mentary change in the coat of animals or the skin of man. Such anomalies have been observed in zinc, protein, and various vitamin deficiencies (7, 12). However, in most of these, generalized symptomatology develops: we have not observed such in the thymectomized mice.

The histological basis of the coat depigmentation described above, could unfortunately not be studied. It is impossible to conclude with certainty that depigmentation of the coat is related directly to thymectomy. Although the hypothesis of a deficiency origin of coat depigmentation may be correct, the exact mechanism remains obscure.

ACKNOWLEDGEMENT

This work has been supported by INSERM (grant no. ATP 19-75-42).

REFERENCES


Phylogenetic Studies with Pemphigus and Pemphigoid Antibodies

Luis A. Diaz, Harlan J. Weiss and Nickolas J. Calvanico

Department of Dermatology, Immunodermatology Unit, University of Michigan Medical School, Ann Arbor, Michigan 48109 and the Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Received July 11, 1978.

Abstract. Indirect immunofluorescence (IF) studies were performed on skin from a variety of vertebrate specimens and IgG fractions from pemphigoid and pemphigus sera. Pemphigoid antigen was present in fish, amphibian, reptilian, avian and mammalian skin, whereas pemphigus antigen was observed in avian and mammalian skin only.

Key words: Epidermal antigens; Phylogenesis; Immunofluorescence; Pemphigus; Pemphigoid

Pemphigus and pemphigoid are two human diseases showing an abnormal epidermal cell adhesion (cell-to-cell in pemphigus and cell-to-basement membrane in pemphigoid) and an autoimmune antigen/antibody reaction, taking place on the surfaces of epidermal cells (differentiating keratinocytes in pemphigus and basal cells in pemphigoid). The defect in cell adhesion and the autoimmune phenomenon seem to be pathogenetically related (5, 7, 10). Pemphigoid antibodies react with an antigen located in the lamina lucida of the dermo-epidermal junction of mammalian skin, producing a linear pattern of staining by IF techniques (1, 4). Pemphigus antibodies, on the other hand, react with an antigenic moiety closely related to the outer leaflet of the cell membrane of differentiating keratinocytes (11). They produce a honeycomb staining of the epidermis by immunofluorescence (1). Pemphigus antibodies react only with stratified squamous epithelia, while pemphigoid antibodies react not only with these epithelia, but also with certain other epithelia, e.g. bladder and gall bladder (1). The

1 This work was presented in part at the American Society of Clinical Investigation, San Francisco, California, 1978.

Abbreviations: PBS = phosphate-buffered saline, IF = immunofluorescence, CF II = Cohn’s Fraction II, FITC = Fluorescein isothiocyanate, BMZ = Basement Membrane Zone.
antigens reacting with these antibodies are found not only in epithelial tissues of patients suffering from these diseases but also in those from normal individuals (1). Early studies showed their wide distribution in mammalian epithelial tissues (1). Recently Diaz et al. (4) have shown that pemphigoid antigen is also present in human saliva and urine. It has been suggested (2, 3, 8, 9) that neoplastic, viral or carcinogenic transformation of epidermal cells is associated with disappearance of these antigens.

This study was undertaken to investigate the distribution of antigenic moieties reacting with pemphigus and pemphigoid autoantibodies in vertebrate skin.

**MATERIALS AND METHODS**

**Vertebrate skin.** The specimens studied were obtained from the museum of Zoology (Dr D. W. Tinkle), the University of Michigan Amphibian Facility (Dr C. W. Nace) or were experimental animals sacrificed by other researchers. Biopsies and tissues from sacrificed animals were snap-frozen in liquid nitrogen and used as substrates for indirect IF studies, on the same day as sacrifice. Pemphigoid and pemphigus sera were heat inactivated and absorbed in human erythrocytes (A, B, O, LeA+, LeB+). The IgG fraction from each serum was purified by ammonium sulfate precipitation and DEAE-cellulose chromatography (5). The same antibodies have been used to monitor purification of the pemphigoid antigen from human skin (4). A stock solution of each of the IgG fractions with an indirect IF titer of 1:320 (human skin or monkey esophagus substrate) was prepared, aliquoted and stored at −20°C.

**Immunofluorescent techniques.** Indirect IF techniques used have been reported previously (1, 4, 5). Briefly, cryostat-cut sections, 4 µm thick, were made from each epithelial tissue under study. These sections were used as substrate tissues for pemphigoid and pemphigus antibodies. Sections were treated with a 1:20 and 1:40 dilution of the purified IgG stock solutions. A triplicate set of slides were simultaneously prepared. Control sections overlaid with normal human IgG (CF-11, Sigma, St. Louis.

**Fig. 1.** Indirect IF studies: quail skin (foot pad) treated with pemphigoid IgG 1:20 (×175). Arrow shows the linear staining of the BMZ.
Mo. 5 mg/ml] and PBS were always included in each experiment. FITC conjugated goat antihuman IgG (Hyland, Division Travenol Laboratories, Costa Mesa, Ca., Molar F/P=3.3) was used as previously described (4, 5). The slides were read with a Zeiss fluorescence microscope and epi-illumination.

RESULTS

The qualitative detection of these antigens by indirect IF using pemphigus and pemphigoid antibodies in various vertebrates is summarized in Table I. It can be seen that antigens reacting with both autoantibodies were found in all mammalian skin examined, suggesting wide distribution in this class. In addition, they were also detected in quail skin (Fig. 1) which suggests that birds also have both antigens although only one member of this class has been examined. The patterns of staining for mammals and birds were similar.

In contrast to mammals, the skin of reptiles, amphibians and fish only reacted with pemphigoid antibodies, exhibiting the usual linear staining of the basement membrane zone (Fig. 2a, 2b, 2c, 3a, 3b). Control tissue sections treated with human IgG (CF-II) or PBS showed negative results.

DISCUSSION

This phylogenetic survey shows that pemphigus and pemphigoid antibodies react with the skin of a wide variety of vertebrates producing the typical "pemphigus" and "pemphigoid" patterns by indirect IF. As seen in Table I, the antigenic moieties reacting with pemphigus antibodies were detected in mammalian and avian skin only, whereas pemphigoid antibodies reacted with all vertebrate skin tested. Preliminary studies in our laboratory indicate that pemphigus and pemphigoid antigens are immunologically related (6). It is possible therefore, that pemphigoid antigen, a more primitive
antigenic moiety, gave rise to the pemphigus antigen during evolution. Another possibility is that the precursor of pemphigus antigen is distinct from the pemphigus antigen detected in mammalian and avian skin and shows no reactivity with our antiserum by indirect IF.

Further studies with other species and antisera will help to elucidate this question. In either case, however, it is clear that the pemphigoid antigen has been preserved during evolution, thus indicating that it may have an important function.

ACKNOWLEDGMENTS
This work has been supported by NIH Biomedical Research Support Grant no. RR-05383.

REFERENCES

Distribution of Complement and Immunoglobulin in Oral Pemphigus Lesions
Erik Dabelsteen
Departments of Oral Pathology and Oral Diagnosis, Royal Dental College, Copenhagen, Denmark
Received March 4, 1978

Abstract. The present report describes four cases of oral pemphigus vulgaris lesions in which complement, in contrast to the situation as reported for skin, was found only in the basal and parabasal layer, whereas IgG was present in all layers of the epithelium.

Key words: Pemphigus vulgaris; Complement; Oral mucosa

Pemphigus is a bullous disease affecting skin and oral mucosa. It is characterized histopathologically by intra-epithelial bullae with loss of cohesion of the epithelial cells (7). Immunopathological circulating antibodies to the cell surfaces of stratified squamous epithelium are found in patients with disease in the active stage and, furthermore, studies on biopsy specimens from affected areas have demonstrated in vivo localization of immunoglobulin and complement at the periphery of the epithelial cells (1, 6).

Biopsies from pemphigus vulgaris lesions in which complement was found only in the basal and parabasal layer, whereas immunoglobulin was present in all layers of the epithelium.

MATERIAL AND METHODS
The clinical and histological data of the patients are given in Table I. All patients were investigated for in vivo bound antibodies and complement in lesions and surrounding normal mucosa and for the presence of circulating antibodies to the cell periphery of stratified squamous epithelium.

Biopsy specimens were taken under local anesthesia, immediately frozen and stored at −70°C. Specimens were processed for detection of in vivo deposits of IgG, IgM, IgA and C3 using conjugates and immunofluorescence staining methods previously described (4, 11). The conju-