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EXPRESSION OF THE APOPTOSIS-INDUCING FAS LIGAND (FASL) IN HUMAN FIRST AND THIRD TRIMESTER PLACENTA AND CHORIOCARCINOMA CELLS

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ABSTRACT

The Fas (Apo-1/CD95) ligand (FasL) belongs to the tumor necrosis factor family and acts through its receptor (FasR/Apo-1/CD95) to induce apoptosis in target cells. FasL is expressed in several immunologically privileged sites. Induction of apoptosis by FasL in invading lymphocytes acts as a mechanism of immune privilege and is important in preventing graft rejection. Furthermore, FasL is expressed in certain malignancies and it has been implicated as a possible key mechanism in immune privilege of these tumors. Since the invading placental trophoblast is another very important site with a privileged immune status, we investigated whether FasL is expressed in the normal and tumoral human placenta. For this purpose, mRNA was extracted from first and third trimester human placental samples as well as from JEG3 choriocarcinoma cells and reverse transcribed to obtain cDNAs. These were used as templates for PCR analysis of FasL expression, in which specific primers were employed to amplify an 853 bp fragment spanning the whole FasL coding region. A product of the appropriate length was amplified from normal placenta as well as from the choriocarcinoma cells. Expression of FasL protein was confirmed by Western Blot and was localized to trophoblast by immunohistochemistry using a FasL-specific antibody. Expression of FasL in the human placenta indicates that induction of apoptosis in lymphocytes by the invading trophoblast could be an important mechanism implicated in the immune tolerance of the fetal semi-allograft.

The Fas (Apo-1/CD95) ligand (FasL) belongs to the tumor necrosis factor family and acts through its receptor (FasR/Apo-1/CD95) to induce apoptosis in cells carrying this receptor (1,2). Expression of FasL is, therefore, a mechanism of allowing immune privilege. It is important in preventing graft rejection (2,3). Furthermore, FasL is expressed by melanomas and it has been implicated in inducing the immune privilege of these tumors (4).

Another very important site of immune privilege is the invading placental trophoblast which, although a semi-allograft, is not rejected by the immune system of the mother. For this reason, we investigated whether FasL is expressed in the human placenta and in placental tumor cells and could thus be implicated in generating the immune tolerance towards this tissue as well.

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Materials and Methods

Human placental tissues and cell lines

Human first trimester and term placentas were collected at the time of therapeutic abortion and birth. JEG3 and JAR human choriocarcinoma cells (ATCC, Rockville, MD) were cultured as recommended.

RNA expression by RT-PCR

Total RNA was extracted and reverse transcribed. For PCR, the following oligonucleotide primers were used for FasL: upstream 5' CC ATG CAG CAG CCC TTC AAT TAC 3' and downstream 5' TTC CTC TTAGAG CTT ATA TAA GCC 3'. These primers generate an 853bp product comprising the whole FasL coding sequence. PCR was carried through 40 cycles (melting, annealing, and extension at 94C, 60C, and 72C).

PCR products were electrophoresed in a 1% agarose gel, purified and subcloned into pCR-Script SK+ (Stratagene, Heidelberg, Germany). After confirmation of insertion and determination of orientation, DNA sequencing was performed by the dideoxy chain termination method.

Protein analysis by PAGE and Western blotting

Whole cell extracts were electrophoresed in a 10% polyacrylamide gel and a 3% stacking gel, transferred to a polyvinylidene difluoride membrane (Immobilon P, Millipore, Eschborn, Germany) and stained with Ponceau S to determine transfer efficiency. After destaining, membranes were incubated overnight at 4C in blocking solution (0.1 M maleic acid, pH 7.5, 0.15 M NaCl, 0.005% Thimerosal, and 1% blocking reagent Boehringer Mannheim, Germany), washed in TBST (Tris-buffered saline with 0.05% Tween 20) and incubated with affinity-purified isoenzyme-specific antibody. Anti-FasL (Santa Cruz Biotechnology, Inc.) was diluted 1:400 with 9:1 TBST/blocking solution, incubated for 1h at room temperature, washed in TBST and incubated with a peroxidase-conjugated secondary antiserum (Sigma) 1:1000 for 1h at room temperature. The reaction was visualized by chemiluminescence

Immunohistochemical localization of FasL protein

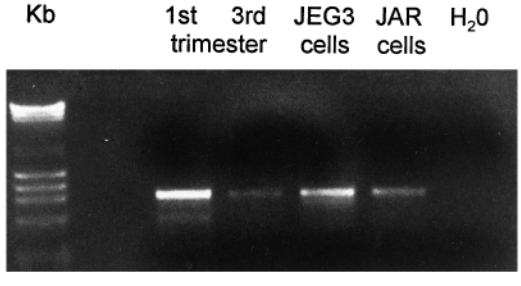
Sections of formalin-fixed paraffin-embedded tissues were pretreated with microwave antigen retrieval. The avidin-biotin technique was performed with a primary polyclonal FasL antiserum (Santa Cruz Biotechnology Inc.) at a dilution of 1:400. The specificity of the reaction was verified by replacing the primary antibody with nonimmune rabbit serum.

Results

RNA expression

RT-PCR demonstrated the presence of FasL mRNA in human first trimester and term placenta. A band of the predicted size (853bp) was visualized after amplification with FasL specific primers (Fig. 1). A band of the appropriate size was also identified using cDNA from JEG3 and JAR cells (Fig. 1). DNA sequencing confirmed the identity of this product with the published FasL sequence.

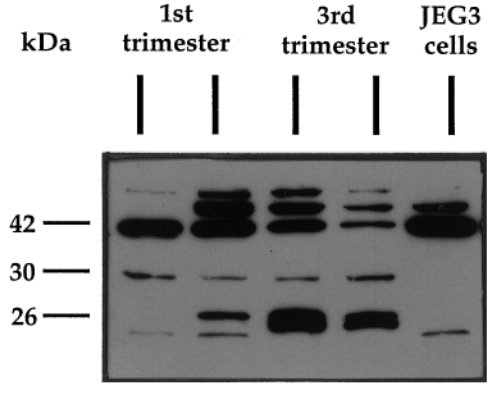
Fig. 1. RT-PCR for FasL in Human Placenta
A band of the expected size, 853 bp, is present in samples of first and third trimester placenta, JEG3 and JAR choriocarcinoma cells but not in the negative control.



Western Blot Analysis of FasL protein

To determine whether FasL mRNA was translated in human placenta and choriocarcinoma cells, we used Western blot to analyze expression of FasL protein. Expected bands of 42 kDa corresponding to mature FasL, 30 kDa corresponding to non-glycosylated FasL, and 26 kDa corresponding to soluble FasL were obtained in all samples (Fig. 2).

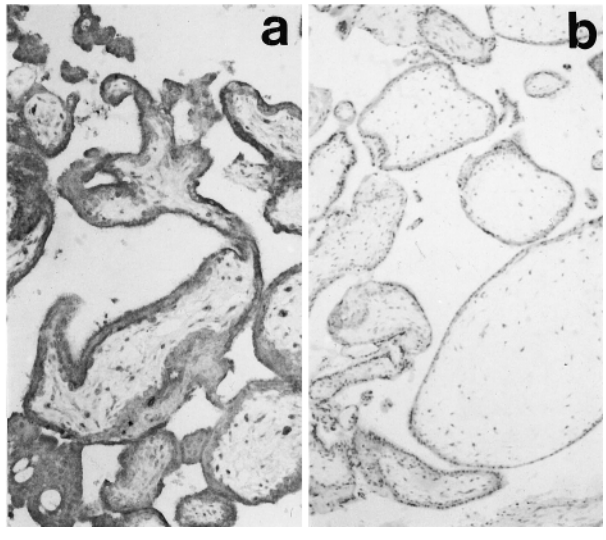
Fig. 2 Western blot for FasL in human placenta
Expected bands of 42, 30 and 26 kDa are present in first and third trimester placenta and JEG3 choriocarcinoma cells.



Immunohistochemistry

To localize FasL in human placenta, immunohistochemical analysis was performed. The protein was localized to cyto- and to syncytiotrophoblast cells (Fig. 3).

Fig. 3. Immunohistochemistry localizes FasL to placental trophoblast (a); staining is abolished when the primary antiserum is replaced with nonimmune rabbit antiserum (b).



Discussion

We demonstrate expression of FasL mRNA and protein in the human first and third trimester placenta and in choriocarcinoma cells using RT-PCR followed by product sequencing, and Western blot. FasL protein was localized to placental trophoblast with immunohistochemistry.

The Fas/FasL apoptotic pathway is used by cytolytic T cells to kill target cells but is also used to eliminate activated T cells during down-regulation of the immune response (4-9). Mice deficient in either FasR (lpr mutation) or FasL (gld mutation) develop a progressive autoimmune disorder that resembles sytemic lupus (10).

In addition to the lymphoid system, FasL has been shown to be expressed in several immunologically privileged sites like the anterior chamber of the eye and the Sertoli cells in the testis (2,3) where it might protect immune privilege by inducing apoptosis in lymphocytes. Also, FasL is expressed by certain malignancies (4,11) where it could also play a role in immune escape of tumor cells.

Another major site of immune privilege is the trophoblast. This is the only fetal tissue which is exposed to maternal uterine decidua and blood. The mechanisms involved in shielding the fetus from immunological rejection are far from clear. One potential mechanism implicates lack of expression of classical MHC antigens by the trophoblast, and production of a non-polymorphic MHC class I antigen, HLA-G (12-15). Its expression is prominent in first trimester villous cytotrophoblasts and is greatly reduced in third trimester cytotrophoblasts (12). It is also expressed by the JEG3 choriocarcinoma cell line (12). This antigen has been implicated in partially mediating immune protection of the trophoblast by allowing it to escape recognition by maternal T-lymphocytes and by protecting it from lysis by activated natural killer (NK) cells (16-18).

Trophoblast and JEG3 choriocarcinoma cells have also been shown to produce an as yet unidentified factor which can block the response of activated T lymphocytes (19,20). Our data suggest that FasL, which is produced in both membrane-associated and soluble forms, may be the substance responsible for this action.

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