Tumor-Promoting Croton Oil Factor Tetradecanoyl-Phorbol-Acetate Stimulates Thymidine Incorporation into Normal and Con A Stimulated Lymphocytes

Tetradecanoyl-phorbol-acetate (TPA) has been found to be mitogenic in dense cultures of 3T3 fibroblasts[1,2], an effect which is accompanied by changes in lipid metabolism[2]. Both observations suggest that lymphocytes may be stimulated by TPA in analogy to plant lectins[3]. In the following paper we report on increased thymidine incorporation into normal and Con A stimulated spleen cells treated with TPA.

Material and methods. Spleens were aseptically removed from 7–8-week-old NMRI mice (Süddeutsche Versuchstierfarm Tuttlingen, SPF). Cells were teased out by slowly passing them 10 times through a disposable syringe without needle (Stylex Syringes Pharmasal Lab. Glensdale, Cal., 1 ml) in about 1 ml medium (MEM, Biocult supplemented with 10% fetal calf serum-Flow). The suspended cells were diluted with 1 ml medium and filtered through sterile gaze (Schweizerische Seidengazefabrik, CH–Zürich; Stabilitex 7–200, corresponding to 200 µm. The cell suspension was diluted to give 10^6 cells/ml and distributed in 200 µl portions into a microtiter plate (Mikrotiterplatte Greiner, Nürtingen, Germany, flat bottom).

TPA (kindly supplied by Dr. E. HECKER) dissolved in dimethylsulfoxide (DMSO) was added to give a final concentration of 0.005% DMSO. Con A (Calbiochem) was diluted in MEM. 3H-thymidine (Amersham, 0.5 µC/culture) was added in saline and the incorporation stopped by the addition of sodiumdodecylsulfate (final concentration 0.2%). The cell lysate was pipetted onto filter paper disks (Whatman 3 mm, 2.3 cm) using an automatic filter paper dispenser[2]. After extraction with 5% TCA (3×) and 5% acetic acid (1×), the dried filter papers were counted in a liquid scintillation counter using 2 ml of a standard toluene scintillator (Liquifluor, New England Nuclear, Boston).

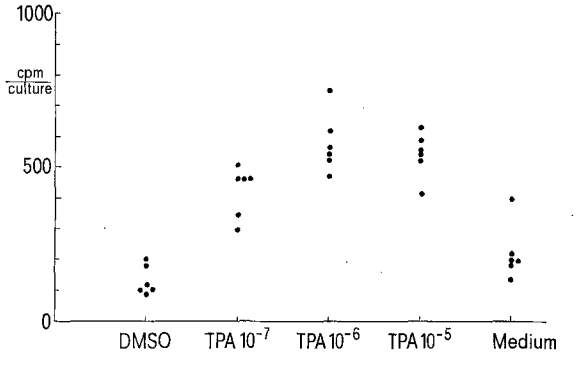

Fig. 1. TPA induced stimulation of thymidine incorporation into mice spleen cells (72 h).

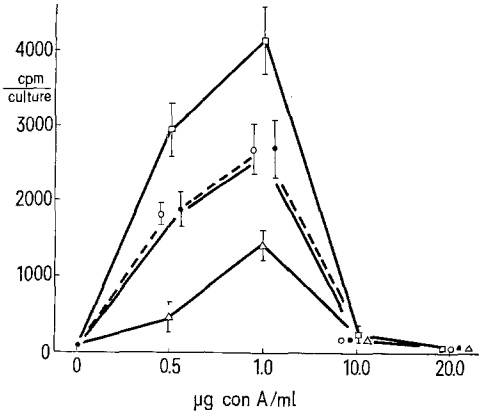

Fig. 2. Con A induced stimulation of thymidine incorporation into mouse spleen cells coincubated with 10^{-6} *M* TPA after 72 h. ○, Con A only (72 h); ●, Con A and TPA simultaneously (72 h); □, Con A preincubated (12 h); △, TPA preincubated (12 h). Standard deviations are given; 6 cultures/point.

Results and discussion. As Figure 1 demonstrates, TPA stimulates thymidine incorporation into spleen cells after a 72 h incubation. This stimulation, however, is only about 1/10 of the effect obtained with Con A, if compared at maximal stimulating concentrations.

If the lymphocytes are incubated simultaneously with TPA (10^{-6} *M*) and Con A (varying concentrations) no potentiating effect is observed (Figure 2). If, however, the cells are preincubated for 12 h with Con A alone, the stimulation is significantly enhanced. On the other hand, lymphocytes preincubated with TPA before adding Con A are less stimulated, provided the preincubation period was longer than 6 h. (A cytotoxic effect of TPA on lymphocytes appears to be ruled out by the observation that TPA, when incubated simultaneously with Con A, does not affect stimulation of thymidine incorporation.)

These observations are consistent with the idea that TPA induces membrane changes[2]. These membrane changes may imply the 'masking' of Con A binding sites. If TPA is added to lymphocytes already triggered by Con A, any masking of Con A receptors has no effect. In this case the weak mitogenic action of TPA can add up to the Con A stimulation. However, a stabilizing effect by TPA as observed by VAN DUUREN[4] and us[5] may explain as well the higher stimulation index measured if Con A triggered lymphocytes are 'promoted' by TPA.

Zusammenfassung. Crotonölfaktor TPA stimuliert den Thymidineinbau in Lymphozyten (Mäusemilzzellen) um den Faktor 10 weniger als Con A. TPA vorbehandelte Milzzellen lassen sich mit Con A weniger hoch stimulieren, während Con A «getriggerte» Lymphozyten durch TPA zusätzlich stimuliert werden können.

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