0021-972X/99/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 84, No. 6
Printed in U.S.A.

Placental Fas Ligand Expression Is a Mechanism for Maternal Immune Tolerance to the Fetus*

SCOTT W. KAUMA, THOMAS F. HUFF, NATALIE HAYES, AND ATHIP NILKAEO

Departments of Obstetrics/Gynecology (S.W.K., N.H.) and Microbiology/Immunology (S.W.K., T.F.H., A.N.), Virginia Commonwealth University/Medical College of Virginia, Richmond, Virginia 23298

ABSTRACT

Fas ligand (FasL) is a peptide that plays an important immunoregulatory role in limiting the host immune response. Several studies have shown that the expression of FasL in the anterior chamber of the eye and the testis allows these tissues to be immunoprivileged sites. Immunotolerance is achieved by binding of FasL to its receptor (Fas) on activated immune cells, which results in cell apoptosis. To determine whether FasL has a role in maternal immune tolerance to the fetus, we looked for the expression of FasL in the human placenta. Immunoperoxidase staining localized FasL to both syncytiotrophoblast and cytotrophoblast in placental villi and chorionic extravillous trophoblast. Western analysis demonstrated FasL in placental villi and a human first-trimester trophoblast cell line (ED_{27}). In contrast, Fas was colocalized to CD45 (leukocyte common antigen) positive cells found in maternal decidua. When isolated peripheral blood lymphocytes were induced to express Fas with phytohemagglutinin (PHA) and interleukin-2 (IL-2) and then cocultured with trophoblast, 30% of the lymphocytes underwent apoptosis, as determined by the *in situ* death (TUNEL) assay. Neutralizing antibodies to FasL inhibited apoptosis by 40% in these studies. In contrast, activated lymphocytes cocultured with non-FasL-expressing fibroblasts or unactivated non-Fas-expressing lymphocytes cocultured with ED_{27} trophoblast showed little evidence of apoptosis. These findings suggest that FasL expressed by fetal trophoblast cells can induce apoptosis in activated lymphocytes there by providing a mechanism for maternal immune tolerance to the fetus. (*J Clin Endocrinol Metab* **84:** 2188–2194, 1999)

THE FETUS is a unique individual, whose genetic makeup is equally derived from both the mother and father. The fetus can be considered a semiallograft to the mother's immune system and consequently should generate a maternal immune rejection response (1). In the majority of cases, however, a maternal immune rejection response against the fetus does not occur, and instead, a process of maternal immune tolerance to the fetus takes place. The main site of immune interaction between the fetus and the mother is where the maternal decidua and fetal placenta are in intimate contact and is called the maternal-fetal interface. The placenta and placental membranes actually provide a barrier between the fetus and the maternal immune system so that the majority of maternal immune interaction is with the placenta and not the fetus (2). The predominant cell type of the placenta that comes into contact with the maternal immune system is trophoblast. Consequently, most discussions concerning maternal immune recognition of the fetus are really concerned with maternal immune recognition of placental trophoblast. Abnormalities of maternal immune tolerance to the fetus have been implicated in several disease processes of pregnancy, including recurrent miscarriage (3) and preeclampsia (4). Unfortunately, the basic mechanisms involved in maternal immune tolerance to the fetal semiallograft are poorly understood.

Fas ligand (FasL) and its receptor, Fas, are known to play an important role in regulation of the immune response. Fas is a type I membrane protein that belongs to the tumor necrosis factor and nerve growth factor receptor family (5). Fas is highly expressed on activated T cells and B cells, NK cells, and macrophages. FasL is a type II membrane protein also related to the tumor necrosis factor family (5). FasL can either be membrane bound (42–48 kDa form) or can be cleaved by metalloproteinases to release the extracellular portion as soluble FasL (sFasL), which is 26 kDa in size (6). FasL is expressed by activated T cells, cytotoxic T lymphocytes, sertoli cells of the testis, and corneal epithelium and endothelium (7–9). The major function of Fas/FasL interaction and Fas activation is the induction of cell apoptosis (10).

Recent studies have demonstrated that the immunoprivileged nature of the testis and anterior chamber of the eye is caused by the expression of FasL in these tissues. When T cells are activated by infection or foreign antigens, they express Fas. Activation of T cells in the testis or anterior chamber of the eye results in apoptosis of these cells secondary to the local production of FasL (8, 9). The immunoprotective effect of FasL has recently been exploited in transplantation biology by transfecting myoblasts with FasL complementary DNA (11). Cotransplantation of these FasL-expressing myoblasts with pancreatic islet cells into donor mice of a different strain resulted in functional islet cells that were not rejected by the host immune system. These studies demonstrate the importance of Fas/FasL in peripheral localized immunotolerance to foreign antigens. These findings also suggest that Fas/FasL could be a very elegant mechanism for local maternal immunotolerance of the fetal semiallograft during pregnancy.

To test the hypothesis that Fas/FasL interaction is a mechanism in human maternal immunotolerance to the fetus, we

Received March 19, 1998. Revision received January 28, 1999. Accepted February 24, 1999.

Address all correspondence and requests for reprints to: Dr. Scott Kauma, Departments of Obstetrics/Gynecology and Microbiology/Immunology, Virginia Commonwealth University/Medical College of Virginia, Box 980034, Richmond, Virginia 23298.

* This work was supported by grants from the NIH/NICHHD (HD-35640, to S.W.K.), NIH/NIAID (AI-25537, to T.F.H.), and the US Army (DAMD-17–93-J-3035, to T.F.H.). E-mail: skauma@hsc.vcu.edu.

first characterized the expression of Fas/FasL in tissues at the maternal-fetal interface. Subsequently, we used human ED_{27} trophoblast cells and lymphocytes from newborn cord blood as a model to determine whether FasL-expressing trophoblast cells could induce apoptosis in activated lymphocytes *in vitro*.

Materials and Methods

Placental tissues and cell lines

The protocol for this study was approved by our institution's Committee for the Conduct of Human Research before obtaining any tissue samples. Term placentas (37–40 weeks gestation) were obtained from patients undergoing uncomplicated repeat cesarean section. Early second-trimester placentas (14–20 weeks gestation) were obtained from patients undergoing elective surgical pregnancy terminations. A first-trimester human trophoblast cell line (ED_{27} cells, a generous gift from Dr. Douglas Kniss, Dept. of Obstetrics and Gynecology, Ohio State University College of Medicine) was maintained in DMEM/F12 (Gibco BRL, Grand Island, NY) with 15% FBS. A fetal skin fibroblast cell line (WS1 cells, ATCC, Rockville, MD) were maintained in MEMα (Gibco BRL) with 10% FBS.

Immunolocalization of Fas, FasL, and CD45

Immunohistochemical staining for Fas was performed on 10-μm frozen sections of placenta (N = 5) post-fixed in acetone at 4 C for 10 min. The primary antibody that was used for Fas detection was a mouse monoclonal antihuman Fas antibody (2 μg/mL, PharMingen, Los Angeles, CA). Immunohistochemical staining for FasL was performed on formalin-fixed, paraffin-embedded 5-μm sections of placenta using a rabbit IgG anti-FasL antibody (0.5 μg/mL; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Localization of the primary antibody was performed using the Elite kit (Vector Laboratories, Inc., Burlingame, CA) and diaminobenzidine as the color substrate. The sections were then counterstained with hematoxylin. Negative controls included preabsorption of the primary antibody with excess FasL peptide (Santa Cruz Biotechnology, Inc.) or substituting the specific primary antibody with nonspecific antibody of the same isotype and concentration.

Colocalization of Fas and CD45 (leukocyte common antigen) was performed using a modification of the EnVison Doublestain System (DAKO Corp., Carpinteria, CA) on 10-μm frozen tissue sections. After blocking the slide for endogenous peroxidase activity and nonspecific binding, 1 μg/mL anti-Fas antibody (PharMingen) was incubated with the tissue sections for 1 h and detected using the HRP-labeled antibody polymer and diaminobenzidine as substrate. The tissue sections were then again blocked for nonspecific binding and for endogenous biotin (Vector Laboratories, Inc.), followed by incubation with anti-CD45 antibody (400 ng/mL, Novocastra Laboratories, Newcastle, UK) for 1 h. The CD45 antibody was detected by incubating the slides with biotinylated antimouse antibody (1:200, Vector Laboratories, Inc.), followed by strepavidin-Texas Red (10 μg/mL, Molecular Probes, Inc., Eugene, OR). Colocalization of Fas and CD45 was accomplished by photographing the tissue section using fluorescence microscopy (CD45), immediately followed by bright-field photomicroscopy (Fas) of the same microscopic field. Specificity controls consisted of substituting the anti-Fas and/or the anti-CD45 antibodies with nonspecific isotype matched antibodies at the same concentration.

FasL Western blot analysis

To perform Western blot analysis for FasL, intact placental villi, 96-h phytohemagglutinin (PHA)/interleukin (IL)-2 activated lymphocytes, ED_{27} trophoblast cells, or WS1 fetal fibroblasts were homogenized in RIPA buffer (5 mmol Tris, 150 mmol NaCl, 1% Nonidet P-40, 0.5% Na deoxycholate, 0.1% SDS, pH 7.5). The homogenate was clarified by centrifugation, and total protein content of the supernate was determined by a Coomassie protein assay using albumin as the standard (Pierce Chemical Co., Rockford IL). For FasL Western analysis, 50 μg of total cellular protein was fractionated in a denaturing 10% SDS-polyacrylamide gel by electrophoresis. The fractionated protein samples were then transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, NH), and nonspecific binding was blocked for 2 h in 5% nonfat dry milk in Tris-buffered saline. The membranes were incubated with 0.3 μg/mL of rabbit IgG antibody (Santa Cruz Biotechnology, Inc.) developed against a synthetic FasL peptide that corresponds to the intracellular portion of the FasL molecule, or 0.3 μg/mL of nonspecific rabbit IgG antibody as a control. Development of the Western blots was performed using the ECL system (Amersham, Arlington Heights, IL).

Peripheral blood lymphocyte isolation

To obtain peripheral blood lymphocytes, mononuclear cells were isolated from heparinized peripheral blood from newborn infants (placental cord blood, N = 4) by Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) density centrifugation. The mononuclear cell layer was recovered and washed in RPMI 1640 media (Gibco BRL). The cells were then placed in plastic culture flasks in RPMI with 5% FCS at a concentration of 2×10^6 for 1 h at 37 C, in 5% CO_2/air, to allow the monocytes to adhere. The nonadherent lymphocytes were then recovered by gentle rinsing with RPMI 1640, and the adherence procedure was repeated. The nonadherent lymphocytes were then recovered and used for study.

FACS analysis for Fas expression in activated lymphocytes

Isolated lymphocytes were activated by incubation in RPMI 1640 with 10% FCS, 10 μg/mL of phytohemagglutinin (Sigma Chemical Co.), and 10 ng/mL of IL-2 (PharMingen) for 24 h at 37 C in 5% CO_2/air. The lymphocytes were then washed and incubated in RPMI 1640 with 10% FCS and 10 ng/mL of IL-2 for an additional 48 h to induce Fas expression, and for 96 h to induce FasL expression (7). For FACS analysis of Fas expression, activated or unactivated lymphocytes were incubated with either 10 μg/mL of fluorescein isothiocyanate (FITC) labeled anti-Fas antibody or FITC-labeled isotype-matched nonspecific antibody control (PharMingen) for 1 h at 4 C. The cells were washed and analyzed for Fas expression using a FACScan flow cytometer (Becton Dickson and Co., Franklin Lakes, NJ).

TUNEL (in situ death) assay for trophoblast-induced lymphocyte apoptosis

ED_{27} trophoblast cells and WS1 fibroblast cells were grown in 6-well plates in their respective growth media. Once confluent, the media was changed to RPMI 1640 with 10% FCS and either 1×10^5 activated or unactivated lymphocytes was added to the wells (N = 3 for each treatment), resulting in a target:effector cell ratio of approximately 1:20. After 24 h of coculture, the wells were gently rinsed to recover the nonadherent lymphocytes. Cytospin cell preparations of the lymphocytes were fixed in 4% formalin at room temperature for 30 min. After fixation, the slides were washed in PBS and treated with acetic acid:ethanol (1:2) at −20 C for 10 min. The slides were washed in PBS and then blocked with 0.1% BSA for 30 min. The slides were washed once in PBS and then in H_2O. The TUNEL reaction mixture (Boehringer Mannheim, Indianapolis, IN) was applied to the slides and then incubated at 37 C for 1 h to label 3′ DNA ends with FITC-labeled deoxyuridine 5′-triphosphate. Apoptotic cells were observed under phase and fluorescence microscopy. Three random 200 × microscopic fields per slide were photographed, and the number of positively fluorescing nuclei and the total number of cells were counted. Occasional nonadherent dead trophoblast cells could be readily distinguished morphologically from lymphocytes by their large sized and substantially increased cytoplasmic-to-nuclear ratio. These experiments were performed on lymphocytes isolated from four different individuals, and the results were analyzed by ANOVA for statistical comparisons. To demonstrate the specificity of the FasL-induced apoptosis by trophoblast on activated lymphocytes, 10 μg/mL of neutralizing anti-FasL antibody (NOK-2, PharMingen) or nonspecific control antibody was added to trophoblast/activated lymphocyte coculture experiments to inhibit FasL activity.

Results

FasL was found to be localized mainly to both syncytiotrophoblast and cytotrophoblast in fetal placental villi by im-

munohistochemistry (Fig. 1A). In addition, extravillous cytotrophoblast in the chorion of the placental membranes also localized immunoreactive FasL (Fig. 1C). These are the sites where fetal tissues would be in contact with the maternal immune system. The specificity of the antibody staining was verified by the lack of any specific staining with anti-FasL antibody preabsorbed with FasL peptide (Fig. 1B) or with nonspecific control antibody (data not shown). Fas localization was seldom seen in the fetal placental villi but instead was found in occasional cells of the maternal decidual tissues (Fig. 1D). The specificity of the Fas antibody staining was verified by the lack of staining with nonspecific control antibody (Fig. 1E). The colocalization experiments for Fas and CD45 demonstrated that some of the Fas-expressing cells in maternal decidua were leukocytes (Fig. 2, A and B). These experiments also showed that not all CD45 positive cells in decidua expressed Fas, and not all Fas positive cells expressed CD45. Substitution of the anti-Fas or anti-CD45 antibodies with nonspecific isotype specific antibody at the same concentration resulted in no specific staining (Fig. 2, C and D), demonstrating the specificity of the colocalization staining procedure.

To verify our immunohistochemistry results for FasL, Western blot analysis was performed. As seen in Fig. 3,

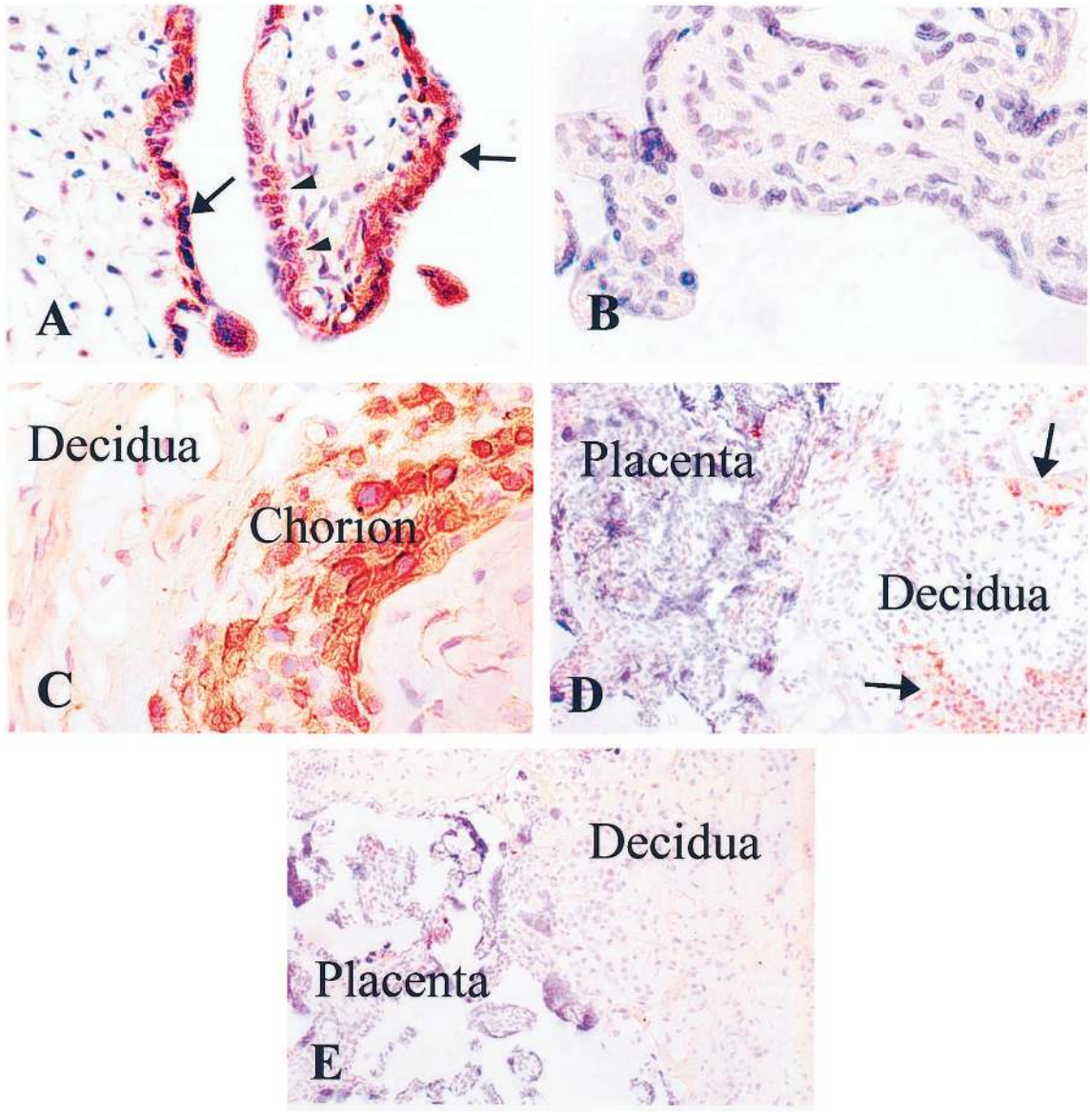

FIG. 1. Localization of FasL and Fas at the maternal-fetal interface. The photomicrographs are representative of the staining seen in other placental samples. Immunohistochemical localization of FasL in placental villi (A, 400× magnification, 19 week gestation) was found mainly in both syncytiotrophoblast (*arrows*) and cytotrophoblast (*arrow heads*). In placental membranes (C, 400× magnification), FasL was localized mainly to the cytotrophoblast of the chorion. Specificity of the FasL staining was demonstrated by preabsorption of the anti-FasL antibody with immunizing FasL peptide (B) or substituting nonspecific rabbit IgG at the same concentration (data not shown) that resulted in no specific staining. Immunohistochemical localization of Fas (D, 100× magnification) was found in isolated cells or groups of cells in the maternal decidua but not in the placental villi or villous trophoblast. Specificity of the Fas staining was demonstrated by substituting the Fas antibody with a nonspecific mouse isotype matched antibody at the same concentration (E) that resulted in no specific staining.

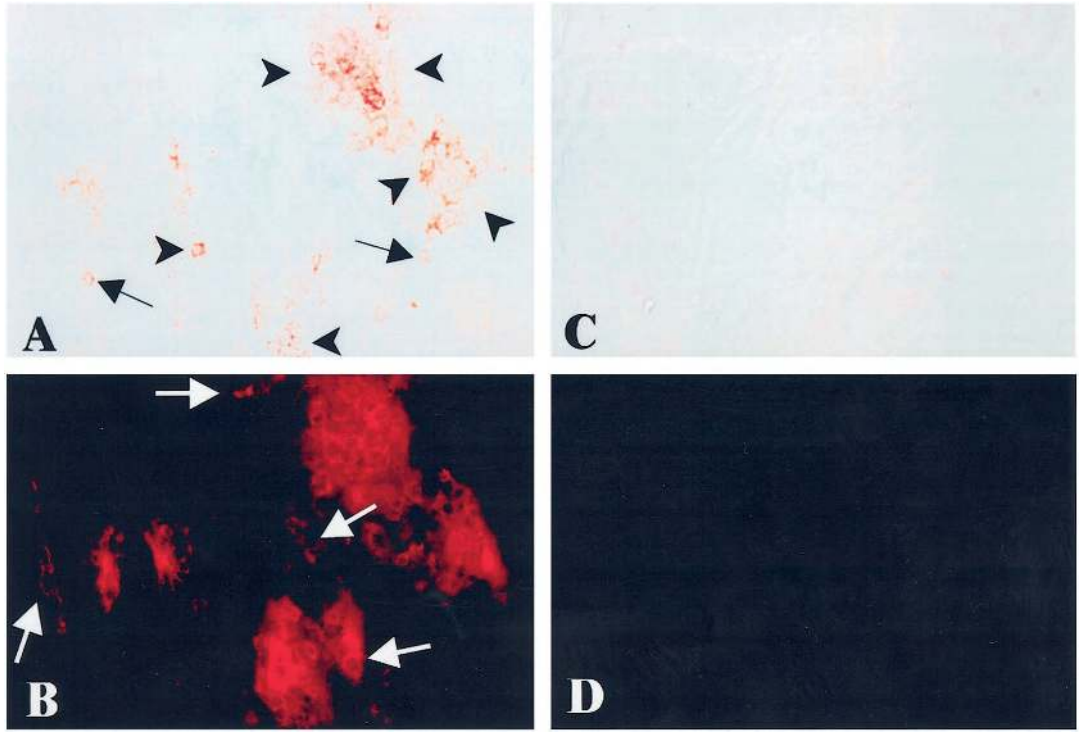

FIG. 2. Colocalization of Fas and CD45 in maternal decidua to determine whether leukocytes in maternal decidua express Fas. The photomicrographs are representative of the staining seen in other decidual samples. A, Immunoperoxidase localization for Fas in decidua from a 14-week pregnancy; B, immunofluorescence localization for CD45 on the same tissue section. The *black arrow heads* in (A) demonstrate Fas positive cells that also localized CD45 by immunofluorescence as seen in B. The *black arrows* (A) demonstrate Fas positive cells that did not express CD45. In B, the *white arrows* demonstrate CD45 positive cells that did not express Fas. In C and D, the primary anti-Fas or CD45 antibodies were replaced with nonspecific antibodies. These control slides showed no specific localization for Fas or CD45 confirming the specificity of the colocalization procedure.

immunoreactive bands for FasL were seen at 46 kDa in placental villi and the ED_{27} trophoblast cell line. These findings are consistent with the localization of FasL to placental trophoblast by immunohistochemistry shown in Fig. 1. Additional immunoreactive bands for FasL were evident at 50 kDa and 29 kDa in placental villi. The WS1 fibroblast cell line, however, did not demonstrate any immunoreactive FasL. Specificity of the Western blot analysis was demonstrated by substituting the FasL antibody with nonspecific rabbit antibody at the same concentration and preabsorbing the primary anti-FasL antibody with FasL peptide, both of which abolished the immunoreactive bands. These results have been reproduced in at least two other separate experiments.

Lymphocytes isolated from newborn peripheral blood were used for studies concerning Fas/FasL-induced apoptosis because these lymphocytes are naïve and do not express Fas antigen (Fig. 4A). After activation with PHA and IL-2 for 72 h, however, a marked up-regulation of Fas antigen expression was seen (Fig. 4B) but not FasL (results not shown). When activated lymphocytes that express Fas were cocultured with FasL-expressing ED_{27} trophoblast for 24 h, 30% of the lymphocytes underwent apoptosis, as determined by the TUNEL assay (Fig. 5B). In these experiments, lymphocyte apoptosis was caused by ED_{27} trophoblast cell FasL activation of Fas expressed on the lymphocytes because apoptosis was inhibited by 40% with the addition of neutralizing antibody to FasL (Fig. 6). In addition, coculture of unactivated non-Fas-expressing lymphocytes with ED_{27} trophoblast (Fig. 5D) or activated lymphocytes with non-FasL-expressing WS1 fetal fibroblast cells (Fig. 5F) resulted in only minimal lymphocyte apoptosis of 4–5%.

Discussion

This study has demonstrated that human fetal trophoblast cells express FasL. These findings are consistent with a recent study that demonstrated FasL messenger RNA in placental villi and choriocarcinoma cell lines (12). In contrast to FasL, Fas was mainly localized to cells found in the maternal decidua, some of which are maternal leukocytes. Furthermore, using a model of ED_{27} trophoblast cocultured with isolated cord blood lymphocytes, we have demonstrated that FasL expressed on trophoblast cells can induce apoptosis in activated peripheral lymphocytes. Because trophoblast form the fetal interface with the maternal immune system, these findings support our hypothesis that expression of FasL on trophoblast provides a local mechanism for maternal immunotolerance to the fetus.

The need for local immune tolerance mechanisms at the maternal-fetal interface comes from numerous studies suggesting that the maternal immune system initiates an immune response directed against the fetus. During pregnancy, there is a large influx of macrophages and lymphocytes into the decidua at the maternal-fetal interface. Leukocytes comprise up to 40% of the cells found in decidua during pregnancy (13). Cytokines such as IL-1 and IL-6 are up-regulated

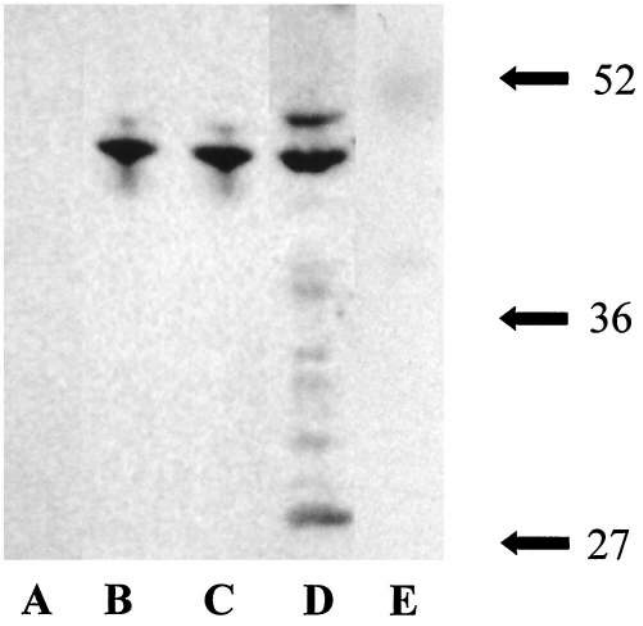

FIG. 3. Western blot analysis for FasL. An immunoreactive band for FasL was found at 46-kDa band in 96-h PHA/IL2 activated lymphocytes (+ control) (B); ED_{27} trophoblast (C); and placental villous tissue (D). No evidence of FasL expression was seen in WS1 fetal fibroblasts (A). Substitution of the anti-FasL antibody with nonspecific antibody resulted in no specific bands with ED_{27} trophoblast (E). Additional immunoreactive bands for FasL were evident at 50 kDa and 29 kDa in placental villous tissue.

in decidua during pregnancy, along with human leukocyte antigen (HLA)-DR (14, 15). Findings of increased leukocytic infiltrates and elevated IL-1 and HLA-DR expression are also found during transplant organ graft rejection (16). Finally, some mothers are known to develop antibodies directed against paternal HLA antigens found in the fetus (16a), in addition to antibodies directed against the placenta (17). These findings are consistent with a maternal immune response directed toward the fetus.

Given the many different effector arms of the immune system, it is not surprising that a number of mechanisms, other than Fas/FasL, have been described for maternal immune tolerance to the fetus. Central to all of these mechanisms is the fact that maternal immunotolerance during pregnancy is a phenomena mainly localized to the maternal-fetal interface. A primary mechanism for maternal immune tolerance to the fetus is the lack of classic major histocompatibility complex (MHC) antigen expression on trophoblast cells (18). Trophoblast do express a unique major histocompatibility complex (MHC) class I antigen designated HLA-G, but this antigen has very limited polymorphism, does not seem to stimulate T cell activation (19), and inhibits NK cell activity (20). A number of immunosuppressive substances are at the maternal-fetal interface. One of the most potent of these immunosuppressive substances is transforming growth factor-β (TGF-β). TGF-β is a multifunctional peptide with potent immunosuppressive activities, including inhibition of IL-1-induced lymphocyte proliferation, lymphokine-activated killer cell activity, cytotoxic T cell activity, natural killer cell activity, and macrophage cytotoxicity (21–24). Both the placenta and placental membranes produce

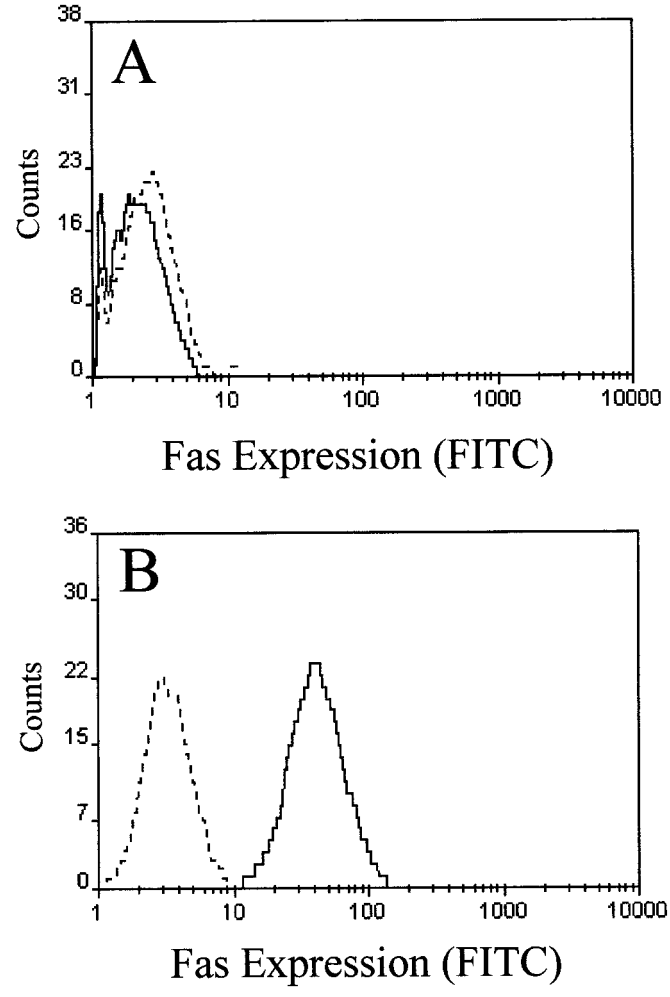

FIG. 4. Fas up-regulation in peripheral lymphocytes, in response to PHA and IL-2 activation. Lymphocytes were analyzed for Fas expression either immediately after purification from peripheral cord blood or after activation with PHA and IL-2 for 72 h. Lymphocytes were stained with Fas specific antibody (*solid line*) or a nonspecific control antibody (*dashed line*). A, Lack of measurable Fas expression on the unstimulated lymphocytes; B, significant up-regulation of Fas expression in 72-h PHA/IL-2 activated lymphocytes.

significant amounts of bioactive TGF-β throughout pregnancy (15). Other substances produced by the placenta that can suppress cell-mediated immunity are PGE_2, prostacyclin, and progesterone (25–27). Protection from maternal humoral immunity comes from several mechanisms. The rapid binding and internalization of antibody by Fc receptors on the trophoblast helps remove maternal antibodies which might result in complement fixation or help mediate antibody-dependent cell-mediated cytotoxicity (28). In addition, trophoblast cells express CD59, which is an inhibitor of the complement cascade (29). Taken together, these findings suggest there are multiple mechanisms to prevent maternal immune rejection of the fetus.

Although Fas/FasL probably plays an active role in maternal immunotolerance to the fetus, the absolute necessity of trophoblast FasL expression for maternal immunotolerance of the fetus has not been demonstrated. Mice with nonfunctional mutations of FasL (*gld*) can reproduce. These mice,

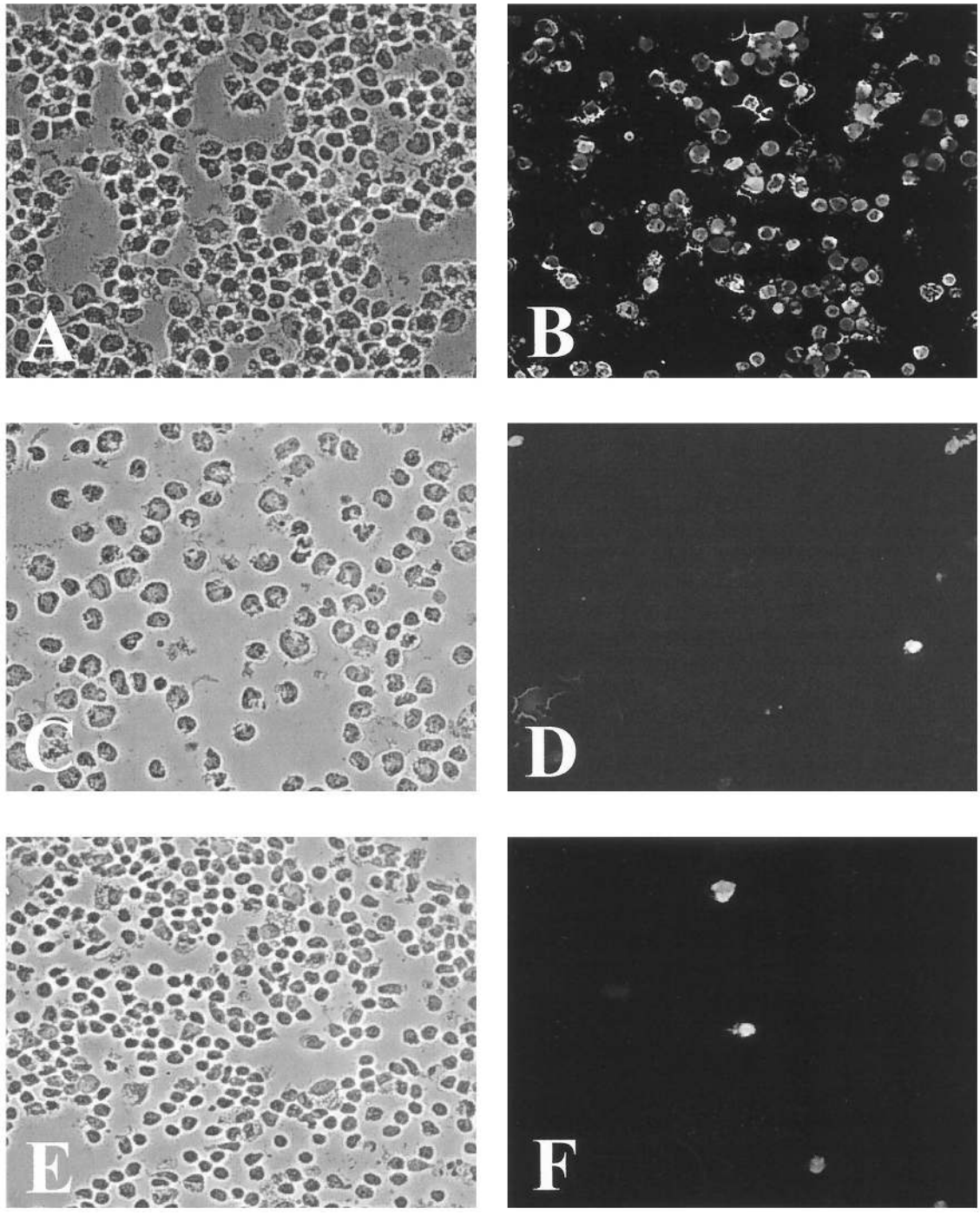

FIG. 5. TUNEL assay for trophoblast-induced apoptosis of activated lymphocytes. Cytospin preparations of activated (+ Fas) or unactivated (− Fas) lymphocytes cocultured with ED_{27} trophoblast (+ FasL) or WS1 fibroblasts (− Fas) were subjected to the TUNEL assay and examined under phase contrast (A, C, and E) and fluorescence microscopy (B, D, and F). A and B are activated lymphocytes (+ Fas) cocultured with ED_{27} trophoblast (+ FasL) and demonstrate numerous apoptotic lymphocytes. C and D are activated lymphocytes (+ Fas) cocultured with WS1 fibroblasts (− FasL). Without the presence of FasL on the WS1 cells, lymphocytes are not induced to undergo apoptosis. E and F are unactivated lymphocytes (− Fas) cocultured with ED_{27} cells (+ FasL). Without the presence of Fas on the lymphocytes, they are unable to respond to the FasL present on the ED_{27} trophoblast and do not undergo apoptosis.

however, lack functional FasL, which is expressed on normal mouse trophoblast and is thought to prevent trafficking of maternal activated lymphocytes to the placenta (30). Consequently, *gld* mice tend to have smaller litters and more fetal resorptions which are associated with placental inflammatory infiltrates. In the absence of a functional Fas/FasL system, other mechanisms for maternal immunotolerance to the fetus are sufficient to prevent absolute pregnancy failure but insufficient to prevent reduced reproductive efficiency. Another function of trophoblast FasL expression could be to

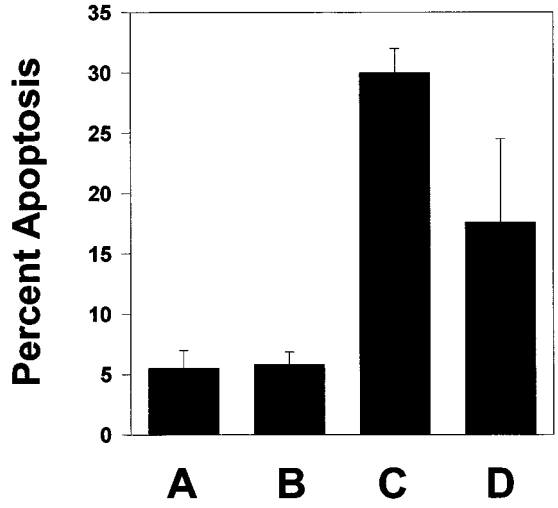

FIG. 6. Comparison of apoptosis in cocultured lymphocytes. To determine the number of lymphocytes that underwent apoptosis in the coculture experiments, photomicrographs were taken under phase contrast (total cells) and corresponding fluorescence views (apoptotic cells), and the cells were counted. The percent of apoptotic cells was then determined. The data represent the mean and SEM of four separate experiments. Both control groups, including activated lymphocytes cocultured on WS1 fibroblasts (A) and unactivated lymphocytes cocultured on ED_{27} trophoblast (B), showed only low levels (6%) of lymphocyte apoptosis. In contrast, activated lymphocytes cocultured on ED_{27} trophoblast (C) had significantly higher levels of apoptosis, compared with the controls (30%, $P < 0.01$, χ-square analysis). The addition of FasL neutralizing antibody (D) at the beginning of the coculture experiments with activated lymphocytes and ED_{27} trophoblast resulted in a 40% decrease in lymphocyte apoptosis, compared with the coculture experiments without the FasL neutralizing antibody ($P < 0.05$, χ-square analysis).

reduce the inflammatory response to vaginal bacteria that gain access to the placental membranes through a partially dilated cervix. Both neutrophils and monocytes constitutively express Fas and undergo apoptosis in the presence of FasL (31). Limiting the inflammatory response to bacteria would be beneficial because chorioamnionitis is associated with increased local production of PGs, which can result in uterine contractions and premature labor and delivery.

In conclusion, our findings, demonstrating the expression of FasL on trophoblast at the maternal-fetal interface and the ability of FasL-expressing trophoblast cells to induce apoptosis in activated lymphocytes, may help to identify a mechanism for maternal immunotolerance to the fetus in human pregnancy.

References

1. **Gill T, Wegmann T.** 1987 Immune recognition, genetic regulation, and the life of the fetus. In: Gill T, Wegmann T, Nisbet-Brown E, eds. Immunoregulation and fetal survival. New York: Oxford University Press; 3–11.
2. **Cross J, Werb Z, Fisher S.** 1994 Implantation and the placenta: key pieces of the development puzzle. Science. 266:1508–1518.
3. **McIntyre JA, Faulk WP, Nichols-Johnson VR, Taylor CG.** 1986 Immunologic testing and immunotherapy in recurrent spontaneous abortion. Obstet Gynecol. 67:169.
4. **Marti JJ, Herrmann U.** 1977 Immunogestosis: a new etiologic concept of essential EPH gestosis with special consideration of the primagravid patient. Am J Obstet Gynecol. 128:489–493.
5. **Nagata S.** 1994 Fas and Fas ligand: a death factor, and its receptor. Adv Immunol. 57:129–144.
6. **Kayagaki N, Kawasaki A, Ebata T, et al.** 1995 Metalloproteinase-mediated release of human Fas ligand. J Exp Med. 182:1777–1783.
7. **Suda T, Okazaki T, Naito Y, et al.** 1995 Expression of the Fas ligand in cells of T-cell lineage. J Immunol. 154:3806–3813.
8. **Bellgrau D, Gold D, Selawry H, et al.** 1995 A role for CD 95 ligand in preventing graft rejection. Nature. 377:630–632.
9. **Griffith T, Brunner T, Fletcher S.** 1995 Fas ligand-induced apoptosis as a mechanism of immune privilege. Science. 270:1189–1192.
10. **Nagata S, Goldstein P.** 1995 The Fas death factor. Science. 267:1449–1456.
11. **Lau H, Yu M, Fontana A, Stoecker C.** 1996 Prevention of islet allograft rejection with engineered myoblasts expressing FasL in mice. Science. 273:109–112.
12. **Bamberger A, Schulte H, Thuneke I, Erdmann I, Bamberger C, Asa S.** 1997 Expression of the apoptosis inducing Fas Ligand in human first, and third trimester placenta and choriocarcinoma cells. J Clin Endocrinol Metab. 82:3173–3175.
13. **Bulmer J, Johnson P.** 1985 Immunohistological characterization of the decidual leukocytic infiltrate related to endometrial gland epithelium in early human pregnancy. Immunology. 55:35–44.
14. **Tabibzadeh S, Kong Q, Babaknia A, May L.** 1995 Progressive rise in the expression of interleukin-6 in human endometrium during menstrual cycle is initiated during the implantation window. Human Reprod. 10:2793–2799.
15. **Kauma S, Matt D, Strom S, Eierman D, Turner T.** 1990 Interleukin-1β (IL-1β), HLA-DRα, and transforming growth factor-β (TGF-β) expression in endometrium, placenta, and placental membranes. Am J Obstet Gynecol. 163:1430–1437.
16. **Halloran P, Cockfield S, Madrenas J.** 1989 The mediators of inflammation (interleukin-1, interferon-α, and tumor necrosis factor) and their relevance to rejection. Transplant Proc. 21:21–30.

16a. **Konoeda Y, Terasaki PI, Wakisada A, Park MS, Mickey MR.** 1986 Public determinants of HLA indicated by pregnancy antibodies. Transplantation. 41:253–259.

17. **Davies M, Brown CM.** 1985 Anti-trophoblast antibody responses during normal human pregnancy. J Reprod Immunol. 7:285–297.
18. **Bulmer J, Johnson P.** 1985 Antigen expression by trophoblast populations in the human placenta and their possible immunobiological relevance. Placenta. 6:127–140.
19. **Kovats S, Librach C, Main E, et al.** 1991 Expression and possible function of the HLA-G α chain in human cytotrophoblasts. In: Chaouat G, Mowbray J, eds. Cellular and molecular biology of the materno-fetal relationship. London: John Libbey Eurotext Ltd.; 212:21–28.
20. **Pazmany L, Mandelboim O, Vales-Gomez M, Davis DM, Reyburn HT, Strominger JL.** 1996 Protection from natural killer cell-mediated lysis by HLA-G expression on target cells. Science. 274:792–795.
21. **Wahl S, Hunt D, Wong H, et al.** 1988 Transforming growth factor-β is a potent immunosuppressive agent that inhibits IL-1 dependent lymphocyte proliferation. J Immunol. 140:3026–3032.
22. **Mule J, Schwartz S, Roberts A, et al.** 1988 Transforming growth factor-β inhibits the *in vitro* generation of lymphokine-activated killer cells and cytotoxic T-cells. Cancer Immunol Immunother. 26:95–100.
23. **Rook A, Rehrl J, Wakefield L, et al.** 1986 Effects of transforming growth factor-β on the functions of natural killer cells; depressed cytolytic activity and blunting of interferon responsiveness. J Immunol. 136:3916–3920.
24. **Tsunawaki S, Sporn M, Ding A, Nathan C.** 1988 Deactivation of macrophages by transforming growth factor-β. Nature. 334:260–262.
25. **Stites D, Bugbee S, Siiteri P.** 1983 Differential actions of progesterone and cortisol on lymphocyte and monocyte interaction during lymphocyte activation-relevance to immunosuppression in pregnancy. J Reprod Immunol. 5:215–228.
26. **Humes J, Bonney R, Pelus L, et al.** 1977 Macrophages synthesize and release prostaglandins in response to inflammatory stimuli. Nature. 269:149–151.
27. **Foegh M.** 1988 Immune regulation by eicosanoids. Transplant Proc. 20:1158–1161.
28. **Lala P, Chatterjee-Hasrouni S, Kearns M, et al.** 1983 Immunobiology of the feto-maternal Interface. Immunol Rev. 75:87–116.
29. **Tedesco F, Narchi G, Radillo O, et al.** 1993 Susceptibility of human trophoblast to killing by human complement and the role of the complement regulatory proteins. J Immunol. 151:1562–1570.
30. **Hunt JS, Vassmer D, Ferguson TA, Miller L.** 1997 Fas ligand is positioned in mouse uterus and placenta to prevent trafficking of activated leukocytes between the mother and conceptus. J Immunol. 158:4122–4128.
31. **Liles WC, Kiener PA, Ledbetter JA, Aruffo A, Klebanoff SJ.** 1996 Differential expression of Fas and Fas ligand on normal human phagocytes: implications for the regulation of apoptosis in neutrophils. J Exp Med. 184:429–440.