Expression of Functional Toll-Like Receptors on Cultured Human Epidermal Keratinocytes

To the Editor:

I read with interest the article by Song *et al* (2002). The authors showed that cultured human epidermal keratinocytes constitutively express CD14 and Toll-like receptor 4 (TLR4), and respond to lipopolysaccharide (LPS) in a CD14- and TLR4-dependent manner (Song *et al*, 2002). Recently, we reported that cultured human epidermal keratinocytes express functional TLR2 (Kawai *et al*, 2002). In contrast to the findings of Song *et al* (2002), we could not detect CD14 or TLR4 expression on cultured human epidermal keratinocytes (Kawai *et al*, 2002). We found that the LPS response of keratinocytes depends on TLR2, rather than CD14 or TLR4 (Kawai *et al*, 2002). Because keratinocytes do not respond to repurified LPS or lipid A (Kawai *et al*, 2002), the LPS response of keratinocytes may be mediated by TLR2-activating non-LPS bacterial components contaminating in commercial LPS preparations.

The discrepancy might be related to the different culture conditions of human epidermal keratinocytes. We have shown that cultured human epidermal keratinocytes can be induced to express TLR4 in response to TLR2-activating bacterial components (Kawai *et al*, 2002). Addition of serum to the growth medium may also regulate TLR4 expression on keratinocytes *in vitro* (Kawai *et al*, 2002). Furthermore, we have demonstrated that normal human epidermal keratinocytes express both TLR2 and TLR4 *in vivo* (Kawai *et al*, 2002). These observations indicate that cultured human epidermal keratinocytes do not express TLR4 constitutively, but culture conditions can modulate TLR4 expression on keratinocytes *in vitro*. Although normal human epidermal keratinocytes cultured in the serum-free growth medium were examined in both studies, serum might be used for the isolation and subculture of keratinocytes and could induce TLR4 expression in the study by Song *et al* (2002). Alternatively, in the study by Song *et al* keratinocytes might be activated through TLR2 with contaminating bacteria/fungi or necrotic cells, which stimulate cells through TLR2 (Li *et al*, 2001).

Our data are consistent with a previous study showing that CD14 is not expressed on cultured human epidermal keratinocytes (Hunyadi *et al*, 1992); however, keratinocytes can be induced to express CD14 *in vitro* in response to interferon-γ (Hunyadi *et al*, 1993). Similarly, CD14 is not expressed on human epidermal keratinocytes in normal skin (Hunyadi *et al*, 1992; Kawai *et al*, 2002), but is expressed on epidermal keratinocytes in several inflammatory skin diseases (Hunyadi *et al*, 1993).

In conclusion, the contradictory data for CD14 and TLR4 expression on cultured human epidermal keratinocytes obtained in these studies might be due to the different culture conditions. These studies, however, clearly demonstrate that human epidermal keratinocytes are able to express functional CD14 and TLRs, and together support the important role for keratinocytes in innate immune responses in the skin.

Kazuhiro Kawai
Department of Dermatology, Niigata University School of Medicine, Niigata, Japan

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Address correspondence and reprint requests to: Dr Kazuhiro Kawai, Department of Dermatology, Niigata University School of Medicine, 1-757 Asahimachi-dori, Niigata 951-8510, Japan. Email: Kawai@med.niigata-u.ac.jp.

Human Normal Keratinocytes Express Biologically Functional CD14 and Toll-Like Receptors

To the Editor:

The role of particular molecular components of the innate immune system in the epidermis is a new and exciting topic in cutaneous immunology. Our group has recently shown that normal human keratinocytes are capable of expressing functional CD14 and Toll-like receptor 4 (TLR4) (Song *et al*, 2002). Keratinocytes were found to express constitutively CD14 and TLR4 mRNA that was augmented by exposure to lipopolysaccharide (LPS). Cell surface expression of keratinocytes CD14 and TLR4 was detected by flow cytometry. LPS binding to keratinocytes CD14 and TLR4 resulted in a rapid intracellular calcium response, nuclear factor-κB nuclear translocation, and the secretion of proinflammatory cytokines and chemokines. We have also shown that human keratinocytes express TLR2 mRNA (Song *et al*, 2001) and that cell surface expression of TLR2 can be demonstrated by flow cytometry (unpublished observation).

The letter by Kawai (2003) raises some important and expected points about how cell culture conditions may alter the constitutive expression of Toll-like receptors and CD14 on human keratinocytes. It is well established that these cells are both extremely sensitive and responsive to culture conditions. We would like to clarify, however, that we did not utilize any type of serum in the isolation or subculture of keratinocytes in our studies. In our study examining calcium influx following stimulation with LPS, which was abrogated by pretreatment with blocking antibodies to CD14 or TLR4, human serum 0.1% was added during the experiment as a source of LPS binding protein. LPS binding pro-

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Address correspondence and reprint requests to: Peter I. Song, Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, U.S.A. E-mail: p-song@northwestern.edu

tein acts as a lipid transfer protein that facilitates the binding of LPS monomers to cellular CD14 (Schumann *et al*, 1990; Wright *et al*, 1990). Our observation that human serum 0.1% alone did not induce keratinocyte calcium influx suggests that other components that may be present in serum were not playing a significant part in the observed results of calcium influx following the addition of LPS. Fortunately, LPS binding protein is now available as a recombinant protein so that it can be utilized directly in subsequent studies.

The studies by both our group and by Kawai and coworkers continue to demonstrate the presence and biologic significance of components of the innate immune system on keratinocytes. The precise role of each molecule in responding to the myriad types of external insults faced by the epidermis will be the focus of many investigators for years to come.

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Peter I. Song, Cheryl A. Armstrong, and John C. Ansel
Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

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