

Letter to the Editor

Lack of filaggrin expression in the human bronchial mucosa

To the Editor:

Why some sensitized atopic subjects develop disease in particular target organs (atopic dermatitis, allergic rhinitis, asthma), or not at all, remains one of the fundamental unanswered questions in allergy. Rather than differences in immunological responses, increasing attention is being paid to pre-existing, heritable abnormalities of the structure of these target organs that may predispose to atopic disease. An example is the *ADAM 33* gene, which encodes a complex metalloproteinase 33/disintegrin involved in normal lung development. Certain single nucleotide polymorphisms of this gene have been linked with increased risk of asthma and airways obstruction.¹ Very recently, lack of expression of the protein filaggrin, produced in the basal epidermal layer of the skin during terminal differentiation of keratinocytes, has been shown to predispose to the development of ichthyosis vulgaris and atopic dermatitis in affected individuals.² Deficient filaggrin expression appears to result principally from the inheritance of 2 loss of function allelic variants termed *R510X* and *2282del4*. In 3 separate cohorts of European children, individuals heterozygous or homozygous for one or the other of these loss of function allelic variants showed a highly significantly increased risk of development of atopic dermatitis. Interestingly, expression of the loss of function alleles was also associated with increased risk of atopic asthma, but only in those children with coexisting atopic dermatitis. The primary function of filaggrin appears to be to aggregate the epidermal cytoskeleton to form a dense protein-lipid matrix, thus regulating permeability of the skin to both water and external particles such as allergens.²⁻⁴

Although the bronchial epithelium is not keratinized, bronchial epithelial cells express many proteins in common with skin keratinocytes, including keratin itself. These observations raise the possibility that filaggrin is also expressed in the human bronchial mucosa and serves a similar role. We therefore hypothesized that filaggrin is expressed in the human bronchial epithelium, and that expression is deficient in patients with asthma compared with controls. As a positive internal control, we also studied filaggrin expression in the epidermis of skin biopsies from atopic patients undergoing cutaneous allergen challenge.

Endobronchial biopsies were obtained at fiberoptic bronchoscopy from second-generation or third-generation bronchi in the right middle or lower lobe in 7 subjects with asthma and 7 normal controls. Asthma was defined as a clear history of typical symptoms, with documented reversibility of the FEV₁ or peak expiratory flow $\geq 15\%$ within the 6 months before the study. Subjects with asthma were (mean [range]) age 34 (28-53) years and had FEV₁ 92.4 (82.3-108.0) % predicted. All were taking inhaled corticosteroids at low dosage (371 [200-800] $\mu\text{g/d}$ beclometasone or equivalent) and short-acting β_2 -agonist

as required. All patients were nonsmokers and had no chronic diseases other than asthma and allergic rhinitis. Nonasthma was defined as lifelong absence of relevant symptoms, with FEV₁/peak expiratory flow within the predicted range and $<10\%$ variable. Normal controls were age 37 (26-41) years and had FEV₁ 122.0 (118.1-124.3) % predicted. Skin biopsies were obtained from 3 sensitized healthy atopic subjects with no history of atopic disease and taking no medications. Thirty biological units of an aeroallergen to which they were sensitized (grass pollen, house dust mite, or cat dander) were injected intradermally into the skin in a total of volume of 0.1 mL sterile saline on the volar aspect of the forearm as in previous studies.^{5,6} A remote control site was injected with saline alone. Six hours after allergen injection, all 3 subjects developed late-phase erythema and induration of diameter 5.6 (3.0-8.2) cm (mean of orthogonal diameters). Injections of saline produced no visible reaction at all. The challenged sites were biopsied under local anaesthesia by using a sterile skin biopsy punch as previously described.^{5,6} Biopsies were fixed (bronchial biopsies, 2 hours; skin biopsies, 4 hours) immediately in freshly prepared 4% paraformaldehyde (BDH, Poole, United Kingdom [UK]) in PBS, pH 7.4, washed twice (1 hour each) with 15% sucrose (Sigma, Poole, UK) in PBS, embedded in optimum cutter temperature compound (Bayer, Basingstoke, UK), snap-frozen, and then stored at -80°C until used.

Sections (nominal thickness, 6 μm) were freshly cut from the frozen biopsies and stained by using previously described techniques.⁷ Two antifilaggrin primary antibodies were used. The first was an affinity-purified rabbit polyclonal IgG antibody (1/500 dilution; ab24584; Abcam PLC, Cambridge, UK) raised against a synthetic peptide corresponding to amino acids 24 to 39 of filaggrin. This was detected with a second-layer mouse antirabbit IgG antibody (1/50 dilution; Dako, High Wycombe, UK) and a third-layer goat antimouse antibody conjugated to alkaline phosphatase (1/50 dilution; Immune Directory, Kidlington, UK). The second primary antibody was a mouse IgG mAb (1/50 dilution; clone 15C10; Novocastra Laboratories Ltd, Newcastle-Upon-Tyne, UK), which was detected with a second-layer donkey antimouse antibody (1/50 dilution; Jackson Immunoresearch Laboratories Inc, West Grove, Pa) and a third-layer mouse alkaline phosphatase antialkaline phosphatase complex (1/50 dilution; Dako). The indicator used in each case was Fast Red (Sigma). The incubation time for the primary antibodies was 4 hours at room temperature. For each primary antibody, sections from all subjects in the entire study were processed together by using identical reagents. Sections were visualized by using an Olympus BX40 microscope (Olympus Optical Co. Ltd., Tokyo, Japan) and an image analysis system as previously described.⁷

The results showed that in skin sections from all 3 healthy atopic subjects, filaggrin expression was visible as a well-demarcated band located at the base of the stratum

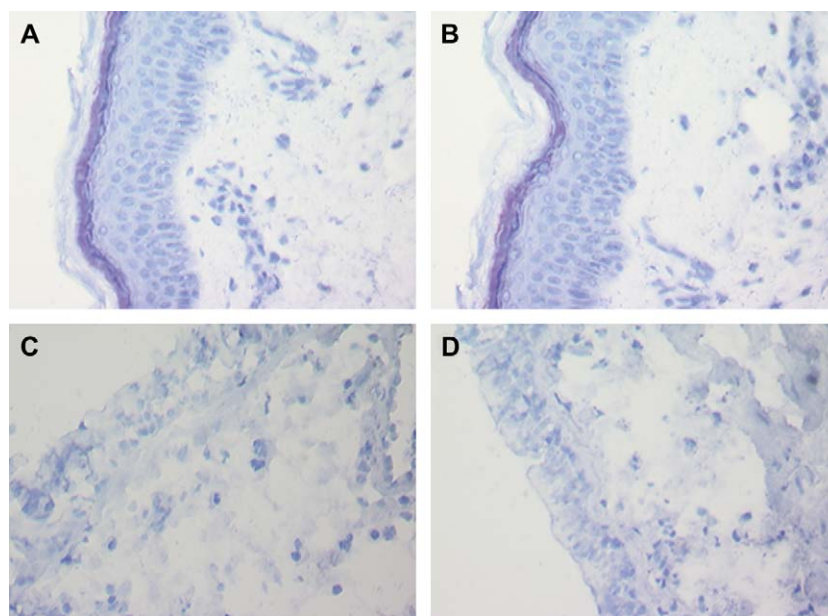


FIG 1. Typical sections of skin (**A,B**) and bronchial (**C,D**) biopsies stained with monoclonal (**A,C**) and polyclonal (**B,D**) antifilaggrin antibodies (see Methods).

corneum of the epidermis (Fig 1). There was no discernable difference in filaggrin expression after allergen, compared with diluent control challenge of the skin as assessed by standard image analysis; we did not think it appropriate for the purposes of this study to search for subtle alterations in filaggrin expression.⁸

In contrast, filaggrin immunoreactivity was undetectable in sections of the bronchial biopsies from all of the subjects with asthma and the normal controls, in both the epithelium and the submucosa (Fig 1). Furthermore, there was no evidence of filaggrin expression in smooth muscle, perivascularly, or in infiltrating cells. Appearances with both primary antibodies were identical.

Our experiments confirm the expression of filaggrin in the basal epidermal layer of normal skin, and clearly show that such expression is absent in the bronchial mucosa in both healthy controls and patients with asthma. It is very unlikely that this lack of staining is a false-negative result, because we observed clear staining with both primary antibodies in the skin, and the skin and bronchial biopsies were processed using identical reagents. The data do not support the hypothesis that filaggrin is expressed in the human bronchial mucosa, or that deficiency of filaggrin expression is associated with asthma. The data therefore detract from the possibility that filaggrin plays a direct role in regulating the permeability of the bronchial mucosa to water, allergens, and other particles. It is possible, however, to hypothesize that deficient filaggrin expression in the skin could predispose to atopic asthma in children by increasing the risk of sensitization to aeroallergens after skin contact. This hypothesis could of course be addressed in future studies.

Filaggrin is clearly not the only protein that affects the risk of development of atopic dermatitis and associated asthma

in atopic individuals. Although deficient filaggrin expression is associated with increased risk of these diseases, their development is not inevitable even in homozygous carriers of the loss of function filaggrin alleles.² Furthermore, even in children, sensitization to aeroallergens is not an inevitable prerequisite for the development of atopic dermatitis or asthma. Nevertheless, if sensitization to aeroallergens with consequent increased risk of atopic asthma does primarily reflect an abnormality of the skin, rather than the bronchial mucosa, this could force a paradigmatic shift in our thinking about mechanisms of allergen sensitization through exposure and its modification by putative inhibitory substances such as endotoxins in early childhood. Perhaps it is not inhalation of but skin contact with these substances that is critical.

In conclusion, filaggrin cannot play a role in regulation of water and allergen permeability in the bronchial mucosa as it does in the skin. However, the fact that filaggrin-deficient patients with atopic dermatitis are also at increased risk of asthma suggests the possibility that the latter may in part reflect transdermal allergen sensitization.

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REFERENCES

1. Holgate ST, Holloway J, Wilson S, Howarth PH, Haitchi HM, Babu S, et al. Understanding the pathophysiology of severe asthma to generate new therapeutic opportunities. *J Allergy Clin Immunol* 2006;117:496-506.
2. Palmer CNA, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
3. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 2005;6:328-40.
4. Jensen JM, Folster-Holst R, Baranowsky A, Schunck M, Winoto-Morbach S, Neumann C, et al. Impaired sphingomyelinase activity and epidermal differentiation in atopic dermatitis. *J Invest Dermatol* 2004;122:1423-31.
5. Ying S, Robinson DS, Meng Q, Barata LT, McEuen AR, Buckley MG, et al. C-C chemokines in allergen-induced late phase cutaneous responses in atopic subjects: association of eotaxin with early 6-hour eosinophils, and eotaxin-2 and MCP-4 with the later 24-hour tissue eosinophilia, and relationship to basophils and other C-C chemokines (MCP-3 and RANTES). *J Immunol* 1999;163:3976-84.
6. Ying S, Meng Q, Barata LT, Robinson DS, Durham SR, Kay AB, et al. Associations between IL-13 and IL-4 (mRNA and protein), vascular cell adhesion molecule-1 expression, and the infiltration of eosinophils, macrophages and T cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Immunol* 1997;158:5050-7.
7. Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, et al. Thymic stromal lymphopoietin (TSLP) expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 2005;174:8183-90.
8. Seguchi T, Chang-Yi C, Kusuda S, Takahashi M, Aisu K, Tezuka T, et al. Decreased expression of filaggrin in atopic skin. *Arch Dermatol Res* 1996; 288:442-6.

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