

The authors revealed that BP sera with autoantibodies for BP230 detected by enzyme-linked immunosorbent assay (ELISA) showed as linearly positive in the basement membrane zone (BMZ) in IIF, which used both ME and NHS as a substrate. In contrast, most sera with antibodies to BP180-NC16A alone showed positive only in IIF using NHS. To elucidate the reason for this discrepancy, the authors conducted further experiments using affinity-purified anti-BP180-NC16A/BP230 polyclonal antibodies from BP sera (BP180-NC16A-AP/BP230-AP). BP180-NC16A-AP showed reactivity to BMZ in IIF using NHS, but this was not observed in IIF using ME. In contrast, reactivity to BMZ was detected in BP230-AP in IIF using both NHS and ME.

The authors also isolated monoclonal antibody for BP180-NC16A (BP180-NC16A-mAb) and BP230 (BP230-mAb) from patients with BP using the phage display method.² BP180-NC16A-mAb was positive in IIF using NHS but negative using ME, and BP230-mAb was positive in IIF using both NHS and ME, consistent with the results of BP180-NC16A-AP and BP230-AP. These results suggested that BP180-NC16A was not expressed well in ME, and that IIF using NHS had an advantage over ME for detecting autoantibodies in BP, because the major role of antibodies for BP180-NC16A in the pathogenesis of BP had been described by previous studies.^{3–5} To support their data, the authors noted the genetic difference in BP180-NC16A between monkeys and humans; there is, in fact, only 71% homology between *Homo sapiens* and rhesus macaques, the main origin of commercially available substrate for IIF. Along with the results of this study, the homology in BP230 and BP180 outside of NC16A is much higher.

Interestingly, BP sera from which antibodies against both BP180-NC16A and BP230 were removed still had reactivity to BMZ in IIF using both NHS and ME. Although it is unclear whether the antibodies that could not be detected by recombinant proteins of BP180-NC16A and BP230 play a role in the pathogenesis of BP, they may contribute to the positivity of IIF in BP sera.

The diagnosis of autoimmune blistering diseases is based on clinical features, histology of a lesional skin biopsy, direct immunofluorescence and serum tests (e.g. IIF, ELISA). For BP, the most common autoimmune blistering disease, specific ELISA kits to detect IgG autoantibodies for BP180-NC16A and BP230 have facilitated diagnosis.^{6,7} However, interpretation of the results can sometimes be difficult when the results of IIF and ELISA are inconsistent. This study revealed why IIF using NHS is more sensitive for detecting autoantibodies in BP sera than ME, and identified the relationship between the results of IIF and ELISA. I believe that it will provide useful information for dermatologists engaged in the diagnosis and treatment of BP.

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Conflicts of interest

None to declare.

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Type VII collagen IgE autoantibodies in epidermolysis bullosa acquisita: more common than suspected

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Pemphigoid diseases are chronic inflammatory diseases of the skin and/or mucous membranes. Clinically, pemphigoid diseases are characterized by subepidermal blistering, which is caused by autoantibodies to structural proteins located along the basement membrane.^{1,2} Depending on the clinical presentation and the specificity of the autoantibodies, pemphigoid diseases are differentiated into bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), pemphigoid gestations, linear IgA disease, p200/laminin γ 1 pemphigoid or epidermolysis bullosa acquisita (EBA). The pathogenicity of the different autoantibodies in pemphigoid diseases has been demonstrated both *in vitro* and *in vivo*.³ However, with few exceptions, these ‘proof-of-pathogenicity’ experiments were performed using IgG autoantibodies. Furthermore, all

commercialized diagnostic assays for pemphigoid diseases are based on the detection of IgG autoantibodies.⁴ Hence, insights into the presence of autoantibodies in pemphigoid diseases, other than IgG, are also limited.

Regarding IgE autoantibodies, several groups confirmed the presence of circulating and tissue-bound IgE autoantibodies in BP, mainly targeting BP180-NC16A. The prevalence of these IgE autoantibodies in patients with BP ranges from 22% to 100%.⁵ Injection of total IgE isolated from patients with BP into human skin grafted onto immunodeficient mice induced dermal infiltration and subepidermal blistering in the grafts, pointing towards a potential pathogenic role of IgE autoantibodies in BP.⁶ This assumption is further supported by the induction of experimental BP in neonatal mice, which are transgenic for the human NC16A as well as the human FcεR1, by injection of human anti-NC16A IgE.⁷ Whereas IgG autoantibodies in BP are predominantly located along the basement membrane,¹ IgE deposits in BP skin may also colocalize with mast cells and eosinophils. In addition, crosslinking of IgE of patients with BP on mast cells leads to their degranulation.⁸ Hence, given the important role of mast cells in BP,⁹ this interaction of mast cells and autoreactive IgE leads to IgE-mediated tissue damage in BP.

In addition, IgE autoantibodies have also been noted in MMP,^{6,10} and very infrequently also in EBA.¹¹ Yet, the exact prevalence of IgE autoantibodies and their pathogenic relevance in MMP and EBA is so far unknown.

To determine the so far undetermined prevalence of circulating IgE autoantibodies in EBA, Koga and colleagues evaluated their prevalence in EBA, a pemphigoid disease characterized and caused by autoantibodies against type VII collagen (COL7).¹² For this, sera from 109 patients with EBA were evaluated for the presence of antibasement membrane autoantibodies by indirect immunofluorescence (IIF) microscopy, modified for the detection of anti-IgE antibodies.¹³ Here, antibasement membrane autoantibodies were detected in 26.6% of the sera. To determine if these autoantibodies are COL7-specific, IIF was repeated using skin from a patient with dystrophic epidermolysis bullosa (DEB), which does not express COL7. All tested sera that showed positive binding in IIF using normal human skin as a substrate did not bind to the DEB skin. Next, a commercial enzyme-linked immunosorbent assay (ELISA) for the detection of COL7 IgG autoantibodies¹⁴ was modified for the detection of COL7-specific IgE. Compared with sera from healthy controls, the overall optical density was higher in the EBA sera. Considering a cut-off of 3 SD, 16 EBA sera were positive for anti-COL7 IgE. Considering both IIF and ELISA, 13 sera were positive in both assay systems, 16 were positive in IIF only and three were positive in ELISA only. This seeming discrepancy is not fully explained. However, as the ELISA specifically detects NC1 and NC2 autoantibodies only, a higher number of positive results in the IIF is likely. Thus, collectively, 32 of 109 (29.3%) of patients with EBA have IgE autoantibodies targeting COL7. Furthermore, as observed for IgA,¹⁵ these COL7-specific IgE autoantibodies were observed more frequently in patients with the

inflammatory variant of EBA. Hence, the presence of IgE autoantibodies may also serve to stratify the diagnosis of patients with EBA, and (in the future) also the selection of therapy.

Collectively, Koga and colleagues have demonstrated a high prevalence of circulating IgE autoantibodies targeting COL7 in a large cohort of patients with EBA. This will shape future research in the field. In this author's personal opinion, the next experiments should confirm these results in an independent cohort, extend these findings to tissue-bound IgE in EBA, evaluate the pathogenic relevance of IgE in EBA, and ultimately test if blockade of IgE has therapeutic effects in patients with EBA.

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Conflicts of interest

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Keratoderma and woolly hair: an important clue for the presence of cardiac pathology

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In this issue of the *BJD*, Maruthappu et al. provide new information on the cutaneous manifestations of the heterozygous carrier state of mutations in *DSP*.¹ It is not very common for the dermatologist to be the one who saves the lives of patients. However, such is the case when examining patients that present with the combination of woolly hair and palmo-plantar keratoderma. This combination can herald the presence of a lethal arrhythmogenic cardiomyopathy due to mutations in genes that encode desmosomal proteins.^{2,3} Most of these conditions are inherited in an autosomal recessive manner, and can be divided into three syndromes:⁴

- 1 Keratoderma with woolly hair (KWWH) type I: caused by mutations in *JUP*, which encodes plakoglobin (Naxos disease);
- 2 KWWH type II: caused by mutations in *DSP*, which encodes desmoplakin (Carvajal disease);
- 3 KWWH type III: caused by mutations in *DSC2*, which encodes desmocollin 2.

Nevertheless, not every case of recessively inherited KWWH is associated with a heart condition, and recently, a fourth

type of KWWH was described (KWWH type IV), which is due to mutations in *KANK2*. This gene encodes a steroid receptor coactivator-interacting protein, and in this syndrome, no cardiac abnormality is found.⁵

So far, almost all the described cases of these cardiocutaneous syndromes were of an autosomal recessive inheritance pattern, and information on heterozygous mutation carriers was sparse and confusing, probably mainly due to lack of proper examination of both the skin and the heart. On this background, the study by Maruthappu et al. in this issue of the *BJD* is unique, as they performed a detailed clinical examination of the skin and hair and cardiac phenotyping of 38 carriers of a dominant loss-of-function mutation in *DSP* in six families.¹ In five of the families, a curly hair phenotype was evident in all mutation carriers, highlighting the importance of proper examination of the hair for making the correct diagnosis, and many of them also had a variable degree of palmo-plantar keratoderma. Of special interest is the fact that the mutations in these families were located to the N-terminal head or rod domains of desmoplakin.

Not only woolly or sparse hair suggests a cardiac disease, but also hypertrichosis. This is seen for example in H syndrome, in which excess hair growth is accompanied by hyperpigmentation, mainly of the lower limbs and abdomen.^{6,7} Cardiac abnormality is seen in many of these patients, and the patients should be evaluated properly for such abnormalities. There are several additional conditions in which the skin can accompany a cardiac pathology, and knowledge of these syndromes is of great importance.⁸

The study by Maruthappu et al. further emphasizes the crucial role of the dermatologist in the assessment of patients with KWWH. Early diagnosis of these cutaneous findings, which usually become clinically apparent before the cardiac manifestations, can be life-saving for the patients, as early treatment and implantation of an automatic cardioverter can prevent premature death. Nowadays, when genetic testing is becoming more and more accessible, diagnosis should always be established by genetic evaluation. Because the number of genes that can be responsible for these syndromes is expanding, whole-exome sequencing should be utilized for rapid diagnosis.

Conflicts of interest

None to declare.

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