

ANTISERUM TO INTERCELLULAR MATERIAL AND BASEMENT MEMBRANE PRODUCED BY IMMUNIZATION WITH ORAL EPITHELIAL CELLS

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Abstract. Antisera to non-enzymatically released oral epithelial cells were produced in rabbits. Reactivity with gingival sections, by immunofluorescence, was observed for intercellular (IC) and basement membrane (BM) zones. Both IC and BM reactivity could be blocked with Concanavalin A (500 and 1000 $\mu\text{g/ml}$ respectively) and anti-human fibronectin serum. Pretreatment with pemphigus serum blocked IC reactivity only. Pemphigus antigen may reside in the material present between coherent epithelial cells and be absent from enzymatically dispersed cells.

Key words: Epithelium; Cells; Experimental antibodies; Pemphigus; Fibronectin

Antisera to enzymatically released epithelial cells have been used to study the distribution of cell surface antigens in the tissue of origin (7, 8, 11) and to determine the degree of tissue specificity of these antisera (7, 12). Thus, antisera to rat epidermal cells were found to possess reactivity with epidermal intercellular material, extending generally from the basement membrane zone of cells to the surface (7, 11); they are of interest because of the apparent similarity to the immunofluorescent (IF) staining pattern observed with pemphigus sera and fluorescein-conjugated concanavalin A (14). However, receptor sites for the epidermal cell antisera differ from those for pemphigus sera and concanavalin A (conA) with regard to solvent resistance and reciprocity of blocking tests (5). In addition, the epidermal cell antisera receptors did not co-precipitate with ConA receptors in cell lysate experiments (8). These findings would suggest that pemphigus sera and conA receptor sites might not be displayed on the cells used for immunization in these investigations (7, 8, 11, 12).

In all of these studies, the epidermal cells were derived with the use of trypsin, so that it is likely that material was removed from the cell surface (2); this material may include receptors for pemphigus

sera and conA. In this investigation we have produced antisera against human oral epithelial cells derived without the use of exogenous enzyme and have examined the reactivity of these antisera with the tissue of origin.

MATERIAL AND METHODS

Anti-epithelial cell sera (AES)

Human gingival fragments removed during surgical treatment of periodontal disease, were finely minced and cultured in TC-199 plus 10% autologous serum for 96 hours. Released cells were washed $\times 3$ in TC-199 and stored in TC-199 plus 10% dimethyl sulphoxide at -80°C . Samples of the released cells were centrifuged (20 g) through 6.5% glutaraldehyde and prepared for examination by electron microscopy. White New Zealand rabbits were injected intravenously with 1.5×10^6 epithelial cells (viability approximately 60% by trypan blue exclusion), 14 days apart and bled 14 days later. Sera were decomplexed, absorbed with glutaraldehyde precipitated human serum protein (the precipitate from 10 ml of whole blood incubated with 5 ml of antiserum for 30 min at 37°C) and stored at -70°C . The antisera were also absorbed with peripheral blood cells of the donors of the specimens described below. Immunoelectrophoresis of the absorbed and unabsorbed antisera was carried out against human serum.

Specimens

Gingival fragments were either (a) frozen in isopentane cooled in liquid nitrogen immediately after removal, or (b) washed in 100 ml of turbid phosphate-buffered saline at 4°C for 48 hours.

Immunofluorescent staining

Sections were stained with the antiserum and sheep anti-rabbit immunoglobulin-FITC (Wellcome Laboratories, F/P molar ratio 4.25, protein concentration 7.6 mg/ml, dilution 1/32, confirmed as anti-rabbit IgG by immunoelectrophoresis).

Examinations were made with an American Optical epi-illuminating fluorescence microscope with BG12 exciter and G515 barrier filters. Blocking tests were carried out as shown in Table I.

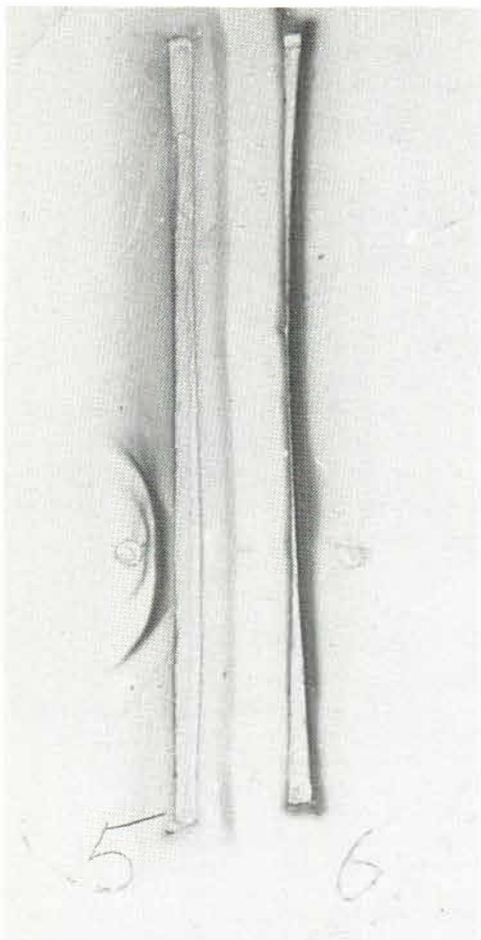


Fig. 1. Immunoelectrophoresis of unabsorbed and absorbed (glutaraldehyde precipitate from 10 ml of whole blood incubated with 5 ml of antiserum for 30 min at 37°C) rabbit anti-oral epithelial cell serum against human serum. 5, well = unabsorbed antiserum; 6, well = absorbed antiserum; troughs = normal human serum.

Enzyme and alcohol treatment of sections

Sections were incubated, separately, with collagenase (0.025–0.0005%) trypsin (0.025–0.0005%) for 20 min at 37°C and alcohol (50–90%) at 37°C prior to immunofluorescent (IF) staining.

RESULTS

Unabsorbed antisera produced two distinct bands upon immunoelectrophoresis against human serum (Fig. 1) which were not detected with the absorbed sera. Electron microscopic examination of the cells used for immunization revealed nucleated and anucleate cells, all with numerous tonofilaments. In un-

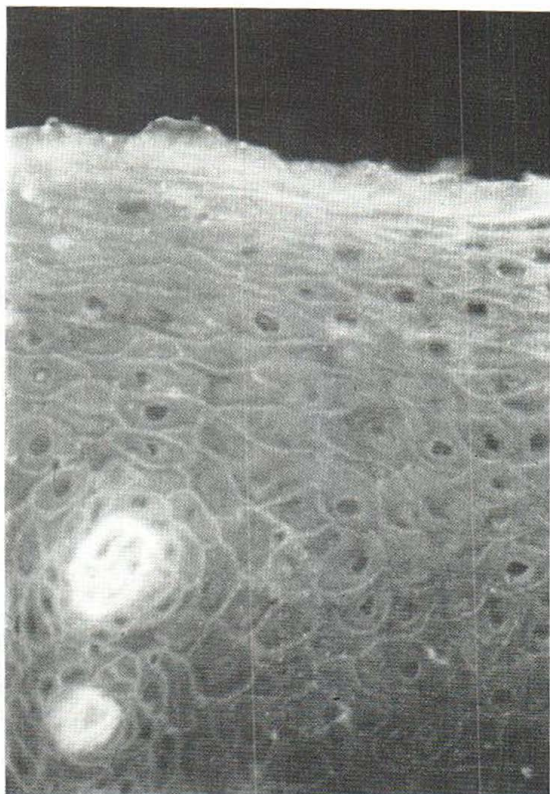


Fig. 2. Human gingiva. Anti-epithelial cell serum (1:16), $\times 280$.

treated gingival specimens, IF staining was observed in the intercellular (IC) and basement membrane (BM) areas (Fig. 2). On prolonged washing the IC staining was removed from the periphery of the specimens (Fig. 3). In both cases, BM staining was present to a higher titre (1/256) than IC staining (1/64). Absorption of the antiserum with epithelial cells (Table I) removed the IC and BM IF. Pretreatment of the sections with conA removed the IC IF (500 $\mu\text{g}/\text{ml}$), Table II, Fig. 4) and IC and BM IF (1000 $\mu\text{g}/\text{ml}$, Table II).

Pretreatment of the sections with anti-human fibronectin serum also removed IC and BM staining, whereas pretreatment with pemphigus serum removed only IC staining (Table II). Absorption with type IV, but not type I or III collagen, removed both IC and BM IF.

DISCUSSION

Antisera to oral epithelial cells derived without the use of enzyme appear to be directed against sites

Table 1. Treatment of the antisera and sections prior to immunofluorescent (IF) staining

Absorption of antisera	Pre-incubation of sections prior to IF staining
Epithelial cells	32 000 cells/ml of 1/32 AES, 30 min at 37°C, ×3
Collagen Type I	2 mg/ml for 30 min at 37°C; further 5 mg/ml for 24 and 48 hours at 4°C
Collagen Type III	2 mg/ml for 30 min at 37°C; further 5 mg/ml for 24 and 48 hours at 4°C
Collagen Type IV	10 mg/ml overnight at 4°C
Concanavalin A (Sigma Ltd., Poole, Dorset)	5–1000 µg/ml, 30 min at 37°C
Pemphigus serum (PS) ^a	Neat, ½, ¼
Pemphigoid serum (PdS) ^{a, b}	Neat, ½, ¼
Rabbit anti-human fibronectin serum (Collaborative Research Inc. Waltham, Mass. USA)	Neat, ½, ¼
Rabbit anti-hamster fibronectin serum	Neat, ½, ¼

^a Titre 1/64 guinea pig oesophagus.

^b Titre 1/32 guinea pig oesophagus.

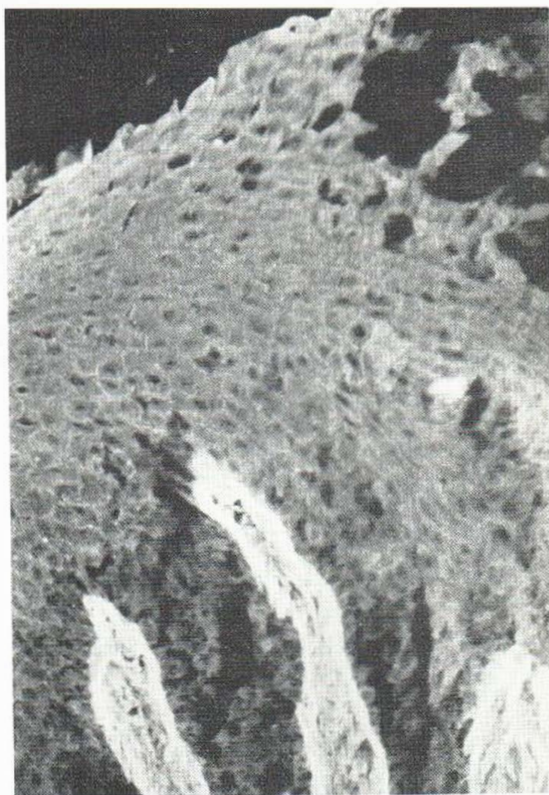


Fig. 3. Human gingiva. 48-hour washing procedure prior to anti-epithelial cell serum (1:16), ×125.

within epithelium which differ from those revealed when enzyme is used to release the cells (7, 8, 11, 12). Thus conA at a concentration of 1 mg/ml blocked the IC and BM sites (Fig. 3), whereas a concentration of 6 mg/ml of lectin did not block IC sites with antisera raised against trypsin-released rat epidermal cells (5). Pemphigus serum also blocked the IC sites, whereas blocking of IC sites does not occur with pemphigus serum and antiserum to rat epidermal cells (5). The sites for the anti-epithelial cell serum were also trypsin labile, soluble in phosphate-buffered saline (Fig. 3), and apparently blocked by antiserum to human (but not hamster) fibronectin.

The BM reactivity of the antiserum, in addition to the IC reactivity, was unexpected. In this respect the antiserum differs markedly from pemphigus serum. The production of basement membrane glycoprotein by human and murine epithelial cells has been reported previously (6). That the material is fibronectin is suggested by the lability with trypsin and the blocking with anti-human fibronectin serum. Blocking was observed with Type IV, but

not Type I or III collagen; however it should be noted that collagenase treatment did not remove the reactivity (Table II), suggesting that an additional component of the Type IV collagen may have been responsible for the blocking effect. Fibronectin is accepted as a major component of normal fibroblasts in culture (13, 15). Fibronectin has also been shown to be produced by epithelial cells in culture (10) and has been detected in association with epithelial crypts cells of the rat (9). In the dermis, in contrast to gut-associated epithelia, there have been reports of the material at the dermo-epidermal junction (3, 4). Only in psoriatic epidermis has it been reported in association with cells—those of the cornified layer (4).

Attempts to demonstrate immuno-reactivity of the anti-epithelial cell antiserum with human fibronectin (Collaborative Research Inc.) were negative; the blocking observed with anti-human fibronectin serum may not have been specific. Although fibronectin bears immunological similarities

Table II. Effect of treatment of sections prior to incubation with anti-epithelial cell serum and immunofluorescent staining

IC = Intercellular, BM = basement membrane staining

Treatment	IC	BM	IC	BM	IC	BM
Trypsin	0.025 %	0.0025 %	0.0005 %			
Collagenase	0.025 %	0.0025 %	0.0005 %			
Concanavalin A	5-250 $\mu\text{g/ml}$	500 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$			
Alcohol	50 %	70 %	90 %			
Anti-human fibronectin serum	$1/2$	$1/4$	$1/8$			
Anti-hamster fibronectin serum	$1/2$	$1/4$	$1/8$			
Pemphigus serum	$1/2$	$1/4$	$1/8$			
Pemphigoid serum	$1/2$	$1/4$	$1/8$			
Normal rabbit serum	$1/2$	$1/4$	$1/8$			

to cold-insoluble globulin (1) the IC and BM reactivity would not appear to have originated from antibody formation to the components of autologous serum used in culture; absorption removed

the two bands demonstrable upon immunoelectrophoresis.

In summary, antisera to epithelial cells derived without the use of exogenous enzyme react with sites in the epithelial intercellular substance: these sites differ from those previously reported, where antisera to trypsin-released cells (7, 8, 11, 12) have been used and the blocking pattern observed with conA and pemphigus serum suggested a similarity to pemphigus serum receptor sites in the intercellular zone. The basement membrane reactivity observed may represent the detection of similar sites or of additional sites.

ACKNOWLEDGEMENTS

We would like to thank Dr R. Colin Hughes for the gift of antiserum to hamster fibronectin, Dr A. J. Bailey for the gift of Type IV human collagen and Dr D. G. Roberts for helpful discussion. We would also like to thank Mrs Aleya Ahmed and Miss Elizabeth Wyld for their technical assistance and the Medical Research Council for support of this work.

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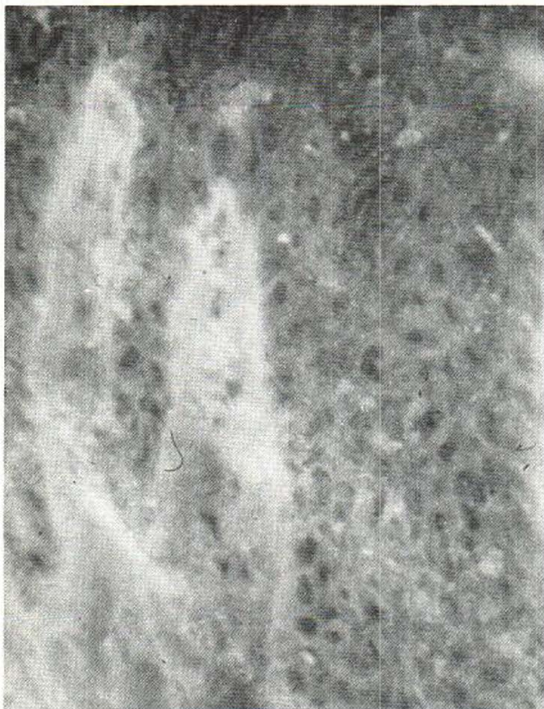


Fig. 4. Human gingiva, 500 $\mu\text{g/ml}$ concanavalin A followed by anti-epithelial cell serum (1:16), $\times 240$.

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Received March 10, 1981

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