

## INVESTIGATIVE REPORT

# Inflammatory Mediators in Normal, Sensitive and Diseased Skin Types

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The role of inflammatory mediators in the pathogenesis and pathophysiology of skin diseases is now widely accepted. We analysed the profiles of inflammatory mediators in normal, sensitive (past history of eczema, but currently patch test negative) and diseased (psoriasis and eczema) skin types to identify the patterns associated with various degrees of inflammatory dermatoses. Compared with normal skin, prostaglandin E<sub>2</sub> was increased approximately 3.8-fold ( $p < 0.0002$ ) and 4.7-fold ( $p < 0.0001$ ) in suction blister fluids from sensitive and diseased skin types, respectively. Leukotriene B<sub>4</sub> and interleukin-1 $\alpha$  showed no differences between normal and sensitive skin types. However, in lesional skin from psoriasis and eczema patients, leukotriene B<sub>4</sub> was increased approximately 6.6-fold ( $p < 0.0001$ ), whereas interleukin-1 $\alpha$  was decreased approximately 3.1-fold ( $p < 0.001$ ). Interleukin 6 and tumour necrosis factor- $\alpha$  could not discriminate between skin types. We conclude that only prostaglandin E<sub>2</sub> showed a significant stepwise increase on progression from normal through sensitive and inflammatory skin diseases. Levels of leukotriene B<sub>4</sub> and interleukin-1 $\alpha$  were also indicative of disease state and may be important in the pathophysiology of these conditions. *Key words: blister fluids; psoriasis; eczema; prostaglandin E<sub>2</sub>.*

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Cytokines and eicosanoids play a crucial role in the maintenance of homeostasis and regulation of skin inflammation (1–3). Dermal and epidermal cell types constitutively produce various cytokines and eicosanoids, levels of which are regulated by physiological and pathophysiological events. The development of psoriatic or eczematous skin lesions also involves the recruitment of cells from the immune system, and these cell populations also produce inflammatory mediators, adding to the overall orchestrated cellular inflammatory response. Due to the complicated nature of bioregulatory positive and negative feedback mechanisms that exist, the significance and contribution of these inflammatory mediators to the overall pathophysiology of a skin lesion remains unclear.

We measured eicosanoid and cytokine levels in normal skin, sensitive skin and inflammatory skin disease. A number of volunteers from each group were blistered on the volar forearm skin. Levels of 5 pro-inflammatory mediators were measured in blister fluids, namely, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>),

leukotriene B<sub>4</sub> (LTB<sub>4</sub>), interleukin-1 $\alpha$  (IL-1 $\alpha$ ), interleukin-6 (IL-6) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ). By assessing patterns or changes in levels of pro-inflammatory mediator in blister fluids from healthy through lesional skin disease we can better understand the role of such mediators in the generation and/or maintenance of inflammatory skin reactions or lesions.

## MATERIAL AND METHODS

### Subjects

At the Colworth Laboratories, Sharnbrook, Bedford, UK, subject recruitment for the normal group ( $n=28$ ; mean age  $\pm$  SD,  $34 \pm 11$  years) was carried out by self-assessment using a Skin Questionnaire. Subject recruitment for the sensitive skin group ( $n=12$ ; mean age  $\pm$  SD,  $46 \pm 10$  years) was carried out by SP at Oxford Dermatology Clinic. Subject recruitment for the psoriasis ( $n=7$ ; mean age  $\pm$  SD,  $43 \pm 10$  years), atopic eczema ( $n=7$ ; mean age  $\pm$  SD,  $34 \pm 7$  years) and asteatotic eczema ( $n=4$ ; mean age  $\pm$  SD,  $69 \pm 8$  years) skin types was carried out by RP and GRS at the Royal Liverpool University Hospital. Subjects with a history of skin disease (other than psoriasis and eczema for the clinical groups) or skin disorders (keloid scar formation, chloasma, etc) were excluded from the trial. Ethical approval and informed consent was obtained for all procedures.

Normal skin was defined by self-assessment using a questionnaire. Family history and past experience with respect to skin irritancy were assessed. The sensitive skin group comprises patients who describe sensitivity to agents such as cosmetics, detergents and toiletries, some of whom had a history of atopic eczema (50%). Despite this sensitivity patch testing in these patients was negative. No attempt was made, apart from clinical history, to distinguish atopic from non-atopic subjects in this group. The skin was either clear, or in some cases there was mild eczema at sites such as the hands or face, but the sites subjected to blistering had never been involved with eczema. The inflammatory skin disease was defined as lesional psoriatic or eczematous skin. Severity of skin lesion was clinically assessed as mild to moderate. Patients with severe skin lesions were excluded from the study. Within the eczema group volunteers were recruited with both atopic eczema and asteatotic eczema.

### Skin sampling

A total of 58 subjects, who satisfied the inclusion and exclusion criteria and who gave witnessed, written informed consent for the procedures involved, were recruited into the study. All procedures were carried out in accordance with the Declaration of Helsinki (1964) and subsequent amendments. The blister cup of the Dermovac Unit (Instrumentarium Corporation, Helsinki) was placed on the volar forearm of the subject and a negative pressure of approximately 250 mmHg pressure applied for up to 2 h (4, 5). In the normal and sensitive group, blisters were raised on normal, healthy forearm skin. For patients with eczema and psoriasis, blisters were raised on

lesional forearm skin. In the psoriatic group no de-scaling techniques were required on the lesional skin areas. On each site 5 blisters of 6 mm diameter were raised. Blister fluid was removed, stored at  $-70^{\circ}\text{C}$ , and assayed by immunoassay for eicosanoids and cytokines.

#### Immunoassay

$\text{PGE}_2$  and  $\text{LTB}_4$  were assayed by enzyme immunoassay (Amersham kits RPN 222 and RPN 223, respectively). Typically, aliquots of 2–30  $\mu\text{l}$  of blister fluid were used. In both cases this is an indirect assay based upon competition of antigen with a peroxidase-labelled antigen for a limited quantity of a specific antibody. Cytokine levels were measured by enzyme-linked immunosorbent assay of  $\text{IL-1}\alpha$ ,  $\text{IL-6}$  and  $\text{TNF-}\alpha$  (British Biotechnology Quantikine). Due to the very low levels of cytokine in normal skin it is difficult to detect these cytokines by standard kits and high-sensitivity kits were used where possible. Protein concentration in the blister fluids was determined using the Bio-Rad microtitre plate assay.

#### Statistics

As the mean  $\pm$  SD of protein concentrations is similar in each group, all mediators were expressed as pg analyte per mg of protein. Simple statistics (mean, SD, etc.) and Pearson correlation coefficients were carried out for the raw data and the log transformed data. The Wilcoxon rank sum test and one-way analysis of variance was carried out for the log transformed data sets to test for differences between different skin types (results from these 2 different statistical methods were compatible). Stepwise discriminant analysis was carried out where possible on combined clinical data sets.

## RESULTS

#### Comparison of normal, sensitive and clinical groups

It was noted that the time required to blister psoriatic skin was longer than for normal, sensitive or eczema skin types. However, no bleeding or rupture of blisters occurred. A comparison of normal and sensitive skin types showed approximately 3.8-fold higher levels of  $\text{PGE}_2$  ( $p < 0.0002$ ) in the sensitive group (Table I). Levels of  $\text{LTB}_4$ ,  $\text{IL-1}\alpha$ ,  $\text{IL-6}$  and  $\text{TNF-}\alpha$  showed no differences between normal and sensitive skin types. For clinically diseased (eczema/psoriasis) lesional skin 4.7-fold higher levels of  $\text{PGE}_2$  were observed ( $p < 0.0001$ ). Levels of  $\text{LTB}_4$  were increased approximately 6.6-fold ( $p < 0.0001$ ), whereas  $\text{IL-1}\alpha$  was decreased approximately 3.1-fold ( $p < 0.001$ ), in lesional skin compared with normal skin. The cytokines  $\text{IL-6}$  and  $\text{TNF-}\alpha$  levels showed minor changes over the spectrum from normal to diseased skin, but these failed to reach significance. Stepwise discriminant analysis indicates that  $\text{PGE}_2$  and  $\text{LTB}_4$ , but not  $\text{IL-}\alpha$ , can be used to discriminate between the normal, sensitive and inflamed skin types.

#### Comparison of psoriasis and eczema subgroups

Analysis of psoriasis and eczema subgroups showed similar results to the combined data sets for  $\text{PGE}_2$ , i.e. a 4.6-fold increase in  $\text{PGE}_2$  for the psoriasis group and a 4.8-fold increase for the atopic group ( $p = 0.0051$  and  $p = 0.0001$ , respectively, Table I). For  $\text{IL-1}\alpha$  a 3.47-fold and a 2.9-fold decrease was measured in psoriasis and eczema group, respectively ( $p = 0.0049$  and  $p = 0.0186$ , respectively). The levels of  $\text{LTB}_4$  were also increased in both the psoriasis (4.2-fold,  $p = 0.0009$ ) and eczema groups (8.2-fold,  $p = 0.0007$ ),

but the 2-fold difference between the 2 groups was not significant. No significant changes were observed in the levels of  $\text{IL-6}$  and  $\text{TNF-}\alpha$  measured.

#### Comparison of atopic and asteatotic subgroups

Although both  $\text{PGE}_2$  and  $\text{LTB}_4$  are increased in eczematous relative to normal skin, the profile observed suggests that levels of these inflammatory mediators can further discriminate between the atopic and asteatotic eczema subgroups. Although the subject number is too small to show a significant difference, the atopic eczema group shows 2.9-fold higher  $\text{PGE}_2$  levels and 2.5-fold lower  $\text{LTB}_4$  levels, relative to the asteatotic group (Fig. 1).

## DISCUSSION

Only  $\text{PGE}_2$  is able to discriminate between all clinical groups studied, showing a continued increase with progression towards the diseased skin state. An increase in levels of  $\text{LTB}_4$  and a decrease in levels of  $\text{IL-1}\alpha$  are potential markers of skin inflammatory dermatoses, but were not able to distinguish normal from sensitive skin subjects.

There is conflicting evidence in the literature as to the role of  $\text{PGE}_2$  in clinical skin disease, particularly in the case of psoriasis. Some groups have shown an increase in  $\text{PGE}_2$  levels in psoriasis (6, 7), whereas others have observed decreased levels of  $\text{PGE}_2$  (3, 8). Our results agree with the former, showing 4- or 5-fold increases in  $\text{PGE}_2$  levels in psoriasis and eczema ( $p < 0.0001$ ). It is important to note that some of these studies have used tape-stripping of psoriatic skin and measured levels in skin chamber fluids, which is an inappropriate model as tape stripping of skin increases  $\text{PGE}_2$  levels and this baseline may obscure potential differences (9). Furthermore, non-steroidal anti-inflammatory drugs either have no effect on psoriasis or are said to exacerbate the condition (10), and topical  $\text{PGE}_2$  gel can even improve psoriatic lesions (11). However, this may reflect the dual role of  $\text{PGE}_2$  in inflammation, i.e. under appropriate conditions it is pro-inflammatory in the acute phase and anti-inflammatory in the chronic phase. This anti-inflammatory effect probably occurs via cAMP mediated mechanisms.

In agreement with our observations,  $\text{PGE}_2$  levels have been reported to be increased in atopic eczema (12, 13). In a study by Ruzicka et al., it was shown that spontaneous levels of  $\text{PGE}_2$  remained equivalent to healthy skin, but there was an enhanced releasability of  $\text{PGE}_2$  following application of stimuli. In contrast to the work of Ruzicka et al., it was subsequently shown by Fogh et al. (14) that there were increased levels of  $\text{PGE}_2$  in biopsies from patients with atopic inflammatory dermatoses.

The levels of the lipoxigenase product  $\text{LTB}_4$  have been reported to be increased in skin of lesional psoriasis and eczema (12, 14, 15). This is most likely a reflection of neutrophil recruitment, or mast cell activation in the case of atopic eczema, as the epidermis does not produce significant quantities of  $\text{LTB}_4$ .

Although both  $\text{PGE}_2$  and  $\text{LTB}_4$  are increased in eczematous relative to normal skin, the profile observed suggests that levels of these inflammatory mediators can further discriminate between the atopic and asteatotic eczema subgroups. The asteatotic eczema has a different pattern of scaling and is less

Table I. Mediator levels in blister fluids from normal, sensitive and diseased skin types. All data are expressed as pg/mg protein (mean  $\pm$  SD) of mediator levels in blister fluids of volunteers

| Skin type                     | n  | PGE <sub>2</sub> | LTB <sub>4</sub>  | Il-1 $\alpha$    | Il-6            | TNF- $\alpha$   |
|-------------------------------|----|------------------|-------------------|------------------|-----------------|-----------------|
| Normal                        | 28 | 135 $\pm$ 133    | 2.6 $\pm$ 1.47    | 3.26 $\pm$ 2.67  | 1.26 $\pm$ 1.92 | 1.34 $\pm$ 1.21 |
| Sensitive                     | 12 | 513** $\pm$ 390  | n.d.              | 3.17 $\pm$ 2.88  | 0.14 $\pm$ 0.08 | 0.71 $\pm$ 0.28 |
| Diseased (psoriasis + eczema) | 18 | 639** $\pm$ 572  | 17.3** $\pm$ 20.3 | 1.05* $\pm$ 1.08 | 1.14 $\pm$ 1.89 | 1.87 $\pm$ 2.35 |
| Psoriatic subgroup            | 7  | 627* $\pm$ 698   | 10.8** $\pm$ 6.2  | 0.94* $\pm$ 1.6  | 0.49 $\pm$ 0.34 | 1.5 $\pm$ 0.99  |
| Eczematous subgroup           | 11 | 647** $\pm$ 514  | 21.4** $\pm$ 25.1 | 1.12* $\pm$ 0.61 | 1.54 $\pm$ 2.35 | 2.11 $\pm$ 2.95 |

PGE<sub>2</sub> = prostaglandin E<sub>2</sub>; LTB<sub>4</sub> = leukotriene B<sub>4</sub>; Il-1 $\alpha$  = interleukin-1 $\alpha$ ; Il-6 = interleukin-6; TNF- $\alpha$  = tumour necrosis factor- $\alpha$ ; n = number of panellists/ patients.; n.d. = not done.

\*  $p < 0.05$ .

\*\*  $p < 0.001$ .

inflammatory than the atopic eczema. The atopic eczema group shows 2.9-fold higher levels of PGE<sub>2</sub>, whereas the asteatotic eczema shows 2.5-fold higher levels of LTB<sub>4</sub>. This suggests a switch in arachidonate metabolism from the cyclooxygenase pathway in atopic eczema to the lipoxigenase pathway of arachidonate metabolism in asteatotic eczema.

The majority of the work on cytokine levels in psoriatic and eczematous skin has been carried out on *in vitro* and *ex vivo* models (e.g. scale extracts, keratinocyte assays, lymphocyte assays, etc) or by immunocytochemistry. Some of the earlier studies included analysis of suction blister fluid, but the detection limits and specificity of these assays were poor, leading to misinterpretation of data. Over the last few years there has been a dramatic increase in the number of publications and reviews with reference to cytokines in inflammatory dermatoses, both *in vitro* and *in vivo* (16, 17).

Il-1 $\alpha$  is decreased in inflammatory dermatoses, including both psoriasis and eczema. Several groups have shown a decrease in Il-1 $\alpha$  in scale extracts of psoriasis patients (18, 19). Similar observations have been reported in suction blister fluids (20, 21), and in studies on organ cultures from psoriatic patients (22). These data are in good agreement with our own results showing a 3-fold decrease in Il-1 $\alpha$  in diseased skin relative to normal, healthy controls.

Earlier studies have reported an increase in Il-6 in psoriatic lesional skin. This has been observed by immunocytochemical staining (19, 23), and psoralen plus UVA treatment was

shown to cause a concomitant decrease in Il-6 immunostaining with resolution of psoriatic lesion (24). Increased levels of Il-6 were also observed in suction blister fluids (25) in isolated monocytes and in keratinocytes from psoriatic skin (26). Analysis of *in situ* hybridization expression of Il-6 in psoriatic epidermis also showed increased levels of Il-6 mRNA (27). Depending on subgroup analysis (psoriatic vs atopic eczema vs asteatotic eczema) some trends in levels of Il-6 in blister fluids were observed, but these were not significant. Further work is required to assess the role of Il-6 in psoriasis and particularly in atopic eczema, where there are few published reports.

TNF- $\alpha$  showed some differences in levels in blister fluids, but these did not show significant trends. From our own results we conclude that TNF- $\alpha$  does not play a role in psoriasis or eczema. Earlier studies could not detect TNF- $\alpha$  in blister fluids from lesional psoriatic skin, although this was due to the detection limits of bioassay systems available at that time (28). Since then there have been conflicting results as to whether TNF- $\alpha$  is changed in psoriatic lesions or remains at baseline levels, as we have shown. In isolated monocytes from atopic patients there appears to be a decrease in TNF- $\alpha$  levels in phytohaemagglutinin- and lipopolysaccharide-stimulated cells (29). In psoriatic skin, TNF- $\alpha$  and receptor levels were shown to be upregulated by immunolocalization studies (30).

In conclusion, we have demonstrated a stepwise increase in tissue levels of PGE<sub>2</sub> from normal skin through sensitive skin to lesional skin of inflammatory dermatoses. None of the other four inflammatory markers measured was able to discriminate between normal and sensitive skin. The lipoxigenase product, LTB<sub>4</sub>, was elevated in psoriasis with an even greater elevation in eczematous skin. Il-1 $\alpha$  was decreased in lesional psoriatic and eczematous skin, whereas Il-6 and TNF- $\alpha$  remained relatively unchanged. These inflammatory mediators are important in the pathogenesis and pathophysiology of these common skin conditions. Further studies are required to define the sequence and relative importance of these and other key mediators in inflammatory dermatoses, such as psoriasis and eczema.

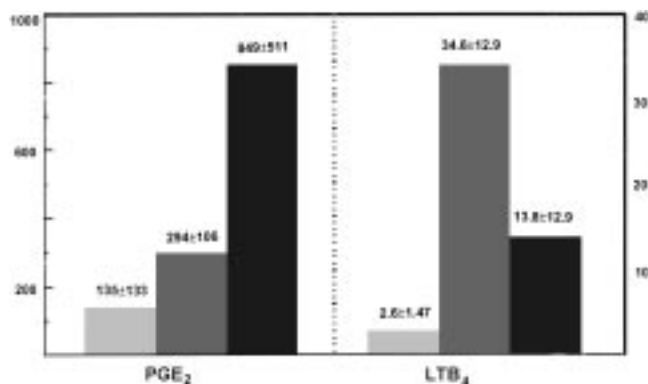


Fig. 1. A comparison of arachidonate metabolites in atopic (dark shading) and asteatotic (medium shading) skin compared to normal skin (light shading). All data are expressed as pg/mg protein (mean  $\pm$  SD) of mediator levels in blister fluids of volunteers. PGE<sub>2</sub> = prostaglandin E<sub>2</sub>; LTB<sub>4</sub> = leukotriene B<sub>4</sub>.

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