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Interleukin 11 blockade during mid to late gestation does not affect maternal blood pressure, pregnancy viability or subsequent fertility in mice

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KEY MESSAGE
Blocking interleukin (IL)11 during mid to late gestation pregnancy in mice did not affect pregnancy viability, including normal decidual, placental, fetal or offspring morphology. These findings highlight the exciting potential of IL11 signalling inhibition as a safe therapeutic to treat pregnancy complications, or as a novel fertility-preserving anti-cancer agent.

ABSTRACT

Interleukin (IL)11 is a crucial regulator during the initiation of pregnancy in humans and mice. Elevated levels are detected in serum, placenta and decidua of women with pre-eclampsia. Elevated IL11 during placentation recapitulates pre-eclampsia in mice, although withdrawal rescues pre-eclampsia features, suggesting that IL11 could provide a novel therapeutic target. The aim of this study was to determine the safety profile of an IL11 antagonist ligated to polyethylene glycol (PEGIL11A) during pregnancy in mice. Blocking IL11 signalling during mid to late gestation pregnancy in mice did not affect pregnancy viability, or alter placental or fetal weight, or morphology. Importantly, decidual area remained unchanged. PEGIL11A did not affect maternal blood pressure, urinary protein or term pup weight. PEGIL11A administration to non-pregnant mice did not affect subsequent fertility; there was no difference in number of implantation sites, or placental or fetal weight between PEGIL11A and PEG-treated mice. These data show that blocking IL11Rα during placentation does not alter the placenta, decidua, fetus, maternal blood pressure or kidneys. These findings highlight the potential of IL11 signalling inhibition as a safe therapy to alleviate pre-eclampsia symptoms and demonstrate the potential for IL11 inhibition as a novel fertility-preserving therapy for women with cancer.

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Introduction

Placental development is highly regulated spatially and temporally by numerous factors, such as cytokines, which can determine the success or failure of pregnancy (Salamonsen et al., 2009). Impaired placentation can be characterized by inadequate extravillous trophoblast (EVT) invasion (Brosens et al., 1972; Pijnenborg, 1994) and remodelling of the decidual arteries, which is completed early in the second trimester in humans (Naicker et al., 2003; Staff et al., 2013). The abnormal placenta, which bathes in maternal blood, releases factors into the blood causing maternal systemic inflammation and widespread endothelial dysfunction, which lead to the symptoms of pre-eclampsia (Redman and Sargent, 2005).

Pre-eclampsia is a pregnancy-induced disorder characterized by hypertension and proteinuria, and is a major cause of maternal and perinatal morbidity and mortality, affecting approximately 8% of pregnancies (Sibai et al., 2005). Alarmingly, those women that survive are at increased risk of developing severe complications, including chronic kidney and cardiovascular disease and have an increased risk of dying within 12 months of giving birth (Eiland et al., 2012). Advances in the prevention and treatment of pre-eclampsia have been hampered by poor understanding of the aetiology (Kaufmann et al., 2003). Nevertheless, there is substantial evidence showing abnormal placentation is crucial to the underlying cause (reviewed by Roberts and Escudero, 2012). Clinically diagnosed most often in the late second or third trimester, the only current treatment for pre-eclampsia is placental delivery.

Interleukin (IL)11 is a member of the IL6 family of cytokines that signals via the IL11 receptor (R)α chain and gp130, to form a heterodimeric complex (Heinrich et al., 1998). It is well established that IL11 activates the janus kinase (JAK) signal transducers and activators of transcription (STAT)3 pathway in the human endometrium (Dimitriadis et al., 2006), primary human EVT and placental villous (Paiva et al., 2007, 2009; Winship et al., 2015c), as well as the mouse placenta and decidua (Winship et al., 2015c). In women, IL11 and IL11Rα localize to the placenta; produced by syncytiotrophoblast and cytotrophoblast cells in the chorionic villi, as well as endovascular EVT during the first trimester (Paiva et al., 2007). In mice, both IL11 and IL11Rα localize to placental labyrinth and endovascular trophoblast and endothelial cells in mouse implantation sites throughout gestation (Winship et al., 2015c), reflecting the expression pattern in women and implying a role in placentation *in vivo*.

IL11 has well-established functional roles in the cycling endometrium and in the early initiation of pregnancy during endometrial stromal cell decidualization (Bilinski et al., 1998; Dimitriadis et al., 2000, 2002; Robb et al., 1998; White et al., 2007). Female mice with a null mutation in IL11Rα are infertile due to defective decidualization that leads to resorption of the embryo by E10 of gestation (Bilinski et al., 1998; Robb et al., 1998). Our research group utilized an IL11 mutein antagonist ligated to polyethylene glycol (PEGIL11A) to block IL11 signalling in the mouse uterine lumen following intraperitoneal administration during early post-implantation, demonstrating that IL11 is only required for decidual formation during early gestation in mice (Menkhorst et al., 2009).

IL11 levels are elevated in serum, placenta (Winship et al., 2015c) and decidua (Basar et al., 2010) of women with pre-eclampsia. *In vitro*, IL11 impedes primary human first trimester EVT cell invasion (Paiva et al., 2007) and placental villous outgrowth (Sonderegger et al., 2011) required for normal placentation. In mice, elevated levels of IL11 during placentation impair trophoblast invasion and spiral artery remodelling and recapitulate the hallmark features of pre-eclampsia and intrauterine growth restriction (IUGR) (Winship et al., 2015c). In the same study, withdrawal of IL11 after the onset of hypertension and proteinuria rescued pre-eclampsia features in mice, suggesting that IL11 signalling inhibition could provide a novel treatment option. In women and mice, decidual IL11Rα protein levels are significantly reduced during the second trimester or mid-gestation, respectively (Winship et al., 2015c), highlighting the potential feasibility of targeting IL11 during mid to late gestation to ameliorate pre-eclampsia in women, without affecting the decidua, or pregnancy viability.

We postulated that blocking IL11 action temporally during mid to late gestation would not alter pregnancy viability. We aimed to determine the effect of antagonizing IL11Rα on the placenta, decidua, fetus and maternal peripheral organs affected during pre-eclampsia. We also examined the effect of blocking IL11Rα on subsequent female fertility, 1 month after the cessation of treatment.

Materials and methods

Animals

Female (virgin 8-week-old) and male C57BL/6J mice (Monash Animal Services, Clayton, Australia) were housed under conventional conditions, with food and water available *ad libitum* and a 12 h light–dark cycle. All procedures were approved by the Monash Medical Centre Animal Ethics Committee on 19 July 2012 (reference number MMCB/2012/17). This study followed the NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. C57BL/6 mice were mated. The time of gestation is denoted by embryonic day 'E', where E0 represents the day of detection of a vaginal plug. Mice were killed by carbon dioxide gas.

Animal treatments and tissue collection

Polyethylene glycol (PEG)-ligated IL11 antagonist (PEGIL11A) (Lee et al., 2008) and PEG control were kindly donated by CSL Ltd (Parkville, Australia). To block IL11Rα during pregnancy, mated female mice were administered twice daily with 20 mg/kg/dose PEGIL11A or PEG (equivalent molarity) in sterile saline by intraperitoneal (IP) injection as established previously (Menkhorst et al., 2009), from E10–13, E10–17 or E15–17 of gestation and killed on the final day of treatment, or allowed to deliver pups. To determine the longer-term effects of PEGIL11A on fertility, non-pregnant female mice were administered with PEGIL11A or PEG as above, then mated 4 weeks after treatment and killed on E13 of pregnancy. Implantation sites were dissected on the final day of treatment to obtain placenta, decidua and fetus and these tissues were weighed and imaged using a dissecting microscope.

Histology and immunohistochemistry

All tissues were fixed in 4% neutral buffered formalin solution for 24 h and paraffin embedded. Tissues were sectioned (5 µm), placed on SuperFrost slides, dried, deparaffinized and rehydrated. Mouse ovaries were stained with periodic acid–Schiff (PAS) and implantation site sections were stained with haematoxylin and eosin (H and E) and

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