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Demonstration of Leukotriene B_4 in the Scale Extracts of Psoriasis and Inflammatory Pustular Dermatoses

Correlation with Leukocyte Chemotactic Activity and C5a Anaphylatoxin

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We carried out quantification of the levels of leukotriene B_4 (LTB_4), a highly potent cell membrane-derived leukocyte chemotactic factor, in scale extracts of psoriasis and related inflammatory dermatoses characterized by sterile subcorneal pustule formation by using radioimmunoassay. A small amount of LTB_4 was demonstrable even in extracts from non-inflammatory stratum corneum, but larger amounts were detected in the scale extract from pustular psoriasis and in those from psoriasis vulgaris. There was a significant correlation between the LTB_4 and C5a levels in scale extracts, suggesting that complement activation and generation of LTB_4 are a closely related event in psoriatic lesions. However, in contrast to highly signficant correlation noted between the amount of C5a and chemotactic activity for polymorphonuclear leukocytes (PMN) detectable in scale extracts, LTB_4 levels correlated only marginally with the chemotactic activity. *Key words: Pustular psoriasis; Pustulosis palmaris et plantaris; Radioimmunoassay.* (Received June 27, 1985.)

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In psoriasis the lesional epidermis is characteristically infiltrated by polymorphonuclear leukocytes (PMN). Generation of leukotactic factors in the lesional epidermis is suspected to be responsible for this PMN infiltrate. Complement fragments have been identified in scales of psoriasis lesions (1). Scales are assumed to contain various trapped chemical mediators that have played a part in the pre-existing inflammatory changes occurring in the epidermis, analogous to the stratum of soil that contains historic remains of the past. In our previous report we demonstrated that scale extracts obtained from psoriasis and related pustular dermatoses contained significantly higher levels of anaphylatoxins, C3a, C4a, and C5a, than in the stratum corneum of non-inflammatory skin (2).

Recently the potential significance of leukotrienes, 5-lipoxygenase arachidonic acid metabolites, has become a focus of interest in the pathogenesis as well as in the treatment of psoriasis (3, 4, 5, 6). LTB_4, the most potent lipid chemoattractant (7), is identified in chamber fluid from psoriatic skin lesions (8), whole skin extracts of psoriatic lesions and even of uninvolved skin as well as in the scales overlying involved skin (9). These features are suggestive that LTB_4 may be derived from the cutaneous tissue of psoriatic patients to initiate the inflammation noted in psoriatic lesions.

In the present investigation, (i) we have examined extracts of scales from patients with psoriasis and related sterile pustular dermatoses for the quantification of LTB_4 using radioimmunoassay: and (ii) we have attempted to determine whether these LTB_4 levels have any correlation with those C5a or chemotactic activity for PMN demonstrable in the scale extracts.

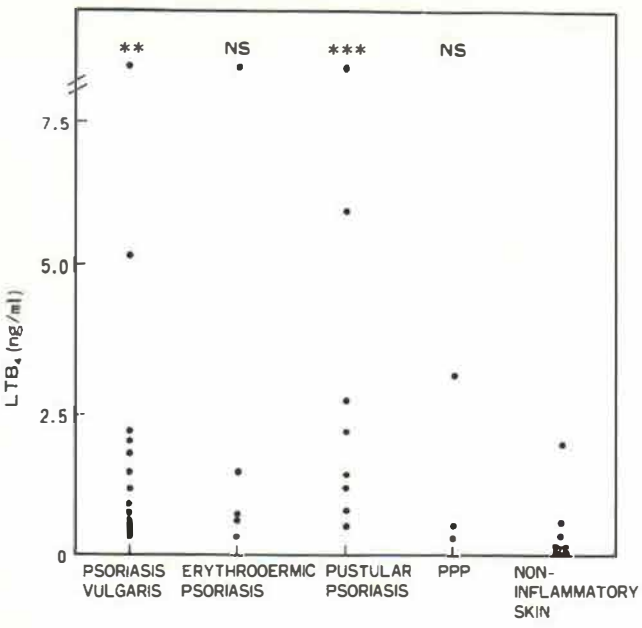

Fig. 1. Comparison of LTB_4 in scale extracts of psoriasis vulgaris, erythrodermic psoriasis, pustular psoriasis, PPP, and non-inflammatory skin. *Stars* denote levels of significance of psoriasis vulgaris or inflammatory pustular dermatoses vs non-inflammatory skin: *=$p<0.05$, **=$p<0.01$. *NS* denotes not significant.

METHODS

We collected scales from skin lesions of 14 patients with psoriasis vulgaris, 5 with psoriatic erythroderma, 8 with pustular psoriasis, 3 with pustulosis palmaris et plantaris (PPP), and from 7 cases of non-inflammatory skin that include tylosis 2, clavus 1, ichthyosis 1, scar 1, and normal skin 2. Scales were also collected from one patient with each of the following: subcorneal pustular dermatosis (SPD), non-psoriatic erythroderma, and pityriasis rubra pilaris (PRP). All these scales were kept at −70°C.

Extracts of stratum corneum

The dried scales were crushed with Freezer/Mill (Spex Industries, Metuchen, New Jersey, USA), weighted, suspended in phosphate-buffered saline (PBS) at 20 times the original dry weight, homogenized with a Potter-Elvehjem device, and adjusted to pH 3.0 with 1 N HCl. Two ml of PBS, containing 10 mg of scales was extracted 3 times into 2 volumes of diethyl ether (Wako Pure Chemical Industries, Osaka, Japan). After evaporation of the diethyl ether phase under nitrogen, the residue dissolved in 50 mM Tris: HCl buffer was tested for radioimmunoassay. For chemotactic assay, the dried scales were suspended in PBS, shaken at 4°C for 1 h, and diluted at 5000 times the original weight.

Radioimmunoassay

Radioimmunoassay for LTB_4 was performed according to the method described by Salmon et al. (10). The kit was obtained from Amersham International plc (United Kingdom) and the assay was carried out according to manufacture instructions. In 32 samples radioimmunoassay for C5a was performed as reported before (2) to study correlation between LTB_4 and C5a levels in scale extracts.

PMN chemotactic assay

Chemotactic activity was measured in Blind-Well Chambers (Labo Science, Tokyo, Japan) as previously described (11). N-formyl-L-methionyl-L-leucyl-L-pheynylalanine (Sigma Chemical Company, St. Louis, Missouri, USA) served as a control chemoattractant. Results are expressed as a number of cells counted in a high power field that completely migrated through the filter after 45 min incubation.

Statistical analysis

Levels of LTB_4 were compared using the Wilcoxon rank-sum test and levels of LTB_4, C5a, and chemotactic activity compared using Spearman rank correlation coefficient.

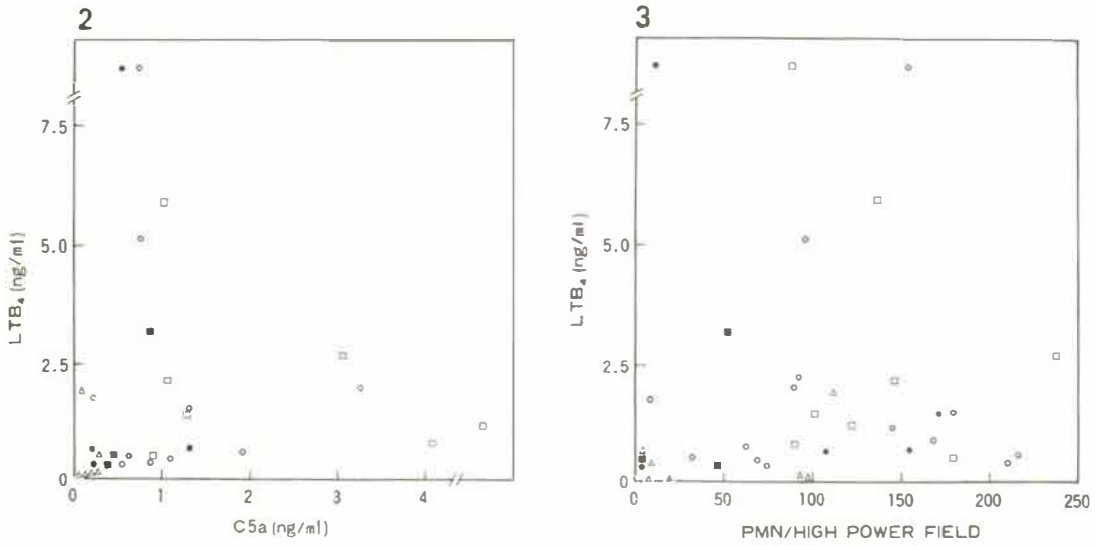

Fig. 2. Relation between the amount of C5a and LTB_4. ○, psoriasis vulgaris; ●, erythrodermic psoriasis; □, pustular psoriasis; ■, PPP; △, non-inflammatory skin.

Fig. 3. Correlation between chemotactic activity and the amount of LTB_4. Each *dot* denotes the same as that in the caption of Fig. 2.

RESULTS

LTB_4 levels in scale extracts

Levels of LTB_4 were significantly elevated in scale extracts from psoriasis vulgaris ($p<0.05$) and pustular psoriasis ($p<0.01$) in comparison with those of non-inflammatory skin, 0.7±0.7 ng/ml (Fig. 1). However, we could not find any significant increase in LTB_4 levels in those from erythrodermic psoriasis and PPP. The level of LTB_4 in SPD was 0.9 ng/ml. In one case of PRP its level, 0.1 ng/ml, fell within the normal range.

Correlation between the levels of LTB_4 or C5a and chemotactic activity

A positive correlation was found between the concentration of C5a and LTB_4 in scale extracts ($r_s=0.48$, $p<0.001$), although there were some cases showing an increase chiefly in LTB_4 or C5a alone (Fig. 2). When these data were compared with those of chemotactic activity assessed in highly diluted scale extracts, we could only find a marginally significant correlation with the detectable chemotactic activity ($p<0.1$) (Fig. 3), in contrast to the highly significant correlation that was noted between the chemotactic activity and C5a levels ($p_s=0.58$, $p<0.001$).

DISCUSSION

Histopathologic demonstration of intraepidermal PMN microabscesses produced by topical application of 100 ng LTB_4 (12) as well as increased LTB_4 levels in psoriatic skin suggests that LTB_4 may play an important role in the pathogenesis of psoriatic PMN infiltrate and of related sterile pustular dermatoses. Our results reported here demonstrate that scale extracts prepared from psoriasis and pustular psoriasis contain significantly higher levels of LTB_4 than the horny tissue extracts of non-inflammatory skin. However, such a feature was unexpectedly less prominent as compared to that observed with

chemotactic anaphylatoxin C5a (2). No significant increase was noted even in scale extracts of pustulosis palmaris et plantaris. Furthermore the correlation of the chemotactic activity noted in crude scale extracts with the amount of LTB_4 contained was found to be much less remarkable than that with the C5a levels. We demonstrated that anaphylatoxins were significantly increased in psoriatic sera as well as in the scales of psoriasis and all the related pustular dermatoses (2, 13). Nevertheless, we must keep in mind the possibility that LTB_4, although existing in the increased amounts, may be more unstable than C5a in the lesional epidermis and metabolized by the time it is pocketed in the stratum corneum. Another possibility is that enough amounts of LTB_4 may not be recovered in our water-soluble scale extracts. Moreover, contamination of less chemotactically potent arachidonate metabolites such as 12-HETE may be also responsible for the results, since there is a slight cross reaction with such agents in the LTB_4 radioimmunoassay (10).

PRP is occasionally difficult to differentiate from psoriasis clinically and histologically but totally lacks the transepidermal migration of PMN. It is interesting that the scale extract of PRP showed only a minute amount of LTB_4 as in the case with C5a which was also within the normal range (2).

Meanwhile, LTB_4 was reported to be increased in other inflamed skin such as allergic contact dermatitis (15) and atopic dermatitis (16) in which the infiltration of PMNs does not constitute a characteristic feature. Therefore it still remains controversial whether LTB_4 is disease specific to psoriasis (17). However, the interrelationship noted between chemotactic activity, LTB_4, and C5a levels in scale extracts suggests at least that activation of complement and generation of lipoxygenase products is a closely related event and that C5a and LTB_4 may act synergistically in the accumulation of PMN in the lesional skin.

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