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Differential Expressions of Fas and Fas Ligand in Human Placenta

To investigate the expressions of Fas and Fas ligand (FasL) in human placenta, we studied the expressions of Fas and FasL in placenta with RT-PCR, immunoblotting and immunostaining. We observed amplified products of Fas and FasL transcripts, the band of Fas (52 kDa) and multiple bands of FasL (42-52 kDa) in placenta. Fas and FasL localized mainly on fetal vessels and on syncytiotrophoblasts respectively. The differential distribution of Fas and FasL in human placenta may reflect intrinsic expressions of them by trophoblasts during differentiation. The increased expression of Fas in trophoblasts may promote apoptosis of placenta in pathologic condition such as preeclampsia.

Key Words : *Fas; Fas ligand; Placenta; Trophoblast*

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INTRODUCTION

Although the fetus is semiallograft to the mother's immune system, a maternal immune tolerance to the fetus takes place instead of immune rejection response (1). Since placenta is the main site of maternal-fetal interaction, it should play an immunosuppressive role for the maintenance of its villous structures. When the maternal-fetal immune interaction becomes pathologic as in pregnancies complicated with preeclampsia and intrauterine growth restriction, however, increased apoptosis in placenta has been reported (2, 3).

The Fas-Fas ligand (FasL) system is one of the major pathways for the induction of apoptosis in cells and tissues (4). Fas (CD95) is a type I membrane protein of 45 kDa that belongs to the tumor necrosis factor (TNF) superfamily and FasL, a type II membrane protein of 42 kDa, belongs to the TNF and CD40 ligand family. Fas has been reported to be expressed widely in many tissues, in T and B cells and in human trophoblast throughout gestation (5). Although Fas is present constitutively on the surface of resting cells in low levels, its expression is enhanced after lymphocyte activation (4). On the other hand, FasL promotes apoptosis of activated Fas-bearing lymphocytes and has been thought to confer immune privilege in the anterior chamber of the eye (6), Sertoli cells of the testis (7), and placenta (8).

Although Fas-FasL system may play a role in maintaining human pregnancy, aberrant activation of this system in trophoblast, the major cell type at the maternal-fetal interface of placenta, may invoke pathologic changes in placenta. In this study, we examined the distribution of Fas and FasL in human placenta throughout gestation. Moreover, we investigated changes in the expression of Fas and FasL in trophoblasts isolated from human term placenta during their differentiation in vitro.

MATERIALS AND METHODS

Placentas were obtained from women who underwent elective abortion from medical problems in first trimester (n=5) and second trimester (n=5), and women (n=22) who delivered at term without obstetric or medical complication. Immediately after delivery, about 10 g of placental tissues was collected from the maternal-fetal interface and washed in saline. All samples were snap frozen in liquid nitrogen and stored at -70℃ until further usage.

RT-PCR

Total RNA was extracted from each of the frozen tissues in Trizol® (Gibco-BRL, U.S.A.) as recommended by the manufacturer and reverse transcripted with 0.5 μg Oligo (dT) 12-18, 5×RT buffer, 1 mM dNTP, 1 U Rnasin, and 1 μg Moloney murine leukemia virus reverse transcriptase in a final volume of 20 μL. The synthesized first strand cDNA was mixed into reaction cocktail (10×Buffer, 1 mM dNTP, 0.25

pM 5′ primer, 0.25 pM 3′ primer, 2.5 U *Taq* DNA polymerase, and distilled water in a final volume of 40 μL) for PCR amplification. The primers for Fas, FasL, and GAPDH were as follows, Fas (5′-GAAGGACATGGCTTAGAAGTG and 3′-ACTTAGTGTCATGACTCCAGC), FasL (5′-TCTCAGA CGTTTTTCGGCTT and 3′-AAGACAGTCCCCCTTGAG GT), and GAPDH (5′-TGAAGGTCGGAGTCAACG GAT TTGGT and 3′-CATGTGGGCCATGAGGTCCAC CAC).

PCR was performed in thermal cycler for 30 cycles: annealing for 45 sec at 60℃, extension for 45 sec at 72℃, and denaturation for 30 sec at 94℃. For internal control, GAPDH primers were put into the reaction mixture along with Fas or FasL primers. PCR products were visualized on 2% agarose gel.

Western blotting and Immunoprecipitation

Each placental tissues were disrupted in RIPA buffer (50 mM TrisCl, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, pH 7.5) containing 10 mM phenylmethylsulfonyl fluoride. After centrifugation, protein concentrations were determined for supernatants and 20 μg of proteins were separated on 10% (w/v) SDS-PAGE under reducing condition. After electrotransfer onto polyvinyldifluoride membrane, the blots were blocked overnight at 4℃ in 5% nonfat dry milk in TBS-T buffer. The blots were incubated for 1 hr at room temperature with 1:500 diluted either mouse monoclonal anti-FasL antibody, G247-4 (Pharmingen, U.S.A.) or rabbit polyclonal anti-Fas antibody, Fas (Ab-1) (Oncogene Research Products, U.S.A.) in Tris-buffered saline-Tween20 (TBS-T) buffer. After washing, membranes were incubated with peroxidase-conjugated secondary antibody at dilution of 1:2,000 for 1 hr at room temperature. The reaction was visualized using ECL western blotting kit (Amersham, U.K.). To confirm the presence of Fas in placenta, placental tissue lysates were subjected to immunoprecipitation with Fas (Ab-1) diluted 100-fold and 10 μL of protein A-agarose (Sigma, U.S.A.). After incubation for 2 hr at 4℃, the pre-cleared lysates were used for western blotting

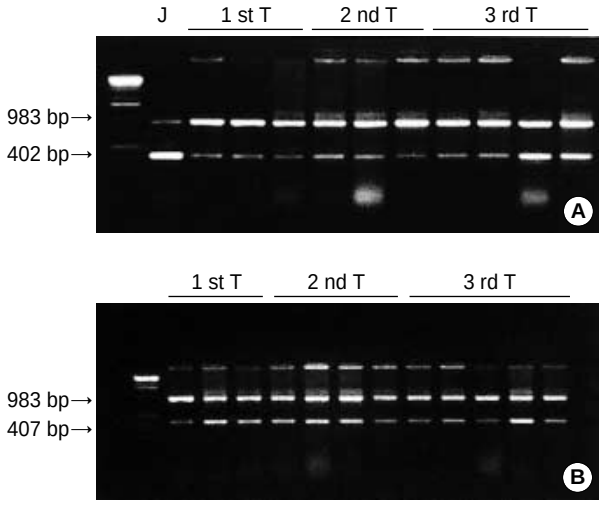

Fig. 1. RT-PCR amplification of Fas and FasL transcripts in human placenta. (A) RT-PCR reveals amplified products of Fas (402 bp) and GAPDH (983 bp) transcripts. (B) RT-PCR reveals amplified products of FasL (407 bp) transcripts and GAPDH (983 bp) transcripts. J; Jurkat cell, T; trimester.

Immunohistochemistry

The immunostaining for Fas and FasL were performed on paraffin-embedded placental sections using Fas (Ab-1) and anti-human Fas Ligand (Santa Cruz Biotechnology, Inc, U.S.A.). Tissue sections were mounted on slides coated with 0.1% poly-L-lysine. After deparaffination and rehydration, tissue sections were blocked with normal serum and incubated for 1 hr with antibodies to Fas and FasL at a dilution of 1:100. Tissue sections were treated in 0.1% Triton X-100 for 15 min and were incubated with a 1:1,000 diluted biotinylated secondary antibody. After incubation in streptoavidin buffer, the antibody complexes were visualized by incubation with stable DAB chromogen (DAKO A/S, Denmark) for 5 min. Sections were counterstained with Autohematoxylin (DAKO A/S, Denmark), dehydrated and mounted.

RESULTS

RT-PCR revealed amplified products of Fas (402 bp) (Fig. 1A) and FasL (407 bp) (Fig. 1B) transcripts along with GAPDH (983 bp) transcripts in all placentas studied. The levels of amplified Fas and FasL products, measured semiquantitatively by densitometry with reference to GAPDH, were not different through gestation.

Immunoblotting showed multiple glycosylated bands of FasL ranging from 42 to 52 kDa. A 26 kDa band, which

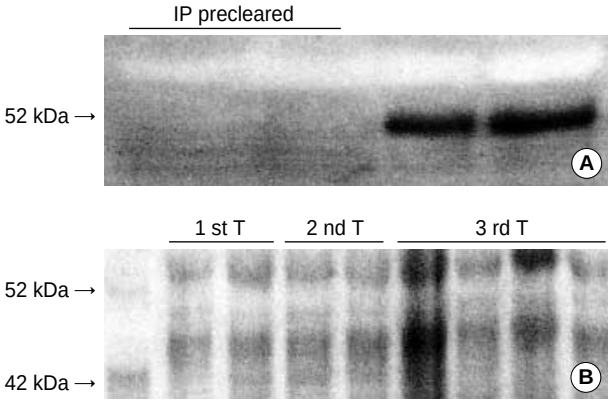

Fig. 2. Immunoblots for Fas and FasL in human placenta. (A) Immunoblot for Fas after preclearing with immunoprecipitation (IP) reveals 52 kDa bands. (B) Multiple bands of glycosylated FasL ranging from 42 to 52 kDa.

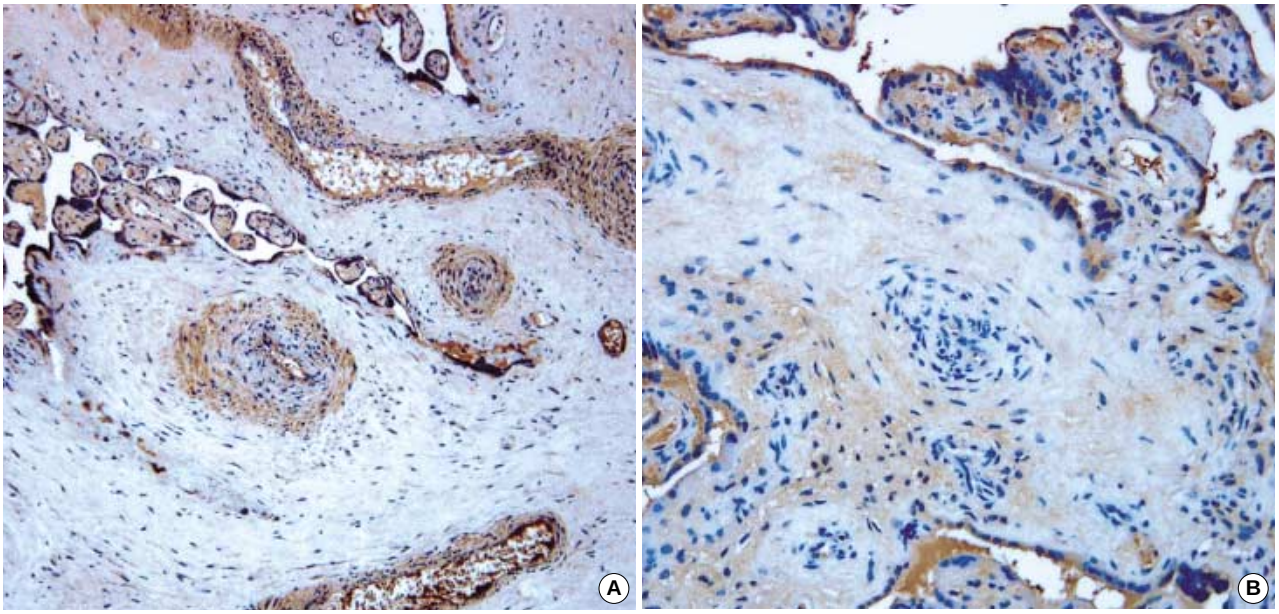

Fig. 3. Immunostainings for Fas and FasL in human placenta at term pregnancy. (A) The immunoreactivity for Fas is detected most strikingly in fetal vessels. Note bare staining for Fas in syncytiotrophoblasts lining villi. (B) The immunostaining for FasL is observed mainly on syncytiotrophoblast (original magnification ×200).

corresponds to soluble form of FasL, was observed after glycosidase treatment of placental extracts. Densitometric measurements of immunoblot revealed no significant change in the level of FasL in placenta from the first trimester to the third trimester of pregnancy (Fig. 2B). The presence of Fas (52 kDa) in human placenta (15 placentas) was also confirmed by preclearing experiments with immunoprecipitation (Fig. 2A).

Immunostaining of placenta from first trimester showed localization of FasL in cytotrophoblastic cell column and extravillous trophoblasts. In placenta from third trimester, the staining for FasL, however, was observed mainly on syncytiotrophoblast (Fig. 3B) and occasionally on intermediate cytotrophoblast. On the other hand, the staining for Fas was observed most strikingly in fetal vessels (Fig. 3A), notably in degenerating villi and occasionally in intermediate trophoblasts throughout gestation. The staining for Fas, however, was barely observed in syncytiotrophoblasts lining villi.

DISCUSSION

There are two potential sites of interaction between trophoblastic cells and maternal lymphocytes in human placenta (9). The first is the intervillous space where cells of floating villi, consisting of an inner cytotrophoblastic layer and an outer layer of syncytiotrophoblasts around a stromal cell core, is bathed by maternal blood. The second site is the placental bed where highly invasive extravillous cytotrophoblasts of the anchoring villi, known as intermediate trophoblasts, invades deciduas and the first third of the myometrium. Fas-FasL system of trophoblast is located in these interactive sites and might play a role in maintaining pregnancy.

In the present study, we observed the expression of FasL most prominently in syncytiotrophoblasts lining microvillar membranes and less frequently in invasive extravillous cytotrophoblasts. Runic et al. (10) demonstrated the presence of FasL in cytotrophoblasts of first trimester human placenta and pronounced staining in syncytiotrophoblasts of term placenta. Therefore, we speculate that in the first trimester of pregnancy the main function of the Fas/FasL system, in this area of the placenta, is more likely to be part of a mechanism regulating placental growth than a strategy for immunological defense. Furthermore, up to the 12th week of pregnancy, maternal plasma alone was found in the intervillous space. Only from the 13th week onwards does the intervillous space contain maternal blood cell (11). Subsequent studies also confirmed the localization of FasL in syncytiotrophoblasts and extravillous cytotrophoblasts by immunohistochemistry (8, 10) and in situ RT-PCR (12) techniques. Our results are consistent with those previous reports in which FasL was detected in trophoblastic cells, namely cytotrophoblasts and syncytiotrophoblasts of placenta and support the concept that FasL in syncytiotrophoblasts and extravillous cytotrophoblasts may play a key role in maintaining immune privilege by repelling the attack of activated maternal lymphocytes at intervillous and placental bed sites.

In contrast to the localization of FasL in human placenta, the expression of Fas was observed most strikingly in fetal vessels, notably in degenerating villi and occasionally in

intermediate trophoblastic cells. This may be the final protection from FasL expressing activated maternal T lymphocytes in intervillous space. The expression of Fas, however, was not evidently observed in syncytiotrophoblasts lining microvillar membranes. In placenta, syncytiotrophoblasts are in direct contact with maternal peripheral blood lymphocytes and FasL, expressed in activated or cytotoxic T-lymphocytes, natural killer cells and neutrophils, might bind to Fas on these target cells and induces apoptosis (13, 14). Although it was reported Fas- but not TNF receptor p55-mediated apoptosis was blocked in primary villous trophoblasts (15), the bare staining for Fas in syncytiotrophoblasts seems to make sense in that the Fas response may be avoided by FasL constitutively expressed on neighboring cyto- or syncytiotrophoblasts and activated maternal lymphocytes. The notable localization of Fas in fetal vessels might have some clinical implications and trophoblast apoptosis is a significant feature of early-onset intrauterine growth restriction (16). It seems possible, in some cases of intrauterine growth restriction, Fas mediated fetal vascular apoptosis would be accentuated resulting in deficit in peripheral villous development when orderly development of the fetal villi is deranged by enhanced apoptosis of trophoblasts.

Our findings are different to data published for the term placenta, where a constitutively high expression of Fas mRNA and protein, and FasL mRNA and protein, was found in the syncytiotrophoblast (12). In this study, we observed main localization of Fas on fetal vessels and that of FasL on syncytiotrophoblasts. The differential distribution of Fas and FasL in human placenta can be explained by intrinsic expressions of them in trophoblasts during differentiation. In pathologic condition such as preeclampsia, the increased expression of Fas in syncytiums may promote apoptosis of placenta.

The regulatory mechanisms for the expression of Fas and FasL in human placenta, however, need further investigations.

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