IL-6 Trans-Signaling System in Intra-Amniotic Inflammation, Preterm Birth, and Preterm Premature Rupture of the Membranes

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IL-6 Trans-Signaling System in Intra-Amniotic Inflammation, Preterm Birth, and Preterm Premature Rupture of the Membranes

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Classic IL-6 signaling is conditioned by the transmembrane receptor (IL-6R) and homodimerization of gp130. During trans-signaling, IL-6 binds to soluble IL-6R (sIL-6R), enabling activation of cells expressing solely gp130. Soluble gp130 (sgp130) selectively inhibits IL-6 trans-signaling. To characterize amniotic fluid (AF) IL-6 trans-signaling molecules (IL-6, sIL-6R, sgp130) in normal gestations and pregnancies complicated by intra-amniotic inflammation (IAI), we studied 301 women during second trimester (n = 39), third trimester (n = 40), and preterm labor with intact (n = 131, 85 negative IAI and 46 positive IAI) or preterm premature rupture of membranes (PPROM; n = 91, 61 negative IAI and 30 positive IAI), ELISA, Western blotting, and real-time RT-PCR were used to investigate AF, placenta, and amniochorion for protein and mRNA expression of sIL-6R, sgp130, IL-6R, and gp130. Tissues were immunostained for IL-6R, gp130, CD15+ (polymorphonuclear), and CD3+ (T cell) inflammatory cells. The ability of sIL-6R and sgp130 to modulate basal and LPS-stimulated release of amniochorion matrix metalloprotease-9 was tested ex vivo. We showed that in physiologic gestations, AF sgp130 decreases toward term. AF IL-6 and sIL-6R were increased in IAI, whereas sgp130 was decreased in PPROM. Our results suggested that fetal membranes are the probable source of AF sIL-6R and sgp130. Immunohistochemistry and RT-PCR revealed increased IL-6R and decreased gp130 expression in amniochorion of women with IAI. Ex vivo, sIL-6R and LPS augmented amniochorion matrix metalloprotease-9 release, whereas sgp130 opposed this effect. We conclude that IL-6 trans-signaling molecules are physiologic constituents of the AF regulated by gestational age and inflammation. PPROM likely involves functional loss of sgp130.

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Interleukin-6 is a multifunctional cytokine involved in regulation of acute-phase responses, hematopoiesis, cell regeneration, and transition from innate to acquired immunity (1–4). IL-6 engages in two types of downstream signaling pathways to achieve these functions: classic and trans-signaling (Fig. 1) (5). In the classic pathway, IL-6 binds to a transmembrane cognate receptor, IL-6R, resulting in homodimerization of a signal-transducing glycoprotein, gp130 (6). This triggers a complex intracellular cascade involving STAT factors, JAK and MAPK (6). The end result is a concerted transcriptional increase of genes with critical roles in inflammation (6). In the trans-signaling pathway, IL-6 complexes to a soluble form of IL-6R (sIL-6R) produced by alternative splicing or proteolytic cleavage of IL-6-R (5, 7). This IL-6–sIL-6R complex then triggers dimerization of gp130 and downstream signaling, thereby circumventing the need for IL-6-R (5, 7).

Unlike gp130, which is ubiquitously expressed, IL-6-R is restricted to a few cell types such as hepatocytes, megakaryocytes, neutrophils, macrophages, leukocytes, and trophoblast (5, 8). Thus, the potential of IL-6 acting through its classical cognate receptor is limited. The molecular interaction of IL-6–sIL-6-R complex with gp130 confers IL-6 sensitivity to many cell types that do not express IL-6-R. Importantly, the induction of acute-phase response genes via trans-signaling pathway is controlled by an inhibitory mechanism (6). Generated by enzymatic shedding and alternative splicing, soluble gp130 (sgp130) is a natural inhibitor of IL-6 trans-signaling by preventing the agonistic IL-6–sIL-6-R complex from interacting with membrane-bound gp130 (6, 9). This selective antagonistic effect of sgp130 distinguishes between downstream effects of IL-6 trans-signaling and those controlled via the classical pathway. In particular, IL-6 trans-signaling defines the nature of inflammatory infiltrates by modulating expression of chemokines and adhesion molecules, polymorphonuclear neutrophil function and apoptosis, and T cell recruitment (1, 10–12). A schematic representation of the interplay among molecular elements involved in downstream IL-6 signaling is provided in Fig. 1.
The height of the inflammatory response elicited by microbial invasion of the amniotic fluid (AF) cavity and amnionchorion is critical for pregnancy outcome (1). For instance, cytokine activation triggers release of matrix metalloproteinases (MMPs), which, in turn, leads to degradation of extracellular matrix and preterm premature rupture of membranes (PPROM) (13). That the levels of AF IL-6 in pregnancies complicated by intra-amniotic inflammation (IAI) are significantly increased remains undisputed (1, 14). Still, our understanding of IL-6’s biological function in AF remains incomplete unless placed in the context of its two signaling pathways. We hypothesized that aside from IL-6, human AF contains IL-6 trans-signaling molecules with independent ability to modulate downstream end points with pathogenic significance for preterm birth (PTB).

In this study, we investigated the presence and activation of IL-6 trans-signaling in AF and reproductive tissues of pregnancies complicated by IAI. Furthermore, we provide functional evidence that sgp130 is a modulator of fetal membrane MMP-9 release, thereby highlighting the involvement of IL-6 trans-signaling in PPROM and PTB.

Materials and Methods

Patient population and AF samples

A flowchart of the women enrolled in the study outlining the subgroups of analyzed samples is presented in Supplemental Fig. 1. AF was retrieved from 301 consecutive women who had an indicated amniocentesis at Yale-New Haven Hospital from March 2004 to December 2008. All women provided informed consent. The study was approved by the Human Investigational Committee of Yale University.

We studied the following clinical groups: 1) second-trimester women undergoing genetic amniocentesis who delivered a healthy baby at term (gestational age [GA] median [interquartile range]: 19 [17–20] wk; n = 39); 2) third-trimester women undergoing fetal lung maturity testing before delivery (GA: 36 [36–37] wk; n = 40); and 3) women presenting with preterm labor or PPROM who had an amniocentesis to rule out infection (GA: 28 [25–31] wk; n = 222). This last group was further divided into women with negative IAI and intact membrane (GA: 28 [25–32] wk; n = 85), women with negative IAI and PPROM (GA: 30 [28–32] wk; n = 61), women with positive IAI and intact membrane (GA: 26 [24–29] wk; n = 46), and women with positive IAI and PPROM (GA: 28 [26–31] wk; n = 30). Of the 85 cases with negative IAI and intact membranes, 31 women delivered a healthy term baby. These cases were analyzed together with the genetic and lung maturity indicated before induction of labor or surgical delivery (cesarean section).

Second- and third-trimester control groups were designed to identify possible GA-mediated changes in AF levels of IL-6, sIL-6, and sgp130. A baby. These cases were analyzed together with the genetic and lung maturity negative IAI and intact membranes, 31 women delivered a healthy term healthy women where confirmation of fetal lung maturity was clinically indicated before induction of labor or surgical delivery (cesarean section). A diagnosis of IAI and/or IAI was established based on biochemical and microbiological test panels. AF was analyzed for glucose concentration, lactate dehydrogenase (LDH) activity, WBC count, and Gram stain. Standard culturing methods for aerobic and anaerobic bacteria, including Prevotella and Mycoplasma species, were used. Positive Gram stain and/or culture results were considered suggestive of intra-amniotic infection. Once the clinical requirements were satisfied, the remaining AF was centrifuged at 3000 × g for 10 min, aliquoted, and stored at −80°C for research purposes. The results for these studies were used for clinical case management of the cases.

Diagnosis of IAI by mass spectrometry and Mass Restricted score

In previous studies, our group demonstrated that proteomic profiling of AF using surface-enhanced laser desorption ionization time-of-flight mass spectrometry was the most accurate research method for diagnosing IAI (14, 16). In brief, the AF Mass Restricted score is composed of four proteomic biomarkers: defensin-2, defensin-1, S100A12 (calgranulin C), and S100A8 (calgranulin A). The Mass Restricted score ranges from 0 to 4, depending on the presence or absence of each of the four protein biomarkers. A value of 1 was assigned if a biomarker peak was present and 0 if absent. A Mass Restricted score of 3 or 4 indicates the presence of IAI. All mass spectrometry tracings were scored by one investigator (I.A.B.) who was unaware of the results of the biochemical or microbiological tests used to diagnose IAI/intra-amniotic infection.

Immunoassays for IL-6, sIL-6R, sgp130, and MMP-9

ELISA assays for IL-6 (eBioscience, San Diego, CA), sIL-6R (eBioscience), and sgp130 (R&D Systems, Minneapolis, MN) were performed to measure their respective levels in AF and explain media. The molar ratio between AF sIL-6R and sgp130 was calculated for each patient as previously described (17). Explant media was also immunoassayed for MMP-9. The assays were run in duplicate according to the manufacturer’s protocol. For all assays, samples were diluted from 1:10 to 1:100 to fall within the range of the standard curves. The interassay and intra-assay coefficients of variation was <10% for all the analytes.

Evaluation of histological chorioamnionitis

Paraffin-embedded tissues were available from 119/146 (82%) of the negative IAI and 76/76 (100%) of the positive IAI patients who provided AF samples. Placental and fetal membranes tissues biopsies were collected immediately after delivery. Tissues were formalin fixed and embedded in paraffin. For clinical purposes, a diagnosis of histological inflammation of the placenta and fetal membranes was based on well-established criteria (18).

Placental and fetal membrane IL-6R, gp130, CD3, and CD15 immunohistochromy

Immunohistochemistry for IL-6R, gp130, CD3, and CD15 (polymorphonuclear marker) (19), and CD3 (mature T cell marker) (1, 20) was performed in tissues of women with idiopathic PTB (negative IAI and absent histological chorioamnionitis, n = 5), PTB in the setting of positive IAI and histological chorioamnionitis (n = 15), and healthy women with cesarean delivery and absence of labor (n = 9). The third-trimester group (GA: 38–40 wk) consisted of healthy, term, nonlaboring women undergoing a scheduled elective cesarean delivery for indications such as fetal mal-

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presentation (i.e., breech) or prior cesarean delivery. All their infants were appropriately grown for GA and had reassuring fetal heart rate patterns before surgery. Clinical characteristics of these cases are provided in Supplementary Table I.

Five-micrometer paraffin tissue sections were deparaffinized in xylene and rehydrated with graded ethanol to potassium-PBS solution, pH 7.2. After Ag retrieval with citrate buffer (pH 6), the sections were pretreated with 1% hydrogen peroxide for 15 min, followed by 1 h blocking with 5% goat serum. The following primary Abs were used: rabbit anti-human IL-6R (sc-661; 1:200, overnight incubation at 4°C; Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-human gp130 (sc-9994; 1:50 dilution, overnight incubation at room temperature; Santa Cruz Biotechnology), rabbit anti-human CD3 (T cell marker; A0452; 1:200, overnight incubation at 4°C; Dako, Denmark), mouse monoclonal CD15 (polymorphonuclear neutrophil marker; M0824; 1:50, overnight incubation at 4°C). After 1 h incubation with appropriate secondary Abs (biotinylated goat anti-rabbit or anti-mouse IgG 1:600 dilution; Jackson ImmunoResearch, West Grove, PA), the sections were developed using the avidin-biotin-peroxidase system (Vectorstain Elite ABC; Vector Laboratories, Burlingame, CA) with VectorNovaRed (Vector Laboratories) as chromogen and hematoxylin as counterstain. The tissue sections were dehydrated in graded ethanol, cleared, and mounted. Speciﬁcity of staining was conﬁrmed by replacing the primary Abs with equivalent concentrations of mouse or rabbit non-immune IgG (Novus Biologicals, Littleton, CO). Immunohistochemical staining of the intensity of the chromogen deposited in the amnion epithelium, choriodecidual and placental villous trophoblast, and stromal and endothelial cells was evaluated semiquantitatively in a blind fashion by examining three ﬁelds per slide and subjectively scoring on a scale from 0 (no staining) to 3 (intense red-brown staining) the intensity of the chromogen deposited in the amnion epithelium, choriodecidua, and placental villous trophoblast, as previously described (21). Vimentin immunostaining was performed to identify decidual cells, whereas cytokeratin staining identiﬁed trophoblast cells (data not shown).

**Quantitative real-time RT-PCR and Western blotting for IL-6R and gp130**

For the RT-PCR experiments, we used tissues (placenta and amniochorion membranes) retrieved from the same cases where the IL-6R level of expression was evaluated by immunohistochemistry. Clinical characteristics of these cases are provided in Supplementary Table I. Immediately after delivery, tissues were frozen in liquid nitrogen and kept at −80°C. RNA was extracted and reverse transcribed into cDNA using random hexamer primers using standard procedures. Quantitative RT-PCR was performed using TaqMan (Applied Biosystems) chemistry in 20 μl reactions composed of 10 μl master mix (TaqMan Fast Universal PCR 2X Master Mix), 8 μl water, 1 μl cDNA template normalized, and 1 μl PCR probe set (TaqMan Gene Expression Assays on Demand). For the detection of IL-6R and gp130 mRNAs, TaqMan probes from Applied Biosystems were used: hs01698424_m1 (IL-6R) (22) and hs01743601_m1 (gp130) (23). For controls, we used TaqMan probes h99999990_1_m1 (β2 microglobulin, B2M) and h00265497_1_m1 (ribosomal protein L30, RPL30). The combination of the two endogenous control mRNAs (B2M and RPL30) was validated in preliminary experiments using pools of cDNA amplified in the TaqMan Human Endogenous Control Plate (Applied Biosystems). Selection of the two reference genes was based on low cycle threshold (Ct) values that were not different among the six cDNA pools.

For each target, amplification was performed in duplicate reactions in a 2-step cycle (denaturation, 95°C for 15 s, annealing/extension at 62°C for 60 s) for 40 cycles. Postprocessing calculations were performed using the StepOne Software (v.2.1). The values obtained were normalized to the geometric mean of the endogenous control mRNAs using calculations of ΔCt (Ct of the target – Ct of endogenous controls). A ΔCt of 0 indicates a ratio of 1 between the target and housekeeping genes. This ratio may be used as an indication of relative abundance between different targets among different tissues. Calculation of ΔDCt (ΔCt of individual sample – ΔCt of same target in reference sample) adds an additional normalization step. Therefore, this method improves the estimate of relative mRNA abundance among different biological groups (24, 25).

For ΔCt calculations, we used our reference sample RNA pools of the same tissue type.

**Western blotting for IL-6R and gp130 isoforms**

We used fetal membrane, placental tissue lysates, and corresponding AF samples of a select group of preterm women with (n = 6) and without (n = 6) IAI and/or histological chorioamnionitis. Western blotting was performed on AF (5 μl/lane), fetal membrane, and placenta tissue lysates (20 μg total protein/lane) prepared by homogenization in a buffer containing 50 mM Tris buffer (pH 7.4), 0.1 mM EDTA, 0.14 μM 2-ML, and complete protease inhibitor mixture (Roche Applied Sciences, Indianapolis, IN). Samples were mixed 1:2 with reducing sample buffer (Bio-Rad, La Jolla, CA), boiled, and applied to 4–20% SDS-PAGE gels. After electrophoretic transfer, nitrocellulose membranes were blocked with 5% milk and then incubated overnight at 4°C with either polyclonal rabbit anti–IL-6R Ab (sc-661; 1:200; Santa Cruz Biotechnology) or mouse monoclonal anti-gp130 Ab (clone B-P4, ab34325; 1:300; Abcam, Cambridge, MA). Detection was performed using appropriate HRP-linked secondary Abs and chemiluminescence (ECL Plus; Amersham Biosciences). OD of the bands of interest (sIL-6R: ~44–55 kDa [26]; sgp130: ~100 kDa [27]) was analyzed with ImageJ software (National Institutes of Health: http://rsb.info.nih.gov/ij).

**Fetal membranes explain culture system**

Fetal membranes were obtained from healthy women (n = 7) without any significant medical history undergoing scheduled, elective cesarean delivery in the absence of labor (GA: 39 [38–40] wk). Indications for abdominal delivery included elective repeat or primary cesarean delivery for fetal malpresentation (i.e., breech) or prior uterine scar. No patient had abnormal placentation (i.e., placenta previa, abruption). All their infants were appropriately grown for GA and had reassuring fetal heart rate patterns before surgery.

The setup for the fetal membrane culture system was established based on previously published procedures (28, 29). Membranes, distal from the point of iatrogenic rupture, were harvested under sterile conditions and dissected free from the placenta within 10 min of delivery. Ten-millimeter-diameter pieces were washed thoroughly to eliminate blood clots using cold HBSS with antibiotic-antimycotic solution (100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B). Two pieces of fetal membrane tissue were placed into each well of a 24-well tissue culture dish in 1 ml culture medium containing a 1:1 mixture of MEM with low glucose, 10% FBS, and antibiotic-antimycotic solution. Prior studies demonstrated that relative cellular quiescence is achieved in untreated membrane cultures after a minimum period of 48 h (30). Therefore, in this study, fetal membranes were initially maintained in the medium for 48 h (37°C, 5% CO2, 95% air) before treatment. Subsequently, the tissues were placed in fresh culture medium and stimulated for 24 h with LPS (50 ng/ml; Sigma, St. Louis, MO), sIL-6R alone (10 ng/ml; R&D Systems), or sgp130 alone (500 ng/ml; R&D Systems). In addition, the effect of sgp130 in the presence of LPS or sIL-6R was also investigated. The LPS dose was chosen based on a dose–response experiment (5, 50, 500 ng/ml LPS) where we determined that the peak of MMP-9 and IL-6 release in the early phase of stimulation was seen after stimulation with LPS (Supplementary Fig. 2). Optimal concentrations of sIL-6R and sgp130 were chosen in our attempt to mimic as close as possible our in vivo data.

After 24 h of incubation, the supernatants were collected, centrifuged to remove cellular debris, and stored at −80°C. The incubated tissue was immediately homogenized in 1 ml cell extraction buffer (20 mMol/Tris-HCl pH 7.4, 150 mmol/l NaCl, 1% Triton X-100, 1 mmol/l PMSF, and complete protease inhibitor mixture: Roche, Indianapolis, IN). Specimens were spun at 1000 × g at 4°C for 15 min, and protein quantification of incubated tissue was performed using bichinchoninic acid protein assay (Pierce BioTechnology, Rockford, IL), according to the manufacturer’s instructions. To correct for variations in tissue incubated per each well, we normalized the explant medium concentration of the sought analytes (MMP-9, IL-6, sIL-6R, sgp130) to total protein in tissue extract. For each experimental condition, values were derived by averaging normalized values from duplicate wells either without (untreated) or with the various treatments. Values were further interpreted as percent change from basal release.

To assess tissue viability during in vitro incubations, we determined the release of the intracellular enzyme LDH into the incubation medium as previously described using the LDH/LiquiUV Assay (Stanbio, Boerne, TX) (31). The interassay and intra-assay coefficients of variation were <5%. We found no significant change in LDH release during the incubation period and after treatments in support of sustained tissue viability.

**Statistical analysis**

Normality testing was performed using the Kolmogorov–Smirnov test. Data were compared with one- or two-way ANOVA followed by Student–Newman–Keuls tests (parametric) or Kruskal–Wallis tests on ranks followed by Dunn’s tests (nonparametric), to adjust for multiple comparisons as appropriate. Statistical analysis of data derived from immunohistochemistry was performed after logarithmic transformation. Spearman or Pearson correlations were used to measure linearity between the selected independent variables, as well as other relevant relationships between dependent and
IL-6 TRANS-SIGNALING SYSTEM AND PRETERM BIRTH

Table I. Demographic clinical and outcome characteristics of the women who provided AF samples for sgp130, sIL-6R, and IL-6 levels (n = 301)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Second-Trimester Genetic Amniocenteses (n = 39)</th>
<th>Third-Trimester Lung Maturity Amniocenteses (n = 40)</th>
<th>Rule-Out Infection Amniocenteses (n = 222)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33 (28–38)</td>
<td>30 (26–36)</td>
<td>28 (22–33)</td>
<td>0.002</td>
</tr>
<tr>
<td>Parity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 (0–1)</td>
<td>1 (0–2)</td>
<td>1 (0–1)</td>
<td>0.017</td>
</tr>
<tr>
<td>Gravidity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 (1–3)</td>
<td>3 (2–4)</td>
<td>2 (1–3)</td>
<td>0.162</td>
</tr>
<tr>
<td>GA, wk&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19 (17–20)</td>
<td>36 (36–37)</td>
<td>29 (25–31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uterine contractions&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>109 (40–49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cervical dilation &gt; 3 cm&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>72 (32)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PPROM&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>91 (41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical chorioamnionitis&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>18 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Outcome characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Term delivery (≥37 wk)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>39 (100)</td>
<td>23 (58)</td>
<td>31 (14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTB at &lt; 34 wk&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>175 (79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GA at delivery, wk&lt;sup&gt;e&lt;/sup&gt;</td>
<td>39 (39–40)</td>
<td>37 (36–37)</td>
<td>31 (27–33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amniocentesis-delivery time, d&lt;sup&gt;e&lt;/sup&gt;</td>
<td>143 (134–154)</td>
<td>1 (1–4)</td>
<td>3 (0–17)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data presented as median (interquartile range) and analyzed by Kruskal–Wallis ANOVA on ranks.

<sup>b</sup>Data are presented as n (%) and analyzed by χ² tests.

Results

Gestational regulation of AF trans-signaling molecules IL-6, sIL-6R, and sgp130

Demographic clinical and outcome characteristics of the women who provided AF samples are presented in Table I. Women in the second-trimester genetic amniocentesis group were significantly older compared with those who had an amniocentesis to rule out infection. Third-trimester control women were of higher parity compared with the second-trimester genetic amniocentesis group and, as expected, were evaluated at a more advanced GA compared with the other two groups. Given the clinical circumstances at the time of recruitment, the rule-out infection group had a greater frequency of uterine contractions, advanced degree of cervical dilation, PPROM, clinical symptoms of chorioamnionitis, and a greater frequency of spontaneous or induced PTB. Seventy-five percent of the third-trimester patients had reassuring AF fetal lung maturity test results (lecithin/sphingomyelin ratio > 2.5). Thus, the third-trimester group experienced a greater frequency of medically indicated PTB and the shortest amniocentesis-to-delivery interval.

Our analysis of GA regulation of IL-6 trans-signaling molecules was limited to genetic (n = 39), lung maturity (n = 40), and rule-out infection amniocenteses from women who ultimately had a term delivery (n = 31). All three trans-signaling analytes (Fig. 1) were constitutively present in human AF across gestation. The AF IL-6 and sIL-6R concentrations did not vary significantly among groups or with GA (Fig. 2A, 2B; absolute levels are provided in Supplemental Table II). Conversely, AF sgp130 was inversely correlated with GA, with lower levels approaching term (Fig. 2C; p < 0.001). Because the balance between the stimulatory sIL-6R and the inhibitory sgp130 may have considerable functional relevance, we were interested in how their relative ratio varied with GA (17). There was a significant GA regulation of sIL-6R/sgp130 molar ratio, which increased toward term (Fig. 2D; p < 0.001).

Changes in AF IL-6 trans-signaling molecules in human pregnancies complicated by IAI, PPROM, or both

The demographic, clinical, and laboratory characteristic of the preterm women are presented in Table II. PPROM patients were significantly older irrespective of IAI status (two-way ANOVA: PPROM: p < 0.001; IAI: p = 0.171). We also determined that both IAI and PPROM impacted on latency interval (time from PPROM to delivery) with significant interaction between the two variables (IAI: p < 0.001; ROM: p = 0.101; interaction: p = 0.002). Moreover, women in the IAI groups delivered at earlier GA, and their newborns had lower birth weights. Both GA and birth weight were synergistically impacted by IAI and membrane status (IAI: p < 0.001; PPROM: p < 0.001; interaction: p = 0.001).

The results of the biochemical and microbiological studies of the AF showed lower glucose and higher LDH activity in the IAI groups, which were significantly influenced by membrane status (IAI: p < 0.001; PPROM: p < 0.001; interaction: p = 0.001).
Greater AF WBC counts were seen in the IAI groups with significant interaction with PPROM (IAI: p < 0.001; PPROM: p < 0.001; interaction: p = 0.005). Lastly, women in IAI groups experienced a higher frequency of positive Gram stain and positive microbial culture results, again significantly influenced by the membrane status (IAI: p < 0.001; PPROM: p < 0.001; interaction: p = 0.004). The pathological examination of the placenta revealed higher stages and grades of histological chorioamnionitis and funisitis in the IAI groups, independent of membrane status.

Table II. Demographic, clinical, laboratory, and outcome characteristics of women with symptoms of preterm labor or PPROM who provided AF samples for sgp130, sIL-6R, and IL-6 levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rule-Out Infection Amniocenteses (n = 222)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No IAI and Intact (n = 85)</td>
</tr>
<tr>
<td>Clinical and outcome characteristics at amniocentesis and at delivery</td>
<td></td>
</tr>
<tr>
<td>Maternal age, y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25 (20–32)</td>
</tr>
<tr>
<td>Parity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 (0–1)</td>
</tr>
<tr>
<td>Gravidity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 (1–3)</td>
</tr>
<tr>
<td>History of PTB&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21 (25)</td>
</tr>
<tr>
<td>GA at amniocentesis, wk&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28 (25–32)</td>
</tr>
<tr>
<td>Expectant management&lt;sup&gt;d&lt;/sup&gt;</td>
<td>72 (87)</td>
</tr>
<tr>
<td>GA at delivery, wk&lt;sup&gt;e&lt;/sup&gt;</td>
<td>33 (28–38)</td>
</tr>
<tr>
<td>Amniocentesis-delivery interval, h&lt;sup&gt;f&lt;/sup&gt;</td>
<td>428 (52–1379)</td>
</tr>
<tr>
<td>Amniocentesis-delivery &lt; 7 d</td>
<td>33 (39)</td>
</tr>
<tr>
<td>Birth weight, g&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2210 (1285–3060)</td>
</tr>
</tbody>
</table>

*Data are presented as median (interquartile range) and analyzed by Kruskal–Wallis ANOVA on ranks.

<sup>a</sup>Data are presented as n (%) and analyzed by χ<sup>2</sup> test.
The absolute values for IL-6, sIL-6R, and sgp130 in AF of preterm women evaluated for IAI are presented in Supplemental Table III. IL-6 was significantly increased in women with IAI, although its levels were lower in IAI with PPROM compared with IAI with intact membranes (two-way ANOVA; Fig. 3A; IAI: \( p < 0.001 \); membrane status: \( p < 0.001 \); interaction: \( p < 0.001 \)). The levels of AF sIL-6R were increased in women with IAI independent of PPROM (Fig. 3B; IAI: \( p < 0.001 \); membrane status: \( p = 0.866 \); interaction: \( p = 0.973 \)). Conversely, PPROM women had decreased sgp130 levels regardless of whether they had IAI (Fig. 3C; IAI: \( p = 0.247 \); membrane status: \( p < 0.001 \); interaction: \( p = 0.129 \)). The sIL-6R/sgp130 molar ratio was significantly increased by PPROM alone and further increased in the setting of PPROM with IAI (Fig. 3D; IAI: \( p < 0.001 \); membrane status: \( p < 0.001 \); interaction: \( p < 0.001 \)). When we restricted our analysis to PPROM cases with negative Gram stain and microbial culture results (n = 40) managed expectantly postamniocentesis, we determined that women with amniocentesis-to-delivery intervals longer than 7 d (n = 16) had greater AF sgp130 concentrations compared with cases with shorter latencies (n = 24; latency ≥ 7 d: 50.1 [38.4–61.4] versus latency < 7 d: 39.2 [31.1–51.2] ng/ml; \( p = 0.034 \)). All results maintained following GA correction.

Transcriptional changes in IL-6R and gp130 in human fetal membranes and placenta

Fig. 4 displays mRNA levels of the full-length IL-6R and gp130 in reproductive tissues of term and preterm women with and without IAI. In the absence of IAI, transcripts of IL-6R and gp130 were present in both placenta and fetal membranes (Fig. 4A). Compared with IL-6R, gp130 mRNA was more abundant (about 3 orders of magnitude) in both tissue types (\( p < 0.001 \)). IL-6R mRNA levels were similar in fetal membranes and placental villous tissues with absent GA regulation (Fig. 4A). The gp130 mRNA was significantly decreased in term amniochorion compared with preterm tissues (\( p = 0.002 \)). This change was not observed in placental villous tissue. We noted a significant increase in IL-6R mRNA (\( p = 0.029 \); Fig. 4B) concurrent with significantly decreased gp130 expression in amniochorion of IAI cases (\( p = 0.018 \); Fig. 4C). The differences remained significant after correcting for GA. Placental IL-6R and gp130 mRNA levels remained unaffected by inflammation (Supplemental Fig. 3).

Immunoreactive forms of sIL-6R and sgp130 in human AF, placenta, and fetal membranes

The Western blots in Fig. 4D depict several specific bands corresponding to IL-6R (80–110 kDa) and sIL-6R (38–55 kDa). The multiple bands likely resulted from posttranslational modifications of both targets as previously described (33). In the setting of IAI, we observed marked differences in banding patterns in both AF (Fig. 4D, lanes 1–4) and fetal membranes (Fig. 4D, lanes 5–8), but not in placental lysates (Fig. 4D, lanes 9, 10). A significant increase in 80- to 110-kDa bands was identified in both AF and fetal membranes of patients with IAI compared with those without IAI (\( p < 0.001 \)). In the absence of IAI, immunoreactive bands corresponding to sgp130 (~100 kDa) were present in the AF (Fig. 4E, lanes 1–4) but absent in the fetal membranes and placenta, which appear to express only the full-length gp130 isoform (~130 kDa; Fig. 4E, lanes 5–10). AF bands characteristic to sgp130 were significantly decreased in the context of IAI (Fig. 4E, lanes 1–4; \( p = 0.026 \)). This phenomenon appeared to be mirrored by a decrease in the expression of gp130 protein in fetal membranes but not placenta (Fig. 4E, lanes 5–10).

Immunostaining of IL-6R and gp130 in human placenta and fetal membranes

Fig. 5A–D shows IL-6R immunostaining in fetal membranes and placental tissue. In preterm fetal membranes without IAI, IL-6R was localized predominantly in extravillous trophoblasts (EVTs) and decidual cells, whereas the amnion epithelium exhibited little positive staining (Fig. 5A). In IAI, a marked increase in IL-6R immunoreactivity was observed in preterm amnion epithelium and in infiltrating inflammatory cells (Fig. 5B). In term fetal membranes, IL-6R remained localized only at the periphery of vacuolated EVT’s, whereas decidual cells maintained their staining...
CD15+ and CD3+ cells populated perivascular areas in the decidua and villous syncytiotrophoblast (Fig. 5). In the placenta, IL-6R was identified in EVT intensity (Fig. 5) without notable changes with either IAI or GA (data not shown). Fig. 5E–H illustrates gp130 immunostaining in fetal membranes and placental tissues in the same tissues as shown for IL-6R. In preterm fetal membranes absent IAI, amnion and choriodcidual cells displayed conspicuous gp130 staining (Fig. 5E), which decreased significantly with IAI (Fig. 5F) and at term (Fig. 5G). In the placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5). The placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5). The placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5).

We explored the functional role of sIL-6R and sgp130 in modulating the release of MMP-9. In AF, fetal membrane (FM), and placental (Plac) protein (Western blot) expression of IL-6R and gp130 are shown. Membranes were probed with compatible polyclonal anti–IL-6R (D) or monoclonal anti-gp130 Ab (E). Specificity was confirmed in identical blots with omitted primary Abs.

Effect of sIL-6R and sgp130 on MMP-9 release by fetal membrane explants

We explored the functional role of sIL-6R and sgp130 in modulating the release of MMP-9 and IL-6 by using an amniochorion explant system. Recombinant sIL-6R alone, but not sgp130, significantly upregulated MMP-9 (IL-6R versus basal: p = 0.035; Fig. 6A), an effect similar to that of LPS alone (LPS versus basal: p = 0.020; LPS versus sIL6-R: p = 0.330). Addition of recombinant sgp130 over sIL-6R or LPS had an antagonistic effect by reversing the MMP-9 release (p < 0.001 for both) to the basal level (sIL-6R + sgp130 versus basal: p = 0.910; LPS + sgp130 versus basal: p = 0.141; Fig. 6A). sgp130, sIL-6R, and their combination had no effect on IL-6 (Fig. 6B). In contrast, LPS alone significantly upregulated IL-6 levels (LPS versus basal: p < 0.001), an effect unaltered by addition of sgp130 (LPS + sgp130 versus LPS: p = 0.706).

Discussion

Considerable progress has been made in understanding the downstream molecular events of a large array of decidual, placental, fetal membranes, and AF cytokines including TNF-α, IL-1, IL-8, and in particular, IL-6 (1, 3, 8, 34, 35). The latter is a pleiotropic factor that belongs to the family of gp130 cytokines (36). Investigation of IL-6’s bioactivity has been focused primarily on its proinflammatory properties given that augmented AF IL-6 levels are associated with PTB (37). However, as our understanding continues to evolve, we have gained a better appreciation of the physiologic and pathologic roles of IL-6 in human gestation. Consistent with previous reports, our study demonstrated that IL-6 was present in AF of pregnancies with normal outcomes and absent infection (38, 39). That the human amnion, choriodcidual, and fetus are potential sites of IL-6 synthesis and probable sources of AF IL-6 has been previously proposed (34, 35). Therefore, a relevant question relates to the physiologic function of AF IL-6 across normal human gestation. This study provides firsthand evidence that, if any, the biological role of AF IL-6 can be exercised through both classic and trans-signaling pathways. In support of the classic pathway, we demonstrated that both IL-6R and gp130 are expressed in resident cells of the fetal

FIGURE 4. mRNA (quantitative real-time RT-PCR) and protein (Western blot) expression of IL-6R and gp130. Real-time quantitative RT-PCR was used to study tissues retrieved from 9 term (T) third-trimester healthy women and a subset of 20 PTB cases (no IAI and no histological chorioamnionitis: n = 5; yes IAI and yes histological chorioamnionitis: n = 15). IL-6R and gp130 mRNA was identified in both fetal membranes and placental villous trophoblast (A). gp130 mRNA was more abundant than the IL-6R mRNA in both fetal membranes and placental villous tissues. In the fetal membranes, the gp130 mRNA levels were significantly decreased at term (T). There was a significant increase in IL-6R mRNA (B) and a significant decrease in the expression of gp130 (C) in the fetal membranes of women with IAI. Relative quantitation (RQ) ΔCt values are reported relative to expression of the housekeeping genes for each tissue (A). ΔΔCt RQ values were reported relative to a reference RNA pool of the same tissue (B, C). Data presented as mean ± SEM and analyzed by one-way ANOVA followed by post hoc Student–Newman–Keuls tests. Means marked with at least one common superscript letter are not statistically different (p > 0.05). D and E, Representative Western blots of AF, fetal membrane (FM), and placental (Plac) proteins are shown. Membranes were probed with compatible polyclonal anti–IL-6R (D) or monoclonal anti-gp130 Ab (E). Specificity was confirmed in identical blots with omitted primary Abs.

FIGURE 5. Expression of IL-6R (A) and gp130 (B–D) in fetomaternal tissues illustrates homing for both IL-6R and gp130 in different tissues and signs of IL-6R and gp130 expression in fetal membranes and placental tissues in the same tissues as shown for IL-6R. In preterm fetal membranes absent IAI, amnion and choriodcidual cells displayed conspicuous gp130 staining (Fig. 5E), which decreased significantly with IAI (Fig. 5F) and at term (Fig. 5G). In the placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5). The placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5). The placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5). The placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5). The placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5). The placenta, the strongest gp130 signal was noted in villous cytotrophoblasts and in EVTs of the basal and chorionic plate without remarkable impact from the maltic-6R mRNA expression of gp130 (Fig. 5).
infiltration of the CD of women affected by IAI. CD15 + inflammatory cytokines (i.e., TNF-α) is facilitated through downregulation in expression of proinflammatory role (1). The anti-inflammatory function of IL-6 is responsible for the presence of these two soluble factors in AF (9, 42). There is a high regulatory complexity of the splicing mechanisms responsible for sIL-6R and sgp130 synthesis (9). Inducers of the IL-6R mRNA splicing process include oncostatin M (cytokine synthesized by activated T lymphocytes and monocytes) and IL-1 (44). The splicing process for the sgp130 mRNA continues to be poorly understood, but oncostatin M, IL-1, and IL-6 may play a role (44). The well-documented expression of oncostatin M, IL-1, and IL-6 in the human decidua (34, 45) suggests that physiologic immune processes occurring at the maternal–fetal interface may be responsible for the synthesis of sIL-6R and sgp130, and their subsequent release into the AF. The contribution of enzymatic cleavage and mRNA splicing mechanisms to the total pool of AF sIL-6R and sgp130, in addition to the rates of metabolism and clearance for the two aforementioned factors, needs to be clarified in the future.

We showed that AF levels of sgp130 progressively decrease toward term. This suggests that the inflammatory modulator effect of this molecule is GA dependent. This observation is remarkable because sgp130 specifically inhibits sIL-6R–reliant responses and does not interfere with the cell-surface expressed IL-6R (6). Our data raise important questions regarding the identity of the inhibitory mechanisms leading to the decreased cellular release or synthesis of sgp130 at term. Understanding the subtleties of this

membranes and placental villous tissues. It is critical to recognize that IL-6 exercises not only a proinflammatory but also an anti-inflammatory role (1). The anti-inflammatory function of IL-6 is facilitated through downregulation in expression of proinflammatory cytokines (i.e., TNF-α, IFN-γ) and induction of soluble IL-1 and TNF-αR antagonists (40). These mediators act then in an autocrine/paracrine fashion and target normal biological processes occurring within villous trophoblast, decidua, and amnion (41). As a result, regardless of its source, the AF and decidual IL-6 could be of significant relevance for fetal development.

Our novel finding that sIL-6R and sgp130 are constituents of the normal human AF supports the notion that any biological role assigned to AF IL-6 could be initiated or repressed via the trans-signaling pathway. Similar to IL-6, the source of sIL-6R and sgp130 in AF is debatable. Our real-time RT-PCR, Western blot, and immunohistochemistry results point to fetal membranes as primary and placenta as secondary sources. Cleavage of the aminoenhorin and villous trophoblast IL-6R or gp130 could be responsible for the presence of these two soluble factors in AF (9, 42, 43). That this mechanism occurs in fetal membranes and placental tissues needs to be confirmed. However, in the case of sIL-6R, the inducers of the shedding process (i.e., TNF-α converting enzyme) are expressed in amnion and choriodecidua of healthy women (42).
We determined that the fetal membrane IL-6R mRNA levels were unknown (35), but angiopoietins may play a significant role (50). The transmembrane passage of IL-6 into AF remains largely unexplored. Therefore, we propose that lower levels of AF sgp130 toward term may predispose fetal membranes to rupture.

The MMP-9 release was inhibited when amniochorion was incubated with sgp130, sIL-6R, and their combination. Recombinant sIL-6R and LPS stimulated the release of MMP-9 (A). Addition of recombinant sgp130 over sIL-6R or LPS reversed the release of MMP-9. Incubation of the tissues with sgp130, sIL-6R, and their combination had no effect of IL-6 release in the conditional media of the amniocoele explant (B). The IL-6 levels were significantly upregulated after incubation with LPS. sgp130 did not impact the stimulatory effect of LPS. These results indicate that sIL-6R and sgp130 modulate MMP-9 activity through mechanisms independent of IL-6 levels in the system. Data are presented as mean ± SEM of seven independent experiments and analyzed by one-way ANOVA followed by post hoc Student–Newman–Keuls tests. Means with at least one common superscript are not statistically significant. 

regulatory process is important because an increased level of sgp130 can render cells unresponsive to IL-6 and the family of gp130 cytokines. Data derived from the human endometrium demonstrated that sgp130 was downregulated in the proliferative but upregulated during the secretory phase of the menstrual cycle (46). This suggests that hormonal factors such as estrogen and progesterone may impact the synthesis and release of sgp130 (46, 47). Whether the functional progesterone withdrawal characteristic to human pregnancy is responsible or linked to the decreased AF levels of sgp130, at term, remains unknown.

Mitchell et al. (48, 49) indicated that IL-6 signaling through the classic pathway was responsible for the increased production of PGs by amnion and decidual cells both term and preterm. The observed increased sIL-6R/sgp130 molar ratio at term implies that the IL-6 trans-signaling pathway becomes more active toward the end of gestation. Withdrawal of the trans-signaling system inhibition could enhance the process of IL-6–induced PG production and subsequently facilitate the onset of term human parturition. The MMP-9 release was inhibited when amniochorion was incubated with sgp130 plus sIL-6R in the absence of LPS stimulation. Therefore, we propose that lower levels of AF sgp130 toward term may predispose fetal membranes to rupture.

There is evidence to sustain the view that the sharp increase in the AF IL-6 levels occurs secondary to microbial invasion of the amnion and choriodendua (34, 35). This is further supported by our in vitro experiments that demonstrate the amplified release of IL-6 after incubation of fetal membranes with LPS. What mediates the transmembrane passage of IL-6 into AF remains largely unknown (35), but angiopoietins may play a significant role (50). We determined that the fetal membrane IL-6R mRNA levels were upregulated, whereas the expression of gp130 was reduced in IAI. First, these results imply that in IAI, the amniochorion is a functional target for IL-6 classic signaling. Second, incubation of the amniochorion with sIL-6R, sgp130, and their combination did not enhance the release of IL-6. From this perspective, components of the IL-6 trans-signaling pathway play a limited role in the process of amniochorion IL-6 protein synthesis or release, or both.

In our study, it was evident that AF sIL-6R levels were upregulated in relation with IAI. The steady-state amniochorion, IL-6R mRNA, and sIL-6R protein levels also were significantly enhanced by IAI. The mechanisms responsible for the proteolytic release of the ectodomain of IL-6R and IL-6R alternative splicing rearrangements are likely to play an integral role in promoting an increase of the AF sIL-6R levels during microbial (i.e., Streptococcus, Escherichia coli) invasion of fetal membranes. The proof of concept that exacerbation of the TNF-α converting enzymatic activity and shedding of the IL-6R could be evoked by bacterial MMPs and toxins (i.e., streptolysin-O, E. coli-hemolysin) was previously provided (51). In addition, bioavailability of the AF sIL-6R could occur through functional amplification of the alternative splicing apparatus in the amnion and choriodendua. Experiments to substantiate this hypothesis are needed.

We found that women with PPROM and IAI have lower IL-6 levels than women with IAI but intact membranes. No prospective study has targeted a direct comparison of the two clinical categories. Yet, studies in PPROM women report lower optimal diagnostic cutoffs for IL-6 than studies limited to intact subjects, which would be in agreement with our data (14). To our knowledge, our finding that PPROM and especially PPROM and IAI women have lower sgp130 in AF is novel. One possible explanation may be that similar to other soluble antagonists (i.e., sRAGE) (21), sgp130 becomes consumed while binding the IL-6–IL-6R complex in the process of trans-signaling inhibition (52). Alternatively, gp130 itself may be downregulated as processes terminating IL-6 signaling become activated (53). Lastly, transcriptional and/or shedding processes responsible for synthesis and release of sgp130 may be altered in a subgroup of women, and these events may facilitate rupture of the membranes.

Real-time RT-PCR and Western blotting results revealed downregulation in expression of fetal membrane gp130 in the setting of IAI. In addition, by Western blotting, we demonstrated a significant downregulation in the synthesis and release of sgp130 protein in relation to IAI. This could be an alternative explanation for the decreased sgp130 in women with PPROM and IAI. Binding of IL-6 to sIL-6-R is known to prolong IL6’s half-life (54). Although relevant for complex systems harboring all IL-6 trans-signaling components (i.e., amniotic cavity), the direct role played by sgp130 in modulating the clearance of IL-6 or IL-6–sIL6-R complexes has not been explored.

A relevant question is whether IL-6 plays a direct role in events leading to PTB. In an infection-induced mouse model of PTB, acute administration of rIL-6 did not result in significant shortening of gestation (55). This observation would argue that IL-6 has limited biological relevance for parturition. However, recent evidence suggests the contrary (56). By using IL-6–deficient animals, Robertson et al. (56) demonstrated that IL-6 deletion results in delayed normal timing of delivery (~24 h). Chronic infusion of IL-6 in IL-6−/− mice restored the normal timing of delivery. Maternal progesterone levels remained unaltered by IL-6 manipulation, despite remarkable changes of a wide array of genes with critical roles in parturition. Together, these data support the argument that IL-6 is instrumental in regulating the timing of delivery in normal gestation and in infection-induced PTB in a manner independent of luteolysis.
We found that women with PPROM and longer latency intervals had greater levels of sgp130. This argues that in these women, the inflammatory process involved in activation of uterine contractility could be suppressed for a longer period. The observation that PPROM women had decreased AF sgp130 levels and an increased sIL-6R/sgp130 molar ratio was provocative. Our in vitro experiments provide evidence for a mechanism through which AF sgp130 may prevent weakening of the fetal membranes in pregnancies complicated by infection. As shown, incubation of the fetal membranes with LPS induced a significant increase in the release of MMP-9. The recombinant sgp130 reversed the process.

The acute inflammatory response involves immediate access of the neutrophils at the site of a bacterial attack (1, 3, 18, 34). Our immunohistochemistry results established concurrent homing of CD15+ neutrophils and CD3+ T cells in the choriodecidua of women with IAI. Given that resident cells of these tissues express IL-6, IL-6R, sIL-6R, gp130, and sgp130, we argue that in chorioamnionitis, the classic and trans-signaling mechanisms are both engaged in the process of leukocyte trafficking and activation (34). Generation of sIL-6R may therefore represent a rate-limiting event in the regulation of these processes distinguishing between physiological and pathological events orchestrated by IL-6.

Decidual T cells are sparse in early pregnancy (57). At term, however, 45–50% of the leukocytes in the decidua basalis are CD3+, representing one of the most abundant leukocyte populations. The choriodecidua T cell population is thought to participate in defense mechanisms against pathogens, particularly during parturition and in regulation of chronic inflammatory processes localized at the fetal–maternal interface (57). Studies have documented key roles for IL-6 trans-signaling in leukocyte trafficking and activation. In particular, IL-6 trans-signaling–drivenSTAT3 activity is important for T cell recruitment during successful resolution of any inflammatory response and transition from innate to acquired immunity (58). Therefore, we posit that downregulation of gp130 approaching term may facilitate IL-6 trans-signaling and physiological IL-6 approach term in the choriodecidua, in anticipation of parturition.

In summary, we found that IL-6 trans-signaling molecules are physiologic constituents of the AF with both GA and inflammatory regulation. The decreased AF levels of sgp130 in women with ruptured membranes and the demonstrated inhibitory effect of sgp130 on MMP-9 release suggest that IL-6 trans-signaling may play a critical role in PPROM. Similar to inflammatory arthritis, where intra-articular injection of recombinant sgp130 effectively prevented joint destruction (59), we suggest that targeting of IL-6 trans-signaling pathway may have potential for preventing PTB and PPROM (56).

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Disclosures

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