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Lipopolysaccharide-Induced Profiles of Cytokine, Chemokine, and Growth Factors Produced by Human Decidual Cells Are Altered by *Lactobacillus rhamnosus* GR-1 Supernatant

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Abstract

The aim of this study was to assess the effects of bacterial lipopolysaccharide (LPS) and *Lactobacillus rhamnosus* GR-1 supernatant (GR-1SN) on secretion profiles of cytokines, chemokines, and growth factors from primary cultures of human decidual cells. Lipopolysaccharide significantly increased the output of proinflammatory cytokines (interleukin [IL]-1B, IL-2, IL-6, IL-12p70, IL-15, IL-17A, interferon gamma [IFN-γ], and tumor necrosis factor [TNF]); anti-inflammatory cytokines (IL-1RN, IL-4, IL-9, and IL-10); chemokines (IL-8, eotaxin, IFN-inducible protein 10 [IP-10], monocyte chemoattractant protein 1 [MCP-1], macrophage inflammatory protein-1α [MIP-1α], macrophage inflammatory protein-1β [MIP-1β], and regulated on activation normal T cell expressed and secreted [RANTES]); and growth factors (granulocyte colony-stimulating factor [CSF] 3, CSF-2, and vascular endothelial growth factor A [VEGFA]). *Lactobacillus rhamnosus* GR-1SN alone significantly increased CSF-3, MIP-1α MIP-1β, and RANTES but decreased IL-15 and IP-10 output. The GR-1SN also significantly or partially reduced LPS-induced proinflammatory cytokines TNF, IFN-γ, IL-1β, IL-2 IL-6, IL-12p70, IL-15, IL-17, and IP-10; partially reduced LPS-induced anti-inflammatory cytokines IL-1RN, IL-4 and IL-10, and LPS-induced VEGFA output but did not affect CSF-3, MIP-1α, MIP-1β, MCP-1, IL-8, and IL-9. Our results demonstrate that GR-1SN attenuates the inflammatory responses to LPS by human decidual cells, suggesting its potential role in ameliorating intrauterine infection.

Keywords

lipopolysaccharide, *Lactobacillus rhamnosus* GR-1, cytokine, chemokine, growth factor, decidual cells

Introduction

Prevention of preterm birth (PTB) remains a major challenge in obstetrics. The condition occurs globally in 9% to 13% of all pregnancies.[1] A strong body of evidence suggests that infection and inflammation are important mechanisms that might account for 25% to 40% of PTBs.[2,3] Bacterial vaginosis (BV), an alteration in the vaginal microbiota, is associated with a 40% increase in the risk of PTB.[4] The consequences of intrauterine infection are not limited to the complication of PTB but are among the leading causes of inflammation-induced neurologic injury resulting in intra- or periventricular hemorrhage and cerebral palsy of the newborn.[5] Antibiotics have been used in an attempt to prevent infection-induced PTB; however, there is little benefit in terms of a reduction in the rate of PTB from antimicrobial administration alone. Indeed, there may be even an increased risk of PTB with metronidazole treatments.[6,7]

Research conducted during the last decade has opened up the possibility that cellular immune effectors in inflammation induced by infection underlie preterm delivery. Dysregulation of the cytokine milieu has been suggested to contribute to preterm labor, and therefore a better understanding of the link between cytokines and PTB may lead to the development of effective

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interventions to prevent infection- or inflammation-related preterm labor. Traditionally and functionally, cytokines are subgrouped according to the paradigm of the T-helper type 1 (Th1):Th2 cell/response dichotomy. The Th1 cells produce proinflammatory cytokines (interleukin [IL]-1, 2, 6, 12, 15, 17, interferon [IFN-γ], and tumor necrosis factor [TNF]) which activate cytotoxic T cells and macrophages, thereby stimulating cellular immunity and inflammation. On the other hand, Th2 cells produce anti-inflammatory cytokines (such as IL-4, 5, 9, 10, and 13) that stimulate antibody production by B cells and act antagonistically with Th1-type cytokines to promote humoral immunity.[8-10] It has been proposed that recurrent spontaneous abortion occurs with Th1 bias, successful term pregnancy is associated with Th2 bias, and an appropriate ratio of Th1-Th2 cytokines is key for successful term pregnancy.[11-15] Recently, a number of studies suggest that regulatory T cell and natural killer (NK) cells are also important for the establishment and maintenance of pregnancy.[16,17] Thus, the balance of proinflammatory cytokines to anti-inflammatory cytokines is necessary for successful maintenance of pregnancy. Consequently, an overexaggerated maternal immune response to microorganisms through pattern-recognition receptors, mainly toll-like receptors (TLRs) has been suggested as one of the underlying causes of PTB with infection.[18,19] Previous studies demonstrated that pregnancies that display signs of infection are characterized by increased proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNF) in the amniotic fluid, myometrium, decidua, fetal membranes, and maternal serum, demonstrating Th1 bias.[20] Furthermore, a disproportionate increase in IL-1β over IL-1RN in the cervicovaginal secretion of pregnant women with an altered vaginal microflora correlates with PTB.[21] However, the profiles of inflammatory-related cytokine, chemokine, and growth factor production, and especially the balance between proinflammatory and anti-inflammatory cytokines in infection-induced PTB, are less well characterized.

Probiotics are potential alternatives to antibiotics or anti-inflammatory drugs to prevent PTB. *Lactobacillus* strains can act as competent immune modulators, enhancing intestinal and systemic immune functions.[22-26] *Lactobacillus rhamnosus* GR-1 in combination with *Lactobacillus reuteri* RC-14 can help return homeostasis to the vagina, suggesting a potential therapeutic option to protect against infection-induced PTB.[27-30] Previously, we showed that *L rhamnosus* GR-1 supernatant (GR-1SN) downregulates the bacterial lipopolysacchride (LPS)-induced TNF output but upregulates IL-10 and CSF-3 output by human placental trophoblast cells in vitro.[31,32] However, the mode of action of *L rhamnosus* GR-1 on a broader profile of cytokine production and on LPS-induced cytokine production profiles in human decidual cells is unknown.

Decidua is the functional layer of the endometrium of pregnancy, containing a high number of immune cells, such as natural killer cells, macrophages, dendritic cells, and T lymphocytes, and the first immunological barrier to microbial infection. Its activation has been suggested to play an important role in labor at term and preterm. Previous studies have demonstrated that LPS induces IL-1RN, IL-6, IL-8, IL-10, IFN-γ, and TNF-α output from human choriodecidual tissues or cells in vitro, using a traditional enzyme-linked immunosorbent assay (ELISA).[33-36] Obviously, studies focused on the actions of individual cytokines may not be sufficient for understanding the mechanism of PTB related to infection or inflammation. Because many cytokines that accompany inflammation have closely related or overlapping biological effects and may be involved in different aspects of immune responses, quantitation of single cytokines may be of limited value. However, the more extensive profiles of cytokine, chemokine as well as growth factor production in response to LPS by human decidual cells have not been reported.

In this study, we employed a high-sensitivity multiplex bead immunoassay technique (Luminex assay), which has been shown to have good correlation to ELISA platform assays.[37] It allows for the simultaneous detection of cytokine multiplex panels and low expressed cytokines not previously recognized in small-volume samples. This allows simultaneous assessment of a large panel of cytokines and growth factors to obtain a "fingerprint" of innate and adaptive immune responses in inflammation. Using such a tool, we examined the secretion of a broad range of cytokines, chemokines, and growth factors from cultured human primary decidual cells challenged with LPS or/and GR-1SN to characterize the features of inflammatory-related cytokine, chemokine, and growth factor production induced by microorganism products in human decidua.

Methods and Materials

Placenta Collection

Placentas with attached fetal membranes were collected from normal term (>37 weeks of gestation) pregnancies after elective cesarean delivery in the absence of labor (n = 13). Informed consent was obtained before tissue collection. The study was approved by the Review Board for Human Subject Research (IRB no. 04-0018-U) at Mount Sinai Hospital (Toronto, Ontario, Canada) and the University of Toronto in accordance with the Canadian Tri-Council Policy Statements on Human Ethics Reviews. Patients who had multifetal gestations, preterm rupture of membranes, chorioamnionitis, chromosomal abnormalities, and/or preeclampsia were excluded. None of the patients had received prostaglandins, corticosteroids, or oxytocin. The indications for elective cesarean section included breech presentation, previous cesarean delivery, and cephalopelvic disproportion. None of the patients had experienced premature uterine activity during their pregnancy.

Lactobacillus Preparation

Lactobacillus rhamnosus GR-1 supernatant (GR-1SN) was prepared as described previously.[25] Briefly, the organism was grown anaerobically for 48 hours to reach the stationary growth phase in de Man, Rogosa, and Sharpe media (MRS). The culture medium was then collected and centrifuged at 6000*g* for 10 minutes at 4°C. Residual bacteria were removed by

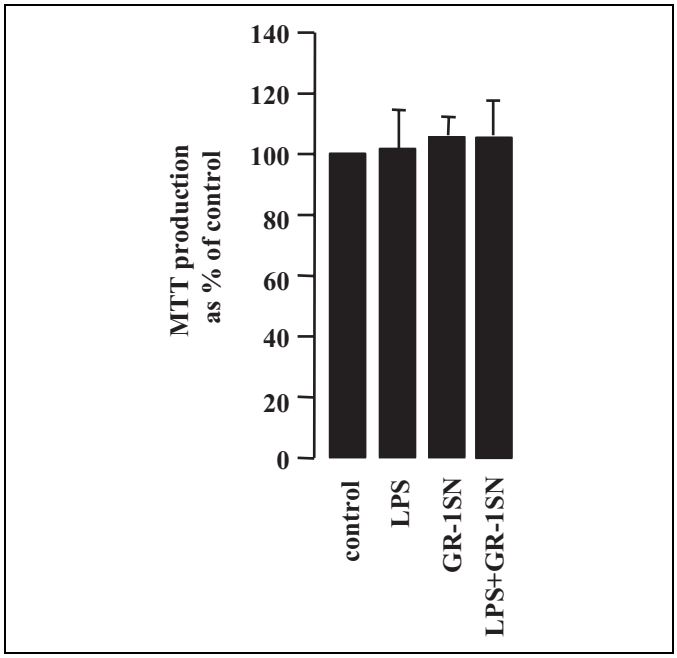

Figure 1. The effect of treatments on decidual cell viability. Cells were treated with lipopolysaccharide (LPS; 10 ng/mL) and/or GR-1SN (1:20) for 8 hours, then the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed as described in the Materials and Methods section. Each bar represents the change relative to control; data are presented as mean ± standard error of the mean.

filtration of the supernatant through a 0.22-μm pore size filter. The supernatant was divided into aliquots and frozen at −80°C.

Decidua Collection and Cell Culture

Decidua, scraped from fetal membranes, was washed with phosphate-buffered saline (PBS) to remove red blood cells, chopped, and digested with DMEM containing collagenase type H (6 mg/g tissue; Roche, Basel, Switzerland) and DNAse type I (200 μg/mL; Sigma, St. Louis) for 1 hour at 37°C. Following centrifugation (650*g*, 10 minutes), the cells were passed through a 40-μm filter. The viability of the cells was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[38] After 8 hours of treatment with LPS and/or GR-1SN, the MTT reagent was added to a final concentration of 0.5 mg/mL and the cells were incubated for 2 to 4 hours. After incubation, the culture medium with MTT reagent was removed and the formazan dye generated was dissolved in dimethyl sulfoxide and the absorbance was measured at 570 nm by a plate reader (TECAN infinite M200; TECAN Group Ltd, Mannedorf, Switzerland. Figure 1 shows that LPS in the presence or absence of GR-1SN did not have any detrimental effect on cell viability.

Treatments

Decidual cells were plated in 24-well plates (0.5×10^6 cells/well) and cultured in DMEM/F-12 (GIBCO Invitrogen, Burlington) supplemented with 10% heat-inactivated fetal bovine serum (Wisent Inc, Montreal) and antibiotics (1000 U/mL of penicillin, 0.1 mg/mL of streptomycin, and 0.23 μg/mL of amphotericin; GIBCO Invitrogen) and estradiol (E_2, 10^{-9} mol/L) at 37°C under 5% CO_2/95% ambient air for 48 hours. The culture medium was replaced with DMEM/F-12 as mentioned earlier, with added progesterone (P_4, 10^{-9} mol/L) for further 48 hours of incubation. Cells were washed with PBS and cultured in serum-free DMEM/F-12 with E_2 (10^{-9} mol/L) + P_4 (10^{-9} mol/L). In preliminary experiments, we found that after LPS treatment of cells cultured in serum-free or serum- or charcoal-stripped serum medium cytokine outputs were similar, and modestly, although not significantly higher in the presence of serum than in the absence of serum (data not shown). For consistency between studies, we used serum-free media at the time described throughout these experiments. After 12 hours of starvation and/or preincubation with GR-1SN at a dilution of 1:20,[28] further treatments of LPS (10 ng/mL, Sigma) or GR-1SN or LPS plus GR-1SN were added, and the cells were incubated for 8 and 24 hours. Vehicle-treated wells (controls) were present in each experiment. After incubation, the medium was harvested, centrifuged to remove cells and debris, and stored at −80°C until further assay. Original samples were used for multiplex assay and for TNF and IL-10 ELISA. Samples were diluted from 60 to 150 times for IL-6, IL-8, and monocyte chemoattractant protein 1 (MCP-1) ELISA.

Cytokine and Chemokine Measurements

ELISA cytokine assays. In preliminary experiments, concentrations of IL-6, 8, 10, TNF, and MCP-1 in conditioned media were determined by ELISA using a commercial kit according to the manufacturer's instructions (eBioscience, Inc, California). Plate reading and curve fitting were performed on plate reader (TECAN infinite M200) using Magellan6 software (TECAN Group Ltd).

Bio-plex cytokine assay. Subsequent cytokine/chemokine measurements were made using the Bio-Plex 200 system (Bio-Rad, Hercules, California) and Bio-Plex Human Cytokine 27-plex assay according to the manufacturer's instructions. The 27-plex assay kit contains beads conjugated with monoclonal antibodies specific for IL-1β (IL-1β), IL-1RN, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, eotaxin, fibroblast growth factor (FGF-2), granulocyte colony-stimulating factor (CSF) 3, granulocyte–macrophage-CSF (CSF-2), interferon gamma (IFN-γ), IFN-inducible protein 10 (IP-10), MCP-1, macrophage inflammatory protein 1α (MIP-1α), macrophage inflammatory protein 1β (MIP-1β), platelet-derived growth factor-BB (PDGFB), regulated on activation normal T cell expressed and secreted (RANTES), TNF, and vascular endothelial growth factor A (VEGFA). Standard curves and the concentration of cytokines within samples were generated with the Bio-Plex Manager 4.1 software.

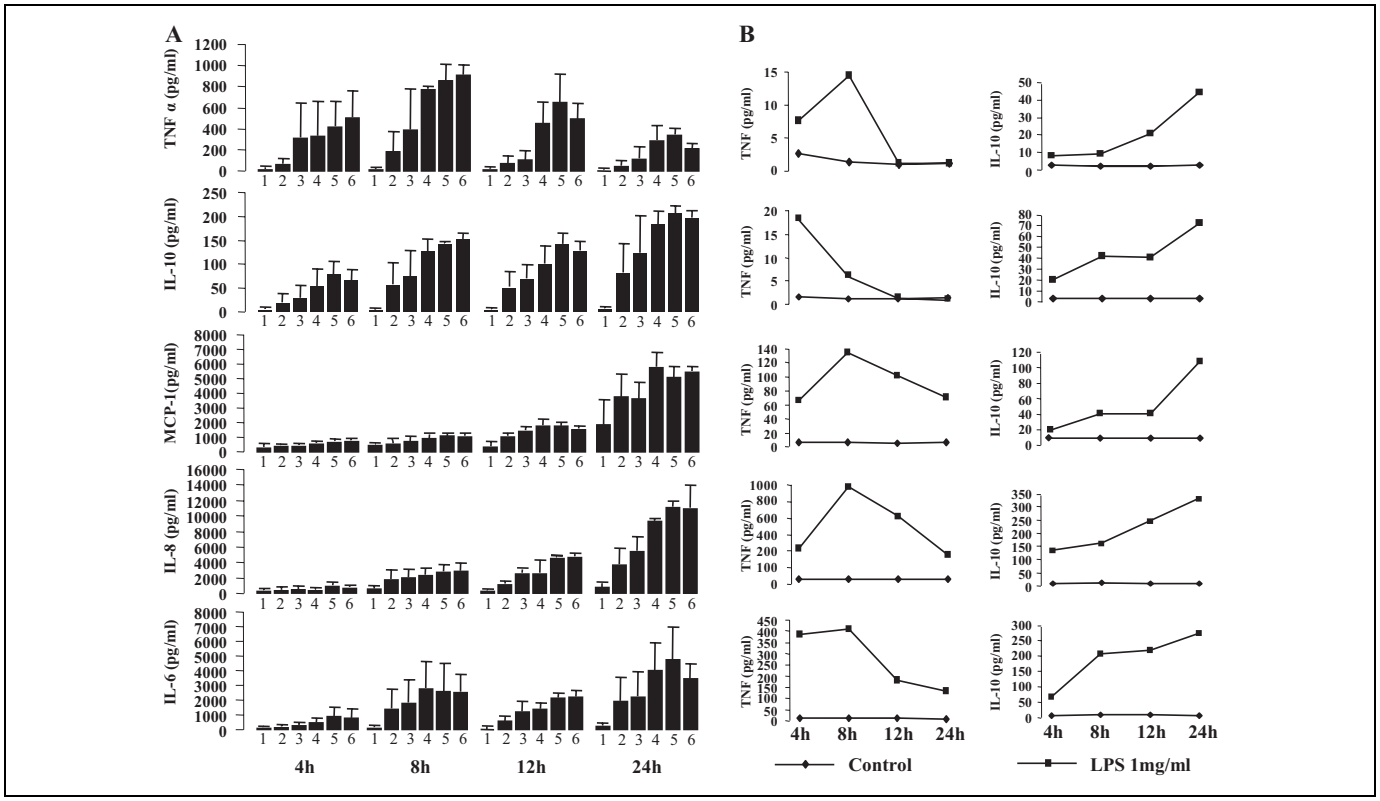

Figure 2. Dose and time curves of tumor necrosis factor (TNF), interleukin (IL) 10, monocyte chemoattractant protein 1 (MCP-1), IL-8, and IL-6 secretion from decidual cells stimulated with lipopolysaccharide (LPS). A, Cells were treated with LPS with different dosages from 0 ng/mL (1); 0.1 ng/mL (2); 1 ng/mL (3); 10 ng/mL (4); 100 ng/mL (5); 1000 ng/mL; (6) and at different times as indicated (n = 2). Each bar represents the concentration of cytokines in medium (pg/mL); data are presented as mean ± standard error of the mean (SEM). B, Cells were treated with vehicle (control) or 1 mg/mL LPS at 4, 8, 12, and 24 hours as indicated. Results are presented as 5 individual experiments.

Statistical Analysis

Data were presented as average values ± standard error of the mean (SEM). Original data were log transformed for statistical analysis, and statistical significance between groups was determined using a 2-way analysis of variance followed by Holm-Sidak method post hoc analysis using SigmaPlot version 9.01 statistical software (Jandel Scientific Software, San Rafael, California). The criteria for statistical significance were set at $P < .05$.

Results

In preliminary experiments using ELISA, we showed that LPS (0.1-1000 ng/mL) stimulated the output of IL-6, IL-8, IL-10, MCP-1, and TNF from decidual cells in a dose- and time-dependent manner (Figure 2A). The TNF response peak reached earlier (8 hour) than that of IL-6, IL-8, IL-10, and MCP-1 (24 hours; Figure 2A). Results from 5 individual experiments also demonstrated that TNF increased to peak levels at 8 hours, which was much earlier than IL-10 at 24 hours (Figure 2B).

Based on the preliminary results, we chose LPS at a concentration of 10 ng/mL and incubation duration of 8 hours for multiplex assay in subsequent experiments. Out of 27 cytokines assayed, 24 cytokines were detectable under our experimental conditions; exceptions were IL-5, IL-7, and IL-13. The concentrations of cytokines, chemokines, and growth factors in the control group demonstrated a large range from 1.2 pg/1 × 10^6 cells (IL-4) to 15 686.5 pg/1 × 10^6 cells (CSF-3).

As the concentration of individual cytokines showed large variations between individual experiments (Figure 3), we converted the results to log data for statistical analysis and comparison between treatment groups. After stimulation with LPS, 22 of the 24 cytokines, chemokines, and growth factors were significantly increased, including cytokines with proinflammatory properties (TNF, IFN-γ, IL-1β, IL-2, IL-6, IL-12p70, and IL-17A; Figure 3A); cytokines with anti-inflammatory properties (IL-1RN, IL-4, IL-9, and IL-10; Figure 3B); growth factors(CSF-3, CSF-2, and VEGFA; Figure 3C); and chemokines (IL-8, MCP-1, MIP-1α, MIP-1β, RANTES, IP-10, and eotaxin; Figure 3D). Of these, TNF, IL-6, and IL-10 were the most highly upregulated compared with the control group. There were no significant changes in PGDFbb and FGF-2 (data not shown).

The GR-1SN significantly increased the output of the chemokines, IL-8, MIP-1α, MIP-1β, RANTES, and growth factors CSF-3 and significantly decreased the output of proinflammatory cytokine IL-15 and chemokine IP-10 in conditioned medium, compared to the control group (Figure 3A-D).

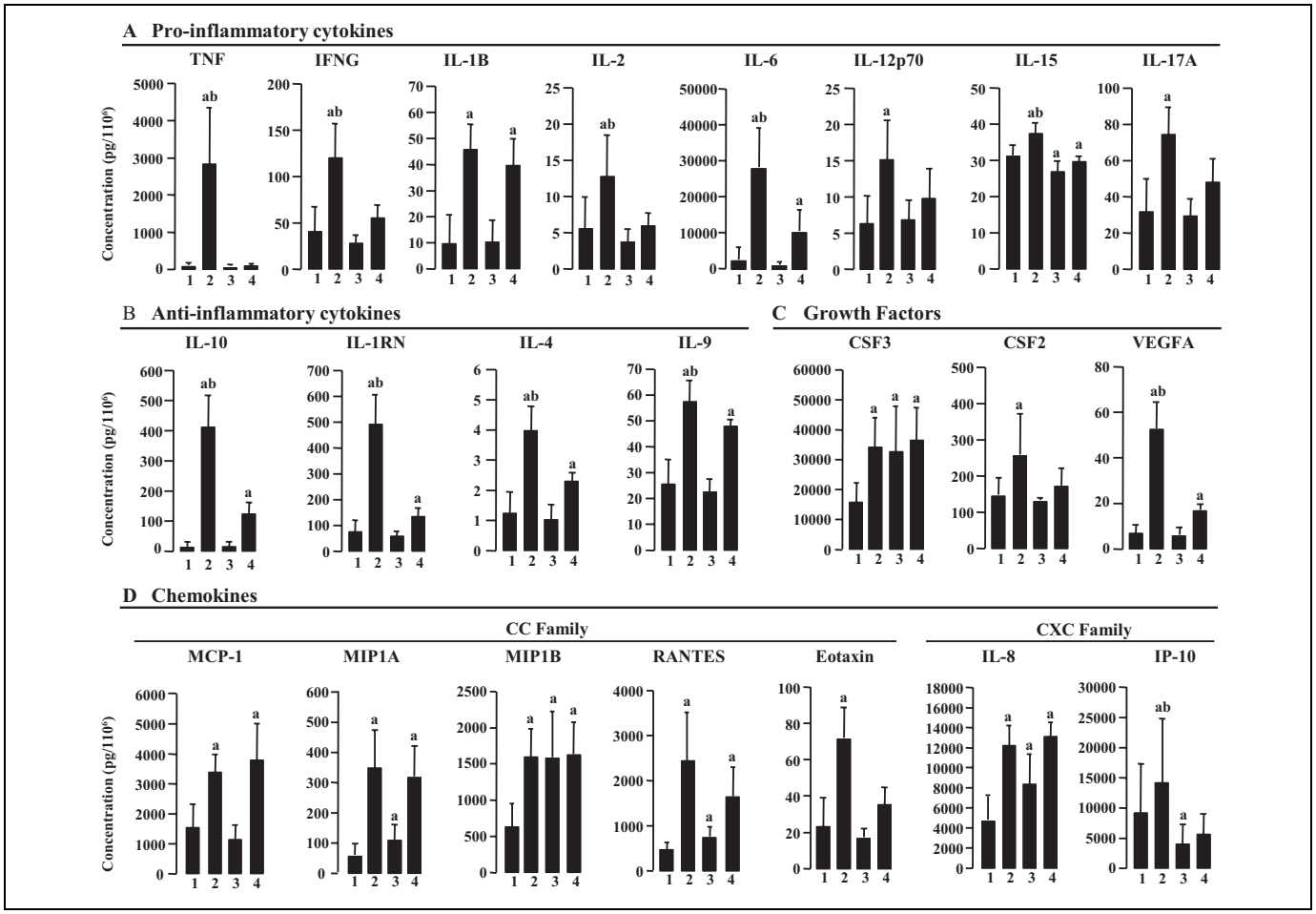

Figure 3. Effect of lipopolysaccharide (LPS) and GR-1SN on secretion of cytokines, chemokines, and growth factors from decidual cells (n = 6). A, Proinflammatory cytokines. B, Anti-inflammatory cytokines. C, Growth factors. D, Chemokines. Cells were treated with LPS (10 ng/mL) and/or GR-1SN (preincubation with GR-1SN (1:20) for 12 hours) for 8 hours. Each bar represents the concentration normalized to cell number; data are presented as mean ± SEM. a, $P < .05$ compared with control; b, $P < .05$ LPS versus LPS + GR-1SN. Control (1); LPS (2); GR-1 (3); and LPS + GR-1SN (4).

The combination of LPS and GR-1SN treatment (preincubation) significantly decreased the output of LPS-induced proinflammatory cytokines, TNF, IFN-γ, IL-2, IL-6, and IL-15. The TNF output was attenuated more than 30-fold, and the outputs of IL-1β, IL-12p70, and IL-17A were reduced partially in conditioned medium, compared with LPS treatment alone (Figure 3A). The outputs of LPS-induced anti-inflammatory cytokines, IL-1RN, IL-4, IL-9, IL-10 were significantly reduced by GR-1SN compared with LPS alone but were still significantly higher than the control group (Figure 3B). The GR-1SN had no significant effect on the output of LPS-increased growth factors CSF-3 and chemokine MCP-1, MIP-1α, MIP-1β, RANTES, and IL-8 but significantly decreased LPS-induced IP-10 (Figure 3C and D).

In order to examine the possible intermediary role of TNF in the stimulatory effects of LPS, we cultured cells in the presence of LPS plus TNF antibody. As shown in Figure 4, TNF significantly stimulated IL-6, IL-8, and MCP-1 secretion from decidual cells in a dose-dependent manner (using ELISA; Figure 4A) while addition of TNF-neutralizing antibody significantly reduced TNF-induced IL-6, IL-8, and MCP-1 secretion (Figure 4B).

Discussion

It is well known that cytokines, chemokines, and growth factors play an important role in inflammatory responses through in situ activation and proliferation of innate and adaptive immune cells as well as recruitment of these immunocompetent cells out of the circulation. In this study, we showed a similar increase in IL-1RN, IL-6, IL-8, IL-10, IFN-γ, and TNF production induced by LPS, consistent with previous studies.[33-36] We extended those observations to show that LPS also increased the secretion of other inflammatory-related cytokines, including proinflammatory cytokines IL-1B, IL-2, IL-12p70, IL-15, and IL-17A and anti-inflammatory cytokines IL-4, IL-9; chemokines MCP-1, MIP-1α, MIP-1β, RANTES, IP-10, eotaxin, and growth factors, CSF-3, CSF-2, and VEGFA. To our knowledge, this is the first description of secretion of these cytokines, chemokines, and growth factors in human decidual cells in

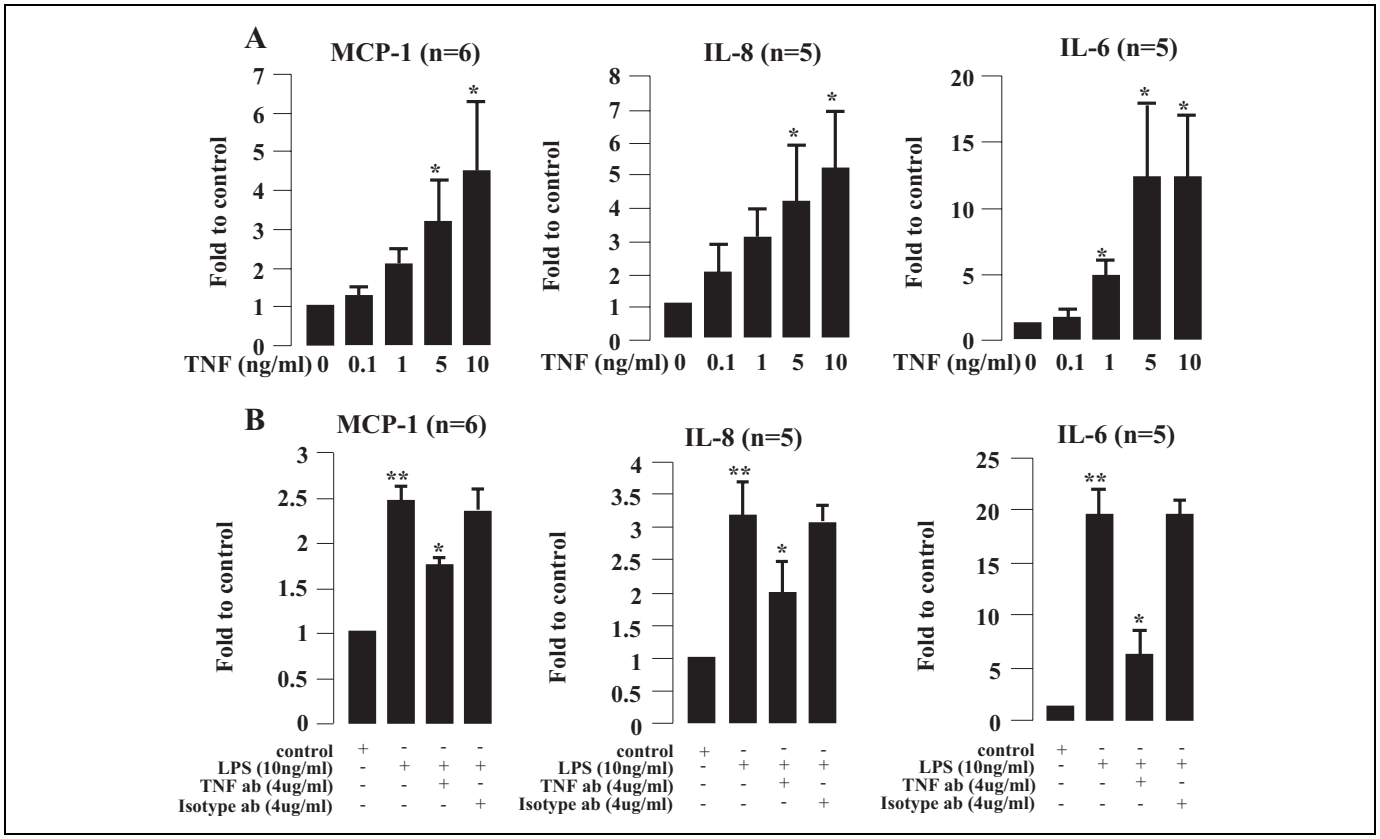

Figure 4. Effect of tumor necrosis factor (TNF) and TNF-neutralizing antibody (TNF ab) in lipopolysaccharide (LPS)-induced monocyte chemoattractant protein 1 (MCP-1), interleukin (IL) 8, and IL-6 secretion. A, Cells were treated with TNF for 24 hours. B, Cells were treated with LPS plus TNF ab or isotype antibody for 24 hours. Each bar represents the concentration fold increase relative to control. Data are presented as mean ± standard error of the mean (SEM). * $P < .05$ versus control (A) or versus LPS (B); ** $P < .01$ versus control.

response to LPS in vitro. The present results do not allow us to delineate the cell type responsible for cytokine secretion in mixed decidual cell preparations. In preliminary studies, we have shown that depletion of CD45+ cells from the mixed decidual preparations abolished LPS responsiveness (unpublished results) consistent with these immune cells being the primary source of different cytokines, but the possibility of cell–cell interaction has not yet been explored and cannot be excluded at this time. Indeed, we deliberately utilized an unpurified decidual cell population in culture, in an attempt to allow the cell–cell interactions that might occur in vivo. Given the results we obtained, it will be informative to evaluate the contributions of different cell types, in subsequent, more extensive studies than we have presented herein.

Taken together, our results suggest that LPS is able to stimulate mixed decidual cells to release a broad range of cytokines, chemokines, and growth factors. Therefore, these effectors may play an important role in inflammatory responses and in the further activation and regulation of the innate immune system and subsequent antigen uptake (TNF, IL-1B, IL-12p70, IL-17A, IFN-γ, IL-10, IL-1RN, and CSF-3) as chemoattractants for various immune cells including monocytes, NK cells, and T cells (MIP-1α, MIP-1β, IL-8, MCP-1 RANTES, IP-10, eotaxin, and VEGFA) and stimulating activation and proliferation of T cells (IL-2, IL-4, IL-12p70, IL-6, IL-9, IL-15, CSF-3, and CSF-2). Notably, IL-15 is now recognized as a cytokine with potent survival and immunomodulatory effects on cells of both the innate and the adaptive immune systems, including activating and expanding NK cells, NKT cells, $CD8^+$ effector memory, and central memory T lymphocytes, which play a central role in defense mechanisms against pathogens, and it is elevated in preterm labor. [39,40] It has also been demonstrated that IL-17A is involved in the development of inflammation by inducing the expression of proinflammatory cytokines, chemokines, antimicrobial peptides, and matrix metalloproteinases from different cells, and it is also the signature cytokine of the recently identified Th17 cell subset.[41] A recent study showed that Th17 cell number is increased in the chorioamniotic membrane of preterm delivery cases with choroamnionitis (CAM) and IL-17 levels in severe CAM preterm delivery cases were significantly higher than those in CAM-negative preterm delivery cases.[42] In this study, we found that LPS-induced IL-15 and IL-17A production in human decidual cells, suggesting that the IL-15 and the Th17 cell subset may be involved in infection-induced preterm labor. Overall, these findings suggest that there may be an overexaggerated maternal inflammatory response to microorganism products and/or microorganisms through proinflammatory cytokines/chemokines and activated innate and adaptive immune systems.

Lipopolysaccharide-induced IL-6, IL-8, and MCP-1 outputs were partially blocked by TNF-neutralizing antibody and stimulation of TNF by LPS, indicating that TNF may be an initial regulator and enhancer with positive feedback in LPS-stimulated cytokine production. The different time courses of TNF and IL-10 responses to LPS indicate subtle temporal differences that need consideration in the design of future studies as well as in interpretation of the underlying biology. For example, it has been suggested that the later rise in IL-10 downregulates TNF output by negative feedback, a response that is abrogated by administration of IL-10 neutralizing antibody.[43] At this time, one can only speculate on the importance that different cell–cell interactions may have in determining these responses

Proinflammatory cytokine bias in pregnancy disorders has led us to consider the therapeutic possibility of manipulating/redirecting the cytokine balance in order to downregulate proinflammatory and/or upregulate anti-inflammatory cytokines, thereby creating a milieu that is favorable toward successful pregnancy maintenance. The *L rhamnosus* GR-1 strain is one of a family of immunoregulatory probiotics. Our data indicate that the stimulated levels of proinflammatory cytokines TNF, IFN-γ, IL-2, IL-15, and VEGFA induced by LPS were significantly attenuated by GR-1SN in human decidual cells. In particular, LPS-induced TNF was substantially downregulated by GR-1SN, consistent with our previous studies on human placental trophoblast cells[31] and amniotic epithelial cells.[44] Conversely, levels of the anti-inflammatory cytokines IL-1RN, IL-4, and IL-10 in decidual cells were significantly higher after exposure to LPS, and LPS-induction of these cytokines was partially reduced by GR-1SN but was still significantly higher than the control group by GR-1SN. The GR-1SN did not significantly impact LPS-induced CSF-3, although it did stimulate secretion of CSF-3, consistent with our previous studies on placental trophoblast cells.[32] Studies have already shown that IL-4, IL-10, CSF-3, and CSF-2 are not only regulators of hematopoiesis with Th2-inducing capacity but are also potent inhibitors of the production of inflammatory mediators such as proinflammatory cytokines and prostaglandin.[45-50] This indicates that GR-1SN can manipulate the LPS-induced proinflammatory cytokine bias, accounting for its anti-inflammatory activity and suggesting that *L rhamnosus* GR-1 may induce a tolerogenic milieu within the fetomaternal interface. Excessive activation of Th2 is harmful for fetal survival, indicating that an appropriate ratio of Th1-Th2 cytokines is key for successful term pregnancy.[15] That GR-1SN partly suppresses LPS-stimulated production of anti-inflammatory cytokine may be favorable to keep an appropriate ratio of proinflammatory to anti-inflammatory cytokine. Moreover, that GR-1SN alone inhibited IL-15 secretion from decidual cells suggests that GR-1SN may play a role in defense mechanisms against pathogens through immunomodulatory effects of IL-15 on cells of both innate and adaptive immune systems.

Chemokines are a group of low-molecular-weight peptides that induce chemotaxis of different leukocyte subtypes to inflammation sites.[51] Here, GR-1SN stimulated secretion of MIP-1α, MIP-1β, RANTES, and IL-8. However, it decreased secretion of IP-10. The GR-1SN did not affect LPS-induced secretion of MCP-1, MIP-1α, MIP-1β, RANTES, and IL-8 but reduced LPS-induced secretion of IP-10 and eotaxin. These results suggest that GR-1SN is able to promote recruitment of phagocytes (including macrophages and neutrophils) and other immune cells by certain chemokines with or without infection. Whether GR-1SN is able to stimulate the activity of phagocytes remains to be investigated.

It has been reported that RANTES, MIP-1α, and MIP-1β are the most potent chemokines with anti-HSV-1 activity [52] and also that they have anti-HIV activity.[53] The IFN-inducible protein 10, a strong Th1 cell chemokine that is positively associated with bacterial and viral-induced tissue damage,[54] was inhibited by GR-1SN, indicating an additional anti-inflammatory property of GR-1SN. We speculate that patients with bacterial or viral infection may benefit from the ability of GR-1SN to promote the production of these chemokines and to inhibit IP-10 production.

In conclusion, bacteria through endotoxin production can induce the production of a broad range of inflammatory-related cytokines, chemokines, and growth factors in human deciduas. This then leads to an overexaggerated inflammatory response potentially resulting in preterm labor. The ability of lactobacilli to produce substances that directly and/or indirectly reduce production of inflammatory mediators, through altering microorganism-activated decidual cell (including immune cells and nonimmune cells) responses or recruiting and activating phagocytes at the decidual interface, suggests a potential mechanism for preventing some cases of PTB.

Declaration of Conflicting Interests

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