British Journal of Dermatology (1985) **113**, 43–52.

Leukotrienes B_4, C_4 and D_4 stimulate DNA synthesis in cultured human epidermal keratinocytes

K.KRAGBALLE, L.DESJARLAIS AND J.J. VOORHEES

Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, U.S.A.

Accepted for publication 16 December 1984

SUMMARY

Leukotrienes in psoriatic skin lesions are potent mediators of inflammation. We have studied the capacity of leukotrienes to stimulate the DNA synthesis of cultured human epidermal keratinocytes. At concentrations ranging from 10^{-12} to 10^{-8} M, LTB_4 produced a 100% increase of DNA synthesis determined both as the incorporation of [^{3}H] thymidine and as the labelling index. In comparison, LTB_4 had no effect on the DNA synthesis of dermal fibroblast cultures. 5S,12S-LTB_4 and 5S,12S-all-trans-LTB_4 did not change the DNA synthesis of keratinocytes, but the effect of LTB_4 was abolished in the presence of 5S,12S-all-*trans* $HLTB_4$. Being less potent than LTB_4 the peptidoleukotrienes (LTC_4, LTD_4) also stimulated keratinocyte DNA synthesis. The effect of the peptidoleukotrienes, but not of LTB_4, was antagonized by FPL 55712. These results show that leukotrienes B_4, C_4 and D_4 exert potent and stereospecific mitogenic effects on cultured human keratinocytes. The presence of these arachidonic acid metabolites in psoriatic skin lesions may be pertinent to both inflammation and aberrant epidermal growth in psoriasis.

Leukotrienes constitute a family of arachidonic acid derivatives with potent biological actions (Samuelsson, 1983). The leukotrienes may be divided into two groups according to their chemical structure: those which have a sulphur linkage and amino acid residues at C-6 (the peptidoleukotrienes) and those which do not. The first group includes leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) while leukotriene B_4 (LTB_4) belongs to the second group. The general biological actions of the leukotrienes also vary between these groups. The peptidoleukotrienes contract vascular (Hedqvist *et al.*, 1980) and nonvascular (Dahlén *et al.*, 1980) smooth muscle and increase vascular permeability (Soter *et al.*, 1983). LTB_4, on the other hand, has potent actions on neutrophils inducing chemotaxis (Malmsten *et al.*, 1980), adhesion (Ford-Hutchinson *et al.*, 1980), degranulation (Feinmark *et al.*, 1981), and a respiratory burst (Serhan *et al.*, 1982). These pharmacological actions of leukotrienes suggest that they may play a role in inflammatory processes.

The best evidence for the involvement of leukotrienes in human disease is in psoriasis. Some

Correspondence: Knud Kragballe, M.D., Dept. of Dermatology, University of Michigan Medical School, R6558 Kresge 1, Ann Arbor, Michigan 48109, U.S.A.

criteria that have been fulfilled to classify psoriasis as a disease mediated by leukotrienes and possibly other lipoxygenase products include: (1) the presence of LTB_4, LTC_4 and mono-hydroxyeicosatetraenoic acids (mono-HETEs) in psoriatic skin lesions (Hammarström *et al.*, 1975; Brain *et al.*, 1982); (2) the ability of these compounds to mediate the accumulation of neutrophils and the vasodilation that characterize psoriatic skin lesions (Soter *et al.*, 1983; Camp *et al.*, 1984; Bisgaard, Kristensen & Sondergaard, 1982); and (3) the improvement of psoriasis by an inhibitor of both 5-lipoxygenase and cyclooxygenase (benoxaprofen) (Allen & Littlewood, 1982; Kragballe & Herlin, 1983). However, these facts raise another set of questions. One of the fundamental questions is how and/or whether the inflammatory events are linked to the abnormal epidermal growth characteristic of psoriasis.

In this study we report the capacity of synthetic leukotrienes to stimulate the proliferation of primary cultures of human epidermal keratinocytes.

METHODS

Reagents

Synthetic LTB_4, 5S,12S-LTB_4, 5S,12S-all-trans-LTB_4, LTC_4, LTD_4 and 20-OH-LTD_4 were provided by Dr J. Rokach (Merck-Frosst Canada Inc., Dorval, Quebec, Canada). FPL 55712 was from Fisons Pharmaceuticals, Bedford, MA. Minimal essential medium (MEM), McCoy's 5A medium, HEPES, fetal bovine serum (FBS), penicillin, streptomycin, Vitrogen 100 purified collagen (bovine type I), 0·4% trypan blue and Lux plastic cover slips were from Flow Laboratories, Inc., Rockville, MD. Trypsin (bovine pancreas type III), collagenase (type II-5) and L-serine were purchased from Sigma Chemical Co., St Louis, MO. [Methyl-^{3}H] thymidine ([^{3}H]TdR) (60 mCi/mmol) and [^{14}C] orotic acid (46·9 mCi/mmol) was obtained from New England Nuclear, Boston, MA. Agarose was from Accurate Chemical Scientific Corp., Westbury, N.Y. Corning plastic products (Corning Glass Works, Corning, N.Y.) were used in all experiments. All other chemicals were reagent grade.

Skin specimens

Informed consent was obtained from adult, healthy volunteers. After intracutaneous injection of 1% lidocaine, strips of buttock skin (epidermis and some papillary dermis) were obtained with a keratome set at 0·2 mm.

Cultures of human epidermal keratinocytes

To establish human epidermal keratinocyte cultures we used a modification of the procedure reported by Liu & Karasek (1978). The keratomed skin was incubated with 0·25% trypsin in PBS containing 5 mM glucose (pH 7·0) for 30–40 min at 37°C. After aspiration of trypsin, MEM with 10% FBS, 50 IU/ml penicillin, 50 μg/ml streptomycin was added. The upper part of the biopsy (most of the epidermis) was separated from the lower part (dermis with some basal epidermal cells) with forceps, and the basal cells released into the medium by gently agitating both the epidermal and the dermal parts of the biopsy. Two millilitres of epidermal cell suspension ($0{\cdot}8 \times 10^6$ trypan-blue excluding cells per ml) were plated on 16-mm culture wells precoated with a collagen type I gel (Vitrogen). The collagen (6·4 ml) was diluted with 0·1 N NaOH (3·8 ml), Hank's balanced salt solution (0·03 ml) and $NaHCO_3$ (1 ml) to a final concentration of 1·7 mg/ml. After layering the diluted collagen in tissue culture wells (0·1 ml/16-mm well), the wells were placed at 37°C for 30 min to allow the formation of a gel. Cultures were incubated at 37°C in 100% humidity in a 95% air/5% CO_2 environment. After 24 h the plating medium was replaced by McCoy's 5A medium supplemented with 10% normal

human type AB serum, 50 IU/ml penicillin, 50 μg/ml steptomycin and 4×10^{-4} M L-serine. The medium was changed twice every week. After 1–3 weeks the cultures became confluent and stratified into 2–4 layers.

Cultures of human dermal fibroblasts

Fibroblasts were isolated by incubating the dermal part of the biopsy with 0·1% collagenase for 30 min at 37°C. After aspirating the collagenase, the plating medium was added and the dermis was scraped. Two millilitres of suspended fibroblasts ($0 \cdot 5 \times 10^5$/ml) were added to each 16 mm well and cultured in the same way as the epidermal cells.

DNA synthesis

DNA synthesis via the salvage pathway was determined by [^{3}H]TdR incorporation into terminally labelled cultures and by autoradiography as previously described (Marcelo *et al.*, 1978). For [^{3}H]TdR incorporation, cultures were pulsed for 6 h with 1 μCi/ml [^{3}H]TdR (60 Ci/mmol). Then, cells were scraped off the dishes with 6% trichloroacetic acid (TCA), and the precipitate was washed twice by centrifugation in 6% TCA. The collected supernatant was counted to determine the [^{3}H]TdR acid-soluble fraction. The precipitate was hydrolysed in 3% perchloroacetic acid (PCA) at 95°C for 15 min. After centrifugation aliquots of the supernatant were counted to determine the [^{3}H]TdR incorporation into DNA and assayed colorimetrically for DNA.

For autoradiography cells were grown on plastic cover slips and labelled for 16 h with 10 μCi/ml [^{3}H]TdR (60 Ci/mmol). The cover slip cultures were then prepared for autoradiography and stained with haematoxylin and eosin. The labelling index was determined by counting the number of labelled nuclei of 500 cells in 25 fields.

The activity of the *de novo* pathway of DNA synthesis was determined by measuring the incorporation of 1 μCi/ml [^{14}C]orotic acid (46·9 mCi/mmol) into cultures terminally labelled for 6 h. Since orotic acid is incorporated into both RNA and DNA, the DNA was separated from RNA in these experiments (Marcelo *et al.*, 1978).

Chemokinesis of human neutrophils

Chemokinesis was measured using the agarose microdrop technique (Smith & Walker, 1980). Neutrophils were isolated from EDTA-blood as earlier described by us (Borregaard & Kragballe, 1980) and resuspended in a minimal volume (0·2 ml) of MEM containing 10% heat-inactivated human serum and 60 mM HEPES at pH 7·3. Neutrophils were then mixed with an equal volume of 0·4% agarose in MEM. A drop (2 μl) of this mixture was placed on the bottom of each well of a microtitre plate. The solidified drop was overlayed with 0·1 ml MEM containing 10% heat-inactivated human serum and 60 mM HEPES at pH 7·3, with and without chemoattractants. After incubation at 37°C for 3 h in a humidified atmosphere, the radius of the neutrophil-agarose drop was measured in an inverted microscope. Movement was expressed as the chemokinetic index (CI) as follows: CI = radius in test wells/radius in control wells. Experiments were done in quadruplicate.

Effect of leukotrienes on epidermal keratinocyte proliferation

At time zero, methanolic solutions of leukotrienes (methanol concentration $< 0 \cdot 1\%$) were added to confluent stratifying cell cultures. After incubation for 72 h the DNA synthesis was determined as described above. Before adding the leukotrienes, these were subjected to high performance liquid chromatography (HPLC) analysis to confirm their purity. Experiments were done in triplicate.

Statistics

The data were analysed by non-parametric methods because the number of experiments performed does not allow us to analyse whether the data follows a normal distribution. Results were expressed as medians and 95% confidence limits. The Wilcoxon Rank–Sum test for paired and unpaired samples was used to assess statistical significance.

RESULTS

Confluent, 2–3-week old cultures of normal human epidermal keratinocytes and dermal fibroblasts were incubated with LTB_4. LTB_4 produced almost a 100% increase of the incorporaton of [^{3}H]TdR, a precursor of the salvage pathway of DNA, into DNA of epidermal keratinocytes, whereas the [^{3}H]TdR incorporation into DNA of dermal fibroblasts was unchanged (Table 1). The acid-soluble pool of [^{3}H]TdR did not change in LTB_4-treated epidermal keratinocyte cultures indicating that the increase of [^{3}H]TdR incorporation was not

TABLE 1. Effect of leukotriene B_4 on the DNA synthesis of terminally labelled (6 h) epidermal keratinocyte and dermal fibroblast cultures

Cell	LTB_4* (M)	*n*	DNA (μg/well)	[^{3}H]TdR incorporation (cpm/μg DNA)	[^{14}C] orotic acid incorporation (cpm/μg DNA)
Epidermal keratinocyte	None	8	9·1 (6·0–12·8)	1880 (1450–2741)	5 (3–7)
	10^{-8}	7	8·3 (5·0–12·0)	3290 (2746–4611)†	6 (4–7)
	10^{-10}	7	8·8 (5·2–12·4)	3460 (2761–4629)†	5 (3–8)
Dermal fibroblast	None	6	9·7 (7·3–13·1)	4705 (3862–5758)	—
	10^{-8}	6	9·6 (6·9–11·5)	4262 (3226–5085)	—
	10^{-10}	6	10·0 (7·7–11·2)	4112 (3236–4994)	—

Values are medians and 95% confidence limits.
* Incubated for 72 h at 37°C.
† LTB_4 *vs* none: $P<0{\cdot}01$.

TABLE 2. Effect of leukotriene B_4 on the labelling index of epidermal keratinocyte cultures developed for autoradiography after labelling with [^{3}H]TdR for 16 h

LTB_4 (M)*	Labelling index (%)
None	11 (7–17)
10^{-8}	15 (11–22)†
10^{-10}	16 (12–22)†

Values are medians and 95% confidence limits for six experiments.
* Incubation for 72 h at 37°C.
† LTB_4 *vs* none: $P<0{\cdot}01$.

TABLE 3. Effect of leukotriene B_4 analogues on the DNA synthesis of epidermal keratinocytes

Agent* (M)	DNA (μg/well)	[³H]TdR incorporation (cpm/μg DNA)
None	8·7 (7·8–11·1)	1786 (1466–2441)
LTB_4 10^{-8}	9·6 (8·2–12·3)	3201 (2848–4052)†
5S, 12S LTB_4 10^{-6}	9·1 (7·8–11·3)	1875 (1520–2561)
5S, 12S LTB_4 10^{-8}	9·1 (7·4–10·8)	1893 (1603–2590)
5S, 12S all-trans-LTB_4 10^{-6}	9·4 (6·6–11·3)	1822 (1511–2488)
5S, 12S all-trans-LTB_4 10^{-8}	9·0 (7·5–11·5)	1840 (1522–2512)
5S, 12S all-trans-LTB_4 10^{-6} + LTB_4 10^{-8}‡	8·9 (7·7–11·4)	1845 (1490–2607)

Values are medians and 95% confidence limits for six experiments.
* Incubation for 72 h at 37°C.
† LTB_4 *vs* none: $P < 0{\cdot}01$.
‡ 5S, 12S all-trans-LTB_4 was added 10 min before LTB_4.

caused by a change of cellular transport (data not shown). The incorporation of [^{14}C] orotic acid, a precursor of the *de novo* pathway of DNA synthesis, was extremely low in epidermal keratinocyte cultures with or without LTB_4 (Table 1). The increase of DNA synthesis measured as [^{3}H]TdR incorporation into epidermal keratinocyte cultures treated with LTB_4 was confirmed by autoradiographic studies (Table 2). LTB_4-induced increase of keratinocyte DNA synthesis was not accompanied by a change of the DNA content of the cultures (Table 1).

To evaluate the stereospecificity of the effect of LTB_4, 5S,12S-LTB_4 (this is LTB_4 except that the configuration of the 12-OH group is S instead of R) and 5S,12S-all-*trans*-LTB_4 (this is 5S,12S-LTB_4 except that the configuration of the triene structure is all-*trans* instead of *cis-trans-trans*) were added to the cell cultures (Table 3). Neither 5S,12S-LTB_4 nor 5S,12S-all-*trans*-LTB_4 had any effect on epidermal keratinocyte DNA synthesis. The effect of LTB_4 was, however, inhibited in the presence of 5S,12S-all-*trans*-LTB_4, which is a competitive inhibitor of the binding of LTB_4 to human neutrophils (Goldman & Goetzl, 1982).

TABLE 4. Effect of peptido-leukotrienes on the DNA synthesis of epidermal keratinocytes

Agent*	M	DNA (μg/well)	[³H]TdR incorporation (cpm/μg DNA)
None		9·2 (7·5–12·0)	1736 (1444–2427)
LTC_4	10^{-8}	9·1 (7·3–11·8)	2747 (2150–3851)†
LTD_4	10^{-8}	9·4 (7·8–12·2)	2651 (2014–3273)‡
20-OH LTD_4	10^{-8}	9·0 (7·3–11·7)	1809 (1500–2446)

Values are medians and 95% confidence limits for six experiments.
* Incubated for 72 h at 37°C.
† LTC_4 *vs* none: $P < 0{\cdot}02$.
‡ LTB_4 *vs* none: $P < 0{\cdot}05$.

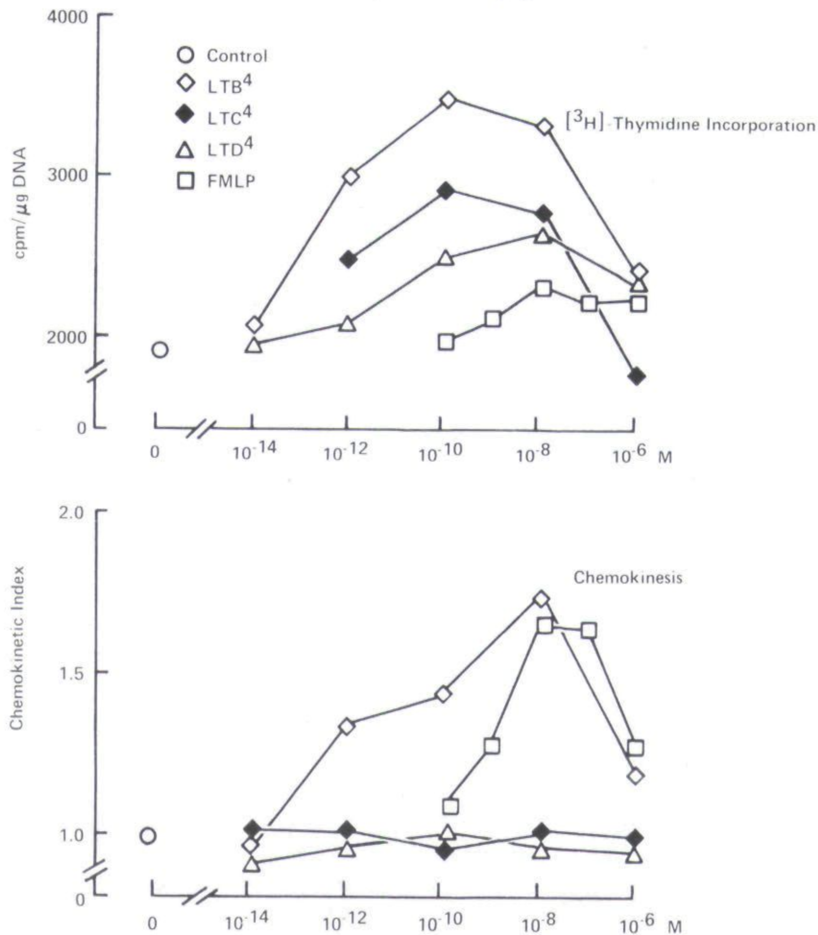

FIGURE 1. Dose-response curves for the effect of leukotrienes B_4, C_4 and D_4 and of *N*-formyl-L-methinonyl-L-leucyl-L-phenylalanine (FMLP) on epidermal keratinocyte DNA synthesis and neutrophil chemokinesis. Each point represents the median value of seven experiments. LTB_4 10^{-8} M and 10^{-10} M *vs* control: $P < 0.01$. LTB_4 10^{-12} M, LTC_4 10^{-10} to 10^{-8} M, LTD_4 10^{-10} to 10^{-8} M *vs* control: $P < 0.02$.

The stimulating effect of LTB_4 on human epidermal keratinocyte cultures was shared with the peptido-leukotrienes (LTC_4 and LTD_4), although their effect was less pronounced (Table 4, Fig. 1). The biologically inactive 20-OH-LTD_4, a putative metabolite of LTD_4, had no effect on mitogenesis (Table 4). The dose-response curve of the leukotriene effect on epidermal keratinocyte DNA synthesis is shown in Fig. 1. For LTB_4, the maximal stimulation was obtained at 10^{-10} M, but even at 10^{-12} M a significant increase was observed. Furthermore, stimulation of epidermal keratinocyte DNA synthesis by LTB_4 occurred at the same concentrations at which LTB_4 stimulated chemokinesis of human neutrophils (Fig. 1). In comparison, FMLP, another potent chemokinetic agent, was without effect on epidermal keratinocye DNA synthesis.

In the presence of FPL 55712, a competitive inhibitor of the binding of the peptidoleukotrienes to lung cells (Augstein *et al.*, 1973), the addition of LTC_4 and LTD_4 did not result in a

TABLE 5. Effect of LTB_4, LTC_4 and LTD_4 on the DNA synthesis of human epidermal keratinocyte cultures in the presence of FPL 55712

Agent* (M)	DNA (μg/well)	[^{3}H]TdR incorporation (cpm/μg DNA)
None	9·4 (6·8–13·0)	1806 (1544–2300)
FPL 55712 10^{-6}	9·9 (7·5–13·4)	1679 (1334–2105)
LTB_4 10^{-8}	10·3 (8·0–13·4)	2995 (2412–3624)†
LTC_4 10^{-8}	10·5 (7·3–13·2)	2700 (2094–3017)‡
LTD_4 10^{-8}	10·1 (7·0–13·3)	2785 (2101–3272)‡
FPL 55712 10^{-6} + LTB_4 10^{-8}	9·4 (6·9–12·4)	3067 (2527–3725)†
FPL 55712 10^{-6} + LTC_4 10^{-8}	9·2 (6·8–12·8)	1733 (1443–2076)
FPL 55712 10^{-6} + LTD_4 10^{-8}	9·6 (6·9–12·1)	1851 (1461–2228)

Values are medians and 95% confidence limits for five experiments.

* FPL 55712 was added 10 min before the LTs. Total incubation was 72 h at 37°C.

† LTB_4 and FPL 55712 + LTB_4 *vs* none: $P < 0{\cdot}01$.

‡ LTC_4 and LTD_4 *vs* none: $P < 0{\cdot}02$.

stimulation of epidermal keratinocyte DNA synthesis (Table 5). In contrast, the effect of LTB_4 was not antagonized by FPL 55712.

DISCUSSION

The pharmacological actions of leukotrienes suggest that they may have significant pathological role. Previously, most research has been fosussed on their actions as mediators of inflammation. In the present study we have demonstrated that both LTB_4, LTC_4 and LTD_4 are potent and stereospecific stimulators of human epidermal keratinocyte DNA synthesis *in vitro*. In comparison with 8-bromo cyclic AMP, LTB_4 and the peptido-leukotrienes are, respectively, 1000- and 100-fold more potent on a molar basis in stimulating DNA synthesis in human epidermal keratinocyte cultures, although the maximal response to leukotrienes is approximately half of the response to 8-bromo cyclic AMP (Marcelo & Tomich, 1983). The increased DNA synthesis induced by leukotrienes was not accompanied by an increased DNA content of the cell cultures. Unchanged DNA content has also been found in human keratinocyte cultures stimulated with the mitogen 8-bromo cyclic AMP. This apparent discrepancy between DNA content and DNA synthesis may be explained by the fact that the usual indicators of culture growth, i.e increases in cell number, DNA or protein, are not readily demonstrated in continually shedding cultures such as confluent, stratifying epidermal keratinocyte cultures.

In our study LTB_4 was equipotent in stimulating epidermal keratinocyte proliferation and neutrophil chemokinesis. These results could seem to indicate that LTB_4 stimulates cell proliferation and cell migraton via the same structural determinant. There is, however, no solid foundation for this belief. Thus, LTB_4 was without effect on fibroblast proliferation in our study, although it has been shown to induce fibroblast chemotaxis (Mensing & Czarnetzki, 1984). The lack of a mitogenic effect on keratinocytes by FMLP, another potent chemoattractant agent, is also against a general relationship between cell proliferation and cell movement.

The actions of LTB_4 generally differ from those of the peptidoleukotrienes with respect to both the cell type and the cell functions affected (Samuelsson, 1983). Our demonstration of a

common action of the two groups of leukotrienes is, however, not unique for human epidermal keratinocyte cultures. Both LTB_4, LTC_4 and LTD_4 have the capacity to mediate helper cell requirements for γ-interferon production (Johnson & Torres, 1984), and LTB_4 and LTC_4, but not LTD_4, enhance the activity of complement receptors on neutrophils (Nagy *et al.*, 1982). The ability of leukotrienes with different chemical structures to affect the same cell function might lead to the assumption that their effect is non-specific and related to their general properties as unsaturated fatty acids. In support of this concept is the ability of unsaturated fatty acids, but not of saturated fatty acids, to stimulate the growth of rat mammary epithelial cells (Wicha, Liotta & Kidwell, 1979). However, the failure of isomers of LTB_4 and LTD_4 to stimulate the DNA synthesis of epidermal keratinocytes does clearly demonstrate that the mitogenic effect of native leukotrienes on keratinocytes are stereospecific like most of the other biological effects of leukotrienes (Malmsten *et al.*, 1980; Lewis *et al.*, 1981).

The structural requirements for the effect of leukotrienes on epidermal keratinocytes suggest that leukotriene-induced keratinocyte mitogenesis is likely to be mediated via receptors. This idea gets support from the inhibition of the effect of LTB_4 and peptido-leukotrienes by 5S,12S-all-*trans*-LTB_4 and FPL 55712, respectively. 5S,12S-all-*trans* is a competitive inhibitor of the binding of LTB_4 to human neutrophils (Goldman & Goetzl, 1982), whereas FPL 55712 competitively inhibits the binding of peptido-leukotrienes to tracheal epithelium (Augstein *et al.*, 1973). The selective inhibition of peptido-leukotrienes by FPL 55712 may indicate that LTB_4 binds to different receptor from LTC_4 and LTD_4. From our data it is not possible directly to compare the potency of LTC_4 and LTD_4. LTC_4 is the direct precursor of LTD_4, which can be further metabolized to LTE_4. The formation of LTD_4 from LTC_4 is catalysed by γ-glutamyl transpeptidase (Örning & Hammarström, 1980). Since this enzyme is universally present in tissues, this conversion is most likely to occur in keratinocyte cultures, but to what extent is unknown. Experiments including specific enzyme inhibitors would help to define the relative potency of LTC_4 and LTD_4.

The mitogenic effect of leukotrienes on human epidermal keratinocytes *in vitro* may be pertinent to the cutaneous lesions of psoriasis. One of the fundamental questions regarding the pathogenesis of psoriasis is how and whether the inflammatory events are linked to the abnormalities of epidermal growth. The property of leukotrienes to affect both epidermal keratinocyte proliferation and inflammation (accumulation and stimulation of neutrophils and vasodilation) could provide a molecular link between the abnormal epidermal growth and inflammation in psoriasis. It has, however, to be emphasized that the proliferation of epidermis *in vivo* may be regulated by many other factors. This is clearly demonstrated by failure of topically applied LTB_4 to produce histological evidence of abnormal epidermal growth (Camp *et al.*, 1984). Other unsolved questions are whether epidermal cells or leukocytes are the cellular sources of leukotrienes in psoriatic skin. While neutrophils are known to be rich sources of leukotrienes, preliminary data indicates that the epidermis itself can produce small amounts of leukotriene B_4-like material and other 5-lipoxygenase products (Brain *et al.*, 1982; Ziboh *et al.*, 1983). Whether epidermal keratinocytes from normals or psoriatics are able to produce leukotrienes in amounts required to attract neutrophils and to affect proliferation and possibly differentiation are unanswered. Studies to answer these questions are under way.

ACKNOWLEDGMENT

Supported in part by the Danish Medical Research Council Grant No. 12-3095. Part of this work was presented at the Society for Investigative Dermatology meeting in Washington D.C., May 7–9, 1984.

REFERENCES

ALLEN, B.R. & LITTLEWOOD, S.M. (1982) Benoxaprofen: effect on cutaneous lesions of psoriasis. *British Medical Journal*, **285**, 1241.

AUGSTEIN, J., FARMER, J.B., SHEARD, P. & TATTERSALL, M.L. (1973) Selective inhibitor of slow reacting substance of anaphylaxis. *Nature New Biology*, **254**, 215–217.

BISGAARD, H., KRISTENSEN, J. & SÖNDERGAARD, J. (1982) The effect of leukotriene C_4 and D_4 on cutaneous blood flow in humans. *Prostaglandins*, **23**, 797.

BORREGAARD, N. & KRAGBALLE, K. (1980) The role of oxygen in antibody-dependent cytotoxicity mediated by monocytes and neutrophils. *Journal of Clinical Investigation*, **66**, 676.

BRAIN S.D., CAMP, R.D.R., LEIGH, I.M. & FORD-HUTCHINSON, A.W. (1982) The synthesis of leukotriene B_4-like material by cultured human keratinocytes. *Journal of Investigative Dermatology*, **78**, 328 (abstract).

BRAIN, S.D., CAMP, R.D.R., CHARLESON, S., DOWD, P.M., FORD-HUTCHINSON, A.W., GREAVES, M.W. & BLACK, A.K. (1984a) The release of LTC_4-like material from the involved lesional skin in psoriasis. *British Journal of Clinical Pharmacology*, **17**, 650P.

BRAIN, S.D., CAMP, R.D.R., CUNNINGHAM, F.M., DOWD, P.M., GREAVES, M.W. & BLACK, A.K. (1984b) Leukotriene B_4-like material in scale of psoriatic skin lesion. *British Journal of Pharmacology*, **83**, 313.

CAMP, R., RUSSELL JONES, R., BRAIN, S., WOOLARD, P. & GREAVES, M. (1984) Production of intraepidermal microabscesses by topical application of leukotriene B_4. *Journal of Investigative Dermatology*, **82**, 202.

DAHLÉN, S-E., HEDQVIST, P., HAMMARSTRÖM, S. & SAMMUELSSON, B. (1980) Leukotrienes are potent constrictors of human bronchi. *Nature*, **228**, 484.

FEINMARK, S.J., LINDGREN, J.A., CLAESSON, H., MALMSTEN, C. & SAMUELSSON, B. (1981) Stimulation of human leukocyte degranulation by leukotriene B_4 and its ω-oxidized metabolites. *FEBS Letters*, **136**, 141.

FORD-HUTCHINSON, A.W., BRAY, M.A., BOIG, M.V., SHIPLEY, M.E. & SMITH, M.J.H. (1980) Leukotriene B, a potent chemokinetic and aggregating substance from polymorphonuclear leukocytes. *Nature*, **286**, 264.

GOLDMAN, D.W. & GOETZL, E.J. (1982) Specific binding of leukotriene B_4 to receptors on human polymorphonuclear leukocytes. *Journal of Immunology*, **129**, 1600.

HAMMARSTRÖM, S., HAMBERG, M., SAMUELSSON, B., DUELL, E.A., STAWISKI, M. & VOORHEES, J.J. (1975) Increased concentrations of nonesterified arachidonic acid, 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid, prostaglandin E_2, prostaglandin $F_{2\alpha}$ in epidermis of psoriasis. *Proceedings of the National Academy of Sciences, U.S.A.*, **72**, 5130.

HEDQVIST, P., DAHLÉN, S-E., GUSTAVSSON, L., HAMMARSTRÖM, S. & SAMUELSSON, B. (1980) Biological profile of leukotrienes C_4 and D_4. *Acta Physiologica Scandinavica*, **110**, 331.

JOHNSON, H.M. & TORRES, B.A. (1984) Leukotrienes: positive signals for regulation of γ-interferon production. *Journal of Immunology*, **132**, 413.

KRAGBALLE, K. & HERLIN, T. (1983) Benoxaprofen improves psoriasis. A double-blind study. *Archives of Dermatology*, **119**, 548.

LEWIS, R.A., FRAZEN, J.M., AUSTEN, K.E., TODA, M., BRION, F., MARGAT, A. & COREY, E.J. (1981) Contractile properties of leukotrienes C and D: role of the polar substituents. *Proceedings of the National Academy of Sciences, U.S.A.* **78**, 4579.

LIU, S.C. & KARASEK, M. (1978) Isolation and growth of adult human epidermal keratinocytes. *Journal of Investigative Dermatology*, **71**, 157.

MALMSTEN, C.L., PALMBLAD, J., UDÉN, A.-M., RÅDMARK, O., ENGSTEDT, L. & SAMUELSSON, B. (1980) Leukotriene B_4: A highly potent and stereospecific factor stimulating migration of polymorphonuclear leukocytes. *Acta Physiologica Scandinavica*, **110**, 449.

MARCELO, C.L., KIM, Y.G., KAINE, J.L. & VOORHEES, J.J. (1978) Stratification, specialization, and proliferation of primary keratinocyte cultures. *Journal of Cell Biology*, **79**, 356.

MARCELO, C.L. & TOMICH, J. (1983) Cyclic AMP, glucocorticoid, and retinoid modulation of *in vitro* keratinocyte growth. *Journal of Investigative Dermatology*, **81**, 64s.

MENSING, H. & CZARNETZKI, B.M. (1984) Leukotriene B_4 induces *in vitro* fibroblast chemotaxis. *Journal of Investigative Dermatology*, **82**, 9.

NAGY, L., LEE, T.H., GOETZL, E.J., PICKETT, W.C. & KAY, B. (1982) Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clinical and Experimental Immunology*, **47**, 541.

ÖRNING, L. & HAMMARSTRÖM, S. (1980) Inhibiton of leukotriene C and D biosynthesis. *Journal of Biological Chemistry*, **255**, 8023.

SAMUELSSON, B. (1983) Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science*, **220,** 568.

SERHAN, C.M., RADIN, A., SMOLEN, J.E., KORCHAK, H., SAMUELSSON, B. & WEISSMAN, G. (1982) Leukotriene B_4 is a complete secretagogue in human neutrophils: A kinetic analysis. *Biochemical and Biophysical Research Communications*, **107,** 1006–1012.

SMITH, M.J.H. & WALKER, J.R. (1980) The effect of some antirheumatic drugs on an *in vitro* model of human polymorphonuclear leucocyte chemokinesis. *British Journal of Pharmacology*, **69,** 473.

SOTER, N.A., LEWIS, R.A., COREY, E.J. & AUSTEN, K.E. (1983) Local effects of synthetic leukotrienes (LTC_4, LTD_4 and LTB_4) in human skin. *Journal of Investigative Dermatology*, **80,** 115.

WICHA, M.S., LIOTTA, L.A. & KIDWELL, W.R. (1979) Effects of free fatty acids on the growth of normal and neoplastic rat mammary epithelial cells. *Cancer Research*, **39,** 426.

ZIBOH, V.A., CASEBOLT, T., MARCELO, C.L. & VOORHEES, J.J. (1983) Enhancement of 5-lipoxygenase activity on soluble preparations of human psoriatic plaque preparation. *Journal of Investigative Dermatology*, **80,** 359.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.