

CORRESPONDENCE

Filaggrin is expressed in the epithelial cells of the buccal mucosae

To the Editor,

We read with great interest the recent article by Tan et al. (1) who looked for the expression of *FLG*, the gene encoding filaggrin, in the epithelial cells of mucosae of the oral cavity. *FLG* non-sense mutations are the strongest genetic risk factor for atopic dermatitis (2) and were shown to increase the risk of food sensitization (3). In the epidermis, filaggrin is expressed in the late step of keratinocyte terminal differentiation. It is a key protein for maintaining the *stratum corneum* properties necessary for the epidermal barrier efficiency (4). The authors hypothesized that if filaggrin is expressed in the epithelia of the oral cavity, the first potential entry port for dietary antigens after birth, *FLG* loss-of-function mutations could be responsible for alterations of the epithelial barrier integrity in the oral cavity as they are in the skin (2, 4). The authors compared the expression of *FLG* by quantitative reverse transcription-PCR in superficial buccal epithelial cells obtained by swab and in matched skin biopsies from eight adult volunteers. Their results indicated that *FLG* is expressed in the buccal samples but at a low level as compared to the skin samples [mean normalized level of *FLG* in the mouth relative to skin equal to 0.19 (0.08–0.45)]. In addition, *FLG* expression in buccal cells was shown to be two times higher in young children than in adults.

Contrary to the authors' allegation, this is not the first study to show that human buccal cells express *FLG*. There are at least two previous reports demonstrating filaggrin expression in these cells at the protein level. In 1986, Beverly Dale and Sally Smith investigated the expression by immunofluorescence microscopy and Western blotting, using a rabbit antiserum and the specific monoclonal antibody AKH1, in thirty biopsies coming from various sites of the oral cavity of healthy

volunteers (5). Expression was analysed in the orthokeratinized squamous epithelium of the hard palate, the parakeratinized squamous epithelium of the gingiva and the non-keratinized squamous epithelium of the mouth (ten biopsies from each site). The orthokeratinized epithelium displayed a granular cytoplasmic labelling of keratinocytes in the upper *stratum spinosum* and *stratum granulosum*, and a diffuse labelling of corneocyte matrix in the *stratum corneum*. Consistently, profilaggrin and filaggrin monomers were detected in epithelial protein extracts. As compared to that observed in the epidermis extracts, the immunoreactivity was weak. The parakeratinized epithelium showed a similar but weaker and scattered pattern of labelling. Most samples of the non-keratinized epithelium displayed a weak, perinuclear and granular labelling of keratinocytes in the upper layers. In the related extracts, only profilaggrin was weakly detected. In 1995, our group analysed human buccal epithelial cells by immunocytology and Western blotting with AKH1 and three other monoclonal antibodies we raised against human filaggrin: AHF1, AHF2 and AHF7 (6). The latter had previously been shown to be specific for different epitopes of epidermal profilaggrin and/or filaggrin (7). The epithelial cells were obtained by scraping the inner side of the cheeks of three healthy volunteers. In the cells analysed after cytocentrifugation, AKH1 and the other antibodies detected perinuclear granules [(6) and Fig. 1a]. When protein extracts of the cells were analysed by Western blot, high molecular weight bands corresponding to profilaggrin were stained [(6) and Fig. 1b]. Filaggrin monomers could not be detected, even when the proteins were extracted in the presence of powerful denaturing agents. Furthermore, the immunofluorescence labelling and

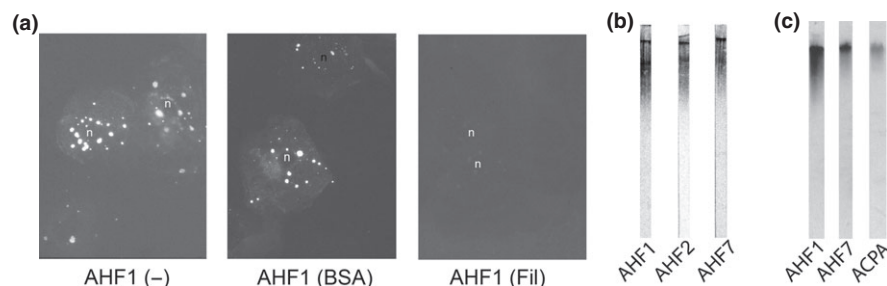


Figure 1 Immunoreactivity of murine monoclonal antibodies and human autoantibodies specific for human filaggrin on human buccal superficial epithelial cells and human buccal cell extracts. (a) Indirect immunofluorescence analysis of human epithelial cells of the oral cavity with AHF1 before (–) and after immunoabsorption on bovine serum albumin (BSA) or human epidermal filaggrin (Fil). Nuclei (n) are indicated. (b) Immunoblotting detection with AHF1, AHF2 and AHF7 of a protein extract of the same cells obtained after direct solubilization in 50 mM Tris pH 7.6 buffer containing 8M urea, 100 mM dithiothreitol and 130 mM 2-mercaptoethanol. (c) Immunoblotting detection of a Tris–Nonidet P-40 protein extract of the same cells (see reference 6 for a detailed buffer composition) with AHF1, AHF7 and human anticitrullinated protein autoantibodies (ACPA). The autoantibodies were purified from a pool of serums of patients with rheumatoid arthritis by affinity chromatography on human epidermal filaggrin.

Western blotting reactivity of AKH1 and AHF1 were completely inhibited after immunoabsorption of the antibodies on purified human epidermal filaggrin but not on bovine serum albumin used as a control [(6) and Fig. 1a]. In addition, it is now clear that at least part of the profilaggrin present in the human buccal mucosa epithelium is citrullinated. In fact, it is extracted in the absence of a denaturing agent, and it is recognized by the human autoantibody called 'antiperinuclear factor'. The latter is specifically found in the serum of patients with rheumatoid arthritis and belongs to the family of autoantibodies that exclusively recognize epitopes generated

by citrullination of arginine residues on the targeted antigenic proteins (8, 9).

In conclusion, these data clearly demonstrate that profilaggrin is expressed by the most differentiated keratinocytes of the oral cavity mucosae, the filaggrin monomers being generated only in the orthokeratinized epithelium of the hard palate.

M. Sebbag; G. Serre & M. Simon

UMR 5165 CNRS, 1056 INSERM, University of Toulouse, Toulouse, France

E-mail: michel.simon@udear.cnrs.fr

DOI:10.1111/pai.12277

References

1. Tan H-TT, Ellis JA, Koplin JJ, et al. Methylation of the filaggrin gene promoter does not affect gene expression and allergy. *Pediatr Allergy Immunol* 2014; doi: 10.1111/pai.12245. [Epub ahead of print].
2. Kezic S, Novak N, Jakasa I, et al. Skin barrier in atopic dermatitis. *Front Biosci (Landmark Ed)* 2013; **19**: 542–56.
3. Tan HT, Ellis JA, Koplin JJ, et al. Filaggrin loss-of-function mutations do not predict food allergy over and above the risk of food sensitization among infants. *J Allergy Clin Immunol* 2012; **130**: 1211–3.
4. Brown SJ, McLean WH. One remarkable molecule: filaggrin. *J Invest Dermatol* 2012; **132**: 751–62.
5. Smith SA, Dale BA. Immunologic localization of filaggrin in human oral epithelia and correlation with keratinization. *J Invest Dermatol* 1986; **86**: 168–72.
6. Sebbag M, Simon M, Vincent C, et al. The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1995; **95**: 2672–9.
7. Simon M, Sebbag M, Haftek M, et al. Monoclonal antibodies to human epidermal filaggrin, some not recognizing profilaggrin. *J Invest Dermatol* 1995; **105**: 432–7.
8. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1998; **101**: 273–81.
9. Girbal-Neuhaus E, Durieux JJ, Arnaud M, et al. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro) filaggrin by deimination of arginine residues. *J Immunol* 1999; **162**: 585–94.

Langerhans cell histiocytosis in IPEX syndrome: Possible role for natural regulatory T cells?

To the Editor,

Langerhans cell histiocytosis (LCH) is generally accepted as a disorder characterized by Langerhans cell (LC) accumulation in single or multiple organs. Nevertheless the pathogenesis of the disorder is not completely understood and it has not yet been precisely clarified whether it is a malignancy or a immunodysregulation (1). The diagnosis is based on the evaluation of the clinical picture with histopathological features, that identify LC's reactivity to CD1a and S100 immunohistochemical staining (2).

The heterogenous clinical course of the LCH -from benign single organ involvement to life-threatening multi-system disorder- suggests that, LC accumulation may be the result of different diseases or conditions, each one caused by a different mechanism. Although the available data strongly suggest a relevant genetic component in the development of the disorder, to our knowledge, there is only one known relationship between LCH and a specific monogenic disease, that is, X-linked lymphoproliferative disease type 1 (XLP) (3, 4).

A 50-day-old boy was admitted to our Pediatric Gastroenterology Department with a history of bloody diarrhea for

10 days. He had erythrodermia in his trunk and extremities and a few papules in the trunk. Additionally, a few cervical (one of them 1 × 1 cm) and axillary lymph nodes were palpable. Laboratory results showed no abnormalities. After cow milk elimination was initiated, the symptoms improved moderately over 2 months, but then the patient readmitted with the complaints of intractable watery diarrhea and maculopapular rash. Lymph node biopsy was performed at the 5 months of age due to the persistence of lymphadenopathy and skin rash. The biopsy revealed LC accumulation as shown in Fig. 1; the patient was eventually diagnosed with LCH as a result of the clinical and pathological findings. The lymphadenopathy and skin rash were resolved soon after the beginning of chemotherapy. On the other hand, during the first 2 months of therapy, the high-volume watery diarrhea did not improve, although several formulas and nutrients were tried. On investigation, no infectious or metabolic causes of the diarrhea were found. The secretory nature of the diarrhea directed us to suspect autoimmune enteropathy and its prototype immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Sequence analysis of the coding region of