

Nicotine Inhibits Cytokine Production by Placenta Cells via NF κ B: Potential Role in Pregnancy-Induced Hypertension

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Pregnancy-induced hypertension (PIH), also known as preeclampsia, is one of the major causes of maternal and fetal death. While the precise cause of PIH is not known, aberrant cytokine production and placenta participation are considered to be important factors. Gestational cigarette smoking, which is widely accepted to be harmful to both the mother and fetus, is protective against PIH. Based on the antiinflammatory activity of nicotine, the major component of cigarettes, we examined the effect of nicotine and other cholinergic agonists on placental inflammatory responses *ex vivo*. We observed that nicotine and other cholinergic agonists significantly suppress placenta cytokine production following stimulation. Placenta cells express the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR), and using cholinergic antagonists, we demonstrated that the antiinflammatory effect of nicotine and other cholinergic agonists is, in part, mediated through the nAChR pathway. By contrast, cholinergic stimulation had no effect on the expression of soluble fms-like tyrosine kinase (sFlt), an antiangiogenic substance implicated in maternal vascular dysfunction during PIH. Mechanistic studies reveal that cholinergic agonists exert their antiinflammatory effects through the NF κ B pathway. Taken together, our results suggest that cholinergic agonists, including nicotine, may reduce cytokine production by placenta cells via NF κ B to protect against PIH.

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INTRODUCTION

Despite the many detrimental effects of smoking on maternal and fetal health, several studies show that maternal smoking is protective against pregnancy-induced hypertension (PIH), also known as preeclampsia (1-6). PIH is one of the leading causes of perinatal morbidity and mortality (7), occurring in 5%-10% of all pregnancies. PIH is characterized by maternal hypertension, proteinuria, and edema during the second half of pregnancy (7,8). Although the pathogenesis of PIH is poorly understood, the role of the placenta in mediating PIH is well accepted, as the condition is resolved upon delivery of the placenta following childbirth. While the association between angiogenic factors and PIH has been recently discovered (9-11), numerous reports highlight the proposed contribu-

tion of leukocyte activation and several proinflammatory cytokines to the development of PIH, including TNF α (TNF), IL-6, IL-1 receptor antagonist, and IL-8 (12-18). Further studies implicate a generalized phenomenon of maternal immune cell activation via NF κ B, a critical regulator of inflammation (19,20).

The systemic antiinflammatory effect of cholinergic agents including nicotine, the major constituent of cigarettes, has been described in numerous reports (reviewed in 21). Nicotine suppresses inflammation during experimental ulcerative colitis (22), improves survival during experimental sepsis via its effect on the production of proinflammatory mediators (23), and blocks leukocyte recruitment *in vivo* (24). Nicotine suppresses TNF production by LPS-stimulated macrophages (25) and microglial cells

(26) through the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR), and inhibits endothelial cell activation *in vitro* and *in vivo* (24). Based on the observations that smoking protects against PIH and nicotine exerts antiinflammatory effects, we examined the effects of cholinergic agonists, including nicotine, on placental inflammatory responses *ex vivo*.

MATERIALS AND METHODS

Reagents

LPS, nicotine, and mecamlamine chloride were obtained from Sigma-Aldrich (St Louis, MO, USA). Cholinergic agonists, GTS-21 and CAP55, were provided by Y. Al-Abed (The Feinstein Institute for Medical Research).

Placenta Cell Isolation and Analysis of Cytokine and sFlt Expression

Anonymous human placentas obtained from normal, term pregnancies at North Shore University Hospital (exempt from IRB review) were processed within three to four hours of delivery. For each pla-

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centa, five random 1×1×1 inch placenta pieces were collected, washed in PBS, and minced, and the resulting pooled mixture was passed through a 40 μM mesh to obtain a single cell suspension. Contaminating erythrocytes were removed by hypotonic water lysis, and isolated cells were washed once with PBS. Placental cells were plated on 0.15% gelatin-coated 96-well plates (2×10⁶ cells/mL) in RPMI containing 10% FBS, penicillin, streptomycin, and glutamine at 37°C and 5% CO₂. Plated placenta cells were stimulated with lipopolysaccharide (LPS) (isolated from *Escherichia coli* serotype 0111:B4, 0-1000 ng/mL) and incubated overnight. To examine the effect of cholinergic agonists on cytokine production, placenta cells were pretreated for 0.5 hours with cholinergic agonists (nicotine, GTS-21, CAP55) prior to LPS stimulation (each sample was assayed in triplicate or quadruplicate). Following an overnight incubation, cell-free supernatants were collected and assayed for cytokine production (IL-1β, IL-6, and IL-8) or sFlt production by ELISA (R&D Systems, Minneapolis, MN, USA). TNF production was assayed by ELISA according to Hesse et al (27). Each ELISA sample was assayed in triplicate. The sensitivities of the IL-1β, IL-6, and IL-8 ELISAs were less than 4 pg/mL. The sensitivity of the sFlt ELISA was 10 pg/mL. The sensitivity of the TNF ELISA was 39 pg/mL. The mean intra- and interassay coefficient of variation for all ELISA assays (i.e. precision) was ≤15%. Additional studies employed pretreatment with the cholinergic antagonist, mecamylamine, prior to treatment with cholinergic agonists and LPS. Cell death was assessed by LDH using the Cytotox96 Cytotoxicity Assay kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. Each experiment was repeated at least three to four times (with different placentas).

Analysis of α7nAChR Expression by Placenta Cells

Western blotting. Placenta cells were resuspended in 50 mM Tris-HCl pH 6.8, and 3% SDS and solubilized at 37 °C for

one hour. Lysates were separated by SDS-PAGE electrophoresis and transferred to PDVF membrane. Membranes were probed with rabbit antihuman α7nAChR (Chemicon, Temecula, CA, USA) followed by incubation with HRP-conjugated goat antirabbit antibody and ECL development (Amersham Biosciences/GE Healthcare, Piscataway, NJ, USA).

RT-PCR. Total RNA was isolated from placenta cells using the RNeasy mini kit (Qiagen, Valencia, CA, USA). Briefly cDNA was prepared using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) from 1 μg RNA. We amplified 2.5-μL aliquots of cDNA by PCR using PCR SuperMix (Invitrogen) in a thermal cycler (model 9600; Perkin Elmer, Waltham, MA, USA) using primer sequences for α7nAChR and α7nAChR duplicate gene (α7 (F-GGCAGATATCAGTGGC-TATA; R-CTTCATTCGCAGGAACC); α7dup (F-CGGTGCCCCTTGCCATTTTC; R-CAGAGTGCTTCTGCACCTTGG)). These experiments were repeated twice (with different placentas) with similar results. Representative data are shown.

Analysis of Placenta Cytokine Expression by Quantitative PCR

Placenta cells were processed and plated (in 6-well plates) as described above. Placenta cells were pretreated with cholinergic agonists for 30 min prior to LPS stimulation (100 ng/mL). Two hours later, total RNA from placenta cells treated with cholinergic agonists (or vehicle) ± LPS was isolated using the RNeasy RNA isolation kit (Qiagen). The relative expression of TNF, IL-1β, IL-8, and IL-6 mRNA was assessed by quantitative real-time PCR using TaqMan technology with GAPDH as an internal control. Reactions (performed in duplicate) were completed using 50 ng RNA, Eurogentec quantitative RTqPCR master mix, and the Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA). Results were expressed as fold increase with respect to the vehicle control. Primer sequences were as follows:

TNF F-TCTTCTCGAACCCCGAGTGA, R-CCTCTGATGGCACCACCAG, probe-

TAGCCCATGTTGTAGCAAACCCTCAA GCT; *IL-8* F-CTAGGACAAGAGCCAGG-AAGAAAC, R-CCACGGCCAGCTTGG, probe-ACCGGAAGGAACCATCT-CACTGTGTGTA; *IL-6* F-CTGCA-GAAAAAGGCAAAGAATCTAG, R-CG-TCAGCAGGCTGGCATT, probe-TGCAATAACCACCCCTGACCCAACC; *IL-1β* F-TGCACCTGTACGATCACT-GAACT, R-TGGACCAGACATCAC-CAAGCT, and probe-CACGCTC-CGGGACTCACAGCA. The respective mRNA levels were calculated using the ΔCt method. Expression levels were normalized to the housekeeping gene GAPDH. Data from three experiments (using three different placentas) are presented as mean fold increase over control (mean ± SD).

NFκB Activation Studies

Placenta cells were prepared as described above and plated in T25 flasks. Placenta cultures were treated (in duplicate) with vehicle or nicotine (10⁻⁴ M) for 15 min prior to LPS stimulation (0-100 ng/mL). After one hour, placenta cells were collected and nuclear lysates were prepared using a nuclear extraction kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's instructions. The nuclear extracts were then analyzed for NFκB activation using the TransAM NFκB p65/NFκB p50 Chemi Transcription Factor Assay Kit (Active Motif), according to the manufacturer's instructions. This assay is based on the binding of nuclear p65/p50 to an oligonucleotide containing an NFκB consensus site bound to a 96-well plate. Binding of p65/p50 to the oligonucleotide is determined using antibodies specific for the p65/p50 subunits of NFκB followed by chemiluminescent detection. The specificity of the assay was confirmed using the appropriate controls. Data from three separate experiments are presented as mean percent control ± SD.

Statistics

Analysis of variance (ANOVA) followed by the Dunnett's Test (to make pair-wise comparisons) and the Student

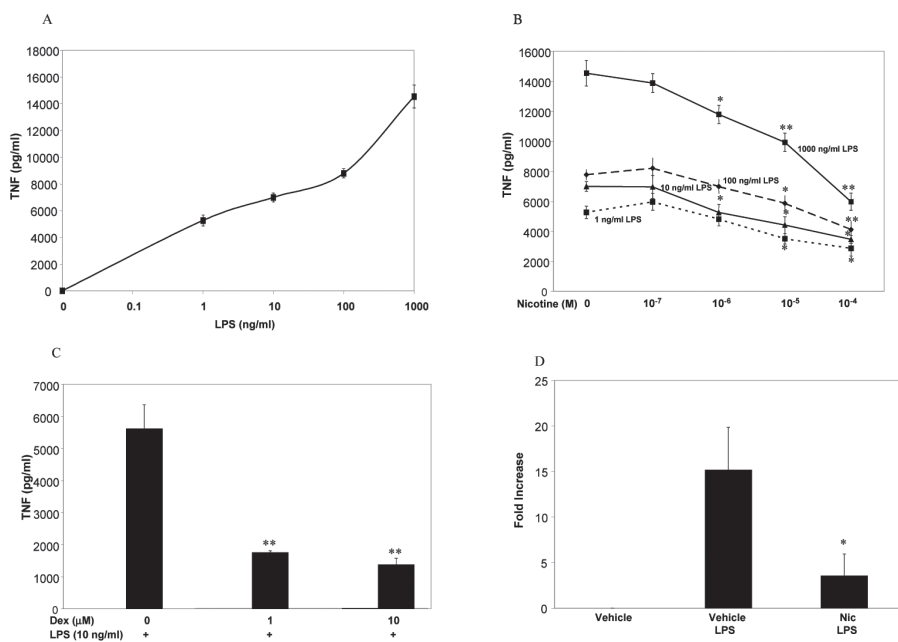


Figure 1. Nicotine reduces TNF expression by LPS-treated placenta cells. (A) LPS stimulation (1-1000 ng/mL) of placenta cells ex vivo induces TNF release. (B) Nicotine or (C) dexamethasone treatment reduces TNF production by LPS stimulated placenta cells. (D) Nicotine (10^{-4} M) treatment suppresses TNF mRNA expression by LPS-stimulated (100 ng/mL) placenta cells. Data from four separate experiments are shown as mean \pm SD. * $P < 0.05$, ** $P < 0.01$ when compared with appropriate vehicle control.

t-test (unpaired, 2 tailed) were employed for comparing groups as appropriate. The differences were considered significant if *P* was less than 0.05.

RESULTS

Nicotine Blocks TNF Production by LPS-Stimulated Placenta Cells Ex Vivo

To mimic inflammation associated with PIH, human placenta cells were treated with LPS to induce TNF production. Under basal conditions, TNF was not detectable in the placenta cell culture supernatants collected after an overnight incubation (Figure 1A). LPS treatment of placenta cells stimulated TNF production, in a dose-dependent manner (Figure 1A). Nicotine treatment ($\geq 10^{-6}$ M) of the placenta cell cultures significantly reduced LPS-induced TNF production over a wide range of LPS concentrations (Figure 1B), with up to 50%-60% inhibition. Similar results were observed with placenta tissue explants (data not

shown). These effects were comparable to the suppressive effects of dexamethasone (Dex, 1-10 μ M), a well-known anti-inflammatory agent, on TNF production following LPS stimulation (Figure 1C). Consistent with these observations, nicotine significantly reduced placenta cell TNF mRNA expression induced by LPS, as determined by Q-PCR (Figure 1D). The concentrations of nicotine and Dex

used for these experiments were not cytotoxic to the placenta cells (Table 1).

Nicotine Reduces Cytokine Expression by LPS-Treated Placenta Cells

Next, we examined the effect of nicotine on the expression of other proinflammatory cytokines by placenta cells following LPS treatment ex vivo. We found that nicotine significantly reduced IL-1 β , IL-8, and IL-6 mRNA expression following LPS stimulation (Figure 2A). Consistent with these observations, we found that nicotine significantly reduced LPS-induced IL-6 production by placenta cells (Figure 2B). However, the inhibitory effects of nicotine on LPS-induced IL-6 mRNA and protein levels were less dramatic than that observed for TNF expression (see Figures 1B and 1D). Similar results were observed for IL-1 β and IL-8 protein expression. Nicotine (10^{-4} M) reduced IL-1 β and IL-8 production by placenta cells following LPS stimulation (100 ng/mL) by 33% (\pm SD) and 34.5% (\pm 5.4%, SD), respectively.

Placenta Cells Express the α 7nAChR

The well-established role of the α 7nAChR in mediating the antiinflammatory effects of nicotine on macrophages, endothelial cells, and microglial cells (24-26) prompted us to examine the expression of the α 7nAChR by placenta cells. We found that placenta cells expressed α 7nAChR and α 7nAChRdup mRNA and protein, as determined by

Table 1. Effect of nicotine and dexamethasone (Dex) on placenta cell viability.*

Nicotine, M	LPS, ng/mL	Dex, M	Mean Viability, %	SD	Significance
0	0	0	100.00	15.40	
0	100	0	97.98	15.03	NS
10^{-6}	100	0	96.35	3.19	NS
10^{-5}	100	0	96.35	3.19	NS
10^{-4}	100	0	89.60	2.40	NS
0	100	10^{-6}	101.35	2.61	NS
0	100	10^{-5}	108.44	3.90	NS

*Placenta cell viability was assessed by measuring LDH cytotoxicity. Data are presented as % control viability + SD. All samples were compared to vehicle treated cells. NS, not significant.

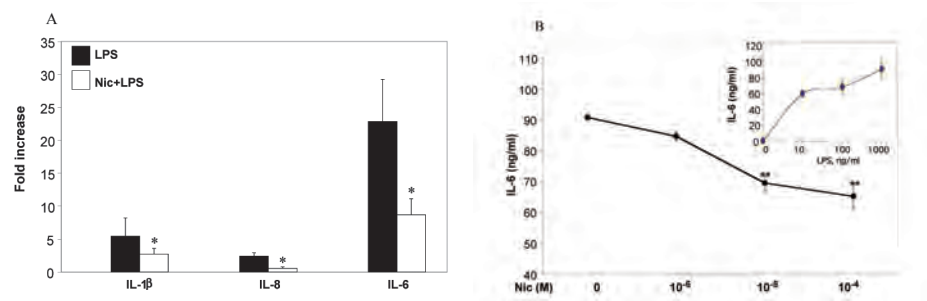


Figure 2. Nicotine inhibits cytokine expression by LPS-treated placenta cells. (A) Nicotine decreases IL-1 β , IL-8, and IL-6 mRNA expression by LPS-stimulated (100 ng/mL) placenta cells. Data are shown as fold increase mRNA expression over vehicle control (no LPS). (B) LPS treatment (100 ng/mL) induces IL-6 protein production by placenta cells (inset), which can be suppressed by nicotine. Data from three separate experiments are shown as mean \pm SD. * P < 0.05, ** P < 0.01 when compared with appropriate control.

RT-PCR and Western blotting methods (Figures 3A and 3B).

α 7nAChR Selective Agonists Suppress Placenta Inflammatory Responses

Based on our observations that nicotine reduced proinflammatory cytokine production by placenta cells expressing the α 7nAChR, we investigated the effects of GTS-21 [3-(2,4)-dimethoxybenzylidene anabaseine or DMXB], a well-described selective α 7nAChR agonist (28-30) and CAP55, a novel α 7nAChR agonist (24) on LPS-induced cytokine expression by placenta cells (LPS = 100 ng/mL). Both GTS-21 and CAP55 significantly reduced TNF, IL-6, and IL-8 mRNA expression induced by LPS (Figure 4A). Likewise, both GTS-21 and CAP55, a novel selective α 7nAChR agonist, significantly in-

hibited both LPS-induced TNF (Figure 4B) and IL-6 production (Figure 4C) by placenta cells in a dose-dependent manner. Treatment of placenta cells with GTS-21 (10⁻⁵ M) also reduced LPS-induced (100 ng/mL) IL-1 β and IL-8 by approximately 35% each. We found GTS-21 to be more effective in reducing cytokine production than nicotine or CAP55 (when used at the same concentrations). Neither GTS-21 nor CAP55 showed any cytotoxic effects on placenta cells at the concentrations used (Table 2).

To further demonstrate the role of the nAChR pathway in mediating the antiinflammatory effects of nicotine, GTS-21, and CAP55, we used nAChR antagonists in our model system. Pretreatment of placenta cells with the cholinergic antagonist mecamylamine (1-5 μ M) reversed

the antiinflammatory effects of nicotine, GTS-21, and CAP55 on LPS-induced TNF production by up to 67%, suggesting that the inhibitory action of these cholinergic agonists is mediated, in part, through the nAChR pathway.

Cholinergic Agonists Do Not Suppress sFlt Production Following LPS Treatment

Several studies suggest that excess soluble fms-like tyrosine kinase (sFlt, also known as soluble VEGFR-1), produced by the placenta, contributes to the pathogenesis of PIH (9-11). Therefore, we examined whether LPS (100 ng/mL) induced sFlt expression by placental cells ex vivo and whether sFlt expression was modulated by cholinergic agonists. We found that LPS significantly induced sFlt production by placental cells ex vivo [Figure 5 (inset)]. The induction of sFlt by LPS was not as dramatic as that observed for cytokines such as TNF. In contrast to what we observed for TNF, we did not observe an inhibitory effect of nicotine on sFlt production following LPS stimulation of placental explants (Figure 5).

Cholinergic Agonists Mediate Their Antiinflammatory Effects through NF κ B

Next, we examined the potential role of the NF κ B pathway in mediating the antiinflammatory effects of cholinergic agonists on placenta cytokine production. To assess NF κ B activation, we utilized the quantitative and sensitive TransAM p65/p50 luminescent assay, which is based on the binding of nuclear NF κ B (p65/p50) to a target oligonucleotide bound to a 96-well plate, followed by incubation with nuclear extract and antibodies specific for p65/p50. LPS (100 ng/mL) significantly induced NF κ B activation by placenta cell explants (Figure 6). We observed that treatment of placenta cells with either nicotine (10⁻⁴ M) or GTS-21 (10⁻⁵ M) inhibited NF κ B activation induced by LPS (100 ng/mL) (Figure 6).

DISCUSSION

In 2002, 11.4% of all women giving birth in the US reported smoking (31). Undoubtedly, smoking during preg-

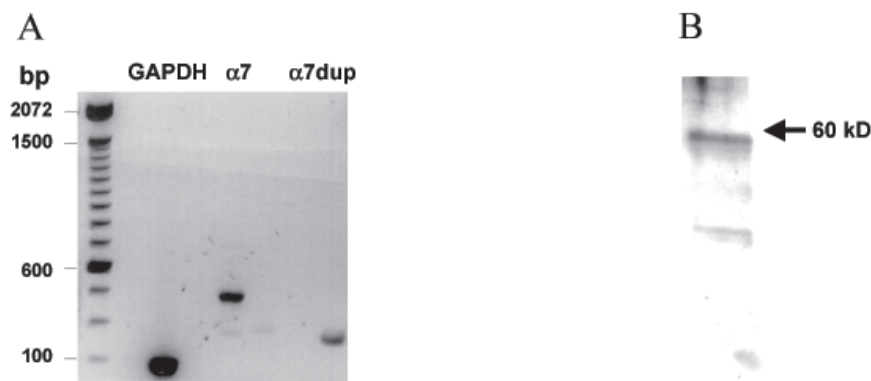


Figure 3. Placenta cells express the α 7-nAChR. Placenta cells express α 7-nAChR mRNA (A) and protein (B) as determined by RT-PCR and Western blotting, respectively.

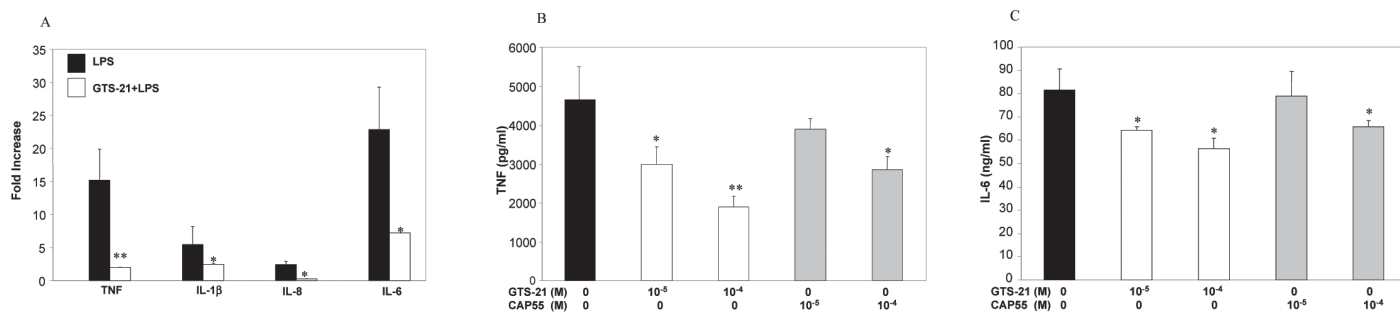


Figure 4. $\alpha 7$ -nAChR-selective cholinergic agonists suppress cytokine expression by placenta cells. (A) GTS-21 inhibits LPS-stimulated (100 ng/mL) TNF, IL-1 β , IL-8, and IL-6 mRNA levels. Data are shown as fold increase mRNA expression over vehicle control. GTS-21 and CAP55 suppress (B) TNF and (C) IL-6 protein production induced by placenta cells following LPS stimulation (100 ng/mL). Data from three separate experiments are shown as mean \pm SD. * $P < 0.05$, ** $P < 0.01$, when compared with appropriate vehicle control.

nancy may be detrimental to both maternal and fetal health. Smoking is associated with preterm birth and preterm premature rupture of membranes (PPROM), placenta abruption, placenta previa-accrta, intrauterine fetal growth retardation, and sudden infant death syndrome (32-36). Despite the adverse effects of smoking on maternal-fetal health, numerous studies demonstrate that smoking is protective against PIH (1-6). Interestingly, a history of cigarette smoking before pregnancy alone is not protective against PIH (37), suggesting that some agent(s) found in cigarettes may mediate the protective effects of smoking during pregnancy. Nicotine, the major constituent of cigarettes, and/or cholinergic stimulation via vagus nerve stimulation inhibit inflammatory cytokine production in vitro and in vivo using several experimental models including sepsis, postoperative ileus, and pancreatitis (23,38,39). In addition, nicotine is effectively used for the treatment of inflammatory bowel disease in the clinical setting (40,41). Our observations showing that nicotine and other cholinergic agents suppress placenta cytokine production ex vivo raise the possibility that nicotine exerts antiinflammatory effects to protect against PIH.

Numerous reports support the hypothesis that aberrant proinflammatory cytokine production, with an excessive maternal inflammatory response in pregnancy, plays a critical role in the development of PIH

(42-49). The immune theory of PIH is further supported by several epidemiological studies and research reports demonstrating the deposition of immune complexes and complement activation preceding maternal vascular injury and systemic inflammation during PIH [reviewed in (50)]. PIH is associated with increased maternal circulating proinflammatory factors, including TNF, IL-6, and IL-8 (12-17), as well as enhanced expression of TNF (protein and mRNA) by PIH placentas (14) and increased IL-1 β , IL-6, and IL-8 production by peripheral monocytes (49).

While our studies demonstrate the production of cytokines including TNF by LPS-treated placenta cell explants, additional studies will be required to determine which cells within the placenta produce cytokines following stimulation.

Several cell types within the placenta, including Hofbauer cells (placenta resident macrophages), syncytiotrophoblasts, and cytotrophoblasts produce cytokines (51-54). Previous studies using experimental model systems reveal the highest expression of TNF by trophoblast cells within the placenta (54).

Using an ex vivo model system with placenta cell explants, we investigated the effects of nicotine and cholinergic stimulation on cytokine production by placenta cells. Our observations are comparable with previous studies demonstrating the production of numerous cytokines by the placenta ex vivo following LPS stimulation and the antiinflammatory effects of glucocorticoids on placenta cell inflammatory responses from cultured normal and PIH placental ex-

Table 2. Effect of GTS-21 and CAP55 on placenta cell viability.*

GTS-21, M	LPS, ng/mL	Mean Viability, %	SD	Significance
0	0	100.00	15.40	
0	100	97.99	15.03	NS
10 ⁻⁶	100	114.88	14.41	NS
10 ⁻⁵	100	94.00	1.79	NS
10 ⁻⁴	100	96.47	3.82	NS
CAP55, M	LPS, ng/mL	Mean Viability, %	SD	Significance
10 ⁻⁶	100	121.38	18.76	NS
10 ⁻⁵	100	119.87	8.91	NS
10 ⁻⁴	100	106.84	14.43	NS

*Placenta cell viability was assessed by measuring LDH cytotoxicity. Data are presented as % control viability + SD. All samples were compared to vehicle treated cells. NS, not significant.

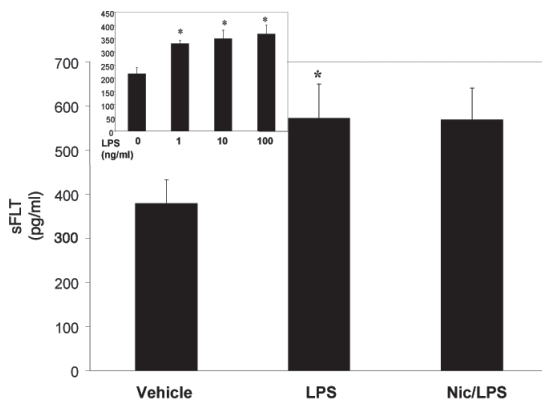


Figure 5. LPS induces sFlt expression by the placenta but nicotine does not inhibit LPS-induced sFlt production. Placenta cells were treated with either vehicle or nicotine (10^{-4} M) prior to LPS stimulation (100 ng/mL) or increasing concentrations of LPS alone (inset). After an overnight incubation, cell-free culture supernatants were collected and assayed for sFlt by ELISA. Data from three separate experiments are shown as mean \pm SD. * $P < 0.05$ when compared with vehicle control.

plants (55). Using this model system, we demonstrate for the first time the anti-inflammatory effects of cholinergic agonists, including nicotine on placental inflammatory responses. Our observations suggest that nicotine contained in cigarettes may confer the protective effect of smoking on PIH by mediating anti-inflammatory responses. However, these findings do not eliminate the possibility that other protective factors may contribute, such as carbon monoxide contained in cigarettes (56). While future studies are required to investigate the effect of nicotine (and cholinergic stimulation) on placental inflammatory responses in vivo, one major advantage of our ex vivo model system is that it allowed mechanistic-based studies to better understand how nicotine and other cholinergic agonists exerts antiinflammatory effects on placenta cells.

Our observations of $\alpha 7$ nAChR expression by placenta cells are consistent with previous studies reporting $\alpha 7$ nAChR expression by the human placenta (57,58). While Kwon and coworkers found no change in $\alpha 7$ nAChR gene expression within the placenta related to PIH, they did report that $\alpha 7$ nAChR protein expression was significantly increased in placentas obtained from severe PIH patients (57). However, their conclusions are

somewhat weakened by their semiquantitative data and small sample sizes. Regardless, this observation raises questions about the modulation of $\alpha 7$ nAChR expression within the placenta and the function of the $\alpha 7$ nAChR with reference to (a) acetylcholine, a potentially important placental signaling molecule; (b) nicotine, which may exert both positive and negative effects on fetal outcome, and (c) other cholinergic agonists. Further investigations to explore the effect of smoking on placenta $\alpha 7$ nAChR expression and to assess both placental

$\alpha 7$ nAChR expression and acetylcholine levels during PIH are warranted.

Recent studies suggest the role of soluble VEGFR-1 or sFlt in mediating the pathogenesis of PIH (9-11). sFlt is a secreted splice variant of Flt that binds angiogenic factors, VEGF and placenta growth factor (PlGF), preventing their binding to Flt in tissues and hence reducing angiogenesis. Numerous studies reveal higher sFlt expression by the placenta during PIH (9,59-61), and increased circulating levels of sFlt are observed during PIH (59). While the placenta was originally described as the sole source of sFlt, a recent study showed that peripheral blood mononuclear cells of pregnant women secrete high concentrations of sFlt (62). We investigated the effect of cholinergic stimulation on sFlt in our ex vivo model system using placenta explants because of its potential role in PIH, the link between sFlt and inflammation (63), and because smoking has been associated with reduced sFlt secretion (64). Our data suggest that sFlt levels are regulated, in part, by inflammation and that sFlt levels are not modulated by cholinergic agonists, such as nicotine. Based on previous studies showing the inhibitory effect of cigarette smoke on sFlt secretion by cultured placental explants (65), it is possible that other con-

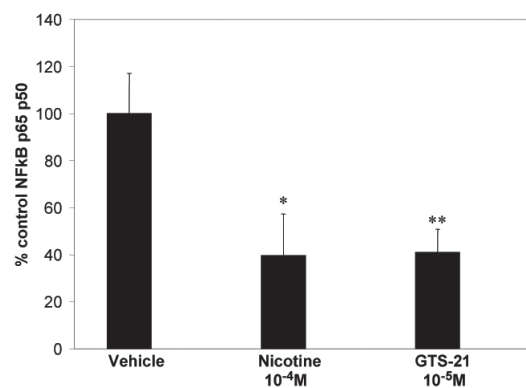


Figure 6. Cholinergic stimulation reduces NFkB activation by LPS-treated placenta cells. Placenta cells were treated with either vehicle, nicotine (10^{-4} M), or GTS-21 (10^{-5} M) prior to LPS stimulation (100 ng/mL). Data from three separate experiments are shown as mean percent control \pm SD. * $P < 0.05$, ** $P < 0.01$ (control = vehicle/LPS treatment).

stituents in cigarette smoke regulate sFlt expression.

Numerous studies implicate a generalized phenomenon of maternal immune cell activation with excessive cytokine production with PIH. NFκB is family of transcription factors associated with inflammation. NFκB activation is triggered by LPS and proinflammatory cytokines, including TNF and IL-1β. Several studies by our group and others have linked the antiinflammatory function of cholinergic stimulation to the NFκB pathway (23,24,66,67). In addition, previous studies support the role of NFκB in labor (19). Further studies reveal the engagement of the NFκB pathway by circulating immune cells during PIH (20). Consistent with observed heightened expression of proinflammatory factors during PIH, antiinflammatory pharmacologics, namely potent glucocorticoids, are used, with some degree of success, to suppress maternal inflammatory responses and to improve maternal and fetal outcomes associated with HELLP Syndrome (50,68-70). Similarly, low-dose aspirin (alone or in combination with dipyridamole, another antiplatelet agent) may be associated with moderate reductions in the relative risk of PIH and birth prior to 34 weeks gestation (71). Together, these observations raise the question whether antiinflammatory agents such as anti-TNF drugs might be useful for the treatment of PIH.

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