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Immunocytochemical labelling of Merkel cells of human oral mucosa by means of antibodies to protein gene product 9.5

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ABSTRACT

Merkel cell (MC) are one of the non-keratinocyte cell populations that reside in oral epithelium. Protein gene product 9.5 (PGP 9.5) is a protein specifically present in the cytoplasm of neurons and neuroendocrine cells. It is demonstrated by immunofluorescence and immunoperoxidase that MC of oral human mucosa express PGP 9.5-like immunoreactivity. This finding is in agreement with the hypothesis that oral MC are paraneurons or neuroendocrine cells. The use of anti-PGP 9.5 serum may be of value for future studies of MC in normal and pathological oral tissues.

KEY WORDS:

Merkel cells - Oral mucosa - Human - Protein gene product 9.5

RÉSUMÉ

Les cellules de Merkel (MC) représentent une des populations cellulaires différentes des Kératinocytes qui résident dans les épithéliums de la cavité orale. Le Protein Gene Product 9.5 (PGP 9.5) est une protéine présente, de façon spécifique, dans les neurones et dans les cellules neuroendocrines. On démontre ici, en utilisant les techniques d'immunofluorescence et d'immunopéroxydase, que les MC de la muqueuse orale humaine présentent une immunoréactivité au PGP 9.5. Cette observation est en accord avec l'hypothèse que les MC de la cavité orale sont des paraneurones ou cellules neuroendocrines. L'emploi d'antisérums anti PGP 9.5 peut être utile pour d'ultérieures études sur les MC dans les tissus de la cavité orale aussi bien en conditions normales qu'en conditions pathologiques.

MOTS-CLÉS:

Cellules de Merkel - Muqueuse orale - Homme - Protein gene 9.5

INTRODUCTION

Merkel cells (MC) are one of the non-keratinocyte cell populations («clear cells») that reside in normal oral epithelia. Recently it has been demonstrated that oral MC may undergo malignant transformation (Reichler and Bourlond, 1986). The exact function (Munger, 1975; Gottschaudt and Vahle-Hinz, 1981) and the site of origin of MC are still a matter of debate (Winckelmann and Breathnach, 1973; English, 1977; Fortman and Winckelman, 1977: Tachinaba e Nawa, 1980; Moll et al., 1986; Ness et al., 1987; Ortonne et al., 1988). Electron microscopical studies have shown that MC can readily be identified in oral mucosa by the presence of cytoplasmic dense-core granules (Nikai et al., 1971; Hashimoto, 1972; Fortman and Winkelmann, 1977). By contrast, MC are difficult to distinguish from keratinocytes by routine light microscopy. Protein gene product 9.5

(PGP 9.5) is a 27 Kd protein that has been proved to be specifically contained in neurons and neuroendocrine cells (Jackson and Thompson, 1981; Thompson et al., 1985). With regard to oral tissues, antibodies to PGP 9.5 have been used to investigate the innervation of human dental pulp by immunocytochemistry (Casasco et al., 1989 a,b). In this study we report that MC of human oral mucosa display PGP 9.5-like immunoreactivity.

MATERIALS AND METHODS

Samples of healthy human gingiva and palatal mucosa were obtained during periodontal surgery from 20 volunteers. The samples were fixed in 4% paraformaldehyde solution in phosphate buffer, pH 7.4, for 6 h. After washing in phosphate buffer, the specimens were dehydrated throught graded alcohols, routinely embedded in paraffin and cut at 10 μm. The sections were then processed for indirect immunofluorescence and immunoperoxidase techniques (Polak and Van Noorden, 1986). All the antisera were diluted in Tris buffer, 0.15 M, pH 7.4.

Immunofluorescence. Rehydrated sections were incubated with the rabbit antiserum to PGP 9.5 diluted 1:1000 overnight at 4° C, washed in Tris buffer and incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Cappel, U.S.A.) diluted 1:100. After washing in Tris buffer, the sections were mounted in Tris buffer-glycerol (1:1) and examined in a Leitz Orthoplan fluorescence microscope.

Immunoperoxidase. Rehydrated sections were incubated with 0.3% hydrogen peroxide for 30 min to remove endogenous peroxidase activity. Possible back-ground staining was reduced by application of normal goat serum, diluted 1:20 in Tris buffer, for 30 min. The sections were then incubated with the rabbit antiserum to PGP 9.5 diluted 1:4000 overnight at 4° C, washed in Tris buffer and incubated with peroxidase-conjugated goat anti-rabbit IgG (Dakopatts, Denmark) diluted 1:100. After washing, peroxidase was visualised by incubation with 0.03% 3,3'-diaminobenzidine tetrahydrochloride solution in Tris buffer, to which hydrogen peroxide (0.03%) was added just before use. When developed the sections were dehydrated, mounted in DPX and examined under a Leitz Orthoplan transmitted light microscope.

Specificity controls. The rabbit antiserum to PGP 9.5 has been previously characterized (Gulbenkian et al., 1987). Pertinent specificity tests were performed, including absorption of the antiserum to PGP 9.5 with related and unrelated antigens, omission of the first layer and substitution of an inappropriate antiserum or a non-immune serum for the specific primary anti-PGP 9.5 serum (Polak and Van Noorden, 1986).

RESULTS

In the epithelium of all samples examined, some suprabasal cells, primarily localized at tips of rete ridges, displayed PGP 9.5-like immunoreactivity (Figs 1, 2). Moreover, nerve fibres and intraepithelial nerve endings closely associated to these cells were sometimes found immunoreactive (Figs 1, 2).

Many nerve fibres displaying PGP 9.5-like immunoreactivity were detectable in the lamina propria of the mucosa (data not shown). In control sections no specific staining could be observed.

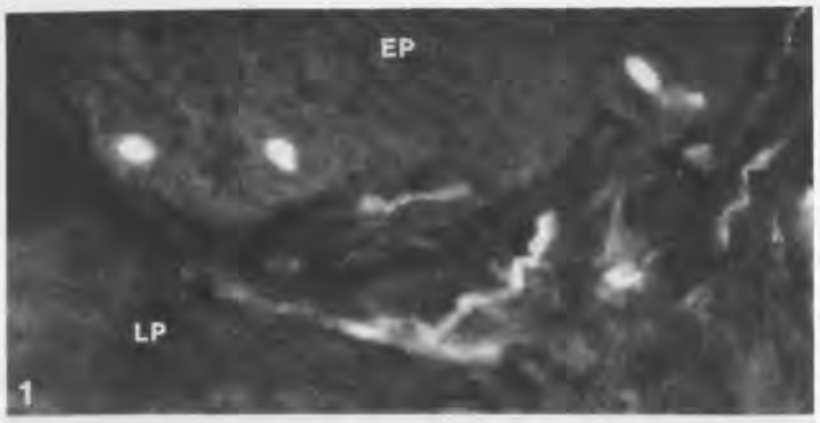
DISCUSSION

In this study we have demonstrated that some cells of human oral epithelium are immunostained by antibodies to PGP 9.5, a cytoplasmic soluble protein present in neurons and neuroendocrine cells. The number, the suprabasal localisation, the morphological features and the close relation with nerve fibres demonstrate that immunostained cells are MC, according to Ness et al. (1987).

Although the origin and function of the MC are still unclear, these cells are regarded as paraneurons, i.e. as a recepto-secretory cells (Fujita et al., 1988; Ortonne et al., 1988; Hartschuh et al., 1989) or as members of the «diffuse neuroendocrine cell system» (Pearse, 1986).

Previous investigations have demonstrated that MC of normal human oral mucosa can be immunocytochemically identified by means of antibodies to chromogranin A, a component of neuroendocrine granules, and with antibodies to 54-kD keratin (Ness et al., 1987). The occurrence of both chromogranin A and PGP 9.5 in the MC of human oral mucosa is compatible with the hypothesis that these cells are paraneurons or neuroendocrine cells. On the other hand, the interesting finding that oral MC contain cytokeratins strongly supports the epithelial origin of these cells (English, 1977; Ness et al., 1987).

No hypothesis about the role of PGP 9.5 in oral MC can be advanced, the function of PGP 9.5 being obscure. However, the use of PGP 9.5 antibodies may be of value for future studies of MC in normal and pathological human oral tissues.



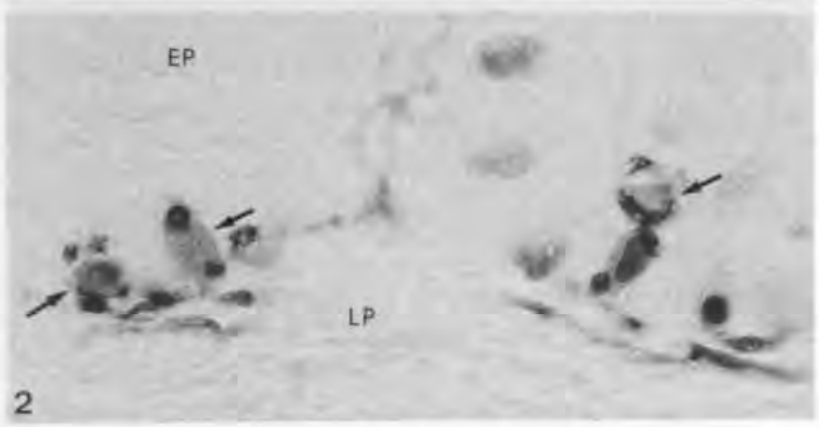


Fig. 1: Immunofluorescence micrograph of human gingiva after incubation with PGP 9.5-antiserum. Three immunoreactive Merkel cells are visible within the epithelium (EP); immunostained nerve fibres close to the immunoreactive cells are also visible in the underlying lamina propria (LP). Magn. 340 ×.

Fig. 1: Micrographie de gencive humaine après incubation avec antisérum anti PGP 9.5. Technique d'immunofluorescence. On peut voir dans l'épithélium (EP) trois cellules de Merkel immunoréactives; dans la lamina propria sous-jacente (LP) on peut aussi reconnaître certaines fibres nerveuses immunoréactive en proximité des cellules marquées. Grossissement 340 ×.

Fig. 2: Immunoperoxidase micrograph of human hard palate epithelium after incubation with PGP 9.5-antiserum. Immunoreactive Merkel cells (arrows) are detectable in association with intraepithelial nerve endings, that appear as immunoreactive dots. EP, epithelium; LP, lamina propria. Magn. 900 ×.

Fig. 2: Micrographie d'épithélium du palais dur après incubation avec antisérum anti PGP 9.5. Technique d'immunopéroxydase. On peut noter des cellules de Merkel immunoréactives (flèches) en association avec des terminaisons nerveuses intraépithéliales qui apparaissent comme des structures punctiformes immunoréactives. EP, épithélium; LP, lamina propria. Grossissement 900 ×.

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