

Soluble receptor for advanced glycation end products (sRAGE) and endogenous secretory RAGE (esRAGE) in amniotic fluid: modulation by infection and inflammation

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Abstract

Objective: The receptor for advanced glycation end products (RAGE) has been proposed to participate in the innate and adaptive immune responses. RAGE can induce production of pro-inflammatory cytokines and chemokines, as well as neutrophil chemotaxis in a manner that may be suppressed or stimulated by soluble, truncated forms of RAGE including the soluble form of RAGE (sRAGE) and endogenous secretory RAGE (esRAGE). The objective of this study was to determine whether intra-amniotic infection/inflammation (IAI) is associated with changes in the amniotic fluid concentration of sRAGE and esRAGE.

Study design: Amniotic fluid (AF) was retrieved from patients in the following groups: 1) mid-trimester (14–18 weeks of gestation; n=68); 2) term not in labor (n=24); 3) term in labor (n=51); 4) preterm labor and intact membranes (n=124); and 5) preterm PROM (n=80). Intra-amniotic infection and inflammation were defined as the presence of a positive amniotic fluid culture for microorganisms and an AF interleukin-6 concentration

≥2.6 ng/mL, respectively. The AF concentration of sRAGE and esRAGE were determined using specific and sensitive ELISAs which measured total immunoreactive sRAGE and esRAGE, respectively. Patients were matched for gestational age at amniocentesis to compare the AF concentration of sRAGE and esRAGE in patients with and without IAI. Non-parametric statistics were used for analysis and a P<0.05 was considered significant.

Results: 1) Patients at term not in labor had higher median AF concentrations of sRAGE and esRAGE than those in the mid-trimester (P<0.001 for both comparisons) and those at term in labor (P=0.03 and P=0.04, respectively); 2) patients with preterm labor and intact membranes with intra-amniotic infection/inflammation (IAI) had higher median AF concentrations of sRAGE and esRAGE than those without IAI (P=0.02 and P=0.005, respectively); 3) similarly, patients with preterm PROM with IAI had higher median AF concentrations of sRAGE and esRAGE than those without IAI (P=0.03 and P=0.02, respectively).

Conclusion: Intra-amniotic infection/inflammation is associated with increased amniotic fluid concentrations of sRAGE and esRAGE. Changes in the amniotic fluid concentration of sRAGE and esRAGE may represent part of the immune response to intra-amniotic infection/inflammation.

Keywords: Amniotic fluid; chorioamnionitis; intra-amniotic infection/inflammation; MIAC; microbial invasion of the amniotic cavity; preterm labor; preterm PROM; preterm delivery.

Introduction

Intra-amniotic infection/inflammation (IAI) is causally linked to preterm labor/delivery and fetal injury [11, 15, 25, 38, 59, 63, 73, 76, 78, 104, 105]. Microbial invasion of the amniotic cavity (MIAC) is a risk factor for impending preterm delivery [28, 76, 78], spontaneous rupture of membranes [8, 26, 36], clinical chorioamnionitis [78], pulmonary edema while receiving tocolysis [31], short-term neonatal morbidity [3, 24, 78], and long-term sequelae, such as chronic lung disease [23, 105] and cerebral palsy [104, 107]. Accumulating evidence indicates that patients with intra-amniotic inflammation but a negative amniotic fluid culture have a similar outcome

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to those with a positive amniotic fluid culture [89, 106]. Therefore, the detection of inflammation may be more practical than the detection of infection in patient management. Intra-amniotic inflammation can be detected with tests such as the amniotic fluid white blood cell count [7, 75, 79, 80], concentrations of chemokines [18, 39, 40, 72, 100], cytokines [1, 2, 16, 32, 41, 44, 58, 66, 71, 74, 77, 83], or antimicrobial peptides and proteins [17, 64, 65].

Non-enzymatic protein glycation modifies existing proteins and lipids yielding a heterogeneous class of compounds that are collectively termed Advanced Glycation End products (AGEs) [62, 87, 95, 96]. AGEs have been implicated in the pathogenesis of diabetes, renal failure and aging [84]. RAGE was first identified as a signal transduction receptor for AGEs species [62, 87]. More recently, RAGE has been described as a receptor for pro-inflammatory molecules including S100/calgranulins [33], high mobility group box 1 (HMGB1) [35, 94], amyloid and β -sheet fibrils [9, 46, 88]. Of note, RAGE has recently been proposed to be part of the pattern recognition receptor system and participate in the innate immune response [53]. Further, recent studies illustrate key roles for RAGE in effective T lymphocyte priming *in vivo* [60].

Interference of the RAGE pathway with soluble RAGE (sRAGE, a truncated isoform of RAGE) can prevent or reduce vascular injury associated with some conditions, presumably by blocking the effect of RAGE [5, 68, 81, 97]. However, recent *in vitro* studies demonstrated that sRAGE can stimulate the production of pro-inflammatory cytokines including interleukin (IL)-6 and TNF- α , it can produce the chemokine MIP-2 by spleen cells via the NF- κ B and Mac-1 pathways [70] and induce neutrophil chemotaxis [70]. Recently, a novel splice variant of RAGE mRNA coding for a C-terminally truncated secretory form was identified as endogenous secretory RAGE (esRAGE) [103]. The authors demonstrated that esRAGE is produced by human microvascular endothelial cells and pericytes, and proposed that the endogenous secretory receptor may be present in the circulation and extracellular fluids in the vascular walls [82, 103].

RAGE is expressed in the amnion epithelium, extravillous trophoblast and decidual cells in patients without chorioamnionitis as well as in the neutrophils in the choriondecidua in cases of histologic chorioamnionitis [6]. The objective of this study was to determine whether intra-amniotic infection/inflammation is associated with changes in the amniotic fluid concentrations of sRAGE and endogenous secretory RAGE (esRAGE).

Study design

Study population

Amniotic fluid (AF) was retrieved from patients in the following groups: 1) women in the mid-trimester of pregnancy (14–18

weeks) who underwent amniocentesis for genetic indications and delivered at term ($n=68$); 2) preterm labor and intact membranes ($n=124$); 3) preterm PROM ($n=80$); 4) term not in labor ($n=24$); and 5) term in labor ($n=51$). Patients with preterm labor and intact membranes and those with preterm PROM with and without intra-amniotic infection/inflammation were matched for gestational age within one week to compare the AF concentration of sRAGE and esRAGE. Inclusion criteria were: 1) singleton gestation; 2) absence of chromosomal or congenital anomalies; and 3) a signed informed consent approved by the Institutional Review Boards of the Sotero del Rio Hospital, Pennsylvania Hospital, and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, DHHS. These samples have been previously used to study the biology of inflammation in amniotic fluid.

Definitions and study procedures

Intra-amniotic infection was defined as the presence of a positive amniotic fluid culture for microorganisms, and intra-amniotic inflammation as an AF IL-6 concentration ≥ 2.6 ng/mL [106]. Preterm labor with intact membranes was diagnosed in the presence of regular uterine contractions of at least 3 in 30 min and cervical change at <37 weeks of gestation that required hospitalization. Rupture of membranes was diagnosed by testing for pooling, nitrazine paper color change, and ferning. Beta-mimetic agents and/or magnesium sulfate were given intravenously for tocolysis, and steroids (betamethasone) were administered between 24 and 34 weeks at the discretion of the attending physician.

Sample collection

Amniocenteses were performed for genetic studies or to determine the microbiological state of the amniotic cavity and/or fetal lung maturity. Amniotic fluid was retrieved by transabdominal amniocentesis under sonographic guidance. The fluid was transported to the laboratory in a capped plastic syringe and cultured for aerobic and anaerobic bacteria, as well as genital *Mycoplasmas*. A white blood cell count, glucose concentration, and Gram stain for microorganisms were performed in amniotic fluid after collection. The results of these analyses were available for patient management. Amniotic fluid not needed for clinical assessment was centrifuged at $700 \times g$ for 10 min at 4°C and the supernatant was aliquoted and stored frozen at -70°C until analysis.

sRAGE, esRAGE, IL-6, and matrix metalloproteinase 8 (MMP-8) immunoassays

Specific and sensitive enzyme-linked immunoassays were used to determine concentrations of sRAGE, esRAGE, IL-6 and MMP-8 in human amniotic fluid. Immunoassay kits for sRAGE, which measure total immunoreactive sRAGE, IL-6 and MMP-8 were obtained from R&D Systems (Minneapolis, MN). Immunoassay kits for esRAGE, which measure the endogenous secretory RAGE, were purchased from B-Bridge International, Inc., (Mountain View, CA). All these assays were specifically validated for human AF in our laboratory. Validation included spike and recovery experiments, which produced parallel curves indicating that amniotic fluid constituents did not interfere with antigen-antibody binding in this assay system.

sRAGE AF samples were incubated in duplicate wells of the micro titer plates, which have been pre-coated with a monoclonal antibody specific for RAGE (extracellular domain). During this incubation, any sRAGE present in the standards or AF samples is bound by the immobilized antibodies. After repeated washing and aspiration to remove all unbound substances, an enzyme-linked polyclonal antibody specific for RAGE was added to the wells. Following a wash to remove excess and unbound materials, a substrate solution was added to the wells and color developed in proportion to the amount of RAGE bound in the initial step.

esRAGE Standards or AF samples and detection antibody (esRAGE antibody conjugated with horseradish peroxidase) were incubated for 16 h at 4°C in duplicate wells of the micro titer plates, which have been pre-coated with a monoclonal antibody specific for human esRAGE. During this incubation, any esRAGE present in the standards or AF samples is bound by the immobilized antibodies. After repeated washing and aspiration to remove all unbound substances, a substrate solution was added to the wells and color developed in proportion to the amount of esRAGE bound in the initial step.

The color development was stopped with the addition of an acid solution and the intensity of color was read using a programmable spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA). The concentrations of sRAGE and esRAGE in AF samples were determined by interpolation from individual standard curves composed of human esRAGE.

The calculated inter-assay coefficients of variation (CVs) for sRAGE, esRAGE, IL-6 and MMP-8 immunoassays in our laboratory were 3.2%, 4.8%, 9% and 4.6%, respectively. Calculated intra-assay CVs for sRAGE, esRAGE, IL-6 and MMP-8 were 4.2%, 2.1%, 7.2% and 3.7%, respectively. The sensitivity was calculated to be 33 pg/mL for sRAGE, 0.028 ng/mL for esRAGE, 2.28 pg/mL for IL-6 and 0.05 ng/mL for MMP-8 assays.

Statistical analysis

The Shapiro-Wilk test was used to evaluate the distribution of data. Amniotic fluid sRAGE and esRAGE concentrations were not normally distributed. Therefore, Kruskal-Wallis and Mann-Whitney *U* tests were used for comparison of continuous variables. First, the amniotic fluid concentrations of sRAGE and esRAGE of patients with a normal pregnancy in the mid-trimester were compared to that of those at term not in labor in order to determine if the amniotic fluid concentrations of sRAGE and esRAGE change with advancing gestational age. Second,

patients not in labor were compared to those in spontaneous labor to test if the amniotic fluid concentrations of sRAGE and esRAGE change in the process of term parturition. Finally, amniotic fluid concentrations of sRAGE and esRAGE were measured and compared between patients with preterm labor and intact membranes, as well as between those with preterm PROM, to determine if they change with and without the presence of intra-amniotic infection/inflammation. Spearman's rho was utilized for the analysis of non-parametric correlations. Patients were matched for gestational age at amniocentesis (within one week) to compare the AF concentrations of sRAGE and esRAGE among patients with preterm labor with (*n*=42) and without intra-amniotic infection/inflammation (IAI) (*n*=82) as well as among patients with preterm PROM with (*n*=40) and without IAI (*n*=40). The statistical package used was SPSS 12 (SPSS Inc., Chicago, IL). A *P*-value of <0.05 was considered statistically significant.

Results

Table 1 displays the demographic and clinical characteristics of patients in the mid-trimester, term not in labor and term in labor groups. Tables 2 and 3 display the demographic and clinical characteristics of patients with preterm labor and intact membranes and those with preterm PROM, respectively. There were no differences in the proportion of patients receiving antibiotic therapy at the time of amniocentesis between patients with IAI and those without IAI, among patients with preterm labor and intact membranes and those with preterm PROM.

Patients at term not in labor had a higher median AF concentration of sRAGE than those in the mid-trimester (median: 24,346 pg/mL, range: 1153.1–56,760 vs. median: 327.6 pg/mL, range: 0–15,069; *P*<0.001) and those at term in labor (median: 24,346 pg/mL, range: 1153.1–56,760 vs. median: 18,819 pg/mL, range: 0–79,754; *P*=0.03) (Figure 1). Similarly, the median amniotic fluid concentration of esRAGE was significantly higher in patients at term not in labor than those in the mid-trimester (median: 8520 pg/mL, range: 0–22,628 vs. median: 59.5 pg/mL, range: 0–699; *P*<0.001) and than in women at term in labor (median: 8520 pg/mL, range:

Table 1 Demographic and clinical characteristics of patients in the mid-trimester, and those at term with and without spontaneous labor.

	Mid-trimester (<i>n</i> = 68)	Term no labor (<i>n</i> = 24)	Term in labor (<i>n</i> = 51)	<i>P</i> -value*
Maternal age (years)	36 (24–42)	28.5 (17–40)	23 (16–37)	<0.01
Gestational age at amniocentesis (weeks)	16 (14–18)	39.3 (38–42)	39.2 (37–41.7)	<0.01
Gestational age at delivery (weeks)	39 (37–41)	39.3 (38–42)	39.2 (37–41.7)	NS
Birthweight (g)	3347 (2809–4180)	3405 (2810–4530)	3250 (2540–4440)	NS

Values are expressed as percentage (number) or median (range).

NS: not significant.

*Kruskal-Wallis test.

0–22,628 vs. median: 6056 pg/mL, range: 0–15,073; $P=0.04$) (Figure 2).

Patients with preterm labor and intact membranes with intra-amniotic infection/inflammation (IAI) had a higher median AF concentration of sRAGE (median: 22,195 pg/mL, range: 0–83,644 vs. median: 16,538 pg/mL, range: 484.5–44,766; $P=0.02$) and esRAGE (median: 10,420 pg/mL, range: 0–35,958 vs. median: 6859 pg/mL, range: 0–19,158; $P=0.005$) than those without IAI (Figures 3 and 4). Among patients with spontaneous preterm labor with intact membranes without IAI included in this study, 76% (62/82) delivered at term and 24% (20/82) delivered preterm. A sub-analysis performed in this subgroup demonstrated that those who delivered preterm had a significantly higher median amniotic fluid concentration of sRAGE (median: 21,391 pg/mL, range: 0–83,644 vs. median: 16,224 pg/mL, range: 1158–44,766; $P=0.02$) and esRAGE (median: 8743 pg/mL, range: 0–35,958 vs. median: 6859 pg/mL, range: 0–17,854; $P=0.03$) than those who delivered at term.

In addition, patients with preterm PROM with IAI had a higher median AF concentration of sRAGE (median: 23,730 pg/mL, range: 1466–52,827 vs. median: 16,077 pg/mL, range: 267–37,070; $P=0.03$) and esRAGE (median: 9242 pg/mL, range: 0–28,337 vs. median: 5451 pg/mL, range: 543–14,996; $P=0.02$) than those without IAI (Figures 5 and 6). There were five patients with gestational diabetes in the group of preterm labor and intact membranes and three patients with gestational

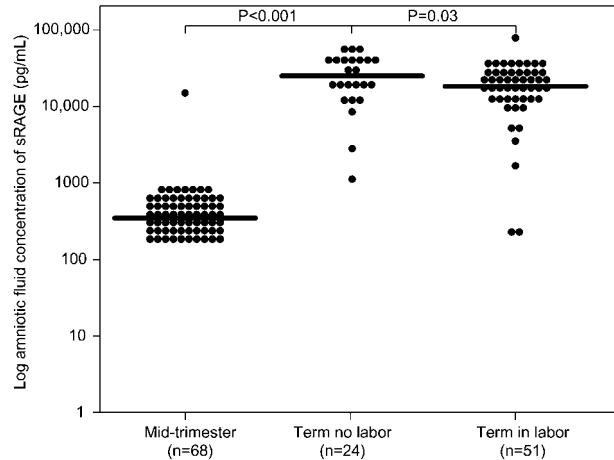


Figure 1 Amniotic fluid concentration of sRAGE in normal pregnancies at mid-trimester and in those at term with and without labor. Patients at term not in labor had a higher median amniotic fluid concentration of sRAGE (median: 24,346 pg/mL, range: 1153.1–56,760) than those in the mid-trimester (median: 327.6 pg/mL, range: 0–15,069; $P<0.001$) and those at term in labor (sRAGE, median: 18,819 pg/mL, range: 0–79,754; $P=0.03$). The logarithmic scale was used to better visualize the midtrimester values.

and pre-gestational diabetes in the preterm PROM group. The results of the study did not change after excluding these patients from the analysis.

Table 2 Demographic and clinical characteristics of patients with preterm labor and intact membranes.

	No intra-amniotic infection/inflammation (n=82)	Intra-amniotic infection/inflammation (n=42)	P-value
Maternal age (years)	22 (15–43)	23 (15–44)	NS
BMI (kg/m ²)	22.6 (15.9–32.9)	24.2 (17.3–32.4)	NS
Gestational age at amniocentesis (weeks)	31.2 (23.1–34.5)	31.2 (23.2–34.5)	NS
Gestational age at delivery (weeks)	38.3 (28.5–41.4)	32 (23.3–39)	<0.001
Birthweight (g)	3130 (730–4470)	1940 (660–3640)	<0.001
Antibiotic therapy at amniocentesis	1.2 (1)	7.1 (3)	NS

Values are expressed as percentage (number) or median (range).

BMI: body mass index; NS: not significant.

Table 3 Demographic and clinical characteristics of patients with preterm PROM.

	No intra-amniotic infection/inflammation (n=40)	Intra-amniotic infection/inflammation (n=40)	P-value
Maternal age (years)	25 (15–40)	29.5 (17–43)	NS
BMI (kg/m ²)	23.7 (19.1–37.3)	24.2 (18.1–34.7)	NS
Gestational age at amniocentesis (weeks)	29.5 (23–34.3)	29.5 (22.9–34.6)	NS
Gestational age at delivery (weeks)	32.1 (24.6–35.6)	30.4 (24.9–34.7)	0.004
Birthweight (g)	1800 (740–2670)	1620 (740–2600)	0.02
Antibiotic therapy at amniocentesis	17.5 (7)	22.5 (9)	NS

Values are expressed as percentage (number) or median (range).

BMI: body mass index; NS: not significant.

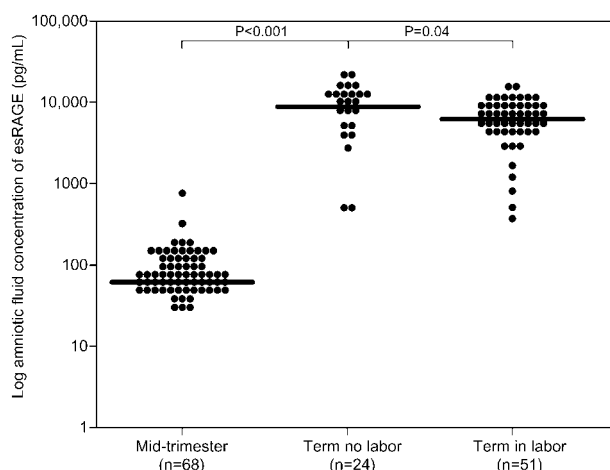


Figure 2 Amniotic fluid concentration of esRAGE in normal pregnancies at mid-trimester and in those at term with and without labor. Patients at term not in labor had a higher median amniotic fluid concentration of esRAGE (median: 8520 pg/mL, range: 0–22,628) than those in the mid-trimester (median: 59.5 pg/mL, range: 0–699; $P < 0.001$) and those at term in labor (median: 6056 pg/mL, range: 0–15,073; $P = 0.04$). The logarithmic scale was used to better visualize the mid-trimester values.

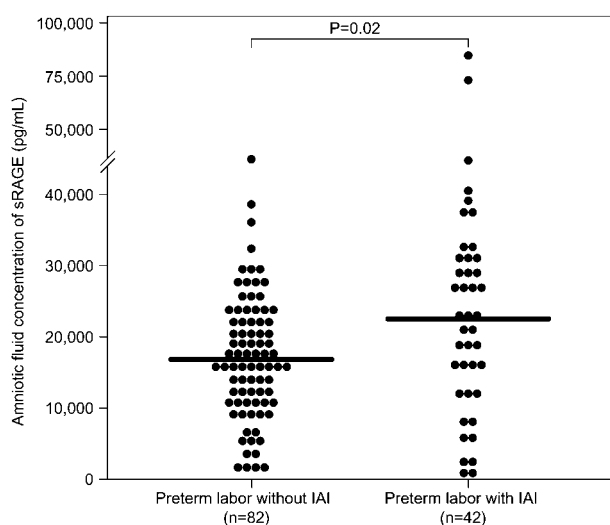


Figure 3 Amniotic fluid concentration of sRAGE among women with spontaneous preterm labor and intact membranes. Patients with preterm labor and intact membranes with intra-amniotic infection/inflammation (IAI) had a higher median AF concentration of sRAGE than those without IAI (median: 22,195 pg/mL, range: 0–83,644 vs. 16,538 pg/mL, range: 484.5–44,766; $P = 0.02$).

Among patients with preterm labor and intact membranes, there was a significant correlation only between the AF concentration of MMP-8 and sRAGE ($r = 0.19$; $P = 0.04$), and almost significant correlation between esRAGE and MMP-8 ($r = 0.18$; $P = 0.053$), but not between the AF concentrations of sRAGE ($r = 0.13$; $P = 0.2$) or esRAGE ($r = 0.12$; $P = 0.2$) and IL-6.

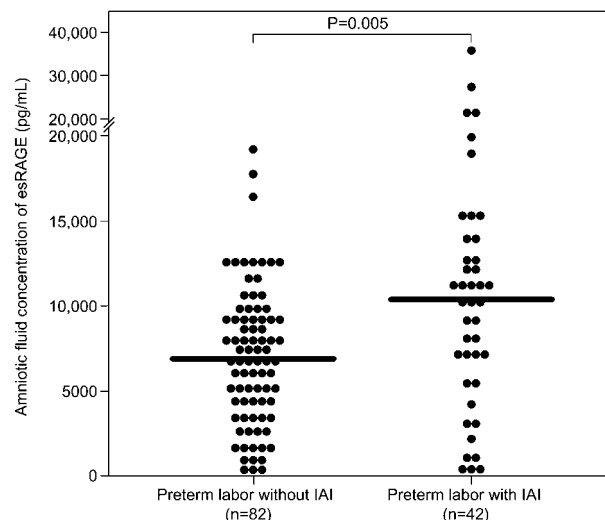


Figure 4 Amniotic fluid concentration of esRAGE among women with spontaneous preterm labor and intact membranes. Patients with preterm labor and intact membranes with intra-amniotic infection/inflammation (IAI) had a higher median AF concentration of esRAGE than those without IAI (median: 10,420 pg/mL, range: 0–35,958 vs. median: 6859 pg/mL, range: 0–19,158; $P = 0.005$).

Discussion

Principal findings of the study

The amniotic fluid concentrations of sRAGE and esRAGE: 1) significantly increase with gestational age; 2) are significantly lower in patients with spontaneous term labor than in women at term not in labor; and 3) intra-amniotic infection/inflammation is associated with increased amniotic fluid concentrations of sRAGE and esRAGE.

The biology of RAGE, sRAGE and esRAGE

RAGE is a member of the immunoglobulin receptor superfamily, which is a 332-amino acid extracellular component consisting of 2 “C”-type domains preceded by 1 “V”-type immunoglobulin-like domain [45, 88, 102]. RAGE has a single trans-membrane domain followed by a highly charged 43-amino acid cytosolic tail [88]. The extracellular domain is essential for ligand binding and the cytosolic tail is central to RAGE-induced intracellular signaling [62, 85, 87, 88]. The human RAGE gene is on chromosome 6 in the major histocompatibility complex [92]. Nuclear binding factor (NF) κ B sites, an interferon- γ response element, and a NF-interleukin-6 DNA binding motif are located on the RAGE promoter [51]. Thus, NF- κ B sites control, in part, cellular expression of RAGE, linking RAGE to the inflammatory response [51]. In adults, RAGE is expressed in multiple cell types, such as neuronal cells and microglia [21, 35, 50, 55, 56, 99, 101], endothelium [4], vascular smooth muscle [4], monocyte-

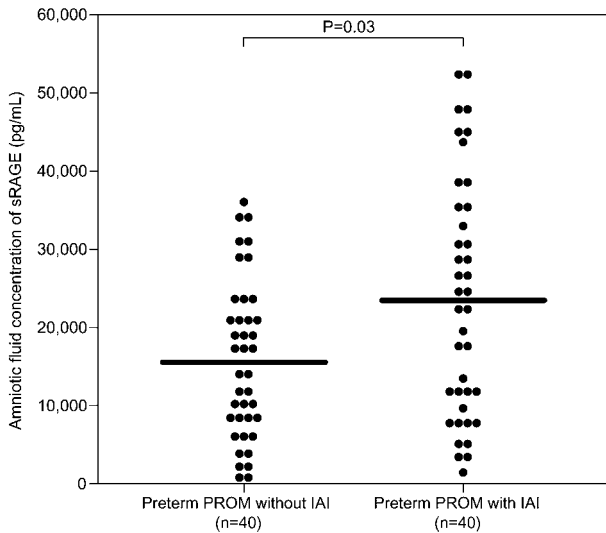


Figure 5 Amniotic fluid concentration of sRAGE in women with preterm prelabor rupture of the membranes (preterm PROM). Patients with preterm PROM with intra-amniotic infection/inflammation (IAI) had a higher median AF concentration of sRAGE than those without IAI (median: 23,730 pg/mL, range: 1466–52,827 vs. median: 16,077 pg/mL, range: 267–37,070; $P=0.03$).

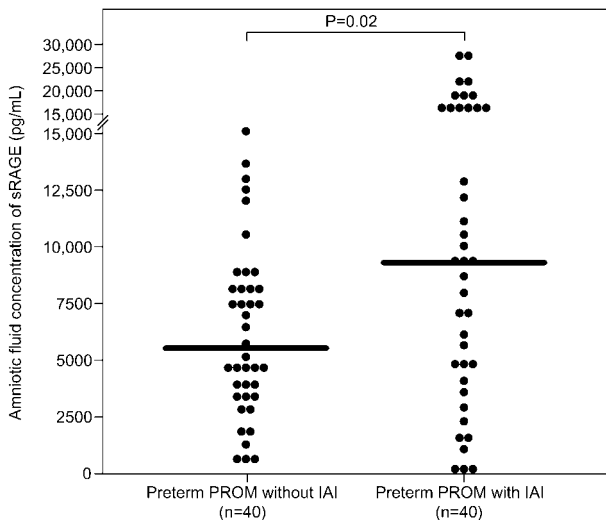


Figure 6 Amniotic fluid concentration of sRAGE in women with preterm prelabor rupture of the membranes (preterm PROM). Patients with preterm PROM with intra-amniotic infection/inflammation (IAI) had a higher median AF concentration of esRAGE than those without IAI (median: 9242 pg/mL, range: 0–28,337 vs. median: 5451 pg/mL, range: 543–14,996; $P=0.02$).

derived macrophages [4], cardiac myocytes [4], hepatocytes [19], podocytes [99], and lung alveolar epithelial cells [90].

Several truncated forms of RAGE have been described [12, 13, 57, 67, 103]. These isoforms may arise from alternative splicing of the pre-mRNA of RAGE [103]. The

mRNA of the C-truncated isoform of RAGE does not contain the exon 10 sequence that encodes the transmembrane-spanning domain [103]. This isoform has been proposed to be secreted extracellularly as endogenous secretory RAGE (esRAGE) and can be detected in human sera [103]. However, Geroldi et al. [22] proposed that esRAGE may not represent the entire pool of soluble RAGE (sRAGE) that is present in the serum, because it remains possible that some RAGE isoforms may result from proteolytic cleavage from the native membranous receptor by enzymes such as MMPs [37].

Soluble isoforms of RAGE have been proposed to play an antagonistic role with RAGE by competing with cell surface RAGE for ligand-binding [22]. Evidence in support of this view includes: 1) administration of sRAGE attenuated alveolar bone loss and decreased the concentration of MMP-9, MMP-2, and TNF- α in gingival tissue extracts in an animal model of periodontitis induced by *Porphyromonas gingivalis* [47]; 2) the administration of sRAGE to diabetic rats blocked vascular leakage in the intestine, skin and kidney [97]; 3) the administration of sRAGE to apolipoprotein E null mice, who develop spontaneous hypercholesterolemia and atherosclerosis, result in a dose-dependent decrease in the atherosclerotic area and complexity compared to vehicle-treated mice [68]; 4) the administration of sRAGE to hyperglycemic rats in whom neointimal expansion was triggered by acute vessel injury reduced the smooth muscle cell numbers and the degree of matrix encompassed within the neointima [81]. This decoy function of sRAGE has been proposed to provide a regulatory negative feedback mechanism to modulate the activity of RAGE [22]. More recently, *in vitro* studies have suggested that sRAGE may have biological effects other than acting as a decoy to trap cell surface-bound RAGE ligands [70]. The authors reported that: 1) sRAGE dose-dependently induced the production of pro-inflammatory cytokines including IL-6 and TNF- α , as well as the production of the chemokine MIP-2 by spleen cells via the NF- κ B and Mac-1 pathways; and that 2) sRAGE induces neutrophil chemotaxis [70]. The authors concluded that sRAGE acts as an important proinflammatory and chemotactic molecule [70]. However, a key limitation of the latter experiments is that they were restricted to the *in vitro* setting.

RAGE and the immune response

Lin [53] proposed that RAGE is a pattern recognition receptor that may use signaling mechanisms parallel to Toll-like receptors during the innate immune response. Evidence in support of this view includes: 1) RAGE may recognize its ligands through their shared three-dimensional structure [53]; 2) RAGE is expressed in phagocytes such as macrophages [4], monocytes [4], and astrocytes [69]; 3) binding of AGEs and other endogenous ligands to RAGE activates the NF- κ B pathway [53]; moreover, blocking the access of the ligand to RAGE

(with excess sRAGE or anti-RAGE) or inhibition of RAGE signaling, suppressed NF- κ B activation in mononuclear phagocytes [33]; 4) RAGE-ligand interaction activates mitogen-activated protein kinase (MAPK) family members, such as Jun-N-terminal kinase (JNK), p38 and extracellular signal regulated kinase (ERK) [61, 91, 94]; and 5) RAGE null mice (RAGE^{-/-}) are protected from the lethal effects of septic shock caused by cecal ligation and puncture, an effect that is largely dependent on the innate immune response [52]. Recent studies have illustrated definitive roles for RAGE in effective T lymphocyte priming *in vitro* and *in vivo*, thereby establishing that RAGE participates in adaptive immune responses as well. Collectively, this evidence supports the role of RAGE signaling in innate and adaptive immune responses [53].

Possible sources of sRAGE in the amniotic cavity

The observations that RAGE is expressed in the amnion epithelium, extravillous trophoblast and decidual cells in patients without chorioamnionitis, as well as in the neutrophils in the choriodecidual of patients with histologic chorioamnionitis [6] suggest that these cells may contribute to the increased amniotic fluid concentration of sRAGE in patients with IAI. Since the chorioamniotic membranes are considered fetal tissues, it is possible that the fetus may contribute to the increased AF concentration of sRAGE in the amniotic fluid in the same manner that the fetus may participate in the innate immune response by increasing the mRNA expression of Surfactant protein-A (SP-A) in the chorioamniotic membranes during histologic chorioamnionitis [29]. SP-A is produced by the fetal lung, participates in innate immunity, and has been proposed to play a role in the initiation of parturition in mice [10].

Changes in the AF concentration of sRAGE and esRAGE with advancing gestational age and spontaneous labor at term in normal pregnancies may represent changes in the innate immunity in preparation for delivery. Indeed, among women with a normal pregnancy, there was a dramatic increase in the amniotic fluid concentration of sRAGE and esRAGE between those in the midtrimester and those at term in the absence of labor (74-fold and 143-fold, respectively). However, there was a significant decrease in the amniotic fluid concentration of sRAGE and esRAGE in the presence of spontaneous labor at term. These results may be unexpected since parturition has been proposed to be an inflammatory process [27, 30, 43] and compelling evidence suggests that RAGE has pro-inflammatory properties. One interpretation of these findings may be that both sRAGE and esRAGE are consumed by an over expression of RAGE ligands during labor. It is possible that sRAGE and esRAGE may play an antagonistic role with RAGE by competing with cell surface RAGE for ligand-binding. This decoy function of sRAGE has been proposed to

provide a regulatory negative feedback mechanism to modulate the activity of RAGE. Moreover, although a significant correlation was found between the AF concentration of MMP-8 and sRAGE, and almost also between esRAGE and MMP-8 ($P=0.053$), there was not a significant relationship between the AF concentrations of sRAGE or esRAGE and IL-6 in patients with spontaneous preterm labor with intact membranes.

Possible roles of sRAGE and esRAGE in the amniotic cavity during intra-amniotic infection/inflammation

To the extent that RAGE represents a novel pattern recognition receptor [53], it is possible that increased amniotic fluid concentrations of sRAGE and esRAGE may participate in the immune response to intra-amniotic infection. Thus, both sRAGE and esRAGE may be added to the list of molecules involved in the innate immune response to intra-amniotic infection/inflammation including chemokines [18, 39, 40, 72, 100], cytokines [1, 2, 16, 32, 41, 44, 58, 66, 71, 74, 77, 83], or antimicrobial peptides and proteins [17, 64, 65]. Alternatively, increased AF concentration of sRAGE and esRAGE may represent additional biomarkers of inflammation during IAI.

RAGE mediates the increased secretion of IL-6 and TNF- α in cultured astrocytes induced by S100B [69]. S100B is a calcium binding protein that can stimulate the expression of proinflammatory cytokines and induce neural apoptosis [69]. Since the AF concentration of S100B is increased in patients with intra-amniotic infection/inflammation [20], it is also possible that increased AF concentration of sRAGE may modulate the pro-inflammatory effects of S100B during IAI.

Conclusions

The AF concentrations of sRAGE and esRAGE increase dramatically with advancing gestational age and decrease with spontaneous labor at term. The observation that AF concentrations of sRAGE and esRAGE increase with intra-amniotic infection/inflammation suggests that these soluble isoforms of the receptor for advanced glycation end products may participate in the innate immune response to intra-amniotic infection/inflammation.

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