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Changing expression of intermediate filaments in fibroblasts and cementoblasts of the developing periodontal ligament of the rat molar tooth

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ABSTRACT

The distribution of vimentin and cytokeratin intermediate filaments within the cells of the dental follicle and developing periodontal ligament is described during eruption of the rat 1st molar tooth. Alcohol-fixed tissues from animals ranging from neonates to 12 wk old were cryosectioned, immunolabelled with monoclonal antibodies against vimentin and a range of cytokeratins and examined by indirect immunofluorescence. Vimentin was observed in follicular and periodontal ligament fibroblasts in all animals and at all stages of eruption. It was also observed in cementoblasts after disruption of the epithelial root sheath (of Hertwig) which is responsible for determining the shape of the developing root. Prior to eruption, cytokeratins were restricted to epithelial components of the developing tooth, including the root sheath. However, they were seen in cementoblasts on disruption of the root sheath at 2 wk and in periodontal ligament fibroblasts at 3 wk after birth, when the tooth was erupting but had not reached occlusion. On occlusion (at 4 wk), fibroblasts no longer labelled for cytokeratins but cementoblasts associated with acellular cementum formation continued to express them. These results demonstrate temporal and spatial changes within the cells of the developing periodontal connective tissues and suggest that the appearance of cytokeratins in periodontal fibroblasts and cementoblasts may be related to mechanical changes during tooth eruption. Further, the results suggest different origins for cementoblasts associated with cellular and acellular cementum formation.

Key words: Cytokeratins; vimentin; tooth development.

INTRODUCTION

The forces responsible for tooth eruption are generated in the connective tissues of the dental follicle and periodontal ligament. The dental follicle is a loose connective tissue which surrounds the developing tooth and differentiates into the periodontal ligament. This structure provides tooth support by attaching to the root of the tooth and the alveolar bone (Moxham & Berkovitz, 1995). Tooth eruption has been divided into three phases—the preeruptive, prefunctional and functional (Sicher, 1942). In the preeruptive phase, the tooth remains in its intraosseous location with any movement in the occlusal plane being attributed to the passive growth of tooth germs (Darling & Levers, 1975). In contrast, the prefunctional phase is characterised by axial movement of the tooth which is accompanied by the development of the periodontium (Scott, 1948, 1953, 1968; Scott & Symons, 1977). Measurements of the rates of emergence of human and ferret teeth into the oral cavity before they reach the occlusal plane suggests that eruption rates are greatest at the time of crown emergence (Hoffman & Schour, 1940; Moxham and Berkovitz, 1988). During the prefunctional phase, the tooth, on emerging into the oral cavity, continues to erupt at a reduced rate to attain its functional position (Moxham & Berkovitz, 1995). This decrease in eruption rate is thought to be either a consequence of changes in the eruptive mechanism or to be related to structural and bio-

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mechanical changes within the periodontium. During eruption, the connective tissues comprising the dental follicle and periodontal ligament must respond to mechanical changes and undergo considerable remodelling to maintain the new position of the tooth. As the cytoskeleton controls cell shape and motility and promotes cell-matrix interactions (Goldman & Steinert, 1990; Amos & Amos, 1991; Skali & Goldman, 1991; Benjamin et al. 1994), it may be important in such remodelling.

The cytoskeleton of most vertebrate cells consists of microfilaments, microtubules and intermediate filaments (Amos & Amos, 1991). Intermediate filaments are a large, multigene family of fibrous proteins that include cytokeratins, which are characteristic of epithelia (Moll et al. 1982) and vimentin, which is typical of cells of mesenchymal origin (Steinert & Roop, 1988). Cytokeratins are the most complex group, with at least 20 different types present in human epithelia (Moll et al. 1982). For each type of epithelium, a characteristic pattern of cytokeratins is expressed (Franke et al. 1978, 1979; Moll et al. 1982, 1983). Cytokeratins have been divided into type I (acidic) and type II (neutral and basic) groups which share only 30% sequence homology (Fuchs et al. 1981). These subclasses compliment each other by forming heterodimers of type I and type II keratin proteins (Steinert et al. 1984; Hatzfield & Franke, 1985; Eichner et al. 1986). In oral tissues, cytokeratin expression has been used primarily as a cell marker during normal tooth development (Lesot et al. 1982; Smith et al. 1990; Lombardi et al. 1992) and in diseased states such as in the epithelia of inflamed gingiva and periodontal pockets (Mackenzie & Goa, 1993). Vimentin is found in a variety of cell types of mesenchymal and nonmesenchymal origin. In oral tissues, it has been immunolocalised in fibroblasts of the periodontal ligament and dental pulp, in odontoblasts and in fibroblasts of the dental papilla and dental follicle during tooth development (Lesot et al. 1982, Pellissier et al. 1990; Lombardi et al. 1992).

Coexpression of cytokeratins and vimentin has been reported in the human eye (Kasper et al. 1989*a*), in the stellate reticulum epithelium of the developing enamel organ (Kasper et al. 1989*b*), in fibroblasts of the human choroid plexus (Kasper et al. 1988), as well as in other mesenchymal cells such as chondrocytes of fish and rat cartilage (Markl & Franke 1988; Benjamin et al., 1994) and regenerating newt limb blastema (Ferretti et al. 1989). Furthermore, the expression of cytokeratins 8 and 18 (commonly accompanied by cytokeratin 19) has been observed in certain non-epithelial tissues (Franke et al. 1989; Jahn et al. 1989; Knapp et al. 1989). The coexpression is often transient and limited to particular developmental stages (Kasper et al. 1989*a*, *b*).

The functions of intermediate filaments are poorly understood, but are presumed to be structural (Lazarides, 1980). There is some evidence to suggest that cells may increase their intermediate filament content in response to mechanical loading (Eggli et al. 1988; Schnittler et al. 1993). Where coexpression of different intermediate filaments occurs, these cytoskeletal elements have been associated with the cell environment, cell shape or the possible secretory activity of the cell (Kasper et al. 1988, 1989*a*, *b*; Benjamin et al. 1994). The specific distribution of different intermediate filaments indicates particular functions for these elements within tissues. For instance, the presence of cytokeratins in chondrocytes of the surface zone of articular cartilage could be related to the combined shear and compressive forces to which they are subjected (Benjamin et al. 1994).

Since cells in tissues such as rat and fish cartilage (Markl et al. 1989), endothelia (Schnittler et al. 1993), and fibrocartilage (Benjamin et al. 1994) can express different intermediate filaments in response to mechanical forces, similar intermediate filament expression may occur in the cells of other tissues subject to mechanical stress. Therefore, it may be expected that the onset and cessation of eruption could be marked by changes in the cytoskeleton of periodontal fibroblasts. In the present study, we report changes in the expression of intermediate filaments within the cells of the dental follicle and the periodontal ligament during different phases of eruption of the rat molar tooth.

MATERIALS AND METHODS

Wistar rats from a range of developmental stages including neonates and 1, 2, 3, 4, 8 and 12-wk-old animals were used and all animals were killed by decapitation following ether anaesthesia. At least 4 rats were used at each stage. The mandibles were dissected out on ice and segments incorporating the periodontal tissues of molar teeth (including the alveolar bone) were obtained by transverse sectioning of the jaws at the level of the 1st molar; segments were also obtained which included the transseptal fibre region in order to compare periodontal ligament and gingival connective tissues. The segments were rinsed in 10 mM phosphate buffered saline (PBS; pH 7.2), fixed in 90% alcohol for 2 h at 4 °C, decalcified in 5% EDTA in PBS for approximately 3 wk at 4 °C and processed for histology or immunocytochemistry.

For histology, the material was dehydrated with a

Table. *Details of intermediate filament antibodies used and a comparison of their distributions in human and rat tissues*

Antibody dilution	Intermediate filaments recognised	Antibody specificity in human tissues (see References)	Antibody specificity in rat tissues	Supplier	References
K8.12 1:20	Type I polypeptides 13, 15, 16	Nonkeratinising stratified squamous epithelia, basal layer of pseudostratified epithelia, transitional epithelia	Oesophageal epithelial cells, basal cells of tracheal epithelium, urothelium	Sigma Chemical Co.	Gigi-Leitner et al. 1986; Moll 1991; Ryder & Weinreb, 1990
2D7 1:20	Type I polypeptide 13	Nonkeratinising stratified squamous epithelia	Occasional cells of oesophageal epithelium	Sigma Chemical Co.	Moll, 1991
K8.13 1:20	Type I polypeptides 10, 11, 18 Type II polypeptides 1, 5, 6, 7, 8	Variety of epithelial tissues; cultured epithelial cells	Epidermis of thick and thin skin, oesophageal, tracheal, gut epithelia, urothelium	Sigma Chemical Co.	Gigi-Leitner et al. 1986
PCK-26 1:300	Type II polypeptides 1, 5, 6, 8	Normal, reactive and neoplastic epithelia	Epidermis of thick and thin skin, oesophageal and gut epithelia	Sigma Chemical Co.	Lane et al. 1992
K8.60 1:160	Type I polypeptides 10, 11	Suprabasal layers of keratinised stratified squamous epithelia, individual cells in other stratified epithelia	Epidermis of thick and thin skin, occasional oesophageal epithelia	Sigma Chemical Co.	Gigi-Leitner et al. 1986; Ryder & Weinreb, 1990
K4.62 1:20	Type I polypeptide 19	Basal cells of stratified nonkeratinising squamous epithelia, transitional epithelia, pseudostratified epithelia, simple epithelia, squamous carcinoma	Basal oesophageal cells, tracheal, urothelium and gut epithelia	Sigma Chemical Co.	Gigi-Leitner et al. 1986; Ryder & Weinreb, 1990
LAS86 1:2	Type I polypeptide 19	Differentiated urothelium	Basal oesophageal cells, tracheal, urothelium, gut epithelia	Chemicon	Vonkoskull et al. 1984
CY-90 1:800	Type II polypeptide 18	Simple epithelia	Tracheal, urothelium, gut epithelia	Chemicon	None
MAB 1673 1:10	Type I polypeptide 8	Simple epithelia	Tracheal, urothelium, gut epithelia	Chemicon	Trayanovskii et al. 1986
VIM 13.2 1:300	Type III vimentin intermediate filament	Fibroblasts, astroglia, chondrocytes	Chondrocytes, fibroblasts	Sigma Chemical Co.	Adams & Watt, 1988

graded series of alcohols, cleared in CM830 and embedded in paraffin wax. Sections were cut at 8 μm and stained with Masson's trichrome, haematoxylin and eosin or toluidine blue. For immunocytochemistry, the specimens were infiltrated with 5% sucrose for 2 h, frozen with dry ice, cryosectioned at 10 μm and mounted on poly-L-lysine coated slides. The sections were immunolabelled using standard procedures for indirect immunofluorescence with a panel of monoclonal antibodies recognising various cytokeratins and vimentin (see Table). The specificity of the cytokeratin antibodies has been previously determined by immunoblotting using isolated human cytokeratin peptides (Table). All antibody dilutions were made in PBS containing 0.1% bovine serum albumen, 0.05% sodium azide and 0.1% polyoxyethlenesorbitanmonolaurate (Tween 20) as wetting agent.

Sections were treated with a 1:20 dilution of normal goat serum for 20 minutes at room temperature to block nonspecific binding of antibodies, and then incubated with primary antibody for 30 minutes. Sections were washed in 3 changes of PBS containing 0.1% Tween 20 for 15 minutes and incubated with secondary antibody (fluorescein-conjugated goat anti-mouse Fab fragments diluted 1:120; Sigma Chemical Co., UK). After 3 further washes, the sections were mounted in an aqueous mountant containing 1, 4 diazobicyclo[2,2,2]octane (DABCO) to act as a fluorescence preservative (Johnson et al. 1982). In control sections, the primary antibody was omitted or the sections were incubated with nonimmune mouse immunoglobulins (20 g/ml). Photomicrographs were taken using a Zeiss photomicroscope III on Ilford HP5 film developed in Microphen.

To test tissue specificity and cross-reactivity of these

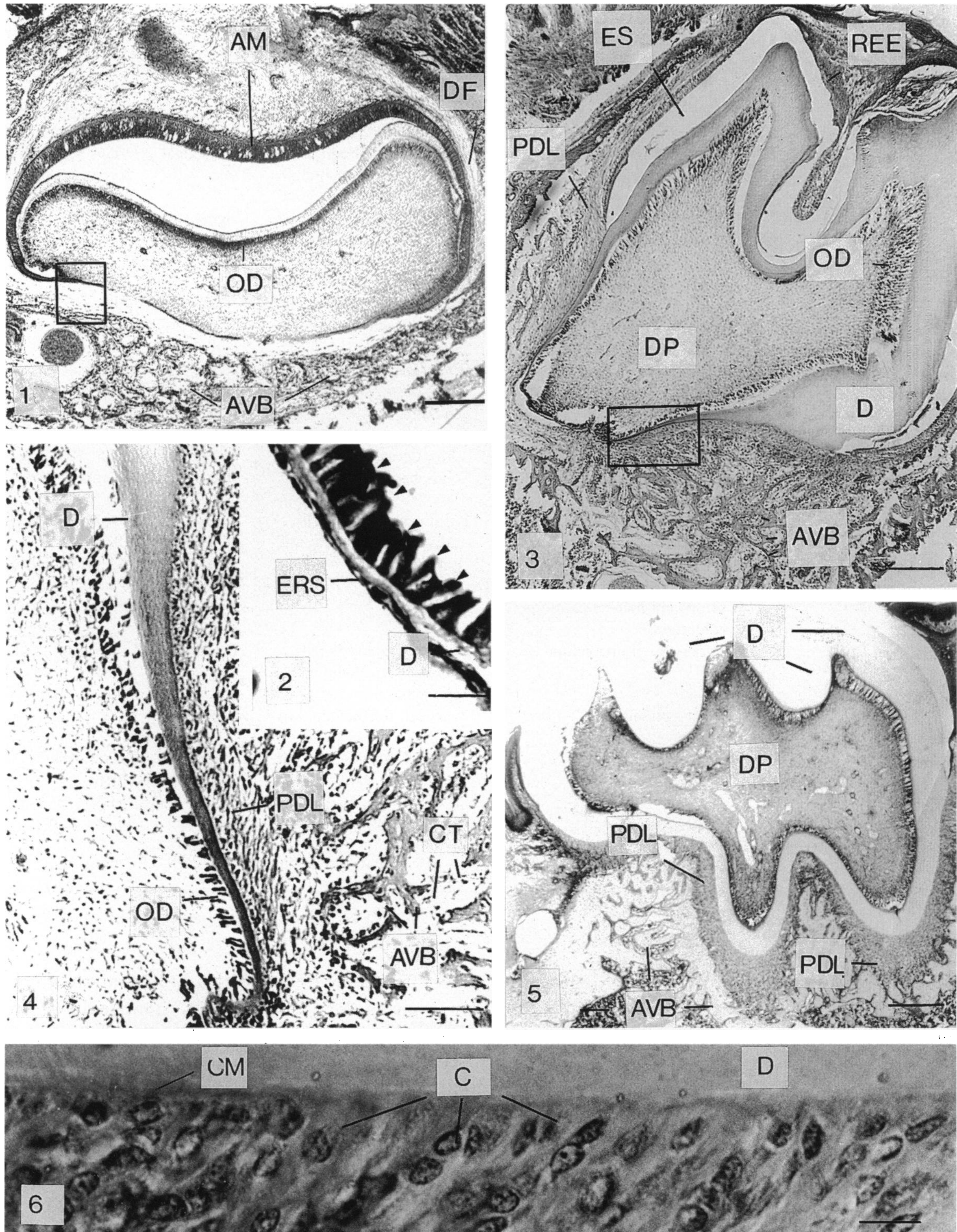


Fig. 1. In the neonatal rat, the tooth germ is surrounded by the loose connective tissue of the dental follicle (DF) and is located within a crypt of alveolar bone (AVB). OD, odontoblasts; AM, ameloblasts. Haematoxylin and eosin. Bar, 0.1 mm.

Fig. 2. High power view of the region enclosed in the rectangle in Figure 1. The root odontoblasts (arrowheads) are differentiated and have begun to secrete dentine (D) which is covered by a thin layer of cells from the epithelial root sheath (ERS). Haematoxylin and eosin. Bar, 25 μm.

Fig. 3. At 2 wk, the dental follicle has begun to form the periodontal ligament (PDL) and greater quantities of dentine (D) has been secreted by the root odontoblasts (OD). Enamel formation is also well advanced, but decalcification of the section has created an artefactual enamel space (ES). All that remains of the enamel organ is the reduced enamel epithelium (REE). AVB, alveolar bone; DP, dental papilla. Masson's trichrome. Bar, 0.75 mm.

antibodies in rat tissues, different types of epithelial and mesenchymal tissues were processed as above and immunolabelled with the panel of anti-intermediate filament antibodies to compare their distribution in rat epithelia with that previously described in man. The epithelia were the keratinised stratified squamous epithelia of thin and thick skin, nonkeratinised stratified squamous epithelium of the oesophagus, pseudostratified epithelium of the trachea, transitional epithelium of the ureter, and the simple columnar epithelial lining of the duodenum. Mesenchymal cells included connective tissue fibroblasts and chondrocytes from mature (12 wk) rats.

RESULTS

Histology of oral tissues

The details of tooth eruption were similar to those described by Trott (1962). Briefly, at the neonate stage and in 1-wk-old rats, the 1st molar was located within a bony crypt, in its preeruptive phase (Fig. 1). At this stage, the epithelial root sheath (of Hertwig) covered the surface of the developing root (Fig. 2). By 2 wk, the tooth had commenced eruption through the bony crypt and the root sheath had begun to fragment (Fig. 3). The periodontal ligament had differentiated, but did not entirely span the periodontal space (Fig. 4). By 3 wk, the tooth had just emerged through the oral mucosa into the oral cavity (Fig. 5). By 4 wk, when the tooth had reached occlusion, the periodontal ligament had numerous, well organised collagen fibres which passed from the root of the tooth to the alveolar bone, with fully differentiated cementoblasts being present at the root surface (Fig. 6).

Antibody specificity in epithelial and mesenchymal tissues

Specificity testing on rat epithelial and mesenchymal tissues showed that binding of the various antibodies to rat tissues was consistent with previous studies of human tissues (see Table and Figs 7–12). No cross reactivity between antibody VIM 13.2 and cytokeratin antibodies was detected.

Intermediate filament expression in oral tissues

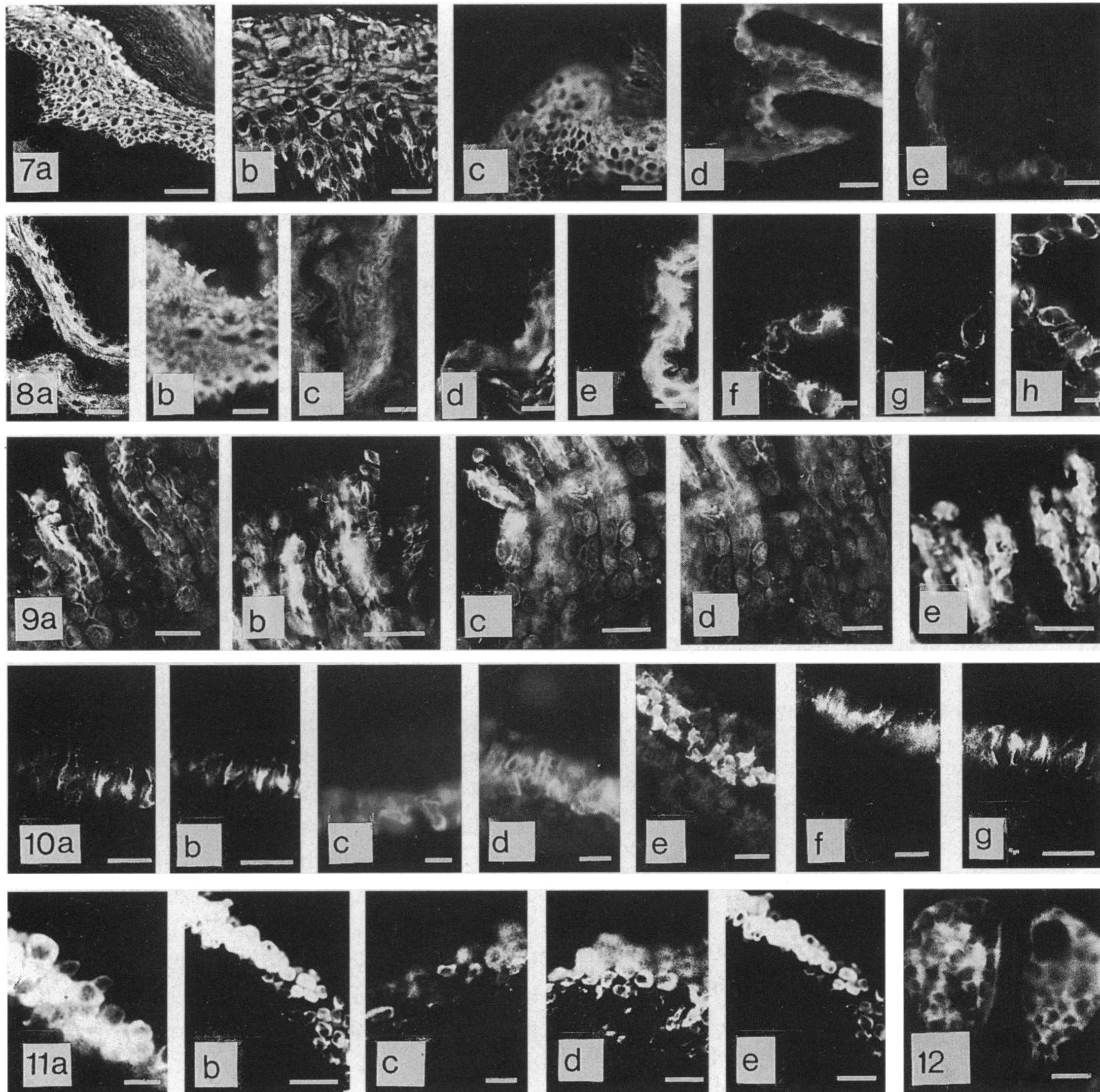
Fibroblasts of the dental follicle and periodontal ligament labelled for vimentin at all stages of development (e.g. Fig. 13). Cytokeratin labelling varied according to age. At 1 wk, prior to tooth eruption, cytokeratins were restricted to the epithelial root sheath and to the enamel organ (Fig. 14). At 2 wk, when eruption had started, labelling for cytokeratins using antibodies K8.13 (cytokeratins 1, 5–8, 10, 11 and 18), MAB 1673 (cytokeratin 8) and CY-90 (cytokeratin 18) was detected in cells at 2 sites in the developing periodontal ligament. One of these was a single layer of cells adjacent to the root cementum, running from the cervical region of the periodontal ligament to a region close to the root apex (Fig. 15). The other site was at the root apex, adjacent to the fragmenting epithelial root sheath, where several layers of fibroblast-like cells labelled with antibody K8.13 (Figs 16, 17). No cytokeratin labelling was detected in fibroblasts located close to the alveolar bone, or in osteoblasts or osteocytes. By 3 wk, labelling for cytokeratins 8 and 18 using antibodies MAB 1673 (Fig. 18*a*), CY-90 (Fig. 18*b*) and antibody K8.13 (not shown) could only be detected in cementoblasts associated with acellular cementum. These cells now also labelled for vimentin. Coexpression of cytokeratin and vimentin intermediate filaments was observed at all subsequent stages. Periodontal ligament fibroblasts labelled transiently for cytokeratin 19 using antibody K4.62 (Fig. 18) and LAS86 at 3 wk. By 4 wk, when the tooth had reached occlusion, this label had disappeared but cytokeratins, as detected by antibodies CY-90, MAB 1673 and K8.13, continued to be expressed in cementoblasts.

At no stage was labelling for cytokeratins detected in the cells of the outer (perifollicular) layer of the dental follicle or within the gingival connective tissues. Labelling with antibodies K8.12 (cytokeratins 13, 15, 16), 2D7 (cytokeratin 13) and K8.60 (cytokeratins 10, 11) was not detected in any of the connective

Fig. 4. High power view of the region enclosed in the rectangle in Figure 3. to show that the periodontal ligament (PDL) does not entirely span the periodontal space. There are areas of loose connective tissue (CT) near the alveolar bone (AVB). Masson's trichrome. Bar, 250 µm.

Fig. 5. At 3 wk, the tooth has erupted into the oral cavity and the periodontal ligament (PDL) is more densely fibrous. AVB, alveolar bone; D, dentine; DP, dental pulp. Masson's trichrome. Bar = 0.9 mm.

Fig. 6. At 3 wk, typical cuboidal cementoblasts (C) are visible and have secreted a thin layer of cementum (CM) which covers the root dentine (D). Toluidine blue. Bar, 20 µm.



Figs 7–12. Immunocytochemical localisation of intermediate filaments in rat epithelial and mesenchymal tissues.

Fig. 7. Cytokeratin localisation in the keratinising stratified squamous epithelium of the epidermis of thin skin. Labelling occurs in the basal and suprabasal layers with antibody K8.13 (*a*), suprabasal layers only with antibody K8.60(*b*), basal and suprabasal layers with antibody PCK-26(*c*), basal layer only with antibody CY-90 (*d*) and basal cell layer only with antibody MAB 1673 (*e*). Bars, 50 µm (*a,c*); 25 µm (*b,d,e*).

Fig. 8. Cytokeratin localisation in the nonkeratinised stratified squamous epithelium of the oesophagus. Labelling occurs in the basal and suprabasal layers using antibody K8.13 (*a*), basal and suprabasal layers with antibody K8.12 (*b*), occasional cells in suprabasal layers with antibody 2D7(*c*), occasional cells in suprabasal layers with antibody K8.60 (*d*), basal and suprabasal layers with antibody PCK-26 (*e*), basal cell layer only with antibody CY-90 (*f*), basal cell layer only with antibody K4.62 (*g*) and basal cell layer only with antibody LAS 86 (*h*). Bars, 50 µm (*a*); 25 µm (*c,d,e*); 20 µm (*b*); 10 µm (*f–h*).

Fig. 9. Positive labelling for cytokeratins in the simple columnar epithelial lining of the duodenum with antibodies K8.13 (*a*), CY-90 (*b*), MAB 1673 (*c*), K4.62 (*d*) and LAS 86 (*e*). Bars, 30 µm (*a,b,e*); 15 µm (*c,d*).

Fig. 10. Positive labelling for cytokeratins in the pseudostratified ciliated columnar epithelial lining of the trachea with antibodies K8.13 (*a*), PCK-26 (*b*), CY-90 (*c*), MAB 1673 (*d*), K8.12 (*e*), K4.62 (*f*) and LAS 86 (*g*). Both columnar and basal cells are labelled except in (*e*) where labelling is restricted to the basal cells. Bars, 25 µm (*e*); 20 µm (*a,b,f,g*); 10 µm (*c,d*).

Fig. 11. Positive labelling for cytokeratins in the transitional epithelium lining the bladder with antibodies K8.13 (*a*), CY-90 (*b*), MAB 1673 (*c*), K4.62 (*d*) and LAS 86 (*e*). Bars, 50 µm (*c*); 25 µm (*b,d,e*); 10 µm (*a*).

Fig. 12. Positive labelling for vimentin with antibody VIM 13.2 in tracheal chondrocytes. Bar, 5 µm.

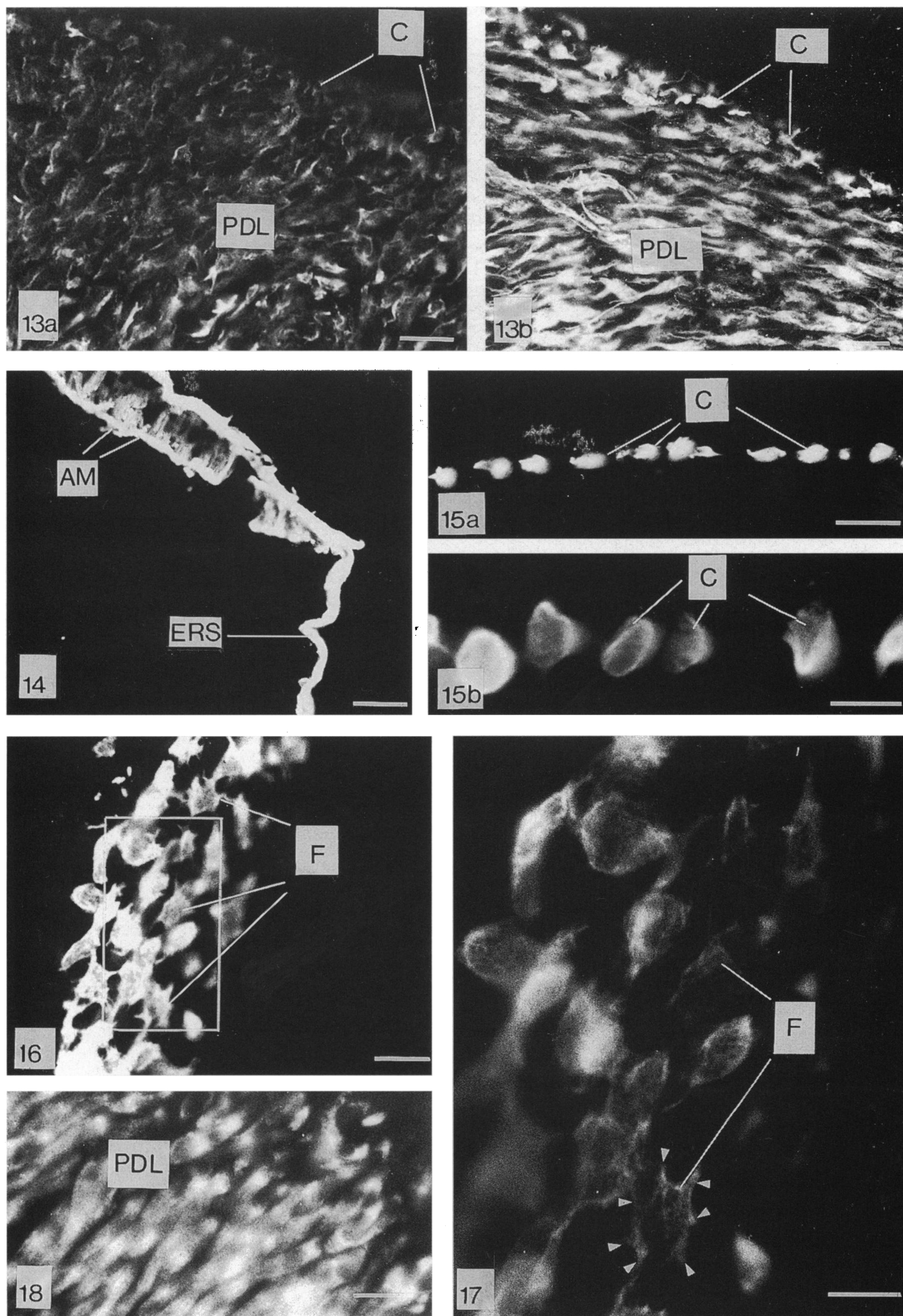


Fig. 13. Vimentin labelling using antibody VIM 13.2 in periodontal ligament fibroblasts and cementoblasts. (*a*) Weak vimentin labelling in cementoblasts (C) and in fibroblasts of the periodontal ligament (PDL) in a 2-wk-old rat. Bar, 50 µm. (*b*) Strong vimentin labelling in cementoblasts (C) and in fibroblasts of the periodontal ligament (PDL) in a 4-wk-old rat. Bar, 25 µm.

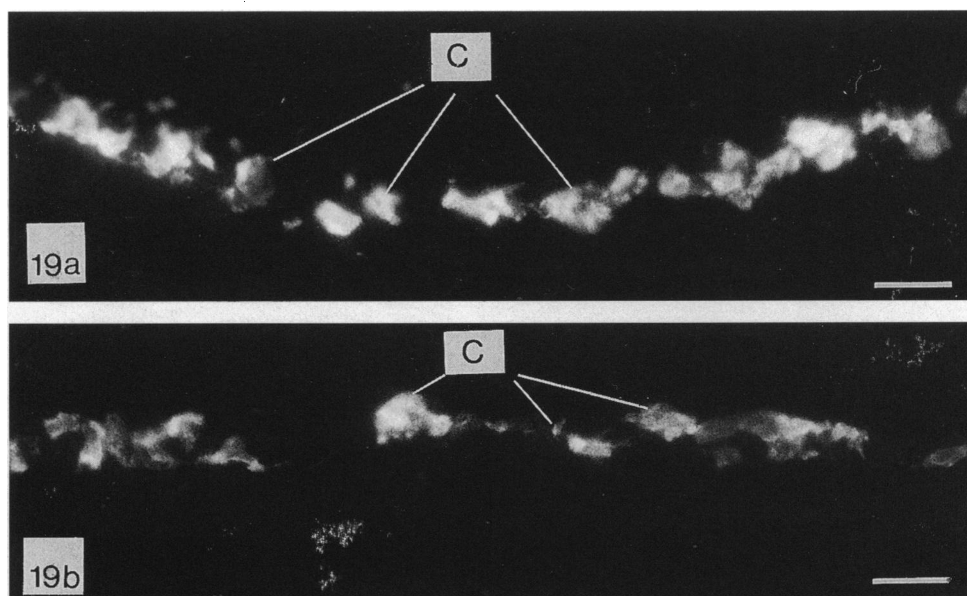


Fig. 19. Immunolocalisation of cytokeratins in cementoblasts (C) of a 2-wk-old rat with antibody CY-90 labelling for cytokeratin 18 (*a*) and antibody MAB 1673 labelling for cytokeratin 8 (*b*). Bars, 15 μm.

tissues of the periodontium but was present in epithelia.

DISCUSSION

The cytokeratin antibodies demonstrated the same range of labelling in rat as in human tissues, suggesting that they bind to homologous cytokeratins. The antibodies did not cross react with the antibody to vimentin as they labelled different sets of filaments in the same cell. Coexpression of cytokeratins and vimentin has been reported previously in several mesenchymal cell types, including those of the regenerating newt limb (Ferretti et al. 1988), fish and rat cartilage (Markl & Franke, 1988) and developing myocardial cells (Kuruc & Franke, 1988).

Typically for cells of mesenchymal origin, periodontal ligament fibroblasts labelled for vimentin. By analogy with other tissues, the accumulation of vimentin may be related to mechanical loading. There is evidence to suggest that it accumulates in cartilage and fibrocartilage and endothelial cells in response to mechanical loading (Ralphs et al. 1992; Schnittler et al. 1993; Benjamin et al. 1994). The presence of vimentin at all ages in periodontal ligament fibroblasts therefore suggests that it may be related in some general way to the loading of the ligament. Transiently, the cells also express cytokeratin 19. The time of expression coincides with the maximum rate of tooth eruption into the oral cavity (Moxham & Berkovitz, 1995), persisting until occlusion at 4 wk. When cytokeratin 19 is present, the periodontal ligament is undergoing a major structural reorganisation of its extracellular matrix (Melcher & Correia, 1971). It has been suggested that intermediate filaments could form part of a mechanotransduction system which enables the cells to respond to external forces and sense changes in the extracellular matrix (for a review see Benjamin et al. 1994). Thus, the expression of an additional type of filament could be related to the effects of periodontal ligament remodelling and the changing conditions during tooth eruption (Pitaru et al. 1987). In epithelia, cytokeratin 19 commonly occurs at sites of rapid cell proliferation

Fig. 14. Immunolocalisation of acidic and basic cytokeratins in the tooth germ of a neonatal rat using antibody K8.13. Cytokeratins were only present in the epithelial components of the developing tooth–ameloblasts (AM) and the epithelial root sheath (ERS). Bar, 20 μm.

Fig. 15. Immunolocalisation of cytokeratins in cementoblasts (C) using antibody K8.13 in a 2-wk-old animal (*a*) and a 3-wk-old animal (*b*). At 2 wk the cells have started to assume a cuboidal shape which is finally attained at 3 wk. Bars, 25 μm (*a*); 10 μm (*b*).

Fig. 16. Immunolocalisation of cytokeratins with antibody K8.13 in cells at the root apex at 2 wk. Several layers of fibroblast-like cells (F) label at the root apex. Bar, 25 μm.

Fig. 17. High power view of the region enclosed in the rectangle in Figure 17. Note the numerous cytoplasmic processes extending from the cells (arrowheads) and the honeycomb network of cytokeratin filaments within the cells. Bar, 10 μm.

Fig. 18. Transient immunolocalisation of cytokeratin 19 in fibroblasts of the periodontal ligament (PDL) of a 3-wk-old rat with antibody K4.62. Bar, 15 μm.

(Gigi-Leitner et al. 1986). This may also be the case in the periodontal ligament. Studies of the periodontal ligament have demonstrated measurable cell proliferation rates during molar eruption in the mouse (Perera & Tonge, 1981*a*, *b*) with peak proliferation rates occurring at the time that tooth eruption is occurring.

Cytokeratins are obligate heteropolymers (i.e. they occur in cells as pairs with acidic and basic partners (Hatzfield & Franke, 1985; Eichner et al. 1986)). Although any pair of acidic and basic keratin polypeptides will form stable filaments in vitro (Steinert et al. 1982; Hatzfield & Franke, 1985), coexpressed pairs in vivo demonstrate a preferential affinity for one another (Hatzfield & Franke, 1985; Eichner et al. 1986). This probably reflects a functionally significant stability of coexpressed polypeptides. Cytokeratin 19 is exceptional in that it does not have a single partner, but can complex with any basic cytokeratin (Hatzfield & Franke, 1985). In the present study, we have been able to screen for 4 of the 8 basic cytokeratins, but we have failed to find a partner for cytokeratin 19 in the periodontal fibroblasts. Whilst one of the other basic cytokeratins may be present, it is also possible that cytokeratin 19 could be interacting with the vimentin network (Lu et al. 1993; Klymkowsky, 1995). However, other evidence has demonstrated that the alignment of vimentin molecules is different from that of keratin filaments, suggesting that they are incapable of assembly into intermediate filaments in vivo (Steinert et al. 1993).

The epithelial root sheath of Hertwig is known to give rise to 'epithelial rests' within the periodontal ligament (Lester, 1969) and both the root sheath and epithelial rests label for the cytokeratin pair 8 and 18. Cementoblasts associated with acellular cementum also label for the same intermediate filaments, suggesting the possibility that they are also derived from the epithelial root sheath. Whilst there is no other direct evidence for this, it is worth noting that cementoblasts associated with acellular cementum differ in other ways from cementoblasts associated with cellular cementum. On the basis of immunolabelling characteristics for growth factors and osteoblast specific markers (Tenerio et al. 1993), it has been suggested that cementoblasts of cellular cementum and osteoblasts share the same precursor cells, and that they differ in labelling characteristics from cementoblasts of the acellular cementum. In addition, Cho et al. (1991) distinguished between the 2 types of cementoblasts on the basis of immunolabelling for epidermal growth factor.

Several authors have provided evidence suggesting that the formation of the root surface in rats is different from other mammalian species (Owens, 1976, 1978). Thus it may be that the role of root sheath epithelia in cementum formation may be species specific. Although our findings should be supported by further studies to demonstrate whether root sheath epithelia are involved cementogenesis, the root sheath has been shown to contain mRNAs for cementum proteins (Breitbart et al. 1987; Slavkin et al. 1988, 1989, 1990) which suggests that these epithelial cells may secrete cementum specific proteins (Bringas et al. 1985). Thus, although several authors suggest that cementoblasts are derived from the cell population of the inner (investing) layer of the dental follicle (Ten Cate et al. 1971; Palmer & Lumsden, 1987), our results suggest that the root sheath in rats may give rise to the population of cementoblasts associated with acellular cementum.

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