophylaxis and therapy in influenza infections. This is described in more detail in the Immunotherapy section of this review.

There are several TLRs expressed on plasmacytoid DCs (pDCs), also known as ''interferon-producing cells.'' The pDCs recognize viral components such as genomic DNA and RNA and secrete copious amounts of IFNs, especially IFN-α.[51] Myeloid differentiation factor 88 is an adapter protein in IL-1 signaling and is involved in most TLR signaling except TLR3.[41,48,52] Through activation of IL-1 signalining, MyD88 promotes the downstream transcription of various proinflammatory cytokines and acute-phase reactants through NFκ-B/activating protein 1.[53-55] Myeloid differentiation factor 88 plays an important role during pDC recognition of ssRNA viruses, which involves TLR7.[48] The endocytic location of viral RNA serves as a molecular recognition signature for RNA viruses, and the strategic localization of TLR7 within the lysosome is important in this pathway of viral detection. By rapidly producing high levels of type-1 IFNs, pDCs rarely become infected with viruses taken up through endocytic pathways, and this resistance enhances their ability to participate in antiviral immunity.[56]

Toll-like receptors 7 and 9 represent a structurally related subfamily that responds to nucleic acids by eliciting type-1 IFN production and are implicated in the response to HIV and influenza viral infections. Toll-like receptors 7 and 9 are exclusively sequestered in the ER in unstimulated cells and rapidly traffic to endolysosomes after ligand stimulation. This translocation is regulated by the ER-localizing protein UNC93B1.[57] This is a 12 membrane-spanning protein, mutation of which has defects in cytokine production and upregulation of costimulatory molecules in response to TLR7 and 9, as well as TLR3 ligands, making them highly susceptible to viral and bacterial infection.[48,58] Production of IFN-α by pDCs in TLR7-deficient mice is impaired after infection with the influenza virus.[59] The TLR9 recognizes unmethylated 2′-deoxyribo cytidine-phosphate-guanosine (CpG) DNA motifs present in bacterial and viral DNA but are rare in mammalian cells. Synthetic CpG oligodeoxynucleotides function as TLR9 ligands directly activating DCs, macrophages, and B cells, thus driving strong T helper 1 (Th1) responses.[48,49]

Additionally, epithelial cells can express intracellular RNA helicases that function as PRRs for actively replicating viruses

and help establish antiviral immunity by triggering type-1 IFN responses.[41] The influenza virus encodes the NS1 protein, which binds to double-stranded RNA and inhibits various antiviral pathways.[60] However, pDCs can still secrete high levels of IFN-α, despite infection with the influenza virus (even in the presence of NS1),[61,62] whereas classical DCs can only do so when NS1 is deleted.[63]

During pregnancy, DCs infiltrate the decidua around invading trophoblasts.[64] Invading trophoblasts (ie, nonimmune syncytiotrophoblast cells) also express TLRs 3 to 6 and 9 intermittently during pregnancy. This expression is temporal rather than constant, with certain TLRs on syncytiotrophoblasts upregulated only in the third trimester.[65-67] Placental expression of TLRs may play a role in pathologic states including preeclampsia and preterm labor as well as infectious morbidity during pregnancy.[68-71]

In human pregnancy, messenger RNA (mRNA) levels of TLRs 2 and 4 in the maternal neutrophils do not significantly change in the maternal circulation longitudinally during pregnancy, compared to nonpregnant controls.[72] Although there is a significant reduction in the percentage of circulating pDCs compared to the nonpregnant state,[73] longitudinal studies have demonstrated increases in protein levels of TLRs 1, 7, and 9 expressed by pDCs throughout pregnancy.[74] It was also noted that these changes in TLR expression were associated with significantly elevated expression of IL-6 and IL-12 and tumor necrosis factor (TNF) α, of which only IL-12 remained elevated in the postpartum period. Moreover, the altered pDC phenotype during pregnancy includes increased expression of activation and antigen-presenting molecules and paradoxically, inhibitory ligands.[73] It could be hypothesized that altered pDC phenotype and its decreased circulating numbers, as opposed to alterations in other inflammatory cells, may affect systemic viral clearance and the exaggerated inflammatory responses to infection during pregnancy. This pregnancy-specific phenomenon needs confirmation in future studies.

Cytokines as the Initial Line of Defense

Influenza infection initially triggers an array of host immune responses in an attempt to limit virus replication and spread. Acute-phase response is defined as the dose-dependent behavioral and physiological response of host organisms to infections and is regarded as a nonspecific host defense response.[75] The acute-phase response includes fevers of different magnitudes, changes in food and water intake in mice as well as decreases in activity and body temperature, which again have been correlated to cytokine activity after influenza virus infection.[75]

On recognition of viral components, PRRs initiate production of a variety of cytokines, mainly type-1 INFs (IFN-β and -α), and induce innate and adaptive immune responses.[40,42,43,46,76,77] It has been shown that type-3 IFNs, which include IFN-λ, contribute to the initial immune response to a viral respiratory infection.[78] Type-1 IFNs induce maturation of DCs by increasing expression of costimulatory molecules such as CD80, CD86, and CD40 as well as antigen presentation through major histocompatibility complex (MHC) class I.[43] Type-1 IFNs also mediate induction of chemokines that cause stimulation and recruitment of lymphocytes and monocytes to inflamed sites. In addition, type-1 IFNs upregulate effector molecules that directly influence protein synthesis and cell growth and survival in the process of establishing an antiviral response. Both IRF3 and IRF7 in particular are activated in response to viral infection and are primarily involved in inducing type-1 IFNs.[79] Apart from respiratory epithelial cells, human peripheral blood mononucleated cells (PBMCs) also produce type-1 and -3 IFNs in response to viral infection.[78] It has been reported that PBMCs from nonvaccinated pregnant women have an attenuated antiviral immune response as evidenced by the production of significantly less IFN-α and -λ compared with nonpregnant women.[80]

Early in the infection, low levels of TNF-α may mediate inflammation through limiting viral replication and direct virus-induced injury. As the infection resolves, TNF-α may also support the tissue repair process, stimulating the growth of fibroblasts and endothelial cells. If viral replication is not inhibited at an early stage, inflammation and infection become widespread, resulting in significant tissue damage.[81,82] When IAV infection progresses to pneumonia, TNF-α can enhance pulmonary inflammation without playing a significant antiviral role.[83] Dysregulation of these innate immune responses involving TNF-α has also been linked to severe influenza infection in the pediatric population.[84]

Natural Killer Cells

Natural killer (NK) cells are a key component of innate immunity. The NK cells have both cytotoxic and cytokine-producing functions controlled by a complex panel of activating and inhibitory receptors. These receptors play a central role in the regulation of NK cell function.[85] The NK cells may play a more significant role than activated effector CD8+ T lymphocytes in controlling viral burden when the host is infected with a new influenza subtype. Among patients severely ill after H1N1/09 infection, reduction in NK cells, but preservation of T lymphocytes, was observed.[86] The NK cells are present in maternal decidua in large numbers where they can destroy virus-infected cells via a perforin-dependent mechanism, leading to apoptosis induction.[36]

Role of Adaptive Immunity

Adaptive immune responses to influenza infection can be homotypic and heterosubtypic based on the mechanism and the cell types involved. In what is called "homotypic immunity," protection against influenza infection may arise from previous exposure to influenza of the same serotype and is dependent on circulating neutralizing antibodies, mainly immunoglobulin (Ig) subclass G (IgG).[87,88] By comparison, in heterotypic immunity, the protection against severe disease arises from previous infection with an influenza virus of a different serotype.[89] Heterotypic immunity is thought to involve multiple

components, including CD8+ T cells, B cells, and CD4+ T cells, which play a role individually and synergistically to provide antiviral immunity. There are variations in the level of these responses in primed individuals based on antigen-specific clonal diversity, age, and infection with other pathogens and the length of time between exposures to the influenza virus. Influenza-specific memory CD4+ T cells can act as helper cells in generating optimal B- and T-cell responses, as regulators of innate immunity, and as direct effectors of protection.[90] They can support protective heterotypic responses in both early and later stages of infection. Although the role of heterotypic immunity in human influenza is controversial, recent pandemic strain outbreaks have demonstrated that seasonal vaccines can provide a degree of protection against an emergent pandemic strain, especially against severe disease. A retrospective analysis of the 1918 epidemic showed the significant impact of heterosubtypic immunity on antiviral response.[91] It is likely that memory CD4+ T cells play a role in this heterosubtypic protection, and these effects merit further investigation.[90]

CD8+ T cells form an integral part of the adaptive immune system and assist host response to viral and specific intracellular bacterial infections. A recent study examined PBMCs from individuals naive to the 2009 pandemic H1N1 virus to assess the role of cross-reactive CD8+ T cells. The PBMCs were stimulated in vitro with a panel of live viruses and the study found that most patients exhibited cytokine-positive CD8+ cells in response to pandemic H1N1 infection.[92] Cytokine-producing cells were predominantly single positive (IL-2, IFN-γ, or TNF-α), and polyfunctional triple cytokine-producing cells are relatively rare. These findings suggested that these cells were functionally limited, possibly because the patients had been recently exposed to either seasonal or pandemic influenza strains. The majority of circulating memory T cells specific for influenza were in a quiescent state with restricted functionality in the absence of a prior priming effect.[92] Recently, a study of the in vitro effects of H1N1/09 infection on PBMCs collected from healthy nonpregnant and pregnant women suggested that pregnancy may enhance phenotypic and functional exhaustion in CD8+ T cells following H1N1/09 infection.[73]

A study of a cohort of pregnant patients infected with the 2009 H1N1 pandemic influenza virus showed that there were significantly lower levels of Igs of the G_2 subclass in the infected group compared to healthy pregnant controls.[93] This infection-related reduction in IgG_2 level in pregnancy may explain the increased severity of H1N1 infection in some but not all pregnant patients with infection. These findings were confirmed in another recently published study from China, where the pregnant H1N1-infected population was shown to have an imbalance of anti-H1N1 Ig subclasses (IgG_1, IgG_2, IgG_3, and IgG_4) and dysregulated cytokines, when compared to nonpregnant as well as uninfected pregnant controls.[94] The anti-H1N1 IgG_2 constituent proportion was decreased and IgG_4 was increased in the pregnant H1N1 group. This was also associated with a reduction in CD4+ and CD8+ T-cell percentages and a low CD4+–CD8+ ratio in the same population. Both IL-10 and IFN-γ were noted to be significantly elevated in the infected pregnant group compared to pregnant controls and thus thought to potentially contribute to the increased severity of infection in this population.

Immune Responses at the Maternal–Fetal Interface

Cytokines play an important role in the regulation of intrauterine functions, including parturition and defense against various infections. They regulate the production of prostaglandins and matrix metalloproteinases, which are postulated to facilitate parturition by increasing myometrial contraction and collagen degradation in fetal membranes.[95] A current thinking suggests that the maternal–fetal interface is in a controlled state of inflammation early during implantation and later during parturition.[96-98] Consistent with this model is a recent longitudinal study where the measured peripheral blood cytokine levels during pregnancy, compared with postpartum, showed significant pregnancy-related alterations in proinflammatory and chemotactic cytokines, including IFN-γ (↓), TNF-α (↑), granulocyte colony-stimulating factor (G-CSF; ↑), and monocyte chemotactic protein 1 (MCP-1; ↓). These changes were especially dramatic in the second and third trimesters.[99] Thus, pregnancy may enhance systemic inflammatory response to influenza infection.

Viremia can result from IAV infection during pregnancy, leading to decidual and placental infection.[29,31,100,101] The preferred environment for viral replication is human decidua (vs placental tissue), and from there, the virus spreads to fetal membranes[35,36] and is likely the basis of adverse pregnancy outcomes related to IAV infection.[37] The IAV infection induces apoptosis and gene expression of proinflammatory cytokines IL-1β, IL-6, and TNF-α in human fetal membranes in culture and may facilitate premature rupture of membranes by collapsing the amniotic epithelial cell layer.[102] In vitro IAV infection of chorion cells induces apoptotic cell death and mRNA expression of proinflammatory cytokines such as IL-1β, IL-6, TNF-α, IFN-β and -γ, and GM-CSF. The IAV affects cultured chorion cells from human fetal membrane tissues directly both cytopathically (such as detachment and cell rounding) and by cellular degradation (eg, oligonucleosomal DNA fragmentation and lactate dehydrogenase leakage in chorion cells), which are characteristics of cells undergoing apoptosis.[37,103] These apoptotic effects were not seen in infected, cultured amnion cells, which in turn induces persistent infection.[37] Synthesis of specific viral macromolecules at an early stage of infection plays a critical role in inducing apoptosis.[104,105] Further, it was apoptotic chorionic cells, not amnion cells, that induced secretion of bioactive IL-6 and TNF-α in vitro.[106] Thus, IAV infection of the fetal chorionic membrane results in a cellular proinflammatory cytokine response with associated apoptosis.

As with choriodecidual cells, macrophages also significantly participate in local innate inflammatory response to influenza infection. Human monocytic leukemia THP-1 cells differentiate into mature macrophages with adhesion and

phagocytic abilities when the cells were incubated with heat-treated culture supernatants of influenza virus-infected chorion cells undergoing apoptosis.[38] Further studies suggested that these apoptotic chorion cells secreted heat-stable monocyte differentiation-inducing (MDI) factors which potentially induce the differentiation of maternal monocytes in decidua tissue into well-matured macrophages.[39] These activated macrophages phagocytize influenza-infected apoptotic cells in a process mediated by a scavenger receptor (scavenger receptor A [SR-A]) on the cell surface of macrophages, which identifies cells undergoing apoptosis. Treatment with MDI factors induced SR-A mRNA expression in THP-1 cells.

Following macrophage phagocytosis, there is an abrupt onset of superoxide production known as "oxidative burst," catalyzed by a reduced nicotinamide adenine dinucleotide phosphate oxidase enzyme complex.[107] The controlled production of superoxide anion by macrophages is known to be necessary for remodeling tissues damaged by infectious agents, and excessive production may be implicated in the pathogenic effects of influenza virus infection. The MDI factors seem to play a critical role in the molecular pathogenesis of influenza virus infection through differentiation induction of monocytes into macrophages capable of phagocytosis and superoxide anion production. Studies have tried to further characterize MDI factors.[39] Interleukin 6 has been shown to induce the differentiation of human monocytic leukemia cell lines, including THP-1 cells, into macrophages contributing to MDI activity derived from influenza virus-infected chorion cells. This IL-6 effect is synergistically enhanced in combination with either TNF-α or IFN-γ. Influenza virus infection induces the secretion of MDI factors containing IL-6 from organ-cultured human amniochorion tissues, which in turn induces the differentiation of maternal decidual monocytes into mature macrophages with superoxide anion production. Interleukin 6 is partly responsible for MDI activity by associating with its receptor α- (gp80) and β-chains (gp130).[39]

Studies involving several known inhibitors of cytokine production suggest that the cellular oxidation process and peroxisome proliferator-activated receptor, MAP kinase, and NFκ-B regulate the induction of proinflammatory cytokine gene expression in fetal membranes during infection.[108-110] This raises the possibility that balanced levels of proinflammatory (IL-1β, IL-6, 8, and TNF-α) and anti-inflammatory (IL-10 and activin A) cytokines are needed to control various intrauterine functions in infectious states, including influenza, during pregnancy.[36]

Fetal membrane tissues produce specific chemotactic cytokines (eg, IL-8, MCP-1, IFN-γ inducible protein 10 [IP-10; IFN-γ inducible protein], and regulated upon activation and normal T-cell expressed and secreted [RANTES]) upon infection with IAV.[111,112] Elevated mRNA expression of chemotactic cytokines (such as macrophage inflammatory protein 1β [MIP-1β] and pulmonary and activation-regulated chemokine [PARC]) are present in the amnion and chorion tissues of patients with chorioamnionitis after preterm labor as compared with nonchorioamnionitis patients.[113] During infection, chemokines may prompt monocyte migration from maternal blood to fetal chorioamnionic tissue.

Other Factors Affecting the Immune Response to Influenza Infection

Adipocytes secrete factors called "adipokines" that can significantly alter inflammatory cell and immune function. Adiponectin is an adipokine that reduces macrophage activity and proinflammatory cytokine production[114] and thus may alter the inflammatory response to IAV. Adiponectin plasma levels and their correlation with H1N1 infection have been studied. Individuals with low adiponectin level (which include those who are obese, pregnant, or have metabolic syndrome) are more likely to mount a more pronounced innate immune response when infected with H1N1.[115] Proinflammatory mediators are more elevated in groups with decreased adiponectin levels, likely resulting in more severe disease.

Stress and depression predict exaggerated responses to biological challenges in humans and animals.[116-118] Studies on the macrophage migration inhibitory factor (MIF) levels in pregnant women vaccinated against the influenza virus link depression and sensitization of the inflammatory immune response.[119] In these studies, depressive symptoms predicted exaggerated MIF production following vaccination. Thus, depression may enhance the pathogenic effect of IAV infection during pregnancy, and modulation of depression or its biological mediators could present a novel therapeutic avenue.

Animal Studies

Many animal models have been used to study different aspects of mammalian influenza infection including mice, cotton rats, Syrian hamsters, guinea pigs, ferrets, dogs, cats, domestic swine, and nonhuman primates such as rhesus, pigtailed, and cynomolgus macaques, and most recently marmosets.[120-125] Studies on animal models of influenza infection in pregnancy are limited. Here, we focus on studies in mice and nonhuman primate (cynomolgus macaque).

Studies With Recombinant Influenza Virus

Studies conducted with reconstructed 1918 (H1N1) influenza virus (r1918) examined the virulence and pathogenesis of the virus responsible for the pandemic.[126-128] BALB/c mice infected with r1918 had more severe weight loss, an earlier mean time of death, and viral titers of lung tissue 10-fold higher on days 1 and 3 compared to mice infected with other viral strains.[129] Gene expression studies confirmed that the virulence correlated with the early and increased expression of immune response-related genes in r1918 infection. Characterizing the functional consequences of this gene expression data by pathway analysis showed the most significant activation of death receptor, IL-6, IF-1, and TLR response pathways. There was also r1918 infection-induced expression of mRNAs for Fas and caspase 8 and -9, which play a central role in apoptosis

induction through the death receptor and mitochondrial apoptosis pathways.[129] Increased mortality of r1918-infected mice was accompanied by increased (more than 200-fold) viral replication, a greater influx of neutrophils into the lungs, an increased number of alveolar macrophages, and increased protein expression of cytokines and chemokines in lung tissues compared to controls.[130] Human influenza H1N1 virus with 1918 HA and NA glycoproteins can induce severe lung inflammatory infiltrates consisting of alveolar macrophages and neutrophils, which play a role in controlling the replication and spread of the r1918 virus after intranasal infection of mice.

The pathogenic potential of the r1918 virus in primates was demonstrated by studies of respiratory infection in a cynomolgus macaque model that resulted in acute respiratory distress and a fatal outcome.[131] The animals demonstrated substantial increase in the serum levels of IL-6 and IL-8, MCP-1 (CC chemokine ligand 2), and RANTES, with no significant changes in IL-2 or -4, IFN-γ, or TNF-α. This study supports the theory that atypical host innate immune responses characterized by dysregulation of the antiviral response (which is insufficient for protection) may contribute to lethality. The ability of influenza viruses to modulate host immune responses was also found to be applicable in cases of infection with H5N1 strains, demonstrating that this may be a shared feature among highly pathogenic influenza viruses. Marked elevation in chemokines and proinflammatory cytokines (including IL-6, TNF-α, and MCP-1) is referred to as a "cytokine storm" and hypothesized as the main cause of mortality in H5N1 influenza viral infections.[132-138] Mice deficient in these important cytokines succumb to infection with H5N1 virus as do wild-type mice treated with glucocorticoids for cytokine suppression, which indicates that cytokine inhibition alone cannot sufficiently reduce the morbidity and mortality of a highly pathogenic H5N1 infection.[139]

Role of Cytokines/Chemokines and Their Receptors

Influenza A virus replicates in lung epithelial cells and leukocytes, resulting in the production of chemokines and cytokines that favor the recruitment of mononuclear cells to the site of infection. Influenza infection leads to the synthesis of major inflammatory mediators, including IL-6, G-CSF, IL-12p40/p70, MCPs, MIPs, and RANTES. Interleukin 6 exhibits multifunctional activities that are largely proinflammatory and their release has been correlated with the same clinical symptoms and signs related to influenza in mice.[75] Interferon γ inducible protein is a chemokine that is being studied for its role in the severity of lung damage after IAV infection. BALB/c mice infected with H5N1 after administration of IP-10 presented with more fulminant and necrotizing diffuse alveolar and bronchiolar damage with lymphocyte infiltration compared to infected mice without IP-10 administration.[140] This chemokine needs further research in IAV infection in pregnancy for its potential role in worsening the lung pathology and thereby increasing the severity.

Chemokine receptor (CCR) 5 and CCR2 (CCRs for MIP-1α and MCP-1, respectively) are both expressed on activated macrophages and T cells, and their role in immune response against IAV has been studied.[141] When infected with IAV, CCR5-deficient mice displayed increased mortality rates associated with acute, severe pneumonitis, and enhanced expression of MCP-1 and RANTES. In contrast, CCR2-deficient mice were protected from early pathological manifestations of influenza possibly because of defective macrophage recruitment. Chemokine receptor 2 deficiency also resulted in high pulmonary viral titers early in infection in CCR2-deficient mice which correlated with a relative delay in T-cell migration. Mice deficient in both CCR2 and MIP-1α paradoxically had the best survival and the highest viral titers among all groups in the study.[141] Absence of CCR2 blocks most of the early pulmonary macrophage accumulation, whereas MIP-1α has been shown to be a major regulator of T-cell trafficking into lymph nodes and specific tissues in response to a variety of antigenic stimuli, including IAV.[142,143] These studies suggest that MCP-1 and MIP-1α make distinct and additive contributions to the pulmonary inflammation induced by influenza virus infection.

As in humans, mice are highly susceptible to secondary bacterial infections after recovery from airway infection from an influenza virus. Mice with postinfluenza pneumonia express strikingly elevated pulmonary concentrations of IL-10 in comparison to mice with primary pneumococcal pneumonia.[26] Interleukin 10 inhibits the production of proinflammatory cytokines and chemokines by potently inhibiting neutrophil functions, including degranulation.[144] Thus elevated levels of IL-10 in mice with postinfluenza pneumonia may be responsible for the relatively reduced neutrophil function observed, resulting in increased susceptibility to secondary bacterial pneumonia. Therefore, therapies aimed at neutralization of IL-10 could play a role in prevention of postinfluenza pneumococcal pneumonia.[26]

Adaptive Immune Responses in Animal Studies

Published research on adaptive immune responses in animal models of influenza infection in pregnancy is very limited. Some of the studies described here outside of pregnancy can provide insights into further research in pregnancy and also in the area of vaccination. In mouse studies, memory CD4+ T lymphocytes impact various stages of antiviral immune response. Studies have shown that virus-specific memory CD4+ T cells may directly regulate innate inflammation within 48 hours after heterotypic challenge, independent of PAMP recognition.[145] In studies on heterosubtypic immunity in mice, clinical outcomes and histopathological changes in the lungs were compared between mice with heterosubtypic immunity and the naive group after lethal influenza virus infection.[146] Weight loss up to the first 4 days after infection followed by weight gain and accelerated clearance of the virus from the lungs were observed in mice with prior exposure to the influenza virus. The reduced clinical manifestations and accelerated viral clearance are correlated with anamnestic cytotoxic T-lymphocyte (CTL) responses in mice with prior priming

infection. It has also been shown that memory CTLs can reside in the lungs which would allow an immediate recall response upon secondary infection.[147-149]

Another important role CD4+ T cells fulfill when responding to a primary influenza challenge is actively supporting the generation of long-lasting functional memory CD8+ T cells, thereby aiding the magnitude of response and function of CD8+ T-cell effectors.[150,151] Interleukin 6 is critical to inducing naive and memory CD4+ T-cell IL-21 production upon T-cell receptor stimulation.[152] CD4+ T-cell IL-21 production is required for IL-6 to promote B-cell antibody production in vitro. Moreover, administration of IL-6 with an inactive influenza virus enhances virus-specific antibody production. Thus, IL-6, through increased IL-21 production, promotes antibody production by supporting the B-cell helper capabilities of CD4+ T cells.[152]

CD8+ CTLs are important effectors responsible for the clearance of viral infections and consequently a valuable population to induce by vaccination. It was noted that proinflammatory cytokine production by effector CD8+ T cells was largely restricted by T cells localized to the pulmonary interstitium in mice with sublethal influenza virus infection.[153] T-cell immunity induced after infection with human IAV may be cross-reactive with avian influenza viruses and could provide some degree of heterotypic protection against these highly pathogenic viruses by involving these virus-specific CD8+ CTLs. Induction of these cross-reactive CTLs may aid in the development of universal vaccines against viral infections.[146]

Although CD8+ T cells are associated with protective responses to IAV infection, it is becoming increasingly evident that they can also be associated with the development of influenza-related immunopathological sequelae.[154] A dysregulated CD8+ T cell response to IAV infection might lead to exacerbation of lung pathology and sustained lung injury.[155] This TLR3-mediated T-cell response has been studied in TLR3-deficient mice, where the excessive IFN-γ recovered from lungs was thought to contribute to a decrease in CD8+ T-cell number and limit T cell-mediated inflammation compared to wild type.[22] Prolonged survival and also increased pulmonary IAV load seen in TLR3-deficient mice after IAV infection were also attributed to this negative feedback regulation of CD8+ T cells by IFN-γ. Dysregulation of CD8+ T cells was also noted in studies of mouse pregnancy complicated by influenza infection. Such dysregulation was thought to be related to altered lung DC function and impaired IFN signaling.[156] It was further hypothesized that estrogen may play a role in this process.

Influenza viruses with HA of the H2 subtype are potent inducers of T cell-independent murine B-cell proliferation, and induction of this response is affected by the expression of cell surface I-E molecules (class II MHC glycoprotein) on B cells.[157] The inactivated H2-bearing influenza virus and purified HA of the H2 subtype induce B-cell proliferation and upregulate costimulatory molecules through a MyD88-dependent pathway independent of any known TLR.[158] Only members of the TLR/IL-1R receptor family have been reported to signal via MyD88-dependent pathways, thus suggesting activation of these innate responses in B cells by influenza HA may involve an unidentified TLR.[158]

Pregnancy Model

In one of the initial mouse models used to study the effects of IAV infection during pregnancy, infections with the WSN strain during the first and third weeks of gestation were found to have a detrimental effect on neonatal growth and development.[159,160] Infections in the third week resulted in significantly increased postnatal mortality in the first 8 weeks of life, with a similar trend observed in mice born to mothers infected in the first week of gestation. C3H inbred and Prince Henry outbred pregnant mice, infected with WSN and MEL strains in the third week of gestation, exhibited a 3-fold higher maternal mortality rate when compared with mice in the first week or with nonpregnant mice.[159] These findings are similar to the higher maternal mortality rates seen in human pregnancies during past influenza pandemics.[15,16]

The effects of wild-type and a mutant 2009 H1N1 influenza virus strain have been compared in pregnant and nonpregnant BALB/c mice.[161] The mutant strain contained a glutamate–glycine substitution at position 222 on viral HA and was found more frequently in patients who had severe pandemic infection. When infected at day 12 to 14 of gestation, mice experienced higher viral titers in the lungs, more severe histologic evidence of pneumonia, and significantly increased mortality rates compared to nonpregnant mice, especially in response to infection with the mutant virus. Moreover, pregnant infected mice expressed higher levels of proinflammatory cytokines and chemokines in lung homogenates. Only IFN-γ was significantly lower in pregnant mice. In addition, CD3+/CD4+ and CD3+/CD8+ peripheral T lymphocytes and specific antiviral serum antibody counts were lower in pregnant mice, leading to the conclusion that both innate and adaptive immune responses to influenza are suppressed during pregnancy.[161]

A similar study recently demonstrated significantly increased maternal mortality and fetal absorptions as a result of pandemic H1N1 infection. In this study, pregnant BALB/c mice had higher viral titers and elevated levels of inflammatory cytokines and chemokines (eg, IL-1α, IL-6, G-CSF, RANTES, and MCP-1) when infected with the pandemic H1N1 virus versus seasonal H1N1.[162] The study attributed fatality from pandemic H1N1 to its high pathogenic characteristics rather than a lack of preexisting immunity against the virus. Another study showed that the increased mortality rate in pregnant mice due to pandemic H1N1 infection was related to reduced regeneration of the respiratory epithelium, suggesting impaired lung repair and not related to the absolute viral burden in the nasal cavities/lungs.[163] This lethal influenza infection during pregnancy was associated with elevated pulmonary chemoattracants (such as MCP-1), enhanced numbers of macrophages and neutrophils, and increased nitrite (a nitric oxide metabolite) in the lungs. These mediators were

associated with decreased regeneration of the pulmonary epithelial lining, indicating an immunopathology-based mechanism of lung injury mediated by elevated cellular recruitment in severe pandemic H1N1 infection in pregnant mice.[163]

Another study in CD-1 mice addressed the immune system in pregnant and nonpregnant states and its contribution to increased maternal mortality in systemic illnesses, such as influenza and pyelonephritis.[164] Using a mouse model of systemic and localized inflammation induced by lipopolysaccharide injection (intraperitoneal or intrauterine) derived from *Escherichia coli* 055: B5, it was shown that the maternal immune response appeared to be dynamic and functionally different in pregnancy compared to nonpregnant state. The response to both systemic and local inflammation during pregnancy was functionally altered with increased serum concentrations of IL-10, IL-6, IL-12, and TNF-α in the pregnant state, correlating with decreased immune reactivity and increased susceptibility to infections. Moreover, response to local infection differed according to gestational age. Significantly lower concentrations of cytokines were observed in at-term versus preterm mice.[164] These results to some extent explain the increased morbidity from influenza infections during pregnancy as well as the associated risk of preterm birth.

Pregnancy Hormones and the Immune Response to Influenza

Progesterone and glucocorticoids, which increase during pregnancy, can have an anti-inflammatory effect.[165] This would explain the increase in severity of infectious agents such as influenza, which require prompt inflammatory responses for the initial control and clearance of pathogens.[15,166,167]

In addition, elevated levels of progesterone during pregnancy can stimulate the synthesis of progesterone-induced binding factor that promotes CD4+ T cell/helper T cell type 2 (Th2) differentiation, with increased serum concentrations of Th2 cytokines, including IL-4, -5, and -10.[168-170] The observed promotion of Th2 responses during pregnancy corresponds with a reduction in Th1 responses both systemically and at the maternal–fetal interface in animal models as well as in humans.[166,171-175] Peripheral regulatory T cells (a subset of CD4+ T cells) are thought to promote immune tolerance in pregnancy by upregulating transforming growth factor β and IL-10 through hemeoxygenase 1.[176-178] It is thought that the apparently tolerant microenvironment of the placenta is supported by these pregnancy-specific alterations in T cell immunity. Further research into the role of regulatory T cells and T helper subset regulation specific to influenza infection during pregnancy is needed.[176] Apart from Th responses, the direct role of progesterone in disease susceptibility and severity, in the context of influenza infection, also requires further investigation.

The effects of estrogens on the severity of influenza infection are more complex, wherein elevated levels imposed on nonpregnant mice are protective; in contrast, elevated levels during pregnancy are not.[179] Estrogen appears to have both anti- and proinflammatory effects depending on the level expressed,[156,179] and this may explain the differences in the severity observed. Additionally, estradiol, through its receptors, has been shown to activate the alveolar epithelial sodium channels promoting alveolar fluid clearance.[180] This mechanism seems to be challenged during influenza infection.[181,182] Influenza infection seems to induce a hypoestrogenic state that affects these sodium channels, decreasing clearance of alveolar fluid, and thereby increasing susceptibility to pneumonia.[180] Thus, estrogen can affect the severity of disease through mechanisms unrelated to immune system modulation.

Behavioral Implications of Influenza Infection

Evidence from animal and human studies has indicated that influenza infection during pregnancy is a risk factor for neuropsychiatric diseases, such as schizophrenia, in offspring.[183-186] This has been further elucidated in a recent study conducted on rhesus monkeys, where influenza infection affected fetal neural development with a reduction in gray matter throughout the cortex and decreased white matter in the parietal cortex.[187] These changes are thought to increase the likelihood of behavioral impairments later in life. The nature and extent of brain volume reductions seen in monkey bore most in common with the structural abnormalities found frequently in schizophrenia. Another interesting observation is that species differences in the extent of behavioral findings reflect the virulence of the influenza strain. The exact mechanism of abnormal brain development is not known. Proinflammatory cytokines generated by a maternal immune response transferred to amniotic fluid entering into the fetal circulation and permeating the immature blood–brain barrier and central nervous system has been implicated rather than direct exposure of the fetus to the virus.[187] There is also recent evidence from a nested case–control study of a population-based birth cohort, showing a significant 4-fold increase in the risk of bipolar disorder in adult offspring when exposed to influenza in utero.[188] An increase in slow-wave sleep accompanies influenza infection in certain strains of mice and has been shown to be related to the levels of the expression of the *If-1* gene.[189] The *If-1* gene codes for high and low production of IFN, which is considered a putative sleep-inducing cytokine. *If-1* has thus been identified as a genetically determined factor that elicits sleep after viral challenge.

Immunization

Vaccination

Immunization is the mainstay of influenza prophylaxis. There are 2 types of influenza vaccine: parenterally administered multivalent inactivated vaccine and intranasal live-attenuated virus vaccine.[190] The split-virus vaccine is the most used inactivated influenza formulation because it induces protective levels of antibody and has few side effects.[191] Whole-virus

vaccine is considered more immunogenic than split virus[192]; however, whole-virus vaccine has been associated with adverse reactions and thus it is currently not widely used.[193]

The American College of Obstetricians and Gynecologists and the Centers for Disease Control and Prevention recommend that all pregnant women and women planning a pregnancy occurring during the influenza season should be vaccinated and state that the multivalent inactivated vaccination is safe in all trimesters of pregnancy.[194-196] The immunization of women during pregnancy with an inactivated trivalent influenza vaccine (seasonal H1N1, H3N2, and influenza B virus) has also been recommended by the World Health Organization since 2005.[197] Population-based studies have not shown any adverse pregnancy effects of vaccination even with receiving the vaccine in the first trimester of pregnancy.[198] A recent study showed significantly decreased low birth weight and preterm birth (both decreased by nearly 25%) in the vaccinated compared to nonvaccinated women.[199] In contrast, other studies did not show any association of vaccination with spontaneous abortion, preterm birth, birth defects, or small for gestational age babies.[200,201] In the recent years, significant improvements in influenza vaccination coverage among pregnant women have been observed, from 11% during the 2001 to 2002 influenza season to 38% to 50% during the 2010 to 2011 influenza season, depending on the survey tool used.[202] Despite these improvements, efforts need to be directed toward identifying the key barriers to immunization and further increasing the acceptance rate of vaccination among pregnant women to reach the 80% vaccination goal established for 2020.[203]

In a randomized study of mothers who received the multivalent inactivated influenza vaccine, the illness caused by influenza virus decreased by 63% in infants, and the rate of respiratory illness with fever decreased by 36% in the mothers.[204] A follow-up study investigated the prevalence of mothers and infants with protective antibodies postvaccination in the third trimester and noticed significant antibody titers against the influenza A subtypes used to create the vaccine in a high proportion of mothers and their newborns, with newborns protected during first 6 months of childhood.[205] A study evaluating the efficiency of influenza vaccination in the second and third trimesters of pregnancy measured the Th1/Th2 balance, maintenance of antibody titers, and transplacental transfer of antibodies to the fetus after vaccination.[206] It was reported that the maintenance of the level of antibody depends on the time elapsed after vaccination, and titers decreased with time irrespective of gestational age. The presence of higher antibody titers in fetal blood may be related to the mechanism of active transfer. The successful immunization rate in the study of approximately 90% was independent of both the Th1/Th2 balance and the stage of gestation. Vaccination at any gestational age yielded sufficient antibody titers to provide protection against infection, showing that influenza vaccination can be administered at any stage of pregnancy.[206] In a study of a nonlethal murine challenge model, 2 doses of split-virus vaccine were found to be most effective in reducing viral shedding and were associated with high concentrations of prechallenge vaccine-induced serum IgG. Also, irrespective of IgG subclass, high concentrations of serum IgG induced by immunization were an important indicator of the efficacy of the vaccine.[193]

Significant protection from lethal challenge of H5N1 influenza viruses was reported in mothers and fetuses in studies conducted on pregnant mice immunized against H5N1 with 1- and 2-dose immunizations.[207] Newborn mice born to immunized mothers were also protected, and the complete protective immunity lasted for as long as 4 weeks after delivery. Protective immunity against the H5N1 influenza virus in newborn mice was closely related to the presence of IgG2a antibody subtype. This study concluded that maternal vaccination was effective and critical in protecting pregnant females, their fetuses, and their newborns from highly pathogenic viruses like H5N1.[207]

Immunotherapy

Although still under investigation, immunotherapy may be beneficial for the prevention and treatment of influenza. It remains uncertain whether administration of Igs to IgG2-deficient patients is likely to be therapeutically beneficial, although some studies report an IgG2-subclass deficiency in H1N1 infections during pregnancy.[93,94] Interestingly, convalescent blood products were administered during the 1918 Spanish influenza pandemic with a reduction in mortality.[208] A vaccination strategy providing broad protection against diverse influenza isolates could eliminate the need for annual vaccines and protect against emergent pandemic strains. One approach towards this goal could involve vaccines that generate strong memory T cells against multiple internal core proteins.[90] The CTL peptides (lipopeptide immunogens), although not significantly superior in inducing primary effector CD8+ T cells, do elicit a much more effective memory cell population, the recall of which may account for their superiority in inducing pulmonary protection after viral challenge, making them candidates for future vaccine incorporation.[150]

The use of TLR7 agonists as an adjuvant for antiviral vaccines and immunological interventions to clear existing viral infections are promising approaches in the search for new antiviral treatments.[56] Synthetic TLR7/8 agonist (3M-011) has been studied as an immunomodulator in a rat model of influenza infection and was noted to be beneficial for populations at risk of influenza infection. The advantage of a small molecule (less than 400 kDa) to act locally via intranasal preparations to induce an antiviral state without causing adverse systemic effects seems to be feasible. Apart from TLR7/8 agonists, TLR3, TLR4, and TLR9 agonists also have been investigated as standalone immunomodulators in murine models of influenza infection.[209] Studies in mice have revealed that poly(I: C), a synthetic TLR3 ligand, can modulate the immune responses including IFN induction and activation of NK cells. These responses provide nonspecific antiviral defense against several viral infections and may therefore provide a broad-spectrum antiviral effect against influenza viruses regardless

Table 1. Influenza Infection-Related Pathology and the Immune Alterations in Pregnancy.[a]

Site	Pathology/Sequelae	Pregnancy-Specific Immune Alterations
Lung	Inflammation-induced lung damage	↑ IL-6, IL-8, RANTES, IP-10
	Impaired lung repair	↑ MCP-1, neutrophils, macrophages, nitrites
	Secondary bacterial infection	Infection induced hypoestrogenic state → decrease alveolar fluid clearance and ↑ risk for pneumonia and secondary infection
Circulation	Viremia	
	↑ severity of infection	↑ Expression of TLRs 1, 3, 6, 7, 9
		↑ IL-10, IFN-γ (↑ susceptibility to secondary infection)
		↑ IL-6, 12
	↓ viral clearance	Altered pDC phenotype/number
		↓ IFN-α, λ
		Altered proinflammatory state ↓MCP-1, IFN-γ, ↑TNF-α
		Progesterone and glucocorticoids induced ↑ Th2 response
		Altered CD8 T-cell phenotype
		↓IgG2 levels
Maternal–fetal interface	Inflammation of placenta and membranes	↑ NK cell activation in maternal decidua
	Labor	↑ Prostaglandin release and ↑ MMP activity
	Apoptosis of chorion with rupture of membranes	↑ IL-6, 8, IL-1β, TNF-α, IFN-β, γ
		↑ chemotactic cytokines (IP-10, MDI factors)
		↑ MDI factors including IL-6 → recruitment of macrophages, ↑MCP-1, RANTES, MIP-1β

Abbreviations: IFN, interferon; IL, interleukin; IP-10; IFN-γ inducible protein 10; MDI, monocyte differentiation-inducing; MIP, macrophage inflammatory protein; NK, natural killer; RANTES, regulated upon activation and normal T-cell expressed and secreted; th2, helper T cell type 2; TLR, toll-like receptor; TNF, tumor necrosis factor; MCP, monocyte chemotactic protein 1; pDCs, plasmacytoid dendritic cells; Ig, immunoglobulin; MMP, matrix metalloproteinase.
[a]Summary of the pathology at different levels (lung, systemic circulation, and maternal fetal interface) in relation to influenza infection in pregnancy and the likely immune mechanisms contributing to these changes.

of the strain or subtype involved.[210] Caution is needed with this approach because multiple high doses of poly(I: C) administered intravenously have been found to produce toxic reactions in humans.[211] In addition, poly(I: C) has been observed to stimulate preterm delivery in pregnant mice[71,212]; therefore, its therapeutic utility during pregnancy is uncertain.

Summary, Conclusion, and Future Directions

During pregnancy, the immune system undergoes important adaptations to accommodate the immune tolerance required by the mother to carry the allogeneic fetus; however, these adaptions induce a potentially dysfunctional response to infections, when compared to the nonpregnant state. Further, these adaptations may account for the increased maternal/fetal morbidity and mortality observed in certain infections, including influenza. The underlying mechanisms contributing to these effects are summarized in Table 1. Existing evidence suggests that there occurs complex regulation of the maternal immune system, including innate and adaptive responses. This regulation occurs at various levels (locally in the lung, systemically, and at the maternal–fetal interface) and appears to differ according to gestational age. Regarding infection, such regulation is activated at various time points after exposure and is influenced by the extent of preexisting (prepregnancy) immunity. Expanded research concentrating on the maternal–fetal interface in influenza infection will likely lead to a better understanding of the mechanisms underlying pregnancy-related complications that occur as a result of infection. Ongoing research addressing the effects of gender and sex hormones on immunity as well as the direct effects of these hormones on the growth, adaptability, and virulence of infectious agents will provide further insights into this apparent altered immune status and susceptibility to infections.

Development of better animal models to more closely replicate the morbidity and mortality observed with human infections is also necessary to advance the study of the effect of infectious diseases during human pregnancy. For example, there is evidence that host genetic variations increase susceptibility to influenza infection.[213] Animal models may help define the role of genetic variability during influenza infection, especially by identifying key polymorphisms. Gene-specific animal models will facilitate identification of at-risk populations and new targets for therapeutic interventions and vaccines.[214] This is an area of growing interest.

In addition to immune system regulation during pregnancy, another important aspect of increased susceptibility in a pregnant host may be pregnancy-related adaptations in other organ systems. Pregnancy-related changes in the lung may be an important example with regard to influenza infection, as it has been reported that progenitor cells present in the bronchiolar epithelium are capable of regeneration after H1N1 infection.[215] Influenza immunopathogenicity during pregnancy is related to impaired lung repair in mice.[163] If confirmed in humans, this could open up new avenues for stem cell therapies in acute and chronic lung injury following severe infections, facilitating faster recovery.

Although influenza infection during pregnancy can result in spontaneous abortions and preterm delivery, the fetus appears

to be rarely infected directly. However, the emerging literature on schizophrenia and bipolar disorder in offspring of mothers exposed to IAV during pregnancy, and the contribution of the immune system to these indirect effects of infection, is worth further exploration.

Vaccination remains the mainstay for prevention of infection, and multivalent inactivated influenza vaccines are recommended during pregnancy irrespective of trimester. Another potentially fruitful area of research will be defining strategies to recognize the key barriers to immunization during pregnancy and improving vaccination rates in this high-risk group.

Influenza infection during pregnancy continues to be a complex and clinically important problem. Continuing efforts to understand the interactions between the mother, the virus, and the immune system, both systemically and locally, will lead to improved strategies for prevention and treatment.

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