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Intrauterine Group A Streptococcal Infections Are Exacerbated by Prostaglandin E₂

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Streptococcus pyogenes (Group A Streptococcus; GAS) is a major cause of severe postpartum sepsis, a re-emerging cause of maternal morbidity and mortality worldwide. Immunological alterations occur during pregnancy to promote maternofetal tolerance, which may increase the risk for puerperal infection. PGE₂ is an immunomodulatory lipid that regulates maternofetal tolerance, parturition, and innate immunity. The extent to which PGE₂ regulates host immune responses to GAS infections in the context of endometritis is unknown. To address this, both an in vivo mouse intrauterine (i.u.) GAS infection model and an in vitro human macrophage–GAS interaction model were used. In C57BL/6 mice, i.u. GAS inoculation resulted in local and systemic inflammatory responses and triggered extensive changes in the expression of eicosanoid pathway genes. The i.u. administration of PGE₂ increased the mortality of infected mice, suppressed local IL-6 and IL-17A levels, enhanced neutrophilic inflammation, reduced uterine macrophage populations, and increased bacterial dissemination. A role for endogenous PGE₂ in the modulation of antistreptococcal host defense was suggested, because mice lacking the genes encoding the microsomal PGE₂ synthase-1 or the EP2 receptor were protected from death, as were mice treated with the EP4 receptor antagonist, GW627368X. PGE₂ also regulated GAS–macrophage interactions. In GAS-infected human THP-1 (macrophage-like) cells, PGE₂ inhibited the production of MCP-1 and TNF-α while augmenting IL-10 expression. PGE₂ also impaired the phagocytic ability of human placental macrophages, THP-1 cells, and mouse peritoneal macrophages in vitro. Exploring the targeted disruption of PGE₂ synthesis and signaling to optimize existing antimicrobial therapies against GAS may be warranted. The Journal of Immunology, 2013, 191: 2457–2465.

Puerperal infections are an important cause of morbidity and mortality in women, and these infections cause 75,000 maternal deaths each year (1). Streptococcus pyogenes, also known as Group A Streptococcus (GAS), is the most common cause of puerperal infections in developing and developed nations (2). Despite preventive measures, including antibiotic use and hospital sanitation efforts, the past two decades have seen a re-emergence of GAS infections worldwide (3, 4). Several factors influence the ability of GAS to establish an infection during the puerperium, including immunological changes in the mother that occur as a result of pregnancy, environmental exposures to the bacterium, bacterial virulence factors, disrupted maternal mucosal barrier, and antibiotic administration during labor and delivery. A better understanding of the pathogenesis of puerperal GAS infections is needed to advance novel, more effective, preventive and therapeutic options.

Pregnancy is accompanied by alterations in maternal immune activation that help to accommodate an immunologically distinct fetus, a process known as maternofetal tolerance (5). Despite this, pregnant women must be able to identify and respond to potentially pathogenic microorganisms; however, several Gram-positive bacterial pathogens are adept at evading the pregnant/postpartum immune system, including Listeria monocytogenes, Streptococcus pneumoniae, Streptococcus agalactiae, and GAS (2, 6, 7). Postpartum women are 25 times more likely than are nonpregnant women to have invasive GAS infections, but the reasons behind this susceptibility are not defined (6). Further, GAS virulence factors alone do not predict the development or severity of GAS infection (8). There is a need for potential preventative therapies or treatment for severe postpartum GAS infections. A challenge of studying infections in the female reproductive tract is the unique anatomical and immunological milieu compared with other mucosal sites.

PGE₂ is an arachidonic acid–derived mediator that modulates cell behavior through four distinct G protein–coupled receptors called E prostanoïd (EP) receptors, which are numbered EP1–4 (9). It has myriad immunomodulatory functions: in, but general, PGE₂ has anti-inflammatory and immunosuppressive actions that limit both the amplitude and duration of immune responses (10–12). For
example, PGE2 can downregulate neutrophil and lymphocyte activity (13), inhibit the production of Th1 cytokines, and enhance Th2 cytokine production (14–16). During gestation, PGE2 supports materno-fetal immune tolerance, whereas at term, PGE2 concentrations increase acutely because it regulates cervical softening and uterine contractions (9, 17–24). The immunological consequences to the mother of this spike in PGE2 levels near term have not been defined.

Previous studies demonstrated the ability of PGE2 to regulate host–microbial interactions in the context of streptococcal infections (25–31). During S. agalactiae sepsis, mice with pharmacologically inhibited PGE2 synthesis had increased survival (32). Cyclooxygenase (COX)-2 is the enzyme that converts arachidonic acid into PGH2, the first committed step on the synthetic pathway to physiologically active “terminal” PGs, like PGE2. Goldman et al. (25, 33) demonstrated that COX-2 is upregulated in human and mouse tissues infected with GAS, and they established that PGE2 signaling via EP2 receptors and cAMP elevation suppressed host defenses against GAS (25). Although none of these studies was conducted in the context of female reproductive tract infection, our previous work revealed that intrauterine (i.u.) administration of the stable PGE2 analog/PGE2 pharmacomimetic compound misoprostol significantly reduced host immune defenses against the reproductive tract pathogen Clostridium sordellii (34). Additional evidence that the cAMP-elevating properties of PGE2 might impair host immune defense against GAS endometritis was recently provided by Soares et al. (35), who reported that the eicosanoid leukotriene (LT)B4 enhanced immune defenses against GAS in the mouse uterus in vivo and in human decidua macrophages and placental macrophages (PMs) in vitro. This is relevant because LTB4 suppressed intracellular cAMP production, counteracting the actions of PGE2 in vitro (35, 36).

Macrophages seem to be major cellular participants in the innate immune response to systemic and deep tissue GAS infections (37–39), but the role of macrophages in the female reproductive tract in controlling GAS infections is unknown. In the uterus, macrophages participate in immunosurveillance against intrauterine pathogens (40). Although macrophage phagocytosis, intracellular killing, and cytokine production are important in the defense against pathogens, the role of PGE2 in GAS infections in the female reproductive tract remains unknown. We hypothesized that elevated PGE2 levels, as experienced during labor and delivery in mice and humans (21, 22), inhibit macrophage action and enhance susceptibility to GAS infections in the uterus. Both in vivo and in vitro models of infection were used to determine the role of PGE2 as an immunoregulator during GAS endometritis.

Materials and Methods

Reagents

RP4M 1640 culture medium, antibiotic solution (penicillin and streptomycin) including an anticoagulant (amphotericin), and 1× PBS were bought from Invitrogen (Carlsbad, CA). THP-1 monocytic cells were obtained from the American Type Culture Collection (TIB-202; Manassas, VA). Charcoal-stripped and dextran-treated PBS was purchased from HyClone Laboratories (Waltham, MA). FITC, BSA, trypsin blue, DNaše from bovine pancreas type IV, hyaluronidase from bovine testes type I-S, collagenase from Clostridium histolyticum type I-A, Percoll, 0.09 M citrate buffer, fucoidan, saponin, PMA, nonenzymatic cell dissociation solution, and sodium bicarbonate were from Sigma-Aldrich (St. Louis, MO). PGE2, GW627636X (EP4 antagonist), Butaprost (free acid) (BFA; EP2 agonist), and L-902,688 (EP4 agonist) were from Cayman Chemicals (Ann Arbor, MI). Todd Hewitt Broth, Tryptic Soy Agar, anti-mouse CD11b Alexa Fluor 488 and its isotype control, and yeast extract were from BD Biosciences (San Jose, CA). Sheep blood was from Remel (Lenexa, CA). Lysis buffer for erythrocytes was from eBioscience (San Diego, CA). Cytochalasin D was from Calbiochem (Billerica, MA). Anti-mouse CD45 Pacific Blue and F4/80 allopolyoctanycin-Cy7 and their isotype controls were from BioLegend. Anti-mouse Ly-6B.2 (Neu7/4) Alexa Fluor 647 and its isotype control were from Serotec (Raleigh, NC). Required dilutions of all compounds were prepared immediately before use, and equivalent quantities of vehicle were added to the appropriate controls. Compounds requiring reconstitution were dissolved in DMSO (Sigma-Aldrich).

Animals and housing

Female C57BL/6 mice, 6–8 wk old, were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice harboring a targeted deletion of both alleles of the Pgrer2 encoding the EP2 receptor were originally generated by Dr. Richard Breyer (Vanderbilt University, Nashville, TN). These 6–8-wk-old female EP2-deficient (EP2−/−) mice, bred on a C57BL/6 background, and age-matched, female wild-type (WT) animals (EP2+/+ mice) were purchased from The Jackson Laboratory and bred in the University of Michigan Unit for Laboratory Animal Medicine (41). nPGES-1 heterozygous mice on a DBA12 background were originally obtained from Pfizer (Gronot, CT) and then backcrossed onto a C57BL/6 background. Homozygous mPGES-1−/− mice and homozygous WT mPGES-1+/+ mice derived from the same heterozygous mPGES-1−/+/ parents were bred in the University of Michigan Unit for Laboratory Animal Medicine. The genotypes of mouse strains were confirmed by tail-snip DNA PCR analyses performed by Transnety (Cardova, TN). All mice were housed under aseptic specific pathogen-free conditions at the University of Michigan, and all protocols were approved by an animal institutional review board.

Intrauterine infections were performed in mice according to a previously published protocol (34, 35). Briefly, GAS cultures were grown for 18 h, shaking at 200 rpm, at 37°C in 10 ml Todd Hewitt Broth supplemented with 5% yeast extract. Dilutions were prepared in 1× PBS to obtain the correct approximate inoculum of CFU/mouse. The actual inoculum was determined by plating serial 10-fold dilutions of the PBS suspension on Tryptic Soy Agar supplemented with 5% sheep blood (TSA) blood overnight at 37°C. In anesthetized animals, a low 2.0-cm midline abdominal incision exposed the right uterine horn, the uterine horn was ligated at the cervical junction to prevent inoculum loss, and 10–40 CFU/mouse were injected directly into the horn. For survival experiments, mice were monitored every 2–4 h on weekdays and two times/d on weekends for 1 wk after infection.

Bacterial culturing and quantification

GAS strain 5448 (M1T1) is a clinical isolate generously provided by the laboratory of Dr. Malak Kotb (University of Cincinnati, Cincinnati, OH) (43–46). Bacteria were grown in Todd Hewitt Broth supplemented with 5% yeast extract at 37°C, shaking aerobically at 200 rpm for 18 h. Bacterial inoculation levels were confirmed by differentially culturing on TSA and counting CFU aerobically at 37°C. Cultured bacteria were heat killed (HK) FITC-labeled GAS was used for phagocytosis experiments. These were generated as previously described for S. pneumoniae (47). Following i. u. mouse infections, mice were euthanized, and the bacterial load was determined in the blood, spleen, and uterus. The blood was serially diluted in sterile 1× PBS and cultured on TSA blood at 37°C overnight. The spleen and uterus were homogenized in 1 ml sterile 1× PBS, serially diluted, and cultured on TSA blood at 37°C overnight. CFU were counted to determine bacterial load.

Cell culture

Human THP-1 cells were cultured in RPMI1640 (antibiotic, antimycotic, and FBS added). Cells were passaged every 2–4 d and were only used through the tenth passage, at which time a new culture was started. Cells were differentiated into macrophage-like cells by culturing with 100 nM PMA (Sigma-Aldrich). Human THP-1 cells were cultured in RPMI+/+ (antibiotic, antimycotic, and FBS added). Cells were detached from the flask by scraping after 5 min of incubation in nonenzymatic cell dissociation solution at 37°C with 5% CO2, as described previously (48, 49).

Measurement of cytokines and chemokines

Mouse serum or uteri were removed at specified time points postinfection to measure immune and eicosanoid responses. Briefly, uteri were homogenized in 1 ml sterile 1× PBS, centrifuged, and stored at −80°C until assayed. PMA-treated THP-1 cells were infected with live GAS at a multiplicity of infection (MOI) of 1:1, and supernatants were collected 24 h later. Immunological ELISAs were performed by the University of Michigan Cancer Center Immunology Core.

Human subjects

Following appropriate informed consent, human placental tissue was obtained from healthy women undergoing caesarean section at the term of

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pregnancy. These studies were reviewed and approved by the University of Michigan Institutional Review Board.

**Isolation and culture of PMs**

Isolation of PMs was adapted from a previously described protocol (35, 50, 51). Briefly, the tissue was collected in 50-ml conical tubes containing 15 ml sterile PBS and washed three times with 50 ml PBS at 1500 rpm for 10 min. The tissue was minced into small pieces with autoclaved scissors and weighed to determine final grams collected. Tissue fragments were placed into 50-ml conical tubes with digestion solution containing 150 μg/ml DNase, 1 mg/ml collagenase, and 1 mg/ml hyaluronidase at 10 ml/g of tissue. Cells were filtered through a 280-μm autoclaved metal sieve, followed by 180- and 80-μm autoclaved nylon screens. Cells were centrifuged again and resuspended in 25% Percoll diluted in cold RPMI+/+ and overlaid onto 50% Percoll plus 2 ml PBS on top of the density gradient. Isolation of CD14+ cells was performed using the magnetic MACS large cell separation column system (Miltenyi Biotec), according to the manufacturer’s instructions.

**Fluorometric phagocytosis assay with FITC-labeled GAS**

Phagocytosis of unopsonized FITC-labeled HK GAS was assessed using PMA-treated THP-1 cells, PMs, or peritoneal macrophages. All cell types were cultured in 384-well tissue culture–treated plates at a concentration of 2 × 10^4 cells/well, and the phagocytosis assay was performed as previously reported (35, 51–53).

**Bacterial killing assay**

PMA-treated THP-1 cells were adjusted to a suspension of 2 × 10^5 cells/well in 100 μl RPMI−/− (no antibiotic, antimycotic, or FBS) in replicates of eight/condition in a 96-well plate and centrifuged at 500 rpm for 30 s to settle all cells into a monolayer. Cells were allowed to adhere for 1 h prior to treatments. One set of replicate conditions within each plate was treated with cytochalasin D for 30 min. Cells were treated with PGE2 (1 μM) for 15 min before infecting them with a 10:1 MOI of live GAS. The plates were then centrifuged again to synchronize bacterial contact with the monolayer and incubated for 30 min. After this time, all media were removed, and plates were washed gently three times with RPMI−/− (no FBS added) to remove nonphagocytosed bacteria. Some cells were incubated for an additional 30 min to allow intracellular killing. After either phagocytosis or killing, the cells were lysed by a 10 min incubation with 100 μl 0.5% saponin in PBS. Serial dilutions were made, the lysates were plated onto TSA blood to quantify bacterial growth. At 24 h post-infection, GAS escaped the uterine horn and spread to the blood and spleen, and it continued to propagate in the uterine horn (Fig. 1C, data not shown). A nonsignificant trend toward enhanced bacterial escape was observed in animals simultaneously injected with GAS and PGE2.

**Flow cytometry**

Murine uterine cells were isolated by a modified protocol that was published previously (50, 51, 55). Uterine horns were collected, weighed, minced with sterile scissors, and digested in a solution containing 1 mg/ml collagenase, 1 mg/ml hyaluronidase, and 150 μg/ml DNase I, as indicated for isolation of human PMs above. A total of 10 ml digestion solution was used per gram of tissue (if tissue was <1 g, 10 ml was still used) and the samples were placed on a shaker at 37°C for 1 h. Samples were washed using RPMI−/− and centrifuged at 1500 rpm at 4°C for 10 min, followed by filtration through a 100-μm nylon mesh to eliminate the remaining particulates. Cells were then washed, as previously described, and the filtrate was resuspended in 25% Percoll in RPMI−/− and overlaid onto 50% Percoll, with 2 ml PBS layers above the 25% Percoll. Cells were washed as before, followed by 10 min at room temperature in RBC lysis buffer (eBioscience). After two more washes, cells were counted, and 1 million cells were aliquoted into flow cytometry tubes. Cells were surface stained for 20 min at 4°C with anti-mouse CD45, CD11b, F4/80, and Ly6B2. Isotype controls were included in separate tubes. After surface staining, all cells were washed with 4 ml PBS containing 1% BSA (FACS buffer), fixed for 15 min at 4°C with 1% paraformaldehyde in PBS, and washed again with FACS buffer. Cells were resuspended in FACS buffer and immediately acquired on a BD FACS Canto II (BD Biosciences). Analysis was performed with BD FACSDiva software (BD Biosciences).

**Quantitative RT-PCR**

Quantification of mRNA levels using high-throughput quantitative PCR was carried out on uterine tissue using tissue homogenization and the TRizol method of RNA isolation (Ambion, Carlsbad, CA). RNA was further purified using the RNeasy Mini Kit (QIAGEN, Germantown, MD) with the optional DNase I step. RNA concentration and quality was determined using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). cDNA was generated using the RT2 First Strand Kit (QIAGEN). Expression levels of genes under investigation were determined using Mouse RT2 Profiler PCR assays, custom-made to contain replicate sets of 32 primer pairs related to eicosanoid biology on each card (QIAGEN). Eicosanoid cards were run on a LightCycler 480 Real-Time PCR system (Roche, Indianapolis, IN). The relative RNA expression levels were determined from the Ct values using the 2^−ΔΔCt method (56). The geometric mean of β-actin and GAPDH levels was used as the internal control for the generation of ΔCt values.

**Statistical analysis**

Data are presented as the mean ± SEM and were analyzed with GraphPad Prism 5.0 software (GraphPad, San Diego, CA). Comparisons among three or more experimental groups were performed with one-way ANOVA, followed by a Bonferroni correction. Comparisons between two experimental groups were performed with a Student t test, unless otherwise stated. Differences in survival were compared with a Mantel–Cox log-rank test. Differences were considered significant at p < 0.05.

**Results**

**Intrauterine GAS infection results in a systemic, sepsis-like illness, and exogenous PGE2 promotes mortality**

Mice were used to model GAS endometritis, as previously reported (35). Infection of the uterus with GAS resulted in a rapid, sepsis-like death that was inoculum dependent (Fig. 1A). To emulate the increased tissue concentrations of PGE2 associated with term pregnancy and the immediate postpartum period, as well as to assess its effect on the severity of GAS endometritis, mice were injected i.u. with both GAS (106 CFU/mouse) and PGE2 (300 μg/kg). This high concentration of PGE2 resulted in significantly increased mortality (p < 0.01) (Fig. 1B). The effect of PGE2 was dose dependent, and PGE2 alone did not result in death (data not shown).

To assess the ability of GAS to evade host defense and disseminate from the infected uterus, mice were sacrificed at 24 h post-infection, and blood, serum, and uteri were differentially cultured on TSA blood to quantify bacterial growth. At 24 h post-infection, GAS escaped the uterine horn and spread to the blood and spleen, and it continued to propagate in the uterine horn (Fig. 1C, data not shown). A nonsignificant trend toward enhanced bacterial escape was observed in animals simultaneously injected with GAS and PGE2.

Systemic perturbation by bacterial infection can be reflected in changes to the number and types of circulating WBCs. To assess the extent to which GAS evoked such shifts in blood leukocytes, analysis was conducted using mouse blood obtained 24 h after inoculation. Mice injected with saline or PGE2 alone had high percentages of lymphocytes (68.28 ± 9.1% and 73.05 ± 4.7%, respectively), whereas mice injected with GAS or GAS + PGE2 had a statistically significant (p < 0.001) decrease in lymphocytes (36.72 ± 13.0% and 39.66 ± 5.6%, respectively) (Fig. 1D). Further, mice injected with GAS or GAS + PGE2 had a statistically significant (p < 0.001) increase in the percentage of neutrophils at 24 h postinfection (58.11 ± 13.8% and 52.31 ± 5.3%, respectively) compared with saline- or PGE2-injected mice (27.11 ± 9.2% and 22.01 ± 5.3%, respectively) (Fig. 1D). A statistically significant decrease in the total numbers of circulating WBCs was observed in GAS-infected mice compared with saline controls; interestingly, this effect was not observed when PGE2 was added during GAS infection (p < 0.05) (Fig. 1E).

**Intrauterine infection results in changes in macrophage and neutrophil populations in the mouse uterus**

Little is known regarding the innate immune response to GAS during endometritis. Therefore, to better quantify changes in neutrophil and macrophage populations during i.u. GAS infection, uterine horns were removed at 24 h postinfection, and flow cytometry was performed. We observed that, although GAS infection alone
or PGE2 injection alone did not significantly alter neutrophil populations, a significant increase in tissue neutrophils was observed when infected mice were treated with PGE2 (Fig. 2A). When CD11b+ cells were excluded from neutrophil analysis, similar results were obtained, suggesting that monocyte contamination in the Neu7/4+ cells in the uterus was not a significant issue (data not shown). Neither PGE2 injection alone nor GAS infection alone significantly reduced CD45+ F4/80+ macrophage populations (Fig. 2B). However, it was surprising to note that the simultaneous injection of GAS and PGE2 resulted in a dramatic reduction in uterine macrophages to levels that were significantly lower than those seen following either treatment alone (Fig. 2B). Representative flow plots demonstrate the gating strategy used for neutrophils and macrophages in the mouse uterus (Supplemental Fig. 1).

Intrauterine infection increases local and systemic inflammation responses that are partially abrogated by PGE2

To assess the host immune response to acute GAS infections, cytokines and chemokines in the uterus and serum were analyzed at 24 h post-i.u. infection by ELISA. GAS i.u. infection resulted in statistically significant increases in IL-1β, IL-6, IL-17a, and MCP-1 in the uterus, indicating a robust proinflammatory response at the local site of infection (Fig. 3A). In the serum, MCP-1 and IL-12p70 were increased. No local or systemic increase was observed in IL-13, IL-10, TGF-β, IL-23, IFN-γ, or TNF-α (Fig. 3A, data not shown). In THP-1 cells in vitro, GAS infection increased IL-10, TNF-α, and MCP-1 levels, whereas exposure to PGE2 during GAS infection significantly increased IL-10 production while inhibiting TNF-α and MCP-1 production (Fig. 3B). Although it altered select inflammatory mediators significantly in infected mice (Fig. 3A), PGE2 injection alone did not significantly alter the global murine response in either uninfected or infected animals. The predominant effect of GAS is seen in the serum and the uterus, where GAS-infected animals or animals infected with GAS in the presence of PGE2 group together, suggesting that the significant changes in cytokine and chemokine responses are due to the GAS infection.

PGE2 impairs macrophage phagocytosis and killing of unopsonized GAS

In light of the importance of macrophages in innate defense against GAS and the observed changes in uterine macrophage populations induced by GAS infection in mice treated with PGE2, we assessed the effects of PGE2 on human macrophage activity ex vivo. Primary human PMs were sensitive to exogenous PGE2, which suppressed the phagocytosis of unopsonized GAS in a concentration-dependent manner (Fig. 4A). Similar to primary PMs, human THP-1 macrophage-like cells had significantly reduced phagocytic ability after PGE2 treatment (Fig. 4B), consistent with previously published data (35). These findings seem to be generalizable to mouse macrophages, because peritoneal macrophages obtained from female WT mice were also sensitive to the inhibitory actions of PGE2 on GAS phagocytosis (data not shown). These data demonstrate the capacity for PGE2 to directly limit the ability of human macrophages to phagocytose GAS and to modulate inflammatory mediator responses to GAS.

Intrauterine GAS infection results in a systemic, sepsis-like illness, and PGE2 promotes mortality. (A) Mice injected with i.u. GAS died in a dose-dependent manner over 1 wk (two combined experiments, n = 4–9/group). (B) Mice injected i.u. with GAS (10^7 CFU/mouse) in the presence of PGE2 (300 μg/kg) had increased mortality (two combined experiments, n = 7–10/group). *p < 0.01, log-rank test. (C) GAS translocates from the uterus to the blood (data not shown) and spleen by 24 h postinfection (10^3 CFU/mouse), as determined by differential culturing on TSA blood (one representative experiment, n = 9–10/group). **p < 0.05, ***p < 0.001 versus saline, two-way ANOVA with Bonferroni correction.

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It is known that PGE₂ increases cAMP in THP-1 cells (35). To investigate the role of the Gαs-coupled EP2 and EP4 receptors on human macrophage functions, we incubated PMs and THP-1 cells with the highly selective EP2 receptor or EP4 receptor agonists BFA or L-902,688, respectively, for 15 min prior to inoculation with GAS. In PMs, EP4 stimulation with L-902,688 resulted in

**FIGURE 3.** GAS i.u. infection increases local and systemic inflammation responses. (A) Uteri and serum were removed from mice at 24 h postinfection (10⁴ CFU) and analyzed by ELISA (one representative experiment of four, n = 5/group). *p < 0.05, **p < 0.01, ***p < 0.001, versus GAS i.u. infected mice, two-way ANOVA with Bonferroni correction. (B) THP-1 cytokine levels were analyzed by ELISA (one representative experiment of six, MOI 1:1, n = 3/group). *p < 0.05, **p < 0.01, ***p < 0.001 versus PBS, one-way ANOVA, with Bonferroni correction.

**FIGURE 4.** Macrophage activity is altered by GAS infection in the presence of PGE₂. Phagocytosis of FITC-labeled HK GAS was quantified by fluorometry after treatment with increasing amounts of PGE₂ for 15 min in human PMs (A) (n = 3–5/group) and PMA-treated THP-1 cells (B) (n = 10–22/group). Human PMs (C) (n = 3/group) and PMA-treated THP-1 cells (D) (n = 5/group) were treated with decreasing doses of the EP2 receptor agonist BFA or the EP4 agonist L-902,688 for 15 min. All data are mean and SEM, with the number of replicate experiments shown on each graph. *p < 0.05, **p < 0.01, ***p < 0.001, versus 150:1 GAS-challenged control, one-way ANOVA with Bonferroni multiple-comparison test. PMA-treated THP-1 cells were treated with PGE₂ (1 nM) for 15 min prior to inoculation with live unopsonized GAS (10 bacteria/cell). (E) Phagocytosis was quantified after 30 min. Intracellular killing was then assayed by washing the THP-1 cells to remove extracellular bacteria, lysing to release intracellular bacteria, and culturing on TSA blood. Data are expressed as the percentage of bacterial CFU relative to the untreated control and are presented as the mean and SEM of five independent experiments. *p < 0.05 versus untreated control, one-way ANOVA.
a significant reduction in phagocytic ability, whereas the EP2 agonist BFA led to a small, but nonsignificant, reduction in phagocytic ability (Fig. 4C). THP-1 cells were also susceptible to these agents, because both EP2 and EP4 agonists significantly reduced phagocytic ability (Fig. 4D). These data suggest that PGE2 binding to EP2 and/or EP4 receptors can suppress human macrophage phagocytosis of GAS.

These studies were limited by their use of heat-inactivated bacteria. It was reported that mouse bone marrow–derived macrophages and human blood monocytes incubated with PGE2 have an impaired capacity to kill live GAS (25). We sought to determine whether THP-1 cells were also susceptible to this immunoregulation of phagocytosis and killing. As anticipated, PGE2 treatment reduced the phagocytic ability of PMA-treated THP-1 cells and inhibited the ability of the THP-1 cells to kill phagocytosed live GAS (Fig. 4E). These data demonstrate the ability of PGE2 to suppress macrophage activity during GAS infection.

EP2 and EP4 receptors mediate the effects of PGE2 on mouse susceptibility to i.u. GAS infection

The above studies addressed a model of exaggerated PGE2 concentrations using exogenously added PGE2 in an attempt to mimic the physiology of pregnancy at the time of parturition. However, we noted that streptococcal endometritis induced significant shifts in the expression of eicosanoid pathway–related genes, including those involved in PGE2 synthesis and signaling (Supplemental Fig. 2), implicating endogenous PGE2 as a regulator of the inflammatory response to infection. We addressed this by infecting mice with impaired PGE2 synthetic and signaling abilities. We first assessed the role of mPGES-1, a critical enzyme involved in PGE2 synthesis in vivo, through comparison of survival of mPGES-1−/− mice (lack the ability to generate PGE2 in response to inflammation) and WT mice after i.u. GAS infection (10⁵ CFU). mPGES-1−/− mice demonstrated a delayed and decreased mortality over time compared with WT mice (Fig. 5D).

We then used EP2−/− mice and a pharmacological EP4 antagonist to dissect the role for these receptors in mediating host defense in vivo. Because EP4 deficiency is an embryonically lethal defect, a pharmacological approach was used to assess the role of EP4 with the EP4 receptor antagonist, GW627368X (EP4RA).

Mice infected i.u. with EP4RA and infected concurrently with GAS (10³ CFU) had increased survival compared with mice infected with GAS alone (Fig. 5C). These data suggest that the EP4 receptor plays a role in susceptibility to i.u. GAS infections. We next assessed the role of the EP2 receptor. We infected EP2−/− mice with GAS (10⁵ CFU) i.u. and compared survival to WT mice (Fig. 5B). EP2−/− mice had increased survival, suggesting that signaling through the EP2 receptor also contributes to GAS-induced mortality. When we exposed EP2−/− mice to EP4RA, we found that they were incrementally more protected from infection than were the EP2−/− mice that were not exposed to EP4RA (data not shown). Collectively, these data suggest that PGE2 signaling through EP2 and EP4 alters host susceptibility to i.u. GAS.

Discussion

S. pyogenes is the most commonly identified cause of postpartum sepsis worldwide (57–59). Despite antibiotic use, this pathogen inflicts significant harm on new mothers, emphasizing the need for novel therapeutic targets. Alterations in the immune system during pregnancy result in increased susceptibility to invasive GAS infections, but the mechanisms behind this remain unknown. To develop optimal preventive or therapeutic strategies, a better understanding of the interactions between the pregnant host and GAS is needed. In this article, we report the novel findings that PGE2, an immunomodulatory lipid and a regulator of cervical softening and contractions, enhanced susceptibility to GAS endometritis and that primary human macrophages obtained from the gravid uterus are susceptible to immunoregulation by this lipid. These studies suggest that PGE2, which is present at elevated levels during the postpartum period, might be important mechanistically in the increased susceptibility of postpartum women to invasive GAS (6). Our work also provides a rationale for further investigations using postpartum animal models and suggests that pharmacological targeting of mPGES1, EP2, and/or EP4 receptors might provide additional therapeutic benefit to standard antibiotic use in the treatment of septic mothers.

A significant amount of research demonstrated the ability of PGE2 to regulate host–microbial interactions during streptococcal infection (25–31). In 1982, Short et al. (32) demonstrated increased survival in animals when PGE2 synthesis was inhibited during Group B Streptococcus sepsis. In 2010, Goldmann et al. demonstrated that COX-2 is upregulated in human and mouse tissues infected by GAS (25, 33). Using a mouse model of GAS bacteremia and in vitro studies of bone marrow–derived macrophages, they established that PGE2 signaling via EP2 receptors suppressed host defenses against GAS (25). Further, they demonstrated that COX-2−/− mice had increased survival compared with WT mice following i.v. infection with GAS (25). Our data add to this by unmasking the importance of EP2 receptor signaling in the context of i.u. GAS infections. EP2/EP4 signaling was demonstrated, in vitro and in vivo, to suppress immune function by increasing cAMP levels (53, 54, 60, 61). Our data demonstrate...
that GAS infections originating in the female reproductive tract upregulate EP2 and EP4 receptor gene expression, and the presence of endogenous PGE2 during this infection suppresses host defense. This may relate to its capacity to alter the phagocytic and bacterial killing activity of macrophages, although future studies are needed to assess the role of macrophages in intrauterine GAS infection. Further, EP2<sup>−/−</sup> mice treated with EP4RA have increased survival, showing an additive protective effect with the absence of EP2/4 signaling (data not shown). Previous work implicated mPGES-1 in mediating susceptibility to GAS infections (46). The present study demonstrates that, in WT mice, the expression of these enzymes is induced at 6 and 24 h post-i.u. infection with GAS. Also, mPGES-1<sup>−/−</sup> mice showed a delayed and decreased mortality over time, suggesting that, without proper PGE2 synthesis, mice are less susceptible to i.u. GAS infections.

We noted that PGE2 modulated inflammatory mediator generation both in vivo and in vitro, although the cytokines (and host species) were different in these two models (Fig. 3). It is unclear whether these changes reflect immunological alterations that are important to the in vivo actions of PGE2 on survival. Extrapolation from the human-derived THP-1 cell experiments (Fig. 3B) to the mouse in vivo work (Fig. 3A) is also difficult given the species difference. Little is understood about which cytokines are causally involved in innate defense against GAS, and future experiments will be needed that address this question. In addition, future studies are needed to parse out the causative pathways underpinning the results of PGE2 observed in this study.

Studies investigating GAS and infected soft tissues established the importance of macrophages in controlling the infection (25, 62–65). In deep tissue infections, macrophages are the cellular population that controls the dissemination of GAS (66). Our data demonstrated that uterine macrophage abundance was suppressed by elevated PGE2 levels in the reproductive tract, particularly during infection. The reasons for this are unclear, and future studies will be needed to determine how macrophage dynamics are affected by the combination of infection and PGE2 excess. For example, it would be interesting to know whether inflammation-dependent signaling is triggering pyroptosis, which, in turn, is augmented by PGE2 (67). Pyroptosis was found to be dependent upon eicosanoid signaling, because mice deficient in COX-1 are resistant to the effects of inflammasome activation and, therefore, alter the later pyroptotic response (67). It also remains speculative whether these actions and/or the suppression of macrophage antimicrobial functions by PGE2 are causally related to outcome during GAS infection; future research is needed.

Our laboratory reported recently that LTβ<sub>4</sub>, a product of the 5-LO metabolic pathway, enhanced phagocytosis and streptococcal killing by macrophages. This macrophage-stimulating action involved signaling through the G<sub>i</sub>-coupled BLT1 receptor and suppression of cAMP, an effect that opposes PGE2's actions in THP-1 cells (35). In this study, we demonstrate that, at 6 and 24 h postinfection, LTβ<sub>4</sub>-associated genes are significantly downregulated (Supplemental Fig. 2). This may promote mortality in our model of GAS i.u. infection as the result of reduced LTβ<sub>4</sub> levels, with consequent reduced phagocytosis and streptococcal killing by resident leukocytes (35).

Although our studies aimed to investigate i.u. GAS infections with in vivo and in vitro systems, our work has limitations. As noted, these studies did not include the use of pregnant or postpartum mice. We chose to administer PGE2 into the uterus of nonpregnant mice to model elevated PGE2 levels, as might be observed during pregnancy and in the immediate puerperium. This approach conserves animal numbers, consistent with efforts to reduce, refine, and replace the use of animals, because newborn pups would not survive after the mother is euthanized. Further, our studies in mice used a ligature of the right uterine horn. Future studies aim to look at pregnant and postpartum mice, as well as to examine cytokine/chemokine changes and histological alterations due to PG upregulation. Further, investigating the immunological changes associated with GAS infections in human placental and decidual tissue are of interest; because of differences in fetal and maternal origin of tissue types, alternative immunological responses may occur.

In summary, this study establishes the ability of PGE2 to negatively regulate the innate immune defenses against GAS during i.u. infections, a major cause of postpartum sepsis. These findings demonstrate that i.u. GAS infection leads to a sepsis-like illness, similar to other models of i.v. or deep tissue GAS infections. These findings also correlate well with previous work from our group demonstrating the ability of LTs to positively regulate macrophage activity against GAS. Our data suggest that EP2/4 signaling plays a vital role in susceptibility to i.u. GAS infections, revealing a potential pharmacological target for preventive or therapeutic interventions in the future. For example, locally or systemically limiting the production and responsiveness to PGE2 directly after delivery could potentially reduce maternal mortality and morbidity. Reducing maternal mortality requires ongoing studies of fundamental mechanisms of disease and potential pharmacological interventions, with a goal of identifying improved novel targets for treatment and prevention.

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Disclosures
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References


