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Failure to Detect Circulating IgG or IgM Antibodies to Basal Cell Carcinoma by Immunofluorescence

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Abstract. Immunofluorescence techniques failed to reveal evidence of anti-tumour antibody in the sera of patients with basal cell carcinoma. Although the presence of such antibodies has previously been associated with the absence of metastasis in malignant melanoma, other explanations for the low metastatic potential of basal cell carcinoma should be sought.

Key words: Basal cell carcinoma; Antibodies; Immunofluorescence

The basal cell carcinoma is the only common invasive tumour of man (outside the cranial cavity) that very rarely metastasizes. This finding remains un-

explained despite its possible considerable relevance to the problem of tumour metastasis in general. The cell-mediated immune response to basal cell carcinoma, as judged by the local inflammatory reaction observed by conventional histology, is usually mild or lacking.

The purpose of the study reported here was to determine whether or not a humoral immune response to basal cell carcinoma could be detected by using immunofluorescence techniques.

The discovery of circulating anti-tumour antibodies in malignant melanoma and their apparent association with the absence of metastatic spread (2) has led to the suggestion that serum antibodies might be involved in the control of metastasis (3). A search for similar antitumour antibodies in patients with basal cell carcinoma therefore appeared to be justified.

MATERIALS AND METHODS

After excision, basal cell carcinomas were placed in tissue culture medium 199 (Wellcome) and stored at 4°C for up to 18 hours; the varying times did not affect the results. From each patient, serum was obtained from a 10 ml clotted blood sample. From one-half of the carcinoma, 8 µm thick cryostat sections were prepared and, when possible, cell smears were made as well. Small or fragmented tumours were excluded so as not to jeopardize the routine histology performed on the remaining half of the tumour. The cell smears were prepared by scraping the surface of the tumour with a cataract knife into a drop of buffered saline on a glass slide. Haematoxylin and eosin staining revealed that this technique consistently provided tumour cells singly and in small clumps. The dried smears were frozen with isopentane in liquid nitrogen at -186°C for 5 min (2), redried and then treated in the same way as the sections.

In about half the cases studied, both fixed and unfixed preparations were used, the fixation being in dry acetone for 10 min at 4°C. As the fixation made no difference to the subsequent staining, all the subsequent preparations were processed unfixed.

The section and smears were then treated for 30 min with the serum, diluted 1:32 with 0.01 M phosphate-buffered saline, from either the same patient or another basal cell carcinoma patient. Control preparations from the same lesion were treated with 0.01 M phosphate-buffered saline only. After washing in buffered saline for 30 min all preparations were treated with fluorescein isothiocyanate-labelled goat antisera to human IgG and IgM (Behringwerke, protein concentration 0.8-1.5 g/100 ml) diluted 1:32 in 0.01 M phosphate-buffered saline. Further details of this sandwich method were published by Nairn (4).

The preparations were mounted in buffered glycerol and examined by darkground ultraviolet light with a Reichert Zetopan fluorescence microscope using excitation filter E2 and absorption filter Sp2.

RESULTS

Ten basal cell carcinoma lesions were studied from patients aged 50 to 82 years and from 5 of them smears were prepared as well as sections. Using fluorescein-labelled anti-IgG, scattered foci of staining were found in the connective tissue of all the sections. In 3 cases of very faint fluorescence was visible in the cytoplasm of the carcinoma cells but was present to the same degree in the control sections too. The carcinoma cells proved easy to identify in the smears, all five of which showed a weak cytoplasmic fluorescence in about 70% of the tumour cells, the remainder being unstained. A similar proportion of cells in the control sections showed the same weak staining. Dilution of the conjugate to 1:64 quenched the fluorescence. There was no difference in staining between preparations treated with autologous serum and those treated with homologous serum. Staining with fluorescein-labelled anti-IgM was negative in all preparations. No surface membrane fluorescence was seen.

The faint cytoplasmic fluorescence was regarded as non-specific staining (4) because it was present in the control groups, was readily removed by diluting the conjugate, and was present to the same extent in preparations treated with homologous sera.

DISCUSSION

We have failed, using immunofluorescence techniques, to find evidence of anti-tumour IgG or IgM antibody in the sera of patients with basal cell carcinoma. Other explanations for this tumour's low metastatic potential should be sought. The high rate of individual cell death in basal cell carcinoma has been proposed (1) as an explanation of its slow growth rate, but the relationship of this finding to metastatic potential is unknown. Although non-immunological factors may well be important in the behaviour of basal cell carcinoma, a search for other immune responses, particularly cell-mediated, would be justified, as this tumour could represent one of the few situations in which host responses are relatively successful in limiting tumour spread.

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Rapid Diagnostic Tests for Cutaneous Eruptions of Herpes Simplex

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Abstract. Comparison was made of the results of virus isolation, indirect immunofluorescent staining (IF-test) and Tzanck smears from 32 patients with cutaneous eruptions clinically diagnosed as herpes simplex. Herpesvirus hominis was isolated from 21 patients; IF-test was positive in 22 patients; Tzanck smears were positive in 20 patients. 23 to 25 of the 25 patients in whom one of the diagnostic tests was positive could be identified by a combination of any two of the diagnostic methods employed. No false-positive reactions were observed for IF-test or Tzanck smear.

Key words: Herpesvirus hominis; Tzanck smear; Immunofluorescence; Herpes simplex

In 1948 Tzanck described a microscopy test for the identification of cutaneous disorders, using scrapings from diseased skin (Tzanck smear). The main area of employment for this test is in the diagnosis of herpes simplex, zoster and varicella (4). Immunofluorescent staining of smears (IF-test) from cutaneous eruptions and eruptions on the mucous membranes has also proven useful for the diagnosis of herpes simplex (1, 3, 7).

The rapid establishment of the diagnosis of a cutaneous eruption of herpes simplex is important when eczema herpeticum is suspected. It is, likewise, important that the diagnosis of genital