Acta Dermatovener (Stockholm) 54: 183–188, 1974

INCIDENCE OF CIRCULATING ANTIBODIES REACTIVE WITH BASAL CELLS OF SKIN IN DRUG REACTIONS

Th. van Joost

From the Department of Dermatology, University, of Amsterdam, Amsterdam, The Netherlands

Abstract. The indirect immunofluorescent (IIF) method revealed the presence of circulating antibodies of the IgG-class reactive with the basal cells of skin in 7 out of 42 patients with skin drug reactions. The impression was gained that the basal cell cytoplasm was the target structure for these antibodies, but binding with the basal cell-membranes could not be excluded. The findings reported in this study may arouse speculation as to immunological reactions which may play a role in toxicodermia.

In the literature there are very few reports of skin-reactive antibodies induced by drugs.

By means of the indirect immunofluorescent (IIF) method, circulating pemphigus-like antibodies were found in two cases of toxic epidermal necrolysis (14) and in thirteen out of twenty cases with morbilliform skin eruptions caused by penicillin (4).

In a direct immunofluorescent (DIF) study the occurrence has been reported of immunoglobulin (IgG) and complement confined to the intercellular spaces of the lower epidermal region of the lesional skin in a patient with a bullous eruption caused by phenolphthalein (11). With the same method the binding of immunoglobulins (IgG, IgM and IgA) and complement β_1A/β_1C) to the basal cells of lesional skin was found in two cases of toxic epidermal necrolysis, due to penicillin and chlorpromazine (12) respectively.

In the present study, sera of patients with various skin–drug reactions were subjected to IIF studies to ascertain whether in these conditions circulating antibodies reactive with skin constituents are detectable. When positive results were obtained, routine IIF tests for detecting antibodies reactive with other tissue antigens were performed. In addition to IIF tests the DIF method was used for the investigation of lesional skin in four cases.

MATERIALS AND METHODS

Sera were collected of 42 patients whose history and clinical manifestations firmly indicated a drug reaction. Histological examination of lesional skin performed in most cases was compatible with this diagnosis. In some of the cases examined positive patch tests or positive provocation tests on drugs used gave support to the diagnosis. Control sera of 10 healthy individuals and 40 persons with skin diseases other than drug reactions were subjected to the same procedure. The sera were stored at -20°C until use.

For the IIF study the standard procedure was used, as previously described in detail (15). In order to detect circulating skin antibodies, FTC-labelled monovalent antisera directed against IgG, IgM and IgA were used. These sera do not contain antibodies against other human plasma proteins detectable by immunoelectrophoresis. The antisera used for preparing the class-specific conjugates were adequately adsorbed in equivalence with both light chain types to eliminate antibody activity other than that directed against the Fc-fragment of the respective immunoglobulin. Staining properties and specificity of the class-specific conjugates were tested in direct immunofluorescent (DIF) tests on normal and monoclonal human bone marrow plasmocytes, using serial dilutions of the conjugate and incident illumination. The sera examined were tested on cryostat sections (4 μm) of guinea pig lip and normal human skin (blood group O).

For DIF study of skin sections (4 μm) of the erythematous area of the lesions the same FTC-labelled antisera were used. For the location of in vivo bound complement and for the in vitro complement fixation test only an unlabelled rabbit anticomplement serum was available. A double-layer technique was consequently used. The anticomplement serum, prepared by the Central Laboratories of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, contained antibodies against C1q, C4, C3 (β1A and α2D) and C5.

The method for the in vitro complement fixation test for circulating skin antibodies (IIF technique) has been described in previous papers (8, 6).

Characteristics of FTC-labelled antisera used for detecting skin antibodies and for DIF study of lesional skin

Batch no.	F/P ratio	(mol. 10^{-3})	Protein content (mg/ml)	Antibody (units/ml)	Working dilution
Rabbit anti-human IgG/FTC (0170)	1.10	2.5	1.0	2	1:32
Goat anti-human IgM/FTC (7–171)	1.25	3.0	16.4	4	1:32
Goat anti-human IgA/FTC (6–171)	0.94	2.4	17.8	4	1:32
Goat anti-rabbit/FTC (3–171)	0.90	2.6	1.37	—	1:64

The qualitative assay of antibodies (units/ml) was carried out in relation to international standard sera. The FTC-labelled antisera were prepared by Nordic Pharmaceuticals and Diagnostics, Tilburg, Holland.

IIF tests for detecting antibodies reactive with tissue other than skin, such as nuclei, thyroid, skeletal muscle, smooth muscle, stomach, parietal cells, supra adrenal gland and mitochondria, were performed in the Central Laboratories of the Netherlands Red Cross Blood Transfusion Service, Department for Autoimmune Diseases (Head: Dr Feltkamp). Methods for these tests are described in detail in (5).

Microscope

The optical system for blue narrow band excitation (with wavelengths between 470 and 490 nm) and epi-illumination has been extensively described in a previous paper (3).

RESULTS

For positive results of the IIF studies see Table III. The results of the IIF tests indicate the presence of serum circulating antibodies reactive with the basal cells of the substrates in 7 out of the 42 patients examined. The data on these 7 cases are summarized in Table I. Table II summarizes 35 cases with drug reactions in which the IIF tests were negative. In none of the control group sera could basal cell antibodoy activity be noted. It is worth noting that normal human serum in low dilutions (< 10) may show false positive staining of the basal cell cytoplasm. Chessboard titrations, however, revealed that in higher dilutions of the sera this non-specific staining became negative. The basal cell antibody activity was of the IgG type only. The impression was gained that the cytoplasm was the target structure for these antibodies, but staining of the basal cell membranes could not be excluded (Figs. 1 and 2). When guinea pig lip was used as the substrate, basal cell antibodies were detectable in all 7 cases. Using normal human skin of blood group O (to exclude the possibility of demonstrating blood group A and B-antibodies), the endpoint titres were lower and the phenomenon could be noted in 6 of the 7 patients.

Different endpoint titrations performed on two sera (I and III) failed to show any correlation between antibody endpoint titres and the clinical individual state of the patients. In case III (highest antibody titre, see Table III) 3 months after the lesions had spontaneously disappeared, basal cell antibodies were no longer detectable.

In vitro complement fixation tests performed in 2 cases (I and III) revealed a basal cell staining of the substrate used (guinea pig lip) in both cases, indicating the in vitro complement fixing ability of the basal cell antibodies in these cases.

In serum of patient III, an antibody of the IgA-class, reactive with the basement membrane zone of skin was also demonstrable (Fig. 3). Unabolished staining after blocking tests with two different sera containing high titre bullous pemphigoid antibodies, indicates that the IgA antibodies were directed against other antigens located in the basement membrane zone than those reactive with the IgG-type bullous pemphigoid antibodies.

Table I. *Data on the 7 investigated patients with circulating basal cell antibodies*

In the cases I, II, III and IV, the diagnosis toxicodermia was supported by positive skin reactions on provocation tests with the drugs respectively mentioned in Table I

Patients	Sex	Age	Diagnosis	Drugs used
I	♂	74	Drug reaction (bullous type)	Chloram-phenicolum
II	♂	54	Drug reaction (dermatitis type)	Cavergot
III	♂	57	Drug reaction (vasculitis type)	Butazolidin
IV	♀	5	Drug reaction (bullous type)	Penicillin
V	♀	38	Drug reaction (vasculitis type)	Hygroton
VI	♂	60	Drug reaction (toxic. eryth. type)	?[a]
VII	♂	53	Drug reaction (eryth. multiform type)	Salicylates

[a] In case VI various drugs were used and the drug or the drugs apparently responsible could not be determined.

Table II. *Data on 35 cases with toxicodermia in which negative IIF results were obtained*

	Number of cases
Morbilliform eruptions caused by Penicillin	5
Urticarial eruptions caused by Salicylates	4
Fixed drug reaction caused by Phenolphthalein	1
Vasculitis-type eruptions	3
Bullous-type eruptions	5
Lichenoid-type eruptions	3
Other types of skin eruptions	14

Table III. *Results of IIF tests in 7 cases of toxicodermia with circulating basal cell antibodies*

T = end point titre

Pat. sera	Indirect immunofluorescent (IIF) studies			
	Circ. basal cell antibodies of the IgG type		Antibod. reactive with other skin constituents as nuclei, bas. membr. zone or intercellular substance	Antibod. reactive with other tissue antigens
	Guinea pig lip	Normal hum. skin (bl.gr.0)		
I	Pos. T 256	Pos T 64	Neg.	Neg.
II	Pos. T 64	Pos. T 16	Neg	Neg.
III	Pos. T 1 024	Pos. T 256	Pos.: circ. antibodies of the IgA class reactive with bas. membr. zone	Pos.: circ. antibodies of the IgG class reactive with skeletal muscle and mitochondria
IV	Pos. T 512	Pos. T 128	Pos.; circ. antibodies of the IgG class reactive with epidermal intercellular substance.	Neg.
V[a]	Pos. T 64	Neg.	Neg.	Neg.
VI	Pos. T 128	Pos. T 32	Neg	Neg.
VII	Pos. T 256	Pos. T 32	Neg.	Neg.

[a] In this case no antibody activity could be shown against the basal cells of normal human skin (blood group O) and the possibility of blood group antibodies was not excluded.

It was of interest that in higher dilutions of the serum (between 64 and 256) in case I, probably due to an interference mechanism (2), antibody activity (IgG) with the intercellular space of the epidermis was also noted.

Circulating antibodies reactive with other tissue antigens. In 6 out of the 7 cases with basal cell antibodies no such antibodies were detectable. In the serum of patient III, however, circulating antibodies reactive with mitochondria and skeletal muscle were demonstrable.

Results of DIF study. DIF tests in addition to IIF tests performed in 4 cases of circulating basal cell antibodies revealed only in one case (III) a basal cell staining (cytoplasm and/or cell membrane) in vivo in the erythematous area of the skin lesion (Fig. 4). In the same case a granular complement staining was noted, but was confined to the lower part of the

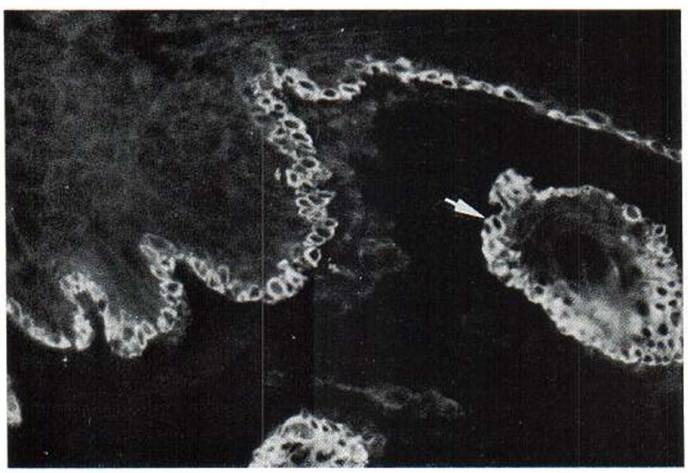

Fig. 1. IIF technique (IgG/FTC). Serum dilution 32. Substrate: guinea pig lip. Note the bright staining of the basal cells. No staining is visible of either the epidermal intercellular substance or of the basement membrane zone. The arrow points to the positive staining of the basal cells of the hair follicles (water immersion 22, obj. 10).

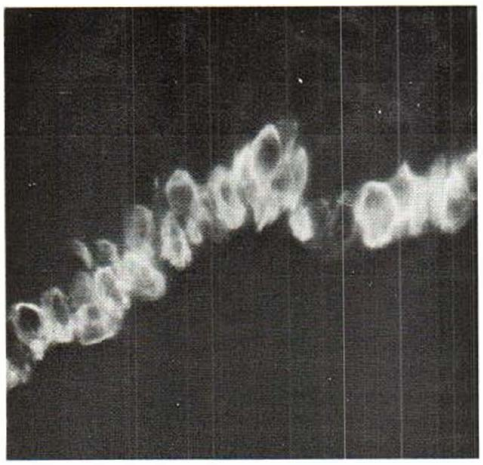

Fig. 2. IIF technique. Serum of patient III (dilution 32). Substrate: guinea pig lip (IgG/FCT staining). Note the bright fluorescence of the basal cells (water immersion 50, obj. 10).

basal cell layer. In this case and also in case V immunoglobulin-complement deposits could be detected focally in the upper dermal vessel walls.

Serum levels of immunoglobulins. Immunoelectrophoresis of the seven positive sera revealed an elevated IgG level in one case only. (III) In the remaining cases occurrence of circulating basal cell antibodies was not reflected by elevated IgG levels in the sera.

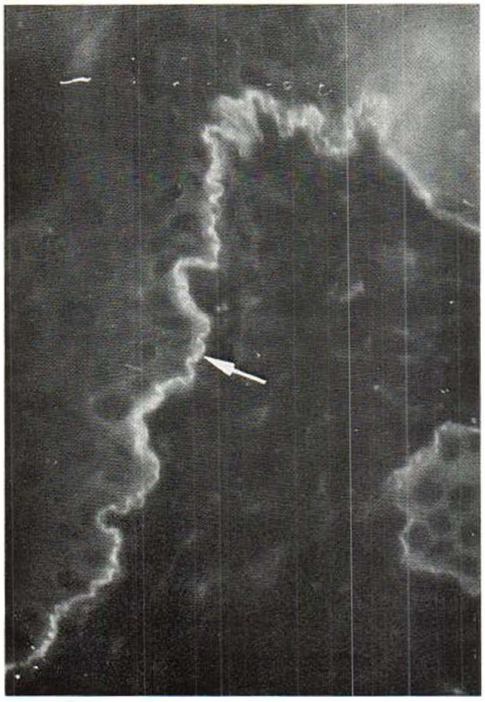

Fig. 3. IIF technique. Serum of patient III (dilution 32). Substrate: guinea pig lip (IgA/FTC staining). In the same serum an antibody of the IgA type was demonstrably reactive with the basement membrane zone (*arrow*) (water immersion 50, obj. 10).

DISCUSSION

It has been postulated in previous papers (2, 12) that some drugs or degradation products of drugs may have a strong affinity for substance located in the intercellular spaces of the basal cells or on the surfaces of the basal cells of human epidermis. The findings in this study may be compatible with this hypothesis and may indicate that some drugs participate in the forming of immunogens (haptene–skin complexes) at the sites of the basal cells, which can result in circulating antibodies with cross-reactivity to basal cells of normal human skin. It is known that immunization with chemicals results not only in antibodies directed against the chemical, but also against carrier proteins (1). The exact localization of the target antigens (cytoplasm and/or cell membrane) and their nature could not be elucidated in this study. If the cell membranes are responsible, the possibility may exist that actinomycin-like substances are the antigenic components (7).

In the serum of patient III, besides two different types of skin-reactive antibodies, mitochondrial antibodies and skeletal muscle antibodies were noted. In this case there was no evidence for existence of myasthenia gravis, thymoma, primary biliary cirrhosis or active hepatitis. From the literature it seems possible that some drugs induce mitochondrial

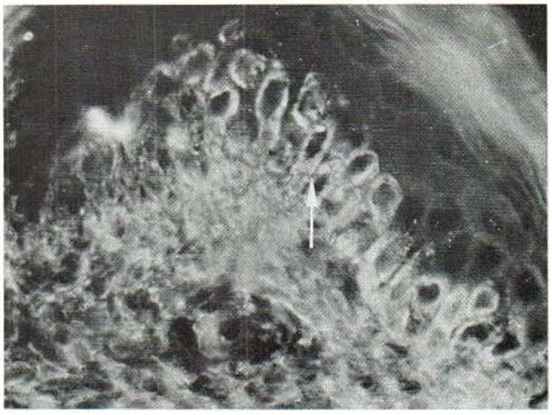

Fig. 4. DIF technique (IgG/FTC). Erythematous area of the lesion (patient III). Note the staining of the basal cells (membrane and/or cytoplasm). Dermis shows non-specific fluorescence. This is a common phenomenon in inflammatory skin (water immersion, 50, obj. 10).

antibodies (9). The skeletal muscle antibodies were of the so-called Zebra-type with alternating muscle fibre fluorescence (personal communications with Dr T. E. W. Feltkamp). This type of antibody has so far been described in sera of mice, experimentally treated with α-methyl-dopa and chlorthalidone (13).

In the majority of the cases—35—with drug reactions examined, and in the control sera, we failed to detect basal cell antibodies. In this respect it is acceptable that only in some drug reactions and under certain circumstances are basal cell antibodies detectable.

In vivo bound IgG and complement at the sites of the basal cells was noted in one case. The fact that routine light-microscopy revealed laterations of the basal cells in this case but also in other cases with circulating basal cell antibodies examined, may arouse speculation as to the role of basal cell antibodies. The lack of direct relationship between antibody titre and the course of the disease has also been described in cases of bullous pemphigoid (10) and may be considered as evidence against the direct harmful role of these antibodies. The fact that in 2 patients examined the basal cell antibodies were able to fix complement in vitro, may give rise to the question—what role can complement play in some drug reactions with respect to tissue injury?

In conclusion it can be said that the present study supports the hypothesis that in drug reactions immunological phenomena may play a role. Further studies are required in order to cast more light on immunological reactions in these conditions.

ACKNOWLEDGEMENTS

I am thankful to Miss A. Kinloch, Mr L. van Steen and Mr B. Vrees for their technical assistance.

REFERENCES

1. Bennaceraf, B. B. & Levine, B. B.: Immunologic specificity of delayed and immediate hypersensitivity reactions. J Exp Med *115:* 1023, 1962.
2. Beutner, E. H., Chorzelski, T. P. & Jordon, R. E.: Autosensitization in Pemphigus and Bullous Pemphigoid, p. 56. Thomas, Springfield, 1970.
3. Cormane, R. H. & Szabó, E.: Immunohistopathology of the skin in pemphigoid. Br J Dermatol *83:* 45, 1970.
4. Fellner, H. J., Fukuyama, K., Moshill, A. & Klaus, H. V.: Intercellular antibodies in blood and epidermis. Br J Dermatol *89*, 115, 1973.
5. Feltkamp, T. E. W.: Idiopathic autoimmune diseases. A study of their serological relationship. Thesis, Amsterdam 1966.
6. Heydenreich, G., From, E. & Diederichsen, H.: Some unusual findings obtained by the immunofluorescence method in bullous pemphigoid and benign mucous membrane pemphigoid. Acta Dermatovener (Stockholm) *52:* 201, 1972.
7. Holborow, E. J.: Smooth muscle auto-antibodies, viral infections and malignant diseases. Proc Roy Soc Med *65:* 481, 1972.
8. Jordon, R. E., Sams, W. M. & Beutner, E. H.: Complement immunofluorescent staining in bullous pemphigoid. J Lab Clin Med *74:* 548, 1969.
9. Paronetto, F. & Popper, H.: Lymphocyte stimulation induced by Halothane in patients with hepatitis following exposure to Halothane. New Eng J Med *283:* 277, 1970.
10. Peck, S. M., Osserman, K. E., Weiner, L. B., Lefkowitz, A. & Osserman, R. S.: Studies in bullous diseases. New Eng J Med *279:* 951, 1968.
11. Shelley, W. B., Schlappner, O. L. A. & Heiss, H. B.: Hysteresis in bullous fixed drug eruption due to phenolphthalein. Br J Dermatol *86:* 118, 1972.
12. Stein, K. M., Schlappner, O. L. A., Heaton, Ch. L. & Dechard, J. W.: Demonstration of basal cell immunofluorescence in drug induced toxic epidermal necrolysis. Br J Dermatol *86*, 246, 1972.
13. Ten Veen, J. H.: Exogenous factors in experimentally induced lupus erythematosus in mice. Thesis, chapter *5:* 81 1973.
14. Thivolet, J., Beyvin, A. J. & André, D.: Anticorpos "pemphigus-like" et "pemphigoid-like" (pemphigus and pemphigoid-like antibodies). Dermatologica *140:* 310, 1970.
15. Weller, T. H. & Coons, A. H.: Fluorescent antibody studies with agents of varicella and herpes zoster propagated in vitro. Proc Soc Biol Med *86:* 769, 1954.

Received October 1, 1973

Th. van Joost, M.D.
Department of Dermatology
Binnengasthuis
Amsterdam
The Netherlands