

IN SITU IDENTIFICATION OF MONONUCLEAR CELLS INFILTRATING CUTANEOUS CARCINOMA: AN IMMUNO-HISTOCHEMICAL STUDY

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Abstract. The present study was undertaken in order to investigate the exact nature of the cells surrounding cutaneous tumours. Formalin-fixed, paraffin-embedded sections were tested with a horse antihuman T lymphocyte serum and with IgG, IgA and IgM rabbit antihuman serum. All the sections were respectively treated with rabbit anti-horse or swine antirabbit peroxidase-labelled serum. The advantages offered by the immunoperoxidase technique are briefly discussed. T cells are in overwhelming proportion in comparison with IgG, IgA and IgM bearing cells. This seems a further demonstration that mononuclear infiltrate surrounding cutaneous carcinoma mainly represents a cell-mediated immune response.

Key words: Cutaneous carcinoma; Lymphocytes; Immunoglobulins; Immunohistochemistry; Peroxidase

The occurrence of differentiated lines of cells involved in immunological processes has given rise to several investigations intended to identify the exact nature of these cells and, moreover, to quantify the humoral and/or cellular involvement of the immunological system. Spreading carcinoma of skin is usually accompanied by a cellular infiltrate, which is regarded as the evidence of a "host-versus-tumour" immunological response. B cells, T cells and histiocytes have been identified in cutaneous infiltrates surrounding tumoral tissues, when employing sheep erythrocytes coated with IgG or IgM antibodies, in presence of complement (7). The presence of immunologic membrane markers has been recently demonstrated in lymphoreticular cells of cutaneous infiltrates (14). Mononuclear cells have been identified in cutaneous tumours, using extractive procedures (5, 6).

In 1974, Taylor & Burns (16) improved the original method proposed by Nakane & Pierce (11, 12) employing peroxidase-labelled antibodies on

formol-fixed, paraffin-embedded sections. This method proved to be almost equally sensitive and more specific, in comparison with the immunofluorescence techniques, with special regard to cell morphology (2). We have therefore been induced to perform the above techniques in skin carcinoma, in an attempt to identify the immunoglobulin producing cells surrounding the tumour within the histological sections, as well as the "T cells".

MATERIALS AND METHODS

Four basal cell carcinomata (bcc), three squamous cell carcinomata (scc) and one squamous cell carcinoma metastasis of a regional lymph node were used. All the specimens were routinely fixed in 10% saline formol and embedded in paraffin. Sections 3-4 μ m thick were deparaffinized, hydrated and treated with methanol and hydrogen peroxide to block the endogenous peroxidases (15). After PBS washing, the slides were incubated for one hour at room temperature with horse antihuman lymphocyte serum, supplied by Farmitalia, Milan, pure and adsorbed to human B lymphocytes. After PBS washing, the sections were incubated with rabbit antihorse peroxidase-labelled serum, for demonstration of T cells. Ig-bearing cells were identified after incubation with rabbit antihuman IgG, IgA and IgM immunoglobulin, obtained from Dakopatt, Denmark, diluted 1:10 and followed by incubation with swine 1:10 peroxidase-labelled antirabbit serum. After PBS washing, all the peroxidase-conjugated antibodies were revealed by an extemporarily prepared solution of diaminobenzidine (DAB, Serva), dissolved in 0.05 M Tris-HCl buffer, pH 7.6, containing 0.01% hydrogen peroxide (8). The development of the immunohistochemical reaction was followed by means of an ordinary light microscope: the best results were obtained after 3-10 min of incubation with DAB solution. After a further PBS washing, the slides were routinely dehydrated and mounted in DPX, without counterstain. Control sections were prepared using DAB incubation without previous contact with antiserum. In two cases (bcc and scc), the above technique and the immunofluorescence technique were performed on frozen sections.

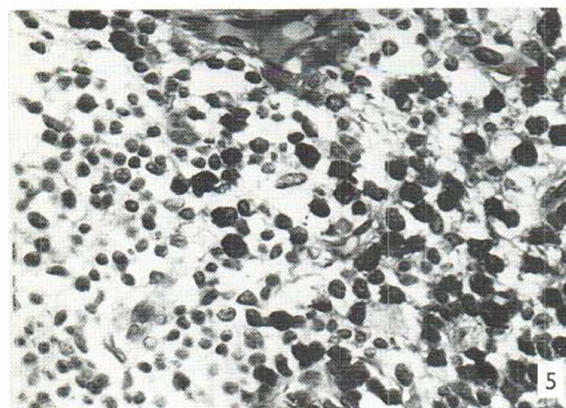
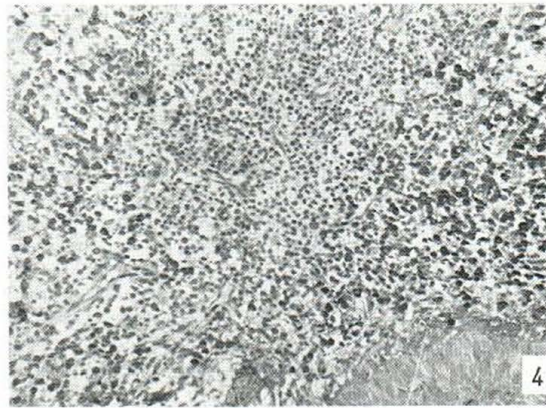
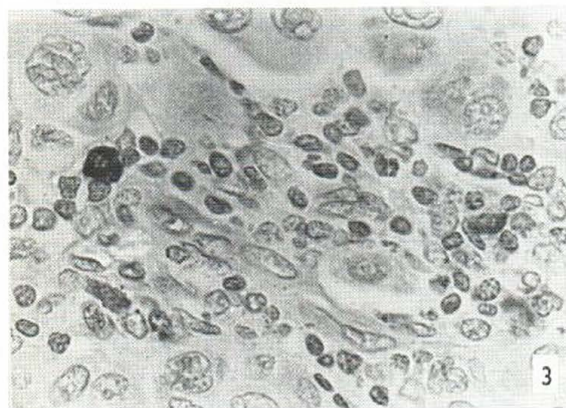
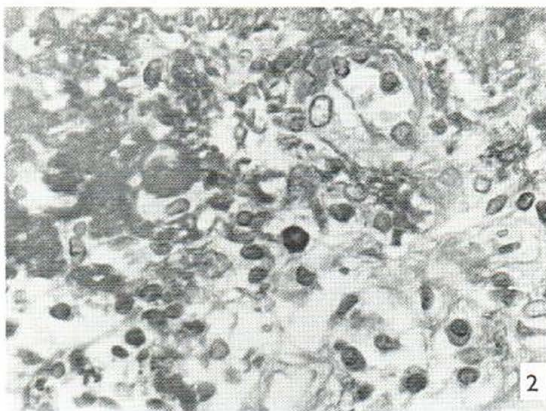
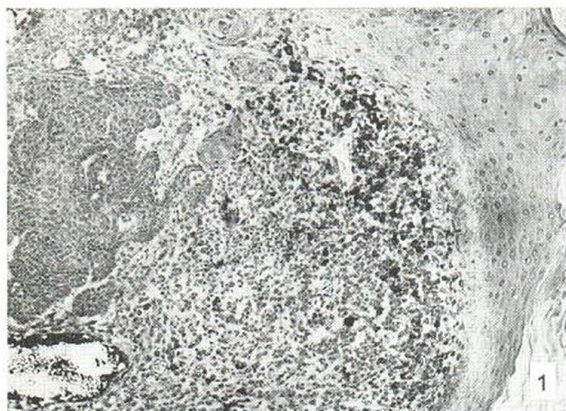


Fig. 1. Basal cell carcinoma. IgA-producing cells.

Fig. 2. Squamous cell carcinoma. An IgG-producing cell, surrounded by unlabelled T cells.

Fig. 3. Squamous cell carcinoma metastasis. IgM-producing cell, among negative lymphoid cells and neoplastic cells.

Fig. 4. Basal cell carcinoma. IgM-producing cells having a follicle-like disposition. Several unlabelled cells are visible in the centre.

Fig. 5. Basal cell carcinoma (same case as in Fig. 4). IgG-producing cells, higher magnification.

RESULTS

T- and Ig-bearing cells resulted markedly dark-brown and their morphology was excellent with regard to either non-specific background and else non-reactive cells. IgG, IgA and IgM-bearing cells were observed in the connective tissue surrounding both bcc and scc (Figs. 1, 2, 4 and 5). IgA-labelled cells (Fig. 1) were generally more numerous than

IgG (Fig. 2) and IgM producing ones. T cells, IgM (Fig. 3) and IgG cells were detected in the lymph node of the scc metastasis. IgG and IgM cells (Figs. 4 and 5) were also demonstrated in a bcc, displaying a putative follicle-like structure. In all instances the mononuclear cell infiltrate appeared chiefly composed of T lymphocytes (Fig. 6), thus demonstrating an exact correspondence with the predominant

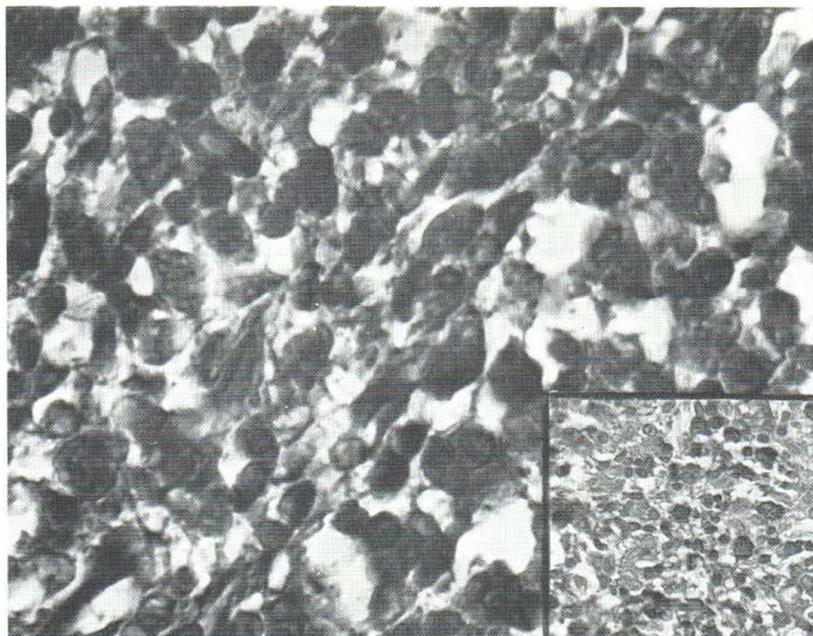


Fig. 6. Squamous cell carcinoma. T lymphocytes, chiefly composing the mononuclear infiltrate. In a serial section of the same area, the inset demonstrates some IgG-bearing cells, surrounded by an overwhelming population of unlabelled cells.

unlabelled cells, as observed in sections treated with the anti-Ig sera (Figs. 1–5 and inset of Fig. 6). Controls on frozen sections were in agreement with the previously described results.

COMMENT AND DISCUSSION

Our results agree with the previously published data appeared in the literature (2, 16). We were able, in fact, to confirm that both T lymphocytes and immunoglobulin-producing cells do keep their antigenic characters, thus giving further evidence that cytoplasmic antigens remain relatively unchanged after formol fixation and embedding procedures even after prolonged storage (3, 9, 10, 17). Several advantages of the immunoperoxidase technique have been confirmed. The stain is permanent and does not show any diminution of intensity, even after some months of storage; it is, obviously, possible to study these preparations by means of an ordinary light microscope and the morphological details are excellent, also without contrast stain, so that labelled and unlabelled cells can be accurately classified. These cells have been detected in paraffin blocks, routinely obtained and, in some cases, stored for years, thus confirming the possibility of performing valuable retrospective studies. Since this immunohistochemical technique can be directly

performed on common histological sections, it allows one to distinguish exactly the immunoglobulin producing cells and, moreover, to ascertain the relationships between the above cells and other non-specific cells as well as to demonstrate the connections with tumoral cells.

Similar conclusions have been recently heralded by Bustamante et al. (4), whose technique differs from ours only in some minor details. By using inhibition of the endogenous peroxidases according to Streefkerk (15), we avoided mistaking macrophages, polymorphonuclear leukocytes and erythrocytes. Moreover, enhancement of the label was obtained in our experience, by using the so-called "sandwich" technique.

It is generally believed that the dense lymphocytic infiltrate surrounding cutaneous carcinoma consists chiefly of T lymphocytes: evidence has been published by Schoorl et al. (13) also in human breast carcinoma. In accordance with their key role, T cells have been regarded as an *in vivo* "host-versus-tumour" immune response, similar to the "in vitro" activity of these cells in the disruption of neoplastic cells (1). Mononuclear cells have in fact been proved to be in a T/B ratio close to that of the delayed type (tuberculin) reaction, after extraction from basal cell carcinoma (6), thus justifying the hypothesis of a cell-mediated immune response in

tumours. The immunoglobulin-producing cells lying in the infiltrate surrounding the tumours probably display the role of a partial interaction or a synergism between cell-mediated immune processes and immunoglobulin production against tumoral antigens. On the basis of some details documented in the present study (Figs. 4 and 5), apparently comparable to analogous findings reported by Schoorl et al. (13), we are inclined to believe that the lymphatic infiltrate having the appearance of a small lymphatic follicle with a germinal centre may represent a partially organized cell reaction against tumoral antigens.

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