Leukotrienes and Thromboxane B₂ Stimulate Normal Human Melanocytes in Vitro : Possible Inducers of Postinflammatory Pigmentation

YASUSHI TOMITA, KAZUHISA MAEDA* and HACHIRO TAGAMI

*Department of Dermatology, Tohoku University School of Medicine, Sendai 980, and *Shiseido Laboratories, Yokohama 223*

TOMITA, Y., MAEDA, K. and TAGAMI, H. *Leukotrienes and Thromboxane B₂ Stimulate Normal Human Melanocytes in Vitro : Possible Inducers of Postinflammatory Pigmentation.* Tohoku J. exp. Med., 1988, 156 (3) 303-304 — Normal human epidermal melanocytes became swollen and more dendritic with an increase in the amount of immunoreactive tyrosinase when they were cultured for 2 days with each one of leukotriene (LT) C₄, LTD₄ and TXB₂. Their effects were stronger than that of prostaglandin E₂, about which we have previously reported. From these data we suggest that arachidonate-derived chemical mediators, especially LTC₄, LTD₄ and TXB₂ may be responsible for the induction of post-inflammatory hyperpigmentation of the skin. — leukotriene C₄; leukotriene D₄; melanocyte; skin pigmentation; thromboxane B₂

Despite such a common phenomenon for the skin to develop hyperpigmentation after inflammation, the regulation mechanisms of melanin synthesis involved in it have not been well clarified. Prostaglandin (PG) E₁, PGE₂ and PGD₂, which are known to be important endogenous regulators of inflammatory diseases in the skin, were reported to stimulate the mammalian pigment cells in vitro (Tomita et al. 1987) and in vivo (Nordlund et al. 1986) and their involvement has been suggested in the post-inflammatory skin pigmentation. However, it is quite possible that synthesis of other arachidonate-derived leukotrienes (LTs) and thromboxanes (TXs) also takes place simultaneously with that of PGs and that they also stimulate the melanocytes. We report here that LTC₄, LTD₄ and TXB₂ have been found to be stronger stimulators of human melanocytes than PGE₂.

Human melanocytes from the roof of suction blisters were cultured as described previously (Tomita et al. 1987). Immunofluorescence staining of cultured melanocytes were carried out using rat monoclonal antibody against tyrosinase and fluorescein isothiocyanate (FITC)-conjugated second antibody directed to the monoclonal antibody (TAGO Inc., Burlingame, CA, USA), and the intensity of fluorescence of FITC in each melanocyte was measured by microphotometer (P-1, Nikon, Tokyo) according to the method described previously (Tomita et al. 1987). The cell perimeter, area as well as number of dendrites of

Received October 3, 1988; revision accepted for publication October 12, 1988.

Supported in part by grants from the Ministry of Education, Science and Culture of Japan (Nos. 62570448 and 63570466).

TABLE 1. *The effect of various arachidonate derivatives on melanocytes in vitro*

Treatment	Number of cells examined	Amount of immunoreactive tyrosinase (Relative fluorescent intensity)		Cell configuration			
		Mean	S.D.	Area (μm^2)		Number of dendrites	
Control	30	3.7	1.2	793	419	2.4	0.7
PGE ₂ (5 μM)	30	5.3 ^a	2.0	1,467 ^b	476	2.9	1.3
LTC ₄ (5 μM)	30	11.6 ^a	6.4	1,564 ^a	622	4.7 ^a	1.5
LTD ₄ (5 μM)	30	7.8 ^a	3.4	1,456 ^b	480	3.4 ^a	1.1
TXB ₂ (5 μM)	30	7.4 ^a	2.1	1,880 ^b	447	2.9 ^c	0.9

Melanocytes were cultured for 2 days in MEM with 5% fetal bovine serum in the presence or absence of various agent. PGE₂, prostaglandin E₂; LTC₄, leukotriene C₄; LTD₄, leukotriene D₄; TXB₂, thromboxane B₂.

^a, $p < 0.01$ against control value by Welch's t -test.

^b, $p < 0.01$ against control value by Student's t -test.

^c, $p < 0.05$ against control value by Student's t -test.

each melanocytes were determined with an image scanner (PC-IN 502, NEC, Tokyo) connected to a microcomputer (PC-9801MV, NEC) and appropriate soft ware as described previously (Tomita et al. 1987).

The elongation and increased number of dendrites of melanocytes are observed in the human epidermis when hyperpigmentation of the skin occurs after sun light exposure. These morphological changes are thought to be required for the transfer of the melanosomes, melanin granules, to the surrounding keratinocytes. In the present studies, we found that LTC₄, LTD₄, and TXB₂ increased remarkably the amount of immunoreactive tyrosinase and also changed the configuration of the melanocytes into more swollen and dendritic ones as compared with PGE₂ (Table 1). The number of dendrites also increased which appeared more slender and extended than those of the controls. The stimulatory effect of various PGs such as PGE₁, PGE₂ and PGD₂ on pigment cells in mammals have already been reported in vitro and in vivo (Nordlund et al. 1986; Tomita et al. 1987). However, little is known about the effect of other arachidonate-derived TXs and LTs, except for the insignificant topical effect of TXB₂ on the epidermal melanocytes of DBA/2 mouse as reported by Nordlund et al. (1986). The disagreement about the effect of TXB₂ between the experiments done by Nordlund et al. (1986) and ours may be due to the differences in the used materials, experimental conditions and methodology.

Based on these results we strongly suggest that arachidonate derived inflammatory chemical mediators such as LTs and TXB₂ as well as PGE₂ play an important role in the induction of post-inflammatory hyperpigmentation in the skin.

References

- 1) Nordlund, J.J., Collins, C.E. & Rheins, L.A. (1986) Prostaglandin E₂ and D₂ but not MSH stimulate the proliferation of pigment cells in the pinnal epidermis of the DBA/2 mouse. *J. invest. Derm.*, **86**, 433-437.
- 2) Tomita, Y., Iwamoto, M., Masuda, T. & Tagami, H. (1987) Stimulatory effect of prostaglandin E₂ on the configuration of normal human melanocytes in vitro. *J. invest. Derm.*, **89**, 299-301.