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Increased Expression of CD44 and Hyaluronate Synthase 3 Is Associated with Accumulation of Hyaluronate in Spongiotic Epidermis

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TO THE EDITOR

Eczema is one the most common skin diseases. At the histopathological level, epidermal spongiosis is the hallmark of eczema. In spongiosis, keratinocytes lose cohesiveness with a decreased expression of cadherins, as a water influx into the epidermal intercellular spaces occurs together with an accumulation of hyaluronate (HA; Ohtani *et al.*, 2009). This observation is in agreement with recent reports in which increased HA production and hyaluronate synthase 3 (HAS3) expression was shown to be associated with inflammation in epithelia, as a result of a positive regulation by inflammatory cytokines *in vitro* and *in vivo* (Sayo *et al.*, 2002; Ohtani *et al.*, 2009; Chow *et al.*, 2010; Mack *et al.*, 2011). In epidermis, HA is naturally present in the intercellular spaces (Tammi *et al.*, 2005). Among the three known HAS variants, HAS3 is the most evident one in epidermis (Tien and Spicer, 2005; Ohtani *et al.*, 2009; Mack *et al.*, 2011). Although several recent data point out HAS3 as a possible key factor in inflammatory processes, its expression pattern at the protein level remains unclear in normal human epidermis (Bai *et al.*, 2005; Mack *et al.*, 2011). In this study we explored the expression of HAS3, HA and its major cell surface receptor, CD44, by immunohistochemistry.

We stained paraffin-embedded skin sections of seven spongiotic lesions and seven healthy donors for CD44 and HA. All spongiotic lesions showed a stronger CD44 expression and an accumulation of HA when compared with healthy skin (Figure 1). Whereas eight other cryopreserved spongiotic lesions were stained for HAS3. The HAS3 staining in normal skin revealed a weak but detectable spotty signal at the intercellular junctions. This staining pattern fits well with the known membrane localization of HAS3, as this enzyme extrudes the newly synthesized HA through the cytoplasmic membrane (Itano and Kimata, 2002; Rilla *et al.*, 2005). In normal epidermis, HAS3 seemed to be expressed in all the layers with a weaker or absent signal in the basal layer, and a stronger signal in the suprabasal layers. This expression pattern is very similar to the one of HA and CD44. In spongiotic epidermis, the HAS3 signal was clearly increased in all spongiotic lesions with spots significantly larger in the suprabasal layers when compared with normal skin (Figure 2).

These results show the unreported expression pattern of HAS3 in normal human epidermis and confirm that HAS3 is strongly induced during inflammation, as recently shown in mouse models (Mack *et al.*, 2011). However, although HAS3 is the most evident HAS variant in the epidermis, any role of HAS1 and HAS2 in human epidermal inflammation still remains to be investigated (Sayo *et al.*, 2002; Ohtani *et al.*, 2009). HAS3 probably has a specific role in human epidermis and in epidermal inflammation. Its expression pattern shows a stronger signal in the suprabasal layers, suggesting that HAS3 may be one of the key factors in the keratinocyte terminal differentiation. In spongiosis, HAS3 expression was already shown to be increased but only by *in situ* hybridization detecting the HAS3 RNA (Ohtani *et al.*, 2009). Here we confirm that the expression of the HAS3 protein is also significantly increased in spongiosis. We also found a strong HA accumulation and a strong CD44 expression in spongiotic epidermis, a result consistent with such an increased HAS3 expression.

Abbreviations: HA, hyaluronate; HAS3, hyaluronate synthase 3

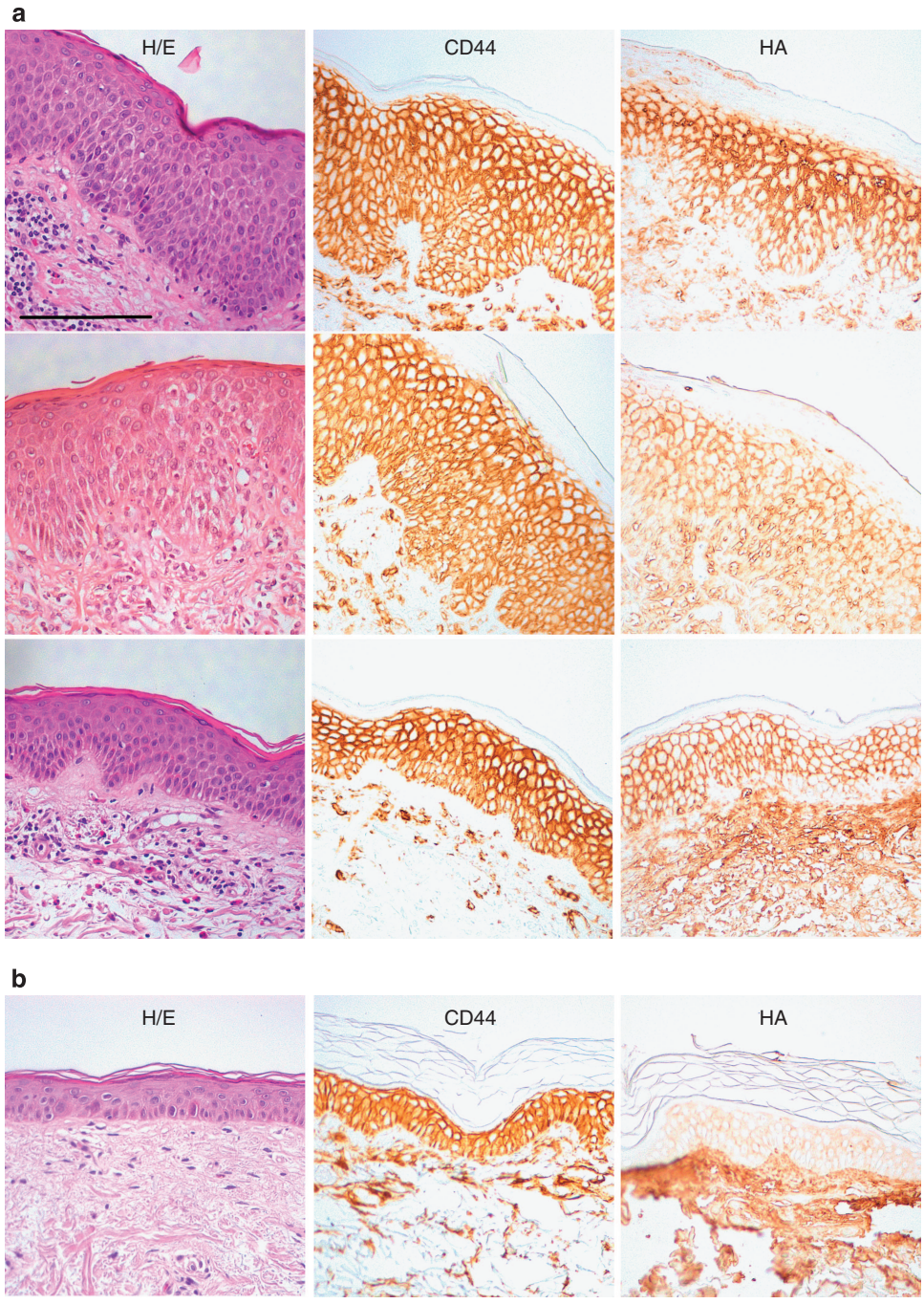

Figure 1. Increased CD44 expression and hyaluronate (HA) accumulation in spongiotic human epidermis. Paraffin-embedded sections of spongiotic (**a**) and normal skin (**b**) were stained with hematoxylin/eosin, an anti-human CD44 antibody (CD44), and a biotinylated HA binding protein. CD44 staining was performed using an anti-human CD44 antibody (Clone F10-44-2, mouse anti-human CD44, MCAP89, AbD Serotec, Dusseldorf, Germany), followed by an anti-mouse secondary antibody (Vectastain Universal Elite ABC kit PK-6200, Vector Laboratories, Burlingame, CA). HA was stained with a biotinylated hyaluronate binding protein (HA, B-HABP 400763 Seikagaku Biobusiness, Tokyo, Japan). Both the immunostainings were visualized using 0.05% 3,3′-diaminobenzidine (Sigma-Aldrich, St Louis, MO). Bar = 150 μm.

HA accumulation has been shown to be associated with a stronger expression of CD44 (Kaya *et al.*, 2005, 2006; Barnes *et al.*, 2010). It seems that increased CD44 expression is necessary as a feedback mechanism to uptake the excess of HA in tissue (Culty *et al.*, 1992; Kaya *et al.*, 1997). This HA uptake is probably crucial for the resolution of inflammation, as shown in lung models (Teder *et al.*, 2002). This HA accumulation may obviously explain a water influx into the epidermal intercellular spaces due to the high water-retention properties of this polysaccharide. We assume that a transcriptional activation of the *has3* gene causes the increased expression

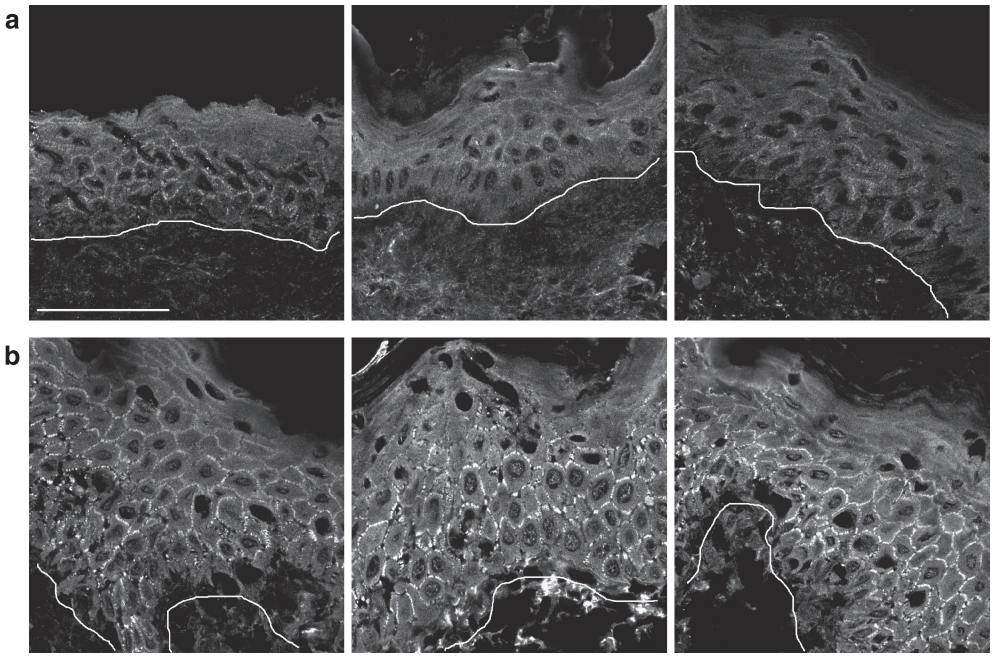

Figure 2. Hyaluronate synthase 3 (HAS3) expression is increased in spongiotic epidermis. Cryosections of healthy (**a**) and spongiotic (**b**) epidermis were stained for HAS3 (rabbit polyclonal antibody, sc-66917, Santa Cruz Biotechnology, Santa Cruz, CA). An anti-rabbit secondary antibody conjugated with Alexa Fluor 488 dye (Molecular Probes, Life Technologies, Paisley, UK) was used to visualize HAS3. Bar = 70 μm.

of HAS3. Several lines of evidence indicate that HA production and HAS3 expression are upregulated by proinflammatory factors (Sayo *et al.*, 2002; Bai *et al.*, 2005; Mack *et al.*, 2011). In lung epithelia, HAS3 has also been shown to be strongly involved in the development of inflammation (Bai *et al.*, 2005). Thus, even if no direct positive regulation by proinflammatory transcription factors of *has3* gene promoter has been shown to date, such a regulation is highly suspected. In total, our results demonstrate the HAS3 expression pattern in normal epidermis and in spongiosis, and underline a probable crucial role for HA in epidermal homeostasis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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