

The role of tumor necrosis factor-receptors in pregnancy with normal and adverse outcome

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Abstract: TNF α receptors, TNF-R1 and TNF-R2, mediate the biological activities of the multifunctional cytokine, tumor necrosis factor alpha, TNF α . These receptors have a central role in human pregnancy. Although each receptor induces distinct intracellular signals, they also have co-operative and overlapping effects. The membrane bound TNF-R1 carries out most of the pro-inflammatory activities of TNF α , especially those that are rapid, while TNF-R2 is involved in the late long-term effects of this cytokine. The soluble forms of these receptors can bind to TNF α , neutralizing its effects. In normal human pregnancy, TNF α receptors are present in the maternal circulation, placenta, amniotic fluid, and coelomic cavity. Changes in TNF α and its receptors are associated with adverse pregnancy outcomes, including miscarriage, preterm labor and preeclampsia. Advances in anti-TNF α therapy may have potential use in the management of complicated pregnancies.

Keywords: TNF α receptors, pregnancy, miscarriage, preeclampsia, preterm labor

Introduction

The role of maternal leucocytes and other immune factors such as cytokines in the trophoblast-decidual interaction remains unclear. There are two major subsets of CD4+ T-helper mediated responses, T-helper Th1 and Th2,¹ which act via different patterns of cytokine production. Th1 cells secrete tumor necrosis factor (TNF) α and β , interferon gamma (IFN γ) and interleukin (IL)-2. This cell-mediated immune response, also known as Type 1 response, involves activation of macrophages and cell-mediated reactions involved in resisting infections due to intracellular pathogens, and cytotoxic and delayed-type hypersensitivity reactions. Th2 type cytokines include IL-4, IL-5, IL-6, IL-10, and IL-13, which are associated with strong antibody responses to infections with extracellular organisms (Type 2 or humoral reactions).² There is evidence that cytokines are pivotal in the reproductive immune response.³⁻⁶ Normal pregnancy is now considered to be a state of controlled mild maternal systemic inflammation, where circulating levels of pro-inflammatory cytokines, including TNF α , are raised compared to the non-pregnant state, in a way similar to what happens during sepsis.⁷ These pro-inflammatory cytokines are produced by monocytes and also by trophoblasts.^{8,9} It has been hypothesized that during normal pregnancy, there is a subtle immunological shift to the Th2-type cytokine responses that would suppress the potential harmful effects of the cell-mediated (Th1-type) immune system.³ Imbalance in the Th1/Th2 cytokine response with an increase in Th 1 cytokines is associated with adverse pregnancy outcome.⁵

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Structure and bioactivities of TNF α receptors

TNF α is a potent, multifunctional cytokine in autocrine and paracrine processes central to reproduction. These processes include gamete, follicle and luteal development, steroidogenesis, uterine cyclicity, placental differentiation, development of the embryo, and parturition.¹⁰ The biological activities of TNF α are mediated via two different TNF α receptors (TNF-Rs): TNF-R1 (also known as p55/p60, Type I, b, TNF-R55, TNF-R β or CD120a) with a molecular mass of 55–60 kDa; and TNF-R2 (or p75/p80, Type II, a, TNF-R75, TNF-R α or CD120b), weighing 75–80 kDa^{11,12} (Table 1).

The differential expression of the two TNF-Rs is regulated by female sex steroid hormones. These two receptors consist of a homologous extracellular, cysteine-rich transmembrane domain, but their intracellular domains are entirely different, and each receptor is independently regulated. Although each receptor induces distinct intracellular signals, they also have co-operative and overlapping effects. In cells responding to TNF α via the TNF-R1, the extracellular part of TNF-R2 captures TNF α , even at low concentrations, and delivers it to TNF-R1, resulting in an enhanced response to TNF α .¹³

Depending on cell type and activation status, the number of receptors per cell ranges from 100 to 10,000 copies.^{14,15} The TNF-R1 is found on most tissues, and seems to be the main mediator of TNF α signaling, leading to pro-inflammatory and programmed cell death pathways, and is therefore associated with cytotoxicity. TNF-R1 carries out most of the activities of TNF α , especially those that are rapid, while TNF-R2 is involved in the late long-term effects of this cytokine. TNF-R2 is more prevalent in immune cells^{11,16} and is primarily associated with lymphocyte proliferation. While TNF-R2 may induce apoptosis,¹⁷ it can also enhance

tissue repair and angiogenesis, thus promoting cell survival.¹⁸ Other biological activities of TNF-Rs include gene induction in endothelial cells, inducing cytokine production, and activation of nuclear factor kappa-light-chain-enhancer of B cells.^{15,19,20}

Both receptors can have their extracellular domains cleaved from the membrane, thus forming soluble TNF-Rs. The soluble TNF-Rs are present in the serum and urine and have been shown to protect against the harmful effects of excessive TNF α by neutralizing this cytokine.²¹ The soluble form of TNF-R2 is cleaved by proteolysis through the metalloproteinase TNF α converting enzyme (TACE, also known as ADAM17).^{22,23} Soluble TNF-R2 is involved in the inactivation of TNF α in the circulation by the formation of high affinity complexes. This subsequently reduces the binding of TNF α to target cell membrane receptors and downregulates the response to TNF α .²⁴ The proteolytic enzyme that releases soluble TNF-R1 is still unknown.⁹ Lack of soluble TNF-R1 leads to autosomal dominant inherited auto-inflammatory syndromes.²⁵

TNF α and its receptors during the normal first trimester

In situ hybridization studies and immunohistochemical analyses have shown that in non-pregnant women, the expression of TNF α protein in the endometrial glands is negligible in the early proliferative phase, then increases and peaks during the late proliferative phase. In the secretory phase, TNF α protein expression remains high, but slightly less than in the late proliferative phase.^{26,27} Both TNF-Rs follow a similar pattern, with the highest expression in the late secretory phase.²⁸

Following decidualisation, TNF α mRNA has been shown to be present in macrophages,²⁹ T-cells,³⁰ uterine NK cells, endothelial cells,³¹ and decidual stromal cells

Table 1 Comparison between TNF-R1 and TNF-R2

	TNF-R1	TNF-R2
Other names	p55/p60, Type I, b, TNF-R55, TNF-R β or CD120a	p75/p80, Type II, a, TNF-R75, TNF-R α or CD120b
Molecular weight	55 kDa	75–80 kDa
Structure	Contains the DD	Does not contain the DD
Expressed cell types	Most tissues, including the proliferating cytotrophoblast of the cell islands and cell columns, the EVT invading the decidual tissue, and villous stromal cells	Immune cells
Functions	The main mediator of TNF α signalling, leading to proinflammatory and programmed cell death pathways, and cytotoxicity; carries out most of the activities of TNF α , especially those which are rapid	Primarily associated with lymphocyte proliferation, may induce apoptosis, can enhance tissue repair and angiogenesis; soluble TNF-R2 is involved in the inactivation of TNF α in the circulation
Signaling pathways	Interacts indirectly with TRAF2 via TRADD	Interacts directly with TRAF2

Abbreviations: DD, death domain; EVT, extravillous trophoblasts; TNF α , tumor necrosis factor alpha; TRADD, TNF- α receptor-associated death domain; TRAF2, TNF-receptor associated factor 2.

in vitro.^{32,33} Other studies have also shown that various decidual cell types express TNF-Rs.⁹ During normal human pregnancy, TNF α gene products have been detected in amniotic fluid^{34–38} and soluble TNF-Rs have been detected in first trimester coelomic fluid.³⁹ Since pro-inflammatory cytokines do not cross normal term placenta,⁴⁰ TNF α and its receptors are probably produced from within the gestational sac from a very early stage in pregnancy. TNF α gene products have been detected in placental supernatants.^{41,42}

During the first trimester, all cell types of trophoblastic lineage express TNF α mRNA. These cell types include villous and proliferating cytotrophoblasts, syncytiotrophoblasts, and the extravillous trophoblasts (EVT) invading the uterine wall.^{43,44} Messenger RNA and protein are found in both fully differentiated syncytiotrophoblasts^{26,45} and proliferating EVT cells⁴⁴ during early human gestation. There is a predominance of TNF α in cell columns during invasion especially in the EVT as it displaces the endothelial cells of the spiral arteries.^{46,47}

TNF-R1 mRNA has been identified in the proliferating cytotrophoblast of the cell islands and cell columns, the EVT invading the decidual tissue⁴⁸ and villous stromal cells.⁴⁹ There is also a non-uniform distribution of TNF-R1 mRNA in villous cytotrophoblasts and syncytiotrophoblasts in the first trimester placenta.⁹ During early gestation, TNF-R1 protein is expressed widely in villous cytotrophoblasts, EVT and cell columns, and trophoblasts.⁴⁸ As for syncytiotrophoblasts, TNF-R1 has been shown to be present during all gestational ages.⁵⁰ Under inflammatory conditions, soluble TNF-R1 may protect the trophoblast from the cytotoxic effects of TNF α .⁴⁸

TNF-R2 mRNA has a similar distribution to TNF-R1 mRNA. TNF-R2 mRNA has been observed in cultures of first trimester trophoblasts, but to a lesser extent than TNF-R1.⁴⁸ It is yet not clear how TNF-R2 is expressed in the placenta.⁹ Studies show that TNF-R2 mRNA is restricted to the trophoblast in early pregnancy and, at later stages, shifts to placental mesenchymal cells.⁴⁹

It has been proposed that placental TNF α derived from macrophages, possibly modulated by TNF α -TNFRI signaling, facilitates trophoblast differentiation.^{48,51} TNF α at the fetal-maternal interface plays an important role in regulating macrophage recruitment by trophoblast cells. It has been shown that media conditioned by TNF α -treated trophoblast cells significantly enhance the ability of the monocyte cell line THP-1 to invade through Matrigel.⁵² TNF α might promote proliferation of trophoblast and

increased human chorionic gonadotrophin secretion by acting as an autocrine growth factor via TNF-RI.⁴⁴

Systemic and placental levels of TNF α and its receptors in pregnancy

Prospective longitudinal studies of cytokine expression in the circulation during normal pregnancy show that as pregnancy progresses, there is an overall decrease in pro-inflammatory cytokines such as TNF α and IFN γ , accompanied by an increase in the anti-inflammatory cytokines such as IL-10 and IL-6.^{53,54} Successful pregnancy requires a delicate balance in Th1/Th2 cytokines. Plasma levels of TNF α and the neutralizing soluble receptor TNF-R2 rise till the second trimester, and then decrease.⁵⁵ This is followed by a shift towards Th2 cytokines in the second trimester with an increase in Th2 cytokines till term.⁵⁴ As pregnancy progresses, there is a change in placental expression of TNF α .²⁶ As for TNF-Rs, TNF-RI mRNA and protein are expressed in essentially all types of cells of the human placenta, with increasing levels as the pregnancy advances to term.³⁹ This suggests that TNF α and its receptors may have a specific role in the process of developmental differentiation. Later in pregnancy, TNF α mRNA is more prominent in placental macrophages within villous stromal cells than in trophoblasts.^{26,56} In the third trimester, there is less expression of TNF α protein in invasive cells, and no expression at all in trophoblast giant cells.⁵⁷ TNF α mRNA and protein are prominent in macrophage-like cells present in term placentas and extraplacental membranes.^{26,29} TNF-R1 mRNA is also present in high amounts in the villous stroma and endothelial cells, and to a lower extent in the syncytiotrophoblasts of the term placenta.⁴⁹ In cultured third trimester villous cytotrophoblasts, cytotoxic effects of TNF α , both alone and in combination with IFN γ , have been demonstrated, predominantly induced through TNF-R1.⁵⁸ There are elevated concentrations of soluble TNF-Rs in the urine of pregnant women.⁵⁹ This can be explained by the in vitro finding that third trimester trophoblast cells rapidly release soluble TNF-R1 and TNF-R2 into the culture medium.⁶⁰ Pregnancy specific glycoproteins derived from the placenta increase the secretion of IL-10 and other anti-inflammatory cytokines. IL-10 downregulates activity of TNF α by inhibiting the release of TNF α , increasing the release of soluble TNF-R1 and -R2, and reducing the surface expression of both TNF-Rs.⁶¹

Total antioxidant activity of amniotic fluid samples from asymptomatic mid-trimester women positively correlate with soluble TNF-Rs.⁶² TNF α is important in the initiation and amplification of inflammation.⁶³ TNF-Rs may reduce oxidative stress due to receptor binding of the inflammatory TNF α .

Many cell types present in the endometrium, placenta and deciduas have been shown to express TNF α and its receptors, implying that multiple autocrine and paracrine interactions can occur.⁹ Although there are complementary roles for the TNF-receptors, TNF-R1 has been shown to be mainly involved in apoptosis in the placenta.⁵⁸

TNF α and its receptors in miscarriage

The balance between pro- and anti-inflammatory cytokines is essential for implantation, placental development and pregnancy outcome. Changes in the Th1/Th2 balance in the feto-maternal interface in favor of Th1 can lead to adverse pregnancy outcome, including recurrent spontaneous miscarriages.⁶⁴ Increased Th1 cytokines, including TNF α , have been found in women suffering from recurrent spontaneous miscarriages.⁶⁵ It was also demonstrated that women with recurrent spontaneous miscarriages had reduced levels of soluble TNF-R1 and TNF-R2, which were then normalized upon administration of progesterone.⁶⁶ Once treated with TNF α inhibitors, this group of women had an increase in the rate of live births.⁶⁷ TNF α is unlikely to be the only mediator and, in most cases of miscarriage, there are additional triggers.⁹ Evidence shows that TNF α , IFN γ and NK cells cannot induce miscarriage separately, but a Th1-NK-macrophage triad may bring about miscarriage, which can in turn be suppressed by a Th2 cytokine response.^{3,68}

Immunohistochemical studies have shown abundant mTNF-R1 expression in the cytotrophoblasts, villous stromal cells and vessel endothelial cells derived from placenta from women with early spontaneous miscarriage. Over-expression of TNF-R1 may mediate TNF α to induce apoptosis in these cells, leading to tissue damage in chorionic villi in non-viable pregnancies.⁶⁹ Mice studies are showing that TNF α via TNFR1 signaling causes placental pathology leading to fetal hypoxia, which can be prevented by TNF α -antagonists.⁷⁰

TNF α and its receptors in preterm labor

Parturition is a complex process, brought about by the right combination of signals, following mechanical and endocrine stimulation.⁷¹ Prematurity occurs in the case of aberrations in these signals, together with inflammation, cervical abnormalities and/or progesterone resistance. However the major mechanism of preterm labor is still unclear. Complications of pregnancy have been associated with deficient conversion of the uterine spiral arteries, leading to abnormal placental perfusion. Placental malperfusion can cause

oxidative stress,⁷² induced by an ischemia-reperfusion-type insult,⁷³ leading to a rise in pro-inflammatory cytokines and anti-angiogenic factors in the maternal circulation.

In the case of late miscarriages and premature labor, TNF α and other pro-inflammatory cytokines have been shown to stimulate uterine activity and cervical ripening by producing prostaglandins⁷⁴ and cortisol,⁷⁵ and degrade the extracellular matrix of chorio-amniotic membranes via MMP-2 and MMP-9.⁷⁶ Oxidative stress and inflammatory cytokines are powerful inducers of apoptosis and necrosis. TNF α , together with other pro-inflammatory cytokines such as IL-1 β , are elevated in the amniotic fluid of women with preterm labor and/or preterm premature rupture of membranes (PPROM), even in the absence of infection.⁷⁷⁻⁷⁹ Pro-inflammatory cytokines can stimulate production of prostaglandins, leading to uterine contractions, and upregulation of MMP activation. Intra-amniotic inflammation may lead to apoptosis, thus weakening fetal membranes and leading to PPRM.^{80,81}

In PPRM, two major apoptotic pathways have been implicated. The first is a TNF α receptor-Fas-mediated pathway. This initiates signal transduction through 2 docking proteins known as TRADD (TNF- α receptor-associated death domain) and FADD (Fas-associated death domain), which in turn activate pro-caspase-8 to active caspase-8 (Figure 1). The other apoptotic pathway is p53-mediated, initiated by DNA fragmentation with activation of caspase-9. Caspase-8 and -9 initiate a cascade of caspase activation, followed by sequential activation of caspases 3, 7 and 6, leading to proteolysis of structural proteins, proteins of homeostasis, and several other target proteins leading to apoptosis.⁸²

Lipopolysaccharide (LPS)-induced apoptosis in macrophages has been attributed to the LPS-mediated induction of pro-apoptotic TNF α acting back on the cells in an auto-crine/paracrine manner.⁸³ LPS triggers TNF α production in fetal membranes.⁸⁴ Elevated endotoxin levels are found in the amniotic fluid of women with preterm labor and PPRM.⁸⁵ Endotoxin is capable of stimulating prostaglandin production in amnion cells, and can initiate preterm labor via the host inflammatory response through activation of immunocytes and release of inflammatory cytokines.⁸⁶ Elevated levels of TNF α , together with other pro-inflammatory cytokines such as IL-1, are found in women with intra-amniotic infection and preterm labor, and, in turn, these cytokines stimulate prostaglandin synthesis in human tissues.^{74,85,87} The mRNA from TNF α and other pro-inflammatory cytokines is expressed in human fetal membranes in response to infection and endotoxin stimulation.⁸⁸ Infection is closely involved in the process of preterm birth, partly through the host response via

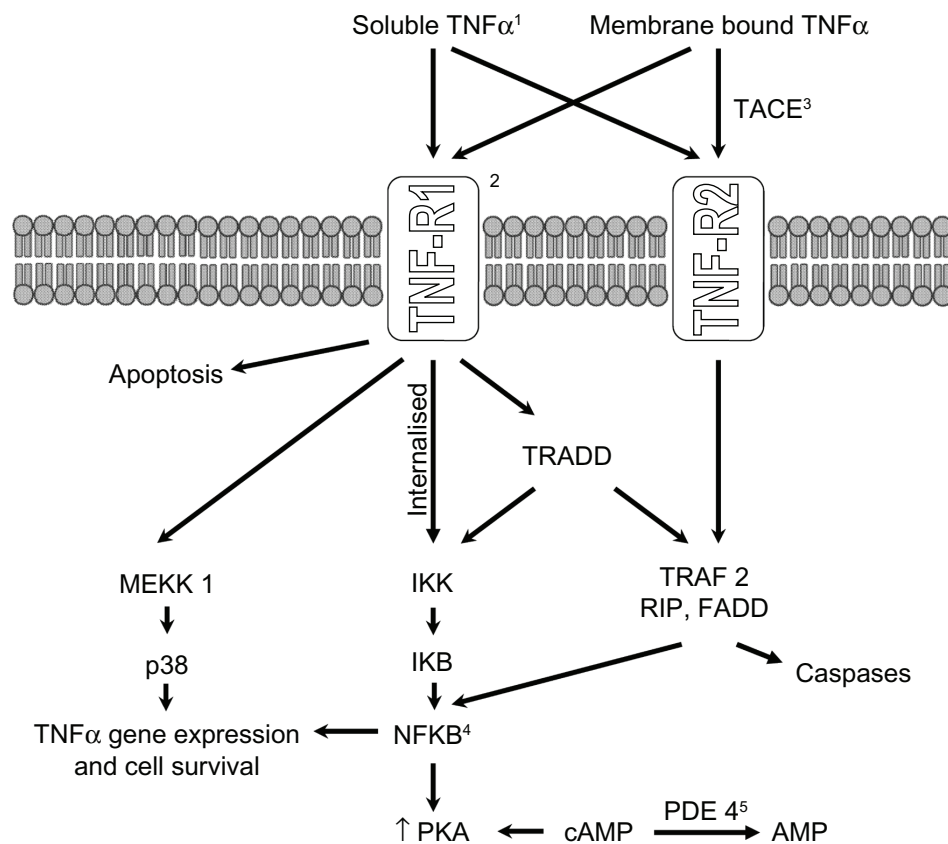


Figure 1 TNF- α signaling and the potential targets for the inhibition of TNF- α -related activities.

Notes: Inhibitors can target the TNF- α molecule (1) or its receptor (2), preventing the resultant signaling pathways. Another target includes TACE (3), which processes the 26-kDa membrane form of TNF- α to the soluble 17-kDa form preventing its release into the circulation. Inhibition of the activation of NF- κ -B (4) prevents the synthesis of NF- κ -B inducible genes, including many pro-inflammatory cytokines. Molecules targeting intracellular TNF- α -related signaling pathways have also been identified, including inhibitors of p38 and PDE4 (5).^{204,205}

Abbreviations: cAMP, cyclic AMP; DD, death domain; IB, inhibitor of NF-B; IKK, IB kinase; MEKK1, mitogen-activated protein kinase kinase kinase 1; NF-B, nuclear factor-B; NIK, NF-B-inducing kinase; PDE4, phosphodiesterase 4; PKA, protein kinase A; RIP, receptor-interacting protein; TACE, TNF-converting enzyme; TNF-, tumor-necrosis factor-; TNFR1, TNF-receptor 1; TRADD, TNFR1-associated death-domain-containing protein; TRAF2, TNFR-associated factor 2.

the inflammatory cytokine release, and its effect on starting uterine activity.⁸⁵ TNF α and other pro-inflammatory cytokine levels in the amniotic fluid increase towards term and in normal labor. However, there is an increase in TNF α released from the amniochorion, together with other pro-inflammatory cytokines in the amniotic fluid of women with preterm labor caused by intra-amniotic infection.

Women with preterm labor or PPRM have an elevated concentration of IL-6⁸⁹ and TNF α ⁹⁰ in the amniotic fluid, compared to women whose preterm labor did not progress to preterm delivery.⁹¹ Increased TNF α , together with IL-6, IL-1 α , IL-1 β , and PGE2 are associated with histologic chorioamnionitis among women who delivered within 1 week of amniocentesis.⁹⁰ Ex vivo incubation of whole unprocessed amniotic fluid may provide a more accurate indication of the cytokine release from amniotic cells, than just measuring the soluble components in the unincubated amniotic fluid supernatant (similar to using whole blood rather than peripheral blood mononuclear cells).⁹²

TNF α levels present in amniotic fluid are in the picogram per milliliter range.⁹³ TNF α peptide is present only in the amnion, but chorionic cells also have mRNA for TNF α .⁸⁸ TNF α has been detected in less than half of amniotic fluid samples in midtrimester, and even less in cases of preterm delivery.⁶² In Caucasians, midtrimester levels of TNF α and soluble TNF-Rs in symptomatic women are not significantly different between patients with preterm birth and those who proceed to term.^{36,62} Elevated TNF α concentration in amniotic fluid is associated with preterm birth and PPRM,^{94,93} and the bioavailability of TNF α and its receptors influences the pathophysiology of these outcomes.⁹⁵ During an ascending infection, the choriodecidua is the first line barrier for pathogens such as *E.coli*, that can cross the amniotic membranes and into the amniotic fluid.⁹⁶ In response to this ascending infection, there is abnormal production of TNF α in the amnion compartment when the pathogen affects both the amniotic membrane and the choriodecidua in vitro (comparable to chorioamnionitis in vivo).⁹⁷

Asymptomatic intra-amniotic infection is confirmed when micro-organisms are cultured in amniotic fluid obtained during amniocentesis. However, since culture results may take several days, measuring pro-inflammatory cytokines in amniotic fluid by enzyme-linked immunosorbent assay, as markers of intrauterine infection, may provide a quicker way of predicting preterm labor. TNF α is not normally detected in amniotic fluid in the 2nd and 3rd trimester, but rises during normal labor or in pathologic conditions such as intrauterine infection.^{80,93,98} IL-6 present in the amniotic fluid has been linked with chorioamnionitis.⁹⁹ Elevated TNF α > 6.6 pg/mL and IL-6 > 99.3 pg/mL levels in amniotic fluid samples obtained in asymptomatic women during second trimester amniocentesis can identify patients at risk for intra-amniotic infection (sensitivity of 78.4% and 91.9% and specificity of 70.1% and 73.8%) and preterm delivery (sensitivity of 81.3% and 89.6% and specificity of 79.2% and 80.3%).³⁷ However, studies have shown that some patients with a positive amniotic fluid culture and low levels of pro-inflammatory cytokines, still had preterm delivery. This could be due to a low maternal response due to functional polymorphism and/or some bacterial endotoxins may not be potent enough to stimulate the pro-inflammatory cytokine response.¹⁰⁰

It is still unclear whether cytokines in the maternal circulation can predict preterm labor, before symptoms of preterm labor or PPRM start. TNF α has been considered as a marker of preterm labor, together with other inflammatory cytokines such as IL-1B, because it can activate uterotonins and increase synthesis of prostaglandins, which can induce labor in non-human primates.^{101–103} A number of studies report elevated levels of pro-inflammatory cytokines in mid-pregnancy amniotic fluid,¹⁰⁴ maternal serum and cervical samples^{105,106} among women with preterm delivery, and even in placental tissues^{107–109} of spontaneous preterm deliveries. In the Preterm Prediction Study, the use of a combination of tests such as maternal serum alpha-fetoprotein, alkaline phosphatase, GM-CSF, fetal fibronectin and cervical length could enhance prediction of spontaneous preterm birth.¹¹⁰

Cytokines in the circulation are more non-specific than amniotic fluid or cervical fluid cytokines, because they might reflect a combination of a maternal acute-phase response accompanying the local inflammation, together with cytokines derived from the feto-placental unit. Therefore, the lack of association between preterm and midterm pregnancy circulatory cytokine levels in asymptomatic women suggest that inflammation occurring in the feto-placental unit may not always be reflected in maternal serum levels of TNF α and other cytokines. Also, the timing of inflammation in

pregnancy is probably very important, with inflammation occurring in the first trimester having a more significant association with adverse pregnancy outcome.¹¹¹

By contrast, plasma cytokine levels have been measured in a case-control study among Danish women at 25 weeks' gestation, using multiplex flow cytometry (Luminex Corporation, Austin, TX). Elevated TNF α levels >75th and >90th percentile do not differ by gestational age at delivery, and therefore are not associated with an increased risk of preterm delivery. There is an increased risk of preterm birth with elevated IFN γ and IL-6.¹¹² Therefore, there appears to be only limited value in using mid-pregnancy cytokines in predicting spontaneous preterm birth. During preterm labor, serum levels of IL-6, IL-8 and TNF α are not increased when compared to normal control women.¹¹³

Bacterial intrauterine infection stimulates maternal immune cells to produce pro-inflammatory cytokines.¹¹⁴ In non-human primates, inoculation of the amniotic cavity with TNF α or IL-1 β induces preterm labour.^{101,115} In mice, TNF α causes preterm birth,¹¹⁶ while TNF α -antibodies block LPS-induced preterm birth.¹¹⁷ Most infections leading to preterm birth are subclinical, and it may be possible that women who undergo a preterm birth have an increased immune response to the causative bacteria. There have been largely conflicting results regarding TNF α gene promoter polymorphisms that may increase the risk of preterm birth,¹¹⁸ and it is likely that either these polymorphisms alone do not cause preterm birth in the absence of infection,¹¹⁹ or else these polymorphisms do not increase TNF α secretion. Women with a history of preterm birth have an elevated TNF α production in response to LPS relative to controls.¹²⁰ However, in another study, peripheral blood mononuclear cells from women with a history of preterm birth have not produced significantly different amounts of TNF α in response to *E.Coli*, Group B Streptococci (*S. agalactiae*) and *U.urealyticum* (bacterial species causing preterm birth in animal species) compared to women with prior uncomplicated term deliveries.¹²¹

Cytokine profiles, especially TNF α , differ between different ethnic groups and by pregnancy outcome.^{36,122} In pregnancy, the function of TNF α is determined by its specific binding to one of its two receptors: MMP activation and apoptosis through TNFR1 and Nk-kB activation, leading to overall enhancement of inflammation through TNFR2.⁸⁰ The soluble forms of these membrane receptors bind to TNF α with high affinity and can neutralize TNF α function.^{95,125–125} There is a difference in the co-ordination between TNF α and its receptors between peoples (African Americans and Caucasians) with respect to preterm versus term delivery.

In Caucasians, midtrimester levels of TNF α and soluble TNF-Rs in symptomatic women are not significantly different between patients with preterm birth and those who proceed to term.^{36,62} Elevated TNF α concentration in amniotic fluid is associated with preterm birth and PPRM,^{93,94} and the bioavailability of TNF α and its receptors influences the pathophysiology of these outcomes.⁹⁵ During an ascending infection, the choriodecidual is the first line barrier for pathogens such as *E.coli*, that can cross the amniotic membranes and into the amniotic fluid.⁹⁶ In response to this ascending infection, there is abnormal production of TNF α in the amnion compartment when the pathogen affects both the amniotic membrane and the choriodecidual in vitro (comparable to chorioamnionitis in vivo).⁹⁷

TNF α is produced by both maternal and fetal tissues, and increases the production of prostaglandins, myometrial activity, induction of MMPs and apoptosis, all of which can lead to preterm labor, irrespective of infection.^{81,93} In amniotic membrane samples taken from the placenta of Caucasian women, there is a pronounced increase in TNF α concentration in response to endotoxin stimulation, compensated by an increase in soluble TNFRs. The latter is not evident in African Americans.¹²⁶ In amniotic fluid taken prior to labor (term or preterm) in African Americans (but not in Caucasians), there is increased TNF α bioavailability (higher TNF α compared to soluble TNFR1 and TNFR2) in women who deliver preterm compared to those who deliver at term.³⁶ Therefore, in Caucasians, but not in African Americans, TNF α changes in preterm labor are compensated by changes in soluble receptors.^{36,122}

This phenotypic difference of African Americans having a significant cytokine imbalance is caused by variation in the genes encoding these proteins, with significant differences between allelic, genotypic and haplotypic frequency differences in TNF α and TNF α receptor genes between different peoples.^{118,127} However, no association has been observed between these single nucleotide polymorphisms, including the TNF α promoter functional variant (–308) and other markers in the TNF α and TNF α receptor genes, and preterm birth.¹²⁷ This may be due to gene-environment interactions, with the effects of some single nucleotide proteins differing as a function of specific environmental factors. The presence of the TNF α risk allele at –308 can modify pregnancy outcome through interactions with bacterial vaginosis and periodontitis, even in the absence of an independent single locus effect.^{98,119,128} Therefore, genetic regulation of TNF α and soluble TNF-Rs concentrations in amniotic fluid is affected by ethnicity and preterm birth.³⁴ In Caucasians, TNF-Rs in the amniotic fluid

are higher in preterm than in term patients; but in African Americans, amniotic fluid TNF-Rs are higher in term versus preterm patients.³⁴ The disparity in inflammatory cytokine profiles found in amniotic fluid can partly explain the higher rate of preterm birth among African Americans and Caucasians in the United States. In African Americans with term birth, TNF α and IL-10 concentrations in amniotic fluid are positively correlated, indicating a generalized inflammatory status during labor, but there is a negative correlation coefficient in preterm birth with an overwhelming increase in TNF α not being co-ordinated by IL-6.¹²⁹ In this ethnic group, preterm labor is mediated predominantly by TNF α and IL-1 β .¹⁰¹ IL-10 levels correlated with soluble TNFR1 and TNFR2 in preterm, confirming immunoinhibitory mechanisms during preterm labor, which are overwhelmed by the increase in TNF α and IL-1. Therefore, the pathways leading to preterm birth may be different in the two ethnic groups.¹³⁰

There are probably other unmeasured (environmental) factors that interact to alter cytokine levels in amniotic fluid. Women with bacterial vaginosis and TNF α promoter polymorphism (–G238A) are at increased risk of delivering preterm, irrespective of ethnicity,¹³¹ further illustrating a potential gene-environment interaction in preterm delivery. The presence of bacterial vaginosis is associated with elevated levels of TNF α and other pro-inflammatory cytokines, such as IL-1, in the vaginal fluid.^{132,133} In vitro experiments with decidual and amniotic cells, these pro-inflammatory cytokines are able to induce the release of prostaglandins and MMPs.^{134,135} Therefore, high levels of TNF α in the presence of bacterial vaginosis may stimulate contractions and/or degradation of membranes.

It has been shown that during maternal infection, TNF α and IFN γ increase the production of prostaglandins, resulting in premature labor.^{136,137} A positive association has been shown between elevated levels of pro-inflammatory cytokines, including TNF α , IFN γ , IL-1, and IL-8.^{138–140} Studies have shown conflicting evidence as to whether increasing levels of TNF α are associated with an increased risk of intra-uterine growth restriction.^{141–143} In a recent study, higher levels of TNF α in umbilical cord blood was associated with preterm delivery, but not with intra-uterine growth restriction.¹⁴⁴ Interestingly, higher levels of other pro-inflammatory markers in the umbilical cord blood, such as IFN γ and interleukin 12p70, are associated with decreased risk of small for gestational age.¹⁴⁴

TNF α and preeclampsia

Preeclampsia is a potentially life-threatening complex multisystem maternal disorder that can occur in the second

half of pregnancy, labor or the early postpartum period. It is characterized by high blood pressure, proteinuria and other systemic disturbances secondary to diffuse maternal endothelial dysfunction.¹⁴⁵ Preeclampsia is considered as a state of exaggerated inflammation, in excess of the baseline inflammatory state of normal pregnancy, with local and systemic changes in Th1/Th2 cytokines.¹⁴⁶ Polymorphisms of cytokine genes may increase the risk of developing preeclampsia.¹⁴⁷ Peripheral blood mononuclear cells and decidual lymphocytes express higher levels of Th1 cytokines, including TNF α , and lower Th2 cytokine expression in preeclampsia compared to normal pregnancy.^{148,149} This is reflected in the maternal circulation, with a further rise in pro-inflammatory cytokines such as TNF α , accompanied by an elevated level of soluble receptor in an attempt to dampen the cytokine response.^{150–153} Increased levels of TNF α and other pro-inflammatory cytokines have also been found in the umbilical serum of pregnancies complicated by preeclampsia, suggesting a role in intra-uterine growth restriction secondary to preeclampsia.¹⁵⁴ The rise in pro-inflammatory cytokine TNF α and TNF-R1 in maternal circulation increases as early as 11–13 weeks, well before the clinical manifestation of preeclampsia,¹⁵⁵ but so far has not proved to be useful in screening.¹⁵⁶

In placental preeclampsia, there is defective placentation with insufficient remodeling of the uterine spiral arteries by the EVT towards the end of the first trimester and in the early second trimester leading to an ischemia-reperfusion phenomenon with subsequent excessive oxidative stress.¹⁵⁷ It has been shown that placentation is better, with a decrease in incidence of preeclampsia, if the trophoblast strongly stimulates maternal uterine NK cells, which in turn secrete pro-inflammatory cytokines to allow proper invasion.¹⁵⁸ Activity of decidual NK cells is in turn regulated by a complex network of cytokines.¹⁵⁹ Pro-inflammatory cytokines such as IL-1 can stimulate MMP-9 and 2¹⁶⁰ and therefore can act as positive regulators of trophoblast differentiation in becoming more invasive. The contrary has been shown for anti-inflammatory cytokines such as IL-10 and transforming growth factor β .^{161,162} As mentioned above, there is a predominance of TNF α in cell columns during invasion, especially in the EVT, as it displaces the endothelial cells of the spiral arteries.^{46,47}

TNF α and its receptors are expressed in excess both systemically and at the feto-maternal interface¹⁴⁶ and may play a key role in the pathophysiology of preeclampsia. In preeclampsia, TNF α , together with IFN γ , has been shown to cause apoptosis of cultured cytotrophoblasts

and syncytiotrophoblasts, together with impairment of syncytialization, especially under hypoxic conditions in term placenta.¹⁶³ In vitro studies have shown that the combination of TNF α and IFN γ inhibit first trimester EVT invasion due to increased apoptosis and reduced proliferation of EVT cells and reduced pro-MMP-2 secretion.¹⁶⁴ Hypoxia/re-oxygenation leading to placental oxidative stress is a potent inducer of TNF α secretion by villous explants.⁷³ Since there is an elevation of both of these pro-inflammatory cytokines in the placenta of preeclamptic patients,^{165,166} they may have a role in abnormal placentation. TNF α may inhibit migration of EVT in the first trimester placenta via elevated plasminogen activator inhibitor-1¹⁶⁷ or via activated macrophages.¹⁶⁸ The sources of TNF α in preeclampsia are the trophoblast cells themselves due to the ischemia-reperfusion insult,^{56,169} as well as the activated maternal monocytes upon adhering to the syncytiotrophoblast.^{170,171} TNF α has also been shown to inhibit the subset of CD4+CD25+ regulatory T lymphocytes.¹⁷² The latter cells promote fetal tolerance during normal pregnancy, and once inhibited, will not be able to produce immunosuppressive cytokines that are important at the feto-placental interface to prevent fetal rejection.¹⁷³

Preeclampsia is associated with a systemic inflammatory response, which is more exaggerated than what happens in normal pregnancy, due to aberrant cytokine expression.¹⁷⁴ In early onset preeclampsia, TNF α /IL-10 findings suggest that an imbalance in pro-inflammatory to anti-inflammatory cytokines ratio is associated with unfavorable pregnancy outcomes.¹⁷⁵ Toll-like receptor (TLR)-4 increases production of TNF α .¹⁷⁶ TLR is the main danger signaling pathway involved in the pathogenesis of preeclampsia.¹⁷⁷ TLR2 and TLR4 single nucleotide proteins appear to alter the maternal susceptibility to preeclampsia.¹⁷⁸

TNF α is a potential mediator of endothelial cell dysfunction, contributing to the systemic effects of preeclampsia.^{150,179} The excess TNF α produced by the placental villous tissue in response to the hypoxia-reperfusion injury affects the endothelial cells by reducing their viability, and upregulating the expression of adhesion molecule E-selectin.⁵⁶ Excess placental production of factors, such as vascular endothelial growth factor receptor-1 (also known as soluble fms-like tyrosine kinase 1 (sFlt-1)), which bind to vascular endothelial growth factor and placental growth factor are anti-angiogenic.^{180,181} They deprive the systemic endothelium of essential survival factors, decreasing the number of adhesion complexes at the cytoplasmic membrane, leading to vascular permeability.¹⁸² However, the role of cytokines to this particular endothelial response to serum factors

is still not clear. Elevated angiotensin II type-1 receptor autoantibodies (AT1-AA), together with cytokines, lead to dysfunctional maternal vascular endothelium.¹⁸³ This in turn leads to increased levels of circulating endothelin, reactive oxygen species, and increased vascular sensitivity to angiotensin II, together with lower levels of vasodilators, such as prostacyclin and nitric oxide.¹⁸⁴ This can lead to multi-organ dysfunction in preeclampsia, including hemolysis, elevated liver enzymes and low platelets syndrome.^{184,185} Sex steroids also play a role in modulating the effect of TNF α on vascular function in preeclampsia. However, in rats, increased levels of ovarian hormones to those observed in pregnancy were not sufficient to induce TNF α -induced vascular changes observed in preeclampsia.¹⁸⁶ Trophoblastic debris, including syncytiotrophoblast membrane microparticles, fetal soluble RNA and DNA, cytokeratin fragments and cytotrophoblast cells, is released into the maternal circulation by apoptotic and necrotic processes in elevated amounts compared to normal pregnancy. This debris is pro-inflammatory and, through the release of cytokines such as TNF α , aggravates maternal inflammation.¹⁸⁷ It has been shown that placental ischemia leading to preeclampsia is associated with raised inflammatory cytokines such as TNF α , and CD4+ T helper cells.¹⁸⁸

Currently, there is no reliable test that can be used for screening or to facilitate informed decision during management of preeclampsia. Therefore, better understanding of the link between abnormal hemostasis and inflammation in preeclampsia may clarify the underlying pathophysiology, and help design primary preventative and therapeutic measures at an early stage.¹⁸⁹

TNF α -inhibitors and their role in pregnancy

Over the past 2 decades, anti-TNF α treatment has been developed, including etanercept (Enbrel), a recombinant soluble TNF-R2, and monoclonal TNF α -antibodies, such as adalimumab (Humira), infliximab (Remicade) and certolizumab pegol (Cimzia). These have been licensed for use in the treatment of autoimmune diseases such as inflammatory arthritis¹⁹⁰ and inflammatory bowel disease,¹⁹¹ and there is also research showing their possible role in the management of recurrent colorectal cancer.¹⁹² Because of the immuno-modulatory action of these biologicals, there have been associated increased risks of infections such as viral, tuberculosis and histoplasmosis, and lymphoma.¹⁹³

In an LPS-induced murine model of preterm birth, the use of anti-TNF α treatment decreased fetal deaths and preterm deliveries.¹¹⁷ Although regulatory agencies encourage

the participation of pregnant and breastfeeding women in randomized controlled trials, this subset of the population has universally been excluded from studies involving the use of anti-TNF α treatment because of unknown or potential risks to the fetus. Thus, strong evidence-based treatment recommendations during pregnancy is lacking, and TNF α inhibitors are listed as Class B, that is, animal reproduction studies have failed to demonstrate fetal risk and there are no well-controlled studies in pregnant women.

Since autoimmune diseases such as Crohn's disease, ulcerative colitis, and rheumatoid, psoriatic, and juvenile idiopathic arthritis are prevalent in women of childbearing age, there have been a number of case reports and registries documenting the effect of the incidental use of anti-TNF α agents in women who inadvertently became pregnant while on treatment.^{194–196} Overall, conflicting results have been produced from these case reports and small case series, partly due to the different timing of when the treatment was taken, other concurrent medication such as methotrexate, and different underlying autoimmune conditions of varying severity. Occurrence of uncommon adverse pregnancy outcomes observed with TNF α inhibitor therapy, such as premature birth, miscarriage, low birth weight, hypertension, and preeclampsia appear to approximate those seen in women not receiving such therapy and may be due to the underlying autoimmune condition itself.¹⁹⁷ While there is data suggesting little to no risk of congenital anomalies,¹⁹⁷ a large independent review of the Food and Drug Administration database reports a higher number of VACTERL anomalies in offspring of mothers who were on TNF α -antagonists at some point during their pregnancy.^{198,199}

VACTERL is a non-random association of birth defects, including vertebral anomalies (V), anal atresia (A), cardiovascular anomalies (C), tracheoesophageal fistula (T), esophageal atresia (E), renal and/or radial anomalies (R) and limb defects (L). So far, the recommendations per observational studies are that women of childbearing age with autoimmune diseases should ideally plan to conceive when their disease is well controlled and while on no medication, and most pregnant patients can discontinue their anti-TNF α treatment early in pregnancy without increasing maternal and fetal risks.¹⁹⁷

Anti-TNF α treatment has been shown to increase live birth rates in women with recurrent spontaneous abortion⁶⁷ and in a subset of patients with a history of >2 failed in-vitro fertilization attempts,²⁰⁰ with the latter study having an impressive 100% pregnancy and 88% take-home baby rate. In both of the cohort-controlled, non-randomized studies,

treatment was generally started a month prior to starting a cycle of conception, and continued until a fetal heart was demonstrable by ultrasound. Minimal side-effects and no birth defects were reported. Pretreatment with anti-TNF α is thought to reduce Th1/Th2 levels in CD3+ cells by upregulating regulatory T-cell activity in women with Th1 driven inflammation.²⁰¹ However, in both studies there could have been a selection bias in the choice of patients, because many of the patients without anti-TNF α treatment lacked the high qualifying ratio of Th1/Th2. The karyotype was not tested in the cases of recurrent miscarriage, and the maternal-fetal genotype was unknown. There are also other factors controlling reproductive outcome, such as autoantibodies and coagulation defects, therefore using Th1/Th2 ratios alone may not be enough to determine who would benefit from anti-TNF α treatment. Also, one needs to define at what level is Th1/Th2 ratio considered high to merit a beneficial effect from anti-TNF α treatment.

Although the observational studies of Winger et al represent important new data in the field of reproductive immunology,^{67,200,202,203} further prospective randomized controlled studies are needed. Studies in mice are showing that targeting placental TNF α using TNF α -antagonists such as etanercept prevents fetal hypoxia and neuroproliferative defects in the fetal brain.⁷⁰ Understanding the mechanism of action of TNF α and its receptors may lead to development of new drugs to decrease the pro-inflammatory effects of this cytokine (Figure 1).

Conclusion

There is still a lot to be learnt about the role of TNF-R1 and TNF-R2 in normal and complicated pregnancies. Recently, studies have shown that altered levels of these receptors in the circulation, in combination with other cytokines and/or hormones, may play a role in predicting miscarriage in patients presenting with threatened miscarriage.²⁰⁶ Future clinical trials are needed to study the possible benefit of anti-TNF treatment in pregnancy complications.

Disclosure

The authors declare no conflicts of interest.

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