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Autoantibody and Tumor Promotion (40749)

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Application of a relatively small dose of carcinogen to the skin of a mouse initiates tumor cells which remain latent and which will produce macroscopic tumors only after repeated applications of a promoting agent. Promoting agents cause increased cell multiplication, hyperplasia, and inflammation, but do not produce tumors unless applied subsequent to the application of carcinogens. The mechanism by which these agents promote tumors is not clear (1–3). The most effective promoting agents are croton oil and 12-*O*-tetradecanoylphorbol-13 acetate (TPA), which produces marked irritation and inflammation of the skin. Skin stripping and multiple incisions are also effective promoters of tumors (4). Because wounding of the skin induces autoantibody (5), it is reasonable that the injury produced by TPA and croton oil may also induce autoantibody.

The premise of our investigation is that promotion causes injury which results in autoantibodies that stimulate tumor cell division. We have demonstrated stimulation of tumor cell division *in vitro* by antibody to normal cells (6, 7). The immunological enhancement of tumor growth *in vivo* in allogeneic mice is known to be due to the development of antibody to histocompatibility antigens (8).

The objectives of this report were to determine (1) if croton oil and TPA induce autoantibodies and (2) if autoantibodies resulting from syngeneic tissue injection will promote tumors in carcinogen-initiated mice.

Materials and methods. Female strain A mice, 6–8 weeks of age, were obtained from Jackson Laboratory. 7,12-Dimethylbenz(a)anthrancene and TPA were applied to the interscapular area of the mice skin in 20 μl of acetone with a Hamilton dispenser. The methods of preparing the mice and measurement of the tumors have been reported previously (9).

The passive hemagglutination procedures as described by Campbell *et al.* (10) were employed to measure autoantibody. Skin antigen for attachment to the human red cells was prepared as follows: Hair was removed from the mice with a depilatory; full thickness skin was obtained and homogenized in a Tissumizer (Tekmar Co.) in cold 0.15 *M* NaCl. The homogenate was centrifuged at 500*g* for 20 min and prepared as described by Boyden (5). Blood samples were obtained by suborbital puncture. The sera were heated at 56° for 30 min and absorbed with type O human red blood cells. All serum samples were frozen at −20° until assayed by passive hemagglutination.

Tissues for injection were prepared from strain A mice (syngeneic) and female, New Zealand, white rabbits (xenogeneic). The tissues were prepared by the procedures described for preparing skin antigen. After protein determination, the preparations were adjusted to 100 μg protein/ml. Twenty micrograms were injected subcutaneously and 10 μg intraperitoneally three times/week for 1 month after which the injections were continued on a once-a-month schedule.

Results. To determine if the promoting agents TPA and croton oil induce autoantibody, strain A mice were shaved and treated with croton oil, TPA, ethylphenylpropiolate (EPP) and acetone. EPP produces hyperplasia but does not promote tumors (3). All treatments were given in 20 μl of acetone twice a week. Each week two mice were bled by suborbital puncture and the serum assayed for antibody to strain A skin by passive hemagglutination. After bleeding, the mice were sacrificed and not used again for antibody titer because the suborbital bleeding procedure produces tissue damage which can stimulate autoantibody synthesis.

The titers in the acetone- and EPP-treated mice were 1:4 or less during the 21

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TABLE I. AUTOANTIBODY LEVELS IN STRAIN A MICE FOLLOWING TREATMENT WITH TPA AND CROTON OIL[a]

Weeks	1	2	3	4	5	6	7	8	13	21
TPA	0	0	0	0	1:64	1:8	1:8	1:16	—	1:32
Croton oil	0	0	0	0	1:64	1:8	1:16	1:32	1:16	1:64

[a] Strain A mice received 3.2 μg of TPA, 500 μg of croton oil, or 1 mg of EPP in 20 μl of acetone three times a week. Another group of mice received 20 μl of acetone three times a week. Three strain A mice were bled at each time interval and not reused. The sera were pooled and assayed. Acetone and EPP treatment gave titers less than 1:4 at all time intervals.

weeks of the study whereas the titers in the TPA- and croton oil-treated mice rose to 1:64 after 5 weeks of treatment (Table I). Since different mice were used each week, the variation is not surprising. However, it is clear that an increase occurs at 5 weeks of treatment.

If TPA and croton oil promote tumors by inducing autoantibody then it may be possible to promote tumors by an alternate method for obtaining autoantibody. The most direct method for producing autoantibody is the injection of syngeneic tissue or, in some instances, by injection of an allogeneic or xenogeneic tissue which shares antigens with the host animal.

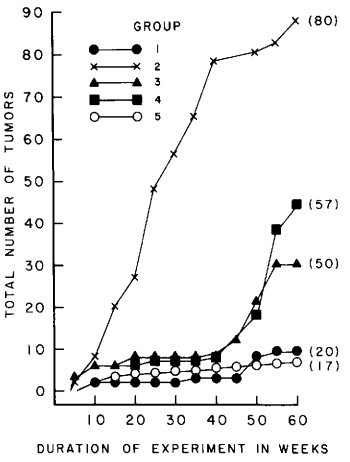

FIG. 1. Tumor promotion by syngeneic tissue. All strain A mice were initiated with 100 μg of DMBA. Thirty mice were employed for each group. Number in parentheses is the percentage of tumor-bearing mice at 60 weeks. Group 1 received no further treatment, Group 2 received twice weekly applications of 3.2 μg TPA, Group 3 received 10 μg of strain A liver protein intraperitoneally and 20 μg subcutaneously once every other week for three injections, then once a month. Group 4 received 10 μg of strain A skin intraperitoneally and 20 μg of strain A skin protein subcutaneously once every other week for three injections, then once a month. Group 5 received 10 μg intraperitoneally and 20 μg subcutaneously of rabbit skin once every other week for three injections, then once a month.

Five groups of mice were used in an experiment to study the effect of tissue injection autoantibody production and tumor promotion. The mice were initiated with 100 μg of DMBA and then injected with syngeneic liver, syngeneic skin, xenogeneic skin, or saline for 40 weeks. Five mice from each group were bled at 2, 4, 8, 16, 20, and 28 weeks. Thirty mice from each group were examined weekly for the development of tumors.

In Fig. 1 is shown the number of tumors that developed in each of the groups. The appearance of tumors in the saline- and TPA-treated groups is the expected rate of tumor development employing these conditions. The mice injected with syngeneic skin and liver had only a few more tumors than the controls until 45 weeks after initiation. At this time, tumors started appearing rapidly in the syngeneic skin and liver groups but not in the xenogeneic skin group. All of the tumors in the TPA-treated group appeared on the back of the mouse in the treated site whereas only 47% of the tumors which developed in the syngeneic skin group appeared on the back of the mouse in the area initiated with DMBA. The remainder appeared on the abdomen in the approximate area of the tissue injection. The liver injected group had a similar distribution of 58% on the back and the remainder on the abdomen where injected. Ninety percent of the tumors were papillomas with proliferating squamous epithelium. The remaining tumors were keratoacanthomas and malignant tumors.

The autoantibody level in each of the groups is shown in Table II. The repeated

TABLE II. AUTOANTIBODY IN STRAIN A MICE INJECTED WITH SYNGENEIC AND XENOGENEIC TISSUE[a]

Weeks	8	16	20	28
TPA	16	16	64	64
Skin (syn)	0	16	28	102
Liver (syn)	0	0	12	12
Skin (xn)	0	0	512	512
Saline	0	0	0	4

[a] Titers are the geometric mean of five determinations. No antibody was measurable at 2, 4, and 6 weeks.

injections of saline did not induce appreciable levels of autoantibody. TPA-Treated mice had autoantibody levels before any of the other groups. Xenogeneic (rabbit) skin probably shares some antigens with mouse skin as evidenced by the much higher titers of autoantibody in this group of mice.

Discussion. The initiation–promotion method of inducing epidermal tumors by carcinogen and croton oil was first described and analyzed by Berenblum (11) and Berenblum *et al.* (12, 13). During this period, Pullinger demonstrated the promotion of tumors by wounding and injury. Repeated injury was found, by him, to be a successful method for promoting tumors (14–16). A more recent study by Clark-Lewis *et al.* (4), using skin stripping with silicon carbide paper as well as multiple incisions, confirms the effectiveness of physical injury as a tumor-promoting agent.

A variety of forms of injury such as burns, wounding, and treatment with carbon tetrachloride have been found to induce autoantibody (17, 18). It is not unexpected, therefore, that the injury produced by TPA or croton oil induces autoantibody formation. Similarly, the injection of syngeneic tissue has been found to induce autoantibody (19, 20). High levels of autoantibody to xenogeneic tissue injections have also been reported (21).

To the best of our knowledge, the promotion of tumors by induction of autoantibody has not been previously reported. Although the promotion of DMBA tumors with tissue injection takes many more weeks than does TPA, it should be noted that it also takes 15 weeks longer to induce autoantibody with tissue injection than with TPA. The appearance of tumors at the site of injections of the xenogeneic and syngeneic tissue is of interest and suggests a number of possibilities. The injection may cause inflammation and increased lymphocytes at the site. It is also possible that the tumors produced at the site of injection are not DMBA initiated. However, since the xenogeneic tissue injection did not induce increased tumors, it is apparent that injection of tissue is not responsible for these tumors.

It is of interest that in spite of the high titers of antibody to skin following xenogeneic skin injections, that tumors were not promoted. However, *in vivo* studies by Prehn demonstrated that high levels of immunity inhibit tumors and lower levels stimulate (22). Similarly, *in vitro* studies show that high levels of antibody inhibit tumors and low levels stimulate (7). Thus, the failure of xenogeneic skin to promote the tumors appears consistent with previous data.

It is proposed that autoantibody promotes tumor growth. The *in vitro* stimulation of tumor cells by antibody, the induction of autoantibody by the promoting agents, and the promotion of tumors by tissue injections support this hypothesis. These findings may provide a possible explanation for the increased incidence of cancer in diseases where autoantibodies commonly arise such as Crohn's disease, Sjogrens's syndrome, ulcerative colitis, and Coeliac disease (23–27), as well as the observations of tumors following chronic injury and burns (28–31).

Summary. Following treatment of Strain A mice with either croton oil or TPA, autoantibodies were detected by passive hemagglutination in the sera of the mice.

Autoantibody to skin was induced in Strain A mice by the repeated injection of syngeneic skin and liver. Higher levels of antibody were achieved by injection of xenogeneic skin.

Mice initiated with DMBA were injected with syngeneic liver and skin. The injected mice developed significantly higher levels of tumors than did noninjected controls. Xenogeneic skin injections produced high

levels of autoantibody but did not promote tumor development.

The results suggest that autoantibody may play a role in the promotion of tumors.

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