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SHORT REPORTS

Human Keratinocytes in Vitro have Receptors for Leukotriene B_4

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We have demonstrated a high-affinity binding site for leukotriene B_4 (LTB_4) on human epidermal keratinocytes in vitro. In substrate saturation studies, one population of binding sites with a dissociation constant (K_d) of 1.03 ± 0.3 nM and a maximal binding capacity (B_{max}) of 148.2 ± 45.3 fmol/mg protein could be demonstrated. On average 5500 binding sites were found on individual keratinocytes in culture. The affinity constant of this binding site correlates well with previous reports on the proliferative effect of LTB_4 on keratinocytes in vitro. These findings confirm that LTB_4 may in part be responsible for epidermal hyperproliferation in inflammatory skin dieseases. ***Key words: LTB_4; Inflammation.***

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Metabolites of arachidonic acid have attracted interest as biologically significant mediators in a variety of inflammatory and hypersensitivity reactions. 5(S)-, 12(R)-dihydroxyeicosa-6-14-cis-8,10-trans-tetranoic acid, or leukotriene B_4 (LTB_4), is a product of the 5-lipoxygenase pathway and a potent mediator for human polymorphonuclear leukocyte (PMN) functions. Nanomolar concentrations of LTB_4 elicit chemotactic and chemokinetic migration, superoxide production, and the release of lysosomal enzymes (for review see ref 1).

Recently, LTB_4 in vitro, in concentrations ranging from 10^{-12} to 10^{-8} M, was found to stimulate the proliferation of human epidermal keratinocytes by 100% as judged by the incorporation of ^{3}H-thymidine and the labeling index (2). Injection of LTB_4 into human skin results in adherence of neutrophils to endothelial cells, diapedesis of inflammatory cells, and, with repeated applications, neutrophilic vasculitis and epidermal hyperproliferation (3). An important role for LTB_4 in the pathogenesis of inflammatory skin diseases, particularly psoriasis, has recently been suggested (4).

For human polymorphonuclear leukocytes (PMN) both high-affinity and low-affinity receptors for LTB_4 have been found with dissociation constants (K_ds) of 0.4 nM and 61 nM, respectively (5). The high-affinity receptor apparently mediates chemotaxis and increased adherence of PMN, whereas the low-affinity receptor mediates the release of lysosomal enzymes and superoxide production (1). For human keratinocytes, an LTB_4-receptor has not been yet demonstrated. In this study, we present evidence for a high-affinity binding site on human keratinocytes, in vitro, which may be involved in the mitogenic effect of LTB_4 on epidermal cells.

MATERIAL AND METHODS

Human epidermal keratinocyte cultures

Epidermal keratinocyte cultures were established from facelift skin using previously described procedures (6). The keratotomed skin was incubated for 40 min at 37°C in 0.3% trypsin solution in GKN (Mg^{2+}-, Ca^{2+}-free solution, containing 1.0 g/l glucose, 0.4 g/l KCl, 8.0 g/l NaCl) to separate epidermis and dermis. Keratinocytes were grown on collagen-coated dishes in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal calf serum at 37°C in 100% humidity in a 95% air/5% CO_2 environment. After reaching confluency within 2–3 weeks, cells were scraped off the dishes using a rubber policeman and collected in phosphate buffered saline (PBS).

Cell membrane preparation

Keratinocytes at a density of 3.5×10^6/ml were homogenized using a Polytron © (Brinkman, Westbury, NY) (setting no. 4 for 30 sec), to a 2% homogenate (w/v) in 0.05 M phosphate buffer (81 mM Na^+, 9 mM K^+, 0.9 mM Ca^{2+}, 0.5 mM Mg^{2+}, pH 7.43 at 4°C). The protein content was established by the method of Lowry et al. (7). Each homogenate was diluted with 20 ml of phosphate buffer and centrifuged at $10000 \times g$ for 15 min in a Beckman J2–21 centrifuge (Beckman Instruments, Inc., Fullerton, CA). Pellets were then suspended in the appropriate volumes of buffer for the binding assays.

Receptor binding studies

Aliquots of the tissue-suspension were added to two sets of triplicate, 2 ml glass culture tubes; one set contained 0.025

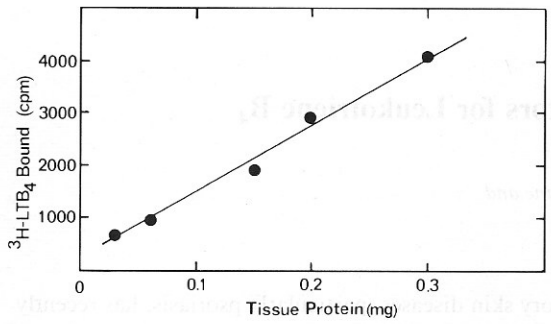

Fig. 1. Specific ^{3}H-LTB$_4$ binding to keratinocyte cell membranes as a function of tissue protein concentration. This study was done at 4°C and 1 nM ^{3}H-LTB$_4$.

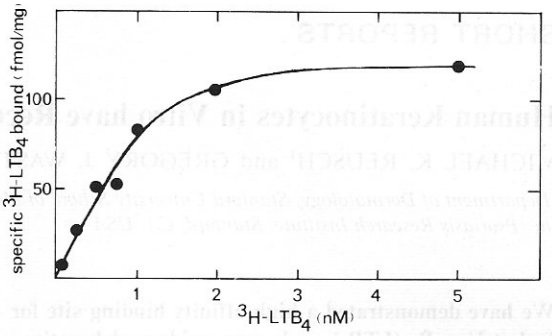

Fig. 2. Specific binding of ^{3}H-LTB$_4$ to cell membrane preparations of human epidermal keratinocytes as a function of the ^{3}H-LTB$_4$ concentration.

nM to 5 nM ^{3}H-LTB$_4$ (specific activity 177 Ci/mmol, Amersham Corporation, Arlington Hts., IL) in 1 ml phosphate buffer. The other set (nonspecific binding) contained an additional 1 μM unlabeled LTB$_4$ (Sigma, St. Louis, MO). After initial experiments to determine the time-dependence of binding at 4°C, tubes were routinely incubated at 4°C for 20 min to ensure steady-state conditions. The suspensions were then diluted with an excess 4 ml cold buffer and filtered through glass-fibre filters (Whatman GF/B, 2.5 cm). The tubes were rinsed again with ice-cold buffer and poured over filters in a 30-well filter box (Steed Engineering Co., Palo Alto, CA). The filters were washed an additional two times, air-dried and placed into liquid scintillation vials containing 5 ml Triton-toluene Omnifluor (1 liter: 2 liters: 16 g) liquid scintillation cocktail. Following extraction, the bound radioactivity was counted in a Beckman LS 7000 liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, CA) with a counting efficiency of 55.4%.

Binding to intact cells

Binding to intact cells was measured using essentially the same protocol. The reaction mixture was put into 1.5 ml clonical polypropylene tubes (Bio-Rad Laboratories, Richmond, CA). Following incubation tubes were centrifuged for 30 s at 8000×g in a Beckman Microfuge B (Beckman Instruments, Inc., Fullerton, CA). After repeated, superficial washing with ice-cold phosphate buffer, the tips of each tube containing the pellet were cut off with a laboratory guillotine and extracted for 12 h in Omnifluor. ^{3}H-LTB$_4$ binding was quantified in a liquid scintillation counter.

RESULTS

^{3}H-LTB$_4$ binding was linear between 0.025 and 0.3 mg of tissue protein per assay at ^{3}LTB$_4$ concentrations between 0.025 and 5 nM (Fig. 1). ^{3}H-LTB$_4$ binding is a time-dependent process. At 4°C and a tissue protein concentration of 0.03 mg/assay equilibration was found in 10 min. In all subsequent assays assays were performed at 4°C for 20 min at a tissue concentration of 0.03 mg/assay.

In saturation studies specific, saturable binding of ^{3}H-LTB$_4$ to membrane preparations of human epidermal keratinocytes grown in cell culture was found. Substrate saturation of LTB$_4$ receptors was measured between 0.05 and 5 nM (Fig. 2). Scatchard analysis of these saturation isotherms gave a K_d of 1.03±0.3 nM and a B_{max} of 148.2±45.5 fmol/mg (Fig. 3). The maximal binding amounts to 5500 receptors per keratinocyte in culture.

^{3}H-LTB$_4$ binding to intact cells had a K_d of 0.9±0.4 nM and a B_{max} of 125±35 fmol/mg protein.

DISCUSSION

These binding data indicate the presence of a population of high affinity binding sites for LTB$_4$ on cell membrane preparations of human keratinocytes grown in culture. The characteristics of this binding site bear similarities to those found for the high affinity receptor for LTB$_4$ on human PMN using a different methodology (K_d 1.03 nM vs. 0.4 nM, number of

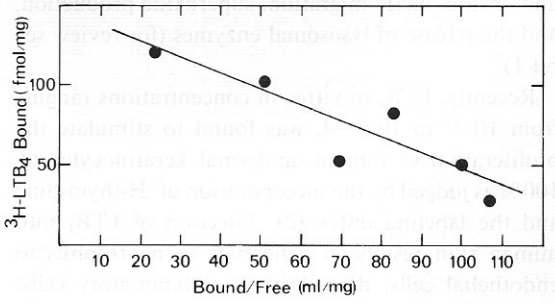

Fig. 3. Scatchard analysis of the saturation isotherm shown in Fig. 2. K_d=1.03±0.3 nM, B_{max}=148.2±45.3 fmol/mg protein, r value=0.90.

receptors per cell 5 500 vs. 4 400) (5). The PMN receptor was also found to be located to the plasma cell membrane (5).

The characteristics of this binding site correlate well with previous studies on the mitogenic effect of LTB_4 on human keratinocytes in vitro (2, 8). LTB_4 stimulated keratinocyte proliferation in culture at concentrations between 10^{-1} and 10^{-8} M, and half-saturable binding occurs at 1.03 nM of the leukotriene. The presence of one population of specific binding sites for LTB_4 on keratinocytes in culture, thus, supports the finding of a direct mitogenic effect of LTB_4 on human keratinocytes in vitro. This may suggest that this arachidonic acid metabolite may directly influence epidermal proliferation in a number of cutaneous inflammatory diseases.

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Skin Extensibility Time in Women. Changes in Relation to Sex Hormones

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The influence of female sex hormones on mechanical properties of the skin has been assessed in an in vivo extensometric study. Twenty young (20 ± 4 years) and 12 middle-aged healthy women (42 ± 3 years) entered the study. Measurements were carried out on the volar surface of the left forearm on the 10th and 25th day of the menstrual cycle. A significantly decreased skin extensibility time in the pre-menstrual phase was found (25th day) when compared with the 10th day in the young group, while the older one did not reveal significant changes. The data are compatible with an increased water content of the skin noticeable in the pre-menstrual phase and more relevant in young women. In studies on mechanical properties of the skin, changes relative to sex hormones and menstrual cycle need to be taken into account. *Key words: Estradiol; Menstrual cycle.*

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Mechanical and viscoelastic properties of the skin are related to many variables, such as skin thickness (1), age (2, 3, 4), sex (1) and environmental conditions (5). Age-related changes are widely studied (2, 3, 4). Most authors agree that the skin becomes less extensible with age. Leveque et al. (4) and Agache et al. (6), using the same torque method, reported decreased extensibility with age, namely after 30 years, associated with an increase of the elastic modulus. Similar

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