PAPERS AND ORIGINALS

Specific Immune Response in Human Skin Carcinoma

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[WITH SPECIAL PLATE]

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Summary

Eight out of nine patients with squamous cell carcinoma of skin have shown immunological reactivity against their own tumour cells by one or more tests with their sera or peripheral blood lymphocytes. The tests included membrane and cytoplasmic immunofluorescence, and, with cultured tumour, complement-dependent serum cytotoxicity and lymphocyte attack. One case examined in depth had an unusually conspicuous lymphocyte and plasma cell reaction on histological examination, and was positive by all four tests; a time-lapse cinephotomicrographic record over seven days was obtained of the attack on the carcinoma cells in culture by the patient's lymphocytes.

Introduction

Current world-wide interest in immunity against cancer in man is sustained by rapidly expanding laboratory information on particular immunological reactions in a variety of tumours (Hellström *et al.*, 1971a). Patients with malignant melanoma (*British Medical Journal*, 1970), Burkitt's lymphoma (Klein, 1971), and neuroblastoma (Bill, 1969) have so far provided the best examples of clinically significant anticancer activity. For most tumours direct immunological evidence is scanty, though in some there is circumstantial support for a host immune response as in squamous cell carcinoma of skin, which is often associated with a notable local lymphocytic reaction.

It seemed reasonable to suppose that this tumour might provide a fruitful subject for detailed laboratory investigation of the presence in the patient of specific serum antibody and lymphocyte activity. We report here positive findings from such a study, in eight out of nine patients with squamous cell carcinoma, including one strongly reactive (Case 1) examined in particular depth for immunological specificity.

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Methods

The immunological investigations were made on cell suspensions of squamous cell carcinomas of skin freshly removed at operation as part of the patient's routine clinical management. Tests on tumour cells for lymphocytotoxicity and complement-dependent serum cytotoxicity in culture, and, by immunofluorescence, for membrane- and cytoplasmic-reactive serum antibodies were performed by methods substantially similar to those developed for the melanoma studies of the Chester Beatty Institute tumour immunology group (Lewis *et al.*, 1969; Ikonopisov *et al.*, 1970; Currie *et al.*, 1971). There were several differences in technical detail which are described.

TUMOURS, LYMPHOCYTES, AND SERA

Nine patients with squamous cell carcinoma of skin were studied, selected only in so far as their resected tumours yielded enough cells for testing.

Histopathological confirmation of diagnoses was obtained on formalin-fixed paraffin sections stained with haematoxylin and eosin, and, as necessary, by phosphotungstic acid haematoxylin; methyl-green/pyronin staining was used to assess plasma cell reactions.

Cell suspensions were made from specimens transported in culture medium 199 within 24 hours of the operation, usually within three hours. The suspension was obtained by mechanical teasing and scraping the tumour in about four times its volume of 199 enriched by 20% fetal calf serum and filtering through a 100-μm mesh copper sieve. Resulting cell counts were of the order of 5×10^6/ml, and viability was assessed by exclusion of trypan blue dye at a concentration of 0·1%. Suspensions were then used either immediately for the immunological tests or as

convenient after storage with 10% dimethylsulphoxide in liquid nitrogen following controlled cooling to −30°C at 1° per minute and to −70°C at 10° per minute. The cell suspensions were observed to contain not less than 80% tumour cells with viability up to 50%.

Lymphocytes were obtained from 20 ml of heparinized blood, taken about the time of the operation, by glass-wool filtration of separated buffy layer; granulocytes are retained on the glass fibres and the lymphocytes, contaminated by less than 5% of other leucocytes, were collected and kept in 199 plus 10% fetal calf serum at 37°C for a few days until required. No difference in reactivity was found between freshly used lymphocytes and those stored as described for up to 12 days at least.

Serum was obtained from 20 ml of clotted blood taken at the same time and was heated at 56°C for 30 minutes to inactivate complement before use in the tests.

IMMUNOFLUORESCENCE TESTS

These were carried out with appropriate controls and serum absorptions by general procedures described elsewhere (Nairn, 1969).

Membrane staining of viable cancer cells in suspension was conducted with the aid of an apparatus permitting partial mechanization and the simultaneous study of up to 40 serum samples (Nairn *et al.*, 1971). In principle, the tumour cell suspension was gently washed free of fetal calf serum and, for stored cells, of dimethylsulphoxide by centrifugation at 250*g* maximum for five minutes in medium 199, repeated twice. The washed suspension was adjusted to a cell count of some 30 × 10^6/ml and drops containing about 1 million cells each were transferred to 1-ml polystyrene tubes and incubated for 20 minutes at room temperature with a drop of patient's serum, diluted 1:5 with phosphate-buffered saline (0·01 M phosphate, pH 7·1). The cells were then washed twice in 199 as before and the centrifuged deposit contained in a drop of the residual medium was incubated for 20 minutes with fluorescein-conjugated goat antihuman-γ-globulin with activity against IgG, IgA, and IgM. The conjugate had a fluorescein to protein molar ratio of 4·5:1 and was used at a globulin concentration of 0·8%; after absorption with bovine liver homogenate and well-washed human group AB erythrocytes it gave by itself no fluorescent staining of any of the microscopical preparations. The cells were again washed once in 199 and then, to remove this medium, which is fluorescent, once in the phosphate buffer, and finally in barbitone-buffered saline (0·05 M sodium barbitone, HCl to pH 8·6), which gives optimum immunofluorescence (Nairn *et al.*, 1969).

The centrifuged cell deposit in a residual drop of the final buffer was mounted with a drop of glycerol (A.R. grade) on a microscope slide using a 2-cm circular coverglass to provide a convenient area for microscopical examination. It is important to use the minimum volume of suspension fluid to fill the area of the coverglass which is sealed with a ring of nail varnish; this permits examination of a "monolayer" of cells by high-power and glycerol-immersion objectives. Examination was by darkground ultraviolet fluorescence microscopy with a colourless barrier filter.

Cytoplasmic staining was studied on cell films. For these, cell suspensions were washed as for membrane immunofluorescence but with 0·5% fetal calf serum in the final washing solution to assist adhesion of the films to microscope slides. The films were air-dried for 10 minutes at 37°C, plunged into isopentane-liquid nitrogen slurry for at least three minutes, and either used immediately or stored at −70°C for a few days in sealed polyethylene bags containing a little isopentane. Before immunofluorescent staining the films were prewashed for 20 minutes in the phosphate buffer with gentle agitation and wiped dry. They were then incubated for 20 minutes at room temperature with test sera, diluted 1:5 in phosphate buffer, rinsed, and washed twice for five minutes each time, treated with the fluorescein-conjugated antihuman-γ-globulin for 20 minutes, again rinsed, and washed twice and mounted in barbitone-buffered glycerol (1:9, pH 8·6) for fluorescence microscopy as before.

CYTOTOXICITY TESTS

Cultural methods were much the same as in the melanoma studies of Lewis *et al.* (1969); cultures in Teflon ring chambers on microscope slides, sealed by coverglasses, were made in triplicate. Sterility was secured by adding benzylpenicillin (200IU/ml) and streptomycin sulphate (125 μg/ml) to the 199 medium, which also routinely includes polymyxin B sulphate (20 IU/ml) and neomycin B sulphate (10 IU/ml).

Complement-dependent serum cytotoxicity tests and control studies made on these cultures closely followed the procedures already described for melanoma. An important feature of this cultural method is that it is designed to select for testing only viable cells from the original tumour suspension which proliferate on glass. In the eight cases where tumour culture was possible, the large majority of cells growing on the coverglass of the chamber were readily identifiable morphologically as carcinomatous.

Lymphocytotoxicity tests were made on similar tumour cell cultures. Lymphocytes were washed twice in fresh 199 plus 10% fetal calf serum and then generally they were added to the tumour cells when the culture was first set up, at twice the concentration of the original tumour inoculum—that is, 2 × 10^6 lymphocytes in 1 ml of 199 plus 10% fetal calf serum were added to 10^6 of the cells from the tumour in 1 ml of the same medium; thereafter 0·4 ml of human group AB serum rich in complement was added. We do not know whether the presence of complement is essential for this lymphocytotoxicity test but on several occasions have had evidence of enhancement of reactivity by it. The mixture, which contained about 2·5 lymphocytes per tumour cell—that is, at least 5 per viable tumour cell—was transferred to three separate culture chambers each holding about 0·7 ml and incubated with repeated examinations over seven days. In Case 1 a second test was performed in which addition of the lymphocytes was delayed until the tumour cell culture had been established for five days. The addition was made by removing the microscope slide closing one side of the chamber, leaving viable tumour cells attached to the coverglass closure. The original culture fluid was replaced by the lymphocyte suspension in fresh medium and the chamber re-closed by a second coverglass which became the bottom of the preparation when inverted. In this experiment the ratio of lymphocytes to tumour cells was estimated to be not less than 10:1. Such a chamber with fresh culture medium and coverglass closures top and bottom is more satisfactory optically for phase-contrast time-lapse cinephotomicrography than the standard preparations.

Time-lapse Cinephotomicrography.—This was largely confined to the study over seven days of the delayed lymphocytotoxicity culture of Case 1; a few control preparations were examined for shorter periods. The apparatus permitted continuous phase-contrast photomicrography with a × 40 objective and one-second exposures at intervals of 5, 10, 20, 40, or 80 seconds. In this way it was possible to observe the attachment of lymphocytes to the tumour cells and the manner in which they are destroyed. Continuity of events was best recorded with a time lapse of 5 or 10 seconds.

IMMUNOLOGICAL SPECIFICITY

Satisfactory study of specificity was possible in Case 1; the material available was insufficient in the others. Two successive serum absorptions with half volumes of tissue homogenates, detailed in the Results section, were made by standard methods (Nairn, 1969) both for the immunofluorescence and the serum cytotoxicity tests. Examination of reactivity of tumour cells

with positive sera before and after absorptions, with normal sera or control sera from other cancer patients, and with lymphocytes from other individuals provided one group of specificity tests. Other tests were made on the serum and lymphocytes with the patient's normal epidermal cells and fibroblasts, and with other tumours, including a squamous cell carcinoma from another patient. In addition, serum cytotoxicity was studied with human blood group AB serum complement before and after inactivation.

Monospecific antihuman-globulin fluorescein conjugates with activity against IgG, IgM, and IgA respectively were used to examine the immunoglobulin class responsible for the cytoplasmic immunofluorescent staining in Case 1.

All the patients' sera were examined by immunofluorescence for possible autoantibody activity against nuclei, mitochondria, smooth muscle, and other normal tissue elements.

Results

Of the nine cases, eight showed at least one type of immunological reactivity between their serum or peripheral blood lymphocytes and their own tumour (Table I); more than one response was observed in four cases. There was no example of globulin binding to the tumour in vivo in any of the patients.

TABLE I—*Immunological Results in 9 Cases of Squamous Cell Carcinoma of the Skin: Reactivity between Patient's Serum or Peripheral Blood Lymphocytes and Own Tumour*

Case No.	Immunofluorescence		Cytotoxicity	
	Membrane	Cytoplasm	Serum	Lymphocyte
1	+	+	+	+
2	−	+	−	−
3	?*	+	+	−
4	+	+	+	+
5	−	+	−	+
6	−	−	−	+
7	−	−	−	+
8	−	−	...	+
9	−	−	...	...
No. positive of No. tested (8/9)	2/9	5/9	3/7	6/8

*Possibly positive but too few viable cells in suspension for confident assessment.
... Not examined.

Repeated tests over some three months were possible in four cases and gave substantially the same results with only minor variations in intensity of reaction. There was nothing in this small series to suggest any influence on the results of age, sex, or tumour grade of the patients. Relevant autoantibodies against normal tissue components were not detected in any of the sera. Lymphocytic infiltration was especially conspicuous in Cases 1 and 4, which were the most clearly reactive by all four tests. The findings in Case 1, from which adequate material was available, were studied in greatest detail and are described below.

Case 1

A woman of 63 years, with 15 years' history of ankylosing spondylitis, had a skin tumour on the mid-anteromedial aspect of the right leg (Special Plate, Fig. 1). This had been present for six months and was apparently a recurrence of a carcinomatous ulcer that had been treated by diathermy five months previously. The current lesion was a raised, solid tumour, 4·5 by 2·5 by 1 cm, mobile, superficially ulcerated and crusted, and without abnormal pigmentation. There were several mobile subcutaneous nodules, up to 5 mm across, within 10 cm around the lesion, but there was no sign of metastatic spread beyond this area. Biopsy specimens were taken from the primary tumour and an adjacent subcutaneous nodule. Megavoltage *x*-ray therapy (6 MeV) was given to the area at a peak dose of 4,500 rads with 1·5 cm tulle gras surface build-up, as 15 treatments over three weeks. There was pronounced resolution on the tenth day after starting treatment, and on completion no tumour could be detected and the ulceration had healed.

Histopathology.—The tumour was a poorly differentiated squamous cell carcinoma (Special Plate, Fig. 2); the presence of tonofibrils was confirmed in sections stained by phosphotungstic acid haematoxylin and there was an attempt at epithelial pearl formation in some areas but no keratinization. Widespread foci of infiltration by lymphocytes were conspicuous and plasma cells were numerous with patchy distribution at the tumour-dermis boundary. This lymphoid reaction was associated with viable tumour cell proliferation, not with necrosis or infection.

IMMUNOFLUORESCENCE

Membrane Staining.—Reactivity between the patient's serum and the surface membrane of her own viable tumour cells in suspension was unequivocally positive (Special Plate, Fig. 3). The staining had the now well-known typical interrupted peripheral distribution of cell surface antigens. This reactivity was reduced but not abolished by serum absorption with homogenate of the patient's normal skin and to much the same extent by absorption with other homogenates of human skin, liver, or two different epidermal squamous cell carcinomas. Total inhibition of reactivity was obtained only by absorption with the patient's own tumour (Table II). A second sample of serum taken three weeks after the first gave weaker staining. Neither serum sample stained the patient's own epidermal cells or a squamous cell carcinoma from another patient (Case 2).

TABLE II—*Effect of Various Serum Absorptions on Immunofluorescent Staining of Tumour Cells of Case 1*

Type of Staining by Patient's own Serum	Absorptions by Human Tissue Homogenates						
	Nil	Normal Liver	Normal Skin	Patient's Normal Skin	Squamous Cell Carcinoma (2 other Cases)	Malignant Melanoma	Patient's Squamous Cell Carcinoma
Membrane	++	+	+	+	+	...	−
Cytoplasmic	++	+	+	+	+	+	−

Cytoplasmic Staining (Table II).—The patient's serum unabsorbed gave strong cytoplasmic staining of the tumour cell films (Special Plate, Fig. 4). With the monospecific antiglobulin conjugates the reactivity was found to be due predominantly to IgG. The absorptions, which also included homogenate of a maligant melanoma here, had much the same selective effects as in the membrane staining: only the patient's own tumour had the capacity to neutralize all serum reactivity. Again the second serum sample gave weaker staining, and neither sample stained the patient's epidermal cells or Case 2 squamous cell carcinoma.

CYTOTOXICITY

Serum Cytotoxicity.—The tumour cells grew well in all cultures not treated with the patient's own serum (Special Plate, Fig. 5). In the presence of the patient's first serum sample, plus either active or inactivated complement, there was virtually total cytodestruction over the course of five days. The second serum sample gave much the same result except that total destruction occurred only in the cultures to which active complement had been added; with the inactivated complement, destruction was severe but not complete. Complement-dependence of the cytotoxicity thus seems only partial, but this could well be attributable to in-vitro production of complement by the lymphocytes inevitably present in a tumour cell suspension prepared from a carcinoma so heavily infiltrated by lymphoid cells as in Case 1. Absorption of the patient's serum with homogenates of (*a*) her own skin had no effect on cytotoxicity, (*b*)

normal human liver reduced cytotoxicity by about 50%, and (*c*) her own tumour homogenate abolished almost all reactivity. The second serum sample was tested against the tumour cells of Case 2, and also against cultures of a malignant melanoma and a colonic carcinoma: there was no evidence of cytotoxicity.

Lymphocytotoxicity.—Whether the patient's lymphocytes were added to the tumour cells at the time the cultures were first set up or five days after, some, within an hour or two, became firmly attached to many of the tumour cells, often no more than one or two per cell (Special Plate, Fig. 6). Progressively over the course of five days such a tumour cell was totally destroyed. The sequence of events in this process could be followed in most detail by time-lapse cinephotomicrography (Special Plate, Fig. 7).

In the single microscope field studied at any one time by this technique, lymphocytes could be seen moving passively in the medium until one, presumably specifically immunoreactive, encountered a tumour cell, to which it became attached. Sometimes actual attachment required several minutes during which the lymphocyte rebounded a few times from the tumour cell and edged unsteadily along its surface, apparently because of superficial cytoplasmic agitation with pseudopod formation in the target region. This local agitation continued for about an hour after firm attachment, which could be recognized by the now synchronous movements in the field of both lymphocyte and tumour cell. The lymphocyte sometimes looked actively mobile and in one instance of attack by another lymphocyte on an already damaged cell (Fig. 7 H) microscopic processes could be detected near the limit of visibility between the lymphocyte and target area.

Within some eight hours of the initial lymphocyte attachment, the tumour cell started to become more spherical, and was usually quite spherical by about 12 hours. At this stage the nuclear definition was diminished and altogether lost within 24 hours, and at the same time peripheral cytoplasmic blebs appeared and became increasingly conspicuous for up to five days. The bleb formation seemed to be responsible for the shedding into the medium of numerous spherical fine membranous 5-8-nm vesicles from the dying tumour cells on about the second and third days. Finally, the vesicles ceased to appear, a day or two later movement of the cytoplasmic blebs stopped, and the tumour cell became an inert amorphous spherical mass with a dull phase-contrast image, but did not disintegrate even after eight days' total observation following the addition of the lymphocytes.

The lymphocytes did not show any of these effects against cultured fibroblasts of the patient or a suspension in culture of her normal epidermal cells (Special Plate, Fig. 8). Furthermore, homologous lymphocytes from a patient with malignant melanoma had no cytotoxic activity against Case 1 tumour cells.

An observation on Case 1 tumour cultures without added peripheral blood lymphocytes, which may be of special theoretical interest, is that some tumour cells became attached to the coverglass of the chamber and remained viable even though partly enveloped by lymphocytes carried over from the original tumour tissue (Special Plate, Fig. 9). After four to five days of culture these lymphocytes often fell away from the tumour cells which continued to survive and proliferate.

When the peripheral blood lymphocytes of Case 1 were added to the cultures of tumours from other patients—namely, two squamous cell carcinomas (Cases 2 and 4), a malignant melanoma, and a colonic carcinoma—no cytotoxicity was detected.

Cross-reactivity with Tumour Cells of Case 2

There were sufficient tumour cells from this case for testing all the squamous cell carcinoma sera, four sera from patients with other tumours, and lymphocytes from three squamous cell carcinoma patients and two with other tumours.

By immunofluorescence, there was no cross-reactivity of membrane staining in any of 16 serum samples from the nine cases of squamous cell carcinoma, and none with sera from one case of basal cell carcinoma and three cases of malignant melanoma; the autologous reaction was also negative. The autologous reaction was positive for the cytoplasmic staining, which showed positive cross-reactivity with two of the four samples of serum from Case 3, but not with the sera of Case 1 or any other of the 16 serum samples mentioned above. Interestingly, the two cross-reacting sera were later samples obtained two and six weeks after many of the multiple skin tumours of Case 3 had been resected.

In the cytotoxicity tests, nine serum samples from each of the nine squamous cell carcinoma cases were tested: eight were negative, including the autologous reaction. Weak cross-reactivity was observed with a serum sample from Case 3. Lymphocytotoxicity was also negative with the autologous lymphocytes and those from Cases 1, 4, and 8, one melanoma, and one colonic carcinoma patient.

Discussion

The data show that immunological reactivity by blood serum and/or lymphocytes is common in patients with squamous cell carcinoma of skin. In particular, lymphocytotoxicity was most often positive of the reactions tested. There can be little doubt that this is likely to be the response most significant clinically. Serum antibodies, even if reactive, as in Cases 1, 3, and 4, with tumour cell surface as demonstrated by cytotoxicity and membrane immunofluorescence tests, will probably have only limited access to solid tumour tissue. If not cytotoxic in vivo, as, for example, when complement is deficient, they could perhaps block the effects of immune lymphocytes (cf. Hellström *et al.*, 1971b). It is noteworthy that Cases 1 and 4, with the most intense local infiltration of tumour by lymphoid cells, also showed the clearest positive in-vitro tests for immunological reactivity.

The failure of the infiltrating lymphoid cells within this carcinoma to destroy cultivated tumour cells or even to prevent cultured tumour growth suggests a local immunological paralysis or other lymphocytic incompetence not shared by some peripheral blood lymphocytes, which were demonstrably cytotoxic. Such non-reactivity of lymphoid tissue in the immediate drainage area of a tumour has also been observed in experimental studies (Alexander *et al.*, 1969) and could explain the thriving of metastatic deposits in regional lymph nodes. Its association with more general lymphocyte reactivity, not necessarily overt, might account for the provoking of immune responsiveness in patients who are otherwise non-reactive (Ikonopisov *et al.*, 1970; Currie *et al.*, 1971).

A low ratio of lymphocytes to tumour cells was used in the lymphocytotoxicity studies, mainly to provide clear negative control results with any homologous lymphocytes from normal individuals. In the event, unequivocal tumour cell destruction by autologous peripheral blood lymphocytes was observed in six of the eight cases tested. Exactly how this is brought about is not clear. Apart from direct interaction between blood lymphocytes and tumour cells, there could conceivably be synergism between these lymphocytes and those, otherwise inert, carried over in the original tumour cell suspension. Among other possibilities, some form of toxic chain reaction in the cultures, starting with destruction of a few tumour cells by initial lymphocyte attack, has not been excluded.

The antibodies against tumour cell cytoplasm demonstrated by immunofluorescence in five of the nine cases are doubtless part of an immune response to internal components which have leaked from tumour cells damaged by whatever cause. Could an immunocytic counterpart be the lymphocyte which later became attached in culture to the dying tumour cell in Case 1, illustrated in the cinephotomicrograph (Special Plate, Fig. 7 H) referred to in the Results? Any immunological reactivity confined to internal cell constituents would be expected in vivo

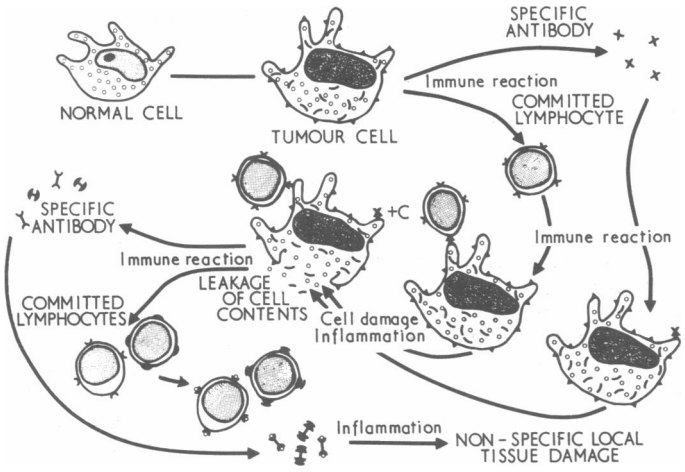

FIG. 10—Hypothetical explanation of membrane and cytoplasmic humoral antibody and lymphocyte reactivity against a tumour cell, and their possible relationship and effects.

to contribute to local immunological and inflammatory events but not to any specific rejection process of viable tumour cells. One speculative explanation of the possible relationship between such surface- and cytoplasmic-reactive immunity is given in (Fig. 10).

Immunological specificity and cross-reactivity were studied mainly in Case 1, in which it was found that the patient's lymphocytes reacted against her tumour cells but not her normal fibroblasts or skin, nor against four other tumours, including two squamous cell carcinomas. The patient's serum reactivity by whatever test was partly limited to her own tumour and could not be abolished except by absorption with her tumour. Absorption by two other squamous cell carcinomas, a melanoma, normal skin, including the patient's own, and normal human liver each reduced immunofluorescent staining, both membrane and cytoplasmic, incompletely and by about the same degree. Serum cytotoxicity, though not diminished by absorption with the patient's skin homogenate, was partially reduced by the liver homogenate absorption. This suggests that the antitumour activity in the serum included antibodies weakly reactive with components common to other human tissues, though no particular autoantibodies to normal tissue were in fact detected. The residual antitumour activity after serum absorption could reasonably be regarded as largely, if not totally, tumour-specific and not cross-reactive with at least two other squamous cell carcinomas. Such partial cross-reactivity, if applicable to other tumour immunity situations, may reconcile the discrepant reports on melanoma discussed by Hellström *et al.* (1971a). It might be identifiable only in seropositive cases with adequate material for comprehensive serum absorption testing.

The cross-reactivity tests carried out against the tumour cells of Case 2 were all negative except for Case 3, which was seropositive by immunofluorescence and cytotoxicity.

The lymphoid infiltration seen histologically in the biopsy specimen of Case 1 looked like a reaction to the proliferating tumour cells themselves and there was no sign of any extraneous cause for it. It seems reasonable to suppose that such lymphoid infiltration in carcinomas of skin is a host attempt at immunological rejection. Almost invariably by the time the tumour is clinically detectable such rejection must be ineffective, the lesion having outpaced the defences; this might be partly due to the lack of local lymphoid reactivity against the tumour, as in Case 1. It may be speculated that the best means of reversing this situation would be extirpation of sufficient tumour to permit recovery from the regional immunological insufficiency and opportunity for the reactive lymphocytes of the body to deal with a small enough number of residual cells to assure their elimination. The favourable immediate outcome of the local *x*-ray therapy in Case 1, in which the prognosis looked initially grave, might be attributable to an unusually vigorous systemic antitumour immunoreactivity.

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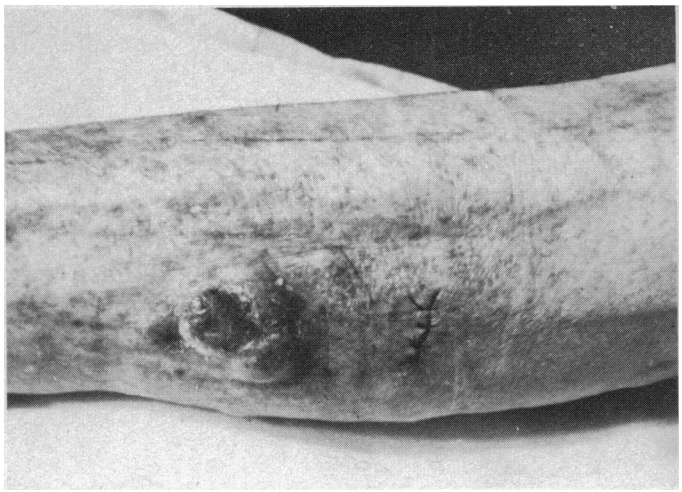

FIGS. 1-9—All from Case 1.

FIG. 1—Appearance of the leg tumour after biopsy.

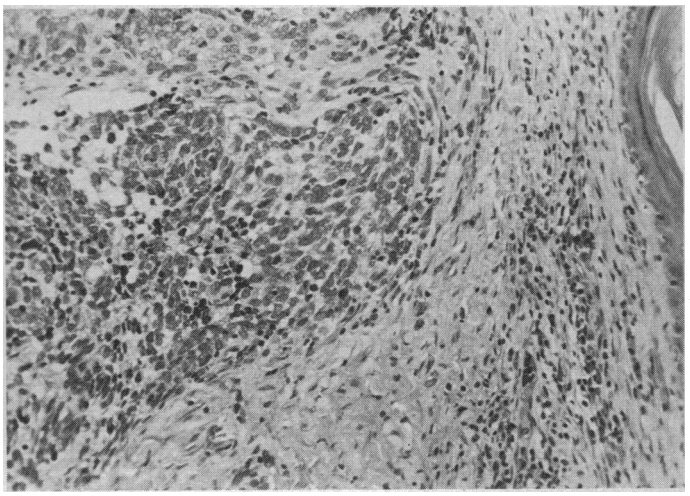

FIG. 2—Histopathology of the biopsy specimen showing sheets of squamous cell carcinoma abundantly infiltrated by lymphoid cells. (Haematoxylin and eosin. × 150.)

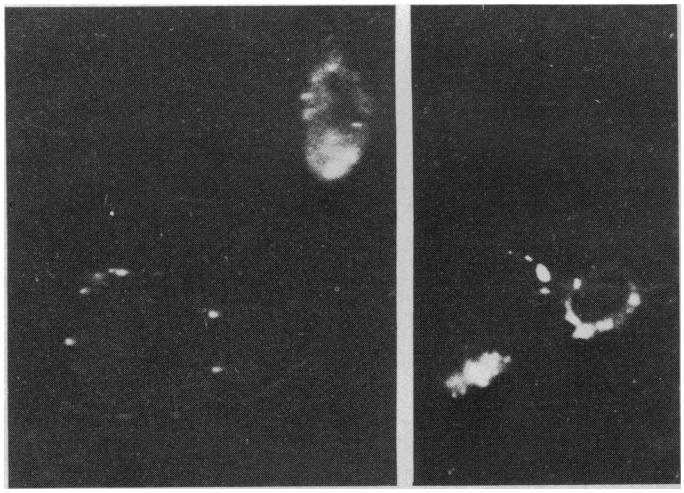

FIG. 3—Membrane immunofluorescence of the living skin carcinoma cells in suspension, by the patient's own serum. Two different fields. Note speckled staining of cell surface. (× 1,200.)

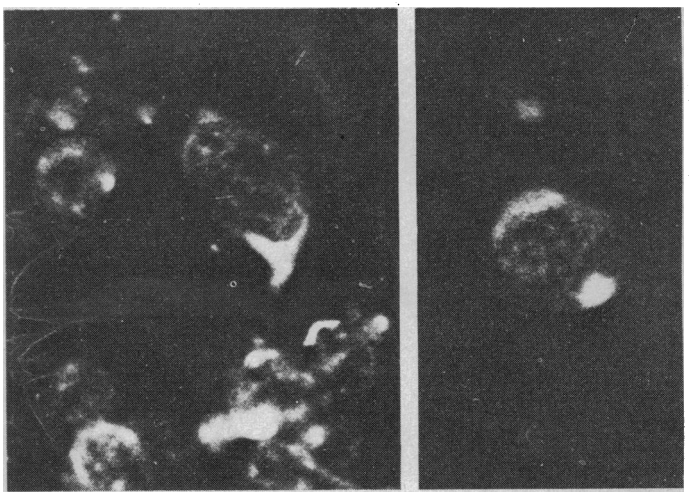

FIG. 4—Cytoplasmic immunofluorescence of dried film of the carcinoma cells, by the patient's own serum. Two different fields. (× 800.)

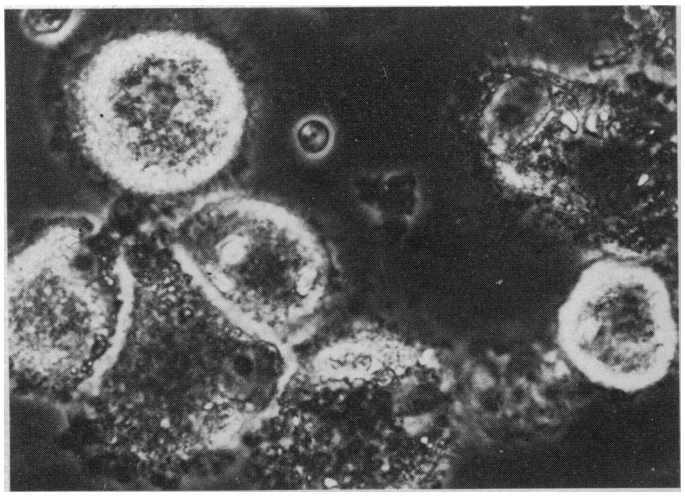

FIG. 5—Phase-contrast photomicrograph showing normal growth in culture of the squamous cell carcinoma. (× 800.)

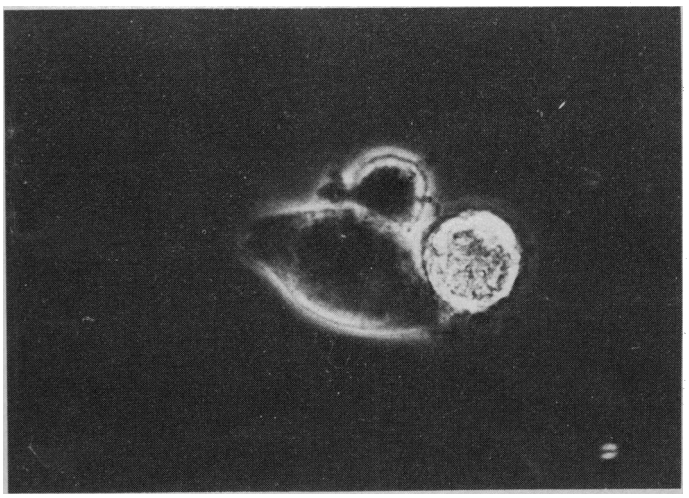

FIG. 6—Similar preparation to Fig. 5 to which the patient's peripheral blood lymphocytes had been added five days previously. Single surviving carcinoma cell on the coverglass in this field with an attached blood lymphocyte above. The white spherical cell to right is probably an adhering dead cancer cell. (× 800.)

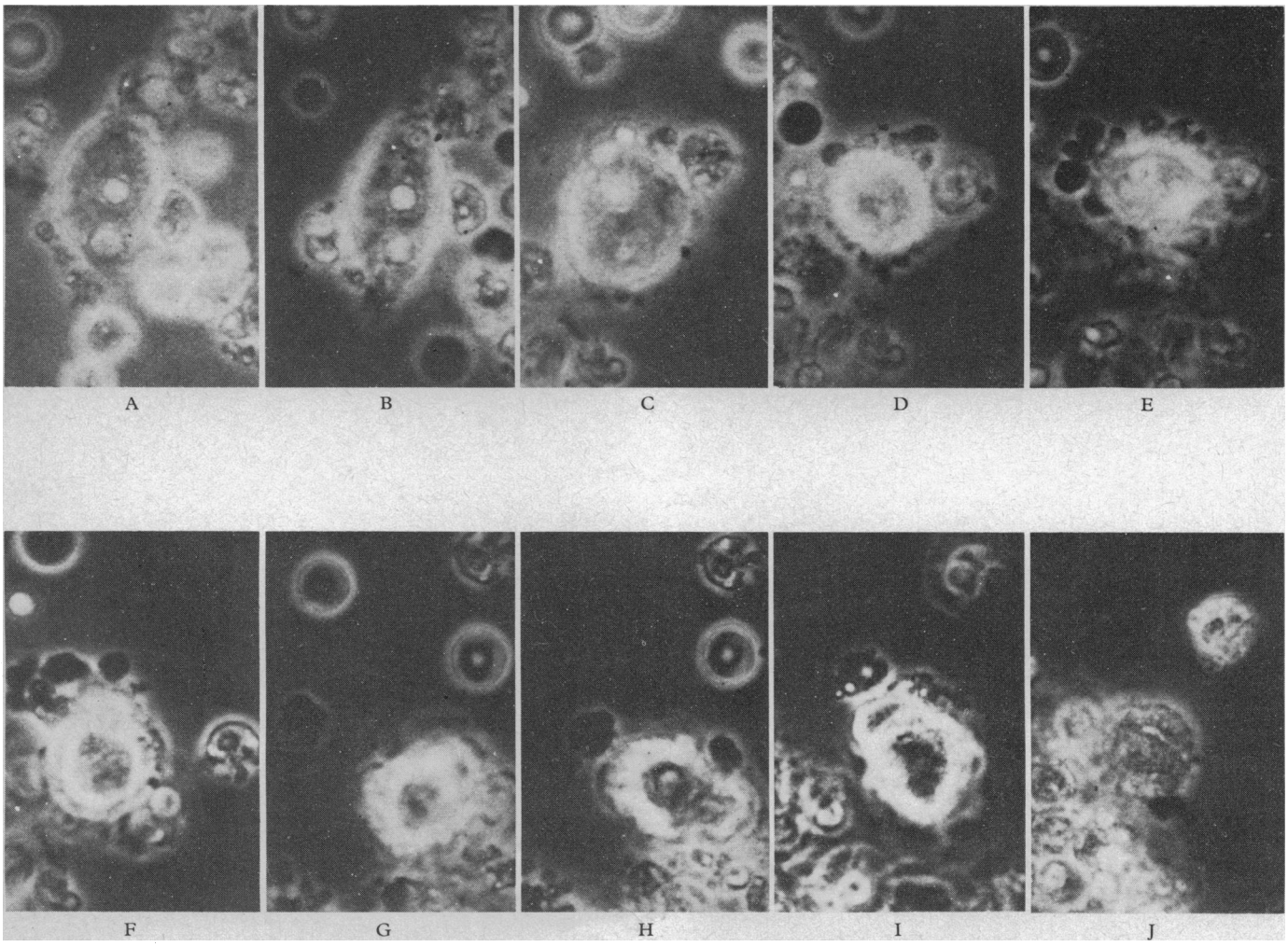

FIG. 7—Time-lapse phase-contrast cinephotomicrographs showing the reaction between the patient's peripheral blood lymphocytes and one cancer cell at different times over the course of seven days after adding the lymphocytes to five-day tumour culture. (× 800.) (A) Central fusiform cancer cell with two prominent nucleoli; attached lymphocyte lower mid-right of cancer cell. Other cells and debris largely out of focus. (1 hour.) (B) Second lymphocyte, partly out of focus, attached to lower left side of the cancer cell. (2 hours.) (C) Cancer cell rounded off. Loss of nucleolar definition and beginning of peripheral cytoplasmic blebs (black spots below and lower right). Only one attached lymphocyte visible, upper right. (12 hours.) (D) Cancer cell small and rounded off with loss of nuclear definition. Peripheral blebs round most of cell except where lymphocyte attached. (20 hours.) (E) Cytoplasmic blebs very conspicuous. (30 hours.) (F) Lymphocyte, mid-right, detached and moving away from dead cancer cell, and itself showing signs of death with nuclear distortion. (36 hours.) (G) New lymphocyte (black and just visible, mid-left of picture) approaching dead cancer cell, which has a fuzzy pale microscopic image; previously detached lymphocyte, now top right corner, showing karyorrhexis. (43 hours.) (H) New lymphocyte (black) firmly attached to upper left of cancer cell; a few fine black processes can just be seen between the two cells. Small black cytoplasmic bleb top right of cancer cell. Earlier detached lymphocyte top right corner. (44 hours.) (I) Further destruction of cancer cell and of detached lymphocyte. The late attached lymphocyte, top left of cancer cell, with three phase-bright spots. (72 hours.) (J) Loss of definition of cancer cell still producing occasional blebs, and of the earlier detached lymphocyte. (7 days.)

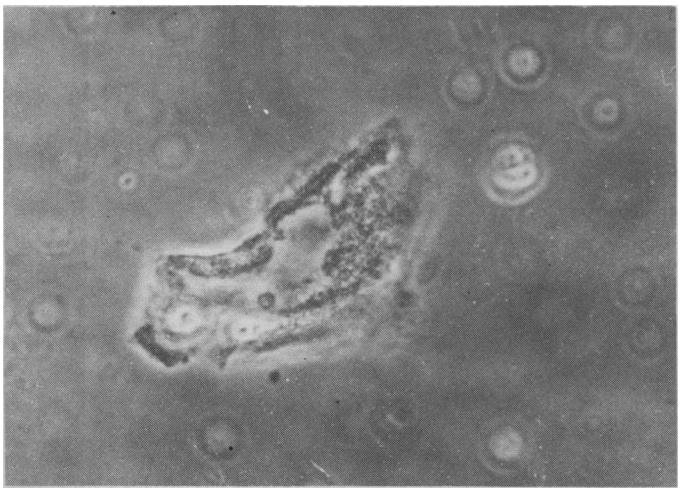

FIG. 8—Normal epidermal cell of the patient five days after addition of peripheral blood lymphocytes, which have not become attached to the cell. (× 800.)

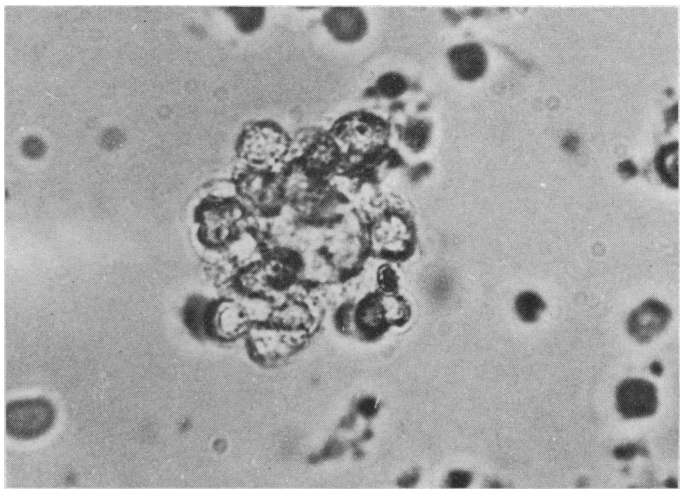

FIG. 9—Cancer cell of the patient attached to culture chamber coverglass and surviving despite investment by many lymphocytes present in the original tumour. No peripheral blood lymphocytes added. (× 800.)