

Evaluation of allergic vesicular reaction to patch test using *in vivo* confocal microscopy

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Background: Confocal microscopy has been successfully applied both in oncologic and inflammatory diseases. In particular, it has been proved as a useful tool for the *in vivo* detection of microscopical changes occurring in allergic reactions.

Aims of the study: To evaluate microscopic changes occurring in positive patch test reactions.

Methods: Eight patients with history of allergic dermatitis and positive patch test reaction were analysed by means of confocal microscopy.

Results: Confocal microscopy showed the presence of spongiotic vesicle preferentially localized around the ad-

nexal ducts that appeared to be in the middle of the spongiotic phenomena.

Conclusion: Confocal microscopy offered for the first time new insight into vesicle formation and development, showing that adnexal ducts can play a role in allergic reaction.

Key words: allergic vesicular reaction – confocal microscopy – adnexal structures

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REFLECTANCE CONFOCAL microscopy (RCM) is a non-invasive tool for *in vivo*, microscopical evaluation already known to be useful for the investigation of both neoplastic and inflammatory skin processes (1–7).

In spongiotic skin diseases, RCM showed the presence of fairly demarked, dark areas at the level of the stratum spinosum/granulosum. The surrounding apparently normal keratinocytes, showed a thickening of the inter-keratinocyte spaces associated with the presence of bright, round cellular structures lower in diameter than keratinocytes corresponding to inflammatory cells (6).

In this paper, we described our experience in RCM evaluation of patch tests reactions to different allergens, reporting new confocal microscopical features of spongiotic vesicle.

Methods and Results

Eight consecutive patients (four male and three female), with age ranging from 18 to 64 years, previous diagnosis of allergic dermatitis and a positive patch reaction after the application of a standard series, were considered.

Patch tests reaction was evaluated after 48 h from chambers application with semi-quantification of the reaction from + to +++++.

Only clinically visible vesicular reaction was selected and underwent to RCM analysis performed using a commercially available Vivascope 1500[®] device (Lucid[®] Technologies, Rochester, New York, NY, USA) for clinical *in vivo* imaging. High-resolution single images (0.5 × 0.5 mm) were obtained from different skin levels (epidermal layer, dermo-epidermal junction and upper dermis) focusing on the vesicular structures. Additionally, the following software programs were used:

- Viva Block[™] (mosaics of 16–64 images generating a 2–4 × 2–4 square mm at the level of the spinous layer).
- Vertical Viva Stack[™] (evaluating the vesicle and adnexal structures from the ostium to the reachable dermis progressing deeper in 5 µm-steps until the fading of the images).

In the eight patients evaluated, different degree of reaction to specific allergens (from + to +++) was seen. RCM was performed in the site of 10 patch test with vesicular reaction (from

++ to ++++) to Dermatophagoides (1), Nickel (6), Palladium (1), thimerosal (1) and disperse blue (1).

In all the cases VivaBlock mosaic analysis disclosed the presence of sparse to several spongiotic vesicles constantly centred by an adnexal structure duct (Fig. 1).

At the level of the stratum corneum, the presence of the adnexal ostium was seen in correspondence of the centre of the vesicles. Considering the RCM morphology, the visible adnexal structure seen in our cases seems to be more suggestive for an glandular infundibulum because of the thin epithelial duct walls and the absence of keratotic material inside the lumina as seen commonly in follicular structures (Fig. 2). Deeper into the stratum spinosum, enlarging spongiotic areas, seen as darker spaces than the background with thickening of the inter-keratinocytes spaces and defined by a virtual wall composed by the epidermal epithelium were seen. These features were associated with round, highly refractile cellular structures corresponding to activated, exocytotic, inflammatory cells. At this magnification, spongiotic vesicles were better seen disposed around the adnexal structure infundibula. Closer to the dermo-epidermal junction level, in the spongiotic areas, the ratio between inflammatory cells and oedema was progressively

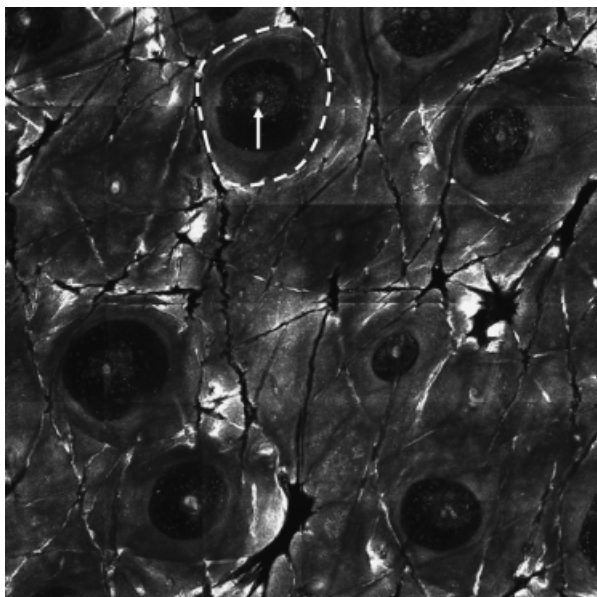


Fig.1. 3 mm by 3 mm VivaBlock mosaic taken at the level of the stratum spinosum discloses the presence of numerous vesicles (white lines) centred by an adnexal structures (white arrow).

in favour to the second. Mildly refractile polygonal structures connected to the vesicle 'wall', probably corresponding to acantholytic keratinocytes, were also evident (Fig. 2).

Discussion

Spongiotic reaction derives from the activation of inflammatory cells, called at the site of allergic process activation, vessels dilatation and the developing of epidermal oedema.

In our cases, adnexal infundibula are constantly seen centring the vesicle lumina disclosing the possibility that the liquid determining the epidermal oedema, and the consequent vesicle formation, could transude directly from the adnexal lumina into the epidermis. Moreover, the location of vesicle suggests that adnexal ducts, in continuity with the external environment throughout the infundibula openings, may represent a minor resistance point for allergens penetration into the skin. Supporting the hypothesis, it is already known that infundibular epithelium is highly populated by Langerhans cells playing a role in allergic reactions (8). Consequently, both eccrine glands duct and the connected follicular epithelium itself may represent the initial site of activation for spongiotic vesicle formation.

To the best of our knowledge, this is the first report of such a microscopical description of spongiotic vesicles; it can be justified because of the vertical cut of conventional histology that limits the possibility to explore the adnexal ducts inside the vesicle lumina. Conversely, the RCM *en face* visualization of the epidermis allowed the evaluation of anatomical and structural relationship between ducts and vesicles.

In our report, RCM gives new microscopical information about spongiotic vesicle features that may be explained by different biological substrates. Probably, also therapeutical options could derive from the understanding of the effective role played by the adnexal duct in the pathogenesis of spongiotic vesicle. Further studies on large case series, are warranted in order to better understand the pathologic changes and related biologic events in vesicle formation as well as the role played by the hair follicles and eccrine gland ducts, not only in patch test allergic reaction, but also in allergic, vesicular contact dermatitis.

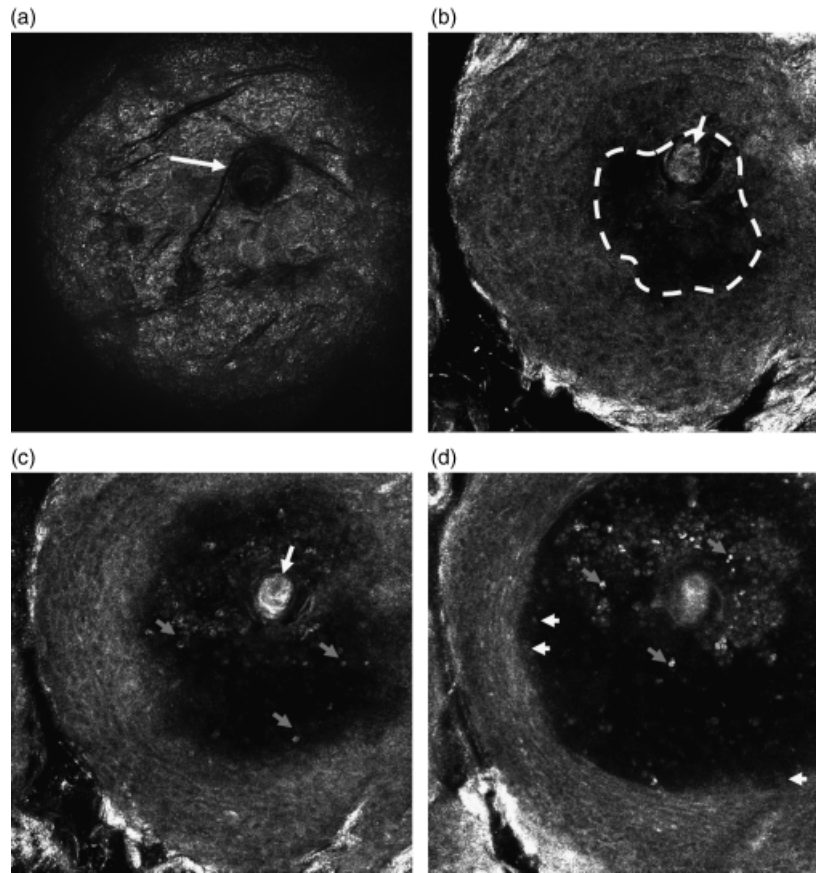


Fig. 2. 0.5×0.5 mm selected images taken from vertical VivaStack evaluation of a vesicle preformed from the stratum corneum to the reachable dermal layer. (a) Frame taken at the level of the stratum corneum with presence of an eccrine gland ostium (white arrow). (b) Frame taken deeper at the level of the stratum spinosum disclosing the presence of a spongiotic area (grey line) seen as darker spaces around the adnexal structure (white arrow). (c) Frame, taken deeper in the stratum spinosum with the presence of a larger and well defined spongiotic area around the eccrine duct (white arrow) and associated with round, highly refractile cellular structures corresponding to inflammatory activated cells (grey arrows). (d) Frame, taken at the level of the stratum spinosum closer to the DEJ, shows the spongiotic vesicle composed by massive spongiosis delimited by a virtual wall composed by the epidermal epithelium and associated with numerous inflammatory cells (grey arrows) and mildly refractile polygonal structures sometimes connected to the vesicles wall and probably corresponding to acantholytic keratinocytes (grey arrows).

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