

# Leukotriene B<sub>4</sub> Receptors on Neutrophils in Patients with Psoriasis and Atopic Eczema<sup>1</sup>

Friedegund Meier<sup>a</sup>, Eva Gross<sup>a</sup>, Karl-Norbert Klotz<sup>b</sup>, Thomas Ruzicka<sup>a</sup>

<sup>a</sup>Department of Dermatology, University of Munich, FRG;

<sup>b</sup>Department of Pharmacology, University of Heidelberg, FRG

**Key Words.** LTB<sub>4</sub> receptors · Neutrophils · Psoriasis · Atopic eczema

**Abstract.** Polymorphonuclear leukocyte (PMNL) infiltration is an important characteristic in psoriatic lesions. Elevated concentrations of the chemoattractant eicosanoid leukotriene B<sub>4</sub> (LTB<sub>4</sub>) are present in psoriatic skin. Its chemotactic activity is mediated via high affinity receptors on PMNL. The goal of our work was to ascertain whether PMNL infiltration in psoriasis can be accounted for by functional abnormalities of the circulating PMNL due to alterations in the LTB<sub>4</sub> receptor density or affinity (or both). No significant difference was found between patients with psoriasis, healthy controls and patients with another inflammatory dermatosis (atopic eczema) with regard to the binding parameters of LTB<sub>4</sub> receptors on PMNL. Our findings suggest that PMNL accumulation in psoriatic skin may be the result of an excess of cutaneous chemoattractant rather than the increased readiness of psoriatic PMNL to migrate towards LTB<sub>4</sub> due to altered LTB<sub>4</sub> receptor density or affinity.

## Introduction

In recent years, the knowledge about the arachidonic acid metabolism in skin has considerably increased. A very complex picture about the physiological and pathophysiological role of arachidonic acid metabolites in skin has emerged. The 5- and 12-lipoxygenase products of arachidonic acid (LTB<sub>4</sub> and 12-HETE) appear to play an important role in the pathophysiology of common inflammatory and hyperproliferative skin diseases such as psoriasis and atopic eczema as

can be deduced from the following observations:

(1) In involved psoriatic skin the levels of LTB<sub>4</sub> and 12-HETE are increased [1-3]. (2) After topical application, LTB<sub>4</sub> and 12-HETE have been shown to induce leukocyte chemotaxis, inflammatory infiltration of the skin, intraepidermal neutrophil infiltrate and epidermal hyperplasia [4-7]. (3) Gluco-

<sup>1</sup> Supported by the Free State of Bavaria (FM) and the Deutsche Forschungsgemeinschaft (TR, Ru 292/3-2).

corticosteroids, the most potent anti-inflammatory drugs in dermatology, inhibit the release of arachidonic acid from the cellular phospholipids [8] and consequently the formation of LTB<sub>4</sub> and 12-HETE. (4) Indometacin, a known cyclooxygenase inhibitor, redirects arachidonic acid into lipoxygenase products and exacerbates psoriasis [9, 10]. (5) The topical application of RS-43179 (lonapalene), an inhibitor of 5-lipoxygenase, has been shown to clear psoriatic lesions [11].

LTB<sub>4</sub> is the most potent polymorphonuclear leukocyte (PMNL) chemotactic factor generated by the lipoxygenation of arachidonic acid [12]. Chemotactic activity of PMNL is mediated by specific LTB<sub>4</sub> receptors [13]. Cutaneous infiltration of PMNL is assumed to be an important early event in the pathogenesis of psoriasis [14].

The goal of the present investigation was therefore to ascertain whether the intraepidermal accumulation of PMNL in psoriasis can be accounted for by functional abnormalities of the circulating PMNL due to alterations in the LTB<sub>4</sub> receptor density or affinity (or both). For this purpose, we measured the binding of LTB<sub>4</sub> to PMNL in patients with psoriasis, and compared these findings with those in another chronic inflammatory dermatosis (atopic eczema) and healthy controls.

## Materials and Methods

### *Patients*

Eleven patients with psoriasis (10 males, 1 female; 32–82 years of age), 10 patients with atopic eczema (6 males, 4 females; 12–67 years of age), and 11 healthy volunteers (4 males, 7 females; 21–49 years of age) participated in the study.

The psoriasis group consisted of 6 patients with the plaque type, 4 patients with the guttate type, and 1 patient with erythrodermic psoriasis.

The extent of cutaneous involvement was 10–25% of the body surface in 3 psoriasis and 5 atopic eczema patients, and greater than 25% in 8 patients with psoriasis and 5 patients with atopic eczema, respectively.

Excluded from the study were patients who had received systemic treatment or phototherapy for their skin disease within the previous 4 weeks.

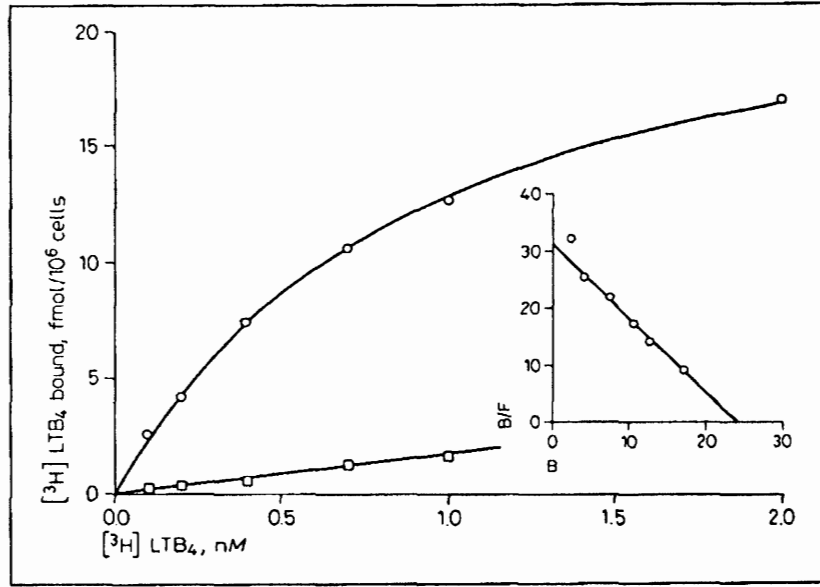
### *Preparation of PMNL*

PMNL were isolated as previously described [15]. Briefly, 40 ml of heparinized blood was centrifuged at 150 g for 15 min at room temperature. The platelet-rich plasma was discarded, the cell pellet was suspended in saline and layered over a discontinuous Percoll gradient (Pharmacia, Freiburg, FRG). After centrifugation at 1,450 g for 20 min at 14 °C the PMNL layer was taken off and washed with saline. Erythrocytes were removed by hypotonic lysis with distilled water, platelets by repeated centrifugation in bovine serum albumin solution (Sigma, Deisenhofen, FRG). The final PMNL pellet was resuspended in Hanks' balanced salt solution with phenol red (HBSS; Flow, Meckenheim, FRG) containing 10 mM Hepes buffer (pH 7.3; Flow). The resulting cell suspension contained more than 90% neutrophils with a viability of greater than 95% as assessed by trypan blue exclusion.

### *Measurement of the Binding of [<sup>3</sup>H]LTB<sub>4</sub> to PMNL*

[5,6,8,9,11,12,14,15-<sup>3</sup>H(N)]-leukotriene B<sub>4</sub> (specific activity 200 Ci/mmol) was purchased from New England Nuclear (Dreieich, FRG). PMNL were incubated with increasing concentrations of [<sup>3</sup>H]LTB<sub>4</sub> to determine the dissociation constant K<sub>d</sub> and the receptor density of the cells. Final [<sup>3</sup>H]LTB<sub>4</sub> concentrations were in the range of 0.1–2 nM. Increasing concentrations of [<sup>3</sup>H]LTB<sub>4</sub> were pipetted into Eppendorf tubes and evaporated under a stream of nitrogen. Then aliquots of PMNL suspension (10<sup>6</sup> cells/200 μl buffer) were added to start the incubation which was performed for 40 min at 4 °C. At this temperature, negligible amounts of [<sup>3</sup>H]LTB<sub>4</sub> were metabolized to oxygenated products as assessed by reversed-phase HPLC (data not shown). Nonspecific binding was determined in the presence of 2 μM unlabelled LTB<sub>4</sub> (Paesel, Frankfurt, FRG) which was pipetted and evaporated together with [<sup>3</sup>H]LTB<sub>4</sub>. Specific binding was defined as total binding (measured in the absence of unlabelled LTB<sub>4</sub>) minus nonspecific binding.

**Fig. 1.** Specific (○) and nonspecific (□) binding of [<sup>3</sup>H]LTB<sub>4</sub> to PMNL as a function of increasing concentrations of [<sup>3</sup>H]LTB<sub>4</sub>. Each point represents the mean of duplicate measurements. The inset shows the Scatchard plot derived from the specific binding data. Curve fitting according to a one-site model revealed a K<sub>d</sub> of 0.76 nM and a receptor density of 14,316 sites/cell.



After incubation, cells were harvested on glass fiber filtermats (Skatron, Düsseldorf, FRG) using a Skatron cell harvester, and washed for 7 s with cold buffer. The filters were then placed into plastic vials containing 2 ml scintillation fluid (Scintillator 299™, Packard, Frankfurt, FRG), and the radioactivity retained in the filters was determined in a TRI-CARB liquid scintillation counter (Packard, Frankfurt, FRG).

*Analysis of Binding Data*

All binding experiments were done in duplicate. Computer analysis of binding data was performed by nonlinear curve-fitting with a modification of the program SCTFIT [16].

Assessment of statistical differences between the proband groups was achieved using the nonparametric Mann-Whitney test.

**Results**

Figure 1 illustrates the specific binding of [<sup>3</sup>H]LTB<sub>4</sub> to PMNL as a function of increasing concentrations of [<sup>3</sup>H]LTB<sub>4</sub>. Curve fitting assuming the presence of a single class of

binding sites gave a K<sub>d</sub> of 0.76 nM and a receptor density of 14,316 sites/cell in the shown example. Curve fitting according to a two-site model did not improve the fit. Consequently, a linear Scatchard plot could be derived from the data, suggesting that [<sup>3</sup>H]LTB<sub>4</sub> interacts with a single class of binding sites under the experimental conditions used.

Table 1 summarizes the binding parameters (i.e. receptor affinity expressed as K<sub>d</sub> in nM, and receptor density expressed as number of receptors per cell) for LTB<sub>4</sub> receptors on PMNL in patients with psoriasis and atopic eczema compared to healthy controls. PMNL from healthy controls expressed LTB<sub>4</sub> receptors with a K<sub>d</sub> of 0.97 ± 0.29 nM and a density of 13,400 ± 3,770 receptors/cell. Virtually identical results were obtained with PMNL from patients with psoriasis and atopic eczema. Their LTB<sub>4</sub> receptors revealed a K<sub>d</sub> of 0.85 ± 0.23 nM and a density of 13,800 ± 4,360 receptors/cell in psoriasis and a K<sub>d</sub> of 0.82 ± 0.27 nM, and a density of

**Table 1.** Binding parameters (means of duplicate measurements) of LTB<sub>4</sub> receptors on PMNL in patients with psoriasis, atopic eczema and healthy controls

Diagnosis and patient No.	Receptor affinity K <sub>d</sub> , nM	Receptor density sites/cell
<i>Psoriasis</i>		
Plaque type		
1	0.70	19,876
2	0.96	11,471
3	0.70	15,991
4	0.75	14,789
5	0.56	8,336
6	0.76	14,316
Guttate type		
7	1.22	10,654
8	1.06	15,886
9	1.23	17,808
10	0.71	16,819
Erythrodermic		
11	0.64	5,306
<i>Atopic eczema</i>		
12	1.08	19,671
13	0.80	16,600
14	0.84	16,321
15	0.72	12,459
16	0.67	15,446
17	1.11	19,163
18	0.46	12,785
19	1.27	7,928
20	0.79	9,672
21	0.42	8,114
<i>Psoriasis</i>		
(n = 11) $\bar{x} \pm SD$	0.85 ± 0.23	13,800 ± 4,360
<i>Atopic eczema</i>		
(n = 10) $\bar{x} \pm SD$	0.82 ± 0.27	13,800 ± 4,310
<i>Controls</i>		
(n = 11) $\bar{x} \pm SD$	0.97 ± 0.29	13,400 ± 3,770

13,800 ± 4,310 receptors/cell in atopic eczema. The difference between healthy controls and patients with psoriasis or atopic eczema was statistically not significant. Within the psoriasis group, binding parameters did not differ significantly between patients with plaque and guttate types of psoriasis. The only patient with erythrodermic psoriasis, however, exhibited a K<sub>d</sub> of 0.67 nM and a remarkably low density of 5,306 receptors/cell, the lowest number measured in any of our probands. No correlation was observed between LTB<sub>4</sub> receptor affinity or density and the severity or extent of psoriasis and atopic eczema (data not shown).

## Discussion

Psoriatic lesions are characterized, among others, by a pronounced inflammatory infiltrate which may be, at least partly, caused by the chemotactic activity of lipoxigenase-derived eicosanoids since elevated levels of LTB<sub>4</sub> and 12-HETE were identified in psoriatic skin [1-3]. The goal of the present investigation was to ascertain whether the inflammatory changes in psoriasis may also be accounted for by functional abnormalities of the circulating PMNL due to alterations in the LTB<sub>4</sub> receptor density or affinity (or both). For this purpose, we performed radioligand binding studies in PMNL of patients with different types of psoriasis. For comparison, we studied a group of patients with another inflammatory dermatosis (atopic eczema) and healthy controls.

Using classical radioligand binding assays for the detection of cell surface receptors, we were able to demonstrate the presence of high-affinity LTB<sub>4</sub> receptors on PMNL.

Their mean  $K_d$  was 0.97 nM, and there were 13,400 binding sites/cell. This is within the range reported in the literature [17, 18]. Unlike previous workers, we were unable to measure low-affinity binding sites. This may be due to our method of separation of receptor-bound from free radioligand since the separation time of 7 s by vacuum filtration may be sufficient to dissociate ligands from low-affinity receptors. The allowable separation time at a  $K_d$  of  $10^{-7}$  nM, for example, is only 0.1 s [19]. On the other hand, there are widely varying results in the literature concerning binding characteristics of LTB<sub>4</sub> to PMNL [17–21], and some other groups were also unable to confirm the presence of low affinity binding sites [20, 21]. Furthermore, PMNL chemotaxis is mediated by the high-affinity subset of receptors [13] which seems thus of greater biologic relevance with regard to psoriasis than the low-affinity component.

No significant difference was found between healthy controls, patients with psoriasis and atopic eczema with regard to their LTB<sub>4</sub> binding parameters. The mean receptor affinities and densities were virtually identical in the three groups of probands. Closer analysis of binding data within the groups showed no correlation between the LTB<sub>4</sub> receptor affinity or density and type, severity or extent of psoriasis and atopic eczema. However, this analysis depends on a small number of subjects, and requires confirmation in greater subgroups of patients. However, it was conspicuous that the only patient with erythrodermic psoriasis, whom we had the opportunity to examine, displayed by far the lowest number of receptors per cell (5,306 compared to 13,400 in healthy controls and 13,800 in psoriatic patients). The receptor affinity was within nor-

mal range in this person. This finding may be chance or reflect down regulation of LTB<sub>4</sub> receptors in the most severely affected patient. A final answer to this question requires analysis of a greater number of patients with psoriatic erythroderma which is in progress.

So far, no other studies of eicosanoid receptors in psoriasis and atopic eczema have been published. Our results, however, complement findings of normal chemotactic response of psoriatic PMNL to LTB<sub>4</sub> [22, 23], suggesting that the accumulation of PMNL in psoriatic skin may be the result of an excess of cutaneous chemoattractant, rather than reflecting the increased readiness of psoriatic PMNL to migrate towards a chemotactic stimulus. Indeed, elevated cutaneous levels of the chemoattractant eicosanoids LTB<sub>4</sub> and 12-HETE were identified in psoriasis and atopic eczema [1–3, 24]. In addition, enhanced 5-lipoxygenase activity was found in skin of psoriatic patients [25] although peripheral PMNL of these patients display normal 5-lipoxygenase activity [15]. Thus, it is likely that elevated cutaneous levels of chemoattractant eicosanoids may play a role in the initiation of the inflammatory infiltrate in both dermatoses, and that eicosanoids released from infiltrating inflammatory cells may contribute to the maintenance of the inflammatory infiltrate and the hyperproliferation observed in chronic inflammatory skin diseases [7]. On the other hand, the altered responsiveness of monocytes from patients with psoriasis and atopic eczema towards LTB<sub>4</sub> as demonstrated by Czarnecki [26] leaves open the question of possible abnormalities of LTB<sub>4</sub> receptors on cell types other than PMNL.

Our findings of normal LTB<sub>4</sub> receptor affinity and density and normal 5-lipoxygen-

ase activity [15] of PMNL do not support the hypothesis of an intrinsic defect in the eicosanoid metabolism of psoriatic PMNL. Still, however, agents interfering with LTB<sub>4</sub> receptors seem to be a promising therapeutic approach in psoriasis. 15-HETE, for example, was recently reported to inhibit LTB<sub>4</sub>-induced chemotaxis of PMNL, possibly by receptor antagonism [27]. This eicosanoid displays antipsoriatic properties when injected intralesionally [28]. Taking into consideration our recent identification of LTB<sub>4</sub> and 12-HETE receptors on keratinocytes [29, 30], the use of eicosanoid receptor antagonists in inflammatory and hyperproliferative skin diseases seems of even greater interest.

## References

- 1 Hammarström S, Hamberg M, Samuelsson B, et al: Increased concentrations of nonesterified arachidonic acid, 12-*L*-hydroxy-5,8,10,14-eicosatetraenoic acid, prostaglandin E<sub>2</sub> and prostaglandin F<sub>2</sub> in epidermis of psoriasis. *Proc Natl Acad Sci USA* 1975;72:5130-5134.
- 2 Brain SD, Camp RDR, Dowd PM, et al: Psoriasis and leukotriene B<sub>4</sub>. *Lancet* 1982;2:762-763.
- 3 Grabbe J, Czarnetzki BM, Mardin M: Chemotactic leukotrienes in psoriasis. *Lancet* 1982;2:1464.
- 4 Dowd PM, Black AK, Woollard PM, et al: Cutaneous responses to 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE). *J Invest Dermatol* 1985;84:537-541.
- 5 Camp R, Russel Jones R, Brain S, et al: Production of microabscesses by topical application of leukotriene B<sub>4</sub>. *J Invest Dermatol* 1984;82:202-204.
- 6 Chan CC, Duhamel L, Ford-Hutchinson AW: Leukotriene B<sub>4</sub> and 12-hydroxy-eicosatetraenoic acid stimulate epidermal proliferation in vivo in the guinea pig. *J Invest Dermatol* 1985;85:333-334.
- 7 Ruzicka T, Burg G: Effects of chronic intracutaneous administration of arachidonic acid and its metabolites. Induction of leukocytoclastic vasculitis by leukotriene B<sub>4</sub> and 12-hydroxyeicosatetraenoic acid and its prevention by prostaglandin E<sub>2</sub>. *J Invest Dermatol* 1987;88:120-123.
- 8 Hong SL, Levine L: Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. *Proc Natl Acad Sci USA* 1976;73:1730-1734.
- 9 Katayama H, Kawada A: Exacerbation of psoriasis induced by indomethacin. *J Dermatol* 1981;8:323-337.
- 10 Ellis CN, Fallon JD, Heezen JL, et al: Topical indomethacin exacerbates lesions of psoriasis. *J Invest Dermatol* 1983;80:362.
- 11 Jones GH, Venuti MC, Young JM, et al: Topical nonsteroidal antipsoriatic agents. 1. 1,2,3,4-Tetraoxygenated naphthalene derivatives. *J Med Chem* 1986;29:1504-1511.
- 12 Goetzl EJ, Pickett WC: The human PMN leukocyte chemotactic activity of complex hydroxyeicosatetraenoic acids (HETEs). *J Immunol* 1980;125:1789-1791.
- 13 Goldman DW, Goetzl EJ: Selective transduction of human polymorphonuclear leukocyte functions by subsets of receptors for leukotriene B<sub>4</sub>. *J Allergy Clin Immunol* 1984;74:373-377.
- 14 Chowaniec O, Jablonska S: Pre-pin-point papules changes preceding pin-point lesions of psoriasis. *Acta Derm Venereol* 1979;59(suppl 85):39-45.
- 15 Ruzicka T, Radspieler H, Strasser T, et al: Polymorphonuclear leukocyte 5-lipoxygenase activity in psoriasis. *Prostaglandins Leukot Med* 1985;18:313-319.
- 16 De Lean A, Hancock AA, Lefkowitz RJ: Validation and statistical analysis of a computer modeling method for quantitative analysis of radioligand binding data for mixtures of pharmacological receptor subtypes. *Mol Pharmacol* 1982;21:5-16.
- 17 Goldman DW, Goetzl EJ: Heterogeneity of human polymorphonuclear leukocyte receptors for leukotriene B<sub>4</sub>. *J Exp Med* 1984;159:1027-1041.
- 18 Lin AH, Ruppel PL, Gorman RR: Leukotriene B<sub>4</sub> binding to human neutrophils. *Prostaglandins* 1984;28:837-849.
- 19 Yamamura HI, Enna SJ, Kuhar MJ: Neurotransmitter receptor binding. New York, Raven Press, 1985.
- 20 Charleson S, Evans JF, Zamboni RJ, et al: Leukotriene B<sub>3</sub>, leukotriene B<sub>4</sub> and leukotriene B<sub>5</sub>: Binding to leukotriene B<sub>4</sub> receptors on rat and human

- leukocyte membranes. *Prostaglandins* 1986;32: 503-516.
- 21 Bomalaski JS, Mong S: Binding of leukotriene B<sub>4</sub> and its analogs to human polymorphonuclear leukocyte membrane receptors. *Prostaglandins* 1987; 33:855-867.
  - 22 Green CM, Ferguson J, MacLeod TM, et al: Polymorphonuclear leukocyte chemotaxis in response to leukotriene B<sub>4</sub> in treated and untreated psoriatics. *Dermatologica* 1989;178:20-22.
  - 23 Cunningham FM, Wong E, Woollard PM, et al: The chemotactic response of psoriatic and normal polymorphonuclear leukocytes to arachidonic acid lipoxygenase products. *Arch Dermatol Res* 1986;278:270-273.
  - 24 Kragballe K, Fogh K, Herlin T: Eicosanoids in skin of patients with atopic dermatitis: Prostaglandin E<sub>2</sub> and leukotriene B<sub>4</sub> are present in biologically active concentrations (abstract). *J Invest Dermatol* 1989;92:464.
  - 25 Ziboh VA, Casebolt T, Marcelo CL, et al: Enhancement of 5-lipoxygenase activity in soluble preparations of human psoriatic plaque preparation. *J Invest Dermatol* 1983;80:359.
  - 26 Czarnetzki B: Increased monocyte chemotaxis towards leukotriene B<sub>4</sub> and platelet activating factor in patients with inflammatory dermatoses. *Clin Exp Immunol* 1983;54:486-492.
  - 27 Ternowitz T, Fogh K, Kragballe K: 15-Hydroxyeicosatetraenoic acid (15-HETE) specifically inhibits LTB<sub>4</sub>-induced chemotaxis of human neutrophils. *Skin Pharmacol* 1988;1:93-99.
  - 28 Fogh K, Sogaard H, Herlin T, et al: Improvement of psoriasis vulgaris after intralesional injections of 15-hydroxyeicosatetraenoic acid (15-HETE). *J Am Acad Dermatol* 1988;18:279-285.
  - 29 Gross E, Ruzicka T, Mauch C, et al: Evidence for LTB<sub>4</sub>/12-HETE binding sites in a human epidermal cell line. *Prostaglandins* 1988;36:49-58.
  - 30 Gross EC, Stolz W, von Restorff B, et al: High affinity binding and lack of growth promoting activity of 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] in a human epidermal cell line (abstract). *J Invest Dermatol* 1989;92:437.

Received: April 27, 1989

Accepted: July 24, 1989

Priv.-Doz. Dr. T. Ruzicka  
 Department of Dermatology  
 University of Munich  
 Frauenlobstrasse 9  
 D-8000 Munich 2 (FRG)