

SHORT COMMUNICATION

Detection of Autoantibodies to Precursor Proteins of Desmogleins in Sera of a Patient with Bowen Carcinoma

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Accepted Oct 2, 2013; Epub ahead of print Jan 28, 2014

Non-pathogenic autoantibodies against desmogleins (Dsg), which do not reflect disease activity, might be detected during disease courses of pemphigus, but are rarely shown in healthy individuals (1). To clarify the significance of these antibodies, using various techniques we examined sera of a patient with Bowen carcinoma, which showed high titres of circulating anti-Dsg1 and anti-Dsg3 IgG antibodies by commercially available enzyme-linked immunosorbent assays (ELISA).

CASE REPORT AND RESULTS

A 78-year-old Japanese female had suffered from an erosive erythematous lesion on the right hand for 9 months. She visited a dermatologist and pemphigus was suspected as a differential diagnosis. Because ELISA analysis showed positive IgG autoantibodies to Dsg3 (index value 117; normal <7), the patient was referred to us for further examination. Physical examination revealed a brownish erythema with infiltration 40 × 18 mm in diameter on the dorsum of the right hand (Fig. 1). Histopathology of the skin lesion demonstrated proliferation of numerous dysplastic cells with abnormal nuclei in the epidermis. Tumour cells showed subepidermal invasion (Fig. S1¹). These findings were consistent with Bowen carcinoma. Direct immunofluorescence



Fig. 1. A brownish, erythematous plaque with scales and crusts on the right dorsal hand.

of both the normally appearing skin and the tumour showed no positive reactivity. Re-examination by ELISA confirmed positive IgG reactivity with both Dsg1 (index value 280; normal <14) and Dsg3 (index value 113; normal <7). Anti-Dsg1 antibody titres were higher than anti-Dsg3 antibody titres.

The study of non-pathogenic pemphigus antibodies by EDTA-treated ELISA. Pathogenic pemphigus antibodies recognise calcium-dependent conformational epitopes of Dsgs, whereas non-pathogenic antibodies react with calcium-independent linear epitopes of Dsgs (1, 2). To investigate whether the patient sera reacted with calcium-dependent or independent epitopes, we measured titres of anti-Dsg IgG autoantibodies in the patient sera by both conventional and EDTA-treated ELISA. ELISA analysis was carried out as previously reported using ELISA kits (MBL, Co., Ltd., Japan) (2, 3). The titres of IgG anti-Dsg1 and anti-Dsg3 autoantibodies in the patient sera were not influenced by the treatment of EDTA, as both sera taken before and after surgical resection of the tumour (Table S1¹). This result suggested that the patient had calcium-independent non-pathogenic antibodies to Dsgs. Therefore, we considered that antibodies in our case might recognise calcium-independent linear epitopes or precursor domain on Dsgs. In the pipetting assay, the patient serum was not capable of inducing the cell dissociation of the keratinocytes *in vitro* (data not shown) (2).

The study of antibodies against precursor domains of Dsgs by immunoprecipitation-immunoblotting (IP-IB). To clarify whether the patient IgG antibodies reacted with the precursor forms of Dsg1 and Dsg3, we performed IP-IB using recombinant proteins (RPs) of mutated forms of Dsg1 and Dsg3, in which the original endoproteolytic cleavage site of Dsg is replaced (4). The original RPs of Dsg1 and Dsg3 were also used as mature forms. These RPs were expressed in Chinese hamster ovary (CHO) cells, which have more efficient post-translational modification than insect cells (baculovirus expression system) used for standard Dsg ELISA analysis. Because the pure precursor and mature forms of Dsg were prepared in CHO cells from their respective plasmid constructs (Fig. S2¹, left panel), we could investigate reactions with the precursor and mature forms of Dsg1 and Dsg3, respectively (5). The IP-IB clearly demonstrated that the patient serum reacted with the precursor forms of Dsg1 and Dsg3, but not with the mature forms of both Dsgs (Fig. S2¹, right panel). Immunoreactivity of Dsg3 was much stronger than that of Dsg1.

The study of antibodies against extracellular (EC) domains of Dsgs by IP-IB using domain-swapped molecules. The domain-swapping approach is useful for conformational epitope mapping in pemphigus (6). To analyse specific domains of Dsgs reacted by the patient serum, IP-IB using swapped molecules of Dsg1/Dsg2 and Dsg3/Dsg2 containing one each of EC1-5 domains of Dsg1 or Dsg3 on the backbone of Dsg2, whose endoproteolytic cleavage sites were not replaced (7, 8), was performed as described previously (9–11), with the following

¹<http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1776>

modifications. We used anti-His-tag mAb, anti-6xHis (28–75) (WAKO, Osaka, Japan) instead of anti-E-tag mAb for IP. In IB, precipitated proteins, which were considered to contain both precursor and mature forms of Dsgs (12), were visualized by a chemiluminescence detection system. IP-IB was performed using 20 μ l of the patient serum, and anti-His tag mAb and normal serum were used as positive and negative controls, respectively. The results indicated that the patient serum failed to react with any domains of Dsg1. In contrast, the patient serum reacted with both the full-length protein and EC1 domain of Dsg3 (Fig. S3¹).

Direct immunohistochemical study of the skin tumour using anti-Dsg1 and -3 antibodies. To examine expression of Dsgs in the tumour, we have performed immunohistochemical staining on formalin-fixed and paraffin-embedded tissue sections. Anti-Dsg1 antibody (prediluted, Progen Biotechnik GmbH, Heidelberg, Germany) and anti-Dsg3 antibody (1:50 diluted, Invitrogen Corporation, Camarillo, CA, USA) were used in the experiment with histofine new fuchsin substrate kit (Nichirei, Tokyo, Japan). Immunohistochemical staining of the tumour tissue showed positive intracytoplasmic staining with weak cell surface staining for both Dsg1 and Dsg3 (Fig. 2). In contrast, surrounding normal epidermis demonstrated much weaker staining than the tumour portion (data not shown). Negative controls were obtained by switching primary antibody to normal mouse IgG (Fig. 2C) or by omitting the primary antibody (Fig. 2D).

DISCUSSION

The results of IP-IB study of RPs of precursor and mature forms suggested that the patient IgG antibodies reacted with precursor segments of Dsg1 and Dsg3, and were non-pathogenic. Furthermore, IP-IB study of domain-swapped molecules showed that the patient serum reacted with both the full-length and EC1-containing RPs of Dsg3. These 2 RPs contain the precursor domain of Dsg3, further suggesting positive reactivity of the patient serum with precursor fragment of Dsg3. The results of EDTA-treated Dsg ELISA analysis indicated that the patient serum reacted with non-conformational epitopes.

In IP-IB studies, the patient serum reacted more strongly with Dsg3 RPs than Dsg1 RPs, and reacted swapped molecule of only Dsg3 but not Dsg1. These results indicated that the patient's IgG antibodies reacted with different types of epitopes on the precursor fragments on Dsg1 and Dsg3.

However, these results were inconsistent with the stronger reactivity with Dsg1 in ELISA analysis. To

account for this discrepancy between IP-IB and ELISA methods the following (unproven) are proposed: (i) ELISA is solid phase reaction in which dried antigen proteins fixed to the bottom of wells react with patient serum, whereas IP-IB is a liquid phase reaction between antigen and serum; (ii) RPs used in ELISA analysis originated from insect cells, while RPs used in IP-IB were produced by CHO cells; (iii) experimental conditions, including molecular structures of RPs, reaction time and secondary antibodies, are different. Accordingly, ELISA index value may not necessarily reflect intensity of the signals in IP-IB.

Immunohistochemical analysis demonstrated that the tumour portion expressed intracytoplasmic Dsg1 and Dsg3 stronger than adjacent normal epidermis. Therefore, the cancer cells may contribute to the development of these proteins.

Because Dsg precursor proteins are not exposed to the immune system, immune tolerance to precursor segments may not be induced. Therefore, since the presence of specific B cells against precursor of Dsg1 in a normal population has been demonstrated previously (13), antibodies to precursor segments may be produced in some specific individuals. Alternatively, large amounts of Dsg precursor proteins were released from Bowen carcinoma cells and were exposed to immune system, causing production of anti-Dsg antibodies. Further studies are needed to clarify the nature of anti-Dsg antibodies in non-pemphigus cases.

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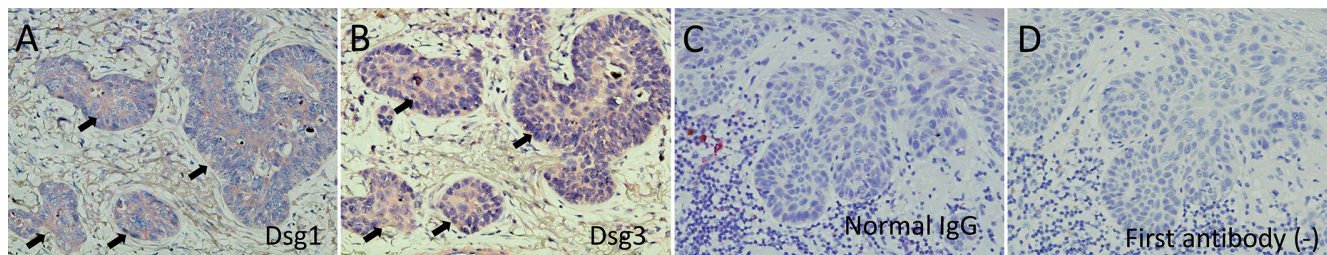


Fig. 2. Immunohistochemical studies of tumour tissue for Dsg1 and Dsg3. Immunohistochemically, Dsgs showed strong cytoplasmic staining with weak cell surface staining. (A) Intracytoplasmic staining of Dsg1 in tumour cells ($\times 200$). (B) Intracytoplasmic expression of Dsg3 in tumour cells ($\times 200$). (C) Negative control obtained by switching primary antibody to normal mouse IgG ($\times 200$). (D) Negative control obtained by skipping primary antibody ($\times 200$).

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