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# Uric Acid Crystals Induce Placental Inflammation and Alter Trophoblast Function via an IL-1–Dependent Pathway: Implications for Fetal Growth Restriction

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**Excessive placental inflammation is associated with several pathological conditions, including stillbirth and fetal growth restriction. Although infection is a known cause of inflammation, a significant proportion of pregnancies have evidence of inflammation without any detectable infection. Inflammation can also be triggered by endogenous mediators, called damage associated molecular patterns or alarmins. One of these damage-associated molecular patterns, uric acid, is increased in the maternal circulation in pathological pregnancies and is a known agonist of the Nlrp3 inflammasome and inducer of inflammation. However, its effects within the placenta and on pregnancy outcomes remain largely unknown. We found that uric acid (monosodium urate [MSU]) crystals induce a proinflammatory profile in isolated human term cytotrophoblast cells, with a predominant secretion of IL-1β and IL-6, a result confirmed in human term placental explants. The proinflammatory effects of MSU crystals were shown to be IL-1–dependent using a caspase-1 inhibitor (inhibits IL-1 maturation) and IL-1Ra (inhibits IL-1 signaling). The proinflammatory effect of MSU crystals was accompanied by trophoblast apoptosis and decreased syncytialization. Correspondingly, administration of MSU crystals to rats during late gestation induced placental inflammation and was associated with fetal growth restriction. These results make a strong case for an active proinflammatory role of MSU crystals at the maternal–fetal interface in pathological pregnancies, and highlight a key mediating role of IL-1. Furthermore, our study describes a novel in vivo animal model of noninfectious inflammation during pregnancy, which is triggered by MSU crystals and leads to reduced fetal growth.** ***The Journal of Immunology*****, 2017, 198: 443–451.**

Prenatal inflammation is an important clinical problem associated with devastating short- and long-term consequences for the developing fetus (1–3). In human pregnancy, exposure to inflammation increases the incidence of stillbirth, and surviving fetuses are at increased risk of fetal growth restriction (FGR) and preterm birth, with increased incidence of neonatal death (4–8). These serious complications of pregnancy affect ∼10–12% of all pregnancies in industrialized countries (4, 5, 7). Furthermore, prenatal exposure to inflammation is associated with fetal programming of cardiovascular diseases and a higher incidence of neurodevelopmental disorders, including cerebral palsy and autism (1, 2). Currently available treatments only aim to alleviate the symptoms of these disorders, as the causes are still speculative.

These effects of prenatal inflammation are most likely mediated through placental dysfunction. The causal link between inflammation and altered placental function has been shown primarily using animal models of infection-induced inflammation (9, 10). Infection is a well-known cause of inflammation but, in many cases of pathological pregnancies, there is no detectable infection although evidence of inflammation, such as increased levels of cytokines, is present (2, 11). Other than infection, a plausible cause of inflammation is alarmins or damage-associated molecular patterns (DAMPs) (12, 13), such as uric acid (monosodium urate [MSU]) crystals, high-mobility group box 1 (HMGB1) and cell-free fetal DNA, among others, which are increasingly associated with pathological pregnancies (14–18). DAMPs are endogenous mediators that can induce inflammation through the same receptors as pathogens, namely the TLR and NOD-like receptors (NLRs) (12, 13, 19, 20). However, little is currently known about the causal link between alarmins and pregnancy pathologies and the mechanisms involved remain mostly unresolved.

The DAMP uric acid has been shown to be elevated in the maternal circulation of many pregnancy pathologies, especially

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Abbreviations used in this article: DAMP, damage associated molecular pattern, FGR, fetal growth restriction; G, gestational day; hIL-1β, human IL-1β; HMGB1, high-mobility group box 1; MSU, monosodium urate; NLR, NOD-like receptor; PE, preeclampsia; rIL-1β, rat IL-1β.

preeclampsia (PE) (14, 15, 21–23). Furthermore, hyperuricemia has been associated with poor maternal and fetal outcomes (21). MSU crystals can exert proinflammatory effects in humans and animals (24, 25); however, their effects at the maternal–fetal interface are still mostly unknown. Studies using human first-trimester trophoblast cell lines showed that IL-1β was produced in response to MSU crystals via the activation of the Nlrp3 inflammasome (26–28). Furthermore, it has been reported that uric acid decreased system A amino acid transporter activity in third-trimester placental explants (29); a decrease in the activity of this transporter in the placenta has previously been associated with FGR (30). Together, mechanistic and clinical evidence points toward a direct effect of uric acid on the placenta, which could lead to inflammation and contribute to the pathogenesis of pregnancy disorders.

Our objective was to investigate the effect of MSU crystals on the human placenta late in gestation, with the hypothesis that MSU crystals cause placental inflammation and dysfunction that leads to FGR. In this study, we show that MSU crystals induce an IL-1–dependent proinflammatory response in human term cytotrophoblasts and placental explants associated with abrogated syncytialization and increased trophoblast apoptosis. Alongside this, administration of MSU crystals in vivo to pregnant rats led to FGR, which was characterized by a proinflammatory profile and immune cell infiltration of the placenta. Hence, our work strongly supports a causal role of uric acid in placental inflammation and dysfunction observed in pathological pregnancies.

## Materials and Methods

### *Ethical approval*

Approval was obtained from North West Research Ethics Committee in Manchester, U.K. (Ref: 08/H1010/55), Yale University's Human Research Protection Program, and the Sainte-Justine Hospital Ethics Board (Ref: 3988) for placentas from uncomplicated term pregnancies. All participants provided written informed consent, except at Yale University where this was not required due to de-identified samples of discarded tissue. Animal work was approved by Institutional Animal Care Committee at the Sainte-Justine Research Center and in line with the guidelines of the Canadian Council of Animal Care.

### *Primary cytotrophoblast isolation*

Primary cytotrophoblast cells were isolated from term placentas from uncomplicated pregnancies obtained after caesarean section without labor using a well-established method developed by Kliman et al. (31) with slight modifications. Briefly, villous tissue was dissected, minced, and rinsed in PBS prior to three enzymatic digestions in HBSS with trypsin and DNase. Cytotrophoblasts were isolated from these digestions after separation by centrifugation on a discontinuous Percoll gradient. Cytotrophoblasts were plated at $2 \times 10^6$ cells/ml in DMEM-F12 supplemented with 10% FBS and penicillin/streptomycin and washed with PBS to remove nonadherent cells after 12 h to remove residual syncytiotrophoblasts. Cell purity was assessed by staining for the trophoblast-specific marker cytokeratin 7 and 98% of the cells were cytokeratin 7+ (data not shown). Additional sets of experiments were performed with added negative purification after Percoll separation with anti-HLA-ABC Ab (Affymetrix, CA) and magnetic beads to ensure removal of immune cells. There was no difference observed in cytokine secretion between cells with or without this purification step and therefore the results were combined. Cytotrophoblast were treated with uric acid crystals [MSU crystals, 100 μg/ml; Invivogen, CA; same concentration as previously published (26) and representing the levels observed in severe PE], recombinant human IL-1β (rhIL-1β, 10 ng/ml; Peprotech, QC, Canada), caspase-1 inhibitor (10 μM; Z-WEHD-FMK, R&D Systems, MN) or IL-1 receptor antagonist (IL-1Ra, 1 μg/ml; Sobi-Swedish Orphan Biovitrum, ON, Canada) for 24 or 48 h in Opti-MEM (Life Technologies, ON, Canada). Supernatants were collected and kept at −80°C until analyzed. For immunohistochemical or immunofluorescence analysis or cytotrophoblasts, cells were fixed with cold methanol and kept at −20°C until processed.

### *Placental explant culture and treatment*

Villous tissue was obtained from term, uncomplicated pregnancies after caesarean section without labor ($n$ = 9) within 30 min of delivery and explants were prepared as previously described (32) with slight modifications. Briefly, biopsies of the chorionic villi were dissected and washed with PBS to remove any blood. Tissue was cut into small pieces (~2–3 mm) and three pieces (i.e., explants) were placed into netwells (74 μm mesh; Corning; Sigma-Aldrich, ON, Canada) in 1.5 ml of culture media (RPMI 1640, 5% heat-inactivated FBS, 100 μg/ml streptomycin, 100 IU/ml penicillin, 1 μg/ml insulin, 0.1 μg/ml hydrocortisone, 0.1 μg/ml retinol acetate, 0.05 mg/ml gentamicin; all chemicals are from Sigma-Aldrich). Explants were cultured at 37°C with 5% $CO_2$ with daily media change. On day 4, explants were treated with MSU crystals (100 μg/ml; Invivogen, CA), rhIL-1β (10 ng/ml; Peprotech), caspase-1 inhibitor (10 μM; Z-WEHD-FMK, R&D Systems) or IL-1Ra (1 μg/ml; Sobi-Swedish Orphan Biovitrum) for 24 or 48 h in Opti-MEM (Life Technologies). Explants were processed for histology (fixed for 24 h in 4% formalin and paraffin embedded) or protein analysis (supernatants and explants frozen and the stored at −80°C until extraction and analysis).

### *In vivo animal model*

Timed mated Sprague-Dawley rats were obtained at gestational day (G) 13 (Charles River Laboratories, QC, Canada) and kept in a controlled 20°C environment with a 12 h light/dark cycle and access to food and water ad libitum. Pregnant dams were injected i.p. every 12 h starting at G18 until G21 with uric acid crystals (MSU crystals; 250, 500 or 100 μg/kg/12 h; Sigma-Aldrich) combined with potassium oxonate (125 mg/kg/d; Sigma-Aldrich). Potassium oxonate is needed to block the enzyme uricase, which is absent in humans but present in rodents and rapidly degrades uric acid (33). No differences between vehicle (PBS) or potassium oxonate–injected animals were observed in term of uric acid levels or inflammatory markers in the placenta (or fetal weight). Therefore, both were combined and are shown as vehicle-injected in the results. Six dams/litters were used in each experimental group.

### *Protein extraction and analysis*

Cells, explants or tissues were homogenized in lysis buffer containing 1% Triton X-100 (Sigma-Aldrich) and protease inhibitor mixture (Calbiochem; Millipore, ON, Canada), and centrifuged at 13,000 rpm for 10 min at 4°C. Supernatants were collected and stored at −20°C until analysis. The protein concentration was determined using the Bradford assay (Bio-Rad, ON, Canada). Levels of human (h) and rat (r) cytokines [hIL-1β, hIL-6, MCP (hMCP)-1, rIL-1β, TNF-α, rIL-6] were determined by DuoSet ELISAs (R&D Systems) following the manufacturer's instructions. Multiplex assay (BioPlex; Bio-Rad) was performed using a custom panel of 16 human cytokines (IL-1β, IL-6, IL-10, IL-17, IFN-γ, TNF-α, G-CSF, GM-CSF) and chemokines (IL-8, MCP-1/CCL2, MIP1α/CCL3, MIP1β/CCL4, RANTES/CCL5, VEGF, GROα/CXCL1, IP-10/CXCL10) on cell supernatants according to the manufacturer's instructions. Caspase-1 activity was detected in culture supernatant using a caspase-1 specific kit following manufacturer instructions (Caspase-Glo 1 Inflammasome Assay, Promega). Western blotting was used to detect caspase-1 using the following protocol. Proteins (50 μg) were loaded on to an SDS-PAGE gel and, after separation, transferred to a 0.45 μm nitrocellulose membrane. Membranes were blocked and incubated overnight at 4°C with the primary Ab (caspase-1; Santa Cruz Biotechnology, CA). After washing they were incubated with goat anti-mouse HRP-conjugated secondary Ab (Bio-Rad) and signal was detected by chemiluminescence (clarity western ECL substrate; Bio-Rad).

### *Histological analysis*

As previously described, 5 μm-thick paraffin sections were processed for H&E staining and immunohistochemistry (17). The following Abs were used: M30 (caspase-cleaved cytokeratin 8; Roche), desmoplakin (Sigma-Aldrich, U.K.), CD68 (Serotec, ON, Canada), IL-1β (Santa Cruz Biotechnology), polymorphonuclear Ab (PMN; Cedarlane, ON, Canada). Matched secondary Ab HRP-conjugated (anti-rabbit-HRP, or anti-mouse-HRP; Bio-Rad, ON, Canada) were used, revealed using 3, 3-diaminobenzidine (DAB; VWR, Canada) and sections were counterstained with hematoxylin. For immunofluorescence, fluo-conjugated secondary Ab was used (anti-mouse Alexa 488; Life Technologies, U.K.) and mounted with mounting media countaining DAPI (Life Technologies). Images were obtained with a slide scanner (Axioscan; Zeiss, ON, Canada) or an Olympus upright BX51, CoolSnap ES camera and MetaVue software, for immunofluorescence.

### *Statistical analysis*

Data are presented as mean ± SEM. Statistical comparison between multiple groups was assessed using one-way ANOVA with Dunnett's multiple comparison test. Statistical analysis was performed with GraphPad Prism 6.0 (GraphPad Software, CA) and a $p$ value $< 0.05$ was considered significant.

## Results

*MSU crystals induced an inflammatory profile in primary-term cytotrophoblasts and placental explants*

We determined the inflammatory profile induced by MSU crystals in primary cytotrophoblasts ($n$ = 6–9) isolated from term human placentas from uncomplicated pregnancies. Exposure of cytotrophoblasts to MSU crystals for 48 h induced the secretion of several proinflammatory mediators, primarily IL-1β (18.6-fold increase, $p < 0.01$ versus vehicle) and IL-6 (143-fold increase, $p < 0.01$ versus vehicle), but also IFN-γ, TNF-α, IL-17, G-CSF as well as the anti-inflammatory cytokine IL-10 (Fig. 1A). No effect was seen on the secretion of GM-CSF (Fig. 1A). Elevated secretion of several chemokines was also observed, especially MCP-1 (5.8-fold increase, $p < 0.01$ versus vehicle), MIP-1β, MIP-1α and GROα (3.9, 15.3, 2.5-fold increase respectively, $p < 0.01$ versus vehicle, Fig. 1B). RANTES and VEGF were also significantly upregulated whereas IP-10 and IL-8 levels were unaffected (Fig. 1B).

Because IL-1β is a well-known mediator of MSU crystal actions in immune cells, and it was strongly induced in term cytotrophoblasts in response to MSU crystals, we addressed the mechanisms of IL-1β production. The secretion of IL-1β induced by MSU crystals in cytotrophoblasts was dependent on the common processor of pro–IL-1β, caspase-1, which was ascertained using a caspase-1 inhibitor (Fig. 1C). The secretion of another inflammatory mediator, MCP-1, was also decreased following caspase-1 inhibition (Fig. 1D) suggesting an upstream role of IL-1 in the induction of inflammatory mediators in cytotrophoblasts in response to MSU crystals. A similar inflammatory effect of MSU crystals was observed in term placental explants in which MSU crystals significantly induced IL-1β, MCP-1, and IL-6 secretion via caspase-1 (Fig. 2A–C) and IL-1 receptor (Fig. 2D–F) as shown

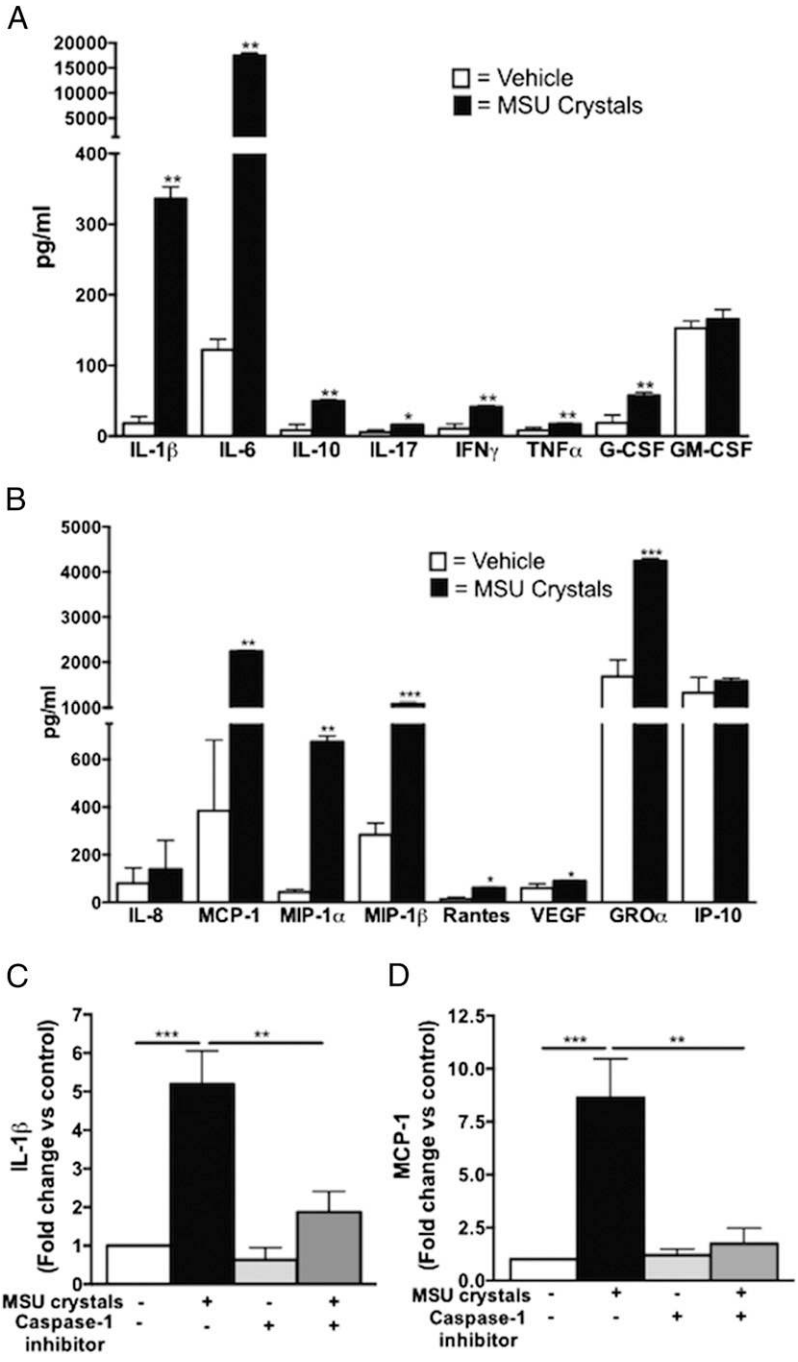


**FIGURE 1.** Inflammatory profile of human term cytotrophoblast after MSU crystal exposure is IL-1 dependent. MSU crystals induced the secretion of multiple cytokines, primarily IL-1β and IL-6 in cytotrophoblast (**A**) as well as chemokines, including MCP-1 and MIP-1α and MIP-1β (**B**). IL-1β secretion by cytotrophoblast exposed to MSU crystals was elevated and this effect was blocked by concomitant treatment with the caspase-1 inhibitor (**C**). This was also observed for secretion of the chemokine MCP-1 (**D**). $n$ = 6. Results presented as mean ± SEM. Statistical analysis by $t$ test with Welch correction (A and B) and one-way ANOVA with Dunnett's posttest (C and D). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

with a caspase-1 inhibitor and IL-1Ra, respectively, confirming the central role of IL-1β in placental inflammation induced by MSU crystals. Furthermore, caspase-1 activation was significantly elevated in the supernatant following MSU crystal exposure (Fig. 2G) concomitantly to increased cleaved caspase-1 in the supernatant and decreased levels within the cells (Fig. 2H). Basal activation of caspase-1 was observed in term placental explants (Fig. 2G–H) due to the constant release of alarmins (i.e., fetal DNA, trophoblast debris) in such a culture setting and to the trophoblast own production of uric acid as previously reported (27, 28).

*MSU crystals induced trophoblast apoptosis and blocked syncytialization in an IL-1–dependent manner*

Both MSU crystals and IL-1β significantly induced cell death in cytotrophoblast cultures, as seen by the 3.3- and 2.7-fold increase, respectively, in the percentage of cells positive for the apoptotic marker M30 at 48 h (Fig. 3A). Treatment of MSU crystal-exposed cytotrophoblasts with IL-1Ra was protective, with decreased percentage of M30+ apoptotic cells (Fig. 3A). MSU crystal exposure also significantly blocked syncytialization (i.e., decreased the percentage of multinucleated cells) to a similar extent of that observed following direct IL-1β exposure (Fig. 3B). Blockade of IL-1 signaling pathway with IL-1Ra increased the percentage of multinucleated cells (syncytialization) (Fig. 3B), although this did not reach significance ($p = 0.069$). Consistent with these findings in cytotrophoblasts, treatment with MSU crystals or IL-1β induced apoptosis in term placental explants, (Fig. 4A, 4B), which was mainly observed in cytotrophoblast cells (arrowheads in Fig. 4C). MSU crystals-induced apoptosis was again demonstrated to be IL-1–dependent using caspase-1 inhibitor and IL-1Ra (Fig. 4A–C).

*Administration of MSU crystals during late gestation induced placental inflammation and FGR in rats*

Pregnant dams exposed to MSU crystals during late gestation presented evidence of decreased fetal weight; there was an overall 20% decrease in fetal weight at G22 (term gestation corresponding

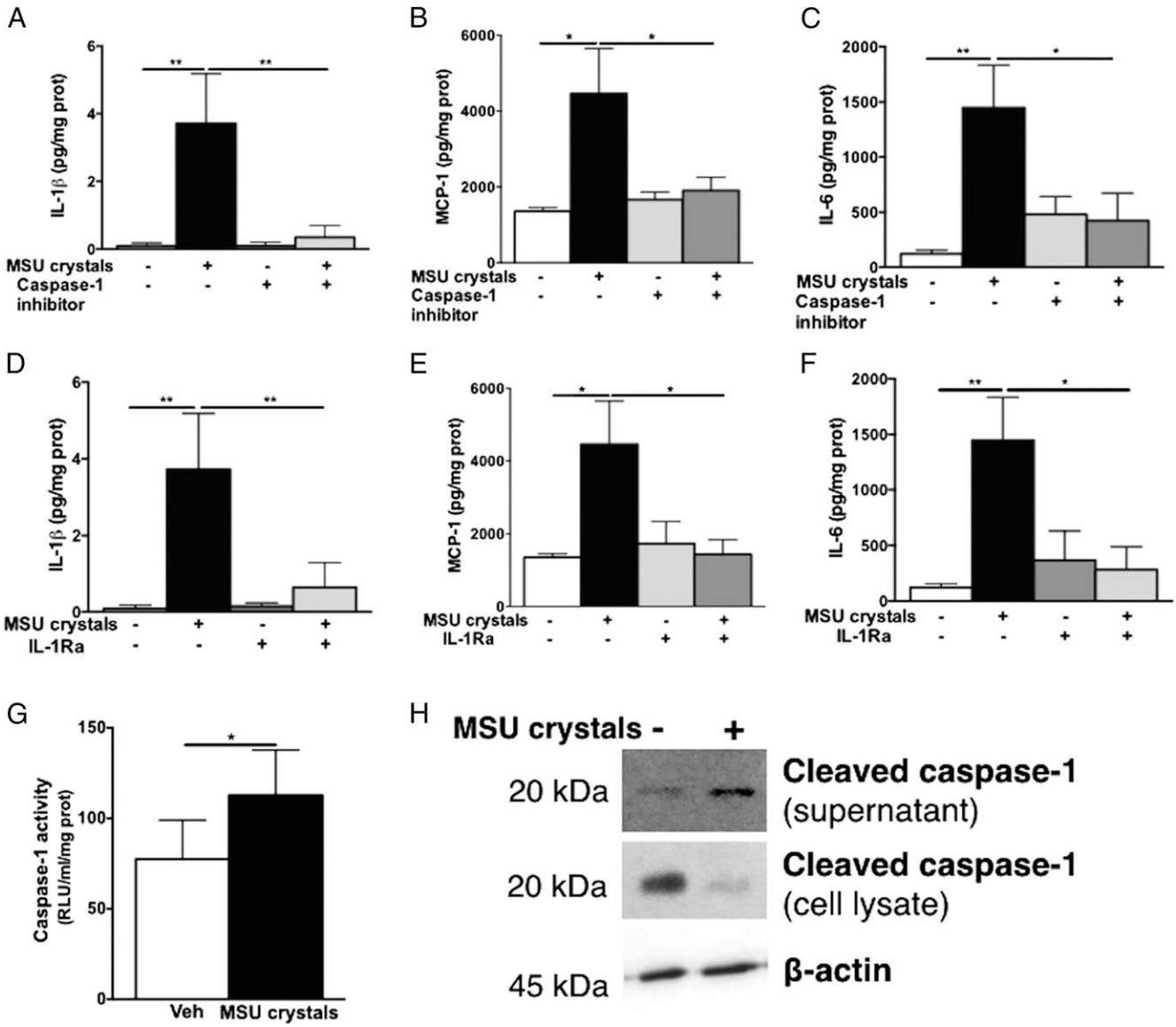


**FIGURE 2.** IL-1 dependent effects of MSU crystals on the inflammatory profile of term placental explants. In human term placental explants, IL-1β (**A**), MCP-1 (**B**), and IL-6 (**C**) secretion were all induced by MSU crystals and abrogated with the use of the caspase-1 inhibitor. The specificity to the IL-1 pathway was confirmed using the IL-1 receptor antagonist (IL-1Ra), which also decreased the secretion of IL-1β (**D**), MCP-1 (**E**) and IL-6 (**F**). Increased caspase-1 activity was detected in the supernatant (**G**) alongside increased cleaved caspase-1 in the supernatant and decreased in the lysate detected by western blot [representative example shown in (**H**)]. $n = 4–6$. Results presented as mean ± SEM. Statistical analysis by one-way ANOVA with Dunnett's multiple comparison posttest (A–F) or $t$ test with Welch correction (G). $^*p < 0.05$, $^{**}p < 0.01$.

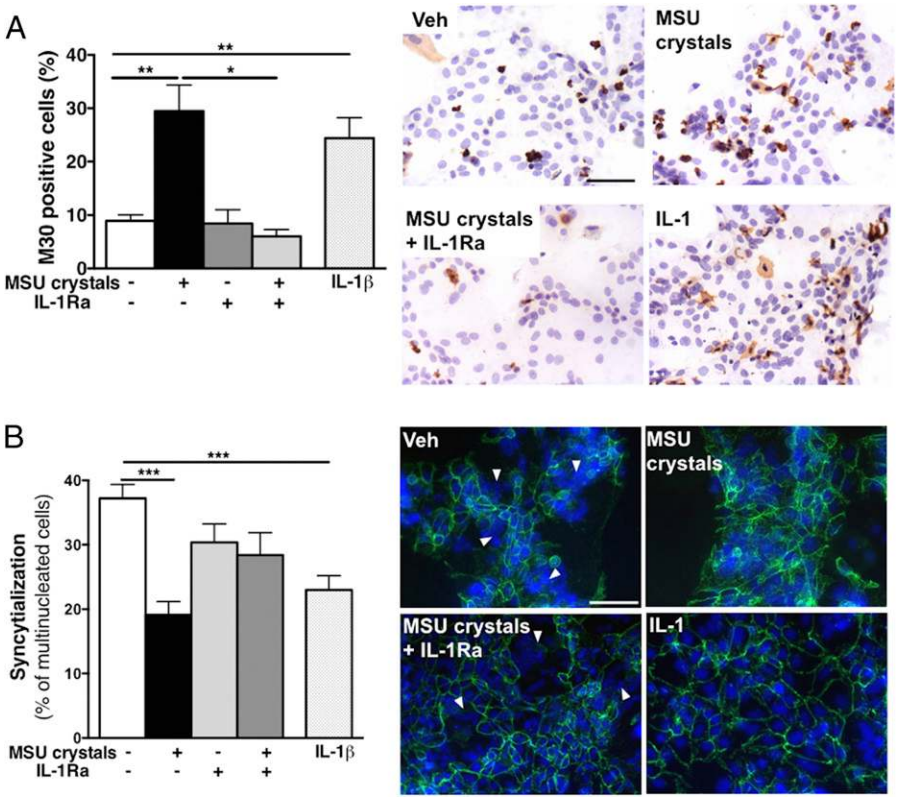


**FIGURE 3.** IL-1–dependent effects of MSU crystals of cytotrophoblast viability and syncytialization. Term human cytotrophoblast exposed to MSU crystals (100 μg/ml) showed increased number of M30+ apoptotic cells [brown staining in (**A**)] to a similar extent has exposure with IL-1β and this effect was dependent on IL-1 because blocking the IL-1 pathway using IL-1Ra protected cytotrophoblast against MSU crystal–induced cell death (A). Syncytialization (addressed by staining for desmoplakin and quantifying the percentage of multinucleated cells counted (white arrows) was decreased by MSU crystals or IL-1β treatment (**B**). $n$ = 4–6. Results presented as mean ± SEM. Statistical analysis by one-way ANOVA with Dunnett's multiple comparison posttest. Scale bar, 50 μm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

to G22–23 in rats) in pups from dams exposed to 500 or 1000 μg/kg/12 h of MSU crystals (4.82 ± 0.45 and 5.03 g ± 0.18 respectively as compared with 6.03 ± 0.09 in those vehicle exposed; Fig. 5A). Over 80% of the pups from MSU crystals exposed dams (1000 μg/kg, Fig. 5B) were below the fifth centile of the weight of pups from vehicle-exposed dams, whereas in those exposed to the lower dose of MSU crystals (250 μg/kg) only 20% of the pups were growth restricted (data not shown). MSU crystal exposure was associated with placental inflammation, significantly increased levels of IL-1β (5.4-fold increase versus vehicle, $p < 0.001$), TNF-α (5.9-fold increase

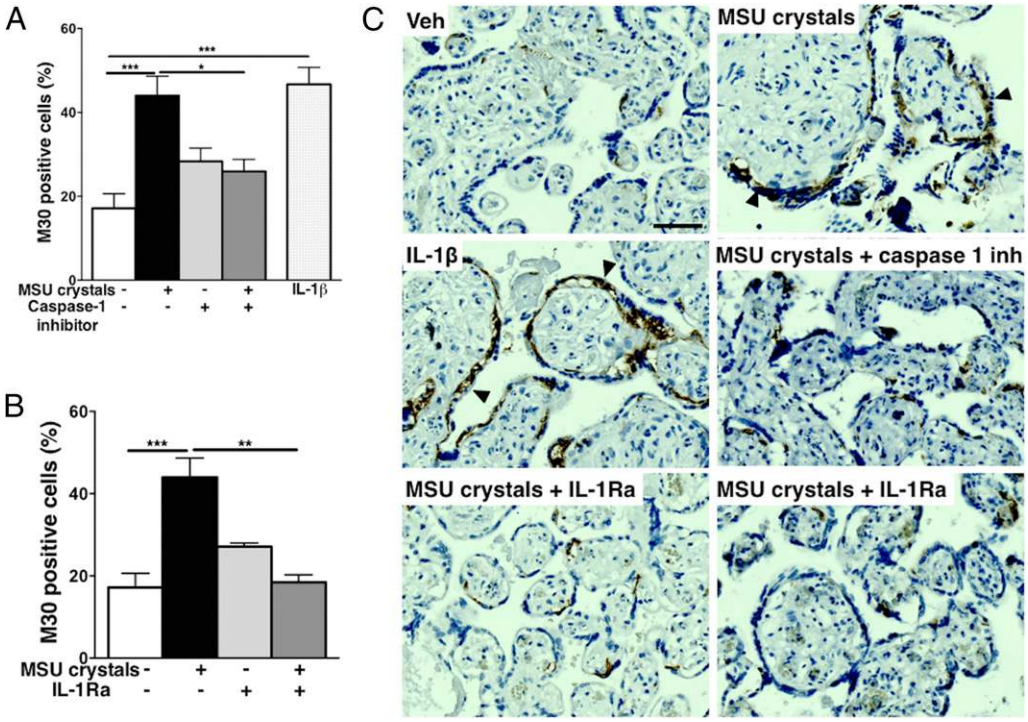


**FIGURE 4.** Effects of MSU crystals on human term placental explants. Term human placental explants exposed to MSU crystals or IL-1β displayed elevated apoptosis, which was blocked by caspase-1 inhibition (**A**) and IL-1Ra treatment (**B**). Representative examples of M30+ cells in explants are shown (**C**) with positive cells being mostly trophoblast (black arrowheads). $n$ = 6. Results presented as mean ± SEM. Statistical analysis by one-way ANOVA with Dunnett's multiple comparison posttest. Scale bar, 50 μm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

versus vehicle, $p < 0.05$), and IL-6 (6.2-fold increase versus vehicle, $p < 0.05$) proteins in placentas from dams exposed to the highest dose of MSU crystals (1000 μg/kg; Fig. 5C–E). Uric acid levels were significantly elevated within the placentas of dams exposed to the highest dose of MSU crystals (1000 μg/kg, $p < 0.001$) (Fig. 5F). However, uric acid levels in the maternal circulation was not significantly elevated by i.p. injection of MSU crystals combined with potassium oxonate (1.6 ± 0.27 mg/dl in dams exposed to MSU crystals 1000 μg/kg versus 1.0 ± 0.39 mg/dl in vehicle exposed, $p = 0.27$). Preterm birth was not observed in any of the experimental conditions.

There were no significant differences in placental weight or gross morphological analysis between control and MSU crystal–treated animals (Fig. 5G, 5H); however, immunohistochemical analysis revealed increased numbers of immune cells within the placenta (Fig. 6). This was predominantly observed within the junctional zone, where all doses of MSU crystals led to a significant elevation of monocytes or macrophages (CD68+ cells) and within the labyrinth (fetal side), where only the highest dose of MSU crystals induced a significant increase in CD68+ monocytes or macrophages (Fig. 6C). There was also an elevated number of macrophages within the labyrinth zone in placenta from dams exposed to the two highest doses of MSU crystals (Fig. 6C). There were only a small number of neutrophils (polymorphonuclear) detected; these cell numbers were similar in all experimental groups (data not shown). No immunoreactivity for CD3 (lymphocytes) was detected (data not shown). Similar to the ELISA data (see Fig. 5C), IL-1β immunoreactivity was elevated within the placentas exposed to MSU crystals, mainly in the junctional zone and in the labyrinth (fetal side) (Fig. 7). The majority of positive cells were CD68+ immune cells, with some immunostaining in trophoblast (data not shown).

## Discussion

Elevated uric acid has been strongly associated with several pathologies specific to pregnancy, such as PE, but also more recently, to FGR (14, 15, 17, 21–23). Even though uric acid (MSU) crystals are a known inducer of inflammation in inflammatory cells, their role at the maternal–fetal interface and potential involvement in prenatal inflammation is mostly unknown. We now provide evidence that this DAMP has a causative role in placental inflammation and dysfunction, as seen by the inflammatory profile, increased trophoblast death and impaired syncytialization in primary cytotrophoblasts and in placental explants. Furthermore, we confirmed the effects of MSU crystals in vivo in a newly developed animal model of noninfectious inflammation during pregnancy that led to reduced fetal growth with placental inflammation.

Inflammation during pregnancy has been strongly associated with pregnancy pathologies. Infection-induced inflammation leading to FGR, preterm birth or PE-like symptoms has been shown in animal models and studied in depth (9, 10, 34). However, in an important

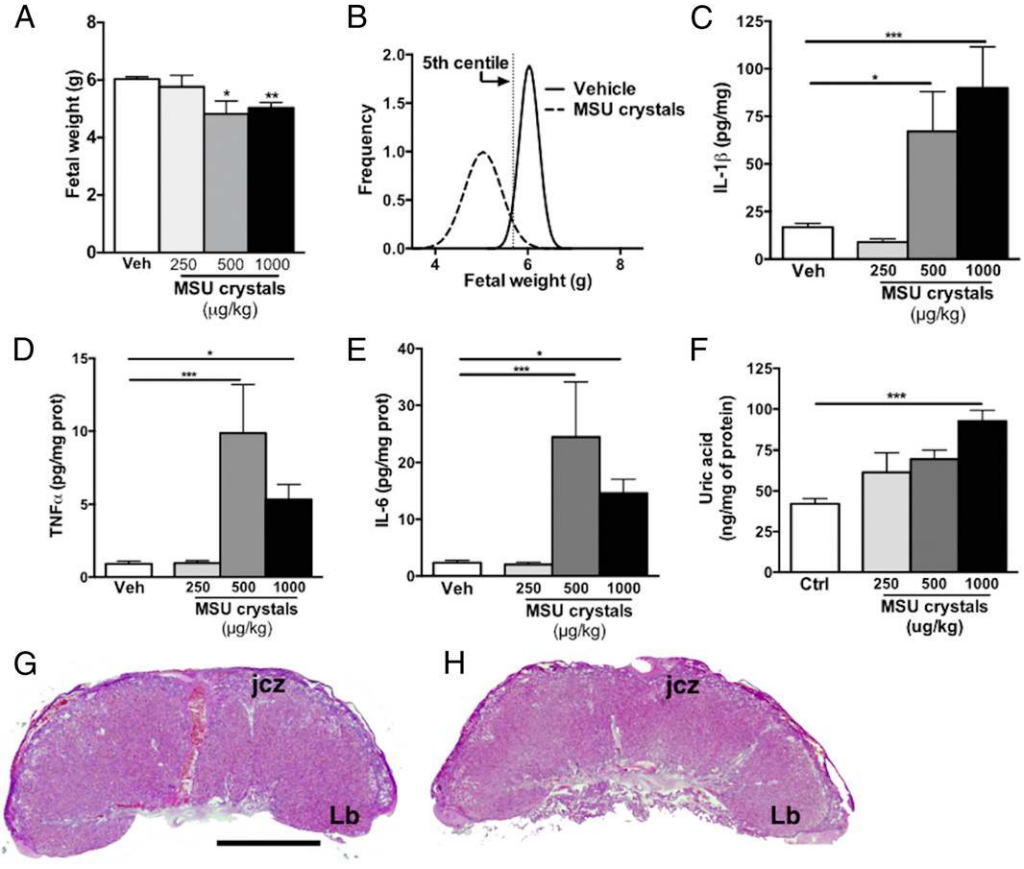


**FIGURE 5.** Maternal treatment with MSU crystals during late gestation in rats led to placental inflammation and FGR. Intraperitoneal administration of MSU crystals (500 μg/kg/12 h or 1000 μg/kg/12 h) at the end of gestation in rats significantly decreased fetal weight (**A**) with over 80% of the pups being below the 5th centile of pups from vehicle-exposed dams (**B**) (R square value for vehicle curve: 0.98 and MSU crystals curve: 0.87). The highest doses of MSU crystals led to increased placental inflammation with increased levels of IL-1β (**C**), TNF-α (**D**) and IL-6 (**E**) proteins. Levels of uric acid were significantly elevated in the placenta from dams exposed to the highest dose of MSU crystals (1000 μg/kg/12 h) (**F**). There was no difference between vehicle (**G**) and MSU crystal (**H**) exposed dams in morphological analysis of their placentas. At least six dams were used in each experimental group (48–84 fetuses in each experimental group). Results presented as mean ± SEM. Statistical analysis by one-way ANOVA with Dunnett's multiple comparison posttest. Scale bar, 1000 μm. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Jcz, junctional zone; Lb, labyrinth.

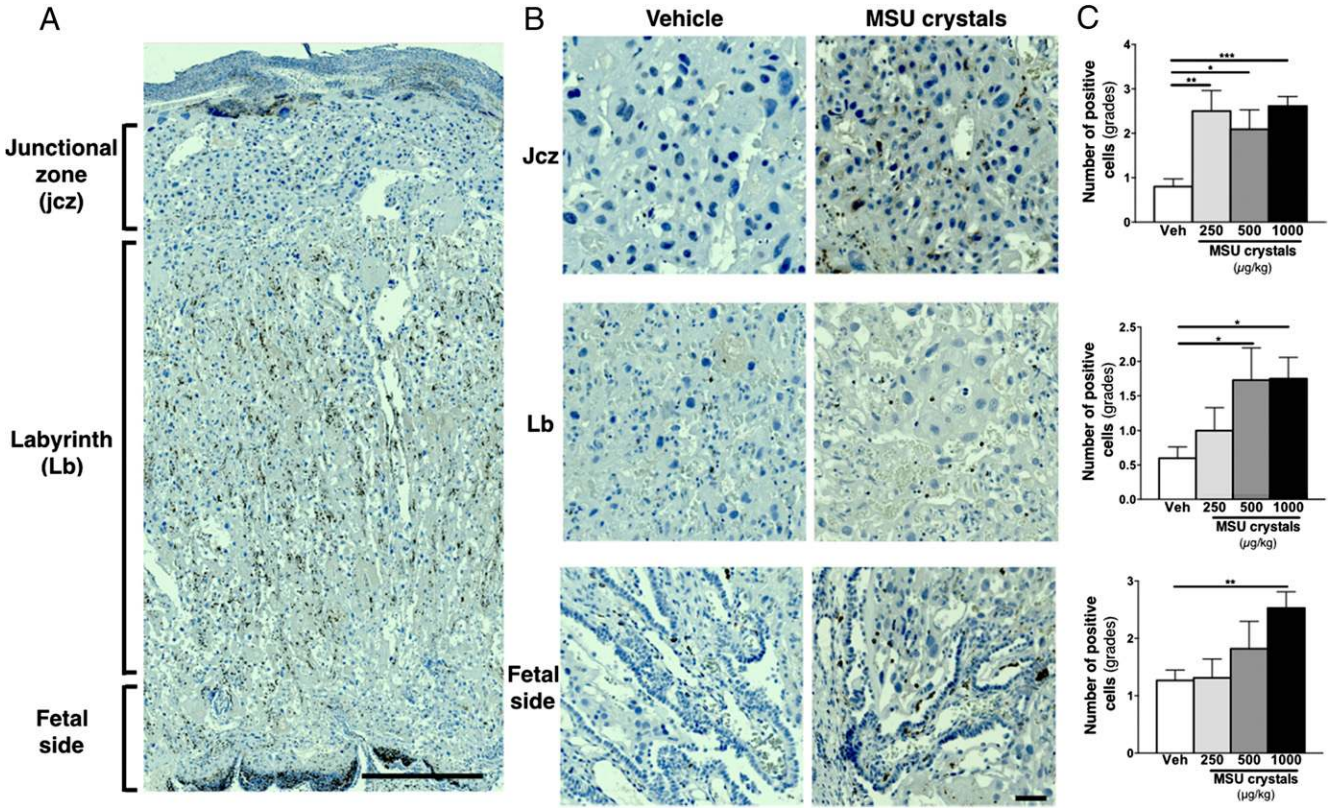


**FIGURE 6.** Histological analysis of CD68+ macrophage in the placenta following MSU crystal treatment. Injection of MSU crystals during gestation in rats increased the number of CD68+ macrophages in the junctional zone, the labyrinth and on the fetal side (**A**) with higher magnification in (**B**) (1000 μg/kg/12 h of MSU crystals shown in the pictures). Semiquantitative analysis of the numbers of CD68+ cells is shown in (**C**). Gradation scale: 0 = <5; 1 = 6–10; 2 = 11–15; 3 = 16–20 and 4 = more than 20 ($n$ = 6 dams per conditions; 3 placentas per dams). Results presented as mean ± SEM. Statistical analysis by one-way ANOVA with Dunnett's multiple comparison posttest. Scale bar, 500 μm (A); 50 μm (B). *$p < 0.05$, **$p < 0.01$.

proportion of women with pregnancy pathologies, infection is not detected even though evidence of inflammation (such as elevated cytokines) is still present. Alarmins or DAMPs can also induce inflammation through TLRs and NLRs, as the same receptors activated by pathogens (12, 13, 19, 20), and are increasingly associated with pathological pregnancies (14–18, 21–23, 29). Uric acid in particular has been strongly associated with PE with elevated levels detected in the maternal circulation (14, 15, 21, 29). Furthermore, our group also showed that uric acid was elevated in other pathologies of pregnancy, such as pregnancies with reduced fetal movements (17), without any hypertension or abnormal renal function. The inflammatory actions of MSU crystals have been described in immune cells, as an activator of the NALP3 inflammasome, upstream of caspase-1, leading to the production of IL-1β (25, 35, 36). Information on the action of MSU crystals at the maternal–fetal interface is scarce but it has been published that it does induce IL-1β secretion in a first-trimester trophoblast cell line through activation of caspase-1 and the NLRP3 inflammasome (26–28). NLRP3 activation by MSU crystals was also observed in monocytes from PE patients (37). This is in line with our current work, wherein we showed the role of caspase-1 in MSU crystal–induced IL-1β secretion in term primary cytotrophoblast cells and placental explants. The fact that caspase-1 inhibition completely blocked cytokine secretion in the presence of MSU crystals suggests that IL-1β, and not IL-1α, is involved because caspase-1 is not needed for IL-1α maturation. These findings also suggest that the secretion and extracellular actions of IL-1β are needed for the negative actions of MSU crystals on placental cells. In terms of placental function, uric acid has been shown to decrease placental system A nutrient transport in explants (29), which is in line with the effects on placental function we observed such as trophoblast apoptosis as well as decreased syncytialization. This strongly supports altered placental function with abrogated syncytiotrophoblast renewal mediated by MSU crystal action on placental cells.

Commonly used animal models of inflammation during pregnancy involve infectious or pathogenic induced inflammation, with bacterial LPS or the viral mimic polyinosinic:polycytidylic acid the most widely used (10, 38). These models have been very useful to show the causal link between prenatal inflammation and the negative effects on fetal development, and highlight the deleterious actions of inflammatory mediators on the placenta, central to the observed negative fetal effects (10, 38, 39). However, these models do not reproduce the most common cases observed in clinical situation in which infection is not detectable but markers of inflammation are still present. Recently, animal models were developed first using fetal DNA as a stimulus leading to inflammation and fetal demise (40) whereas another group used HMGB1 administration in the amniotic fluid, which induced preterm labor (41). Both HMGB1 and cell-free fetal DNA were shown by us and others to be elevated in pregnancy pathologies (14–18) in association with placental dysfunction. This evidence points toward an important role of endogenous mediators of inflammation in pregnancy pathologies. In the current study, we showed that MSU crystal administration to the rat reduces fetal growth in a dose-dependent fashion. To our knowledge, this is the first demonstration of such an effect and strongly suggests that uric acid is a potent inducer of placental inflammation or dysfunction leading to FGR, rather than just being elevated as a consequence of the pathology. This is supported by human studies that reported uric acid to be associated with the levels of inflammatory mediators in PE patients (42), and the levels of uric acid in the first and second trimester were shown to correlate with pregnancy complications and birthweight (42, 43, 44).

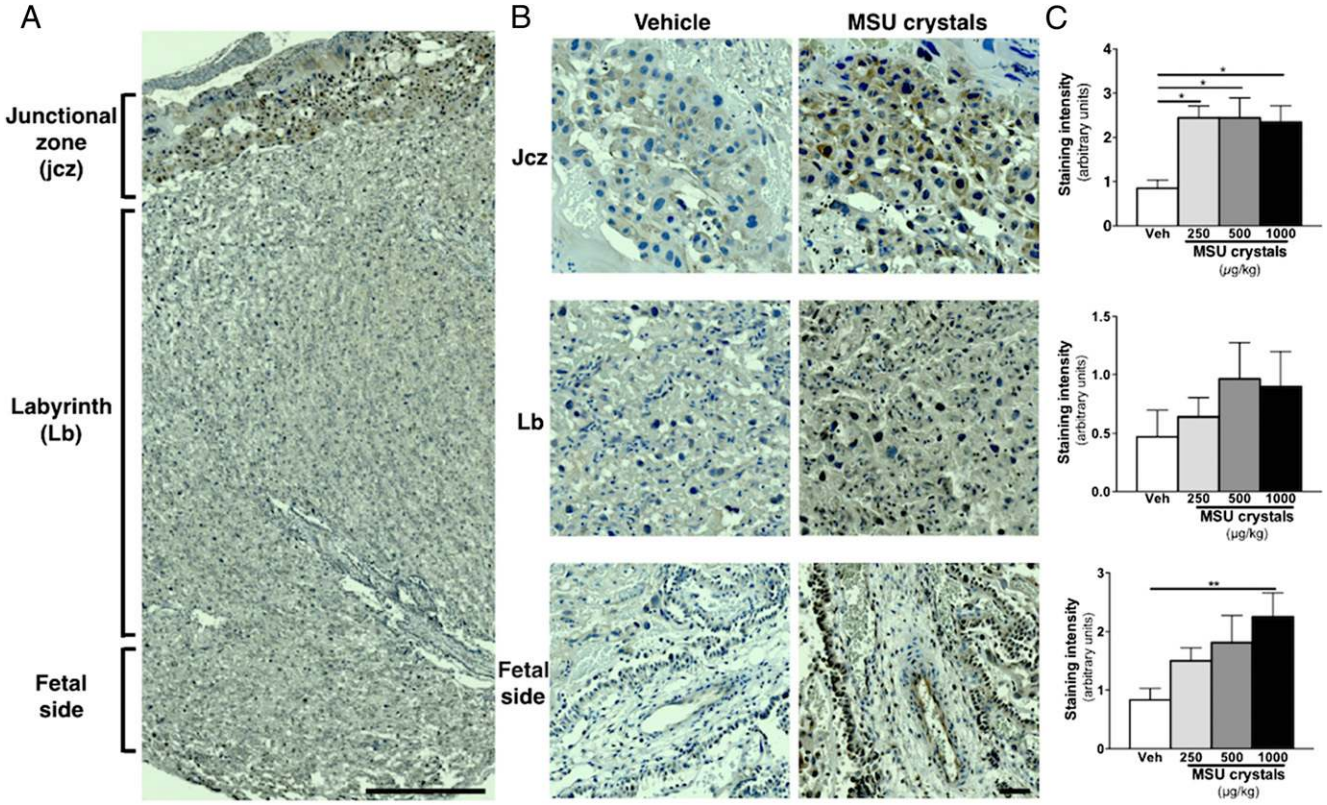


**FIGURE 7.** Histological analysis of IL-1β staining in the placenta following MSU crystals treatment. Injection of MSU crystals during gestation increased the staining intensity of IL-1β primarily in the junctional zone but also on the fetal side and in the labyrinth. Representative image shown in (**A**), with higher magnification of each region in (**B**) and quantification in (**C**) ($n$ = 6 dams per conditions; 3 placentas per dams). Results presented as mean ± SEM. Statistical analysis by one-way ANOVA with Dunnett's multiple comparison posttest. Scale bar, 500 μm (A); 50 μm (B). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

In conclusion, this study support the hypothesis that MSU crystals cause placental inflammation and dysfunction that leads to FGR. The work demonstrates the negative effects of MSU crystals within the human placenta, inducing inflammation and apoptosis while blocking syncytialization. Furthermore, we showed a causal role of MSU crystals in reducing fetal growth in an animal model of noninfectious inflammation during pregnancy. Importantly, we showed that the deleterious effects of MSU crystals were mediated via IL-1, which has many implications for the development of an effective therapy to tackle obstetrical disorders with placental dysfunction. Together these results strongly suggest an important role of MSU crystals and IL-1 in triggering placental inflammation with potential involvement in placental dysfunction leading to FGR.

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## Disclosures

The authors have no conflicts of interest to disclose.

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