SHORT COMMUNICATION

Chemical carcinogens and antigens contribute to cutaneous tumor promotion by depleting epidermal Langerhans cells

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Epidermal Langerhans cells (LC) are an integral component of the skin immune system as they initiate immune responses to a variety of antigens, including tumor antigens. When skin is exposed to carcinogenic doses of ultraviolet-B irradiation, chemical carcinogens or tumor promoters there is a significant reduction of LC density. This causes the skin to be immunocompromised and provides an opportunity for aberrant cells to escape immune detection and develop into tumors. Consequently LC depletion is a key event associated with the pathogenesis of skin cancer. We propose that LC depletion contributes to tumor promotion and therefore any agents that reduce LC number, e.g. the contact sensitizing antigen 2,4,6-trinitrochlorobenzene (TNCB), may also contribute to tumor promotion. This proposal was evaluated in cutaneous carcinogenesis by treating mouse skin with a tumor initiating dose of the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) followed by a tumor promoter. The initiating dose of DMBA did not cause LC depletion or tumor development. However, if the DMBA-treated skin was then exposed to a concentration of TNCB that caused LC depletion, skin tumors developed. This is analogous to the classical initiator/promoter system with an LC-depleting dose of TNCB contributing to tumor promotion. Further, this promotion effect was independent of the commencement time of the promoter application, as 2% TNCB applied either 1 or 12 weeks after DMBA initiation induced tumor development. Analysis of the association of LC depletion with immunosuppression and tumor promotion, showed that these events were linked, irrespective of the agent that caused the depletion. It is therefore concluded that LC depletion and local immunosuppression are important aspects of tumor promotion in cutaneous carcinogenesis and non-carcinogenic agents may have tumor promoter activities.

Langerhans cells (LC*) are a major component of the skin immune system. They are antigen presenting cells that recognize and transport antigen to the local lymph node where it is presented in a processed form to antigen specific T-lymphocytes (1–3). It is essential that the skin has an adequate density of LC; otherwise an immune response is not generated (4,5). Exposure of mouse skin to the complete chemical carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) (6), the tumor promoters 12-O-tetradecanoylphorbol-13-acetate (TPA) (7) or ultraviolet-B (UVB) irradiation (4,8) causes significant LC depletion from the epidermis, leaving the skin immunocompromised. Application of antigen through this immunocompromised skin results in antigen specific immunosuppression. LC depletion has therefore been proposed as a key event associated with the pathogenesis of skin cancer as it will allow aberrant cells to escape immune detection (2,6,7,9).

The ability of carcinogens to cause LC depletion is partly due to the carcinogens being treated as antigens. This in turn triggers LC migration to the local lymph node to induce an immune response (10) and leaves the epidermis depleted of LC and immunologically compromised. Contact sensitizing antigens have also been shown to induce LC depletion from murine skin (8). We have recently demonstrated that such antigens induce LC depletion in a dose-dependent manner. Skin exposed to a high concentration of antigen is immunologically compromised as any new antigen applied through this skin generates active immunosuppression rather than a protective immune response (11). These findings indicate that antigens have the ability to modulate the skin immune system in such a way that the treated skin becomes immunocompromised similar to that caused by chemical and physical carcinogens.

Carcinogenesis, including cutaneous carcinogenesis, can be classified into initiation and promotional phases (12,13). The initial stage, tumor initiation, will occur following exposure to a tumor initiator such as urethane and results in phenotypically silent alterations including somatic mutations and epigenetic events (12,14,15). However, the local antigen presenting cells, i.e. the LC, are unaffected and their density remains unaltered (7). No detectable tumors develop at this stage. The latter stage, tumor promotion, will occur following repeated exposures to tumor promoters such as TPA, involves many different cellular effects including the ability to promote skin inflammation and hyperplasia (16) and activate protein kinase C leading to protein phosphorylation and proliferation (17). Tumor promoters also deplete LC from the epidermis (7).

The fact that carcinogens and tumor promoters may behave as antigens and induce an immune response (10) combined with the observation that antigens may cause LC depletion (8,11), similar to carcinogens and tumor promoters indicates that these agents share a number of properties. As both tumor promoters and antigens can induce LC depletion, then antigens may also have tumor promoting capacities. The present study investigated this proposal by evaluating the importance of LC depletion in tumor development, as part of the tumor promotion process. Tumor initiation/promotion experiments were undertaken using the shaved dorsal trunk skin of female BALB/c mice treated with either the complete chemical carcinogen DMBA, the tumor promoter TPA (both from Sigma) or the antigen TNCB (from Tokyo-Kasei) in appropriate combinations and concentrations. The density of LC in the epidermis was determined by preparing epidermal sheets using a modified EDTA separation procedure, and LC were identified with an anti Ia monoclonal antibody (TIB120, American Type Culture Collection) using immunohistochemistry as previously...
or (B) the antigen TNCB. Dose ‘0’ represents control groups where mice were treated with the vehicle alone. Results represent mean ± standard deviation from six mice in each group. Student’s unpaired t-test was used to compare the control group with each treated group. NS = not significant.

Fig. 1. Dose-dependent reduction in epidermal LC density following a single application of different concentrations of (A) the carcinogen DMBA or (B) the antigen TNCB. Dose ‘0’ represents control groups where mice were treated with the vehicle alone. Results represent mean ± standard deviation from six mice in each group. Student’s unpaired t-test was used to compare the control group with each treated group. NS = not significant.

Reduction in epidermal LC density following multiple applications of 0.1% DMBA (solid column, left panel) or 0.005% TPA (solid column, right panel). The open columns represent the vehicle control and the results are the mean ± standard deviation from six mice in each group. Student’s unpaired t-test was used to compare the control group with each treated group. NS = not significant. Described (18). Tumor development (usually papillomas but occasionally squamous cell carcinomas) were scored at weekly intervals and the average number of tumors per mouse (from a group of six mice) was determined. The mice were observed for a maximum of 20 weeks following the first treatment. Mice were killed earlier if they showed signs of discomfort.

There was a direct correlation between LC depletion and tumor promotion. This was first highlighted by the observation that LC depletion induced by DMBA or TNCB was dose-dependent (Figure 1A and B). When analyzing DMBA it was found that a single high dose (100 µl of 1%) caused LC depletion from the epidermis but not a single low dose (100 µl of 0.1%). Further investigations revealed that two applications of this low dose of DMBA were required to cause LC depletion (Figure 2). As shown in Figure 3A, the doses of DMBA which caused LC depletion (i.e. 100 µl of 1% or two applications of 100 µl of 0.1%) also induced tumor formation, thus providing support for the correlation between LC depletion and tumor development.

This association was further extended to the tumor promoter TPA, which required multiple applications following DMBA initiation to cause tumor development (Figure 3A). It should be noted that multiple applications of TPA were also necessary to cause LC depletion (Figure 2). The requirement for multiple applications of TPA to promote tumor formation is a characteristic of tumor promoters. Further, as tumor promotion is reversible, the effects of tumor promoters can be overcome. For example, after cessation of tumor promoter (e.g. TPA) treatment, LC have been shown to return to the epidermis (7) and the immune response reverts to normal. This provides additional support for the proposal that immune suppression is a key element of tumor promotion.

To confirm that LC depletion is a critical component of the tumor promotion process, experiments were undertaken with LC depleting doses of TNCB. After initiation with 100 µl of 0.1% DMBA, multiple applications of 100 µl of 2% TNCB induced tumor development (Figure 3B), whereas 20 weeks after the mice were treated with only a single dose of 0.1% DMBA, there was no LC depletion and no tumor development. This demonstrates that an LC depleting dose of TNCB has tumor promoter activity and that LC depletion, even if caused by antigens, can account for this aspect of promotion.

The tumor promotion effect of LC-depleting doses of TNCB was independent of the commencement time, as applications of 2% TNCB either 1 week or 12 weeks after DMBA initiation, caused tumor development (Figure 3B). Consequently, once a cell has undergone initiation by 0.1% DMBA, the effect is long term and LC depletion is sufficient to help drive the process through the promotion phase. During the latency period between the initial DMBA treatment and TNCB application it is tempting to speculate that the LC are actively involved in protection against tumor development. Although the evidence indicates that DMBA- and TPA-induced LC depletion is a key component of the tumor promotion process (6, 19) it could be argued that as both of these agents are carcinogens, their involvement in tumor growth was merely through their carcinogenic activity, and that LC depletion was coincidental. Our initial studies with DMBA showed that LC repopulation of the depleted epidermis coincided with tumor regression (6) suggesting an active role for LC in tumor immunity. More direct evidence that LC present tumor antigens was provided by Grabbe and colleagues who demonstrated that LC could present fibrosarcoma cells to induce a protective immune response (20). Thus LC may be expected to play a vital role in anti-tumor immunity.
Tumor promotion involves a number of biochemical events e.g. protein kinase C activation (17) and altered expression of genes involved in the cell cycle (14). Our studies indicate that LC depletion, which leads to immunosuppression, is also a major component of the promotional phase. The concept developed in this study is that non-carcinogenic antigens that induce LC depletion may have a role in tumor promotion by establishing an immunosuppressive environment. Once a reduction in anti-tumor immunity occurs through a reduction in the number of available LC, the pre-malignant cells may obtain a proliferative advantage and develop unhindered.

Acknowledgements
This work was supported by the Tasmanian Cancer Committee and M. Qu was the recipient of a University of Tasmania Writing-Up Scholarship.

References

Received on August 28, 1996; revised on February 10, 1997; accepted on February 11, 1997.