

Extensive accumulation of eosinophil extracellular traps in bullous delayed-pressure urticaria: a pathophysiological link?

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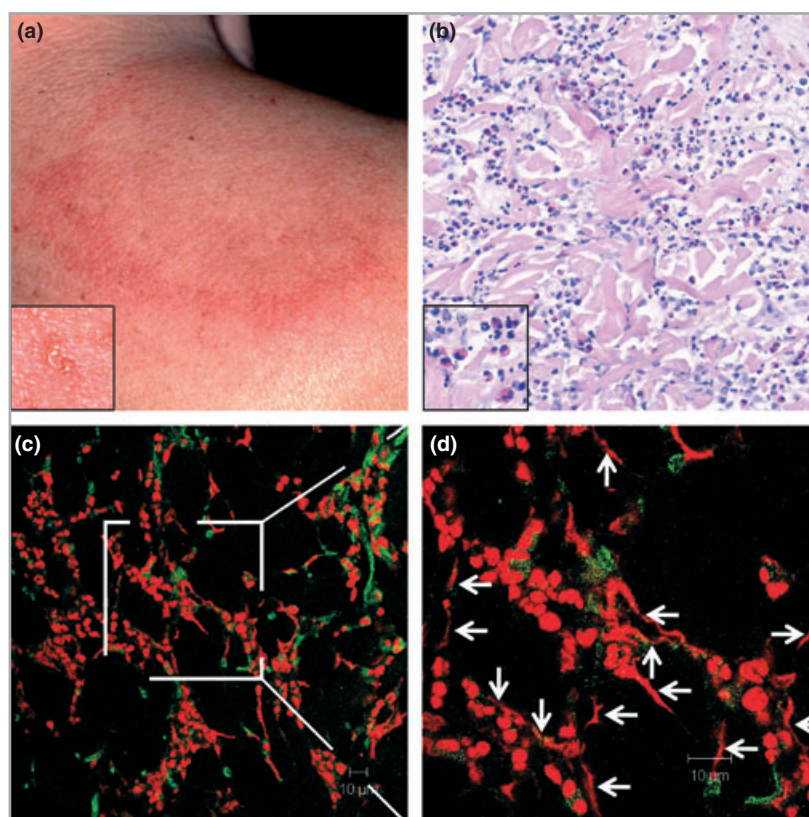
MADAM, Eosinophil extracellular traps (EET) are structures containing mitochondrial DNA in association with eosinophil cationic protein (ECP) and other granule proteins. EET were recently identified as an important antibacterial mechanism operating in the extracellular space.^{1,2} EET were subsequently demonstrated in a variety of inflammatory skin diseases including, allergic contact dermatitis, bullous pemphigoid or Well's syndrome.³ Bullous delayed-pressure urticaria (DPU) is regarded as a rare and maximal variant of DPU, which is histologically characterized by epidermal blisters filled with eosinophils and an extensive dermal eosinophil infiltration.⁴ The aetiology of DPU is unclear, but the abundance of eosinophils might point to a pathogenic role of these cells.⁵ However, the knowledge for potential eosinophil effector mechanisms in this disease remains elusive to date.

Here, we followed up a previously reported case of a 72-year-old man with bullous DPU⁵ and investigated biopsy specimens for the presence of EET. The clinical diagnosis of bullous DPU was verified by pressure challenge with a hanging 10-kg weight belt of 2 cm in width over his right shoulder

for 20 min. The arising painful deep erythematous swelling showed numerous blisters within the test area after 24 h and 48 h (Fig. 1a). Histological examination showed intraepidermal blisters and a marked eosinophil infiltration of the upper and lower dermis accompanied by few lymphocytes and neutrophils (Fig. 1b). We performed immunofluorescence stainings using a monoclonal mouse anti-human ECP antibody (Pharmacia Diagnostics AB, Uppsala, Sweden) in conjunction with the appropriate Alexa Fluor 488-labelled secondary antibody (Invitrogen Molecular Probes, Paisley, U.K.) and propidium iodide for labelling DNA. Using confocal laser scanning microscopy, these studies revealed large numbers of EET (Fig. 1c,d). Intriguingly, the proportion of DNA- plus ECP-releasing eosinophils in the dermis was much higher than in previously reported inflammatory skin diseases,³ such as Well's syndrome (range 10–30%), and reached 80–90% (Fig. 1d).

Because EET are not routinely found in ordinary urticaria,³ it is conceivable to speculate that they might play a pathogenic role in bullous DPU. EET are regarded as an ancient defence mechanism against bacteria and other pathogens, so that the release of EET in bullous DPU might represent the tip of a dysregulated innate immune response fostering the maintenance of inflammation and resulting in epidermal blister formation. The hypothesis is supported by the finding that, in addition to their antimicrobial properties, EET might simultaneously cause cell and tissue injury.^{2,3,6} This hypothesis has been exemplified for bullous pemphigoid, where EET were

Fig 1. Bullous delayed-pressure urticaria. (a) Urticarial plaque on the shoulder 48 h after pressure challenge test. Inset shows blisters and vesicles at the margins of the plaque. (b) Extensive eosinophil infiltration (mean $\pm$ SD 103 ± 50.9 cells mm^{-2}) of the reticular dermis (see also inset for larger magnification). (c, d) Eosinophil extracellular traps (arrows). Accumulation of eosinophils releasing DNA (red) and eosinophil cationic protein (green). The indicated area in (c) (original magnification $\times 400$) was again photographed at a larger magnification (d, $\times 1000$).



found in close proximity to apoptotic keratinocytes, suggesting involvement of EET in blister formation.³ Furthermore, we speculate that the massive deposition of EET in loco results in enduring high tissue concentrations of cytotoxic ECP and other granule proteins. In turn, this may explain the prolonged inflammation, which results in clinically relevant persistent swellings for several days in patients with DPU. However, as the frequencies of eosinophils are lower in DPU than in bullous DPU,⁷ the involvement and role of EET formation in DPU remains to be determined.

Eosinophils rapidly form EET upon stimulation, whereby interleukin (IL)-5 often acts as a priming factor.² In our previously reported case,⁵ up to 50% of skin-infiltrating CD4+ and CD8+ T lymphocytes expressed IL-5 pointing to a role for T lymphocytes to trigger and/or maintain the generation of and deposition of EET in bullous DPU. Future studies, including serial biopsies, are needed to determine the precise role of the possible cross-talk between IL-5-secreting T cells and eosinophils through enhancing eosinophil effector functions by producing EET in DPU.

In summary, our case report documents enhanced eosinophil effector functions that translate in secretion of DNA and cytotoxic ECP to form EET in bullous DPU. These findings point to a possible mechanistic insight for the persistent swellings clinically observed in DPU. However, the precise pathophysiological role of EET in bullous DPU (and DPU) remains to be determined.

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News and Notices

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Annual Evidence Based Update Meeting 2012: Acne and Rosacea

Thursday 10th May 2012: 10am–4.15pm
Location: Holywell Park, Loughborough
Organiser: Professor Hywel Williams
Cost: £90/£100

For further information please contact: Carron Layfield on carron.layfield@nottingham.ac.uk

National Skin Centre Dermatology Update 2012 May 11th–13th 2012

Meritus Mandarin Orchard, Singapore.
For further information contact:
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Applied Photodermatology course 21st–22nd May 2012, BAD House, London

The 2012 Applied Photodermatology course will be held at the Headquarters of the British Association of Dermatologists in Central London, 21st–22nd May 2012, organised by Professor Alex Anstey, Royal Gwent Hospital, Newport.

The 2 day course meets a number of educational objectives teaching delegates: how to evaluate patients with photosensitivity; how to identify the pros and cons of pre-phototherapy phototesting in PUVA and UVB; and how to identify currently accepted best practice in the practice of phototherapy for it's most common applications and to identify how this differs from practice in their own unit/department.