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Superantigen Staphylococcal Enterotoxin B Induces Release of IL-1β in Human Epidermis

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Lesional skin in patients with inflammatory skin diseases is often colonized with *Staphylococcus aureus*, which is capable of releasing superantigens. We therefore studied whether application of superantigen on the skin led to release of cytokines, especially IL-1β. Suction blisters were raised on vehicle- and superantigen-treated skin and IL-1β protein levels measured in suction blister fluid and supernatant from blister roofs. In all volunteers studied, application of the superantigen Staphylococcal enterotoxin B led to increased release of IL-1β protein from suction blister roofs ($n=7$). In contrast, we did not detect any difference in IL-1β in the blister fluid ($n=5$). IL-1β is known as a mediator of inflammation, and the increase in IL-1β may be involved in the aggravation of inflammatory skin diseases seen following *Staphylococcus aureus* colonization. *Key words: cytokine; Staphylococcus aureus; suction blister; ELISA.*

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Lesional skin in patients with inflammatory skin diseases, such as atopic eczema, is often colonized with *Staphylococcus aureus* (1, 2), which may cause aggravation of the skin disease. Furthermore, there is a link between the degree of colonization of atopic skin with *Staphylococcus aureus* and the intensity of the inflammation (1, 3). It has been shown that staphylococcus isolated from atopic skin produces exotoxins with superantigen properties (4, 5). We have previously demonstrated that the superantigen Staphylococcus enterotoxin B (SEB) induces eczema when applied on human skin (6). Superantigens directly cross-link the antigen-presenting cell with T-cells, which express certain T-cell receptor V-β-chains, leading to polyclonal T-cell activation (7). In addition, superantigens are shown to bind directly to monocytes and macrophages and induce cytokine release, including IL-1β (8, 9). To determine whether superantigen induces cytokine release from cells in the skin, we applied SEB on normal skin. Suction blisters were performed and IL-1β protein levels measured in suction blister fluid and supernatant from blister roofs.

MATERIAL AND METHODS

Subjects

Nine volunteers were recruited after approval had been obtained from the local ethics committee (KA95222). All subjects were aged between 21 and 27 (mean 25) years, with no history of chronic disease.

Superantigen application

Staphylococcal enterotoxin B (SEB) (Toxin Technologies INC, Sarasota, Florida, USA) was dissolved in phosphate-buffered saline (PBS). The subjects were patch tested with SEB and vehicle on the volar aspect of the skin of their forearms. The SEB and vehicle (PBS) were applied using 12-mm-diameter Finn chamber taped on their skin (Epitest Ltd Oy, Tuusula, Finland). Filter disks were soaked with 50 μl of test substance, vehicle or SEB. All volunteers were tested with 25 μg/cm^2 SEB, and 3 volunteers also with 6 and 12 μg/cm^2 SEB. After 24 h, the patches were removed, and the arm was rinsed gently in cold water.

Suction blister

Suction blisters were raised on vehicle- and SEB-treated skin 48 h after the patches were applied. The blister fluid was collected and stored at −70 °C. The roofs were floated in 0.5 ml RPMI with antibiotics, glutamine and 10% human serum and incubated at 37°C in 5% CO_2. After 24 h the supernatants were harvested and stored at −70 °C. IL-1β protein levels in the blister fluid and the supernatant were determined by ELISA (Human IL-1β, Endogen, Cambridge, MA, USA) following the instructions of the manufacturer. The minimal detection limit of
IL-1β was <1 pg/ml.

Statistical analysis

The Wilcoxon matched-pairs signed-rank sum test was used to compare the cytokine concentration in supernatant from vehicle- and SEB-treated skin of each subject. The analysis was performed using SYSTAT for Windows (Systat Inc., Evanston, IL, USA).

RESULTS

IL-1β protein release in vitro following SEB application in vivo

In all volunteers the application of SEB led to induction of dermatitis, as described previously (6). There was no clinical reaction following vehicle application. In 7 out of 9 subjects we were able to obtain suction blister roofs from both SEB- and vehicle-treated skin in the same subject. In 6 out of 9 subjects we were able to obtain suction blister fluid from both SEB- and vehicle-treated skin in the same subject. Application of 25 μg/cm^2 of SEB on human skin led to a significant release of IL-1β in supernatants from suction blister roofs (Fig 1). The IL-1β values are expressed as pg IL-1β per mg wet-weight of the suction blister roofs. The mean value for vehicle-treated skin was 0.08 ± 0.09 pg/mg compared with 1.19 ± 0.77 pg/mg for SEB-treated skin (mean ± SD, $n=7$, $p=0.02$). Application of 6 and 12 μg/cm^2 also led to a slight increase in IL-1β in the supernatant ($n=3$, data not shown).

In contrast to the increase in IL-1β found in the supernatant from the blister roof, we did not find any increase in IL-1β in the suction blister fluid following SEB

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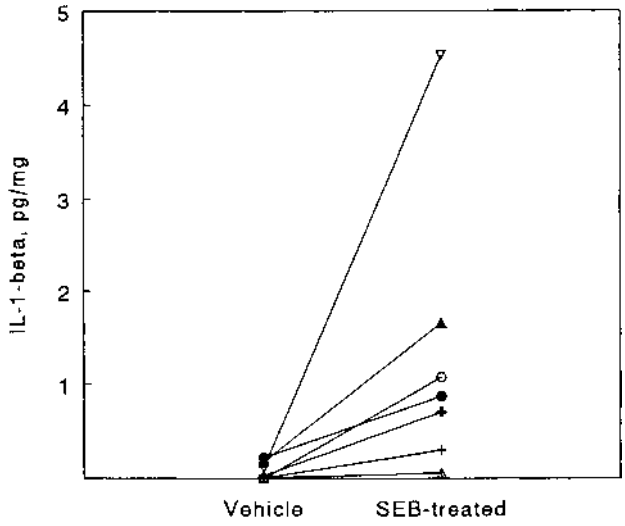

Fig. 1. IL-1β concentration in supernatant from suction blister roof. Normal volunteers were treated with vehicle and Staphylococcus enterotoxin B (SEB) (25 μg/cm^2). After 48 h blisters were raised at the treated sites. The blister roofs were floated in media and the IL-1β protein level in the supernatant was determined using a commercial ELISA kit and expressed as pg/mg roof. Values for vehicle- and SEB-treated skin for the same person are identified via the same symbol connected with a line.

application. In one volunteer the levels of IL-1β were below the detection limit in suction blister fluid from both SEB- and vehicle-treated skin. In the rest, there was no difference in IL-1β levels in blister fluid from SEB-treated (3.8 ± 2.91 pg/ml) and vehicle-treated skin (4.3 ± 2.02 pg/ml) (mean $\pm$ SD, $n = 5$).

DISCUSSION

This is the first study that looks at superantigen application on human skin and induction of IL-1β. Previous studies have shown that superantigens bind directly to blood macrophages and monocytes and induce IL-1β release *in vitro* (8, 9). Here we found that, in all volunteers tested, application of SEB on the skin led to increased release of IL-1β protein from suction blister roofs. We do not know the source of IL-1β; however, since we did not detect any increase in IL-1β concentrations in the suction blister fluid following application of SEB, it is likely that the cytokine is released from cells in the epidermis. However, IL-1β receptors in the blister fluid may also bind released IL-1β. Human monocytes and macrophages are known to be capable of producing bioactive IL-1β and in mice Langerhans' cells are shown to produce IL-1β (10). Until recently, Langerhans' cells and monocytes were believed to be the major source of IL-1β in human skin, since keratinocytes were shown only to produce the 31 IL-1β precursor protein (11) and not the IL-1β converting enzyme. Recent data, however, indicate that inflammatory and immunological stimuli may induce keratinocytes to produce the IL-1β converting enzyme (12). Furthermore, biologically active IL-1β alternative processed through mechanisms not involving the IL-1β converting enzyme, has been found in human epidermis (13). IL-1β is known as a major mediator of inflammation. The increase in IL-1β may be involved in the development of eczema seen following application of SEB and thereby involved in the aggravation of inflammatory skin diseases induced by superantigen-producing *Staphylococcus aureus.*

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