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REVIEW

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

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Abstract: TNFα receptors, TNF-R1 and TNF-R2, mediate the biological activities of the multifunctional cytokine, tumor necrosis factor alpha, TNFα. 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 TNFα, 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 TNFα, neutralizing its effects. In normal human pregnancy, TNFα receptors are present in the maternal circulation, placenta, amniotic fluid, and coelomic cavity. Changes in TNFα and its receptors are associated with adverse pregnancy outcomes, including miscarriage, preterm labor and preeclampsia. Advances in anti-TNFα therapy may have potential use in the management of complicated pregnancies.

Keywords: TNFα 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 (IFNγ) 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 TNFα, 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 TNFα receptors

TNFα 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 TNFα are mediated via two different TNFα receptors (TNF-Rs): TNF-R1 (also known as p55/p60, Type I, b, TNF-R55, TNF-Rβ or CD120a) with a molecular mass of 55–60 kDa; and TNF-R2 (or p75/p80, Type II, a, TNF-R75, TNF-Rα 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 TNFα via the TNF-R1, the extracellular part of TNF-R2 captures TNFα, even at low concentrations, and delivers it to TNF-R1, resulting in an enhanced response to TNFα.[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 TNFα 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 TNFα, 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 TNFα by neutralizing this cytokine.[21] The soluble form of TNF-R2 is cleaved by proteolysis through the metalloproteinase TNFα converting enzyme (TACE, also known as ADAM17).[22,23] Soluble TNF-R2 is involved in the inactivation of TNFα in the circulation by the formation of high affinity complexes. This subsequently reduces the binding of TNFα to target cell membrane receptors and downregulates the response to TNFα.[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]

TNFα 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 TNFα 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, TNFα 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, TNFα 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-Rβ or CD120a	p75/p80, Type II, a, TNF-R75, TNF-Rα 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 TNFα signalling, leading to proinflammatory and programmed cell death pathways, and cytotoxicity; carries out most of the activities of TNFα, 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 TNFα in the circulation
Signaling pathways	Interacts indirectly with TRAF2 via TRADD	Interacts directly with TRAF2

Abbreviations: DD, death domain; EVT, extravillous trophoblasts; TNFα, 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, TNFα 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] TNFα and its receptors are probably produced from within the gestational sac from a very early stage in pregnancy. TNFα gene products have been detected in placental supernatants.[41,42]

During the first trimester, all cell types of trophoblastic lineage express TNFα 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 cells[44] during early human gestation. There is a predominance of TNFα 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 tissue[48] 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 TNFα.[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 TNFα derived from macrophages, possibly modulated by TNFα-TNFRI signaling, facilitates trophoblast differentiation.[48,51] TNFα at the fetal-maternal interface plays an important role in regulating macrophage recruitment by trophoblast cells. It has been shown that media conditioned by TNFα-treated trophoblast cells significantly enhance the ability of the monocyte cell line THP-1 to invade through Matrigel.[52] TNFα 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 TNFα 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 TNFα and IFNγ, 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 TNFα 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 TNFα.[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 TNFα and its receptors may have a specific role in the process of developmental differentiation. Later in pregnancy, TNFα 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 TNFα protein in invasive cells, and no expression at all in trophoblast giant cells.[57] TNFα 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 TNFα, both alone and in combination with IFNγ, 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 TNFα by inhibiting the release of TNFα, 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] TNFα is important in the initiation and amplification of inflammation.[63] TNF-Rs may reduce oxidative stress due to receptor binding of the inflammatory TNFα.

Many cell types present in the endometrium, placenta and deciduas have been shown to express TNFα 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]

TNFα 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 TNFα, 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 TNFα inhibitors, this group of women had an increase in the rate of live births.[67] TNFα is unlikely to be the only mediator and, in most cases of miscarriage, there are additional triggers.[9] Evidence shows that TNFα, IFNγ 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 TNFα to induce apoptosis in these cells, leading to tissue damage in chorionic villi in non-viable pregnancies.[69] Mice studies are showing that TNFα via TNFR1 signaling causes placental pathology leading to fetal hypoxia, which can be prevented by TNFα-antagonists.[70]

TNFα 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, TNFα and other pro-inflammatory cytokines have been shown to stimulate uterine activity and cervical ripening by producing prostaglandins[74] 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. TNFα, together with other pro-inflammatory cytokines such as IL-1β, 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 PPROM.[80,81]

In PPROM, two major apoptotic pathways have been implicated. The first is a TNFα 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 TNFα acting back on the cells in an autocrine/paracrine manner.[83] LPS triggers TNFα production in fetal membranes.[84] Elevated endotoxin levels are found in the amniotic fluid of women with preterm labor and PPROM.[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 TNFα, 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 TNFα 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

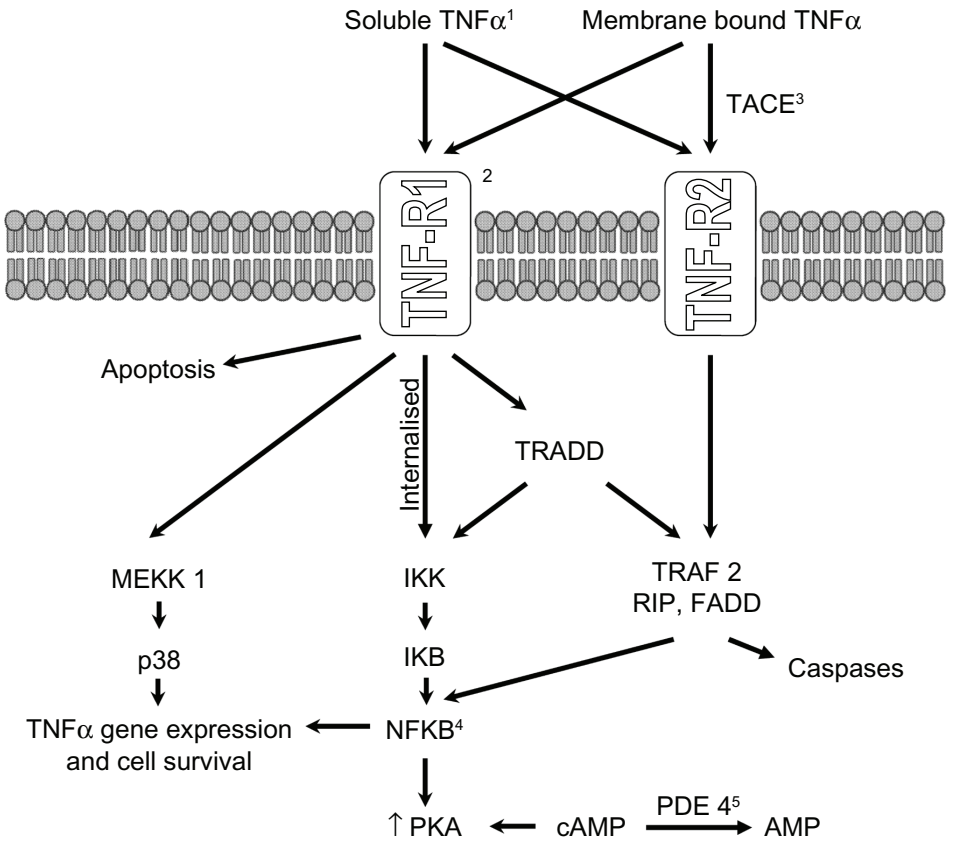

Figure 1 TNF-α signaling and the potential targets for the inhibition of TNF-α-related activities.
Notes: Inhibitors can target the TNF-α molecule (**1**) or its receptor (**2**), preventing the resultant signaling pathways. Another target includes TACE (**3**), which processes the 26-kDa membrane form 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-coverting 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] TNFα and other pro-inflammatory cytokine levels in the amniotic fluid increase towards term and in normal labor. However, there is an increase in TNFα 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 PPROM have an elevated concentration of IL-6[89] and TNFα[90] in the amniotic fluid, compared to women whose preterm labor did not progress to preterm delivery.[91] Increased TNFα, 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]

TNFα levels present in amniotic fluid are in the picogram per milliliter range.[93] TNFα peptide is present only in the amnion, but chorionic cells also have mRNA for TNFα.[88] TNFα 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 TNFα 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 TNFα concentration in amniotic fluid is associated with preterm birth and PPROM,[94,93] and the bioavailability of TNFα 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 TNFα 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. TNFα 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 TNFα > 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 PPROM start. TNFα 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 TNFα 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 TNFα 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 IFNγ 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 TNFα 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 TNFα or IL-1β induces preterm labour.[101,115] In mice, TNFα causes preterm birth,[116] while TNFα-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 TNFα 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 TNFα secretion. Women with a history of preterm birth have an elevated TNFα 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 TNFα 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 TNFα, differ between different ethnic groups and by pregnancy outcome.[36,122] In pregnancy, the function of TNFα 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 TNFα with high affinity and can neutralize TNFα function.[95,125–125] There is a difference in the co-ordination between TNFα and its receptors between peoples (African Americans and Caucasians) with respect to preterm versus term delivery.

In Caucasians, midtrimester levels of TNFα 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 TNFα concentration in amniotic fluid is associated with preterm birth and PPROM,[93,94] and the bioavailability of TNFα 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 TNFα in the amnion compartment when the pathogen affects both the amniotic membrane and the choriodecidua in vitro (comparable to chorioamnionitis in vivo).[97]

TNFα 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 TNFα 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 TNFα bioavailability (higher TNFα 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, TNFα 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 TNFα and TNFα receptor genes between different peoples.[118,127] However, no association has been observed between these single nucleotide polymorphisms, including the TNFα promoter functional variant (−308) and other markers in the TNFα and TNFα 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 TNFα 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 TNFα 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, TNFα 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 TNFα not being co-ordinated by IL-6.[129] In this ethnic group, preterm labor is mediated predominantly by TNFα and IL-1β.[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 TNFα 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 TNFα 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 TNFα 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 TNFα in the presence of bacterial vaginosis may stimulate contractions and/or degradation of membranes.

It has been shown that during maternal infection, TNFα and IFNγ 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 TNFα, IFNγ, IL-1, and IL-8.[138–140] Studies have shown conflicting evidence as to whether increasing levels of TNFα are associated with an increased risk of intra-uterine growth restriction.[141–143] In a recent study, higher levels of TNFα 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 IFNγ and interleukin 12p70, are associated with decreased risk of small for gestational age.[144]

TNFα 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 TNFα, 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 TNFα, accompanied by an elevated level of soluble receptor in an attempt to dampen the cytokine response.[150–153] Increased levels of TNFα 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 TNFα 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 2[160] 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 TNFα in cell columns during invasion, especially in the EVT, as it displaces the endothelial cells of the spiral arteries.[46,47]

TNFα and its receptors are expressed in excess both systemically and at the feto-maternal interface[146] and may play a key role in the pathophysiology of preeclampsia. In preeclampsia, TNFα, together with IFNγ, has been shown to cause apoptosis of cultured cytoblasts and syncytiotrophoblasts, together with impairment of syncytialization, especially under hypoxic conditions in term placenta.[163] In vitro studies have shown that the combination of TNFα and IFNγ 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 TNFα 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. TNFα may inhibit migration of EVT in the first trimester placenta via elevated plasminogen activator inhibitor-1[167] or via activated macrophages.[168] The sources of TNFα 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] TNFα 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, TNFα/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 TNFα.[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]

TNFα is a potential mediator of endothelial cell dysfunction, contributing to the systemic effects of preeclampsia.[150,179] The excess TNFα 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 TNFα on vascular function in preeclampsia. However, in rats, increased levels of ovarian hormones to those observed in pregnancy were not sufficient to induce TNFα-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 TNFα, 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]

TNFα-inhibitors and their role in pregnancy

Over the past 2 decades, anti-TNFα treatment has been developed, including etanercept (Enbrel), a recombinant soluble TNF-R2, and monoclonal TNFα-antibodies, such as adalimumab (Humira), infliximab (Remicode) and certolizumab pegol (Cimzia). These have been licensed for use in the treatment of autoimmune diseases such as inflammatory arthritis[190] 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-TNFα 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-TNFα treatment because of unknown or potential risks to the fetus. Thus, strong evidence-based treatment recommendations during pregnancy is lacking, and TNFα 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-TNFα 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 methotraxate, and different underlying autoimmune conditions of varying severity. Occurrence of uncommon adverse pregnancy outcomes observed with TNFα 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 TNFα-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-TNFα treatment early in pregnancy without increasing maternal and fetal risks.[197]

Anti-TNFα treatment has been shown to increase live birth rates in women with recurrent spontaneous abortion[67] 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-TNFα 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-TNFα 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-TNFα treatment. Also, one needs to define at what level is Th1/Th2 ratio considered high to merit a beneficial effect from anti-TNFα 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 TNFα using TNFα-antagonists such as etanercept prevents fetal hypoxia and neuroproliferative defects in the fetal brain.[70] Understanding the mechanism of action of TNFα 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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