Trophoblasts express Fas ligand: a proposed mechanism for immune privilege in placenta and maternal invasion

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Cross-linking of Fas (CD95, APO-1) and Fas ligand (FasL; CD95L) induces apoptosis of Fas-bearing cells. Recent evidence suggests that FasL expression plays an important role in maintenance of immune privilege in murine testis and eye and in tumour escape from immune rejection in colon cancer, melanoma and hepatocellular carcinoma. Bcl-2 is a membrane protein that suppresses apoptosis in response to a variety of stimuli. In this paper we describe abundant expression of Fasl protein and mRNA transcripts within the immune privileged environment of the placenta by immunohistochemistry and reverse transcription-in-situ polymerase chain reaction methods. The syncytiotrophoblast layer, the main site of feto–maternal interface, and extravillous trophoblasts, demonstrated consistent immunoreactivity for FasL in term placentae. Co-occurrence of Fas and Bcl-2 were detected with a similar pattern of distribution with FasL. The TUNEL method revealed evidence of apoptosis in the placental tissues. We speculate that abundant presence of FasL in the trophoblast contributes to immune privilege in this unique environment, perhaps by fostering apoptosis of activated Fas-expressing lymphocytes of maternal origin. An apoptotic process mediated by FasL may also play a role in placental invasion during implantation and underscores similarities between the trophoblast and neoplastic cells.

Key words: apoptosis/Fas ligand/immune privilege/placenta/trophoblast

Introduction

During pregnancy, the semi-allogeneic fetus is in direct contact with the uterine and blood-borne cells of the mother, yet the survival of the paternal antigen-expressing fetus in the potentially hostile environment of the uterus is not understood. A number of mechanisms to explain immune privilege during gestation have been suggested. The role of the membranes in partial separation of embryo from the mother; the presence of layers of cells between two circulations in placenta (one layer in human, six in pigs); low levels of major histocompatibility complex (MHC) on fetal cells (Simpson, 1996); placental production of the anti-inflammatory cytokines, transforming growth factor (TGF)-β2, interleukin (IL)-4 and IL-10 to counteract the deleterious inflammatory cytokines IL-2, interferon (IFN)-γ and tumour necrosis factor (TNF)-α (Moulton, 1993; Chaouat et al. 1995); weakening of cell-mediated immunity and strengthening of humoral immunity (Wegmann et al., 1993), and the characteristics of the uterus as an immune-privileged site (Wilbanks and Streilein, 1992) have all been suggested to play a vital role in the survival of fetal allograft to term. Recently, acquisition of a transient state of tolerance specific for paternal alloantigens has been demonstrated in murine transgenic studies, suggesting that immune privilege at the maternal–fetal interface is expressed systemically, is actively acquired, can be transient (as in pregnancy) and is necessary for the survival of the fetus to term (Streilein, 1995; Tafuri et al., 1995; Simpson, 1996).

Fas (CD95) is a cell surface protein belonging to the TNF receptor family. Cross-linking of Fas with its natural ligand (FasL; CD95L) induces apoptosis of Fas-bearing cells (Nagata and Goldstein, 1995). Fas and FasL mRNAs are widely expressed in several lymphoid and non-lymphoid tissues and have been found to be co-expressed in thymus, lung, spleen, small intestine, large intestine, seminal vesicle, prostate and uterus (French et al., 1996). As well as its role in clonal death, tolerance acquisition (Nagata and Suda, 1995), immune response termination, activation-induced cell death (Lynch et al., 1995) and T cell-mediated cytotoxicity (Berke, 1995), recent evidence suggests an important role for Fas–FasL interaction is the maintenance of immune privilege in the testis (Bellgrau et al., 1995), the eye (Griffith et al., 1995) and in tumour escape from immunological rejection in colon cancer (O’Connell et al., 1996), melanoma (Hahne et al., 1996) and hepatocellular carcinoma (Nagata, 1996; Strand et al., 1996). T and B lymphocytes become activated when their receptors bind foreign antigens. This activation induces Fas expression which delivers an apoptotic signal to the cell when bound by its ligand, allows disposal of lymphocytes and suppresses the immune response (Vaux, 1995; Scott et al., 1996). FasL expression in the immune-privileged tissues has been proposed as inducing apoptosis of the target cells, the
activated Fas-expressing lymphocytes, providing a powerful mechanism to down-regulate a deleterious immune response (Streilein, 1995; Vaux, 1995). We postulate that a similar mechanism mediated by Fas–FasL interaction may play a role in the protection of the semi-allogeneic fetus from maternal immune attack and may make a critical contribution to the immune-privileged environment of the placenta.

An anti-apoptotic membrane-associated protein, Bcl-2, has been shown to prolong cell survival of various cell types under defined conditions. Recent reports suggest protection from Fas-mediated apoptosis by Bcl-2 expression or up-regulation (Itoh et al., 1993; Jaatela et al., 1995; Weller et al., 1995; Barcena et al., 1996). Bcl-2 is expressed and appears to act as a survival factor in fetal tissues and in a restricted manner in some adult tissues characterized by apoptotic cell death (Hockenbery et al., 1991; Baer, 1994). Hormonally responsive regulation of Bcl-2 expression has also been suggested by its presence in the syncytiotrophoblast (Lue et al., 1993) and its differential expression in the endometrium during the menstrual cycle. Similarly, Fas expression has been described in endometrium (Tabibzadeh et al., 1995a) and granulosa/lutea cells of the ovaries and has been related to apoptosis (Quirk et al., 1995).

In the present study, we investigated FasL protein expression and mRNA transcripts in human term placenta by immunohistochemistry (manual and automated) and by reverse transcription (RT) in-situ polymerase chain reaction (PCR) to gain an insight into whether FasL expression in the placenta contributes to immune privilege. In addition, Fas expression was studied as a related protein and Bcl-2 as a candidate to modify the Fas–FasL induced response. In situ, hybridization by the terminal deoxytransferase-mediated deoxyuridine 5'-triphosphate nick end labelling (TUNEL) method was applied to detect the extent of apoptosis in term placenta in relation to the expression of these molecules that play a significant role in immune regulation and apoptosis.

Materials and methods

Tissues

Placentae from normal term pregnancies (n = 10) were obtained at elective Caesarean sections carried out for breech delivery or cephalo-pelvic disproportion between 38–40 weeks gestation. Informed consent and Institutional Review Board approval were obtained. All the mothers had an uncomplicated delivery and delivered healthy infants without malformations or complications. The placenta were removed according to standard obstetric practice, with the umbilical cord being clamped immediately after delivery. The umbilical cord was cut at ~5 cm from the fetal–placental surface. Placentae were received in a fresh state and samples were immediately dissected. Tissue blocks (1 × 1 cm) of whole thickness placenta were taken from areas close to the central cotyledon to avoid sampling from the placental margins. All samples were fixed in 10% neutral buffered formalin (C.M.S., Swedesboro, NJ, USA) for automated overnight processing (Tissue-Tek VIP, Miles/Sakura, Mishawake, USA) followed by embedding in paraffin blocks. Tissue sections were cut at 3 µm (microtome), floated on a water batch, mounted on Superfrost/Plus (Fisher, Pittsburg, PA, USA) slides and dried in an oven at 60°C for 1 h. A single first trimester placenta (spontaneous abortion) and hydatiform mole were similarly processed.

Immunohistochemistry

Anti-Fas (N-18), anti-FasL (N-20) and anti-Bcl-2 (N-19) are rabbit polyclonal immunoglobulin (Ig) G (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

An immunohistochemical protocol for manual and automated application (Steele et al., 1996) was developed for use on paraffin sections of placenta. Incubations were performed at room temperature. Following high heat antigen retrieval (10 min at 100°C) by an electric steamer (Black and Decker, Santa Barbara, CA, USA), endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in methanol for 30 min following by avidin–biotin and protein blocks. Sections were then incubated with the primary antibody, biotinylated secondary antibody (Multilink/Biogenex, San Ramon, CA, USA) and horseradish peroxidase-conjugated streptavidin (Label/Biogenex, CA, USA) for 60 min respectively. 3,3'-diaminobenzidine (DAB; DAB Stable DAB/Research Genetics, Huntsville, AL, USA) was used as chromogen. Final optimized dilution for primary antibodies was 1:300 in phosphate-buffered saline (PBS) and for Link and Label 1:60 in streptavidin–peroxidase diluent (Biogenex). Sections were counterstained using haematoxylin.

Automatic staining was performed using the Techmate 500 (Biotech Solutions, Santa Barbara, CA, USA) computerized immunostainer. Tween-20 solution (Polyoxyethylene Sorbitan Monolaurate) (Sigma, St Louis, MO, USA) was used in all reagents and buffered washes prepared in order to facilitate capillary gap draw and thus ensure reagent coverage and staining regularity of the entire tissue section.

Negative control staining was performed by: (i) substituting PBS for the primary antibody and (ii) peptide neutralization. Primary antibodies were neutralized by reacting the antibody with a 10 × (1 µg/ml) excess of the peptide antigen in PBS followed by incubation at room temperature for 2 h for complete immunoprecipitation for specific inhibition of staining. Liver sections (hepatitis +), previously shown to be strongly positive for Fas, served as a positive control. Lymph nodes demonstrating reactive hyperplasia or follicular lymphoma served as controls for Bcl-2. A human eye section was used as control tissue for FasL staining. The placental tissues were graded with respect to Fas, FasL and Bcl-2 expression with a semi-quantitative scoring of the proportion of cells showing positive reaction: (+ = 1–24%); (++ = 25–49%); (+++ = 50–74%) and (++++) = >75%). Intensity of staining was assessed as absent, weak, moderate or intense. The results of immunohistochemical staining experiments were confirmed by using both manual and automated system sample repeats.

Double-labelling

Double-labelling immunohistochemistry experiments were performed on the DAB-stained slides (horseradish peroxidase-conjugated streptavidin–enzyme system; Biogenex) using an alkaline phosphatase–conjugated streptavidin enzyme system (Biogenex) with Nitroblue Tetrazolium (NBT)/5-bromo–4-chloro–3-indolyl phosphate (BCIP) (Boehringer Mannheim, Indianapolis, IN, USA) as the subsequent chromogen. Antibodies employed were purchased from Zymed (South San Francisco, CA, USA). The monoclonal antibody (AE1) anticytokeratin is directed against low-molecular weight cytokeratins and is reactive with both normal and neoplastic epithelial tissues; this was used to identify trophoblast cells. The monoclonal antibody (KP-1) is anti-macrophage (CD68) and was used to detect Hofbauer cells. The monoclonal antibody (A6) CD45RO was used to identify T cells. Incubations were carried out at room temperature for 60 min unless otherwise specified. Endogenous alkaline phosphatase activity was suppressed using a heat block for 2 min at 90°C. Sections were then pre-incubated with avidin–biotin for 5 min each, protein was blocked for 10 min, and pre-diluted antibody was applied followed by...
cytoplasmic and membranous pattern of immunoreactivity was detected with no nuclear staining or background interference (Figure 1A). Specificity of the staining was confirmed by demonstration of total loss of staining reactivity following incubation with neutralizing peptide used as the competitive inhibitor (Figure 1B). Reactivity of the syncytiotrophoblast layer was uniform among different villi regardless of maturity level or location. However, the intensity of the staining was denser in terminal villi, the main functional units of the fetoplacental unit. Terminal villi were characterized by their numerous dilated capillaries close to the trophoblast surface, the relatively little stroma, smaller villus diameter and reduced number of Hofbauer cells. Syncytial knots were intensely reactive with anti-FasL antibodies. Within the cell components of the villous stroma, Hofbauer cells were scarce, but were identified by their triangular, or spindle shape, anti-CD68 positivity and their reactivity to anti-FasL antibody (+ + +). Anti-cytokeratin staining did not reveal significant numbers of cytotrophoblast cells within the chorionic villi in term placenta.

The results of immunohistochemical staining were confirmed by demonstration of FasL mRNA transcripts and subsequent amplification of cDNA in the syncytiotrophoblasts by using RT in-situ PCR technique. The distribution of the signal pattern for FasL mRNA mimicked the pattern for gene product localization obtained by immunohistochemistry. Dense signals were localized on the free surface of the syncytiotrophoblasts that directly faced the intervillous space of the maternal circulation (Figure 1C). No evidence for transcription and amplification of mRNA transcripts was found in the negative control. The non-DNase-treated positive systems control section showed signals in >50% of all nuclei (Figure 1D).

**Extravillous trophoblasts express FasL**

The distribution of FasL-stained cells within the decidual tissue closely matched that of extravillous trophoblast (EVT) (Figure 2A) as determined by cytokeratin staining (Figure 2B). The FasL reactivity was membranous and cytoplasmic. Decidual cells were characterized by their large size, polygonal shape, prominent nucleolus and often granular and vacuolar cytoplasm. Besides their staining for cytokeratin, extravillous trophoblast cells were distinguished from decidual cells by morphology; cytotrophoblasts are small uniform mono-nucleate cells which are smaller than the adjacent decidual cells; syncytiotrophoblasts are large, multinucleate giant cells with dense cytoplasm and dark nucleus; intermediate trophoblasts range from round or polyhedral to spindle-shaped, bipolar-, mono-, bi- or multinucleate cells (Kurman, 1994). Expression of FasL by the trophoblastic cells was further confirmed by immunohistochemical staining of tissue sections of a first trimester placenta (Figure 2C,D) and a hydatiform mole (Figure 2E). Maternal T cells within decidua were not apparent using anti-T cell antibody (CD45RO). However, variable numbers of cells in the maternal intervillous spaces showed reactivity demonstrating the specificity of the immunohistochemical reaction.

**Trophoblastic cells co-express Fas and Bcl-2**

In all specimens examined (n = 10) homogenous expression (++ + +) of Fas and Bcl-2 was detected in the syncytiotropho-
Fas ligand expression of syncytiotrophoblast at term gestation. Immunohistochemistry with 3,3′-diaminobenzidine (DAB)/haematox: (A) FasL reactivity (original magnification ×600); (B) loss of reactivity with neutralizing peptide inhibition (original magnification ×200). Reverse transcription in-situ polymerase chain reaction Nitroblue Tetrazolium/no counterstain (C) FasL mRNA transcripts, note dense signals on the surface facing the maternal intervillous spaces (original magnification ×1000); (D) non-DNase treated system control demonstrating the effects of DNA repair; signal present in > 50% nuclei (original magnification ×600).

blast layer of the chorionic villi (Figure 3A,B) respectively, with a similar reactivity pattern to FasL staining which was cytoplasmic and membranous. Neutralizing peptides inhibited staining completely. The cell components of villous stroma reacted with anti-Fas antibody (+ +) while only scattered positivity was detected for FasL and Bcl-2. Endothelial lining of the fetal vessels in the chorionic villi were reactive with anti-Fas, FasL and Bcl-2 antibodies. RT in-situ PCR technique applied for Fas and Bcl-2 (n = 1 each) confirmed the immunohistochemical findings by demonstrating mRNA transcripts with a similar distribution as described.

Fas reactivity of the cells in the basal plate showed a patchy distribution with membranous and cytoplasmic pattern (Figure 3C). EVT, distinguished by characteristic morphology, was uniformly stained with both antibodies. Although reactivity of the fibroblastoid decidual cells was weak or absent, epithelioid-type decidual cells were positive (+ +).

Umbilical vessel endothelium (Figure 3D) and amniotic membrane epithelial cells (Figure 3E) express Fas, FasL and Bcl-2 proteins. Endothelial lining of the umbilical vessels was reactive (+ + + +) with all three antibodies. FasL and Bcl-2 reactivity was not detected on the media layer cells, but moderate expression of Fas was present (+ +) in the smooth muscle cells. The loose connective tissue cells of the Wharton’s jelly and amniotic membrane epithelial cells showed uniform (+ + + +) staining pattern for all three determinants.

Immunoreactivity patterns for the control tissues were as follows: FasL reactivity was detected in human eye (Figure 3F); lymph node specimens from reactive node lacked Bcl-2 reactivity in the germinal centres whereas the germinal centres in the lymphoma specimen were Bcl-2 positive; liver tissue with hepatitis showed intense and uniform immunoreactivity (hepatocytes) with anti-Fas antibody.

Addition of the appropriate competitive peptide completely inhibited staining of the placenta verifying the specificity of the antibody reactions in the tissues as described. The intervillous blood spaces, as internal control for placenta sections, showed scattered cells positive for Fas, FasL and Bcl-2. Neutrophils demonstrated consistent reactivity for FasL.
Apoptosis in human term placenta

Apoptotic cells were detected by staining of DNA fragments with the TUNEL technique. Although we performed this method to detect apoptosis of activated T lymphocytes of maternal origin, CD45RO staining failed to show the presence of T lymphocytes in term placentae. However, TUNEL-stained nuclei were present in almost every structure of placental tissues. Extent of apoptosis varied between different samples and appeared to be highest in the villus stroma, where more than half of the stromal cell population of the chorionic villi were stained (Figure 3G). Immunoreactivity to Fas, FasL and Bcl-2 did not correlate with the extent of apoptosis in the placenta sections examined \((n = 4)\). Although these proteins were expressed constitutively in the syncytiotrophoblast layer, the frequency of occurrence of apoptotic nuclei was highly variable among different fetal villi and sections \((20\% \text{ to } 50\%)\). Syncytial knots and sprouts consisting of several apoptotic bodies and nuclei were noted frequently. A small minority of knots contained intact nuclei. These nuclei might represent the tangential sectioning of the trophoblast surface since the syncytial sprouts at term placenta are aggregations of degenerate nuclei. Stained cells were frequent in the basal plate of the placenta with patchy distribution \((>50\%)\). Apoptotic nuclei were scarce in the amniotic membrane cells. In contrast, several degenerate nuclei were contained in the media of the umbilical artery. Scattered endothelial and intimal cells were also stained. Apoptotic nuclei were rarely noted in the loose connective tissue of the umbilical cord.

The control post-weaning rat mammary gland tissue and dexamethasone-treated mouse thymocytes demonstrated extensive apoptosis. Double-labelling of human thymic tissue demonstrated apoptotic nuclei (TUNEL) within the KP-1 stained macrophages, besides the scattered apoptotic cells. Where TdT was omitted, all negative control sections, including those for placenta, failed to demonstrate any stained nuclei (Figure 3H).
Discussion

In the present report, we have described the constitutional expression of Fas ligand protein and mRNA transcripts in human term placenta at several locations, most notably in the syncytiotrophoblast layer. In addition, co-expression and localization of other apoptosis-related molecules, Fas and Bcl-2, are described.

Our findings of the conjoined presence of those determinants that are involved in programmed cell death (apoptosis) at a number of locations in the placenta are most provocative. We initiated the present investigations in order to elucidate the persistent puzzle of the immunological relationship between the fetus and the mother. Why is the fetus, despite being allogenically haplotype identical to the mother, regularly tolerated and not rejected outright? A number of mechanisms to explain these phenomena have been presented, but their explanations have not been entirely satisfactory. These include the local mechanical and hormonal barriers in placenta, antigenic deficiency of fetal tissues, down-regulation of histocompatibility antigens and suppression of certain specific and non-specific maternal immune responses (Fox, 1978; Wegmann et al., 1993; Simpson, 1996). Human placental trophoblasts demonstrate an unusual MHC class I expression: suppressed class Ia production and expression of HLA-G, a class Ib molecule (McMaster et al., 1995; Yang et al., 1995). However, studies demonstrating suppressed cytoxicity of maternal lymphocytes in trophoblast cultures and a transient state of tolerance specific for paternal antigens during pregnancy support the possibility of an active participatory role for the fetus in the development of maternal tolerance. Fetal survival from a potential maternal assault has been attributed to a reduction in the antigenicity of the fetal membranes, particularly involving a fail-safe mechanism and, at the local decidua–placental level, inhibition of maternal immunological activity (Fox, 1978; Cherry and Merkatz, 1991; Tafuri et al., 1995). Although the role of decidualized endometrium as an immune-privileged site similar to the eye or brain has been implicated in fetal survival (Fox, 1978; Wilbanks and Streilein, 1992), factors such as deficiency in either vascular supply or lymphatic drainage that are possessed by other immune-privileged sites are not applicable to placenta. Recently, a novel mechanism has been described in murine testis and eye. Immune privilege is postulated to be attributable to apoptosis of activated T lymphocytes which express Fas initiated by Fas ligand expression upon exposure of these tissues to antigen (Bellgrau et al., 1995; Griffith et al., 1995). In the present study, we investigated whether FasL is expressed in human placenta and whether a similar mechanism of immune down-regulation could contribute to fetal survival.

In the placenta, the trophoblasts represent the fetal tissues that are most prone to immunological reactions from maternal factors and are the only component of the placenta that is in direct intimate contact with maternal tissues (Fox, 1978). Our results show that FasL mRNA is expressed and FasL is extensively deposited at the syncytiotrophoblast layer of placenta, the main site of feto–maternal interaction where the surfaces of chorionic villi are bathed in maternal blood (Kurman, 1994). If maternal lymphocytes become activated toward fetal antigens, the lymphocytes would be expected to express Fas determinants. Interaction of such specifically activated Fas-expressing T lymphocytes with Fas-expressing syncytiotrophoblasts might be expected to lead to apoptosis of maternal lymphocytes and, thus, blunt or prevent inflammatory destruction of the fetus. The presence of abundant expression of FasL in the trophoblast cells would be quite compatible with this scenario.

Fas and Bcl-2 were also present constitutively in term placenta. In studies with normal and neoplastic tissues, constitutive expression of Fas and its co-expression with FasL have been described (French et al., 1996; O’Connell et al., 1996). The ability of the Fas system to promote apoptosis of T cells depends on their activation stage. Cell surface expression of Fas alone is not sufficient to render T lymphocytes susceptible to anti-Fas monoclonal antibody-mediated apoptosis, suggesting that other signals might be involved in the events that lead to cell death. Over-expression of Bcl-2 protein in cell lines partially protects against apoptosis induced by growth factor deprivation and by Fas (Barcena et al., 1996). A differential susceptibility of the syncytiotrophoblast to undergo apoptosis by adjusting the critical level of Fas expression is reasonable. The expression of Bcl-2 in syncytiotrophoblasts may represent a partially protective role against apoptotic signals that are likely to be induced by autocrine Fas–FasL interactions or, alternatively, the findings may suggest involvement of other molecules which can regulate the direction of the apoptotic response. Studies have suggested that hormone-dependent regulation of Bcl-2 expression occurs in normal endometrium (Gompel et al., 1994) and menstrual shedding is related to apoptotic signals induced by TNF-α, possibly Fas, and the loss of protective effect of Bcl-2 (Otsuki et al., 1994; Tabibzadeh et al., 1995a).

We found no evidence of apoptosis of lymphocytes in the placental sinusooids that contain maternal blood cells or in the decidua. In spite of the very large blood flow through the placenta, lymphocytes are not always detected in the placental tissues (Tafuri et al., 1995). Anti-CD45RO staining to identify activated maternal T lymphocytes was negative in the placental bed. It could be that the Fas–FasL interaction of activated T lymphocytes and trophoblast cells did take place as postulated, but that the programmed cell death occurs at a distant site and that the characteristic TUNEL staining and phagocytosis of the apoptotic cells would be seen elsewhere, for example, in spleen, bone marrow, liver or even lungs. Furthermore, apoptotic lymphocytes may have been phagocytosed by placental macrophages or trophoblasts and thus escape histological detection. Exploration of these possibilities, although urgent, will require further analysis of apoptosis occurring during pregnancy in placenta and distant sites, using as models gld or lpr mice defective in FasL and Fas respectively (Nagata and Goldstein, 1995). It could well be that the Fas–FasL interaction of maternal T lymphocytes is initiated in placenta, but that apoptosis and phagocytosis of apoptotic cells occurs elsewhere. However, these results show that syncytiotrophoblasts express FasL abundantly and are located where they are likely to make contact with any activated T cells that express Fas. This is very relevant to our original hypothesis and might
be a basis for the postulated fail-safe mechanisms by which the fetus is guarded from any assault activated by maternal lymphocytes. Furthermore, Fas, FasL and Bcl-2 interactions may be involved in the role of the placental membranes in implantation and/or embryonic development. Cytokines play an important role in the preparation of the endometrium for implantation (Tabibzadeh and Babaknia, 1995; Tabibzadeh et al., 1996). The cell-specific pattern of distribution and hormone-dependent expression of IL-6 and other proteins suggest their role in menstrual shedding and implantation (Tabibzadeh et al., 1995b). In rodents, differential expression of Fas and FasL during embryogenesis (French et al., 1996), together with progressive continuous induction of apoptosis in the maternal tissues lining the conceptus, have been demonstrated (Abrahamsohn and Zorn, 1993; Piacentini and Autuori, 1994). Degeneration of the maternal tissues has been shown to be limited to the sites that first make contact with trophoblastic cells, facilitating trophoblast access to maternal blood vessels (Welsh, 1993). Based on our observations of marked expression and the co-ordinated presence of Fas, FasL and Bcl-2 proteins in the trophoblast cells, including the cytokertatin-positive invasive trophoblasts in the deeper basal plate, it may also be speculated that the interactions of these determinants may be involved in the maternal invasion and implantation process by inducing apoptosis in decidua and enabling trophoblast giant cells to gain access to maternal vessels. Recent studies have suggested an important role for FasL in tumour escape from immune clearance (Hahne et al., 1996; O’Connell et al., 1996; Strand et al., 1996). The resemblance to tumour cell behaviour of the invasive nature of trophoblast cells has been pointed out by several investigators. An addition to several other shared characteristics of trophoblasts and malignant cells (Lalani et al., 1987; Loke and King, 1990; Kilman et al., 1990; St. Jacques et al., 1993; Welsh, 1993; Roncalli et al., 1994; Marzusch et al., 1995), is the novel finding of constitutional FasL expression in the trophoblast.

In conclusion, we report constitutive expression of FasL in the trophoblasts of term placentae. The presence of this protein was confirmed by demonstration of mRNA transcripts by RT in-situ PCR technique. We speculate that the constitutional expression of FasL in the trophoblast cells and Fas-FasL, and possibly Bcl-2 interactions, may contribute to maintenance of immune privilege in pregnancy by inducing apoptosis of activated maternal lymphocytes, thus preventing a deleterious immune response towards the conceptus. Although most of our observations have been made on term placenta, analysis of a single first trimester placenta supported expression of FasL on the trophoblastic cells also at this early stage. Investigations of Fas–FasL interactions at earlier stages of gestation and in animal models may contribute to resolution of a challenging and long-enduring immunological puzzle. Furthermore, FasL expression in the trophoblast cells may suggest a non-immune role for the maternal invasion process during implantation, abnormalities of which may contribute to development or invasion of trophoblastic tumours. Thus, constitutional expression of FasL underscores similarities between the trophoblastic and neoplastic cells.

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