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.

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REFERENCES


Manuscript received October 15, 2002; accepted for publication November 18, 2002
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LETTERS TO THE EDITOR

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-