Skin Levels of Arachidonic Acid-Derived Inflammatory Mediators and Histamine in Atopic Dermatitis and Psoriasis

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Since the biochemical events leading to cutaneous inflammation in atopic dermatitis and psoriasis are unknown, we studied the levels of arachidonic acid-derived mediators of inflammation as well as histamine in the suction blister fluid obtained from lesional and nonlesional skin of patients with these dermatoses. Mediator levels were determined radioimmunologically. Skin from healthy controls and uninvolved skin from patients contained very low or unmeasurable levels of the 5-lipoxygenase metabolite of arachidonic acid, leukotriene (LT) B\(_4\). In contrast, higher levels of LT\(_B4\)-like immunoreactivity were detected in suction blister fluid from lesional atopic dermatitis skin, and even higher concentrations occurred in psoriasis lesions. LT\(_B4\)-like immunoreactivity from atopic dermatitis suction blister fluid chromatographed on reverse-phase high-pressure liquid chromatography with authentic LT\(_B4\), thus excluding cross-reaction of the LT\(_B4\)-antibody with arachidonic acid or monohydroxyicosatetraenoic acids. In contrast, suction blister concentrations of the cyclooxygenase metabolite of arachidonic acid prostaglandin (PG) \(_E_2\) showed no significant differences between lesional and nonlesional patient skin and healthy control skin. PGD\(_2\) determined as a stable metabolite could not be detected in these samples. Histamine concentrations in lesional skin were within normal range. The elevated levels of the potent proinflammatory and immunomodulating mediator LT\(_B4\) could be involved in the pathogenesis of cutaneous inflammation in atopic dermatitis and psoriasis. In addition, they might explain the therapeutic efficiency of glucocorticosteroids, which among other actions inhibit the release of arachidonic acid from phospholipid stores by blocking the enzyme phospholipase A\(_2\). However, the specificity of disease expression in atopic dermatitis and psoriasis must be due to factors other than cutaneous LT\(_B4\) elevation. J Invest Dermatol 86:105–108, 1986

The biochemical events underlying inflammatory changes in atopic dermatitis skin are unknown. Since itch is a predominant symptom of this disease, histamine involvement has been suspected [1,2]. Antihistamines, however, display only weak therapeutic activity in this disease. In contrast, the therapeutic efficiency of glucocorticosteroids in atopic dermatitis must be taken into consideration when interpreting results from studies concerned with pathobiology of the disease. Recently, arachidonic acid-derived mediators of inflammation evolved as potential candidates to assume a pathogenetic role in cutaneous inflammation due to their potent proinflammatory activities (reviewed in [3]). This assumption is strengthened by the fact that glucocorticosteroids suppressed in in-vitro studies both cyclooxygenase and lipooxygenase products of arachidonic acid metabolism via indirect inhibition of the enzyme phospholipase A\(_2\) [4].

Metabolic transformations of arachidonic acid can proceed along several pathways. Cyclooxygenase pathway leads to the formation of, among others, classical prostaglandins (PG) such as PGE\(_2\), which has been identified as the major cyclooxygenase product in human skin [5]. Alternatively, arachidonic acid can be metabolized by lipooxygenases, of which 5-lipoxygenase has attracted particular interest due to its formation of substances with extremely potent biologic activities, the leukotrienes (LT). 5-Lipoxygenase enzyme activity has also been identified in epidermis [6]. We therefore decided to investigate cutaneous levels of arachidonic acid-derived mediators of inflammation in atopic dermatitis skin. We measured the concentrations of the main cyclooxygenase metabolites PGE\(_2\) and PGD\(_2\), the 5-lipoxygenase-derived product LT\(_B4\), and in addition histamine in suction blister fluid obtained from lesional and nonlesional atopic dermatitis skin. Results were compared with those measured in another inflammatory dermatosis, psoriasis. Part of these results was published in a preliminary communication [7].

MATERIALS AND METHODS

Patients, Suction Blisters Sixteen patients with atopic dermatitis, aged 13–69 years, 8 male and 8 female, were studied. Diagnosis was made according to Hanifin and Rajka [8]. The psoriasis group comprised 9 patients aged 18–78 years, 6 male and 3 female. Six of these patients suffered from chronic plaque type psoriasis. 2 patients had erythrodermic disease, 1 patient showed psoriasis guttata. Twelve volunteers aged 21–72 years, 6 male and 6 female, were used as controls. All probands were without any medication for at least 14 days. Some of the atopic dermatitis subjects used topical emollients up to the evening before examination. Suction blisters were raised using the Dermovac suction blister device [9]. In atopic dermatitis subjects, suction blisters were raised on clinically most affected areas, usually in the antecubital fossa. The eczematous lesions sampled were...
usually of the acute, inflammatory type. In eczematous skin, intact blisters were not always obtained and, in these cases, interstitial fluid was collected from the vacuum bell of the device. Control blisters were taken from clinically uninvolved skin, usually in the periumbilical area. Corresponding areas were chosen in the control subjects. The blister fluid was removed, immediately frozen in liquid nitrogen, and stored at −70°C until assay.

Radioimmunoassays of arachidonate metabolites PGE_{2}, PGD_{2}, and LTB_{4} were determined radioimmunologically in unextracted blister fluids. The details of the radioimmunoassay for PGE_{2} have been described previously [10,11]. PGD_{2} is unstable in the presence of albumin [12] and was, therefore, determined after conversion to a stable degradation product [13].

The anti-LTB_{4}, antiserum [14] was obtained from Wellcome Diagnostics, Beckenham, Kent, U.K. [5,6,8,9,11,12,14,15]. [(n)-^{3}H]LTB_{4} (sp act 32 Ci/mmol) was purchased from Amersham International.

Further confirmation of the identity of the immunoreactive LTB_{4} in suction blister fluid from atopic dermatitis patients was obtained by high-pressure liquid chromatographic (HPLC) separation of the samples prior to radioimmunoassay. HPLC was performed according to Fitzpatrick et al [15]. Briefly, 4 samples of suction blister fluid from involved atopic dermatitis skin containing LTB_{4}-like immunoreactivity were pooled, yielding a volume of approximately 1 mL. The pooled sample was brought up to 10 mL with 0.01 M Tris/HCl buffer, pH 7.4, and acidified to pH 3.0. The sample was applied to a C18-Sep Pak column (Waters) conditioned sequentially with 20 mL ethanol, 20 mL H_{2}O, and 5 mL 0.01 M Tris/HCl buffer, pH 3.0. The sample was eluted with 10 mL hexane, then with 8 mL methylformate (this fraction contains LTB_{4}), and finally with 5 mL methanol (this fraction contains the sulfidopeptide leukotrienes). The methyl-formate fraction was evaporated, redissolved in methanol, and injected on a C18-Nucleosil column (Macherey-Nagel, Düren). The mobile phase consisted of methanol:H_{2}O:acetic acid 68:32:0.01, pH 5.5. One-milliliter fractions were collected and tested for the presence of immunoreactive (ir) LTB_{4} by radioimmunoassay.

Histamine Assay Histamine in suction blister fluid was determined using a radioenzymatic assay according to Beaven et al [16].

**Serum Immunoglobulin E Determination** Serum IgE was measured by a paper-radio-immuno-sorbent-test (PRIST) (Pharmacia, Uppsala).

**RESULTS**

**Skin Levels of LTB_{4}-Like Immunoreactivity (Fig 1)** The levels of LTB_{4}-like immunoreactivity in suction blister fluids on skin of healthy probands were not measurable or were very low. In 7 of 9 samples from antecubital skin and 10 of 12 samples from abdominal skin, ir LTB_{4} was below the detection limit of 194 pg/mL. In only 2 samples from each location, low ir LTB_{4} levels could be detected (197–268 pg/mL). Likewise, in 8 of 13 samples from uninvolved atopic dermatitis skin, ir LTB_{4} was undetectable. The values in the remaining 5 samples were 199, 283, 291, 299, and 388 pg/mL, respectively. The mean ± SEM value was 231 ± 62 pg/mL. For this calculation, the value of 194 pg/mL was assumed in case of undetectable levels, a method which probably overestimates the mean value. In sharp contrast, markedly elevated ir LTB_{4} concentrations were found in lesional skin from atopic dermatitis patients. Only 5 of 16 samples contained no measurable ir LTB_{4}. The remaining values ranged up to 1524 pg/mL, the mean ± SEM value was 426 ± 85 pg/mL. The difference between lesional and nonlesional atopic dermatitis skin was statistically significant (p < 0.05, Wilcoxon rank sum test). No positive or negative correlation was seen between cutaneous ir LTB_{4} concentrations and disease severity, i.e., extent of disease or intensity of inflammation. In addition, no correlation was seen between ir LTB_{4} levels and serum IgE concentration, which was elevated in 10 of 13 serum samples.

**Figure 1.** Concentrations of LTB_{4}-like immunoreactivity in suction blister fluids from controls, patients with atopic dermatitis and psoriasis. Concentrations are shown in pg/mL. Horizontal line depicts the detection limit of 194 pg/mL. Horizontal and vertical bars show mean and SEM, respectively.

In 5 of 7 suction blister fluid samples from nonlesional psoriasis skin, ir LTB_{4} was below detection limit. In 2 samples, ir LTB_{4} concentrations were 373 and 730 pg/mL, respectively. In contrast, in only 1 of 9 samples from psoriasis lesions, ir LTB_{4} was below the detection limit. The remaining values ranged from 239–1042 pg/mL with a mean value of 649 ± 102 pg/mL. No correlation was observed in this small patient group between the type of psoriasis, extent of disease, and cutaneous LTB_{4} concentration. The ir LTB_{4} was further characterized by its chromatographic properties on reverse-phase HPLC (Fig 2). Pooled samples from lesional skin of 4 patients with atopic dermatitis were separated by HPLC, and immunoreactivity for LTB_{4} was determined in 1-mL eluate fractions. LTB_{4}-like immunoreactivity was detected only in fractions chromatographing with an authentic LTB_{4} standard, thus excluding the possibility of cross-reactions with 12-HETE or arachidonic acid.

**Skin Levels of PGE_{2} and PGD_{2}-Like Immunoreactivity (Table I).** Suction blisters from antecubital skin of normal controls contained 12.9 ± 3.7 ng/mL of PGE_{2}-like immunoreactivity. The values for abdominal skin were 17.5 ± 3.3 ng/mL (p > 0.05).
Lesional atopic dermatitis skin contained 7.3 ± 0.30 ng/ml of PGE₂ whereas in uninvolved skin, 13.0 ± 3.6 ng/ml were found. The corresponding values for involved and uninvolved psoriasis skin were 9.5 ± 1.4 and 15.2 ± 4.9 ng/ml, respectively. The differences between all these values were statistically not significant. Even though exogenously added PGD₂ could be detected in blister fluid after incubation at alkaline pH [13] (detection limit 360 pg), no PGD₂ was found in any of the samples analyzed using a radioimmunoassay for a stable degradation product of PGD₂ formed under such conditions.

**Histamine Skin Levels (Table II)**

Histamine levels in suction blister fluid from healthy controls were 3.11 ± 0.48 ng/ml on the forearm and 2.25 ± 0.20 on the abdomen (difference not significant). Lesional atopic dermatitis skin contained 2.71 ± 0.59 ng/ml histamine, the corresponding values in unaffected skin were 2.66 ± 0.23. Lesional and nonlesional psoriatic skin contained 2.32 ± 0.23 and 2.98 ± 0.46 ng/ml histamine, respectively. None of the differences was statistically significant.

**DISCUSSION**

In an attempt to elucidate biochemical events underlying cutaneous inflammation in atopic dermatitis, we measured suction blister concentrations of several potent mediators of inflammation, including LT보, PGE₂, PGD₂, and histamine. Skin of healthy controls contained no measurable or very low levels of ir LT보, a 5-lipoxygenase metabolite of arachidonic acid. Similar results were obtained in uninvolved skin of subjects suffering from atopic dermatitis. In striking contrast, elevated levels of LT보-like immunoreactivity were detected in lesional skin of these patients. Their magnitude did not correlate with clinical disease severity or serum IgE concentrations. On the other hand, arachidonic acid metabolites formed via the competing cyclooxygenase pathway were found in normal levels in atopic dermatitis skin: PGE₂ concentrations were almost identical in all groups studied, and PGD₂ was undetectable. Similar results were obtained in a second group of patients suffering from psoriasis. Uninvolved psoriatic skin contained no or only slightly elevated levels of ir LT보. In lesional psoriasis skin, levels of ir LT보 were found which even exceeded those measured in atopic dermatitis skin. No correlation was seen between the type of psoriasis and ir LT보 concentrations. In psoriasis, too, PGD₂ was not detectable and PGE₂ was within normal limits.

Histamine concentrations were not elevated in any of the conditions examined.

Our findings fit well with the therapeutic efficiency of glucocorticosteroids in both atopic dermatitis and psoriasis, since these drugs at least in vitro inhibit the formation of both cyclooxygenase and lipooxygenase products.

Our results showing elevated cutaneous levels of LT보-like immunoreactivity in lesional psoriasis skin are in accordance with findings of Brain et al [17] and Grabbe et al [18], who were the first to demonstrate elevated LT보 levels in psoriatic plaques using different methodology. This phenomenon, however, seems not to be specific for psoriasis, since elevated levels could now be shown in atopic dermatitis skin as well. These findings could have important pathogenetic implications. Although LT보 is known as a predominant chemoattractant of neutrophil and eosinophil granulocytes, its topical application in human skin led only in the early stages of the reaction to dermal and epidermal neutrophil infiltrates, which lacked further characteristics of the psoriatic lesion. At later time points, however, a predominantly mononuclear infiltrate was observed, thus pointing to the possible attraction of mononuclear cells into atopic dermatitis skin by LT보 [19].

The LT보-derived chemotactic activity for mononuclear cells in normal skin might be further enhanced in atopic dermatitis. The interesting observations of Czarnetzki [20] showed increased monocyte sensitivity to the chemotactic action of LT보 in patients with atopic dermatitis and psoriasis. This mechanism could confer selectivity for mononuclear cells to LT보-derived chemotactic signals and thus contribute to the predominant accumulation of mononuclear cells in atopic dermatitis skin. Since mononuclear cells themselves seem the most likely cellular source of LT보 in dermis [21] (although its production by keratinocytes cannot be excluded from our results), a self-perpetuating amplification circle of macrophages producing LT보, and thus attracting further macrophages into the inflammatory skin site could be operative in atopic dermatitis. The lack of specificity of elevated LT보-levels in various inflammatory dermatoses such as atopic dermatitis and psoriasis (as and recently reported, also in allergic contact dermatitis [22]) is not surprising and does not, in our opinion, lessen their pathogenetic implications. The biochemical events occurring in inflammatory lesions in all these dermatoses might well be quite similar in view of the uniform therapeutic efficiency of glucocorticosteroids in these diseases which may further implicate, but does not prove, an involvement of the arachidonic acid cascade. Additional factors unrelated to findings reported here should account for the different clinical disease expressions. Histamine, however, seems to assume a minor role, if any, in the pathogenesis of inflammation in atopic dermatitis.

The normal values measured in suction blister fluid are in accordance with our recent report on normal histamine levels in biopsies obtained from atopic dermatitis skin. Highly elevated histamine levels were found only in a patient suffering from the hyper-IgE syndrome [23]. Previously, however, elevated levels of histamine were found in atopic dermatitis skin using less specific methods [24]. In previous studies from our group, increased plasma levels of histamine were found in atopic dermatitis patients during acute disease stages, returning to normal during remission [25].

Recently, the question was raised as to whether elevated levels of LT보-like immunoreactivity in suction blister fluid represented true LT보 or could be partly due to cross-reaction with arachidonic acid or its 12-lipoxygenase metabolite, 12-HETE [26], which

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**Table I.** Concentrations of PRE₂-Like Immunoreactivity (mean ± SEM) in Suction Blister Fluids

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>PGE₂ Concentration (ng/ml)</th>
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<tbody>
<tr>
<td>Controls</td>
<td></td>
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<tr>
<td>Forearm</td>
<td>12.85 ± 3.72</td>
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<tr>
<td>Abdomen</td>
<td>17.47 ± 3.30</td>
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<tr>
<td>Atopic dermatitis</td>
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<tr>
<td>Lesional skin</td>
<td>7.28 ± 2.98</td>
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<tr>
<td>Unaffected skin</td>
<td>13.01 ± 3.64</td>
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<tr>
<td>Psoriasis</td>
<td></td>
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<tr>
<td>Lesional skin</td>
<td>9.48 ± 1.39</td>
</tr>
<tr>
<td>Unaffected skin</td>
<td>15.21 ± 4.91</td>
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</tbody>
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**Table II.** Histamine Concentrations (mean ± SEM) in Suction Blister Fluids

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Histamine Concentration (ng/ml)</th>
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</table>
were found in lesional psoriatic skin by Hammarström et al [5]. This explanation seems unlikely for several reasons. After separation of pooled samples of suction blister fluids from atopic dermatitis patients by reverse-phase HPLC, LTβ-like immunoreactivity cochromatographed with an authentic LTβ standard. Furthermore, the anti-LTβ antibodies used cross-react only to a small extent with arachidonic acid and 12-HETE [14]. Thus, very high concentrations of these components must be present to significantly interfere with the LTβ radioimmunoassay. Such high concentrations, however, should also cause cross-reaction in the radioimmunoassays for PGE₂ and PGD₂, which was not observed.

Finally, chemotactic biologic activity which can now be assumed to be due to LTβ was recovered from affected, but not unaffected atopic dermatitis skin by Dierskmeier et al [27].

In summary, elevated levels of LTβ-like immunoreactivity were identified in affected atopic dermatitis skin, which might be of central importance in the pathogenesis of the disease in view of the potent proinflammatory activities of this mediator and the anti-inflammatory efficiency of glucocorticoid acting as possible inhibitors of LTβ synthesis. Due to the lack of specificity of these drugs’ actions, however, further clarification of the role of LTβ in the pathogenesis of cutaneous inflammation must await the availability of more selective inhibitors of LTβ synthesis or functional LTβ antagonists.

REFERENCES