Keratin Polypeptides Distribution
in Normal and Diseased Human Epidermis and Oral Mucosa

Immunohistochemical Study on Unaltered Epithelium and Inflammatory,
Premalignant and Malignant Lesions

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Summary. Immune sera against total keratin and keratin polypeptide subunits were induced in guinea pigs, using the different bands of SDS polyacrylamide gel electrophoresis of fibrous proteins of stratum corneum, derived from normal human epidermis.

The distribution of the different polypeptides was studied in numerous human biopsies of normal epidermis, normal oral mucosa and epidermal and mucosal inflammatory, premalignant and malignant lesions using the indirect immunoperoxidase method.

Antisera against total keratin (TK) and against the keratin polypeptide of M.W. 55,000 dalton (55K) labelled all keratinocytes in normal and pathological conditions. These antisera may be useful for the histodifferentiation in diagnostic pathology.

Antisera against the keratin polypeptides of M.W. 67,000 (67K) and 62,000 dalton (62K) identified only keratin antigens in the spinous, granular and keratinized layer of normal epidermis and oral mucosa. No labelling of the basal layer was achieved with these immune sera. However, there were important differences in the distribution of these keratin antigens in altered epithelia which may be of value in the differential diagnosis of inflammatory, premalignant and malignant lesions of the skin and oral mucosa.

Key words: Keratin polypeptides – Epidermis – Oral mucosa – Epithelial differentiation – Malignant transformation.

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Introduction

The cytokeratins of epidermal cells consist of a number of polypeptide subunits (molecular weight 40,000 to 67,000 dalton; Matoltsy 1975; Baden et al. 1976; Brysk et al. 1977; Baynes et al. 1978; Thivolet 1980). Anti-keratin sera have been used for the study of epithelial differentiation in in vitro cultures of epithelia (Sun and Green 1978; Franke et al. 1978), in animal experiments (Schmid et al. 1979; Franke et al. 1979) and in human tissue of various epithelial origin (Oberle et al. 1979; Schlegel et al. 1980). Recently, specific antibodies against different polypeptides of the intermediate-sized filament system have been induced in guinea pigs and tested on normal human and rabbit epidermis (Viac et al. 1980). Some differences in filament distribution in the various compartments of the epidermis were thereby observed (Viac et al. 1980; Viac et al. 1980).

The demonstration of the cytokeratins in normal tissue and pathological conditions of the epidermis and oral mucosa is valuable, for the following reasons: 1. It allows analysis and comparison of the keratinization processes in normal epidermis and oral mucosa. 2. It provides an additional morphological substrate for the identification and classification of epidermal and mucosal premalignant and malignant lesions.

In this study, the distribution of total keratin (TK) and three different cytokeratin polypeptides of molecular weight 67,000, 62,000 and 55,000 dalton (67K, 62K, 55K) was examined in 82 cases of normal epidermis and oral mucosa and in various inflammatory, premalignant and malignant lesions (lichen planus, leukoplakia, premalignant dyskeratosis, Bowen's disease, keratoacanthoma, basal cell carcinoma and squamous cell carcinoma) using the indirect immunoperoxidase technique (Bustamante et al. 1978; Thivolet et al. 1980).

Materials and Methods

1. Antigen Preparation. Keratin proteins were isolated according to the method of Baden and Lee (1978), modified by Viac et al. (1980). The fibrous proteins were analyzed by SDS polyacrylamide gel electrophoresis as described by Laemmli (1970). The distribution of the different keratin polypeptides is shown in Fig. 1.

2. Antibody Preparation. Keratin proteins purified from human stratum corneum (150 μg) were emulsified with Freund's complete adjuvant and injected intraperitoneally in adult female Hartley guinea pigs (400 g) using a method previously described (Viac et al. 1978). Keratin polypeptide antibodies were obtained by a recently published procedure (Staquet et al. 1979). The immune sera were absorbed with human erythrocytes and liver powder (Olson et al. 1972). There was no cross reactivity of immune sera with human serum albumin.

3. Material. The 82 specimens of the epidermis and oral mucosa were collected during the years 1979-1980 from the surgical department of the Dermatological Clinic (Pr. J. Thivolet) and from the Clinic of Maxillo-Facial Surgery (Pr. Dumas) of the Ed. Herriot Hospital (Lyon).

4. Immunoperoxidase Staining. The skin and mucosal biopsies were fixed in Bouin's solution for 24 h and embedded in paraffin. After cutting at 5 μ sections, keratin antigens were demonstrated by the indirect immunoperoxidase technique (Bustamante et al. 1978; Schmitt 1979; Thivolet et al. 1980) using the specific antisera described (diluted at 1/200) and a peroxidase-conjugated goat-anti-guinea pig-immunoglobulin serum (Nordic - diluted at 1/50). 3,3' diaminobenzidine (Sigma) was used to reveal peroxidase activity (Graham and Karnovsky 1966). Control reactions with preimmune sera instead of the primary specific antisera were included.