Acta Derm Venereol (Stockh) 1996; 76: 399–415

LETTERS TO THE EDITOR

Enhanced Release of Interleukin-8 from Human Epidermal Keratinocytes in Response to Stimulation with Trichophytin *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 chemoattractant 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 trichophytin, 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% CO_2 in air. Trichophytin was prepared using *Trichophyton mentagrophytes* SM 0111=RV 27961 (*Arthroderma vanbreuseghemii*), and this trichophytin 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/cm^2 in KGM. When the cells were 70–80% confluent, fresh medium was added, and then the keratinocytes were exposed to trichophytin (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).

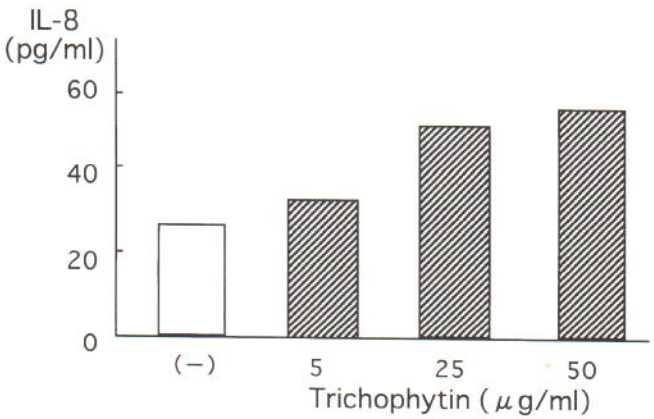

Fig. 1. IL-8 release by human epidermal keratinocytes in response to trichophytin in vitro.

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 trichophytin (Fig. 1). The optimal stimulation concentration of trichophytin 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 trichophytin-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 trichophytin significantly enhanced the release of IL-8 from keratinocytes.

The mechanism of IL-8 enhancement by trichophytin is unclear. Trichophytin may be directly responsible for enhancing IL-8 release from keratinocytes. It is also possible that trichophytin 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 trichophytin 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 trichophytin-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

1. Wagner DK, Sohnle PG. Cutaneous defenses against dermatophytes and yeasts. Clin Microbiol Rev 1995; 8: 317–335.
2. Kondo S, Kono T, Sauder DN, McKenzie RC. IL-8 gene expression and production in human keratinocytes and their modulation by UVB. J Invest Dermatol 1993; 101: 690–694.
3. Mohamadzadeh M, Müller M, Hultsch T, Enk A, Saloga J, Knop J. Enhanced expression of IL-8 in normal human keratinocytes and human keratinocyte cell line HaCaT in vitro after stimulation with contact sensitizers, tolerogens and irritants. Exp Dermatol 1994; 3: 298–303.
4. Wilmer JL, Burleson FG, Kayama F, Kanno J, Luster MI.

Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemical-induced inflammation in mouse skin. J Invest Dermatol 1994; 102: 915–922.
5. Koga T, Ishizaki H, Matsumoto T, Hori Y. In vitro release of interferon-γ by peripheral blood mononuclear cells of patients with dermatophytosis in response to stimulation with trichophytin. Br J Dermatol 1993; 128: 703–704.
6. Takematsu H, Tagami H. Mode of release of interleukin-8 from proliferating human epidermal keratinocytes in vitro. Exp Dermatol 1993; 2: 121–124.

Accepted March 13, 1996.

Tetsuya Koga,[1] Hiroshi Ishizaki,[2] Tadahiko Matsumoto[3] and Shoji Toshitani[1]
Departments of Dermatology, [1]School of Medicine, Fukuoka University, Fukuoka 814–80, [2]Kanazawa Medical University, Ishikawa, and [3]Toshiba Hospital, Tokyo, Japan