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The Fetal Inflammatory Response Syndrome

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Abstract: The fetal inflammatory response syndrome (FIRS) is a condition characterized by systemic inflammation and an elevation of fetal plasma interleukin-6. This syndrome has been observed in fetuses with preterm labor with intact membranes, preterm prelabor rupture of the membranes, and also fetal viral infections such as cytomegalovirus. FIRS is a risk factor for short-term perinatal morbidity and mortality after adjustment for gestational age at delivery and also for the development of long-term sequelae such as bronchopulmonary dysplasia and brain injury. Multiorgan involvement in FIRS has been demonstrated in the hematopoietic system, thymus, adrenal glands, skin, kidneys, heart, lung, and brain. This article reviews the fetal systemic inflammatory response as a mechanism of disease. Potential interventions to control an exaggerated inflammatory response in utero are also described.

Key words: preterm labor, premature birth, prematurity, funisitis, chorioamnionitis, intrauterine infection; PROM

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Introduction

The fetal inflammatory response syndrome (FIRS) is a condition characterized by systemic activation of the fetal innate immune system. FIRS was originally defined in fetuses with preterm labor and preterm prelabor rupture of the membranes (PROM) by an elevation of the fetal plasma interleukin-6 (IL-6) concentration.[1] Affected fetuses had evidence of multiorgan involvement, had a higher morbidity rate after adjustment for gestational age, and were more likely to have

a subsequent spontaneous preterm delivery in cases of preterm PROM.

FIRS is the fetal counterpart of the systemic inflammatory response syndrome (SIRS),[1] described in adults.[2] SIRS and sepsis are the main causes of death of patients admitted to intensive care units, with mortality rates ranging from 30% to 70% despite more than 20 years of intensive basic and clinical research.[3,4]

The original definition of SIRS in adults was proposed in 1992 by the American College of Chest Physicians and the Society of Critical Care Medicine[2] based on the presence of ≥ 2 of the following findings: (1) changes in temperature [$> 38^{\circ}C$ (fever) or $< 36^{\circ}C$ (hypothermia)]; (2) heart rate changes [> 90 bpm (tachycardia)]; (3) respiratory rate or $PaCO_2$ changes [> 20 breaths/min (tachypnea) or $PaCO_2 < 32$ mm Hg (hypocapnia)]; and (4) white blood cell count changes [$> 12{,}000$ cells/mm^3 (leukocytosis) or < 4000 cells/mm^3 (leukopenia)]. A high or low white blood cell count could meet the criteria of SIRS without a left shift, which is defined as $> 10\%$ immature (band) cells in the differential count.[5] This definition of SIRS cannot be applied to the human fetus because the vital signs (with the exception of heart rate) cannot be readily determined before birth or the intrapartum period. This is the reason we elected to define FIRS in 1997[6] on the basis of a change in the concentration of a cytokine (IL-6), which is readily detectable in peripheral blood and easy to measure. In 2001, the American College of Chest Physicians and the Society of Critical Care Medicine noted that elevation of the plasma concentration of certain mediators, such as IL-6, may be associated with SIRS and that this observation may bring about a new definition of the syndrome in adult patients, as the clinical and laboratory findings originally proposed to characterize SIRS were nonspecific.[7]

Despite the similarities between FIRS and SIRS, the unique circumstances of the "patient" (fetus) and of its environment (uterus) pose challenges, which are *sui generis* for the diagnosis, management, and treatment of FIRS. In this review, we will describe the proposed pathophysiology, diagnostic criteria, multisystemic involvement, and short-term and long-term consequences of FIRS. This contribution is based on previous articles and chapters on the subject of FIRS published by our group in recent years.[8,9]

What is Inflammation?

Inflammation is the basic process by which tissues of the body respond to insults.[10] The first comprehensive description of the clinical signs of inflammation has been attributed to Celsus,[11] who introduced 4 of the 5 classic signs (*calor*, *dolor*, *rubor*, *and tumor*), which translate to heat, pain, redness, and swelling. Galen added the fifth sign (*function laesa*), which means impaired function.[11] Since that time, clinical inflammation has been classically defined by the presence of these 5 cardinal signs, all of which reflect the effects of cytokines, chemokines, and other inflammatory mediators on local blood vessels and tissues.[12,13] Vasodilatation and increased permeability account for the changes in temperature, redness, and swelling (to some extent), whereas the migration of cells into tissue and the action of their mediators on the nerve endings account for pain and swelling.[13] In contrast, histologic inflammation is defined by infiltration of the tissue by neutrophils, macrophages, and lymphocytes.[11] The type of infiltrating cell classifies inflammation into acute or chronic. However, this classification may have some limitations in reproductive tissues in which there is physiologic infiltration of inflammatory cells. For example, neutrophils are normally present in menstrual endometrium,[14] and the differential

diagnosis between acute endometritis and perimenstrual endometrium requires examination of the magnitude of the infiltration.[15] Pathologic examination has been the gold standard for the diagnosis of inflammation. However, chemotactic signals must be present for the white blood cells to migrate to the site of injury or infection. Thus, there is a window of time in which a "molecular signature of inflammation" is present before histologic evidence is observed. For example, analysis of the transcriptome[16] or the detection of inflammatory markers in body fluids (eg, plasma, cerebrospinal fluid, or amniotic fluid) may allow both detection of early signs of inflammation that may not be detectable by conventional pathology and diagnosis without tissue samples.

A clear understanding of the spectrum of inflammation is important because a common misconception is that inflammation (and sometimes infection) is unlikely in the absence of systemic clinical signs, such as fever, chills, leucocytosis, etc. The evidence and the understanding of pathophysiology suggest that the opposite is true. Most cases of histopathologic inflammation are subclinical in nature. This is the case in histologic chorioamnionitis, both at term (Yoon and Romero et al, unpublished observations) and preterm.[17,18]

The Role of Inflammation in Physiology and Pathology

Inflammation is widely regarded as the fundamental mechanism by which multicellular organisms deal with insults, both of infection-related and non–infection-related. Inasmuch as the primary force shaping the evolution of the immune system is defense against microorganisms, it is not surprising that the mechanisms of inflammation were discovered when studying infectious diseases.[19] However, inflammation also plays a central role in physiologic processes, particularly in the reproductive tract. The rupture of an ovarian follicle,[20] the implantation of the blastocyst,[21,22] menstruation,[23] and parturition[24,25] are characterized by cellular and molecular events which are found in pathologic inflammation (ie, that associated with disease).

The Benefits of an Inflammatory Response

In the presence of invading microorganisms, inflammation accomplishes 3 main goals: (1) to deliver cells and molecules to suppress the infection; (2) to generate a physical barrier to the spread of the infection; and (3) to promote repair of the injured tissue.[13]

Cells called to the site of injury include macrophages, neutrophils, and lymphocytes. Molecules released during the course of inflammation include antimicrobial peptides, cytokines, chemokines, and other inflammatory mediators such as prostaglandins, leukotrienes, complement, etc.[13] Some of these molecules change the state of activation of macrophages and neutrophils so that microbial killing is enhanced (ie, through the release of reactive oxygen species). For example, activation of nicotinamide adenine dinucleotide phosphate oxydase converts molecular oxygen into superoxide, which in turn is transformed by superoxide dismutase into hydrogen peroxide. This molecule has antimicrobial properties and can be transformed by peroxidase to hypochlorite and hydroxyl radicals. Superoxide, hydrogen peroxide, and hydroxyl radicals are called reactive oxygen species or reactive oxygen metabolites, and are important in microbial killing.[13] When these molecules are released outside of phagocytes, they can injure other host cells. The state in which there is an excess generation and activity of reactive oxygen

metabolites is called oxidative stress and is a mechanism of disease in systemic inflammation (eg, sepsis[26] and preeclampsia[27–30]).

A second major goal of inflammation is to prevent the spread of microorganisms, and this is often accomplished by activation of the coagulation system and formation of thrombi in blood vessels draining the infected/inflamed sites. Thrombin, the rate-limiting step of coagulation, has also proinflammatory properties. Indeed, in vitro experiments had demonstrated that thrombin enhances the secretion of lipopolysaccharide (LPS)-induced IL-1 and tumor necrosis factor-α (TNF-α) in monocytes.[31]

Inflammation as a Response to "Danger Signals" (Microbial or Not)

Injury can be the result of exposure to microorganisms or non–microbial-related insults. The consequences of microbial invasion and proliferation are well known and, therefore, will not be discussed in this section. However, the means by which nonmicrobial insults can injure and be recognized by the host are less well known. For example, exposure to an allergen or a transplanted organ can cause disease because the immune system recognizes the allergen or the organ as nonself. How is this accomplished? The immune system has evolved to identify the nonself using pattern recognition receptors (PRRs) which are receptors that can identify repeating patterns of molecular structure, common to most microorganisms.[13] However, it is now realized that these PRRs can be used not only to sense the presence of microorganisms but also to identify danger signals.[32] The basic premise of the "danger model" is that the immune system is more concerned with damage than with foreignness ("nonself"), and that an immune reaction is set into action because of "alarm signals" from injured tissues rather than by the recognition of nonself.[32]

Examples of alarm signals are those released by necrotic cells of the host that have been injured by microbial or nonmicrobial insults. The first PRRs identified where Toll-like receptors (TLRs)[33,34] and the ligands for these receptors were originally thought to be of microbial origin (eg, endotoxin, peptidoglicans, viral RNA, etc).[13,33,34] It is now known that TLRs can recognize not only microbial products but also host signals produced in the context of injury, such as heat-shock proteins.[35] Thus, the danger model of immunity provides a framework to understand the nature of the immune response and it releases it from the previously held paradigm, which was dependent largely on the self versus nonself concept.[32]

The immune response has 2 components: the innate and the adaptive. The innate mechanisms act immediately, are nonspecific, and lack immunologic memory. The adaptive immune response, on the other hand, is specific, takes time to develop, and has memory.[13] Inflammation is part of the innate immune response. However, it is now known that innate immunity orchestrates an appropriate adaptive immune response, and inflammation is also part of the adaptive response.[13] In conclusion, inflammation can be thought as central to maintaining tissue homeostasis. Exaggerated or prolonged inflammation or lack of an adequate inflammatory response can lead to disease.[9]

Innate and Adaptive Immune Responses

The immune system evolved primarily for host defense against infection. Host defense is accomplished by means of the innate and adaptive limbs of the immune system. The innate component

is nonspecific, whereas the acquired or adaptive response improves with repeated exposure to a specific antigen.[36] The cells of the immune system are derived from pluripotential hematopoietic stem cells, which are first present in the yolk sack and then migrate to the liver, spleen, and bone marrow. In response to inductive signals, these hematopoietic stem cells undergo differentiation along 2 major pathways: (1) the innate immune system, whose cellular components include phagocytes (neutrophils, monocytes, macrophages), cells that release inflammatory mediators (eg, basophils, mast cells, eosinophils) and natural killer cells; and (2) the adaptive immune system, composed of 2 major classes of lymphocytes (B and T cells).[12]

Innate Immunity

The first line of defense against infection is provided by the innate immune system. Epithelial surfaces (skin and mucous membranes) represent the first physical barrier between the body and microorganisms. Injuries to the epithelial surface provide a point of entry of microorganisms. These injuries can result from accidents or physiologic processes (eg, menstruation). Thus, a sexually transmitted microorganism may cause infection if it gains access to the endometrial wound during menstruation. However, bacteria can cross-intact epithelial barriers. There is experimental[37] and clinical evidence[38,39] that bacteria can cross-intact chorioamniotic membranes. Epithelium, however, represents more than a physical barrier against microorganisms. Most epithelia produce natural antimicrobial peptides (eg, α-defensins and β-defensins),[40] which can kill bacteria by damaging their cell membrane.[41–44] For example, the fetal lung produces surfactant proteins (SP-A[45,46] and SP-D[45]), that belong to the collectin family, which can bind microorganisms and facilitate phagocytosis (opsonization). Moreover, SP-A and SP-D have been shown to be involved in clearance of bacteria, fungi, and apoptotic and necrotic cells, down-regulation of allergic reaction, and resolution of inflammation.[47]

Another mechanism of host defense against infection derives from the metabolic products of bacteria. For example, lactobacilli, which colonize the vagina shortly after birth, produce lactic acid and lower the pH of the vagina. This unique partnership between vaginal tissues and species-specific strain of lactobacilli has been considered responsible for enabling internal fertilization in the evolution of mammals from amphibians.[48] In addition to the low pH, some strains of lactobacilli also produce antimicrobial products (bacteriocinlike compounds), which prevent the growth of pathogenic bacteria.[49,50]

The innate component of the immune system also provides immediate protection from microbial challenge by recognizing the presence of microorganisms, thus preventing tissue invasion and/or eliciting a host response to limit microbial proliferation (inflammation).[13] One of the mechanisms by which the innate immunity recognizes microorganisms is by using PRRs, which bind to repeating patterns of molecular structures present in the surfaces of microorganisms.[13] PRRs which are classified by their function and subcellular localization into the following groups: (1) soluble PRRs such as "the acute-phase proteins" mannan binding lectin and C-reactive protein (CRP), which act as opsonins to neutralize and clear pathogens through the complement and phagocytic systems; (2) transmembrane PRRs, which include scavenger receptors, C-type lectins, and the TLRs; (3) intracellular PRRs, including Nod1 and Nod2, RIG-1, and MDA-5, which mediate recognition of intracellular pathogens (eg, viruses).[51]

Ten different TLRs have been recognized in humans.[13] TLR-4 recognizes the presence of LPS (Gram-negative bacteria), TLR-2 recognizes peptidoglycans, lipoproteins, and zymosan (Gram-positive bacteria, mycoplasmas, and fungi). TLR-3 recognizes double-stranded RNA (viruses). The ligand for TLR-5 is flagellin.[13,52,53]

Ligation of TLRs results in activation of NF-κB, which, in turn, leads to the production of cytokines, chemokines, and antimicrobial peptides.[13] Moreover, activation of the Toll pathway also induces surface expression of costimulatory molecules such as CD80 and CD86, required for the induction of adaptive immune responses. These molecules, in combination with antigenic microbial peptides, presented by major histocompatibility complex class II proteins in dendritic cells and macrophages, can activate naïve CD4 T cells, which, in turn, initiate most adaptive immune responses.[13]

Given that TLRs are crucial for the recognition of microorganisms, it could be expected that defective signaling through this PRRs will impair bacteria-induced preterm labor. Mice with a spontaneous mutation for TLR-4 are less likely to deliver preterm than wild-type mice after intrauterine inoculation of heat-killed bacteria or LPS administration.[54,55] In pregnant women, TLR-2 and TLR-4 are expressed in the amniotic epithelium.[56] In addition, spontaneous labor at term or preterm with histologic chorioamnionitis, regardless if the membrane are intact or ruptured, is associated with an increased mRNA and protein expression of TLR-2 and TLR-4 in the chorioamniotic membranes.[56] These observations suggest that the innate immune system plays a role in parturition.

Cytokines and Chemokines

Cytokines are small soluble peptides or glycoproteins, whose primary function is intercellular communication. They are produced by and act on leukocytes, endothelium, and other cells through autocrine and paracrine mechanisms. The half-life of cytokines is short, and repeated stimuli are generally required for continued production. Cytokines play a major role in the control of the inflammatory response through the regulation of: (1) the innate immune response; (2) the adaptive immune response; and (3) the growth and differentiation of hematopoietic cells.

Cytokines exert their effects by binding to specific receptors. In general, the affinity of binding between cytokines and their receptors is high and, thus, cytokines have effects at picomolar concentrations. Often, a cytokine binding to its receptor results in the expression of other cytokines and this is the reason the term "cytokine network" has been used extensively in the literature. For example, IL-1 and TNF-α activate NF-κB, a transcription factor, which in turn, stimulates expression of IL-6.

Individual cytokines have multiple functions (pleiotropy), and also, the same biologic function can be induced by several cytokines (redundancy). The broad categories include: (1) interferons (IFN), (2) interleukins, (3) TNF and related molecules, (4) transforming growth factors, (5) hematopoietic growth factors, and (6) chemokines.

Chemokines (ie, IL-8, RANTES, IP-10) attract leukocytes to the site of inflammation. A subset of cytokines is proinflammatory in nature (IFN-γ, IL-1, IL-12, and TNF-α). Anti-inflammatory cytokines include IL-4, IL-10, IL-11, IL-13, and transforming growth factor-β.

Cytokines are classified by their primary cellular origin; either T-helper type 1 (T_H1) or T_H2 cells. T_H1 cytokines (ie, IFN-γ, IL-2, and TNF) function mainly to promote cell-mediated immunity for protection against intracellular bacteria and viruses. The role of T_H2 cytokines (ie, IL-4, IL-6, IL-10, and IL-13) is to promote humoral immunity for protection against extracellular pathogens.

The proinflammatory cytokines IL-1-β and TNF-α are considered to be central mediators of septic shock and the administration of these cytokines to animals elicits many of the clinical manifestations of sepsis such as fever, hypotension, leukocytosis, etc (Fig. 1). The reader is referred to reviews on the subject for a full discussion of the role of cytokines in sepsis and SIRS.[57–69] IL-6 is a major mediator of the acute-phase response to tissue injury. It is detectable in the peripheral circulation and can stimulate the production of acute-phase reactants such as CRP by the liver.

A Role for Cytokines and Chemokines in the Onset of Preterm Parturition

The current view is that during the course of ascending intrauterine infection, microorganisms may reach the decidua, where they can stimulate a local inflammatory reaction and the production of proinflammatory cytokines and inflammatory mediators. If this inflammatory process is not sufficient to signal the onset of labor, microorganisms can cross intact membranes into the amniotic cavity, where they can also stimulate the production of inflammatory mediators by resident macrophages and other host cells. Finally, microorganisms that gain access to the fetus may elicit a systemic inflammatory response syndrome.

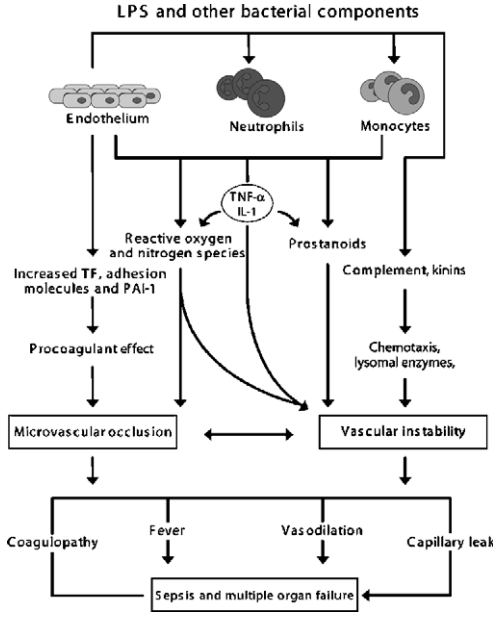

FIGURE 1. Reprinted with permission from Macmillan Publishers Ltd: *Nat Rev Drug Discov*. 2003;2:635–645.[68] Scheme for main effector pathways activated by sepsis that contribute to end-organ dysfunction.

Compelling evidence supports a role for inflammatory mediators in the mechanisms of preterm parturition. IL-1 was the first cytokine to be implicated in the onset of spontaneous preterm labor associated with infection.[70] Evidence in response of the participation of IL-1 includes: (1) IL-1 is produced by human decidua in response to bacterial products[71]; (2) IL-1 can stimulate prostaglandin production by human amnion and decidua[72]; (3) IL-1 concentration and bioactivity was increased in the AF of women with preterm labor and infection[70]; (4) IL-1 could stimulate myometrial contractions[73] (C. Bulletti, personal communication 2002); and (5) administration of IL-1 to pregnant animals induced preterm labor and preterm birth,[74] a phenomenon that could be blocked by the administration of its natural antagonist: IL-1 receptor antagonist (IL-1ra).[75] Similarly, evidence supporting the role of TNF-α in the mechanisms of preterm parturition includes: (1) TNF-α stimulates prostaglandin production by the amnion, decidua, and myometrium[76]; (2) human decidua can produce TNF-α in response to bacterial products[77,78]; (3) AF TNF-α bioactivity and immunoreactive concentrations are elevated in women in preterm labor and intra-amniotic infection[77]; (4) in women with PPROM and intra-amniotic infection, TNF-α concentrations are higher in the presence of labor[79]; (5) TNF-α can stimulate the

production of MMPs,[80,81] which may play a role in membrane rupture[82–84] and cervical ripening[81,85,86]; (6) TNF-α application on the cervix induces changes that resemble cervical ripening[87]; and (7) TNF-α is involved in the mechanisms of bacterial-induced preterm parturition in animal models.[88–90]

Can the Fetus Mount an Inflammatory Response?

During intrauterine life, several mechanisms operate so that the fetus can mount an immune response to pathogens while preserving self-tolerance. Such mechanisms include: (1) clonal deletion of T lymphocytes in the thymus[91]; (2) functional inactivation and tolerance mediated by a subset of T regulatory cells or $CD4^+$ $CD25^+$ Tr cells,[91] which have the ability to inhibit T-cell proliferation in vitro[92,93]; and (3) activity of functional regulatory cells in the thymus and secondary lymphoid organs, which have been identified in the human fetus from 14 to 17 weeks of gestation.[94]

Pioneering studies of the fetal immune system, published more than 50 years ago by the group of Sir Peter Medawar, suggested that antigen presentation in the fetus induces tolerance, rather than immunity, and that embryonic and neonatal lymphocytes are hyporesponsive.[95] This view, however, is not consistent with well-established observations which demonstrate that the fetus can mount an innate and also an adaptive immune response. Evidence in support of this includes: (1) fetuses with preterm labor or preterm PROM can have neutrophil and monocyte activation, as demonstrated by immunophenotyping with flow cytometry[96]; (2) elevations of fetal plasma concentrations of IL-6[1] and CRP[97] have been demonstrated in a subset of fetuses with preterm labor and preterm PROM; (3) in mice, fetal T lymphocytes can mount T_H1 (cell-mediated) and T_H2 (antibody mediated) immune responses upon stimulation with an appropriate amount of antigens, antigen presenting cells, and adjuvants[98]; (4) umbilical cord blood of human neonates with clinical evidence of infection, has a higher proportion of T_H1 cells than that from uninfected neonates. Furthermore, among patients with preterm PROM, the percentage of T_H1 cells in the infected neonates correlates with the duration of membrane rupture before the onset of labor[99]; and (5) umbilical cord blood from neonates with PROM has an increased proportion of IFN-γ–secreting cells (T_H1-polarized response)[100]; (6) fetuses with congenital infection with rubella or cytomegalovirus can have an antibody response which has been used for diagnostic purposes.[101–104] Collectively, this evidence indicates that fetuses are capable of mounting an immune response.

FIRS was originally described in pregnancies complicated by preterm labor and preterm PROM, and was operationally defined as a fetal plasma concentration of IL-6 > 11 pg/mL. Fetuses with FIRS had a higher rate of severe neonatal morbidity [respiratory distress syndrome (RDS), suspected or proven neonatal sepsis, pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage, periventricular leukomalacia (PVL), or necrotizing enterocolitis][1] and a shorter cordocentesis-to-delivery interval.[1,105]

The original work describing FIRS was based on fetal blood samples obtained by cordocentesis.[1,105] Many of the findings have since been confirmed by studies on umbilical cord plasma at the time of birth, which have demonstrated elevation of proinflammatory cytokines and the relationship between these cytokines and the likelihood of clinical and suspected sepsis.[106–108] Pathologic examination of the umbilical cord is an alternative approach for determination of whether a fetal inflammation was present before birth.

Funisitis and chorionic vasculitis are the histopathologic hallmarks of FIRS.[109] Funisitis is associated with endothelial activation, a key mechanism in the development of organ damage,[110] and neonates with funisitis are at increased risk for neonatal sepsis[17] and long-term handicaps, such as bronchopulmonary dysplasia[106] and cerebral palsy.[111] Another approach to the detection of FIRS is measurement of CRP concentration in umbilical cord blood, which is elevated in patients with amniotic fluid infection, funisitis, and congenital neonatal sepsis.[112] In addition, as neutrophils in the amniotic fluid are predominantly of fetal origin,[113] the amniotic fluid white blood cell count can be used as an indirect index of fetal inflammation.[113] Intra-amniotic inflammation is a risk factor for impending preterm delivery and adverse perinatal outcome in women with preterm PROM, even in the absence of documented intra-amniotic infection.[114]

Fetal Target Organs During FIRS

Fetal microbial invasion or other insults can result in a systemic fetal inflammatory response that can progress toward multiple organ dysfunction, septic shock, and perhaps death in the absence of timely delivery. Evidence of multisystemic involvement in cases of FIRS includes increased concentrations of fetal plasma matrix-metalloproteinase-9 (MMP-9),[115] an enzyme involved in the digestion of type IV collagen and in the pathophysiology of preterm PROM.[84] Moreover, several fetal organs including the hematopoietic system, the adrenals, heart, brain, lungs, and skin have been proposed to be target organs during FIRS (Fig. 2).

THE HEMATOPOIETIC SYSTEM

The hematologic response of the human fetus with FIRS is characterized by significant changes in the granulocyte and red blood cell lineages.[116] Indeed, two thirds of fetuses with FIRS have neutrophilia, defined as a neutrophil blood count above the 95th percentile for the gestational age.[116] In contrast, only 7.1% of these fetuses (3/42) had neutropenia.[116] The mechanisms responsible for fetal neutrophilia are not completely understood. However, it has been proposed that granulocyte colony stimulating factor, the primary physiologic regulator of neutrophil

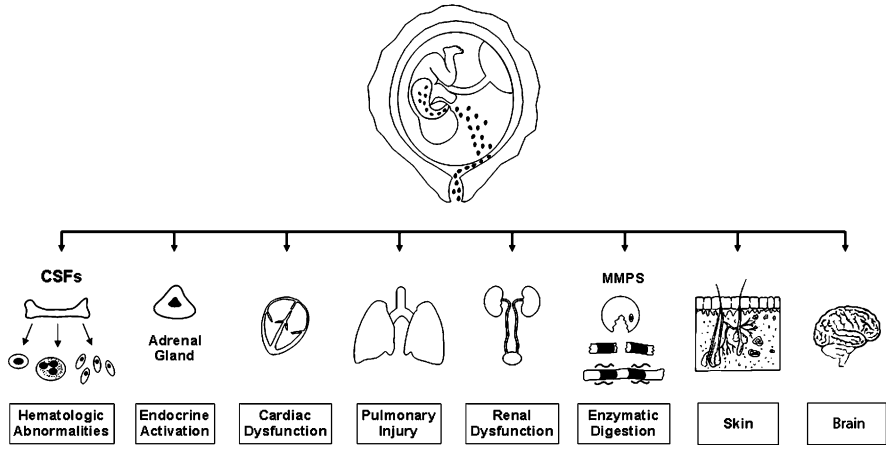

FIGURE 2. Fetal target organs during the FIRS. CSFs indicate colony-stimulating factors.

production, may participate in these mechanisms.[117] Indeed, fetuses with FIRS had a higher median plasma concentration of granulocyte colony stimulating factor than those without FIRS (median: 714.4 pg/mL, range: 23.3 to 4229.2 vs. median: 55.7 pg/mL, range: 7.7 to 411; $P < 0.01$).[117]

Fetuses with FIRS had a higher median nucleated red blood cell count than those without FIRS (median count: 2.42, range: 0 to 35 vs. median count: 1.38, range: 0 to 63.6; $P < 0.05$).[116] These changes are not associated with changes in the umbilical vein pH or PO_2 levels.[118] Thus, metabolic acidemia is unlikely to be the cause of these hematologic changes, as described in fetuses with intrauterine growth restriction and abnormal Doppler velocimetry in the middle cerebral artery, inferior vena cava, and ductus venosus.[119] Thus, the possibility that elevated nucleated red blood cell counts are a consequence of FIRS should be considered. Evidence in favor of this has been recently reported, indicating that the IL-6 concentration in umbilical cord blood may be an independent explanatory variable for the prediction of high-nucleated red blood cell count.[120]

FIRS has also been associated with changes in markers of monocyte and neutrophil activation.[96] Indeed, fetuses were delivered within 72 hours of cordocentesis had a higher expression of CD11c, CD13, CD15, and CD67 than those delivered at term. In contrast, there were no significant differences in the percentages of CD14 and CD63 between the 2 groups. Collectively, these results indicate that fetuses destined to deliver prematurely have phenotypic evidence of activation of the monocyte-neutrophil system.[96]

THE FETAL THYMUS

Recent evidence indicates an association between thymus involution and infection in both fetuses and neonates.[121–123] Di Naro et al[124] found a sonographically small thymus in cases with intra-amniotic infection/inflammation, among patients with preterm labor and intact membranes. The fetal thymus perimeter measured less than the fifth percentile for gestational age in 10 out of 10 cases with microbial invasion of the amniotic cavity (MIAC), in contrast to 5 of 21 (23.8%) cases with negative cultures ($P < 0.01$). Moreover, the fetal thymus measured less than the fifth percentile for gestational age in 8 of 8, 5 of 7 (71.4%), and 2 of 16 (12.5%) fetuses with funisitis, isolated chorioamnionitis, and absence of histologic signs of inflammation, respectively. Depletion of thymocytes probably results from glucocorticoid-induced apoptosis of the lymphoid tissue during the acute-phase response or from the effect of cytokines (see below).[125] Thymic involution has been proposed to be the result of lymphocyte depletion from both the thymic cortex and medulla, possibly mediated by activation of the hypothalamo-pituitary-adrenal axis.[125]

In 89 preterm newborns (< 28 wk of gestation), Kuban et al[126] studied whether the interval between birth and thymus involution relates to cerebral white matter damage. The time interval to thymus involution was determined by serial chest x-rays, and cerebral white matter damage was estimated by echolucency on postnatal cranial ultrasound scans. Infants with white matter echolucency were more likely to have undergone early involution (involution before the median interval for the gestational age group). The authors propose that early thymus involution can be attributed to 2 phenomena: stress and/or inflammation. The role of stress is supported by findings in mice, documenting a relationship between elevated glucocorticoid concentrations and acute thymus involution. On the other hand, there are reports on the association between histologic chorioamnionitis and fetal vasculitis/funisitis, with thymus involution.[98] This process can be attributed

to the effects on the thymus of proinflammatory cytokines such as IL-1, directly or via steroid production.[127,128]

THE ADRENAL GLANDS

Fetuses with FIRS have endocrine evidence of "stress" expressed as an abnormal cortisol/dehydroepiandrosterone ratio.[129] Indeed, Yoon et al[129] reported a significant correlation between fetal plasma cortisol and fetal plasma IL-6 ($R = 0.3$, $P < 0.05$) and a significant association between fetal plasma cortisol/dehydroepiandrosterone sulfate (DHEA-S) ratio and a shorter interval from cordocentesis to delivery [hazards ratio: 2.9, 95% confidence interval (CI): 1-8.4; $P < 0.05$]. Fetal plasma cortisol, but not maternal cortisol, was an independent predictor of the duration of pregnancy, after adjusting for gestational age and the results of amniotic fluid cultures (hazards ratio: 2.9, 95% CI: 1.3-6.7; $P < 0.05$). Patients with preterm PROM, who went into spontaneous labor and delivered within 7 days of cordocentesis, had a significantly higher median fetal plasma concentration of cortisol but not of DHEA-S than those delivered after 7 days (for fetal plasma cortisol: median 8.35 μg/dL, range: 4.7 to 12.4 μg/dL vs. median 4.75 μg/dL, range: 3.0 to 10.4 μg/dL; $P < 0.0001$; for fetal plasma DHEA-S: median 154.4 μg/dL, range: 8.6 to 333.8 μg/dL vs. median 194.6 μg/dL, range: 96.7 to 402.5 μg/dL; $P = 0.09$).[129] Collectively, these results indicate that an elevation in fetal plasma cortisol, but not DHEA-S, was followed by the onset of spontaneous preterm labor in patients with preterm PROM. Adult patients admitted to an intensive care unit with burns[130] or pancreatitis, have elevation of the cortisol/DHEA-S ratio just as human fetuses with FIRS. These metabolic changes may have short-term and long-term implications given recent observations about the effect of glucocorticoids in fetal programming of several metabolic functions.[131–135]

THE FETAL SKIN

The fetal skin is another target organ during FIRS. Indeed, Kim et al[136] studied the expression of TLR-2 and TLR-4 in skin samples from fetuses delivered between 21 and 24 weeks of gestation who died shortly after delivery, and reported that: (1) the skin from fetuses born to mothers without chorioamnionitis expressed TLR-2 and TLR-4 in the epidermis (TLR-2: median 3%, range 0.4% to 7.2% and TLR-4: median 99.5%, range 91% to 100%); (2) there was a dramatic increase in the expression of TLR-2, but not in TLR-4, in the epidermis of fetuses born after chorioamnionitis (TLR-2: median 19.6%, range 10.3% to 89.6%; $P = 0.007$ and TLR-4: median 100%, range 89.4% to 100%; $P = 0.5$); and (3) TLR-2 and TLR-4 were also expressed in the mononuclear inflammatory infiltrate of the dermal-epidermal junction. The authors proposed that the fetal skin is capable of recognizing the presence of microorganisms through the expression of "pattern recognition receptors" and, thus, participates in a fetal inflammatory response to microbial products.[136] The clinical manifestation the involvement of the fetal skin during FIRS would be a fetal dermatitis.

THE FETAL KIDNEYS

Yoon et al[137] reported that oligohydramnios is associated with FIRS among patients with preterm PROM. Indeed, patients with an amniotic fluid index ≤ 5 cm had (1) significantly higher IL-6 concentrations in umbilical cord plasma at birth (fetal response); (2) higher concentrations of amniotic fluid proinflammatory cytokines, such as IL-6, IL-1-β, and TNF-α (intra-amniotic inflammatory response); and (3) higher rates of histologic and clinical chorioamnionitis

(maternal response) than did those with an amniotic fluid index >5 cm.[129,137] These observations are consistent with the report that fetuses with fetal bacteremia, diagnosed by cordocentesis, had oligohydramnios (amniotic fluid index <5 cm) more frequently than did those with a sterile blood culture.

The reasons oligohydramnios in preterm PROM is associated with a higher rate of fetal infection/inflammation remain unclear. Yoon et al proposed that since the amniotic fluid has antimicrobial properties,[138,139] oligohydramnios may reduce the protective effect of this component of innate immunity. Alternatively, a redistribution of blood flow away from the kidneys may take place as part of the host response to microbial products, leading to oligohydramnios.[129,137]

THE FETAL HEART

A recent report indicates that fetuses with preterm PROM have changes in the parameters used to evaluate diastolic function of the heart when compared with fetuses of women with uncomplicated pregnancies.[140] The changes in the Doppler velocimetry in fetuses with preterm PROM are consistent with a high left ventricular compliance, particularly among fetuses with proven intra-amniotic infection. These changes include a higher ratio between the early filling delta early diastolic filling/atrial contraction ratio in both ventricles and a higher delta diastolic filling/atrial contraction velocity time integral in the left ventricle. It is possible that these changes represent a compensatory mechanism, similar to that observed in adults with sepsis. It is also possible that fetuses unable to change cardiac compliance, in the context of a fetal SIRS, may not be able to maintain ventricular stroke volume and cardiac output and, hence, may not perfuse the brain adequately, predisposing to hypotension and brain ischemia in utero, which could create conditions for the development of PVL. These changes in diastolic function may, therefore, have protective and even survival value. In cases of overwhelming fetal sepsis (the pathophysiologic counterpart to septic shock in adults), myocardial depression may lead to fetal death, which we have observed in cases with preterm PROM. The mechanism by which sepsis induces myocardial depression is not completely understood. The most likely explanation is the action of soluble factors, such as bacterial products and cytokines, which are elevated in the circulation of patients with septic shock.[141–143]

The observation that fetuses with preterm PROM and intra-amniotic infection undergo changes in cardiac function are consistent with the findings of Yanowitz et al.[144] The authors reported that neonates born with histologic chorioamnionitis had several hemodynamic abnormalities, including a decreased mean and diastolic blood pressure, and that there was a correlation between mean blood pressure and umbilical cord IL-6 concentrations.[144] It is possible that some of these hemodynamic changes are present in utero and may contribute to the pathophysiology of PVL and cerebral palsy.[145] These conditions were originally considered to be due to ischemia/hypoxia and have recently been linked to chorioamnionitis, infection, and fetal inflammation. In the context of FIRS, the combination of inflammatory changes in the brain and fetal systemic hypotension may increase the likelihood of brain injury. Recent evidence in support of this view is the observation that histologic chorioamnionitis with histologic evidence of placental hypoperfusion (poorly vascularized villi, multiple capillary lumina in some villi, increased intervillous volume, and reduced total capillary bed) is associated with and odds ratio (OR) of 15.2 (95% CI: 1.3-181) to have abnormal neurologic outcome at the corrected age to 24 months.[146]

THE FETAL LUNG

Amniotic fluid can be inhaled during "fetal breathing movements" in normal pregnancy. Indeed, it is possible to demonstrate influx and efflux of amniotic fluid from the fetal upper respiratory tract with color Doppler ultrasound.[147,148] It is less clear whether amniotic fluid reaches the distal part of the airways and the alveoli under normal circumstances. Meconium has been detected in the alveoli in cases of fetal death, suggesting that, under certain circumstances, amniotic fluid can reach the fetal lung (ie, fetal gasping under conditions of hypoxia).

Examination of tracheobronchial fluid obtained through an endotracheal tube placed shortly after birth shows the presence of white blood cells and microorganisms (eg, *Ureaplasma urealyticum*) in patients with intra-amniotic infections. These observations suggest that infected/inflamed amniotic fluid can reach the distal respiratory tract.

Several lines of evidence suggest that intrauterine inflammation is associated with the subsequent development of chronic lung disease, often referred to as bronchopulmonary dysplasia.[149–152] Watterberg et al[153] studied the association between lung inflammation and chorioamnionitis in 53 low-birth-weight infants who required intubation. Lung inflammation was evaluated on days 1, 2, and 4 of intubation, by measuring the concentration of IL-1-β, thromboxane B2, leukotriene B4, and prostaglandin E2 in tracheal lavage samples. Infants exposed to chorioamnionitis had a higher concentration of IL-1-β in their tracheal fluid from day 1 of intubation forward and were more likely to develop bronchopulmonary dysplasia. It is noteworthy that the authors also reported that these very low-birth-weight infants had fewer instances of RDS than controls. Thus, fetuses exposed to chorioamnionitis seem to have less RDS but more bronchopulmonary dysplasia than controls.[154]

Antenatal exposure to proinflammatory cytokines is also a risk factor for the development of bronchopulmonary dysplasia.[155] Ghezzi et al[155] measured IL-8 concentration in the amniotic fluid of women with preterm labor with intact membranes and preterm PROM (n = 47) who delivered between 24 and 28 weeks of gestation. Bronchopulmonary dysplasia was diagnosed in 23.4% (11/47) of the cases. The prevalence of a positive amniotic fluid culture was 44.7% (21/47). IL-8 concentrations were higher in the amniotic fluid of neonates who subsequently developed bronchopulmonary dysplasia compared with those who did not develop bronchopulmonary dysplasia. The majority of mothers whose fetuses developed bronchopulmonary dysplasia had an amniotic fluid IL-8 concentration greater than 11.5 ng/mL, and this relationship remained significant even after correction for the effect of gestational age and birth weight (OR: 11.9; $P < 0.05$). The relationship between amniotic fluid IL-6, TNF-α, IL-1-β, and IL-8 and the occurrence of bronchopulmonary dysplasia was further examined in a subsequent study[156] of 69 neonates delivered preterm ($\leq$33 wk) within 5 days of amniocentesis. Bronchopulmonary dysplasia was diagnosed in 19% (13/69) of the neonates and the median amniotic fluid concentrations of IL-6, IL-1-β, and IL-8 were each significantly higher in the amniotic fluid of infants who developed bronchopulmonary dysplasia compared with those who did not.

Yoon et al[106] studied the relationship between IL-6 concentration in umbilical cord plasma at birth and the occurrence of bronchopulmonary dysplasia in 203 preterm births (25 to 34 wk). Bronchopulmonary dysplasia was diagnosed in 17% (34/203) of the cases. Neonates who developed bronchopulmonary dysplasia had a significantly higher median IL-6 concentration in umbilical cord plasma at birth than those in whom bronchopulmonary dysplasia did not

develop [median: 68.3 pg/mL (95% CI: 0.3-6150.0 pg/mL) vs. median: 6.9 pg/mL (95% CI: 0-19,230.0 pg/mL); $P < 0.001$]. This difference remained significant after adjusting for gestational age at birth (OR: 4.2, 95% CI: 1.6-11.2). The same authors[157] have also observed an association between a fetal inflammatory response (defined as the presence of funisitis or umbilical cord plasma IL-6 concentration > 17.5 pg/mL or amniotic fluid MMP-8 concentration > 23 ng/mL) and the development of atypical chronic lung disease (defined as chronic lung disease in the absence of RDS). Among 70 newborns with chronic lung disease, a fetal inflammatory response was present in 76% (53/70) of the cases and was more common among those with atypical chronic lung disease [90% (27/30) vs. 65% (26/40); $P < 0.05$].

The mechanisms responsible for lung injury after exposure to microbial products have been the subject of a series of elegant studies by the group of Jobe and Newnham,[158,159] as well as Bry et al.[160]

The first experimental study on the effects of antenatal inflammation on the fetal lung was reported by Bry et al.[160] Intra-amniotic injection of IL-1-α to pregnant rabbits resulted in improved neonatal lung function, increased mRNA, and protein expression for surfactant proteins A and B and also surfactant lipids. Injection of IL-1-α also induced preterm delivery. These seminal observations suggested that fetal lung inflammation accelerates fetal lung maturation in preparation for preterm birth.

Subsequently, intra-amniotic endotoxin administration to pregnant sheep and the inoculation of bacteria have been used as models of intrauterine inflammation. The administration of *Escherichia coli* endotoxin induces histologic chorioamnionitis and overexpression of IL-1-β, IL-6, and IL-8 mRNA in cells from amniotic fluid.[159,161] In addition, intra-amniotic endotoxin induces fetal lung inflammation, which can be documented by a dramatic increase in the number of mononuclear cells and granulocytes in the bronchoalveolar lavage fluid and mRNA for IL-1, IL-8, and IL-6 in lung tissue. There is also overexpression of IP-10 and MIG, chemokines with proinflammatory and antiangiogenic properties. Apoptosis of lung cells is increased and lung cell proliferation decreased. This is accompanied by inhibition of microvascular development and decreased expression of vascular endothelial growth factor and other angiogenic factors such as platelet endothelial cell adhesion molecule, tyrosine kinase epidermal growth factor-2, and vascular endothelial growth factor receptor-2. The net effect is that 7 days after the administration of endotoxin, the number of alveoli is decreased while the alveolar size is increased. There is a concomitant increase in the median thickness of the arteriolar walls. Collectively, these findings resemble those observed in infants with bronchopulmonary dysplasia[162] and demonstrate an effect of microbial products and inflammation on lung development. These studies also demonstrated that endotoxin increased mRNA and protein expression for surfactant proteins A, B, C, and D in fetal sheep. This was associated with an improvement in lung compliance, gas exchange, and oxygenation.[163–165] IL-1 seems to be the major cytokine responsible for lung injury because TNF-α and IFN-γ did not elicit the same degree of lung inflammation. Moreover, administration of the IL-1 receptor antagonist into the amniotic fluid before the administration of endotoxin and IL-1-α prevented lung inflammation and maturation.[166] The effects of IL-1 seem to be mediated through NF-κB because the administration of an NF-κB inhibitor (perthenolide) prevented many of the effects of LPS in a mouse model of chorioamnionitis.[167]

Intra-amniotic inoculation of *Ureaplasma* in sheep has generated a model

for chronic exposure to these microorganisms and yields results similar to those observed after the inoculation of endotoxin into the amniotic cavity.[168]

In summary, exposure to microbial products and intra-amniotic inflammation induces fetal lung maturity, which favors survival in the context of preterm delivery. However, chronic exposure to microbial products and inflammatory mediators induces profound changes in the developmental program of the lung (alveoli and vasculature). Therefore, the short-term gain in lung maturity can extract a price, which is a predisposition to the development of chronic lung disease. Clearly, the pathogenesis of bronchopulmonary dysplasia results from a combination of immaturity, oxygen toxicity, and mechanical damage during the course of ventilation, and also antenatal inflammation.

THE FETAL BRAIN

Strong evidence links brain injury with exposure to perinatal infection and inflammation.[169–173] In 1955, Eastman and DeLeon[174] reported that intrapartum maternal fever conferred a 7-fold increased risk for cerebral palsy. Nelson and Ellenberg,[175] using data from the Collaborative Perinatal Project, showed that among low-birth-weight infants, chorioamnionitis was associated with an increased incidence of cerebral palsy from 12 to 39 per 1000 live births. These observations were confirmed by several other investigators.[169,170,176–179]

Dammann and Leviton proposed that intrauterine infection leads to a fetal inflammatory response, which, in turn, contributes to adverse outcomes such as preterm labor and delivery, intraventricular hemorrhage, white matter damage, and neurodevelopment disability (mainly cerebral palsy).[180] Several lines of evidence support this concept (1) a fetal inflammatory response precedes spontaneous preterm delivery in the context of infection[1,105]; (2) clinical and histologic chorioamnionitis are associated with increased risks of cerebral palsy[169,170,176–179,181]; (3) white matter lesions are associated with intrauterine inflammation and infection in women with spontaneous preterm labor[182–185]; (4) intrauterine infection has been experimentally linked to white matter damage[172]; (5) elevated concentrations of cytokines in amniotic fluid and fetal plasma are associated with intraventricular hemorrhage, white matter damage, and cerebral palsy[171,180,186–191]; and (6) fetal vasculitis (chorionic and umbilical cord vessel inflammation) is associated with increased risks for the development of intraventricular hemorrhage, white matter damage, and cerebral palsy.[111,192–195]

Prematurity and cerebral palsy are strongly associated[196] and, indeed, approximately one third of all neonates who later have signs of cerebral palsy have birth weights less than 2500 g.[197] Newborns whose birth weights are < 1500 g have a rate of cerebral palsy that is 25 to 31 times higher than those with normal birth weights.[197] A possible mechanism contributing to the increased risk of cerebral palsy among extremely premature infants is not only an exaggerated fetal systemic inflammatory response but a limited ability to buffer the effect of proinflammatory cytokines[173,198–204] and other inflammatory mediators, or prevent/reduce oxidative stress.[204,205] In addition, some fetuses may have an increased genetic susceptibility to develop cerebral palsy in the setting of intrauterine infection/inflammation.

Nelson et al[206] have recently examined the association of genetic polymorphisms and cerebral palsy in premature infants. In a case-control study of 96 infants with cerebral palsy and 119 control children delivered before 32 weeks, an association with cerebral palsy was observed in heterozygotes for the following single nucleotide polymorphisms: endothelial

nitric oxide synthase: A-922G (OR: 3.0, 95% CI: 1.4-6.4); factor VII: arg353gln and del[-323]10bp-ins (OR: 2.7, 95% CI: 1.1-6.5); plasminogen activator inhibitor factor-1: 4G(-675)5G and G11053T (OR: 3.2, 95% CI: 1.2-8.7); and lymphotoxin A: thr26asn (OR: 2.1, 95% CI: 1.0-4.6). These single nucleotide polymorphisms are related to nitric oxide, thrombosis or thrombolysis, and cytokine function, respectively.

The association between clinical/histologic chorioamnionitis and subsequent development of cerebral palsy has been extensively investigated.[111,171,183,207–213] A recent meta-analysis found that clinical chorioamnionitis is associated with an increased risk of both cerebral palsy and white matter damage [RR 1.9 (95% CI: 1.5-2.5) and 2.6 (95% CI: 1.7-3.9), respectively].[214]

Nelson et al reported an association between clinical chorioamnionitis and cerebral palsy in near-term infants.[170,215] Clinical chorioamnionitis is a maternal host response. However, we have recently generated evidence of fetal involvement in patients with clinical chorioamnionitis.[107] The median umbilical venous plasma concentration of IL-6 was higher in neonates born to mothers with clinical chorioamnionitis than in those born to women without chorioamnionitis. Sixty-two percent (16/26) of the neonates born to women with clinical chorioamnionitis had elevated plasma concentrations of IL-6 >11 pg/mL in the umbilical vein. The observation that the concentration of IL-6 was higher in the blood from the umbilical artery than in the umbilical vein suggests a fetal origin of the excess plasma IL-6.[107]

Evidence that a fetal inflammatory response is involved in the pathophysiology of cerebral palsy in the preterm infant comes from the study of Yoon et al,[111] who followed 123 preterm children to the age of 3, observing that the odds of developing cerebral palsy were higher in the presence of funisitis (OR: 5.5, 95% CI: 1.2-24.5), increased amniotic fluid IL-6 concentrations (OR: 6.4, 95% CI: 1.3-33.0), and increased amniotic fluid IL-8 concentrations (OR: 5.9, 95% CI: 1.1-30.7). All 14 children who subsequently developed cerebral palsy had evidence of white matter damage and 11 had evidence of intrauterine inflammation. Fifty percent (7/14) of the children had positive amniotic fluid cultures.[111]

Other studies supporting a link between fetal inflammation and brain injury have documented higher concentrations of IL-6,[216–218] TNF-α,[217] and MMP-8[219] in the umbilical cord blood and amniotic fluid[111,171] of fetuses with white matter damage who subsequently develop cerebral palsy. Recently, Kaukola et al[220] performed a case-control study which included 19 children with cerebral palsy and 19 controls matched by gestational age at birth and measured the serum concentrations of eight cytokines (ciliary neutotrophic factor, IL-5, IL-12p40, IL-12p70, IL-13, IL-15, macrophage migration inhibitory factor, and TNF-related apoptosis inducing ligand), which were found to be higher in cord blood samples of neonates who subsequently developed cerebral palsy. Similarly, serum concentrations of epidermal growth factor and 3 chemokines (B-lymphocyte chemoatractant, MCP-3, and monokine induced by IFN-γ) were higher in cord blood samples from infants with cerebral palsy than in controls. Infants with cerebral palsy that were born preterm had a different pattern of cytokines in the cord blood than infants with cerebral palsy that delivered at term, suggesting that the pathophysiology of cerebral palsy may vary according to the gestational age at birth.

White matter damage identified by neonatal brain ultrasound is currently considered the best predictor of long-term disability in preterm infants.[221] Adverse outcomes associated with

white matter damage include cognitive limitations,[185] behavioral problems,[222] visual-spatial difficulties,[223] and cerebral palsy.[224] White matter damage is more common among children of pregnancies complicated by chorioamnionitis[177] and purulent amniotic fluid,[176] and also among neonates with bacteremia.[196]

Experimental evidence indicates that intrauterine infection results in white matter damage and neuronal lesions.[172,225–230] Yoon et al[172] experimentally induced ascending intrauterine infection with *E. coli* in 31 pregnant rabbits and inoculated 14 controls with sterile saline solution. Histologic evidence of brain white matter damage was identified in 12 fetuses born to 10 *E. coli*-inoculated rabbits compared with none in the control group ($P < 0.05$). All cases with white matter damage had evidence of intrauterine inflammation. Similar findings were reported by Debillon et al.[225,229] Increased cytokine expression in the white matter (mainly TNF-α[231–234] and, to a lesser extent, IL-6,[233] IL-1-β,[233,234] and IL-2[235]) has been demonstrated by immunohistochemistry studies performed in neonatal brains with PVL and increased immunoreactivity for TNF-α has been reported in the neocortex, hippocampus, basal ganglia, and thalamus of neonatal brains with PVL.[236]

Leviton[190] proposed that inflammatory cytokines (TNF-α) released during the course of intrauterine infection could participate in the pathogenesis of PVL by 4 different mechanisms: (1) induction of fetal hypotension and brain ischemia[188]; (2) stimulation of the tissue factor production and release, which activated the hemostatic system and contributed to coagulation necrosis of white matter[237]; (3) induction of the release of platelet activating factor, which could act as a membrane detergent causing direct brain damage[186]; and (4) a direct cytotoxic effect of TNF-α on oligodendrocytes and myelin.[187,189] Yoon et al[171] proposed a mechanism by which inflammatory cytokines could lead to white matter damage and cerebral palsy. MIAC (which occurs in approximately 25% of preterm births) results in congenital fetal infection/inflammation that stimulates fetal mononuclear cells to produce IL-1-β and TNF-α. These cytokines increase the permeability of the blood-brain barrier, facilitating the passage of microbial products and cytokines into the brain.[238,239] Microbial products then stimulate the human fetal microglia to produce IL-1 and TNF-α, with subsequent activation of astrocyte proliferation and production of TNF-α. TNF-α damages oligodendrocytes, which are the cells responsible for the deposition of myelin. IFN-γ and LPS also increase the permeability of the blood-brain barrier and this increase in permeability is, at least in part, dependent on cyclic guanosine monophosphate and nitric oxide.[239] For a detailed review of the evidence linking prenatal exposure to LPS and brain injury, the reader is referred to the excellent review by Hagberg and Mallard.[240]

Evidence for involvement of the adaptive arm of the immune system in the pathogenesis of white matter damage comes from the study of Duggan et al,[217] who proposed that activated memory T cells may be involved in brain injury among neonates born between 23 and 29 weeks. These investigators found that the percentage of $CD45RO^+$ cells is higher among neonates with cerebral lesions detected by magnetic resonance imaging (MRI) when compared to neonates with normal MRI results. MRI abnormalities included germinal layer or intraventricular hemorrhage, discrete periventricular lesions, and/or cystic lesions in the caudate nucleus. The authors proposed that that high fetal cytokinemia may be because of antigen exposure and is not secondary to the brain injury, hypoxia, or parturition.[217]

It should be stressed that some studies have not demonstrated an association between inflammation and brain damage in the preterm infant.[241–244] The apparent discrepancy between studies has been attributed to the difficulty of adjusting for the potential effects of gestational age and other pregnancy complications such as preeclampsia in the frequency of cerebral palsy. These investigators have found, however, that cerebral palsy is associated with inflammation in term pregnancies.

Hypoxic/ischemic damage and inflammation are proposed to have a synergistic role in causing fetal brain injury.[245–247] Recently, the administration of *N*-acetylcysteine has been demonstrated to restrict brain damage in an animal model which combined inflammation induced by LPS and ischemia. This has potential therapeutic effects in humans, given that *N*-acetylcysteine crosses the placenta.[248–250]

Why Does the Fetus Mount an Inflammatory Response?

Exploring the purpose of biologic phenomena almost always carries the risk of teleology. However, it is plausible to assume that in the context of intrauterine infection, the onset of preterm labor would have survival value for both mother and fetus and that it would be part of the repertoire of host defense mechanisms against infection.[1,105] The fetus would take an active role in the induction of labor by secretion of proinflammatory cytokines to signal the onset of labor and exit a hostile intrauterine environment. Indeed, the frequency of a histologic fetal response to infection (chorionic vasculitis with or without funisitis) was significantly higher in infants who survived the neonatal period as compared with those who died in the perinatal period.[251]

A Role of the Fetus in the Onset of Labor

Among women with preterm PROM, FIRS is associated with the impending onset of preterm labor, regardless of the inflammatory state of the amniotic fluid.[1] This suggests that the human fetus plays a role in initiating the onset of labor. However, maternal cooperation must occur for parturition. Thus, it is possible that systemic fetal inflammation may occur in the absence of labor when the inflammatory process does not involve the chorioamniotic membranes and decidua. Such instances may occur in the context of hematogenous viral infections (ie, cytomegalovirus infection) or other disease processes (ie, alloimmunization).

Fetal Death and Maternal/Fetal Systemic Inflammation

The rate of maternal inflammation is 9 times more frequent than that of fetal inflammation in stillbirth. This could have 2 potential explanations. First, it is possible that fetal infection occurs, but fails to trigger a fetal inflammatory response and the onset of preterm labor. In this case, in utero fetal death would represent failure of the host response mechanisms dealing with intrauterine infection. This concept is supported by evidence that, in some cases of intrauterine death due to group B streptococci (with intact membranes), there may be absence of both a maternal and a fetal inflammatory response despite widespread fetal infection.[252,253] Similar observations can be derived from the study of Tafari and colleagues[254] with intrauterine infection by *Ureaplasma urealyticum*. Despite the fact that intrauterine infection was documented by culture of microorganisms from fetal lung tissue at postmortem examination, fetal death could occur before the onset of labor. The second possible explanation for the

discrepant rate of maternal and fetal inflammatory responses is that inflammation of the placental membranes may occur after fetal death and be etiologically unrelated to the fetal demise.[255] The possibility that fetal death represent a failure of host defense requires further consideration given the association between homozygosity for the IL-1 receptor antagonist (IL-1ra) allele 2 and the risk of fetal death.[256] Carriage of this allele is associated with increased production of IL-1ra.[257,258] An excess of IL-1ra in the fetal compartment may limit the ability of the fetus to deploy a proinflammatory response and thus limit the repertoire of mechanisms available for host defense, including the ability to exit a hostile intrauterine environment by initiating the onset of labor.[1,105,259] However, further studies are required to test this hypothesis.

Short-term Consequences of FIRS

NEONATAL MORBIDITY

FIRS is an independent risk factor for the occurrence of severe neonatal morbidity. Fetuses with FIRS have a higher rate of neonatal complications, including RDS, suspected or proven neonatal sepsis, pneumonia, intraventricular hemorrhage, PVL, and necrotizing enterocolitis,[1] and are frequently born to mothers with subclinical MIAC.[1]

PRETERM PARTURITION

Among women with preterm PROM, FIRS is associated with the impending onset of preterm labor, regardless of the inflammatory state of the amniotic fluid.[1] This finding further supports the notion of an active role of the human fetus in initiating the onset of labor. However, maternal cooperation must occur for parturition. Fetal inflammation is linked to the onset of labor in association with ascending intrauterine infection. Moreover, FIRS in the context of hematogenous viral infections or other disease processes (ie, alloimmunization) is not associated with an increase rate of PTL.

PERINATAL DEATH

An association between placental inflammation and perinatal death has been reported by several investigators.[251,260–264] Moyo et al[260] compared the frequency of vasculitis in the placental chorionic plate between stillbirths and live births in a case-control study. The investigators showed that 9% (6/66) of the stillbirths but none of the live births (0/66) had evidence of vasculitis in the chorionic plate (OR: 14, 95% CI: 2.8-72). Others found that acute placental inflammation (defined as inflammation involving the membranes, decidua, villi, or umbilical cord) was an independent risk factor for preterm low birth weight, stillbirth, and perinatal death.

Strategies for the Management of the FIRS

Several approaches can be used to interrupt the course of FIRS: (1) delivery; (2) antimicrobial treatment of women in whom the FIRS is due to microbial invasion of susceptible bacteria; (3) administration of agents that down-regulate the inflammatory response; (4) combination of the above-mentioned options. Preterm delivery places the unborn child at risk for complications of prematurity. Therefore, the risks of prematurity and intrauterine infection must be balanced.

The administration of antimicrobial agents may eradicate MIAC in cases of preterm PROM. The results of the ORACLE I trial[265] suggest that antibiotic administration may not only delay the onset of labor, but improve neonatal outcome as well.

Agents that down-regulate the inflammatory response, such as anti-inflammatory cytokines (ie, IL-10),[266,267] antibody to macrophage migration inhibitory factor[268,269] and antioxidants, may also play a role in preventing preterm delivery, neonatal injury, and long-term perinatal morbidity.[270] A combination of antibiotics and immunomodulators (dexamethasone and indomethacin) was effective in nonhuman pregnant primates to eradicate infection, suppress the inflammatory response, and prolong gestation in experimental premature labor induced by intra-amniotic inoculation with group B streptococci.[271] Collectively, this evidence indicates that immunomodulation may be an effective intervention in preventing fetal injury and prolonging gestation among patients with inflammation/infection-induced preterm labor.

Parts of this article have been previously published elsewhere.[9,272,273]

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