

Streptococcal and Staphylococcal Superantigens (ETA, SEB): Presentation by Human Epidermal Cells and Induction of Autologous T Cell Proliferation *In vitro*

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Streptococcal and staphylococcal toxins are responsible for skin-related clinical conditions, e.g. scarlet fever and toxic shock syndrome. Skin involvement may result from a hypersensitivity reaction to these toxins; however, their precise mode of action has still to be elucidated. The aim of the present study was to investigate the capacity of human epidermal cells to present streptococcal erythrogenic toxin A (ETA) or staphylococcal enterotoxin B (SEB) to autologous T-lymphocytes *in vitro*. We found a significant T-lymphocyte proliferation response to minute amounts of ETA ($p < 0.01$) and SEB ($p < 0.02$) when co-cultured with freshly isolated autologous human epidermal cells. We suggest that human skin may serve not only as an entry for microbial toxin-producing strains but also as an important target for streptococcal and staphylococcal toxin-binding and subsequent T cell proliferation. **Key words:** Erythrogenic toxin A; Staphylococcal enterotoxin B; Scarlet fever; Toxic shock syndrome; Pathogenesis.

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Streptococcal erythrogenic toxins and staphylococcal enterotoxins are of dermatological interest because they may cause scarlet fever and toxic shock syndrome. In addition to their toxic skin effects (1, 2), they may cause profound alterations in the immune system homeostasis (3). Streptococcal and staphylococcal toxins belong to the recently characterized group of immunocytotropic bacterial superantigens that are potent mitogens for human T cells (3, 4). They bind to MHC class II molecules and stimulate the development of large quantities of T cells bearing particular TCR V β gene products (5). The subsequent release of a variety of cytokines may play a major role in the pathogenesis of scarlet fever and toxic shock syndrome (3, 6, 7). It was the aim of the present study to investigate the capacity of human epidermal cells to present streptococcal erythrogenic toxin A (ETA) and staphylococcal enterotoxin B (SEB) to autologous human T-lymphocytes.

MATERIAL AND METHODS

Sheets of epidermis were obtained from 5 healthy adult volunteers using a suction blister device (8). Informed consent was obtained from all the volunteers. The epidermal sheets were dissociated by incubating with collagenase (type "Worthington" CLSII 135 U/mg, Biochrom, Germany) and DNase (20 μ g/ml, Biochrom, Germany) for 3 h at 37°C (9). Peripheral blood mononuclear cells (PBMC) were first

obtained by centrifugation on ficoll density gradient. T-lymphocyte-enriched suspensions were then prepared by rosetting with neuraminidase-treated sheep erythrocytes (10, 11). 50,000 T-lymphocytes were co-cultured with 10 μ g/ml ETA and 10 μ g/ml SEB either with or without autologous epidermal cells for 4 days at 37°C, 5% CO₂. A control series was undertaken with concanavalin A (ConA, 5 μ g/ml) and in the absence of ETA, SEB, epidermal cells (EC) or T-lymphocytes. ETA was isolated from culture supernatants of *Streptococcus pyogenes*, NY 5-Dochez strain, and purified as previously described (12, 13). SEB was purchased from Sigma, Germany. RPMI 1640 with glutamine (Biochrom, Germany), 10% FCS and 1% penicillin / streptomycin (10,000 IE/ml / 10,000 μ g/ml, Biochrom, Germany) served as culture medium. 3H-thymidine (0.1 μ Ci/well, NEN-Dupont, Germany) was added 4 h before harvesting, which was performed with a semiautomatic cell harvester (Skatron, Norway). Incorporation was measured by means of a liquid scintillation counter (Zinsser, Germany) and is expressed as mean DPM \pm SE of triplicates. Statistical analysis was performed by using Student's *t*-test, with $p < 0.05$ indicating significant differences.

RESULTS

For both superantigens (ETA, SEB), there was a significant increase in lymphocyte proliferation response when epidermal cells were co-cultured (ETA $p < 0.01$; SEB $p < 0.02$). Fig. 1 shows the influence of epidermal cells (EC) added to the cultures on the T-lymphocyte response to ETA, SEB and

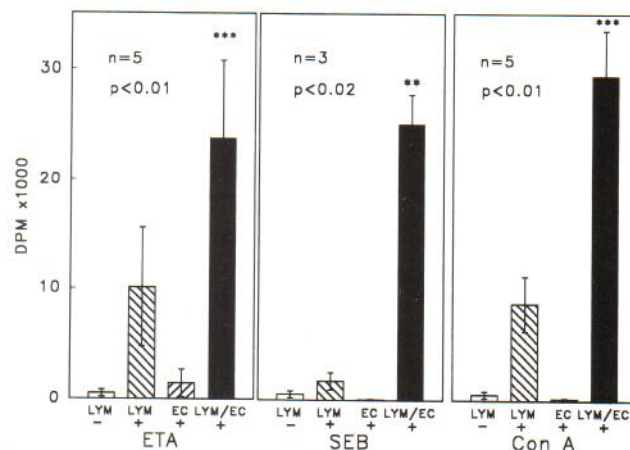


Fig. 1. Proliferation response of blood-derived human T cells co-cultured with erythrogenic toxin A or enterotoxin B and with or without autologous epidermal cells. LYM: T-lymphocytes; ETA: erythrogenic toxin A; SEB: staphylococcal enterotoxin B; EC: epidermal cells; Con A: concanavalin A.

ConA. The results represent the mean values of 3 (SEB) and 5 (ETA, ConA) experiments.

DISCUSSION

Our results show that minute amounts of erythrotoxic toxin A (ETA) derived from *Str. pyogenes* A and staphylococcal enterotoxin B (SEB), derived from *S. aureus*, induce significant enhancement of T cell response in the presence of autologous epidermal cells. Both toxins belong to the group of "microbial superantigens" (3). They, unlike other microbial antigens, can stimulate T-lymphocytes after they have bound to MHC class II-bearing cells but without prior internalization and processing (4). In contrast to these findings, Kushnaryov and co-workers reported toxic shock syndrome toxin 1 (TSST-1) internalization by receptor-mediated endocytosis in human epidermis (14). Though we have no direct proof, it is possible that ETA and SEB act as "superantigens" in the test system used.

Epidermal Langerhans' cells are well known as MHC class II-expressing antigen-presenting cells (15). They do not require the presence of significant numbers of keratinocytes to exert this function after stimulation by microbial antigens (e.g. *Candida albicans* and HSVI) (16). Langerhans' cells were a possible candidate for binding and presentation of ETA and SEB in our experiments.

Human keratinocytes express MHC class II molecules after stimulation by IFN γ (17), and HLA-DR expression on keratinocytes is a common phenomenon in diseased skin (18, 19). Only recently we were able to demonstrate binding of ETA to OKT6-positive and OKT6-negative human epidermal cells (20). Therefore, we cannot exclude the possibility that keratinocytes were involved in our experiments. Further investigations with isolated Langerhans' cells and keratinocytes are needed.

In conclusion, our data suggest that human skin may serve not only as an entry for microbial toxin-producing strains but also as an important target for streptococcal and staphylococcal toxin-binding and subsequent T cell stimulation. This mechanism may play a role in the pathogenesis of skin involvement in scarlet fever and toxic shock syndrome. A *Str. pyogenes*-derived keratinocyte- and lymphocyte-proliferating factor has been purified and characterized (21). This proliferating factor has the same physicochemical properties as erythrotoxic toxin A. Femtomolar concentrations of erythrotoxic toxin A and C induce significant T cell proliferation response in guttate psoriasis (22). It is therefore interesting to speculate whether erythrotoxic toxins play a role in streptococcal-induced psoriasis, e.g. via binding to human epidermis and subsequent T cell proliferation.

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