

## Correspondence

### Effective removal of sea-urchin spines by erbium: YAG laser ablation

SIR, Sea-urchins are found along the coastline, including in many tourism sites. Their spines consist of calcium carbonate crystals covered by a layer of epithelium.<sup>1</sup> Injuries from sea-urchins result from penetration of the spines into the dermis or subcutis, leading to pain of several days' duration. Complications include local inflammation and infection or a delayed granulomatous foreign-body reaction that can develop weeks or months later. The latter may often be followed by long-lasting pain and even by loss of function.<sup>2,3</sup> To prevent these sequelae, most authors favour the excision of spines rather than waiting for spontaneous recovery as recommended by others.<sup>4</sup> At present, no uniformly accepted successful treatment method is available. We found the erbium:YAG laser to be a suitable tool for effective removal of the spines.

Four patients presented to our outpatient department about 1 week after a sea-urchin spinous injury during a vacation. They all suffered from severe pain and loss of function of the injured extremities. Previous attempts to remove the spines mechanically had not been successful. Inspection disclosed multiple black spines (> 50) up to 1 mm in diameter in the soles of the feet (Fig. 1A) in three of the patients. Mobility at the distal interphalangeal joints was reduced due to an accompanying erythematous oedema. The fourth patient had spines in the left index finger, with swelling and reduced flexibility.

We decided to use the erbium:YAG laser (Medilas E, Dornier, Munich, Germany; spot size 2.5 mm, 300 mJ,

fluence  $6 \text{ J cm}^{-2}$ , repetition rate 5–10 Hz) to remove the foreign material. Three of the patients were treated under local infiltration anaesthesia (1% lidocaine without epinephrine), and one under general anaesthesia. The foreign bodies were destroyed leaving circumscribed crater lesions with tiny pinpoint areas of bleeding (Fig. 1B). Postoperatively, an antiseptic wound dressing was applied. In two of the patients we took a 2-mm skin biopsy; histological examination revealed penetration of the spines into the deep dermis and even subcutis. Two treatment sessions were necessary to remove all the widespread spines in two patients. The wounds re-epithelialized at the latest within 2 weeks after treatment; in some locations we saw focal hyperkeratosis but there were no signs of scarring. Figure 1(C) shows the typical appearance 4 weeks after treatment. Mobility and function of the distal phalanges were restored completely. During 1 year of follow-up no delayed granulomatous foreign-body reactions or other complications have been observed.

Sea-urchins are relatively sedentary creatures often attached to rocks or corals. The porous spines penetrate the skin when stepped on.<sup>5</sup> Mechanical extraction is almost ineffective because the spines fragment easily; surgical excision has been recommended, even though this is difficult with widespread injuries.<sup>4,6,7</sup> Leaving spines in place leads to acute pain, local inflammation or infections and a risk of delayed granulomatous foreign-body reactions. There are reports of granulomatous inflammation in the dermis and subcutaneous tissues, with severe involvement of bone and cartilaginous surfaces followed by chronic pain and even loss of function.<sup>2,3</sup>

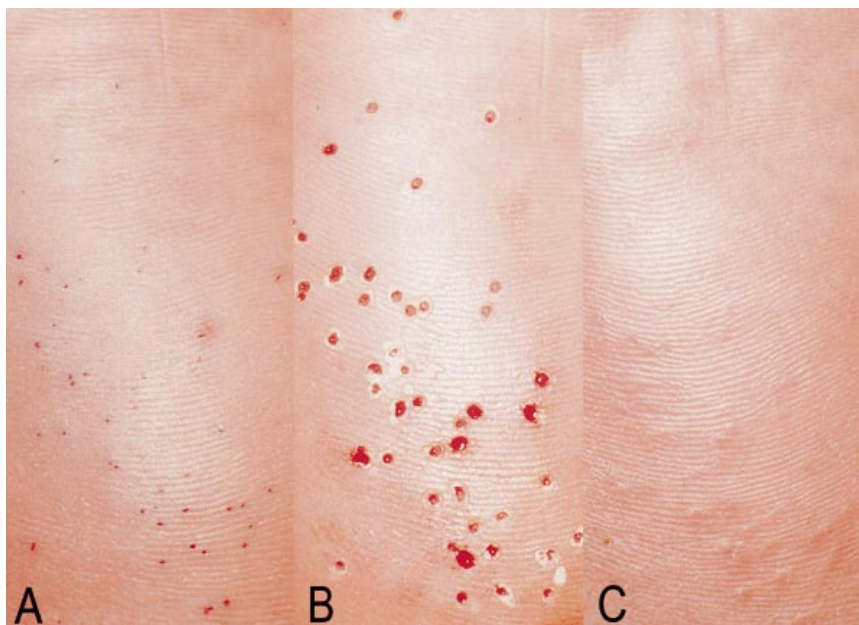


**Figure 1.** (A) Multiple black sea-urchin spines are evident on the sole of the foot. (B) Appearance immediately after erbium:YAG laser ablation. (C) Appearance 4 weeks after treatment.

Conservative treatments such as salicylic acid plasters may work in superficial spinous injuries, but are inadequate with the deep dermal and subcutaneous involvement revealed by histological examination in our patients. As it was impossible to remove the spines mechanically, the alternatives were excision or physical destruction. Due to the multitude of spines and their localization next to joints, the former approach was inappropriate.

We decided to use the unique absorption characteristics of the erbium:YAG laser. As the emission wavelength (2940 nm) exactly matches the absorption peak of water, the energy absorption is 10-fold higher than for carbon dioxide laser light.<sup>8</sup> Thus a precise and clean ablation is possible, avoiding thermal injury and tissue necrosis. As with bony material, the spines were easily destroyed by erbium:YAG laser treatment. The lesions healed within 2 weeks without complications or scarring. This quick recovery could be explained by the unimpaired blood supply and the lack of tissue necrosis after erbium:YAG ablation. One or two treatment sessions were enough to remove the spines. We did not observe delayed granulomatous reactions during several months of follow-up. We recommend erbium:YAG laser ablation as a precise, effective and minimally traumatic approach for the removal of sea-urchin spines.

Zentrum der Dermatologie und Venerologie,  
Klinikum der J.W.Goethe-Universität,  
Theodor-Stern-Kai 7,  
60590 Frankfurt,  
Germany  
E-mail: boer@em.uni-frankfurt.de

A.BÖER  
F.R.OCHSENDORF  
C.BEIER  
R.KAUFMANN

## References

- 1 Heatfield BM, Travis DE. Ultrastructural studies of regenerating spines of the sea urchin *Strongylocentrotus purpuratus*. I. Cell types without spherules. *J Morph* 1975; **145**: 13–49.
- 2 Cooper P, Wakefield MC. A sarcoid reaction to injury by sea urchin spines. *J Pathol* 1974; **112**: 33–6.
- 3 Wilson GE, Curry A, Kennaugh JH *et al*. Severe granulomatous arthritis due to spinous injury by a 'sea mouse' annelid worm. *J Clin Pathol* 1990; **43**: 291–4.
- 4 Baden HP, Burnett JW. Injuries from sea urchins. *South Med J* 1977; **70**: 459–60.
- 5 McWilliam LJ, Curry A, Rowland PL, Watson JS. Spinous injury caused by a sea urchin. *J Clin Pathol* 1991; **44**: 428.
- 6 Falkenberg P. Sea urchin spines as foreign bodies – an alternative treatment. *Injury* 1985; **16**: 419–20.
- 7 Killpacks WS. Injury from spines of sea urchins. *Lancet* 1974; **i**: 1342[Letter].
- 8 Kaufmann R, Hartman A, Hibst R. Cutting and skin-ablative properties of pulsed mid-infrared laser surgery. *J Dermatol Surg Oncol* 1994; **20**: 112–18.

## Human dermatophilosis mimicking ringworm

SIR, Dermatophilosis is a contagious zoonotic skin infection caused by *Dermatophilus congolensis*, a Gram-positive

facultative anaerobic actinomycete. *D. congolensis* infections are world-wide in distribution and are common in cattle, sheep, goats, horses, deer and other domesticated and wild animals.<sup>1–3</sup> They are characterized by an exudative dermatitis that is followed by the formation of scabs, crusts and alopecia.<sup>1,4</sup> A survey of the literature revealed that the organism rarely causes human infections. Human infections reported previously were traced to contact with infected animals.<sup>2,3,5</sup> We report *D. congolensis* infection clinically resembling ringworm in a 44-year-old veterinarian with a known history of relevant contact.

A 44-year-old man presented to the dermatology department with a 10-day history of a slowly enlarging, crusted, scaly, erythematous oval lesion on the dorsum of his right hand. Symptoms were mild itching and burning; he was otherwise well and had no personal or family history of note except that he was a veterinarian and described contact with infected sheep and goats. Physical examination revealed a crusted, scaly, erythematous oval lesion 1.5 × 2.5 cm in size. The lesion was slightly elevated, particularly at the border, where it was more inflamed and crusted than at the central part (Fig. 1).

Potassium hydroxide preparations were negative for fungi, but revealed many branched septate filaments and motile coccoid organisms. Gram and acid-fast stains showed that the organism consisted of branching septate filaments measuring 1–5 nm in diameter, and of coccoid cells that were Gram-positive and non-acid-fast. Many of the filaments showed both transverse and longitudinal septation. A diagnosis of *D. congolensis* infection was made on the basis of these findings. Confirmatory diagnosis of the disease was



**Figure 1.** Well-demarcated, scaly, crusted, erythematous oval lesion on the right hand presenting a 'ringworm' appearance.

made by culturing the organism on blood agar and identification by conventional methods such as biochemical reactions.

Treatment with oral tetracycline hydrochloride 500 mg four times daily, and mupirocin ointment topically twice daily for 2 weeks, resulted in complete improvement of the lesion, and there has been no evidence of recurrence during a 2-month follow-up period.

*D. congolensis* is an obligate parasite isolated from animals, and its mechanical or traumatic inoculation into human skin seems to be an occupational disease. The disease is acquired by direct contact, but it can also be spread indirectly from debris of infected animals, or it can be transmitted by flies and ectoparasites.<sup>3</sup> Dean *et al.*<sup>5</sup> first described the isolation of *D. congolensis* from four of 10 individuals who developed furunculosis after handling a 23-week-old infected deer. It has been suggested that certain predisposing factors are of great importance for the establishment of human infection because many humans have regularly handled infected animals without ever developing the disease.<sup>1</sup> Pathogen, host and environmental factors must be considered in order to understand the pathogenesis of dermatophilosis. A frequently cited sequence of events involves physical damage to the skin, bacterial multiplication in the epidermis, infiltration by neutrophils, and exudate, regeneration of epidermis, and reinvasion.<sup>6</sup>

Human disease has a wide clinical spectrum that includes pitted keratolysis, pustules, exudative scaly lesions, intertriginous lesions with maceration and fissuring of the skin, folliculitis, subcutaneous nodules, and hairy leucoplakia.<sup>3</sup> The latter two conditions are associated with an immune deficiency.<sup>7,8</sup> *D. congolensis* has been isolated from cases of pitted keratolysis.<sup>9,10</sup> *D. congolensis* is a skin pathogen and is usually confined to the living epidermis. Long-term systemic complications as seen with other actinomycoses have not been reported in humans.

The diagnosis of dermatophilosis in humans is primarily based on a history of contact with infected animals, consistent clinical signs, and the demonstration of the organism in a direct microscopic examination. However, confirmatory diagnosis of the disease can be made by culturing the organism on bacteriological media and identification by conventional methods such as biochemical reactions. *D. congolensis* grows in most ordinary laboratory media, but grows best on blood agar, brain–heart infusion agar and tryptone broth. Bacteriological examination of numerous strains isolated from various animal species demonstrated only slight variation in the biochemical reactions of *D. congolensis* strains. The organisms generally have little saccharolytic activity, with the production of acid from glucose and fructose, but not from lactose or sucrose.<sup>1</sup> The clinical differential diagnosis includes fungal infection caused by dermatophytes, particularly *Trichophyton* species.

Systemic antibiotics are the preferred treatment. Penicillin, oxytetracycline, streptomycin, erythromycin and chloramphenicol are known to be effective against dermatophilosis. Topical antibiotics alone are not generally recommended

because of the impossibility of introducing them into infected layers of skin.<sup>1</sup> Topical antibiotics including erythromycin, clindamycin phosphate, mupirocin, gentamicin sulphate and tetracycline hydrochloride are effective in cases of pitted keratolysis.<sup>11,12</sup>

Department of Dermatology and Venereology,  
Faculty of Medicine,  
University of Dicle,  
Dermatoloji Anabilim Dalı,  
21280 Diyarbakir,  
Turkey

M. HARMAN  
S. SEKİN\*  
S. AKDENİZ

\*Department of Internal Medicine,  
Faculty of Veterinary Medicine,  
University of Dicle,  
Diyarbakir,  
Turkey  
E-mail: mharman@dicle.edu.tr

## References

- 1 Zaria LT. *Dermatophilus congolensis* infection (dermatophilosis) in animals and man! An update. *Comp Immun Microbiol Infect Dis* 1993; **3**: 179–222.
- 2 Kaminsky GW, Suter II. Human infection with *Dermatophilus congolensis*. *Med J Aust* 1976; **1**: 443–7.
- 3 Towersey L, de Martins EC, Londero AT *et al.* *Dermatophilus congolensis* human infection. *J Am Acad Dermatol* 1993; **29**: 351–4.
- 4 Erickson EL. *Dermatophilus congolensis* infection in man. *Cutis* 1975; **16**: 83–4.
- 5 Dean DJ, Gordon MA, Severinghaus CW *et al.* Streptothricosis; a new zoonotic disease. *NY State J Med* 1961; **61**: 1283–7.
- 6 Ambrose NC. The pathogenesis of dermatophilosis. *Trop Anim Health Prod* 1996; **28** (Suppl. 2): 29–37S.
- 7 Albrecht R, Horowitz S, Gilbert E *et al.* *Dermatophilus congolensis* chronic nodular disease in man. *Pediatrics* 1974; **53**: 907–12.
- 8 Bunker ML, Chewning L, Wang SE *et al.* *Dermatophilus congolensis* and 'hairy' leukoplakia. *Am J Clin Pathol* 1988; **89**: 683–7.
- 9 Rubel LR. Pitted keratolysis and *Dermatophilus congolensis*. *Arch Dermatol* 1972; **105**: 584–6.
- 10 Gillum RL, Qadri SM, Al-Ahdal MN *et al.* Pitted keratolysis: a manifestation of human dermatophilosis. *Dermatologica* 1988; **177**: 305–8.
- 11 Burkhart CG. Pitted keratolysis: a new form of treatment. *Arch Dermatol* 1980; **116**: 1104[Letter].
- 12 Vazquez-Lopez F, Perez-Oliva N. Mupirocine ointment for symptomatic pitted keratolysis. *Infection* 1996; **24**: 55[Letter].

## Novel intracytoplasmic inclusion bodies in human papillomavirus-associated warts

SIR, We report two patients with cutaneous warts in which novel intracytoplasmic inclusion bodies (ICBs) were observed by histopathological examination. The warts were also analysed by immunohistochemistry and polymerase chain reaction (PCR).

*Patient 1* A 30-year-old Japanese man had had a pigmented warty lesion on his left forearm for about 5 years.

Examination revealed a well-demarcated, rough-surfaced, oval, brownish-black nodule, 4 × 5 mm in diameter, on his left forearm. He had received a renal transplant 1 year earlier, and subsequently had been taking oral immunosuppressive therapy. Biopsy of the pigmented warty lesion showed

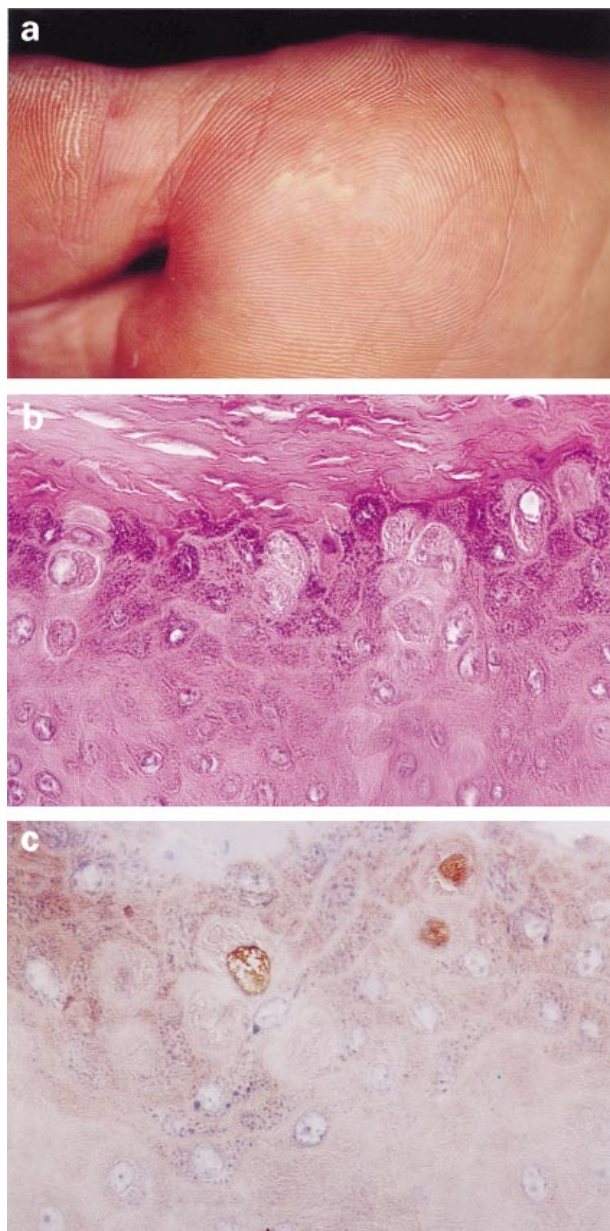


**Figure 1.** (a) Numerous slightly exophytic whitish warty lesions are evident on the sole of the right foot (patient 2). (b) Characteristic eosinophilic fibrillary structures were found in the upper layer of the epidermis (patient 2; haematoxylin and eosin, original magnification × 200). (c) The antihuman papillomavirus antibody K1H8 stained positively in the nuclei of keratinocytes with novel intracytoplasmic inclusion bodies (patient 2; original magnification × 300).

numerous characteristic fibrillary structures in the upper layer of the epidermis.

**Patient 2** A 36-year-old Japanese woman developed multiple warty lesions on the sole of her right foot, which had been noted 3 years previously. Examination revealed numerous, slightly exophytic, whitish warty lesions on the sole of her foot (Fig. 1a), and a punch biopsy was performed. Histologically, it showed a large number of characteristic eosinophilic fibrillary structures in the granular layer of the epidermis (Fig. 1b).

Total cellular DNA was extracted from frozen tissue in each case. The presence of human papillomavirus (HPV) DNA was examined by PCR using F22/F11 and HVP2/B5 primer sets,<sup>1</sup> which can amplify HPV types 1, 4, 60, 63 and 65. However, PCR products could not be obtained from the lesions using these primers. Immunohistochemistry to detect HPV capsid antigen was performed for the two specimens using the avidin–biotin–peroxidase complex method with an anti-HPV antibody K1H8 (Dako, Tokyo, Japan). The nuclei of cells with the intracytoplasmic eosinophilic fibrillary structures found in the upper layers of the epidermis stained positively with the anti-HPV antibody in sections of both specimens (Fig. 1c).

In recent studies, based on histological findings, some novel ICBs in warts were discovered and their full sequences compared with known HPV types: they were designated HPV 60, 63 and 65.<sup>2,3</sup> Keratinocytes infected with cutaneous HPVs usually have characteristic histological features, the so-called cytopathic effect, in the upper epidermis and/or within the stratum corneum. In myrmecia warts, which are related to HPV-1, characteristic eosinophilic granular ICBs are found in the upper epidermis.<sup>4</sup> The warts induced by HPV 4, 60 and 65 display a characteristic histopathology which includes homogeneous eosinophilic ICBs.<sup>4</sup> On the other hand, filamentous ICBs are found in HPV-63-induced warts.<sup>4</sup> These findings strongly suggest that certain cutaneous HPV types correlate with those characteristic ICBs.

In our patients, the histopathological findings were distinctly different from known ICBs. Egawa *et al.* recently reported a new type of cutaneous wart that had characteristic ICBs with an eosinophilic fibrillary substance of wire loop- or knitting wool-like appearance.<sup>5</sup> Histologically, our patients are similar to that case. In addition, in our patients the anti-HPV antibody K1H8 reacted with nuclei of keratinocytes that had the eosinophilic fibrillary substance, indicating that there was a high copy number of the HPV genome in these lesions. Our PCR analysis was unable to amplify the viral genome in our specimens, suggesting that these novel ICBs contain an unknown HPV type. Analysis of the full sequence of the HPV harboured in these novel ICB-containing warts will be required. Furthermore, this novel type of wart may be observed elsewhere in the future.

#### Acknowledgement

We thank Ms M.Sakuma for excellent technical assistance.

Department of Dermatology,  
Tokyo Women's Medical University,  
School of Medicine,  
8-1 Kawada-cho,  
Shinjuku-ku,  
Tokyo 162-8666, Japan

\*Department of Dermatology,  
Kumamoto University School of Medicine,  
Kumamoto, Japan

†Department of Pathology,  
National Institute of Infectious Diseases,  
Tokyo, Japan

E-mail: tmitsu@derm.twmu.ac.jp

T.MITSUISHI  
M.KAWASHIMA  
K.EGAWA\*  
T.SATA†

## References

- 1 Shamanin V, Glover M, Rausch C *et al.* Specific types of human papillomavirus found in benign proliferations and carcinomas of the skin in immunosuppressed patients. *Cancer Res* 1994; **54**: 4610–13.
- 2 Matsukura T, Iwasaki T, Kawashima M. Molecular cloning of a novel human papillomavirus (type 60) from a plantar epidermoid cyst with characteristic pathological changes. *Virology* 1992; **190**: 561–4.
- 3 Egawa K, Delius H, Matsukura T *et al.* Two novel types of human papillomavirus, HPV 63 and HPV 65: comparison of their clinical and histological features and DNA sequences to other HPV types. *Virology* 1993; **194**: 789–99.
- 4 Egawa K. New types of human papillomaviruses and intracytoplasmic inclusion bodies: a classification of inclusion warts according to clinical features, histology and associated HPV types. *Br J Dermatol* 1998; **138**: 381–9.
- 5 Egawa K, Honda Y, Ono T, Kitasato H. A case of viral warts with particular fibrillar intracytoplasmic inclusion bodies. *Dermatology* 2000; **200**: 275–8.

## Acquired perforating collagenosis: is it due to damage by scratching?

SIR, Atopic dermatitis leading to a secondary perforating disorder of the skin has rarely been reported. Perforating disorders of the skin are a heterogeneous group of conditions on which no general consensus concerning a classification exists. Traditionally, four main primary entities<sup>1</sup> are distinguished: Kyrle's disease, elastosis perforans serpiginosa, hereditary reactive perforating collagenosis and perforating folliculitis.<sup>2,3</sup> In addition, an increasing number of patients with a perforating dermatosis in association with a systemic disorder<sup>1</sup> have been reported in the last two decades,<sup>4</sup> in whom the clinical and histological picture often presents a combination of features from the four traditional perforating skin disorders as well as from prurigo nodularis of Hyde.<sup>3</sup> For these conditions, whose prevalence is much higher than suggested by the few reports in the literature,<sup>4</sup> the term acquired perforating collagenosis (APC) was coined.<sup>5</sup>

Our patient, a 56-year-old man, presented with a 3-week history of typical perforating lesions following a severe flare-up of atopic dermatitis, a condition which he had had in mild form for more than 20 years. Prior to presenting at our

department, several treatment regimens, including local steroids, topical anaesthetic creams, ultraviolet (UV)B therapy and thalassotherapy, all aimed at controlling his severely exacerbated atopic eczema had been ineffective. On admission, apart from skin changes typical for atopic dermatitis, he had multiple erythematous, partly hyperpigmented papules ranging from 4 to 8 mm in diameter with central adherent keratotic plugs. The extensor surfaces of the extremities and the trunk were affected predominantly and showed in part a linear distribution pattern. The clinical diagnosis of acquired perforating collagenosis was confirmed by histology (Fig. 1). A van-Gieson's stain, which is not shown here, proved the perforating fibres to be strands of collagen and also demonstrated elastic fibres in the perforations. Apart from several type IV contact allergies we did not find any other underlying illnesses. He was started on local steroids and polidocanol-containing ointment as well as a course of bath-psoralen UVA therapy. After 8 weeks on this regimen both the eczema and the papular eruption had markedly improved.

This observation is, in our opinion, a further piece of evidence<sup>6,7</sup> that an important link between perforating lesions and the underlying condition is the scratching, such as was recently elucidated by the clinical course of a case of APC triggered by scabies and subsiding after appropriate treatment.<sup>8</sup>

The spectrum of systemic illnesses that are associated with a secondary perforating dermatosis is vast, including one case with atopic eczema. Intense pruritus and subsequent scratching is a common clinical feature in many of these illnesses and can cause the superficial trauma that is regarded as the starting point for histopathological changes for APC.<sup>9,10</sup> However, there is no conclusive explanation so far why some people with a particular systemic illness develop APC in response to scratching whereas others do not.

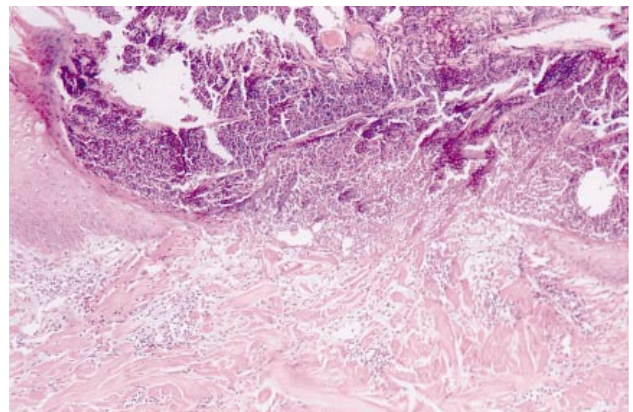


**Figure 1.** Haematoxylin and eosin stain of histological features from a typical lesion over the left scapula (original magnification  $\times 100$ ). Irregular acanthosis of the epidermis with ulcer formation. Numerous neutrophils are seen in the adjacent dermis. Collagen fibres are extruded from the dermal compartment through the disrupted basement membrane into the ulcerous lesion.

Ultrastructural studies have given some insight into the development of APC lesions,<sup>2</sup> but so far they do not provide a straightforward explanation for the formation of transepithelial channels around extruded dermal matrix material in the affected patients. Proposed explanations include a foreign-body reaction against collagen,<sup>2</sup> although it appears normal on electron microscopy,<sup>1</sup> and an abnormal keratinization secondary to this reaction, although electrophoresis does not show abnormal keratin bands.<sup>2,3,11</sup> There is some support for the assumption that patients with diabetes mellitus might, in general, be more prone to such an abnormal pattern of wound healing and altered handling of the exposed collagen matrix after trauma.<sup>10</sup> In this condition, storage phenomena and glycosylation of matrix components have been described, and linked with morphological and biochemical changes reflecting changes in matrix degradation and reassembly.<sup>12</sup> Whether these findings could apply to other medical conditions described in conjunction with APC is not clear.

Another focus of interest is the inflammatory infiltrate of leucocytes.<sup>2,3,13</sup> It has been proposed that the release of matrix metalloproteases and other serine proteases initiated by neutrophils,<sup>11</sup> which transgress the epithelium, could contribute to the formation of lesions by digestion of essential extracellular matrix components.<sup>2,13</sup> This hypothesis fits the finding that serine proteases released by granulocytes degrade elastic fibres and destroy basement membrane structure by cleaving anchoring fibres and collagen IV,<sup>14</sup> and would be an explanation for the elastic fibres found regularly in initial lesions of APC. Furthermore the infiltrating leucocytes are initiators of the release of various inflammatory cytokines in the surrounding tissue, amongst which some, such as IL-1 and TGF- $\beta$ , are important modifiers of matrix metalloprotease activation.

### Acknowledgements

S.Theile-Ochel and L-A.Schneider contributed equally to this manuscript.

Department of Dermatology and Venerology,  
University of Cologne,  
D-50924 Cologne,  
Germany

E-mail: karin.scharffetter@uni-koeln.de

Correspondence:

Prof. Dr med.

Karin Scharffetter-Kochanek

S.THEILE-OCHE

L-A.SCHNEIDER

K.REINHOLD

N.HUNZELMANN

T.KRIEG

K.SCHARFFETTER-KOCHANEK

### References

- Heilman ER. Degenerative diseases and perforating disorders. In: *Lever's Histopathology of the Skin* (Elder D, Elensitas R, Jaworsky C, Johnson B, eds), Philadelphia: Lippincott-Raven, 1997: 343–7.
- Patterson JW, Brown PC. Ultrastructural changes in acquired perforating dermatosis. *Int J Dermatol* 1992; **31**: 201–5.
- Sehgal VN, Jain S, Thappa DM *et al.* Perforating dermatoses: a review and report of four cases. *J Dermatol* 1993; **20**: 329–40.
- Morton CA, Henderson IS, Jones MC, Lowe JG. Acquired perforating dermatosis in a British dialysis population. *Br J Dermatol* 1996; **135**: 671–7.
- Beck HI, Brandrup F, Hagdrup HK *et al.* Adult, acquired reactive perforating collagenosis. Report of a case including ultrastructural findings. *J Cutan Pathol* 1988; **15**: 124–8.
- Kawakami T, Saito R. Acquired reactive perforating collagenosis associated with diabetes mellitus: eight cases that meet Faver's criteria. *Br J Dermatol* 1999; **140**: 521–4.
- Chan LY, Tang WY, Lo KK. Treatment of pruritus of reactive perforating collagenosis using transcutaneous electrical nerve stimulation. *Eur J Dermatol* 2000; **10**: 59–61.
- Kurschat P, Kröger A, Scharffetter-Kochanek K, Hunzelmann N. Acquired perforating collagenosis triggered by scabies infection. *Acta Derm Venereol* 2000; **80**: 384–5.
- Poliak SC, Lebwohl MG, Parris A, Prioleau PG. Reactive perforating collagenosis associated with diabetes mellitus. *N Engl J Med* 1982; **306**: 81–4.
- Kyriaki A, Ephtichia Z, Anna L, Panagiotis D. Reactive perforating collagenosis and acquired perforating dermatosis: presentation of two cases. *J Dermatol* 1997; **24**: 170–3.
- Briggs PL, Fraga S. Reactive perforating collagenosis of diabetes mellitus. *J Am Acad Dermatol* 1995; **32**: 521–3.
- Moczar M, Allard R, Ouzilou J *et al.* Structural and biochemical alterations of human diabetic dermis studied by H-lysine incorporation and microscopy. *Pathol Biol Paris* 1976; **24**: 329–36.
- Zelger B, Hintner H, Aubock J, Fritsch PO. Acquired perforating dermatosis. Transepidermal elimination of DNA material and possible role of leukocytes in pathogenesis. *Arch Dermatol* 1991; **127**: 695–700.
- Briggaman RA, Schechter NM, Fraki J, Lazarus GS. Degradation of the epidermal–dermal junction by proteolytic enzymes from human skin and human polymorphonuclear leukocytes. *J Exp Med* 1984; **160**: 1027–42.

### Treatment of acquired reactive perforating collagenosis with allopurinol

SIR, Reactive perforating collagenosis (RPC) is one of the perforating disorders,<sup>1</sup> originally described by Mehregan *et al.* in 1967.<sup>2</sup> It is now recognized that the condition exists in two variants: an inherited or childhood form and an acquired or adult type (acquired RPC, ARPC) which is associated with diabetes mellitus, chronic renal failure, hypothyroidism, hyperparathyroidism, Hodgkin's disease, liver disorders, neurodermatitis, IgA nephropathy and AIDS. Resolution of lesions in ARPC by oral allopurinol was initially described in two patients with diabetes and hyperuricaemia,<sup>3</sup> and more recently in a patient with diabetes and normal uric acid levels.<sup>4</sup> We report a further patient with ARPC associated with non-insulin-dependent diabetes mellitus who was successfully treated with allopurinol despite having a normal serum uric acid level.

A 57-year-old Turkish woman presented with itchy keratotic papules of 4 weeks' duration, preceded by generalized pruritus. These affected the extremities, mainly the dorsal aspects, the lower back and the abdomen, and were umbilicated papules measuring up to 3 mm in diameter with a central keratotic plug. On the abdomen the distribution of the lesions was linear, demonstrating the Koebner

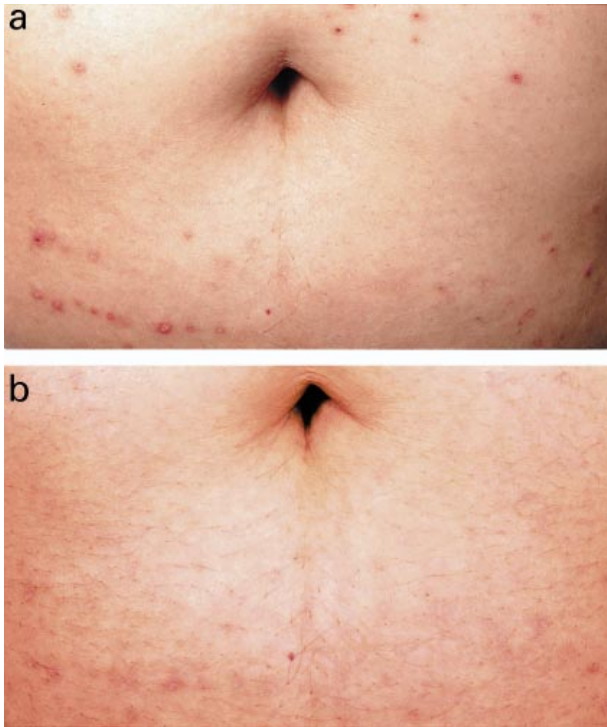


**Figure 1.** (a) Acquired reactive perforating collagenosis before treatment. The linear configuration of the papules demonstrates the Koebner phenomenon. (b) Seven weeks after treatment with allopurinol.

phenomenon (Fig. 1a). The patient's past medical history revealed non-insulin-dependent diabetes mellitus treated with glibenclamide.

Histopathological examination of formalin-fixed and haematoxylin and eosin-stained tissue specimens showed an ulcer filled with a mixture of parakeratotic material, collagen strands and a mainly lymphocytic infiltrate. The lower border of the ulcer was interrupted by eosinophilic staining strands in a perpendicular orientation. Elastica–van Gieson stain showed these to be collagen. Microscopic examination with polarizing light of alcohol-fixed sections did not reveal urate crystals in the biopsy.

The following laboratory parameters were elevated: plasma glucose  $215 \text{ mg dL}^{-1}$  (normal  $70\text{--}100$ ), haemoglobin  $A_{1c}$   $7.6\%$  (normal  $3.9\text{--}5.7$ ), antinuclear antibodies (ANA) with a speckled pattern of immunofluorescence  $1:400$  and ANA with a nuclear pattern of immunofluorescence  $1:400$  (normal  $1:\leq 100$ ). Full blood count, hepatic and renal function and uric acid were normal. The level of antibodies against double-stranded DNA was  $1.3 \text{ mg L}^{-1}$  (normal  $0\text{--}5$ ). Microbiological examination of a swab from a single lesion revealed *Staphylococcus epidermidis*.

Two weeks before admission, treatment with topical corticosteroids and systemic antihistamines was started, but without effect. On the day of admission selective ultraviolet B phototherapy was started combined with oral antihistamines, but phototherapy had to be stopped after five exposures

because of deterioration of pruritus and eruption of new papules. Eight days after hospitalization oral allopurinol  $100 \text{ mg}$  daily was started and led to nearly complete remission of the skin lesions within 2 weeks. During hospitalization the oral antidiabetic medication, consisting of glibenclamide  $5.25 \text{ mg}$  daily, was improved: acarbose  $50 \text{ mg}$  was added on the sixth day after admission and increased up to  $50 \text{ mg}$  three times daily within 3 days. Seven weeks after starting allopurinol the lesions completely cleared, leaving residual pigmented macules (Fig. 1b).

Since the first description of RPC, there have been various hypotheses of the pathogenesis of the skin lesions. Mehregan *et al.*<sup>2</sup> postulated that superficial trauma precipitates the skin lesions in genetically susceptible persons, such that changes take place in the connective tissue of the papillary layer that finally lead to necrobiosis and elimination of the necrobiotic material, together with degenerating inflammatory cells and normal-appearing collagen bundles.<sup>2</sup> Cochran *et al.*<sup>5</sup> suggested that diabetic vasculopathy accompanied by trauma from scratching caused by diabetes and renal insufficiency is the underlying factor. Trauma leads to a dermal necrosis due to a poor blood supply as a consequence of the diabetic vasculopathy.

According to Fretzin *et al.*<sup>6</sup> the extruded collagen is histochemically altered but ultrastructurally intact. The ultrastructural integrity is also stated by Beck *et al.* who observed collagen fibres with a regular periodicity under the electron microscope.<sup>7</sup> Herzinger *et al.* provided evidence that the eliminated collagen may be derived from the basement membrane zone: fluorescent antibodies detected the collagen as type IV collagen.<sup>8</sup>

Yanagihara *et al.*<sup>9</sup> tried to reconstruct the mechanism of formation of the skin lesions in ARPC. They observed that in excoriated wounds the collagen bundles in the reticular dermis were continuous with the collagen bundles in the necrotic mass through epidermal tunnels, and found that in the developing stage, the regeneration of the epidermis progresses between the necrotic mass and the reticular dermis and the collagen bundles that remain in the tunnels. In the next stage, the regenerated epidermis forms a thick horny layer. As a consequence, the necrotic masses are lifted up and the collagen bundles are pulled up from the dermis through the epidermal channels.

There has to be an explanation for the continuity of the collagen bundles between the epidermis and the reticular dermis. Nearly all patients with ARPC, like the patient presented here, suffer from diabetes or chronic renal failure. It is known that high blood glucose levels *in vitro* and *in vivo* result in collagen cross-linking by advanced glycation end-products (AGE).<sup>10</sup> Introduction of foreign AGE into proteins might alter their tertiary structure. Besides a modified function, an immune response with production of specific antibodies might follow the alteration.<sup>11</sup> Collagen cross-linking *in vitro* can be uncoupled from glycation by the use of antioxidants,<sup>12</sup> and Krüger *et al.* suggested that successful treatment of two patients suffering from ARPC with allopurinol might be explained on this basis.<sup>3</sup> Both of these patients, who also had diabetes and hyperuricaemia, were

given allopurinol 100 mg daily. Allopurinol is an inhibitor of xanthine oxidase. After 2 weeks of treatment, complete remission was observed in both cases. The authors came to the conclusion that the result might be due to the antioxidative effect of allopurinol on collagen glycation. Allopurinol is also able to protect against collagen damage: it has been reported that oxygen free radicals generated by hypoxanthine and xanthine oxidase trigger collagen damage in cardiac ischaemia–reperfusion. Free radical scavengers can effectively limit oxygen free radical-induced collagen loss.<sup>12</sup> Rees *et al.*<sup>13</sup> measured increased xanthine oxidase activity in necrotic skin areas in animals and showed a reduction of skin necrosis after treatment of the animals with allopurinol. It may be possible that oxidative stress due to hyperglycaemia in diabetes mellitus acts as the causal factor in the development of lesions in ARPC. Thus, the function of allopurinol as a free-radical scavenger might be relevant in the treatment of ARPC. The effectiveness of treatment with allopurinol in our patient and in a previously reported patient<sup>4</sup> with a normal serum uric acid level, supports the hypothesis of Krüger *et al.*<sup>3</sup>

Opponents might argue that the resolution in our patient, as in the two patients of Krüger *et al.*<sup>3</sup>, was due to the improvement in the diabetic metabolism. However, collagen cross-linking following hyperglycaemia is a long-term process. The levels of AGE in skin collagen are considered to be a sum of accumulated glycation over time, and have a positive correlation with the duration of diabetes.<sup>14,15</sup> It is therefore unlikely that reduction of blood glucose has an immediate influence on the level of AGE in skin collagen. The positive effect of allopurinol on oxygen free radical-induced collagen loss and skin necrosis, however, could be apparent within a short interval.<sup>12,13</sup> This case confirms that allopurinol can be effective in treatment of ARPC.

Department of Dermatology and Allergology,  
Klinikum Augsburg,  
Stenglinstr. 2,  
86156 Augsburg,  
Germany  
E-mail: K.Querings@gmx.de

K. QUERINGS  
B.-R. BALDA  
D. BACHTER

## References

- 1 Rapini RP, Hebert AA, Drucker CR. Acquired perforating dermatosis. Evidence for combined transepidermal elimination of both collagen and elastic fibers. *Arch Dermatol* 1989; **125**: 1074–8.
- 2 Mehregan AH, Schwartz OD, Livingood CS. Reactive perforating collagenosis. *Arch Dermatol* 1967; **96**: 277–82.
- 3 Krüger K, Tebbe B, Krenkel S *et al.* [Acquired reactive perforating dermatosis – successful treatment of two cases with allopurinol]. *Hautarzt* 1999; **50**: 115–20.
- 4 Munch M, Balslev E, Jemec GBE. Treatment of perforating collagenosis of diabetes and renal failure with allopurinol. *Clin Exp Dermatol* 2000; **25**: 615–16.
- 5 Cochran RT, Tucker SB, Wilkin JK. Reactive perforating collagenosis of diabetes mellitus and renal failure. *Cutis* 1983; **31**: 55–8.
- 6 Fretzin DF, Beal DW, Jao W. Light and ultrastructural study of reactive perforating collagenosis. *Arch Dermatol* 1980; **116**: 1054–8.
- 7 Beck H-I, Brandrup F, Hagdrup HK *et al.* Adult, acquired reactive perforating collagenosis. Report of a case including ultrastructural findings. *J Cutan Pathol* 1988; **15**: 124–8.
- 8 Herzinger T, Schirren CG, Sander CA *et al.* Reactive perforating collagenosis – transepidermal elimination of type IV collagen. *Clin Exp Dermatol* 1996; **21**: 279–82.
- 9 Yanagihara M, Fujita T, Shirasaki A *et al.* The pathogenesis of the transepithelial elimination of the collagen bundles in acquired reactive perforating collagenosis. *J Cutan Pathol* 1996; **23**: 398–403.
- 10 Monnier VM, Glomb M, Elgawish A, Sell DR. The mechanism of collagen cross-linking in diabetes: a puzzle nearing resolution. *Diabetes* 1996; **45**: 67–72.
- 11 Sensi M, Pricci F, Andreani D, Di Mario U. Advanced non-enzymatic glycation endproducts (AGE): their relevance to aging and the pathogenesis of the late diabetic complications. *Diabetes Res* 1991; **16**: 1–9.
- 12 Lonn E, Factor SM, van Hoeven KH *et al.* Effects of oxygen free radicals and scavengers on the extracellular collagen matrix during ischaemia–reperfusion. *Can J Cardiol* 1994; **10**: 203–13.
- 13 Rees R, Smith D, Li TD *et al.* The role of xanthine oxidase and xanthine dehydrogenase in skin ischaemia. *J Surg Res* 1994; **56**: 162–7.
- 14 Dominiczak MH, Bell J, Cox NH *et al.* Increased collagen-linked fluorescence in skin of young patients with type 1 diabetes mellitus. *Diabetes Care* 1990; **13**: 468–72.
- 15 Freitas JP, Filipe P, Guerra Rodrigo F. [Glycosylation and lipid peroxidation in skin and in plasma in diabetic patients]. *CR Seances Soc Biol Fil* 1997; **191**: 837–43.

## An alopecic phenotype of canine pemphigus vulgaris?

SIR, Pemphigus vulgaris (PV) is an autoimmune blistering skin disease associated with the generation of autoantibodies that target transmembrane desmosomal proteins in the epidermis. In humans, early manifestations of PV are characterized by a marked predilection of lesions for mucous membranes and by circulating IgG autoantibodies binding to desmoglein (Dsg) 3.<sup>1</sup> With disease progression, the distribution of lesions shifts from mucosal to mucocutaneous, while autoantibodies recognize both Dsg3 and Dsg1.<sup>1</sup> Dsg3 has recently been identified as a major component of murine and human hair follicle desmosomes.<sup>2,3</sup> Targeted disruption of the *DSG3* gene in knockout mice yielded animals with cyclical hair loss, defective follicular keratinocyte adhesion (acantholysis) and separation of telogen clubs from the surrounding outer root sheath.<sup>4</sup> Similar alopecic lesions also have been observed in mice with spontaneous mutations of the *DSG3* gene ('balding phenotype')<sup>5</sup> as well as in experimental murine PV.<sup>6</sup> These murine models have provided compelling evidence that abnormalities in *DSG3* gene expression or protein adhesion are followed by defective anchoring of hair shafts within telogen follicles and clinical manifestation of alopecia.

We report an adult dog that exhibited spontaneous alopecia, microscopic suprabasal acantholysis of the hair

follicle isthmus as well as skin-fixed and circulating IgG autoantibodies targeting both Dsg1 and Dsg3. The diagnosis of alopecic PV was therefore proposed.

A 10-year-old female spayed Boston Terrier presented with a 2-month history of generalized alopecia and multifocal erosions, scales and crusts. These erosive lesions responded to amoxicillin–clavulanate and low-dose prednisone, and the dog was referred to our university hospital. At the time of presentation to the dermatologist, this dog exhibited

multifocal patches of erythema and alopecia (Fig. 1a) that affected predominantly the trunk and head. Hair could be plucked easily from perilesional skin. Erosions and ulcers were not seen on the skin, mucosae or mucocutaneous junctions.

Histopathological examination of skin biopsy specimens revealed suprabasal acantholysis of hair follicles. Intra-epithelial clefts occurred at either or both inferior and isthmus (Fig. 1b) segments of anagen hair follicles. Suprabasal acantholysis of the epidermis was not observed. Periodic

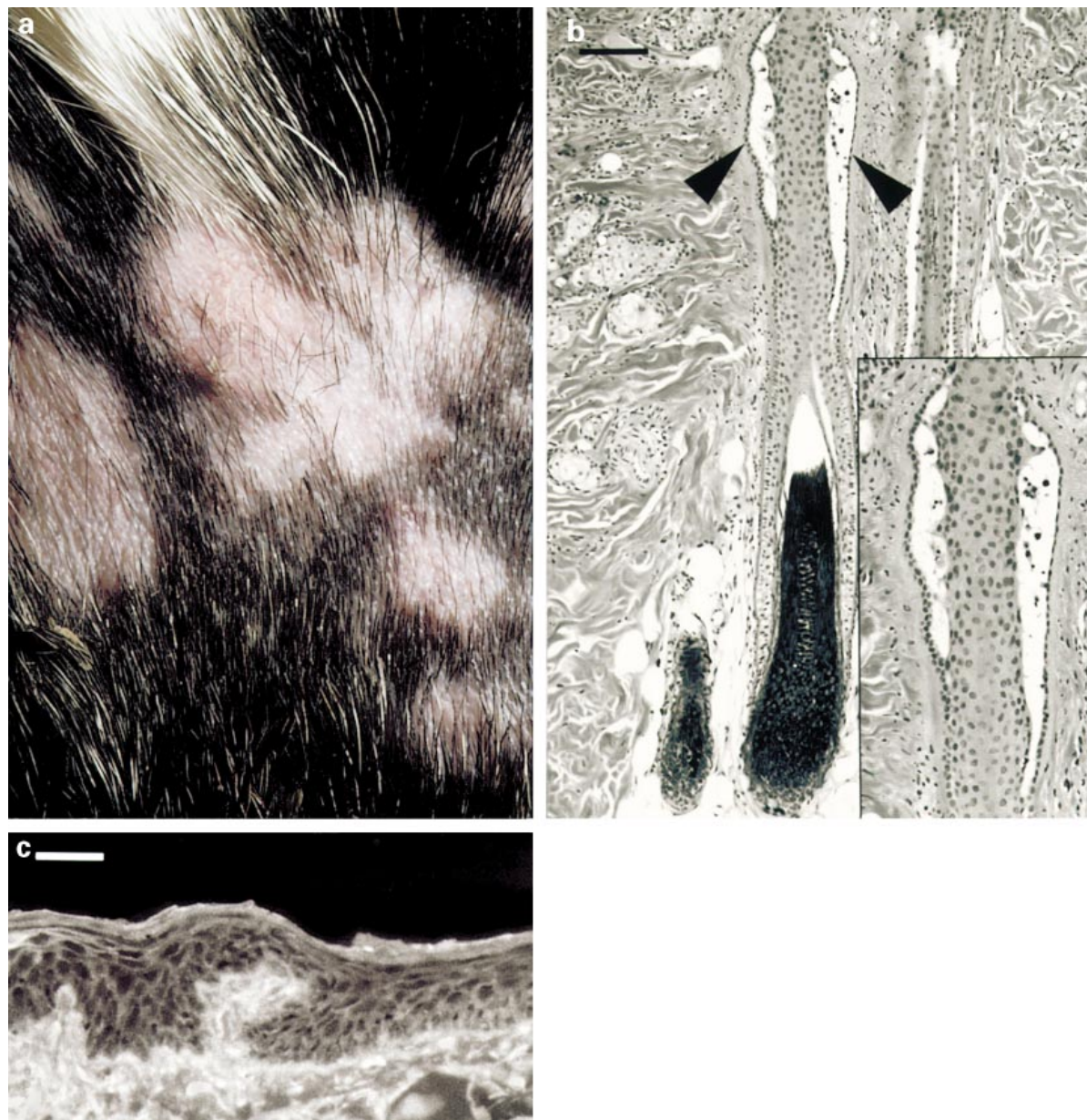


**Figure 1.** (a) Well-demarcated patches of alopecia can be seen on the lateral thorax. (b) Suprabasal cleft formation (black arrowheads) in the isthmus of an anagen hair follicle. Occasional free-floating acantholytic keratinocytes can be seen in the clefts (inset) (haematoxylin and eosin; bar = 200  $\mu$ m, inset 140  $\mu$ m). (c) Direct immunofluorescence confirms the presence of skin-fixed IgG autoantibodies deposited around epidermal keratinocytes (frozen skin sections; anticanine IgG-fluorescein; bar = 45  $\mu$ m).

acid-Schiff staining did not reveal fungal arthrospores in affected or unaffected follicles. A dermatophyte culture of plucked hair shafts was unremarkable.

To confirm the suspicion of autoimmune PV, immunological investigations were performed as previously described.<sup>7</sup> Direct immunofluorescence examination of paraffin-embedded and frozen skin specimens revealed patchy IgG deposition around epidermal and follicular keratinocytes (Fig. 1c). Intercellular epidermal deposits of IgA, IgM and activated complement were not seen. Indirect immunofluorescence permitted the detection of circulating IgG autoantibodies that recognized basal epidermal cells of the normal canine substrates canine lip and oesophagus (titre 1 : 50). Serum autoantibodies were confirmed to bind to the extracellular segments of recombinant human Dsg1 and Dsg3 using a commercially available enzyme-linked immunosorbent assay kit (MBL-Rhigene, IL, U.S.A.) adapted to the canine species.<sup>7</sup>

Because of the coexistence of acquired hair loss, follicular suprabasal acantholysis and Dsg-specific antikeratinocyte antibodies, the existence of an 'alopecic' variant of canine PV was therefore suspected. The clinical features of this canine dermatosis are comparable with the anagen effluvium described recently in three human patients with PV.<sup>8</sup> It is of interest that the suprabasal acantholysis seen in rodents with *DSG3* mutations takes place around the club of telogen hair follicles. In contrast, the acantholysis in this canine case was seen primarily in the isthmus segment of anagen follicles. The reason for this variation in targeted follicular regions is not known. A possible explanation is that this difference could be due to the concurrent existence of autoantibodies targeting both Dsg3 and Dsg1 in this case, in contrast to the single alteration in Dsg3 expression in rodent genetic alopecias. Further determination of the mechanisms involved in the generation of hair loss in this case awaits the performance of passive transfer experiments in rodent species.

Department of Clinical Sciences,  
College of Veterinary Medicine,  
North Carolina State University,  
4700 Hillsborough Street,  
Raleigh,  
NC 27606,  
U.S.A.  
E-mail: Thierry\_Olivry@ncsu.edu

T. OLIVRY  
H.A. JACKSON

## References

- 1 Amagai M, Tsunoda K, Zillikens D *et al.* The clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile. *J Am Acad Dermatol* 1999; **40**: 167–70.
- 2 Kurzen H, Moll I, Moll R *et al.* Compositionally different desmosomes in the various compartments of the human hair follicle. *Differentiation* 1998; **63**: 295–304.
- 3 Koch PJ, Mahoney MG, Cotsarelis G *et al.* Desmoglein 3 anchors telogen hair in the follicle. *J Cell Sci* 1998; **111**: 2529–37.
- 4 Koch PJ, Mahoney MG, Ishikawa H *et al.* Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. *J Cell Biol* 1997; **137**: 1091–102.
- 5 Montagutelli X, Lalouette A, Boulouis HJ *et al.* Vesicle formation and follicular root sheath separation in mice homozygous for deleterious alleles at the balding (*bal*) locus. *J Invest Dermatol* 1997; **109**: 324–8.
- 6 Amagai M, Tsunoda K, Suzuki H *et al.* Use of autoantigen-knockout mice in developing an active autoimmune disease model for pemphigus. *J Clin Invest* 2000; **105**: 625–31.
- 7 Olivry T, Alhaidari Z, Ghohestani RF. Anti-plakin and desmoglein autoantibodies in a dog with pemphigus vulgaris. *Vet Pathol* 2000; **37**: 496–9.
- 8 Delmonte S, Semino MT, Parodi A *et al.* Normal anagen effluvium: a sign of pemphigus vulgaris. *Br J Dermatol* 2000; **142**: 1244–5.

## The use of two substrates for indirect immunofluorescence in the diagnosis of pemphigus

SIR, We read with great interest a clinically important paper by Harman *et al.* on the use of two substrates for indirect immunofluorescence (IIF) in the diagnosis of pemphigus.<sup>1</sup> We fully agree with the authors that two substrates should be used for IIF screening, one rich in desmoglein (Dsg)1 and the other rich in Dsg3, as the sensitivity is not only related to the quantity of pemphigus antibodies in the serum, but is also determined by the quantity of Dsg1 and Dsg3 in the epithelial substrate used for IIF. The authors also quote early papers which stressed that IIF titres do not always correlate with disease severity,<sup>2,3</sup> but we<sup>4</sup> and others found an excellent correlation with disease activity. These disagreements could be due mainly to the use of inappropriate substrates.

We agree with Harman *et al.* that monkey oesophagus (MO), rich in Dsg3, is a good substrate for pemphigus vulgaris (PV), and human skin (HS), rich in Dsg1, is a sensitive substrate for pemphigus foliaceus (PF). However, in our experience the best results are obtained by using human oesophagus (HO) and guinea pig oesophagus (GPO), as HS is a less reliable substrate for PF than is GPO. In the authors' study, the sensitivity of IIF on HS was 83% in both types of pemphigus, whereas it was 90% on MO and almost 100% on the two substrates. In our laboratory, we have used GPO and HO for diagnosis and differentiation of PV and PF for about 20 years, and in our large series (over 300 cases) we had only three cases confirmed by direct immunofluorescence that were negative by IIF. Two of these were PV localized exclusively on oral mucosa, and the other was on the skin and oral mucosa. We also found that use of these two substrates was best for monitoring disease activity, with an almost perfect correlation with clinical findings. In some cases positive in IIF, with no visible cutaneous or oral involvement, we detected lesions localized to other mucous membranes, mainly the oesophagus.

In addition, we noticed that the patterns on MO or HO enabled differentiation between PV and PF (PV, staining of the whole epithelium; PF, staining of the upper parts only), reflecting the distribution of Dsg3 and Dsg1 in epithelium. This is in accordance with the findings of Sabolinski *et al.* who stressed these differences on MO.<sup>5</sup> In our experience, the

results are clearer on HO, which even allows differentiation between PV and PF using only this substrate. The titres of PF sera on HO, MO and GPO are similar, with stronger intensity of staining on GPO. PV sera have comparable patterns and titres on HO and MO, and the titres on MO are much higher than on GPO.<sup>5</sup>

To compare our results with those of Harman *et al.* we performed a simultaneous IIF study on 15 PV and 19 PF sera using four substrates including HS. Typical results are illustrated in Figure 1. We found that HS is a less sensitive substrate than GPO for PF. In our study of 19 PF sera positive on GPO, the IIF titres on HS were, in general, lower; five sera were negative, and in two, strong background staining (prozone phenomenon) enabled evaluation as positive only at the 1 : 40 dilution (Table 1). In general, in our experience, background staining is frequent when using HS as a substrate. We agree with Harman *et al.* that HS is not an appropriate substrate for PV: their study gave 75% positive results, and in our study nine of 15 sera were negative, and others weakly positive.

In conclusion, we fully agree with the authors that IIF study in pemphigus with the use of two substrates increases sensitivity and helps to differentiate the main pemphigus subtypes. Our comparative study with the use of four substrates for IIF in PV and PF also showed the best sensitivity by the use of two substrates: HO and GPO. On HO (also on MO) a characteristic pattern allowed differentiation of PV and PF, whereas on GPO the titres in PF were usually somewhat higher than in PV. In our experience, HS is a less reliable substrate for PF than is GPO, and for PV HO and MO are comparable, although HO as a substrate appears to be somewhat superior to MO due to a more characteristic immunofluorescence pattern (for both PV and PF).

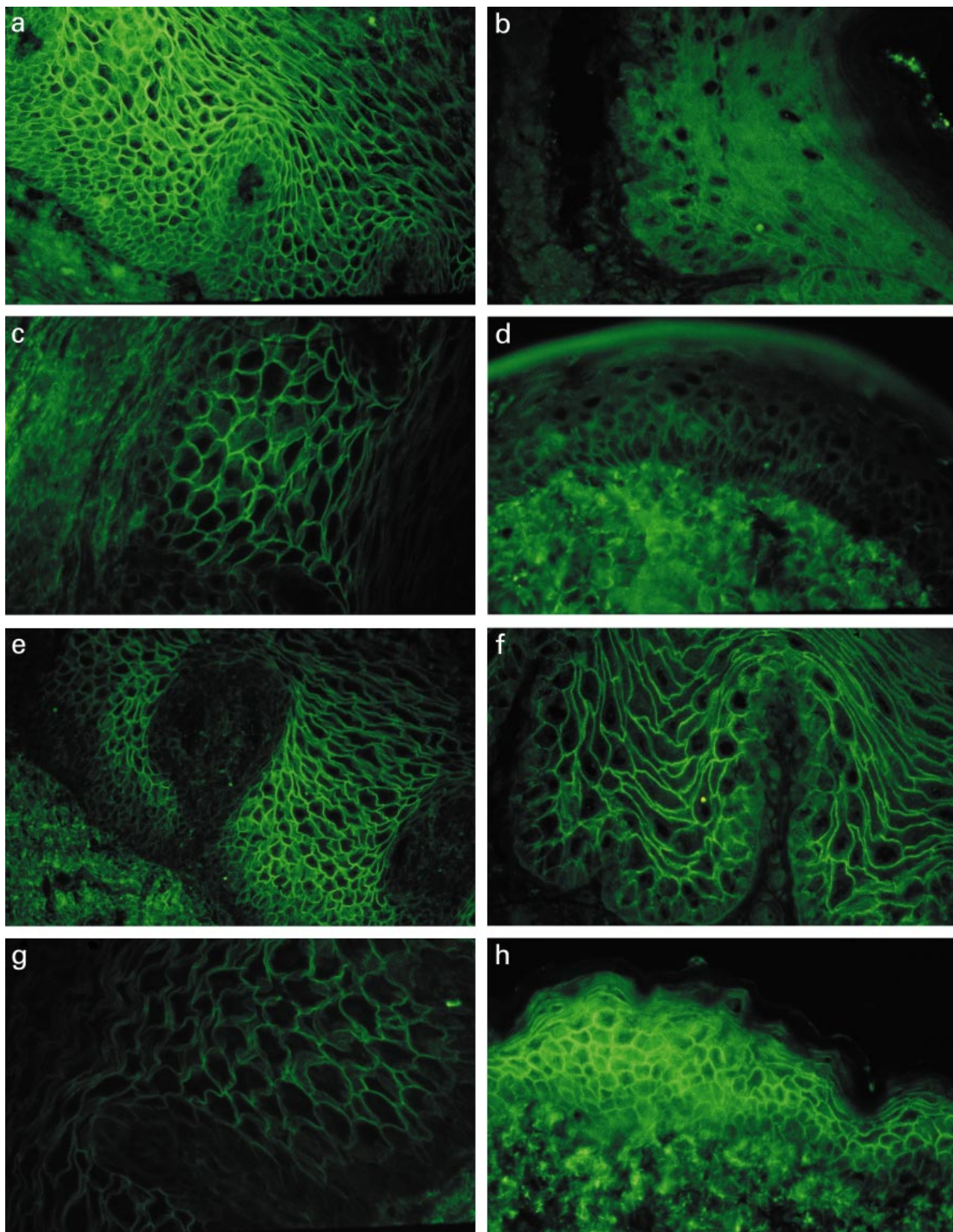
Department of Dermatology,  
Warsaw School of Medicine,  
Koszykowa 82a, 02-008 Warsaw,  
Poland  
Correspondence: S.Jablonska

M.JARZĄBEK-CHORZELSKA  
Z.STRASZ-KOLACIŃSKA  
J.SULEJ  
S.JABLONSKA

**Table 1.** Comparative indirect immunofluorescence study on 34 pemphigus sera with the use of different substrates

|       | Human<br>oesophagus titre | Guinea pig<br>oesophagus titre | Monkey<br>oesophagus titre | Human skin titre |
|-------|---------------------------|--------------------------------|----------------------------|------------------|
| PV 1  | 320                       | 20                             | 320                        | Negative         |
| PV 2  | 160                       | 20                             | 320                        | Weak 20          |
| PV 3  | 320                       | Negative                       | 320                        | Negative         |
| PV 4  | 640                       | 20                             | 640                        | Negative         |
| PV 5  | 640                       | 40                             | 640                        | Prozone, weak 80 |
| PV 6  | 320                       | Negative                       | 320                        | Weak 20          |
| PV 7  | 640                       | 80                             | 640                        | Negative         |
| PV 8  | 640                       | 160                            | 640                        | Weak 80          |
| PV 9  | 160                       | 10                             | 160                        | Weak 80          |
| PV 10 | 80                        | 10                             | 40                         | Negative         |
| PV 11 | 320                       | 40                             | 320                        | Negative         |
| PV 12 | 320                       | 20                             | 160                        | Negative         |
| PV 13 | 320                       | 80                             | 160                        | Negative         |
| PV 14 | 160                       | 40                             | 160                        | 160              |
| PV 15 | 320                       | 320                            | 320                        | Negative         |
| PF 1  | 160                       | 320                            | 320                        | Prozone 40       |
| PF 2  | 1280                      | 5120                           | 1280                       | 2560             |
| PF 3  | 160                       | 320                            | 320                        | 80               |
| PF 4  | 160                       | 640                            | 320                        | 80               |
| PF 5  | 160                       | 320                            | 160                        | Negative         |
| PF 6  | 640                       | 640                            | 320                        | 640              |
| PF 7  | 40                        | 160                            | 80                         | Prozone, weak 40 |
| PF 8  | 320                       | 640                            | 320                        | 80               |
| PF 9  | 5120                      | 5120                           | 5120                       | 2560             |
| PF 10 | 640                       | 640                            | 320                        | 160              |
| PF 11 | 160                       | 160                            | 160                        | Negative         |
| PF 12 | 160                       | 160                            | 80                         | Negative         |
| PF 13 | 640                       | 1280                           | 320                        | 640              |
| PF 14 | 1280                      | 1280                           | 640                        | 640              |
| PF 15 | 320                       | 1280                           | 320                        | 640              |
| PF 16 | 160                       | 640                            | 160                        | Negative         |
| PF 17 | 160                       | 320                            | 160                        | Negative         |
| PF 18 | 640                       | 640                            | 320                        | 640              |
| PF 19 | 320                       | 320                            | 320                        | 320              |

PV, Pemphigus vulgaris; PF, pemphigus foliaceus.



## References

- 1 Harman KE, Gratian MJ, Bhogal BS *et al.* The use of two substrates to improve the sensitivity of indirect immunofluorescence in the diagnosis of pemphigus. *Br J Dermatol* 2000; **142**: 1135–9.
- 2 Judd KP, Lever WF. Correlation of antibodies in skin and serum with disease severity in pemphigus. *Arch Dermatol* 1979; **115**: 428–32.
- 3 Judd KP, Mescon H. Comparison of different epithelial substrates useful for indirect immunofluorescence testing of sera from patients with active pemphigus. *J Invest Dermatol* 1979; **72**: 314–16.
- 4 Chorzelski TP, Jablonska S, Beutner EH. Relationship of autoimmunity to clinical findings in pemphigus. In: *Immunopathology of the Skin* (Beutner EH, Chorzelski TP, Kumar V, eds), 3rd edn. New York: Wiley, 1987: 249–68.
- 5 Sabolinski ML, Beutner EH, Krasny S *et al.* Substrate specificity of anti-epithelial antibodies in pemphigus vulgaris and pemphigus foliaceus in immunofluorescence tests on monkey and guinea pig esophagus sections. *J Invest Dermatol* 1987; **88**: 545–9.

## The use of two substrates for indirect immunofluorescence in the diagnosis of pemphigus: reply from authors

SIR, We thank Jarzabek-Chorzelska *et al.* for the interest shown in our paper.<sup>1</sup> They concur that two substrates should be used for indirect immunofluorescence (IIF) in order to detect antibodies to both desmoglein (Dsg) 1 and Dsg3 in pemphigus sera. We read with interest that the use of human oesophagus (HO) and guinea-pig oesophagus (GPO) has proved to be the most sensitive combination of substrates in their experience over many years. In our study, we used a combination of monkey oesophagus (MO) and human skin (HS).

The aim of our study was to compare IIF titres on these two substrates in the knowledge not only of the pemphigus subtype, i.e. pemphigus vulgaris (PV) or pemphigus foliaceus (PF), but more specifically the serum content of Dsg1 and Dsg3 antibodies, which were quantitatively measured by enzyme-linked immunosorbent assay (ELISA). We established that variation in relative titres on these two substrates could be explained by the content of Dsg1 and/or Dsg3 antibodies in sera. HS proved to be more sensitive for detection of Dsg1 antibodies, which are found in all PF sera and approximately 60% of PV sera.<sup>2</sup> MO was better for the detection of Dsg3 antibodies which are characteristic of PV. Combining data from both substrates enhanced the overall sensitivity of IIF for PV and PF sera. In addition, the relative titres on each substrate helped differentiate PV and PF.

Our study did not aim to identify the optimum tissue substrates for IIF. Therefore, the comparative data of Jarzabek-Chorzelska *et al.* using four tissue substrates is valuable, showing the overall superiority of GPO for PF sera and HO for PV sera. However, it is striking that HS was the least sensitive substrate for PF sera, even in comparison with MO, which is at odds with our data. In contrast, in a study of five tissue substrates, Judd and Mescon demonstrated the superiority of HS over MO for PF sera,<sup>3</sup> a finding that concurs with ours. So who is correct? One explanation for these apparent contradictions is the variability in expression of Dsg molecules in the skin according to site. For example, the IIF titre using PF serum on HS can vary six-fold according to site, with the face and upper torso providing the highest titres.<sup>4</sup> It is also possible that there is interindividual variation in antigen expression for both skin and other substrates. IIF is also a subjective technique but it seems unlikely that observer bias alone could produce such divergent data. The use of recombinant human Dsg molecules as the basis for an ELISA has overcome some of these difficulties and allows automated measurement of Dsg1 and Dsg3 antibodies separately.<sup>5</sup> Another advantage is that animal or human sources of tissue are not required. In the current sociopolitical climate in the U.K., it is unlikely that a continuous supply of HO for IIF could be readily obtained.

We agree that the pattern of antibody staining is different in PV and PF and this can also be observed in human epidermis where Dsg1 antibodies stain the upper layers and Dsg3 antibodies stain the basal layers. A combined pattern is seen in PV sera containing both Dsg1 and Dsg3 antibodies. In the long term, it is likely that ELISA will supersede IIF because it offers many advantages.<sup>6</sup> However, in those laboratories relying on IIF for detection of pemphigus autoantibodies in serum, we recommend the routine use of two substrates to enhance sensitivity. MO or HO are sensitive substrates for Dsg3 antibodies. The optimum substrate for Dsg1 antibodies is less clear cut and we plan to evaluate GPO in comparison with HS.

St John's Institute of Dermatology,  
St Thomas' Hospital,  
and \*Department of Oral Medicine and  
Pathology,  
Guy's Hospital,  
Guy's, King's and St Thomas' School of  
Medicine and Dentistry, King's College,  
London, U.K.

K.E.HARMAN  
M.J.GRATIAN  
B.S.BHOGAL  
S.J.CHALLACOMBE\*  
M.M.BLACK

**Figure 1.** Indirect immunofluorescence staining using four different substrates. (a–d) Serum from pemphigus vulgaris (PV). (a) Human oesophagus as a substrate; dilution 1 : 80. Intercellular (IC) staining of the entire epithelium (original magnification  $\times 200$ ). (b) Guinea pig oesophagus as a substrate; dilution 1 : 20. No IC staining (original magnification  $\times 200$ ). (c) Monkey oesophagus as a substrate; dilution 1 : 80. IC staining of the entire epithelium (original magnification  $\times 400$ ). (d) Human skin as a substrate; dilution 1 : 40. No staining (original magnification  $\times 400$ ). (e–h) Serum from pemphigus foliaceus. (e) Human oesophagus as a substrate; dilution 1 : 80. IC staining of the epithelium except for three to four basal and suprabasal cell layers (original magnification  $\times 200$ ). (f) Guinea pig oesophagus as a substrate; dilution 1 : 40. Strong IC staining of the entire epithelium (original magnification  $\times 400$ ). (g) Monkey oesophagus as a substrate; dilution 1 : 80. Strong IC staining except for two basal and suprabasal cell layers. This serum in lower dilutions produced IC staining of the whole epithelium, similar to PV (original magnification  $\times 400$ ). (h) Human skin as a substrate; dilution 1 : 40. IC staining of the whole epidermis, with slight background staining (original magnification  $\times 400$ ).

Correspondence: Dr K.E. Harman,  
Department of Dermatology,  
King's College Hospital, Denmark Hill,  
London SE5 9RS, U.K.  
E-mail: karen.harman@kcl.ac.uk

## References

- 1 Harman KE, Gratian MJ, Bhogal BS *et al.* The use of two substrates to improve the sensitivity of indirect immunofluorescence in the diagnosis of pemphigus. *Br J Dermatol* 2000; **142**: 1135–9.
- 2 Harman KE, Gratian MJ, Bhogal BS *et al.* A study of desmoglein autoantibodies in pemphigus vulgaris: racial differences in frequency and the association with a more severe phenotype. *Br J Dermatol* 2000; **143**: 343–8.
- 3 Judd KP, Mescon H. Comparison of different epithelial substrates useful for indirect immunofluorescence testing of sera from patients with active pemphigus. *J Invest Dermatol* 1979; **72**: 314–16.
- 4 Ioannides D, Hytiroglou P, Phelps RG, Bystryn JC. Regional variation in the expression of pemphigus foliaceus, pemphigus erythematosus, and pemphigus vulgaris antigens in human skin. *J Invest Dermatol* 1991; **96**: 159–61.
- 5 Amagai M, Komai A, Hashimoto T *et al.* Usefulness of enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. *Br J Dermatol* 1999; **140**: 351–7.
- 6 Harman KE, Gratian MJ, Seed PT *et al.* Diagnosis of pemphigus by ELISA: a critical evaluation of two ELISAs for the detection of antibodies to the major pemphigus antigens, desmoglein 1 and 3. *Clin Exp Dermatol* 1999; **25**: 236–40.

## $\gamma$ -Gliadin: a presumptive allergen causing wheat-dependent exercise-induced anaphylaxis

SIR, Food-dependent exercise-induced anaphylaxis is a distinct form of allergy involving anaphylaxis induced by exercise in

**Table 1.** Patients with wheat-dependent exercise-induced anaphylaxis

| Patient | Age (years) | Gender | Antigluten IgE (UA mL <sup>-1</sup> ) |
|---------|-------------|--------|---------------------------------------|
| 1       | 43          | M      | 0.70                                  |
| 2       | 44          | M      | 0.90                                  |
| 3       | 39          | M      | 1.51                                  |
| 4       | 53          | M      | 1.42                                  |

combination with food ingestion. Although various foods have been reported as allergens, including wheat, shellfish, vegetables, nuts and fish,<sup>1,2</sup> wheat is the most frequent causative food in Japan.<sup>2</sup> Wheat protein consists of water/salt-soluble proteins and water/salt-insoluble proteins; the former comprise albumins, globulins and several inhibitors, and the latter proteins are collectively termed as gluten and comprise gliadins and glutenins. Water/salt-soluble proteins have been identified as allergens in baker's asthma,<sup>3</sup> whereas water/salt-insoluble proteins (gluten) are allergens for wheat-dependent exercise-induced anaphylaxis.<sup>4</sup> Among the wheat water/salt-insoluble proteins, Palosuo *et al.*<sup>5</sup> have identified  $\alpha$ -gliadin (40 kDa) and a novel  $\gamma$ -gliadin-like protein (65 kDa) as allergens in European patients with wheat-dependent exercise-induced anaphylaxis. Our previous study<sup>6</sup> on two Japanese patients with wheat-dependent exercise-induced anaphylaxis indicated that  $\gamma$ -gliadin was an important allergen for wheat-dependent exercise-induced anaphylaxis in these patients.<sup>6</sup> In the present study, using a RAST inhibition test to gluten by using purified  $\gamma$ -gliadin, we show that  $\gamma$ -gliadin is a dominant allergen in these and two other patients with wheat-dependent exercise-induced anaphylaxis.

Serum samples were obtained from four patients with wheat-dependent exercise-induced anaphylaxis (clinical characteristics shown in Table 1). As controls, serum samples were also obtained from two patients with atopic dermatitis who had specific IgE for gluten and a patient with hayfever who had

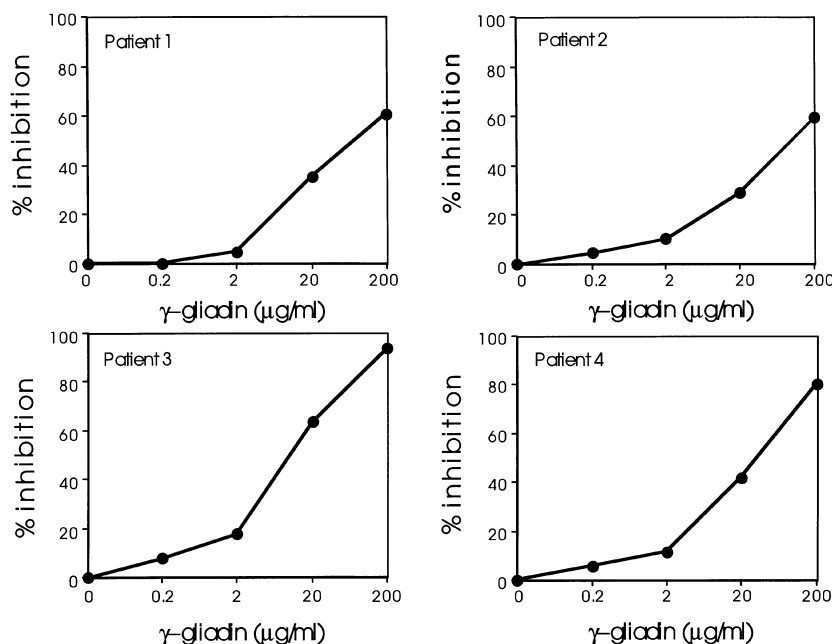


**Figure 1.** Inhibition of IgE binding to solid-phase wheat gluten following preincubation of serum with purified  $\gamma$ -gliadin in four patients with wheat-dependent exercise-induced anaphylaxis. Patients 1 and 2 have been reported previously.<sup>6</sup>

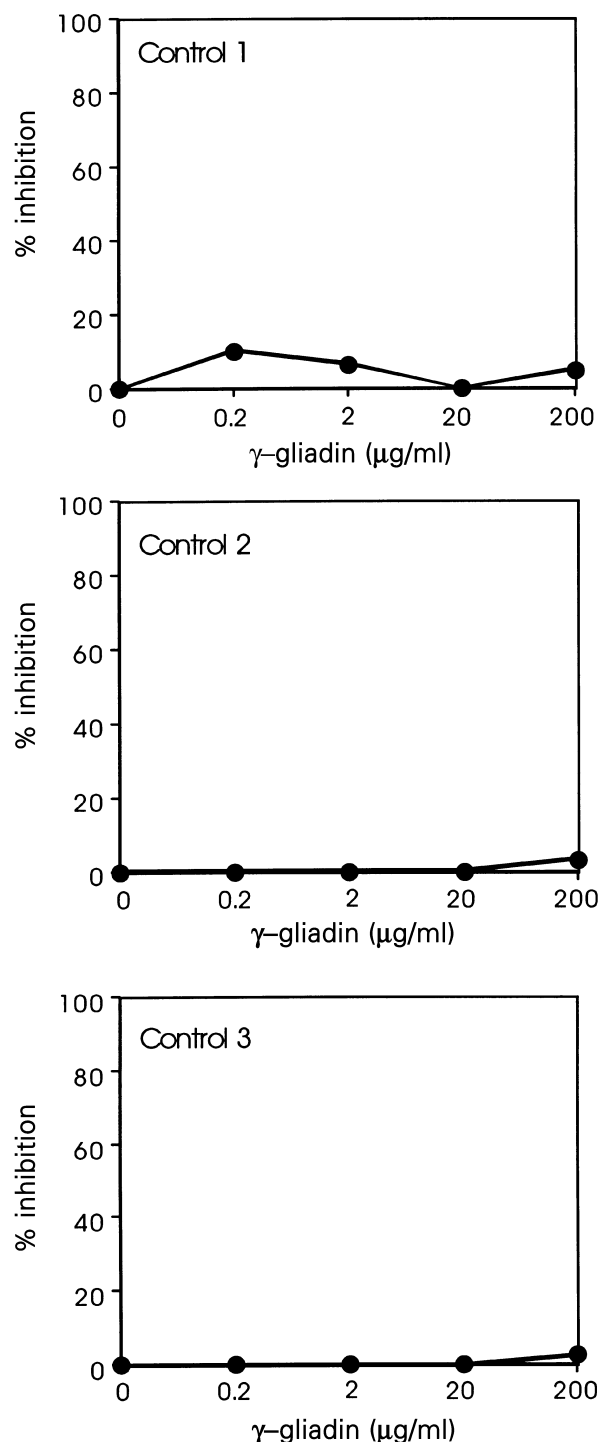


**Figure 2.** Inhibition of IgE binding to solid-phase wheat gluten in three controls following preincubation of serum with purified  $\gamma$ -gliadin. Control 1, patient with atopic dermatitis (gluten RAST  $1.51 \text{ UA mL}^{-1}$ ); control 2, patient with atopic dermatitis (gluten RAST  $1.11 \text{ UA mL}^{-1}$ ); control 3, patient with hayfever (Japanese cedar pollen RAST  $6.77 \text{ UA mL}^{-1}$ ).

specific IgE for Japanese cedar pollen.  $\gamma$ -Gliadin was purified as previously described,<sup>7</sup> with modification. Briefly, commercial gliadin from wheat (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was dissolved in  $50 \text{ mmol L}^{-1}$  acetate buffer (pH 4.8) with  $2 \text{ mol L}^{-1}$  urea, and the soluble proteins were separated by cation exchange chromatography (Mono S HR 5/5; Amersham Pharmacia Biotech, High Wycombe, U.K.). Fractions containing  $\gamma$ -gliadin were applied to a reversed-phase high-performance liquid chromatography column (Sephacil Protein C4 4.6/20; Amersham Pharmacia Biotech), and eluted with a gradient of acetonitrile containing  $0.1\% \text{ v/v}$  trifluoroacetic acid. Sodium dodecyl sulphate electrophoresis of the  $\gamma$ -gliadin preparation showed two bands: a major band with  $M_r$  36 kDa, and a minor band with  $M_r$  30 kDa. N-terminal amino acid sequencing revealed that the major band corresponded to  $\gamma$ -gliadin,<sup>8</sup> and the minor band to a fragment. Specific IgE antibodies (RAST) in serum were determined by a fluoroenzyme immunoassay (CAP system; Pharmacia Diagnostics, Osaka, Japan).<sup>6</sup> For RAST inhibition by  $\gamma$ -gliadin, before determination of RAST to gluten the patients' sera were mixed with various amounts of purified  $\gamma$ -gliadin, and incubated at  $37^\circ \text{C}$  for 30 min.

In inhibition of RAST by purified  $\gamma$ -gliadin, the  $\gamma$ -gliadin caused dose-dependent inhibition of the patient's serum IgE binding to solid-phase gluten proteins in all four patients with wheat-dependent exercise-induced anaphylaxis (Fig. 1). At  $200 \text{ } \mu\text{g mL}^{-1}$  of  $\gamma$ -gliadin (final concentration), 60–90% of the total binding was inhibited. In the two patients with atopic dermatitis who had IgE reacting to gluten, no marked inhibition was seen in IgE binding to gluten proteins following pre-exposure to purified  $\gamma$ -gliadin (Fig. 2), suggesting that IgE specific for  $\gamma$ -gliadin existed exclusively in the patients with wheat-dependent exercise-induced anaphylaxis. The specificity of the  $\gamma$ -gliadin-related inhibition was also confirmed by using serum with IgE to Japanese cedar pollen (Fig. 2).

In the present study we have demonstrated that in four Japanese patients with wheat-dependent exercise-induced anaphylaxis, binding to gluten was inhibited to a large part by purified  $\gamma$ -gliadin, suggesting that  $\gamma$ -gliadin is a dominant allergen causing the anaphylactic symptoms in these patients.

Department of Dermatology,  
Faculty of Medicine,  
Hiroshima University,  
Kasumi 1–2–3,  
Minami-ku,  
Hiroshima 734–8551, Japan  
E-mail: emorita@hiroshima-u.ac.jp

E.MORITA  
Y.KAMEYOSHI  
S.MIHARA  
T.HIRAGUN  
S.YAMAMOTO

## References

- 1 Kushimoto H, Aoki T. Masked type I wheat allergy. Relation to exercise-induced anaphylaxis. *Arch Dermatol* 1985; **121**: 355–60.
- 2 Dohi M, Suko M, Sugiyama H *et al.* Food-dependent, exercise-induced anaphylaxis: a study on 11 Japanese cases. *J Allergy Clin Immunol* 1991; **87**: 34–40.
- 3 Savolainen J. Baker's asthma: diversity of allergens. *Clin Exp Allergy* 1997; **27**: 1111–13.

- 4 Varjonen E, Vainio E, Kalimo K. Life-threatening, recurrent anaphylaxis caused by allergy to gliadin and exercise. *Clin Exp Allergy* 1997; **27**: 162–6.
- 5 Palosuo K, Alenius H, Varjonen E *et al.* A novel wheat gliadin as a cause of exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1999; **103**: 912–17.
- 6 Morita E, Yamamura Y, Mihara S *et al.* Food-dependent exercise-induced anaphylaxis: a report of two cases and determination of wheat- $\gamma$ -gliadin as the presumptive allergen. *Br J Dermatol* 2000; **143**: 1059–63.
- 7 Sjöström H, Friis SU, Noren O, Anthonsen D. Purification and characterization of antigenic gliadins in coeliac disease. *Clin Chim Acta* 1992; **207**: 227–37.
- 8 Scheets K, Hedgcock C. Nucleotide sequence of a  $\gamma$ -gliadin gene: comparisons with other  $\gamma$ -gliadin sequences show the structure of  $\gamma$ -gliadin genes and the general primary structure of  $\gamma$ -gliadins. *Plant Sci* 1988; **57**: 141–50.

### Systems dermatology and clinical dermatological research

SIR, I read with great interest the article entitled 'Genetics, past and present, and the rise of systems dermatology' by J.L. Rees.<sup>1</sup> There were several highly important aspects in that article. I was impressed by the writer's comments on genetics and gene therapy. The writer rightly pointed out the hyperbole presented in the media and by scientists about the ability of genetics to produce new and effective therapies for various diseases. It is very honest to acknowledge that even though huge amounts of research money and effort have been invested into genetics and gene therapy, very little has been achieved, if we look at how the findings have been applied to clinical work. As pointed out by the author and also by other authors, the genetics revolution has had surprisingly few practical consequences for the diagnosis and treatment of diseases.<sup>1,2</sup>

In a recent article, Holtzman and Marteau conclude that new genetics will not revolutionize the way in which common diseases are identified or prevented.<sup>2</sup> They present evidence that the highly acclaimed Human Genome Project will have only a limited impact on clinical medicine. They acknowledge that the mapping and sequencing of the human genome will help to identify more disease genes causing mendelian disorders and to enable the development of diagnostic and predictive tests for them. They argue, however, that only a small proportion of the population has mendelian disorders, which in fact will limit the impact of the Human Genome Project. One argument to support the hyperbole on the Human Genome Project has been that it will be possible to predict each individual's risk of getting certain diseases simply by looking at the individual's genome. Unfortunately, however, reality is not so simple, as pointed out by Holtzman and Marteau. Even in monogenic disorders, there may not be a complete correlation between genotype and phenotype; the situation is far more complicated in complex diseases. Simple gene analysis may not allow any more accurate prediction of the individual's risk of getting, for example, atopic diseases than a careful family history. In

addition, how many people really want to know all the possible disease genes they have right from birth? The knowledge that you have a 1.5-fold increased risk of developing psoriasis will not be of any help for the possible prevention or therapy of psoriasis.

The hyperbole of scientists and the media concerning possible gene therapy and its capacity to cure diseases has been quite unrealistic. In numerous articles and symposia, we have had an opportunity to listen to how close we are to gene therapy and that, within the near future, monogenic diseases and subsequently polygenic diseases will be able to be cured by transferring genes into tissues or cells. This exaggeration started in the mid-1980s and is still going on. The over-enthusiasm about the possibilities of gene therapy has not been avoided in dermatology either. As an example, in a preface to the symposium 'The Genetics of Skin Diseases' held in Colorado in 1993, there was a statement that 'we are on the threshold of gene therapy trials to correct the central genetic defects in several forms of epidermolysis bullosa'.<sup>3</sup> As we now know, there are not many successful gene therapy achievements in the whole field of medicine. Even in monogenic diseases such as cystic fibrosis, gene therapy has not worked. There are numerous obstacles to be solved before gene therapy will be useful in clinical practice even for monogenic diseases: the vector problem, the stem-cell problem (very important in treating skin diseases), the targeting of genes into the right position, the possible hazards of the transferred gene, and so on. It can be seen clearly that most gene therapy trials have been started prematurely due to the ambitious competition between scientists and companies, while there is still no solution to the above-mentioned obstacles.

The hyperbole on genetics and gene therapy has also had a negative impact on clinical research and medicine. One effect is that traditional clinical research has not gained resources or appreciation among sponsors. It has been much easier to get money for a research project that includes a mention of gene therapy than for a clinical study. The situation has been similar in publication policy. If a study involves modern technologies such as molecular medicine or genetics, it has a better chance of being accepted by a highly ranked journal than a study in which mostly clinically oriented methods have been utilized. This has caused researchers to focus on molecular medicine, while clinically oriented research has suffered. However, one should bear in mind that all genetics, gene therapy and applications of modern techniques are based on sound clinical work. If the patient is not diagnosed correctly, relevant clinical genetics is not possible. As stated by Judith Vaitukaitis, director of the U.S. National Institutes for Health general clinical research program, in an article entitled 'The future of clinical research', "nothing is more demanding, more difficult, more frustrating, more time-consuming and requiring more creativity than clinical research".

One thing that makes clinical medicine even more demanding is that we should be able to connect the achievements of modern biology to real patient-oriented practice.<sup>4</sup> This is not possible if we focus on one approach, i.e. genetics, but we should instead look at the problems from a

much broader perspective, as discussed by Rees in his article. We really need a kind of 'systems dermatology', in which we could combine different subspecialties and technologies to solve clinically and scientifically relevant questions. For this, we will need more research money to enable the training of our younger colleagues in relevant and clinically significant research.

Department of Dermatology,  
Oulu University Hospital,  
FIN-90220 Oulu, Finland

A.OIKARINEN

## References

- 1 Rees JL. Genetics, past and present, and the rise of systems dermatology. *Br J Dermatol* 2000; **143**: 41–6.
- 2 Holtzman NA, Marteau TT. Will genetics revolutionize medicine? *N Engl J Med* 2000; **343**: 141–4.
- 3 Norris DA, Uitto J, Epstein E Jr, Yohn Y. Preface. *J Invest Dermatol* 1994; **103**: 15.
- 4 Lindahl S. Academic medicine sets focus on clinical research. *Lancet* 2000; **356**: 54.

## Cutaneous malignancy in arsenicosis

SIR, Arsenicosis or chronic arsenic toxicity due to consuming groundwater containing arsenic above the safe limit of 0.05 mg L<sup>-1</sup>, as laid down by the World Health Organization,<sup>1</sup> was discovered by the author in 1982<sup>2</sup> in patients from West Bengal, India, and in 1984 in a patient from Bangladesh.<sup>3</sup> Cancer from medicinal arsenic used in the treatment of psoriasis was first detected by Hutchinson<sup>4</sup> in 1887. Among 20 places in different countries where a high concentration of groundwater arsenic was found, West Bengal and Bangladesh are suffering from the biggest arsenical calamity in the world.<sup>5</sup>

A previous study reported clinical features of 4865 patients with arsenical dermatosis from 1206 villages of nine districts in West Bengal during the period 1983–87.<sup>6</sup> The present study addresses neoplasia developing during 1983–99 in the same subjects. In this group, 250 subjects (5.1%) developed a malignancy: 212 had skin cancers (84.8%) and the remaining 38 had internal cancers (15.2%). Skin cancers comprised 161 cases of squamous cell carcinoma and 51 cases of Bowen's disease or intraepidermal carcinoma. The cancers were mostly monocentric but one patient had 26 separate skin cancers. No basal cell carcinomas or malignant melanomas were seen in this series of cases. Among the 38 cases of internal cancers, lung carcinoma was most common (25 cases), followed by bladder and genital tract carcinoma (five cases each); only three cases of gastric cancer were found.

Cancers usually develop 10–20 years after onset of symptomatology of arsenicosis; large keratotic nodules are forerunners of skin cancer. That cancer is due to arsenicosis can only be inferred if the associated features of arsenicosis are present, e.g. melanosis and keratosis. High levels of arsenic in nails, hair or skin scales confirm the clinical stage of arsenicosis. Therapeutically, the chelating drugs

dimercaptosuccinic acid<sup>7</sup> or dimercaptopropane sulphonate<sup>8</sup> gave only a little benefit when administered at a late clinical stage; the melanosis was eliminated and further complication could be prevented, but the keratosis remained unchanged. The drugs were of no use once malignancy had developed. The author encountered one case of arsenicosis where cancer developed 15 years following chelation drug therapy with demercaprol and D-penicillamine.

## Acknowledgments

The author thanks D.Chakraborti, Department of Chemical Engineering, School of Environmental Studies, Jadavpur University, and G.Poddar, Department of Chemistry, The School of Tropical Medicine, Calcutta, India. Research during 1984–86 was supported by a grant from the Indian Council of Medical Research.

The School of Tropical Medicine, EC-21,  
Sector 21, Bidhannagar,  
Calcutta 700 064, India

K.C.SAHA

E-mail: poddars@cal3.vsnl.net.in; debasaha@yahoo.com

## References

- 1 World Health Organization. *Environmental Health Criteria 18: Arsenic* Geneva: World Health Organization, 1981; 93–105.
- 2 Saha KC. Melanokeratosis from arsenic contaminated tube well water. *Indian J Dermatol* 1984; **29**: 37–46.
- 3 Saha KC. Skin disease from arsenic contaminated tube well water in West Bengal. *Foundation Souvenir Calcutta School Trop Med* 1995; **8**: 24.
- 4 Hutchinson J. Arsenic cancer. *Br Med J* 1887; **ii**: 1280–1.
- 5 Chowdhury UK, Biswas BK, Chowdhury TR *et al.* Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ Health Perspect* 2000; **108**: 393–7.
- 6 Saha KC. Chronic arsenical dermatosis from tube well water in West Bengal during 1983–87. *Indian J Dermatol* 1995; **40**: 1–12.
- 7 Graziano JH. Role of 2,3-dimercaptosuccinic acid in the treatment of heavy metal poisoning. *Med Toxicol* 1986; **1**: 155–62.
- 8 Aposhian HV, Maiorino RM, Gonzalez-Ramirez D *et al.* Mobilization of heavy metals by newer, therapeutically useful chelating agents. *Toxicology* 1995; **97**: 23–38.

## Antioxidant characteristics of chronic wound fluid

SIR, Some hypotheses of venous ulceration suggest that leucocytes induce agents, probably reactive oxygen species (ROS), which result in tissue damage.<sup>1</sup> The high reactive potential of ROS is balanced by the presence of antioxidants<sup>2</sup> and, in a normally healing wound, tissue concentrations of enzymatic and non-enzymatic antioxidants have been reported to fall.<sup>3</sup> However, little is known of the antioxidant environment of chronic wounds. We have undertaken a preliminary assessment of antioxidant activity in fluid collected from chronic leg wounds.

Methods have been developed<sup>4,5</sup> to assess the total antioxidant ability of a given solution. They are designed

to give a predictable response for the major serum antioxidants, particularly uric acid, albumin, vitamin C, vitamin E and bilirubin. We applied two of these assays, the trolox-equivalent antioxidant capacity (TEAC)<sup>4</sup> assay and the ferric-reducing ability of plasma (FRAP)<sup>5</sup> assay to wound fluid from patients with chronic wounds. Fluid was collected from patients attending a leg ulcer clinic (the Wound Healing Institute has ethical approval to collect wound fluid) by covering the wound with a transparent occlusive dressing (Opsite, Smith and Nephew Medical Limited, Hull, U.K.) and keeping the leg dependant for 30–40 min. Fluid was withdrawn from beneath the dressing with a needle, centrifuged and stored at  $-70^{\circ}\text{C}$ . Both the TEAC and FRAP assays were measured on a Roche Cobas Fara autoanalyser (Roche Ltd, Lewes, U.K.).

The TEAC assay was applied to five wound fluids from five patients (median age 69 years, range 65–88, three men, two women, median wound area  $50.7\text{ cm}^2$ , range  $2.5\text{--}100\text{ cm}^2$ ). In each case patient assessment and clinical history suggested venous disease as a cause of the chronic wound. The median TEAC in the wound fluid was  $2510\text{ }\mu\text{mol L}^{-1}$  (range  $2430\text{--}2580\text{ }\mu\text{mol L}^{-1}$ ). These values are well above those in plasma (95% confidence interval for adults<sup>4</sup> was  $1320\text{--}1600\text{ }\mu\text{mol L}^{-1}$ ). Three individual antioxidants known to contribute significantly to TEAC are uric acid, albumin and bilirubin. We measured these three components in the wound fluid with standard autoanalyser techniques. Based on the known TEAC reaction rates, these antioxidants accounted for less than 20% of the observed activity while in plasma they contribute approximately 78%.<sup>4</sup>

The FRAP assay was applied to wound fluids collected from nine patients with chronic venous ulcers (median age 72 years, range 65–89 years, three men, six women, median wound area  $34\text{ cm}^2$ , range  $2.5\text{--}100\text{ cm}^2$ ). The median FRAP in nine specimens of chronic wound fluid was  $1177\text{ }\mu\text{mol L}^{-1}$  and range  $858\text{--}1819\text{ }\mu\text{mol L}^{-1}$ , slightly higher than expected for plasma ( $612\text{--}1634\text{ }\mu\text{mol L}^{-1}$ ).<sup>5</sup> Therefore both antioxidant assays, but particularly the TEAC, suggest that the antioxidant activity of wound fluid is higher than that in plasma.

Uric acid, a component known to contribute to FRAP, was also measured in this group of patients. Median uric acid concentration was  $224\text{ }\mu\text{mol L}^{-1}$ , range  $184\text{--}384\text{ }\mu\text{mol L}^{-1}$ , which represents a median of 45.3% and range 20.5–65.3% of the measured FRAP activity.

For the nine patients whose wound fluid antioxidant status was measured with the FRAP assay we were able to assess the wound for evidence of healing. The ulcer was defined as healing if there had been complete wound closure or a reduction in wound area by greater than 50% 12 weeks after fluid collection. In three of the nine patients the wounds healed (median age 67 years, range 67–89 years, median wound area  $26.4\text{ cm}^2$ , range  $2.5\text{--}60.0\text{ cm}^2$ ). These patients had a median FRAP activity of  $1661\text{ }\mu\text{mol L}^{-1}$  and range  $1214\text{--}1819\text{ }\mu\text{mol L}^{-1}$ . The six patients with non-healing wounds (median age, 74 years, range 65–86 years, median wound area  $35.0\text{ cm}^2$ , range

$21.0\text{--}100.0\text{ cm}^2$ ) had a median FRAP of  $1134\text{ }\mu\text{mol L}^{-1}$  and range  $858\text{--}1666\text{ }\mu\text{mol L}^{-1}$ . The differences between FRAP in healing and non-healing groups was not statistically significant (Mann–Whitney non-parametric *U*-test,  $P = 0.12$ ) but FRAP activity was generally lower in non-healing wounds.

Two patients whose wounds failed to heal had a second fluid specimen collected and FRAP re-measured. In one patient the FRAP in wound fluid was  $1177\text{ }\mu\text{mol L}^{-1}$  on the first occasion, and  $1136\text{ }\mu\text{mol L}^{-1}$  on the second, 6 months later. In the second patient the FRAP was  $1278\text{ }\mu\text{mol L}^{-1}$  on the first occasion and  $1007\text{ }\mu\text{mol L}^{-1}$  on the second, 6 weeks later. Thus the FRAP activity remained at, or fell to concentrations lower than those observed in the healing wounds.

Methods available to assess total antioxidant activity are based on different theoretical principles and for plasma do not produce numerically similar results.<sup>6</sup> However, they have been used in many clinical contexts to provide an insight into pathophysiological changes associated with disease. Our initial observations of chronic wound fluid suggest a localized increase in antioxidant activity at the site of a chronic wound, not accounted for by individual antioxidants known to be significant contributors in plasma. In a small number of patients, fluid collected from chronic healing wounds had a non-statistically higher antioxidant activity than that from non-healing wounds. This might suggest that improving the local antioxidant activity might be beneficial to healing outcome. More work is required to identify the individual components of antioxidant activity in wound fluid and clarify the role of ROS in impaired healing.

Department of Clinical Biochemistry,  
Oxford Radcliffe Hospital,  
Oxford OX3 9 DU, U.K.  
\*Wound Healing Institute,  
Department of Dermatology,  
The Churchill Hospital,  
Oxford, U.K.

Correspondence: Dr R.P.Taylor.

E-mail: richard.taylor@orh.nhs.uk

T.J.JAMES  
M.A.HUGHES\*  
D.HOFMAN\*  
G.W.CHERRY\*  
R.P.TAYLOR

## References

- Coleridge Smith PD, Thomas P, Scurr JH, Dormandy JA. Causes of venous ulceration: a new hypothesis. *Br Med J* 1988; **296**: 1726–7.
- Vervaaert P, Knight KR. Oxidative stress and the cell. *Clin Biochem Rev* 1996; **17**: 3–16.
- Shukla A, Rasik AM, Patnaik GK. Depletion of reduced glutathione, ascorbic acid, vitamin E and antioxidant defence enzymes in a healing cutaneous wound. *Free Rad Res* 1997; **26**: 93–101.
- Miller NJ, Rice-Evans C, Davies MJ *et al.* A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature infants. *Clin Sci* 1993; **84**: 407–12.
- Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem* 1996; **239**: 70–6.

6 Cao G, Prior RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin Chem* 1998; **44**: 1309–15.

### Atypical presentation of adult Still's disease with generalized rash and hyperferritinaemia

SIR, Still's disease is a multisystemic inflammatory disorder of unknown aetiology that was first reported in children but may also affect adults.<sup>1,2</sup> It is characterized by high spiking fever, sore throat, articular involvement, a salmon-pink evanescent rash, lymphadenopathy, hepatosplenomegaly, serositis, leucocytosis with neutrophilia, and negative rheumatoid factor and antinuclear antibody tests.<sup>3,4</sup> The diagnosis of adult-onset Still's disease (AOSD) is still based on the presence of compatible clinical findings and the exclusion of other diseases, as there is no diagnostic test.<sup>5</sup> However, recent reports have noted a striking elevation of serum ferritin in patients with active AOSD in comparison with other inflammatory disorders or with inactive AOSD.<sup>6–8</sup> We report a patient with a generalized persistent rash characterized by erythematous papules and plaques and hyperferritinaemia as initial manifestations of AOSD.

A 39-year-old healthy white man presented with a 9-day history of a non-pruritic persistent rash, severe sore throat, fever, chills, weight loss, polyarthritides, myalgia, fatigue and lymphadenopathy. On admission, his temperature was 39.5 °C. Examination revealed a diffuse redness of the pharynx, cervical lymphadenopathy and hepatosplenomegaly. He had a widespread erythematous eruption, with confluent papules and plaques on the neck, trunk and limbs (Fig. 1a). The palms and soles were spared. He had symmetrical synovitis of the elbows, wrists, knees and ankles. The remainder of the physical examination was normal. The only abnormal laboratory studies were white blood cell count of  $16 \times 10^9 \text{ L}^{-1}$  with a left shift, an erythrocyte sedimentation rate of 85 mm in the first hour, aspartate aminotransferase 68 U L<sup>-1</sup>, alanine aminotransferase 73 U L<sup>-1</sup>,  $\gamma$ -glutamyl-transferase 62 U L<sup>-1</sup>, C-reactive protein 13 mg L<sup>-1</sup> (normal < 5) and a serum ferritin level of 65 950  $\mu\text{g L}^{-1}$  (normal 20–250). Tests for rheumatoid factor, antinuclear antibody, antineutrophil cytoplasmic antibodies, anticardiolipin antibody, cryoglobulins,  $\alpha_1$ -antitrypsin, angiotensin-converting enzyme, antistreptolysin O, serum complement C3 and C4 levels and circulating immunocomplexes were all negative or normal. Serology for *Salmonella* spp., *Shigella* spp., *Brucella* spp., *Chlamydia* spp., Q fever, *Borrelia burgdorferi*, *Bartonella henselae*, *Yersinia enterocolitica*, group B *Streptococcus*, *Treponema pallidum*, hepatitis A, B and C, human immunodeficiency virus, parvovirus B19, herpes simplex, herpes zoster, varicella-zoster virus, Epstein–Barr virus and cytomegalovirus was negative. Mantoux test was non-reactive. Blood, urine, stool and throat cultures were negative. An electrocardiogram, chest X-ray, an echocardiogram and joint X-rays were normal. A computed tomographic scan of the abdomen revealed hepatosplenomegaly. Biopsy of the lymph nodes showed plasma cells and a polymorphonuclear infiltrate with signs of reactive hyperplasia, but no other characteristic

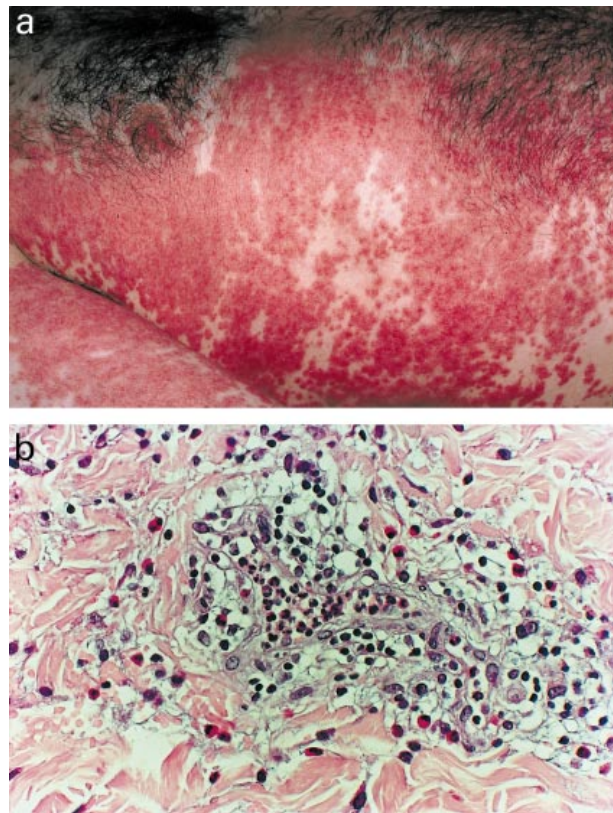


**Figure 1.** (a) Confluent erythematous papules and plaques are evident on the trunk and upper right arm. (b) Photomicrograph showing perivascular infiltrate of dermal vessels with eosinophils, histiocytes, lymphocytes and neutrophils within the vascular lumen (haematoxylin and eosin).

findings. Skin biopsy revealed perivascular infiltration of the small vessels in the dermis by eosinophils, histiocytes and lymphocytes (Fig. 1b). Direct immunofluorescence was negative for immunoglobulin and complement deposition. Stains for fungi and acid-fast bacilli were negative. There was no response to empirical antibiotics.

AOSD was diagnosed and the patient was initially started on a regimen of salicylates and later on indomethacin, with minimal improvement. Treatment with prednisone 80 mg daily (1 mg kg<sup>-1</sup> daily) was started with a remarkable decrease of disease activity, and serum ferritin returned to normal values. The patient was maintained on low-dose prednisone for 1 year because he had several relapses characterized by an evanescent, erythematous, maculopapular rash and arthralgia, which were controlled by increasing the oral prednisone dose. He is currently asymptomatic without therapy, and without recurrence, 41 months later.

Our patient fulfilled the criteria of Yamaguchi *et al.* for AOSD.<sup>5</sup> He had four major criteria, including high spiking fever, arthralgia, rash and leucocytosis, and four minor ones, which were sore throat, lymphadenopathy and splenomegaly, liver dysfunction, and the absence of rheumatoid factor and antinuclear antibody. Although the major forms of

cutaneous vasculitis, including urticarial vasculitis, were initially considered in the differential diagnosis, the lack of diagnostic criteria and the absence of vasculitis in the cutaneous biopsy specimen excluded them. On the other hand, our patient did not have characteristic manifestations of Kawasaki syndrome such as conjunctival congestion, inflammation of oral mucous membranes, involvement of palms and soles by the rash, desquamation of the finger and toes, cardiovascular involvement or thrombocytosis. Other infectious, neoplastic or rheumatological disorders were excluded by appropriate laboratory, radiological and pathological investigations.

In 1956, Isdale and Bywaters<sup>1</sup> described the rash of Still's disease, in a study of 46 children, as a salmon-pink, macular or maculopapular eruption. It is frequently evanescent and is most likely to be observed with the fever spike. The rash is most commonly found on the limbs and over the trunk, less often occurring on the face and neck. Pouchot *et al.*<sup>4</sup> found that in one-third of patients the rash is mildly pruritic and thus may be mistaken for drug allergy. It may appear in areas of mechanical irritation. In one-third of patients it can be precipitated by rubbing or, less commonly, by a hot bath.<sup>4</sup> Histologically, the eruption consists of mild perivascular inflammation of the superficial dermis with prominent lymphocytes and histiocytes associated with dermal oedema. Direct immunofluorescence is usually negative for immunoglobulin and complement.<sup>4</sup> A search of the literature revealed only a few reports of atypical cutaneous manifestations of AOSD, including a typical rash together with erythematous confluent papules that coalesced to form large, irregular, erythematous plaques,<sup>9</sup> generalized peau d'orange appearance of the skin,<sup>10</sup> urticaria,<sup>11</sup> and an evanescent rash and fixed papular lesions.<sup>12</sup> The last three patients had serum ferritin levels between 514 and 3800  $\mu\text{g L}^{-1}$ .<sup>10–12</sup>

Ferritin is mainly found in the reticuloendothelial system, including the liver, spleen and bone marrow; other locations include the macrophage lineage of white blood cells, including histiocytes.<sup>13</sup> Very high serum ferritin levels have been found in patients with active AOSD.<sup>6–8</sup> Other conditions associated with high serum ferritin levels, such as haemochromatosis, polytransfusion, hepatocellular necrosis and malignant histiocytosis, usually have typical clinical or laboratory abnormalities to aid in diagnosis.<sup>8</sup> In patients with rheumatoid arthritis, systemic lupus erythematosus or vasculitis, serum ferritin was found to be normal or modestly elevated.<sup>8</sup> Increased serum ferritin levels, but below 500  $\mu\text{g L}^{-1}$ , have been reported in patients with acute bacterial infections, whereas normal levels of ferritin were found in patients with lung and pancreatic cancer.<sup>8</sup> The origin of the elevated ferritin levels in AOSD remains unclear. It has been suggested that liver necrosis, activation of cytokines, increased release and/or decreased clearance by Kupffer cells and viral-associated haemophagocytic syndrome may be the source of hyperferritinaemia.<sup>6,13,14</sup>

In conclusion, a persistent rash characterized by erythematous papules and plaques and hyperferritinaemia may be an atypical presentation of AOSD.

Departments of Internal Medicine and  
\*Pathology, Hospital Virgen del Camino,  
Irunlarrea 4,  
31008 Pamplona,  
Spain  
Departments of †Dermatology and  
‡Rheumatology,  
Hospital Provincial de Navarra,  
Pamplona,  
Spain  
E-mail: cperezg@meditex.es

C. PEREZ  
M. MONTES\*  
M. GALLEGÓ†  
E. LOZA‡

## References

- 1 Isdale IC, Bywaters EGL. The rash of rheumatoid arthritis and Still's disease. *Q J Med* 1956; **25**: 377–87.
- 2 Bywaters EGL. Still's disease in the adult. *Ann Rheum Dis* 1971; **30**: 21–33.
- 3 Ohta A, Yamaguchi M, Kaneoka H *et al.* Adult Still's disease: review of 228 cases from the literature. *J Rheumatol* 1987; **14**: 1139–46.
- 4 Pouchot J, Sampalis JS, Beaudet F *et al.* Adult Still's disease: manifestations, disease course, and outcome in 62 patients. *Medicine* 1991; **70**: 118–36.
- 5 Yamaguchi M, Ohta A, Tsunematsu T *et al.* Preliminary criteria for classification of adult Still's disease. *J Rheumatol* 1992; **19**: 424–30.
- 6 Schwarz-Eywill M, Heilig B, Bauer H *et al.* Evaluation of serum ferritin as a marker for adult Still's disease activity. *Ann Rheum Dis* 1992; **51**: 683–5.
- 7 van Reeth C, le Moel G, Lasne Y *et al.* Serum ferritin and iso-ferritins are tools for diagnosis of active adult Still's disease. *J Rheumatol* 1994; **21**: 890–5.
- 8 Akritidis N, Giannakakis Y, Sakkas L. Very high serum ferritin levels in adult-onset Still's disease. *Br J Rheumatol* 1997; **36**: 608–9.
- 9 Kaur S, Bamberg P, Dahr S. Persistent dermal plaque lesions in adult onset Still's disease. *Dermatology* 1994; **188**: 241–2.
- 10 Phillips WG, Weller R, Handfield-Jones SE, Kobza-Black A. Adult Still's disease. *Br J Dermatol* 1994; **130**: 511–13.
- 11 Setterfield JF, Hughes GRV, Kobza-Black A. Urticaria as a presentation of adult Still's disease. *Br J Dermatol* 1998; **138**: 906–8.
- 12 Lübke J, Hofer M, Chavaz P *et al.* Adult-onset Still's disease with persistent plaques. *Br J Dermatol* 1999; **141**: 710–13.
- 13 McFarlane M, Harth M, Wall WJ. Liver transplant in adult Still's disease. *J Rheumatol* 1997; **24**: 2038–41.
- 14 Coffernils M, Soupart A, Pradier O *et al.* Hyperferritinemia in adult-onset Still's disease and the hemophagocytic syndrome. *J Rheumatol* 1992; **19**: 425–7.

## Linear focal elastosis

SIR, Linear focal elastosis (LFE) occurring solely on the legs of a young female is rare. A review of the English literature has revealed only one other case reported in a young Vietnamese female and occurring exclusively on the legs.<sup>1</sup> The authors of that report used a video-measuring method for quantification of elastic fibres (EF) in lesional and normal skin. EF were

found to be increased by about 100% in lesional skin compared with unaffected skin.

A 13-year-old girl was referred with a 12-month history of 'stretch marks' on her thighs. She was mainly concerned about its cosmetic appearance as she regularly participated in troupe dancing. There was no history of rapid change in weight or height. On examination, she was noted to have horizontal, linear, palpable, yellow-red striae arranged in parallel mostly on her thighs (Fig. 1a) but also on the legs and extending down to the ankles. A skin biopsy of affected skin showed dermal focal clumping of EF with haematoxylin and eosin staining. Orcein and Giemsa staining showed fragmentation of elastic fibres with apparent increase in numbers (Fig. 1b). This is in contrast to atrophic striae

distensae where the elastic fibres are lacking. A Von Kossa stain for calcification was negative.

Linear focal elastosis, first described by Burket<sup>2</sup> in 1989, tends to occur in elderly males, occurring mainly on their backs and associated with striae distensae. Histologically, Burket described numerous fine wavy bundles of EF separating the collagen in the middle dermis.

Although our patient's peculiar localization of striae is not that of classical LFE, the clinical and histological features are consistent with this entity. We feel that this condition may be under-diagnosed; in our patient the features that alerted us to the diagnosis of linear focal elastosis were the absence of a precipitating cause of striae distensae and that the stretch marks were elevated and palpable. We suggest that

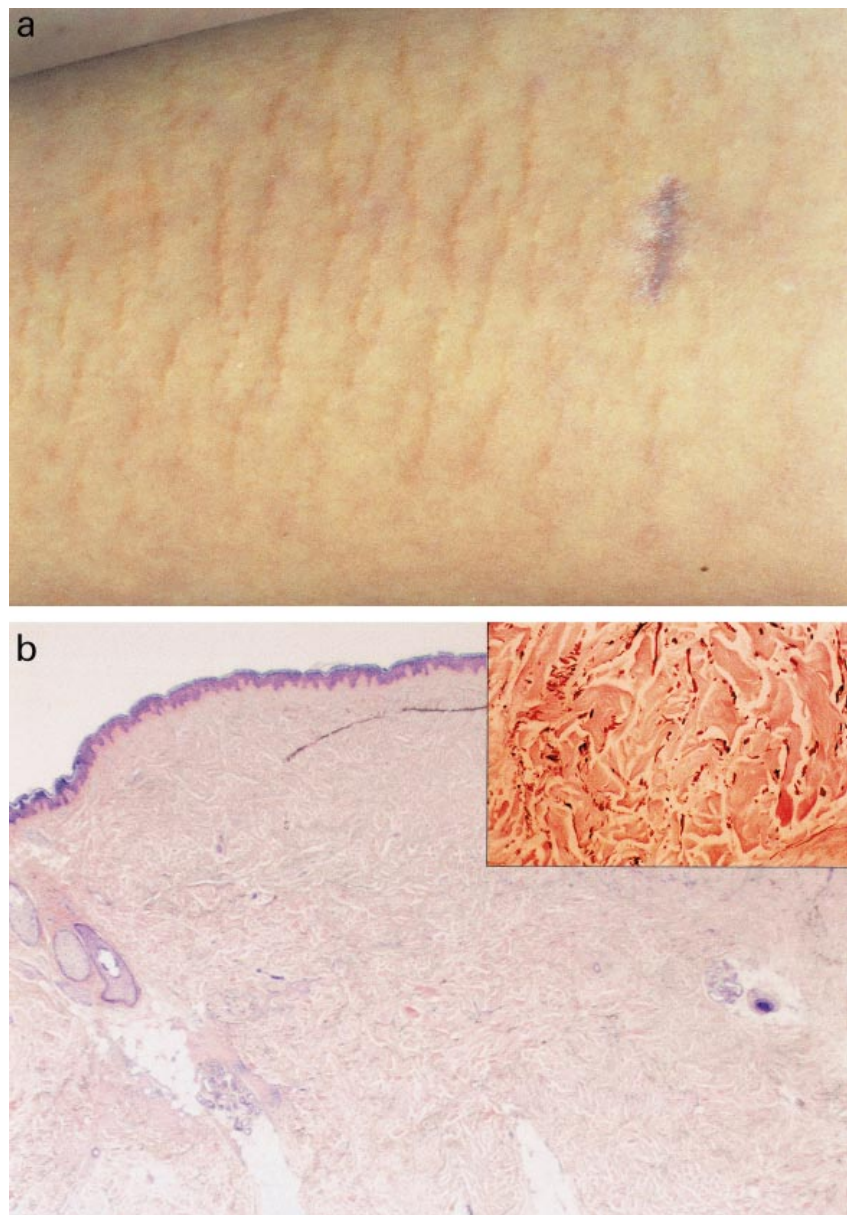


**Figure 1** (a) Elevated, palpable, yellow-red striae arranged in parallel on the thigh. (b) Photomicrograph showing increase of elastic fibres in the middle dermis (orcein and Giemsa staining; original magnification  $\times 40$ ). Inset: highlighting fragmentation and increase of elastic fibres (Orcein and giemsa staining; original magnification  $\times 200$ ).

histological confirmation should be considered in individual cases.

Department of Dermatology,  
Central Out-Patients,  
North Staffordshire Hospital,  
Stoke-on-Trent ST4 7PA, U.K.

D.RAMLOGAN  
B.B.TAN  
M.GARRIDO

## References

- 1 Breier F, Trautinger F, Jurecka W *et al.* Linear focal elastosis (elastotic striae): increased number of elastic fibres determined by a video measuring system. *Br J Dermatol* 1997; **137**: 955–7.
- 2 Burket JM, Zelickson AS, Padilla RS. Linear focal elastosis (elastotic striae). *J Am Acad Dermatol* 1989; **20**: 633–6.

## Erratum

González-Beato MJ, Moyano B, Sánchez C, González-Beato MT, Pérez-Molina JA, Miralles P and Lázaro P. Kaposi's sarcoma-like lesions and other nodules as cutaneous involvement in AIDS-related visceral leishmaniasis. *Br J Dermatol* 2000; **143**: 1316–18.

The authors for reference 16 were supplied incorrectly as Murph RC, Matulis WS, Hernández JE. The correct authors are Scaglia M, Malfitano A, Douville H *et al.*

## Book Reviews

**Textbook of Pediatric Dermatology** (2000). Edited by J.HARPER, A.ORANJE and N.PROSE. Blackwell Science, Oxford. ISBN 0865429391, 2064 pages. Price £295.00

From the size and cover design to the layout and typefaces, everything about the format of this weighty two-volume work declares its expectation to be treated as a companion to the latest Rook *Textbook of Dermatology*. Nonetheless, differences of editorial approach are apparent. The authorship is much more international, even if the un-British spelling of 'pediatric' dictated by marketing considerations has been confined to the title. There are also proportionately many more authors (185 dermatologists, paediatricians, geneticists and others) and about 150 chapters. An advantage of this inclusive approach is that large topics such as atopic eczema are shared, and particular expertise brought to individual aspects: epidemiology, diet, infection and so on. Specific expertise is also applied to the multiplicity of less common conditions. Many contributions are very brief. There is a potential for fragmentation and inconsistency inherent in this approach, and in any work of this size, there must be some weaker contributions, but in practice such problems are very few. They are more than countered by chapters in which undisputed experts exploit the opportunity to provide detailed and well-illustrated reviews on specific topics. However, the allocation of topics to sections is sometimes questionable; surely most of us would expect to find alopecia areata in the section on 'Hair and Nails', not tacked on at the end of the book as a 'Miscellaneous Disorder'? The content is comprehensive, including for example extended sections on surgery and tropical dermatology, and is as up to date as one could expect in a printed textbook. Although intended as a text rather than an atlas, most chapters are profusely, and in the

case of meningococcal disease, even prodigally, illustrated, with colour photographs of excellent quality. Disproportionate space is sometimes devoted to adult conditions that happen occasionally to occur in childhood, but this is defensible in a dermatology work intended to serve as the sole major text in a paediatric unit. Conversely, for dermatologists, much more space than possible in a general text is devoted to paediatric issues with cutaneous aspects, such as neonatal medicine, multisystem conditions, generalised genetic disorders, or child abuse, or to specifically paediatric aspects of common skin diseases. This book can be recommended to paediatric departments as a stand-alone text on skin disease, and as a reference work for dermatologists who treat children. For the latter, its additional content may indeed justify thinking of it as a companion to the 'Rookbook' but at 75% of the price for half the length, and with at least some overlapping material, it is more likely to join departmental or library than personal copies of Rook.

COLIN S.MUNRO

**Fisher's Contact Dermatitis** 5th edn. (2001). Edited by R.L.RIETSCHEL and J.F.FOWLER. Philadelphia: Lippincott Williams and Wilkins. ISBN 0-7817-2252-7, 1088 pp. Price £83.

It is hard to believe that 6 years have elapsed since publication of the fourth edition of Alexander Fisher's *tour de force* on contact dermatitis. With the first edition now 34 years ago, this text has undoubtedly stood the test of time and remains a practical and readable account of the discipline.

The new edition has altered in format to have a larger page size and slimmer shape, which means that the frustration of having to rotate the book through 90° to read most of the

tables has been reduced, although not eradicated. However, the metamorphosis in shape rather obscures the fact that in many areas there is actually very little change in content from the previous edition. Most chapters have had some updating of basic references, and some have had several new topics added (for example, a variety of 'new' agents causing contact urticaria are described). The ubiquitous tea-tree oil has entered the text, but is not in the appendix of contactants. Cross-reactions between latex and foods are still glossed over by comparison with the attention given to some food issues, such as low nickel diets. Cetrimide and benzoxonium chloride have been added to the disinfectants and antiseptics chapter. The patient information literature has been expanded by inclusion of brief summaries of allergy to chemicals such as glutaraldehyde, parabens, diazolidinyl urea, tosylamide formaldehyde resin, Euxyl K and some corticosteroids. However, most of the trademark 'boxed points' have survived unaltered, as have most of the tables. Some of the chapter titles have altered slightly, but the sequence and numbering is reassuringly unchanged (a positive point for those who do not wish to spend time getting to grips with a fundamentally new layout).

Use of new nomenclature since the last edition is one reason why this further edition was required. However, it might have been prudent to have both old and new names in the index for some agents in order to make life easier for those of us who do not spend the majority of our time in a contact dermatitis clinic. International cosmetic ingredient labelling terms are not specifically discussed, and although the authors state that they have included some non-USA agents such as some Japanese topical antifungal agents, the majority of tables involving trade names are specific to the USA. But then, this always was a personal account of the subject, and acknowledged as such.

Whether this edition adds sufficient new information to warrant upgrading from the previous edition is uncertain, but Fisher's book remains one of the most important manuals of contact dermatitis, and every general dermatologist and contact allergy specialist will need a copy available.

NEIL H. COX

---

## News and Notices

### **Third International Symposium 'Lasers in Dermatology' 1–2 October 2001, Ulm, Germany**

The Third International Symposium, 'Lasers in Dermatology' will discuss all methods of laser applications in dermatology. The Directors are Professor R. Steiner and Professor R. U. Peter. For further information, e-mail: [events@ilm.uni-ulm.de](mailto:events@ilm.uni-ulm.de) Website: <http://www.uni-ulm.de/ilm/lid>

### **Fifty-third Annual Meeting: Pacific Dermatologic Association 3–7 October 2001, Pointe South Mountain Resort, Phoenix, AZ, USA**

The meeting will include the traditional Clinicopathologic Conference, and other topics such as an update on HIV and Hepatitis C, atopic dermatitis, psoriasis, eczema, nail lesions, retinoids, drug synergism, and more. The winner of the Nelson Paul Anderson Memorial Contest will present their paper at the meeting. For further information, please contact: Cathy Powers, Pacific Dermatologic Association, 930 N. Meacham Road, Schaumburg, IL 60173, U.S.A. Tel.: +1 847 1330 9830; fax: +1 847 1330 1135; e-mail: [cpowers@aad.org](mailto:cpowers@aad.org).

### **Twenty-Second Symposium of The International Society of Dermatopathology 4–6 October 2001, Stresa, Italy**

For further details contact: Organizing Secretariat, Congress Team Project, via D. Sacchi 12, 27100 Pavia, Italy. Tel.: +39 382 22650; fax: +39 382 33822; email: [ctp@mbox.medit.it](mailto:ctp@mbox.medit.it); website: [www.congressteam.com](http://www.congressteam.com)

### **Eleventh South Zone Conference of the Indian Association of Dermatologists, Venereologists & Leprologists 5–7 October 2001, PSG Hospitals, Coimbatore, India**

For further information, contact Reena Rai, Organizing Secretary, or C.R. Srinivas, Scientific Chairman, Department of Dermatology, PSG Hospitals, Peelamedu, Coimbatore, Tamil Nadu, India. Tel.: +91 570170 176, ext. 472; fax: +91 422 594400; e-mail: [psgimsr@md3.vsnl.net.in](mailto:psgimsr@md3.vsnl.net.in)

## **Announcements**

### **British Skin Foundation 2001 Awards: Call for Grant Applications**

The Trustees of the British Skin Foundation wish to announce that funding is again available for skin disease research. This year there are four categories of award as listed below. Applicants are asked to apply for funding from the category that they feel is most appropriate. The closing date is **17th September 2001**.

**BSF Research Awards:** One- or 2-year project grants of up to £50,000 per annum.

**The BSF Fellowship:** A grant of £40,000 to support a specialist registrar in dermatology through one year of research. Candidates should submit details of their proposed research and a C.V. in the manner described below. Selection will be by interview in late 2001. It is envisaged that the successful candidate will already hold an NTN or will have just completed their training.

**BSF Small Grant Awards:** One-off payments of up to

£10,000. May be used to purchase equipment or for less costly projects.

**BSF Studentship:** A 3-year package, value £44,000, covering tuition fees, expenses and some consumables.

If you wish to receive an application form for a British Skin Foundation grant, please contact the office, specifying the type of grant you are interested in at:

British Skin Foundation, 19 Fitzroy Square, London W1T 6EH, U.K. Tel: 20 7383 0266; fax: 7388 5263.

#### **Fondation René Touraine Fellowships 2001**

The Fondation René Touraine invites applications for four fellowships up to €4573 (FF 30 000) for short exchange, and one fellowship of up to €18 293 (FF 120 000) for a long exchange, to be given towards supporting a period spent in a research laboratory or clinical department of a different country, in order to promote international collaboration.

Application forms should be requested from the secretariat of the Foundation at [fond.r.touraine@chu-stlouis.fr](mailto:fond.r.touraine@chu-stlouis.fr) or by mail from: Mme Pitton, Fondation René Touraine, Hôpital St Louis, Pavillon Bazin, 1 avenue Claude Vellefaus, 75475 Paris cedex 10, France. Tel.: + 33 1 5372 2060; fax: + 33 1 5372 2061.

#### **Ignaz Philipp Semmelweis Research Prize 2002**

In appreciation of the work of Ignaz Philipp Semmelweis, the German Society for Hospital Hygiene (DGKH), together with

BODE CHEMIE, is for the second time calling for entries in the competition for the Ignaz Philipp Semmelweis prize. Innovative scientific achievements throughout Europe in the field of hand hygiene and disinfection, body hygiene and antisepsis as a result of patient-friendly measures are to be rewarded. The prize for specific work in this field is worth €15 000. Entrants may submit articles that have been published in Europe within the last 3 years, or manuscripts whose receipt has been confirmed by a publisher. Completed dissertations and postdoctoral theses, as well as defended research projects, may also be submitted. The final date for submission is **30 September 2001**. Further information can be obtained from <http://www.bode-chemie.delnews> or by fax from Scicom Public Relations on: + 49 401 2532 8608.

#### **L'Oréal 2001 Grant for Skin Research**

Applications are invited for the L'Oréal 2001 Grant, in partnership with La Société Française de Dermatologie and La Société de Recherche Dermatologie. The grant is aimed at financing a project in the field of clinical or basic research on the physiology of normal skin. The deadline for application for the grant, worth €45 000, is **30 September 2001**. Further information and online applications are available from <http://www.skingrant.loreal.com>