TUMOR PROMOTION AND THE ARACHIDONATE CASCADE

S.M. FISCHER, G.S. CAMERON, K.E. PATRICK, J. LEYTON, and R.J. MORRIS

University of Texas Cancer Center, Science Park, Smithville, Texas 78957

INTRODUCTION

Experimental chemical carcinogenesis studies in animals, particularly the multistage model in mouse skin (1-3) are valuable in identifying those biological events or agents that play either an essential or modulatory role in the development of tumors. The promotion stage is most often accomplished by using 12-O-tetradecanoyl phorbol-13-acetate (TPA), although a variety of agents have been identified as skin-tumor promoters (3,4). Among the many morphological and biochemical responses of the skin to promoters, the induction of inflammation followed by increased epidermal cell proliferation (hyperplasia) are events common to all promoters and appear to be required events (5).

There is now substantial evidence that TPA induces eicosanoid synthesis in epidermal cells in vivo and in vitro (6). Since it is well established that eicosanoids are one of the major mediators of inflammation, questions have arisen as to the role and/or requirement for these metabolites in the tumor process. Using several approaches, the conclusion of studies by several different laboratories indicate that (i) exogenous application of prostaglandins with TPA can modify tumor development (7), (ii) use of inhibitors of various parts of the arachidonate cascade causes an inhibition in tumor development (8) and (iii) reduction of arachidonic acid levels in epidermal membranes by altering dietary fatty acid intake results in a reduction in tumor number (9).

Current work in our laboratory is now aimed at addressing three questions concerning the relationship of TPA and eicosanoid synthesis and subsequent alterations in skin function. First, what is the mechanism by which TPA causes arachidonate release from the membrane? Second, are there differences between basal and differentiated cells with regard to arachidonate metabolism? Third, what is the relationship of inflammation to proliferation of the overlying epidermis.

METHODS AND MATERIALS

Cell culture.

Primary cultures of epidermal cells from newborn SSIN mice were established as previously described and labelled overnight with $^{14}$C-arachidonic acid (10). These prelabelled cultures were then treated with TPA or other agents for 3 hr after which the media was removed and extracted. Eicosanoid release was measured as amount of released radiolabel; prostaglandin E$_2$ was likewise measured following TLC of the extracted media (10).
Separation of epidermal cells.

Epidermal cells isolated from trypsinized adult SSIN mouse skins were layered on a 50% Percoll gradient as previously described (11). Separation into three fractions, based on their state of differentiation, were obtained following centrifugation. The cells in these fractions were incubated with $^{14}$C-arachidonic acid and the synthesis of radiolabelled prostaglandins and HETEs measured.

Induced Inflammation/Hyperplasia Studies.

The epidermis of the inside of the ear of SSIN mice was removed by abrasion with fine emery paper. At various times following the procedure, histological cross-sections of the ear were made and the extent of hyperplasia of the outer ear epidermis evaluated. In another experiment, 10 µl of 2% carrageenan was injected subcutaneously into the dorsal skin. Histological sections were evaluated for hyperplasia of the overlying epidermis.

RESULTS

In order to determine whether protein kinase C (PKC) mediates the TPA induced release of arachidonic acid, several approaches were taken. First, as shown in Figure 1, the PKC inhibitor H-7 (from Seikagaku America) was found to inhibit both the TPA response and the response to the diacylglycerol (DAG) 1,2-dioctanoyl-sn-glycerol. DAG is believed to be the natural ligand or activator of PKC; TPA performs this function with even greater efficacy (12). The observation (data not shown) that $4\alpha$-TPA, a nonpromoting analog of TPA (13) also causes arachidonic acid release (at a level comparable to TPA if used at a 10-fold higher dose) that is not inhibited by H-7, further supports PKC involvement.

Additionally, prior treatment with TPA or DAG suppresses the release of arachidonate upon subsequent treatment 15 to 18 hr later, as shown in Figure 2. This is interpreted as being due to the down-regulation of PKC (12). If the treatment protocol is TPA first, followed by $4\alpha$TPA, such down-regulation of arachidonic acid release is not observed (data not shown). Collectively, these results support a conclusion that TPA induces arachidonic release via PKC activation.

To better understand the cellular source and function of specific eicosanoids in the skin, it is of value to know if all or only specific populations of epidermal cells synthesize and/or respond to eicosanoids. To address the first question, epidermal cells were separated on the basis of their state of differentiation, with fraction 1 denoting the most differentiated, fraction 2 those committed to differentiation and fraction 3 having the most basal, least differentiated characteristics. When these populations of cells, used either as viable preparations or freeze-thawed (this eliminates the competing reaction of incorporation of arachidonate into lipids) were analyzed for their ability to metabolize exogenous arachidonate, the most differentiated cells were found to have the greatest synthetic activity with respect to both prostaglandins and HETEs (Figure 3). The basal cells have relatively very little synthetic activity, suggesting that the expression of prostaglandin synthetase and the lipooxygenases are a function of commitment to differentiation.