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The Regulation of CD11b Integrin Levels on Human Blood Leukocytes and Leukotriene B4-Stimulated Skin by a Specific Leukotriene B4 Receptor Antagonist (LY293111)

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ABSTRACT. CD11b is part of the β2-integrin Mac-1 and plays an important role in neutrophil adhesion. Leukotriene B_4 (LTB_4) is an active upregulator of neutrophil CD11b-expression, acts as a potent chemoattractant to neutrophils and is also known to upmodulate epidermal proliferation. We performed a placebo-controlled study on LY293111, an oral LTB_4 receptor antagonist. Twenty healthy male volunteers were randomised over three treatment groups that received placebo, 48 mg, or 200 mg drug twice daily for 10 days. Before and after treatment, flow cytometrical CD11b assessment was performed on *in vitro* LTB_4-stimulated peripheral blood neutrophils. Additionally, skin biopsies were taken at 24 and 72 h after epicutaneous LTB_4 application, before and after treatment. The effects on skin were assessed immunohistochemically using various markers. All observed effects were dose related. CD11b upregulation on blood neutrophils was significantly suppressed in both treatment groups compared to placebo. In skin, a significant suppression of inflammation and hyperproliferation occurred. Pronounced inhibition was observed on neutrophil migration into the epidermis and the inflammatory infiltrate was decreased. A similar but weaker response was seen in the dermis. The number of cycling cells as well as suprabasal keratin-16 expression were decreased in both treatment groups. LY293111 proved to be a potent inhibitor of LTB_4-induced cutaneous inflammation and hyperproliferation. The potent antiinflammatory effect *in vivo* and the fact that in the present study the compound showed no clinically significant side effects make it an interesting drug in the future treatment of inflammatory conditions predominated by neutrophils. BIOCHEM PHARMACOL 53;7:1005–1012, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. LY293111; skin; inflammation; neutrophils; integrins, leukotriene B_4

The arachidonic acid cascade plays an important role in inflammation. It is the precursor of prostaglandins, thromboxanes, hydroxyeicosatetraenoic acids, and leukotrienes [1]. This latter group is important in the induction of polymorphonuclear leukocyte (PMN)¶ aggregation and adhesion, production of superoxides, and chemotaxis [2, 3]. Leukotrienes are 5-lipoxygenase products of arachidonic acid and consist of various inflammatory eicosanoids [4]. Leukotriene B_4 (LTB_4) is a potent chemo attractant for PMN and might together with 12-hydroxyeicosatetraenoic acid play an important role in many inflammatory processes [4]. Indeed, inhibitors of the enzyme 5-lipoxygenase have proven to be beneficial in the treatment of several inflammatory conditions [5–7].

PMN play an important role in acute inflammation. These cells have an established role in the initiation and possibly the maintenance of various inflammatory diseases [8–10]. PMN are attracted by numerous chemokines and metabolites of the arachidonic acid cascade, interleukins, and GRO-α [11]. Migration of PMN from the intravascular compartment to the extravascular space is a mechanism that requires interaction through adhesion molecules on the cell surface of PMN as well as on tissue cells and matrix. Human PMN adherence to endothelial cells can be enhanced by a variety of inflammatory mediators *in vitro* [12]. For PMN interaction with other cells and intercellular matrix, the $\alpha_m\beta_2$-integrins, and in particular CD11/CD18, are considered to be important [12–14]. The significance of

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¶*Abbreviations:* LTB_4, Leukotriene B_4; PMN, polymorphonuclear leukocytes; ICAM-1, intercellular adhesion molecule 1; EDTA, ethylene-diamine-tetra-acetic-acid; RAM, rabbit-antimouse antibody; AEC, 3-amino-9-ethyl-carbazole; PAP, peroxidase-antiperoxidase technique; HUVEC, human umbilical vein endothelial cells.

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individual integrin heterodimers in the various stages of PMN migration *in vivo* is not yet fully clarified. The clinical relevance, however, is demonstrated in Leukocyte Adhesion Deficiency syndrome, in which PMN lack surface expression of β2-integrins, and is associated with a short life expectancy due to severe inflammatory complications [15]. CD11b/CD18 is usually referred to as the Mo-1 or Mac-1 receptor. Known ligands are C3bi, Fx, Fb, and intercellular adhesion molecule 1 (ICAM-1) [16, 17]. ICAM-1 is mainly expressed by endothelial cells and to a lesser extent by keratinocytes [18].

Epicutaneous application of LTB_4 on human skin results in a reproducible dose-dependent cutaneous inflammatory response, initially dominated by PMN with a maximum presence at 24 hr, and followed by a dermal T-cell infiltrate, which is most pronounced after 72 hr [19]. Epidermal hyperproliferation occurs, reaching a maximum after 72–96 hr. Therefore, this model has been used previously to study sequential changes in aspects of cutaneous inflammation and interference in this process by antiinflammatory therapies [3].

Recently, the new specific oral LTB_4 receptor antagonist LY293111 (Lilly Research Laboratories, Indianapolis, IN, USA) has been developed. *In vitro* it has proven to be a potent inhibitor of the LTB_4 receptor [20]. In addition, it specifically inhibits chemotactic activity, calcium mobilisation, chemiluminescence, superoxide generation, and induction of CD11b/CD18 integrin upmodulation in LTB_4-stimulated neutrophils *in vitro* [20].

In this study, the following questions were addressed: first, is LTB_4-induced PMN accumulation in normal skin inhibited by a 10 days treatment course with LY293111 as compared to placebo; second, does LY293111 reduce CD11b expression on PMN in blood or skin and to what extent; third, are late effects of epicutaneous LTB_4 application modified (epidermal hyperproliferation and T-cell influx); and last, what is the clinical tolerability of the drug?

MATERIALS AND METHODS

Study Design

Prior to initiation of the study, approval from the Medical Ethics Committee was obtained. The trial was performed in a double-blind, placebo-controlled randomised fashion with three parallel groups. Twenty healthy male volunteers (20–42 years of age, mean age 27) participated in this study. Informed consent was obtained from all volunteers, none of whom had any history or signs of skin disease. No medication other than the compound was to be administered for at least 7 days before and during the study. For minor complaints paracetamol was allowed.

All volunteers were treated with LY293111 or placebo for 10 days. Six volunteers received LY293111 orally at a dose of 200 mg twice daily, seven volunteers received the compound at a dose of 48 mg twice daily, and seven volunteers received placebo. The study period lasted 32 days.

Clinical and Laboratory Safety Measurements

Prior to starting medication, a general physical examination and laboratory measurements were performed. The laboratory measurements consisted of: haematology (total and differential white blood cell counts, erythrocyte count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, haemoglobin, haematocrit, and platelet count); blood chemistry (ASAT, ALAT, bilirubin, alkaline phosphatase, gamma-GT, urea, creatinin, uric acid, phosphorus, calcium, total protein, creatin kinase, and thyroid function tests); electrolytes (sodium, potassium, chloride, bicarbonate); and random blood glucose. In addition, urinalysis was performed (specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, and sediment). During the study period, the haematology, blood chemistry, and urinalysis were repeated at day 1, 15, and the last day of the study.

LTB_4 Application and Biopsy Procedures

LTB_4 was applied epicutaneously to all volunteers before treatment and after 8 days of treatment. A 4-mm punch biopsy was taken on each voluteer before application to assess histology of the unchallenged skin. Aliquots of 100 ng LTB_4 (Paesel GmbH, Frankfurt, Germany) dissolved in 10 µl of ethanol were applied on the skin of the upper part of the back of the volunteers via a plastic cylinder (6.5 mm diameter) and the ethanol was evaporated under a stream of nitrogen. The test sites were covered with impermeable dressings (Silver patch, van der Bend BV, Brielle, The Netherlands) and held in place with leukosilk tape (Beiersdorf, Hamburg, Germany).

Biopsies were taken on days 2 and 4 (before administration of the compound), and on days 16 and 18 (during the 10 day treatment course with the compound) after injection of a local anaesthetic. The biopsies were washed in phosphate-buffered saline (PBS), embedded in Tissue Tek OCT compound (Miles Scientific, Elkhart, IN, USA), snap frozen in liquid nitrogen and stored at −80°C until use.

CD11b Integrin Upregulation Assay

Blood specimens for assessment of *ex vivo* CD11b surface expression were obtained on day 1 prior to the first LTB_4 challenge and on day 15 (after 7.5 days of treatment with LY293111 or placebo) prior to the second LTB_4 challenge.

Peripheral blood (4 ml) was collected through venepuncture, kept in ethylene-di-amine-tetra-acetic-acid (EDTA) at 4°C and processed within 3 hr after collection to prevent nonspecific upregulation of CD11b surface expression as a result of neutrophil activation. Blood samples were processed in triplicate using 90 µL aliquots that were incubated with LTB_4 (10 µl 1×10^{-7} M) in Hanks' balanced salt solution (HBSS, Sigma Chemical Corp., St. Louis, MO, USA) containing 0.1% bovine serum albumin (BSA), or with HBSS (10 µl) alone for 30 min at 37°C.

Samples were then cooled and incubated in the dark for 30 min at 4°C with 10 μL (0.045 g/l) antihuman CD11b-fluorescein conjugate (Mo-1-FITC, Coulter Corp., Hialeah, FL, USA). Erythrocytes were lysed and the remaining cells were washed with HBSS-BSA, fixed in 1% paraformaldehyde solution and stored at 4°C until analysis. The analysis was always performed within 1 week after preparation of the leukocyte suspensions.

Flow Cytometric Analysis

All specimens were analysed on an Epics Elite Flow Cytometer (Coulter, Luton, UK). Cells were excited with an air-cooled 488 nm argon laser set at 15 mW. FITC fluorescence was measured through a 525 nm (band width 30 nm) band pass filter. Calibration and sensitivity were checked by using FITC-labelled beads (Standard-Brite, Coulter Source, Hialeah, FL, USA). Forward and side scatter were used for gating granulocytes only. For each sample 5,000 gated cells were analysed.

Immunohistochemical Staining Procedures

Cryostat sections of 7 μm were cut and fixed for 10 min in acetone/ether (60/40 vol.%) for Mib-1, or in acetone for staining with the other antibodies. Table 1 depicts the various markers used. Staining with Ks8.12, Mib-1, anti-elastase, anti-CD11b, and anti-ICAM-1 was performed using an indirect peroxidase technique. Slides were incubated with the monoclonal antibodies for 30 min, and after two washes with PBS incubated with rabbit-antimouse antibody (RAM, Dakopatts, Copenhagen, Denmark) conjugated with peroxidase for 30 min.

Staining with T11 and T6 was done using a peroxidase-antiperoxidase technique (PAP technique). Slides were incubated with the monoclonal antibodies for 60 min. After two washes with PBS, the slides were incubated with RAM immunoglobulins (RAM-lg, Dakopatts, Copenhagen, Denmark) and after two more washes with PBS, PAP complexes (Dakopatts, Copenhagen, Denmark) were added. The incubation with RAM-lg and PAP was repeated. After two more washes with PBS and preincubation with sodium acetate buffer, pH 4.9, slides were stained with sodium acetate buffer containing 200 mg/l 3-amino-9-ethyl-carbazole (AEC solution) and 0.01% H_2O_2 for 10 min at 37°C in the dark.

All slides were washed in demineralised water and slightly counterstained with Mayer's Haematoxylin (Sigma, St. Louis, MO, USA). Slides were finally mounted in glycerin gelatin and studied by light microscopy.

Histological Examinations

Epidermal proliferation was measured by counting the number of Mib-1-positive nuclei per mm length of section. Ks8.12 binding of the epidermis was assessed in the basal and suprabasal compartment using a seven-point scale: 0 = no staining, 1 = sporadic staining, 2 = minimal staining, 3 = moderate staining, 4 = moderate/pronounced staining, 5 = pronounced staining, 6 = complete staining.

The density of PMN (elastase and CD11b stainings), T-lymphocytes, and Langerhans cells were assessed semi-

TABLE 1. Markers used for histology and in the CD11b upregulation assay

Cell type	Antibody	Ig-type	Ligand	Clone	Specificity	Concentration (μg/mL)	Manufacturer
PMN	anti-elastase	IgG_1 kappa	neutrophil elastase	NP57 mouse	neutrophil elastase in PMN, sporadic in monocytes	61.5	Dakopatts, Copenhagen, Denmark
T-lymphocytes	T11	IgG_1 kappa	CD2	MT910 mouse	CD2	155	Dakopatts, Copenhagen, Denmark
Langerhans cells	T6	IgG_{2a} kappa	CD1a	NA 1/34 mouse	CD1a	337	Ortho Diagnostics Systems, Raritan, NJ, USA
Cycling cells	Mib-1	IgG_1	Ki67	Mib-1 mouse	nuclear antigen present in the late G_1, S and G_2 + M phases of the cell cycle	200	Immunotech, Marseille, France
Cytokeratin 16-positive cells	Ks8.12	IgG_1	Cytokeratin 16	K8.12 mouse	hyperproliferative epidermis, cross reaction with cytokeratin 13, which is mainly present in hair follicles		Sigma, St. Louis, MO, USA
CD11b-positive cells (in skin)	anti-CD11b	IgG_1	CD11b	Bear-1 mouse	PMN, some monocytes, and macrophages		Monosan, Uden, The Netherlands
ICAM-1-positive cells	ICAM-1	IgG_1	CD54	84H10 mouse	CD54	200	Immunotech, Marseille, France
CD11b-positive cells (in blood)	anti-CD11b	IgM kappa	CD11b	94	PMN, some monocytes, and macrophages		Monosan, Uden, The Netherlands

quantitatively in epidermis and dermis using a seven-point scale: epidermis: 0 = no positive cells observed, 1 = sporadic staining, 2 = minimal presence, 3 = moderate presence, 4 = moderate/pronounced presence, 5 = pronounced presence, 6 = complete staining. Dermis: 0 = no positive cells, 1 = sporadic, 2 = 1–25% of infiltrate cells stained, 3 = 26–50%, 4 = 51–75%, 5 = 76–99%, 6 = 100%. The dermal infiltrate was subdivided in perivascular and diffuse localization. ICAM-1 staining was quantified in epidermis and dermis using a five-point scale: 0 = no staining, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%. In addition, an estimation of the total infiltrate was made using a four-point scale: 0 = no infiltrate present, 1 = minimal infiltrate, 2 = moderate infiltrate, 3 = pronounced infiltrate.

Statistical comparisons between groups were carried out before and after LY293111 treatment. After treatment, the placebo group was compared to both LY293111-treated groups. For statistical evaluation the Mann-Whitney test was used.

RESULTS

Clinical Results

No clinically significant toxicities were observed. There were no dropouts. The safety urine and blood measurements remained within normal ranges. LTB_4 application was well tolerated. The histological observations within each subgroup proved to be reproducible.

Flow Cytometry

Ex vivo challenge of blood PMN with LTB_4 before treatment showed a highly reproducible and consistent upregulation of CD11b expression. Before treatment, no statistically significant difference was seen in the relative neutrophil CD11b expression (ratio challenged/unchallenged, expressed as a fold increase) between the various treatment groups (placebo: 3.12 ± 0.49 [mean ± SEM], low-dose LY293111: 2.60 ± 0.27, and high dose: 2.63 ± 1.02, Fig. 1).

After systemic treatment, the placebo group showed no difference in the relative neutrophil CD11b expression compared to before administration (2.34 ± 0.24). A highly significant reduction was reached with high dose LY293111 (1.02 ± 0.02, p = 0.0027). The low dose group also showed a significant reduction (1.17 ± 0.05, p = 0.0017) (Fig. 1).

The mean relative expression of CD11b after *ex vivo* LTB_4 challenge on blood neutrophils permitted a total separation between placebo-treated and LY293111-treated volunteers.

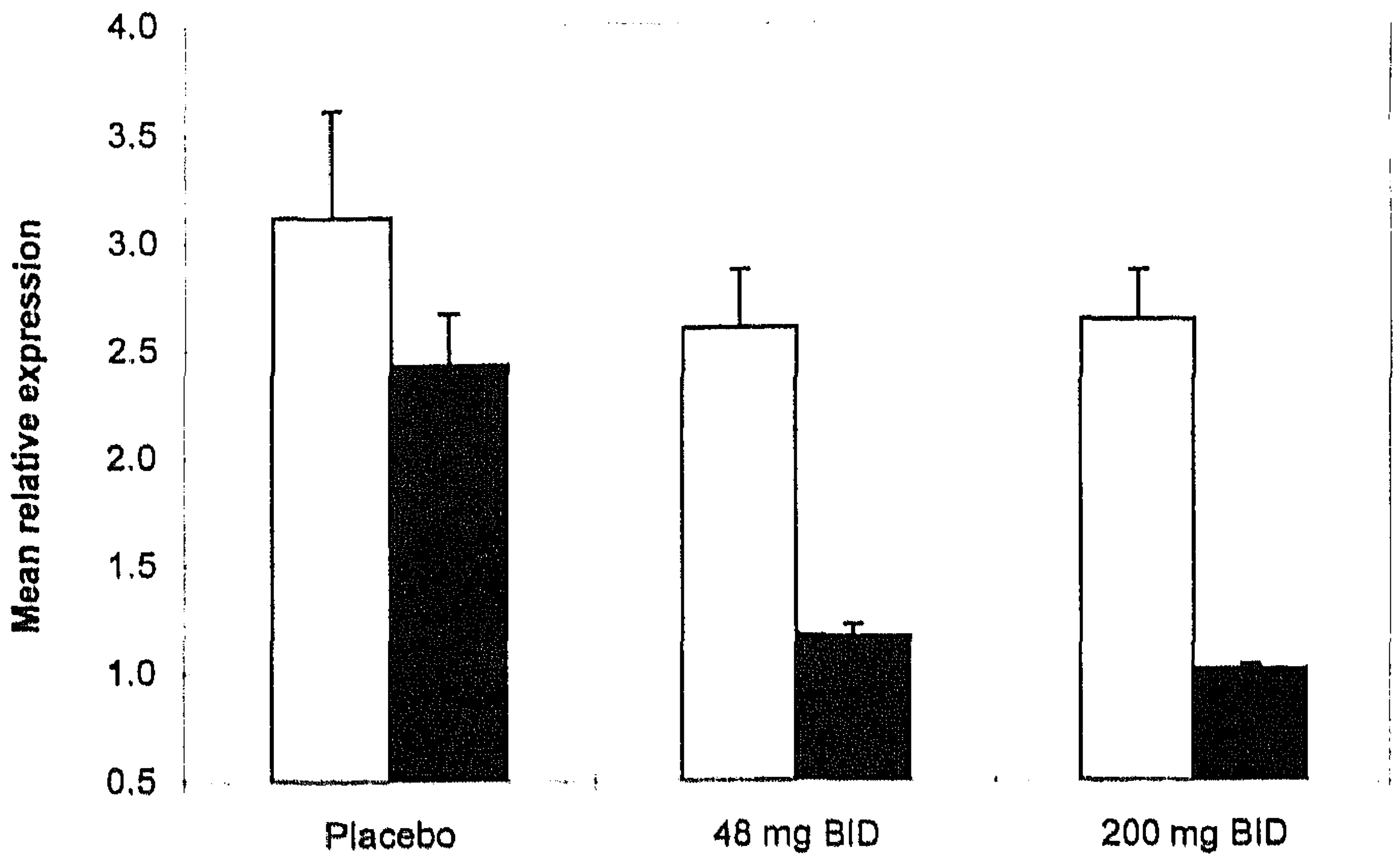

FIG. 1. *Ex vivo* CD11b expression in unstimulated and LTB_4-stimulated peripheral blood neutrophils, before and after treatment. The results are expressed as a ratio $CD11b_{stimulated}/CD11b_{unstimulated}$ per treatment group (mean ± SEM). □: before treatment, ■: after treatment.

Immunohistochemistry

EPIDERMAL PROLIFERATION: CYCLING CELLS. The number of cycling epidermal cells showed a reproducible response to LTB_4 before systemic treatment: following a slight reduction after 24 hr, a marked increase was observed at 72 hr after LTB_4 challenge (Fig. 2). During placebo treatment, LTB_4-induced Mib-1 expression was comparable to the response before treatment, whereas the induction of Mib-1 staining was decreased in the low dose group and virtually absent in the high-dose group (p = 0.01 and p = 0.003 respectively, Figs. 2 and 3).

CYTOKERATIN 16. Ks8.12 staining was sporadically present in normal unchallenged skin. After LTB_4 application, a statistically significant increase of keratin staining had already occurred after 24 hr. Skin specimens from volunteers receiving LY293111 showed a significant decrease of keratin staining after 24 hr compared to the placebo group (p = 0.02 in the low and p = 0.004 in the high dose group). In the high dose group, this decrease persisted until 72 hr after LTB_4 application (p = 0.005).

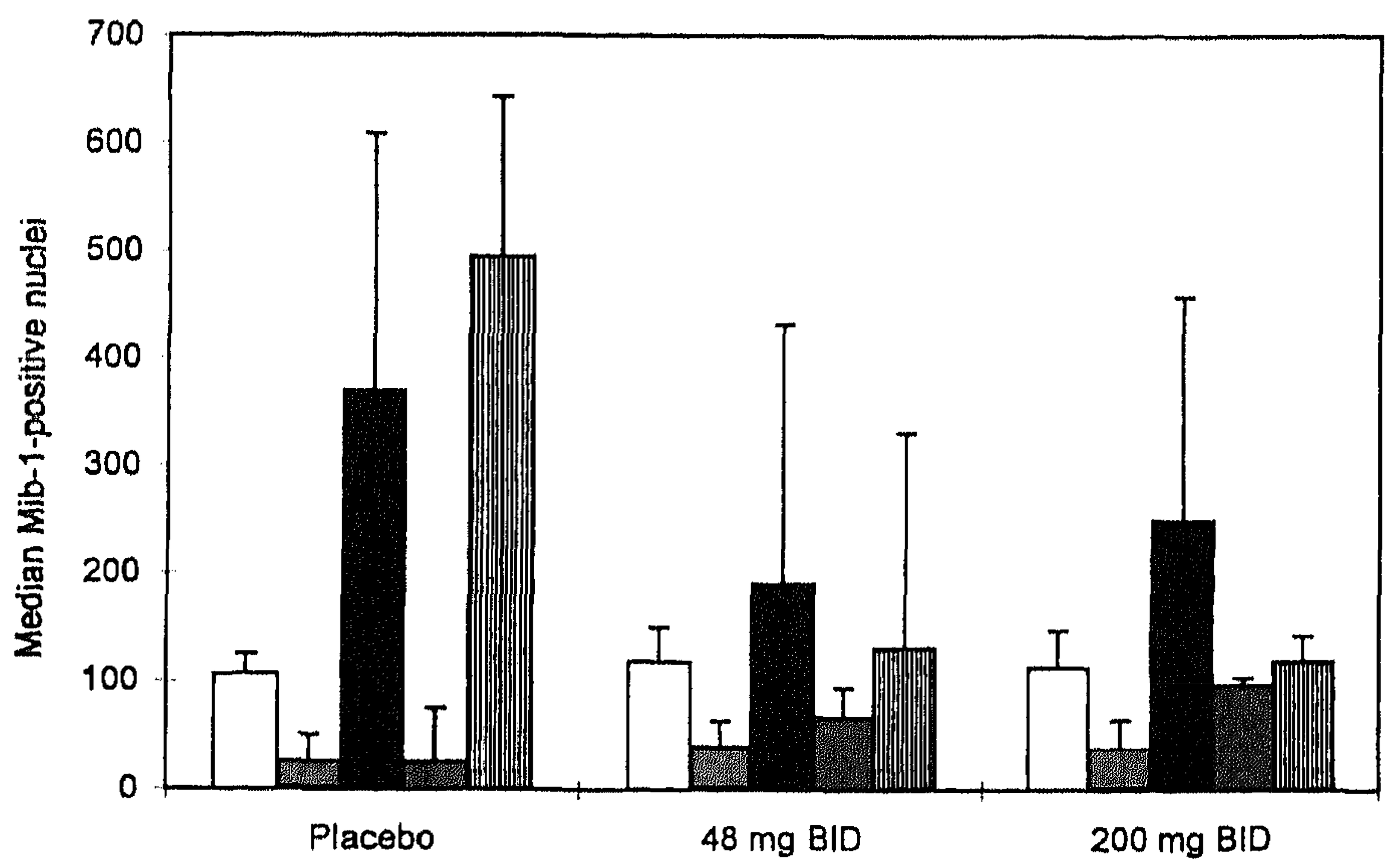

FIG. 2. Number of Mib-1–positive nuclei indicating cycling cells. The results are expressed per mm length of epidermis, before and after treatment with LY293111 (median ± SD). open column: normal epidermis, horizontal rule column: before treatment 24 hr after LTB_4 stimulation, solid column: before treatment 72 hr after LTB_4 stimulation, slanted rule column: after treatment 24 hr after LTB_4 stimulation, vertical rule column: after treatment 72 hr after LTB_4 stimulation.

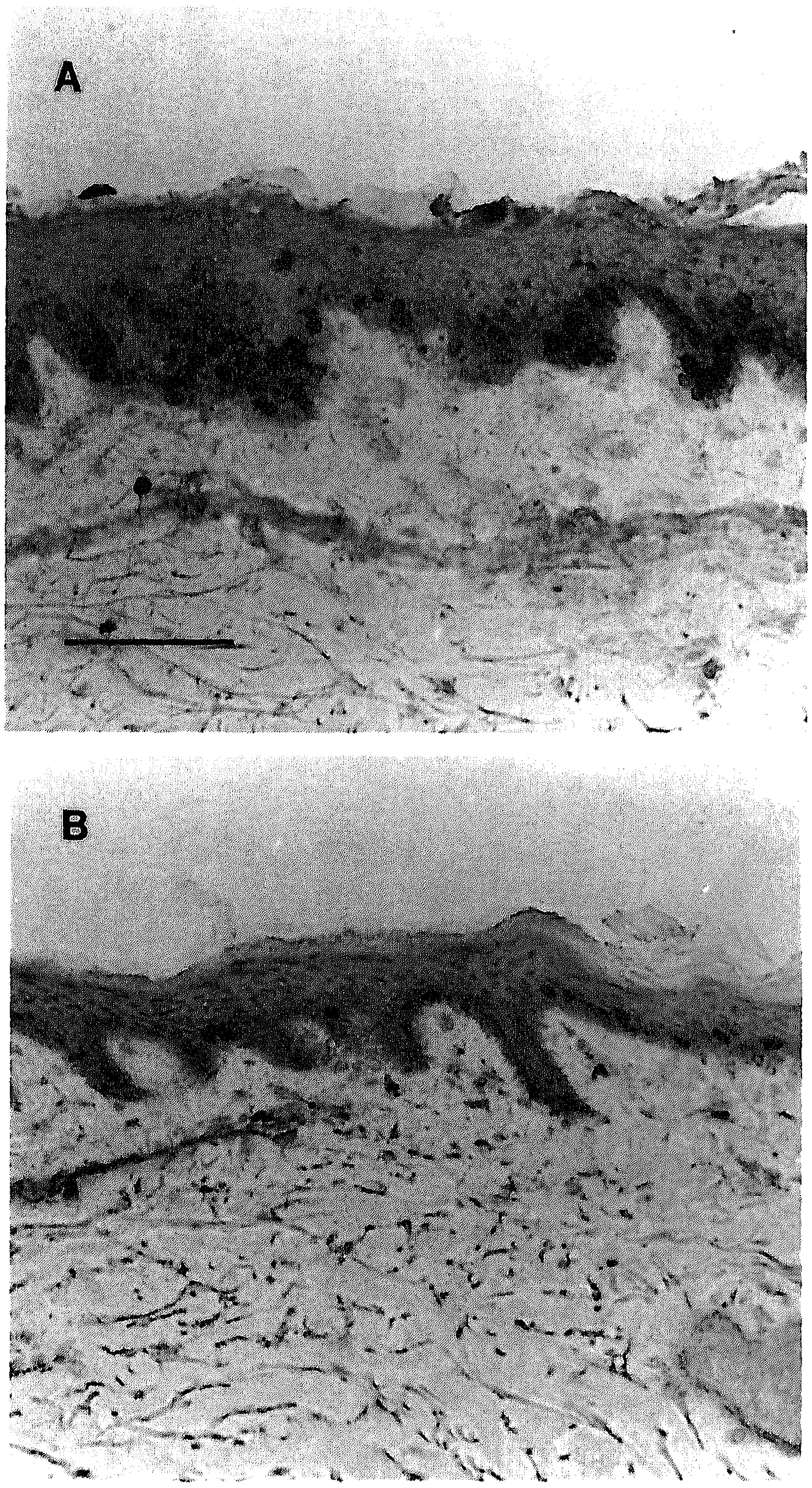

FIG. 3. Mib-1-stained nuclei 72 hr after application of LTB_4 on normal skin (scale bar: 100 μm). (A): placebo, (B): 200 mg LY293111 BID.

INFLAMMATION: TOTAL INFILTRATE CELLS. A substantial increase was observed at 24 and 72 hr following LTB_4 challenge in all three volunteer groups before systemic treatment. After treatment with placebo, this increase was virtually identical. In contrast, high-dose LY293111 induced a pronounced reduction of cutaneous inflammation which was most expressed 72 hr after LTB_4 challenge (p = 0.012). The low-dose group had an intermediate response pattern (Fig. 4).

ELASTASE STAINING. LTB_4 challenge induced a maximum elastase expression after 24 hr in epidermal and dermal skin compartments, one that decreased substantially after 72 hr. Again, all three volunteer groups showed a similar response pattern before systemic treatment. In contrast, during systemic treatment, the accumulation of elastase-positive cells was inversely correlated to the dose of

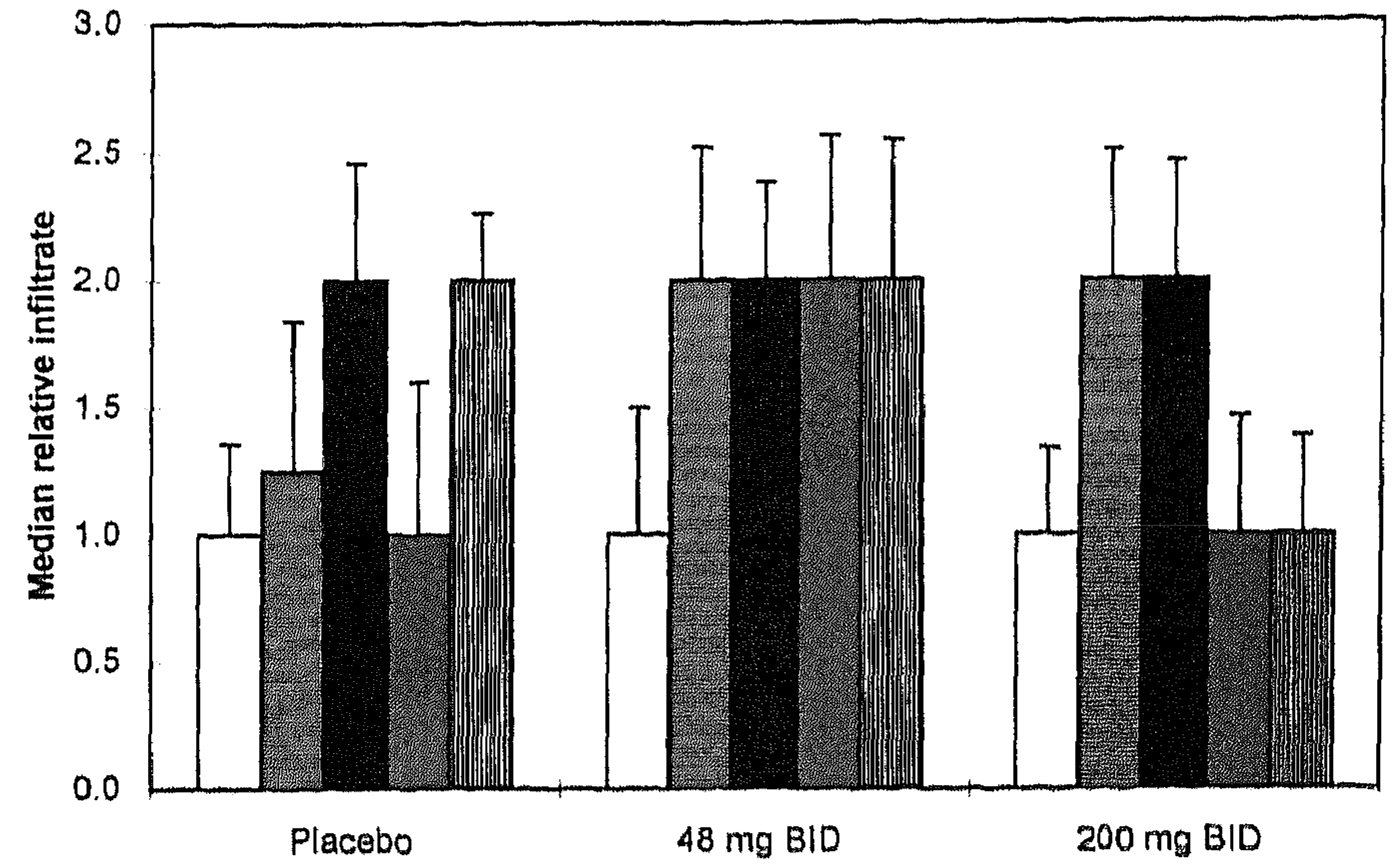

FIG. 4. Infiltrate cells, before and after treatment (median ± SD). Open column: normal unchallenged skin, horizontal rule column: before treatment 24 hr after LTB_4 stimulation, solid column: before treatment 72 hr after LTB_4 stimulation, slanted rule column: after treatment 24 hr after LTB_4 stimulation, vertical rule column: after treatment 72 hr after LTB_4 stimulation.

LY293111: a significant reduction in the epidermal accumulation was seen during low-dose treatment 24 hr after LTB_4 challenge (p = 0.03), whereas in the high-dose group epidermal PMN accumulation was virtually completely suppressed (p = 0.005). At 72 hr, the differences between the three groups had diminished considerably, but there was still a decreased expression in the high-dose group (p = 0.05). Twenty-four hours after LTB_4 challenge, the accumulation of PMN diffusely in the dermis was suppressed during high-dose treatment (p = 0.04). PMN accumulation was not suppressed in the dermal perivascular infiltrate, but remained present directly adjacent to the endothelium.

CD11B STAINING. The accumulation of CD11b-positive cells mimicked the pattern of elastase-positive cells. Before systemic treatment, all three groups showed a maximal accumulation after 24 hr in all skin compartments. Again, during systemic treatment with high-dose LY293111, the epidermal accumulation of CD11b-positive cells was completely suppressed both at 24 and 72 hr (p = 0.01, and p = 0.05, Figs. 5 and 6). Diffusely located dermal CD11b-positive cells were inhibited at 24 hr after LTB_4 application (p = 0.02). Low-dose LY293111 resulted in a mitigated accumulation of epidermal CD11b-positive cells.

T-LYMPHOCYTE STAINING. In contrast to the marked interference of LY293111 with PMN migration, the effects on T-lymphocytes were modest. Accumulation of T-lymphocytes in the epidermis and diffusely in the dermis was low in all three treatment groups. However, 72 hr after LTB_4 application, a reduction in the number of T-lymphocytes in the dermal perivascular compartment was accomplished in the high-dose group (p = 0.01).

LANGERHANS CELL STAINING. T6-positive cells in the epidermis showed a decrease 72 hr after LTB_4 application before systemic treatment. All three treatment groups showed a similar pattern after administration of the com-

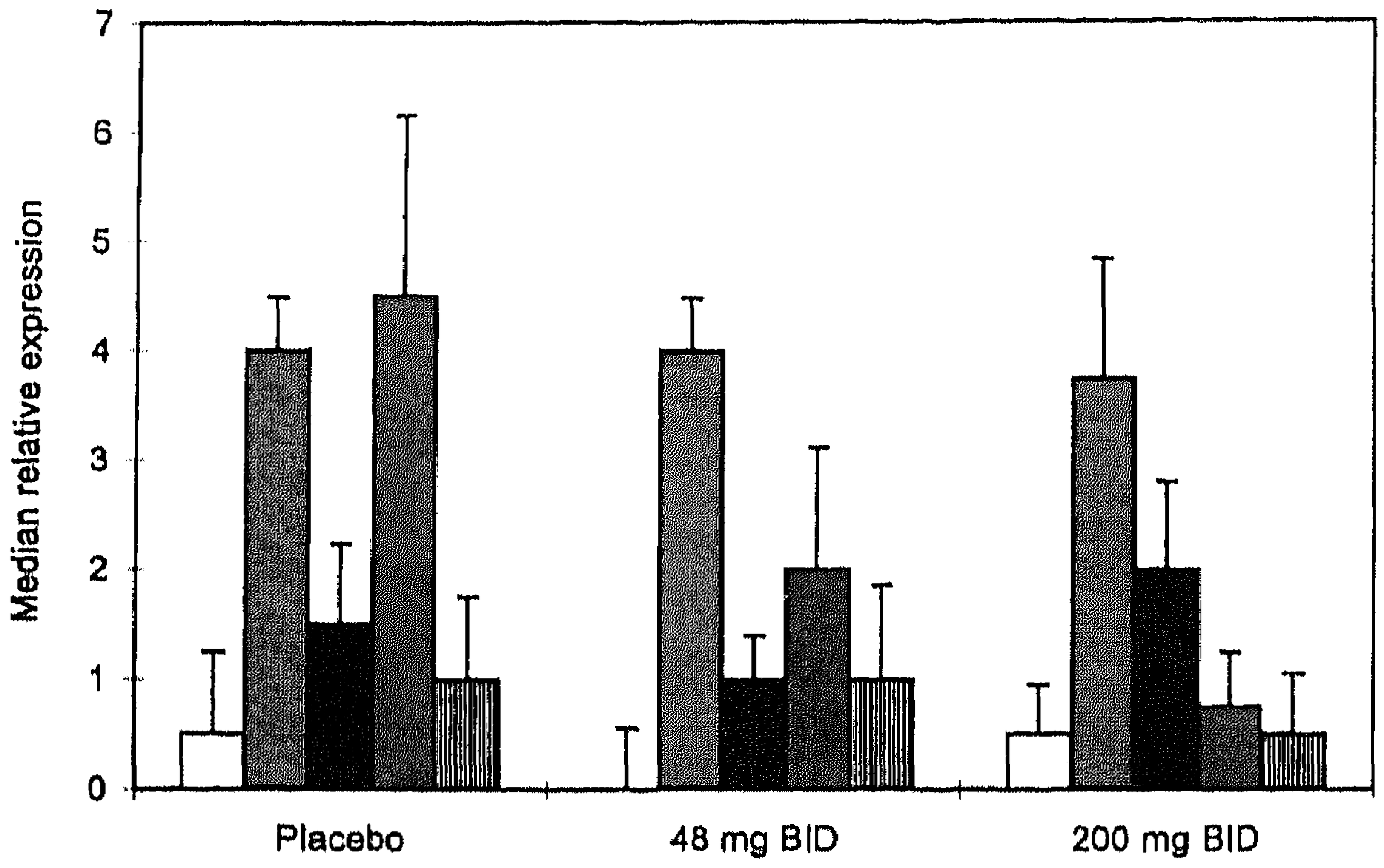

FIG. 5. Epidermal CD11b staining before and after treatment with LY293111 (median ± SD). Open column: normal epidermis, horizontal rule column: before treatment 24 hr after LTB_4 stimulation, solid column: before treatment 72 hr after LTB_4 stimulation, slanted rule column: after treatment 24 hr after LTB_4 stimulation, vertical rule column: after treatment 72 hr after LTB_4 stimulation.

pound. No significant changes in the epidermal T6-positive cells were recorded. The dermal compartment reflected a slight increase in T6-positive cells at 72 hr in the untreated and placebo-treated groups. At this time point, the subgroup receiving high-dose LY293111 demonstrated a significantly lower number of perivascular T6-positive cells (p = 0.04).

ICAM-1 STAINING. Epidermal ICAM-1 staining was focally present in normal unchallenged skin. In the pretreatment specimen, a consistent increase of ICAM-1 in epidermis and dermis was seen at 24 and 72 hr after LTB_4 application. After treatment, a remarkable overexpression of epidermal ICAM-1–positive cells was observed in the high-dose group at 24 hr (p = 0.01). After 72 hr, however, ICAM-1 expression in the epidermis was comparable to the other two groups and equivalent to before treatment.

In contrast, the increase of ICAM-1 diffusely in the dermis, as seen in the placebo-treated and untreated skin, was substantially inhibited by high dose treatment at 72 hr (p = 0.02). Perivascular ICAM-1 expression remained identical before and after treatment.

DISCUSSION

A 10-day treatment course with LY293111 showed marked influences on peripheral blood PMN, epidermal proliferation, and cutaneous inflammation. The treatment showed no significant side effects. A consistent observation was the potent inhibition of CD11b upregulation on peripheral blood PMN. LTB_4-induced epidermal hyperproliferation was virtually completely blocked, and hyperproliferation-associated keratins showed a decreased expression following high-dose treatment.

Infiltrate cells were modulated selectively. LY293111 had a reproducible dose–response effect on LTB_4-induced PMN CD11b expression, resulting in a complete suppression of CD11b upregulation in volunteers treated at the high dose, and a significant reduction in the low-dose treatment group. Migration of CD11b-positive cells and elastase-positive cells was blocked at the postperivascular level, resulting in an inhibition of PMN accumulation in the epidermis and diffusely in the dermis after 24 and 72 hr. The discrepancy between marked inhibition of epidermal and diffuse dermal PMN accumulation on the one hand and the unaffected perivascular presence of PMN on the other suggests an effect of LY293111 on PMN migratory capacities from the perivascular space into stroma and epidermis. In the perivascular compartment, the number of Langerhans cells was slightly decreased and T-lymphocytes were prevented from accumulating in the high-dose treat-

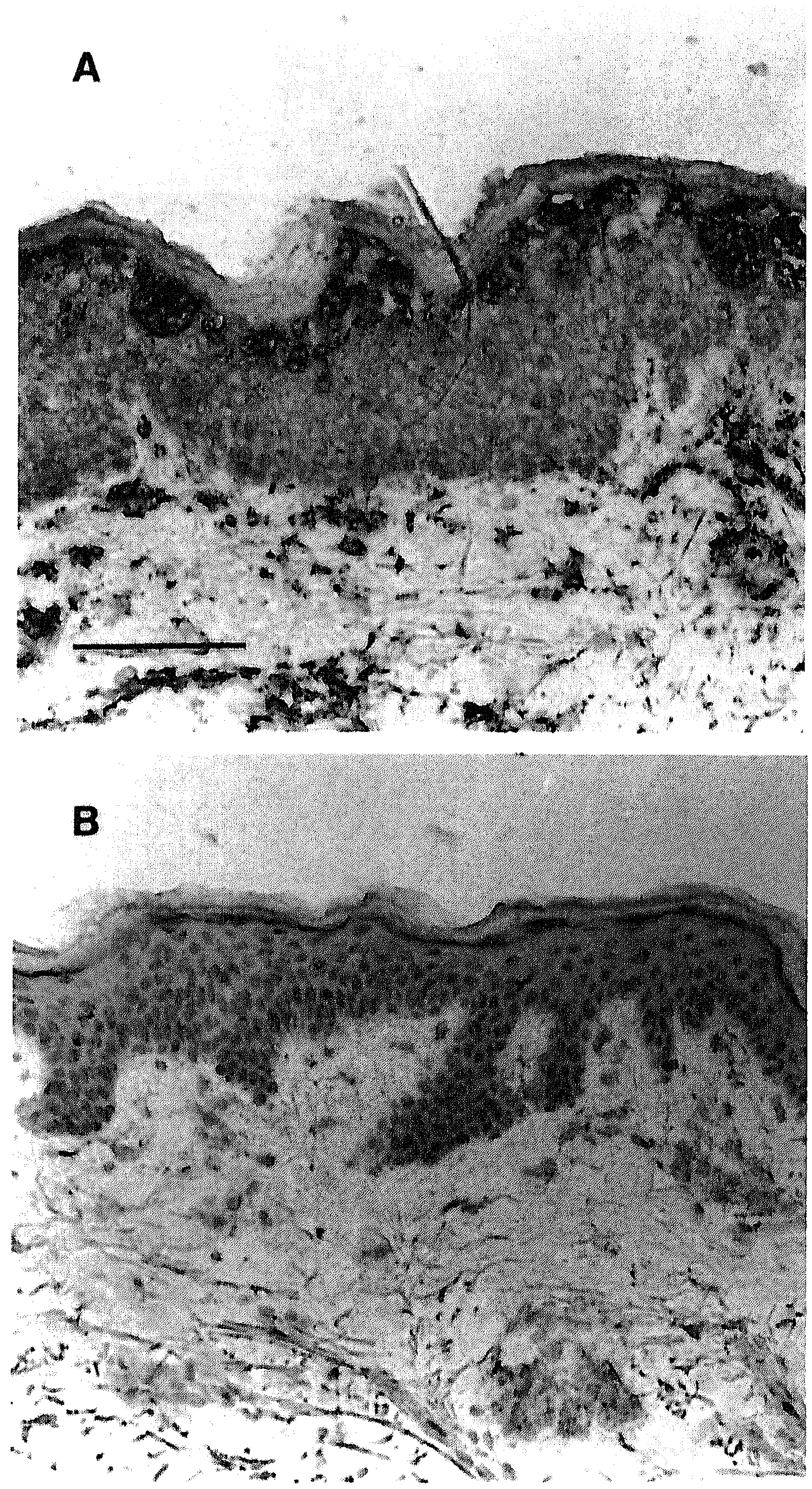

FIG. 6. CD11b staining 24 hr after application of LTB_4 on normal skin (scale bar: 100 μm). (A): placebo, (B): 200 mg LY293111 BID.

ment group 72 hr after LTB_4 application. Epidermal ICAM-1 was increased 24 hr after LTB_4 challenge in the high-dose group, and decreased diffusely in the dermis 72 hr after application, in contrast to the other two treatment groups. Perivascular ICAM-1 upregulation persisted after treatment with LY293111. Based on these observations, we may construct the following response pattern to LY293111 in LTB_4-induced cutaneous inflammation. PMN (as assessed by elastase) accumulate in the perivascular zone but fail to migrate into the stroma and epidermis. CD11b-positive cells showed the same distribution pattern. During treatment, the decreased presence of T-lymphocytes in the perivascular zone suggests inhibition of accumulation of T-lymphocytes. Diffusely in the dermis, the relative sparsity of PMN and T-cells is accompanied by a lack of ICAM-1 expression. The late events following LTB_4 application (i.e. epidermal hyperproliferation and expression of keratin 16) are prevented by pretreatment with LY293111.

LY293111, a specific LTB_4 receptor antagonist, proved to suppress CD11b induction on peripheral blood PMN. The observation that PMN in skin keep their ability to adhere to endothelium and move through the vessel walls, but lose their ability to migrate through the stroma, gives rise to speculation concerning specific functions of CD11b. Indeed, Furie *et al.* [21] studied the adhesion to and migration of neutrophils across human umbilical vein endothelial cells (HUVEC) in an *in vitro* model. Monoclonal antibodies to CD11b substantially inhibited migration of neutrophils. Monoclonal antibodies to ICAM-1 decreased transendothelial chemotaxis. This effect is mediated by binding of the antibody to ICAM-1 on HUVEC and not by a direct effect of the antibody on neutrophils. Therefore, migration seems dependent on CD11b and transendothelial chemotaxis on ICAM-1. In addition, Vedder *et al.* [22] showed that ICAM-1 is essential in adherence of PMN to endothelium, and that increased expression of CD11b/CD18 is not. This might explain why PMN still adhere to endothelium and show diapedesis but fail to migrate into the stroma when CD11b is decreased during treatment with LY293111. It is striking that late events in the LTB_4 model do not take place when PMN are restricted to the perivascular compartment, although LTB_4 itself might induce these late effects. In the high-dose group, hyperproliferation and epidermal PMN influx were strongly inhibited. By contrast, hyperproliferation did not occur in the low-dose group, whereas a small percentage of PMN were still able to migrate into the epidermis. One might speculate that epidermal hyperproliferation and associated features are possibly modulated by the LTB_4 receptor antagonist and not exclusively secondary to the intraepidermal migration of PMN.

ICAM-1 has been reported to be expressed by activated endothelial cells, monocytes, B- and T-lymphocytes, and keratinocytes [23]. Increased ICAM-1 expression has been described on cells under inflammatory conditions such as psoriasis, atopic dermatitis, lichen planus, and in rheumatoid synovium [24–26]. In psoriasis, the development of manifest lesions is linked with the appearance of ICAM-1 positivity on keratinocytes [27]. After LTB_4 application, an increase of dermal diffuse ICAM-1 was observed after 72 h, one that is prevented by LY293111. It is feasible that LY293111 is capable of preventing upregulation of ICAM-1 in incipient psoriatic lesions, thereby preventing the induction or spreading of the disease. There is a substantial variability in epidermal ICAM-1 expression possible between different individuals. This could be an explanation for the profound increase in epidermal ICAM-1 expression in the high-dose group. However, the difference with the other two treatment groups is striking.

Blocking of CD11b using monoclonal antibodies prevents PMN adhesion to keratinocytes [28]. Furthermore, functional CD11b has been reported to be decreased in the development of autoimmune neutropenia [29]. Wu *et al.* [30] showed that CD11b/CD18 may participate in the acute expression of glomerular damage in nephrotoxic nephritis. In addition, CD11b/CD18 has been reported to be of importance in septic liver injury [31] and in reperfusion-induced albumin leakage inducing microvascular dysfunction [30].

Therefore, CD11b likely plays an important role in many diseases and interference with upregulation may form an important clinical target in management of these diseases.

A total separation between placebo-treated volunteers and LY293111-treated volunteers was possible with respect to PMN CD11b upregulation in blood. In skin, however, high-dose treatment is necessary to achieve complete blocking of PMN influx. LY293111 decreases LTB_4-induced cutaneous proliferation, prohibits spreading of PMN and decreases extravasation of T-lymphocytes. LTB_4 levels are increased in psoriatic skin lesions [32], and immunohistochemical changes as seen after epicutaneous LTB_4 application have been found in lesional skin of psoriatic patients [24, 33]. Various compounds that interfere with LTB_4 metabolism such as 5-lipoxygenase inhibitors proved to be effective in psoriasis [5, 6]. Unfortunately, side effects have limited their use until now. The present study suggests that the pharmacological profile of LY293111 is very attractive: substantial effects on cutaneous inflammation, and absence of clinically significant side effects in the 20 subjects at the dosage used. In diseases that are initiated, dominated or maintained by PMN, LY293111 may well be beneficial.

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