PREMATURE RUPTURE OF THE FETAL MEMBRANES: MECHANISM OF DISEASE

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The membranes surrounding the amniotic cavity are composed of the amnion and the chorion, which are closely adherent layers consisting of several cell types, including epithelial cells, mesenchymal cells, and trophoblast cells, embedded in a collagenous matrix. They retain amniotic fluid, secrete substances both into the amniotic fluid and toward the uterus, and guard the fetus against infection ascending the reproductive tract. The membranes normally rupture during labor. Premature rupture of the fetal membranes is defined as rupture of the membranes before the onset of labor.' Premature rupture of the membranes occurring before 37 weeks' gestation is usually referred to as preterm premature rupture of the membranes. Despite advances in perinatal care, premature rupture of the membranes and preterm premature rupture of the membranes continue to be important obstetrical complications. At term, 8 to 10 percent of pregnant women present with premature rupture of the membranes; these women are at increased risk for intrauterine infection when the interval between the membrane rupture and delivery is prolonged.' Preterm premature rupture of the membranes occurs in approximately 1 percent of all pregnancies and is associated with 30 to 40 percent of preterm deliveries. It is thus the leading identifiable cause of preterm delivery (after less than 37 completed weeks' gestation) and its complications, including respiratory distress syndrome, neonatal infection, and intraventricular hemorrhage.

Obstetricians have traditionally attributed rupture of the membranes to physical stress, particularly that associated with labor. However, more recent evidence suggests that membrane rupture is also related to biochemical processes, including disruption of collagen within the extracellular matrix of the amnion and the chorion and programmed death of cells in the fetal membranes. It has been proposed that the fetal membranes and the maternal uterine lining

(decidua) respond to various stimuli, including membrane stretching and infection of the reproductive tract, by producing mediators, such as prostaglandins, cytokines, and protein hormones, that govern the activities of matrix-degrading enzymes. We review here the association between the degradation of the extracellular matrix within the fetal membranes and premature rupture of the membranes, in an effort to understand better the pathophysiology of such ruptures and identify potentially effective interventions.

STRUCTURE OF THE FETAL MEMBRANES

The human amnion is composed of five distinct layers (Fig. 1).2 It contains no blood vessels or nerves; the nutrients it requires are supplied by the amniotic fluid. The innermost layer, nearest the fetus, is the amniotic epithelium. Amniotic epithelial cells secrete collagen types III and IV and noncollagenous glycoproteins (laminin, nidogen, and fibronectin) that form the basement membrane, the next layer of the amnion.

The compact layer of connective tissue adjacent to the basement membrane forms the main fibrous skeleton of the amnion. The collagens of the compact layer are secreted by mesenchymal cells in the fibroblast layer. Interstitial collagens (types I and III) predominate and form parallel bundles that maintain the mechanical integrity of the amnion. Collagen types V and VI form filamentous connections between the interstitial collagens and the epithelial basement membrane. There is no interposition of amorphous ground substance between collagen fibrils in amniotic connective tissue at term, so the amnion maintains its tensile strength throughout the late stages of normal pregnancy.

The fibroblast layer is the thickest of the amniotic layers, consisting of mesenchymal cells and macrophages within an extracellular matrix. The collagens in this layer form a loose network with islands of noncollagenous glycoproteins.
The intermediate layer (spongy layer, or zona spongiosa) lies between the amnion and the chorion. Its abundant content of hydrated proteoglycans and glycoproteins gives this layer its

"spongy" appearance in histologic preparations, and it contains a nonfibrillar meshwork of mostly type III collagen. The intermediate layer absorbs physical stresses by permitting the amnion to slide on the underlying chorion, which is firmly adherent to the maternal decidua.

Although the chorion is thicker than the amnion, the amnion has greater tensile strength. The chorion resembles a typical epithelial membrane, with its polarity directed toward the maternal decidua. As pregnancy progresses, trophoblastic villi within the chorionic layer of the reflected fetal membranes (free of the placenta) regress. Beneath the cytotrophoblast layer (closer to the fetus) are the basement membrane and the chorionic connective tissue, which is rich in collagen fibrils.

The fetal membranes display regional variation that distinguishes the membranes overlying the placenta from the reflected membranes. Although there is no evidence of preset weak points where the membranes break, care must be taken to avoid overlooking localized changes in the membrane structure and composition in studies of premature rupture of the membranes.

NATURAL HISTORY AND MANAGEMENT OF FETAL-MEMBRANE RUPTURE

After premature rupture of the membranes at term, 70 percent of women begin to labor within 24 hours, and 95 percent within 72 hours. After preterm premature rupture of the membranes, the latency period from membrane rupture to delivery decreases inversely with advancing gestational age. For example, at 20 to 26 weeks' gestation, the mean latency period is 12 days; at 32 to 34 weeks' gestation, it is only 4 days.
Given the natural history of the relatively rapid progression to labor after premature rupture of the membranes at term, the goal of management is to minimize the risk of intrauterine infection without increasing the incidence of cesarean delivery. In published series, the rate of neonatal sepsis after preterm premature rupture of the membranes ranges from 2 to 20 percent, and the incidence of neonatal death caused by infection is approximately 5 percent. When the fetal membranes rupture at term or before, the options are expectant management (with close ob-

servation for signs of labor, non-reassuring fetal-heart-rate patterns, or intrauterine infection) or induction of labor.

MECHANISMS OF FETAL-MEMBRANE RUPTURE PRECEDING AND DURING LABOR

Intrapartum rupture of the fetal membranes has been attributed to generalized weakening due to uterine contractions and repeated stretching. The tensile strength of the membranes is reduced in specimens obtained after labor as compared with those obtained during cesarean delivery without labor. Generalized weakness of the membranes has been more difficult to establish when prematurely ruptured membranes have been compared with membranes that were artificially ruptured during labor.8 Membranes that rupture prematurely, however, appear to be focally defective rather than generally weakened. The area near the rupture site has been described as a "restricted zone of extreme altered morphology" that is characterized by marked swelling and disruption of the fibrillar collagen network within the compact, fibroblast, and spongy layers.[9] Because this zone does not include the entire rupture site, it may appear before membrane rupture and represent the initial breakpoint.

Despite the divergent characteristics of premature rupture of the membranes and intrapartum rupture of the membranes, there is little evidence to suggest that the mechanisms that predispose women to the former are not identical to those that normally precede labor. This has led to the view that premature rupture of the membranes represents an acceleration or exaggeration of the processes precipitating spontaneous rupture of the membranes during labor. Consequently, investigators have frequently combined instances of preterm premature rupture of the membranes, premature rupture of the membranes at term, and rupture of the membranes during labor when describing mechanisms of membrane rupture. This practice, however, may obscure important differences among these events.

Changes in collagen content, structure, and catabolism

The maintenance of the tensile strength of fetal membranes appears to involve equilibrium between the synthesis and the degradation of the components of the extracellular matrix. It has been proposed that changes in the membranes, including decreased collagen content, altered collagen structure, and increased collagenolytic activity, are associated with premature rupture of the membranes.

Connective-Tissue Disorders and Nutritional Deficiencies Risk Factors

Although there are conflicting data regarding changes in the composition of fetal-membrane collagen in association with the length of gestation and membrane rupture, a decline in membrane collagen content or a change in collagen structure probably precedes rupture of the membranes. Connective-tissue disorders are associated with weakened fetal membranes and an increased incidence of preterm premature rupture of the membranes. Ehlers—Danlos syndrome, a group of at least 11 heritable disorders of connective tissue characterized by hyperelasticity of the skin and joints, is caused by various defects in the synthesis or structure of collagen. Among 18 patients with Ehlers— Danlos syndrome whose birth histories were available, 13 (72 percent) were delivered prematurely after preterm premature rupture of the membranes, as compared with 1 of 16 unaffected siblings, and this one instance occurred in a twin gestation in which the other twin had Ehlers—Danlos syndrome.'[3] Thus, pregnancies in which the fetus is affected with Ehlers—Danlos syndrome are dramatic examples of preterm premature rupture of the membranes associated with abnormal collagen content and structure.

Nutritional deficiencies that predispose women to abnormal collagen structure have also been associated with an increased risk of preterm premature rupture of the membranes. Collagen cross-links, which are formed in a series of reactions initiated by lysyl oxidase, increase -the tensile strength of fibrillar collagens. Lysyl oxidase is produced by amniotic mesenchymal cells, which lay down the collagenous compact layer of the amnion. Lysyl oxidase is a copper-

dependent enzyme, and women with premature rupture of the membranes have lower copper concentrations in maternal and umbilical-cord serum than women whose fetal membranes are artificially ruptured during labor.'[5] Similarly, women with low serum concentrations of ascorbic acid, which is required for the formation of the triple helical structure of collagen, have a higher rate of premature rupture of the membranes than those with normal serum concentrations. Tobacco smoking, which in-dependently increases the risk of preterm premature rupture of the membranes, has been associated with decreased serum concentrations of ascorbic acid. In addition, the cadmium in tobacco has been found to increase the metal-binding protein metallothionein in trophoblasts, which may result in sequestration of copper. These data suggest that the decreased availability of copper and ascorbic acid may contribute to an abnormal structure of fetal-membrane collagen in smokers. Collectively, reduced collagen cross-linking (possibly due to dietary deficiencies or behavioral activities) may predispose women to premature membrane rupture.

Increased Collagen Degradation

The degradation of collagen is mediated primarily by matrix metalloproteinases, which are inhibited by specific tissue inhibitors and other protease inhibitors. The matrix metalloproteinases are a family of enzymes produced by various types of cells that hydrolyze at least one component of the extracellular matrix. Because of the various substrate specificities of matrix metalloproteinases, effective catabolism of the many component molecules in the extracellular matrix requires the concerted actions of several enzymes. The interstitial collagenases matrix metalloproteinase-1 (MMP-1) and MMP-8 cleave the triple helix of the fibrillar collagens (types I and III), which are then further degraded by the gelatinases MMP-2 and MMP-9. These gelatinases also cleave type IV collagen, fibronectin, and proteoglycans. In human fetal membranes, MMP-1 and MMP-9 messenger RNA and protein have been colocalized to amniotic epithelial cells and chorionic trophoblasts. Thus, the compact (collagenous) layer of the fetal membranes is sandwiched between two layers of cells that produce matrix metalloproteinases.

Tissue inhibitors of metalloproteinases form 1:1 stoichiometric complexes with matrix metalloproteinases and inhibit their proteolytic activity. Tissue inhibitor of metalloproteinase- 1 (TIMP- 1) binds to activated MMP-1, MMP-8, and MMP-9, and TIMP2 binds to latent and active forms of MMP-2. The more recently described TIMP-3 and TIMP-4 appear to inhibit matrix metalloproteinases as efficiently as TIMP- 1. Coordinated activities of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases are essential to the process of extracellular matrix remodeling.

The integrity of the fetal membranes remains unaltered throughout most of gestation, perhaps in part because of a combination of low matrix-metalloproteinase activity and a relatively higher concentration of TIMP-1. Near the time of delivery, however, the balance between activated matrix metalloproteinases and their tissue inhibitors shifts toward proteolytic degradation of the extracellular matrix of the fetal membranes. In the amnion of rats, activities of interstitial collagenase and MMP-9 increase before the onset of active labor. In human amnion and chorion, MMP-9 activity increases and TIMP-1 concentrations decrease dramatically with labor. Analyses of membranes collected from women at the time of cesarean delivery (with and without labor) and after spontaneous labor and delivery suggest that MMP-1 activity increases before labor, MMP-9 and MMP-3 activities increase during labor, and TIMP-1 concentrations increase after delivery. These changes may reflect a coordinated progression of events preceding and during parturition, resulting in the controlled degradation of collagen within the fetal membranes.

Premature rupture of the membranes may also be caused by an imbalance between the activities of matrix metalloproteinases and their tissue inhibitors, leading to inappropriate degradation of the membranes' extracellular matrixes. Collagenase activity is increased in prematurely ruptured membranes at term. Overall, protease activity is increased in membranes of women with preterm premature rupture of the membranes, the predominant activity being that of MMP-9 Furthermore, gelatinolytic activity corresponding to latent and active forms of MMP-9 is increased and the concentration of TIMP-1 is low in amniotic fluid obtained from women whose pregnancies were complicated by preterm premature rupture of the membranes.[20] However,

because specimens in these studies were obtained after membrane rupture, we cannot conclude with certainty that collagen degradation in the fetal membranes precedes membrane rupture.

Other observations suggest that physiologic and pathologic degradation of the extracellular matrix is associated with labor and delivery. Interstitial-collagenase activity increases dramatically in cervical tissue during cervical dilation in human parturition Periodontal disease, in which there is increased matrix-metalloproteinase activity in gingival tissues, has been reported to be an independent risk factor for preterm delivery. This finding raises the interesting possibility that some women have a genetic predisposition to extracellular-matrix degradation due to increased matrix-metalloproteinase activity that may be manifested clinically as periodontitis, premature cervical dilatation, or premature rupture of the membranes.

CLINICAL FACTORS ASSOCIATED WITH COLLAGEN DEGRADATION AND PREMATURE RUPTURE OF THE MEMBRANES

Infection

Obstetricians have long debated whether intrauterine infection is a cause or a consequence of premature rupture of the fetal membranes. There is indirect evidence that genital tract infection precipitates rupture of the membranes in animals and humans. In pregnant rabbits, cervical inoculation with *Escherichia coli* resulted in positive cultures for *E. coli* in the amniotic fluid and decidual tissue of 97 percent of the treated animals and preterm delivery in half the treated animals. In contrast, cervical inoculation with saline resulted in no infections or preterm births.[27] The identification of pathologic microorganisms in human vaginal flora soon after membrane rupture provides support for the concept that bacterial infection may have a role in the patho-genesis of premature membrane rupture. Epidemiologic data demonstrate an association between colonization of the genital tract by group B streptococci, *Chiamydia trachomatis, Neisseria gonorrhoeae,* and the microorganisms that cause bacterial vaginosis (vaginal anaerobes, *Gardnerella vaginalis,* Mobiluncus species, and genital mycoplasmas and an increased risk of preterm premature rupture of the membranes. Furthermore, in some studies treatment of infected

women with antibiotics decreased the rate of preterm premature rupture of the membranes. Intrauterine infection may predispose women to rupture of the fetal membranes through any of several mechanisms, each of which induces degradation of the extracellular matrix. Several organisms that are commonly present in the vaginal flora, including group B streptococci, *Staphylococcus aureus, Trichomonas vagina/is,* and the microorganisms that cause bacterial vaginosis, secrete proteases that can degrade collagen and weaken the fetal membranes. In an in vitro system, proteolysis of the fetal membrane matrix can be inhibited by the addition of an antibiotic.

The host inflammatory response to bacterial infection constitutes another potential mechanism that may partly account for the association between bacterial infection of the genital tract and premature rupture of the membranes. The inflammatory response is mediated by polymorphonuclear neutrophils and macrophages that are recruited to the site of infection and produce cytokines, matrix metalloproteinases, and prostaglandins. Inflammatory cytokines, including interleukin-1 and tumor necrosis factor a, are produced by stimulated monocytes, and these cytokines increase MMP-1 and MMP-3 expression at the transcriptional and posttranslational levels in human chorionic cells.

Bacterial infection and the host inflammatory res**ponse** also induce prostaglandin production by the fetal membranes, which is thought to increase the risk of preterm premature rupture of the membranes causing uterine irritability and by collagen degradation within the membranes. Certain strains of vaginal bacteria produce phospholipase A_2, which releases the prostaglandin precursor arachidonic acid from membrane phospholipids within the amnion. Furthermore, the immune response to bacterial infection includes the production of cytokines by activated monocytes that increase prostaglandin E2 production by chorionic cells. Cytokine stimulation of prostaglandin E2 production by the amnion and chorion appears to involve induction of cyclooxygenase II, the enzyme that converts arachidonic acid into prostaglandins. The precise regulation of prostaglandin E2 synthesis in relation to bacterial infection and the host inflammatory response is not understood, and a direct link between prostaglandin production and

premature rupture of the membranes has not been established. However, prostaglandins (specifically prostaglandin E2 and prostaglandin F2a are considered to be mediators of labor in all mammals, and prostaglandin E2 diminishes collagen synthesis in fetal membranes and increases MMP-1 and MMP-3 expression in human fibroblasts.

Another component of the host response to infection is the production of glucocorticoids. In most tissues the antiinflammatory action of glucocorticoids is mediated by suppression of prostaglandin production. However, in some tissues, including the amnion, glucocorticoids paradoxically stimulate prostaglandin production. Furthermore, dexamethasone reduces the synthesis of fibronectin and type III collagen in primary cultures of amniotic epithelial cells. These findings suggest that glucocorticoids produced in response to the stress of microbial infection facilitate rupture of the fetal membranes. Despite these findings, there has been no conclusive demonstration that infection precedes premature rupture of the fetal membranes in humans. Nonetheless, microbial infection and the host inflammatory response may at the very least increase the activity of matrix metalloproteinases in the fetal membranes and be involved in the pathogenesis of some membrane ruptures.

Hormones

Progesterone and estradiol suppress extracellular matrix remodeling in reproductive tissues. Both hormones decrease concentrations of MMP-1 and MMP-3 and increase the concentrations of tissue inhibitors of metalloproteinases in the cervical fibroblasts of rabbits. High concentrations of progesterone decrease the production of collagenase in the cervical fibroblasts of guinea pigs, although lower concentrations of progesterone and estradiol stimulate the production of collagenase in pregnant guinea pigs. Relaxin, a protein hormone that regulates the remodeling of connective tissues, is produced locally in the decidua and placenta and reverses the inhibitory effects of estradiol and progesterone by increasing MMP-3 and MMP-9 activities in fetal membranes." Expression of the relaxin gene is increased before labor in human fetal membranes at term. Although it is important to consider the roles of estrogen, progesterone, and relaxin in reproductive processes, their involvement in the process of fetal-membrane rupture remains to be

defined.

Programmed Cell Death

Programmed cell death, or apoptosis, has been implicated in the remodeling of various reproductive tissues, including those of the uterus and cervix. Apoptosis is characterized by the nuclear DNA fragmentation and catabolism of 285 ribosomal RNA subunits that are required for protein synthesis. In rats (which have a 21-day gestation), amniotic epithelial cells undergo apoptotic cell death as labor approaches. This cell death appears to follow the start of extracellular- matrix degradation, suggesting that it is a consequence and not a cause of catabolism of the extracellular matrix of the amnion. Human amnion and chorion obtained at term after premature rupture of the membranes contain many apoptotic cells in areas adjacent to the rupture site and fewer apoptotic cells in other areas of the membranes. Furthermore, in cases of chorioamnionitis, apoptotic amniotic epithelial cells are seen in conjunction with adhesive granulocytes, suggesting that the host immune response may accelerate cell death in fetal membranes. Although apoptotic changes have been identified in fetal membranes immediately before delivery, the mechanisms regulating apoptosis and the subsequent effects on the tensile strength of fetal membranes have yet to be elucidated.

Membrane Stretch and Premature Rupture of the Membranes

Uterine overdistention due to both polyhydramnios and multifetal gestation induces membrane stretch and increases the risk of premature rupture of the membranes. Mechanical stretching of the fetal membranes up-regulates the production of several amniotic factors, including prostaglandin E2 and interleukin-8. Stretch also increases MMP-1 activity within the membranes. As stated above, prostaglandin E2 increases uterine irritability, decreases synthesis of fetal-membrane collagen, and increases production of MMP-1 and MMP-3 by human fibroblasts. Interleukin-8, which is produced by amniotic and chorionic cells, is chemotactic for neutrophils and stimulates collagenase activity. The production of interleukin-8, which is present in low concentrations in the amniotic fluid during the second trimester but in much higher concentrations late in gestation, is inhibited by progesterone. Thus, amniotic production of

interleukin-8 and prostaglandin E, represents biochemical changes in the fetal membranes that may be initiated by physical forces (membrane stretch), reconciling the hypothesis of force-induced and biochemically induced membrane rupture.

PREDICTING PRETERM PREMATURE RUPTURE OF THE MEMBRANES

Markers of degradation of the extracellular matrix of fetal membranes could be used to identify women who are at risk for premature rupture of the membranes and preterm delivery. The most extensively studied candidate marker is fetal fibronectin, which is present in the extracellular matrix of fetal membranes and is structurally different from the fibronectin of adult tissues. The production of fetal fibronectin by human amniotic cells is stimulated by inflammatory mediators (including interleukin-1and tumor necrosis factor a) that are considered important in initiating preterm labor.

In the second and third trimesters of pregnancy, the presence of fetal fibronectin in cervicovaginal secretions probably reflects degradation of the extra-cellular matrix at the interface of the chorionic and decidual layers. Measurements of fetal fibronectin in these secretions have been used to identify a subgroup of women at high risk for preterm delivery. Fetal fibronectin is most sensitive for predicting preterm birth at less than 28 weeks' gestation (sensitivity, 63 percent); however, the positive predictive value for preterm birth is less than 33 percent at all gestational ages, and there is no evidence that this test can be used to predict preterm premature rupture of the membranes and reduce the rate of preterm birth. Tests based on the detection of other molecules, such as specific matrix metalloproteinase, have not yet been applied to clinical practice.

PREVENTION OF PRETERM PREMATURE RUPTURE OF THE MEMBRANES

There has been considerable interest in the development of general and specific inhibitors of

matrix metalloproteinases for the treatment of periodontal disease and arthritis and for the prevention of tumor metastasis. These agents include tetracycline antibiotics, synthetic matrix-metalloproteinase inhibitors such as batimastat (which selectively chelates the zinc atom at the active site of the enzymes), and the native inhibitors TIMP-1 and TIMP-2. The ability of such substances to prevent or retard changes in the extracellular matrix of fetal membranes before preterm premature rupture occurs has yet to be evaluated.

CONCLUSIONS

The cause of premature rupture of the fetal membranes is almost certainly multifactorial. Traditionally, rupture of the fetal membranes has been attributed to increasing physical stresses that weaken the membranes. At the molecular level, premature rupture of the membranes appears to result from diminished collagen synthesis, altered collagen structure, and accelerated collagen degradation, possibly in association with concurrent cellular changes within the fetal membranes. These hypotheses are not mutually exclusive, and biophysical stresses may amplify these biochemical changes.

Present research priorities include elucidation of the normal biologic processes of the fetal membranes, including extracellular-matrix remodeling, programmed cell death, and the response to membrane stretch as pregnancy progresses. We need to learn how exogenous risk factors, including nutritional deficiencies, smoking, and infection, promote premature rupture of the membranes. A more thorough understanding of extracellular- matrix degradation in the amnion and the chorion may allow us to reduce the incidence of preterm delivery due to preterm premature rupture of the membranes, possibly with agents that delay matrix degradation.