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Blister Fluid Cytokines in Cutaneous Inflammatory Bullous Disorders

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Cytokines are important regulators of immune and inflammatory reactions in the skin, and may contribute to inflammatory blister induction. We examined the profiles of interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) in fluid of spontaneous blisters in the immune-based inflammatory disorders bullous pemphigoid (8 patients), allergic contact dermatitis (5 patients) and toxic epidermal necrolysis (5 patients). These were compared with levels in 9 patients with burns, i.e. inflammatory blisters of non-immune aetiology, and 4 patients with blisters of physical origin. Very high levels of IL-6 were found in bullous pemphigoid and toxic epidermal necrolysis ($p < 0.001$) compared with non-inflammatory and burn blisters. TNF-α levels were high in bullous pemphigoid and burns, but undetectable in non-inflammatory blisters. The pattern in bullous pemphigoid (very high IL-6, high TNF-α) differed substantially from toxic epidermal necrolysis (very high IL-6, low TNF-α), while burns and allergic contact dermatitis showed lesser elevation of both cytokines. Hence, differences in cytokine profiles were identified, although the relevance to underlying pathomechanisms is uncertain. ***Key words: interleukin-6; tumour necrosis factor-α; blister formation.***

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The blistering disorders are characterized by different patterns of cellular infiltration. It is possible that the profile of cytokines in each disorder reflects the cell-type specific effector mechanisms. Interleukin-6 (IL-6) has many functions including activation of T- and B-lymphocytes (1), and has been shown to induce peripheral lymphocyte migration *in vitro* (2). Tumour necrosis factor-α (TNF-α) is another multifunctional cytokine, whose functions include pivotal effects on neutrophil activation and adhesion, also stimulation of T-lymphocytes, prostaglandin release and induction of further cytokines, including IL-6 (3, 4). Both IL-6 and TNF-α additionally stimulate protein synthesis in the acute phase response, which represents a systemic reaction to significant stress of various aetiology (1, 3, 5).

We have examined the blister fluid content of IL-6 and TNF-α in patients presenting with the immune-mediated conditions bullous pemphigoid (BP), allergic contact dermatitis (ACD) and toxic epidermal necrolysis (TEN), and have compared these with the non-immune inflammatory blisters induced by burns, and with physical blisters caused by friction or gravitational oedema. All samples were collected within 24 h of blister formation and before treatment commenced. In order to assess whether blister levels represented local production or plasma-derived transudation, serum levels of the cytokines were examined simultaneously. Our aim was to determine whether disease-related cytokine patterns could be identified, which might give an indication of the cell-type specific effector patterns.

MATERIALS AND METHODS

Subjects

Thirty-one adults (19 men and 12 women) presented with acute skin blistering to the Accident and Emergency or Dermatology departments of the Royal Liverpool University Hospital, over a-3-year period. The blistering conditions comprised BP ($n = 8$), ACD ($n = 5$), TEN ($n = 5$), small localized thermal burns ($n = 9$), and non-inflammatory blistering disorders ($n = 4$). The non-inflammatory blistering conditions were gravitational oedema ($n = 2$) and mechanical friction ($n = 2$). The diagnosis of BP was made following skin biopsy, with the characteristic histological ±immunofluorescence findings. Other conditions were diagnosed on the basis of characteristic clinical features ±skin biopsy.

Blister fluid and blood sampling

Blister fluid and blood samples were taken via needle puncture at the initial presentation of the patients, before treatment was commenced. The blister fluid and serum were stored at −70°C until use.

Cytokine measurement

IL-6 was determined using an in-house radioimmunoassay, the primary antibody being goat anti-human IL-6. Standardization was performed using IL-6 international reference preparation 88/514 (National Institute for Biological Standard and Control, South Mimms, UK). The detection limit of the assay was 90 pg/ml, imprecision <10%. The normal range of serum IL-6 was <15 pg/ml. TNF-α was measured by ELISA technique as previously described (6). This was based on a murine IgG1 monoclonal antibody and a rabbit polyclonal antiserum raised to recombinant human TNF-α (the human TNF-α was a gift from Dr G. R. Adolf, Ernst Boehringer Institut fur Arzneimittel Forschung, Wien, Austria). The normal range in serum was <5 pg/ml.

Statistics

Differences between the samples were assessed by one way analysis of variance (ANOVA).

RESULTS

Interleukin-6

Low levels of blister fluid IL-6 were seen in the non-inflammatory blisters, while the levels were moderately raised in burns and ACD (Table I). BP and TEN showed markedly elevated levels of blister fluid IL-6, which were significantly raised compared with the levels in both burn blisters and non-inflammatory blisters, $p < 0.001$.

Serum IL-6 levels were detectable in all diagnostic categories, but these were always considerably lower than the corresponding blister fluid levels, confirming that the blister cytokine IL-6 contents were attributable to local production.

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Table I. *Cytokine profiles in blister fluid and serum (pg/ml)*

	Blister fluid[a]		Serum[a]	
	IL-6	TNF-α	IL-6	TNF-α
Bullous pemphigoid ($n=8$)	16,188[b] ± 33,099	425 ± 429	1,394 ± 1,678	4 ± 9
Allergic contact dermatitis ($n=5$)	613 ± 491	58 ± 88	100 ± 111	
Burns ($n=9$)	452 ± 545	248.9 ± 385	53 ± 65	3.3 ± 8
Toxic epidermal necrolysis ($n=5$)	51,760[b] ± 43,852	26 ± 40	4,545 ± 2,382	42.5 ± 33
Non-inflammatory blisters ($n=4$)	177 ± 42	0	49 ± 55	0

[a] Data are expressed as mean ± SD.
[b] $p<0.001$ compared with IL-6 levels in burn and non-inflammatory blisters.

TNF-α

The highest levels of TNF-α were seen in BP and burn blisters, although this did not reach statistical significance due to the pronounced inter-subject variation (Table I). TNF-α was only slightly increased in TEN, in marked contrast to the grossly raised levels of IL-6 in this condition. No TNF-α was detectable in the non-inflammatory blisters.

Elevated serum TNF-α levels were seen in TEN, in keeping with the systemic illness that occurs in this disorder.

DISCUSSION

Our observations confirm that different, disease-related patterns of cytokines IL-6 and TNF-α occur in a range of blistering disorders. Since serum levels are low, the cytokines are present in the blister fluids due to local production, rather than transudation. The pattern seen in BP confirms earlier reports of a pronounced increase in blister fluid IL-6 (7–9) and high levels of TNF-α (10). The inter-individual variation in cytokine levels was high, and the magnitude of the IL-6 rise also differs substantially between studies. These differences could reflect age of the blisters (9) or disease severity, and different assay methods using different antibodies, respectively. The undetectable TNF-α in pressure blisters contrasts with levels in suction blisters (10), suggesting the former are a more suitable control since the suction process may itself induce cytokine release.

It is conceivable that the various cytokine patterns may reflect different effector mechanisms used by the respective infiltrating cells. In BP, the autoimmune reaction in the dermo-epidermal junction attracts and activates neutrophils and eosinophils (11, 12). Neutrophil elastase cleaves the lamina lucida (13), but the mechanisms resulting in fluid accumulation are unknown. Although the inflammatory infiltrate is mainly granulocytic, T-cells and macrophages are also found. In TEN, extensive keratinocyte necrosis is associated with accumulation of CD8+ T-lymphocytes and macrophages in the epidermis and CD4+ T-lymphocytes and macrophages in the dermis (14). In ACD, T-lymphocytes of the $CD4^+$ Th1 subtype respond to specific exogenous antigens, producing the characteristic lymphocytic infiltrate in both epidermis and dermis (15).

The cellular patterns of infiltration suggest that IL-6 may be a major product when granulocytes and/or macrophages are involved, i.e. in BP and TEN. Since granulocytes are not a feature of TEN, it seems that IL-6 is more likely derived from the macrophage; alternatively, it may be produced by keratinocytes. It is conventionally held that TNF-α is a major product of macrophages, but BP and TEN blisters contained many macrophages and differed greatly in TNF-α content. Hence, TNF-α may be produced by a different cell type, or the mechanism of release could be multifactorial. In burns and BP, which had the highest TNF-α levels, mast cells are a potential source (13). In ACD, neither cytokine was markedly elevated, although TNF-α has been identified as a mediator in ACD reactions (16); this may be attributable to the short half life of TNF-α.

Disease effector mechanisms are likely to involve several cytokines, and examination of 2 candidates gives only a partial picture. Moreover, individual cytokines have different actions depending on the cytokine microenvironment. Defining the functional contribution of individual cytokines to skin blistering will therefore probably require experimental injection of pure, recombinant materials into skin, or the application of specific cytokine antagonists or anti-cytokine antibodies (5, 17, 18).

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