Regression Kinetics of Mouse Skin Papillomas

Fredric J. Burns, Martin Vanderlaan, Andrew Sivak, and Roy E. Albert

Institute of Environmental Medicine, New York University Medical Center, New York, New York 10016

SUMMARY

The persistence and proliferation rate of mouse skin papillomas were studied in HA/ICR mice initiated with 7,12-dimethylbenz(a)anthracene and promoted three times weekly with phorbol myristate acetate. When the promoter treatments were stopped, rapid (half-time, 24 days) and slow (half-time, >140 days) components of papilloma regression were observed. When the promoter dose was increased, the major effect was an increase among the rapidly regressing papillomas. Increases in the epidermal pulse-labeling index and the number of dermal inflammatory cells produced by phorbol myristate acetate in normal skin were reversible when the phorbol myristate acetate was stopped, but high pulse-labeling index values in papillomas were not reversible. Antithymocyte serum had no effect on regression, although ethylphenylpropionate, a nonpromoting irritant, slowed the regression sufficiently to increase the half-time from 24 to 57 days. The action of the promoter in overcoming the regression tendency of the papillomas may explain certain features of the role of nonspecific irritation and the importance of promotion frequency in determining tumor yield.

INTRODUCTION

Epithelial papillomas are the most frequently observed type of neoplastic lesion produced in mouse skin as a result of initiation and promotion, i.e., 2-stage carcinogenesis. Although the significance of the papillomas in carcinogenesis has been a subject of much controversy, there is evidence that at least some of them are precursors of carcinomas (2, 5, 10, 13). There is also evidence that some of the papillomas are unstable, and spontaneous regressions were often noted during promotion with croton oil which is a complex mixture of an active promoter and many other compounds, some with antineoplastic properties (6, 9). Van Duuren et al. (14, 15) isolated PMA,3 the active promoter from croton oil, and found that regressions during promotion with PMA were much less frequent than with croton oil.

The regression of papillomas is an interesting, but poorly understood, phenomenon. Methylcholanthrene-induced papillomas regress when the skin containing them is transplanted to an autologous host, but fewer regressions occur when the graft recipients are immunosuppressed which suggests that regression may be the result of immune rejection (7, 12). It is also possible that papillomas regress because their cellular proliferation rate, without the stimulus provided by the promoter, is not sufficient to overcome the cell loss rate and they, in effect, destroy themselves (4, 11). Other explanations are possible (1), and there is clearly a need for experimental information on rates of proliferation and regression of papillomas in relation to the dose and duration of the promoting agent in order to begin to draw distinctions between possible explanations.

MATERIALS AND METHODS

Female HA/ICR mice (Sprague-Dawley, Madison, Wis.) were initiated by pipet with recrystallized DMBA (Eastman Kodak, Rochester, N. Y.) in 0.2 ml acetone. The hair was clipped prior to initiation. Two weeks after initiation promotion was started with PMA applied by pipet 3 times/week in 0.2 ml acetone and continued for various intervals as indicated in Chart 1. In some groups promotion was stopped and restarted, and in 1 group the promoter dose was increased after being restarted at the original dose. At the times indicated by the arrows in Chart 1, groups of about 5 mice were given i.p. injections of 50 μCi of [3H]thymidine (Schwarz/Mann, Orangeburg, N. Y.) 2 days after their last PMA dose. One-half hr later they were killed, and the skin was removed, fixed in Carnoy's solution, stained with a DNA-specific Feulgen procedure, and then cleared in methyl salicylate. Aggregations of nuclei, easily recognized as deep pink foci, were excised and sectioned, and autoradiographs were prepared of all lesions.

Mice given promoter treatments for the 1st 100 days were assigned to treatment groups at the end of promotion. Because of the wide variability in tumor response, mice were not randomly assigned to their postpromotion treatment groups. Instead mice were assigned so that the distribution of tumors in each subgroup was roughly the same as in the composite group.

Rabbit anti-mouse ATS and NRS (Microbiological Associates, Inc., Bethesda, Md.) were injected in 0.25-ml quantities on the last day of promotion and on Days 2, 5, 7, and 14 thereafter (see Group 6 in Chart 1). The same schedule of injections produced a 12-day rejection time for NRS or no treatment and a 30-day rejection time for ATS for skin transplanted from DBA mice to either C57BL or HA/ICR mice. EPP at a concentration of 5% was applied in 0.2 ml acetone.
Regression Kinetics of Mouse Papillomas

PMA dosage was increased to 10 μg/application, and there was an almost immediate increase to a new plateau of 6.5 ± 1.8 papillomas/mouse (50% of the increase occurred within 5 days of the PMA dosage increase).

The data in Chart 2 are based on counts of papillomas visible to the naked eye, i.e., papillomas generally greater than about 1.0 to 2.0 mm in diameter. Whenever autoradiographs were prepared, the skin remaining after visible papillomas was excised was cleared in methyl salicylate and searched for "microtumors" in the size range from 0.3 to 1.0 mm. When the microtumors were included, the curves of papillomas per mouse were displaced about 1.5 weeks earlier than the curves shown in Chart 2 and the number of papillomas per mouse was about doubled. No microtumors were found 3 weeks after the promoter treatments were stopped or when initiator or promoter treatments were given alone.

Chart 3 shows a semilogarithmic plot of the persistence of papillomas as a function of time after the end of the PMA treatments. The circles are taken from Curve 3 in Chart 2, except that time is measured from the incidence peak instead of the end of the PMA treatment. The initial straight lines indicate that papilloma disappearance was approximately exponential with half-times of 21 to 28 days for both

RESULTS

The dependence of papillomas per mouse on time for a single initiating dose of DMBA and PMA doses of 1.0 (Curve 1) and 2.5 μg (Curve 2) per application is shown in Chart 2. The shapes of the curves are nearly identical in a temporal sense. Both curves have initial tumor-free intervals of about 40 days and times to 50% of final yield of about 50 days. The mean ± S.E. of the papillomas per mouse values at 100 days were 4.6 ± 1.1 and 7.5 ± 0.7 for PMA doses of 1.0 and 2.5 μg, respectively.

The number of papillomas per mouse as a function of time for the treatment group where the 2.5 μg dose of PMA was stopped on Day 42 and then resumed on Day 100 is also shown in Chart 2 (Curve 3). New tumors continued to appear for about 10 days after the end of the PMA treatments. All but a few of the tumors regressed by Day 100. When the PMA treatments were resumed, papillomas began to reappear and reached 50% of their ultimate plateau level of 4.7 ± 1.3 papillomas/mouse within about 27 days. On Day 172 the
F. J. Burns et al.

PMA doses. Both PMA doses show components of slowly (half-time greater than 140 days) and rapidly regressing tumors as indicated by the slope changes. The higher PMA dose initially produced about twice as many tumors as the lower dose, but the number of persisting tumors per mouse at 24 weeks was about equal for the 2 doses. The regression rate in mice with many papillomas was essentially the same as the rate in mice with relatively few papillomas.

Chart 4 shows a semilogarithmic plot of tumor persistence for NRS-, ATS-, and EPP-treated mice. Regression half-times were nearly equal for the NRS and ATS groups (18 and 14 days, respectively), and neither differed statistically from the half-times observed in the untreated controls shown in Chart 3. The regression half-time in the EPP-treated group, 57 days, was greater than the half-times of the NRS and ATS groups at the 1% significance level on the basis of a regression analysis.

LI's are shown in Chart 5 as a function of time after the start of promotion for papillomas and for PMA-treated and untreated epidermis. Initiated and uninitiated groups showed no difference and were combined in the curve for control epidermis. The LI of the PMA-treated epidermis declined slightly between 25 and 63 days but generally remained 3 to 4 times as high as the LI of the control epidermis. After the PMA was stopped (Chart 5, Curves A and B), epidermal LI values had returned to control levels by 3 weeks. When the PMA was stopped and ATS was given, the epidermal LI also returned to control level (Chart 5, Curve C), although when EPP was given only a partial return to control was observed (Chart 5, Curve D). In the papillomas the LI values were much higher than in PMA-treated epidermis, but when the PMA was stopped (Chart 5, Curve A) the papilloma LI values remained high and ATS (Chart 5, Curve C) and EPP (Chart 5, Curve D) had no effect (see also Figs. 1 and 2).

Epidermal cell numbers expressed as the number of Feulgen-positive nuclei per 100 μm of epidermal length on histological sections closely followed the pattern seen for the epidermal LI in Chart 5. Sustained PMA treatments produced epidermal hyperplasia with a cell count roughly 2 or 3 times as great as the control value, but if the PMA treatments were stopped the epidermal cell number returned to control levels within 3 weeks.

The cell count in the dermis is shown in Table 1 for control and PMA-treated skin near to (within 100 μm) and distant from tumors. No attempt was made to classify the cells, but most of the observed increases in dermal cellularity resulted from infiltration by inflammatory cells. The PMA at a dose of 2.5 μg produced about a 5-fold increase in dermal cellularity both near to and distant from tumors. The lower PMA dose produced about the same increase near tumors but only about a 3-fold increase distant from tumors. When PMA was stopped, the dermal cell count dropped close to control value by 3 weeks in regions distant from tumors but regions adjacent to tumors remained elevated. For NRS- and ATS-treated mice, dermal cell counts dropped significantly but remained about 2-fold higher than control levels. The EPP partially prevented the decrease in dermal cell count observed in the NRS and ATS mice.

DISCUSSION

The cell population changes induced in mouse skin by promotion with PMA were generally reversible and returned to control levels when the promoter treatments were stopped. Reversibility was observed for the increased labeling index and cell number in the epidermis and for the

<table>
<thead>
<tr>
<th>Time</th>
<th>Near tumors</th>
<th>Distant from tumors</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>During PMA treatment (2.5 μg)</td>
<td>33.5 ± 2.0*</td>
<td>25.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>During PMA treatment (1.0 μg)</td>
<td>27.1 ± 1.5</td>
<td>15.0 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>3 wk after PMA treatment (2.5 μg)</td>
<td>32.0 ± 2.0</td>
<td>7.0 ± 0.5</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>3 wk after PMA treatment (1.0 μg)</td>
<td>26.0 ± 2.0</td>
<td>10.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>3 wk after ATS started</td>
<td>23.8 ± 2.5</td>
<td>14.5 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>3 wk after EPP started</td>
<td>26.9 ± 1.4</td>
<td>19.9 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S.E.
Regression Kinetics of Mouse Papillomas

degree of inflammatory cells infiltrating in the dermis. Even the papillomas that occurred in initiated skin were partially reversible, and a large proportion of them regressed when the promotion was stopped. The LI of the papillomas, while considerably higher than the LI in hyperplastic epidermis, was not readily reversible and remained at high levels (about 30%) in surviving papillomas 3 weeks after the PMA was stopped. At 3 weeks about 50% of the initial papillomas had regressed, and eventually about 95% of the papillomas regressed which implies that about 90% of the papillomas sampled at 3 weeks were destined to regress at some later time, and yet no deficit in their LI values could be detected. The lack of an LI deficit suggests that regression probably occurred in spite of continued vigorous cell proliferation in the papillomas.

While it is possible, in principle, that the high LI values in the papillomas at 3 weeks after the PMA was stopped could have been associated with clonal expansion of immunocytes as part of an immune rejection process, such a possibility is considered extremely unlikely because of the generally uniform distribution of the labeled cells in what corresponds to and resembles morphologically the basal layer in each papule. Relatively few labeled cells could be found in the inflammatory infiltrate or in the differentiating layer in the epithelium. Since the labeled cells are morphologically and functionally identical to epithelial basal cells, it seems likely that the high LI values in the papillomas are not part of an immune rejection process but instead represent the proliferative behavior of the papillomatous epithelium.

The papillomas were growing relatively slowly in comparison to their potential growth rate estimated from LI values. If all newly formed papilloma cells were retained within the mass of the tumor, the expression for potential doubling time $T_p$ is

$$T_p = \frac{0.69}{T_s/LI}$$

where $T_s$ is assumed to be about 10 hr, potential doubling times were about 1 day, while actual doubling times were at least 30 days and probably longer (9). Such a large difference between potential and actual doubling time indicates that papilloma growth was sustained by an excess of cell production over cell loss of only about 5%. Such lesions are nearly in equilibrium where relatively small changes in cell production or cell loss could influence the balance toward growth or regression.

Indirect evidence indicates that the “microtumors” detected microscopically in cleared whole skin were probably in equilibrium, neither growing nor regressing. Microtumors were found in abundance at a time when the papilloma incidence had reached a plateau indicating that the microtumors were unlikely to be converted into papillomas in spite of the continued application of PMA at a given dosage. However, when the PMA dosage was increased from 2.5 to 10 μg, a rapid rise in papilloma incidence was observed. Generally, the induction of new papillomas would require at least 40 days, but the new tumors occurred within 7 days of the dosage increase, and it is reasonable to conclude that the effect of the increased PMA dosage was to stimulate the growth of previously static microtumors.

The regression of a papilloma means that a previously existing tumor mass has disappeared or been resorbed as a result of some cytotoxic process which could include destruction by thymocytes or some other mechanism. The lack of an effect of rabbit anti-mouse ATS on papilloma regression tends to suggest that immune rejection may not be the basis for regression (6). On the other hand, papilloma cells are very weakly antigenic in comparison to allogeneic skin transplants which were used to establish the ATS potency, and the ATS dosage, while sufficient to reduce circulating thymocytes, may have been insufficient to reduce the number of thymocytes already present in the tissue. Even so, considering the precarious equilibrium of cell proliferation in the papillomas, prevention of the entry of new thymocytes into the papillomas should have been sufficient to produce an effect on regression if in fact thymocytes are significantly involved. EPP is a potent skin irritant unlikely to be similar to the action of ATS and yet EPP significantly reduced the regression rate of the papillomas. Skin irritation is a complex reaction involving infiltration with inflammatory cells, increased cellular proliferation in the epidermis, as well as other effects; nevertheless the results suggest the possibility that irritation could be involved in promotion by helping to prevent regression by some as yet unspecified mechanism.

The nearly exponential nature of the regression curve suggests that there may exist a broad spectrum of lesions with different tendencies to regress. A small proportion of the papillomas showed little or no tendency to regress, and accordingly the regression curves were biphasic. Papillomas with a minimal or absent tendency to regress were produced in about equal numbers at the 2 PMA doses which indicates that the increase in number of lesions at the higher PMA dosage was caused mainly by an increase in the number of papillomas with a stronger tendency to regress. Roe et al. (8) have published results for regressions obtained among papillomas produced under conditions very similar to those used in the present experiment, except the DMBA dose was 100 μg. They noted that the papilloma incidence continued to rise for about 5 weeks after the PMA was stopped and subsequently only about 20% regression was noted in 3 months. Their papilloma yield was not appreciably different from the one reported here which suggests that, while the higher DMBA dose did not produce a proportional increase in the yield, the papillomas that did occur were somewhat less likely to undergo regression than those produced at the lower DMBA dose.

Certain features of the effect of PMA dose and application frequency on tumor incidence might be explained by the regression tendency of the tumors. Van Duuren et al. (15) found that the yield of papillomas per mouse was reduced by about 25% for a weekly PMA dose of 5.0 μg when the PMA was given only once instead of 3 times a week. The half-time of the rapidly regressing papillomas in the present experiment was about 24 days which converts to a regression rate of about 20%/week and is sufficiently close to Van Duuren’s observed value to suggest that the tendency to regress may play an important role in the frequency of application effect on promotion.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent technical assistance of Betty Skocik.
REFERENCES

Fig. 1. Radioautograph of typical papilloma during PMA treatment. Feulgen stained, hematoxylin toned, × 100.
Fig. 2. Radioautograph of typical papilloma in ATS-treated group 3 weeks after PMA was stopped. Feulgen stained, hematoxylin toned, × 100.
Regression Kinetics of Mouse Skin Papillomas

Fredric J. Burns, Martin Vanderlaan, Andrew Sivak, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/36/4/1422

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/36/4/1422. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.