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# Tobacco smoke condensate cutaneous carcinogenesis: changes in Langerhans' cells and tumour regression

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**Summary.** Tobacco smoke condensate was painted on the skin of BALB/c mice. It increased the density and changed the morphology of Langerhans' cells (LC). LC number in epidermal sheets of treated mice was significantly higher (1793 LC/mm$^2$) than in controls (946 LC/mm$^2$) ($P < 0.0001$) and remained elevated for 35 weeks. LC became less dendritic, or even rounded in shape, and smaller in size. The function of the morphologically altered LC was impaired when assessed by the contact hypersensitivity response. These changes were associated with skin tumour development in all treated mice.

Ten weeks after stopping the TSC treatment, LC number in skin tumours and in skin around these lesions had not decreased, but significantly increased ($P < 0.0001$). During this period tumour regression occurred in 23% of tumours; the remaining tumours showed a 50% reduction in size. At 45 weeks, the LC number in epidermal sheets around skin papillomas was $2274 \pm 14.14$/mm$^2$ and in invasive squamous cell carcinomas was $2088 \pm 183$/mm$^2$. This was associated with reversible changes in LC morphology, where cells became fully dendritic. This also correlated with lymphocytic infiltration into tumours, tumour necrosis, reduction in tumour size and/or tumour regression. It is concluded that the influx of normal LC into the skin tumours allowed the development of an immune response with tumour regression.

*Keywords:* Langerhans' cells, tobacco smoke condensate, carcinogens, squamous cell carcinoma, tumour regression

Benzo[*a*]pyrene and catechols are the most important of the 71 carcinogens in tobacco smoke condensate (TSC) (Dube & Green 1982). Skin papillomas and squamous cell carcinomas of the skin have been reported in various animals, e.g. guinea-pigs, Swiss mice, C57 black mice, after treatment with TSC (Wynder *et al.* 1953; Wynder 1961; Druckrey 1961; Day 1966).

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Ruby *et al.* (1989) and Andrews *et al.* (1991) studied the effect of the tobacco derived benzo[*a*]pyrene (BP) and co-carcinogen catechols on the number, morphology and function of epidermal Langerhans' cells (LC). They reported a significant increase in number of the Ia positive LC, changes in their morphology and impairment of their function. This was associated with the development of skin tumours after 24 weeks of treatment (58% were squamous cell papilloma and 42% squamous cell carcinoma).

The various reports on the density of LC in squamous cell carcinomas of human skin are conflicting. Gatter *et al.* (1984) used NA1/34 monoclonal antibody to identify LC in human skin tumours and reported changes in LC morphology and depletion in their number in squamous cell carcinomas. Smolle *et al.* (1986) used OKT-6 to study LC in human skin tumours and reported a low density of LC in squamous cell carcinomas compared to normal skin. McArdle and co-workers (1986) used an antibody to S100 protein to study LC in various skin lesions and reported a high LC density in keratoacanthoma, squamous cell carcinomas and basal cell carcinomas compared to the normal epidermis. Korenberg and his colleagues (1988) also used an antibody to S100 protein to study LC and supported McArdle *et al.* (1986) in regard to the high density of LC in keratoacanthoma but found a low density of S100 positive LC in squamous cell carcinomas. Alcalay *et al.* (1989) used adenosine triphosphate to study LC and reported changes in LC morphology but no increase in LC number in squamous cell carcinomas.

Analyses of LC in cervical intraepithelial neoplasia likewise are conflicting. The majority of reports indicate high LC number in cervical intraepithelial neoplasia (Morris *et al.* 1983, Caorsi & Figueroa 1984; 1986; Bonilla-Musoles *et al.* 1987; Xie X. 1990) but Tay *et al.* (1987) reported low LC numbers in this lesion. The variation in the number of LC in squamous cell carcinoma in these reports may reflect the different methods used including counting techniques.

The present investigation arose out of a previous study where we demonstrated an increase in LC density in well and moderately differentiated human lung squamous cell carcinomas in association with increased patient survival (Zeid & Muller 1993). As tobacco smoke is linked to the cause of these tumours (Auerbach *et al.* 1957), the present investigation was designed to examine the effect of TSC on LC during carcinogenesis in murine skin, a well established model to analyse the sequential development of squamous tumours.

## Materials and methods

### *Animals*

Two hundred and four BALB/c male mice 6–8 weeks old were obtained from the central animal house at the University of Tasmania after ethical approval. A group of mice (48) were used to assess the function of LC, while the remaining 156 mice were divided into two groups of 78 mice each, experimental and control. Control mice were treated with olive oil and acetone, while the experimental mice were treated with TSC in acetone and olive oil.

### *Experimental design*

After shaving the dorsal skin of control mice, this group was treated with 200 $\mu$l of olive oil and acetone (1 : 1). The skin of the experimental mice was prepared as for the control and treated with 100 $\mu$l of olive oil and 100 $\mu$l of tobacco smoke condensate in acetone (1 : 1). The treatment was applied every second day including the weekends. At 35 weeks TSC treatment was stopped, and the mice were kept for a further 10 weeks to observe changes in tumour size and relation to LC numbers. Six mice from the control and test group were killed by cervical dislocation at 3 days, 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36 and 45 weeks. Skin sections were stained with haematoxylin and eosin and immunoperoxidase anti-Ia staining to visualize and count LC in the prepared epidermal sheets.

### *Preparation of tobacco smoke condensate*

The TSC was prepared by condensation of cigarette smoke by a cold trap (Bentley & Burgan 1961). TSC was collected in jars and the water content evaporated by placing the jars, after gradual warming, in a water bath maintained at 100°C. TSC was then dissolved in an equal volume of acetone. Each 550 cigarettes gave 55 g (35 ml) of condensate. After dilution with an equal volume of acetone, the total amount of diluted condensate was 70 ml.

### *Preparation of epidermal sheets*

A modification of the method of Scaletta and MacCallum (1972) was used to prepare epidermal sheets. The dorsal skin was shaved using electrical clippers and then depilated with a thioglycolate cream (Veet, Reckitt & Colman, UK). Adhesive cellophane tape was then applied to the dry skin, the skin was removed and incubated at 37°C for 3 hours in tetra-sodium ethylene-diamine tetraacetic acid solution (0.38 g EDTA in 50 ml PBS, pH 7.4; Serva, 11282). Using fine forceps the epidermis was separated from the dermis and stained with anti-Ia to demonstrate epidermal LC.

### *Immunoperoxidase anti-Ia staining of LC in epidermal sheets*

Epidermal sheets were fixed in acetone at room

temperature for 20–30 minutes and then incubated overnight at 4°C with mouse monoclonal anti-Ia (MK-D6). After the sheets had been washed in PBS for 20–30 minutes, they were incubated at room temperature for 2 hours with horse-radish peroxidase-conjugated rabbit anti-mouse immunoglobulin (HRPO-anti-Ig, Dakopatts, Denmark), diluted 1:80 in PBS, pH 7.4. Epidermal sheets were washed again in PBS for 30 minutes and incubated for 10 minutes with diaminobenzidine (DAB; Sigma; 0.5 mg/ml of PBS containing 0.02% hydrogen peroxide, pH 7.4). The epidermal sheets were mounted in glycerine gel after washing in tap water.

The Ia positive LC were identified in the epidermal sheets as brown dendritic cells with various numbers of dendrites extending from the cell body (Ruby *et al.* 1989).

### *Immunoperoxidase anti-Ia staining of LC in tumour sections*

Frozen sections (8 $\mu$m thick) of skin tumours were fixed in acetone for 20–30 minutes, washed three times in PBS over 20–30 minutes, incubated with anti-Ia for 30 minutes at room temperature, washed in PBS as before and incubated with HRPO-anti-Ig for 30 minutes at room temperature. Sections were washed again in PBS before incubation with DAB at room temperature (Ruby *et al.* 1989) for 10 minutes and counterstained in Mayer's haematoxylin after washing in running tap water for 5 minutes. Finally, sections were mounted in glycerine gel.

### *Enumeration of Ia positive LC in epidermal sheets*

LC were assessed as the number of Ia positive LC/mm$^2$. The mean and the standard deviation were calculated.

### *Histology*

Skin sections of mouse tumours and normal control skin were processed for routine haematoxylin and eosin staining.

### *Contact hypersensitivity assessment*

The functions of LC were assessed by inducing a contact hypersensitivity reaction at the 2nd and the 6th weeks, after either treatment with TSC in a 1:1 solution of acetone:olive oil or, in the control mice, acetone:olive oil alone. The dorsal skin of the test mice was initially sensitized with 10 $\mu$l of a 1% solution of 2,4,6-trinitrochlorobenzene (TNCB; Tokyo-Kasei Lot FCV61, 0.01 g/ml).

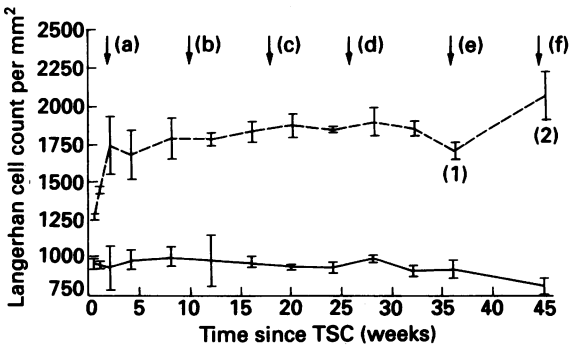


**Figure 1.** Skin lesions and changes in LC number induced by TSC. a, Epidermal hyperplasia; b, dysplastic changes; c, skin papilloma; d, early invasive squamous cell carcinoma; e, treatment with TSC stopped; f, 10 weeks post ceasing TSC treatment. Difference in LC number in epidermal sheets of both – – –, test and ——, control groups is significant ($P < 0.0001$). The increase in LC number in the test mice, 10 weeks after stopping TSC treatment (2) is significant compared with LC number at 35 weeks (1), $P < 0.0001$.

The right ears of both control and test mice were challenged with 10 $\mu$l of a 1% TNCB solution 5 days after sensitization. The thickness of the right and the left (unchallenged) ear was measured 24 and 48 hours later using a spring-loaded engineers' micrometer. The percentage increase in ear swelling was calculated according to the following formula:

$$\% \text{ increase} = \frac{\text{thickness of right ear} - \text{thickness of left ear}}{\text{thickness of left ear}} \times 100$$

The average percentage increase and the standard deviation were calculated for each group of mice.

## Results

### *Skin pathology induced by tobacco smoke condensate (TSC)*

The histopathological changes induced by the TSC in the skin of the treated mice are summarized in Figure 1. Epidermal hyperplasia was observed 2 weeks after treatment; the epidermis was composed of 6–8 cell layers compared to 2–3 layers of control epidermis.

Dysplastic changes in the hyperplastic epidermis were observed in 25% of mice after 10 weeks of TSC treatment. The cells had enlarged hyperchromatic nuclei, and a high nuclear:cytoplasmic ratio. By 18 weeks, about one-third of the treated mice developed macroscopic skin tumours, small wart-like papillomas, up to 2 mm in diameter.

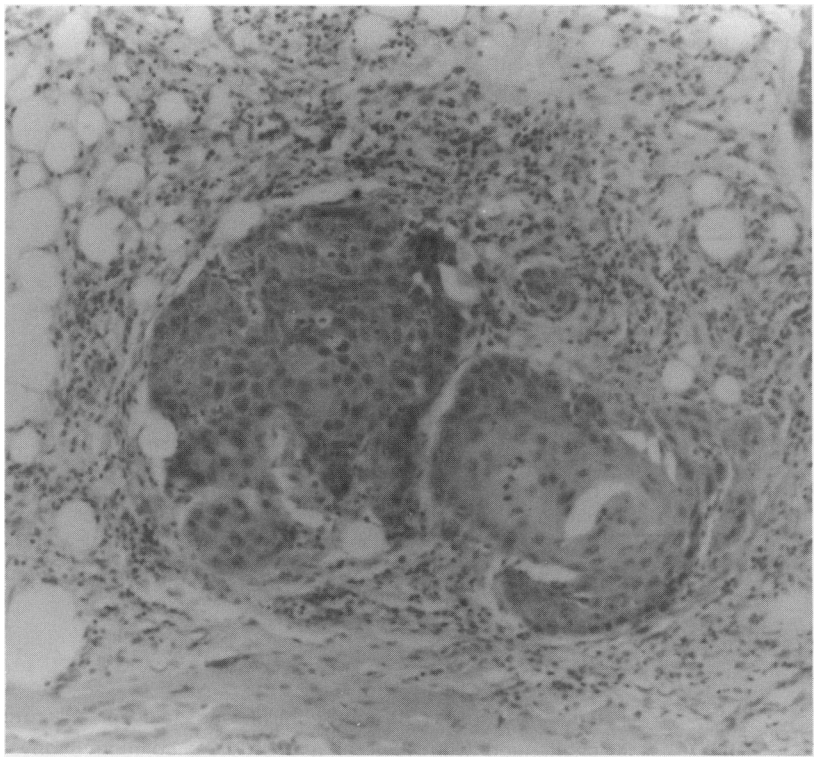

**Figure 2.** Nest of invasive squamous cell carcinoma with mononuclear cell reaction around tumour; 10 weeks after ceasing TSC treatment. H&E. ×200.

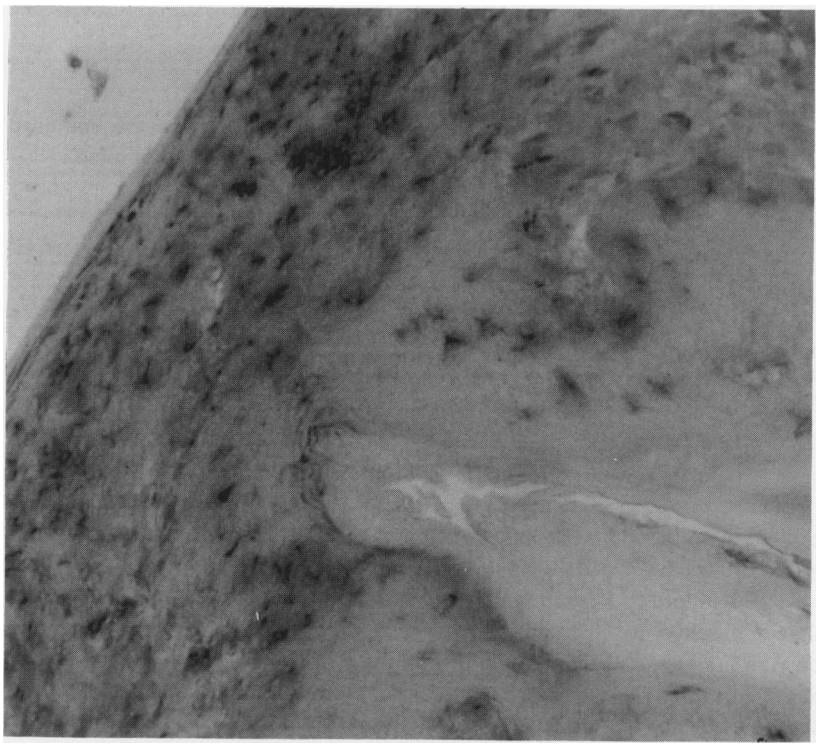

**Figure 3.** Ia positive LC in frozen section of skin tumour. Immunoperoxidase anti-Ia staining. ×200.

After 26 weeks of treatment, the skin of the remaining 50 mice showed skin tumours of various sizes, between 1 and 6 mm in diameter. The histology of the largest skin tumour showed early invasive squamous cell carcinoma. By 35 weeks, the sizes of the skin tumours of all treated mice were between 0.2 and 1.3 cm in diameter and one-third of the mice had developed multiple tumours. The histology of skin tumours of the six mice killed at this stage showed invasive squamous cell carcinoma.

Application of TSC was stopped at 35 weeks and 48 mice were kept under observation for a further 10 weeks. Macroscopically, 23% of tumours spontaneously disappeared and the remaining tumours showed about 50% reduction in their size. Histopathologic examination of these lesions revealed invasive squamous cell carcinomas of the skin in 50% of lesions with varying degrees of tumour necrosis and lymphocytic infiltration (Figure 2). Skin papillomas were observed in 27% of skin lesions; the remaining 23% showed various degrees of hyperplasia and dysplasia.

### *Epidermal LC enumeration in tobacco smoke condensate treated mice*

During the first two weeks there was an increase in the density of the Ia positive LC in the skin of mice treated with TSC and the highest LC level was achieved 28 weeks after treatment. The number of LC in the skin of the treated mice during this period was significantly higher (1793 LC/mm$^2$) than in the controls (946 LC/mm$^2$) ($P < 0.0001$) (Figure 1).

At 45 weeks, almost 10 weeks after stopping the treatment with TSC, the number of LC in frozen skin tumours (Figure 3) and in epidermal sheets from skin around the lesions (Table 1) had not decreased and in general remained elevated (Figure 1). The exceptions were hyperplastic and dysplastic lesions. The difference in LC number before and after stopping TSC treatment was significant ($P < 0.0001$).

**Table 1.** LC in epidermal sheets around skin lesions 10 weeks after ceasing TSC treatment

| Skin lesion | No. | Mean of LC/mm$^2$ | s.d. |
|---|---|---|---|
| (1) Skin papilloma | 13 | 2274 | 14.14 |
| (2) Invasive SCC | 13 | 2088 | 183 |
| (3) SCC with necrosis | 11 | 1960 | 61.25 |
| (4) Hyperplasia and dysplasia | 11 | 1650 | 153.53 |

Using Scheffe's test at the 0.05 level, 2 *vs* 1, 3 *vs* 1, 1 *vs* 2 and 1 *vs* 3 no significant difference; 4 *vs* 1, 2 and 3 all significant.

**Table 2.** Cutaneous contact hypersensitivity response to TNCP of mice treated with TSC

| Group | No. | Time | Mean increase in ear thickness (%) |
|---|---|---|---|
| After two weeks | | | |
| Control | 6 | 24 HR | 110 ± 19.96 |
| TSC | 6 | 24 HR | 54 ± 9.95 |
| Control | 6 | 48 HR | 81 ± 6.96 |
| TSC | 6 | 48 HR | 42 ± 8.06 |
| After 6 weeks | | | |
| Control | 6 | 24 HR | 105 ± 6.59 |
| TSC | 6 | 24 HR | 54 ± 9.95 |
| Control | 6 | 48 HR | 76 ± 7.07 |
| TSC | 6 | 48 HR | 50 ± 7 |

Differences between the percentage increase in ear thickness of test and control group is significant ($P \ll 0.0001$) at all times.

### *Epidermal LC morphology in tobacco smoke treated mice*

After 2 weeks of TSC treatment, 25% of LC in epidermal sheets became smaller in size and the dendrites were blunt and short when compared with normal LC (Figure 4a). By 6 weeks many of the LC cells were rounded or oval in shape (Figure 4b).

Ten weeks following cessation of application of TSC the morphological changes reversed and the LC became more dendritic with more than 3 branched dendrites (Figure 5).

### *Contact hypersensitivity response after TSC treatment*

Table 2 summarizes the percentage increase in ear thickness of both TSC treated and control mice at 2 and 6 weeks following sensitization and challenge with TNCB.

The percentage increase in thickness of the right ear of the TSC treated mice when challenged after sensitization with TNCB was significantly less than that of the control group ($P \ll 0.0001$), at both 24 and 48 hours. This suggests an impairment in the contact hypersensitivity response in the TSC treated mice and indicates a compromised function of the morphologically altered LC.

## Discussion

TSC increased the density of the epidermal LC assessed in epidermal sheets; the highest LC level was achieved 28 weeks after treatment. The density of the epidermal LC in the treated skin was double the control value; the high level of the epidermal LC around lesions remained

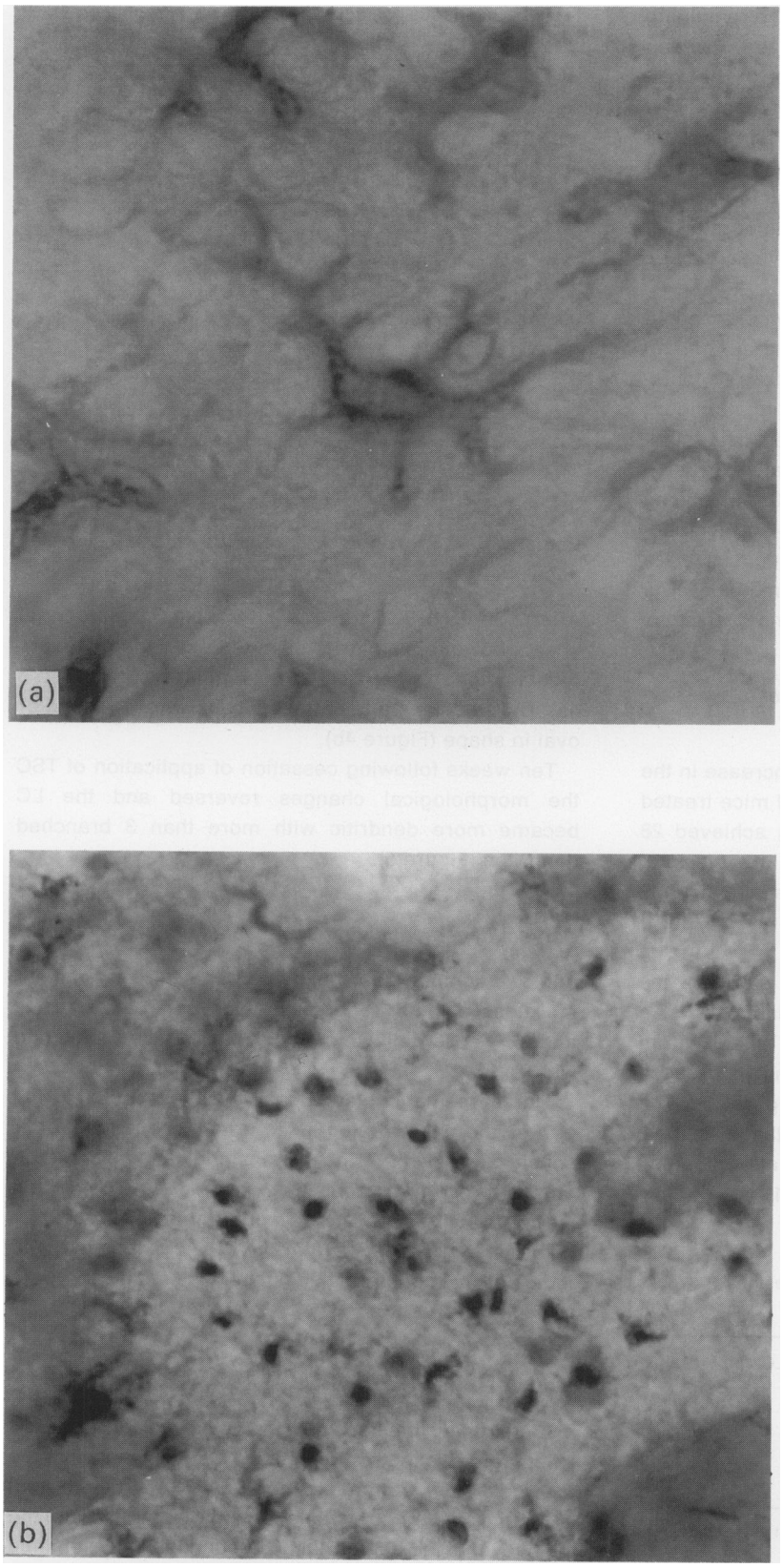


**Figure 4.** a, Normal fully dendritic LC in epidermal sheet of control mice. Immunoperoxidase anti-Ia staining. ×1000. b, Rounded LC 6 weeks after TSC treatment. Immunoperoxidase anti-Ia staining. ×400.

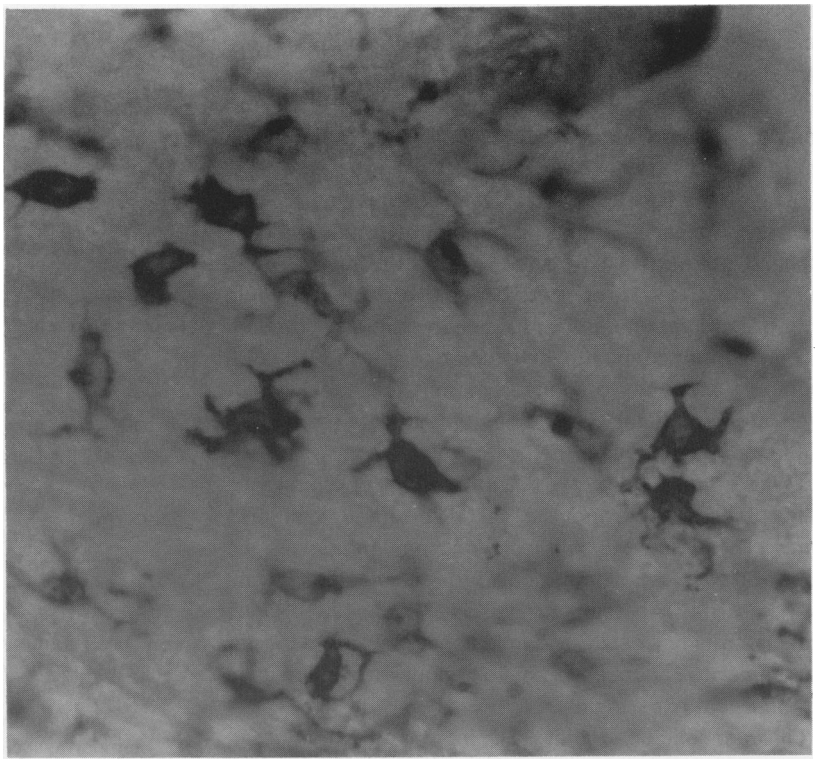

**Figure 5.** LC returned to their dendritic morphology, with more than 3 branched dendrites 10 weeks after stopping TSC treatment. Some LC still abnormal in shape. Immunoperoxidase anti-Ia staining. ×400.

elevated even after stopping TSC treatment. The morphology of epidermal LC in the treated skin was altered. Cells became smaller and the dendrites became shorter and blunt. The functions of the morphologically altered LC were impaired when assessed by the contact hypersensitivity response. The initial increase in epidermal LC number, the morphological changes and the impairment in their function observed during TSC treatment were also associated with tumour development.

These findings support similar observations reported by Ruby *et al.* (1989) and Andrews *et al.* (1991) when they investigated the effect of benzo[*a*]pyrene on murine epidermal LC. Andrews *et al.* (1991) demonstrated that implantation of the prostaglandin synthetase inhibitor, indomethacin, beneath mouse skin before treatment with the tobacco derived carcinogen, benzo[*a*]pyrene, delayed the onset of tumour development and reduced tumour size. Andrews and co-workers (1991) proposed that the cutaneous carcinogenesis induced by benzo[*a*]pyrene may be related to the suppression of cellular cutaneous immunity by prostaglandins.

It is of interest that while the density of epidermal LC decreased ($1650 \pm 154$ cells/mm$^2$) in the skin of mice with hyperplastic and dysplastic lesions 10 weeks after stopping treatment with TSC, the density increased ($P < 0.0001$) in epidermal sheets around both squamous cell papillomas ($2274 \pm 14$ cells/mm$^2$) and squamous cell carcinomas ($2088 \pm 183$ cells/mm$^2$). The LC at this stage appeared fully dendritic. This was associated with spontaneous regression of 23% of skin tumours and 50% reduction in tumour size of the remaining lesions. The high density of the fully dendritic epidermal LC after stopping TSC application was accompanied by increased LC number in frozen sections of skin tumours. This was associated with marked lymphocytic infiltration and necrosis of these lesions.

Muller *et al.* (1985) also reported skin tumour regression after cessation of application of the chemical carcinogen, 7,12-dimethylbenz[*a*]anthracene (DMBA). DMBA depletes epidermal LC and this is associated with impaired skin immune function and skin tumour development (Muller *et al.* 1992). Repopulation of epidermal LC was associated with tumour regression (Muller *et al.* 1985).

The increase in LC density in epidermal sheets around tobacco smoke induced squamous cell skin carcinomas (Table 1) supports our previous report on the increase in S100 positive LC in squamous cell carcinomas of the lung (Zeid & Muller 1993). This is in agreement with the results of previous workers. McArdle *et al.* (1986) and Korenberg *et al.* (1988)

reported an increase in LC density in squamous cell carcinomas of the skin. Caorsi and Figueroa (1984), together with Bonilla-Musoles *et al.* (1987), reported a similar LC elevation in cervical squamous cell carcinoma, and Matsuda *et al.* (1990) in squamous cell carcinoma of the oesophagus.

LC are the best known antigen presenting cells. Their increase in certain tumours may follow recognition by the LC of antigen expressed by tumour cells. Grabbe *et al.* (1991; 1992) reported Ia positive LC are capable of presenting tumour associated antigen derived from a murine spindle cell tumour inducing an anti-tumour immune response manifested by tumour rejection and induction of delayed-type hypersensitivity in immunized animals.

While the extent of LC infiltration of cutaneous and other tumours may be linked to tumour antigens, how LC arrive in the skin lesions remains unclear. One possibility is that skin tumour derived cytokines attract LC into the skin lesions (Halliday *et al.* 1992). Once functionally normal LC are in lesions, they could trigger an immune response leading to tumour regression as in the present experiment.

It was concluded that TSC increased LC number, altered their morphology and impaired LC function; this was associated with tumour development. These events may be related to suppression of cellular cutaneous immunity by prostaglandins as related to the effects of benzo[a]pyrene already described. The further increase in epidermal LC number after stopping TSC treatment was associated with reversible changes in LC morphology; the cells became fully dendritic. These changes correlated with lymphocytic infiltration, tumour necrosis, reduction in tumour size and/or tumour regression, presumably due to an effective anti-tumour response.

## References


Alcalay J., Goldberg L.H., Wolf J.E. & Kripke M.L. (1989) Variation in the number and morphology of LC in epidermal component of squamous cell carcinomas. *Arch. Dermatol.* **125,** 917–920.

Andrews F.J., Halliday G.M. & Muller H.K. (1991) A role for prostaglandins in the suppression of cutaneous cellular immunity and tumour development in benzo(a)pyrene but not dimethylbenz[a]anthracene-treated mice. *Clin. Exp. Immunol.* **85,** 9–13.

Auerbach O., Gere J.B., Forman J.B., Petrick T.G., Smolin H.J., Muehsam G.E., Kassoundy D.Y. & Stout A.P. (1957) Changes in the bronchial epithelium in relation to smoking and cancer of the lung; a report of progress. *N. Engl. J. Med.* **256,** 97–104.

Bentley H.R. & Burgan J.G. (1961) Cigarette smoke condensate: preparation and routine laboratory estimation. Tobacco manufacturers, standing committee, Research papers, No. 4, 1–14.

Bonilla-Musoles F., Castells A., Simon C., Serra V., Pellicer A., Ramirez A. & Pardo G. (1987) Importance of Langerhans cells in the immune origin of carcinoma of the uterine cervix. *Eur. J. Gynaec. Oncol.* **8,** 44–60.

Caorsi I. & Figueroa C.D. (1984) Langerhans cells in squamous cells exocervical carcinoma; a quantitative and ultrastructural study. *Ultrastruct. Pathol.* **7,** 25–40.

Caorsi I. & Figueroa C.D. (1986) Langerhans cells density in the normal exocervical epithelium and in the cervical intraepithelium neoplasia. *Br. J. Obstet. Gynaecol.* **93,** 993–998.

Day T.D. (1966) Carcinogenic action of cigarette smoke condensate on mouse skin. *Br. J. Cancer* **21,** 56–81.

Druckrey H. (1961) Experimental investigation on the possible carcinogenic effects of tobacco smoking. *Acta Med. Scan.* **170 (Suppl. 369),** 24–42.

Dube M.F. & Green C.R. (1982) Methods of collection smoke for analytical purposes. *Recent Adv. Tob. Sci.* **8,** 42–102.

Gatter K.C., Morris H.B., Roach B., Mortimer P., Fleming K.A. & Mason D.Y. (1984). Langerhans cells and T cells in human skin tumours: an immunohistologic study. *Histopathology* **8,** 229–244.

Grabbe S., Bruvers S., Gallo R.I., Nazareno R. & Granstein R.D. (1991) Tumour antigen presentation by murine epidermal cells. *J. Immunol.* **146,** 3656–3661.

Grabbe S., Bruvers S., Gallo R.I. & Granstein R.D. (1992) Effects of immunomodulatory cytokines on the presentation of tumour-associated antigens by epidermal Langerhans cells. *J. Invest. Dermatol.* **99,** 66S-68S.

Halliday G.M., Lucas A.D. & Barnetson R. St C. (1992) Control of Langerhans cell density by a skin tumour derived cytokine. *Immunology* **72,** 13–18.

Korenberg R., Penneys N.S., Kowalczyk A. & Nadji M. (1988) Quantitation of S100 protein positive cells in inflamed and non-inflamed keratoacanthoma and squamous cell carcinoma. *J. Cutan. Pathol.* **15,** 104–108.

Matsuda H., Mori M., Tsujitani S., Ohno S., Kuwano H. & Sugimachi K. (1990) Immunohistochemical evaluation of squamous cell carcinoma antigen and S100 protein positive cells in human malignant oesophageal tissues. *Cancer* **65,** 2261–2265.

McArdle J.P., Knight B.A., Halliday G.M. & Muller H.K. (1986) Quantitative assessment of Langerhans cells in actinic keratosis, bowen's disease, keratoacanthoma squamous cell carcinoma and basal cell carcinoma. *Pathology* **18,** 212–216.

Morris H.H., Gatter K.C., Sykes G., Casemore V. & Mason D.Y. (1983) Langerhans cells in human cervical epithelium: effects of wart virus infection and intraepithelial neoplasia. *Br. J. Obstet. Gynaecol.* **90,** 412–420.

Muller H.K., Bucana C. & Kripke M.L. (1992) Antigen presentation in the skin: modulation by u.v. radiation and chemical carcinogens. *Semin. Immunol.* **4,** 205–215.

Muller H.K., Halliday G.M. & Knight B.A. (1985) Carcinogen-induced depletion of cutaneous Langerhans cell. *Br. J. Cancer* **52,** 81–85.

Ruby J.C., Halliday G.M. & Muller H.K. (1989) Differential effects of benzo[a]pyrene and dimethylbenz[a]anthracene on Langerhans cells distribution and contact sensitization in murine epidermis. *J. Invest. Dermatol.* **92,** 150–155.

Scalettta L.J. & MacCallum D.K. (1972) A fine structural study of divalent cation-mediated epithelial union with connective tissue in human oral mucosa. *Am. J. Anat.* **133,** 431–454.

SMOLLE J., SOYER H.P., EHALL R., BARTENSTEIN S. & KERL H. (1986) Langerhans cell density in epithelial skin tumours correlates with epithelial differentiation but not with peritumoral infiltrate. *J. Invest. Dermatol.* **87,** 477–479.

TAY S.K., JENKINS D., MADDOX P., CAMPION M. & SINGER A. (1987) Subpopulation of Langerhans cells in cervical neoplasia. *Br. J. Obst. Gynae.* **94,** 10–15.

WYNDER E.L. (1961) Laboratory contributions to the tobacco-cancer problem. *Acta Med. Scan.* **170,** 63–101.

WYNDER E.L., GRAHAM E.A. & CRONINGER A.B. (1953) Experimental production of carcinoma with cigarette tar. *Cancer Res.* **13,** 855–864.

XIE X. (1990) Assay of S100+ Langerhans cells in cervical neoplasia. *Chung Hua Chung Liu Isa Chih (China)*, **12,** 406–409.

ZEID N.A. & MULLER H.K. (1993) S100 positive dendritic cells in human lung tumours associated with cell differentiation and enhanced survival. *Pathology* **25,** 338–343.