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# TNF-$\alpha$ in Pregnancy Loss and Embryo Maldevelopment: A Mediator of Detrimental Stimuli or a Protector of the Fetoplacental Unit?

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***Purpose*:** Tumor necrosis factor alpha (TNF-$\alpha$), a multifunctional cytokine, has been identified in the ovary, oviduct, uterus, and placenta, and is expressed in embryonic tissues. For many years TNF-$\alpha$ was mainly considered to be a cytokine involved in triggering immunological pregnancy loss and as a mediator of various embryopathic stresses. However, data collected during the last decade has characterized TNF-$\alpha$ not only as a powerful activator of apoptotic, but also antiapoptotic signaling cascades, as well as revealed its regulatory role in cell proliferation. This review summarizes and conceptualizes the studies addressing TNF-$\alpha$-activated intracellular signaling and the possible functional role of TNF-$\alpha$ in embryonic development.
***Methods*:** Studies addressing the role of TNF-$\alpha$ in intercellular signaling, *in vivo* studies addressing the functional role TNF-$\alpha$ in spontaneous and induced pregnancy loss, and studies addressing the role of TNF-$\alpha$ in fetal malformations were reviewed. Comparative studies in TNF-$\alpha$ knockout and TNF-$\alpha$ positive mice were performed to evaluate embryonic death, structural anomalies in fetuses, the degree of apoptosis and cell proliferation, and the activity of molecules such as caspases 3 and 8, the NF-$\kappa$B, (RelA), I$\kappa$B$\alpha$ in some target embryonic organs shortly after exposure to embryopathic stresses.
***Results*:** It is proposed that the possible essential function of TNF-$\alpha$ may be to prevent the birth of offspring with structural anomalies.
***Conclusions*:** TNF-$\alpha$ will boost death signaling to kill the embryo if initial events (damages) triggered by detrimental stimuli may culminate in structural anomalies, and stimulate protective mechanisms if the repair of these damages may prevent maldevelopment.

**KEY WORDS:** Apoptosis; embryo; pregnancy loss; teratogenesis; TNF-$\alpha$.

## INTRODUCTION

Tumor necrosis factor alpha (TNF-$\alpha$), a multifunctional cytokine, was identified in the ovary, oviduct, uterus, and placenta (1), and it is expressed in embryonic tissues (2) practically at all stages of development. Early studies addressing its functional role in reproduction revealed that that injection of TNF-$\alpha$ or endotoxin (lipopolysaccharide, LPS) to pregnant females results in embryonic death (3,4). Further experiments in the CBA/J $\times$ DBA/2J mouse combination (a model with a high incidence of embryonic death, possibly due to rejection of the semiallogeneic fetoplacental unit) have revealed an increased level of TNF-$\alpha$ in the supernatants of decidual cell cultures (3). TNF-$\alpha$ expression has also been found to be raised in the placentae of CBA/J $\times$ DBA/2J mice (5). The TNF-$\alpha$ level has also been shown to be significantly elevated in the amniotic fluid of women with uterine infections, and its increased production correlates with the incidence of preterm labor (6). These

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observations have implicated TNF-α as a cytokine involved in triggering immunological pregnancy loss (7,8), i.e. death of embryos owing to failure of defense mechanisms preventing rejection of the semiallogeneic fetoplacental unit.

Additionally, TNF-α has been implicated in embryopathies caused by developmental toxicants, various stresses, and maternal metabolic imbalances. Elevated TNF-α expression has been observed in the uterine epithelium and stroma, and in the giant and spongiotrophoblast cells of the placenta of mice exposed to the DNA-damaging agent cyclophosphamide (CP) (9). Increased production of TNF-α by decidual NK cells and/or macrophages has been observed in mice treated with LPS (4). TNF-α producing cells located at the fetomaternal interface have been observed to be activated and to increase the local production of TNF-α in mice exposed to various stresses (10). Studies in diabetic animals, which demonstrate a dramatic decrease in pregnancy rate (11), have revealed that the synthesis of TNF-α increases in the uterine cells from the beginning of implantation onward (12), and it has been revealed that mouse blastocysts exposed to TNF-α *in vitro* have an increased death rate when transferred into pseudopregnant mice (13,14). Finally, elevated levels of TNF-α mRNA and protein expression have been observed in the embryo itself after exposure to CP in doses, which induce structural anomalies (15).

These findings as well as the ability of TNF-α to act as a cytotoxic agent in various cells (16) gave rise to the hypothesis that TNF-α may act as a mediator of detrimental stimuli inducing embryonic death and inborn structural anomalies (14,17). This hypothesis was also supported by results of early studies addressing mechanisms of TNF-α cytotoxicity, which revealed that the cytokine acting in a cell type and stimulus dependent manner, may activate the process of programmed cell death (apoptosis) (18).

Apoptosis is an indispensable event at all stages of embryonic development (19,20). It occurs as early as the blastocyst, during the formation of extraembryonic tissues, and continues throughout organogenesis. Apoptosis is involved in such developmental phenomena and processes as sculpting structures, deleting unneeded structures and eliminating abnormal, misplaced, nonfunctional, or harmful cells. It is clear, therefore, that tight regulation of apoptosis in embryonic cells as well as in cell populations residing at the fetomaternal interface is necessary for normal embryogenesis.

This paradigm was clearly supported by studies demonstrating that embryonic death induced by some developmental toxicants is preceded by the increase in the level of apoptosis in uterine and placental cells (21). An increased level of apoptosis was also detected in blastocyts developing *in vitro* and *in vivo* in hyperglycemic conditions inducing embryonic death during or shortly after implantation (14). Finally, gross structural anomalies were often preceded by excessive apoptosis in the structures of postimplantation embryos destined to be malformed (22,23). In the light of this evidence, the ability of TNF-α to activate apoptosis suggests a mechanism for its role as a mediator of embryopathic stimuli.

However, apoptosis is a genetically regulated process, which is regulated by signaling cascades comprising of molecules, which act as activators, effectors, and negative regulators of the process (24,25). The fact that TNF-α may not only initiate, but also prevent apoptosis has been well established in many different *in vitro* and *in vivo* models (26) and the molecular mechanisms contributing to the antiapoptotic effect of this cytokine have also been described (27). These observations have obliged us to question the concept that TNF-α is unambiguously detrimental for embryonic development. This review outlines some TNF-α activating intracellular signaling cascades, and discusses the concept that TNF-α may function not only as a mediator of detrimental stimuli, but also activate mechanisms which resist and repair the injures induced by these stimuli. Since there is a plethora of reviews addressing the role of TNF-α in activation of life and death signaling cascades, this topic will not be addressed here in any detail, but the review will focus only on the points, which seem to be the most relevant to the topic of the review.

## TNF-α AS AN ACTIVATOR OF INTRACELLULAR DEATH SIGNALING

Cell death is practically always involved in the process leading to embryonic death, or the formation of structural birth defects, regardless of the nature of the stresses triggering these processes (28). There is evidence that embryopathic stresses first disturb the apoptotic process by increasing the rate of cell death in target structures (22,23). Therefore, the main reason that TNF-α has been considered to be a mediator of stimuli inducing embryonic death and maldevelopment is its ability to activate apoptotic signaling cascades (29).

According to current theory (24,25), there are two basic apoptotic pathways: the death-receptor-mediated (extrinsic) signaling pathway and the mitochondria-mediated (intrinsic) signaling pathway. The former is activated by binding of death receptors (30) to their ligands, followed by recruitment of adapter proteins (29) and pro-caspases (31) (mainly, pro-caspase 8) to the cytoplasmic domains of the receptors, resulting in the formation of a death-inducing signal complex (DISC). In this complex, pro-caspase 8 is processed to active caspase 8, an initiator caspase, which cleaves and activates caspase 3, an effector caspase, which, in turn, cleavages caspase target cellular proteins.

The second pathway is triggered by apoptotic signals such as DNA damages and oxidative stress, which induce cytochrome *c* release from the mitochondria into the cytoplasm (32). In the cytoplasm, cytochrome *c* binds to apoptosis-protease activating factor-1 (Apaf-1), which, in turn, binds to pro-caspase 9, forming an apoptosome. Pro-caspase 9 is activated within the apoptosome and then activates caspase 3. There is also convincing evidence that some proapoptotic members of the Bcl-2 family of proteins (33), especially, Bid and Bax act as a positive regulators of this apoptotic cascade promoting cytochrome *c* release from mitochondria.

TNF-$\alpha$ has the potential to activate both these apoptotic pathways (27,34). The death-receptor-mediated signaling pathway is activated by TNF-$\alpha$ mainly through binding to the cell surface TNF receptor type 1 (TNFR1), which contains a cytoplasmic death domain (DD). This event is followed by recruitment of adapter proteins such as TNFR1-associated death domain protein (TRADD) and Fas-associated death domain protein (FADD) with further activation of caspases 8 and 3. The role of TNF receptor type 2 (TNFR2), which lacks a cytoplasmic DD, in TNF-activating apoptotic signaling is not completely understood, but some mechanisms have been suggested by which TNFR2 may potentiate apoptosis mediated by TNF /TNFR1 signaling (27,34).

The existence of mechanisms, involving TNF-$\alpha$ in the activation of the mitochondria-mediated signaling pathway, have also been demonstrated (27,34). They include activation of Bid and Bax proteins via TNF/TNFR1 apoptotic signaling cascade and a direct influence of TNF-$\alpha$ on mitochondrial function resulting in the release of cytochrome *c*.

Along with these basic apoptotic-signaling pathways, which may be activated (extrinsic pathway) or promoted (intrinsic pathway) by TNF-$\alpha$, the results of *in vitro* studies with cell cultures have implicated the involvement of the tumor suppressor protein p53 in TNF-$\alpha$-dependent apoptosis (35,36). The p53 protein plays a key role in the protection of the genome from the consequences of various stresses, such as DNA damage, hypoxia, heat shock, loss of normal growth and survival signals, and others (37). These stresses induce stabilization and activation of the protein resulting, depending on the cellular context, in cell cycle arrest or apoptotic cell death (37). Evidence has accumulated that p53 may stimulate both death-receptor-mediated apoptotic signaling, while regulating the membrane expression of certain death receptors, and mitochondrial-mediated apoptotic signaling, including cytochrome *c* release and activation of some proapoptotic members of the Bcl-2 family proteins (references in 37).

The functional role of p53-mediated apoptosis in the response to embryopathic stress will be discussed later. Here, we only want to mention that excessive apoptosis in the embryo, regardless of its nature, if uncompensated for, ultimately leads to maldevelopment or embryonic death. Therefore, the evidence implicating p53 in TNF-$\alpha$-activating apoptosis suggests an additional mechanism, which may be used by TNF-$\alpha$ to boost apoptotic signaling.

Finally, a new concept of "silencing of survival signals" (SOSS) seems relevant to this review (38). The concept complements and develops a theory on the system of intercellular signaling proteins, pro- and antiinflammatory cytokines, which controls the life and death of neurons while inhibiting the synthesis of each other (references in 38). Detrimental stimuli inducing neurodegeneration may shift the cytokine balance towards dominance of proinflammatory cytokine production, and hence, boost death signaling. The concept of SOSS proposes that pro- and anti-inflammatory cytokines may regulate the signaling of each other, not only indirectly, by inhibiting the synthesis of a cytokine, but also directly, by regulating each other's signal transduction pathways mediated by their receptors in a single cell. The existence of this mechanism is suggested by data demonstrating that TNF-$\alpha$ (a proinflammatory cytokine) at insufficient concentrations to activate the TNF-$\alpha$/TNFR1 apoptotic cascade, promotes neuronal death via TNF-$\alpha$/TNFR1-mediated inhibition (silencing) of the survival signaling induced by insulin-like growth factor (IGF)-1 (an antiinflammatory cytokine) through the type 1 IGF receptor. IGF-1 is a survival factor preventing the onset of apoptosis and this activity of IGF is mediated by the type 1 IGF receptor (39,40).

Evidence suggesting that this mechanism may also be involved in TNF-$\alpha$-mediated embryotoxicity may briefly be formulated as follows. The balance between proinflammatory T helper (Th) 1 cytokines such as interferon (IFN)-$\gamma$, interleukin (IL)-2, TNF-$\alpha$, and antiinflammatory Th 2/3 cytokines, such as IL-4, IL-10, and transforming growth factor (TGF)-$\beta$ is an essential condition for normal, successful pregnancy (8,41). Pro-inflammatory cytokines are necessary at the initial steps of implantation, but shortly thereafter the Th1 to Th2/3 shift is essential to prevent their possible embryotoxic effect (41). The IGF system, including IGF-1 and IGF-2, their receptors, and IGF binding proteins, was found to be essential for normal embryonic and placental growth as early as implantation (39,40). Also, a number of studies suggest that the IGF system may be involved in regulation of the apoptotic process during organogenesis, and that in some types of cells, IGF-1 has the potential to inhibit the action of the death receptors for TNF-$\alpha$ and Fas ligand (references in 40).

Thus, the above data suggest the existence of numerous molecular mechanisms, activation of which allows TNF-$\alpha$ to exert its cytotoxic effect, and hence act as a mediator of detrimental stimuli with the potential to induce embryonic death or maldevelopment. The evidence presented below suggests that TNF-$\alpha$ may also activate the mechanisms which may counteract the process of embryonic maldevelopment.

## TNF-$\alpha$ AS AN ACTIVATOR OF INTERCELLULAR MECHANISMS WITH THE POTENTIAL TO PROTECT THE EMBRYO

Our recent studies with TNF-$\alpha$ knockout (TNF-$\alpha^{-/-}$) mice exposed to CP (Torchinsky *et al*., submitted) have suggested that TNF-$\alpha$ may act as a protector of embryos exposed to teratogenic stress. It was observed that the proportion of fetuses with craniofacial, trunk, and severe limb reduction anomalies were significantly higher in TNF-$\alpha^{-/-}$ females, than in TNF-$\alpha^{+/+}$ mice. The embryonic brain, which is very sensitive to the teratogen (42), was used to evaluate early events occurring in CP-induced teratogenesis. It was observed that CP-induced excessive apoptosis and the suppression of cell proliferation were more prominent in the brain of TNF-$\alpha^{-/-}$ than TNF-$\alpha^{+/+}$ embryos.

This study has also shown that suppression of NF-$\kappa$B DNA-binding activity was more prominent in the brain of CP-treated TNF-$\alpha^{-/-}$ embryos than in their TNF-$\alpha^{+/+}$ counterparts and the restoration of NF-$\kappa$B DNA-binding activity in the former was compromised. The choice of NF-$\kappa$B as a target molecule was based on evidence demonstrating that TNF-$\alpha$ may act as a powerful activator of antiapoptotic signaling cascades (27). The most effective of these cascades is associated with the activation of the NF-$\kappa$B transcription factor (27).

NF-$\kappa$B is a collective name for transcription factors belonging to the Rel family, which are comprised of several related proteins (subunits) including c-rel, RelA, RelB, p50/p105, and p52/p100 (43). In most cell types, NF-$\kappa$B exists in an inactive form in the cytoplasm bound to several I$\kappa$B inhibitor proteins (I$\kappa$Bs) (44). In response to a variety of internal and external stimuli (45), such as proinflammatory cytokines TNF-$\alpha$ and IL-1, viruses, various cell stresses, I$\kappa$Bs are phosphorylated by the I$\kappa$B kinase (IKK) complex, followed by ubiquitination and degradation leading to the release and nuclear translocation of the freed NF-$\kappa$B, where NF-$\kappa$B controls the expression of target genes by binding to their DNA regulatory elements known as $\kappa$B sites (44,45).

TNF-$\alpha$ may activate NF-$\kappa$B through binding to both TNFR1 and TNFR2, but in most cell types, it activates NF-$\kappa$B via TNFR1 (27). Binding to TNFR1 is followed by recruitment of TRADD, which serves as a platform to recruit two signaling proteins, receptor-interacting protein (RIP) and TNF-receptor-associated factor-2 (TRAF2) (27). Signaling via TNFR2 results in direct recruitment TRAF2, which then recruits TRAF1 ( 27). These events are followed by activation of the IKK complex, one of the central steps in the NF-$\kappa$B activation pathway (44).

Convincing evidence has now accumulated demonstrating that NF-$\kappa$B plays a crucial role in regulating apoptotic cell death acting firstly as a blocker of apoptosis in a stimulus and cell type-dependent fashion (46). Studies with NF-$\kappa$B knockout mice have revealed that RelA$^{-/-}$ embryos die on day 15 of gestation from massive hepatocyte apoptosis (47). Subsequent experiments (48–52) have shown that RelA acts in the embryonic liver as a protector against TNF-$\alpha$ induced physiological apoptosis. These observations suggested that NF-$\kappa$B might be involved in the regulation of the response to embryopathic stresses, which induce excessive apoptosis in target cell populations. A study with a DNA-damaging agent (CP) has shown (53) that excessive apoptosis in embryonic organ structures designed to be malformed (e.g., the brain) is accompanied with the suppression of NF-$\kappa$B DNA-binding activity, whereas there was no suppression in the embryonic liver, in which CP-induced

apoptosis was transient and not followed by dysmorphogenesis. However, excessive apoptosis was present in the liver and followed by prominent histopathological changes if the embryos were exposed to combined treatment with CP and sodium salicylate (an NF-$\kappa$B inhibitor) in a dose that suppressed NF-$\kappa$B DNA-binding activity. It appears that suppression of NF-$\kappa$B DNA-binding activity converted the liver into a sensitive organ to the teratogen and increased the intensity of CP-induced apoptosis in other embryonic organs and the severity of CP-induced structural anomalies.

The results of these studies suggest that NF-$\kappa$B may function not only as a protector from TNF-$\alpha$ induced physiological apoptosis, but also, possibly, counteract the apoptotic stimuli induced by stresses damaging embryonic development. The antiapoptotic activity of NF-$\kappa$B may be realized by the activation of genes whose products inhibit apoptosis (54). There are a large number of such NF-$\kappa$B regulated antiapoptotic proteins (54) and those involved in the teratogenic response, or suspected to be targets of NF-$\kappa$B, are described below.

1. One such target may be cellular inhibitors of apoptosis (c-IAPs) (55). These proteins have been shown to bind and inhibit effector caspases such as caspase 3 and 7 and to prevent activation of pro-caspase 9 and pro-caspase 6 (56). Another NF-$\kappa$B-activated inhibitor of apoptosis is caspase-8–c-FLIP (FLICE inhibitory protein), which inhibits procaspase 8 activation (57). The involvement of these caspases has been demonstrated in induced teratogenesis. Thus, activation of caspases 3 and 9 has been observed in embryos developing "*in vitro*," which responded to numerous chemical teratogens and heat shock (58,59). Increased activity of caspase 3 and caspase 8 has also been observed in our study in embryos exposed *in vivo* to cyclophospamide (53) and in murine embryos developing in a medium with a glucose concentration that induced excessive apoptosis and anomalies such as open neural tube defects (Torchinsky *et al*., in preparation).

Whether NF-$\kappa$B-mediated c-IAPs and c-FLIP mechanisms inhibiting caspase activation act in embryos exposed to embryopathic stress still remains to be determined. The results of our studies seem to suggest such a possibility. It has been observed (53) that embryos treated with CP demonstrate a significant and lasting increase in the activity of caspases 3 and 8 in the brain, which is very sensitive to the teratogen, but not in the liver, in which a less intensive and short time increase in the level of the active caspases was found. These events were associated with the suppression of NF-$\kappa$B DNA binding activity in the brain but not in the liver. It has been demonstrated that apoptogenic stimuli not only activate NF-$\kappa$B-mediated mechanisms counteracting caspase activation but also activated caspases cleave (deactivate) some key components in the NF-$\kappa$B activation pathway, NF-$\kappa$B itself and certain NF-$\kappa$B activated antiapoptotic proteins (e.g., c-IAP1) (46,54,60). Hence, it is conceivable that a "NF-$\kappa$B-caspases" negative feedback mechanism might function in an organ type dependent fashion in embryos exposed to the teratogen, and result in the suppression of NF-$\kappa$B DNA-binding activity in the brain but prevention of excessive activation of caspases in the liver. This scenario may explain the resistance of the liver and the sensitivity of the brain to CP.

2. Antiapoptotic members of the Bcl2 family such as Bcl-$x_1$ and Bcl-2 itself may also be activated by NF-$\kappa$B (46). There is evidence that NF-$\kappa$B has the potential to inhibit the expression of Bax, a proapoptotic member of Bcl2 family (references in 46). Decreased expression of Bcl-2 has been observed in embryos exposed to the teratogen urethane(61) and in the placentas of females with an increased incidence of induced pregnancy loss (21). Bax has been shown to be involved in mediating apoptosis, which increases in blastocysts developing in a medium with a high glucose concentration or *in vivo* in diabetic females (62). It is interesting that NF-$\kappa$B-mediated inhibition of Bax expression may only play a role in the survival of cancer cells that show constitutive NF-$\kappa$B activity (46). Although the normal cell types used in most studies of molecular biology seem to have a constitutively low basal level of active NF-$\kappa$B, this level seems to be high enough in organogenesis (52,53), i.e., in the period of their higher sensitivity to stresses inducing gross structural anomalies (63).

3. NF-$\kappa$B has also been shown to have the potential to regulate the expression of the tumor suppressor gene p53 (64,65). The role of this regulation is not well understood. Nevertheless, NF-$\kappa$B has been shown to be essential in p53-mediated cell death (65). The involvement of p53 in regulating the teratogenic response has been well demonstrated in studies with different chemicals (66–68) and ionizing radiation (69,70). Although these studies have shown that the induction of p53-mediated apoptosis in response to DNA damage may not only prevent, but also promote the formation of structural anomalies, it is

believed that p53-mediated apoptosis is an event that promotes the repair of DNA injuries, by removing cells with irreparable DNA damage, and hence, p53 acts as a teratological suppressor (71). The results of our study (42) indicating that the accumulation of p53 protein in the organs of embryos responding to a teratogenic insult is associated with a higher resistance of these organs to the teratogen, support this concept.

It must be mentioned, however, that excessive apoptosis might not be followed by maldevelopment of a targeted structure (42,53,66). As the embryo has the ability to compensate for excessive cell death (72) and the activation of a cell death program is accompanied by the activation of molecules, which suppress this program, the transient apoptosis, which does not lead to embryonic death or the formation of structural anomalies, may be an adaptive response aimed at removing lethally damaged cells and stimulating the proliferation of neighboring cells. Therefore, the possibility that TNF-$\alpha$ may act as an activator of apoptosis in the embryo exposed to an embryopathic stress, cannot be excluded, regardless of the signaling pathways involved or may in some cases function as a teratological protector.

4. Finally, NF-$\kappa$B has the potential to activate the expression of TNF-$\alpha$ itself as well as TRAF 2, an adaptor protein, which is required for optimal NF-$\kappa$B activation (references in 46). This mechanism is one pathway for augmenting the activation of NF-$\kappa$B (46).

As mentioned above, excessive apoptosis in the embryo, regardless of its nature, if uncompensated, ultimately leads to maldevelopment or embryonic death. NF-$\kappa$B seems to have a critical role as a regulator of cell-cycle progression (73). It is especially important that NF-$\kappa$B has been found to be indispensable in promoting cell proliferation in the liver of adult animals exposed to hepatotoxic stimuli (references in 73). In the embryonic liver NF-$\kappa$B has been demonstrated to be a protector against TNF-$\alpha$ apoptotic signaling, but is not critical for liver development (46). Our study in embryos exposed to CP (53) have revealed that the suppression of NF-$\kappa$B DNA binding activity in the liver of these embryos, at the time in which the liver regenerates after CP-induced apoptosis, was associated with a prominent decrease in liver size, but not with massive apoptosis, as was observed in the liver of RelA-knockout embryos (47). The differences in the pattern of liver degeneration demonstrated by these models seem to provide a basis for investigating whether NF-$\kappa$B may act in embryonic liver cells surviving the teratogenic insult, as an inducer of cell proliferation.

Other defensive mechanisms, which may be switched on by TNF-$\alpha$, will be mentioned briefly. TNF-$\alpha$-induced activation of mitogen-activated protein kinases (MAPK) (74), have been reported to be involved in TNF-$\alpha$-stimulated cell proliferation (27). TNF-$\alpha$ has also been reported to be involved in the regulation of the redox status (16), which seems to be important as many teratogens have the potential to induce oxidative stress (75). The above evidence suggests, that elevated TNF-$\alpha$ expression in the embryo and embryonic vicinity in response to a "danger" signal may reflect an adaptive response aimed at preventing the process of maldevelopment.

## CONCLUDING REMARKS

TNF-$\alpha$ knockout mice (TNF-$\alpha^{-/-}$) demonstrated neither alterations in indices such as litter size, sex ratio, and weight gain and no structural anomalies indicating that the cytokine hardly plays an essential role in regulating normal embryogenesis (76,77). However, whether the embryo develops normally or not, depends not only on the mechanisms regulating embryonic development, but also on the mechanisms acting to resist and repair injures in the embryo due to harmful maternal stimuli or exposure to environmental embryopathic stresses. The evidence presented in this review suggests that TNF-$\alpha$ may activate some such defense mechanisms, and may activate mechanisms with the potential to induce embryonic death.

It seems that the dual role of TNF-$\alpha$ in embryogenesis is explainable, the possible essential function of TNF-$\alpha$ may be to prevent the birth of offspring with structural anomalies. In simple terms, TNF-$\alpha$ will boost death signaling to kill the embryo if initial events (damages) triggered by detrimental stimuli may culminate in structural anomalies, and stimulate protective mechanisms if the repair of these damages may prevent maldevelopment. In fact, the sensitivity of the embryo to embryopathic stresses depends on the developmental stage at the time of exposure, as well as on the intensity and nature of the stress (63). Furthermore, embryonic structures demonstrate striking differences in their sensitivity to embryopathic stress (23). Therefore, the involvement of TNF-$\alpha$-mediated protective mechanisms may manifest itself from the no-effect, if the stress has the potential

to induce minimal developmental deviations, to a decrease in the incidence of malformed fetuses in a litter, or in the incidence and severity of structural anomalies in the fetus. The involvement of TNF-$\alpha$-mediated death signaling may manifest itself from the totally lethal effect, to the increase in embryonic loss due to death of embryos having damage incompatible with life.

This paradigm seems to be supported by the results of our recent studies. It seems that the decrease in the severity of structural anomalies in CP-treated TNF-$\alpha^{+/+}$ embryos compared to their TNF-$\alpha^{-/-}$ counterparts might result from the involvement of TNF-$\alpha$-mediated protective mechanisms (Torchinsky *et al.*, submmitted). A dramatic decrease in the pregnancy rate in diabetic TNF-$\alpha^{+/+}$ females as compared to that in diabetic TNF-$\alpha^{-/-}$ females might result from TNF-$\alpha$-mediated death signaling contributing to the early death of embryos developing in a teratologically dangerous environment (Torchinsky *et al.*, in preparation).

It seems that the paradigm implicating TNF-$\alpha$ in mechanisms preventing the occurrence of malformed offspring may eliminate certain discrepancies concerning the role of TNF-$\alpha$ in embryogenesis. This paradigm may also be useful in interpreting the results of studies addressing the mechanisms underlying "occult" pregnancy loss and for developing therapies for their prevention.

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## REFERENCES

1. Terranova PF, Hunter VJ, Roby KF, Hunt JS: Tumor necrosis factor-$\alpha$ in the female reproductive tract. Proc Soc Exp Biol Med 1995;209:325–342
2. Kohchi C, Noguchi K, Tanabe Y, Mizuno DI, Soma GI: Constitutive expression of TNF-$\alpha$ and -$\beta$ genes in mouse embryo: Roles of cytokines as regulator and effector on development. Int J Biochem 1994;26:111–119
3. Chaouat G, Menu E, Wegmann TG: Role of lymphokines of the CSF family and of TNF, gamma interferon and IL-2 in placental growth and fetal survival studied in two murine models of spontaneous resorptions. *In* Cellular and Molecular Biology of the Maternal-Fetal Relationship, G Chaouat, JF Mowbray (eds), Paris, INSERM/John Libbey Eurotext, 1991, pp 91–104
4. Gendron RL, Nestel FP, Lapp WS, Baines MG: Lipopolysaccharide-induced fetal resorption in mice is associated with the intrauterine production of tumor necrosis factor-alpha. J Reprod Fert 1990;90:395–402
5. Tangri S, Raghupathy R: Expression of cytokines in placentas of mice undergoing immunologically mediated spontaneous fetal resorptions. Biol Reprod 1993;49:850–856
6. Romero R, Manogue KH, Mitchell MD, Wu YK, Oyarzun E, Hobbins JC, Cerami A: Infection and labor. IV. Cachectin-tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor. Am J Obstet Gynecol 1989;161:336–341
7. Clark DA, Arck PC, Chaouat G: Why did your mother reject you? Immunogenetic determinants of the response to environmental selective pressure expressed at the uterine level. Am J Reprod Immunol 1999;41:5–22
8. Raghupathy R: Pregnancy: Success and failure within the Th1/Th2/Th3 paradigm. Semin Immunol 2001;13:219–227
9. Gorivodsky M, Zemliak I, Orenstein H, Savion S, Fein A, Torchinsky A, Toder V: Tumor necrosis factor alpha mRNA and protein expression in the uteroplacental unit of mice with pregnancy loss. J Immunol 1998;160:4280–4288
10. Arck PC, Meroli FS, Manuel J, Chaouat G, Clark DA: Stress-triggered abortion: Inhibition of protective suppression and promotion of tumor necrosis factor-$\alpha$ (TNF-$\alpha$) release as a mechanism triggering resorptions in mice. Am J Reprod Immunol 1995;33:74–80
11. Torchinsky A, Toder V, Carp H, Orenstein H, Fein A: In vivo evidence for the existence of a threshold for hyperglycemia-induced major fetal malformations: Relevance to the etiology of diabetic teratogenesis. Early Pregnancy 1997;3:27–33
12. Fein A, Kostina E, Savion S, Orenstein H, Shepshelovich J, Ornoy A, Torchinsky A, Toder V: Expression of tumor necrosis factor-$\alpha$ in the uteroplacental unit of diabetic mice: Effect of maternal immunopotentiation. Am J Reprod Immunol 2001;46:161–168
13. Pampfer S: Apoptosis in rodent peri-implantation embryos: Differential susceptibility of inner cell mass and trophectoderm cell lineages: A review. Placenta 2000;21 Suppl A:S3–10
14. Pampfer S: Dysregulation of the cytokine network in the uterus of the diabetic rat. Am J Reprod Immunol 2001;45:375–81
15. Ivnitsky I, Torchinsky A, Gorivodsky M, Zemliak I, Orenstein H, Savion S, Shepshelovich J, Carp H, Fein A, Toder V: TNF-alpha expression in embryos exposed to a teratogen. Am J Reprod Immunol 1998;40:431–440
16. Beyaert R, Fiers W: Tumor necrosis factor and lymphotoxin. Cytokines 1998;24:335–359
17. Clark DA, Chaouat G, Gorczynsky RM: Thinking outside the box: Mechanisms of environmental selective pressures on the outcome of the materno-fetal relationship. Am J Reprod Immunol 2002;47:275–282
18. Robaye B, Mosselmans R, Fiers W, Dumont JE, Galand P: Tumor necrosis factor induces apoptosis (programmed cell death) in normal endothelial cells in vitro. Am J Pathol 1991;138:447–453
19. Jacobson MD, Weil M, Raff MC: Programmed cell death in animal development. Cell 1997;88:347–354
20. Vaux DL, Korsmeyer SJ: Cell death in development. Cell 1999;96:245–254
21. Savion S, Lepsky E, Orenstein H, Carp H, Shepshelovich J, Torchinsky A, Fein A, Toder V: Apoptosis in the uterus of mice with pregnancy loss. Am J Reprod Immunol 2002;47:118–127

22. Knudsen TV: Cell death. *In* Drug Toxicity in Embryonic Development I, RJ Kavlock, GP Daston (eds), Berlin, Springer-Verlag, 1997, pp 211–244
23. Mirkes PE: 2001 Warkany lecture: To die or not to die, the role of apoptosis in normal and abnormal mammalian development. Teratology 2002;65:228–239
24. Green DR: Apoptotic pathways: The roads to ruin. Cell 1998;94:695–698
25. Pomerantz JL, Baltimore D: Signal transduction. A cellular rescue team. Nature 2000;406:26–27, 29
26. Idriss HT, Naismith JH: TNF alpha and the TNF receptor superfamily: Structure-function relationship(s). Microsc Res Tech 2000;50:184–195
27. Baud V, Karin M: Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol 2001;11:372–377
28. Menkes B, Sandor S, Ilies A: Cell death in teratogenesis. *In* Advances in Teratology, DH Woollam (ed), New York, Academic Press, 1970, pp 169–215
29. Sidoti-de Fraisse C, Rincheval V, Risler Y, Mignotte B, Vayssiere JL: TNF-alpha activates at least two apoptotic signaling cascades. Oncogene 1998;17:1639–1651
30. Baker SJ, Reddy EP: Modulation of life and death by the TNF receptor superfamily. Oncogene 1998;17:3261–3270
31. Chang HY, Yang X: Proteases for cell suicide: functions and regulation of caspases. Microbiol Mol Biol Rev 2000;64:821–846
32. Adrain C, Martin SJ: The mitochondrial apoptosome: A killer unleashed by the cytochrome seas. Trends Biochem Sci 2001;26:390–397
33. Tsujimoto Y, Shimizu S: Bcl-2 family: Life-or-death switch. FEBS Lett 2000;466:6–10
34. Gupta S: Molecular steps of tumor necrosis factor receptor-mediated apoptosis. Current Mol Med 2001;1:317–324
35. Cai Z, Capoulade C, Moyret-Lalle C, Amor-Gueret M, Feunteun J, Larsen AK, Paillerets BB-d, Chouaib S: Resistance of MCF7 human breast carcinoma cells to TNF-induced cell death is associated with loss of p53 function. Oncogene. 1997;15:2817–2826
36. Rokhlin OW, Gudkov AV, Kwek S, Glover RA, Gewies AS, Cohen MB: p53 is involved in tumor necrosis factor-$\alpha$-induced apoptosis in the human prostatic carcinoma cell line LNCaP. Oncogene 2000;19:1959–1968
37. Vousden KH: p53: Death star. Cell 2000;103:692–694
38. Venters HD, Dantzer R, Kelley KW: A new concept in neurodegeneration: TNF$\alpha$ is a silencer of survival signals. Trends Neurosci 2000;23:175–180
39. Rutanen E-M: Insulin-like growth factors in obstetrics. Curr Opin Obstet Gynecol 2000;12:163–168
40. van Kleffens M, Groffen C, Lindenbergh-Kortleve DJ, van Neck JW, González-Parra S, Dits N, Zwarthoff EC, Drop SLS: The IGF system during fetal-placental development of the mouse. Mol Cell Endocrin 1998;140:129–135
41. Coulam CB: Understanding the immunology of pregnancy and applying it to treatment of recurrent pregnancy loss. Early Pregnancy 2000;IV:019–029
42. Torchinsky A, Ivnitsky I, Savion S, Shepshelovich J, Gorivodsky M, Fein A, Carp H, Schwartz D, Frankel J, Rotter V, Toder V: Cellular events and the pattern of p53 protein expression following cyclophosphamide-initiated cell death in various organs of developing embryo. Teratog Carcinog Mutagen 1999;19:353–367
43. Chen FE, Ghosh G: Regulation of DNA binding by Rel/NF-$\kappa$B transcription factors: Structural views. Oncogene 1999;18:6845–6852
44. Karin M: Minireview. The beginning of the end: IkB kinase (IKK) and NF-$\kappa$B activation. J Biol Chem 1999;274:27339–27342
45. Pahl HL: Activators and target genes of Rel/NF-$\kappa$B transcription factors. Oncogene 1999;18:6853–6866
46. Karin M, Lin A: NF-$\kappa$B at the crossroad of life and death. Nut Immunol 2002;3:221–227
47. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D: Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. Nature 1995;376:167–170
48. Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, Jonson R, Karin M: The IKK$\beta$ subunit of I$\kappa$B kinase (IKK) is essential for Nuclear Factor $\kappa$B activation and prevention of apoptosis. J Exp Med 1999;189:1839–1845
49. Li Q, Van Antwerp D, Mercuric F, Lee KF, Verma IM: Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. Science 1999;284:321–325
50. Doi TS, Marino MW, Takahashi T, Yoshida T, Sakakura T, Old LJ, Obata Y: Absence of tumor necrosis factor rescues RelA-deficient mice from embryonic lethality. Proc Natl Acad Sci USA 1999;96:2994–2999
51. Tanaka M, Fuentes ME, Yamaguchi K, Durnin MH, Dalrymple SA, Hardy KL, Goeddel DV: Embryonic lethality, liver degeneration, and impaired NF-kappa B activation in IKK-beta-deficient mice. Immunity 1999;10:421–429
52. Rosenfeld ME, Prichard L, Shiojiri N, Fausto N: Prevention of hepatic apoptosis and embryonic lethality in RelA/TNFR-1 double knockout mice. Am J Pathol 2000;56:997–1007
53. Torchinsky A, Lishanski L, Wolstein O, Shepshelovich J, Orenstein H, Savion S, Zaslavsky Z, Carp H, Brill A, Dikstein R, Toder V, Fein A: NF-kappaB DNA-binding activity in embryos responding to a teratogen, cyclophosphamide. BMC Dev Biol 2002;2(1):2
54. Barkett M, Gilmore TD: Control of apoptosis by Rel/NF-kappaB transcription factors. Oncogene 1999;18:6910–6924
55. Deveraux QL, Reed JC: IAP family proteins-suppressor of apoptosis. Genes Dev 1999;13:239–252
56. Goyal L: Cell death inhibition: Keeping caspases in check. Cell 2001;104:805–808
57. Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer J-L, Schröter M, Burns K, Mattmann C, Rimoldi D, French LE, Tschopp J: Inhibition of death receptor signals by cellular FLIP. Nature 1997;388:190–195
58. Mirkes PE, Little SA: Teratogen-induced cell death in postimplantation mouse embryos: differential tissue sensitivity and hallmarks of apoptosis. Cell Death Differ 1998;5:592–600
59. Mirkes PE, Little SA: Cytochrome c release from mitochondria of early postimplantation murine embryos exposed to 4-hydroperoxycyclophosphamide, heat shock, and staurosporine. Toxicol Appl Pharmacol 2000;162:197–206
60. Aggarwal BB: Apoptosis and nuclear factor-kappaB: A tale of association and dissociation. Biochem Pharmacol 2000;6:1033–1039
61. Sharova L, Sura P, Smith BJ, Gogal RM Jr, Sharov AA, Ward DL, Holladay SD: Nonspecific stimulation of the maternal immune system. II. Effects on gene expression in the fetus. Teratology 2000;62:420–428

62. Moley KH: Hyperglycemia and apoptosis: mechanisms for congenital malformations and pregnancy loss in diabetic women. Trends Endocrinol Metab 2001;12:78–82
63. Wilson JG: Current status of teratology: general principles and mechanisms derived from animal studies. *In* Handbook of Teratology: General Principles and Etiology, JG Wilson, FC Fraser (eds), New York, Plenum Press, 1977, Vol. 1, pp 47–74
64. Campbell KJ, Chapman NR, Perkins ND: UV stimulation induces nuclear factor kappaB (NF-kappaB) DNA-binding activity but not transcriptional activation. Biochem Soc Trans 2001;29(Pt6):688–691
65. Ryan KM, Ernst MK, Rice NR, Vousden KH: Role of NF-kappaB in p53-mediated programmed cell death. Nature 2000;404:892–897
66. Wubah JA, Ibrahim MM, Gao X, Nguyen D, Pisano MM, Knudsen TB: Teratogen-induced eye defects mediated by 53-dependent apoptosis. Curr Bio 1996;6:60–69
67. Nicol CJ, Harrison ML, Laposa RR, Gimelshtein I, Wells PG: A teratologic suppresser role for p53 in benzo[a]pyrene-treated transgenic p53-deficient mice. Nat Genet 1995;10:181–187
68. Moallem SA, Hales BF: The role of p53 and cell death by apoptosis and necrosis in 4-hydroperoxicyclophosphamide-induced limb malformations. Development 1998;125:3225–3234
69. Norimura T, Nomoto S, Katsuki M, Gondo Y, Kondo S: p53-dependent apoptosis suppresses radiation-induced teratogenesis. Nat Med 1996;2:577–580
70. Wang B: Involvement of p53-dependent apoptosis in radiation teratogenesis and in the radioadaptive response in the late organogenesis of mice. J Radiat Res (Tokyo) 2001;42:1–10
71. Choi J, Donehower LA: p53 in embryonic development: Maintaining a fine balance. Cell Mol Life Sci 1999;55:38–47
72. Snow MHL, Tam PPL: Is compensatory growth a complicating factor in mouse teratology. Nature 1979;279:555–557
73. Chen F, Castranova V, Shi X. New insights into the role of nuclear factor-kappa B in cell growth regulation. Am J Pathol 2001;159:387–397
74. Tibbles LA, Woodgett R: The stress-activated protein kinase pathways. Cell Mol Life Sci 1999;55:1230–1254
75. Wells PG, Kim PM, Laposa RR, Nicol CJ, Parman T, Winn LM: Oxidative damage in chemical teratogenesis. Mutat Res 1997;396:65–78
76. Pasparakis M, Alexopoulou L, Episkopou V, Kollias G: Immune and inflammatory responses in TNF alpha-deficient mice: A critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. J Exp Med 1996;184:1397–1411
77. Marino MW, Dunn A, Grail D, Inglese M, Noguchi Y, Richards E, Jungbluth A, Wada H, Moore M, Williamson B, Basu S, Old LJ: Characterization of tumor necrosis factor-deficient mice. Proc Natl Acad Sci USA 1997;94:8093–809