Acta Derm Venereol 2000; 80: 171–174

INVESTIGATIVE REPORT

Inflammatory Mediators in Normal, Sensitive and Diseased Skin Types

D. M. REILLY[1], R. PARSLEW[2], G. R. SHARPE[2], S. POWELL[3] and M. R. GREEN[1]

[1]*Biosciences Division, Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, Departments of Dermatology,* [2]*Royal Liverpool University Hospital, Liverpool and* [3]*Churchill Hospital, Old Road, Headington, Oxford, UK*

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_2 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_4 and interleukin-1α showed no differences between normal and sensitive skin types. However, in lesional skin from psoriasis and eczema patients, leukotriene B_4 was increased approximately 6.6-fold ($p < 0.0001$), whereas interleukin-1α was decreased approximately 3.1-fold ($p < 0.001$). Interleukin 6 and tumour necrosis factor-α could not discriminate between skin types. We conclude that only prostaglandin E_2 showed a significant stepwise increase on progression from normal through sensitive and inflammatory skin diseases. Levels of leukotriene B_4 and interleukin-1α were also indicative of disease state and may be important in the pathophysiology of these conditions. ***Key words: blister fluids; psoriasis; eczema; prostaglandin E2.***

(Accepted 31 January, 2000.)

Acta Derm Venereol 2000; 80: 171–174.

David M. Reilly, Cell Biology and Physiology, Unilever Research, Colworth Laboratories, Sharnbrook, Bedford, MK44 1LQ, UK.

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_2 (PGE_2), leukotriene B_4 (LTB_4), interleukin-1α (Il-1α), interleukin-6 (Il-6) and tumour necrosis factor-α (TNF-α). 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 ± SD, 34 ± 11 years) was carried out by self-assessment using a Skin Questionnaire. Subject recruitment for the sensitive skin group ($n = 12$; mean age ± SD, 46 ± 10 years) was carried out by SP at Oxford Dermatology Clinic. Subject recruitment for the psoriasis ($n = 7$; mean age ± SD, 43 ± 10 years), atopic eczema ($n = 7$; mean age ± SD, 34 ± 7 years) and asteatotic eczema ($n = 4$; mean age ± SD, 69 ± 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

 ISSN 0001-5555

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 $-70^{\circ}C$, and assayed by immunoassay for eicosanoids and cytokines.

Immunoassay

PGE_2 and LTB_4 were assayed by enzyme immunoassay (Amersham kits RPN 222 and RPN 223, respectively). Typically, aliquots of 2–30 µl 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 Il-1α, Il-6 and TNF-α (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 ± 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 PGE_2 ($p<0.0002$) in the sensitive group (Table I). Levels of LTB_4, Il-1α, Il-6 and TNF-α showed no differences between normal and sensitive skin types. For clinically diseased (eczema/psoriasis) lesional skin 4.7-fold higher levels of PGE_2 were observed ($p<0.0001$). Levels of LTB_4 were increased approximately 6.6-fold ($p<0.0001$), whereas Il-1α was decreased approximately 3.1-fold ($p<0.001$), in lesional skin compared with normal skin. The cytokines Il-6 and TNF-α levels showed minor changes over the spectrum from normal to diseased skin, but these failed to reach significance. Stepwise discriminant analysis indicates that PGE_2 and LTB_4, but not Il-α, 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 PGE_2, i.e. a 4.6-fold increase in 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 Il-1α 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 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 Il-6 and TNF-α measured.

Comparison of atopic and asteatotic subgroups

Although both PGE_2 and 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 PGE_2 levels and 2.5-fold lower LTB_4 levels, relative to the asteatotic group (Fig. 1).

DISCUSSION

Only 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 LTB_4 and a decrease in levels of Il-1α 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 PGE_2 in clinical skin disease, particularly in the case of psoriasis. Some groups have shown an increase in PGE_2 levels in psoriasis (6, 7), whereas others have observed decreased levels of PGE_2 (3, 8). Our results agree with the former, showing 4- or 5-fold increases in 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 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 PGE_2 gel can even improve psoriatic lesions (11). However, this may reflect the dual role of 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, 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 PGE_2 remained equivalent to healthy skin, but there was an enhanced releasibility of 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 PGE_2 in biopsies from patients with atopic inflammatory dermatoses.

The levels of the lipoxygenase product 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 LTB_4.

Although both PGE_2 and 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 ± SD) of mediator levels in blister fluids of volunteers*

Skin type	*n*	PGE_2	LTB_4	Il-1α	Il-6	TNF-α
Normal	28	135 ± 133	2.6 ± 1.47	3.26 ± 2.67	1.26 ± 1.92	1.34 ± 1.21
Sensitive	12	513** ± 390	n.d.	3.17 ± 2.88	0.14 ± 0.08	0.71 ± 0.28
Diseased (psoriasis + eczema)	18	639** ± 572	17.3** ± 20.3	1.05* ± 1.08	1.14 ± 1.89	1.87 ± 2.35
Psoriatic subgroup	7	627* ± 698	10.8** ± 6.2	0.94* ± 1.6	0.49 ± 0.34	1.5 ± 0.99
Eczematous subgroup	11	647** ± 514	21.4** ± 25.1	1.12* ± 0.61	1.54 ± 2.35	2.11 ± 2.95

PGE_2 = prostaglandin E_2; LTB_4 = leukotriene B_4; Il-1α = interleukin-1α; Il-6 = interleukin-6; TNF-α = tumour necrosis factor-α; *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_2, whereas the asteatotic eczema shows 2.5-fold higher levels of LTB_4. This suggests a switch in arachidonate metabolism from the cyclooxygenase pathway in atopic eczema to the lipoxygenase 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-1α is decreased in inflammatory dermatoses, including both psoriasis and eczema. Several groups have shown a decrease in Il-1α 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-1α 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.

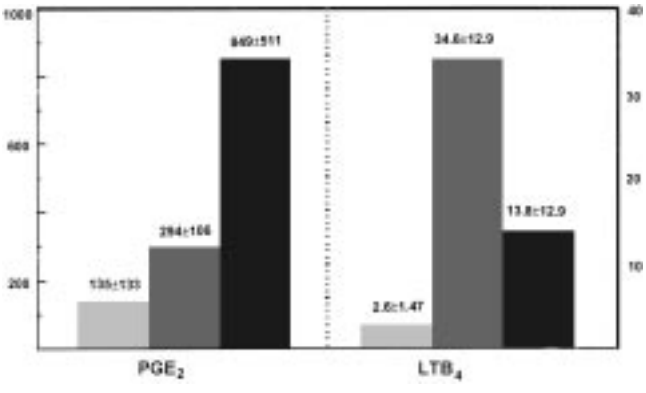

Fig. 1. A comparison of arachidonate metabolites in atopic (dark shading) and asteatotic (medium shading) skin compared to normal skin (light shading). All data are expressed as pg/mg protein (mean ± SD) of mediator levels in blister fluids of volunteers. PGE_2 = prostaglandin E_2; LTB_4 = leukotriene B_4.

TNF-α showed some differences in levels in blister fluids, but these did not show significant trends. From our own results we conclude that TNF-α does not play a role in psoriasis or eczema. Earlier studies could not detect TNF-α 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-α 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-α levels in phytohaemagglutinin- and lipopolysaccharide-stimulated cells (29). In psoriatic skin, TNF-α 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_2 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 lipoxygenase product, LTB_4, was elevated in psoriasis with an even greater elevation in eczematous skin. Il-1α was decreased in lesional psoriatic and eczematous skin, whereas Il-6 and TNF-α 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.

REFERENCES

1. Billingam MEJ. Cytokines as inflammatory mediators. Br Med Bull 1987; 43: 350–370.
2. Salmon JA, Higgs GA. Prostaglandins and leukotrienes as inflammatory mediators. Br Med Bull 1987; 43: 285–296.

3. Greaves MW, Camp RDR. Prostaglandins, leukotrienes, phospholipase, platelet activating factor and cytokines: an integrated approach to inflammation of human skin. Arch Dermatol Res 1988; 280: S33–S41.
4. Kiistala U. Suction blister device for separation of viable epidermis from dermis. J Invest Dermatol 1968; 50: 129–137.
5. Kiistala U, Mustakallio KK. Dermoepidermal separation with suction. Electron microscopic and histochemical study of the initial events of blistering of human skin. J Invest Dermatol 1968; 48: 466–477.
6. Hammerstrom S, Hamberg M, Samuelsson B, Duell EA, Stawiski M, Voorhees JJ. Increased concentrations of non-esterified arachidonic acid, 12L-HETE, prostaglandin E_2 and prostaglandin F2α in epidermis of psoriasis. Proc Natl Acad Sci 1975; 72: 5130–5134.
7. Fogh K, Herlin T, Kragballe K. Eicosanoids in acute and chronic psoriatic lesions: Leukotriene B_4, but not 12-HETE, is present in biologically active amounts in acute guttate lesions. J Invest Dermatol 1989; 92: 837–841.
8. Barr RM, Wong E, Mallet AI, Olins LA, Greaves MW. The analysis of arachidonic acid metabolites in normal, uninvolved and lesional psoriatic skin. Prostaglandins 1984; 28: 57–65.
9. Reilly DM, Green MR. Eicosanoid and cytokine levels in acute skin irritation in response to tape stripping and capsaicin. Acta Derm Venereol 1999; 79: 187–190.
10. Katayama H, Kawada A. Exacerbation of psoriasis induced by indomethacin. J Dermatol 1981; 8: 323–327.
11. Remy W, Sigl MD, Leipold B. Prostaglandin E_2 gel improvement of psoriatic lesions. Pharm Therapeut 1986; 25: 266–268.
12. Ruzicka T, Ring J. Enhanced releasability of prostaglandin E_2 and leukotriene B_4 from leukocytes of patients with atopic eczema. Acta Derm Venereol 1987; 67: 469–475.
13. Ruzicka T, Simmet T, Peskar BA, Ring J. Skin levels of arachidonic acid derived inflammatory mediators and histamine in atopic dermatitis and psoriasis. J Invest Dermatol 1986; 86: 105–108.
14. Fogh K, Herlin T, Kragballe K. Eicosanoids in skin of patients with atopic dermatitis: prostaglandin E_2 and leukotriene B_4 are present in biologically active concentrations. J Allergy Clin Immunol 1989; 83: 450–455.
15. Brain S, Camp R, Dowd P, Black AK, Greaves M. The release of leukotriene B_4-like material in biologically active amounts from the lesional skin of patients with psoriasis. J Invest Dermatol 1984; 83: 70–73.
16. Gearing AJH, Fincham NJ, Bird C, Wadhwa M, Meager A, Cartwright JE, et al. Cytokines in skin lesions of psoriasis. Cytokine 1990; 2: 68–75.
17. Kapp A. Immunologic alterations in atopic eczema – role of cytokines: cytokines in atopic eczema. In: Ruzicka T, Ring J, Przybilla B (eds). Handbook of atopic eczema. Springer, Berlin, 1991: 256–262.
18. Takematsu H, Suzuki R, Tagami H, Kumagai K. Interleukin-1-like activity in horny layer extracts: decreased activity in scale extracts of psoriasis and sterile pustular dermatoses. Dermatologica 1986; 172: 236–240.
19. Takematsu H, Tagami H. Lack of correlation between interleukin 6 and interleukin 1 levels in psoriatic lesional skin. Tohoku J Exp Med 1994; 172: 243–252.
20. Gruaz D, Didierjean L, Grassi J, Frobert Y, Dayer J-M, Saurat J-H. Interleukin 1 alpha and beta in psoriatic skin: enzyme immunoassay, immunoblot studies and effects of systemic retinoids. Dermatologica 1989; 179: 202–206.
21. Takematsu H, Ohmoto Y, Tagami H. Decreased levels of Il-1α and Il-1β in psoriatic lesional skin. Tohoku J Exp Med 1990; 161: 159–169.
22. Yoshinaga Y, Higaki M, Terijima S, Ohkubo E, Nogita T, Miyasaka N, et al. Detection of inflammatory cytokines in psoriatic skin. Arch Dermatol Res 1995; 287: 158–164.
23. Castellas-Rodellas A, Castell JV, Ramirez-Bosca R, Nicolas JF, Valcuende-Cavero F, Thivolet J. Interleukin 6 in normal skin and psoriasis. Acta Derm Venereol 1992; 72: 165–168.
24. Oxholm A, Oxholm P, Staberg B, Bendtzen K. Interleukin 6 in the epidermis of patients with psoriasis before and during PUVA treatment. Acta Derm Venereol 1989; 69: 195–199.
25. Prens EP, Benne K, van Damme J, Bakkus M, Brakel K, Benner R, et al. Interleukin-1 and interleukin-6 in psoriasis. J Invest Dermatol 1990; 95: 121S–124S.
26. Neuner P, Urbanskin A, Trautinger F, Moller A, Kirnbauer R, Kapp A, et al. Increased Il-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 1991; 97: 27–33.
27. Ohta Y, Nishiyama S, Nishioka K. In situ expression of interleukin-6 in psoriatic epidermis during treatment. J Dermatol 1994; 21: 301–307.
28. Takematsu H, Ohta H, Tagami H. Absence of tumour necrosis factor-α in suction blister fluids and stratum corneum from patients with psoriasis. Arch Dermatol Res 1989; 281: 398–400.
29. Kapp A, Textor A, Krutmann J, Moller A. Immunomodulating cytokines in atopic dermatitis and psoriasis: production of tumour necrosis factor and lymphotoxin by mononuclear cells in vitro. Br J Dermatol 1990; 122: 587–592.
30. Kristensen M, Chu CQ, Eedy D, Feldmann M, Brennan FM, Breathnach SM. Localization of tumour necrosis factor-alpha (TNF-α) and its receptors in normal and psoriatic skin: epidermal cells express the 55-kD but not the 75-kD TNF receptor. Clin Exp Immunol 1994; 94: 354–362.