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SHORT COMMUNICATION

Mass Spectrometry of Flame Figures

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In 1971, George Wells first described flame figures as foci of amorphous material associated with dermal collagen fibres in patients exhibiting recurrent granulomatous dermatitis with eosinophilia, so-called eosinophilic cellulitis or Wells syndrome (1). Flame figures, however, are also observed in other eosinophil-related diseases such as bullous pemphigoid and insect bites (2). They appear as variably sized, poorly circumscribed foci of intense eosinophilia of collagen bundles. A few studies have investigated the composition of flame figures and found them to contain abundant eosinophil granule major basic protein (MBP), encrusting intact collagen fibres (3, 4). Despite these attempts, the exact nature of flame figures in humans remains elusive. This study aimed to identify the major components of flame figures in an unbiased manner. A secondary goal was to assess the feasibility of mass spectrometry (MS) on haematoxylin and eosin (H&E)-stained and microdissected frozen material.

MATERIALS AND METHODS

This study is based on 2 fresh frozen punch biopsies. The first biopsy was taken from a 27-year-old man who presented with recurrent itchy, erythematous papules and nodules on the lower legs; 2 similar episodes in the past 10 years had resulted in spontaneous regression. Blood analysis showed increased IgE-levels; constitutional symptoms were lacking and cultures were negative. The second biopsy was from a 37-year-old woman who presented with erythema and oedema of the right ankle as well as itchy, erythematous oedematous plaques of the left thigh and dorsum of the right foot in the absence of epidermal changes. The lesions developed following flu-like symptoms with fever, coughing, nausea and myalgia. Ultrasonography of the ankle showed induration of subcutaneous tissue and no signs of arthritis. Blood eosinophilia of 3,800/µl (39%) was noticed.

Cryosections 10-µm thick were fixed in ice-cold ethanol for 1 min and stained with H&E. Flame figures, and uninvolved dermis were microdissected using a Leica DM6000B (Leica Microsystems). Isolated fragments were captured on AdhesiveCap tubes (Carl Zeiss, Zaventem, Belgium).

The microdissected material was digested overnight with trypsin and the resulting peptide mixture was cleaned with ZipTip C18 and analyzed on an ABI 4800 MALDI Tof/Tof MS. Database search was performed on a Mascot 2.2 server, with the following settings: Uniprot_sprot database, taxonomy: homo sapiens (20,308 sequences), variable modification: oxidation methionine, missed cleavages: 1, peptide mass tolerance: 0.2 Da, fragment mass tolerance: 50 ppm, Decoy database: yes.

Five-µm thick cryosections were fixed in ice-cold acetone for 10 min and stained for Histone H2A (Cell Signaling Technology) and eosinophil peroxidase (Calbiochem), followed by visualization with Alexa Fluor 488 (1/100, Invitrogen). Samples were counterstained with propidium iodide (PI; 20 µg/ml). Fluorescent signals were observed using Zeiss Axioplan (Carl Zeiss, Oberkochen, Germany).

RESULTS

In both biopsies, H&E staining revealed a marked superficial and deep dermal infiltrate of eosinophils, lymphocytes and histiocytes; in addition, large areas of highly eosinophilic material, consistent with flame figures, were found (Fig. 1A). Diagnoses of persistent insect bite reaction and Wells syndrome, respectively, were made.

After a series of preliminary experiments, we established that H&E staining of frozen tissue sections does not interfere with analysis and identification of proteins by MS. Flame figures, and uninvolved dermis of both patients were microdissected from serially cut slides (Fig. 1B) and subsequently analyzed by MS. This strategy successfully identified 5 proteins in the flame figures of patient 1 (Table SI[1]): actin (smooth muscle), eosinophil peroxidase, histone H2A (type 1-A), bone marrow proteoglycan (also known as eosinophil granule MBP) and a tyrosine tRNA ligase. In the corresponding control sample, these 5 proteins were not found. In patient 2, histone H2A was again present exclusively in the flame figures (Table SI[1]), but below the significance level. An expected protein, such as collagen α-1, was detected both in flame figures and in uninvolved dermis, but also below the significance threshold. Finally, MS data were validated by immunofluorescence staining for a selection of the proteins. Nuclear DNA and eosinophils within the dermis served as positive, internal controls of histone H2A and eosinophil peroxidase, respectively. Consistent with the MS data, flame figures showed strong immunoreactivity for both eosinophil peroxidase and histone H2A (Fig. 1C). In addition, these flame figures displayed mesh-like structured propidium iodide-positive DNA.

DISCUSSION

Our finding of eosinophil MBP by MS on microdissected flame figures is in line with previous immunofluorescent studies (3, 4). Similarly, the presence of

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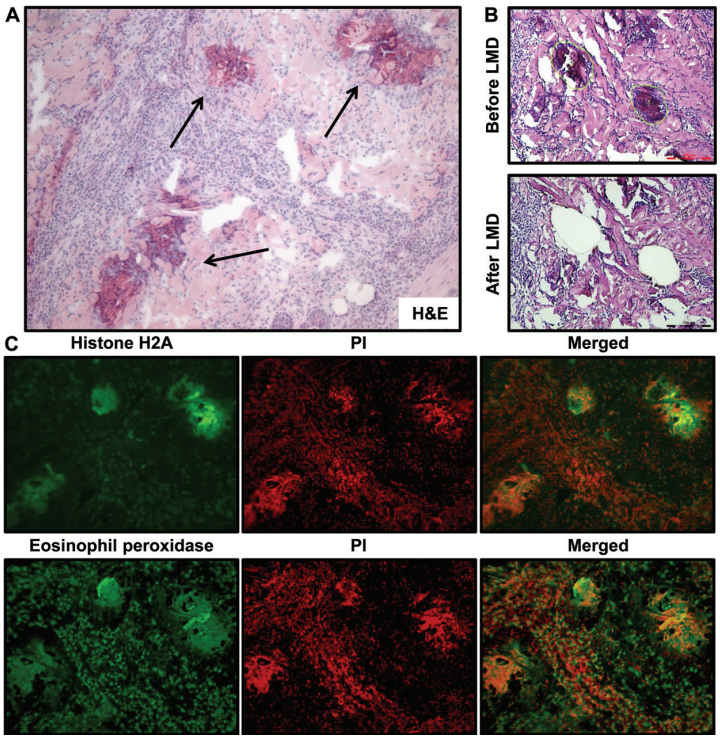

Fig. 1. Characterization of flame figures (patient 1). (A) H&E staining of a frozen punch biopsy showing 3 flame figures surrounded by a mixed inflammatory infiltrate (arrows, flame figures; 100×, original magnification). (B) Representative H&E stained section with 3 flame figures (*top*) of which 2 were microdissected (*bottom*) (LMD: laser microdissection; 100×, original magnification). (C) Protein expression by flame figures showing intense green fluorescence for histone H2A and eosinophil peroxidase (*left*). Flame figures also contain extracellular DNA (*centre*), identified by propidium iodide (PI) positivity (*red*) (100×, original magnification). Merged pictures (right) verified colocalization of staining.

eosinophil peroxidase is in agreement with the abundance of infiltrating eosinophils (1). The identification of collagen α-1 in flame figures, the major constituent of fibrillar collagen, can also be expected, as it was reported earlier by George Wells himself (1). These corroborating data demonstrate the feasibility of MS on H&E-stained and microdissected frozen specimens.

In addition, we identify high levels of histone H2A and mesh-like DNA chains in flame figures. These components have previously been found in eosinophil extracellular DNA traps (EETs) (5–7), which were first described in the context of neutrophils (8), but later also other immune cells, such as eosinophils. Most likely, EETs are formed in 2 phases. Initially, viable eosinophils acutely release mitochondrial DNA in an energy-dependent, catapult-like manner (6). In a later phase, eosinophils can undergo eosinophil cytolytic cell death, resulting in the discharge of free eosinophil granules and chromosomal DNA containing histones (7). The filamentous network of DNA in EETs is coated with cell-free eosinophil granule proteins, such as eosinophil peroxidase and MBP (5–7). The formation of EETs by eosinophils is believed to be part of their function in the innate immune response (5–7). The antimicrobial functions of eosinophil peroxidase and MBP, and their toxic effects on membranes are well-established (reviewed in (9)). Likewise, histone H2A has an acknowledged role in the innate defence against extracellular microbes (10–13). And, finally, extracellular DNA is pivotal for the anti-bacterial role of EETs (6), possibly serving as a web for capturing pathogens/antigens, and as a backbone for eosinophil granule proteins. Simon et al. (5) have previously documented the differences between EETs and flame figures, but also noticed that EETs may form larger aggregates, particularly in samples with clusters of eosinophils. In view of the similar composition of flame figures and EETs and occurrence in similar eosinophil-rich skin diseases, their potential relationship warrants further investigation.

REFERENCES

1. Wells GC. Recurrent granulomatous dermatitis with eosinophilia. Trans St Johns Hosp Dermatol Soc 1971; 57: 46–56.
2. Beer TW, Langtry JA, Phillips WG, Wojnarowska F. Flame figures in bullous pemphigoid. Dermatology 1994; 188: 310–312.
3. Peters MS, Schroeter AL, Gleich GJ. Immunofluorescence identification of eosinophil granule major basic protein in the flame figures of Wells' syndrome. Br J Dermatol 1983; 109: 141–148.
4. Stern JB, Sobel HJ, Rotchford JP. Wells' syndrome: is there collagen damage in the flame figures? J Cutan Pathol 1984; 11: 501–505.
5. Simon D, Hoesli S, Roth N, Staedler S, Yousefi S, Simon HU. Eosinophil extracellular DNA traps in skin diseases. J Allergy Clin Immunol 2011; 127: 194–199.
6. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, et al. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. Nat Med 2008; 14: 949–953.
7. Ueki S, Melo RC, Ghiran I, Spencer LA, Dvorak AM, Weller PF. Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion-competent eosinophil granules in humans. Blood 2013; 121: 2074–2083.
8. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science 2004; 303: 1532–1535.
9. Malik A, Batra JK. Antimicrobial activity of human eosinophil granule proteins: involvement in host defence against pathogens. Crit Rev Microbiol 2012; 38: 168–181.
10. Cho JH, Park IY, Kim HS, Lee WT, Kim MS, Kim SC. Cathepsin D produces antimicrobial peptide parasin I from histone H2A in the skin mucosa of fish. FASEB J 2002; 16: 429–431.
11. Fernandes JM, Kemp GD, Molle MG, Smith VJ. Antimicrobial properties of histone H2A from skin secretions of rainbow trout, Oncorhynchus mykiss. Biochem J 2002; 368: 611–620.
12. Kim HS, Cho JH, Park HW, Yoon H, Kim MS, Kim SC. Endotoxin-neutralizing antimicrobial proteins of the human placenta. J Immunol 2002; 168: 2356–2364.
13. Li GH, Mine Y, Hincke MT, Nys Y. Isolation and characterization of antimicrobial proteins and peptide from chicken liver. J Pept Sci 2007; 13: 368–378.