Cultured Human Amniotic Fluid Cells Characterized with Antibodies against Intermediate Filaments in Indirect Immunofluorescence Microscopy

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ABSTRACT Cells cultured from second trimester human amniotic fluid were characterized in indirect immunofluorescence (IIF) microscopy using specific antibodies against the subunit proteins of different types of cytoskeletal intermediate filaments.

Most of the amniotic fluid cell cultures contained only epithelial cells as indicated by the positive keratin-fluorescence in IIF. Five distinct types of keratin-positive cells could be characterized. A dominating cell type (E-1) in most cultures were rapidly proliferating epithelial cells, previously called amniotic fluid cells (AF-cells). These cells showed a fibrillar cytoplasmic fluorescence both with keratin antibodies and with antibodies against vimentin, the fibroblast type of intermediate filament protein. E-1 cells did not show the typical cell-to-cell arrangement of keratin fibrils between the adjacent cells, a characteristic previously found in most cultured epithelial cells. Most of the cultures also contained large epitheloid cells (E-2), showing a fine fibrillar cytoplasmic organization of both keratin- and vimentin filaments, clearly different from that seen in E-1 cells. Several cultures contained two additional epithelial cells both showing the typical cell-to-cell arrangement of keratin fibrils (E-3 and E-4). These two cell types could be distinguished because of their distinct difference in size. E-4 cells typically grew as small cell islands among other epitheloid cells. Amniotic fluid cell cultures occasionally contained also large multinucleated cells (E-5), which appeared to contain large amount of fibrillar keratin.

Fibroblastic cells, identified by their decoration only with antibodies against vimentin, were rarely found in amniotic fluid cell cultures. Interestingly, in such cultures some cells with a fibroblastoid appearance were identified as epithelial cells on the basis of the positive keratin-fluorescence.

The results show the suitability of IIF with cytoskeletal antibodies in characterization of heterogenous cell populations and indicate that normal amniotic fluid cell cultures mostly contain epithelial cells.

INTRODUCTION

Cultured cells derived from second trimester amniotic fluid are widely utilized in prenatal diagnosis of chromosomal and inherited metabolic disorders (cf. 1–3). Although much effort has been devoted to a morphologic characterization of the cells found in amniotic fluid cell cultures (2, 3, cf. 4–7), only little is known of their site of origin or of their state of differentation.

Based on clonal characteristics and cellular morphology Hoehn et al. (5) could divide the amniotic fluid cells into three main types: E (epithelial), F (fibroblastoid) and AF (amniotic fluid)[1] cells. The presence of fibroblasts in AF cell cultures has been the most conflicting problem. It has been claimed that true fibroblasts are only occasionally found in AF cell cultures (4, 5). On the other hand, as already noted by Hoehn et al. (5), the AF-type of cells and fibroblasts may be difficult to distinguish using morphological criteria only.

Attempts have also been made to characterize cultured AF cells on the basis of their production of extracellular matrix proteins, detectable both in the

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[1] *Abbreviations used in this paper*: AF, amniotic fluid; FITC, fluorescent isothiocyanate; IIF, indirect immunofluorescence; TRITC, tetramethyl rhodamine isothiocyanate.

culture fluid and in the cell layers (7–13). These results have also provided evidence for the presence of both epitheloid and fibroblastoid cells in the AF.

We have previously shown in a case of anencephaly, the presence of glial cells in AF using antibodies against glial specific cytoskeletal intermediate filaments (14). This prompted us to characterize the nature of the cells detectable in normal AF cell cultures using specific antibodies against different types of intermediate filaments in indirect immunofluorescence (IIF).

METHODS

AF cell cultures. AF cell cultures were derived from AF samples taken for fetal karyotyping at the 15th or 16th wk of gestation, mainly due to advanced maternal age. The experiments of the present study were carried out with 90 AF samples, revealing a normal karyotype in the diagnostic study. All amniotic fluid samples had normal α-fetoprotein concentration.

Both primary and secondary cultures were studied. The primary cultures were initiated from a small aliquot (2 ml) of AF by inoculating the centrifuged cells on small glass coverslips in tissue culture dishes. The secondary cultures were obtained by trypsinization of confluent primary culture flasks—after completion of the chromosome studies. Ham F10 growth medium, supplemented with 30% fetal calf serum (Microbiological Associates, Walkersville, Md.) and antibiotics were used in all cell cultures of AF.

Cultured human embryonal fibroblasts were obtained from a local source and cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. PTK_2 cells, a potaroo kidney cell line, were obtained from the American Type Culture Collection (Rockville, Md.) and were cultured in minimal essential medium (MEM) with 10% fetal calf serum and antibiotics. Human amnion epithelial cells were isolated as described by Valle and Penttinen (15) and were cultured as PTK_2 cells.

Indirect immunofluorescence microscopy (IIF). For IIF the cells cultured on small glass coverslips were fixed in −20°C methanol for 30 min, washed thoroughly in phosphate-buffered saline (PBS, pH 7.4) and processed for IIF staining.

Antibodies used in IIF microscopy

(a) Rabbit antibodies against the subunit protein of fibroblast intermediate filaments (anti-vimentin). These antibodies were raised in rabbits against the 58,000-mol wt subunit protein of intermediate filaments (cf. 16), isolated by preparative gel electrophoresis from detergent-resistant cytoskeletons of cultured human fibroblasts as described elsewhere in detail (Fig. 1, lanes 1, 2; 17, 18). The antibodies were immunologically purified using a vimentin-Sepharose CL4B absorbent and they gave a single line of reaction on electrophoretically separated polypeptides from cultured human fibroblasts using the immunoblotting technique of Towbin et al. (19, Fig. 1, lane 3).

(b) Rabbit and sheep antibodies against purified human keratin polypeptides (anti-keratin). Human keratin polypeptides were isolated from human plantar callus using the method of Sun and Green (20). Four to five major polypeptides could be resolved in gel electrophoresis of the purified polypeptide preparations (Fig. 1, lane 4). Antibodies were raised against this polypeptide preparation both in rabbits and in sheep and were further purified immunologically using a keratin-Sepharose-CL4B absorbent as described elsewhere in detail (18, 21). The purified anti-keratin antibodies decorated only cultured epithelial cells in IIF (cf. 18). Using the immunoblotting technique (19), the keratin antibodies were shown to react with cytokeratins from cultured amnion epithelial cells (Fig. 1, lanes 5, 6).

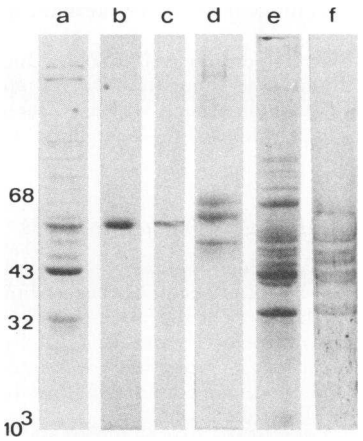

FIGURE 1 Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate of cytoskeletons from cultured human fibroblasts (lane 1). Subunit protein of intermediate filaments from cultured human fibroblasts isolated by preparative gel electrophoretic technique (lane b). Using the rabbit anti-vimentin antibodies a single line of reaction is seen on fibroblast polypeptides transferred to a nitrocellulose sheet (lane c) comigrating with the purified vimentin-polypeptide (cf. lane b). Purified keratin polypeptides from human plantar callus (lane d). Polypeptides of human amnion epithelial cells transferred to a nitrocellulose sheet (lane e). Immunoblotting of human amnion epithelial cells using the purified keratin antibodies (lane f).

(c) Rabbit antibodies against the 55,000 polypeptide from chicken gizzard (anti-desmin). The muscle type of intermediate filament protein (cf. 22) was isolated from chicken gizzard as described earlier (23). These antibodies were also immunologically purified with a chicken gizzard 55,000-polypeptide immunoabsorbent (23). The antibodies were kindly provided by R. A. Badley (Unilever Research, Sharnbrook, England).

(d) Rabbit antibodies against the subunit of glial intermediate filaments (anti-GFA). Glial fibrillary acidic protein was isolated from human spinal cord and antibodies were raised in rabbits and affinity purified as described (24). The antibodies were kindly provided by D. Dahl (Harvard University, Boston, Mass.).

For IIF microscopy the cells were first reacted with the cytoskeletal antibody (anti-keratin, anti-desmin, anti-vimentin or anti-GFA) at a protein concentration of ca 100 μg/ml for 30 min, washed thoroughly in PBS and then reacted with the second antibody, either fluorescein isothiocyanate (FITC)-coupled goat anti-rabbit IgG (Cappel Laboratories, Cochraneville, Pa.) or, in double fluorescence assay, with tetramethyl rhodamine isothiocyanate (TRITC)-coupled swine anti-rabbit IgG (Dako Laboratories, Copenhagen, Denmark). In double immunofluorescence experiments with keratin antibodies the cells were first reacted with rabbit anti-vimentin antibodies, followed by TRITC-swine anti-rabbit IgG and with normal rabbit serum. Thereafter the coverslips were reacted with sheep anti-keratin antibodies followed by FITC-rabbit anti-sheep IgG (Cappel Laboratories). After washing, the speci-

mens were embedded in sodium barbital-glycerol buffer, pH 8.4.

A Zeiss Universal microscope (Carl Zeiss, Inc., New York) equipped with an epi-illuminator III RS and filters for FITC- and TRITC-fluorescence was used for photography. For phase-contrast microscopy a Neofluar phase-contrast optic was used.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis in the presence of SDS was carried out in the presence of SDS according to Laemmli (25) using 8% slab gels. For the immunoblotting technique the method of Towbin et al. (19) was used; briefly, the electrophoretically separated polypeptides were transferred on nitrocellulose sheets using a commercial destainer apparatus (Pharmacia, Inc., Uppsala, Sweden).

For immunostaining, the nitrocellulose sheets were first reacted with the rabbit anti-cytoskeleton antibodies, followed by swine anti-rabbit immunoglobulins (Dako) and then with rabbit antiperoxidase-peroxidase complex (Dako). The enzyme reaction was developed as described by Towbin et al. (19).

RESULTS

All AF cell cultures ($n = 90$) of the present study showed several types of cells in IIF. Cells of epithelial origin were overwhelmingly dominating, while fibroblastic cells were only occasionally detected. Five distinct classes of epithelial cells could be characterized and they have been designed here as E-1 to E-5. The epithelial nature of the cells was judged on their binding ability for keratin-specific antiserum as described for a variety of epithelial cells both in vivo and in vitro (18, 26–31, cf. 32). Both in cultured PTK_2 cells and human amnion epithelial cells a fine fibrillar cytoplasmic keratin-specific fluorescence was seen in IIF (Fig. 2a, b). Especially in human amnion epithelial cell cultures the cells typically showed an abundant cell-to-cell arrangement between the adjacent cells (Fig. 2b). Fibroblastoid cells were identified by their staining with vimentin antibodies but not with keratin antibodies (16, 18, 32).

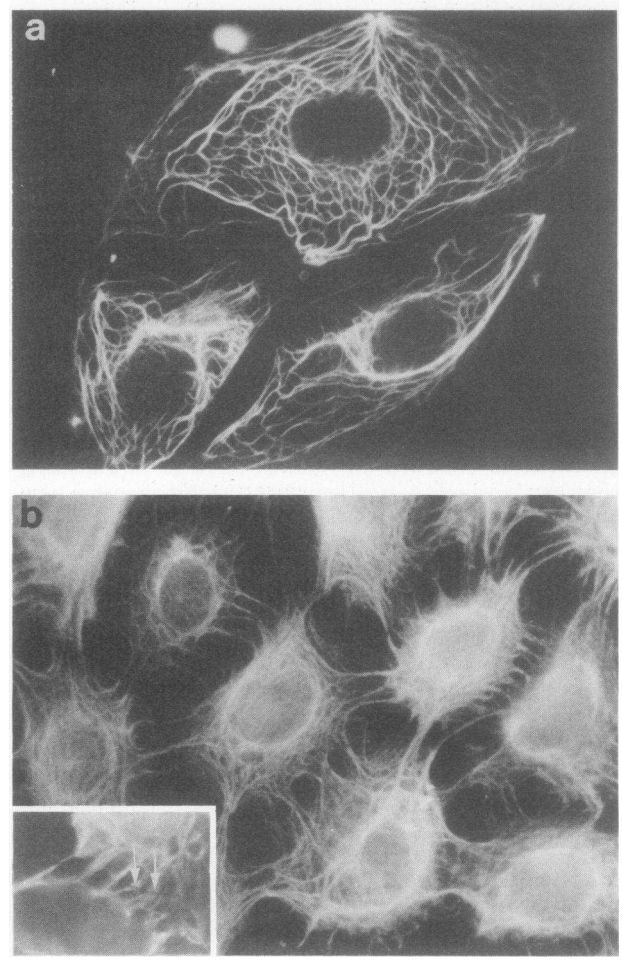

FIGURE 2 Potaroo kidney epithelial cells (a, PtK2) and human amnion epithelial cells (b) stained for indirect immunofluorescence technique with rabbit anti-keratin antibodies. A typical cytoplasmic staining of coiling bright bundles is seen in PtK2 cells (a) whereas the human amnion epithelial cells show a distinct cell-to-cell organization of keratin fibers (b). At higher magnification typical gaps (arrows) of fluorescence are seen between the keratin fibers of adjacent cells (b, the insert).

Epithelial cell types. E-1; the most common cell type present in all cell cultures of the present study was a middle-sized pleomorphic cell that often was quite fibroblastoid in morphology showing a bright fibrillar keratin-positive cytoplasmic staining in IIF (Fig. 3a, b). These cells did not show any cell-to-cell arrangements of keratin fibers, a characteristic previously reported typical for most cultured epithelial cells (18, 26–28, 30, cf. Fig. 2b). In double IIF a bright fibrillar fluorescence was seen in E-1 cells also with anti-vimentin antibodies (results not shown). E-1 cells have previously been classified as AF cells by several workers (cf. 5).

E-2; another distinct cell type of epithelial nature, quite regularly found in AF cell cultures, were the large flattened cells showing a fine, fibrillar organization of both keratin and vimentin filaments (Fig. 4a, b). Similarly to E-1 cells, the keratin fibers in E-2 cells lacked the typical cell-to-cell organization. Slow growth and low proliferation capacity were characteristics of E-2 cells. E-2 cells apparently correspond to E cells by the classification of Hoehn et al. (5).

E-3; these cells were indistinguishable by size and morphology from E-1 cells, but could easily be classified on the basis of their keratin organization. The E-3 cells showed keratin fibers extending from one cell to another (Fig. 4c, d). A typical gap in the fibrillar keratin staining could be seen between the cells (Fig. 4c, d the insert), earlier shown to correspond to the location of the desmosomes in epithelial cells (27).

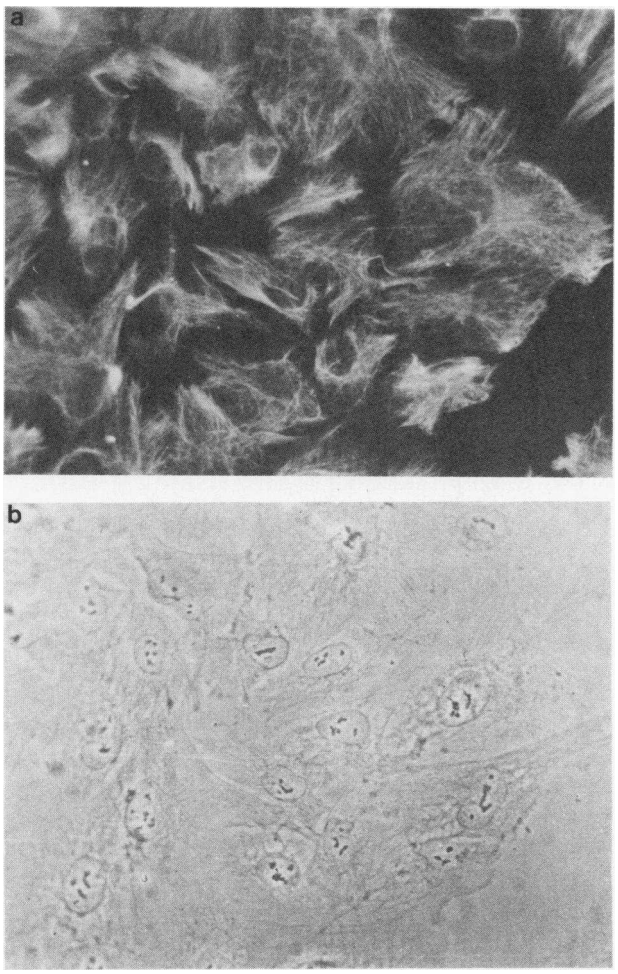

FIGURE 3 Type I epithelial cells from AF stained for indirect immunofluorescence microscopy with rabbit anti-keratin antibodies (a, b). Type I epithelial cells show a pleomorphic appearance with scattered cytoplasmic keratin filaments. Note the absence of any cell-to-cell arrangement of the keratin fibers. a, b ×340.

E-3 cells also showed vimentin fibrils as a diffuse cytoplasmic location.

E-4; small rapidly growing epithelial cells in dense colonies could occasionally be found in AF cell cultures (Fig. 4e, f). These cells could be differentiated from other cell types already by their typical growth pattern and morphology. They have been earlier classified as AF II cells (5). The keratin fibers in E-4 cells also had the cell-to-cell organization with desmosome gaps (Fig. 4e, f). E-4 cells were present only in a small number of cell cultures.

E-5; a small number of large multinucleated cells were found dispersed among other cell types in most of the cultures. These cells exhibited bright bundles of keratin fibers in addition to the vimentin-specific staining (Fig. 4g, h).

Fibroblastic cells in amniotic fluid cell cultures. Fibroblastoid cells (F-cells, 5), identified by their positive vimentin but negative keratin-staining (Fig. 5a, b), were found only in a small number of cultures. In the present study only three amniotic fluid cultures had F-cells as the dominating cell type. Interestingly, even such cultures also showed keratin-positive E-1 and E-3 cells morphologically indistinguishable from the surrounding fibroblasts (Fig. 5 c, d).

Several cell cultures were assayed also for antibodies against glial fibrillary acidic protein (23) or for antibodies against desmin, the muscle type intermediate filament protein (cf. 21), but no positive cells were found in any of the amniotic cell cultures. On the other hand, the cellular content of the AF of the present study showed extensive variation regarding both the number of cells and the proportions of the various cell types seen in the cultures. The scope of the work did not allow a quantitative evaluation of the relative abundance of the different cell types but approximate quantitation is given in Table I.

DISCUSSION

We have shown in the present study that the majority of the cells cultured from second trimester AF are epithelial cells, whereas fibroblastic cells are only occasionally found.

The presence of both epithelial and fibroblastic cells in AF cell cultures has been suggested earlier on the basis of several criteria (4, 5). Results with histidase assays on AF cell cultures demonstrated two types of cells, the epithelial cells being histidase positive while no enzyme was found in fibroblasts (4, 33). Priest and his collaborators (7–9) and Crouch et al. (10–13) were able to distinguish fibroblasts among amniotic fluid cell cultures based on the production of type I and type III collagen and fibronectin (8, 10, 11), similarly to the cultured human fibroblast cell lines (34). Epithelial cell clones in AF cell cultures, on the other hand, secreted type IV collagen (7, 8, 11–13), found in various basement membranes (cf. 34) and also a non-collagenous basement membrane protein (9).

A number of recent reports have shown that different types of cells, both in culture and *in situ* (16, 18, 22, 27–31) can be distinguished based on the type of intermediate filament protein found in their cytoskeleton. Cultured fibroblasts (16, 18, 27), muscle cells (18, 22), epithelial cells (18, 27–31), glial cells (24) and neuronal cells (18, 32–35) contain intermediate filaments, which are both immunologically and biochemically distinguishable. Cultured cells also maintain their specific type of intermediate filament protein, characteristic for cells in tissues and in primary cultures, although they appear to acquire expression of vimentin filaments during adaptation into culture conditions (16, 18, 36).

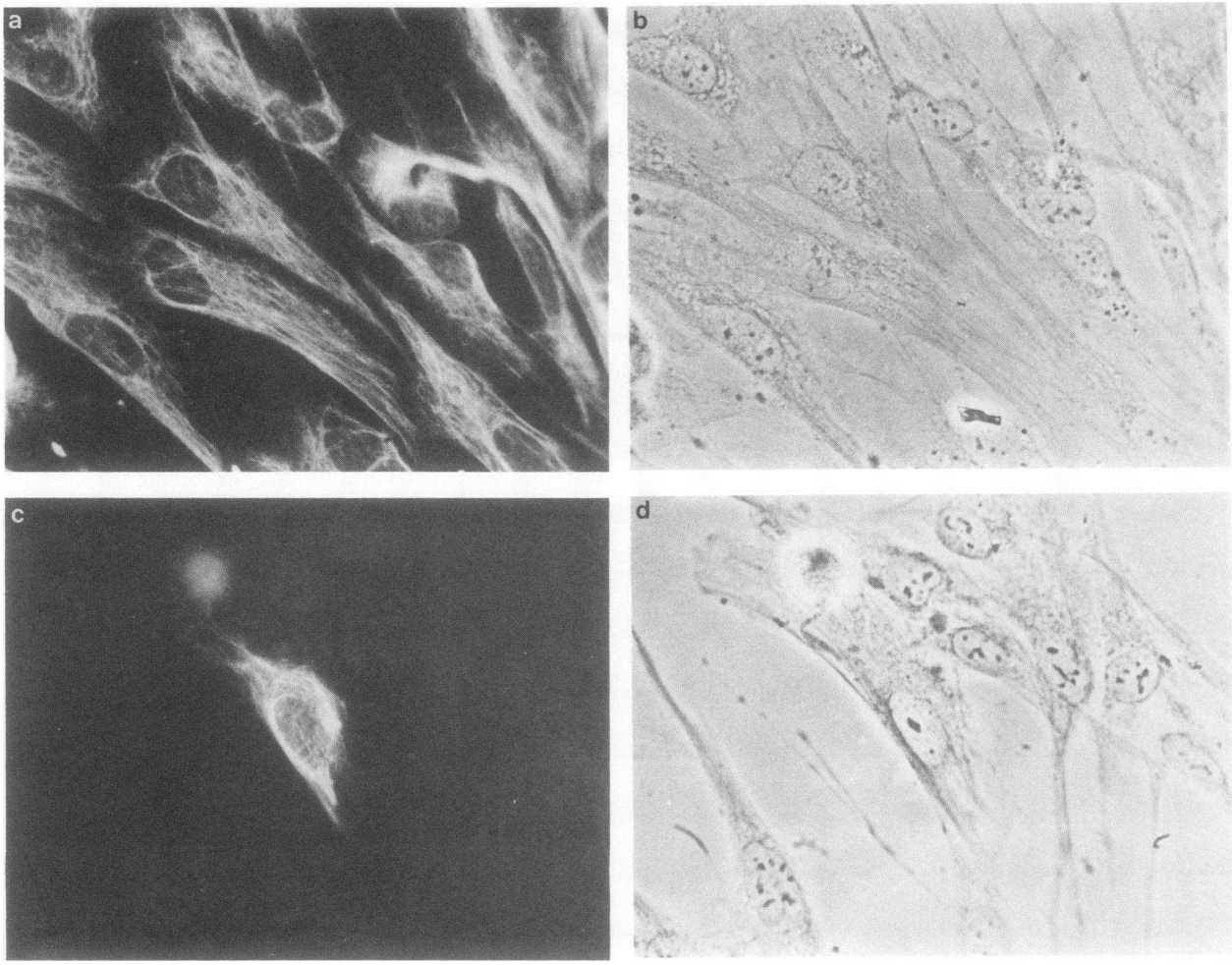

FIGURE 4 Type II (a, b), III (c, d), IV (e, f), and V (g, h) epithelial cells from normal human amniotic fluid stained for keratin in IIF microscopy. Type II epithelial cells consist of large flattened cells with a fine fibrillar cytoplasmic organization of both keratin (a) and vimentin fibers. Type III and IV epithelial cells are distinctly different in size but typically show the cell-to-cell organization to keratin fibers between the adjacent cells (c, e). At higher magnification typical gaps of fluorescence are seen in the keratin fibers between the adjacent cells (c, arrowheads in the insert). Type V epithelial cells (g, h) consist of huge multinucleated cells with thick cytoplasmic keratin fibers. a–f ×340; g, h ×200.

Five distinct epithelial cell types could be characterized in the present study in AF cell cultures. The most common epitheloid cells (E-1 and E-2), closely correspond to the AF and E cells, respectively, in the classification by Hoehn et al. (5). The E-1 is the characteristic cell type in the diagnostic AF cell cultures and the fetal karyotyping is mainly done on mitoses of this cell type. Biochemical analyses of the cultured AF cells also rest to a large extent on E-1 cells. E-3 and E-4 cell types, characterized by the typical arrangement of keratin filaments from cell-to-cell between the adjacent cells (27), not present in E-1 or E-2 cells, were also quite often seen in AF cell cultures. The large multinucleated cells (E-5) containing large amount of fibrillar keratin were recently suggested to rise by fusion of E-1 cells (37). It is clear that such a

FIGURE 5 Fibroblastic cells, characterized by their typical fibrillar cytoplasmic staining only with vimentin antibodies in IIF microscopy (a, b) were only occasionally seen in normal human AF cell cultures. Even among such cells, keratin-positive epithelial cells were consistently encountered indistinguishable in morphology from the surrounding fibroblastic cells (c, d). a–d ×340.

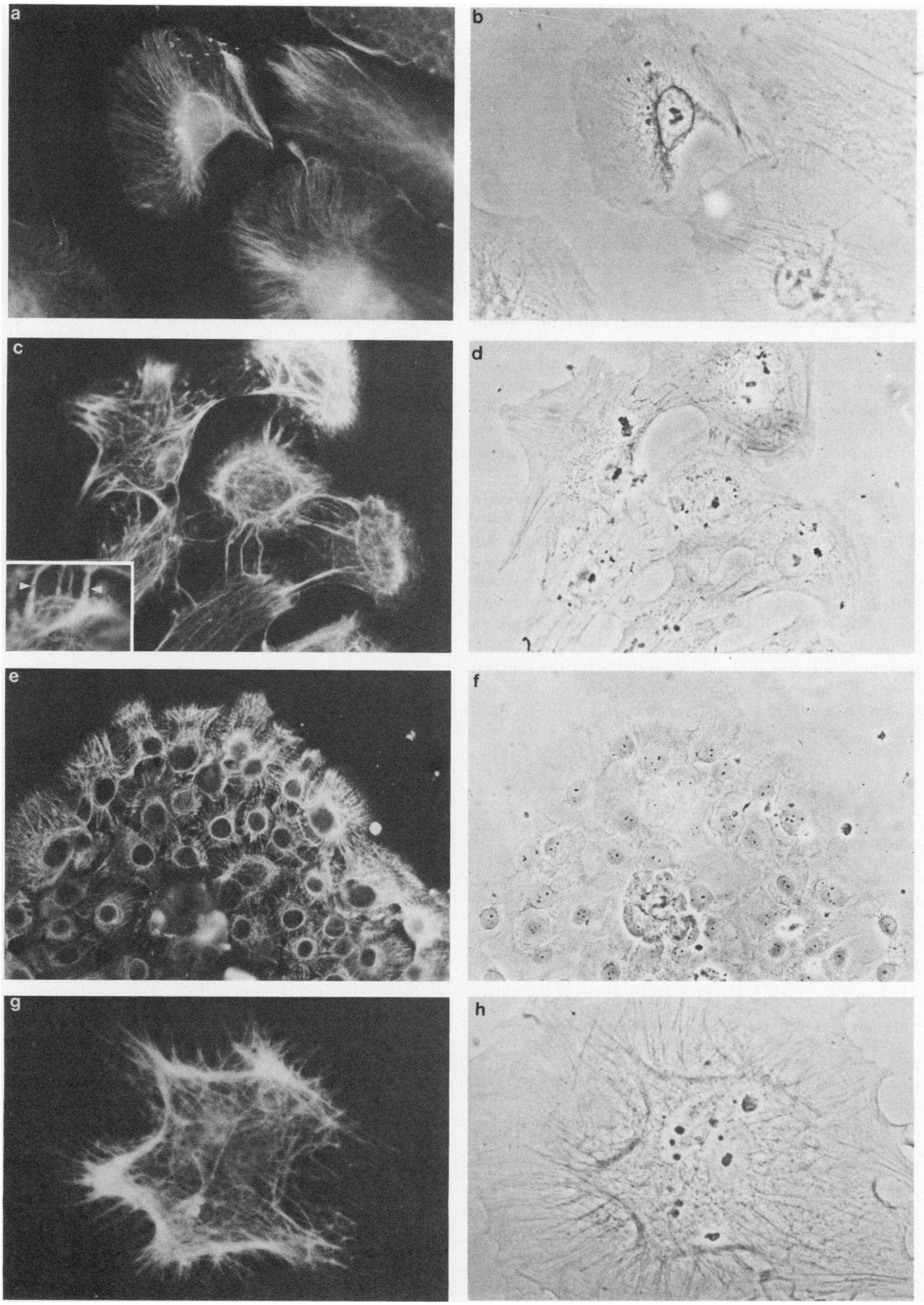
a
b
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TABLE I
Cell Types in AF Cultures

Cell type	Presence in AF cell cultures
E-1	Present in all AF-cultures as the major cell type
E-2	Present in low numbers in all cultures
E-3, E-4	Present in isolated clones in a small proportion of cultures
E-5	Present occasionally in cultures
Fibroblasts	Present exceptionally as major cell type

separation of five different epithelial cell types would not have been possible using morphological criteria only. The absence of glial cells and cells of muscle origin in AF cell cultures suggests that such cells have no access to the AF in normal pregnancy although they can be found in abnormal pregnancies, as in cases with anencephaly (14). Other fetal disorders may also be associated with specific cellular markers.

The site of origin of AF cells has remained unknown although both fetal urine (38, 39) and fetal membranes (40) have been proposed as the origin of the cells in normal pregnancies. Cytoskeletal antibodies in combination with other tissue-specific markers should offer new possibilities for exploring the origin of these cells. Distinction of various cell types in amniotic fluid cell cultures may also facilitate developing of selective cell culture systems for the optimal cell type in each purpose. This applies particularly to the biochemical assays in cultured cells for prenatal diagnosis of metabolic disorders.

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REFERENCES

1. Milunsky, A. 1973. The Prenatal Diagnosis of Hereditary Disorders. Charles C Thomas, New York.
2. Martin, A. O. 1980. Characteristics of amniotic fluid cells *in vitro* and attempts to improve culture techniques. *Clin. Obstet. Gynaecol.* **7**: 143–173.
3. Galjaard, H. 1980. Genetic Metabolic Diseases. Early Diagnosis and Prenatal Analysis. North Holland Biomedical Publishers, Amsterdam.
4. Gerbie, A. B., S. B. Melancon, C. Ryan, and H. L. Nadler. 1972. Cultivated epithelial-like cells and fibroblasts from amniotic fluid. Their relationship to enzymatic and cytologic analysis. *Am. J. Obstet. Gynecol.* **114**: 314–320.
5. Hoehn, H., E. M. Bryant, L. E. Karp, and G. M. Martin. 1974. Cultivated cells from diagnotic amniocentesis in second trimester pregnancies. I. Clonal morphology and growth potential. *Pediatr. Res.* **8**: 746–754.
6. Gosden, C. M., and D. J. H. Brock. 1977. Morphology of rapidly adhering amniotic-fluid cells as an aid to the diagnosis of neural-tube defects. *Lancet.* **II**: 919–922.
7. Priest, R. E., K. M. Marimuthu, and J. H. Priest. 1978. Origin of cells in human amniotic fluid cultures. Ultrastructural features. *Lab. Invest.* **39**: 106–109.
8. Priest, R. E., J. H. Priest, J. F. Moinuddin, and A. J. Keyser. 1977. Differentiation in human amniotic fluid cell cultures. I. Collagen production. *J. Med. Genet.* **14**: 157–162.
9. Megaw, J. M., J. H. Priest, R. E. Priest, and L. D. Johnson. 1977. Differentiation of human amniotic fluid cell cultures. II. Secretion of an epithelial basement membrane glycoprotein. *J. Med. Genet.* **14**: 163–167.
10. Crouch, E., G. Balian, K. Holbrook, D. Duksin, and P. Bornstein. 1978. Amniotic fluid fibronectin. Characterization and synthesis by cells in culture. *J. Cell Biol.* **78**: 701–715.
11. Crouch, E. and P. Bornstein. 1978. Collagen synthesis by human amniotic fluid cells in culture. Characterization of a procollagen with three identical pro l(I) chains. *Biochemistry.* **17**: 5499–5509.
12. Crouch, E., and P. Bornstein. 1979. Characterization of a type IV procollagen synthetized by human amniotic fluid cells in culture. *J. Biol. Chem.* **254**: 4197–4204.
13. Crouch, E., H. Sage, and P. Bornstein. 1980. Structural basis for apparent heterogeneity of collagens in human basement membranes. Type IV procollagen contains two distinct chains. *Proc. Natl. Acad. Sci. U.S.A.* **77**: 745–749.
14. Aula, P., H. von Koskull, K. Teramo, O. Karjalainen, I. Virtanen, V-P. Lehto, and D. Dahl. 1980. Glial origin of rapidly adhering amniotic fluid cells. *Br. Med. J.* **281**: 1–5.
15. Valle, M., and K. Penttinen. 1962. Routine culture of human amnion cells. *Ann. Med. Exp. Fenn.* **40**: 342–351.
16. Franke, W. W., E. Schmid, M. Osborn, and K. Weber. 1979. Widespread occurrence of intermediate filaments of the vimentin-type in cultured cells from diverse vertebrates. *Exp. Cell Res.* **123**: 25–46.
17. Lehto, V-P., I. Virtanen, and P. Kurki. 1978. Intermediate filaments anchor the nuclei in nuclear monolayers of cultured human fibroblasts. *Nature (Lond.).* **272**: 175–177.
18. Virtanen, I., V-P. Lehto, E. Lehtonen, T. Vartio, S. Stenman, P. Kurki, O. Wager, J. V. Small, D. Dahl, and R. A. Badley. 1981. Expression of intermediate filaments in cultured cells. *J. Cell Sci.* In press.
19. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. U.S.A.* **76**: 4350–4354.
20. Sun, T-T., and H. Green. 1978. Keratin filaments of cultured human epidermal cells. Formation of intermolecular disulfide bonds during terminal differentiation. *J. Biol. Chem.* **253**: 2053–2060.
21. Keski-Oja, J., V-P. Lehto, and I. Virtanen. 1981. Keratin filaments of mouse epithelial cells are rapidly affected by epidermal growth factor. *J. Cell Biol.* **90**: 537–541.
22. Lazarides, E., and D. R. Balzer. 1978. Specificity of desmin to avian and mammalian muscle cells. *Cell.* **14**: 429–438.
23. Badley, R. A., C. W. Lloyd, A. Woods, L. Carruthers, C. Allcock, and D. A. Rees. 1978. Mechanisms of cellular adhesion. III. Preparation and preliminary characterization of adhesions. *Exp. Cell Res.* **118**: 231–244.

24. Bignami, A., D. Dahl, and D. C. Rueger. 1980. Glial fibrillary acidic protein (GFA) in normal neural cells and in pathological conditions. *Adv. Cell. Neurobiol.* **7**: 285–310.
25. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* **227**: 680–683.
26. Franke, W. W., E. Schmid, K. Weber, and M. Osborn. 1979. HeLa cells contain intermediate-sized filaments of the prekeratin type. *Exp. Cell Res.* **118**: 95–109.
27. Franke, W. W., E. Schmid, M. Osborn, and K. Weber. 1978. Different intermediate-sized filaments distinguished by immunofluorescence microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **75**: 5034–5038.
28. Franke, W. W., K. Weber, M. Osborn, E. Schmid, and C. Freudenstein. 1978. Antibody to prekeratin. Decoration of tonofilament-like arrays in various cells of epithelial character. *Exp. Cell Res.* **116**: 420–445.
29. Schmid, E., S. Tapscott, G. S. Bennett, J. Croop, S. A. Fellini, H. Holtzer, and W. W. Franke. 1979. Different location of different types of intermediate-sized filaments in various tissues of the chicken embryo. *Differentiation.* **15**: 27–40.
30. Sun, T-T., and H. Green. 1978. Immunofluorescent staining of keratin fibers in cultured cells. *Cell.* **14**: 469–476.
31. Sun, T-T., C. Shih, and H. Green. 1979. Keratin cytoskeletons in epithelial cells of internal organs. *Proc. Natl. Acad. Sci. U.S.A.* **76**: 2813–2817.
32. Lazarides, E. 1979. Intermediate filaments as mechanical integrators of cellular space. *Nature (Lond.).* **283**: 249–256.
33. Melancon, S. B., S. Y. Lee, and H. L. Nadler. 1971. Histidase activity in cultivated human amniotic fluid cells. *Science (Wash. D. C.).* **173**: 627–629.
34. Bornstein, P., and H. Sage. 1980. Structurally distinct collagen types. *Annu. Rev. Biochem.* **49**: 957–1004.
35. Shelanski, M., and R. K. H. Liem. 1979. Neurofilaments. *J. Neurochem.* **33**: 5–13.
36. Osborn, M., W. W. Franke, and K. Weber. 1980. Direct demonstration of the presence of two immunologically distinct intermediate-sized filament systems in the same cell by double immunofluorescence microscopy. Vimentin and cytokeratin fibers in cultured epithelial cells. *Exp. Cell Res.* **125**: 37–46.
37. Priest, R. E., J. H. Priest, C. H. Laundon, and P. W. Snider. 1980. Multinucleate cells in cultures of human amniotic fluid form by fusion. *Lab. Invest.* **43**: 140–144.
38. Hoehn, H., E. M. Bryant, A. G. Fantel, and G. M. Martin. 1975. Cultivated cells from diagnostic amniocentesis in second trimester pregnancies. III: The fetal urine as a potential source of clonable cells. *Humangenetik.* **29**: 285–290.
39. Felix, J. S., and J. W. Littlefield. 1979. Urinary tract epithelial cells cultured from human urine. *Int. Rev. Cytol.* **10**(Suppl.): 11–23.
40. Priest, R. E., J. H. Priest, J. F. Moinuddin, and D. S. Sgoutas. 1979. Differentiation in human amniotic fluid cell cultures: chorionic gonadotropin production. *In Vitro.* **15**: 142–147.