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Interleukin-8 Immunoreactivity in Malignant Tumours of the Skin

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In the past, interleukin-8 (IL-8) could be demonstrated within keratinocytes in normal epidermis and inflammatory skin diseases, like psoriasis and eczema. Using monoclonal antibodies, the distribution of IL-8 immunoreactivity was inversely related to the density of inflammatory infiltrate. Other in vitro observations indicated IL-8 to be a growth factor for keratinocytes. These results prompted an immunohistochemical examination of IL-8 immunoreactivity in malignant and semimalignant epithelial tumours of human skin. Whereas IL-8 could not be detected within the transformed cells of epithelial tumours or melanoma, some tumour cells within well differentiated squamous cell carcinoma and Bowen's disease showed IL-8 immunoreactivity. Thus, loss of IL-8 immunoreactivity can be a sign of malignant transformation. This indicates an important role in growth regulation as well as terminal differentiation of human keratinocytes. ***Key word: immunohistochemistry.***

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Interleukin-8 (IL-8) is a member of the chemokine family, which comprises a number of mediator peptides with shared biochemical and functional characteristics (1 for ref). Two sub-families could be established based on two cysteine residues located aminoterminally. In the chemokine α or C-X-C family, the two cysteines are separated by another amino acid, whereas in the chemokine β or C-C family, the cysteines are adjacent to each other.

IL-8, as a representative of the C-X-C family, was shown to exert both chemotactic and mitogenic activity on a number of different cell types, including neutrophilic granulocytes (2), T-cells, (3), keratinocytes (4) and melanoma cells (5). It is produced by endothelial cells, monocytes, lymphocytes and fibroblasts (6 for ref). Apart from these, keratinocytes (6) and melanoma cells (5) were shown to produce IL-8 in vitro as well.

Using monoclonal antibodies, raised specifically against IL-8 (7), distinct intraepidermal IL-8 immunoreactivity could be detected in normal skin, as well as inflammatory skin diseases such as psoriasis and eczema by immunohistochemical methods (8, 9).

Considering the differential staining pattern of basal and squamous cell layer in normal epidermis by these antibodies (8, 9), and the growth-activating properties of IL-8 for keratinocytes in vitro, the IL-8 immunoreactivity in keratinocyte-derived tumours (squamous cell carcinoma, basal cell carcinoma and Bowen's disease) was investigated. In comparison, malignant melanoma, a non-epithelial tumour, was tested for IL-8 immunoreactivity in vivo, as cells derived from this tumour are able to produce IL-8 in vitro (5).

MATERIALS AND METHODS

Tumour tissue samples were obtained from surgical specimens of basal cell carcinoma ($n=17$), squamous cell carcinoma ($n=7$), Bowen's disease ($n=7$) and malignant melanoma ($n=11$). Normal human skin was used as a control.

Within the group of basal cell carcinoma, solid, superficial and fibrosing variants can be found. The group of squamous cell carcinoma comprised well-differentiated and non-differentiated (grade III according to Broders (10)) tumours. Furthermore, nodular malignant melanoma ($n=1$), superficial spreading melanoma ($n=4$) and melanoma metastases ($n=6$) were studied. In all cases, diagnosis was confirmed histologically on haematoxylin eosin-stained sections.

Specimens were snap frozen and stored in liquid nitrogen until further processing. For immunohistochemistry, acetone-fixed cryostat sections were incubated with monoclonal anti-IL-8 antibodies 46E5 and 52E8 (7). Staining was performed using the Avidin-Biotin-Peroxidase-Complex (ABC) method (Vectastain ABC-kit, Vector, Burlingame) and DAB (3,3'-diaminobenzidine) or AEC (3-amino-9-ethylcarbazole) as chromogen, as described previously (8,9). Negative control was performed by substitution of the primary antibody by isotype-matched irrelevant antibodies or buffer.

RESULTS

In normal skin, monoclonal antibody 46E5 stained all living keratinocytes, leaving the horny layer unstained. In contrast, with monoclonal antibody 52E8 all suprabasal keratinocytes, including the horny layer, were marked.

Using monoclonal anti-IL-8-antibodies, 46E5 and 52E8, morphologically normal keratinocytes adjacent to tumour tissue show IL-8 immunoreactivity comparable to normal skin (Fig. 1) and are thus clearly discernible from tumour cells. However, IL-8 immunoreactivity could not be detected in any tumour cell of basal cell carcinoma (Fig. 1) or non-differentiated squamous cell carcinoma (Fig. 2) by either of the monoclonal anti-IL-8 antibodies used. Neither primary melanoma nor melanoma metastasis showed any IL-8 immunoreactivity within tumour cells (Fig. 3).

In basal cell carcinoma, distribution of IL-8 immunoreactivity was similar among the investigated histological variants of this tumour, e.g. solid, superficial or fibrosing basal cell carcinoma.

In contrast, a distinct difference can be seen between well-differentiated and non-differentiated squamous cell carcinoma. Within well-differentiated forms, one to two layers of 52E8- and 46E5-positive epithelial cells were found around horn pearls (Fig. 4).

Interestingly, in sections of Bowen's disease stained with monoclonal antibody 52E8, IL-8-positive and -negative cells were found side by side in the affected epidermis. These negative cells morphologically appeared to be malignant, with atypical cell formation and pleomorphisms of their nuclei. A similar pattern of immunoreactivity was obtained with moAb 46E5 (Fig. 5).

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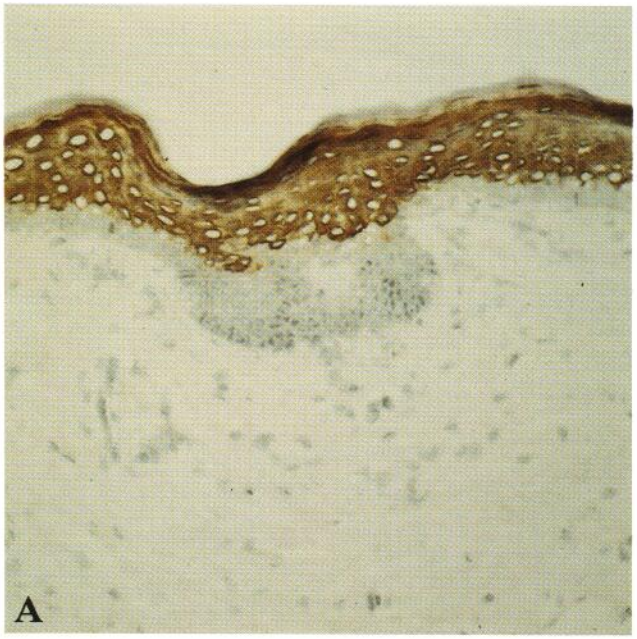


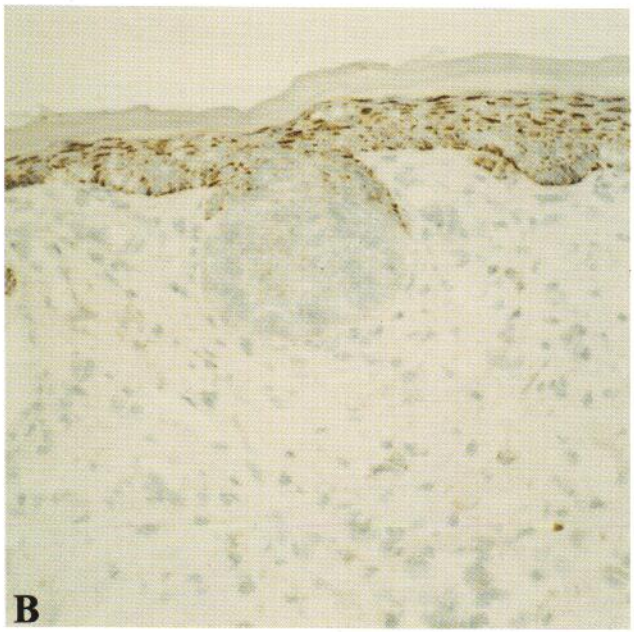


Fig. 1. IL-8 immunoreactivity in basal cell carcinoma with monoclonal antibody 52E8 (A) and 46E5 (B). Immunoreactivity is present only in adjacent normal keratinocytes; all tumour cells are negative with both antibodies (×400, chromogen DAB).

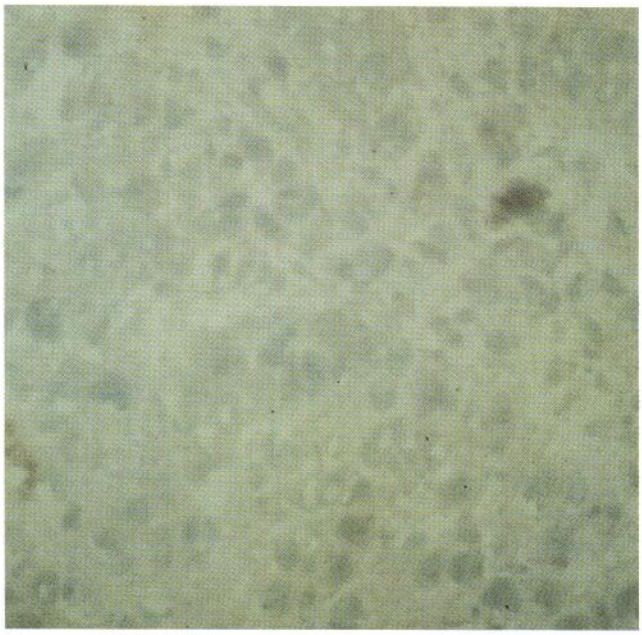


Fig. 2. IL-8 immunoreactivity in non-differentiated squamous cell carcinoma. All tumour cells are negative, as demonstrated here with monoclonal antibody 52E8 (×400, chromogen DAB).

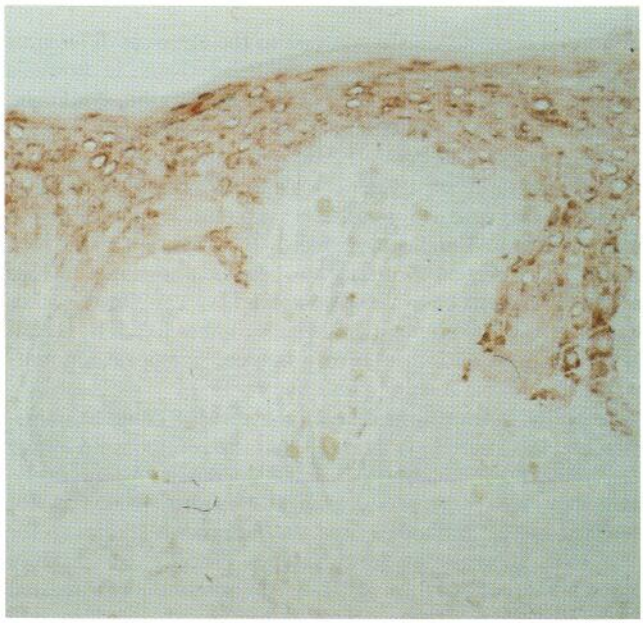


Fig. 3. IL-8 immunoreactivity in primary malignant superficial spreading melanoma with monoclonal antibody 52E8. All tumour cells are negative. Only normal keratinocytes show immunoreactivity (×400, chromogen AEC).

DISCUSSION

In previous studies from our laboratory using IL-8-specific monoclonal antibodies, distinct immunoreactivity was demonstrated within normal epidermis exclusively confined to differentiating keratinocytes (8). The monoclonal antibodies 46E5 and 52E8 are known to detect two different epitopes of the IL-8 molecule (7). It may be postulated that in normal keratinocytes preformed IL-8 will be processed during keratinocyte differentiation, resulting in a different accessibility of the two relevant epitopes. Under inflammatory conditions like psoriasis vulgaris (8) and different forms of eczema (9), immunoreactivity was reduced or even completely absent and inversely related to the degree of inflammation (9).

As demonstrated in this study, in malignant, transformed keratinocytes intracytoplasmic IL-8 immunoreactivity was absent with both antibodies used, whereas normal-appearing keratinocytes adjacent to tumour cells retained their IL-8 immunoreactivity and were similar to epidermal keratinocytes

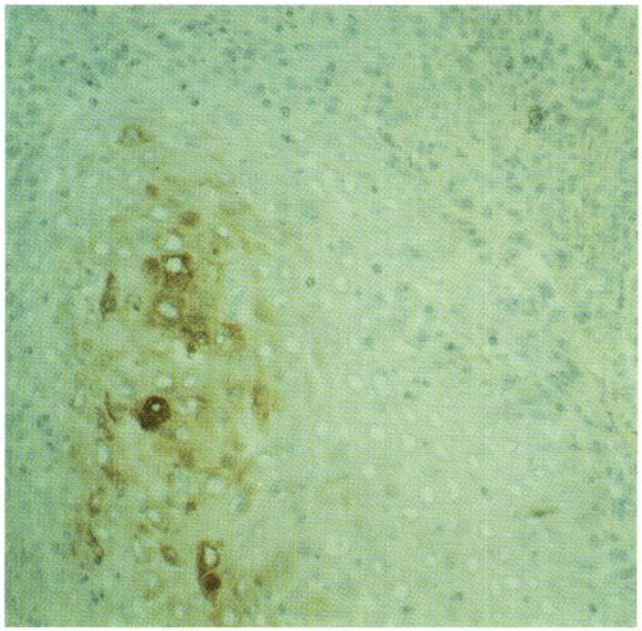


Fig. 4. IL-8 immunoreactivity in well-differentiated squamous cell carcinoma. Except some positive cells around a horn pearl, there is no staining of tumour tissue with monoclonal antibody 52E8 (×400, chromogen DAB).

in normal human skin. In contrast to the clear-cut differences between normal and malignant epithelial tissue, in Bowen's disease atypical cells scattered within the stratum Malpighii were identified as cells without IL-8 immunoreactivity.

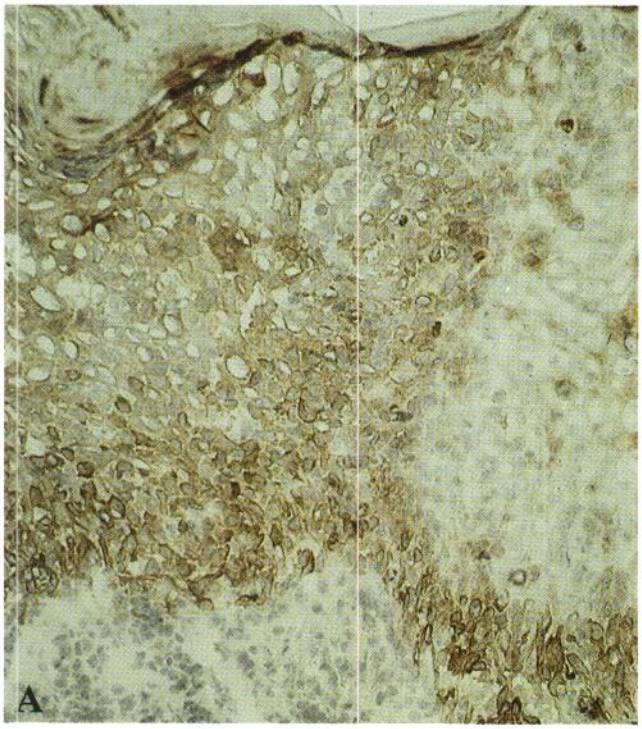
There are several possible causes for the absence of IL-8-related immunoreactivity in neoplastic excrescences of the skin.

Firstly, decrease or loss of IL-8 immunoreactivity in malignant cells could indicate reduced IL-8 synthesis. In fact, reduction in protein synthesis has been demonstrated in cells of semimalignant basal cell carcinoma (11, 12). On the other hand, transformed human epithelial cell lines like KB, HaCat and A431 were shown in vitro to secrete significant amounts of the chemokine (own unpublished results).

Secondly, in transformed keratinocytes IL-8 could be immediately released after synthesis so that the peptide is no longer detectable by immunohistochemical methods. Alternatively, malignant cells may produce an IL-8-isoform which is not detected by the antibodies used. In fact, recent studies (6 for ref) have shown that various types of cells can produce differently processed forms of IL-8. Aminoterminally truncated forms like the 69 amino acid, 77 amino acid and 72 amino acid IL-8 are secreted by endothelial cells, fibroblasts and monocytes, respectively. However, all these truncated forms were shown to be detected by monoclonal antibodies used in this study (13).

Interestingly, similar to the above-mentioned results, in malignant cells of primary melanoma and melanoma metastasis IL-8 immunoreactivity was found to be absent. On the other hand, recent in vitro studies have demonstrated that melanoma cells are able to produce and to release IL-8 in vitro (5, 14). Thus, similar to neoplastic keratinocytes, intracytoplasmic IL-8 immunoreactivity is lacking in malignant melanomas, although in vitro these cells are capable of synthesis and secretion of IL-8.

Thus, irrespective of explanations given, a loss of IL-8



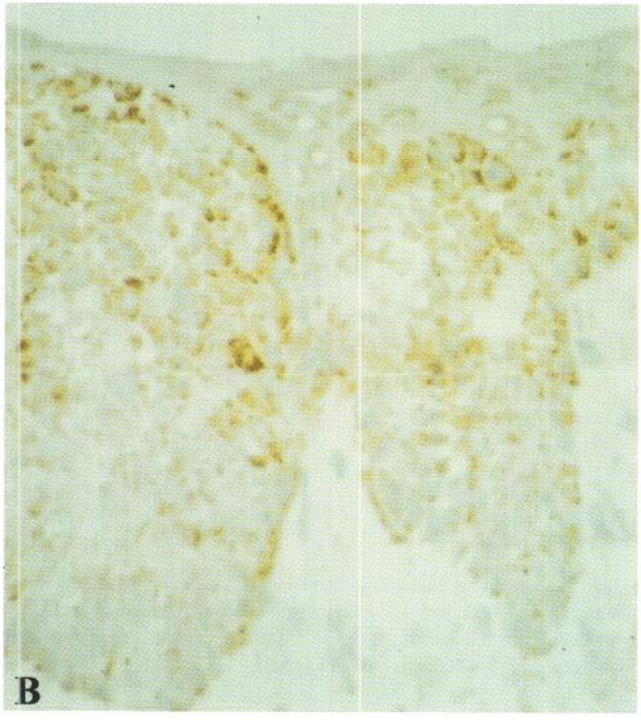


Fig. 5. IL-8 immunoreactivity in Bowen's disease with monoclonal antibody 52E8 (A) and 46E5 (B). Positive and negative cells are side by side in the affected epidermis (×400, chromogen DAB).

immunoreactivity is very probably a sign of malignant transformation. Not only has IL-8 immunoreactivity been shown to be a sensitive marker of inflammation affecting keratinocytes, but it is also absent in keratinocyte neoplasia irrespective of investigated tumour type. Both observations

point toward a central role of IL-8 in growth regulation as well as terminal differentiation of keratinocytes. Studies are under way that will more clearly define the biochemical nature of IL-8 immunoreactivity.

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