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Trophoblast Interaction with Fibrin Matrix

Epithelialization of Perivillous Fibrin Deposits as a Mechanism for Villous Repair in the Human Placenta

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The authors have used morphometric, immunocytochemical, and electron optical techniques to study fibrin deposits associated with villi from 14 normal term placentas, and have examined the response of cultured cellular trophoblast to fibrin matrix in vitro. ***Morphometric analysis of 3477 villous profiles showed that 5.5% of villi examined had fibrin deposition at sites of syncytial denudation and that fibrin deposition was highly associated with villous epithelial denudation, as evidenced by loss of cytokeratin staining. The perivillous fibrin deposits were strongly immunoreactive for the B β chain of fibrin II, consistent with local thrombolytic cleavage of fibrinogen to fibrin. Deposits were frequently surfaced by a discontinuous layer of cytokeratin-positive trophoblastic cells that showed type IV basement membrane collagen immunoreactivity at the interface between trophoblast and fibrin. Ultrastructurally, damage to the syncytial trophoblast was apparent at the edge of some deposits, where syncytial denudation was accompanied by a fibrin coating of residual cellular trophoblast and the trophoblastic basal lamina. Other deposits were surfaced by syncytial trophoblast with underlying cellular trophoblast and a new basal lamina external to the basal lamina of the villous core. Cultured cellular trophoblast grown on a fibrin matrix, but not on uncoated plastic, showed morphologic differentiation into a trophoblast layer like that on term villi. The authors suggest that epithelialization of perivillous fibrin deposits is a form of villous repair and that trophoblast-fibrin interactions can modulate trophoblastic differentiation. (Am J Pathol 1990, 136: 855–865)***

Perivillous deposits of fibrin are a common histologic feature of the human placenta delivered after a complicated pregnancy, but also are observed in term human placentas delivered after normal pregnancies.[1,2] Fibrin deposits at the basal plate frequently enmesh multiple villi, filling the intervillous space and sometimes producing macroscopically visible plaques.[1–4] More localized deposits of fibrin are often observed in the central region of the placenta, between the chorionic and basal plates. These localized deposits frequently show partial or complete epithelialization by trophoblast. We have studied the perivillous fibrin deposits of normal term human placental villi using immunocytochemistry, morphometric analysis, and electron microscopy. We also examined the morphology of cultured human cellular trophoblast grown in the presence or absence of a fibrin matrix. The results of these studies suggest a novel mechanism of villous repair in the human placenta.

Materials and Methods

Placentas

Human placentas from uncomplicated term pregnancies of 38 to 41 weeks' gestation were obtained immediately after labor and vaginal delivery (n = 7) or repeat cesarean section under epidural anesthesia without prior labor (n = 7). All women were normotensive throughout preg-

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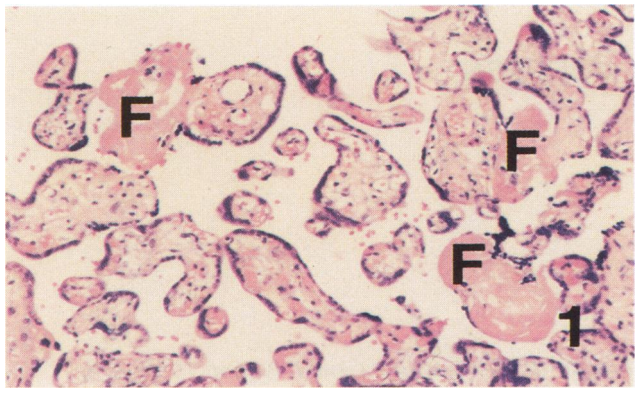
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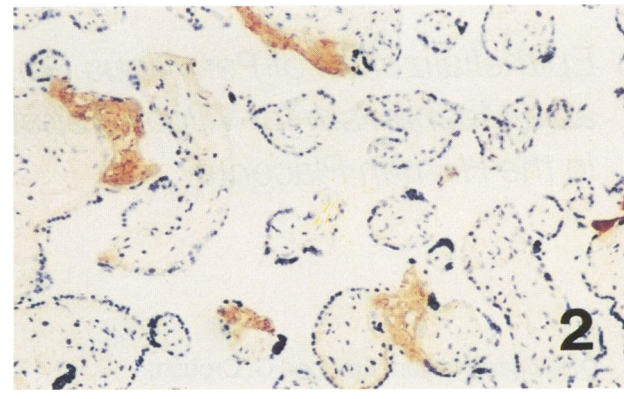
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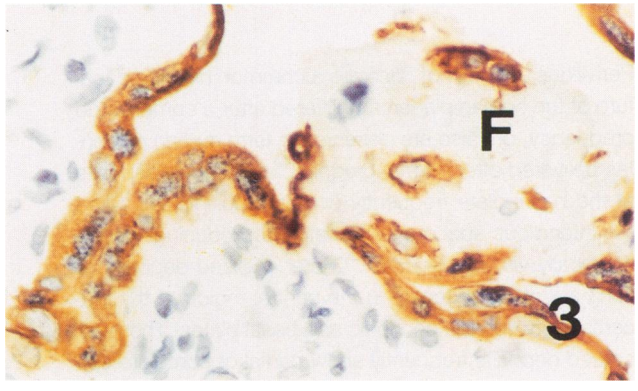
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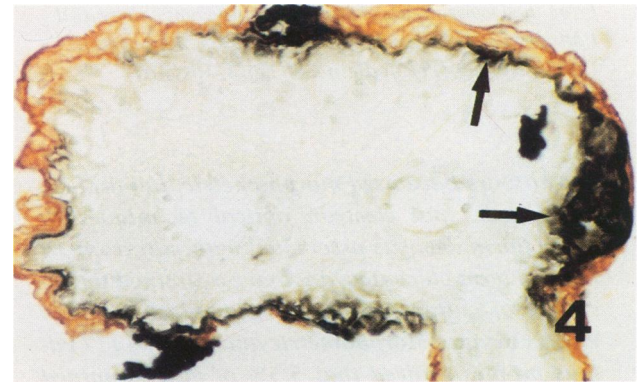
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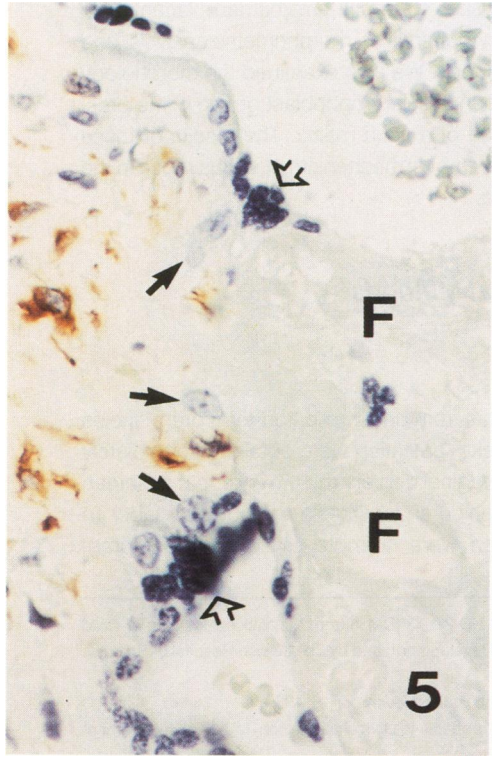
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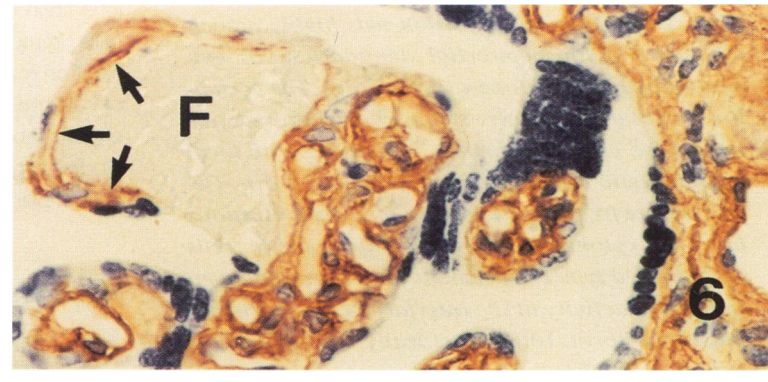
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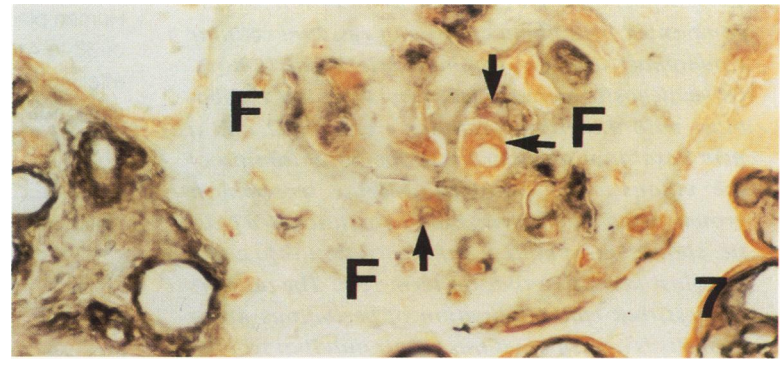
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Figure 1. *Photomicrograph of a cross section of term human placental villi stained with hematoxylin and eosin. Perivillous fibrin deposits (F) are hypocellular, eosinophilic masses attached to the surface of villi, often at sites with a discontinuity in the syncytial trophoblast. ×45.* **Figure 2.** *Photomicrograph of a cross section of term human placental villi immunocytochemically stained to localize the B β chain of fibrin II. Immunoreactivity for this monomer, indicated by the brown reaction product, is localized to the hypocellular perivillous masses. Biotin streptavidin peroxidase primary stain, hematoxylin counterstain. ×45.* **Figure 3.** *Photomicrograph of a cross section of a placental villus stained for cytokeratins. These intermediate filaments are localized to both syncytial and cellular trophoblast. Cells associated with the fibrin deposit (F) are also immunoreactive for the cytokeratins, indicating their trophoblastic origin. Biotin streptavidin peroxidase primary stain, hematoxylin counterstain. ×350.* **Figure 4.** *Photomicrograph of a cross section of a placental villus stained sequentially for the B β chain of the fibrin II monomer (black) and for the cytokeratins (brown). Fibrin is present on the villous surface at sites of discontinuity in the syncytial trophoblast layer. Fibrin was also localized along the trophoblastic basement membrane under the syncytial trophoblast layer in areas adjacent to such discontinuities (arrows). Biotin streptavidin peroxidase with (fibrin II) or without (cytokeratins) nickel chloride. ×270.* **Figure 5.** *Photomicrograph of a cross section of a placental villus immunostained for vimentin intermediate filaments. Clusters of pyknotic nuclei in the syncytial trophoblast (open arrows) are at the edge of a fibrin deposit (F) that is attached to the villous core. Immunoreactivity for vimentin intermediate filaments is localized to villous core cells (brown). Neither cellular trophoblast (closed arrows) nor cells associated with the fibrin deposits contained vimentin immunoreactivity. Biotin streptavidin peroxidase primary stain, hematoxylin counterstain. ×350.* **Figure 6.** *Photomicrograph of a section of placental villi immunostained to localize type IV basement membrane collagen. The basement membranes of the trophoblast layer and of fetal vessels show intense immunoreactivity, with a less intense, although specific, reaction in the villous core connective tissue. Immunoreactivity for type IV collagen (arrows) is also associated with the layer of trophoblast cells surfacing the fibrin deposit (F). Biotin streptavidin peroxidase primary stain, hematoxylin counterstain. ×350.* **Figure 7.** *Photomicrograph of a section of placental villi sequentially stained for type IV collagen (black) and the cytokeratins (brown). The cytokeratin-positive trophoblastic cells (arrows) within a fibrin deposit (F) are also associated with the black reaction product indicative of type IV collagen immunoreactivity. Biotin streptavidin peroxidase with (type IV collagen) or without (cytokeratins) nickel chloride. ×350.*

nancy, were not taking medications, denied smoking or alcohol use, and had no medical illnesses.

Immunocytochemistry

Villous tissue free of visible infarct, calcification, or hematoma was taken from at least four cotyledons sampled midway between the chorionic and basal plates. Four to eight tissue blocks approximately 1 cu cm each were fixed in three changes of 4% (wt/vol) formaldehyde for 2 hours at 24°C before routine embedding in paraffin. The fixative was freshly prepared from paraformaldehyde (Electron Microscopy Sciences, Warrenton, PA), in 0.1 M phosphate buffer at pH 7.2. Four-micron-thick sections of specimens were mounted on slides, deparaffinized in xylene, rehydrated in an ethanol series, and immersed in phosphate-buffered saline (PBS). Sections were covered for 10 minutes at room temperature with 0.75% hydrogen peroxide in PBS (vol/vol) to block endogenous peroxidase activity, rinsed with two changes of PBS, and incubated 20 minutes at room temperature in 1% trypsin (wt/vol; type II from porcine pancreas, Sigma, St. Louis, MO) in PBS to expose antigenic sites, and rinsed with two changes of fresh PBS. Nonspecific immunoglobulin binding sites were blocked by covering sections with 3% (vol/vol) goat serum in PBS for 20 minutes at 24°C. Excess serum was drained and sections were incubated 2 hours at 37°C in a moist chamber with the following mouse monoclonal antibodies to: cytokeratins AE1 and AE3 (prediluted; BioGenex, Dublin, CA), vimentin (prediluted; BioGenex, Dublin, CA), the amino terminal crosslinking domain of pepsin-treated type IV basement membrane collagen from human placenta prepared in our laboratory, or MAb/T2G1s directed to an epitope on the B β chain in fibrin II.[5] The antibodies to type IV collagen and fibrin were diluted in 1% (wt/vol) bovine serum albumin (BSA; Sigma, St. Louis, MO) in PBS at pH 7.6. Negative control sections of human placenta were incubated with mouse nonimmune serum instead of the primary antibody. Sections of human lung provided positive control specimens. All specimens were sequentially incubated with biotinylated goat anti-mouse immunoglobulin (Ig) (Bethesda Research Laboratories, BRL, Gaithersburg, MD) and streptavidin peroxidase (BRL). Color was developed using diaminobenzidine (DAB) as substrate. Specimens were lightly stained with Harris' hematoxylin, dehydrated in ethanol and xylene, and mounted with Permount (Fisher Scientific, St. Louis, MO).

Some specimens were sequentially immunostained for type IV collagen or fibrin followed by cytokeratin. Briefly, antibodies bound in the first step were visualized by reaction with DAB in the presence of nickel chloride to yield a dense black precipitate. After washing and reblocking with serum, sections were incubated with the cytokeratin antibody, and antibodies bound in the second step were visualized using DAB as substrate.

Morphometry

A grid with 25 square fields of 1 sq mm each was positioned over a series of cytokeratin-stained sections from

Table 1. *Morphometric Analysis of Syncytial Denudations and Fibrin Deposits in Term Human Placentas*

Villous profiles	With syncytial denudation	Without syncytial denudation	Total
With fibrin	191	21	212
Without fibrin	67	3198	3265
Total	258	3219	3477

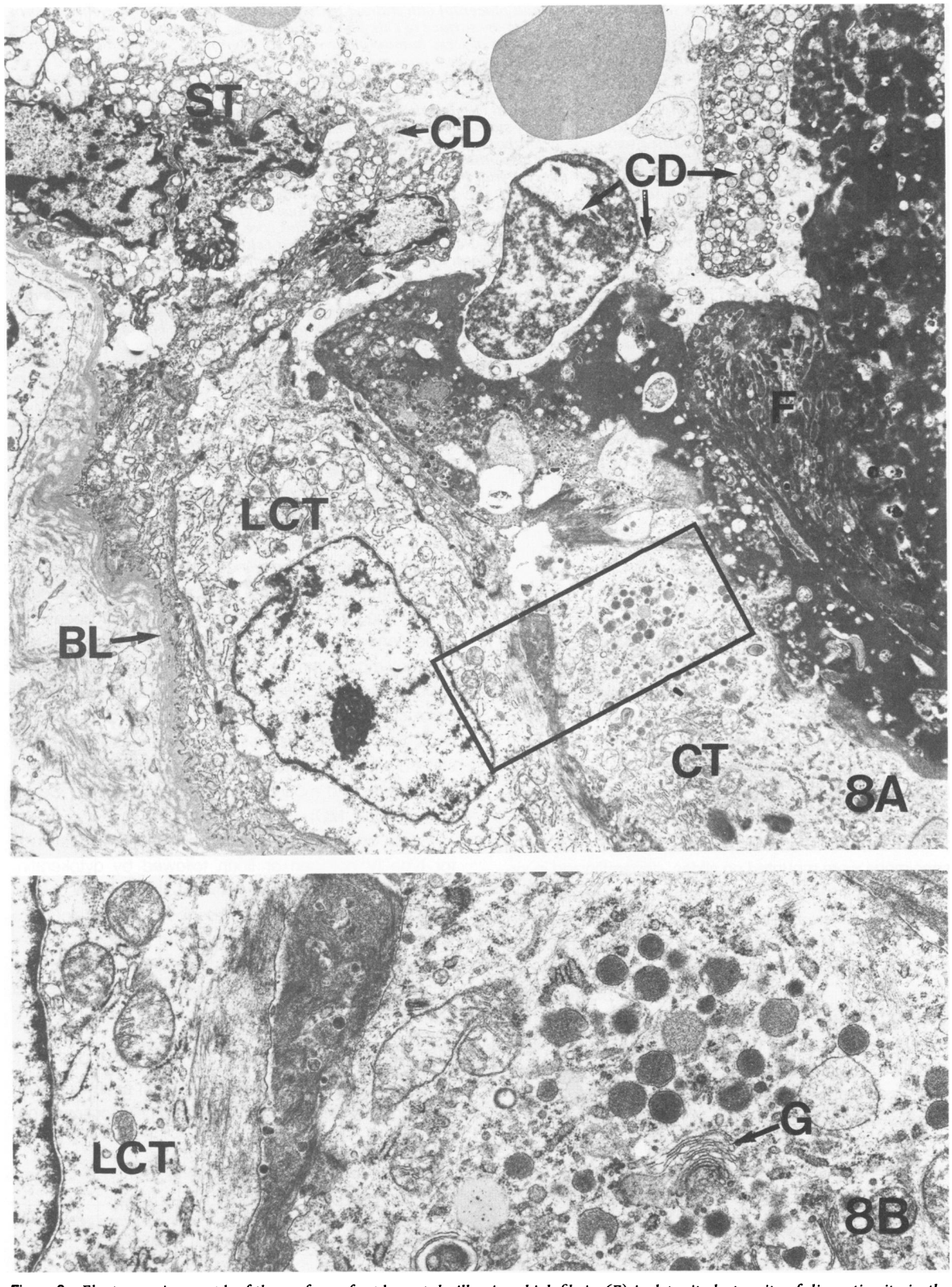

Figure 8. *Electron micrograph of the surface of a placental villus in which fibrin (F) is deposited at a site of discontinuity in the syncytial trophoblast (ST) layer* **A**. *The cytoplasm of the syncytium is vacuolated and cell debris (CD) is present on the apical surface of the syncytial trophoblast and in the intervillous space adjacent to the fibrin deposit. A Langhans cellular trophoblast (LCT) rests on an intact trophoblastic basal lamina (BL). An additional cellular trophoblast (CT) is enmeshed within the fibrin. The area indicated with the box is illustrated at higher magnification in* **B** *to compare the cytology of the typical Langhans cytotrophoblast with the cellular trophoblast enmeshed in the fibrin. The latter has a well-developed Golgi (G) with many electron-dense granules.* **A**: ×4700; **B**: ×9700.

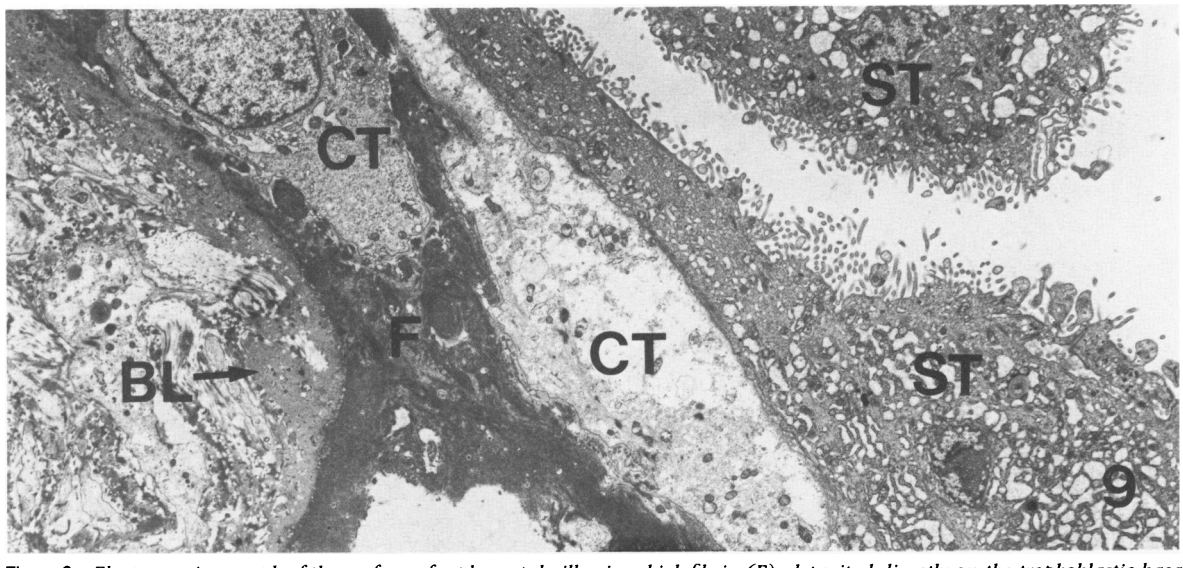

Figure 9. *Electron micrograph of the surface of a placental villus in which fibrin (F), deposited directly on the trophoblastic basal lamina (BL), is surfaced by syncytial trophoblast (ST). (CT, cellular trophoblast) ×3500*

five placentas. Twelve equidistant fields from each section were systematically photographed at a final magnification × 100 in an Olympus BH2 brightfield microscope (Scientific Supply, Chicago, IL) equipped with an Olympus C53AD camera containing Kodacolor Gold 100 film (Eastman Kodak Co., Rochester, NY). Each color print was enlarged to a final magnification of × 370. The total number of villous profiles and the number of villous profiles greater than 100 μ in maximal diameter was recorded. In addition, the number of villous profiles with and without denudation of the syncytial trophoblast and the number of villi with and without perivillous fibrin deposition were recorded.

Culture of Cellular Trophoblast on Fibrin Matrix

Stock solutions of human fibrinogen (2.5 mg/ml, wt/vol; Sigma, St. Louis, MO) and thrombin (5 NIH units/ml, wt/vol; Sigma, St. Louis, MO) were made in Hank's balanced

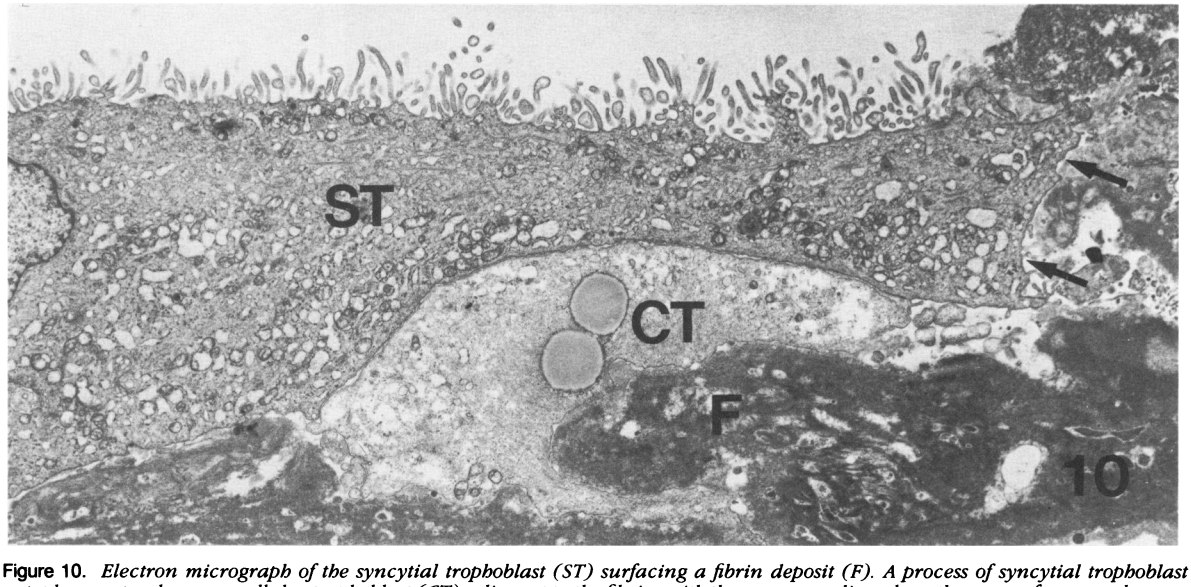

Figure 10. *Electron micrograph of the syncytial trophoblast (ST) surfacing a fibrin deposit (F). A process of syncytial trophoblast cytoplasm extends over a cellular trophoblast (CT) adjacent to the fibrin, with the process ending abruptly as a surface-membrane-bound leading edge (arrows). ×4700*

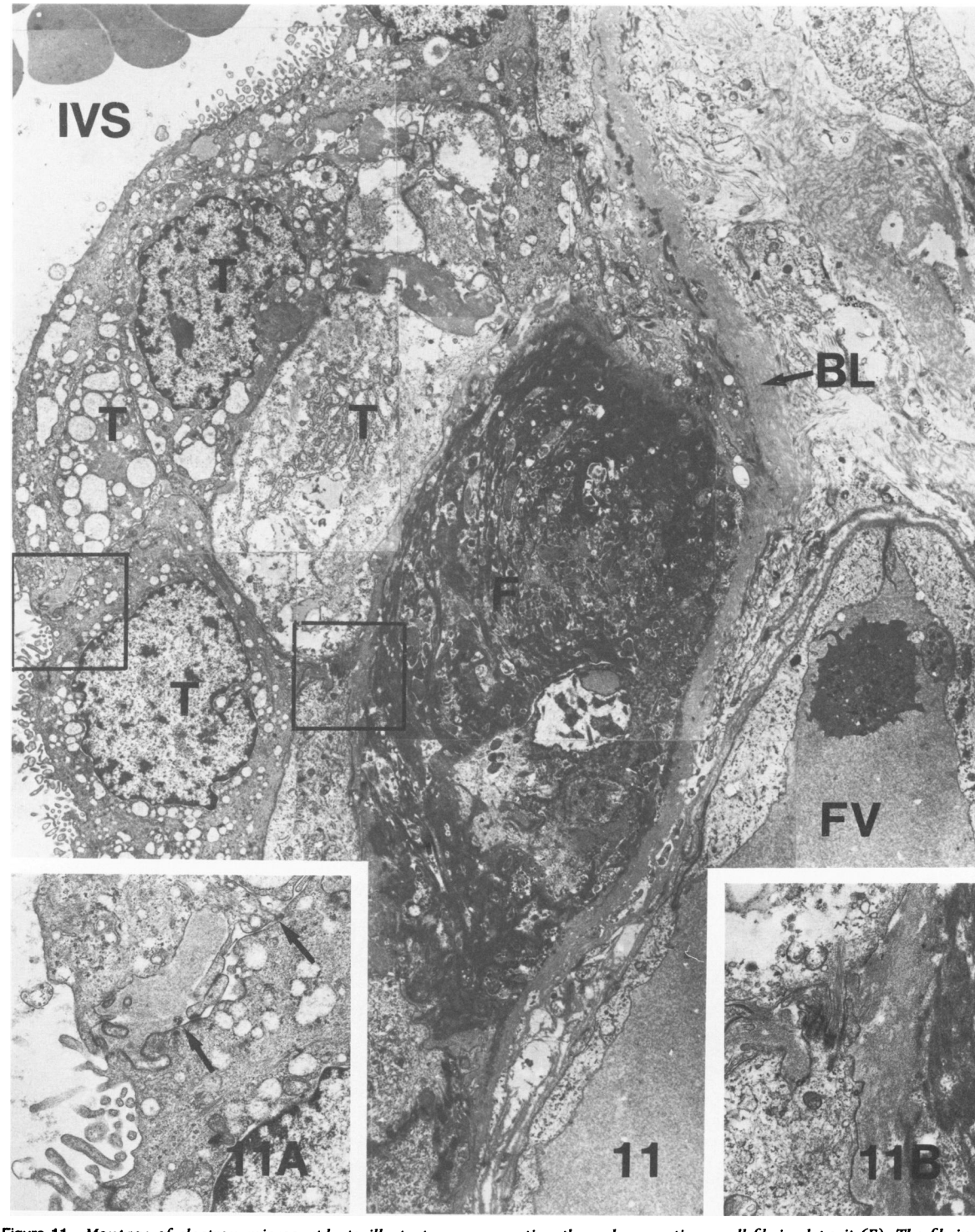

Figure 11. *Montage of electron micrographs to illustrate a cross section through an entire small fibrin deposit (F). The fibrin is deposited on an intact trophoblastic basal lamina (BL), which is adjacent to a fetal vessel (FV) in the villous core. The boxed area on the left is shown at higher magnification in* **A**. *The surfacing trophoblastic cells (T) have lateral cell borders (arrows,* **A**) *and are exposed to the intervillous space (IVS) containing maternal red blood cells. The boxed area on the right shows amorphous material with the electron density of basal lamina* (**B**) *covers the surface of the fibrin, underlying the layer of trophoblastic cells.* ×4600; **A** *and* **B**, ×12,500

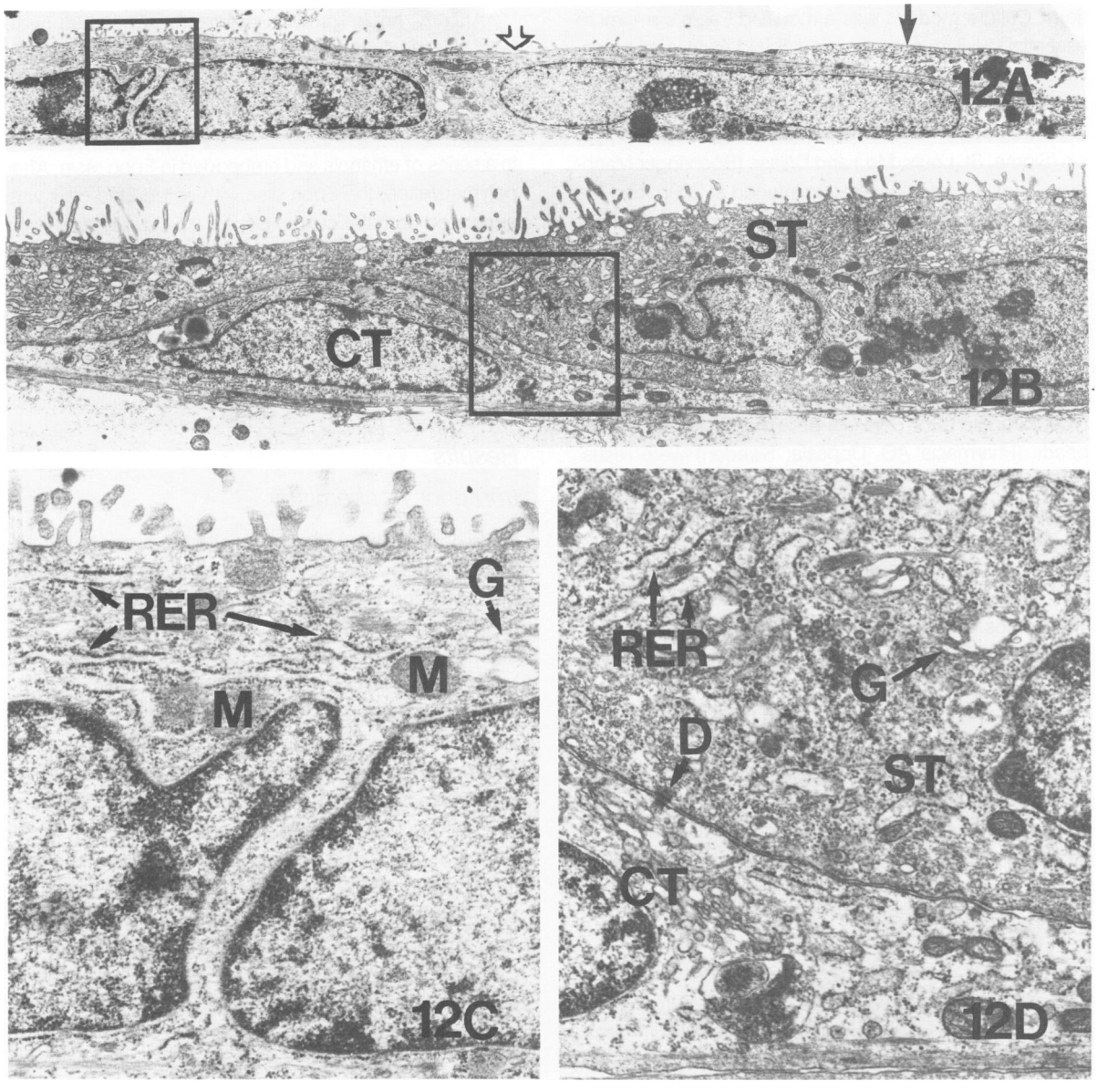

Figure 12. **A:** *Montage of electron micrographs to illustrate the cross-sectional appearance of aggregates of trophoblastic cells cultured 3 days on uncoated plastic. A single cell layer is present, with mononucleated (closed arrow) and multinucleated (open arrow) cells adjacent to each other (×3400).* **B:** *Montage of electron micrographs to illustrate the cross-sectional appearance of aggregates of trophoblastic cells cultured 3 days on a fibrin matrix. Many apical microvilli are apparent on the syncytial trophoblast (ST) layer that overlies a mononucleated cellular trophoblast (CT) (×3400).* **C:** *High-magnification electron micrograph of the boxed area in* **A** *to illustrate the cytology of the trophoblastic cells cultured on uncoated plastic. The cytology of the multinucleated cells on plastic was identical to adjacent mononucleated cells, with profiles of undilated RER dispersed among mitochondria (M) and a poorly developed Golgi complex (G) (×17,190).* **D:** *High-magnification electron micrograph of the boxed area in* **B** *to show the cytology of syncytial trophoblast (ST) and cellular trophoblast (CT) cultured on a fibrin matrix. The syncytial trophoblast contains many profiles of dilated RER and many Golgi complexes (G) within an electron-dense ground cytoplasm. Desmosomes (D) are shared with subjacent cellular trophoblast whose RER and associated mitochondria are located in an electron-lucent ground cytoplasm (×17,190).*

salt solution (HBSS) buffered to pH 7.4 with Hepes and sterilized through a 0.2-μ Millipore filter (Millipore, Bedford, MA). On the day of cell isolation, 675 μl of stock fibrinogen solution and 30 μl of stock thrombin solution were added to 35-mm plastic petri dishes (Corning, Corning, NY) and swirled at room temperature to facilitate fibrin polymerization. The fibrin matrix-coated dishes were stored in a humidified incubator at 37°C and were rinsed with culture medium plus 5% chemically defined-fetal bovine serum (Hyclone, Logan, UT) prior to use.

Cellular trophoblast were isolated from term placentas by the method of Kliman et al[6] with the following modifica-

tions: Culture medium was a modified Eagle's minimum essential medium containing a 1.5-fold excess of essential amino acids, 2.0-fold excess of nonessential amino acids, and a 1.5-fold excess of vitamins (MEMB, Sigma, St. Louis, Mo). The supernatants from digestion with trypsin (Sigma, St. Louis, Mo.) and DNase (Boehringer Mannheim, West Germany) were centrifuged at 200*g*, and the cell suspensions were filtered through four layers of moist cheesecloth and two layers of moist nylon mesh to remove small villous fragments. A continuous gradient was made by centrifuging a 38% solution of Percoll (Pharmacia AG, Uppsala, Sweden) in HBSS at 30,000*g* for 15 minutes in a Beckman model L5-65 ultracentrifuge (Beckman Instruments, Arlington Heights, IL) equipped with a type 35 angle rotor. The cell pellets and density marker beads (Pharmacia AG, Uppsala, Sweden) were resuspended in fresh medium, layered onto separate Percoll gradients, and centrifuged for 20 minutes at 400*g*. The gradient's top zone containing tissue debris was aspirated and discarded and the cell layer at 1.050 to 1.060 g/ml was collected, suspended in MEMB, centrifuged for 10 minutes at 200*g*, and the cell pellets were resuspended in MEMB with 15% (vol/vol) chemically defined fetal bovine serum (FBS; Hyclone, Logan, UT).

Cellular trophoblast were seeded at 10^6 cells per dish in 2 ml MEMB with 15% FBS (vol/vol) onto 35-mm petri dishes with or without a fibrin matrix. Cultures were grown in a humidified 5% CO_2 atmosphere at 37°C in a Lunaire incubator (Lunaire Environmental Inc., Williamsport, PA) and were examined by inverted phase microscopy daily.

Electron Microscopy

Twenty-five 3-cu-mm specimens of villous tissue from each of six placentas were fixed for 1 hour at room temperature in three changes of 3% glutaraldehyde (vol/vol; Electron Microscopy Sciences, Warrenton, PA) in 0.1 M cacodylate buffer at pH 7.2, postfixed at 4°C in 1% osmium tetroxide (wt/vol; Electron Microscopy Sciences, Warrenton, PA) in 0.1 M cacodylate buffer at pH 7.2, dehydrated through a graded series of cold ethanols, and embedded in Epon resin (Electron Microscopy Sciences, Warrenton, PA). One-micron plastic sections from 15 to 20 blocks per placenta were stained with 1% toluidine blue, and villi with associated fibrin were identified. Thin sections with silver-gold interference colors were cut with a Sorvall MT-2 ultramicrotome (Research and Manufacturing Co. Inc., Tuscon, AZ), mounted on formvar-coated copper slot grids (Ernest F. Fullam, Schenectady, NY), stained 15 minutes with 2% (wt/vol) uranyl acetate in 50% ethanol and 3 minutes with Reynolds lead citrate, coated with carbon, and examined in a Phillips 201 electron microscope (Phillips Electronics, Schaumburg, IL) at 60 kV.

After 72 hours' growth, cultures were washed twice with MEMB, fixed 1 hour *in situ* on their growth surface with 3% glutaraldehyde (vol/vol) in 0.1 M cacodylate buffer at pH 7.4, rinsed with buffer, postfixed 1 hour in 1% osmium tetroxide (wt/vol) in the same buffer, dehydrated in a series of ethanols and embedded in Epon resin. After polymerization, the Epon disc with embedded cells was separated from the culture dishes, 0.5-um sections were cut perpendicular to the dish surface, and cells were stained with 1% toluidine blue. Thin sections were prepared and examined as described above.

Photographs of overlapping fields of fibrin deposits and cultured cells were made and prints were arranged as montages for detailed study.

Results

Immunocytochemistry

Fibrin deposits were readily identified as hypocellular, eosinophilic masses in sections of placenta stained with hematoxylin and eosin (H&E) (Figure 1). The presence of fibrin within the deposits was confirmed by immunocytochemical localization using a monoclonal antibody specific for the B β chain in fibrin II (Figure 2). This antibody recognizes an epitope exposed after thrombolytic cleavage of fibrinogen.[5] Placental villi stained for cytokeratin intermediate filaments showed strong immunoreactivity of cellular and syncytial trophoblast, including trophoblastic cells associated with and overlying the fibrin deposits (Figure 3). The fibrin frequently was attached to the villous core at sites showing disruption of the syncytial epithelium on otherwise normal appearing villi (Figures 3 to 5). Sections stained sequentially for fibrin and cytokeratins localized fibrin along the trophoblastic basement membrane and under the syncytial trophoblast in areas adjacent to a discontinuity in the syncytial trophoblast layer (Figure 4). Cellular trophoblasts frequently were present at sites of fibrin attachment to the villous core (Figure 5). Clusters of pyknotic syncytial trophoblastic nuclei also were noted at the edge of some fibrin deposits (Figure 5). Villi stained for placental type IV basement membrane collagen showed intense immunoreactivity of the basement membranes of the trophoblast and the fetal vessels and a less intense staining of the villous stroma (Figure 6). Type IV basement membrane collagen immunoreactivity also was localized adjacent to the trophoblastic cell layer on the free surface of some deposits (Figure 6), and to cytokeratin-positive trophoblastic cells apparently embedded within other fibrin deposits (Figure 7). Villi immunostained for vimentin showed immunoreactivity of fetal endothelium and stromal cells, consistent with the mesenchymal origin of these villous components (Figure 5). However, none of the cells surfacing the perivillous fibrin deposits expressed immu-

noreactivity for vimentin. Vascularization of the deposits by fetal vessels was never observed.

Morphometry

Table 1 summarizes the analysis of perivillous fibrin deposits in term placentas. Fibrin deposition at sites of syncytial denudation occurred on 191 villous profiles, which is 74% (191/258) of all villi with syncytial denudation and 5.5% (191/3477) of all villi examined. Fibrin deposition occurred on an additional 21 villi without syncytial denudation, which is 0.7% (21/3219) of all villi without syncytial denudation and 0.6% (21/3477) of all villi examined. Thus, fibrin deposition was highly associated with villous epithelial denudation, with 90% (191/212) of the villi with fibrin also having syncytial denudations. Ninety-two percent (3198/3477) of villous profiles examined were normal, with an intact syncytial epithelium and no associated fibrin.

Villi $>100\ \mu$ in diameter were more likely to exhibit perivillous fibrin associated with an area of syncytial denudation than were villi $<100\ \mu$ in diameter. Fibrin deposits were present on 55 of the 116 villi $>100\ \mu$ in diameter included in the analysis, while fibrin deposits were associated with syncytial denudation on 136 of the 3361 villi $<100\ \mu$ in diameter.

Electron Microscopy

Ultrastructurally, the deposits consisted of an electron-dense mass of fibrin with associated cell debris (Figure 8A). No platelets were identified in the deposits examined. An intact trophoblastic basal lamina was always present where fibrin was deposited directly on the villous core (Figures 8, 9). The discontinuous layer of cytokeratin-positive cells identified by light microscopy on the surface of some deposits was characteristically a layer of syncytial trophoblast (Figures 9, 10). The syncytial trophoblast at the edge of some fibrin deposits was damaged, with disruption of the syncytium and vesiculation of the rough endoplasmic reticulum (RER; Figure 8A). Fragments of cell debris were adjacent to and mixed with the fibrin deposits (Figure 8A). The syncytium at the edge of other fibrin deposits had an ultrastructure (Figure 9) similar to that of syncytial trophoblast on term villi. This included numerous microvilli, a well-developed RER, and an electron-dense ground cytoplasm. The syncytial trophoblast layer extended over the surface of the fibrin as a tongue of cytoplasm that abruptly ended with a surface membrane-bound leading edge in others (Figure 10).

Cellular trophoblast remained as a discontinuous layer of cells covered by fibrin and resting on the trophoblastic basal lamina (Figures 8, 9). Other cellular trophoblast were enmeshed within the fibrin and not directly located on the trophoblastic basal lamina (Figure 9). The cellular trophoblast resting on the trophoblastic basal lamina had an undifferentiated cytology typical of the Langhans cells of chorionic villi (Figure 8B). Cellular trophoblast enmeshed in the fibrin had cytologic features typical of secretory cells, with a well-developed Golgi complex and multiple electron-dense cytoplasmic granules (Figure 8B). Cellular trophoblast also was present on the surface of the fibrin deposits, usually underlying the syncytial trophoblast exposed to the intervillous space (Figures 9, 10). However, the surface of some fibrin deposits had cellular trophoblast directly exposed to the intervillous space (Figures 11 and 11A). Accumulations of amorphous electron-dense material with the appearance of basal lamina were frequently (Figures 11 and 11B), but not always (Figures 9, 10), present underlying the trophoblastic layer on the fibrin surface. This material was separate from the intact trophoblastic basal lamina at the base of the fibrin deposit.

Cellular Trophoblast Cultured on Fibrin Matrix

Cells isolated from term villi were identified as trophoblast by their staining for cytokeratin-intermediate filaments and their secretion of human chorionic gonadotropin. The cells initially were dispersed uniformly and were rounded during the 3 to 5 hours required for attachment. They subsequently flattened during the next 24 hours, extended numerous cytoplasmic processes after 48 hours in culture, and formed large cell aggregates by 72 hours. The histology of cross sections of the aggregates formed on uncoated plastic consisted of a single cell layer with both mononucleated and multinucleated cells in the aggregates (Figure 12A). In contrast, cell aggregates formed during growth on a fibrin matrix reproduced the histologic pattern typical of the trophoblast layer on term villi, with a syncytial trophoblast layer overlying a discontinuous layer of mononucleated cellular trophoblast (Figure 12B). Both trophoblast components shared the fibrin surface, although no basal lamina was identifiable. The cytology of the multinucleated cells formed on plastic was the same as that of adjacent mononucleated cells, with dispersed profiles of undilated RER and a poorly developed Golgi complex (Figure 12C). However, the mononucleated trophoblast present on the fibrin matrix had a cytology identical to cellular trophoblast on term villi, with multiple free ribosomes, an undilated RER associated with mitochondria, an electron-lucent ground cytoplasm, and numerous

desmosomes shared with the overlying syncytial trophoblast (Figure 12D). Cytologic differentiation in the syncytial trophoblast also was apparent (Figures 12B and 12D), with numerous microvilli and coated pits on the apical surface, an extensive RER, multiple Golgi profiles, and an electron-dense ground cytoplasm, all features strikingly similar to syncytial trophoblast of term villi.

Discussion

This study demonstrates an association between perivillous fibrin deposition and denudation of the syncytial trophoblast layer of otherwise normal-appearing placental villi. We believe there is a cause and effect relationship in this association, although we recognize our morphometric analysis does not establish causality. We favor the hypothesis that damage to the syncytial trophoblast results in exposure of the trophoblastic basement membrane, localized activation of coagulation factors, thrombolytic cleavage of fibrinogen, and fibrin deposition. This response is observed in other situations when basement membrane components are exposed to fibrin.[7] The perivillous fibrin deposits provide a substratum or matrix that is subsequently epithelialized, predominantly by newly formed syncytial trophoblast. The cytologic changes observed in cells cultured on fibrin indicate that a fibrin matrix can modulate the morphologic differentiation of human trophoblast toward a syncytial trophoblast phenotype. We suggest that epithelialization of fibrin deposits with differentiation to form new syncytial trophoblast is a form of villous repair.

At least three patterns of perivillous fibrin deposition have been described in the human placenta:[8] layering of fibrin near the chorionic and basal plates and around stem villi (eg, massive perivillous fibrin deposition); agglutination by fibrin, a pathologic process often accompanying chronic villous inflammation; and fibrinoid necrosis of villi. The latter lesion is identified as periodic-acid-Schiff–positive material underlying syncytial trophoblast and external to the basement membrane.[9] The deposits containing the B β fibrin II monomer in our study are histologically similar in distribution and staining properties to the deposits described as fibrinoid necrosis. In addition, the 5.5% incidence of these deposits in term placentas from our study is similar to the 3% incidence reported for fibrinoid necrosis.[9] We prefer the phrase "perivillous fibrin" because it unambiguously identifies fibrin as a component of these deposits and implicates abnormalities of the villous surface in their pathogenesis. We suggest that fibrinogen from the maternal blood is cleaved to fibrin and deposited on the trophoblastic basement membrane and on residual cellular trophoblast at sites where the continuity of the syncytial trophoblast is lost.

The syncytial trophoblast is a unique nonendothelial epithelium that lines a vascular space filled with blood. The suggestion that the blood protein fibrin deposits at sites of syncytial denudation was first offered by Tighe and Curran[10] and later by Enders.[11] The syncytial trophoblast with a membrane-bound leading edge extending over the fibrin deposit, the new basement membrane underlying the trophoblast cells surfacing the fibrin, and the polarized ultrastructural features of trophoblast cultured on fibrin matrix all indicate that trophoblast actively responds to fibrin.

Conditions suggested to contribute to perivillous fibrin deposition include obliterative endarteritis of fetal vessels with syncytial degeneration, maternal platelet thrombi transformed into fibrin clots, and stasis of maternal blood in the intervillous space, as reviewed.[2,12] In our studies, we found no evidence of fetal vascular thrombosis or platelet thrombi. Emboli of trophoblastic origin are well documented to enter the systemic circulation during normal pregnancy,[13] with estimates of 150,000 trophoblastic fragments entering the systemic circulation each day.[14] Mechanical effects of blood flow in the intervillous space, or stasis of maternal blood with local clotting may injure the trophoblastic layer, predisposing to syncytial denudation and release of trophoblastic emboli.

Fibrin deposition is a feature of ulcerations with tissue damage in many organs. Fibroblast infiltration with granulation tissue formation typically accompanies healing of such wounds. With ulcerations, the integrity of the basement membrane is disrupted, allowing connective tissue cells to migrate into the fibrin clot. The persistence of an intact trophoblastic basal lamina at sites of perivillous fibrin deposition probably accounts for the absence of vimentin-positive fibroblasts or fetal vessels in the fibrin deposits. In addition, the retention of the trophoblastic basement membrane during the process of syncytial denudation and repair may protect the fetus by preventing exposure to the maternal circulation of villous stromal cells known to express fetal class I and II major histocompatibility complex (MHC) antigens.

The phenotypic modulation of trophoblast by the fibrin matrix is analogous to the responses of other epithelia cultured on extracellular matrix components.[15] Further studies will determine if perivillous fibrin formation influences the development of pathologic pregnancies, and if fibrin can modulate the functional and biosynthetic properties of human trophoblast.

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