(Supplementary Figure S2 online). Here, the largest difference concerned 4-fold lowered anti-IsaA levels in wound fluid. This implies that future studies on anti-staphylococcal immune responses in EB patients can be based on noninvasively sampled wound fluid.

In conclusion, EB patients are highly challenged with very diverse *S. aureus* types, and carriage of multiple *S. aureus* types seems to elicit the highest humoral responses in these patients. However, we cannot exclude the alternative possibility of increased humoral and reduced cell-mediated immunity in EB patients, which might have an impact on *S. aureus* carriage. Notably, EB patients do not frequently suffer from *S. aureus* bacteremia, and none of the patients who donated blood was treated for staphylococcal bacteremia in the 5 years before blood donation. This suggests that their high anti-staphylococcal antibody titers may be protective against invasive *S. aureus* infections, which would be consistent with the protective effects of IsaA-specific antibodies in mice (Lorenz *et al.*, 2011).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Keratin Intracellular Concentration Revisited: Implications for Keratin Function in Surface Epithelia

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TO THE EDITOR

In 1978, Sun and Green carried out a densitometry-based analysis of PAGE samples and reported that keratin proteins account for 25–35% of total cellular proteins in human epidermal keratinocytes serially passaged in primary culture. This represents an astounding figure with very significant implications for the structural support role of keratin intermediate filaments in epidermis, and its regulation by post-translational modifications and/or interaction with associated proteins. We report here on an effort to investigate

this issue further, using a distinct method of keratin quantitation in sorted but otherwise native cell populations, and taking advantage of the significant progress made in our understanding of the structure of keratin filaments and differential regulation of keratin expression in epidermis.

Freshly isolated keratinocytes were obtained directly from 2-day old C57Bl/6 newborn mice, labeled with fluorochrome-conjugated antibodies to the surface marker integrin β-1, and then sorted by flow cytometry according to the expression level of this antigen (Jones and Watt, 1993). As an essential component of cell surface receptor for extracellular matrix ligands (Hynes, 1987), this integrin is primarily expressed in basal layer keratinocytes, and only poorly so in differentiating keratinocytes in the suprabasal layers of epidermis. We collected cells that express intermediate levels of integrin β-1 at their surface (Figure 1a). Such integrin β-1 ''dim'' cells (Jones *et al.*, 1995) consist of basal cells with transit-amplifying proliferation status and represent the majority of basal layer keratinocytes. The less abundant cells that express surface integrin β-1 at a higher level (designated ''bright'') are enriched in epidermal stem cells (Jones *et al.*, 1995). Ultrastructural analysis shows that the sorted integrin β-1-dim cells are homogeneous, with a round shape and a smooth surface, and features a round and centrally located nucleus surrounded by thick bundles of keratin filaments in the cytoplasm (Figure 1b). The appearance of keratin filaments, in particular, is very reminiscent of those seen in basal layer keratinocytes *in situ* (Coulombe *et al.*, 1989).

Sorted basal cells were lysed in urea lysis buffer and the resulting protein extracts were analyzed to measure the concentrations of K5, K14, and K15, the three major keratins in the basal progenitor cells of the epidermis. Serially diluted aliquots of native protein lysates were resolved by PAGE, and K5, K14, and K15 antigens were quantitated by infrared western blot analysis (Li-Cor Biosciences, Lincoln, NE). Calibration curves were established using purified recombinant forms of human K5, human K14, and mouse K15 as standards, taking advantage of the linear relationship between western signal intensity and protein concentration (Figure 2a–c). Relevant experimental details are given in Supplementary Materials online.

The quantitative data obtained are related in part in Figure 2d and e and otherwise *in toto* in Supplementary Tables S1–3 online. We estimate that the average sorted basal keratinocyte contains 36.81 ± 4.34 pg of total protein, of which 4.91 ± 1.07, 2.18 ± 0.80, and 0.98 ± 0.01 pg, respectively, represent K5, K14, and K15 (Supplementary Table S3 online). This yields a 1.27:1 molar ratio for type II vs. type I keratin proteins, rather than the expected 1:1 ratio (Kim, 1984). This difference could be because of the presence of K17 (but

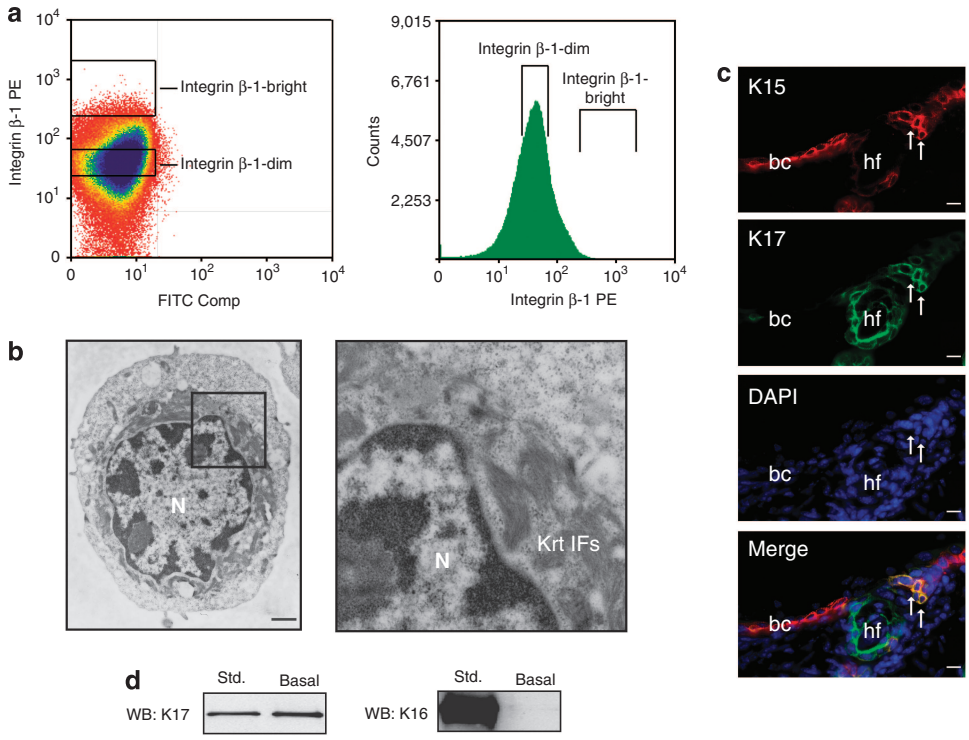

Figure 1. Isolation and characterization of epidermal basal keratinocytes. (**a**) Keratinocytes harvested from C57Bl/6 P2 mouse skin were stained with fluorochrome-conjugated antibody to the surface protein integrin β-1 and sorted using flow cytometry. An integrin β-1-dim status reflects a transit-amplifying population of basal keratinocytes, whereas an integrin β-1-bright status denotes a subpopulation enriched in stem cells. (**b**) Transmission electron micrograph of a representative sorted basal (integrin β-1-dim) keratinocyte. Bar = 1 μm. (**c**) Immunofluorescence micrographs of P2 skin cross-sections highlight the occurrence of K17-positive basal cells in the hair follicle–proximal interfollicular epidermis (see arrows). Bar = 10 μm. (**d**) Western blots (WBs) show the presence of K17 but not K16 in FACS-sorted basal cells, given the protocol used (see **a**). ''Std.'' refers to relevant purified keratin standard. bc, basal cell; hf, hair follicle; Krt IFs, keratin intermediate filaments; N, nucleus; PE, phycoerythrin.