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IMPAIRED IMMUNOLOGICAL SURVEILLANCE BY 7,12-DIMETHYLBENZ(A)ANTHRACENE AUGMENTS ITS SKIN TUMORIGENICITY IN C3H MICE

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Summary: 7,12-dimethylbenz(a)anthracene (DMBA) is a polyaromatic hydrocarbon with potent mutagenic, carcinogenic and immunomodulatory activities. It has been postulated that the immunosuppressive effects of DMBA may contribute to the carcinogenicity of this agent. We investigated this issue by examining whether the pre-administration of DMBA at one skin site would augment its carcinogenic activity at a distant skin site. In animals placed on a cutaneous chemical carcinogenesis protocol to the skin of the back, pre-sensitization with DMBA to the abdomen augmented skin tumorigenicity whether the tumor data are considered as cumulative number of tumors, percent of mice with tumors, number of tumors per mouse, or numbers of tumors per tumor bearing mouse as a function of weeks on test. These data demonstrate that pre-treatment with DMBA augments its skin tumorigenicity by impairing immunological surveillance.

The polyaromatic hydrocarbon DMBA is a potent mutagen and carcinogen in experimental animals and has been used extensively to investigate mechanisms of chemical carcinogenesis in the skin (1). In addition to its carcinogenic effects, DMBA has been observed to modulate immunological reactivity (2,3). In experiments evaluating cell-mediated immunity in animals, treatment with this compound has been reported to prolong skin and heart allograft rejection (4), decrease proliferative responses to the mitogens phytohemagglutinin and concanavalin A (5,6), impair the generation of cytotoxic T cell responses (7), and inhibit the induction of delayed type hypersensitivity to trinitrochlorobenzene (8).

It has been postulated that an impairment of immunological surveillance against tumors is an additional consequence of PAH-induced alterations in cell-mediated immunity (9). According to this hypothesis, the contribution of DMBA to cutaneous carcinogenesis would include both an ability to transform target cells and also an ability to impair those

Abbreviations used: DMBA, 7,12-dimethylbenz(a)anthracene; PAH, polyaromatic hydrocarbon; TPA, 12-O-tetradecanoylphorbol-13-acetate; UVB, ultraviolet B.

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immune responses necessary to eradicate the neoplastic cells it has transformed. The purpose of this study was to examine this issue by evaluating whether pre-administration of DMBA influenced its tumorigenic activity at a distant skin site.

MATERIALS AND METHODS

Animals and Chemicals: Female C3H/HeN MTV$^-$ mice were obtained from Charles River Breeding Laboratories (Kensington, NY) and were 6-8 weeks old when the studies were begun. DMBA was purchased from Sigma Chemical Company (St. Louis, MO) and was of the highest purity commercially available.

Pre-immunization of mice with DMBA: Animals were pre-treated with DMBA by applying 100 microliters of 0.1% DMBA (100 micrograms) in acetone topically to the shaved abdominal skin. Once the acetone had evaporated, a hydrocolloid dressing was placed over the area to prevent animals from ingesting the carcinogen. Treatment of animals in this manner results in the development of cell-mediated immunity to this agent (10).

Induction of skin tumors by DMBA: Skin tumors were induced in C3H mice according to an initiation and promotion protocol, the details of which were described earlier (11). One hundred micrograms of DMBA (0.1% in acetone) was applied to dorsal skin that had been shaved and treated with a dipilatory 24 hours earlier. Beginning one week later, 40 nmoles of TPA was applied biweekly to the area of dorsal skin that had been previously treated with DMBA. Animals were examined for the presence of skin tumors on a weekly basis. Only those tumors greater than 1 mm in diameter and present for two or more weeks were recorded.

RESULTS AND DISCUSSION

When C3H mice were treated with DMBA on the abdomen and then placed on the DMBA skin tumorigenesis protocol to the back six days later, significantly more tumors developed compared to animals that had not been pre-immunized (Fig. 1A). Clinically

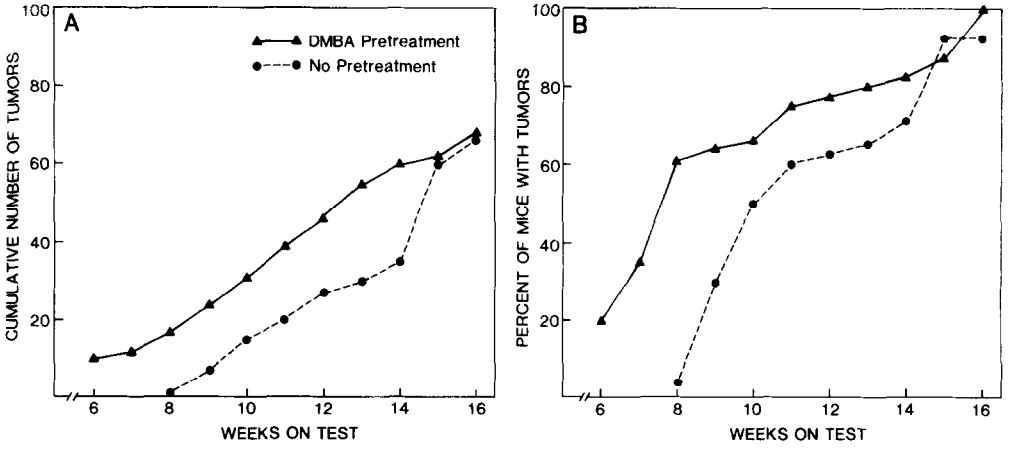

Fig. 1. The effect of pre-immunization with DMBA on DMBA-induced skin tumorigenesis in C3H mice. The cumulative number of tumors (Panel A) and the percent of mice with tumors (Panel B) were plotted as a function of the number of weeks on the test. Mice pre-treated with DMBA (solid line) were compared to mice that received no pre-treatment (dashed line).

apparent skin tumors were present in the pre-immunized group 6 weeks after the start of the skin tumorigenesis protocol whereas no tumors could be found in the non-pre-immunized group until 8 weeks. After 8 weeks on the test, there were 17 tumors in the pre-immunized group of 25 animals and only one in the non-pre-immunized group of 23 animals. Though most striking at earlier time points, the differences between the two groups persisted until 14 weeks. Tumors did not develop in mice that had been pre-immunized with DMBA but had not received DMBA or TPA according to the tumorigenesis protocol or in panels of mice that were neither pre-treated with DMBA nor subjected to the tumorigenesis protocol.

When the data were examined as the percentage of mice with tumors, at 8 weeks only 4% of mice that had not been pre-immunized developed skin tumors compared to 61% of mice that had been pre-immunized (Fig. 1B). A similar increase in tumorigenicity in the pre-treated group was observed when the skin tumor data were considered as tumors per mouse and tumors per tumor bearing mouse (Table 1).

In this study, we have found that pre-administration of DMBA at one skin site augments its tumorigenic activity at a distant site. In this regard, DMBA appears to act in a manner similar to that of UVB radiation, another cutaneous oncogen that has immunomodulatory activities. As with DMBA, exposure of the skin to UVB radiation enhances UVB-induced (12) and 3-methylcholanthrene-induced cutaneous carcinogenesis at unexposed skin sites (13,14). The augmentation in 3-methylcholanthrene-induced tumor formation has been shown to result from an effect on tumor promotion (14).

The mechanism by which pre-treatment with DMBA augments skin tumorigenesis at a distant site is speculative. In previous studies, we have shown that the epicutaneous administration of DMBA results in development of antigen specific cell-mediated immunity to that agent (10). It is possible that application of DMBA to the skin in pre-immunized mice produces a vigorous inflammatory response that enhances the growth of DMBA-induced tumors. In this regard, Prehn and Prehn (15) have found that a non-specific augmentation in the immune response can have a stimulatory effect on PAH-induced tumor growth. If this is the case, our studies would extend their observations by demonstrating that the growth of tumors can be enhanced by specific immunization against these agents. These findings would also suggest that procedures which produce immunological tolerance to DMBA might delay the development of DMBA-induced tumors.

TABLE 1

Effects of pre-treatment with DMBA on DMBA-induced skin tumorigenicity in C3H mice

Weeks On Test	Tumors/Mouse[a]		Tumors/Tumor Bearing Mouse	
	DMBA Pretreatment[b]	No Pretreatment	DMBA Pretreatment	No Pretreatment
6	0.25 ± 0.04[c,d]	0	1.25 ± 0.01[d]	0
7	0.45 ± 0.06[d]	0	1.50 ± 0.5[d]	0
8	0.94 ± 0.12[d]	0.07 ± 0.00	1.55 ± 0.25[d]	1.00 ± 0.07
9	1.20 ± 0.24[d]	0.28 ± 0.05	2.14 ± 0.35[d]	1.10 ± 0.03
10	1.60 ± 0.56[d]	0.75 ± 0.04	2.40 ± 0.45[d]	1.25 ± 0.06
11	2.23 ± 0.60[d]	0.91 ± 0.06	2.90 ± 0.70[d]	1.54 ± 0.70
12	2.40 ± 0.80[d]	1.12 ± 0.25	3.25 ± 0.95[d]	1.75 ± 0.90
13	2.90 ± 0.62[d]	1.30 ± 0.45	3.30 ± 0.58[d]	1.95 ± 0.65
14	2.98 ± 0.75[d]	1.46 ± 0.36	3.35 ± 0.92[d]	2.06 ± 0.46
15	3.28 ± 1.10[d]	2.50 ± 0.95	3.47 ± 0.85[d]	2.73 ± 0.85
16	3.67 ± 0.95[d]	2.87 ± 0.80	3.67 ± 0.97	3.14 ± 1.35
17	5.00 ± 1.02	4.71 ± 1.20	5.00 ± 1.02	4.71 ± 1.05
18	5.28 ± 1.30	5.62 ± 1.20	5.28 ± 1.30	5.62 ± 1.25

[a]Female C3H mice were maintained on food and water ad libitum.
[b]Animals were pretreated with DMBA as described in Methods.
[c]Data are mean ± SEM of 20-25 animals in each group.
[d]Statistically significant from no pretreatment ($P < 0.01$).

A number of investigators have demonstrated that the parenteral administration of any one of a variety of carcinogenic PAHs results in profound immunosuppression (2,3). Thus, an alternative explanation for these results is that the pre-administration of DMBA impairs immunological surveillance against DMBA-induced tumors and thereby facilitates tumor growth. However, we have demonstrated previously that these mice possess antigen-specific cell-mediated immunity to DMBA at the time of tumor induction (10). To reconcile these findings would require either that (1) epicutaneous application of DMBA results in sensitization to DMBA followed at a later time by immunosuppression, or that (2) topical application of DMBA produces sensitization to DMBA but unresponsiveness to other

antigens, including tumor associated antigens on DMBA-induced tumors. Studies investigating these possibilities are currently in progress.

It is also possible that the augmentation in tumorigenic activity is due to non-immunologic factors. The mechanism by which this would occur is unclear since the dose of DMBA employed for epicutaneous sensitization in this study is not adequate to induce the activity of these enzymes required for carcinogen metabolism (16). Furthermore, studies have shown that the skin possesses the enzymes necessary to convert DMBA to its ultimate carcinogenic metabolite (16).

In summary, these studies suggest that the carcinogenic PAH, DMBA, in addition to its ability to act as a tumor initiator, promotes skin tumorigenesis by impairing immunological surveillance.

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