

The cellular origin of fibronectin in the basement membrane zone of developing tooth

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SUMMARY

The cellular source of fibronectin in the dental epitheliomesenchymal interface was studied in interspecies combinations of mouse and quail tissue. Species-specific fibronectin antibodies were produced by immunizing rabbits with purified mouse or chicken fibronectin and by absorbing both antisera with purified heterologous fibronectin and insoluble tissue extract. The absorbed antisera to mouse and chicken fibronectin showed fluorescent staining only in mouse and chicken tissue sections, respectively, but not *vice versa*. When the mouse mesenchymal dental papilla was combined and cultured either with the mouse enamel organ or with the quail pharyngeal epithelium, mesenchymal cell differentiation was initiated and typical alignment of mesenchymal cells along the basement membrane was seen. Examination with transmission electron microscope revealed a typical bilaminar basal lamina with adherent fibrillar matrix on its mesenchymal aspect. Immunofluorescent localization of fibronectin with the mouse-specific fibronectin antiserum showed a brilliant staining in the mesenchymal tissue and in the basement membrane zone. When the chicken-specific fibronectin antiserum was used, no staining was detected in either tissue recombinations. We have suggested earlier that fibronectin in the dental basement membrane plays an important role during the differentiation of mesenchymal cells into odontoblasts. The present study demonstrates that fibronectin in the basement membrane of the developing tooth is produced exclusively by the differentiating mesenchymal cells.

INTRODUCTION

The extracellular matrix at the interface between the dental epithelium and mesenchyme plays an important role in the differentiation of the mesenchymal cells into odontoblasts (Thesleff & Hurmerinta, 1981; Ruch *et al.* 1982). Experimental transfilter studies have indicated that a close association between the differentiating cells and the basement membrane is a prerequisite for odontoblast differentiation (Thesleff, Lehtonen & Saxén, 1978), and several descriptive studies have shown that compositional changes in the basement membrane zone are temporally related to odontoblast differentiation. The density of fibrillar material at the mesenchymal aspect of the basal lamina increases as the mesenchymal cells make close contacts with the basal lamina (Meyer, Karcher-Djuricic, Osman & Ruch, 1978; Hurmerinta & Thesleff, 1981). Furthermore, autoradiographic

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studies have indicated enhanced turnover of basement membrane macromolecules at this time (Osman & Ruch, 1981a; Hurmerinta, 1982). The immunohistological findings on an increase particularly in the fibronectin content (Thesleff, Stenman, Vaheri & Timpl, 1979; Thesleff *et al.* 1981; Lesot, Osman & Ruch, 1981) have prompted speculations about a possible active role of fibronectin in the process of odontoblast differentiation.

The dental basement membrane contains type IV collagen, heparan sulphate proteoglycan and laminin (Thesleff *et al.* 1981) which are likely to be produced by the epithelial cells. However, the origin of fibronectin in the basement membrane is not known. Fibronectin may not be an integral basement membrane component (Boselli *et al.* 1981; Martinez-Hernandez *et al.* 1981), but in the dental basement membrane fibronectin has been localized to the mesenchymal aspect of the basal lamina (Andujar, Magloire & Grimaud, 1984). In the present experimental study interspecies combinations of mouse and quail tissue as well as species-specific antibodies were used to trace the cellular source of fibronectin in the dental basement membrane. The results show that fibronectin in the dental basement membrane is produced exclusively by the differentiating mesenchymal cells.

MATERIALS AND METHODS

First mandibular molars were dissected in phosphate-buffered saline (PBS), from 16-day-old C57B1/CBA mouse embryos (day of vaginal plug = day 0). Quail eggs (*Coturnix coturnix*) were incubated at 37°C for 4½ days and the embryos were selected corresponding to Hamburger-Hamilton stages 23–25 (Hamburger & Hamilton, 1951). Mandibular pharyngeal tissue was dissected from the surrounding tissues in phosphate-buffered saline. The epithelia were separated from the mesenchymal tissue by treatment with cold 2.25 % trypsin/0.75 % pancreatin solution for 30–45 min at 4°C. After 15 min of incubation in culture medium the tissue components were mechanically separated and combined on a Nuclepore-filter (pore size 1.0 µm, Nuclepore Co., Pleasanton, Calif.) on a metal grid. The mesenchymal tissue component of each species was combined either with its own epithelium or with epithelial tissue of the other species. The explants were cultured for 4 days *in vitro*. The culture medium consisted of Minimum Essential Medium supplemented with 10 % horse serum (Microbiological Associates, Inc., Bethesda, Md.) and with 2 mM-glutamine.

For immunohistochemical reactions the explants were fixed with cold alcohol, and embedded in a paraffin and, subsequently, serially sectioned at 7 µm. After quick deparaffinization the sections were treated with a predetermined dilution of each antiserum, washed in PBS and finally treated with fluorescein–isothiocyanate-conjugated sheep antibodies against rabbit immunoglobulins (Cappel, Cohranville, PA, 1:40). Species-specific antibodies to chicken and mouse fibronectins were prepared as described (Sariola, Kuusela & Ekblom, 1984). Briefly, rabbit antiserum to chicken fibronectin was absorbed with mouse tissue extract and purified mouse fibronectin. Accordingly, antiserum to mouse fibronectin was absorbed with chicken tissue extract and purified chicken fibronectin. The species specificity of both antisera was determined by using ELISA for chicken or mouse fibronectin (Sariola *et al.* 1984). At the working dilution (1:200) both antisera revealed a slight reactivity against the heterologous fibronectin that was used for absorption, but at the immunohistological level no cross reactivity was detected. The antisera against laminin and type IV collagen were kindly donated by Dr J. M. Foidart (Liège, Belgium). For light microscopy the explants were fixed with Zenker solution and stained with haematoxylin and eosin or with the Feulgen stain (Feulgen & Rossenbech, 1924). After photography in the fluorescence microscope, all sections were post-stained with the Feulgen stain and studied with the light microscope. For transmission electron microscopy explants were fixed in 2.5 % glutaraldehyde in 0.1 M-phosphate buffer, pH 7.4, for

3 h at room temperature, postfixed with 1% OsO₄ in 0.1 M-phosphate buffer for 1 h at 4°C and embedded in Epon 812. These sections were stained with uranyl acetate and lead citrate and examined with a Jeol JEM-100CX transmission electron microscope.

RESULTS

First the capacity of heterotypic epithelia to support the mouse mesenchymal cell differentiation was studied in interspecies combinations. When the mouse dental papilla mesenchyme was combined either with the mouse enamel epithelium or with quail pharyngeal epithelium and cultured for 4 days *in vitro*, the mesenchymal cells aligned along the epitheliomesenchymal interface (Fig. 1A,B). This is considered as the initial stage of the differentiation of mesenchymal cells into odontoblasts. The structure and composition of the basement membrane were then observed. Examination in the electron microscope showed that a bilaminar basal lamina with associated fibrillar material at its mesenchymal aspect had been restored in all eight homo- and heterotypic combinations. In the latter combinations, however, the structure of the basal lamina was more diffuse (Fig. 1C,D). The antisera against type IV collagen and laminin produced clear linear staining in the epitheliomesenchymal interface of all these explants (Fig. 1E,F).

To study the cellular origin of fibronectin in the basement membrane the explants were reacted with the species-specific antisera against mouse or chicken fibronectin. The enzymic treatment used for the separation of epithelial and mesenchymal tissues resulted in the loss of fibronectin from the basement membrane, as has been shown previously (Lesot *et al.* 1981). In the combinations of mouse dental papilla with mouse enamel epithelium or with quail pharyngeal epithelium, the mouse-specific fibronectin antiserum stained the mesenchymal tissue and the basement membrane area in all 15 explants examined (Fig. 2A,C). The intensity of fluorescence in the basement membrane was weaker in the interspecies combinations. The antiserum species specific for chick fibronectin produced no staining in these explants (Fig. 2B,D). When quail pharyngeal mesenchyme was combined and cultured either with quail pharyngeal epithelium or with mouse enamel epithelium the antiserum species specific for mouse fibronectin revealed no fluorescent staining (Fig. 2E). When the chicken-specific fibronectin antiserum was used, the mesenchymal tissue and the basement membrane stained intensely in all 12 homotypic and heterotypic explants examined (Fig. 2F).

DISCUSSION

By using interspecies tissue combinations and species-specific antibodies we have shown that fibronectin in the developing dental basement membrane zone is produced by mesenchymal cells. Although various cell types including epithelial cells synthesize fibronectin in culture (Hynes, 1981), the cellular origin of fibronectin in tissues is known only in a few cases. In glomerular basement membrane

fibronectin has its origin in endothelial and mesangial cells (Sariola *et al.* 1984). Although a mesenchymal origin for fibronectin in the dental basement membrane has been suggested earlier (Brownell, Bessem & Slavkin, 1981) it has not been convincingly demonstrated.

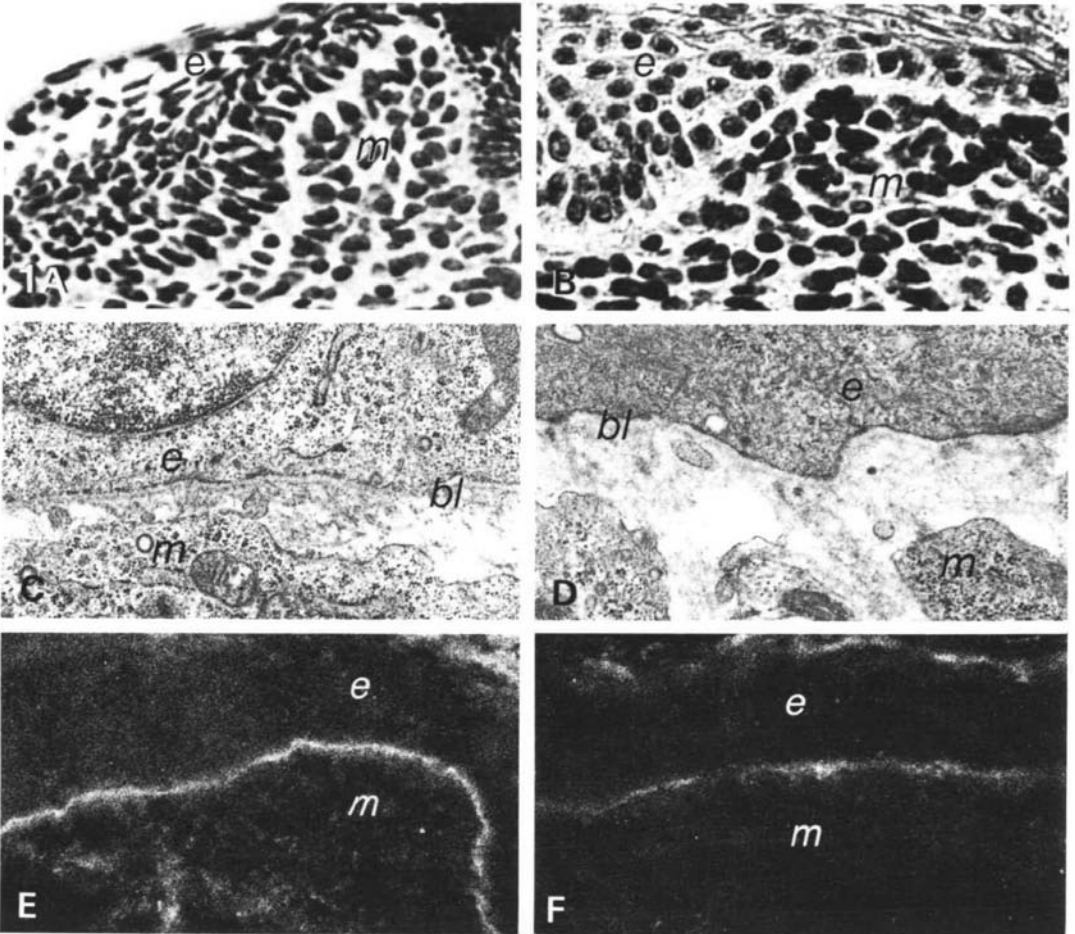


Fig. 1. Micrographs of explants in which enzymically separated mouse dental papilla was combined either with the mouse enamel epithelium (left) or with quail pharyngeal epithelium (right) and then cultured for 4 days *in vitro*. *bl*, basal lamina; *m*, mesenchyme; *e*, epithelium.

(A,B) Light micrographs. The mesenchymal cells have aligned along the epithelio-mesenchymal interface in both homotypic (A) and heterotypic (B) combinants. $\times 120$; Feulgen stain.

(C,D) Electron micrographs. The basal lamina with filamentous material has been deposited at the mesenchymal aspect of the epithelial cells in both homotypic (C) and heterotypic (D) combinants. The filamentous material is more diffuse in the heterotypic combinants. Mesenchymal cell processes make close contacts with the basal lamina. $\times 11\ 000$.

(E,F) Immunofluorescent staining with laminin. The intensity of staining at the epithelio-mesenchymal interface is similar in homotypic (E) and heterotypic (F) explants. $\times 120$.

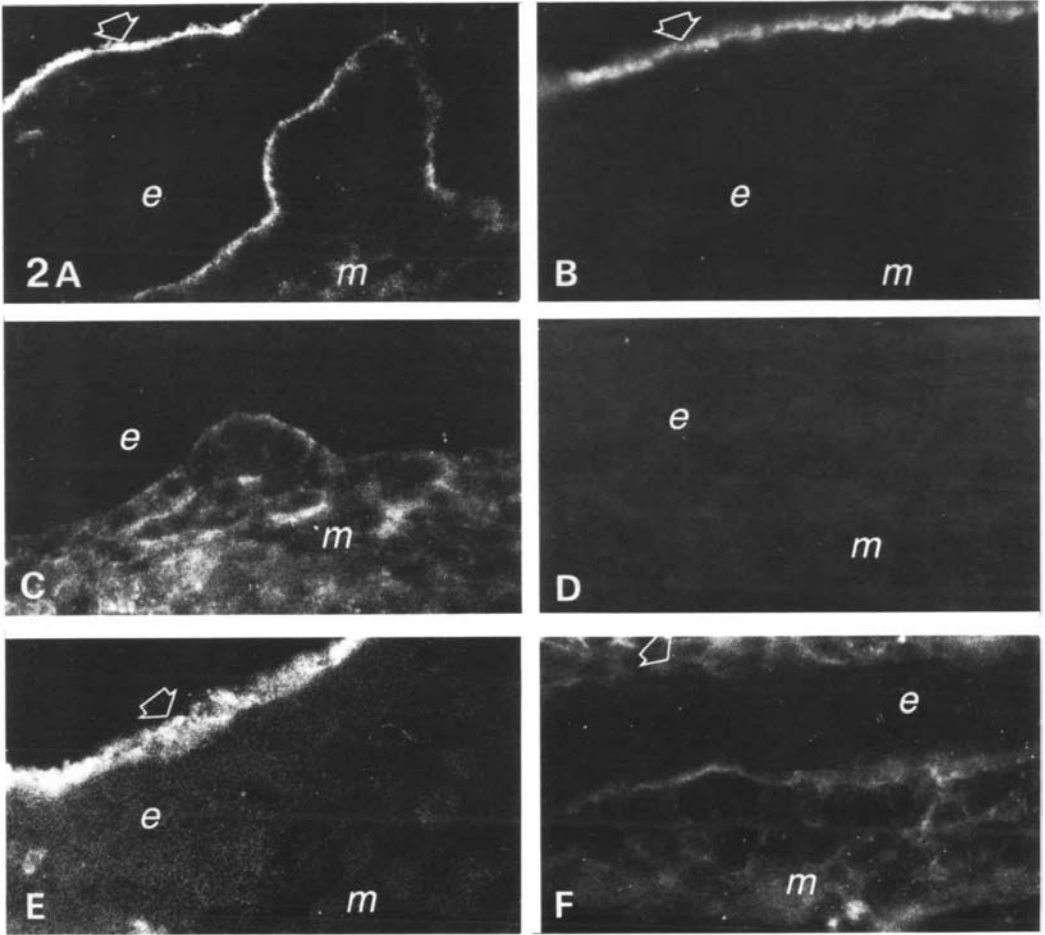


Fig. 2. Immunofluorescence micrographs demonstrating the cellular source of fibronectin in tissue combinations cultured for 4 days *in vitro*. The sections were stained with species-specific antisera against mouse (left) and chick (right) fibronectin. *m*, mesenchyme; *e*, epithelium. $\times 120$.

(A,B) The combination of mouse dental mesenchyme with mouse dental epithelium (the light micrograph of the same explant is shown in Fig. 1A). The mouse-specific antibody against fibronectin (A) reacts with the basement membrane region and the mesenchymal stroma but chick-specific antibody (B) reveals no staining. The epithelial tissues are negative in both sections, but the positive fluorescence at the outer surface of explants (arrows) is probably caused by horse serum fibronectin incorporated from the culture medium.

(C,D) The combination of mouse dental mesenchyme with quail pharyngeal epithelium (the light micrograph of the same explant is shown in Fig. 1B). Mouse-specific antibody against fibronectin (C) stains the epitheliomesenchymal interface and mesenchyme, whereas the chick-specific antibody (D) shows no reaction.

(E,F) In the combinations of quail pharyngeal mesenchyme with mouse dental epithelium the mouse-specific antibody against fibronectin (E) shows no staining whereas the chick-specific antibody (F) reacts with the basement membrane region and with quail mesenchyme. The positive fluorescence at the outer surface of explants (arrows) is probably caused by horse serum fibronectin incorporated from the culture medium.

The possibility of tissue contaminations in interspecies combinants could be ruled out on the basis of the difference in the staining of the quail and mouse nucleus (Le Douarin, 1973). Plasma fibronectin has been shown to be incorporated into basement membranes and connective tissues *in vivo* (Oh *et al.* 1981), but in a similar organ culture system as was used in this study fibronectin was not incorporated from the culture medium to embryonic teeth or kidneys (Thesleff *et al.* 1983). Thus, although the mouse and chick-specific antibodies were not absorbed with horse fibronectin, the detected positive fluorescence does not represent the penetration of the horse serum fibronectin from the culture medium. However, the fluorescence at the surface of explants probably results from the incorporation of horse fibronectin.

Besides fibronectin, the basement membrane area could also be stained with antibodies against laminin and type IV collagen in both homo- and heterotypic combinations. These components are apparently produced by the dental epithelium (Frank, Osman, Meyer & Ruch, 1979; Osman & Ruch, 1981*b*). However, the electron-dense basal lamina and particularly the fibrillar material attached to it was more diffuse in the hybrid combinants than in the control explants. In spite of this, the interspecies tissue combination resulted in normal tooth development up to the stage of mesenchymal cell alignment along the epitheliomesenchymal interface, as also previously reported (Kollar & Fisher, 1980; Cummings, Bringas, Grodin & Slavkin, 1981; Arechaga, Karcher-Djuricic & Ruch, 1983). Thus the newly restored basement membrane in interspecies combination also apparently has the potential of triggering odontoblast differentiation.

Although there is so far no direct evidence for an active role of fibronectin in tooth development, our earlier experimental and descriptive data as well as our recent cell culture studies suggest that fibronectin is involved in the process of odontoblast differentiation. First, the amount of fibronectin in the basement membrane region is increased at the time when mesenchymal cells align, acquire the columnar shape of differentiating odontoblasts and start predentin secretion (Thesleff *et al.* 1979, 1981; Lesot *et al.* 1981). Secondly, odontoblast differentiation can be prevented by interfering with the deposition of fibronectin (Thesleff & Pratt, 1980). Thirdly, our recent studies where the behaviour of mesenchymal dental papilla cells has been observed in monolayer culture have indicated that these cells differ from all other dental-related mesenchymal cells in aspects of fibronectin metabolism (Thesleff, 1986; Thesleff, Partanen, Kuusela & Lehtonen, 1986). The data obtained in the present study showing that the fibronectin at the epithelial-mesenchymal interface has its origin in the mesenchymal dental papilla cells is important for further studies on the molecular mechanism of the tissue interaction leading to odontoblast differentiation.

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