FIXATION OF ANTIBODIES OF PATIENTS WITH PEMPHIGUS VULGARIS AND PEMPHIGUS FOLIACEUS IN THE SNAKE EPIDERMIS

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Autoantibodies of patients with pemphigus vulgaris are known to react specifically with antigens of certain types of epithelium of ectodermal origin and, in particular, the Hassall's corpuscles of the human and animal thymus [1]. The serum of patients with other systemic autoimmune diseases (myasthenia gravis) has been shown to contain antibodies against antigens of myoid cells of the reptilian thymus [2]. Reptiles are the first group of animals on the evolutionary ladder which, like birds or mammals, are characterized by separation of a rejected graft [5]. Antigens with which the autoantibodies (Igb) of patients with true pemphigus react are present in the stratum germinativum of stratified squamous epithelium of man, mammals, and birds ("pemphigus" antigens). Under normal conditions the immune system is tolerant of these antigens. Loss of tolerance, developing for unknown reasons, leads to the onset of acantholysis – loss of connections between cells of the malpighian layer of the epithelium, leading to separation of the superficial layers of the epidermis and of the mucous membranes in patients with true pemphigus. The possibility cannot be ruled out that acantholysis is based on the same mechanism of skin rejection as the seasonal change of the outer cover (molting) in some representatives of the animal kingdom. This suggestion formed the basis for the present investigation, the aim of which was to look for "pemphigus" antigens in the epidermis of snakes.

EXPERIMENTAL METHOD

Experiments were carried out by the usual [6] indirect immunofluorescence method (IIF) using luminescent antibodies against human Igb (Amersham, England). Antibodies were obtained from sera of untreated patients with pemphigus vulgaris and pemphigus foliaceus, with Igb titers of 1/1280 to 1/5120. The titer of serum autoantibodies was determined by IIF tests, using the mucous membrane of the guinea pig esophagus as the substrate [3]. Frozen sections of skin from the grass snake (Natrix natrix) 3-5 μ thick served as the test object. Skin samples were taken from the snakes a few days before the next molting, during molting, and 3, 5, 10, and 30 days after molting. The imminence of molting was shown by opacity of the cornea and the appearance of a whitish color of the grass snake's skin.

EXPERIMENTAL RESULTS

Autoantibodies from patients with pemphigus vulgaris bound with antibodies of the stratum germinativum of the grass snake during the period preceding molting, and also during molting, but the IIF test after the appearance of the new skin gave negative results (Table 1). The skin of the snakes reacted unequally with sera from patients with different clinical forms of pemphigus vulgaris. Before molting, epidermal antigens bound with antibodies from patients with pemphigus vulgaris, but during molting they bound with antibodies obtained from patients with pemphigus foliaceus. Luminescence of antibodies from patients with pemphigus vulgaris was diffuse in character and was observed in all layers of the snake's epidermis located above the basement (Fig. 1). Antibodies of patients with pemphigus foliaceus were fixed in skin samples obtained from molting snakes only in the surface layers of the epidermis (Fig. 2). Fixation of antibodies from patients with pemphigus vulgaris was not ob-

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Fig. 1. Epidermis of grass snake a few days before molting, treated with serum from patient with pemphigus vulgaris. Diffuse deposition of class Igb autoantibodies in stratus germinativum of epidermis. Intensity of fluorescence ++++. Here and in Figs. 2 and 3: IIF, 1175×.

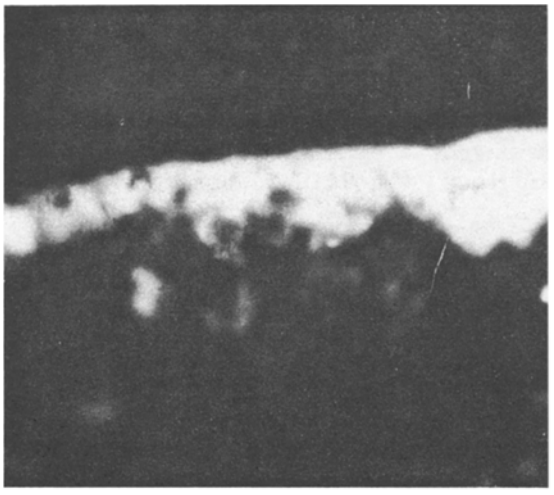

Fig. 2

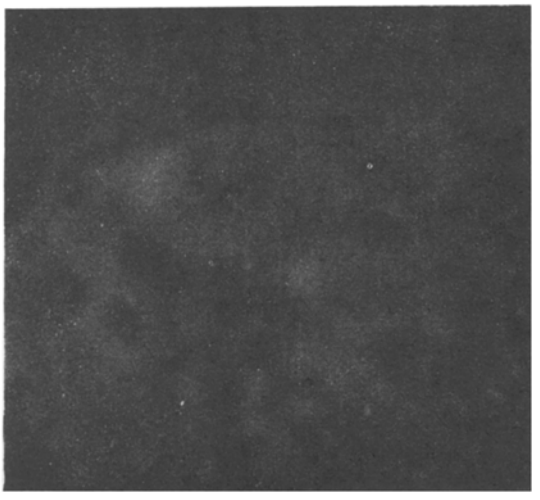

Fig. 3

Fig. 2. Grass snake skin rejected during molting and treated with serium from patient with pemphigus foliaceus. Luminescence of upper layers of epidermis. Intensity of fluorescence ++++.

Fig. 3. Grass snake skin renewed after molting and treated with serum from patient with pemphigus vulgaris. Absence of immunofluorescence in epidermis.

served in the regenerating epidermis of the renewed skin (Fig. 3). These data, in our opinion, reflect transformation of the "pemphigus" antigen during death of the upper layers of the epidermis during molting.

If the results of these experiments are extrapolated to immunopathological processes taking place in the affected skin of the patients, it can be fairly confidently postulated that a glycoprotein (receptor?) expressed on the surface of the epitheliocytes of patients with pemphigus vulgaris [4] and the antigen appearing in the snake epidermis before molting is the same substance. Moreover, the structure of this substance evidently changes: in patients depending on its location in the epidermis, in the snakes depending on death of the upper layers of the epidermis. On the basis of the fact that autoantibodies of patients

TALBE 1. Results of Immunofluorescence Investigation of Snake Skin

Period of investigation	Titer of autoantibodies in sera tested from patients with pemphigus vulgaris			pemphigus foliaceus		
	1/1280	1/1280	1/2560	1/1280	1/2560	1/5120
Before molting	++++ ++ +++ ++++	++++ +++ +++ +++	++ ++++ +++ ++++	+ − − +	− + − −	+ − − −
During molting	− − + −	− − − −	+ + − +	++++ +++ ++++ ++++	++++ +++ +++ +++	++++ ++++ +++ +++
Time after molting, days:						
3	−	−	+	−	−	−
5	−	−	−	−	−	+
10	−	+	−	−	−	−
30	+	−	−	−	+	−

Legend. "Plus" and "minus" signs indicate intensity of fluorescence.

with pemphigus vulgaris directed against the antigen located in the stratum spinosum of the epidermal cells reacts specifically with the skin of snakes in the initial phase of molting, whereas antibodies against the antigen of the stratum granulosum, obtained from patients with true pemphigus are specifically bound by the dying epidermis of the molting snakes, it can be tentatively suggested that the "molting" antigen appears initially on the surface of cells located above the stratum basale of the epidermis, but later, during differentiation of the keratinocytes and their transformation into keratin scales, modifies its structure and, together with the dying cells, is displaced into the upper layers of the epidermis. The results of the present investigation, showing the presence of common antigens in the molting grass snake skin and the epidermis of patients with true pemphigus, in that case will be evidence in support of a single mechanism of skin rejection, probably under the control of a humoral factor coded by the same gene. Accordingly, loss of tolerance to antigens of their own skin by pateints with true pemphigus might be interpreted as atavism, expressed as the triggering of synthesis (or secretion) of a humoral factor, "prohibited" for man, and responsible for the appearance of "pemphigus" ("molting") antigens on the surface of the stratified squamous epithelial cells, attracting cytotoxic antoantibodies to themselves.

Expression of the same antigen by epidermocytes of snakes and man thus is, in the first case, a physiological phenomenon essential for renewal of the skin, whereas in the second case, it leads to the development of a severe and potentially lethal autoimmune bullous dermatosis, namely pemphigus vulgaris.

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