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The matrix metalloproteinases (MMPS) in the decidua and fetal membranes

Amir Weiss [1], Shlomit Goldman [1] and Eliezer Shalev [1,2]

[1]Laboratory for Research in Reproductive Sciences, Department of Obstetrics and Gynecology, Ha'Emek Medical Center, Afula, Israel, [2] Rappaport Faculty of Medicine, Technion – Institute of Technology, Haifa, Israel

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1. ABSTRACT

The role of the matrix metalloproteinases (MMPs) in the decidua, fetal membranes and amniotic fluid (AF) has been receiving more and more attention. The MMPs are not only important intermediaries in pathological processes leading to preterm labor but it seems that they also play a crucial role in the activation of labor at term. During normal gestation MMP-1, -2, -3, -7 and –9 are found in the amniotic fluid and fetal membranes. MMP-2 and MMP-3 are expressed constitutively while MMP-9 is barely detectable until labor. At labor, while MMP-9 is the major MMP responsible for gelatinolytic activity in the membranes, MMP-2 is dominant in the decidua. MMP-7 (AF) increases with gestation but does not appear to play a major role in labor. The expression of MMPs is attenuated through the expression of relaxins, integrins and extracellular matrix metalloproteinase inducer (EMMPRIN). Spontaneous preterm delivery (PTD) may be a product of preterm labor (PTL), preterm premature rupture of membranes (P-PROM) or placental abruption. Each of these processes may have differing pathways but the presence of an intrinsic inflammatory response with or without infection seems to involve all etiologies. The inflammatory response is mediated with cytokines such as interleukines –1, -6 and –8 and tumor necrosis factor alpha. MMP-3, MMP-7 and MMP-8 appear to be important in these processes. MMP-9, which is the major MMP involved in normal labor, plays an important role in pathological labor as well. Finally, apoptosis seems to play a role in pathological labor, particularly deliveries involving P-PROM. African-American are at greater risk of PTD than white or Hispanic Americans. Environmental differences may not suffice to explain this phenomenon. Genetic polymorphisms of the MMP genes may help explain the greater risk among this population. Finally, manipulating MMPs may have a role in the prevention of PTD. Agents suggested include indomethacin, N-acetylcysteine, progesterone and specific inhibitors of phosphodiesterase 4.

2. INTRODUCTION

Human pregnancy is characterized by rapid growth of the gravid uterus, fetus and amniotic fluid. These changes are accompanied with remarkable quiescence in the physiological state. The developing fetus is surrounded by amniotic fluid, enclosed in the fetal membranes (the amnion and chorion) and is attached to the uterus through the decidua in those regions where the placenta is absent. This state is maintained until fetal maturity is attained at 38 weeks gestation when labor may safely ensue. The commencement of labor involves a series of processes, which include sequential, or at times concomitant softening and ripening of the cervix, weakening of the fetal membranes, contractions of the uterus and, after expulsion of the newborn, detachment of the membranes and placenta from the decidua lined inner surface of the uterus. Normally, dilation of the cervix, along with the forces of the uterine contractions on the fetal membranes, causes the membranes to rupture at some point during labor. But it is apparent that there is a concomitant weakening of the fetal membranes, as often, the membranes rupture before contractions begin. The elucidation of these processes is essential to our understanding of labor and the pathological mechanisms behind the premature rupture of membranes (PROM), the preterm PROM (P-PROM) and preterm labor (PTL) and delivery (PTD).

The avascular amnion, lying between the amniotic fluid and the chorion, has been described as being constructed from five layers: the amniotic epithelium, the basement membrane, the compact layer, the fibroblast layer and the spongy layer (1). The chorion consists of a reticular layer and a basement membrane on a bed of trophoblasts facing the decidua. While the chorion is thicker than the amnion, the tensile strength is attributed to the amnion. The strength of the amnion is provided essentially by the compact layer, rich in collagen types I and III with smaller amounts of collagen types V, VI and VII (2). The smaller amounts of collagen V, VI and VII are nevertheless essential for membrane strength since they provide the backbone and anchor for the other extra-cellular components. The source of these collagens in the compact layer has been a source of controversy. It appears that both the amniotic epithelial cells and the mesenchymal stromal cells contribute to collagen production, though the relative contribution of each cell type during each stage of gestation is still under investigation (2). The fetal membranes contain a mixture of elastins, microfibrils, fibronectins and elastinins, which confer upon it its unique properties.

The human decidua is a maternal organ, as opposed to the amnion and chorion, which are genetically derived from the fetus. The decidua is rich in extracellular matrix, decidual cells, macrophages and lymphocytes. While, on the one hand, an immunological barrier exists at the maternal-fetal interface, some hormones, such as prolactin, originating from the decidual cells, transverse the fetal membranes to the amniotic fluid (3).

MMPs are undoubtedly involved in the normal growth and remodeling of the fetal membranes during gestation and in the weakening and rupture of the membranes as contractions begin and labor ensues or in the pathological processes of PROM, P-PROM and PTL.

3. THE MATRIXMETALLOPROTEINASES

Matrix metalloproteinases (MMPs), also known as matrixins, play an essential role in the breakdown and remodeling of the extracellular matrix (ECM). Combined, they are capable of degrading all the components of the ECM. There is great overlap in substrate specificity and they are expressed both in physiological and in disease processes in most tissues. This fact makes their study and pharmacological manipulation a challenge. (4)

There are 24 known MMPs in humans and several more homologues in other species. In humans, the MMPs are subdivided according to their structural and functional properties into five groups: collagenases (MMP-1, -8, -13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, -7, -10, -11), membrane type (MT) and a fifth heterogeneous group (5, 6, 7). They are zinc dependant endopeptidases with specific domain structures. At a minimum, they consist of a propeptide and a catalytic domain (MMP-7 and MMP-26). Most MMPs contain a hemopexin-like, four-bladed beta propeller domain connected by a linker or hinge region, as well (MMP-1, -3, -8, -11, -12, -13, -18, -19, -20, -21, -27, -28). MMP-2 and MMP-9, in addition to the aforementioned, have a fibronectin like domain. Other MMPs contain additional variations such as a transmembrane region with a cytoplasmic tail for membrane bound MMPs (MT-MMP or MMP-14, -15, -16, -24), a glycosylphosphatidyl anchor (MMP-17 and MMP-25) or a cysteine and proline rich IL-1 receptor type II like domain (Figure 1).

The combination and structures of the domains, provide each MMP the properties necessary for its function, while as already mentioned, there is great overlap in the substrate specificity between the MMPs. For example, the hemopexin-like domain, provides the MMPs with the ability to cleave fibrillar collagens. The fibronectin-like domain, found in MMP-2 and MMP-9, allow them to bind denatured collagen and gelatin substrates (8).

The MMPs are synthesized and secreted as latent prepro-enzymes (zymogens) that require cleavage of the propeptide for activation (with the exception of the MT-MMPs). The propeptide contains a conserved sequence PRCG(V/N)PD containing a “cysteine switch” motif, whereby the cysteine residue interacts with the catalytic domain to maintain its inactive state (9). The catalytic domain contains a zinc-binding motif (H*E*XXH*X*XG*X*XH) consisting of three essential histidine residues that must be exposed for activity after removal of the propeptide (4). Calcium ions are essential for maintaining MMP stability and activity (8).

After activation, a family of unique MMP inhibitors labeled tissue inhibitors of MMPs or TIMPs can still inhibit MMPs. Presently, four TIMPs are known:

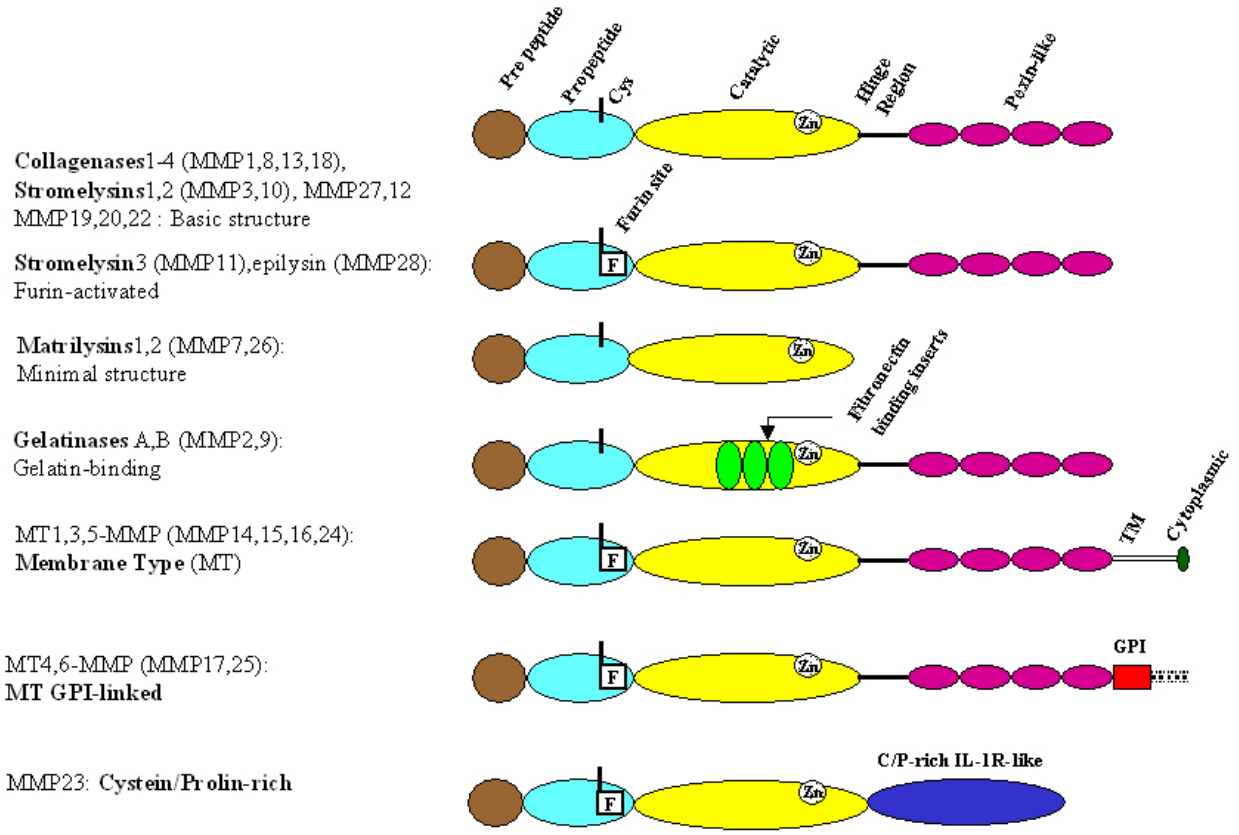

Figure 1. The human MMPs grouped according to their domain arrangement (Modified with permission from 53).

TIMP-1, TIMP-2, TIPM-3 and TIMP-4. TIMPs interact with MMPs catalytic domain to inhibit its activity. There appear to be additional interactions between TIMPs and the hemopexin-like domains for MMP-2 and MMP-9, as well. The TIMPs display differential affinity for the differing MMPs. MMPs are also inhibited by alpha2-macroglobulin, a 722 kDa plasma proteinase which is produced by hepatocytes but may be produced by other tissues as well. It is believed that while TIMPs are MMPs primary inhibitor in the ECM, alpha-macroglobulin is its primary inhibitor in plasma (10).

Transcription is influenced through the actions of cytokines, growth factors, hormones and other agents; meaning that the MMP activity will be influenced by physiological processes as well as pathological ones, such as inflammation and neoplasm. Furthermore, MMP expression has a degree of tissue specificity. For example, MMP-8 is found primarily in neutrophils. Finally, MMPs may influence the activity of other MMPs, as is the case with pro-MMP-2, which requires MT1-MMP (MMP-14) for its activation (11). It is apparent that MMP regulation transpires at many levels. Beginning with the differential tissue expression and compartmentalization to control over its transcription, transfer to the ECM, activation, inhibition, degradation and availability of zinc and calcium ions, there are many targets for MMP activity manipulation.

4. THE MMPs IN TERM LABOR

A gradual softening and dilation of the cervix, weakening of the fetal membranes, uterine contractions and membrane rupture, characterize normal term labor. Rupture of the fetal membranes prior to the commencement of uterine contractions characterizes approximately one-tenth of all deliveries and has been labeled premature rupture of membranes (PROM). While labor generally commences shortly after PROM, prolonged PROM exposes the uterus and fetus to infection. MMPs play a crucial role in the generation of labor and the weakening of the fetal membranes.

Before the onset of contractions, the decidua has the highest production of MMP 2 and 9 and TIMP-1. We demonstrated that contractions, as well as the exposure to PGF2alpha dramatically increased the production of MMP 9 and 2, whereas TIMP-1 was decreased resulting in a shift of the balance between collagenolysis and its inhibition (12). The decidua is known to produce phospholipases, which in turn lead to PG production from the arachidonic acid reservoir in the fetal membranes. According to our findings, PG increases the ability of the decidua to degrade its own extracellular matrix by increasing MMPs 2 and 9 and reducing TIMP-1 secretion. Thus, the shift in MMP/TIMP-1 ratio reflects the stimulatory effect of

Table 1. Basal and changes in expression of MMP 2, MMP 9 and TIMP 1 in the decidua, fetal membrane and amniotic fluid with contractions

Compartment	Decidua	Decidua	Chorion	Chorion	Amnion	Amnion	Am. Fluid	Am. Fluid
Contractions	-	+	-	+	-	+	-	+
MMP 9	-	-	-	-	+	↑	+	↑
MMP 2	-	↑	+	↑	-	-	+++	↓
TIMP 1	++	↑	+	↑	+++	↓	+++	↓

No expression: -, Minimal: +, Moderate: ++, Maximal: +++, Increase expression: ↑, Decrease expression: ↓, Am..= Amniotic

PGF2alpha on membrane rupture. In an additional study (13) we documented that MMP-2 and MMP-9 levels differentially increased after contractions began. In the decidua obtained after the initiation of contractions, MMP-2 was the most active MMP while MMP-9 was the most active MMP in the amnion. In the chorion both MMP-2 and -9 expression was not affected by labor (Table 1).

MMP-9 (92-kd gelatinase B) has been found to increase significantly in the fetal membranes after the onset of contractions (14,15,16). Before labor, MMP-9 is barely detectable. MMP-9 has been localized to the amnion epithelium, macrophages, chorion laeve trophoblast and decidual cells. This is in contrast to MMP-2 (72-kd gelatinase A), which is detectable both before and after contractions began (14). In another study, it was found that both MMP-9 and MMP-3 increased significantly after labor, though MMP-1 was dominant before contractions began (17).

MMP-7, also known as matrilysin, has been found in the amniotic fluid in the second trimester of pregnancy and its concentration was noted to increase in the third trimester. The amniotic fluid concentration of MMP-7, though, was not affected by term labor. In this context, it is still not clear the source of MMP-7 in the amniotic fluid nor its significance in non-complicated labor and delivery (18).

MMP-13 is expressed and produced by the amniochorion, but a role in labor, PTL or PROM has not been found (19). MMP-2 was the predominant gelatinase in amniotic fluid in non-laboring women and was identified along with TIMP-1. After the onset of contractions, MMP-9 replaced MMP-2 in the amniotic fluid and TIMP-1 levels were reduced (13).

RT-PCR has demonstrated that all four known TIMPs are expressed in the fetal membranes before and after labor. TIMPs were localized to the amnion, chorion and to scattered cells in the connective tissue. TIMP-4 expression was only barely visible in all tissues tested (20). TIMP production and localization before and during labor was further studied. It was found that the chorion laeve and the decidua parietalis are the primary producers of TIMPs secreting all four known types into the amniotic fluid. During active labor, this secretion is reduced (21). Reduced TIMP secretion along with increased MMP production, particularly MMP-9, renders the membranes vulnerable to membrane rupture.

A hint that the actual stretching of the membranes, as occurs during uterine contractions and cervical dilation, could in itself cause collagen breakdown through the activation of collagenases, was provided by a study which exposed whole fetal membranes, or amnion alone to 2 and 4 hours of stretching. Stretching of the membranes caused a significant increase in interleukin-8 production and collagenase activity (22).

The role of MMP-3, MMP-9 and TIMP-1 was further elucidated as MMP-3 was immunolocalized to the cells of the amniotic epithelium, fibroblasts and macrophages of the amniotic and chorionic matrix and those of the chorionic cytotrophoblast before labor. MMP-3 was not found in the maternal decidua. TIMP-1 was localized to the same targets but to the maternal decidua as well. Relaxins (H1 and H2), produced by the decidua, caused a significant increase in MMP-3 expression and secretion as well as MMP-9. MMP-2 and TIMP-1 were not affected by the relaxins (23).

It has been proposed that changes taking place at the fetal membranes differ near the cervix, where membrane rupture eventually will occur. It was found, that at term but prior to the commencement of labor, the 92 kDa MMP-9 pro-enzyme was significantly higher at the amniotic membrane near the cervix than it was 10 cm away from the cervical edge. The MMP-2 pro-enzyme did not differ at the two spots. After delivery, there was a significant increase in the level of both MMP-2 and MMP-9 pro-enzymes as well as the appearance of the truncated active enzymes with no difference in concentration relative to the distance of the samples from the cervical edge (24). A proposed explanation for this finding is the preparation of the fetal membranes for rupture during delivery through increased MMP-9 production near the cervix, as the uterus advances from the quiescent phase to the activation phase of labor. At labor itself, the MMP-9 production is maximized along the entire membrane regardless of its distance from the cervix. This temporal association between MMP-9 production near the cervix initially, and maximally later on along the entire membranes, may be a reflection of the fact that initially MMP-9 weakens the membranes in preparation of membrane rupture, while later, MMP-9 production is necessary along the entire chorio-amnion for membrane separation from the decidua and uterine surface.

Indeed, it has been shown that the weakest spots along the fetal membranes, as far as tensile strength is concerned, are those spots with the highest concentration of

MMP-9 or the highest MMP-9/TIMP-1 ratio. Tensile strength was not correlated to MMP-2 concentration (25).

Utilizing immunohistochemistry in term human fetal membranes and placenta, MMP-2 could be localized to cells in the amnion mesenchyme, but primarily in the chorion laeve trophoblast, decidualis parietalis and the vessels in the placental villi. By contrast, MMP-9 stained strongly in the amnion epithelium, chorion laeve trophoblast, decidual parietalis and placental syncytiotrophoblasts. Epithelial cell cultures from the amnion produced only MMP-9 while mesenchymal cell cultures from the amnion produced primarily MMP-2 and very little MMP-9 (16).

After the onset of contractions, both MMP-2 and MMP-9 proenzymes were found to increase. An additional 130 kDa gelatinase band was also elevated in the amnion and decidua which was presumed to be a heterodimer of MMP-9. TIMP-1 secretion was also reduced with the onset of contractions in the amnion and decidua further tipping the MMP/TIMP ration in favor of ECM breakdown. The active form of MMP-9 was found only in media conditioned by amnion and in amniotic fluid after contractions began. By contrast, the active form of MMP-2 was found in media conditioned by chorion and decidua after the commencement of contractions. One drawback of zymography, which is the traditional technique used for detecting gelatinase activity, is that it measures both active and inactive forms of MMPs by separating activator-inhibitor complexes during electrophoresis. A newer technique utilizes biotinilated gelatin as a soluble substrate measuring the net effect of MMPs and their inhibitors. While zymography demonstrated high levels of gelatinolytic activity before contractions began, utilizing soluble biotinylated gelatin demonstrated that endogenous gelatinolytic activity was minimal in all three compartments (amnion, chorion and decidua). With the onset of contractions, it was shown that only the amnion and decidua (and not the chorion) displayed gelatinolytic activity. Utilizing specific inhibitors, it was shown that MMP-9 was the primary contributor to gelatynolytic activity in the amnion while MMP-2 was responsible for gelatinolytic activity in the decidua (13).

A recently discovered MMP-inducing factor termed extracellular matrix metalloproteinase inducer (EMMPRIN) has been characterized and found at the fetal maternal interface. EMMPRIN is a glycoprotein with a molecular mass of 44-66 kDa, depending in the degree of glycosylation of the native 30 kDa protein. This MMP inducer was found in the placenta and fetal membranes and its total level was not changed with labor, though the highly glycosylated 65 kDa EMMPRIN was greatly increased after the initiation of labor. It is postulated that the degree of EMMPRIN glycosylation determines its MMP inducing properties and that the prevalence of the highly glycosylated forms with labor is consistent with the increased MMP activity seen at this time (26).

Integrins are a family of transmembrane glycoproteins providing a link between the ECM and the intracellular cytoskeleton. The integrin $alpha_v beta_6$ has been shown to enhance MMP-9 secretion and its expression increases, as represented by immunohistochemistry intensity of staining, increased in the amnion epithelium after normal delivery as opposed to non-laboring women taken at elective cesarean section (27).

5. MMPs IN PRETERM LABOR

Labor and delivery is the culmination of a cascade of events and signals bringing about the eventual softening and dilation of the cervix, weakening and rupture of the fetal membranes, myometrial contractions leading to birth and subsequent separation of the placenta and uterus from the decidua and inner lining of the uterus. Each of these processes may malfunction or occur prematurely leading to cervical incompetence, PROM and P-PROM, preterm labor and placental abruption. The outcome will generally be preterm delivery and increased neonatal morbidity and mortality. The role of matrix metalloproteinases in preterm labor has been recently reviewed (28).

Bacterial infection is the single most common etiological agent associated with both PTL and P-PROM. Bacteria produce collagenases that weaken the fetal membranes and predispose them to premature rupture (29).

Both term labor and P-PROM have been found to be associated with increased levels of MMP-9 in the amniotic fluid. Amniotic fluid from women with P-PROM was found to have the lowest levels of TIMP-1, further favoring the balance towards greater matrix breakdown (30). The presence of MMPs and TIMPs in amniotic fluid does not explain their origin or whether they are caused or are a cause of preterm labor. A comparison of the levels of MMP-2 and MMP-9 in cultured fetal membranes between non-laboring women to those with proven intra-amniotic infection, found that MMP-2 was present in both groups while MMP-9 was not present in non-laboring women but appeared in those with infections. MMP-9 was produced from the membranes of non-laboring women when exposed to lipopolysaccharide (LPS) or peptidoglycan polysaccharide, two products of infection. TIMP-1 and TIMP-2 were found in membranes from non-laboring women and women with amniochorionic infections (31).

The mechanism for PROM in the absence of infection is elusive. The possibility of an endogenous pathway for membrane rupture has been proposed. It was found that TIMP free MMP-2 and MMP-9 levels were increased in amniotic fluid taken from women with PROM as compared to women at term or women with PTL. TIMP-1 levels were increased while TIMP-2 levels were decreased (32).

While the emphasis has been placed on MMP-2 and MMP-9 (gelatinase A and gelatinase B), other MMPs have been studied in the fetal membranes. The stromelysins are enzymes that cleave several components of the extracellular matrix, but more interestingly they cleave the pro-enzyme forms of MMP-1 and MMP-8 and so, their

presence has an indirect effect stimulating the breakdown of the ECM. All three stromelysins 1, 2, and 3 (MMP-3, MMP-10 and MMP-11) were found in the amnion, chorion and extracellular matrix when stained utilizing *in situ* hybridization. MMP-3 was increased in the amniotic fluid from patients with P-PROM as compared to women at term with unruptured membranes. LPS, a product of infection, stimulated the release of MMP-3 from amniochorionic membranes, though the levels of MMP-3 from the amniotic fluid of women with positive cultures and P-PROM did not differ significantly from those with negative culture. This suggests that MMP-3 production is a product of the inflammatory process regardless of etiology (i.e. infectious vs. non-infectious) (7).

Matrilysin or MMP-7 is a unique MMP in that it does not contain the hemopexin-like domain. Unlike other MMPs, which are produced by cells of the stroma and secreted to the ECM, MMP-7 is produced by epithelial cells. It has been shown to degrade fibronectin, gelatin, laminin, elastin, casein and several proteoglycans. MMP-7 was found in the amniotic fluid in samples taken during the midgestation as well as at term, while at term the levels were significantly higher. MMP-7 levels did not increase at the time of delivery for women at term, though they were higher for women with preterm labor and subsequent preterm delivery. Preterm delivery with infection was associated with even higher levels off MMP-7. While it has been shown that MMP-7 levels were increased with preterm delivery, particularly in the presence of infection, the source of the enzyme was not ascertained, and may be of fetal origin, from the fetal membranes or amniotic macrophages. Since term spontaneous delivery was not associated with increased MMP-7 levels, it appears that its role is primarily in pathological labor; i.e. preterm labor and labor associated with infection (33).

MMP-8 is unique in that it is solely produced by macrophages and therefore, its detection may be evidence of infection. MMP-8 was not found in the amniotic fluid of women with uncomplicated pregnancy in midgestation or at term or during labor. MMP-8 was more likely to be found in the amniotic fluid of women with PTL or P-PROM if amniotic cultures were positive than if they were negative (34).

Further evidence that infection or inflammation triggers MMP production is provided by studies that test the response of membrane explants to LPS, an endotoxin released by *Escherichia coli* bacteria. It was shown that transcription of MMP-2 and MMP-9 messenger RNA was increased dramatically, particularly for MMP-2, after exposure to LPS. Protein product was significantly higher for MMP-2 but remained statistically unchanged for MMP-9. TIMP-2 levels decreased after LPS exposure, further balancing the MMP/TIMP ratio in favor of gelatinolysis (35).

Preterm delivery of an infant is a major cause of neonatal morbidity and mortality. Preterm delivery may be induced by the medical staff for obstetric complications of pregnancy such as severe preeclampsia, but spontaneous delivery will generally follow either PTL or P-PROM. Both processes are often, but not always associated with infection, and a maternal and fetal inflammatory response diagnosed histologically as chorioamnionitis. This response involves the production of inflammatory cytokines (IL-1, IL-6, IL-8 and TNF-alpha) and the production of prostaglandins. While the inflammatory response is common to both PTL and P-PROM it is interesting to note where these processes differ and what will determine if a woman experiences PTL or P-PROM. The participation of MMPs and their inhibitors differed significantly in these two pathways towards PTD. It was found that the expression of MMP-2 and MMP-9 as well as MT1-MMP (MMP-14), which activates MMP-2 by cleaving the pro-enzyme, were significantly higher in amniochorionic membranes from women with PROM as compared to PTL. By contrast, TIMP-2 levels were unchanged. Similar findings were found in the amniotic fluid. MMP-9 was found in the amniotic fluid primarily in patients with P-PROM as compared to PTL while the level of TIMP-2 was significantly decreased tilting the MMP/TIMP balance in favor of gelatinolysis more strongly in women with P-PROM. MMP-2 and MMP-3 levels were not significantly different in the amniotic fluid from women with P-PROM as compared to PTL (36).

Apoptosis may play a role in P-PROM. Expression of pro-apoptotic genes p53 and bax was significantly higher in the amniochorionic membranes from women with PROM relative to PTL, while the level of the anti-apoptotic protein *Bcl2* was significantly lower. Fas, an inducer of apoptosis was seen in most cases of P-PROM while detected in only half of the membranes taken from women with PTL, while, caspase 8, a protein also involved in apoptosis was detected in most of the cases of P-PROM and only a few of the cases of PTL. IL-18, which is also pro-apoptotic and an inflammation cytokine was elevated in the amniotic fluid from women with P-PROM as compared to PTL (36).

Simulating inflammation *in vitro* by exposing fetal membranes from non-laboring women is a strategy used to explore the membranes' response to infection, a primary etiology for P-PROM and PTL. While intra-amniotic infection may cause degradation of the fetal membranes directly through the production of proteases from bacteria, an alternative mechanism is through the initiation of an inflammatory maternal reaction must be considered. Proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-alpha) and interleukin 1beta (IL-1beta), are produced during inflammation by immune cells (such as macrophages) as well as amniotic epithelial cells and chorionic cells. These cytokines are expressed during normal labor as well. Amniochorionic membranes, amnion and chorion tissue explants were exposed to LPS. The MMP-9 proenzyme was significantly increased by amnion but not by chorion. TIMP-1 levels were not elevated. Likewise, MMP-9 increased significantly when amnion was exposed to TNF-alpha or IL-1beta, while its secretion levels from the chorion was unchanged. TNF-alpha and IL-1beta demonstrated an additive effect on MMP-9 production from amnion. Though, when amnion

explants were treated with LPS in the presence of neutralizing antibodies to TNF-alpha, the MMP-9 response was cancelled. This was not the case when neutralizing antibodies to IL-1beta were added. TNF-alpha and IL-1beta secretion was increased significantly from the chorion when exposed to LPS (37).

Likewise, the p55 TNF receptor (TNF-R1) was detected with immunostaining on the surface of amniotic epithelial cells and chorionic cells in women with normal labor and PROM, with PROM displaying the heaviest staining. By contrast, the p75 TNF receptor (TNF-R2) was found in the membranes from women with normal labor, or not in labor at all (during elective cesarean section) but not in the membranes taken from women with PROM. Membranes taken from women after normal labor displayed characteristics compatible with classical apoptosis (type I apoptosis) such as flattened nuclei and compacted chromatin that was attached to the inner nuclear membrane. Non-classical (type II) apoptotic changes, such as vacuolization, cellular and DNA fragmentation and late nuclear condensation; were found in the amniotic epithelium taken from membranes of women with PROM. The main gelatinolytic bands taken from these cell extracts was of the MMP-2 and MMP-9 pro-enzymes. The chorion taken from women with PROM had an intense band correlating with active MMP-9, as well. MMP-9 expression in the chorion and amnion was greatly increased in the membranes taken from women with labor as opposed to non-laboring women (2-4 times greater) and was even more increased in women delivering after PROM (5-6 times greater). This study shows that a connection may be drawn between the expression of a TNF receptor to the process of apoptosis, the production of MMP-9 and labor or PROM. TNF-R2, which is associated with cell proliferation and survival, was found on the membranes of non-laboring women and women with normal labor; while TNF-R1, which has been associated with TNF's cytotoxic properties was not found in the membranes of non-laboring women, but was found in the membranes of women in labor or women with PROM. The authors suggest a TNF receptor switch from TNF-R2 to TNF-R1, which is strongest in the membranes of women with PROM. This switch may induce apoptosis and MMP-9 production seen in labor and in PROM (38).

In order to ascertain the intermediaries responsible for the MMP-9 production in decidual cells, decidual cell cultures were exposed to phorbol 12-myristate 13-acetate (PMA) and MMP-2, MMP-9 and TIMP-1 were measured. Cells exposed to PMA exhibited a dose-dependant increase in MMP-9 and TIMP-1 secretion but no change in MMP-2 production. PGE_2, measured as a positive control, was also increased after PMA exposure. This indicates that the increased MMP-9 production may be a result of increased protein kinase C (PKC) activity (38).

MMP-3 was found constitutively in the amniotic fluid at all stages of gestations checked from the midtrimester on, without any gestational age related changes in its concentration. MMP-3 concentrations were significantly increased during labor whether preterm or term or if amniotic infection was present. Spontaneous rupture of membranes without labor, whether term or preterm, was not associated with an increase in MMP-3 (40).

MMP-1 (collagenase-1) concentrations in amniotic fluid were considerably higher at term than in the midtrimester regardless of the presence of labor. Parturition, whether term or preterm, was not associated with an increase in MMP-1. On the other hand, when PTD was preceded by P-PROM with or without infection, MMP-1 was significantly increased. Surprisingly, for women at term, PROM was associated with lower levels of MMP-1 than of those with unruptured membranes (41).

While infection is a major contributor to the etiology of PTD with or without P-PROM, many cases are not associated with infection but with other factors. Placental abruption increases the likelihood of P-PROM and PTD. Thrombin, added to cell cultures of leukocyte-free decidual cells induced increased expression of MMP-3. This increase was attenuated in the presence of medroxy-progesterone acetate (42).

Recent findings have indicated that there may be a genetic predisposition to P-PROM mediated through polymorphisms on the MMP genes and their promoters. These studies have been carried out primarily among African Americans who have a higher rate of P-PROM than Caucasians. Insertion of a guanine nucleotide (G) in the MMP-1 promoter at position 1607 (producing the 2G allele) increases promoter activity. The neonates of African American women with P-PROM were more likely to be heterozygotes or homozygotes for the 2G allele than a control group homozygous for the 1G, wild type, allele (43).

Many polymorphisms have been found in the promoter region of the MMP-9 gene, while two of them are known to affect promoter activity. One is a CA-repeat micro satellite polymorphism at position –55 to –13, and the other is C to T substitution at –1562. Neonates of African American mothers born after P-PROM were significantly more likely to be carriers of a 14 CA-repeat allele than controls without P-PROM. The 14 CA-repeat allele was also associated with greater MMP-9 promoter activity in amnion epithelial cells. There was no difference in the likelihood of carrying the 1562 C→T substitution. And, at least in this study, this polymorphism was not associated with greater promoter activity (44).

While genetic predisposition is an attractive theory, it clearly cannot explain all, or even most cases of P-PROM, since the stimuli leading to P-PROM are multifactor. At best, a predisposition to P-PROM can be hypothesized. Rather than search for the numerous potential polymorphisms associated with genes that may be associated with P-PROM (such as MMPs), another approach would be to test the reactivity of amniochorionic membranes taken from women of different ethnic backgrounds, to stimuli thought to bring about P-PROM. Amniochorionic membranes, taken from non-laboring

women at term, exposed to LPS showed a dramatic increase in TNF-alpha whether the membranes came from white or African-American women. However, membranes from white women produced significantly more TNF-alpha both before and after LPS stimulation than did the membranes from African-American women. TNF-alpha may bind two receptors: TNF-R1 and TNF-R2. Cleavage of these receptors produces soluble receptors (sTNF-R1 and sTNF-R2), which may bind TNF-alpha and attenuate its activity. The ethnic differences in response to LPS were more apparent when measuring sTNFR1 and sTNFR2, producing opposite results. In whites, sTNFRs was increased at the high concentration of LPS, while in African American women it was decreased. A significant increase in MMP-9 was noted only in the membranes taken from African American women after stimulation with LPS. Despite the high increase in TNF-alpha in whites after LPS stimulation, no significant increase in MMP-9 was noted for them. A significantly different response, though not entirely predictable or easily explainable, to infectious stimuli was demonstrated for different ethnic group women (45).

6. INTERVENTIONS FOR THE ATTENUATION OF MMPS

The role MMPs play in parturition, make them an attractive candidate for attenuation in an attempt to prevent PTD or P-PROM, or to induce labor when indicated. Unfortunately, there are many potential pitfalls in this approach. Most disturbing is that MMPs play a role in most organs and in many physiological processes, of particular concern, in the developing fetus. Therapeutics, which may attenuate their expression or activity, may have untoward effects on the developing fetus and may have implications for the offspring's future. Nevertheless, the morbidity associated with preterm birth, particularly of very low birthweight infants, is often so severe, and interventions to prevent preterm delivery have thus far been disappointing, that it may be worthwhile to invest further research in this avenue. Furthermore, some of the drugs already used in the treatment or prevention of preterm labor, may exert their influence on the expression or activity of MMPs.

Indomethacin, a prostaglandin inhibitor, is a known tocolytic agent in clinical use for the prevention of PTD (46). Its inhibitory effect on MMP-2 and MMP-9 activity has been demonstrated in amnion and chorion explants (12). Indomethacin may therefore have a role in the prevention of P-PROM as well.

N-acetylcysteine, a glutathione precursor and anti-oxidant, has been shown to inhibit amniochorionic matrix metalloproteinase activity (47), and this inhibition is through two mechanisms. A direct inhibition on MMP-2 and MMP-9 activity regardless of tissue origin, and a differential effect on secretion depending on the tissue of origin and the concentration of N-acetylcysteine (48). Furthermore, N-acetylcysteine has been shown to suppress the secretion of LPS stimulated factors from fetal membranes including type II phospholipase A_2 (type II PLA_2) as well as PGF2alpha, IL-6, IL-8, TNF-alpha, and 8-isoprostane. MMP-9 activity was reduced, as was urokinase-type plasminogen activator enzyme. Nuclear factor kappaB (NF-kappaB) binding activity was suppressed (49). The NF-kappaB signaling pathway is a regulator of phospholipid metabolism, proinflammatory cytokines and ECM-remodeling enzymes. All these changes lead to the conclusion that N-acetylcysteine are able to attenuate the cascade of reactions mediated by NF-kappaB and caused by reactive oxygen species stimulated by LPS. Further research is necessary to ascertain if there is a role for N-acetylcysteine in the treatment or prevention of P-PROM, PTL or PTD.

Renewed interest in progesterone for preventing PTD has surfaced following two clinical trials where progesterone was used prophylactically for high risk women and was shown to prevent PTD relative to a control group receiving placebo (50,51). Indeed, MMP-3 secretion from fetal membranes stimulated with thrombin *in vitro* was reduced when progesterone (in the form of medroxy-progesterone acetate) was added to the medium (42). Surely, further research is necessary on the effects of progesterone on MMP dynamics.

Finally, it may be economically feasible in the future to perform genetic analysis on high-risk population groups to determine which individuals are at greatest risk of PTD based on genetic polymorphisms in those genes related to MMPs and membrane weakening and to direct prophylactic therapies towards them even during their first pregnancy. In this way, we may eliminate the greatest disadvantage to all potential prophylactic therapies: those women at greatest risk of PTD are those who have delivered prematurely in the past. Even if prophylactic therapies prove to be efficient on recurrent trials, they will really only eliminate the burden of PTD if those at greatest risk can be identified before their first pregnancy.

Cyclic AMP (cAMP) is an important second messenger in regulating the inflammatory response. The phosphodiesterase (PDE) superfamily functions to degrade cAMP into its inactive metabolites. The PDE4 family was shown to be the major cAMP-PDE expressed in human fetal membranes stimulated with LPS. PDE4 inhibition of LPS stimulated membranes reduced the release of the pro-inflammatory cytokine TNF-alpha, while increasing the release of the anti-inflammatory cytokine IL-10. Cyclooxygenase-2 protein expression and PGE_2 production was reduced. Pro-MMP-9 mRNA expression and pro-MMP activity was reduced as well. It was therefore suggested that selective PDE4 inhibitors such as cilomilast and roflumilast may be efficacious in the prevention of inflammation induced PPROM and PTL (52).

7. SUMMARY AND PERSPECTIVE

The mechanisms controlling labor and delivery are still poorly understood. It is clear the MMPs play a critical role in the induction and propagation of labor, including cervical ripening and dilation, membrane weakening and rupture and detachment of the placenta and membranes after delivery. As more data is collected

concerning the expression and control of MMPs in normal labor, we learn more of its role in pathological labor as well. The prevention or delay of preterm labor is of cardinal importance to society. Recognizing those persons at greatest risk, either through their own personal history or through known environmental or genetic risk factors, allows us to target prophylactic therapies to those women at greatest risk. The development of safe prophylactic therapies, therefore, is essential. Further research into the factors that induce the expression and activity of MMPs; will guide us to those therapies which have a chance of being effective.

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Abbreviations: cAMP: cyclic adenosine monophosphate, ECM: extracellular matrix, EMMPRIN: extracellular matrix metalloproteinase inducer, IL: interleukin, LPS: lipopolysaccharide, MMP: matrix metalloproteinase, MT: membrane type, NF: nuclear factor, PDE: phosphodiesterase, PG: prostaglandin, PKC: protein kinase C, PMA: phorbol 12-myristate 13-acetate, P-PROM: preterm premature rupture of membranes, PROM: premature rupture of membranes, PTD: preterm delivery, PTL: preterm labor, RT-PCR: reverse transcriptase polymerase chain reaction, sTNF-R: soluble TNF-R, TIMP: tissue inhibitors of MMP, TNF: tumor necrosis factor, TNF-R: TNF receptor

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Send correspondence to: Eliezer Shalev MD, Department of Obstetrics and Gynecology, Ha'Emek Medical Center, Afula 18101, Tel: 972-4-6494031, Fax: 972-4-6494032, E-mail: shaleve@tx.technion.ac.il

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