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Suppression of Delayed-Type Hypersensitivity in Mice by 12-Tetradecanoylphorbol-13-acetate

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The effect of a potent tumor promoter, 12-tetradecanoylphorbol-13-acetate (TPA), on delayed type hypersensitivity (DTH) to sheep red blood cells (SRBC) in ddy mice was investigated. The mice were immunized with 10^8 SRBC, and footpad reaction (FPR) was measured after challenge with 10^8 SRBC. When 5 μg of TPA was administered to the mice 24 h before the challenge by painting on the skin. FPR was markedly suppressed. The suppression was greater in the painting of TPA on the footpads of the challanged leg than on the footpad of the other leg. These data suggest that the suppression of FPR involves not only a systemic process, but also a local effect.

The application of 4 phorbol related compounds (TPA, phorbol 12,13-didecanoate, 4α-phorbol 12,13-didecanoate and phorbol) showed that the suppressive effect was on DTH virtually paralleled the tumor-promoting activity. Treatments with λ-carrageenan and formaldehyde did not suppress the FPR. These data suggest that the present effect may not be related to the phlogistic effect.

Keywords——tumor promoter; 12-tetradecanoylphorbol-13-acetate; immunosuppression; delayed hypersensitivity

Introduction

Tumor promoters are compounds that induce malignant tumors when administered after treatment with a small quantities of carcinogens, but do not induce tumors when were administered alone.[1-3] 12-Tetradecanoylphorbol-13-acetate (TPA) is a potent promoter of mouse skin tumors.[4-6]

It has been proposed by many reseachers that immune mechanisms may control the development of tumors[4,7] (The so-called immunosurvaillance theory). Therefore, the suppression of immune function may be related to the development of tumors. However, the effects of tumor promoters on immune function have not been investigated in detail. Some researchers have observed that TPA suppressed immune activities, *i.e.* T cells[8] or natural killer cells in mice.[9]

Recently it was reported that the growth of tumors was suppressed when tumor cells were grafted with an antigen into mice which had been immunized with the antigen.[10-13] These data suggest that delayed hypersensitivity (DTH) is of importance in the mechanisms of control of tumors. On the other hand, a contribution of Lyt 1^+2^- T cells, which include T_D cells, in the process of tumor rejection was suggested by the adoptive transfer of the spleen cells of mice,[14-16] and an increase of Lyt 1^+2^- T cells in tumors was observed.[17] However, the effect of tumor promoters on DTH has not been studied. In this work, the effect of TPA on DTH to sheep red blood cells (SRBC) was investigated by means of the footpad reaction (FPR) in mice. The effects of some phorbol type compounds, λ-carrageenan and formaldehyde were also studied.

Materials and Methods

Animals——Male ddy mice were purchased from the Sankyo Labo Service (Shizuoka, Japan) and used at 12 weeks of age. They were given the basal diet CE-2 and water *ad libitum*.

Reagents——TPA was obtained from Chemicals for Cancer Research Inc. (U.S.A.). Phorbol 12,13-acetate (PDD), 4α-phorbol 12,13-acetate (4α-PDD) and phorbol in the "Phorbol ester research kit" (LCS 58-1513-27) were used.

Assay of Footpad Reaction (FPR)——Mice were immunized by subcutaneous injection of 10^8 of SRBC in 0.05 ml of saline into the back. After 7 d, 0.025 ml of a suspension of 4×10^9 SRBC in saline was injected into the footpad of the left hind leg of each mouse. The thickness of the footpads was measured with a dial thickness gauge (Teclock Corp., SM-528, Japan) before and 24 h after 2nd injection of SRBC. The difference of the thickness before and after the 2nd injection was recorded as a measure of the swelling. Data are given as mean ± S.D. of 4 mice.

Treatment with TPA or Related Compounds——At 24 or 72 h before the 2nd injection of SRBC, 12.5 μl of acetone solution of TPA or a related compound was painted on the footpads or the dorsal region.

Treatment with λ-Carrageenan or Formaldehyde——At 24 h before the 2nd injection of SRBC, 0.05 ml of 0.5% λ-carrageenan or 0.5% formaldehyde in saline was injected into the footpad which was to be challenged with SRBC.

Results

Effect of TPA Painting on FPR

Male ddy mice (12 weeks) were immunized with 10^6, 10^7 or 10^8 SRBC in 0.05 ml of saline, and FPR was measured at 24 h after the 2nd injection of 10^8 SRBC. At 24 or 72 h before the 2nd SRBC injection, 5 μg (8.1 nmol) of TPA in 12.5 μl of acetone was painted on the footpad of the left hind leg which was to be challenged by SRBC. As shown in Table I, FPR was markedly suppressed by TPA treatment at 24 h before the 2nd injection, but no significant effect was observed when TPA was painted at 72 h before the 2nd injection.

Method of TPA Administration

At 24 h before the 2nd SRBC injection, 5 μg of TPA was painted on the footpads or dorsal region. As shown in Table II, the greatest suppression of FPR was observed in the

TABLE I. Effect of TPA Painting on DTH to SRBC

Number of SRBC in 1st injection	Footpad swelling 24 h after 2nd injection of 10^8 SRBC (mm)		
	Control	TPA treatment 24 h before 2nd injection	TPA treatment 72 h before 2nd injection
10^6	0.581 ± 0.057	0.246 ± 0.025[a)]	0.477 ± 0.035
10^7	0.671 ± 0.041	0.253 ± 0.046[a)]	0.586 ± 0.032
10^8	0.786 ± 0.028	0.305 ± 0.031[a)]	0.741 ± 0.059

Each result is the mean ± S.E. (4 mice). *a)* $p < 0.05$ (difference from the control group).

TABLE II. Comparison of Various Locations of TPA Treatment

Group	Footpad swelling 24 h after 2nd injection (mm)
Control	0.601 ± 0.070
Painting on	
footpad of challenged leg	0.167 ± 0.024[a)]
footpads of other legs	0.368 ± 0.049[a)]
dorsal region	0.291 ± 0.062[a)]

Each result is the mean ± S.D. (4 mice). *a)* $p < 0.05$ (difference from the control group).

TABLE III. Age Dependence of Effect of TPA on DTH in Male Mice

Age of mice (weeks)	Footpad swelling 24 h after 2nd injection (mm)	
	Control	TPA treatment 24 h before 2nd injection
5	0.654±0.154	0.442±0.043
6	0.612±0.109	0.377±0.043
8	0.570±0.027	0.386±0.077[a]
9	0.631±0.177	0.302±0.043[a]
10	0.689±0.113	0.252±0.014[a]
12	0.675±0.033	0.234±0.047[a]

Each result is the mean±S.D. (4 mice). *a)* $p<0.05$ (difference from the control group).

TABLE IV. Sex Difference in the Effect of TPA on DTH

Sex of mice	Footpad swelling 24 h after 2nd injection (mm)	
	Control	TPA treatment 24 h before 2nd injection
Male	0.530±0.097	0.285±0.051[a]
Female	0.642±0.045	0.362±0.036[a]

Each result is the mean±S.D. (4 mice). The age of the mice was 8 weeks. *a)* $p<0.05$ (difference from the control group).

TABLE V. Effect of Phorbol-Related Compounds, λ-Carrageenan, and Formaldehyde

Reagent	Footpad swelling 24 h after 2nd injection (mm)	Reagent	Footpad swelling 24 h after 2nd injection (mm)
Control	0.660±0.124	Phorbol	0.623±0.125
TPA	0.167±0.024[a]	λ-Carrageenan	0.907±0.205
PDD	0.456±0.058	Formaldehyde	0.483±0.195
4α-PDD	0.651±0.081		

Each result is the mean±S.D. (4 mice). *a)* $p<0.05$ (difference from the control group).

painted footpad of the challenged leg. However, positive results were also obtained in the painting of the footpads of the other legs or dorsal region.

Age and Sex Difference

The suppressive effect of TPA painting on FPR of male ddy mice of various ages (5—12 weeks) was observed. As shown in Table III, FPR was suppressed more in adult mice (7—12 weeks) than in younger mice. The effect in female ddy mice (12 weeks) was also measured and the suppression was at virtually the same level as in the male mice (Table IV).

Effect of Phorbol Related Compounds, λ-Carrageenan and Formaldehyde

The effects of 4 phorbol-related compounds with different tumor-promoting activities were compared. At 24 h before the 2nd injection, 8.1 nmol of TPA, PDD, 4α-PDD or phorbol was painted on the footpad of the challenged leg of each male ddy mouse. TPA, which is a potent tumor promoter, showed the greatest suppressive effect on FPR. PDD, a weak tumor promoter, showed a weak suppressive effect, while 4α-PDD and phorbol, which are inactive as tumor promoters,[3,6] lacked any suppressive effect. On the other hand, the effects of λ-carrageenan and formaldehyde, typical phlogistic agents, were also investigated. These reagents were injected into the footpad of the challenged leg at 24 h before the 2nd SRBC injection. In both cases, no suppressive effect was observed.

Discussion

The data presented in this paper show that a potent tumor promoter, TPA, can suppress FPR of mice injected with SRBC. FPR was lowered by TPA painting at 24 h bebore the 2nd

injection of the antigen. The positive results after the painting on regions other than the footpad challenged by the 2nd SRBC injection suggest that DTH activity in the whole body was lowered by this treatment. However, the greatest effect was obtained by painting on the footpad of the challenged leg, and these data suggest that TPA suppresses the local processes of FPR.

Occurrence of inflammation often suppresses DTH[18] and TPA is known to be a phlogistic agent.[5] In order to examine the possibility that the suppressive effect of TPA on FPR derived from the phlogistic action, λ-carrageenan and formaldehyde were administered at 24 h before the 2nd SRBC injection. Negative results were obtained in these experiments. On the other hand, the suppressive effect virtually paralelled the tumor-promoting activity in 4 phorbol related compounds. These data suggest that this effect may be related to the tumor-promoting activity.

Recently the significance of DTH in immune surveillance against tumors has been investigated by several researchers[10,12-14] who suggested that the decline of DTH activity might be advantageous to the growth. Thus, the suppressive effect of TPA on DTH is of interest.

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