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.

(Submitted August 10 1999.)
L. Skov, Department of Dermatology, Gentofte Hospital, University of Copenhagen, Niels Andersens Vej 65, DK-2900 Hellerup, Denmark. E-mail: losk@gentoftehosp.kbhamt.dk

**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/cm² SEB, and 3 volunteers also with 6 and 12 μg/cm² 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% CO₂. 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/cm² 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/cm² 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.
converting enzyme (12). Furthermore, biologically active IL-1 released by keratinocytes may induce IL-1 receptors in the skin to produce the IL-1 receptor. However, inflammatory and immunological stimuli may induce keratinocytes to produce the IL-1 receptor, and this 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.

**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 human skin led to increased release of IL-1β protein from suction blister roofs. We do not know the source of IL-1β; however, in the rest, there was no difference in IL-1β levels in blister fluid from SEB-treated skin. In the rest, there was no difference in IL-1β levels in blister fluid from SEB-treated skin. In the rest, there was no difference in IL-1β levels in blister fluid from SEB-treated skin (3.8 ± 2.91 pg/ml) and vehicle-treated skin (4.3 ± 2.02 pg/ml) (mean ± SD, n = 5).

**ACKNOWLEDGEMENT**

This work was in part supported by the Danish Research Council 9602307.

**REFERENCES**