Maternal Serum Levels of Transforming Growth Factor ß1 (TGF-ß1) in Normal and Preeclamptic Pregnancies

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ABSTRACT

Background: Successful pregnancy in allopregnant women depends upon the control of graft rejection mechanisms. It has been suggested that some immunosuppressive cytokines contribute to successful pregnancy and transplantation. Transforming growth factor beta (TGF-β) exhibits potent immunoregulatory and anti-inflammatory properties which might prolong graft survival. Recent studies suggest a role for TGF-β in the generation of T-regulatory lymphocytes which preserves the tolerance to peripheral self antigens and may control the response to allogenic tissues and thereby promote the transplantation tolerance. Also, the function of TGF-β in trophoblast differentiation and hypertension is reported. **Objective:** To evaluate the maternal serum TGF-β1 level in normal allopregnant women and in pregnancies complicated by preeclampcia (PE). **Methods:** Sixty one pregnant preeclamptic women (32 cases with severe and 29 with mild PE), 22 normotensive healthy pregnant, and 20 non-pregnant controls constituted the studied groups. The active form of TGF-β1 in serum from all cases was investigated by indirect ELISA technique. **Results:** The results showed that TGF-β1 level was higher in all three pregnant groups as compared with the non-pregnant controls. No significant changes in serum levels of TGF-β1 were found in PE as compared with the normal pregnancy. **Conclusion:** TGF-β1 may function as a regulatory factor in fetal allograft survival during pregnancy, and TGF-β1 does not have a pathophysiological role in PE.

Keywords: Fetal Allograft Survival, Preeclampsia, Transforming Growth Factor-β

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INTRODUCTION

The mammalian fetus will express paternally inherited antigens that are allogenic to the mother, and the mother is exposed to fetal antigens during pregnancy. This is exemplified by the detection of antibodies against paternal major histocompatibility complex (MHC) molecules, in multiparous women (1). In essence, the fetus is a naturally occurring allograft; nevertheless, fetuses are not normally rejected by the mother immune system. Protection of the fetus against the maternal immune system probably involves several mechanisms. One explanation for the lack of rejection is achieved by contribution of immunosuppressive cytokines such as TGF-β (1,2). Involvement of the TGF-β in human pregnancy is suggested by the presence of many of the TGF-β superfamily members (TGF-β1, TGF-β2, and TGF-β3) in amniotic fluid. Additionally, TGF-β1 has been localized to the placental villi (3,4). The principal action of TGF-β in the immune system is to inhibit the proliferation and differentiation of lymphocytes by controlling the activation of other leukocytes (5). Cytokines in the TGF-β superfamily are thought to have multiple functions. The ability of the TGF-β isoforms to modulate cell-cell adhesion, cell migration, and tissue remodeling has led some authors to suggest that this molecule may control trophoblast invasion and implantation in pregnancy (6). Other possible roles include regulation of fetal growth and suppression of the maternal immune responses. Experimental evidence suggests that the amniotic fluid concentration of the pro-inflammatory cytokines interleukin (IL)-1, IL-6 and tumor necrosis factor rises during labor. Furthermore, pro-inflammatory cytokine production accompanying intrauterine infection has been associated with fetal rejection or preterm labor (7,8). TGF-β1 has been shown to suppress production of pro-inflammatory cytokines by macrophages and lymphocytes, respectively (9). The immunosuppressive effects of TGF-β1 in pregnancy (10), and in trophoblast differentiation and spiral artery transformation (11), have attracted the attention to the possible role of TGF-β1 in the normal and pathological form of pregnancy. Preeclampsia (PE) is the most common hypertensive disorder during pregnancy and represents life–threatening situations for the mother and fetus. PE is characterized by increased blood pressure, proteinuria and end organ damage, and is associated with impaired trophoblast invasion (12). Alteration of TGF-β plasma concentration has been found in preeclamptic women compared with controls suggesting the involvement of TGF-β in the pathophysiology of PE (13).

SUBJECTS AND METHODS

This study comprised of 83 pregnant women in the late third trimester (36-40 gestational age), who attended the antenatal clinic of OB/GYN affiliated to hospitals of Shiraz University of Medical Sciences. They were divided into three groups. The first group included 22 normotensive healthy pregnant women. The second group consisted of 29 women with mild PE, and the third group consisted of 32 women with severe PE. Twenty serum samples were also obtained from healthy non-pregnant women who served as controls. PE were diagnosed clinically on the basis of elevated

blood pressure, proteinuria and edema. Mild and severe PE was subgrouped according to the criteria of Cunningham et al. (14). Mild PE was defined as an absolute systolic blood pressure of at least 140 mmHg or an increase at least 30 mmHg systolic or 15 mmHg diastolic after 28 weeks of pregnancy accompanied with proteinuria (> 300 mg per 24 hours). The criteria for severe PE were systolic blood pressure of at least 160 mmHg or at least 100 mmHg diastolic and proteinuria over 5g per 24 hours, in addition to oligouria (< 400 ml in 24 hours), cerebral or visual disturbances, epigastric pain, or pulmonary edema. Normal pregnant woman were matched with those with PE in terms of maternal age, and time of blood sampling. Patients with history of chronic hypertension, diabetes, renal or cardiovascular disease were excluded. Conventional laboratory methods for assaying renal function were done in all cases and included urine analysis, serum creatinine, and uric acid.

A blood sample was taken from each participant before administration of any drug known to affect blood pressure and before any medical and surgical intervention. Sera from blood samples were separated by centrifugation and were kept at -80ºC until the time of analysis. The active form of TGF-β1 in the serum of all cases was investigated by indirect enzyme immunosorbant assay technique. Reagent kit (Quantikine human TGF-β1) was purchased from Bender Medsystems. The minimum detectable dose of TGF-β1 was 25 pg/ml. Data were analyzed using SPSS software (version 11.5.0; SPSS Inc., Chicago, IL, USA). The statistical companions between studied groups was done using Kruskal-Wallis test with P value < 0.05 taken as being significant.

RESULTS

Maternal serum TGF-β1 levels in preeclamptic women, normal pregnant women and in non-pregnant controls are summarized in Table 1. The mean serum concentration of TGF-β1 in women with PE was not statistically different from those of normotensive pregnant women. It was 696.5 pg/ml in mild PE, 595.3 pg/ml in severe PE, and 721.5 pg/ml in normotensive pregnant women. These results show that TGF-β1 is present in high concentration in all three pregnant groups. No significant differences in the expression of TGF-β1 were found in the severe or the mild forms of PE patients when compared with normotensive pregnant group ($p > 0.05$). The mean serum concentration of TGF-β1 in non pregnant women was 360 pg/ml, which is significantly different from all groups of pregnant women ($p < 0.0001$).

Table 1. Maternal serum transforming growth factor beta-1 (TGF-β1) levels in three pregnant groups and normal controls

Group	No. of patients	Range	Mean (pg/ml)	P-value
Normal pregnant	22	25-850	721.59	<0.0001
Mild preeclampsia	29	250-1100	696.55	<0.0001
Severe preeclampsia	32	25-1150	595.31	<0.0001
Normal	20	100-650	360.0	-

DISCUSSION

An understanding of how the fetus escapes the maternal immune system may be relevant to the prevention of rejection in transplantation. Protection of the fetus against the maternal immune system probably involves several mechanisms. One explanation is that the uterine decidua may be an immunologically privileged site. Several factors may contribute to the immune privilege. For example, the anterior chamber of the eye may contain high concentration of immunosuppressive cytokines such as TGF-β (1,15). There is evidence that cultured decidual cells directly inhibit macrophage and T cell functions, perhaps by producing inhibitory cytokines, such as TGF-β (15,16). Some of these inhibitory decidual cells may be resident regulatory T cells, although the evidence for this suggestion is limited (15). Some experiments have led to the suggestion that Th2 cytokines are produced at the maternal-fetal interface and are responsible for local suppression of Th1 responses to fetal antigens (17). However, this idea is not supported by some data that IL-4 and IL-10 knockout mice (but not IL-2 deficient mice) had normal pregnancies (18,19). Other experiments indicate that a subset of T cells, so called "Th3" CTLA-4+ regulatory cells, are thought to have a role in preventing autoimmune disease and in moderating various forms of immunopathology (20-22). These regulatory T cells are potent suppressors and play important roles in the induction of tolerance to self antigens and in transplantation tolerance. This regulatory function is determined by expression of a critical transcription factor foxP3 (23,24). Recent reports suggest a role for TGF-β in the generation of regulatory T-cells from CD4+ CD25- precursors. These data suggest that regulatory T cells maintain transplantation tolerance through a TGF-β-dependent foxP3 induction. Thus, TGF-β is a key regulator of the signaling pathways that initiate and maintain foxP3 expression and suppressive function in CD4+CD25- precursors (25). The regulatory effect of TGF-β on T cell function provides insight into immunological role of this molecule in pregnancy. There are some studies on the relationship between successful pregnancy outcome and influence of TGF-β1 cytokine. One important role of TGF-β1 may be to dampen the inflammatory process in the placenta and surrounding tissues, as inflammatory mediators have a central role in labor (26). A reduction in TGF-β1, as we observed in samples from non-pregnant women compared with those of pregnant women, might confirm the efficacy of TGF-β1 in controlling the development and function of the immune system during pregnancy.

The failure of trophoblast invasion and spiral artery transformation has been documented in pregnancies complicated by PE, one of the leading causes of maternal death (12). Plasma concentration of TGF-β1 has been found to increase in PE women compared with controls suggesting the involvement of TGF-β1 in the pathophysiology of PE (27). However, some experiments have led to the suggestion that TGF-β1 has no role in hypertension in PE (28,29). The present study examined the hypothesis that the elevated level of TGF-β1 would be protective for the fetus survival during pregnancy-induced hypertension such as PE. We have found that the maternal serum concentration of TGF-β1 was high in all three pregnant groups. No significant differences in the production of TGF-β1 were found in the severe or the mild forms of PE patients compared with normotensive pregnant group. The findings conclude that the TGF-β1 does not have a pathophysiological role in PE and should be considered as a

regulatory factor in fetal allograft survival during pregnancy. The effect of TGF-β1 on immune responses indicates that TGF-β1 negatively regulates the primary immune response (proliferation and cytokine production), but only Th1 memory cells are subject to this negative regulation (30). Thus, TGF-β1 in the fetal circulation could serve to limit the development of primary immune responses by the fetus. In the maternal circulation, it has been suggested that TGF-β1 can limit primary immune responses and favor regulatory T cell responses. However, the biological relevance of circulating levels of locally acting cytokines, such as TGF-β1 remains unclear. As TGF-β1 is markedly elevated in gestation-associated tissues, circulating levels might reflect the levels present at the maternal-fetal interface and serve as a surrogate marker for levels at the maternal-fetal interface, including the placenta.

It is clear that circulating levels of cytokines and other factors with immunosuppressive properties are altered during immune responses. Understanding their effects on maternal-fetal immune functions and on transplantation tolerance is vital but it must be taken into account that these mediators have far reaching properties. Thus, it is impossible to consider their role in pregnancy solely from the immunological perospects. However, these experiments would serve as a good starting point.

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