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AN IMMUNOFLUORESCENCE STUDY OF LIGHT CHAIN IN PEMPHIGUS

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Abstract. Immunofluorescence studies were made on light (L) chain of immunoglobulins in patients with pemphigus. These patients fell into two groups: a subcorneal bulla group, consisting of 10 patients with Senear-Usher syndrome, and one with pemphigus foliaceus, and a suprabasal bulla group consisting of 8 patients with pemphigus vulgaris. In the former group there was deposition of L chains of either type alone in the intercellular spaces of the upper epidermis: kappa type alone in 9 and lambda type alone in 2 patients. Deposition of L chains of both types was observed in the interce llular spaces of the lower epidermis in all patients of the latter group. No significant difference existed between groups as to the titre of circulating intercellular antibodies of either type. Some comments are made on the significance of the L chain of immunoglobulins in pemphigus.

Key words: Immunofluorescence studies; Pemphigus; Light chain of immunoglobulin; Kappa; Lambda

The immunological features of pemphigus include IgG deposition in the intercellular spaces of the epidermis and the presence of circulating intercellular substance antibodies (IC antibodies). There is at present no known immunohistologic difference between subcorneal and suprabasal lesions presenting as early histopathological changes associated with pemphigus. As regards the maturation and differentiation of epithelial cells, however, it is likely that some difference is present in antigenicity between intercellular substances of the upper and the lower epidermis. Bystrin et al. (2) found that antibodies reacted specifically with antigens present only in the subcorneal intercellular substance of the epidermis of patients with pemphigus foliaceus. There are no studies in the literature, however, of the immunohistochemical difference between the subcorneal and the suprabasal bulla group in vivo.

Immunoglobulins consist of heavy (H) and light (L) chains. It has been reported that kappa and lambda types of L chains have a constant ratio in various autoantibodies (6) and that different types of L chains are produced by different cells (1). These previous studies led us to make a study of L chains in patients with pemphigus by means of immunofluorescence (IF) techniques and the results obtained are now presented. A difference between the subcorneal and the suprabasal bulla group with regard to L chain deposition in the epithelial intercellular spaces in vivo is demonstrated.

MATERIALS AND METHODS

Ten patients with Senear-Usher syndrome, one with pemphigus foliaceus, and 8 with pemphigus vulgaris were studied. The first two diseases are hereinafter designated the subcorneal and the latter the suprabasal bulla group in the study. Skin biopsies were obtained from the most recent possible skin lesions containing clinically normal skin. Skin tissue and serum specimens collected were kept frozen until subjected to IF study. The direct IF staining procedure was employed to examine for deposition in the patient's cutaneous tissue, and the indirect IF technique for the presence of circulating IC antibodies.

Direct IF technique

Cryostat cut sections 4–5 μm in thickness were prepared, air-dried for 15–30 min and then rinsed with phosphate-buffered saline (PBS), pH 7.2, for 15 min. After treatment with fluorescein-conjugated antisera, the slides were incubated in a moist chamber at room temperature for 30 min. After a second rinse with PBS for 20 min the slides were mounted with buffered glycerol before being examined in a fluorescence microscope (Chiyoda Co., Japan) under UV excitation.

Indirect IF technique

Doubling dilutions with PBS of the patient's sera were prepared, starting with a 4-fold dilution. Clinically normal skin of an operated patient with benign tumour or rabbit esophagus served as the substrate. These were kept frozen until required for use, when 4–5 μm cryostat-cut sections were prepared. After being air-dried for 15–30 min, the slides were incubated with the dilutions of the patient's serum in a moist chamber at room temperature for about an hour. After a rinse with PBS for 20 min the

Table I. *Characteristics of FITC-labelled anti-IgG, κ and λ*

	FITC content (μg/ml)	Protein content (ng/ml)	F/P molar ratio
Anti-IgG (specific for γ-chain)	23.4	5.0	1.9
Anti-kappa	25.7	5.4	2.0
Anti-lambda	27.3	4.6	2.4

tissue specimens were incubated with fluorescein-conjugated antiserum at room temperature for 30 min. The subsequent procedure was the same as in the direct IF method.

Conjugates

The fluorescein isothiocyanate (FITC)-labelled antihuman antisera used in this study include anti-IgG (γ chain specific), anti-kappa and anti-lambda antisera (all manufactured by Dakopatts Co., Denmark). The characteristics of these antisera are shown in Table I. The conjugates were diluted 1:20 before use. Their specificity was assessed by immunoelectrophoresis and Ouchterlony's method. A blocking test with unlabelled antihuman anti-IgG (γ chain specific), anti-kappa and anti-lambda antisera (all manufactured by Dakopatts Co., Denmark) was also performed to confirm the specificity of positive results.

Specimens treated with FITC-labelled antihuman antisera alone, incubated with normal human sera and subjected to the blocking test, served as a control in the indirect IF procedure.

RESULTS

1. *IF findings of the patient's skin*

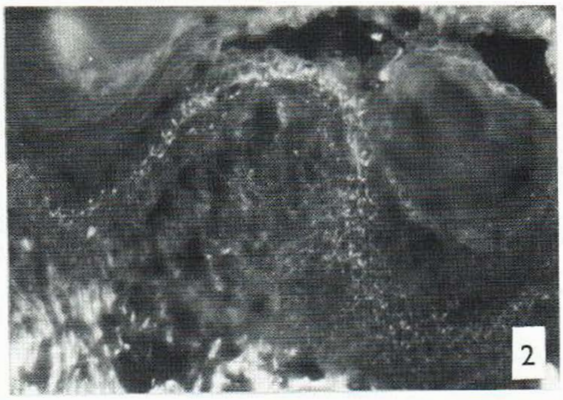
For convenience's sake the epidermis was divided into two layers, upper and lower, for the detection of immunoglobulin deposits. The IF findings in individual patients are shown in Table II.

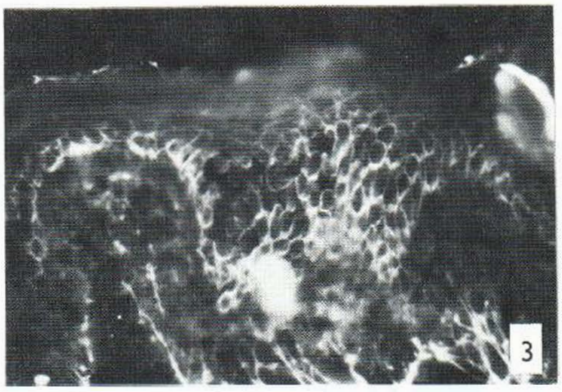
(A) *Subcorneal bulla group.* Deposition of L chains in the intercellular spaces of the epidermis was generally of two different types. In 9 of 11 cases (Cases 1–9) there was more extensive deposition of L chains of kappa type than of lambda type, whereas in the remaining 2 cases (10 and 11) the reverse was true. In this group, deposits in the intercellular spaces of the upper epidermis as the site of acantholysis were positive for the kappa type and negative for the lambda type in cases 1, 2 and 4–9 (Figs. 1, 2) and positive for the lambda type and negative for the kappa type in cases 10 and 11. Concurrent deposition of L chains of both types in the intercellular spaces of the upper epidermis was observed in a localized fashion in some cases but never constituted a consistent finding. In other words, L chains

Table II. *Direct IF stainings in pemphigus*

Cases 1–11: Subcorneal bulla group, cases 12–19: Suprabasal bulla group

Case no.	Sex	Age (y.)	Diagnosis	Direct IF stainings: The upper epidermis γ	κ	λ	The lower epidermis γ	κ	λ
1	F	17	Senear-Usher syndrome	+	+	−	+	−	−
2	M	61		+	+	−	+	+	+
3	F	64		+	±	−	+	+	−
4	F	44		+	+	−	+	−	−
5	M	37		+	+	−	+	+	−
6	F	57		+	+	−	+	−	−
7	F	27		+	+	−	+	+	+
8	F	70		+	+	−	+	+	+
9	M	43	Pemphigus foliaceus	+	+	−	+	+	−
10	F	44	Senear-Usher syndrome	+	−	+	+	−	+
11	M	67		+	−	+	+	±	+
12	M	44	Pemphigus vulgaris	+	+	+	+	+	+
13	F	52		+	+	+	+	+	+
14	F	72		+	+	+	+	+	+
15	F	18		+	+	+	+	+	+
16	F			−	−	−	+	+	+
17	F	60		−	−	−	+	+	+
18	M	47		−	−	−	+	+	+
19	F	40		−	−	−	+	+	+





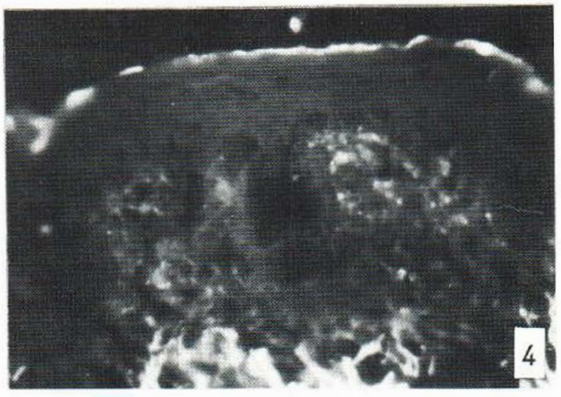


Fig. 1. Direct IF staining of the epidermis in Senear-Usher syndrome, showing deposition of kappa in the intercellular spaces of all layers of the epidermis (case 8, ×130).

Fig. 2. Direct IF staining of the epidermis in Senear-Usher syndrome. Deposition of lambda in the intercellular space of the epidermis is negative in the upper layer of the epidermis, whereas it can be seen in the lower layer (same patient as in Fig. 1, ×260).

Fig. 3. Direct IF staining of the epidermis in Senear-Usher syndrome, showing intercellular deposition of kappa (case 5, ×260).

Fig. 4. Direct IF staining of the epidermis in Senear-Usher syndrome, showing no deposition of lambda in the intercellular spaces (same patients as in Fig. 3, ×260).

deposited in the intercellular spaces of the upper epidermis were of either kappa or lambda type in this group, the deposit consisting solely of L chains of kappa type in most cases (Figs. 3, 4).

IgG (γ chain) deposits in the intercellular spaces were located at either kappa- or lambda-positive sites.

(B) *Suprabasal bulla group.* IgG (γ chain) and L chains of both kappa and lambda type were all detected in the intercellular spaces of the upper epidermis in cases 12–15 in this group, but all of these were absent in cases 16–19. IF staining of the intercellular spaces of the lower epidermis as the site of acantholysis in this group was positive for IgG (γ chain) as well as L chains of both types in all cases. In other words, there was no deposition of L chains of either type alone in this group.

The IF features of L chains detected in the intercellular spaces of the upper epidermis in the subcorneal bulla group and in those of the lower epidermis in the suprabasal bulla group are summarized in Table III.

2. *Circulating IC antibodies*

The titre of circulating IC antibody was determined on sera collected at the peak of skin lesions. In cases where no evidence of the presence of IC antibodies could be obtained with normal human skin (cases 3, 4, 5, 10 and 11), rabbit esophagus was used as the substrate since it is more sensitive. The

Table III. *Summary of direct IF studies*

	Combination of IF staining of the intercellular space o		No. of positive cases/ No. of cases examined
	kappa	lambda	
Subcorneal bulla group (upper epidermis)	+	−	9/11
	−	+	2/11
	+	+	0/11
	−	−	0/11
Suprabasal bulla group (lower epidermis)	+	−	0/8
	−	+	0/8
	+	+	8/8
	−	−	0/8

IC antibody titre in individual cases is shown in Table IV.

In general, IC antibody titre tended to be higher in the suprabasal bulla group. In 5 of 10 cases in the subcorneal bulla group the titre of IC antibodies of kappa type was higher by 2 or more tubes than that of IC antibodies of lambda type. The titres of IC antibodies of kappa type were roughly as high as those of lambda type in the suprabasal bulla group. No difference in IC antibody titre was found between the subcorneal and the suprabasal bulla group.

DISCUSSION

Structurally, immunoglobulins are composed of H and L chains. H chains are designated γ, α, μ, δ, or ε, corresponding to respective immunoglobulin classes. L chains are common to all immunoglobulin classes and classified into kappa and lambda types. There are few studies available concerning the relationship between L chains and autoantibodies. Mannik & Kunkel (6) and Leddy & Bakemeier (5) found one of the types of L chains to be more common than the other. This finding is of interest, since it is significative of the unbalanced incidence of different clones producing autoantibodies.

Few studies have been performed investigating the L chains of immunoglobulins in patients suffering from dermatological disease. Provost & Tomasi (11) investigated L chains in herpes gestationis, bullous pemphigoid and lupus erythematosus but without subdividing the L chains into kappa and lambda types. Our present study appears to have been the first to demonstrate the difference with which deposition of kappa and lambda chains takes place in pemphigus.

Table IV. *Indirect IF findings (circulating IC antibody titre) in pemphigus*
Substrate: Normal human skin (*rabbit esophagus). ND: not done

Classes of IC antibody	Case no.										
	1	2	3*	4*	5*	6	7	8	9	10*	11*
(a) Subcorneal bulla group											
IgG type	1 : 64	1 : 64	1 : 16	1 : 32	1 : 16	1 : 32	1 : 512	ND	1 : 512	1 : 16	1 : 64
Kappa type	1 : 32	1 : 32	1 : 16	1 : 16	1 : 16	1 : 32	1 : 128	ND	1 : 256	1 : 8	1 : 16
Lambda type	1 : 4	1 : 8	(–)	1 : 4	1 : 8	1 : 16	1 : 64	ND	1 : 64	1 : 8	1 : 16

(b) Suprabasal bulla group	12	13	14	15	16	17	18	19
IgG type	1 : 512	1 : 256	1 : 1 024	1 : 32	1 : 64	1 : 128	1 : 256	1 : 16
Kappa type	1 : 64	1 : 64	1 : 256	1 : 8	1 : 16	1 : 32	1 : 64	(–)
Lambda type	1 : 64	1 : 32	1 : 256	1 : 8	(–)	1 : 64	1 : 32	1 : 4

Of great interest is the way in which L chains are deposited in the intercellular spaces of the epidermis of pemphigus patients. There was a difference in the deposits of L chains between the subcorneal and the suprabasal bulla group—in the former being of either kappa or lambda type, but never of both types together in the intercellular spaces of the upper epidermis as the site of acantholysis, while in the latter group, concurrent deposition of both types of L chains in the intercellular spaces of the lower epidermis as the site of acantholysis was invariably observed in all cases.

Immunoglobulins deposited in the intercellular spaces of the epidermis in patients with pemphigus are generally considered to be combined with circulating IC antibodies (10). Our results presented above appear to suggest that in the subcorneal bulla group, IC antibodies of either kappa or lambda type and in the suprabasal bulla group IC antibodies of both types may be involved in the mechanism of acantholysis.

One of the major characteristics of the immunoglobulin molecule is repetition of a structural unit consisting of more than 100 amino acids. Both H and L chains are composed of variable and constant regions. Isolation of a fragment of the molecule consisting solely of variable regions of both chains demonstrated that this is the antigen-binding site of the antibody (3). It is also known that antigen-binding activity is present mainly in H chains and to a lesser degree in L chains as well (9) and that L chains play a part in stabilizing the structure of H chains (13). The functional difference between kappa and lambda types, however, remains largely obscure

The kappa/lambda ratio of the immunoglobulins in normal human serum varies from race to race. Such a difference in the incidence of kappa and lambda types is reflected in the relative incidence of myeloma of respective types (8). It is also postulated that the genes for immunoglobulins in human germ cells represent pooled genes for kappa, lambda and H chains (4). These findings suggest that L chains exhibit a marked ethnic difference and are closely related to genes.

In the process of keratinization, epithelial cells differentiate into horn cells through mitosis of basal cells. It is conceivable that as differentiation of epithelial cells proceeds the epithelial intercellular substance or pemphigus antigen in the upper layer becomes different from that in the lower layer of the epidermis. Characterization (if possible) of an ethnically or genetically defined intercellular substance in the upper epidermis where epithelial cells undergo maturation might help explaining the L chain deposition observed in our subcorneal bulla group.

In the subcorneal bulla group, circulating IC antibodies of kappa type often had a higher titre than those of lambda type. In the suprabasal bulla group, the IC antibody titre was comparable for both types. No relationship between the IC antibody titre and L chain deposition in the patient's skin was established. These results may imply that either of the two types of IC antibodies produced is directed against the intercellular substance of the upper epidermis, according to its degree of antigenicity, in the subcorneal bulla group.

Somewhat contradictory biochemical data on pemphigus antigen have been published (7, 12). Further attempts at obtaining purified antigen and, if possible, at comparing intercellular substances in the upper and the lower layer of the epidermis should be made in order to elucidate the immunological differences between different forms of pemphigus.

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