Production and regulation of Interleukin-1 family cytokines at the materno-fetal interface.

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Keywords

Cytokines, IL-1 family, Placenta, Preterm labour, Reproductive Immunology.

Abbreviations

IL-1α, interleukin-1alpha; IL-1β, interleukin-1beta; IL-18, interleukin-18; IL-33, interleukin-33; IL-37, interleukin-37; IL-1Ra, IL-1 receptor agonist; IL-18BP, IL-18 binding-protein; IL-36Ra, interleukin-36 receptor agonist; IL-36R, interleukin-36 receptor; IL-38, interleukin-38; IL-1R, interleukin-1 receptor; ST2, interleukin-1 receptor-like 1; (s)ST2, soluble interleukin-1 receptor-like 1; IL-1RAcP, interleukin-1 receptor accessory protein; (s)IL-1RAcP, soluble interleukin-1 receptor accessory protein; PROM, premature rupture of membranes; PPROM, premature preterm rupture of membranes; ECS, elective caesarean section

Abstract

IL-1 family members regulate innate immune responses, are produced by gestation-associated tissues, and have a role in healthy and adverse pregnancy outcomes. To better understand their role at the materno-fetal interface we used a human tissue explant model to map lipopolysaccharide (LPS)-stimulated production of IL-1α, IL-1β, IL-18, IL-33, IL-1Ra, IL-18BPa, ST2 and IL-1RAcP by placenta, choriodecidua and amnion. Caspase-dependent processing of IL-1α, IL-1β, IL-18, and IL-33 and the ability of IL-1α, IL-1β, IL-18, and IL-33 to regulate the production of IL-1RA, IL-18BPa, ST2 and IL-1RAcP was also determined. LPS acted as a potent inducer of IL-1 family member expression especially in the placenta and choriodecidua with the response by the amnion restricted to IL-1β. Caspases-1, 4 and 8 contributed to LPS-stimulated production of IL-1β and IL-18, whereas calpain was required for IL-1α production. Exogenous administration of IL-1α, IL-1β, IL-18, and IL-33 lead to differential expression of IL-1Ra, IL-18BPa, ST2 and IL-1RAcP across all tissues examined. Most notable were the counter-regulatory effect of LPS on IL-1β and IL-1Ra in the amnion and the broad responsiveness of the amnion to IL-1 family cytokines for increased production of immunomodulatory peptides and soluble receptors. The placenta and membranes vary not only in their output of various IL-1 family members but also in their counter-regulatory mechanisms through endogenous inhibitory peptides, processing enzymes and soluble decoy receptors. This interactive network of inflammatory mediators likely contributes to innate defence mechanisms at the materno-fetal interface to limit, in particular, the detrimental effects of microbial invasion.

1. Introduction

Cytokines, including IL-1β, IL-8 and TNFα, produced by gestation-associated tissues (placenta and fetal membranes) play key roles in both physiological and pathophysiological labour (Patni, Flynn et al. 2007). PROM and preterm delivery of the baby is influenced by the overproduction of pro-inflammatory cytokines in response to bacterial infection (Peltier 2003). IL-1β was one of the first cytokines evaluated in association with intrauterine infection (Romero, Brody et al. 1989) and has been shown to weaken the fetal membranes by up-regulating apoptosis and essential metalloproteinases (Hoang, Potter et al. 2014).

IL-1β is a member of the larger 11-membered IL-1 superfamily of cytokines (Netea, Veerdonk et al. 2015). The IL-1 subfamily is comprised of IL-1α, IL-1β and IL-33, characterised by their larger precursor peptide length. The IL-18 subfamily members display smaller pro-peptides and include IL-18 and IL-37. Members of the IL-36 subfamily have the smallest pre-processed peptides and this family includes IL-36α, IL-36β, IL-36γ, IL-36Ra and IL-38 (Dinarello, Arend et al. 2010). In addition to these three subfamilies of cytokines, IL-1Ra is also included within the IL-1 superfamily.

A common feature of IL-1 family members is their signalling mechanisms, which are initiated through a collection of structurally similar receptors containing intracellular Toll/IL-1 receptor (TIR) and extracellular immunoglobulin-like (Ig) domains (Sims and Smith 2010). Despite these similarities, IL-1 superfamily members differ in how they are processed. IL-1β is initially translated as inactive pro-IL-1β that requires cleavage by a caspase-containing inflammasome for both secretion and biological activity (Mariathasan, Newton et al. 2004). In contrast, IL-1α does not require the removal of a signal peptide for bioactivity and processing of pro-IL-1α precursor occurs primarily by calpain, a calcium-dependent cysteine protease (Miller, Schattenberg et al. 1994). The processing of pro-IL-18 occurs in a similar inflammasome-dependent manner to IL-1β (Guo, Callaway et al. 2015). Like other IL-1 family cytokines, IL-33 is synthesized as a pro-peptide but the role of caspases, calpain and/or other enzymes awaits clarification (Cayrol and Girard 2009, Hayakawa, Hayakawa et al. 2009, Zhao and Hu 2010, Lefrançais, Roga et al. 2012).

IL-1β, IL-18, IL-33, IL-1Ra, IL-18BP, ST2 and IL-36Ra all have been implicated in physiology and pathophysiology of pregnancy. The placenta and/or fetal membranes variably express them constitutively or upon activation (Romero, Brody et al. 1989, Ida, Tsuji et al. 2000, Granne, Southcombe et al. 2011, Topping, Romero et al. 2013, Southcombe, Redman et al. 2015). As cytokine production is critical to the success of human pregnancy but also implicated in unfavourable obstetric outcomes such as preterm birth and preeclampsia, better understanding of the role that IL-1 family cytokines play at the materno-fetal interface is

essential. To examine this, an explant model of term placenta, choriodecidua and amnion was utilised to determine the production, processing and counter regulation of various IL-1 superfamily members.

2. Methods

2.1. Placental samples

Placenta and fetal membrane samples were collected from healthy term newborns (>37 weeks of gestation) delivered by ECS at Singleton Hospital, Swansea, UK. Written consent was obtained from all study participants following recruitment at the antenatal day assessment unit. Ethical approval for this study was given by Wales Research Ethics Committee 6 (REC No. 11/WA/0040).

2.2. Tissue explant culture

Choriodecidua and Amnion. The membrane was detached from the placenta, separated by blunt dissection and washed repeatedly in sterile Ca^{2+} / Mg^{2+} free phosphate buffered saline (PBS; Life Technologies) to remove blood. Explants were cut with an 8-mm biopsy punch (Stiefel): two discs of choriodecidua placed into 0.5ml Advanced RPMI supplemented with 2mM Glutamax, 2mM penicillin streptomycin fungisone (PSF), 5mM 2-mercaptoethanol (2-ME; all Life Technologies) and 2% fetal bovine serum (FBS; Hyclone) and 5mM 2-mercaptoethanol; and four discs of amnion placed into 0.5 ml Advanced DMEM (Life Technologies) supplemented with 2mM Glutamax, 2mM PSF and 2% FBS.

Placenta. 1cm^3 pieces of placental tissue were taken from different sites across the placenta. Tissue pieces were washed in PBS and further diced into smaller 1-2mm^3 segments. Diced placenta tissue pieces (0.2g) were placed into 1ml Advanced RPMI supplemented with 2mM Glutamax, 2mM PSF, 2% FBS and 5mM 2-ME.

Explant cultures were exposed to different stimuli as detailed in the results; an unstimulated control was always included. The following concentrations of stimuli were used; LPS (10 ng/ml; ultrapure from *E. coli 0111:*B4, Invivogen, USA), rhIL-1α, rhIL-1β, rhIL-33 (all 10 ng/ml; Miltenyi Biotec, UK) and rhIL-18 (10 ng/ml; Invivogen, USA). For inhibition experiments cultures were treated with inhibitors for caspases 1, 4 and 8 (Z-WEHD-FMK, Z-YVAD-FMK, Z-IETD-FMK; 5 µM, R&D Systems) or calpain (PD 150606; 1 µM, Tocris) 30 min before the addition of LPS. DMSO was used as a vehicle control. Cellular cytotoxicity, determined by lactate dehydrogenase assay (Abcam), was not observed with addition of inhibitors or DMSO (data not shown). All treatments were performed in duplicate. Cultures were incubated for 24 hours at 37°C in 5% CO_2. Tissue free supernatants were collected by centrifugation for 7 minutes at 4°C, 515 x g and stored at -20°C until analysis using cytokine specific ELISAs.

2.3. Cytokine production

IL-1α, IL-1β, IL-33, IL-1Ra, IL-18BP, IL-1RAcP, and ST2 in tissue free supernatants of placenta, choriodecidua and amnion explant cultures collected after 24 h were measured using commercially available ELISA kits (DuoSet, R&D Systems) as per manufacturer's instructions. For IL-18, a sandwich ELISA using monoclonal anti-human IL-18 antibody (2 µg/ml; R&D Systems) and a biotinylated anti-human IL-18 antibody (0.5 µg/ml; R&D Systems) was performed as described previously with slight modification (Jitprasertwong, Jaedicke et al. 2014).

2.4. Data analysis

All experiments were performed a minimum of three times in duplicate with data presented as mean ± SEM. Statistical significance was calculated using GraphPad Prism (Version 6, GraphPad Software Inc, USA) with appropriate statistical testing determined for each experimental output as detailed in the relevant figure legends. A *p*-value of ≤ 0.05 was considered significant.

3. Results

3.1. Cytokine output of gestational tissues following lipopolysaccharide stimulation

To examine the production of IL-1 family cytokines by gestation-associated tissues we utilised LPS at a concentration of 10 ng/ml (Patni, Wynen et al. 2009). The effects of LPS on levels of IL-1α, IL-1β, IL-18, IL-33, IL-1Ra, IL-18BPa, (s)ST2 and IL-1RAcP from the placenta, choriodecidua and amnion are summarised in Table 1. In the placenta and choriodecidua, there was a significant LPS-stimulated increase in the production of IL-1α, IL-1β, IL-18, and IL-33 (Figure1 A-H); in the amnion only IL-1 β was increased significantly (Figure 1 I-L). IL-1Ra, IL-18BPa, (s)ST2 and IL-1RAcP were produced from unstimulated cultures for all three tissue types. LPS induced a significant increase in IL-1Ra in the placenta (Figure 2A) and choriodecidua (Figure 2E) but a decrease in the amnion (Figure 2I). There was no effect of LPS on IL-18BPa levels from the placenta, choriodecidua or amnion (Figure 2 B, F & J). LPS down-regulated ST2 levels in the placenta (Figure 2C) but not in the choriodecidua or the amnion (Figure 2G&K). IL-1RAcP levels were decreased in response to LPS in the placenta (Figure 2D) and amnion (Figure 2L) but the choriodecidua showed no change (Figure 2H).

3.2. Caspase-dependent and -independent processing of IL-1 family cytokines by gestational tissues

Processing of IL-1β and IL-18 is associated with activity of several initiator caspases (caspase-1, -4, -5, -8 and -12) either directly by canonical and non-canonical inflammasomes or indirectly by modulating the activation of these inflammasomes (Man and Kanneganti, 2016). Caspase activity can also modulate the production and /or activity of IL-1α and IL-33 (Cayrol and Girard 2009, Madouri, Guillou et al. 2015, Man and Kanneganti 2016). To determine caspase involvement in the production of IL-1 family cytokine production by gestation-associated tissues pharmacological inhibitors were used (caspase-1, -4 and -8). As amnion produced IL-1β only, IL-1α, IL-18 and IL-33 were not analysed for this tissue. Caspase inhibition had no effect on LPS-stimulated IL-1α levels in the placenta (Figure 3A) or choriodecidua (Figure 3E). LPS-stimulated levels of IL-1β and IL-18 in all tissues studied were reduced significantly in the presence of inhibitors for each of the caspases of interest (Figure 3B, C, F, G & I). Inhibition of caspases-1, 4 and 8 had no effect on LPS-stimulated IL-33 in either the placenta (Figure 2D) or choriodecidua (Figure 2H).

The involvement of calpain in gestational IL-1α processing was examined using a selective inhibitor (PD150606, (Low, Karunan Partha et al. 2014)) (Figure 4). As expected, inhibition of calpain reduced LPS-stimulated IL-1α levels in the placenta and choriodecidua (the amnion was not studied). There was no effect on the production of IL-1β, IL-18 and IL-33.

3.3. Counter-regulation of IL-1 family cytokines

IL-1 family cytokines exhibit a broad spectrum of potent biological activities across a range of different cells (Afonina, Muller et al. 2015) so their activity is tightly regulated via a number of mechanisms. The production of soluble (and ligand) decoy receptors and cytokine receptor antagonists provides an effective and efficient means of controlling their inter-cellular signalling following the generation of an immune response and in a range of physiological processes. Here we examined the effects of IL-1α, IL-1β, IL-18 and IL-33 on IL-Ra, IL-18BPa, (s)ST2 and (s)IL-1RAcP levels in gestational tissues. These effects are summarised in Table 2. For the placenta, IL-1α and IL-1β induced a significant increase in IL-1Ra (Figure 5A) and IL-18 an increase in IL-18BPa (Figure 5B); there were no other effects seen. The choriodecidua and amnion showed more responsiveness than the placenta. For the choriodecidua IL-1α, IL-1β and IL-18 induced a significant increase in IL-1Ra (Figure 5E), IL-1α, IL-1β a significant increase in IL-18BPa (Figure 5F), IL-1α, IL-1β and IL-33 a significant increase in ST2 (Figure 5G), and IL-33 a significant decrease in IL-1RAcP (Figure 5H). For the amnion, IL-1α, IL-1β, IL-18 and IL-33 all induced a significant increase in IL-1Ra (Figure 5I), IL-1 β, IL-18 and IL-33 induced an increase in IL-18BPa (Figure 5J), and all four cytokines induced an increase in IL-1RAcP (Figure 5L) but had no effect on ST2 levels (Figure 5K).

4. Discussion

The placenta, choriodecidua and amnion were all responsive to LPS as expected (Patni, Wynen et al. 2009, Miller and Loch-Caruso 2010, Hoang, Potter et al. 2014). IL-1α expression by choriodecidua has been reported (Zaga-Clavellina, Garcia-Lopez et al. 2007, Holcberg, Amash et al. 2008, Hoang, Potter et al. 2014) but greater abundance of IL-1α in the decidua (Huleihel, Amash et al. 2004) contrasts with our study with similar levels in placenta and choriodecidua. This likely reflects differences in the experimental approach. Basal production of IL-18 by various cell types within the placenta has been described in a perfusion model but with no stimulating effect of LPS (Amash, Huleihel et al. 2007). The difference to the placental LPS-stimulated IL-18 production seen in our study might reflect the experimental model or mode of delivery (Ida, Tsuji et al. 2000). Constitutive expression of *IL-18* mRNA and immunohistochemical localisation within the choriodecidua also has been described but the effects of LPS were not considered although PPROM or histological chorioamnionitis did not alter levels (Polettini, Vieira et al. 2012). IL-33 expression has been described previously in various cell types within the placenta and choriodecidua (Fock, Mairhofer et al. 2013, Topping, Romero et al. 2013) but we are the first to show that LPS increases IL-33 levels. Placental and decidual macrophages are key IL-33 producers and increase production in response to IL-1β (Fock, Mairhofer et al. 2013) so it is perhaps not surprising that LPS also increases IL-33. IL-33 might have a protective effect in pregnancy, perhaps mediated through enhancing the anti-oxidant environment (Zhang, Xie et al. 2012), with down-regulated IL-33-dependent signalling through increased (s)ST2 described in preeclampsia (Granne, Southcombe et al. 2011).

Some IL-1 superfamily members require enzymatic processing from an immature to mature form for biological activity. Caspase-1 inflammasomes have been studied extensively but additional caspases allow inflammasomes to operate in a caspase-1-independent manner (Kayagaki, Warming et al. 2011). Therefore, we considered the effects of inhibiting caspases-1, 4 and 8 in gestation-associated tissues. Each inhibitor led to decreased expression of LPS-stimulated IL-1β in all tissues and IL-18 in placenta and choriodecidua but had no effect on IL-1α or IL-33. Caspase-1 is reported to inactivate IL-33 (Cayrol and Girard 2009) and caspase-4 modulates the expression of IL-1α in monocytes (Vigano, Diamond et al. 2015) but we saw no evidence of these in our setting. Caspases might have role in adverse pregnancy outcomes: caspase-4 is increased in preeclamptic syncytiotrophoblast villi undergoing release of apoptotic debris (Burton and Yung 2011); caspase-3 and caspase-8 have been implicated in apoptosis in the placenta (Sokolov, Kolobov et al. 2009).

There is much interest in the natural and therapeutic capability of anti-inflammatory cytokines to modulate the inflammatory response within gestation-associated tissues (Bryant, Spencer-Harty et al. 2017). Therefore, regulation of IL-1Ra, IL-18BP, sST2, and IL-1RAcP was investigated. All of these were produced in relative abundance under basal conditions. While this could reflect a damage response in the tissue in response to delivery and handling we suggest, based on observations of the abundance of IL-1Ra in amniotic fluid (Romero, Sepulveda et al. 1992), that it represents genuine constitutive production of these mediators by the amnion. LPS stimulated IL-1Ra in the placenta and choriodecidua as described already (Fidel, Romero et al. 1994, Holcberg, Amash et al. 2008, Girard, Tremblay et al. 2010) but decreased amnion IL-1Ra. Basal expression of IL-1Ra by amnion has been described (Holcberg, Amash et al. 2008) but this is the first time the effects of LPS have been shown.

IL-18BPa, sST2, and IL-1RAcP are much less studied members of the IL-1 family with broad counter-regulatory effects. The endogenous inhibitory protein IL-18BPa acts in a similar manner to IL-1Ra but binds with high affinity to the IL-18R complex (Garlanda, Dinarello et al. 2013). The (s)ST2 splice variant acts as a decoy receptor, inhibiting ST2/IL-1RAcP signalling (Garlanda, Dinarello et al. 2013). IL-1RAcP is required for IL-1 cytokine binding to receptors and downstream signalling and activation of IL-1 responsive genes. (s)IL-1RAcP contributes to counter-regulation through increasing the affinity of IL-1 cytokines for the IL-1R2 decoy receptor (Garlanda, Dinarello et al. 2013). IL-18BPa, sST2, and IL-1RAcP were produced by all three tissue types. To date, the expression of IL-18BPa, sST2, and IL-1RAcP has been observed in the placenta (Granne, Southcombe et al. 2011, Topping, Romero et al. 2013, Southcombe, Redman et al. 2015), yet only sST2 mRNA expression has been noted in the chorio-amniotic membranes (Topping, Romero et al. 2013, Stampalija, Chaiworapongsa et al. 2014). This is the first study to identify basal expression of IL-18BPa and IL-1RAcP by the amnion and choriodecidua. Where LPS had an effect – placental ST2 and IL-1RAcP, and amnion IL-1RAcP – it was inhibitory. Given that these molecules tend to inhibit the inflammatory response it is not surprising that a potent pro-inflammatory agent such as LPS caused a decrease in anti-inflammatory mediators. Other than choriodecidua IL-1RAcP, any effect of IL-1α, IL-1β, IL-18 and IL-33 on IL-1RA, IL-18BPa, ST2, and IL-1RAcP tended to be stimulatory. This indicates that LPS-mediated inhibition of counter-regulatory IL-1 family members is not driven by the inflammatory members of the IL-1 family through negative feedback but via some other, as yet undetermined, pathway. Differences in the responsiveness of the placenta, choriodecidua and amnion were noted and this might warrant further investigation of receptor complexes and downstream signalling pathways. Also, given the abundance of IL-1Ra, IL-18BP, ST2 and IL-1RAcP under basal conditions studies wherein these are neutralised during the response to LPS or other inflammatory stimuli would provide

insight into their role in endogenous regulation of inflammation within gestation-associated tissues.

Other IL-1 family members, including IL-36, IL-37 and IL-38, were not analysed in this study. Expression of all members of the IL-36 subfamily (IL-36α, IL-36β, IL-36γ, IL-36Ra and IL-38) has been described for the placenta, with increased circulating IL-36Ra observed in pregnant women (Southcombe, Redman et al. 2015). The effect of LPS on IL-36 sub-family expression has yet to be documented in gestational tissues and would be worth further consideration especially given their potential contribution to an anti-inflammatory milieu. Similar to IL-1α and IL-33, processed IL-37 acts as a dual function cytokine with intracellular (nuclear translocation) and extracellular (secreted) anti-inflammatory properties (Nold, Nold-Petry et al. 2010, Bulau, Nold et al. 2014). The placenta expresses IL-37 with a reported increase in preeclampsia placentas (Southcombe, Redman et al. 2015).

5. Conclusion

LPS is a potent inducer of IL-1 family cytokine production in gestation-associated tissues *ex vivo.* Once stimulated, the expression profiles and regulatory systems of bioactivity vary in each tissue suggesting difference in receptor clustering and tissue-specific negative regulatory processes. As one of the most biologically potent families of cytokines the IL-1 family is subject to tight regulation through the presence of signal peptides, natural receptor antagonists, signalling inhibitors and soluble decoy receptors. A role for IL-1 family cytokine dysregulation in various diseases and obstetric complications such as pre-term birth, PPROM and preeclampsia is implicated in many studies (Romero, Sepulveda et al. 1992, Polettini, Vieira et al. 2012, Stampalija, Chaiworapongsa et al. 2014, Southcombe, Redman et al. 2015). Understanding not only the link between IL-1 family members and the maternal environment, but also the role played in orchestrating both adaptive and innate immune responses may be important for a range of translational outlooks in both pregnancy and inflammatory disorders.

Acknowledgments

We are grateful to all the staff in the antenatal day assessment unit and on the delivery suite at Singleton Hospital Swansea for their tireless help in the collection of samples. Thanks also to all the women who agreed to the use of their placentas for this study. This research was supported by an Abertawe Bro Morgannwg University Health Board NHS Research & Development Pathway to Portfolio grant.

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Figure 1. Lipopolysaccharide-induced production of IL-1 family agonists by term non-laboured gestation-associated tissues. Explants of placenta (A-D), choriodecidua (E-H) and amnion (I-L) were cultured for 24h in basal media ± LPS, with supernatants examined for IL-1α, IL-1β, IL-18 and IL-33 using specific ELISAs (pg/ml ± SEM; n=10). Statistically significant differences were determined by paired t-test; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001, **** ≤ 0.001.

Figure 2. Lipopolysaccharide-induced production of IL-1 family antagonists by term non-laboured gestation-associated tissues. Explants of placenta (A-D), choriodecidua (E-H) and amnion (I-L) were cultured for 24h in basal media ± LPS, with supernatants examined for IL-1Ra, IL-18BPa, ST2 and IL-1RAcP using specific ELISAs (pg/ml ± SEM; n=10). Statistically significant differences were determined by paired t-test; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001, **** ≤ 0.001.

Figure 3. The involvement of different caspases in lipopolysaccharide-induced IL-1 family cytokine production by term non-laboured gestation-associated tissues. Explants of placenta (A-D), choriodecidua (E-H) and amnion (I) were cultured for 24h in basal media ± LPS, Z-WEHD-FMK (caspase-1 inhibitor), Z-YVAD-FMK (caspase-4 inhibitor), Z-IETD-FMK (caspase-8 inhibitor) or vehicle (DMSO) control; supernatants were examined for IL-1α, IL-1β, IL-18 and IL-33 using specific ELISAs (pg/ml ± SEM; n=4). Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparison test; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001.

Figure 4. The involvement of calpain in lipopolysaccharide-induced cytokine production by term non-laboured gestation-associated tissues. Explants of placenta (A-D), choriodecidua (E-H) and amnion (I) were cultured for 24h in basal media ± LPS, PD150606 (calpain inhibitor) or vehicle (DMSO) control; supernatants were examined for IL-1α, IL-1β, IL-18 and IL-33 production (pg/ml ± SEM; n=4). Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparison test; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001.

Figure 5. Balancing innate immune responses and uncontrolled inflammation in term non-laboured gestation-associated tissues. Explants of placenta (A-D), choriodecidua (E-H) and amnion (I-L) were cultured for 24h in basal media ± IL-1α, IL-1β, IL-18 and IL-33 (10 ng/ml). Supernatants were examined for the production of IL-1Ra, IL-18BPa, ST2, and IL-1RAcP using specific ELISAs (pg/ml ± SEM; n=5-10). Statistically significant differences were determined by one-way ANOVA with Dunnett's multiple comparison test; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001. **** ≤ 0.001.

Figure 1

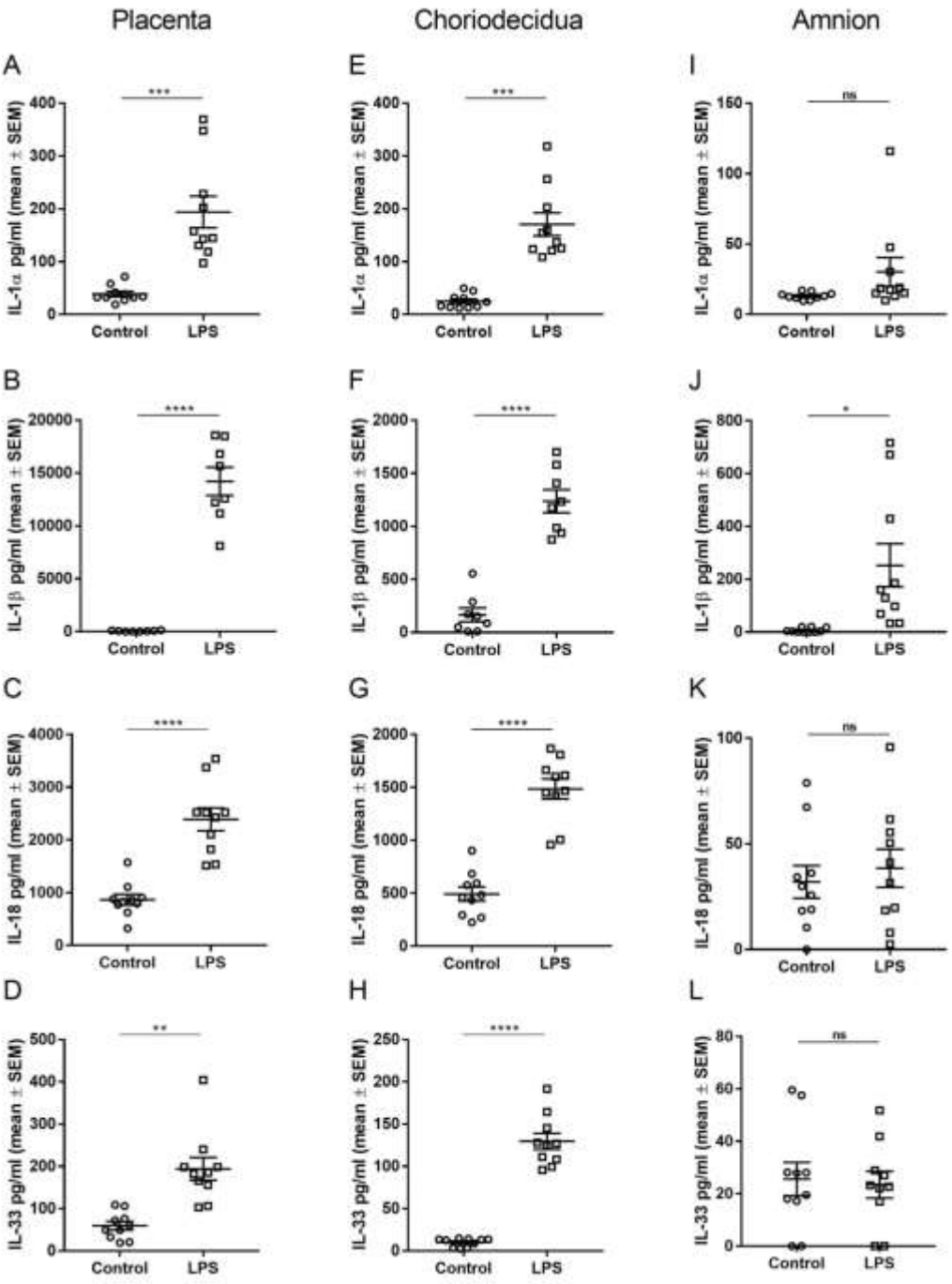

Placenta
Choriodecidua
Amnion
A
E
I
B
F
J
C
G
K
D
H
L
IL-1α pg/ml (mean ± SEM)
IL-1β pg/ml (mean ± SEM)
IL-18 pg/ml (mean ± SEM)
IL-33 pg/ml (mean ± SEM)
Control
LPS
***
****
**
ns
*

Figure 2

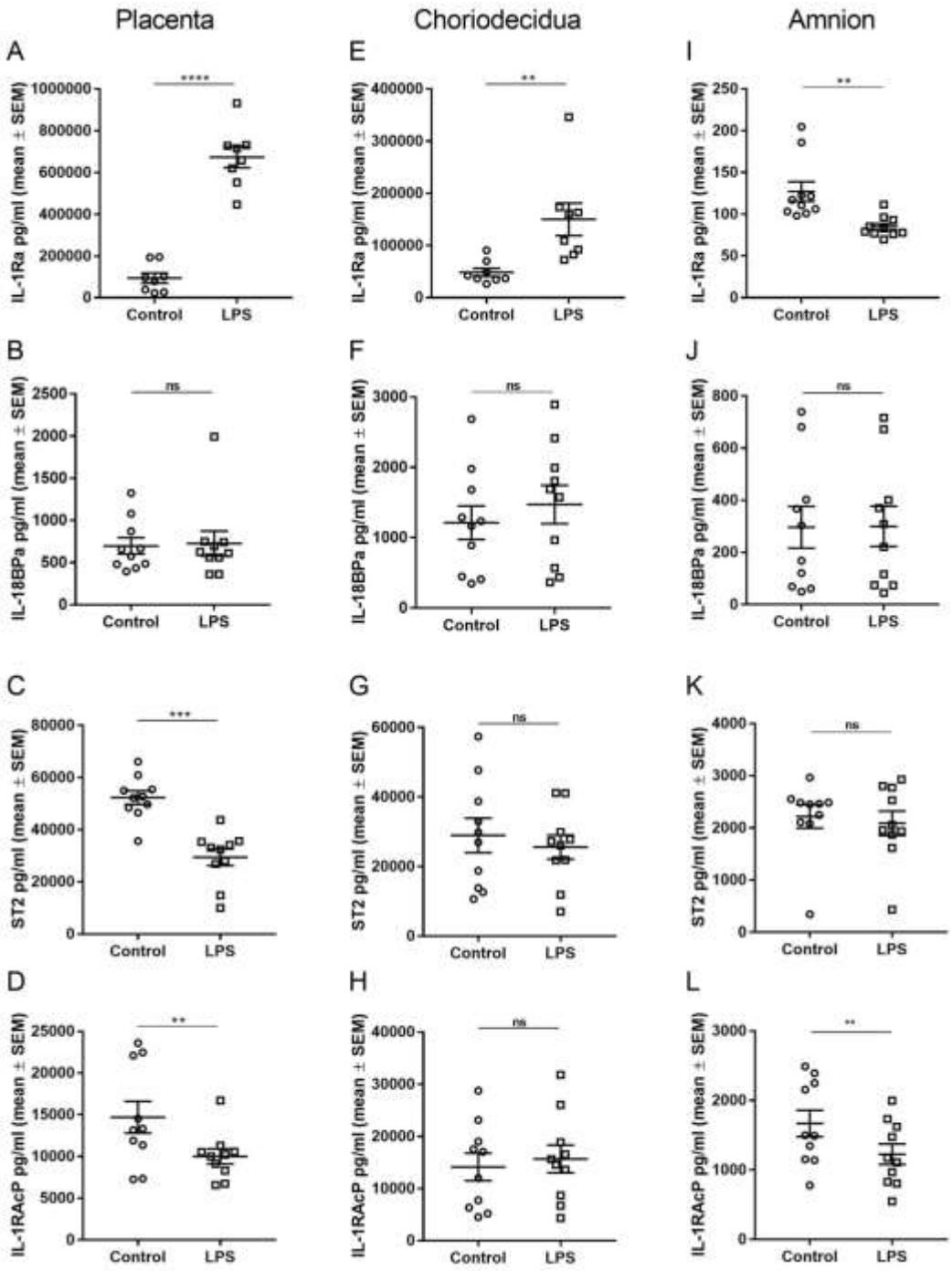

Placenta
Choriodecidua
Amnion
A
E
I
IL-1Ra pg/ml (mean ± SEM)
****
**
**
Control
LPS
B
F
J
IL-18BPa pg/ml (mean ± SEM)
ns
C
G
K
ST2 pg/ml (mean ± SEM)
***
ns
D
H
L
IL-1RAcP pg/ml (mean ± SEM)
**
ns
**

Figure 3

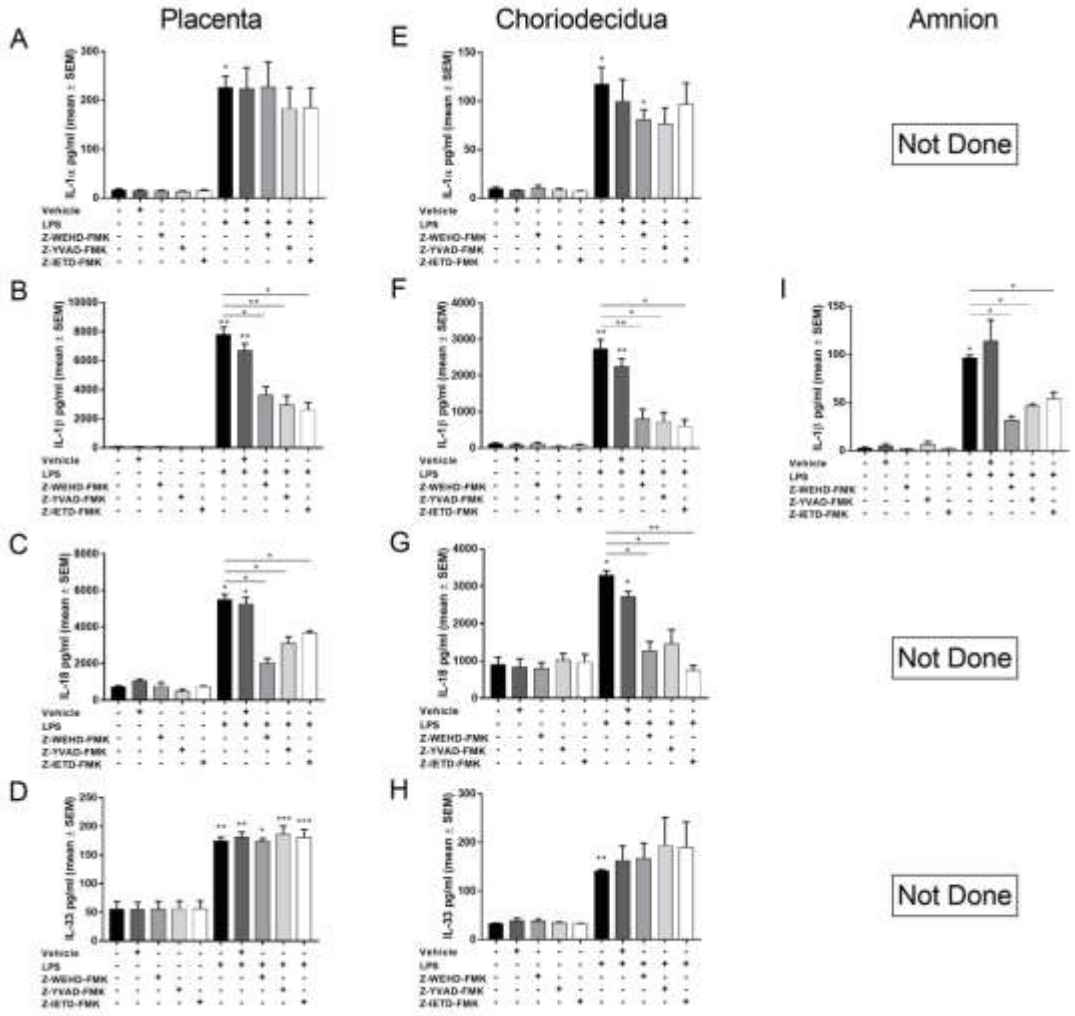
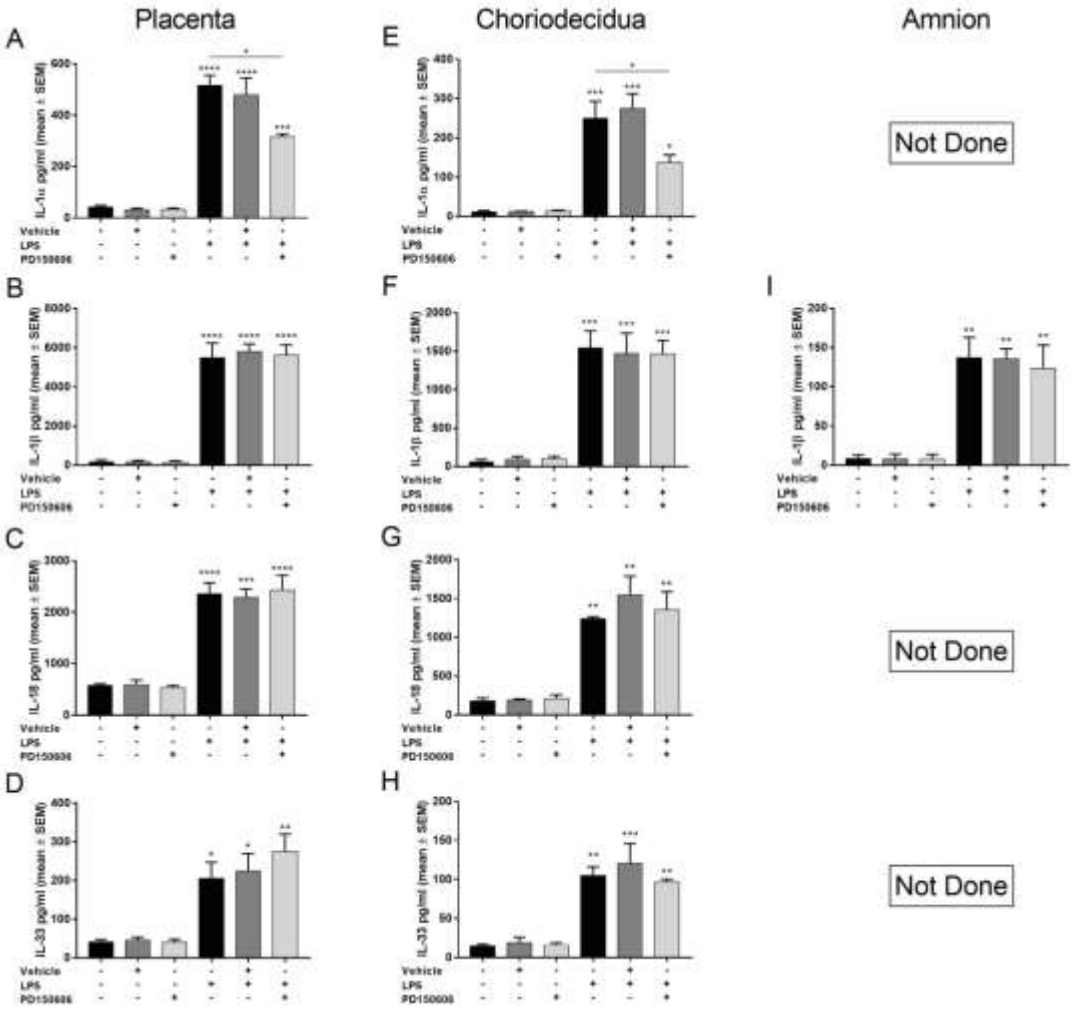

Placenta
Choriodecidua
Amnion
A
B
C
D
E
F
G
H
I
Not Done
Not Done
Not Done
IL-1α pg/ml (mean ± SEM)
IL-1β pg/ml (mean ± SEM)
IL-18 pg/ml (mean ± SEM)
IL-33 pg/ml (mean ± SEM)
Vehicle
LPS
Z-WEHD-FMK
Z-YVAD-FMK
Z-IETD-FMK

Figure 4


Placenta
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Amnion
A
B
C
D
E
F
G
H
I
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Not Done
Not Done

Figure 5

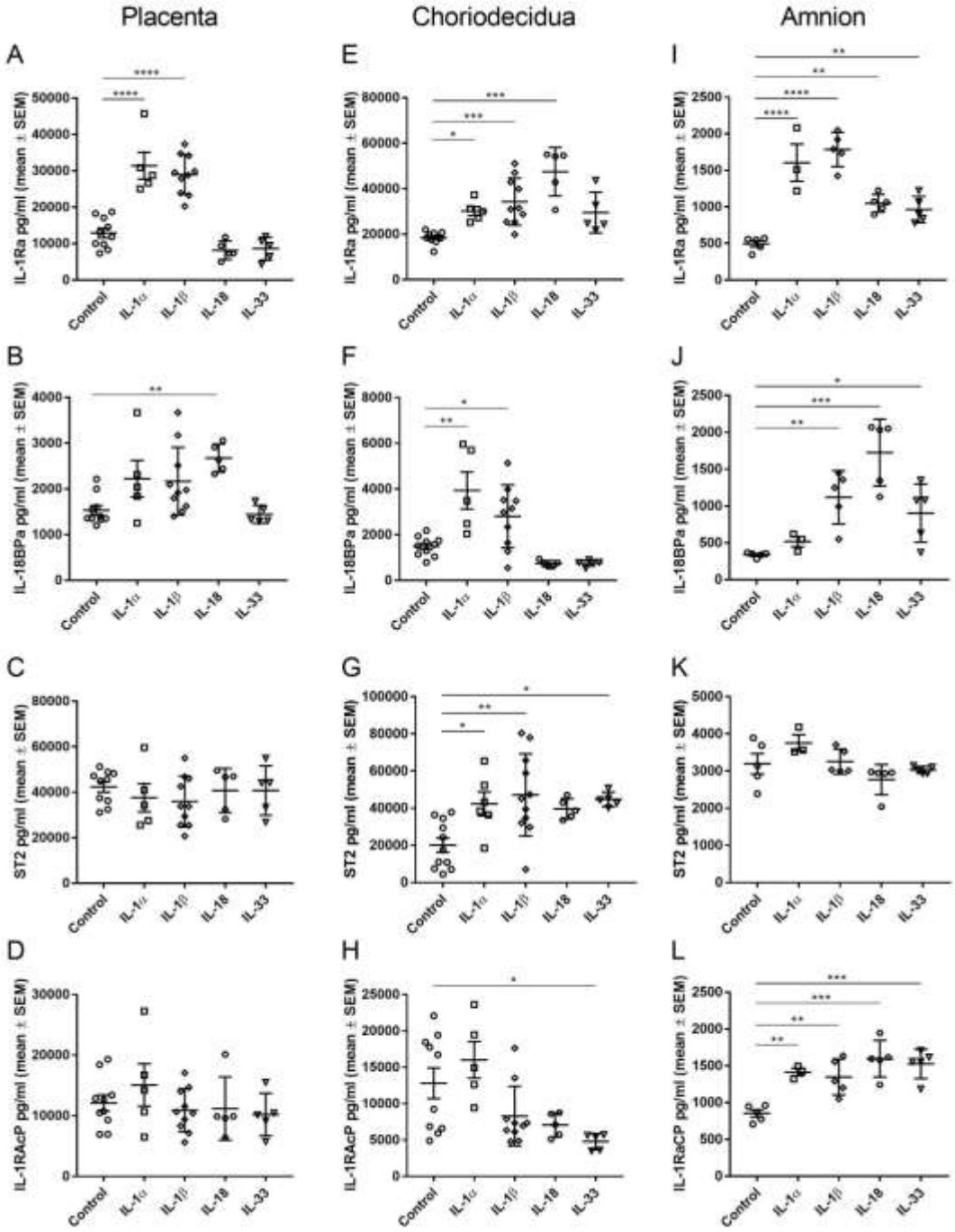
Placenta
Choriodecidua
Amnion
A
B
C
D
E
F
G
H
I
J
K
L
IL-1Ra pg/ml (mean ± SEM)
IL-18BPa pg/ml (mean ± SEM)
ST2 pg/ml (mean ± SEM)
IL-1RAcP pg/ml (mean ± SEM)
IL-1RaCP pg/ml (mean ± SEM)
Control
IL-1α
IL-1β
IL-18
IL-33

Table 1. Lipopolysaccharide-induced production of IL-1 family cytokines.

	IL-1α	IL-1β	IL-18	IL-33	IL-1RA	IL-18BP	ST2	IL-1RAcP
Placenta	↑	↑	↑	↑	↑	–	↓	↓
Choriodecidua	↑	↑	↑	↑	↑	–	–	–
Amnion	–	↑	–	–	↓	–	–	↓

Table 2. The effects of IL-1 family cytokines on production of regulatory molecules.

	IL-RA			IL-18BP			ST2			IL-1RAcP		
	P	C	A	P	C	A	P	C	A	P	C	A
IL-1α	↑	↑	↑	–	↑	–	–	↑	–	–	–	↑
IL-1β	↑	↑	↑	–	↑	↑	–	↑	–	–	–	↑
IL-18	–	↑	↑	↑	–	↑	–	–	–	–	–	↑
IL-33	–	–	–	–	–	↑	–	↑	–	–	↓	↑

'–' denotes no change