

THE SPONTANEOUS REGRESSION OF KERATOACANTHOMA IN MAN

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Abstract. This study was undertaken to search for immune mediated mechanisms which could possibly be involved in the spontaneous regression of keratoacanthomas. In eleven patients with solitary keratoacanthoma, immuno-fluorescence studies did not reveal significant staining patterns, while skin tests, using autologous extracts of keratoacanthomas, were not compatible with true delayed cutaneous hypersensitivity reactions.

Key words: Keratoacanthoma; Papilloma; IF investigation; Skin tests; Non-immunological regression

The name keratoacanthoma was introduced by Rook & Whimster in 1950 (26). Keratoacanthoma is a rapidly growing skin tumour which originates from the hair follicle and resolves spontaneously over a period of months (6, 7, 15).

It is well known that humoral as well as cellular immune responses play a part in the defence mechanism against tumours.

An infiltrate of mononuclear cells at the base of the tumour or within the tumour epithelium is a fairly constant feature in the regression phase of keratoacanthoma (17). This certainly suggests that immune mechanisms are involved in the spontaneous regression.

This paper describes a study on the humoral and cellular immune responses in keratoacanthoma made with the fluorescent antibody technique and delayed cutaneous hypersensitivity reactions (D. C. H. reactions) using autologous extracts of keratoacanthoma.

MATERIAL AND METHODS

Eleven patients with keratoacanthoma were involved in this study. Diagnosis was based primarily on clinical features. Age, sex, localisation and duration of the lesion, are summarized in Table I.

Tissue specimens

The lesions were removed *in toto* with the curette under local ethylchloride anaesthesia, or by plastic surgery.

Immediately after removal, a central cross sectional part of the tumour (24) was fixed in 10% buffered neutral formalin and embedded in paraplast according to a standard procedure. For histological confirmation of the diagnosis, hematoxylin-eosin and Weigert-van Gieson stains were used. The two other parts of the tumour were immediately snap-frozen in liquid nitrogen. The frozen tumour tissue was stored at -90°C until further use.

In 9 patients D. C. H. reactions were carried out. For this purpose, normal skin was taken from each patient as a control by two punch biopsies ($\varnothing$ 5 mm) or by plastic surgery. These specimens were transported and stored in the same way as the tumour tissue.

Human sera

From each patient a blood sample was taken by venous puncture. The blood was allowed to clot for 30 minutes at room temperature and subsequently centrifuged at 3000 r.p.m. The sera were stored at -30°C in small aliquots until needed.

Immunofluorescent techniques

Tissue specimens were cut and stained according to the direct and indirect I.F. technique, and the required

Table I. Some clinical features of the patients

No.	Patient		Duration (weeks)	Localisation
	Sex	Age		
1	♂	92	12	Lower lip
2	♂	76	6	Left cheek
3	♂	65	8	Left auricle
4	♂	58	12	Left cheek
5	♂	72	8	Left forearm
6	♀	73	6	Nose
7	♂	76	16	Below right eye
8	♂	88	24	Right auricle
9	♂	67	5	Left cheek
10	♂	73	4	Left auricle
11	♂	73	8	Left forearm

Table II. Response to skin testing with standard antigens

Read at 24 hours

No. pat.	Hem. strep.	Staph. aureus	Hem. influenza	Strep. viridans	C. albicans	P.P.D.
3	+	++ ○	++ ○	+	+	+++ ○○
4	+	+	+	+	—	++ ○
5	—	+	+++ ○	—	—	++ ○
6	+	++	+++ ○○	+	++ ○	—
7	+	++	++ ○	—	++ ○	—
8	+	+	++ ○	++	—	—
9	—	+++	++ ○	++	—	—
10	—	++	+++ ○	—	—	—
11	++	+++ ○	+++ ○○	—	—	+

+: erythema >5<10 mm. ++: erythema >10<20 mm. +++: erythema >20 mm. ○: induration <10 mm.
○○: induration >10<20 mm.

specificity tests performed as described earlier (2, 19). They were examined with a Zeiss Standard G.F.L. microscope with a dark field condensor and a mercury super pressure lamp (Osram HBO 200W/4), with appropriate filters, and by means of a Leitz Orthoplan microscope, with epi-illumination and interchangeable dichroic mirrors, equipped with a Xenon arc (X.B.O., 75W) (23). Micrographs were made on Kodak Tri-X, 27° Din film.

Conjugates and antisera

The following conjugates and antisera were used for I.F. staining: Rabbit antisera to human IgG, IgA, IgM, light chains kappa and lambda, albumin, Clq, C4, C3b, C3c, C3d, C5 and C4+C3c+C3d (C4/C3) and a F.I.T.C. labelled horse anti-rabbit globulin serum, all obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. The anti-fibrin(ogen) conjugate was obtained from Hoechst Holland N.V., Amsterdam, The Netherlands.

Extracts

Tissue specimens and normal skin as control, were processed in the following manner.

The tumour and normal skin fragments of each patient were suspended in 2 ml distilled water and thoroughly homogenized in a Potter Elvehjem tissue grinder. The homogenates were stored for 2 days at 4°C under regular shaking, then centrifuged at 3000 g for 10 minutes to remove cell membranes and tissue debris. The clear supernatant was decanted and, after freeze drying, stored in ampules. Before use, the dry weights of tumour and

normal skin extracts were determined. Subsequently the extracts were resuspended in a fixed amount of normal saline, so that 0.1 ml of tumour and control extract corresponded to 0.1 mg of dry tissue.

In patients with a variety of tumours, the average test dose concentration required for positive reactions was 1.04 mg/ml as described by Oren & Herberman (22).

Bluming et al. (4) observed maximally evident positive D.C.H. reactions at a protein concentration of 1 mg/ml in Burkitt lymphoma. Although this does not imply that 1 mg/ml is the best choice with regard to keratoacanthoma, this concentration was chosen as the critical antigenic challenge.

The extracts were sterilized through millipore filters (Sartorius Membrane filters, pore size 35 mm); 0.1 ml of tumour extract and 0.1 ml of normal skin extract were injected intradermally with a 27 gauge needle in the flexor side of the forearm, each patient receiving the autologous extracts.

The reactions were read at 20 minutes, 6, 24, 48 and 72 hours. Clinically positive D.C.H. reactions were biopsied at 24 and 48 hour intervals for histological examinations.

0.1 ml of a 0.01% solution of histamine phosphate was injected intradermally as a control on immediate type reactions.

In addition, all patients were tested with 0.1 ml intradermal injections of six standard recall antigens, as a screening for general cellular reactivity. These were: Hemolytic streptococcus 100 million germs (M.G.)/ml; Staphylococcus aureus 1000 M.G./ml; Hemophilus influenzae 1000 M.G./ml; S. viridans 100 M.G./ml; purified protein derivative of tuberculin (P.P.D.) 0.0002 mg; all

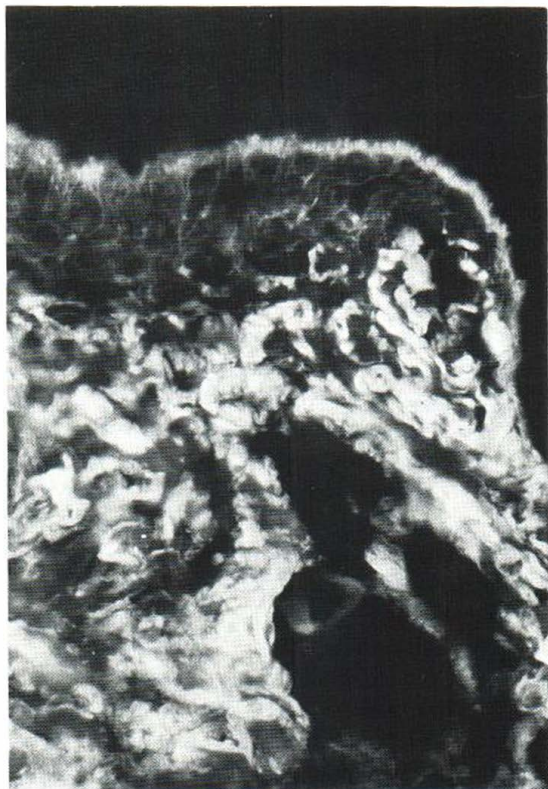


Fig. 1. Homogeneous deposits of IgG in a granular pattern in the epidermal basement zone. $\times 215$.

obtained from the R.I.V. (National Institute of Public Health, Bilthoven) and *Candida albicans* 0.1%, obtained from H.A.L. (Harlems Allergens Laboratories, Haarlem). All patients revealed D.C.H. reactions to one or several of these standard antigens. (See Table II.)

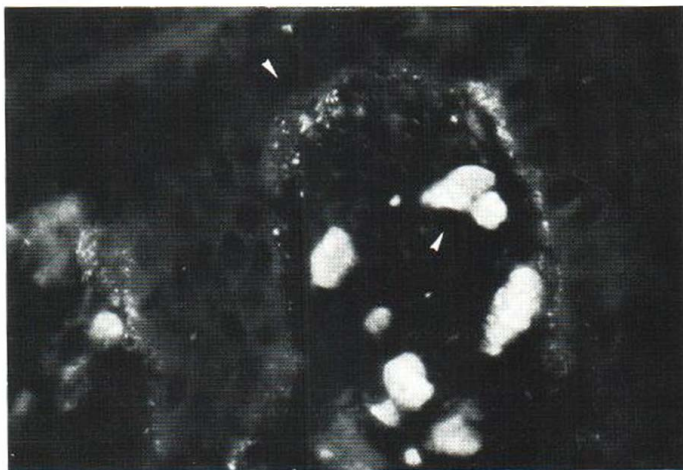


Fig. 2. Small amounts of complement in a discontinuous granular pattern at the dermal-epidermal junction. In the subepidermal region, colloid bodies are visible. $\times 400$.

RESULTS

Immunofluorescence

Epidermal basement zone: Patient 6 (Fig. 1) and patient 9 showed discontinuous homogenous deposits of IgG and IgM in a granular pattern. The immunoglobulins were of both classes of light chains. Granular deposits of C3d (patient 6) and some granular fluorescence with the polyvalent antiserum directed against C4, C3c, C3d (C3/4) was observed in patient 9. In patient 5 fibrin could be demonstrated in the epidermal basement zone, together with small amounts of C3d, C3c and C5 (Fig. 2) in a discontinuous, granular pattern, at this side.

Epidermis. In none of the patients was a specific fluorescence staining pattern seen in the intercellular spaces, nor around the epidermal cell nuclei. In some keratinized partly degenerated areas of epithelium, small deposits of IgM (patient 2) and IgG (patient 1) (Fig. 3) were seen. The immunoglobulins were of both classes of light chains. Small amounts of C3/4 were traced in this area in both patients. In some lesions colloid bodies were demonstrable. They stained for IgA, IgG, IgM and C3/4.

Blood vessels. In 2 patients (nos. 1, 2) deposition of fibrin was found around the vessels in the upper dermis. Fine granular deposits of IgA, IgM and C3c (patient 6), IgM (patient 9) and C3d (patient 5) were observed in some vessel walls.

Connective tissue. Some traces of IgG, IgM, C3/4 and C3d were occasionally demonstrable in several specimens, mostly alongside elastic fibres (background staining).

In vivo, tumour fixation of immunoglobulins and

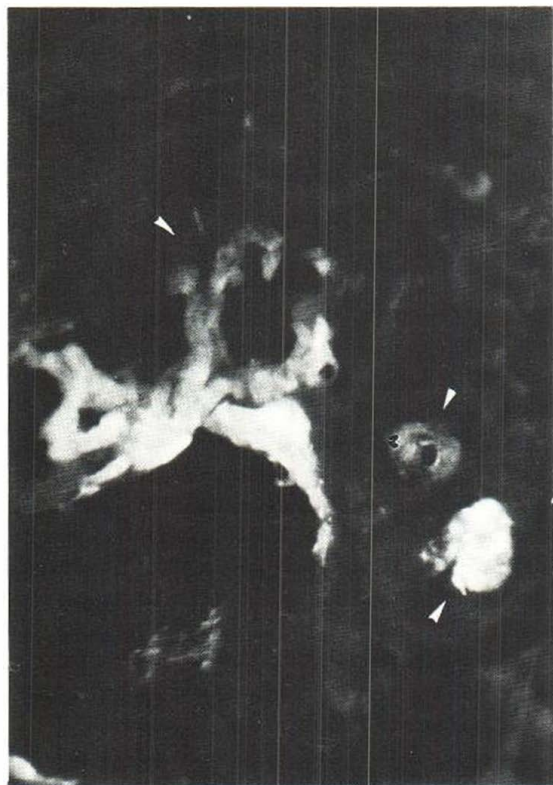


Fig. 3. Staining of IgG in partly degenerated areas of epithelium and epidermal cells, showing individual keratinization. $\times 465$.

complement were not detected in any of the biopsies.

Extracts

The results of the skin tests are summarized in Table III. All patients showed a clinical skin reaction of erythema and induration after 20 minutes, while the control was only slightly erythematous or negative. After 24 hours most reactions were still erythematous, but, except in patient 6, not indurated, in general subsiding after 48 hours. The histological pictures of biopsy specimens taken after 24 and 48 hours were similar in most respects (Figs. 4, 5): oedema of the dermis, and a moderate infiltrate of lymphocytes and neutrophils with some eosinophils. The infiltrate was mainly perivascular, with more or less prominent swelling of the vascular endothelial cells. It was conspicuous that in biopsy specimens taken after 48 hours the neutrophil component of the infiltrate was still present.

DISCUSSION

Recently there has been accumulating evidence for the existence of humoral and cell-mediated immunological reactions against antigens associated with human neoplasms. Delayed cutaneous hypersensitivity reactions (D.C.H. reactions) to tumour extracts have been observed in a variety of human tumours.

Hughes & Lytton (14) and Stewart (27) saw positive reactions in about 25% of patients to their autologous cell-free tumour extracts. Herberman & Oren (11) and Oren & Herberman (22) reported D.C.H. reactions to autologous membrane extracts in 50% of non-aneuric patients with leukemia, lymphoma and carcinoma. A correlation between the D.C.H. reactions and the clinical status of the patient has been reported: in Burkitt lymphoma and in acute lymphocytic leukemia, positive reactions were usually obtained when the disease was in clinical remission (4, 8); in the case of melanoma, on the other hand, positive reactions were only seen in patients with localized disease (9).

D.C.H. reactions are seldom applied in human epidermal skin tumours. In the dermatological literature there are two papers concerning D.C.H. reactions in keratoacanthoma (18, 21). Both papers report immediate and delayed type reactions in patients with solitary keratoacanthoma, with autologous as well as heterologous extracts. In the study of Nicolau et al. (20), D.C.H. reactions in guinea pigs were seen with keratoacanthoma extracts after passive transfer of white blood cell suspension.

Our results are not in agreement with these two papers. Although the skin tests did reveal an immediate-type reaction, no real delayed-type reactions were ever noted. The observed reactions were partly subsiding after 24 and 48 hours, instead of reaching a peak at these times. Histological examination of the late reactions showed in all cases a considerable quantity of polymorphonuclear cells in the infiltrate, which is not in accordance with a pure delayed type reaction (Figs. 4, 5). We used the method of extraction described by Oettgen et al. (21) with slight modifications.

Despite the fact that the antigens of clinically induced tumours have a rather weak immunizing capacity (25), Oettgen et al. (21) noted delayed hypersensitivity reactions in the guinea pig with extracts of carcinogen-induced fibrosarcomas. Sterilization of extracts through millipore filters did not affect their immunizing capacities.

Table III. Skin tests

No. pat.	Histamine phosphate 0.01 % 20 min	Tumour extract 0.1 ml			Control extract 0.1 ml			Dry tumour weight
		20 min	24 h	48 h	20 min	24 h	48 h	
3	++ ○	+++ ○○	++	—	+	—	—	17
4	++ ○	++ ○	+	—	—	—	—	21
5	++ ○○	+++ ○○	+++ ○	++	~	~	~	3.4
6	+++ ○○	++ ○○	+++ ○○	++ ○	—	+	—	35.6
7	+++ ○	++ ○	+++ +	+	—	—	—	50
8	++ ○	+++ ○	+++	—	—	—	—	74
9	+++ ○○	+++ ○○	++	—	—	—	—	14
10	++	++ ○	++	—	—	—	—	8
11	+++ ○	++ ○	+++	—	+	—	—	4

+: erythema < 10 mm. ++: erythema > 10 mm < 20 mm. +++: erythema > 20 mm. ○: induration < 10 mm. ○○: induration > 10 mm < 20 mm. ~: no material available.

A more refined method of extraction, such as described by Fass et al. (8) and Herberman et al. (13), is difficult to carry out with keratoacanthoma, since it is almost impossible to obtain a suspension of cells of one kind.

Our results are not comparable to those obtained by Nicolau et al. (20), who used a pooled extract of keratoacanthomas from different patients. The method of extraction described by Maggiora (18) is also essentially different from ours.

The fact that all controls gave negative or slightly erythematous reactions suggests a specificity of the skin reactions observed with the tumour extracts. Can the immediate-type reaction be explained as a humoral antitumour response?

Brown et al. (5) demonstrated *in vivo* fixation of IgG, IgM and C3 in areas of tumour adjacent to the dermis and in tumour tissue undergoing necrotic degeneration. In some areas immunoglobulin and complement were deposited in intercellular spaces of tumour tissue. It was interpreted that an immunologically mediated mechanism could possibly participate in the spontaneous resolution of keratoacanthoma.

In two patients we found *in vivo* fixation of IgG,

IgM and C3 at the epidermal basement zone, while we too found some small deposits of IgM and IgG with third complement factor in areas of partly keratinized epithelium undergoing necrotic degeneration. None of the patients revealed staining of the intercellular spaces of tumour tissue.

In a recent paper of Baart de la Faille-Kuyper et al. (3) however, IgG, IgM, and C3 were demonstrated along the rootlets of the basal epidermal cells in the skin of healthy individuals. We found the deposits in the same localisation of the dermal epidermal junction in keratoacanthomas. Thus, specificity, with regard to immunologically mediated mechanism, seems doubtful. Perhaps the immediate-type reactions can be explained on the basis of a non-immunological activation process of humoral mediator systems by products of the tumour extracts, resulting in release of chemotactic factors, which might also explain the presence of polymorphonuclear cells in the infiltrate after 24 hours and 48 hours.

Keratoacanthomas originate from the hair follicle. Experimentally induced keratoacanthomas of types 2 and 3, as introduced by Ghadially (10), are clinically and histologically closely related to kerato-

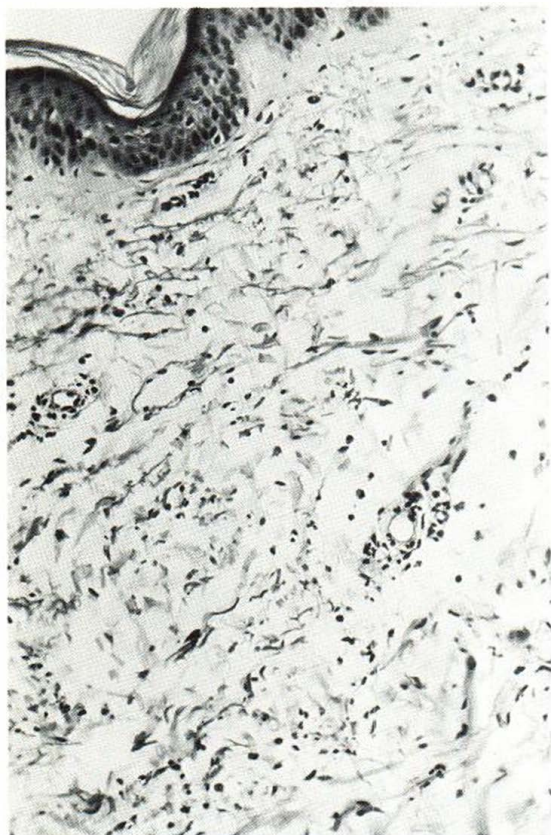


Fig. 4. Patient 5: biopsy, 48 hours after tumour extract: oedema of the dermis. Lymphocytes with neutrophils scattered throughout the dermis. H and E, $\times 90$.

acanthoma in man. Their behaviour—rapid onset and regression—follow the normal physiology of the hair follicle cycle, i.e. the rapid growth of the hair follicle in the anagen phase, and regression in the telogen phase (11). Andrews (1) found regression of papillomas in 80% of 3 methylcholanthrene-treated allografts in mice, when placed in maximally immunosuppressed recipients (thymectomy, 450 R irradiation and weekly antithymocyte serum injection). He concluded that the regressions could be the result of a non-immunological mechanism. However, Lappé (16) found that regression of papillomas by isogenic skin grafting was delayed in thymectomized, irradiated recipients. In rabbits, injected with autologous tumour extracts prepared from 7–12-dimethyl benz(a) anthracene-induced keratoacanthomas, we found no delayed hypersensitivity reaction, while immunofluorescence did not reveal

any significant staining which might suggest a humoral antitumour response (to be published).

The present study suggests that, as in the experimentally induced keratoacanthomas of Andrews (1), mediators other than immunological factors may be responsible for the regression of keratoacanthoma in man.

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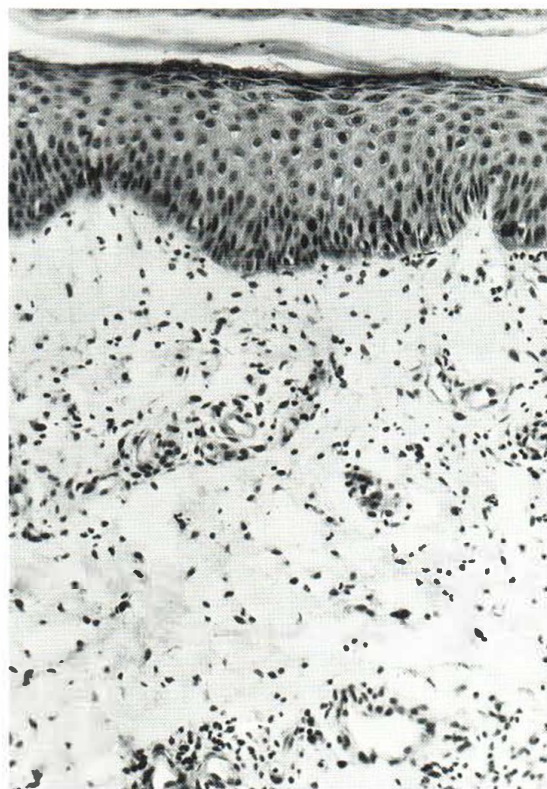


Fig. 5. Patient 10: biopsy, 24 hours after tumour extract: infiltrate diffuse and perivascular in arrangement, composed of lymphocytes, intermingled with neutrophils. H and E, $\times 90$.

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