Review paper

Dew drops on spider web appearance: a newly named pattern of IgG4 deposition in pemphigus with direct immunofluorescence

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Abstract
Novel appearances in cutaneous pathology as well as mucocutaneous clinical signs are being described which indicate that this is still an attractive area for exploration. The H + E histology terms of “decorated tomb stoning” and “undecorated tomb stoning”, advocated by some pathologists, are misleading and as such should be avoided. Here, an appearance of IgG4 pemphigus deposits examined cost-effectively with direct immunofluorescence and suggested to be called “dew drops on spider web” is depicted in depth.

Key words: pemphigus, pathology, autoimmunity.

Introduction to the issue of diagnosing pemphigus
Pemphigus is a group of autoimmune blistering dermatoses. Pemphigus diseases are manifesting a surprisingly rich spectrum of clinical signs. Pemphigus diseases are potentially life threatening and require prompt clearcut diagnosis as usually aggressive immunosuppression, having serious side-effects, is needed. The key diagnostic technique is still subjective imaging direct immunofluorescence (DIF) of perilesional tissue as it detects local organ-specific autoimmunity. At present, indirect immunofluorescence (IIF) techniques, including those using biochip mosaics, are being replaced with biochemical-molecular techniques, particularly multiparametric ELISA (developed within a framework of a multicenter project: “A European prospective study on serum antibodies against target antigens of bullous autoimmune diseases and genetic susceptibility” headquartered in Lübeck, Germany) [1].

The Tzanck cytological smears using May-Grünwald-Giemsa stain and H + E histology have merely a supportive value as the immunological nature of pemphigus cannot be detected with them. Moreover, cutaneous non-neoplastic diseases, which are numerous, present a very limited number of patterns in H + E histology. Acantholysis is a nonspecific finding [2], and can be seen in diverse dermatoses presenting disseminated, nevoid and focal arrangement; it can even be accidental. Still, H + E histology of a lesion in question undergoing spatial-temporal evolution, if evaluated properly, can provide a hint suggesting necessary immunological investigations. H + E histology, evaluated by a skillful pathologist, can be invaluable for differentiating pemphigus lesions from malignant ones, especially on mucous membranes.

Discussion of the issue of appearances in pemphigus pathology with an emphasis on “dew drops on spider web” appearance of IgG4 deposition
Numerous appearances in cutaneous pathology have been described aiming at easing memorization. Those appearances are pathological accompaniments to mucocutaneous clinical signs and novel appearances as well as clinical signs are being described which indicate that this is still an attractive area for exploration [3, 4]. In the context of pemphigus, we cannot agree with the H + E histology appearance named “undecorated tomb stoning” for images seen for example in porphyrias in opposition to “decorated tomb stoning” characterizing pemphigus vulgaris and known for decades. For us, tombstones...
mean keratinocytes, so if there are no keratinocytes in the evaluated pathological area, the term "undecorated tomb stoning" is simply misleading.

We are evaluating IgG4 deposits with DIF as their images are generally easier to interpret compared to those obtained while evaluating IgG deposits. This is particularly true, if tissue examined is fragmented and/or crushed as it happens with mucosal specimens. Regarding DIF patterns in pemphigus, while evaluating deposits of IgG4 using a single-step approach [5], we noticed that IgG4 deposition is dotty under magnification in the range of 400–600. This dotty pattern resembles dew drops on spider web [6], so we propose calling this dew drops on spider web appearance (Figure 1) in opposition to the textbook pattern of IgG deposition known as a fish net or chicken wire appearance. We hypothesize that this dotty appearance in both extrafollicular and follicular epithelium with DIF, as it is a repetitive finding, is not a technical procedure artifact or epiphenomenon, but has true pathological meaning being pathognomonic for pemphigus.

Certain previous studies stated that a punctate or granular fluorescence may be appreciated at higher magnifications by both DIF and IIF in pemphigus in case of...
IgG deposits [7, 8]; however, without the molecular explanation of this phenomenon. Some researchers [9, 10] observed it especially in the lower layers of epidermis. It is known that dotted or granular staining of IgG does not reflect the physiological distribution of desmogleins, which should be present as a normal smooth pattern within intercellular spaces of epidermal keratinocytes. The reason for this event could be related to the clumping of desmosomal cadherins [10]. Fascinatingly, one of the possible clarifications of granular IgG staining was proposed by Oktarina et al. [10], who indicated that the IgG-induced clustering of the pemphigus autoantigens (desmogleins) underlies the granular IgG deposition in the patient skin undergoing spatial-temporal remodeling of keratinocyte surface leading to the formation of invaginations of one cell into another [11]. Moreover, this group of researchers showed that in pemphigus foliaceus and in mucocutaneous pemphigus vulgaris only desmoglein 1 clustering correlates with nonacantholytic intercellular widening between desmosomes. The sequestration of desmogleins from desmosomal components, fitting in with the desmoglein non-assembly depletion hypothesis, as well as targeting non-junctional desmogleins that are no longer available to be incorporated into desmosomes structures are processes leading this way to the disturbed assembly of desmoglein-rich desmosomes [10]. The issue is still more complex, as regarding IIF on monkey esophagus it should be noted that pemphigus-like antibodies of IgG class misleadingly can give a granular appearance, instead of a pemphigus-characterizing honeycomb appearance, whereas such antibodies are not detectable in IgG4 subclass and such a finding is not related to the presence in serum of examined antibodies reacting with blood groups antigens expressed in the substrate tissue [12] (Figure 2). Obvious explanation might be that the substrate influences fluorescence images.

However, in light of the above, the intriguing question arises why IgG4 deposits in DIF are less continuous than IgG ones at the same magnification. The explanation may be that IgG gives more disease-nonspecific background staining involving less desmoglein-rich areas on a keratinocyte’s surface whereas IgG4 is responsible for more pemphigus-specific pathology staining desmoglein-rich desmosomes and extradesmosomal desmogleins. This speculation requires investigational verification as putative non-desmoglein targets might be pathogenetically relevant [13].

Putting aside detailed pathogenesis, we suggest that this dew drops on spider web appearance of IgG4 pemphigus deposits as newly described in depth here, that has already been mentioned just incidentally by us in a PubMed accessible reference [14], should be known by both laboratory workers and dermatology clinicians dealing with pemphigus. The reason for being familiar with this appearance is that evaluating IgG4 antibodies if one still uses IIF on monkey esophagus in the differential diagnosis of pemphigus...
ease experience a relapse following rituximab [15]. The relapse of this kind might be related to the survival of low memory B cells after rituximab use which was noticed in patients with pemphigus [16].

Furthermore, in drug-induced pemphigus, the pattern of immunostaining of perilesional tissue may be used as a prognostic marker since the absence of patchy staining with an antibody to desmoglein 1 and 3 is considered an indicator of favorable prognosis [17].

Finally, advanced imaging techniques such as atomic-force microscopy/scanning-force microscopy, multi-photon microscopy, super/high resolution techniques known under acronyms STED (STimulated Emission Depletion) and GSDIM/dSTORM (Ground State Depletion followed by Individual Molecule return/direct Stochastic Optical Reconstruction Microscopy) would expand our understanding of what is going on when pemphigus autoimmune recognition occurs on the surface of keratinocyte and, if adjusted to routine diagnostic use, provide novel appearances more precisely rendering reality.

**Conflict of interest**

The authors declare no conflict of interest.

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