

ORIGINAL ARTICLE

Dermal dendrocytes FXIIIa+ phagocytizing extruded mast cell granules in drug-induced acute urticaria

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Abstract

Background Few authors have been attempting between mast cells and dermal dendrocytes interactions on urticaria.

Objective To describe the extruded mast cell granules and dermal dendrocytes in drug-induced acute urticaria.

Methods Seven patients with drug-induced acute urticaria were enrolled in the study. We taken skin biopsies of urticaria lesion and perilesional skin. The 14 fragments collected were processed to immunogold electron microscopy using single stains to tryptase and FXIIIa, besides double immunogold labeling with both.

Results Some sections demonstrated mast cells in degranulation process, both in anaphylactic and piecemeal degranulation types. After double immunogold staining, 10 nm (FXIIIa) and 15 nm (Tryptase) gold particles were present together over the granules in mast cells indicating that tryptase and FXIIIa are each localized within the granules of these cells. Interestingly, we found a strong evidence of than the exocytosed mast cell granules contents both FXIIIa and tryptase immunolabeled are phagocytized by dermal dendrocytes.

Conclusions The current observations provide morphological evidence that the exocytosis-phagocytosis mechanisms of mast cell granules represents one pathophysiological example of mast cells-dermal dendrocytes interactions in urticaria.

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Conflict of interest

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Introduction

The knowledge about the cell types involved in urticaria is an essential element for understanding the physiopathology of this disease. In the medical literature, just a few reports using light microscopy have been published in urticaria.^{1–5} Only a few authors have attempted to the role of the dermal dendrocytes (DD) addressing the micro morphology of urticaria.^{6,7}

The dermal micro vascular unit (DMU) is involved in the inflammatory response of the urticaria⁸ and CD34⁺ fibroblast plus FXIIIa⁺ histiocytes dermal dendrites cells are subsets of the DD.⁸ These cells are believed to be critical to the fibrohistiocytic cellular building apparatus of the dermal micro vascular unit, which is responsible for tissue healing and remodelling.⁸ The mast cells are intimately associated to and activate FXIIIa⁺ DD;⁹ after mast cells degranulation, there is an accumulation of FXIIIa⁺ DD in the skin expressing von-Willebrand factor receptor.^{9,10}

We have already studied the ultra structure of this intricate interrelation among the elements of the DMU in drug-induced acute urticaria (DIAU). We observed signs of a functional activity of DMU, represented by mast cells degranulation and the intimate relationship between DD and mast cells.⁷ These findings prompted us to deeper the study of these interactions, using immune histochemistry (IH) and immunoelectron microscopy (IMEM) techniques, in search of a clue of a possible active function in the physiopathology of urticaria in the interactions between mast cells and DD.

Patients and methods

Subjects

Seven patients with DIAU seen at the Dermatology Outpatient Clinic of the Clinics Hospital, School of Medicine, Sao Paulo

Table 1 Clinical and demographic data

Patients	Sex	Age (years)	Ethnic group	Urticaria duration (history)	Drugs associated
Case 1	F	42	C	4 weeks	Potassium diclofenac
Case 2	F	55	A	3 weeks	Acetylsalicylic acid, dexchlorpheniramine, phenylephrine and caffeine
Case 3	F	35	C	2 weeks	Acetylsalicylic acid, dexchlorpheniramine, phenylephrine and caffeine
Case 4	F	29	C	8 days	Potassium diclofenac
Case 5	F	29	C	3 days	Ferrous sulphate
Case 6	F	32	A	7 days	Sodium dipyrone, ranitidine, hioscine, dimenidrate, pyridoxine, ascorbic acid and chlorpheniramine
Case 7	F	49	A	4 weeks	Sodium dipyrone and potassium diclofenac

A, Afro-American; C, Caucasian; F, female.

University, Brazil were enrolled in the study. The mean age was 38.7 years. We estimate an average of 24–48 h interval between the onset of the urticarial lesions and the skin biopsy. Clinical data are summarized in Table 1. The patients with DIAU, according to the criteria established by Moore *et al.*,¹¹ were included in the study. For safety and ethical reasons, provocation tests were not performed. Pregnant women, individuals less than 18 years old and those presenting any simultaneous cardiac, respiratory, neurological, infectious or metabolic diseases were excluded from the study. The research was approved (n. 047/06) by the local Research Ethics Committee and the patients signed an informed consent prior to taking part in the study.

Techniques

Skin fragments from the urticarial lesion and from the normal skin (distant at least 5.0 cm of the biopsied urticarial lesion) were obtained through a 6-mm punch, under anaesthesia with 2% lidocaine without vasoconstrictor, followed by haemostasis with a 4.0 Mononylon® (Ethicon, Johnson & Johnson, São Paulo, Brazil) suture. The fourteen collected fragments were splitted in three pieces: one was routinely processed for HE, one for Toluidine blue stain and the other for IMEM method [the sample was fixed in 0.2% glutaraldehyde (Sigma®, No.G-5882, Sigma-Aldrich Corp., St Louis, MO, USA) /4% paraformaldehyde (LADD no 5-55080)

in 0.1 mol/L phosphate buffer (pH 7.2) and included in LR White Resin (London Resin Company Ltd)]. The late sections were examined in a transmission electron microscope JEOL 1010 (JEOL Ltd, Tokyo, Japan) in the Department of Pathology of the School of Medicine, Sao Paulo University. The ultra structural examination was performed and representative photographs were taken.

Deparaffinized sections of 14 specimens from routinely processed tissue fragments from seven cases of DIAU patients were immunostained with two antibodies by the Streptavidin-Biotin-Peroxidase method to perform IH. The commercial sources, specificities and dilutions of primary antibodies are show in Table 2. For confirmation of mast cells, a specific monoclonal antibody anti-mast cells tryptase was employed.¹²

For single staining, tissue sections were incubated with the primary antibodies for 12 h at 4°C in a sealed, moist chamber. Subsequently, we performed the incubation with the secondary antibody (anti-mouse and anti-rabbit immunoglobulin) for 40 min at room temperature in a sealed, moist chamber. The immunoreactions were detected using the kit LSAB+ System-HRP (DAKO, Carpinteria, CA, USA, code K0690), for 40 min at room temperature. The 3,3'Diaminobenzidine chromogen (Sigma, St. Louis, MO, USA) was used. Immunostained sections were rinsed in distillate water, counterstained with Carazzi's haematoxylin for 10 min and mounted in Entellan (Merck, Darmstadt, Germany).

A double immune staining technique was performed on the 14 sections. In the first sequence, they were stained for tryptase, as previously described. The attached mouse anti-human tryptase antibody was revealed by 3,3'Diaminobenzidine (Sigma, St. Louis, MO, USA) with 40 mg of Nickel Chloride. The primary and secondary antibodies were removed from the tissue after three rinses, first in water and subsequently, double rinse using PBS buffer. The Diaminobenzidine reaction remained in the tissue. In a second sequence, the sections were stained with rabbit anti-human polyclonal antibody anti-FXIIIa (Calbiochem, La Jolla, CA, USA), for 30 min at room temperature, and was revealed by LAB-alkaline phosphatase technique with naphthol-AS-BI phosphate

Table 2 Primary antibodies used at immune histochemistry and immunoelectron microscopy studies

	Monoclonal (M) or Polyclonal (P)	Source	Dilution (immunoelectron microscopy)
Tryptase	M (AAA3) (mouse anti-human)	DAKO, Carpinteria, CA, USA	1 : 200
Factor XIIIa (FXIIIa)	P (rabbit anti-human)	Calbiochem, LaJolla, CA, USA	1 : 100

M, monoclonal; P, polyclonal.

(Sigma, St. Louis, MO, USA) and Liquid Permanent Red (DAKO, Carpinteria, CA, USA, code K0640). The cells that reacted with tryptase become visible black or brown, whereas those reacting with FXIIIa tainted red. The double stained displayed brown mast cells granules inside the red DD cytoplasm.

To do the immune staining for FXIII, the screens containing the sections were incubated with Tris Buffer Saline (TBS) 0.05 mol/L, pH 7.2, twice for 5 min and then incubated in TBS with 3% bovine serum albumin (BSA) at pH 7.2 for 5 min. Subsequently, the TBS was drained on Whatman filter paper and incubated for 30 min at room temperature with normal goat serum (NGS, dilution 1 : 30). After the NGS was drained without washing, the screens were incubated with 1 : 100 primary polyclonal antibody anti-factor XIII (Calbiochem) – see Table 2 – diluted in 0.05 mol/L TBS pH 7.2 plus 3% BSA overnight at 4°C. Afterwards, they were washed in 0.05 mol/L TBS with 1% BSA plus 0.1% Tween 20 four times, for 5 min. After that, the screens sections were incubated in TBS with 1% BSA (pH 8.2) for 30 min at room temperature. Next, the screens were incubated with the secondary antibody, the Goat anti-rabbit IgG gold labelled with colloidal gold particles of 10 nm in diameter (Electron Microscopy Sciences - USA), diluted 1 : 20 in TBS buffer with 1% BSA, pH 8.2, for 1 h at room temperature. The screens were then washed in TBS with 0.05% BSA, 0.1% Tween 20 (pH 7.2), four times for 5 min and in TBS containing 0.1% Tween 20 (pH 7, 2), twice for 5 min. Finally, the screens were washed three times in distilled water for 2 min and sections were fixed in 2% aqueous glutaraldehyde for 10 min. The contrast was done with uranyl acetate and lead citrate and the grids were observed under a transmission electron microscope JEOL 1010 of the Department of Pathology, Faculty of Medicine, University of Sao Paulo.

The same procedures employed for FXIII immune staining were applied to the immune disclose of Tryptase. But, in this case, we used as primary antibody a Monoclonal Mouse anti-human Mast cell Tryptase (DAKO, Carpinteria, CA, USA) diluted 1 : 200 and as secondary antibody a Goat anti-mouse IgG gold labelled with colloidal gold particles of 15 nm in diameter (Electron Microscopy Sciences - USA) diluted 1 : 20 in TBS buffer with 1% BSA, pH 8.2, for 1 h at room temperature. All solutions were filtered with Millipore filter 0.22 µm.

The incubations for double immune staining were done the same way, adapting the procedures of Craig *et al.*¹³ the primary antibodies were put together a mixture of two antibodies diluted in TBS plus BSA 3% 0.05 mol/L (1 : 100 to 1 : 200, respectively for factor XIII and Tryptase). The incubation with the secondary antibodies was done by diluting Goat anti-mouse IgG and Goat anti-rabbit IgG in TBS 0.05% plus BSA 1% 1 : 20.

Analysis of IH: the cells in superficial and mid dermis, positive to primary antibodies anti-FXIIIa, anti-Tryptase and anti-CD34 were counted under light microscopy, OM 400×, in 12 optical fields around dermal vessels, and the results expressed as the total

number of positive cells per square millimetre of the biopsy specimen. The following formula was applied:

Density of cells/mm² = (total number of immune labelled cells in 12 optical fields/12) /0.0625

The blood vessels in these fields were also counted. The coefficient variability of the duplicate counts obtained from all slides was less than 5%. No reactivity was observed in the sections stained with the omission of the primary antibody or substitution of this antibody with an irrelevant antibody of the same type.

Data were analyzed with a statistical package (Graph Pad Prism version 3.00 for windows, GraphPad Software, San Diego, CA, USA). The Mann-Whitney *U* test was used for comparison between two groups (normal skin and urticarial lesion). For all tests, *P* values of less than 0.05 were considered statistically significant.

Results

Clinical aspects

The urticarial lesions consisted of wheals randomly distributed all over the skin, with lesions varying in diameter from 3 mm to 8 cm in all cases, sparing the genital region and the scalp. The symptoms were referred to as feelings of 'stinging', 'burning' and/or 'itching'.

HE

DIAU cases showed a mild to moderate perivascular lymphocytic skin infiltrate, with oedema (Fig. 1a) and scattered neutrophils and eosinophils in the dermis.

Toluidine blue stain

Mast cells were identified around vase and annexes in the superficial and mild dermis in the normal skin sections of all patients, displaying several metacromathic granules into their cytoplasm. The urticarial disclosed partially depleted mast cells (Fig. 1b) around superficial blood vessels and papillary dermis, side by side to intact mast cells.

IH – tryptase staining

Tryptase positive immune labelling cells were identified around annexes and vases in the superficial and mid dermis in the normal skin of DIAU patients (Fig. 1c), and there were no difference in tryptase positive cells number between normal skin and urticarial lesions (Table 3; *P* = 0.7104). In the urticarial lesions there were some intact mast cells and numerous partially granule content depleted mast cells around superficial blood vessels and papillary dermis.

IH-factor XIIIa staining

There were multiple FXIIIa⁺ DD in upper and mid dermis, dispersed in sub epidermal areas and around blood vessels, in

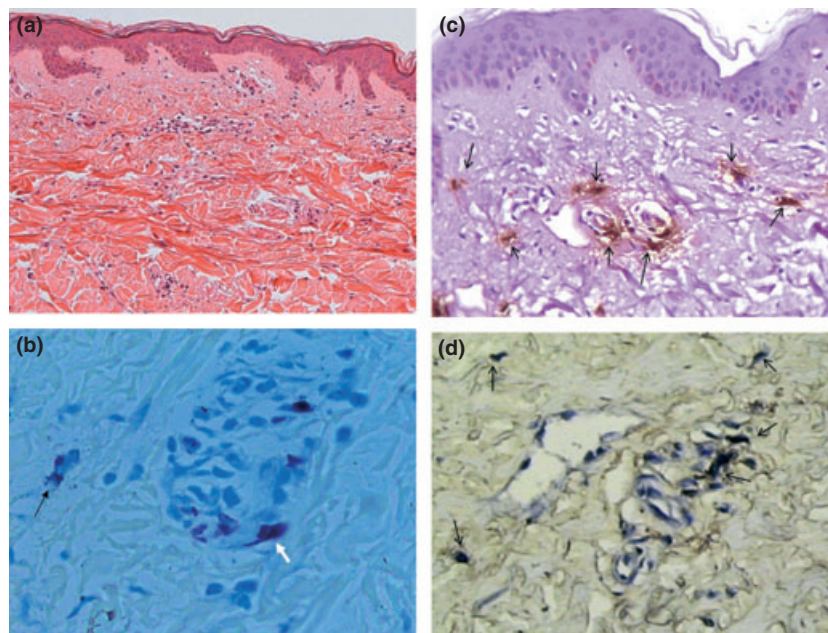


Figure 1 (a) Urticarial lesion (case 1); oedema dissociating the collagen fibers of the superficial dermis and mild perivascular inflammatory infiltrate (Hematoxylin-eosin staining). (b) Urticarial lesion (case 7); presence of non-degranulated mast cells (white arrow) and degranulated mast cells (black arrow) (Toluidine blue staining). (c) Urticarial lesion (case 3); Tryptase immunolabeled mast cells (arrows) showing degranulation halo around the cells (Immunohistochemistry). (d) Urticarial lesion (case 4); FXIIIa immunolabeled dermal dendrocytes (arrows) around superficial dermal vessels. (Immunohistochemistry), Original magnifications: x200 (a), x1000 (b), x400 (c) and x600 (d).

Table 3 Counting of immunolabeled cells with FXIIIa and/or Tryptase in normal skin and urticarial lesions in seven patients with drug-induced acute urticaria

Antibody	Normal skin (number of cells)							Urticarial lesion (number of cells)						
Cases	1	2	3	4	5	6	7	1	2	3	4	5	6	7
FXIIIa ⁺ cells	66.66	109.33	114.66	37.33	117.33	94.66	53.33	62.66	96.00	68.00	101.33	54.66	57.33	60.00
Tryptase ⁺ cells	77.33	61.33	116.00	68.00	66.66	53.33	78.66	68.29	112.0	92.00	38.66	54.66	52.00	73.33
Tryptase ⁺ and FXIIIa ⁺ cells	40.00	42.66	24.00	65.33	33.33	36.00	44.00	28.00	24.00	24.00	17.33	17.33	36.00	18.66

normal skin and urticarial lesions (Fig. 1c). The number of these cells was similar in both groups (Table 3). In urticarial lesions, the number of FXIIIa⁺ dermal dendrocytes per mm² was similar to the normal skin ($P = 0.5350$).

IH-double immune staining (anti-Tryptase and anti-FXIIIa)

In the sections of urticarial lesions, all cases presented FXIIIa⁺ immunostained dermal dendrocytes, Tryptase⁺ immunostained mast cells and double immunostained FXIIIa⁺ and Tryptase⁺, as back-brown engulfed granules into DD cytoplasm (Fig. 2). There was difference ($P = 0.0070$) in double labelled cells number

between normal skin and urticarial lesions, showing a higher number of double labelled cells in normal skin (Table 3).

IMEM-single immunoelectron transmission microscopy

In sections of urticarial lesions at IMEM there was a slight vascular dilatation, with platelets, red blood cells and/or leucocytes in the vessel's lumen, and mast cells, polymorph nuclear cells, lymphocytes and dendrite cells close to the vessels. A few mast cells appeared normal, whereas others presented as granule-depleted. In some areas, mast cells, macrophages, lymphocytes and satellite dendrite cells were closely associated (Fig. 3). Some macrophages were full of melanin. A variable number of eosinophils and

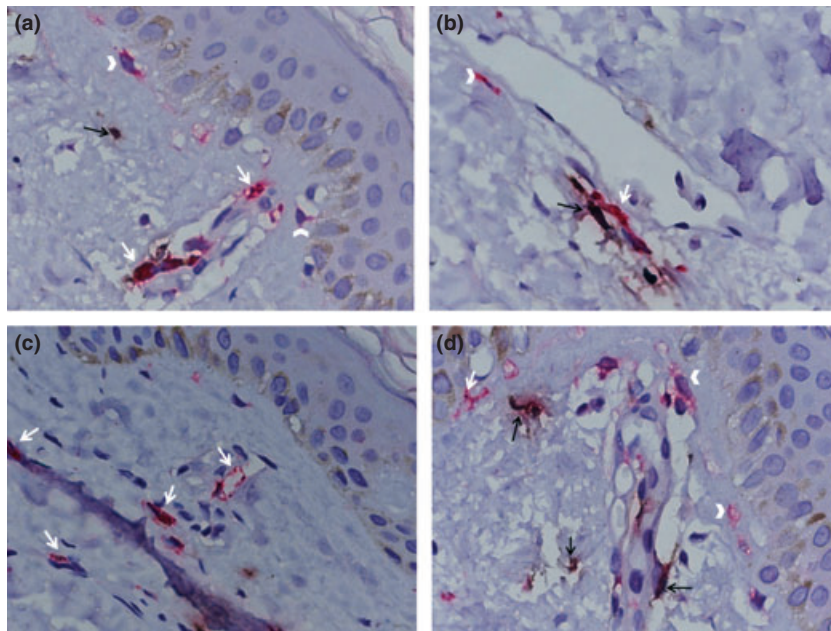


Figure 2 Double immunohistochemistry of sections from urticarial lesions and normal skin using anti-FXIIIa antibodies counter-stained with permanent red (red) and anti-tryptase antibodies counterstained with diaminobenzidine (DAB)-nickel (dark brown). (a) Case 3 (urticarial lesion). Subepidermal dermal dendrocyte FXIIIa+ (arrowhead), mast cells (black arrow) and dermal dendrocytes showing double staining (white arrows). (b) Case 5 (normal skin). Perivascular dermal dendrocyte FXIIIa+ (arrowhead), mast cells (black arrow) and dermal dendrocyte showing double staining (white arrow). (c) Case 5 (urticarial lesion). Subepidermal dermal dendrocytes showing double staining (white arrows). (d) (urticarial lesion). Perivascular and subepidermal dermal dendrocyte FXIIIa+ (arrowhead), mast cells (black arrow) and dermal dendrocyte showing double staining (white arrow). Original magnifications: x1000 (a, b, c and d).

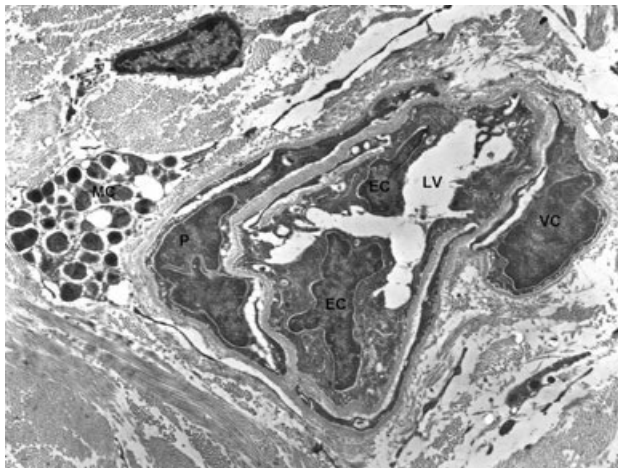


Figure 3 Dermal microvascular unit. Capillary vessel showing endothelial cells (EC) protruded into vascular lumen (LV), pericyte (P), veiled cell (VC) and partially degranulated mast cell (MC). (Immunoelectron transmission microscopy; original magnification, x12,500).

polymorph nuclear neutrophils were observed, besides a significant presence of lymphocytes and macrophages. The epidermis and the basal membrane zone were preserved. We observed

mast cell granules into the cytoplasm and extruded mast cell granules between dermal collagen fibbers showing tryptase and FXIIIa markers.

IMEM-double immunoelectron transmission microscopy

After double immunogold staining, 10 nm (FXIIIa marker) and 15 nm (Tryptase marker) gold particles were present together over the granules in mast cells (Fig. 3) indicating that both Tryptase and FXIIIa are located in the granules of these cells, in the sections of normal skin and urticarial lesions, in all seven cases. Extruded granules in between extracellular matrix showed also a double immunogold staining (Fig. 3). The extruded granules were membrane-free and exhibited reduced matrix density. Contrast and delineation of the granule substructure were less than optimal after the immunogold procedure. FXIIIa⁺ DD were displayed in perivascular location both in normal skin and urticarial lesions. The FXIIIa expression was observed dispersed in the cytoplasm (Fig. 3) and in the DD pseudopods. In a few number of DD, immunogold FXIIIa labelled could be found attached to nuclear chromatin. In urticarial lesions and in the normal skin, we found signs of Tryptase immunogold label into DD cytoplasm, suggesting previous engulfment of the mast cell granule remnants. We found extruded mast cell granules contents both FXIIIa and

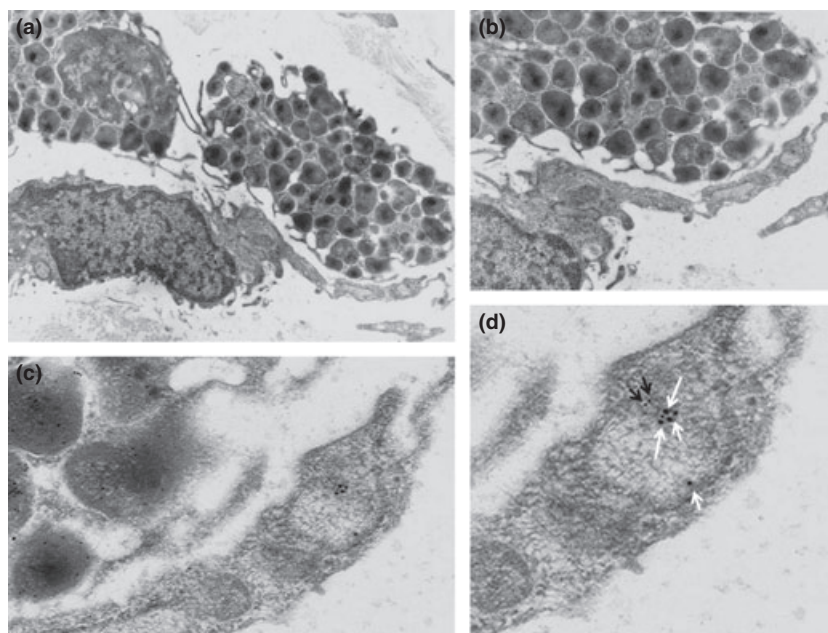


Figure 4 Double immunoelectron transmission microscopy. (a) (case 5) apparently normal skin. Two mast cells in apposition to dermal dendrocyte (original magnification $\times 20,000$). (b) (case 5). Apposition of mast cell under degranulation process to cytoplasm process of dermal dendrocyte (nuclei on the inferior left corner) (original magnification $\times 37,500$). (c) (case 5) Normal skin. Observe mast cell granule phagocytosed by dermal dendrocyte showing double immunostaining to tryptase (gold particles, 15 nm, white arrow) and FXIIIa (gold particles, 10 nm, black arrow) (original magnification $\times 62,500$). (d) Detail of the phagocytosed granule (gold particles, 15 nm, white arrow) and FXIIIa (gold particles, 10 nm, black arrows).

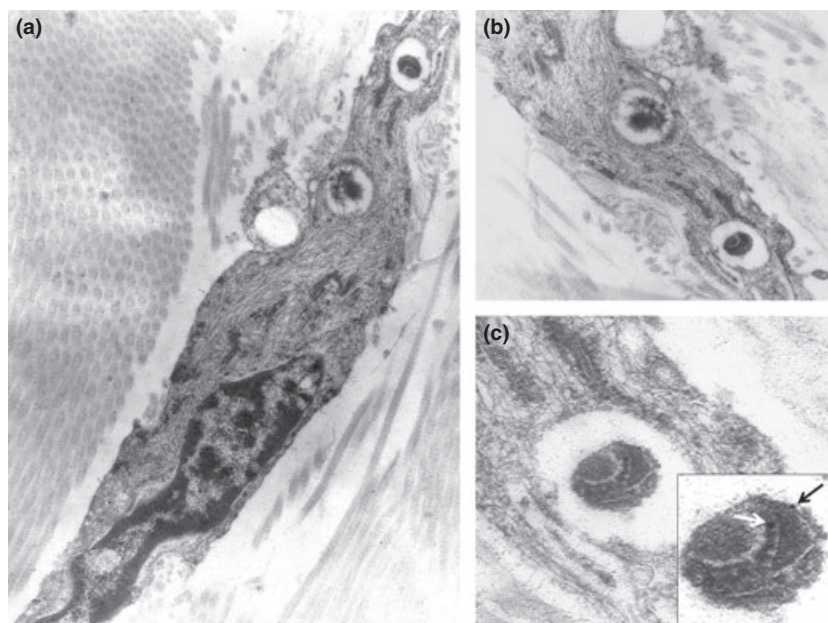


Figure 5 Double immunoelectron transmission microscopy (case 3) urticarial lesion. (a) Dermal dendrocyte under phagocytosis process (original magnification $\times 30,000$). (b) Dermal dendrocyte under phagocytosis process. (original magnification $\times 62,500$). (c) Detail. Observe the presence of 10 nm gold particles (FXIIIa) on dermal dendrocyte cytoplasm and on phagocytosed granule (black arrow) and of 15 nm gold particle (tryptase, white arrow).

Tryptase immune gold labelled inside DD (Fig. 4), disclosing the co-localization of FXIIIa and Tryptase-like molecules in the DD phagocytized material. There was also large dendrites extending from the DD to the site where the extruded mast cell granule remnant were once located (Fig. 5).

Discussion

We describe some features in the mast cells and dermal dendrocytes in patients with DIAU, comparing the normal skin to the urticarial lesions.

As we observed FXIIIa immune labelling in dermal dendrocytes both under IH and IMEM, we can suggest that FXIIIa is distributed inside DD, either in the pseudopods or diffuse throughout the cytoplasm. Furthermore, this confirms previously observed data.^{14,15}

The presence of FXIIIa in monocytes/macrophages is a result of direct synthesis.¹⁶ We observed in the DD some gold particles marking the presence of FXIIIa attached to microfilaments in the cytoplasm, both in the sections of urticarial lesions and normal skin. This strongly suggests that FXIIIa has a role in catalyzing interactions between macromolecules of the locomotor cytoskeleton system of these cells. This assumption well agrees with the experimental findings that different cytoskeleton proteins (as actin, myosin and vinculin) serve as substrates for FXIIIa.¹⁵ Moreover, we found sometimes an expression of FXIIIa inside nuclear DNA-containing areas of DD, as it has been reported in the early phase of macrophage differentiation in long term cultures of human monocytes.¹⁵

The macrophage subtypes in different types of urticaria was once studied and a subtype of activated macrophages reactive to antibody 27E10 was more frequently found in acute urticaria than in chronic urticaria.⁶ The expression of FXIIIa is related to cell activation and inducible via interleukin 4 in alternatively activation of macrophages.^{16,17} FXIIIa is also expressed in migratory dermal dendrite cells, monocyte-derived dendrite cells, hematopoietic progenitor cell-derived DDC-like, and in a population of MHC class II⁺ dendrite-like cells of the dermis, named DD.¹⁷

In urticaria, the FXIIIa expression on DD could be due to a mast cell degranulation, knowing that the mast cells granules contain preformed IL4 and activated mast cells also release newly synthesized IL-4, when stimulated.¹⁸

However, the use of FXIIIa to characterize DD (macrophages) in urticaria has never been described until now.

We found extruded mast cell granules containing both FXIIIa and Tryptase immune gold labelled inside DD, suggesting co-localization of FXIIIa and Tryptase-like molecules in the phagocytized material by DD. There was also large dendrites extending from the DD to the site where the extruded mast cell granule remnant were located.

In some fields, the immunogold marked FXIIIa⁺ DD appears to be in the process of engulfing the remnant granule of a mast cell

and there is the presence of extruded mast cell granules, no matter the section comes from the urticarial lesions or the normal skin. This led us to the suspicion that there is no such thing as normal skin, but an apparently normal skin, in a ready state of giving an immune response to any stimuli at any time. Probably, this is due to the interaction between activated mast cells and DD that occurs all over the skin of a person with DIAU.

Due to the limitations of the chosen research method, that is, electron microscopy, the number of patients had to be small and, this time, we decided to restrict our research to the DIAU,¹¹ although pharmacovigilance criteria cannot replace drug allergy testing in the assessment of drug hypersensitivity reaction.¹⁹ Nevertheless, some ethics issues concerning to drug allergy testing and/or clinical trials are closely knotted. Thus, consideration of ethical and practical issues are critical to choose the assessment of a patient.²⁰

We presented morphological evidence through IH and IMEM techniques of phagocytosis of the extruded mast cell granules by DD, we observed DD with mast cell granules inside the cytoplasm, IMEM study we displayed the immune-expression of tryptase and FXIIIa into granules that had been internalized by DD. Taking all these data together, we suggest that a functional interaction between DD and mast cells in DIAU do occur. Our belief is supported by previous *in vitro* observation that mouse peritoneal macrophages were internalized by phagocytosis of mast cell granules²¹ and that phagocytosis of peritoneal mast cell granules by phagocytes and neutrophils in the course of anaphylactic reactions in the rat had the capacity to scavenge important amounts of mast cell granule products released by anaphylaxis.²²

Similarly, our finding that the remnants of granules expelled into extracellular fluid in normal skin and urticarial lesions of DIAU were subsequently phagocytized by DD rely on earlier results²³ that during mast cell degranulation the soluble component of the granule is released into the extracellular fluid, whereas two neutral proteases and heparin proteoglycans form the extracellular granule remnants, negatively charged and with high affinity bind to low density lipoprotein and other basic molecules¹⁹. Also, mast cell granule phagocytosis by DD was found others immune conditions, such as toxic epidermal necrolysis and graft-versus-host reaction.²⁴

One out of many question remains: what is the functional significance of the granule phagocytosis? Several possibilities were speculated:²¹ (i) this DD mast cell granule phagocytosis has simply a function of scavengers and discharges of the extruded granules and their contents; (ii) it could be possible that phagocytized mast cell granules have a role in the regulation of some DD functions, as: inactivation of certain proteases, e.g. Tryptase that may regulate and participate in cellular inflammatory reactions and in neuronal induced inflammation; (iii) the granule uptake could be an effective and specific mean of inhibiting enzyme systems of the DD, as the blood-coagulation system. These theories explain the uptake of mast cell granules by mouse peritoneal macrophages and we

think they could be applied to clarify the role of the DD granule uptake in DIAU.

Conclusions

The presence of a higher number of DD showing phagocytosis of extruded granules of mast cells in normal skin reinforces the concept that DIAU could result from a process that involves all the skin at different times during its temporal evolution, with wheals in certain areas that appear, soon disappear and appear again, sometimes in a previously normal clinical skin portion. Despite a normal clinical appearance, this region of the skin was probably being affected to some extent by the degranulation of mast cells, by the time of the biopsy, acting as a possible mechanism of modulation of the inflammatory process, maintaining the homeostasis and down regulating the wheals and flare reactions. The phagocytosis of mast cell granules by DD in DIAU could represent a self-limiting process reducing an over-release of mast cell mediators.

This first ultra structural observation in the human being skin provides morphological evidence that the exocytose-phagocytosis mechanism of mast cell granules represents one of the pathways of mast cells-DD interactions in urticaria. Besides the involvement of the traditional cellular inflammatory cells (lymphocytes, monocytes, eosinophils and neutrophils), the FXIIIa⁺ DD display a relevant function in DIAU.

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