Acta Dermatovener (Stockholm) 60: 129–133, 1980

IMMUNOHISTOCHEMICAL STUDY ON 70 HUMAN EMBRYOS CONCERNING THE EPIDERMAL BINDING OF PEMPHIGUS AND PEMPHIGOID ANTIBODIES

A. Varelzidis, A. Tosca, E. Coumantakis, G. Nicolis, J. Hatzis, S. Papavasiliou and J. Stratigos

Department of Dermatology, "Andreas Sygros" Hospital, The University of Athens School of Medicine, Athens, Greece

Abstract. In the present study, the development of pemphigus and bullous pemphigoid antigens was investigated by means of the indirect immunofluorescence technique using sera of pemphigus and bullous pemphigoid patients, respectively, on human foetus skin antigenic substrate. Seventy skin specimens from embryos of 9–38 weeks of gestation were studied. Both pemphigus and bullous pemphigoid antigens were observed for the first time at about 16 weeks of gestation. Pemphigus antigen has a slower rate of evolution. Between 30 and 38 weeks both antigens were detected as strongly positive.

In recent years, a deeper understanding of the structural and physical properties of intercellular spaces, cell junctions and the dermo-epidermal junction as well as of their embryonic development has been achieved by electron microscopy studies (2, 3, 12, 17, 18). Special attention has been paid to these structures because of the immunological role attributed to them in some autoimmune disorders, e.g. pemphigus and bullous pemphigoid, and the abnormalities observed in the course of malignant transformation in a variety of tissues of epithelial or other origin (17, 20).

The diminished intercellular adhesion in solid tumours has been assumed to be a contributory factor in carcinogenesis, either by abolition of the process of "contact inhibition of locomotion" and the increased ability of tumour cells to invade and metastasize, or by abolition of "contact inhibition of growth" (1, 14). The intact dermo-epidermal junction is considered to be a functional barrier against cell movement, since its disruption facilitates the transformation of a pre-invasive into an invasive carcinoma (15, 19). The above concepts are in agreement with the previously proposed "immunological theory of cancer" of Green (6) and Green & Anthony (7), in which great attention was accorded to the surface tissue-specific antigens (T.S.A.) whose presence ensures a kind of immunological control favouring tissue homeostasis. Pemphigus and bullous pemphigoid antigens are considered to be T.S.A. partly or completely absent from premalignant tumours and skin cancers (4, 16). These antigens have been the object of extensive work in recent years. Bullous pemphigoid antigen has been found in the basal lamina, the lamina lucida and in hemidesmosomes and is probably of a proteinaceous nature, having a molecular weight of 20 000. Pemphigus antigen is located exclusively in the intercellular spaces of squamous cell epithelium of the epidermis and is probably also a protein, with a molecular weight of 68 000 D (5, 13).

The existence of these antigens at the dermo-epidermal junction and in the intercellular substance and their disappearance depending on the stage of malignant transformation have led us to study their ontogeny and to try to correlate their presence with the level of epidermal differentiation in the course of embryonic development.

MATERIALS AND METHODS

The aim of the present study was to search for the presence or absence of the intercellular substance and basement membrane antigens, by means of the indirect immunofluorescence technique, using sera of pemphigus and bullous pemphigoid patients with high titres of autoantibodies against the intercellular substance and basement membrane antigens respectively. Tissue sections from the epidermis of 70 human foetuses aged 9–38 embryonic weeks were studied by the indirect immunofluorescence method as described by Nairn (10).

Frozen punch biopsy specimens were stored at −28°C and cryostat sectioned at −40°C within one week. Control sections of normal human skin were included with each assay. Commercially available fluorescein-labeled goat–

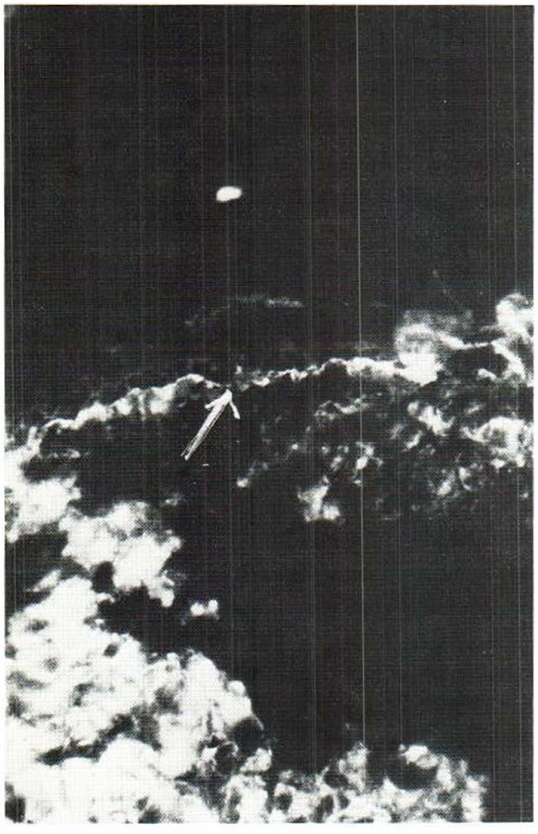

Fig. 1. Strongly positive immunofluorescence at the dermo-epidermal junction in a 17-week human embryo. ×85.

antihuman antisera were used (Hyland Laboratories, California). One kind of antiserum contained antibodies against human immunoglobulins (F/P ratio 8.7) and the second was a predominantly anti-human IgG preparation (F/P ratio 3.4). The antisera were absorbed before use with a pig liver powder (Wellcome) to eliminate non-specific agents. The conjugated antisera gave no staining reaction when applied alone to test sections.

Five high-titre pemphigus sera and five high-titre bullous pemphigoid sera were used. Prior to use the pemphigus sera were absorbed with human group AB red blood cells. Sera were used at a dilution of 1 : 10 in phosphate-buffered saline (0.01 phosphate-buffered saline, pH 7.1). Besides pemphigus and bullous pemphigoid sera, normal human serum and phosphate-buffered saline were included for control purposes (10).

Specificity of the staining reaction was established by neutralization absorptions of the pemphigus and bullous pemphigoid sera with normal human epidermis. Such treatment inhibited the immunofluorescent staining of normal skin. An aliquot from each pemphigus or bullous pemphigoid serum sample was allowed to react with the tissue section and was then overlaid either with antiserum against human immunoglobulins or against IgG. The fluorescent antisera were overlaid at a dilution of 1 : 4. At the end of the procedure, preparations were read blind, using an ultraviolet fluorescence microscope (Zetopan Richert) illuminated with a CS 200 W lamp, excitation filter E_1 (UGI/3 mm+BG 38/2.5 mm) 3-lens glass condenser and protective filter Sp_2 (GG/3/1+3 mm+Wratten foil 2A).

RESULTS

The results of the present study are presented in detail in Table 1. The earliest foetal age at which the intercellular substance antigen was observed was the 16th embryonic week. When the intercellular fluorescence was bright and pervaded the entire epidermis the pemphigus antigen was characterized as strongly positive, but when not bright or localized to isolated islets of the epidermis, it was characterized as weakly positive. The pemphigus antigen was strongly positive in 3 out of 11 foetuses aged 16–20 weeks, weakly positive in 4 and absent in another 4 foetuses within the same age group (fig. 2).

Between 21 and 30 weeks, the pemphigus antigen proved strongly positive in 1 out of 38 foetuses (Fig. 3) weakly positive in 12 and absent in 9. Finally 12

Table 1. *The results of detection of the intercellular substance and basement membrane antigens by indirect immunofluorescence in 70 human foetuses, divided into four age groups*

Negative, weakly positive, and strongly positive results are expressed in both absolute numbers and percentages

Weeks of gestation	Number of foetuses	Intercellular substance antigen			Basement membrane antigen		
		Negative	Weakly positive	Strongly positive	Negative	Weakly positive	Strongly positive
9–14	7	7 (100%)	–	–	7 (100%)	–	–
16–20	11	4 (36.36%)	4 (36.36%)	3 (27.2%)	4 (36.36%	1 (9.09%)	6 (54.54%)
21–30	38	9 (23.68%)	12 (31.5%)	17 (44.73%)	12 (31.5%)	5 (13.15%).	21 (55.26%)
30–38	14	2 (14.28%)	–	12 (85.71%)	1 (9.09%)	–	13 (92.8%)

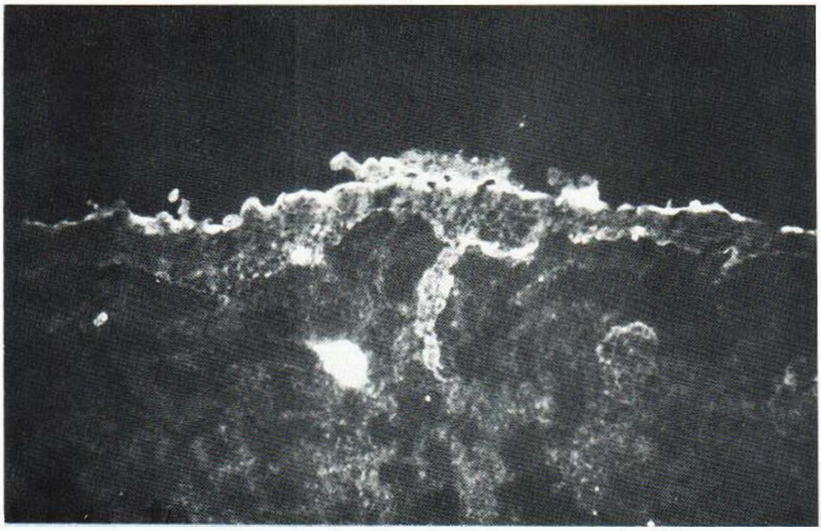

Fig. 2. Negative intercellular fluorescence in a 14-week human embryo. ×85.

out of 14 foetuses had strongly positive pemphigus antigen and two lacked the antigen in the age group 30–38 weeks. There were no weakly positive results in the latter group.

The earliest foetal age at which the basement membrane antigen was detected was likewise the 16th embryonic week. When a thin fibrillar or granular, continuous fluorescent dermo-epidermal zone was observed it was considered to be strongly positive (Fig. 1) and when discontinuous fluorescent zone was observed the bullous pemphigoid antigen was considered weakly positive.

The bullous pemphigoid antigen was strongly positive in 6 out of 11 foetuses aged 16–20 weeks, weakly positive in one and absent in 4 foetuses within the same age group. Between 21 and 30 weeks, the bullous pemphigoid antigen proved strongly positive in 21 out of 38 foetuses, weakly positive in 5 and absent in 12. Finally, 13 out of 14 foetuses had strongly positive basement membrane antigen and one lacked the antigen in the age group 30–38 weeks. There were no weakly positive results in the latter group. An interesting morphological finding was the fluorescence along the vertical mar-

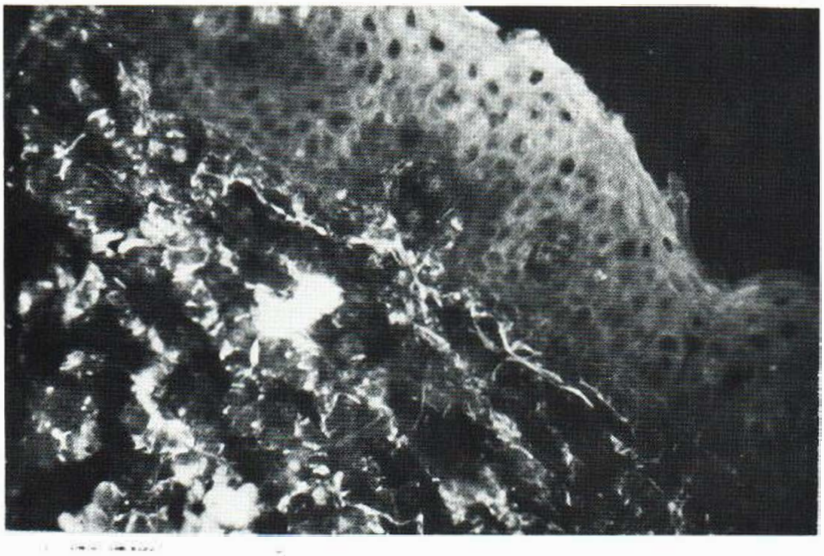

Fig. 3. Strongly positive intercellular fluorescence in a 36-week human embryo. ×214.

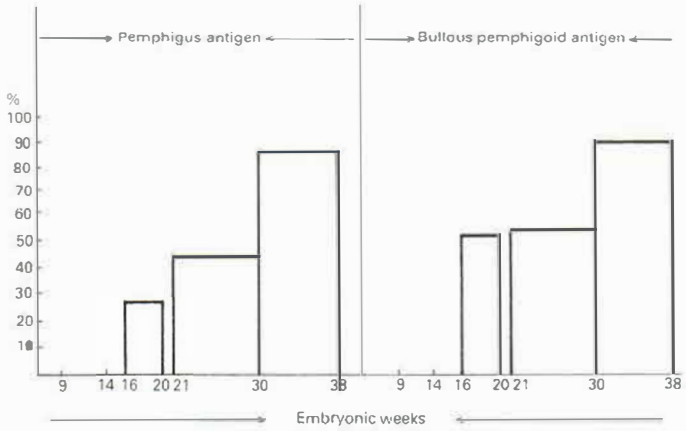

Fig. 4. Percentage of strongly positive intercellular substance and basement membrane antigens as detected by the indirect immunofluorescence method.

gins of juxtaposed basal epidermal cells in addition to the fluorescent pattern of the dermo-epidermal junction.

DISCUSSION

Human epidermis at 3 weeks of gestation consists of a single layer of undifferentiated cells of ectodermal origin; at about 6 weeks a bilaminar epidermis appears and at approximately 9 weeks a new layer appears—the stratum intermedium (2). At 17 weeks, the epidermis has already developed all adult characteristics. The basal lamina can be detected as early as at the 6th embryonic week but a fully developed dermo-epidermal junction is not observed until the 16th week of gestation. The origin of the basement membrane has not been clarified. It has been suggested that the basal lamina is of mainly epidermal origin. On the other hand the anchoring fibrils of the dermo-epidermal junction are believed to derive from the mesenchyme (3).

In the present study, the detection of the basement membrane antigen at about 16 weeks, when a completely developed basement membrane has already been established, favours the concept of the *in situ* production of this antigen. The intercellular fluorescence of the overlying basal cells might be further comfirmation of this view. The results of the present study have revealed that (*a*) of the two antigens, the pemphigus one has the slower developmental rate (Fig. 4). It has previously been suggested that this same antigen is the first to be lost in malignant transformation (16). Thus the pemphigus antigen appears to be a sensitive index of a well differentiated epithelium (*b*). The bullous pemphigoid antigen is observed at about 16 embryonic weeks, almost simultaneous with the appearance of a fully developed basement membrane. It is thus concluded that the presence of pemphigus and bullous pemphigoid antigens is directly correlated with a completely differentiated epithelium and a fully established basement membrane.

The results of this study are not in agreement with those reported by Muller et al. (9), who found that the intercellular antigen appears for the first time at 9 weeks and becomes strongly positive at 12 weeks, whereas the basement membrane antigen is only discernible at 12 weeks, to become positive after 23 weeks of gestation. No explanation, however, can be offered for this discrepancy between the time of establishment of the basement membrane antigen and the time of formation of the basement membrane. The differing results of the present study vis-à-vis the work of Muller et al. (9) might be attributable to various parameters such as differing section thickness, site of biopsy, genetic variations, different racial and/or ethnic origin of the foetuses studied, as well as the conditions of specimen handling before procedure. A possibility of an antigenic heterogeneity of the tissue constituents with which the pemphigus and bullous pemphigoid autoantibodies react cannot be excluded, since pemphigus antigens with different physicochemical properties have been isolated from different organs and animal species (8, 11, 13). Assuming that all other factors are equal, including the physicochemical properties of the pemphigus antigen, then differences in affinity of antibodies directed against these antigens might explain the variance of our results from data previously published regarding the age at which the pemphigus antigen first appears. More specifically, an antibody with

greater affinity than that present in our sera would bind more strongly to the pemphigus antigen, thus producing a positive result, even at low concentrations of the latter.

Further quantitative characterization of the physicochemical properties of the bullous pemphigoid and pemphigus antigens is required before the issue of the ontogenetic development of the pemphigus and bullous pemphigoid antigens can be settled.

REFERENCES

1. Abercombie, M. & Ambrose, E. J.: The surface properties of cancer cells: A review. Cancer Res *22:* 525, 1962.
2. Breathnach, A. S. & Robins, J.: Ultrastructural feature of epidermis of a 14 mm (6 weeks) human embryo. Br J Dermatol *81:* 504, 1969.
3. Briggaman, R. A. & Wheeler, C. E.: The epidermal–dermal junction. J Invest Dermatol *65:* 71, 1975.
4. De Moragas, J. M., Winkelman, R. K. & Jordon, R. E.: Immunofluorescence of epithelial skin tumors. I. Patterns of intercellular substance. Cancer *25:* 1399, 1970.
5. Diaz, L. A. Heaphy, M. R., Calvanico, N. J. & Tomasi, T. B., Jr et al.: Bullous pemphigoid antigen: isolation from normal human skin. J Immunol *118 (2):* 455, 1977.
6. Green, H. N.: An immunological concept of cancer: A preliminary report. Br Med J *ii:* 1374, 1954.
7. Green, H. N. & Anthony, H. M.: The immunological theory of cancer. Practitioner *190:* 705, 1963.
8. Miyagawa, S., Hojo, T., Ishii, H., Yoshioka, J. & Sakamoto, K.: Isolation and characterization of soluble epidermal antigens reactive with pemphigus antibodies. Acta Dermatovener (Stockholm) *57:* 7, 1977.
9. Muller, H. K., Kalnins, R. & Sutherland, R. C.: Ontogeny of pemphigus and bullous pemphigoid antigen in human skin. Br J Dermatol *88:* 443, 1973.
10. Nairn, R. C.: Fluorescein Protein Tracing, 3rd ed. Livingstone, Edinburgh, 1969.
11. Ogawa, H., Taneda, A. & Morioka, S.: Characterization of pemphigus antigen. J Invest Dermatol *70:* 194, 1978.
12. Omar, A. & Krebs, A.: Mode of dermal-epidermal adhesion. Dermatologica *150:* 321, 1975.
13. Shu, S. Y. & Beutner, F. H.: Isolation and characterization of antigens reactive with pemphigus antibodies. J Invest Dermatol *61:* 270, 1973.
14. Stoker, M.: Aspects of medical virology. Br Med Bull *23* 105, 1967.
15. Tarin, D.: Sequential electron microscopical study of experimental mouse skin carcinogenesis. Int J Cancer *2:* 195, 1967.
16. Tosca, A., Varelzidis, A., Nicolis, G., Hadzis, J. & Capetanakis, J.: Antigenic alterations in tumours of epidermal origin. Cancer. Accepted for publication 1979.
17. Weinstein, R. S., Merk, F. B. & Alroy, J.: The structure and function of intercellular junctions in cancer. Adv Cancer Res *23:* 23, 1976.
18. Wolff, K. & Schreiner, E.: Ultrastructural localisation of pemphigus autoantibodies within the epidermis. Nature *229:* 59, 1971.
19. Woods, D. & Smith, C.: Ultrastructure of the dermal epidermal junction in experimentally induced tumours and human oral lesions. J Invest Dermatol *52:* 259, 1969.
20. Yeh, S.: Skin cancer in chronic arsenicism. Hum Pathol *4:* 469, 1973.

Received December 15, 1978

A. G. Varelzidis, M.D.
Department of Dermatology
"Andreas Sygros" Hospital
1. Dragoumi 5, St.
Athens 612
Greece