LETTERS TO THE EDITOR

Enhanced Release of Interleukin-8 from Human Epidermal Keratinocytes in Response to Stimulation with Trichophyton In vitro

Sir,

Although dermatophytes generally invade the most superficial layers of the skin or its appendages, these infections may sometimes represent an acute inflammatory response that is characterized by an accumulation of neutrophils beneath the stratum corneum. The mechanism by which neutrophils are attracted to the sites of dermatophyte infection has already been partially clarified (1). Dermatophytes have been shown to be capable of activating the complement system by an alternative pathway, which thus produces chemotactic activity for neutrophils. In addition, dermatophytes also produce chemotactic factors by themselves.

It has recently been found that keratinocytes can synthesize and release significant amounts of the proinflammatory cytokine interleukin-8 (IL-8) upon stimulation with a variety of environmental agents (2-4). IL-8 is a potent chemotactic agent for neutrophils and can also activate neutrophils after they have arrived at the sites of infection. The production of IL-8 by keratinocytes during a dermatophyte infection could thus result in the recruitment of neutrophils to the lesions of such a dermatophyte infection. Therefore, in order to study the role of IL-8, we stimulated normal human epidermal keratinocytes (NHEK) with trichophyton, a potent dermatophyte antigen, to investigate whether or not the release of IL-8 after stimulation in vitro can be enhanced.

Secondary cultures of commercially available NHEK derived from foreskins (Epipack, Clonetics Corp., San Diego, CA, U.S.A) were grown in culture using a defined keratinocyte growth medium (KGM) at 37°C in a humidified atmosphere of 5% CO₂ in air. Trichophyton was prepared using Trichophyton mentagrophytes SM 0111=RV 27961 (Arthrodema vanbreuseghemii), and this trichophyton could induce cytokines from cultured peripheral blood mononuclear cells, as reported previously (5). NHEK were cultured in 6-well plates at a seeding density of 5,000 cells/cm² in KGM. When the cells were 70-80% confluent, fresh medium was added, and then the keratinocytes were exposed to trichophyton (5-50 μg/ml) for 24 h. Cell-free supernatants were collected and stored frozen at -80°C. IL-8 concentrations in the culture supernatant were determined by ELISA (Toray Fuji Bionics Corp., Tokyo, Japan).

The level of IL-8 in culture supernatant of unstimulated NHEK was low. But the release of IL-8 was observed to markedly increase after 24 h of in vitro stimulation with trichophyton (Fig. 1). The optimal stimulation concentration of trichophyton was 25-50 μg/ml.

The initiation of skin inflammation involves the release of a number of proinflammatory cytokines. However, in a dermatophyte infection it is not known whether trichophyton-stimulated keratinocytes directly participate in the amplification of inflammation by producing one or more of these cytokines. The present study thus showed that the stimulation of trichophyton significantly enhanced the release of IL-8 from keratinocytes.

The mechanism of IL-8 enhancement by trichophyton is unclear. Trichophyton may be directly responsible for enhancing IL-8 release from keratinocytes. It is also possible that trichophyton can induce TNF-α or IL-1, which can then bind to keratinocytes and enhance IL-8 release.

A small amount of IL-8 was released from NHEK without trichophyton in the present culture, and this finding correlates with a previous report in which it was shown that NHEK proliferating in KGM can release IL-8 without any added agents (6).

Although various components of the host-dermatophyte relationship have been explored, the initial phase of infection, the contact of dermatophyte antigen to keratinocytes, has not yet been investigated in detail. Our study concentrated on the interaction between dermatophyte antigen and keratinocytes in order to account for the accumulation of neutrophils beneath the stratum corneum. The capacity of trichophyton-stimulated keratinocytes to release an enhanced level of IL-8 thus suggests that these cells can indeed help induce the acute inflammatory response seen in dermatophyte infection. It therefore appears that keratinocytes not only play an important structural role in the formation of a physical barrier to dermatophytes but may also play an important functional role in initiating cutaneous inflammatory reactions, which might be involved in the host defense against dermatophytes (1).

REFERENCES

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Fig. 1. IL-8 release by human epidermal keratinocytes in response to trichophyton in vitro.
Transitory Hyperpigmentation after Calcipotriol Ointment and PUVA Therapy in Psoriatic Patients

Sir,

In 1995, Kokelj et al. (1) reported 3 patients who had developed hyperpigmentation after a combined treatment of calcipotriol ointment and heliotherapy. It seems interesting to communicate that we have also encountered secondary hyperpigmentation after a combined treatment with calcipotriol and PUVA. As far as we know, there are no reports of a similar side-effect in patients treated with calcipotriol ointment and exposed to ultraviolet A or B radiation.

We have observed 2 patients treated with calcipotriol ointment and PUVA for psoriasis vulgaris, who developed obvious hyperpigmentation around the psoriatic plaques. No other associated symptomatology was found and erythema, pruritus or irritation were not evident prior to the pigmentation. In both cases, calcipotriol ointment (50 mcg/g, Daivonex (r)) was used twice a day. The patients also applied calcipotriol on the morning of PUVA treatment. PUVA therapy was given 3 times weekly; oral 8-methoxypsoralen was given 0.6 mg/kg 2 h before UVA irradiation, and in both cases the initial UVA dosage was 2 J/cm, with an increment of 0.5 J/cm at each treatment.

The first patient was a 54-year-old man with phototype III, who had suffered psoriasis vulgaris for 8 years. Pigmentation was observed after 18 PUVA treatments. The cumulative UVA dose was 112 J/cm. Calcipotriol had then been applied for 50 days. The second patient was a 42-year-old man with phototype IV, who had suffered psoriasis vulgaris for 20 years. When the pigmentation was evident, he had received 11 PUVA treatments. The cumulative UVA dose was 48 J/cm. Calcipotriol was applied for 25 days. No other systemic or biochemical abnormalities were detected in either of the patients and other secondary effects of 8-methoxypsoralen or UVA were not recorded. In both cases, the pigmentation subsided in less than 1 month after the treatment was stopped.

We think that, as in the cases described by Kokelj et al. (1), the pigmentation observed in our patients is probably secondary to the treatment and not a post-inflammatory hyperpigmentation since: 1) this pigmentation was present only around the calcipotriol-treated plaques; 2) both patients displayed some untreated plaques at the top of the back which did not show any obvious hyperpigmentation; 3) the patients had used both calcipotriol ointment and PUVA as single treatments without the presence of such hyperpigmentation; 4) neither of the patients had received any other systemic or topically applied drug and we found no other reasons that could explain the hyperpigmentation; and 5) as Kokelj et al. have pointed out, post-inflammatory hyperpigmentation is unusual after psoriasis.

REFERENCE


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