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# Production and regulation of interleukin-1 family cytokines at the materno-fetal interface

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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 [30]. PROM and preterm delivery of the baby is influenced by the overproduction of pro-inflammatory cytokines in response to bacterial infection [32]. IL-1β was one of the first cytokines evaluated in association with intrauterine infection [34] and has been shown to weaken the fetal membranes by up-regulating apoptosis and essential metalloproteinases [15].

IL-1β is a member of the larger 11-membered IL-1 superfamily of cytokines [28]. 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 [7]. 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 [36]. 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 [25]. 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 [26]. The processing of pro-IL-18 occurs in a similar inflammasome-dependent manner to IL-1β [13]. Like other IL-1 family cytokines, IL-33

*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

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is synthesized as a pro-peptide but the role of caspases, calpain and/or other enzymes awaits clarification [6,14,44,21].

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 [12,18,13,40,38]. 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

#### 2.2.1. 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 0.5 ml Advanced RPMI supplemented with 2 mM Glutamax, 2 mM penicillin streptomycin fungisone (PSF), 5 mM 2-mercaptoethanol (2-ME; all Life Technologies) and 2% fetal bovine serum (FBS; Hyclone) and 5 mM 2-mercaptoethanol; and four discs of amnion placed into 0.5 ml Advanced DMEM (Life Technologies) supplemented with 2 mM Glutamax, 2 mM PSF and 2% FBS.

#### 2.2.2. Placenta

1 $cm^3$ pieces of placental tissue were taken from different sites across the placenta. Tissue pieces were washed in PBS and further diced into smaller 1–2 $mm^3$ segments. Diced placenta tissue pieces (0.2 g) were placed into 1 ml Advanced RPMI supplemented with 2 mM Glutamax, 2 mM PSF, 2% FBS and 5 mM 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 h at 37 °C in 5% $CO_2$. Tissue free supernatants were collected by centrifugation for 7 min at 4 °C, 515*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 [19].

### 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 $\leq 0.05$ 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 [31]. 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 (Fig. 1A–H); in the amnion only IL-1β was increased significantly (Fig. 1I–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 (Fig. 2A) and choriodecidua (Fig. 2E) but a decrease in the amnion (Fig. 2I). There was no effect of LPS on IL-18BPa levels from the placenta, choriodecidua or amnion (Fig. 2B, F and J). LPS down-regulated ST2 levels in the placenta (Fig. 2C) but not in the choriodecidua or the amnion (Fig. 2G and K). IL-1RAcP levels were decreased in response to LPS in the placenta (Fig. 2D) and amnion (Fig. 2L) but the choriodecidua showed no change (Fig. 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 [24]. Caspase activity can also modulate the production and/or activity of IL-1α and IL-33 [6,23,24].

**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 | – | ↑ | – | – | ↓ | – | – | ↓ |

'–' denotes no change.