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T Cell Subsets and Langerhans Cells in Skin Tumours

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Abstract—*The purpose of this study was to examine the phenotype of the cutaneous immunocompetent cells and Langerhans cells in malignant and benign tumour infiltrates (basal cell and squamous cell carcinomas, malignant melanoma, seborrheic keratosis and naevus) by the use of monoclonal antibodies directed against T cell populations and Langerhans cells. This* in situ *investigation indicates that the lymphocytes participating in the inflammatory reaction around skin tumours are mainly of the cytotoxic/suppressor class. It suggests, moreover, that interaction occurs in the skin between T lymphocytes and HLA-DR suppressing cells. The* in situ *study of the inflammatory immune response should be a useful complement to* in vitro *investigations in the exploration of immune reaction against tumours of the skin.*

INTRODUCTION

LYMPHOCYTIC infiltration is quite common in cutaneous tumours. This inflammatory reaction is composed of a large proportion of immunologically competent cells.

IgA-, IgG- and IgM-producing cells have been observed in basal cell and squamous cell carcinomas and malignant melanomas [1].

Other authors have demonstrated that in weakly malignant tumours the infiltrate is characterized by an elevated number of T lymphocytes and numerous plasma cells which secrete all classes of IgG. In highly malignant tumours it is characterized by a reduced number of T lymphocytes (E rosette) [2–4].

The purpose of the present study was to examine the phenotype of the cutaneous infiltrate of basal cell and squamous cell carcinomas, malignant melanoma, seborrheic keratosis and naevus by the use of monoclonal antibodies directed against T cell populations [5, 6] and Langerhans cells [7, 8].

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MATERIALS AND METHODS

Material

Fifteen malignant skin tumours (8 basal cell carcinomas, 2 squamous cell carcinomas and 5 malignant melanomas) and 6 benign skin tumours (4 seborrheic keratosis and 2 naevus) were studied.

Monoclonal antibodies

Monoclonal antibodies (Ortho Pharmaceutical Corp., Raritan, NJ, U.S.A.) directed against various human T cell antigens were produced as already described [9] by mouse hybridomas obtained from cell fusion between mouse myeloma cells and normal spleen cells from mice immunized with T lymphocytes.

Previous studies have demonstrated that some of these antibodies recognize all peripheral T cells among P.B. lymphocytes. Four monoclonal antibodies named OKT3, OKT4, OKT8 and OKT6 were used: T3 reacts with all peripheral T cells, T4 identifies only peripheral T cells with helper/inducer functions, T8 is directed against a T cell subset with both suppressor/cytotoxic and cytotoxic activity [9, 10] and T6 identifies Langerhans cells [5, 11, 12].

Another monoclonal antibody used was produced by J. Brochier (INSERM U 80, Hôpital E. Herriot, Lyon, France) using the same technique. This antibody, named BL2, is specific for HLA-

Table 1. Malignant tumours: phenotype of cutaneous immunocompetent cells

Antibodies	Cases														
	BCC*	BCC	BCC	BCC	BCC	BCC	BCC	BCC	SCC	SCC	MM	MM	MM	MM	MM
OKT3	E+† D+++	D+++	D+++	E+ D+++	D++	E+ D++	E+ D++	D++	D+++	D++	E++ D+++	E++ D+++	D+++	E+ D+++	E++ D+++
OKT4	D+	D+	D+	D+	D+	D+	D+	D+	D++	D++	D+++	D+	D++	D+	D+
OKT8	E+ D+++	D+++	D+++	E+ D+++	D++	E+ D++	E+ D++	D++	D++	D++	E++ D+++	E++ D+++	D+++	E+ D+++	E++ D+++
OKT6	E+++ IPT+ D APT++	E+++ IPT+ D APT+	E+++ IPT- D APT+	E+++ IPT+ D APT++	E+++ IPT+ D APT++	E+++ IPT+ D APT++	E+++ IPT+ D APT+	E+++ IPT++ D APT++	E+++ IPT- D APT+	E+++ IPT- D APT+	E+++ D+	E+++ D-	E+++ D++	E+++ D+	E+++ D+
MAS036	E+++ IPT+ D APT++	E+++ IPT+ D APT+	E+++ IPT- D APT+	E+++ IPT+ D APT++	E+++ IPT+ D APT++	E+++ IPT+ D APT++	E+++ IPT+ D APT+	E+++ IPT++ D APT++	E+++ IPT- D APT+	E+++ IPT- D APT+	E+++ D+	E+++ D-	E+++ D++	E+++ D+	E+++ D+
BL2 (HLA-DR)	E+++ D+	E++ D+	E++ D++	E+++ D++	E+++ D++	E++ D+++	E+ D+	E+++ D++	E+++ D+	E+++ D+	E+++ D+	E++ D+++	E+++ D++	E++ D++	E++ D++

*BCC: basal cell carcinoma; SCC: squamous cell carcinoma; MM: malignant melanoma; E: epidermis; D: dermis; IPT: in proliferative tissue; APT: around proliferative tissue.
†+++: Strongly positive; ++: positive; +: weak; –: negative.

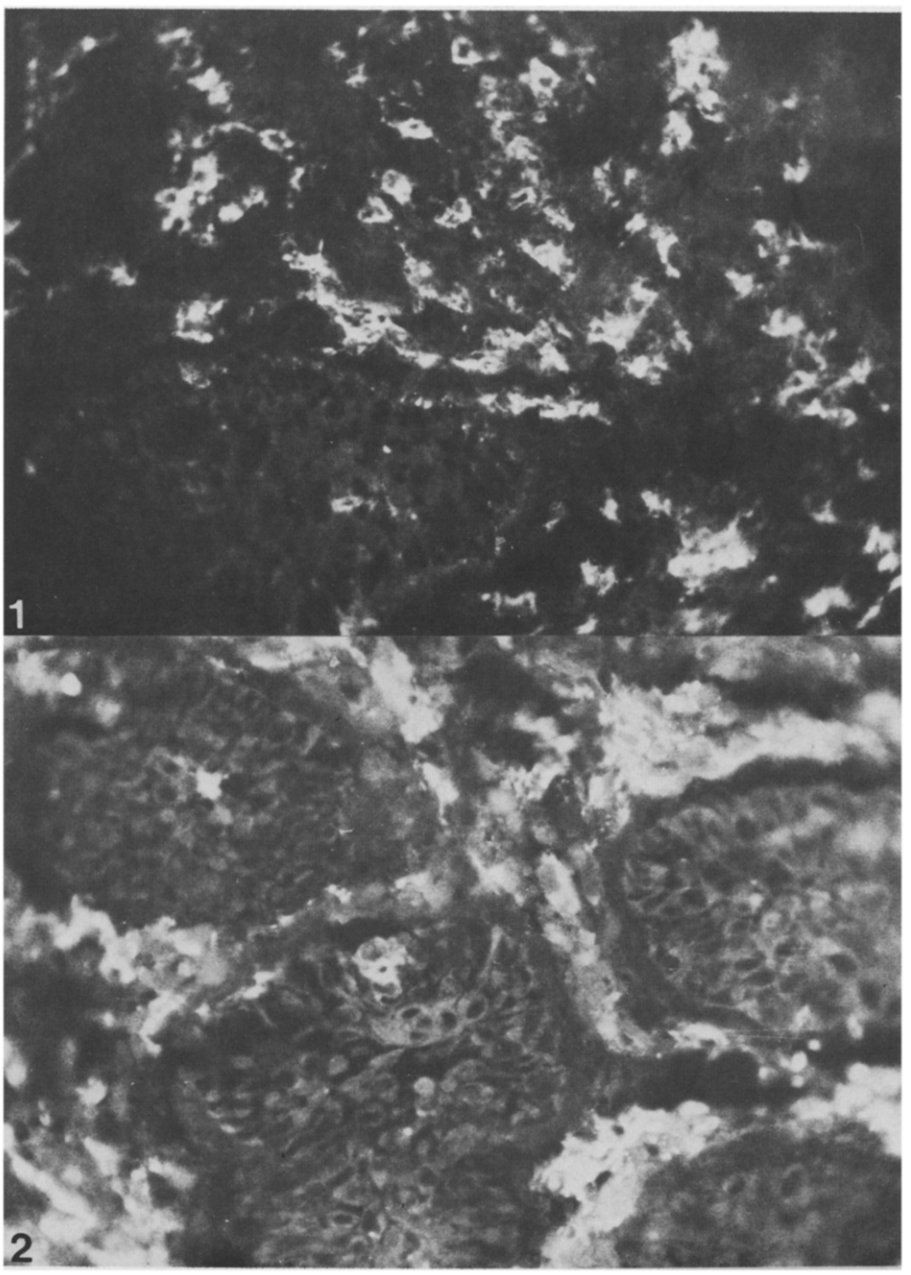

Fig. 1. *Basal cell carcinoma: specific identification of suppressor/cytotoxic cells (OKT8+) observed in dermal infiltrate.*

Fig. 2. *Basal cell carcinoma: specific identification of Langerhans cells (OKT6+) around proliferative tissue.*

DR antigen [10]. Also, a monoclonal antibody produced by SERA-LAB, U.K., named MAS 036 and reactive against thymocytes, was used.

Immunocytochemical procedure on skin tumours

Five-micron frozen sections were prepared, air-dried and fixed in acetone for 10 min at 4°C. Indirect immunofluorescence was performed, first-layer antibodies were added (monoclonal antibodies at dilution 1:5) and the sections incubated for 30 min at 37°C. They were then washed in PBS (pH 7.2, 20 min). Fluorochrome-conjugated second-layer antiserum (Meloy, goat anti-mouse IgG labelled with FITC, dilution 1:20) was next added, incubated and washed as above. Slides were mounted in a glycerol–PBS mixture. The sections were examined under a Zeiss fluorescence microscope.

RESULTS

Light microscopic examination confirmed the diagnosis of the different tumours.

Malignant tumours

Helper cells (OKT4+) and suppressor/cytotoxic cells (OKT8+) were observed in all cutaneous infiltrates (Table 1, Fig. 1).

OKT4+ cells are present only in dermal infiltrate, the OKT8+ cells being observed in both epidermal and dermal infiltrates.

The phenotypes OKT6+, MAS 036+ and BL2+ (HLA-DR) of the epidermal and dermal dendritic cells enables them to be identified as Langerhans cells. Although some of these cells were present within the tumours, by far the greatest concentration of Langerhans cells was present immediately outside the proliferative tissue (Table 1, Fig. 2).

OKT6, MAS 036 and BL2 (HLA-DR) are all capable of staining epidermal dendritic cells, whereas the staining with OKT6 and MAS 036 was identical, a consistent difference being observed in the number of cells marked by OKT6 and BL2 (HLA-DR).

Benign tumours

No OKT4+ cells were observed in benign tumour infiltrates and few OKT8+ cells are present in the dermis (Table 2). The distribution of OKT6, MAS 036 and BL2 (HLA-DR) phenotypes is similar to that seen in malignant tumours (Table 2).

DISCUSSION

Previous studies have demonstrated that in weakly malignant tumours the infiltrate is characterized by an elevated number of T lymphocytes and that in highly malignant tumours the number of T lymphocytes, detected by the E rosette method, is reduced [13].

We have confirmed the presence of T cell lymphocytes in all tumour infiltrates.

The T3+, T4+ and T3+, T8+ phenotypes are characteristic of malignant tumours and we have noted and increased number of T8+, demonstrating an important suppressor/cytotoxic activity in these lesions.

In benign tumours, T3+, T4– and T3+, T8+

Table 2. Benign tumours: phenotype of cutaneous immunocompetent cells

	Cases					
Antibodies	SK*	SK	SK	SK	N	N
OKT3	D+†	D+	D++	D+	D+	D+
OKT4	D–	D–	D–	D–	D–	D–
OKT8	D+	D+	D++	D+	D+	D+
OKT6	E+++ IPT++ D APT++	E+++ IPT++ D APT++	E+++ IPT– D APT+	E+++ IPT– D APT+	E+++ D++	E+++ D+
MAS 036	E+++ IPT+ D APT++	E+++ IPT++ D APT++	E+++ IPT– D APT+	E+++ IPT– D APT+	E+++ D++	E+++ D+
BL2 (HLA-DR)	E+++ D++	E++ D++	E++ D++	E++ D+	E+++ D++	E+++ D+

*SK: Seborrheic keratosis; N: naevus; E: epidermis; D: dermis; IPT: in proliferative tissue; APT: around proliferative tissue.

†+++: Strongly positive; ++: positive; +: weak; –: negative.

phenotypes are present, showing increased lymphocyte activity.

Ultrastructural studies have demonstrated the presence of Langerhans cells in basal cell carcinoma [14], malignant melanoma and naevus [9]. In seborrheic keratosis an unusually large number of Langerhans cells have been observed within the epidermis [15].

Using ATPase techniques, other authors have demonstrated an increased number of Langerhans cells in basal cell carcinomas and seborrheic keratosis. In squamous and intermediary cell carcinoma the Langerhans cells are mostly in the periphery of neoplastic tissue [16].

DR-expression has been demonstrated in basal cell carcinoma by using the monoclonal antibody specific for human Ia-like antigen [17].

Our results confirm, by the use of monoclonal antibodies [OKT6, MAS 036 and BL2 (HLA-DR)], the presence of large numbers of Langerhans cells in the epidermis of malignant and benign skin tumours.

In dermis, OKT6+ and MAS 036+ cells are present in lower numbers and are preferentially located around the proliferative tissue.

In conclusion, this *in situ* investigation indicates that the thymocytes participating in the inflammatory reaction around skin tumours are mainly of the cytotoxic/suppressor class. It suggests, moreover, that interactions occur in the skin between T lymphocytes and HLA-DR-expressing cells. The *in situ* study of inflammatory immune response should be a useful complement to *in vitro* investigations in the exploration of immune reaction against skin tumours.

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