Placental Secretion of Interleukin-1 and Interleukin-1 Receptor Antagonist in Preeclampsia: Effect of Magnesium Sulfate

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Preeclampsia is a pregnancy-specific disorder characterized by hypertension and systemic endothelial dysfunction. Interleukin (IL)-1 β is a possible mediator of maternal endothelial dysfunction in preeclampsia. Serum IL-1 β as well as its natural inhibitor IL-1 receptor antagonist (IL-1Ra) were reported to be increased in women with preeclampsia. In the current study, we addressed the role of the placenta in controlling the circulatory levels of IL-1 β and its natural inhibitor IL-1Ra in preeclampsia, and the possible effect of magnesium sulfate (MgSO₄) on these levels. Using an *ex vivo* placental perfusion system, placentas from preeclamptic ($n=9$) and normotensive ($n=6$) pregnancies were perfused in presence or absence of MgSO₄. Perfusate samples were collected from the maternal and the fetal circulations of the perfusion system, and IL-1 β and IL-1Ra were examined by enzyme-linked immunoassay (ELISA). Preeclamptic placentas secreted higher levels of IL-1 β ($P<0.001$), and a tendentious higher levels of IL-1Ra, mainly into the maternal circulation, as compared with normotensive placentas, although no differences in IL-1 β :IL-1Ra ratio were detected. However, there was only tendentious increase in the secretion levels of IL-1 β or IL-1Ra into the fetal circulation of preeclamptic placentas, when compared with normotensive placentas. Administration of MgSO₄ to preeclamptic placentas resulted in an attenuation of the increased secretion of IL-1 β into the maternal circulation ($P<0.001$), and in a tendentious reduction in IL-1Ra. However, IL-1 β :IL-1Ra ratio in preeclamptic placentas was not affected by MgSO₄. Interestingly, exposure of normotensive placenta to MgSO₄ resulted only in increased levels of IL-1Ra in the maternal circulation, without affecting IL-1 β levels or IL-1 β :IL-1Ra ratio. These findings suggest that the placenta may contribute to the elevation in serum IL-1 β and IL-1Ra in preeclampsia by increased secretion of these cytokines into the maternal circulation, and that MgSO₄ is able to attenuate this increased secretion of IL-1 β , and possibly IL-1Ra, in preeclampsia.

Introduction

FOR CENTURIES, PREECLAMPSIA has considered as the Goliath of obstetric diseases. It continues to be a leading cause for maternal and fetal morbidity and mortality worldwide (Sibai and others 2003; Feinberg 2006). Preeclampsia is characterized by new onset of maternal hypertension, proteinuria, and additional symptoms, including headache, visual disturbances, epigastric pain, thrombocytopenia, and kidney and liver dysfunction (ACOG practice bulletin 2002). Although the etiology of this disorder is still unclear, the placenta is known to play a pivotal role in preeclampsia. In accordance, preeclampsia has been described in cases of molar and abdominal pregnancies and only complete removal of the placenta could attenuate the symptoms associated with this disorder (Shembrey and Noble 1995; Walker 2000).

It has been suggested that defective placentation during the first stages of pregnancy may lead to a reduction in *in utero* placental perfusion rate. As a result, the hypoxic/ischemic placenta releases increased amounts of different circulating factors into the maternal circulation leading to the systemic vascular dysfunction defined in preeclampsia (Page 2002; Kharfi and others 2003). Proinflammatory cytokines are one of these circulating factors, which are known to be actively involved in controlling endothelial, cardiovascular, and renal function under physiological and pathological conditions (LaMarca and others 2007). As the human placenta is known to produce a wide variety of cytokines and hormones over gestation (Rusterholz and others 2007), cytokines are considered as one of the critical mediators of vascular dysfunction in preeclampsia. Serum levels of proinflammatory cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-6, are increased in

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women with preeclampsia (Conrad and Benyo 1997; Conrad and others 1998).

The IL-1 system is one of the key cytokines in mammalian reproduction consisted of 2 agonistic cytokines, IL-1 α (intracellular form) and IL-1 β (the secreted form); their natural antagonist IL-1 receptor antagonist (IL-1Ra); and a receptor family that includes IL-1 receptor type I and type II (Dinarello 1994; Krussel and others 2003; Paulesu and others 2005). IL-1 β is a pivotal component of the proinflammatory response, with multiple functions in the immune system. In addition to its protective effects, this response can also be harmful to the host. IL-1Ra, one of the major antiinflammatory cytokines and the natural inhibitor of IL-1 β activity, reduces IL-1 β -induced inflammatory processes by competing for binding sites on the IL-1 receptor on target cells. The relative levels of IL-1 and IL-1Ra at an inflammatory site determine whether a proinflammatory response will be initiated and persist or will be terminated (Arend 1991).

Correspondingly to TNF- α and IL-6, IL-1 β has been suggested as a potential mediator of maternal endothelial dysfunction in preeclampsia (Rusterholz and others 2007). However, serum IL- β levels in women with preeclampsia seem to be unchanged (Greer and others 1994; Opsjn and others 1995; Stallmach and others 1995; Kimya and others 1997; Conrad and others 1998; Heyl and others 1999; Paulesu and others 2005; Szarka and others 2010), although other groups have recently reported elevated IL-1 β levels in those women (Koyigit and others 2004; Kalinderis and others 2011). Moreover, placental expression levels of IL-1 β in preeclampsia also remain very controversial (Opsjn and others 1995; Estells and others 1998; Rinehart and others 1999). On the other hand, IL-1Ra was also reported as involved in preeclampsia as IL-1Ra serum levels are increased in women with preeclampsia (Greer and others 1994; Kimya and others 1997; Szarka and others 2010). Further, IL-1Ra gene polymorphism was shown to be associated with the disease (Faisel and others 2003).

Cytokine production by the dysfunctional maternal endothelium and peripheral blood mononuclear cells in preeclampsia is obvious (Rusterholz and others 2007). For that reason, the serum cytokine milieu does not reflect the strict contribution of the placenta. However, it is undoubted that placental contribution is likely to be significant, as cytokine imbalance and elevated expression of proinflammatory cytokines is also evident in preeclamptic placentas (Rinehart and others 1999). Recently, we showed that preeclamptic placentas secrete increased amounts of TNF- α and IL-6 into the maternal and the fetal circulations in an *ex vivo* placental perfusion model, suggesting a significant contribution of the placenta to the elevated levels of these cytokines in the maternal serum (Amash and others 2010a, 2010b).

Magnesium sulfate (MgSO₄) is widely used as an anticonvulsant agent for the treatment of severe preeclampsia and prevention of eclampsia (Lucas and others 1995; Witlin and Sibai 1998). MgSO₄ may selectively attenuate the vasoconstrictive effect of angiotensin II and endothelin-1 on placental vasculature (Holcberg and others 2004). Although its widespread use as an anticonvulsant agent in preeclampsia/eclampsia, the mechanism of action of MgSO₄ is still not clearly understood. Nevertheless, potential mechanisms, such as competitive antagonism with calcium ions and glutamate N-methyl-D-aspartate receptor, vasodilatation of cerebral vasculature, and inhibition of platelet aggregation and

free-radical-induced cellular damage, have been proposed (Roberts 1995). However, further investigation on the effect of MgSO₄ on other organs, such as the placenta, and on other cytokines is required in order to improve our understanding about the involvement of the cytokine network in mechanism of action of MgSO₄ in preeclampsia. In the study, we also addressed the possible effect of MgSO₄ on the secretion of IL-1 β and IL-1Ra by the placenta.

The current study was designed to address the possible contribution of the placenta to the imbalance in IL-1 β and IL-1Ra levels in the maternal circulation in preeclampsia by comparing the capacity of *ex vivo*-perfused placentas from preeclamptic pregnancies to secrete IL-1 β and IL-1Ra, as compared with placentas from normotensive pregnancies. Moreover, we examined the effect of MgSO₄ on the capacity of preeclamptic placentas to secrete IL-1 β and IL-1Ra.

Materials and Methods

Participants

After obtaining the appropriate local institutional approval (no. 4188 and 4543), placentas from 6 term (37–40 weeks) normotensive pregnancies and 9 severe preeclamptic pregnancies (33–40 weeks) were collected immediately after vaginal or caesarean deliveries. All preeclamptic placentas were collected after 24–48 h after the onset of preeclampsia. Preeclampsia was defined as following: (1) a new onset of hypertension (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg on 2 occasions at least 4 h apart after 20 weeks of gestation); (2) proteinuria (more than 300 mg per 24 h or 2+ or greater on urine dipstick). Women with preexisting complications, such as chronic hypertension, diabetes mellitus, autoimmune, and renal diseases, were excluded. Women with intrauterine fetal death or women with preterm (<37 weeks of gestation) delivery were also excluded. The clinical data of participants are summarized in Table 1.

Ex vivo placental perfusion

The perfusion experiments were performed using the method previously described (Amash and others 2010a, 2010b). About 3 normotensive placentas and 4 preeclamptic placentas were perfused for 6 h with control medium (without MgSO₄), and another 3 normotensive placentas and 5 preeclamptic placentas were perfused for 6 h with medium containing MgSO₄ (6–7 mg%) in the maternal reservoir (maternal administration of standard doses of MgSO₄ for preeclampsia results in plasma levels of 4.8–7 mg%).

Briefly, within 15–20 min of delivery, an intact cotyledon (lateral cotyledons containing part of the membranes) was chosen of dual perfusion (closed circuit perfusion) of 6 h in a 37°C chamber. Perfusion medium consisted of 2 L of M-199 cell culture medium (Sigma Chemicals Co.), enriched with bovine serum albumin (BSA) (1 g/L) (Sigma), glucose (1 g/L) (Sigma), heparin (10 IU/mL) (Beit Kama), and gentamycin (40 µg/mL) (Teva). The maternal and the fetal reservoirs were placed into heated water baths at 37°C, and were equilibrated with a prehumidified gas mixture of 95% oxygen and 5% carbon dioxide (CO₂) in the maternal reservoir and 95% N₂ and 5% CO₂ in the fetal reservoir. Perfusion pressure of 20–40 mmHg, giving a flow rate of 6–8 mL/min in the fetal circulation and 10–12 mL/min in

TABLE 1. CHARACTERISTICS AND NEONATAL OUTCOMES OF STUDY PARTICIPANTS

Characteristics	Normotensive ^a	Preeclamptic ^a	P Value ^b
Number of cases	6	9	
Control group	3	5	
MgSO ₄ group	3	4	
Maternal age (years)	25.2±2.3	25.9±2.1	NS
Gravidity (No. of pregnancies)	2.4±1	1.6±0.2	NS
Parity (No. of deliveries)	2.4±1	1.1±0.1	NS
Body mass index (kg/m ²)	28.6±0.4	31.3±2	NS
Mode of delivery (PS ^c :CS ^d)	6:0	6:3	NS
Gestational age (W)	39.6±0.8	37.4±0.8	NS
Systolic BP (mmHg)	128.4±4.4	159.2±4.4	<0.001
Diastolic BP (mmHg)	76.4±1.2	104.3±3.3	<0.0001
Proteinuria (urine dipstick)	0	+3	<0.01
Neonatal outcome			
Newborn weight (g)	3,096±168	2,677±253	NS
Mean apgar 1	9	7.9±0.8	NS
Mean apgar 5	10	9.4±0.4	NS
Umbilical pH	7.31±0.05	7.27±0.02	NS
Placental weight (g)	582±52	535±45	NS
Cotyledon weight (g)	30±2.9	23.9±2.2	NS

^aPlus-minus (±) values are mean±standard error.

^bStatistical analysis between the normotensive and the preeclamptic groups was made using the Student's *t*-test. Categorical parameters were summarized using frequency measures, and statistical analysis was made using Fisher's exact test.

^cVaginal delivery.

^dCesarean delivery.

BP, blood pressure; MgSO₄, magnesium sulfate; NS, not significant.

the maternal circulation, was established using an internal pressure monitor (series 50 IP-2; Philips Medizinsystems). Perfusate samples from the fetal and the maternal circulations were collected in each experiment every 30 min through the perfusion, and stored at -70°C until examined for IL-1β and IL-1Ra levels by enzyme-linked immunoassay (ELISA).

To minimize possible effects of labor on placental release of cytokines and in order to remove fetal and maternal blood, each normotensive or preeclamptic placental cotyledon, either after vaginal or after caesarean delivery, was preperfused for 30 min with lactated ringer's [Hartman solution (Teva Medical)] followed by 30 min of preperfusion with medium enriched with albumin (1 g/L), glucose (1 g/L), and heparin (10 IU/mL). Subsequently, the perfusion medium in the fetal and maternal compartments was exchanged with fresh enriched medium. To minimize the error that may result from variations in perfused cotyledon weights from different placentas, each perfused cotyledon was weighed at the end of the perfusion and ELISA results were normalized for perfused cotyledon weight. Validation of placental integrity of each experiment was established throughout the experimental period by ensuring that the rate of perfusate input in both the maternal and fetal circuits equaled the rate of output, and that histological examination of the cotyledon at the end of each experiment revealed no significant morphological changes.

Examination of IL-1β and IL-1Ra by ELISA

IL-1β and IL-1Ra levels in the perfusate samples were measured by ELISA. For IL-1β evaluation, we used mouse monoclonal anti-human IL-1β antibodies (first antibodies) and biotin-conjugated mouse monoclonal anti-human IL-1β antibodies (second antibodies) (Biosource). Sensitivity of the kit was <16 pg/mL, and standard curve range was 4–2,000 pg/mL of recombinant human IL-1β (Peprotech, Inc.). For IL-1Ra evaluation, mouse monoclonal anti-human IL-1Ra (first antibodies) and biotin-conjugated goat monoclonal anti-human IL-1Ra (second antibodies) (R&D Systems) were used. Sensitivity of the kit was <20 pg/mL, and standard curve range was 5–2,500 pg/mL of recombinant human IL-1Ra (R&D Systems).

First antibodies were incubated overnight in 96-well ELISA plates at 4°C (IL-1β) or room temperature (IL-1Ra), followed by washing and addition of blocking buffer, consisted of 10% fetal calf serum (Beit HaEmek) (IL-1β) or 1% BSA (IL-1Ra) in phosphate saline buffer (Beit HaEmek) for 2 h at 37°C (IL-1β) or room temperature (IL-1Ra). Thereafter, blocking buffer was removed, and samples or recombinants were added for 1-h incubation at 37°C (IL-1β) or 2 h at room temperature (IL-1Ra). After washing, second antibodies were added and plates were incubated for additional 1 h at 37°C (IL-1β) or 2 h at room temperature (IL-1Ra). After incubation, plates were washed and streptavidin horseradish peroxidase was added for 30 min at 37°C (IL-1β) or 20 min at room temperature (IL-1Ra). After washing, tetramethyl benzidine (DakoCytomation, Inc.) was added for 15–20 min and reaction was stopped by adding 2 N H₂SO₄. Optical absorbance was read by ELISA Reader (Model 550; Biorad) at 450 nm.

IL-1β:IL-1Ra ratio was calculated for each time point and each placental circulation according to the following equation: ratio = [IL-1β]/[IL-1Ra].

Statistical analysis

Statistical analysis was performed using GraphPad Prism (Version 5.04; GraphPad Software, Inc.). For characteristics and neonatal outcomes of study participants, continuous parameters were summarized as mean±standard error (SE), and examined using the Student's *t*-test and the Mann-Whitney test. Categorical parameters were summarized using frequency measures, and statistical analysis was performed using the Fisher's exact test. For comparisons of IL-1β and IL-1Ra levels and IL-1β:IL-1Ra ratios between the different placental groups, statistical analysis was performed using the 2-way analysis of variance. *P*<0.05 was considered statistically significant.

Results

As shown in Table 1, maternal age, body mass index, number of pregnancies and number of deliveries, gestational age, and mode of delivery were all comparable between the normotensive and preeclamptic groups. Obviously, the preeclamptic group had significantly higher values of blood pressure as well as proteinuria. Moreover, no differences were detected in neonatal outcome parameters or in placental weight, between the 2 groups.

Placental IL-1β and IL-1Ra secretion in preeclampsia

To evaluate the capacity of the human preeclamptic placenta to secrete IL-1β and IL-1Ra, placentas from

FIG. 1. IL-1 β levels in the fetal (A) and the maternal (B) circulations of normotensive ($n=3$) and preeclamptic ($n=5$) placentas after perfusion with control medium (without MgSO_4). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests ($***P < 0.001$). IL-1 β , interleukin-1 β ; MgSO_4 , magnesium sulfate; SE, standard error; ANOVA, analysis of variance.

preeclamptic and normotensive pregnancies were perfused with medium alone (without MgSO_4) for 6 h, using an *ex vivo* placental perfusion system. Perfusate samples were collected from the fetal and the maternal circulations and cytokine levels were tested. IL-1 β levels in the fetal and the maternal circulations of normotensive and preeclamptic placentas perfused with medium alone increased with time starting from basal levels at the beginning of the perfusion and reaching the highest peak levels toward the end of the perfusion (after 6 h). Although there was a tendency of increased secretion of IL-1 β into the fetal and the maternal circulations of preeclamptic placentas as compared with normotensive placentas, this was statistically significant only in the maternal circulation (Fig. 1A, B). After 6 h of perfusion, IL-1 β levels in the maternal circulation of preeclamptic placentas reached higher peak values [$11.48 \pm 2.74 \text{ pg}/(\text{mL} \cdot \text{g}^{-1})$ cotyledon; mean $\pm$ SE], as compared with IL-1 β levels in the maternal circulation of normotensive placentas [$3.59 \pm 1.17 \text{ pg}/(\text{mL} \cdot \text{g}^{-1})$ cotyledon; $P < 0.001$; Fig. 1B].

IL-1Ra in the fetal and the maternal circulations of normotensive and preeclamptic placenta also increased with time reaching the highest peak levels toward the end of the perfusion. Similarly to IL-1 β , IL-1Ra secretion by preeclamptic placentas into the fetal and the maternal circulations showed a tendency

FIG. 2. IL-1Ra levels in the fetal (A) and the maternal (B) circulations of normotensive ($n=3$) and preeclamptic ($n=5$) placentas after perfusion with control medium (without MgSO_4). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests. IL-1Ra, interleukin-1 receptor antagonist.

toward higher levels as compared with normotensive placentas, although this tendency did not reach statistical significance in both circulations (Fig. 2A, B). At the end of perfusion, IL-1Ra levels in the fetal and the maternal circulations of preeclamptic placentas reached $7.18 \pm 1.39 \text{ pg}/(\text{mL} \cdot \text{g}^{-1})$ cotyledon and $25.3 \pm 9.84 \text{ pg}/(\text{mL} \cdot \text{g}^{-1})$ cotyledon, respectively, as compared with IL-1Ra levels in the fetal and the maternal circulations of normotensive placentas [$2.29 \pm 0.16 \text{ pg}/(\text{mL} \cdot \text{g}^{-1})$ cotyledon and $7.07 \pm 1.71 \text{ pg}/(\text{mL} \cdot \text{g}^{-1})$ cotyledon, respectively; Fig. 2A, B].

Although there are changes in IL-1 β and IL-1Ra secretion by preeclamptic placentas, IL-1 β :IL-1Ra ratios in the fetal and the maternal circulations of preeclamptic placentas and normotensive placentas were comparable in the 2 groups. The final IL-1 β :IL-1Ra ratios in the fetal and the maternal circulations of preeclamptic placentas were 0.14 ± 0.08 and 0.54 ± 0.1 (respectively), as compared with IL-1 β :IL-1Ra ratio in the fetal and the maternal circulations of normotensive placentas (0.13 ± 0.03 and 0.56 ± 0.21 , respectively; Fig. 3A, B).

Moreover, normotensive as well as preeclamptic placentas secreted significantly higher levels of IL-1 β and IL-1Ra into their maternal circulations as compared with the levels in their corresponding fetal circulations (Figs. 1 and 2). As summarized in Table 2, final IL-1 β levels in the maternal circulations of normotensive [$3.59 \pm 1.17 \text{ pg}/(\text{mL} \cdot \text{g}^{-1})$ cotyledon] and preeclamptic placentas [$11.48 \pm 2.74 \text{ pg}/(\text{mL} \cdot \text{g}^{-1})$ cotyledon] were higher as compared with IL-1 β levels in the corresponding fetal

FIG. 3. IL-1 β :IL-1Ra ratio in the fetal (A) and the maternal (B) circulations of normotensive ($n=3$) and preeclamptic ($n=5$) placentas after perfusion with control medium (without MgSO₄). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests.

circulations [0.3 ± 0.09 pg/(mL $\cdot$ g⁻¹) cotyledon and 1.43 ± 0.69 pg/(mL $\cdot$ g⁻¹) cotyledon; respectively; $P < 0.001$]. In the same manner, final IL-1Ra levels in the maternal circulations of normotensive [7.07 ± 1.71 pg/(mL $\cdot$ g⁻¹) cotyledon] and preeclamptic placentas [25.3 ± 9.84 pg/(mL $\cdot$ g⁻¹) cotyledon] were significantly elevated as compared with IL-1Ra levels in the corresponding fetal circulations [2.29 ± 0.16 pg/(mL $\cdot$ g⁻¹) cotyledon and 7.18 ± 1.39 pg/(mL $\cdot$ g⁻¹) cotyledon, respectively; $P < 0.05$]. Consequently, IL-1 β :IL-1Ra ratios were also higher in the maternal circulations of normotensive and preeclamptic placentas, as compared with the ratios in their corresponding

TABLE 2. COMPARISON OF FINAL IL-1 β AND IL-1Ra LEVELS AND IL-1 β :IL-1Ra RATIO IN THE FETAL VERSUS THE MATERNAL CIRCULATION OF NORMOTENSIVE AND PREECLAMPTIC PLACENTAS

		Fetal	Maternal	P Value
IL-1 β	Normotensive	0.3 ± 0.09	3.59 ± 1.17	< 0.001
	Preeclamptic	1.43 ± 0.69	11.48 ± 2.74	< 0.001
IL-1Ra	Normotensive	2.29 ± 1.6	7.07 ± 1.71	< 0.05
	Preeclamptic	7.18 ± 1.39	25.3 ± 9.84	< 0.05
IL-1 β :IL-1Ra ratio	Normotensive	0.13 ± 0.03	0.56 ± 0.21	< 0.05
	Preeclamptic	0.14 ± 0.08	0.54 ± 0.1	< 0.001

IL-1 β , interleukin-1 β ; IL-1Ra, interleukin-1 receptor antagonist.

FIG. 4. IL-1 β levels in the fetal (A) and the maternal (B) circulations of normotensive placentas after perfusion with control medium (without MgSO₄; $n=3$) or with medium containing MgSO₄ (6–7 mg%; $n=3$). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests.

fetal circulations. At the end of perfusion, IL-1 β :IL-1Ra ratios in the maternal circulations of normotensive and preeclamptic placentas reached higher peak values of 0.56 ± 0.21 and 0.54 ± 0.1 (respectively), as compared with IL-1 β :IL-1Ra ratios in the corresponding fetal circulations [0.13 ± 0.03 ($P < 0.05$) and 0.14 ± 0.08 ($P < 0.001$), respectively; Fig. 3A, B and Table 2].

Effect of MgSO₄ on placental IL-1 β and IL-1Ra secretion in preeclampsia

To investigate whether MgSO₄ administration in preeclampsia can affect the placental capacity to secrete IL-1 β and IL-1Ra, MgSO₄ (6–7 mg%) was added to the maternal reservoir of preeclamptic and normotensive perfused placentas and the levels of IL-1 β and IL-1Ra secreted into the fetal and the maternal circulations were compared with placentas perfused with medium alone. Addition of MgSO₄ into the maternal circulation of normotensive placentas did not affect IL-1 β levels secreted neither into the fetal nor into the maternal circulations (Fig. 4A, B). However, administration of MgSO₄ in preeclamptic placentas did not affect IL-1 β levels in the fetal circulation (Fig. 5A) on one hand, but resulted in reduced IL-1 β secretion into the maternal circulation, on the other hand (Fig. 5B). IL-1 β levels in the maternal circulation of preeclamptic placentas perfused with

FIG. 5. IL-1 β levels in the fetal (A) and the maternal (B) circulations of preeclamptic placentas after perfusion with control medium (without MgSO₄; $n=5$) or with medium containing MgSO₄ (6–7 mg%; $n=4$). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests (*** $P < 0.001$).

medium containing MgSO₄ reached 6.15 ± 1.51 pg/(mL $\cdot$ g⁻¹) cotyledon at the end of perfusion, as compared with IL-1 β levels in the maternal circulation of preeclamptic placentas perfused with medium alone [11.48 ± 2.74 pg/(mL $\cdot$ g⁻¹) cotyledon; $P < 0.001$; Fig. 5B].

Further, administration of MgSO₄ in the maternal circulation differently affected IL-1Ra secretion by normotensive and preeclamptic placentas. In normotensive placentas, exposure to MgSO₄ resulted in increased secretion of IL-1Ra into the maternal but not the fetal circulations (Fig. 6A, B). IL-1Ra levels in the maternal circulation of normotensive placentas exposed to MgSO₄ reached the highest peak values of 18.08 ± 3.68 pg/(mL $\cdot$ g⁻¹) cotyledon, as compared with IL-1Ra levels in the maternal circulations of normotensive placentas perfused with medium alone [7.07 ± 1.71 pg/(mL $\cdot$ g⁻¹) cotyledon; $P < 0.01$], toward the end of perfusion (Fig. 6B). On the other hand, exposure of preeclamptic placentas to MgSO₄ did not affect IL-1Ra levels in the fetal circulation (Fig. 7A). In the maternal circulation, administration of MgSO₄ resulted in a tendency of decreased IL-1Ra levels although it did not reach statistical significance (Fig. 7B). However, MgSO₄ administration in normotensive and in preeclamptic placentas did not affect IL-1 β :IL-1Ra ratios in the fetal or the maternal circulations (Figs. 8 and 9, respectively).

FIG. 6. IL-1Ra levels in the fetal (A) and the maternal (B) circulations of normotensive placentas after perfusion with control medium (without MgSO₄; $n=3$) or with medium containing MgSO₄ (6–7 mg%; $n=3$). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests (* $P < 0.05$; ** $P < 0.01$).

Discussion

IL-1 β is a major inducer of proinflammatory immune responses. Current reports suggest that serum levels of IL-1 β and its natural inhibitor IL-1Ra may be elevated in pregnancies complicated by preeclampsia. The placenta is known to be an important source for these 2 cytokines during normal and pathological pregnancies (Holcberg and others 2008). Given that cytokine production by the dysfunctional maternal endothelium and peripheral blood mononuclear cells in preeclampsia is obvious, the question regarding the contribution of the placenta to the elevation in the levels of IL-1 β and IL-1Ra in women with preeclampsia remains to be answered. In the current study, we addressed this question using an *ex vivo* placental perfusion system that enables us to distinguish placental cytokine secretion into the 2 different placental circulations: the fetal and the maternal. Our findings provide evidence that the placenta in preeclampsia secretes higher levels of IL-1 β and IL-1Ra into the maternal circulation than it does in normal pregnancy, although the changes in IL-1Ra secretion in preeclampsia were tendencies and did not reach statistical significance.

Although the reports regarding serum IL-1 β levels are still controversial (Greer and others 1994; Opsjn and other 1995; Stallmach and others 1995; Kimya and others 1997; Conrad and others 1998; Heyl and others 1999; Koyigit and others

FIG. 7. IL-1Ra levels in the fetal (A) and the maternal (B) circulations of preeclamptic placentas after perfusion with control medium (without MgSO₄; $n=5$) or with medium containing MgSO₄ (6–7 mg%; $n=4$). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests.

2004; Szarka and others 2010; Kalinderis and others 2011), our current findings highlight the inflammatory state in the placenta during preeclampsia and also suggest a significant contribution of the placenta to the possibly elevated maternal serum IL-1 β in this disorder. Accordingly, Rinehart and others (1999) also reported increased IL-1 β mRNA expression levels in the placenta, although others failed to show that (Opsjn and others 1995; Estells and others 1998; Benyo and others 2001). Increased placental secretion of IL-1 β in preeclampsia could be of clinical relevance as IL-1 β was shown to mediate endothelial dysfunction by inducing structural and functional alterations in endothelial cells (Rusterholz and others 2005), which is a hallmark of the maternal syndrome in preeclampsia.

The tendentious elevation in placental secretion of IL-1Ra in preeclampsia may represent a protective response to increased proinflammatory cytokine activity and be a marker for overt inflammation. Elevated serum IL-1Ra levels in preeclampsia have been reported by different groups (Greer and others 1994; Kimya and others 1997; Szarka and others 2010), suggesting a role for this antiinflammatory cytokine in this disorder. The current results suggest that the placenta may contribute to the elevation in serum IL-1Ra in preeclampsia by increased secretion of IL-1Ra into the maternal circulation. Interestingly, women who are homozygous for allele 2 of the IL-1Ra gene (IL1RN*2), which is believed to be

FIG. 8. IL-1 β :IL-1Ra ratio in the fetal (A) and the maternal (B) circulations of normotensive placentas after perfusion with control medium (without MgSO₄; $n=3$) or with medium containing MgSO₄ (6–7 mg%; $n=3$). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests.

associated with higher IL-1Ra protein expression levels, have more severe preeclampsia than preeclamptic women who possess other IL-1RN genotypes (Hefler and others 2001). The IL-1Ra gene is polymorphic, resulting in quantitative differences in both IL-1Ra and IL-1 β . Consequently, people with high levels of IL-1Ra have been shown to have a more prolonged and more severe proinflammatory immune response than people with other IL-1Ra genotypes (Witkin and others 2002). Thus, while having high IL-1Ra levels may be beneficial when combating infections or malignancies, it might be detrimental for people with chronic inflammatory conditions or who are pregnant. IL1RN*2 homozygosity may also be associated with recurrent spontaneous abortion, preterm birth, and severity of preeclampsia. Although it is still not clear how IL-1Ra may be involved in the development of preeclampsia, these data suggest that increased IL-1Ra production in preeclampsia may play an important role in this disorder by upregulating the production of IL-1 β .

IL-1Ra is a competitive inhibitor of IL-1 β bioactivity. Therefore, the relative levels of IL-1 β and IL-1Ra (IL-1 β :IL-1Ra ratio) at an inflammatory site will thus determine whether a proinflammatory response will be initiated and persist or will be terminated. In this study, the tendentious elevation in IL-1Ra secretion into the maternal circulation of preeclamptic placenta seems to be sufficient in order to maintain normal IL-1 β :IL-1Ra ratio values in the placental maternal

FIG. 9. IL-1 β :IL-1Ra ratio in the fetal (A) and the maternal (B) circulations of preeclamptic placentas after perfusion with control medium (without MgSO₄; $n=5$) or with medium containing MgSO₄ (6–7 mg%; $n=4$). Results are displayed as mean $\pm$ SE. Statistical significance was determined using 2-way ANOVA and Bonferroni post tests.

circulation, although IL-1 β levels are significantly increased. This result suggests that although the contribution of the placenta to increased maternal serum levels of IL-1 β and IL-1Ra is obvious in preeclampsia, it does not seem to play a direct role in disrupting the IL-1 β :IL-1Ra ratio in the circulation. However, the elevated amounts of IL-1 β and IL-1Ra secreted into the maternal circulation may affect this ratio by regulating the production of IL-1 β and IL-1Ra by different maternal tissues, such as the endothelium. Alternatively, other maternal sources, such as circulating leukocytes, may also have a role in the imbalance in IL-1 system in preeclampsia. In this context, circulating monocytes from women with preeclampsia were shown to spontaneously secrete higher amounts of proinflammatory cytokines, including IL-1 β (Luppi and Deloia 2006).

On the other hand, our results also show that placental secretion of IL-1 β into the fetal circulation is possibly increased in preeclampsia, compared with normal pregnancy, although the elevation in the levels of this cytokine failed to reach statistical significance. These findings suggest that the changes in IL-1 β levels seen in the maternal circulation of preeclamptic placentas may also excite in the fetal circulation. However, this increase seems to develop in a slower manner, as compared with the maternal circulation. This observation suggests that the fetus may also be affected by the cytokine imbalance in preeclampsia. Similarly, the se-

cretion of IL-6 and TNF- α into the fetal circulation was also shown to be increased in preeclampsia (Amash and others 2010a, 2010b). The possibly increased placental secretion of IL-1 β into the fetal circulation in preeclampsia may have a clinical significance as IL-1, IL-6, and TNF- α have been found to be associated with neuronal damages in newborns (Dammann and Leviton 1997; Yoon and others 1997; Nelson and others 1998). Therefore, it is possible that the placenta may contribute to the increased risk for neonatal morbidities in preeclampsia, by oversecretion of proinflammatory cytokines, such as IL-1, into the fetal circulation. However, IL-1Ra was recently shown to protect against placental and neuro-developmental defects induced by maternal inflammation (Girard and others 2010). Our results also show an insignificant trend of increased secretion of IL-1Ra, a phenomena that may reflect a placental protective response to prevent possible fetal damage that might be resulted of the increased IL-1 β levels. Accordingly, we did not observe any changes in IL-1 β :IL-1Ra ratio in the fetal circulation of preeclamptic placentas, as compared with normotensive placentas.

Currently, MgSO₄ is the drug of choice in treating preeclampsia, although the mechanism of its therapeutic effects is not fully understood. Recently, we showed that MgSO₄ may reduce placental secretion of TNF- α and IL-6 in preeclampsia (Amash and others 2010a, 2010b), suggesting a role for those 2 proinflammatory cytokines in the mechanism of action of MgSO₄. In the current study we examined the effect of MgSO₄ administration on the capacity of the preeclamptic placenta to secrete IL-1 β and IL-1Ra, as compared with normotensive placentas, by addition of MgSO₄ to the perfusion media of these placentas. Our results show that exposure of preeclamptic placentas to MgSO₄ resulted in decreased IL-1 β and IL-1Ra secretion into the maternal circulation, although the effect on the later was only tendentious. However, IL-1 β :IL-1Ra ratio in the maternal circulation of preeclamptic placentas is not affected by MgSO₄. The decrease in IL-1 β secretion after exposure to MgSO₄ is in accordance with our previous reports regarding the effect of MgSO₄ on proinflammatory cytokine production (Amash and others 2010a, 2010b). Moreover, Rochelson and others (2007) showed that MgSO₄ inhibits endothelial cell activation by reducing the nuclear translocation of nuclear factor kappa B and by protecting its cytoplasmic inhibitor from degradation. On the other hand, the reduction in IL-1Ra secretion in preeclamptic placentas after MgSO₄ administration may be related to the decrease in IL-1 β levels. Together, these data support a potential antiinflammatory role for MgSO₄ within the ischemic preeclamptic placenta, and also suggest that attenuation of placental IL-1 β secretion into the maternal circulation is one of the antiinflammatory effects of MgSO₄ in preeclampsia. However, the unaffected IL-1 β :IL-1Ra ratio puts the clinical relevance of this effect in a question.

Exposure of normotensive placentas to MgSO₄ resulted in different effects on IL-1 β and IL-1Ra secretion when compared with those seen in preeclamptic placentas. MgSO₄ increased IL-1Ra secretion into the maternal circulation and resulted only in a tendentious increase in IL-1 β secretion. However, this tendentious increase in IL-1 β levels in presence of MgSO₄ was enough in order to keep IL-1 β :IL-1Ra ratio unchanged. Although we do not have a good explanation on the reason behind these differences in MgSO₄ effect between preeclamptic and normotensive placentas, these

findings may highlight different regulation mechanisms for those cytokines between the normal placental tissue and the ischemic preeclamptic placenta. Moreover, the overt inflammation and the possibly increased placental production and secretion of IL-1 β and IL-1Ra in preeclampsia could also be a reason behind the unchanged/increased IL-1 β and IL-1Ra secretion by normotensive placentas after exposure to MgSO₄.

According to our results, IL-1 β and IL-1Ra are also secreted by the normotensive placenta during normal gestation into the maternal, as well as the fetal, circulations. This data demonstrates the pivotal role of the placenta in controlling the maternal immune response during normal pregnancy. Moreover, the secretion of these cytokines into the fetal circulation may also indicate a physiological importance for these cytokines as growth factors that may be involved not only in the fetal immune system function. Moreover, IL-1 β and IL-1Ra levels, as well as IL-1 β :IL-1Ra ratios, in the maternal circulations of normotensive and preeclamptic placentas are significantly higher, compared with the fetal circulation, demonstrating the unique role of the placenta in maintaining high IL-1 β :IL-1Ra ratio in the maternal circulation, on one hand, and low IL-1 β :IL-1Ra ratio in the fetal circulation, on the other hand. This phenomenon enables the immunological and physiological effects of IL- β on the maternal side, while preventing possible IL-1 β -mediated damage to the fetus by high expression and secretion of IL-1Ra. This data is also in accordance with our previous reports (Holcberg and others 2008).

In summary, the present study provides evidence that increased levels of IL-1 β , and maybe IL-1Ra, are secreted by the preeclamptic placenta into the maternal circulation, suggesting the placenta as an important source for the elevated serum levels of IL-1 β and IL-1Ra seen in women with preeclampsia, although IL-1 β :IL-1Ra ratio seems to be unchanged. Moreover, the results of the current study show that MgSO₄ administration attenuates IL-1 β , and more moderately IL-1Ra, secretion by the preeclamptic placenta, suggesting these 2 cytokines as possible targets in the mechanism of action of MgSO₄ in preeclampsia. However, since the IL-1 β :IL-1Ra ratio does not seem to be affected by MgSO₄, further investigations are needed in order to understand the clinical value of this reduction in placental secretion of IL-1 β and IL-1Ra after administration of MgSO₄ in preeclampsia.

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