

Immunological Reaction in Keratoacanthoma, a Spontaneously Resolving Skin Tumor^{1, 2}

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SUMMARY

Keratoacanthoma, a skin tumor that undergoes spontaneous resolution, was studied immunohistologically by the fluorescent antibody technique. *In vivo* fixation of immunoglobulin G, immunoglobulin M, and third component of complement were detected in areas of tumor adjacent to dermis or in tumor tissue undergoing necrotic degeneration. In some areas, immunoglobulin and complement were deposited in intercellular spaces of tumor tissue. Deposits of fibrinogen-fibrin were also present, but they were in the dermis and bore no spatial relationship to immunoglobulin and complement deposits. The characteristics of immunoglobulin and complement fixation in keratoacanthoma suggest that immunologically mediated mechanisms might participate in its spontaneous resolution.

INTRODUCTION

Keratoacanthoma is a rapidly growing tumor of skin which is composed of keratinizing squamous cells and which resolves spontaneously in a period of months (14). The close histological resemblance to more aggressive forms of squamous cell carcinoma is well known (1), and one must often rely on the presence of an infiltrate of mononuclear cells in the dermis, invasion of the base of the tumor by eosinophils, and formation of microabscesses to make the diagnosis of keratoacanthoma (10). The manner in which this rapidly proliferating tumor resolves is not understood, but the presence of an infiltrate of mononuclear cells within the tumor tissue and its spontaneous resolution suggest that immunological mechanisms may be involved. This paper reports an immunohistological study of keratoacanthoma in which immunoglobulin, complement, and fibrin were detected in specific locations in tumor tissue, and it is suggested that these findings may be related to immunological mechanisms causing regression of the tumor.

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MATERIALS AND METHODS

Nineteen lesions, clinically diagnosed as keratoacanthoma, were surgically excised under local anesthesia. The 19 patients bearing these skin tumors were predominantly males in good health, although many had skin that was actinically damaged. One-half of each excised lesion was sent to the laboratory for routine histological processing while the remainder was placed in a glass tube and immersed in liquid nitrogen where it was maintained frozen for less than 0.5 hr before embedding in Tissue Tec (Ames Laboratories, Elkhart, Ind.) medium for frozen sections. Sections were cut at -20° to a thickness of $4\ \mu\text{m}$. Individual sections were picked up on glass slides and allowed to air dry.

For direct fluorescent antibody study, the air-dried slides were washed for 10 min in PBS⁴ and then drained and wiped dry, avoiding contact with the tissue. The tissue was then covered with specific fluorescein-conjugated antibody and incubated for 30 min in a moist chamber at room temperature. After incubation, the slides were washed again for 10 min in PBS, drained, wiped dry, and sealed with coverslips using a 50:50 mixture of glycerol and PBS as mounting medium.

Specific staining reagents were aIgG, goat anti-human $\beta 1\text{C}/\beta 1\text{A}$, and sheep anti-human fibrinogen-fibrin. Also used were rabbit anti-human IgM and rabbit anti-human IgA (aIgA).

Antisera to immunoglobulins (IgG, IgM, and IgA) were obtained by immunization of rabbits with purified preparations of monoclonal IgG, IgA, and IgM from patients with multiple myeloma and were absorbed with k and λ light chains to produce antisera with the respective heavy-chain specificities. Antisera to $\beta 1\text{C}/\beta 1\text{A}$ (C3) and to human fibrinogen-fibrin were purchased from Hyland Laboratories, Costa Mesa, Calif. All these antisera were conjugated with fluorescein isothiocyanate according to the method of Frommhagen (4) so as to give a final fluorescein to protein molar ratio of 1:5 to 2:0.

In testing the specificity of reaction of the fluorescein-conjugated antisera, 2 different absorption techniques were used. Tissue sections were cut in the usual manner and picked up individually on slides in sequential order with no more than one $4\text{-}\mu\text{m}$ section lost between each slide. One slide was stained with fluorescein-conjugated aIgG as described above and served as a reference section. The tissue

⁴The abbreviations used are: PBS, 0.01 M phosphate:0.15 M NaCl, pH 7.4; aIgG, rabbit anti-human IgG; C3, 3rd component of complement.

section immediately preceding the reference section was washed for 10 min in PBS and then incubated for 30 min with fluorescein-conjugated aIgG that had been previously absorbed in a mg:mg ratio with human IgG. The section was then washed and mounted as described above. For further evaluation of the specificity of the results, the section immediately following the reference section was incubated with unconjugated aIgG and then stained with fluorescein-conjugated aIgG. The specificity of reaction demonstrated by fluorescein-conjugated anti-C3 and anti-fibrin was studied for a limited number of tissue sections. Absorption of the conjugates with C3 and fibrinogen-fibrin, respectively, removed previously observed staining.

Frequently, after fluorescent antibody staining had been observed in a specific site and photographed, the coverslip was soaked off and the slide was fixed in 10% formalin prior to staining with hematoxylin and eosin. The photographs were then compared to the hematoxylin and eosin slide for better orientation and identification of the specific structures that were reactive in the fluorescent antibody study.

At the time of tumor excision, serum was also obtained from the patient and indirect staining was carried out by reacting serum with autochthonous tumor, allogeneic tumor, and autochthonous and allogeneic normal skin.

In a 16-month period spanning the current study, 52 skin biopsies from patients with various connective tissue diseases such as systemic lupus erythematosus, scleroderma, and dermatomyositis, and skin and muscle biopsies from patients with myopathies were studied by the techniques described above. As reported previously, deposits of immunoglobulin and C3 were present at the dermal-epidermal junction in systemic lupus erythematosus (15). Neither in connective tissue diseases nor in the myopathies was there fixation of immunoglobulin and/or C3 in intercellular spaces of squamous epithelial cells as is described below for keratoacanthoma.

All fluorescent antibody slides were examined on a Leitz Ortholux microscope with a dark-field condenser and a xenon light source with appropriate filters. Photographs of fluorescent specimens were taken with Polaroid ASA 3000 film using time periods of 3 to 4 min.

RESULTS

Routine hematoxylin and eosin slides of biopsy material were reviewed by 2 histopathologists, and at least 1 of the reviewers had to make an unequivocal diagnosis of keratoacanthoma before the material was included in the study. A total of 19 biopsies, each from a different patient, were processed for immunopathological examination. In Table 1 the histological and immunohistological findings are presented. In 14 biopsies, both histopathologists made a diagnosis of keratoacanthoma but in 5 this diagnosis was made by one pathologist but not by the other. The other diagnoses in these 5 patients were squamous cell carcinoma (F. S., H. G., and D. C.) and wart (E. D. and R. McB.). These differences in diagnoses in the 5 patients were not surprising, since there are no generally accepted rigid histological criteria for keratoacanthoma, and the diagnosis is often made only with additional information obtained from the clinical

Table 1
Histological and immunohistological findings in keratoacanthoma

Patient	WBC infiltration	Micro-abscesses	Cell membrane staining for			Fibrin deposits
			IgG	IgM	C3	
J. A.	+	+	+	+	+	+
W. R.	+	0	+	+	+	+
H. B.	+	+	+	+	+	+
G. S.	+	+	+	+	+	+
R. B.	+	+	+	+	+	+
E. G.	+	+	+	+	+	+
T. P.	+	+	+	+	+	+
D. E.	+	+	+	+	+	+
H. E.	+	+	+	0	+	+
W. A.	+	0	+	+	+	+
F. S.	+	+	+	0	+	+
L. W.	+	0	+	+	+	0
E. D.	+	0	+	0	0	+
R. McB.	+	+	+	0	0	+
H. G.	0	0	0	0	0	+
C. M.	0	0	0	0	0	+
B. B.	+	0	0	0	0	+
D. C.	0	0	0	0	0	+
J. T.	+	+	0	0	0	+

history such as sudden appearance of skin tumor, rapid growth, and a characteristic clinical appearance of the lesion.

Fourteen of the 19 keratoacanthoma tissues showed staining for IgG which was distributed in a fashion suggestive of cell membrane staining as will be described later. Staining for IgG could be correlated with mononuclear cell infiltration in keratoacanthoma and presence of micro-abscesses, although a few exceptions were noted. Staining for IgM and C3 were detected in 10 and 12 biopsies, respectively, in similar morphological localization as IgG, and again they appeared to be found largely in tissues with cell infiltration and microabscesses. Fibrin deposits were detected in 18 of 19 biopsies, but there was no correlation either with cell infiltrate and microabscesses or with IgG staining.

Fig. 1a is a representative example of one pattern of IgG localization which was seen (Patient J. A.). Fluorescence was observed as thin linear streaks, frequently circular or polygonal in shape, surrounding nonfluorescent central areas. This pattern of staining was seen within the tumor at the regions of invasive invaginations into the dermis and was frequently but not consistently noted to be adjacent to microabscesses. This was considered to be the more "active" area of tumor where characteristically infiltration by inflammatory cells and widening of tumor intercellular spaces were seen. The arrows in Fig. 1 point to the approximate areas where tumor tissue was contiguous with dermis. Linear staining for IgG corresponded to tumor cell margins or to intercellular spaces as was demonstrated when the fluorescent-stained sections were counterstained with hematoxylin and eosin as illustrated in Fig. 1b. When adjacent sections of tumor tissue were stained with conjugate absorbed with IgG, staining of cell margins was abolished (Fig. 1c).

The 2nd pattern of IgG fixation in keratoacanthoma is

illustrated in Fig. 2A (Patient D. E.). This was an area showing breakdown of epidermal architecture, beginning necrosis of individual keratoacanthoma cells and infiltration by a few mononuclear cells. Staining for IgG was stronger in these areas than in those illustrated in Fig. 1a. In these regions of early keratoacanthoma cell necrosis (Fig. 2a), IgG staining could be observed in occasional areas to be linear and circular or polygonal in shape but were mostly lumpy in character. Adjacent invaginations of healthy looking Ka cells (*starred areas*) did not stain for IgG. An adjacent section was stained with hematoxylin and eosin (Fig. 2b), and the histological characteristics of cellular disintegration were evident in the area in Fig. 2a which stained for IgG. Absorption of aIgG conjugate with IgG resulted in abolition of staining in an adjacent section of this tumor (Fig. 2c).

In vivo tumor fixation of IgM and C3 shown in Table 1 was present only in tumor tissue which had shown fixation of IgG and was localized in keratoacanthoma in the patterns described for IgG. No IgA was detected in any of the biopsies. There was no staining for IgG, IgM, or C3 in nuclei or cytoplasm of cells.

Figs. 3a and 3b were biopsies from 2 different patients stained with anti-human fibrinogen-fibrin and were representative of most positive sections. Staining for fibrinogen-fibrin was usually quite strong and the deposits were clearly in the dermis but close to margins of acanthotic projections of tumor tissue. No extension of fibrinogen-fibrin staining into the intercellular spaces of tumor simulating patterns of cell margin staining was detected. Staining for fibrinogen-fibrin was not associated with immunoglobulin deposition nor appendigeal structure such as sweat glands.

DISCUSSION

Many human neoplasms possess tumor-specific antigens that are capable of inducing immune responses in their hosts. They have been shown to include cell-mediated cytotoxic reactions and humoral responses consisting of cytotoxic and opsonizing serum antibodies (2, 3, 7, 8, 9). In certain tumors such as malignant melanoma and colonic carcinoma, humoral antibodies have been demonstrated by immunofluorescent studies to be directed against antigenic determinants on tumor cell membranes (6, 11). In the present study, a number of features may be related to the findings of previous investigators. The pattern of *in vivo* localization of immunoglobulins within keratoacanthoma tissue may be due to reaction with tumor cell membranes since IgG and IgM were clearly present in intercellular spaces of the tumors. Concomitant detection of C3 component of complement in the majority of tumors staining for IgG and IgM supports the likelihood of antigen-antibody reactions occurring at these sites with fixation or activation of complement.

Another feature was the observation that IgG fixation on tumor cell membranes or intercellular spaces occurred primarily within the advancing edge of tumors in areas close to dermis and were frequently adjacent to microabscesses. The fixation of IgG with complement at these sites could

contribute to destruction of the advancing edge tumor cells. This possibility does not rule out participation of cell-mediated immune reactions. Nicolau *et al.* (13) showed that patients with keratoacanthoma demonstrated delayed-type skin reactions to inoculation with keratoacanthoma cells and Nairn *et al.* (12) have shown both humoral antibody-mediated and lymphocyte-mediated cytotoxicity to tumor cells in skin cancer. We were unable to demonstrate reaction with sections of tumor cells using keratoacanthoma sera in the indirect immunofluorescent technique. However, we did not tease apart tumor cells and cause them to react in suspension with sera as was done by Nairn.

Fibrinogen or fibrin was fixed in tissues in the dermis close to the margins of some acanthotic projections of tumor but did not extend into the intercellular spaces of tumor tissue itself. There was no detectable morphological relation between fibrin deposition and areas showing IgG, IgM, or C3 fixation.

Keratoacanthoma might be regarded as an example of an "experiment of nature" (5) and provides a unique opportunity to study mechanisms regulating a neoplasm which proliferates and later spontaneously undergoes regression and resolution. The current studies suggest that part of the explanation for spontaneous regression might be due to *in vivo* reaction of antibodies and complement with tumor cell membranes.

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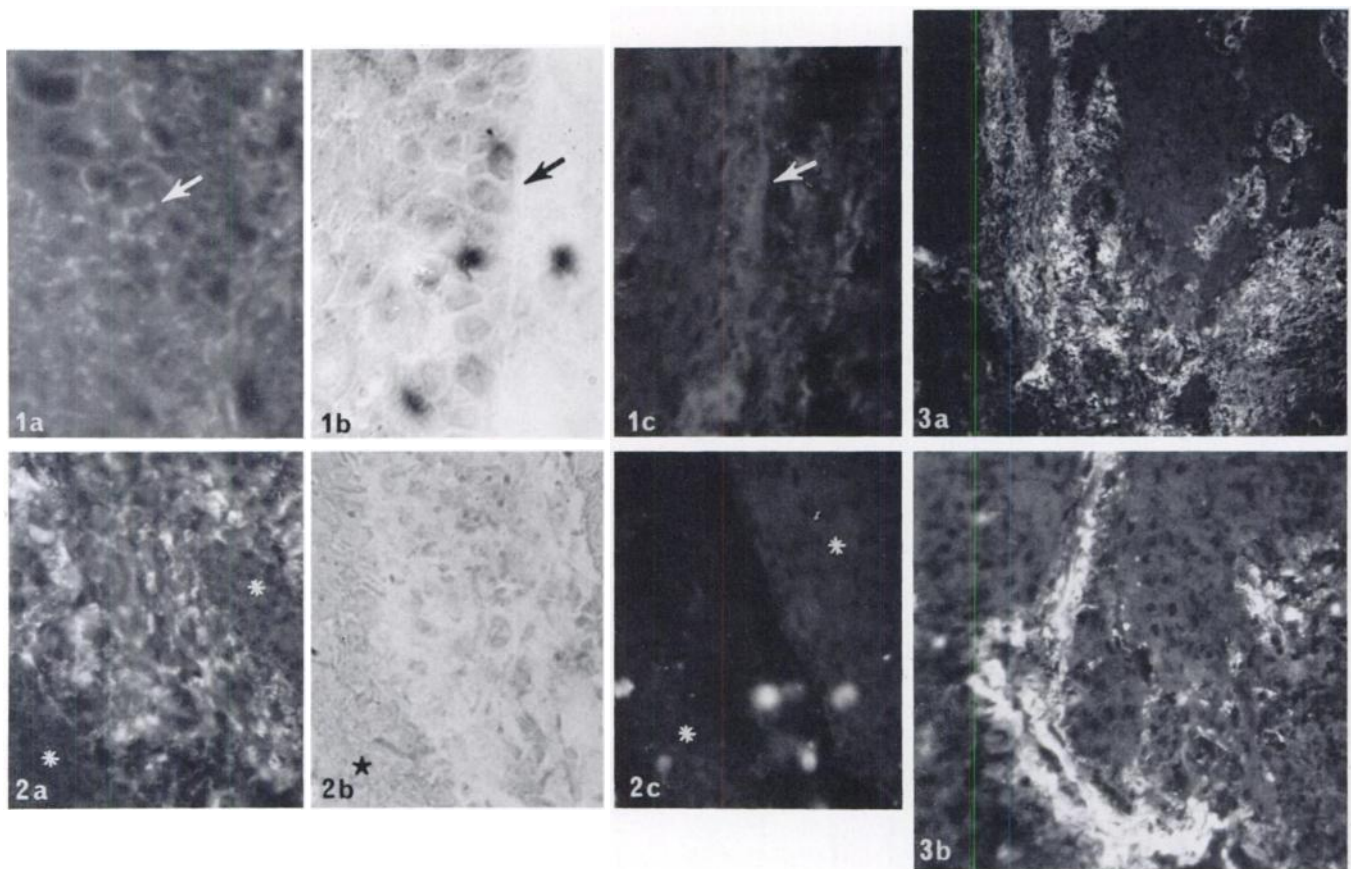


Fig. 1. Sections of keratoacanthoma from Patient J. A. examined by immunofluorescence for *in vivo* fixation of IgG. *a*, area of keratoacanthoma adjacent to dermis where infiltrating mononuclear cells were present. *Arrows*, approximate regions separating tumor tissue from dermis. In *a*, staining was observed as linear fluorescence that was circular or polygonal, surrounding nonfluorescent central areas. The fluorescent regions corresponded to widened intercellular spaces of keratoacanthoma cells as illustrated by H & E staining of the tissue section (*b*) after the coverslip was floated off. *c*, adjacent tissue section stained with fluorescein conjugated absorbed with IgG. Approximately $\times 600$.

Fig. 2. Immunofluorescence of an area of keratoacanthoma showing breakdown of epidermal architecture, beginning necrosis of individual keratoacanthoma cells and infiltration by a few mononuclear cells. *a*, deposits of IgG that were more dense and widespread than in Fig. 1*a*. In this area of early necrosis (*a*) staining was often lumpy. Contiguous areas of healthy keratoacanthoma cells (*starred areas*) on either side showed no staining. *b*, adjacent section stained with H & E showing scattered fragments of cells and cell debris in the area that stained for IgG. *c*, another adjacent section stained with conjugated absorbed with IgG. Approximately $\times 600$.

Fig. 3. *a* and *b*, biopsies from 2 patients with keratoacanthoma stained with antifibrinogen-fibrin conjugate. Large deposits were present in dermis without extension into adjacent keratoacanthoma tissue. Staining for fibrinogen-fibrin was present in majority of keratoacanthoma examined. There was no association between deposits of fibrinogen-fibrin and IgG or C3. *a*, approximately $\times 300$; *b*, approximately $\times 600$.