RESEARCH ARTICLE

Effect of Inflammatory Signals on Progesterone Production at the Maternal-Fetal Interface

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Abstract

Introduction: In explant cultures of human fetal membranes (FM) granulocyte-macrophage-colony-stimulating-factor (GM-CSF) mediates the inflammation-induced FM weakening seen in preterm premature rupture of the membranes (pPROM) and exogenous progesterone (P4) inhibits GM-CSF and inflammation-induced FM weakening. Here we report that GM-CSF induces P4 production within the FM which then acts in a paracrine manner to counteract GM-CSF-induced weakening.

Methods: FM explants mounted in Transwell inserts were cultured with control media and increasing GM-CSF, RU486 (blocks P4 action), or trilostane (blocks P4 production). P4 production, matrix metalloproteinase-2 (MMP-2) and FM rupture strength were determined. Effects of GM-CSF on P4 production and abundance of the 3ß-hydroxysteroid dehydrogenase (3ßHSD) enzyme in the BeWo human trophoblast cell line were also determined.

Results: GM-CSF induced P4 production in both FM explants and BeWo cells in a concentration-dependent manner. GM-CSF also increased 3βHSD protein in BeWo cells. Incubation of FMs with RU486, or trilostane, each caused increased FM weakening. Trilostane also increased MMP-2. Exogenous P4 with trilostane repressed MMP-2 and restored FM strength.

Conclusion: GM-CSF induced P4 production by FM and trophoblastic cells suggesting that locally produced P4 is increased by factors that weaken FM. Inhibition of local P4 production or action resulted in FM weakening with concomitant MMP-2 induction suggesting local P4 maintains FM structural integrity. This weakening is reversed by exogenous P4. These data are consistent with a negative-feedback system whereby P4 induced by GM-CSF, the mediator of inflammation-induced FM weakening, counteracts GM-CSF, inhibiting both its production and downstream action with resultant preservation of FM structural integrity.

Keywords: Fetal membranes, biomechanical weakening, GM-CSF, progesterone, TNFα, thrombin, cytotrophoblast, pPROM, MMP-2

Introduction

Inflammation/infection and decidual bleeding/abruption are major associations of preterm premature rupture of the fetal membranes (pPROM) [1-5]. Each weakens the fetal membranes (FM) by initiating a cascade which degrades the amnion-chorion extracellular matrix (ECM), a prerequisite for rupture [6-9]. To explore this process, we developed an ex-vivo explant culture system in which biomechanical rupture strength, and the biochemical and tissue structure properties of human FM, can be studied under controlled experimental conditions in response to stimuli simulating infection, inflammation and chorion-decidua bleeding [8,10]. Using this model we determined that tumor necrosis factor (TNF) and interleukin 1ß (IL-1ß), modeling inflammation/ infection, and thrombin modeling decidual bleeding, each weaken FM in a concentration-dependent manner [6,11].

Importantly, each agent also markedly induces granulocyte-macrophage colony-stimulating factor (GM-CSF) production by decidual stromal cells [9,12]. We subsequently found that inhibition of GM-CSF activity with a GM-CSF neutralizing antibody inhibits TNF- and thrombin-induced weakening, and that GM-CSF alone is sufficient to induce the entire FM weakening process [13]. Those data suggest that GM-CSF mediates inflammation- and bleeding-induced FM weakening[13]. We have also reported that GM-CSF increases the expression of matrix metalloproteinases (MMPs), most markedly MMP-2, as well other proteases (Cathepsin-S, Proteinase- 3, Elastase-2) and suppresses expression of tissue inhibitors of MMPs (TIMPs) as well as other protease inhibitors (NGAL, Cystatin-C, HE4 and Thrombospondin1) in the FM [14,15]. Based on these findings we propose that GM-CSF, produced by decidual cells in response to inflammatory stimuli, weakens the FM by increasing net proteolytic activity that degrades the amnion ECM[15].

Left unchecked, this cascade would weaken the FM and increase the risk for premature spontaneous rupture, especially at focal areas of decidual bleeding or in response to ascending pathogens through the cervix. Therefore, we reasoned that a locally acting, counteracting pathway exists to maintain the FM structural integrity for most of pregnancy. A prime candidate was progesterone (P4). Consistent with this hypothesis we have previously shown that P4 effectively inhibits the inflammation-induced FM weakening pathway, specifically, that exogenous treatment of FM with P4 blocks weakening induced by TNF, thrombin and GM-CSF [16]. Finally we have shown that P4 inhibits basal, and induced expression and secretion of the inflammation pathway critical intermediate, GM-CSF, by decidual stromal cells [9,12,16].

The purpose of this study was to determine whether P4, produced locally within FM, affects FM structural integrity, and also how its production is affected by inflammatory stimuli that weaken the FM. We found that GM-CSF induces P4 by full thickness FM and by the BeWo trophoblast cell line. Furthermore, we found that inhibition of endogenous P4 production or P4 action in FM weakens full thickness FM. The data support the hypothesis that locally produced P4 plays a key role in maintaining the structural integrity of the FM.

Materials

Unless specified elsewhere, all reagents were purchased from Sigma Chemical Company; St. Louis, MO. Consumables were obtained through Fisher Scientific, Hampton, NH. BeWo (ATCC® CCL-98™cells) were obtained from the ATCC, Manassas, VA. Trilostane was obtained from MedChemExpress USA, Monmouth Junction, NJ. Mouse monoclonal antibody against human 3ß-hydroxysteroid dehydrogenase (3ßHSD) enzyme Type 1:HSD3B1 (ab55268) protein, secondary antibody, and standard were purchased from Abcam, Cambridge, MA. Gel electrophoresis and blotting reagents were from Bio-Rad, Hercules, CA.

Methods

Tissue Acquisition and Culture.

We have described our model system for the study of FM weakening in detail in many recent reports [8-10,14,17-20]. Briefly, FM from uncomplicated repeat cesarean deliveries (37-38wk gestations: N=5), were collected after patient consent and approval by MetroHealth Medical Center's Institutional Review Board (# IRB10-00861). None of the patients had a previous history of spontaneous preterm birth. Intact FM tissue was washed in 2 x 250-ml changes of ice-cold

phosphate-buffered saline (pH 7.2-7.4) following dissection from the placental disc. At term the FM region overlying the cervix is morphologically different and is weaker. Using our procedure to mark the peri-cervical region after delivery of the newborn and prior to the delivery of the placenta we are able to identify the peri-cervical region[21]. This region was avoided in all studies but those described in Figure 5. Intact tissue fragments were mounted in 2.4cm Transwell inserts (Corning, Corning, NY) after removal of the Transwell membrane as previously described [10] and cultured in 6-well dishes with 2ml phenol-free MEM α containing antibiotic-antimycotic and 50mg/L gentamycin sulfate in each of the compartments (amnion(AM)/upper and choriodecidua(CD)/ lower).

GM-CSF induced P4 production in FM explants.

Explants were conditioned for 24h in serum-free medium as described above (5% CO_2, 37 °C, 100% relative humidity). Medium was removed and replaced in both compartments with GM-CSF (0-200ng/ml) added to the CD compartment for an additional 48h. After culture, medium was collected from each compartment, clarified by centrifugation at 12,000 x g/15min/10^{O}C and supernatants were stored at -70^{O}C. P4 was then determined as described below.

FM Rupture (Strength) Testing.

The structural integrity of the FM explants (rupture strength) was tested according to our published protocol[10]. Briefly, Transwell-mounted FM were secured in a 2.5 cm diameter fixture between the aligned horizontal plates of the rupture testing equipment. A motor-driven 1cm diameter spherical-head plunger aligned perpendicular to the FM was then forced against the AM side. Plunger force and concomitant membrane displacement were recorded continuously to determine the force (rupture strength in newtons) and maximum displacement (cm) needed to cause FM rupture.

GM-CSF induced P4 production by BeWo Cells.

BeWo cells were sub-cultured in 6-well plates at 0.2 × 10^6 cells/well in 3ml Ham's F-12K medium with 10% fetal bovine serum and 1X antibiotic-antimycotic. After 48h culture (5% CO_2, 37 °C, 100% relative humidity) cultures were rinsed twice with 3ml serum-free medium, then incubated in serum-free medium for 4h. Cells were then incubated in 3ml fresh serum-free medium containing increasing doses of GM-CSF (0-300ng/ml) for 6 or 24h. Media were then clarified at 12,000 x g for 15 min at 10°C and stored at−70°C. Monolayers were washed twice with cold PBS, then cells were solubilized in 2ml cold lysis buffer (50 mM Tris–HCl [pH 7.5], 150 mM NaCl,1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) containing 1X Protease Inhibitor Set III (Calbiochem/Millipore Sigma, Burlington, MA) by scraping and 2 x 5s sonication (Fisherbrand Model 60 Dismembrator:setting 40). Lysates were frozen at −70°C then thawed on ice and the extracts were clarified by centrifugation at 12,000 × g for 15min. Lysate supernatants were stored at −70°C.

Progesterone Assay.

Progesterone was measured by competitive ELISA in duplicate samples from thawed medium from triplicate cultures/treatment group using a commercial kit (#582601) according to the Manufacturers protocol (Cayman Chemical, Ann Arbor, MI). Developed plates were read after 90min incubation on a Perkin Elmer VICTOR Nivo plate reader at 405nM. Unknown concentrations were determined from comparison with known standards analyzed

by four parameter logistic curve fit using Sigmaplot Software (Systat Software, Inc, Chicago, IL). Sensitivity was typically greater than 10pg/ml with a range of 7.8-1000pg/ml and inter-assay/intra-assay variations of 2.9/7.5 %CV at 125pg/ml. Cross reactivity: pregnenolone (14.0%), 17β-estradiol (7.2%), 5β-pregnan-3α-ol-20-one (6.7%), 17α-hydroxyprogesterone (3.6%).

MMP-2 Assay: MMP-2 was measured by ELISA in diluted duplicate samples from thawed medium from triplicate cultures/treatment group using a commercial kit (#KHC3081) according to the Manufacturers protocol (Invitrogen/Thermofisher, Waltham, MA). Developed plates were read after addition of STOP Solution on a Perkin Elmer VICTOR Nivo plate reader at 450nM. Unknown concentrations were determined from comparison with known standards analyzed by four parameter logistic curve fit using Sigmaplot Software (Systat Software, Inc, Chicago, IL). Sensitivity was <0.15ng/ml. In the range of 0.78-50ng/ml the kit demonstrated inter-assay/intra-assay variations of 6.0/3.5%CV. Recombinant MMP-9 demonstrated 4% cross-reactivity.

Western Blotting for 3βHSD (Type1: HSD3B1 protein) in BeWo cells treated with GM-CSF.

BeWo cell lysates (30ul) from cells treated with increasing doses of GM-CSF for 6h were thawed, mixed with 4X Laemmli Buffer (10ul) and heated in a boiling water bath for 5min. Protocols, reagents and equipment for electrophoresis and western blotting were from Bio-Rad (Hercules, CA). Standards prepared similarly at a concentration of 1ng/ul and 40ul volumes, along with samples, were loaded and run in respective 10% TGX mini-Protean gels for 1hr at 150V. Separated proteins were semi-dry transferred to PVDF membranes for 90min at 14V. Blots were washed briefly in Tris-buffered saline:0.5% Tween-20 (TBST) then blocked in TBST containing 5% non-fat dry milk (TBSTM) for 1h. Blots were then incubated in sealed bags containing HSD3B1 primary antibody diluted 1:1000 in 5ml TBSTM and incubated overnight on a rocking platform at 10°C. After incubation, blots were rinsed and rocked in three 15min washes TBST (50ml each). Blots were then incubated with Goat anti-mouse IgG-HRP diluted 1:2000 in TBSTM (20ml) and incubated for 1hr at room temperature on a rocking platform. After incubation, blots were again washed in three 15min changes of TBST, drained, bagged and subjected to chemiluminescence detection using the BioVision ECL Western Blotting Detection System (Fisher Scientific, Hampton, NH). Proteins were visualized by exposure to FUJI Film (5-30sec) then development and data were quantified after scanning using IMAGEJ Software (NIH, Bethesda, MD).

Inhibition of P4 action or production.

To determine the effect of inhibition of P4 action with RU486 or P4 production with Trilostane on FM strength, full thickness fetal membrane (FM) fragments were mounted in 2.4cm diameter Transwells (Corning, Corning, NY) secured by an o-ring[10]. Triplicate cultures were pre-incubated with or without 10^{-7}M RU486 or, with or without Trilostane (1uM) ± 10^{-7}M P4 for 24hr, added to the CD compartment in MEMα (2ml/compartment in 6 well plates) containing antibiotic-antimycotic solution with 50mg/L gentamicin sulfate at 36.5°C/5% CO_2/100% relative humidity. Medium was removed after 24h culture then replaced, as above, with or without 200ng/ml GM-CSF for an additional 48h. Concentrations of RU486, Trilostane and P4 used in these experiments were based on our previous publications and the literature[16,20,22,23]. Following incubation, FM

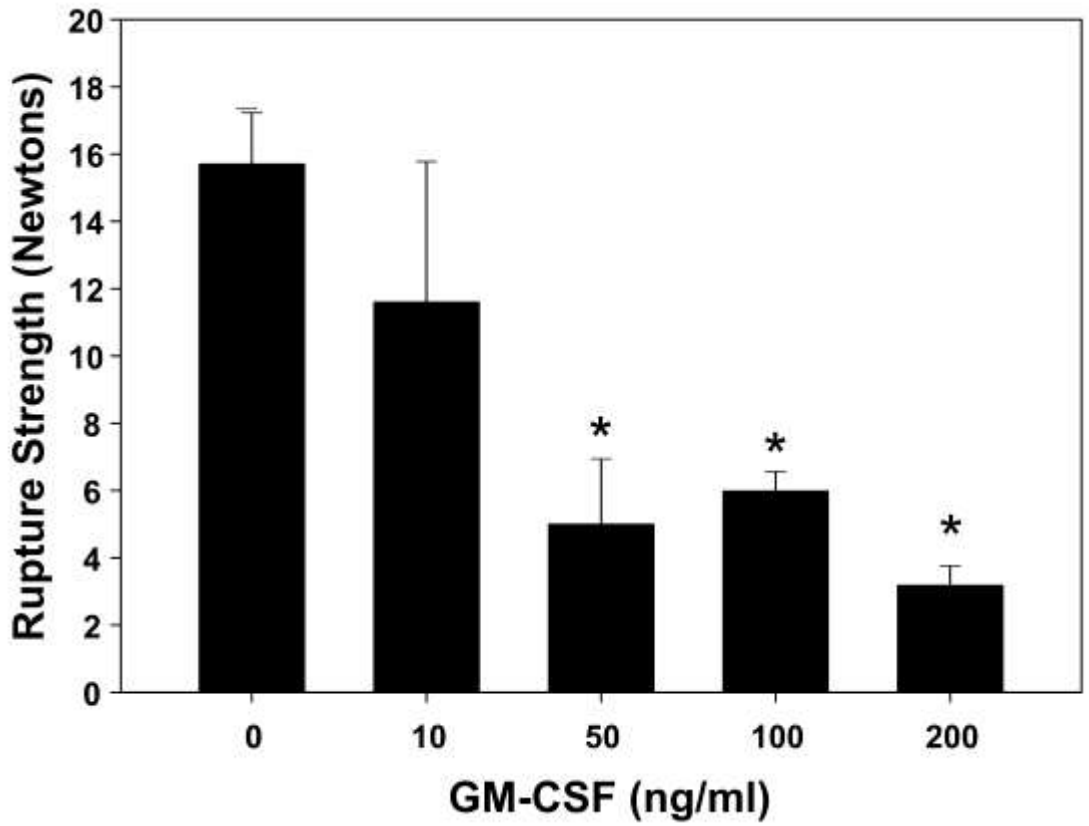
fragments were rupture tested and data analyzed as outlined above.

Statistical Analysis: Data were analyzed by ANOVA followed by post-hoc pair-wise comparisons (Holm-Sidak method) using Sigmaplot (Systat Software, Inc, Chicago, IL). Differences were considered significant when $p<0.05$.

Results

Effect of GM-CSF on FM strength and Progesterone induction.

Compared with controls GM-CSF (0-200ng/ml) weakened intact FM fragments in a concentration dependent manner. Rupture strength (15.8±0.9N-Control versus 3.6±0.6N 200 ng/ml-GMCSF; $p<.01$) **(Figure 1A)**. These findings confirm our previous reports [13,14,19]. Concomitant with the weakening GM-CSF induced a concentration dependent increase in P4 in the explants media ($p<.01$). **(Figure 1B)**



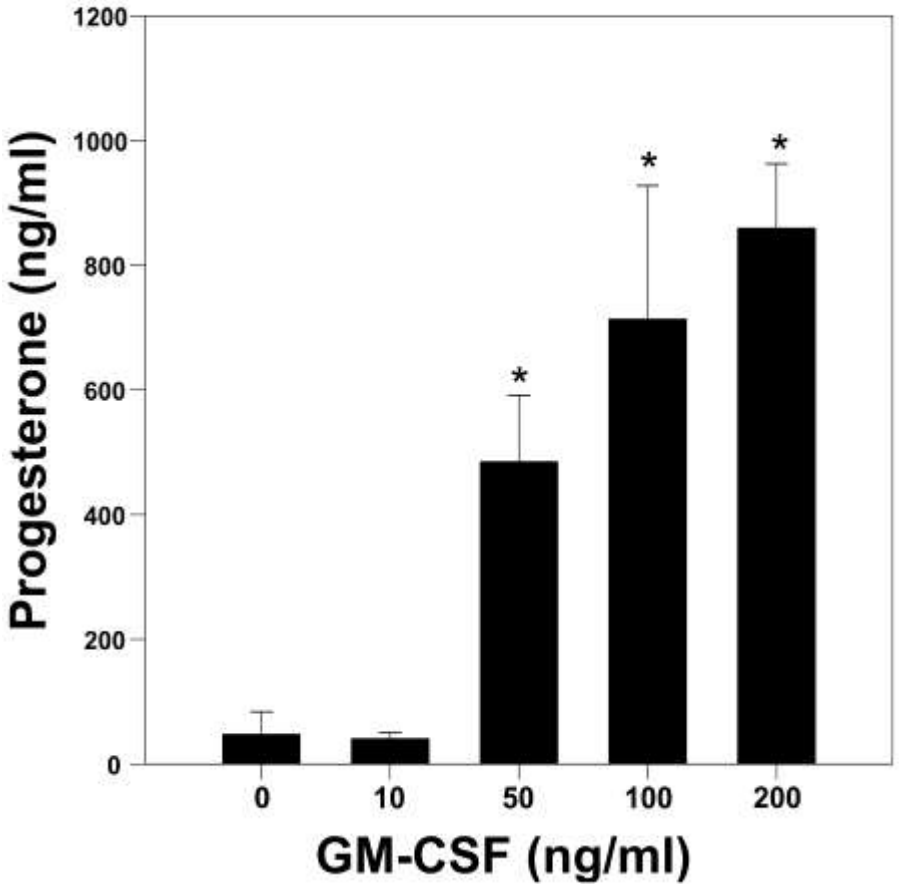


Figure 1: Effect of GM-CSF on FM strength and Progesterone induction A: GM-CSF induces weakening of intact fetal membrane (FM). Intact FM fragments were treated with the indicated concentrations of GM-CSF for 48h. Data are presented as mean rupture strength in Newtons ± SD; N=3 replicates/treatment group from a representative experiment. This experiment was repeated with FM obtained from four uncomplicated c-sectioned patients. In each case the same results were obtained with the significance indicated in this example. (* indicate $p < 0.01$ vs controls). B: GM-CSF induced concentration dependent P4 secretion in intact FM; Media (from the experiment described in A) were analyzed by ELISA for P4 using commercial kits. Data are presented as mean ± SD; N = 3 replicates. (* indicate $p < 0.01$ vs controls).

Inhibition of P4 action or P4 production results in FM weakening.

Incubation of intact FM fragments with RU486 (10^{-7}M) with resultant blockade of the action of endogenously produced P4 action resulted in FM weakening ($p<.01$). Incubation with RU486 and GM-CSF together further weakened FM. Incubation of intact FM fragments with Trilostane (1μM), blocking P4 production in FM by inhibiting 3βHSD, also resulted in FM weakening ($p<.01$). Incubation with Trilostane and GM-CSF together further weakened FM. Co-incubation of FM with Trilostane (1μM) and exogenous P4 (10^{-7}M) prevented the Trilostane induced weakening. **(Figure 2)**.

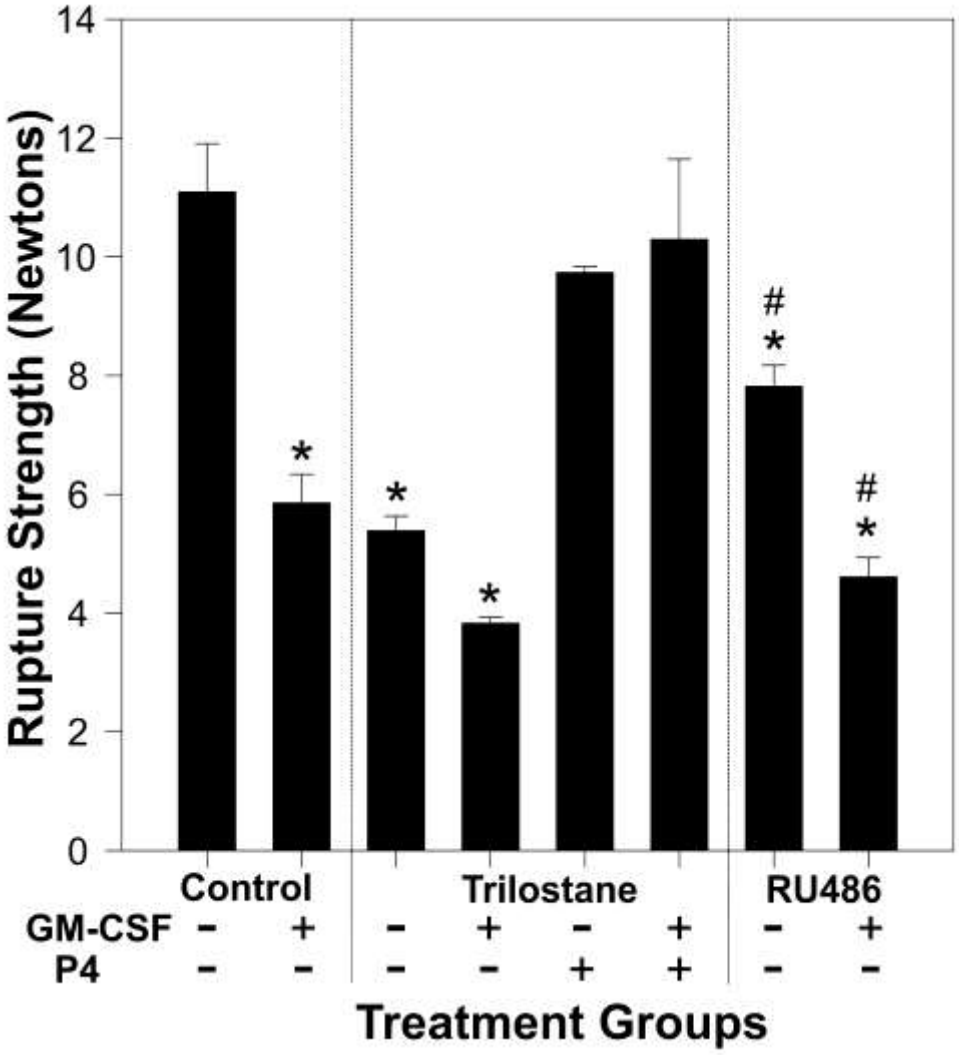


Figure 2: Blockade of P4 Production or Action Weakens FM: Explants of FM were incubated with media alone, trilostane (1 μM) ± progesterone (100 nM), or RU486 (10nM) for 24h. The medium was then refreshed with the same and GM-CSF (200 ng/ml) was added where indicated for another 48h. Strength testing was performed as indicated in Methods. Data are presented as mean rupture strength in Newtons ± SD; N=3 replicates/treatment group from a representative experiment. (* indicate $p<0.01$ vs control; # indicate $p< 0.01$)

Inhibition of P4 production increases MMP-2 in FM.

In our previous studies GM-CSF promoted the greatest increase in MMP-2 compared to all MMPs tested[14]. Incubation of intact FM with either the inflammatory mediator GM-CSF or the 3βSHD inhibitor Trilostane (blocking P4 production) increased MMP-2 protein in the choriodecidua media. Incubation with both GM-CSF and Trilostane synergistically increased MMP-2. Exogenous P4 inhibited the MMP-2 increase due to Trilostane alone or Trilostane and GM-CSF together. **(Figure 3)**

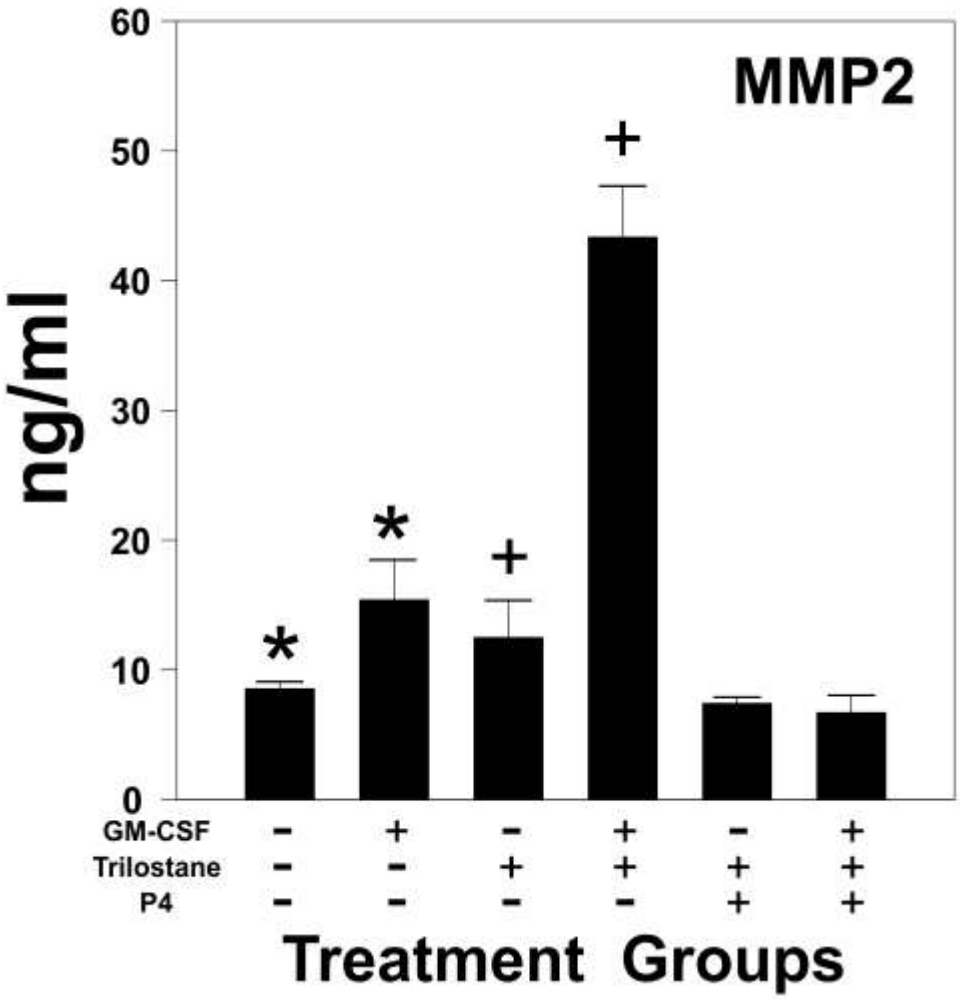


Figure 3: Blockade of P4 Production on MMP-2: Explants of FM were incubated with media alone, trilostane (1μM) ± progesterone (100 nM) for 24h. The medium was then refreshed with the same and GM-CSF (200 ng/ml) was added where indicated for another 48h. MMP-2 testing was performed as indicated in Methods. Data shown are triplicate cultures, m±SD. N=3 patients.(* indicate $p<0.05$ vs controls; + indicate $p< 0.01$)

GM-CSF induced concentration dependent increase in P4 and 3βHSD protein in BeWo cells.

BeWo cells have been used as a model for FM cytotrophoblast cells[24] which are known to produce P4 in fetal membranes[25,26]. GMCSF (0-300ng/ml) induced P4 production in BeWo cells in a concentration dependent manner**. (Figure 4A)**. GM-CSF also increased 3-beta-hydroxysteroid dehydrogenase (3βHSD (Type 1)/HSD3B1 protein), the rate-limiting enzyme in P4 production in a concentration dependent manner **(Figure 4B)**. This is consistent with GM-CSF increasing P4 synthesis by increasing 3β-HSD (Type 1).

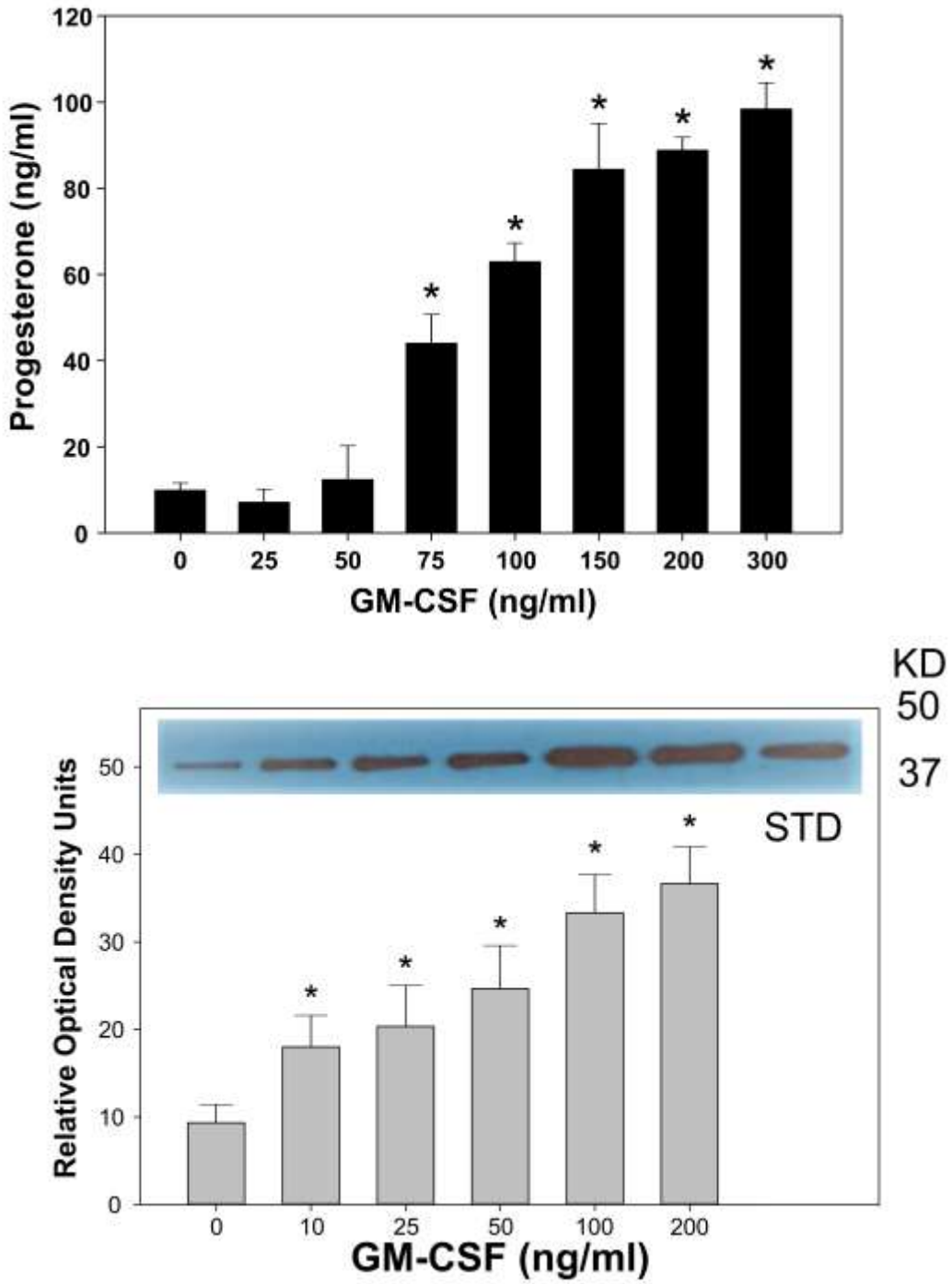


Figure 4: GM-CSF induced concentration dependent increase in P4 and 3βHSD protein in BeWo cells. A.GM-CSF induced P4. Media from BeWo cells plated as described in Methods and treated with increasing concentrations of GM-CSF (0-300 ng/ml) for 24 h were analyzed by ELISA for P4. Data are presented as mean ± SD; N = 4 replicates. (* indicate $p < 0.01$ vs controls). **B.** GM-CSF induced increases in 3β-HSD protein. a) Western blot of 3β-HSD cellular protein BeWo cells. Cells were pretreated with increasing doses of GM-CSF (0-200 ng/ml) for 6 h**.** b) Densitometric analysis of Western blots for 3β-HSD from four experiments using BeWo cell isolates (n =4). Data are presented as mean ± SD. Columns with symbols (*) indicate data are significantly different. KD=molecular weight markers in kilodaltons.

Pericervical (weak zones) of FM produce less P4 than FM segments distal to the cervix.

The FM region which overlays the cervix develops at term into a distinctive area known as the region of "high morphological change" or the "weak zone" because of its relative depletion in cytotrophoblastic cells and its and its decreased rupture strength[21,27-30]. FM segments from the peri-cervical "weak zone" [21] of fresh term FM produce less P4 than that produced from strong FM segments from the same repeat Cesarean delivered FM. **(Figure 5)**

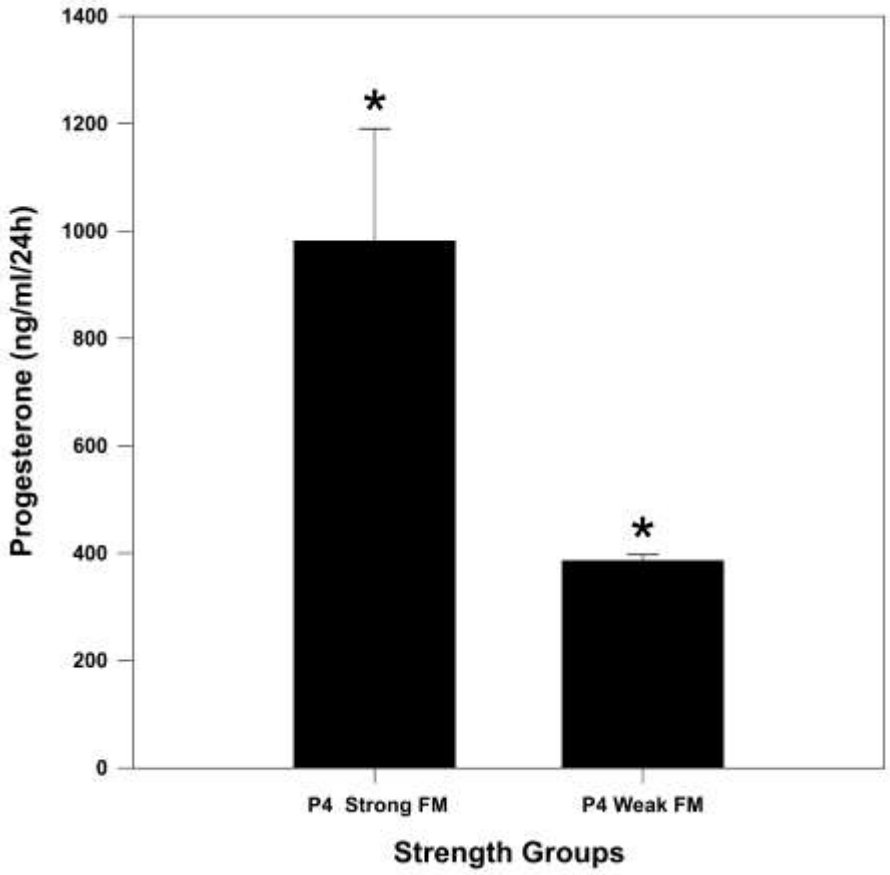


Figure 5: P4 production in fresh FM from pericevical weak zone vs other areas: Explants from 4 fresh term repeat Cesarian deliveries were used for these studies. P4 production from two FM segments from the peri-cervical **weak** zone (rupture strength 3.05 ±0.57) was compared with two FM segments from **strong** areas distal from the peri-cervical area (rupture strength 16. 09 ±2.07) of each placenta (eight segments in each group). Rupture strength and P4 production were as stated in Methods. Incubations were 24h. (* indicate $p<0.01$ vs control) from control; $P < 0.001$.

Discussion

We investigated whether P4 generated within the FM at the maternal-fetal interface might act to abrogate spontaneous and inflammation-induced FM weakening and breakdown. We found that: 1. GM-CSF, in addition to mediating inflammation- and bleeding-induced FM weakening, also induces P4 production by FM in a concentration dependent fashion; 2. Blockade of either the action of P4 with RU486, or the synthesis of P4 with Trilostane

causes FM weakening. Furthermore, FM weakening caused by inhibition of P4 synthesis with Trilostane is reversed with exogenous P4. 3. GM-CSF or Trilostane both increase MMP-2, the most prevalent MMP in FM and a proteolytic enzyme which targets fibular collagen. Furthermore, endogenous P4 reverses MMP-2 induction. 4. GM-CSF induces P4 in immortalized trophoblast BeWo cells, which we have used as a model for the cytotrophoblast sublayer in FM. In parallel, GM-CSF increases 3βHSD (Type1) protein production, the rate limiting enzyme in the P4 synthesis, in BeWo cells in a concentration dependent fashion. Taken together these data are consistant with a negative feedback system whereby local inflammatory challenges induce P4 production in cytotrophblast cells (chorion) within the FM maternal-fetal interface. The P4 generated then counteracts inflammation-induced FM weakening, thus preserving FM integrity.

As no animal model exists for FM weakening and rupture (or pPROM), we have utilized an ex-vivo, full thickness human fetal membrane explant system to study FM weakening and rupture [10,20,31]. With this system we have shown that TNF and Il-1β, modeling inflammation/infection, and thrombin, modeling decidual bleeding, the two common associations with pPROM, each weaken FM in a concentration dependent manner [6 11]. Additionally, we have shown that GM-CSF is a critical intermediate for all of these processes, as its induction in decidual stromal cells is both necessary and sufficient for FM weakening [13]. Recently we have shown that GM-CSF induced FM weakening is concomitant with GM-CSF increases in proteases, and decreases in protease inhibitors in FM[14,15]. Interestingly, the GM-CSF induced changes in proteases and protease inhibitors seen in our explant model system mirror that seen in weak FM explant pieces (obtained from the para-cervical region, the rupture zone, of FM), when compared with strong FM explant pieces, (obtained more distally from the cervix) of fresh untreated term FM[15]. As small areas of inflammation and bleeding are thought to occur in FM during pregnancy, this paradigm for FM weakening could lead to spontaneous weakening and pPROM.

We therefore reasoned that an inhibitory system must be present at the maternal-fetal interface to counteract this inevitability. Because we have previously shown that exogenous P4 and its analogs inhibit inflammation-induced FM weakening by TNF, thrombin and also their critical intermediate GM-CSF[16], we investigated whether P4 produced locally within FM exerts feedback inhibition to counteract inflammation induced FM weakening. The data in this report show that P4 produced within FM is directly induced by inflammatory mediators, most specifically by GM-CSF the critical mediator of inflammation induced FM weakening. Furthermore, we show that blockade of the action of P4 in FM with RU486 or blockade of the production of P4 with trilostane results in spontaneous FM weakening. Importantly, exogenously applied P4 reverses the trilostane weakening action, showing that locally applied P4 compensates for the blockage of local production. MMP-2 is the most abundant MMP in FM and is important because it breaks down fibular collagen, the major strength component of FM [32-35]. Here we show that concomitant with weakening, FM exposed to either Trilostane or GM-CSF show increased MMP-2. Furthermore, MMP-2 is synergistically increased with both agents together. Again, exogenous P4 reverses all of these changes in MMP-2. These data are consistent with a P4 mediated negative feedback system induced by inflammatory challenges in FM which protects them from spontaneous breakdown. **Figure 6** shows how this new finding

integrates into our previous work on the FM weakening pathway.

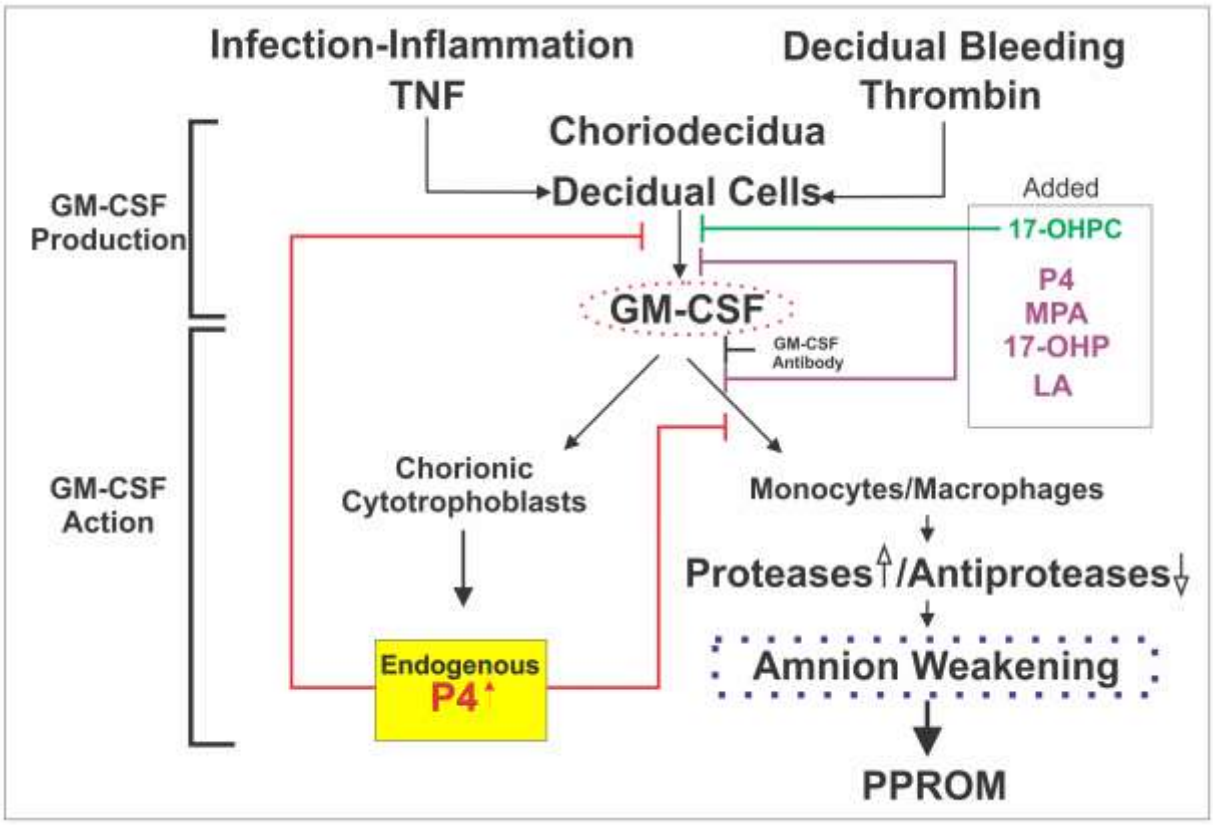


Figure 6: Fetal membrane (FM) weakening Model System Pathways including P4 feedback 1. TNF (modeling inflammation) and Thrombin (modeling decidual bleeding/abruption) weaken FM *in-vitro*[6,11]. 2. TNF and Thrombin induce choriodecidual production of the critical intermediate GM-CSF[9,12]. GM-CSF neutralizing antibody blocks both TNF and Thrombin induced weakening[13]. 3. We hypothesize that GM-CSF recruits and activates mononuclear cells, then activates macrophages which produce proteases to cause FM weakening. 4. GM-CSF increases many proteases and inhibits many protease inhibitors[14,15]. 5. Progestogens (P4, MPA and 17-OHP) inhibit FM weakening by blocking both GM-CSF production and GM-CSF downstream action[16]. HPC only inhibits GMCSF production (not GM-CSF action) and thus may be less efficacious in clinical use than other agents[22]. 6. LA inhibits both GM-CSF production by TNF/thrombin and also GM-CSF downstream weakening activity[13,14,18]. 7. We now show that GM-CSF also induces P4 in chorion trophoblast cells which we speculate inhibits FM weakening in the manner of exogenously added P4. P4, Progesterone; MPA, Medroxyprogesterone acetate; 17-OHP, 17α-Hydroxy-progesterone; 17-OHPC, 17α-Hydroxy-progesterone caproate; LA, Lipoic acid.

Progesterone is biosynthesized from cholesterol via pregnenolone by cholesterol side chain cleavage enzyme and 3βHSD respectively [26,36,37]. Although the production of progesterone by the FM was reported decades ago[25], the large volume of progesterone produced by the placental disc has received the major focus of attention. P4 production in the placental disc has taken prominence in spite of the fact that at least one study suggested that the gradient of P4 showed higher levels closer to the FM, suggesting at the P4 produced in FM might be more significant.[38] Progesterone produced by the placental disc exits the placenta and uterus via the uterine vein and becomes

available to act at the fetal-maternal interface only after transiting and being diluted in the maternal systemic circulation, an endocrine mode of action. There is no evidence that the placental progesterone production is controlled such that it could respond to a specific inflammatory challenge at the fetal-maternal interface. Although progesterone produced in the placental disc may contribute to the maintenance of FM integrity, it cannot provide a focal, and proportional response to a local inflammatory challenge as would be possible by an inducible P4 generating system within FM.

Progesterone synthesis in FM was first described by Mitchell and colleagues who used an explant culture similar to that utilized in our early studies (except that biomechanics was not included [25,26,39]. These reports determined that the chorion FM sublayer was the major site of P4 production within the FM, producing over 100 times the P4 of the amnion or decidua. We show here that P4 in FM is induced by the inflammation signal mediator GM-CSF. In previous studies we found that exogenously applied P4, as well as the P4 analogs MPA and 17-OH progesterone, inhibited inflammation induced FM weakening by inhibiting both GM-CSF production by TNF and thrombin, and GM-CSF downstream action[16,22].

To further investigate the mechanisms by which P4 is induced in FM we used the BeWo trophoblast cell line to model the FM cytotrophoblast [40,41]. We found that P4 production is induced by GM-CSF in BeWo cells concomitant with increases in 3βHSD protein, the rate limiting enzyme step in P4 biosynthesis. This suggests that the increase in P4 seen with the inflammatory mediator GM-CSF is due to induction of 3βHSD in the chorion cytotrophoblast.

The chorion trophoblast sublayer of FM is thought to have a major role in FM integrity. The chorion layer of FM is significantly diminished in normal term FM in the area overlying the cervix, the physiological rupture site, [21,30,42,43]. Consistent with this we found that P4 produced by FM segments from the area overlying the cervix was significantly less than from FM segments distal to the cervix from the same placenta. Importantly, the chorion has been described as "prematurely destroyed", thinner and more apoptotic, in pPROM [44,45]. We suggest that FM rupture, in these circumstances, may be facilitated, in part, due to the absent or damaged chorion's inability to produce P4 at the maternal-fetal interface with a resultant decrease in FM strength.

Conclusions

GM-CSF is the mediator of the inflammatory cytokines TNF, IL-1β, and thrombin in the FM weakening process. It primarily acts on the choriodecidua to induce an array of proteases which weaken the FM and make them susceptible to rupture. However, GM-CSF concomitantly induces P4 production in the chorion of FM in a concentration dependent manner. Based on studies in the BeWo trophoblast cell line, GM-CSF likely induces P4 production by inducing 3βHSD in FM chorion cytotrophoblast. P4 produced locally acts to counteract the FM weakening process and protect the FM from localized, inflammatory challenges that threaten FM integrity.

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