

- 12 Heald PW, Glusac EJ. Unilesional cutaneous T-cell lymphoma: clinical features, therapy, and follow-up of 10 patients with a treatment-responsive mycosis fungoides variant. *J Am Acad Dermatol* 2000; **42** (2 Pt 1): 283–5.
- 13 Hodak E, Phenig E, Amichai B et al. Unilesional mycosis fungoides: a study of seven cases. *Dermatology* 2000; **201**:300–6.
- 14 Palmer RA, Keefe M, Slater D, Whittaker SJ. Case 4: pagetoid reticulosis (Woringer–Kolopp type) or unilesional mycosis fungoides (MF). *Clin Exp Dermatol* 2002; **27**:345–6.
- 15 Yoo SS, Viglione M, Moresi M, Vonderheid E. Unilesional mycosis fungoides mimicking Bowen's disease. *J Dermatol* 2003; **30**:417–19.
- 16 Alsaleh QA, Nanda A, Baker H et al. Unilesional (segmental) mycosis fungoides presenting in childhood. *Pediatr Dermatol* 2004; **21**: 558–60.
- 17 Wilson LD, Jones GW, Smith BD. Cutaneous lymphomas – radiotherapeutic strategies. *Front Radiat Ther Oncol* 2006; **39**:1–15.
- 18 Ohtani T, Kikuchi K, Koizumi H et al. A case of CD30+ large-cell transformation in a patient with unilesional patch-stage mycosis fungoides. *Int J Dermatol* 2009; **48**:623–6.
- 19 Muniesa C, Estrach T, Pujol RM et al. Folliculotropic mycosis fungoides: clinicopathological features and outcome in a series of 20 cases. *J Am Acad Dermatol* 2010; **62**:418–26.

Funding sources: no external funding.

Conflicts of interest: none to declare.

Protease–antiprotease imbalance may be linked to potential defects in profilaggrin proteolysis in atopic dermatitis

DOI: 10.1111/j.1365-2133.2011.10750.x

MADAM, Most recent studies on atopic dermatitis (AD) have focused on the importance of the keratin-binding protein filaggrin in skin barrier function. Loss-of-function mutations of the filaggrin gene *FLG* are the major predisposing genetic factor for AD.¹ Nonetheless, the majority of patients with AD have an apparently normal *FLG*. Some patients with wild-type AD have reduced filaggrin protein expression,² implying the presence of other modifying factors. Profilaggrin, the 400-kDa precursor protein, is unable to bind keratin until it is proteolysed to release the functional 37-kDa filaggrin monomer.³ Knowing the importance of profilaggrin processing, we conducted a small study to determine whether defective profilaggrin proteolysis may be found in patients with AD with wild-type *FLG*. Although profilaggrin processing is presumed to occur intracellularly, extracellular proteases such as matrilysin^{4,5} also influence the expression of filaggrin monomers.

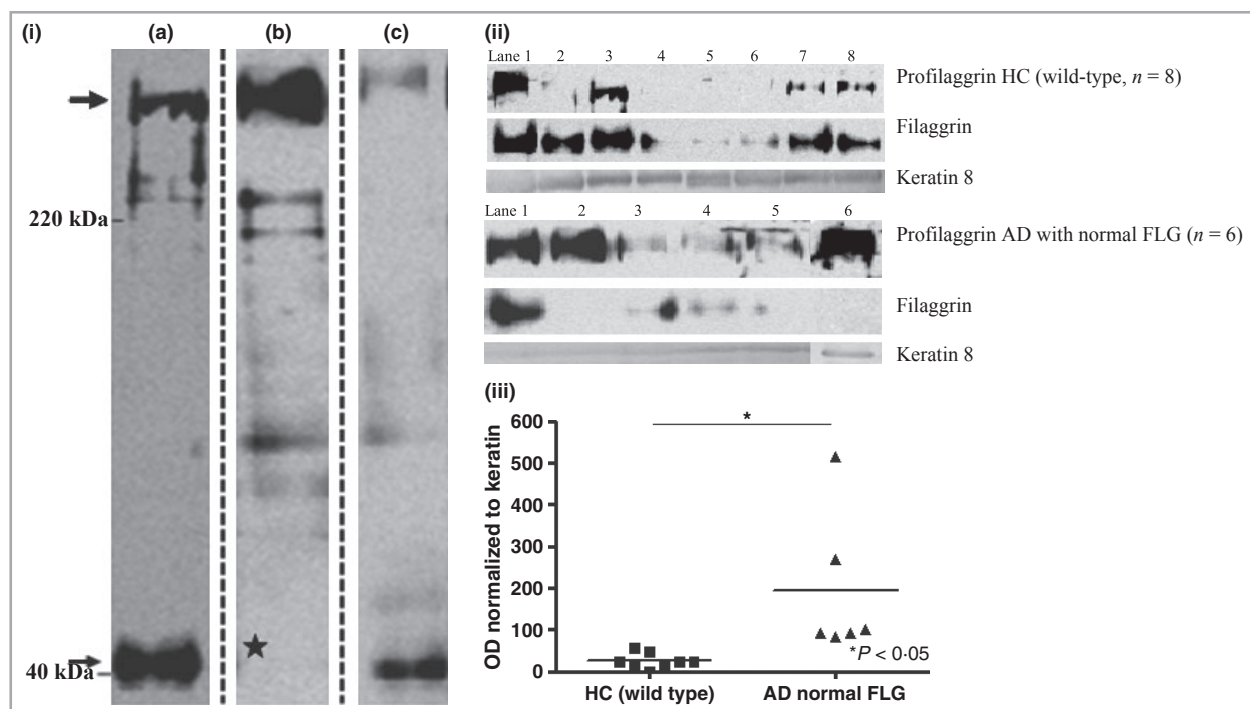


Fig 1. Differences in (pro)filaggrin protein expression between HC with normal FLG and patients with AD by Western blotting. (i) Representative Western blots showing profilaggrin (thick arrow, > 220 kDa) and filaggrin (thin arrow, ~40 kDa). (a) HC with normal FLG; (b) AD with normal FLG showed increased amount of profilaggrin and filaggrin intermediate products and lacked the 37-kDa filaggrin protein (★, absent filaggrin band). (c) AD with FLG mutation (heterozygous for 2282del4 mutation) with reduced amount of (pro)filaggrin. Two FLG heterozygotes (including the one compound heterozygote) had no filaggrin protein on Western blotting and therefore are not shown. (ii) AD with normal FLG exhibited an overall increased profilaggrin and reduced filaggrin monomers in comparison with HC with normal FLG. (iii) Densitometric band analysis of the Western blots showed accumulation of the profilaggrin in AD with normal FLG ($P < 0.05$). HC, healthy controls; AD, atopic dermatitis.

We further investigated whether the extracellular/interstitial protease–antiprotease balance is deranged in patients with AD.

We recruited 11 healthy controls (HC; age 23.5 ± 6.5 years; seven females) with no inflammatory skin conditions and 10 patients with mild-to-moderate AD (age 27.8 ± 11.5 years; eight females). In the AD group, the disease severity score (Six Area, Six Sign Atopic Dermatitis, SASSAD) ranged from 3 to 16 for the wild type and from 0 to 24 for those with FLG mutations. Genotyping for common caucasian FLG polymorphisms (2282del4, R2447X, R501X, S3247X) was performed as described elsewhere.⁶ One of the 11 HC was heterozygous for the FLG 2282del4 mutation. Four of the 10 patients with AD were heterozygous for FLG 2282del4, of whom one was a compound heterozygote (2282del4/R501X).

With a PTC3300 VAC Vacuum Unit (InnoKas Medical Oy, Kempele, Finland), we induced suction blisters on the flexural surfaces of our volunteers' arms. We excluded those with active disease or inflammation at the test area and kept the suctioning to the minimum to reduce any secondary inflammation. Suction blistering has been validated as a way of obtaining epidermal protease inhibitors such as elafin, SLPI and LEKTI, which are secreted into the interstitium.⁷ Western-blot band densitometric analysis using the epidermal skin caps (normalized to housekeeping protein keratin 8) showed that in two of the six patients with wild-type AD (Fig. 1ii, lower panel; lanes 2 and 6), no filaggrin monomers were apparent despite a significant presence of profilaggrin (see also Fig. 1ib). The six patients with AD with wild-type FLG had a higher amount of profilaggrin compared with eight HC (192.52 ± 173.61 vs. 25.96 ± 17.87 , Fig. 1iii). Patients with wild-type AD had, on average, a higher profilaggrin : filaggrin ratio, compared with HC (2.25 ± 2.20 vs. 0.75 ± 0.33) (Fig. 1). Skin caps were not obtained from three HC and one AD with FLG mutation. The higher profilaggrin : filaggrin ratio in patients with wild-type AD, especially that of lanes 2 and 6, suggests defective profilaggrin processing. The absence of pro- and processed filaggrin in some lanes (both HC and wild-type AD) may imply other filaggrin mutations not screened for in this study. It is also known that some people with filaggrin mutations do not develop AD. Suction-induced blister skin caps were stained for (pro)filaggrin and cytokeratin using general double immunohistochemical staining technique with primary antibodies raised against filaggrin (mouse monoclonal antibody from Novocastra, Newcastle, UK) and pan-cytokeratin (mouse monoclonal antibody from Sigma, Poole, UK). Representative images using samples obtained from HC with wild-type FLG, wild-type AD and AD patients with FLG mutations were shown in Fig. 2.

Blister fluid (protein concentrations 62.4 ± 8.9 mg mL⁻¹, measured with BCA Protein Assay Reagent; Thermo Scientific, Barrington, IL, U.S.A.) was mixed with polyethylene glycol (PEG-400; Fluka, Gillingham, U.K.) in a 50 : 50 ratio before storage at -20 °C. PEG-400, added to preserve existing protease activity, also resulted in precipitation of larger-molecular-weight proteins on thawing that were removed by centrifugation (as observed by Coomassie Blue staining of the

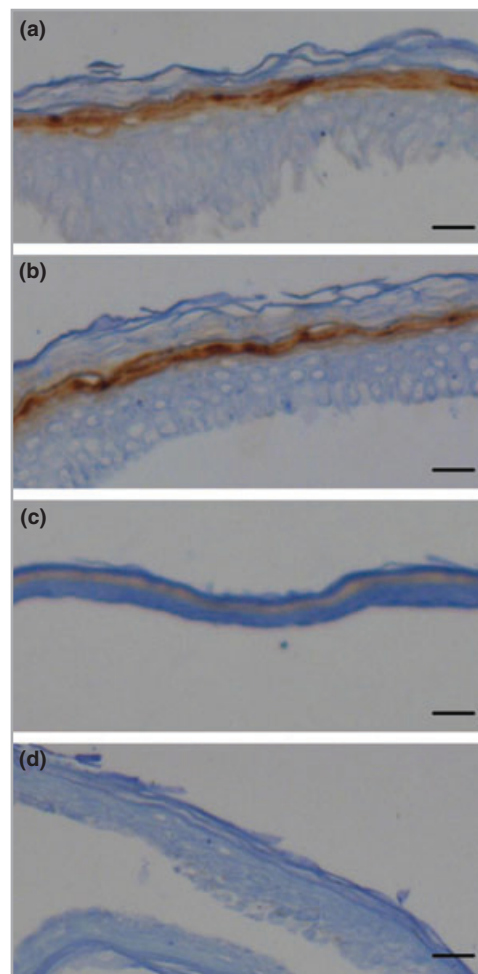


Fig 2. Immunohistochemical staining of suction-induced blister skin caps for (pro)filaggrin and cytokeratin. Suction-induced blister skin caps from 9 AD patients (6 WT-AD, 3 FLG heterozygotes for 2282del4 mutation) and 8 HC with wild-type FLG were stained for (pro)filaggrin (brown) and cytokeratin (blue). (a) A wild-type HC. (b) A WT-AD patient. Note: The thicknesses of skin cap and (pro)filaggrin layers of WT-AD were comparable to HC with wild-type FLG. (c) A FLG-AD heterozygote (FLG +/- 2282del4) showing reduced filaggrin staining. (d) A FLG-AD heterozygote (FLG +/- 2282del4) showed no obvious filaggrin staining. Scale bar = 20 µm.

protein gels). This partial fractionation allowed assessment of the lower-molecular-weight proteins (typically < 80 kDa in size) that remained in suspension. Using chromogenic and fluorogenic peptides, we detected no significant elastolytic, chymotryptic, tryptic or papain-like activity within the blister fluid samples, indicating the presence of potent inhibitors. Blister fluid was therefore analysed for inhibitory activity against commercially available serine (elastase, trypsin, chymotrypsin) and cysteine (papain) proteases.

Compared with HC, blister fluid from patients with AD resulted in a higher inhibition of trypsin ($25 \pm 9\%$ vs. $18 \pm 4\%$, $P < 0.05$), papain ($69 \pm 5\%$ vs. $62 \pm 8\%$, $P < 0.03$) and, perhaps, elastase ($40 \pm 21\%$ vs. $25 \pm 21\%$, $P < 0.16$) (Fig. 3a). Partially fractionated samples consisting of predominantly

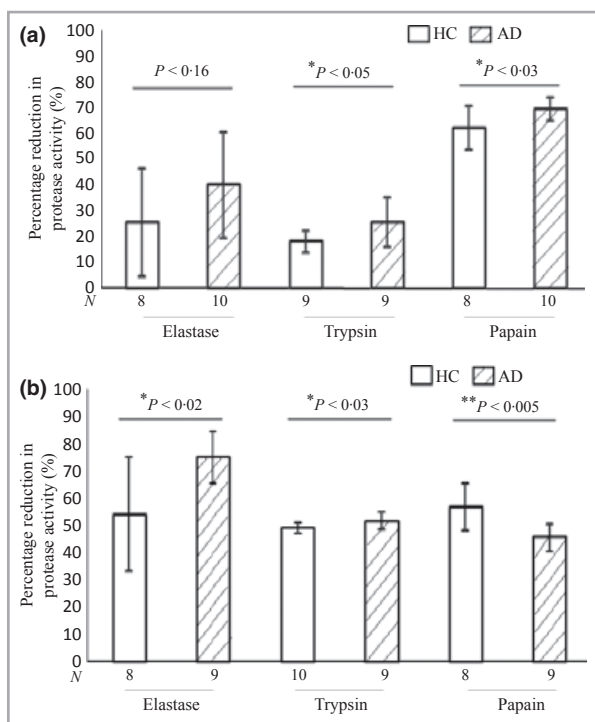


Fig 3. Differences in the protease inhibition profile between HC and AD. (a) Percentage reduction in the initial enzymatic reaction velocity caused by the addition of blister fluid. Patients with AD showed higher antitrypsin and antipapain activities. (b) Percentage reduction in the initial enzymatic reaction velocity caused by the addition of partially purified blister fluid that contained predominantly lower-molecular-weighted inhibitors. Fractionated samples from patients with AD were found to have higher anti-elastase and antitrypsin, but lower antipapain activities compared with HC. Data were presented as mean \pm SD. HC, healthy controls; AD, atopic dermatitis.

lower-molecular-weight inhibitors from patients with AD were found to have higher anti-elastase ($75 \pm 9\%$ vs. $54 \pm 21\%$, $P < 0.02$) and antitrypsin ($52 \pm 3\%$ vs. $49 \pm 2\%$, $P < 0.03$), but lower antipapain ($46 \pm 5\%$ vs. $57 \pm 9\%$, $P < 0.005$) activities compared with HC (Fig. 3b). No difference in the antichymotrypsin activity of blister fluid between AD and HC was found.

While previous studies suggest that excessive proteolysis in the skin is causally linked to skin barrier dysfunction,^{8–10} we propose that abnormally raised protease inhibition demonstrated in the interstitium of patients with AD could be linked to the clinical manifestations of AD, possibly by affecting proflaggrin processing through inhibition of proteases such as matriptase. Our findings are indirectly consistent with a previous study suggesting an association between decreased larger precursors and increased filaggrin monomers with increased protease activities.¹¹ Although our small sample size does not allow a robust relation between genotype and individual antiprotease activity to be drawn, the disproportionately raised protease inhibition and defective proflaggrin processing provide potentially useful pathomechanistic insights into the aetiology of AD.

Acknowledgments

Genotyping work was by our collaborators Professor W.H.I. McLean and Linda E. Campbell at the Epithelial Genetics Group, Colleges of Life Sciences and Medicine Dentistry and Nursing, University of Dundee. We are grateful to the following people at the Queen's Medical Research Institute, University of Edinburgh for their help: Mr Bob Morris for his assistance in the immunohistochemistry work, Ms Olga Lucia and Ms Lesley Farrell for their help in performing the ELISAs for SLPI and elafin. Most importantly, we would like to thank our participants for their contribution.

*MRC Centre for Inflammation Research,
Queen's Medical Research Institute,
University of Edinburgh, 47 Little
France Crescent, Edinburgh EH16 4TJ, U.K.
†Department of Dermatology,
University of Edinburgh,
Lauriston Building, Lauriston Place,
Edinburgh EH3 9HA, U.K.
‡Department of Dermatology,
Ninewells Hospital, Dundee DD1 9SY, U.K.
E-mail: s.p.tan@sms.ed.ac.uk

S.P. TAN*†
S. ABDUL-GHAFFAR‡
R.B. WELLER*†
S.B. BROWN*

References

- Palmer CN, Irvine AD, Terron-Kwiatkowski A 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.
- Howell MD, Kim BE, Gao P et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2009; **3** (Suppl. 2):R7–12.
- Sandilands A, Sutherland C, Irvine AD, McLean WHI. Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* 2009; **122**:1285–94.
- List K, Szabo R, Wertz PW et al. Loss of proteolytically processed filaggrin caused by epidermal deletion of Matriptase/MT-SP1. *J Cell Biol* 2003; **163**:901–10.
- List K, Bugge TH, Szabo R. Matriptase: potent proteolysis on the cell surface. *Mol Med* 2006; **12**:1–7.
- Sandilands A, Terron-Kwiatkowski A, Hull PR et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007; **39**:650–4.
- Groth S, Staberg B. Suction blisters of the skin: a compartment with physiological, interstitium-like properties. *Scand J Clin Lab Invest* 1984; **44**:311–16.
- Vasilopoulos Y, Cork MJ, Murphy R et al. Genetic association between an AACC insertion in the 3'UTR of the stratum corneum chymotryptic enzyme gene and atopic dermatitis. *J Invest Dermatol* 2004; **123**:62–6.
- Wiedow O, Wiese F, Streit V et al. Lesional elastase activity in psoriasis, contact dermatitis, and atopic dermatitis. *J Invest Dermatol* 1992; **99**:306–9.
- Hachem JP, Man MQ, Crumrine D et al. Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. *J Invest Dermatol* 2005; **125**:510–20.

11 Descargues P, Deraison C, Bonnart C et al. Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat Genet* 2005; **37**:56–65.

Funding sources: S.P. Tan is sponsored by the University of Edinburgh College of Medicine and Veterinary Medicine PhD Studentship and the Scottish Overseas Research Students Awards Scheme.

Conflicts of interest: none declared.

Successful management of severe infant bullous pemphigoid with omalizumab

DOI: 10.1111/j.1365-2133.2011.10748.x

MADAM, Bullous pemphigoid (BP) is exceptional during childhood.¹ Autoantibodies directed against the hemidesmosomal proteins BP180 and BP230 are found in most adult patients with BP (review in reference²). In infants with BP (IBP), as in adult patients, topical corticosteroids are usually effective for disease control,³ but sometimes may require additional treatments^{4,5} in the most severe form.

A 5-month-old male infant was referred to our hospital (considered as day 0) for the management of IBP that failed to respond to oral prednisolone 2.5 mg kg⁻¹ daily. At 4 months, IBP had been diagnosed (Fig. 1) and confirmed by histopathological examination showing a subepidermal blister with a dermal infiltrate of eosinophils and a few lymphocytes. Direct immunofluorescence (IF) showed linear IgG, C3 deposits on the basement membrane zone (BMZ), and a few linear IgA deposits. No IgE deposits were observed on the BMZ. The blood cell count showed major eosinophilia (11.5×10^9 L⁻¹). The total IgE serum level was elevated (636 KU L⁻¹), as were the anti-BP180 and anti-BP230 serum antibody enzyme-linked immunosorbent assay (ELISA) values (Fig. 2). IgG2 and IgG3 circulating anti-BMZ antibodies were detected by indirect IF on normal human salt-split skin. No circulating anti-BP180 or anti-BP230 IgE antibodies were detected by immunoblot on human epidermal extract, or by indirect IF on normal or 1 mol L⁻¹ NaCl-split human skin. Despite increasing the prednisolone dose to 3 mg kg⁻¹ daily, and administering three intravenous (IV) pulses of methylprednisolone 120 mg, topi-

cal betamethasone 0.05%, dapsone 2 mg kg⁻¹ daily and azithromycin 10 mg kg⁻¹ daily, the disease remained uncontrolled. The patient was then treated with a 100-mg subcutaneous injection of omalizumab (calculated from the asthma chart) on day 17. During the following days, the number of new blisters and urticarial lesions decreased dramatically. Disease control was achieved by day 25. Omalizumab injections were continued every 2 weeks for a 3-month period and were then administered monthly for 4 months. After a 7-month follow-up period, no clinical relapse had occurred (Fig. 1). Anti-BP180 (but not anti-BP230) antibody ELISA values decreased after treatment (Fig. 2).

Some experimental and clinical data suggest that eosinophils and IgE may play an important role in BP physiopathology. Up to 86% of adult patients with BP have anti-BP180 IgE autoantibodies.⁶ In an animal model using nude mice grafted with human skin, it was shown that injection of purified IgE isolated from patients with BP induced similar clinical and histological lesions.⁷ Omalizumab is approved by the U.S. Food and Drug Administration in severe atopic asthma management. It is a recombinant humanized monoclonal antibody that binds to the Cε3 domain of IgE and prevents free IgE from binding to the basophils and the mast cell high-affinity IgE receptor.⁸

Given the successful use of omalizumab in a previous case of adult BP⁹ and our patient's high total IgE level, elevated eosinophil blood count and dermal eosinophilic infiltrate, we hypothesized that blocking IgE–eosinophil pathways could be a potential alternative to immunosuppressive agents. We made this assumption before knowing that the patient did not have anti-BP180 or anti-BP230 IgE autoantibodies.

The mechanisms by which omalizumab was effective in this case remain unclear. It does not seem to involve the specific IgG2 and IgG3 humoral response, as BP180 autoantibodies were still detectable 4 months after the first injection.² The efficacy of omalizumab is paradoxical as no anti-BMZ IgE or IgG4 antibodies were detected. Although two techniques failed to detect anti-BMZ IgE antibodies, we cannot rule out that anti-BMZ IgE antibodies were present but not detected for technical reasons. We therefore hypothesize that omalizumab might interfere with cells involved in the BP-specific immune response, such as T lymphocytes or eosinophils. Indeed, *in vitro* experiments have shown that omalizumab is able to induce eosinophil apoptosis and downregulation of proinflammatory cytokines

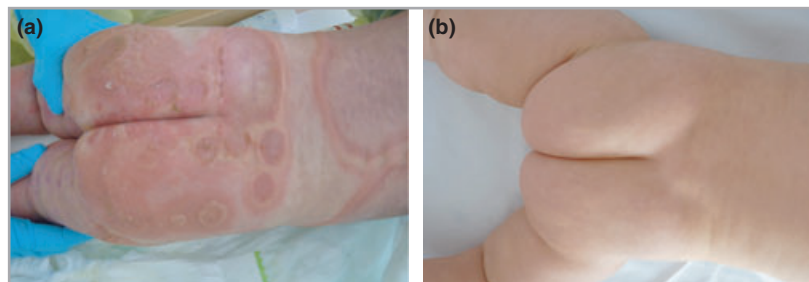


Fig 1. (a) Skin lesions in a 5-month-old boy suffering from pemphigoid bullous; large urticarial areas and bullae are spread over the back and buttocks. (b) The same infant at 10 months of age after seven injections of omalizumab.