

tion after further washing also indicates that the nickel was, in fact, bound to the cells.

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## Cell-mediated Immune Response to Basal Cell Carcinoma

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**Abstract.** The role of cell-mediated immunity in controlling Basal Cell Carcinoma (BCC) growth was studied by measuring the transformation of lymphocytes when challenged *in vitro* with aqueous extracts of the patients' BCCs and of control skin. Tests were carried out in the presence of autologous and heat-treated plasma. Three patients out

of 8 showed significantly raised thymidine uptake ratio (TUR). Heat-treated plasma produced higher TURs, indicating the presence of an inhibitory factor.

**Key words:** Basal cell carcinoma; Aqueous tumour extract; Lymphocyte transformation; Cell-mediated immunity

Tumors may develop as a result of exposure to carcinogens (chemical, physical or viral), defects in DNA repair, and deficiency of immunological surveillance. The common Basal Cell Carcinoma (BCC) of the skin is slow growing, has limited invasive properties, and is only locally malignant. Histologically the tumour is associated with a variable degree of lymphocytic infiltrate. Does the tumour grow autonomously in an "indifferent" environment or is there an active contest between host and tumour, involving a cell-mediated immune response? We report here experiments involving challenge of patient's lymphocytes with extracts of their BCCs and assessment of the resulting blast transformation.

#### MATERIALS AND METHODS

BCCs were excised under local anaesthetic (1% lignocaine) from 8 patients. At the same time a control biopsy was obtained (with informed consent) from a skin site remote from the tumour (upper outer arm). Histological confirmation of the diagnosis was obtained from a portion of the tumour. A portion of the remainder (100–400 mg) was weighed, minced, homogenized in 2 ml physiological saline using a tissue disintegrator, and the extract filtered through a 0.45  $\mu$ m Millex filter. The normal skin sample was treated similarly.

20 ml heparinized venous blood was obtained from each patient and was separated under gravity for 1–2 hours. The white cell layer was removed and total and differential cell counts carried out which confirmed a lymphocyte content of 50–70%. The plasma was separated from the remaining cells by centrifugation at 150 g for 10 min. Half the plasma was heated at 56°C for 30 min to destroy complement. Cells were washed in Medium 199 (Wellcome) and re-suspended in Medium 199 to contain  $2 \times 10^6$  cells per ml.

110- $\mu$ l aliquots of this cell suspension were placed in microwells, supplemented with 30  $\mu$ l plasma and 10  $\mu$ l of the following constituents: (a) physiological saline (as baseline control); (b) BCC extract (BCC 1); (c) BCC extract, 1 in 10 dilution in physiological saline (BCC 2); (d) control skin extract (Cont. 1); (e) control skin extract, 1 in 10 dilution in physiological saline (Cont. 2). All samples were set up in quadruplicate. The series was duplicated using the heat-treated plasma instead of the normal plasma.

After incubation at 37°C for 6 days, 2  $\mu$ Ci [<sup>3</sup>H]thymidine (RCC Amersham) in 10  $\mu$ l Medium 199 was added to each

Table I. Thymidine uptake ratios

Patient no.	Plasma TUR				Heart-treated plasma TUR			
	BCC 1	BCC 2	Cont. 1	Cont. 2	BCC 1	BCC 2	Cont. 1	Cont. 2
1	0.6	1.7	0.7	1.5	1.0	2.0	1.3	0.7
2	0.6	0.6	0.5	0.5	1.4	1.5	0.8	1.3
3	0.8	1.0	0.8	1.6	0.7	1.3	0.5	0.8
4	14.3	5.7	0.5	0.6	32.8	5.4	1.0	1.0
5	11.1	1.0	0.5	0.6	4.6	1.0	1.0	0.8
6	0.6	0.6	0.4	0.6	0.9	2.8	1.4	0.9
7	1.3	0.7	1.0	0.8	0.6	0.9	0.8	0.9
8	0.7	1.1	0.6	1.0	1.1	0.9	1.0	0.9

well, 4 hours prior to harvest. Cells were harvested (Titer-tek Harvester) and the samples assayed by liquid scintillation counting.

Results were expressed as

$$\text{Thymidine Uptake Ratio (TUR)} = \frac{\text{cpm (Cells + "Stimulant")}}{\text{cpm baseline cells}}$$

## RESULTS

Table I shows the TURs of the BCC and control extracts of the 8 patients studied, each value representing the mean of each set of four cultures.

In 2 patients (nos. 4 and 5) BCC extracts in plasma produced lymphocyte transformation (TUR > 2). In 3 patients (nos. 4, 5 and 6) extracts in heat-treated plasma resulted in transformation. All control samples had TURs in the range 0.4–1.6. Normal PHA transformation in all cases indicated T cell immunocompetence.

With heat-treated plasma the TURs were greater in the 6-day PHA cultures and in all BCC and control cultures (with the exception of no. 5). In one patient (no. 6) a higher TUR was obtained with heat-inactivated plasma with the higher dilution of tumour extract (BCC 2 > BCC 1).

## DISCUSSION

Continuous active division is a feature of cells of the basal layer of human skin. The slow growth and locally invasive nature of the BCC suggests that some mechanism is operating to curtail autonomous growth of the tumour. There is evidence that the mononuclear cells infiltrating BCCs are predominantly T cells (3, 8).

These experiments were designed to evaluate a possible cell-mediated immune mechanism operating against tumour-associated antigens. Peripheral

blood lymphocytes have been shown to be stimulated and transformed *in vitro* on challenge with aqueous tumour extracts, thus marking them as antigen-sensitive memory cells. That the existence of such sensitized lymphocytes is more than an interesting epiphenomenon is suggested by the wealth of literature indicating immunological reactivity against tumour cells by peripheral blood lymphocytes. Such reactivity has been demonstrated in squamous cell carcinoma of human skin (7) and melanoma of the skin (5). Although immunoglobulin-producing cells have been demonstrated in the inflammatory infiltrates of BCCs (2) it has not been possible to detect circulating antibodies to BCC (1) and so the question of the part played by cell-mediated immunity in limiting tumour cell growth could be important.

The finding that lymphocyte transformation is demonstrable more readily with tumour extracts in the presence of heat-treated plasma suggests that a plasma factor (possibly complement) may be limiting their stimulatory activity *in vitro*.

Lower TURs with PHA were also noted in 3 subjects using heat-treated plasma. Inhibitory plasma factors have frequently been noted in the context of tumour immunology and their presence has been suggested as a mechanism allowing tumours to escape immunological destruction (11). In some situations the plasma factor has been heat stable, but in others it has been heat labile and its properties defined (4). Another finding of relevance is that homogenates from human skin suppress mitogen-induced transformation of lymphocytes (6). Such a suppression effect would of course operate equally in the test and control situations, but could account for the possible masking of stimulatory activity by tumour extracts. Moreover, in melanomas, extracts from cultured cells have been

shown to be more stimulatory than fresh tumour extracts (9), so the experimental conditions may have been less than optimal to demonstrate maximal stimulation. Any inhibitory effect of local anaesthetic or heparin, or stimulatory effect of possible bacterial antigens on T cell function is eliminated by employing control extracts.

Tumour growth in the presence of tumour-directed immunity remains a paradox. Although experiments involving the culture of human peripheral blood lymphocytes with autologous tumour extracts have, in some reports, demonstrated stimulation only in tumours with a favourable prognosis (10), it seems unlikely that the results we have obtained strongly support the concept of cell-mediated immunity as an important mechanism in limiting tumour spread.

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### DOPA in Sympathetic Nerve Tissue—a Possible Source of Serum DOPA in Albino Animals

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**Abstract.** Dopa, catecholamines, and dopac were determined in superior cervical ganglia of albino rats. The average amount of dopa in ganglia of control animals was 1.1–1.6  $\mu\text{g/g}$ . The concentrations of catecholamines and dopac were similar to values reported by others. The tyrosine hydroxylase inhibitor  $\alpha$ -methylparatyrosine methyl ester caused marked decrease in the dopa concentration in the ganglia. The effect of reserpine was less pronounced. The aromatic amino acid decarboxylase inhibitor NSD 1015 markedly increased the dopa concentration.

**Key words:** Dopa; Dopac; Dopamine; Noradrenalin; Adrenalin; Sympathetic ganglion; Serum; Albino

Recent studies on guinea pigs of different colours suggest that much of the dopa present in the serum originates from the melanocytes (6). Albino animals too have some dopa in the serum. Dopa has been found in the heart, spleen, and a cervical ganglion of guinea pig (1). These findings suggest that sympathetic nerve tissue can store and secrete not only catecholamines but also dopa.

We now report the presence of large amounts of dopa in the superior cervical ganglion of albino rats.