Commentary

Autoantibodies against Adhesion Molecules and Structures in Blistering Skin Diseases

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Even by clinical and histologic findings alone it is reasonable to suspect that the pathogenesis of certain blistering skin diseases might involve abnormalities in adhesion structures. The best defined bullous diseases in this regard are pemphigus (of which there are two basic types: vulgaris and foliaceus) and bullous pemphigoid (BP) (1–3). Histology shows that the blisters in pemphigus result from a loss of cell-to-cell adhesion, a pathologic process called acantholysis. In pemphigus vulgaris (PV) loss of adhesion occurs deep in the epidermis, just above the basal layer, whereas in pemphigus foliaceus (PF) the defective adhesion is more superficial, in the granular layer. In BP blisters result from loss of adhesion of the basal cells to the basement membrane.

The next clue that adhesion structures might be involved in these diseases came when immunofluorescence studies demonstrated that both pemphigus and BP patients have autoantibodies in their skin as well as in their serum (4). In pemphigus the autoantibodies were shown to be directed against the keratinocyte cell surface. In fact, investigators were so sure that these antibodies interfered directly with adhesion, they were termed, somewhat fancifully, anti-intercellular cement substance antibodies (5). In BP the antibodies are directed against the epidermal basement membrane zone. In both instances, the findings of autoantibodies in areas of defective epidermal cell adhesion suggested that these antibodies might be directed against adhesion structures or molecules.

The next stage in the evolution of our knowledge about these diseases came when models were developed to test whether patients' autoantibodies are pathogenic. In pemphigus of both types the answer became clear: pemphigus IgG autoantibodies can cause disease (i.e., loss of cell adhesion) without the additional contribution of complement or inflammatory cells. This was shown by both a skin organ culture model (6) and passive transfer of autoantibodies to neonatal mice (7,8). However, in BP the pathogenicity of the antibodies is not so clear-cut. Probably fixation of complement and recruitment of inflammatory cells is necessary for blister formation (9). Until recently, there has not been a good animal model to test this hypothesis. Now, however, it has been shown that rabbit antibodies raised to a major antigenic epitope of one of the BP antigens (defined below as BPAG2) can induce disease when passively transferred to neonatal mice (10).

From the above data, we know that pemphigus and BP are diseases with pathogenic autoantibodies directed against areas of the epidermis in which loss of cell-cell or cell-substrate adhesion occurs. Therefore, it was reasonable to hypothesize that these autoantibodies are directed against adhesion molecules or structures. This hypothesis has been confirmed (Table 1). Immunochemical studies demonstrated that PF antigen is desmoglein 1 (Dsg1), a transmembrane glycoprotein of desmosomes (11–13). Molecular cloning of Dsg1 indicated that it is a member of the cadherin supergene family of calcium-dependent cell adhesion molecules (14). Molecular cloning also revealed that PV antigen is a desmoglein (Dsg3) (14a). Both Dsg1 and Dsg3 have been localized by immunoelectron microscopy to desmosomes (15, 16) and, in keratinocytes, both bind plakoglobin, a desmosomal plaque protein (17). Autoantibodies from PV patients, affinity purified on the extracellular region of Dsg3, cause blisters when passively transferred to neonatal mice, and the extracellular region of Dsg3 can completely absorb all pathogenic antibodies from PV sera (18, 19).

Immunochemical studies indicated that BP autoantibodies actually identify two distinct proteins, now termed BPAG1 and BPAG2. BPAG1 was characterized by SDS-PAGE as an approximately 230-kD polypeptide, whereas BPAG2 was approximately 180 kD (20, 21). Molecular cloning has confirmed that these two BP antigens are distinct gene products. BPAG1 was found to be homologous in amino acid sequence and structure to desmoplakins I/II, alternatively spliced desmosomal plaque proteins (22–24). Antibodies raised against BPAG1 have been localized by immunoelectron microscopy to the plaque of the hemidesmosome (25), a cell-basement membrane adhesion junction. Therefore BPAG1 and desmoplakins I/II were the first members of a gene family of cell adhesion junction plaque proteins. Molecular cloning of BPAG2 indicated that it is a transmembrane protein with a COOH-terminal extracellular collagenous domain, also localized by immunoelectron microscopy to the hemidesmosome (26–30).

Once the antigens in the classical autoantibody-mediated blistering diseases were defined at a molecular level, it became clear that certain diseases defied easy classification, and new diseases were thereby defined. One of these is paraneoplastic pemphigus (31). This disease shares clinical and histological features of PV and erythema multiforme (32). These patients have in vivo–bound antibodies against their epidermal basement membrane as well as cell surface, and they have serum antibodies that immunoprecipitate a distinct set of polypeptides from epidermal extracts, clearly different from pemphigus antigens. Among these immunoprecipitated polypeptides are desmoplakins I/II as well as BPAG1, but the others are not

Table 1. *Antigens in Autoimmune Blistering Diseases*

Disease	Autoantigen	Related molecules	Adhesion structure
PF	Dsg1	Most closely: desmogleins. More distantly: desmocollins, classical cadherins	Desmosomes
PV	Dsg3	Most closely: desmogleins. More distantly: desmocollins, classical cadherins	Desmosomes
BP	BPAG1	Desmoplakins I/II	Hemidesmosome
	BPAG2	Collagens	Hemidesmosome
Paraneoplastic pemphigus	Desmoplakins I/II, BPAG1, others	Desmoplakins I/II and BPAG1 are homologous	Desmosomes, Hemidesmosomes
Erythema multiforme, major	Desmoplakins I/II	BPAG1	Desmosomes

yet defined (33). Both desmoplakins I/II and BPAG1 are intracellular plaque proteins, as discussed above, and it is not clear that these are the crucial molecules involved in pathogenesis because it has not been shown that antibodies penetrate living cells to cause pathology.

In this issue of the *The Journal of Experimental Medicine*, Foedinger et al. (34) define another novel and potentially significant autoantibody in patients with erythema multiforme major. In this disease, patients do not exhibit defects in cell-to-cell adhesion, but they develop necrosis of the epidermis with dyskeratosis of keratinocytes. Blisters develop because the necrotic epidermis detaches from the underlying dermis. These authors could detect antibodies against desmoplakins I/II in 6 of 6 patients with this disease. Antibodies were detected in epidermis by direct immunofluorescence of lesional skin, but not perilesional skin. Immunoblotting and immunoprecipitation studies showed there were anti-desmoplakin I/II antibodies in the sera of these patients. By direct and indirect immunoelectron microscopy, these antibodies bound the plaque of the desmosomes (i.e., the same location as desmoplakins I/II). When serum for these patients was injected subcutaneously, but not intraperitoneally, in neonatal mice these antibodies could be detected in keratinocytes by direct immunofluorescence. The authors speculate that when these antibodies enter the cell they might interfere with keratin filament binding to the desmosomal plaque and result in dyskeratosis (i.e., collapsed keratin filament networks).

However, a key question remains regarding these newly defined autoantibodies: are they pathogenic? It is important to keep in mind that the authors only present a correlation between anti-desmoplakin antibodies and erythema multiforme major. The finding that, in one patient, these antibodies were seen during the first episode is not convincing evidence that the antibodies are pathogenic. Just as with BPAG1 in BP and paraneoplastic pemphigus, the question is whether autoantibodies reactive with intracellular components of adhesion structures can enter living cells and cause pathology, or is formation of these antibodies an epiphenomenon? The fact that circulating anti-desmoplakin antibodies were only seen in erythema multiforme major, but not the less severe erythema multiforme minor (which can have identical skin lesions), suggests that they may be a result (rather than cause) of disease, with more severe disease releasing more intracellular antigens which elicit an immune response. It seems unlikely that these antibodies enter undamaged cells and cause disease. In the studies reported in this issue of the *Journal*, the antibodies entered keratinocytes when injected subcutaneously, but not intraperitoneally, in neonatal mice. This paradox could be explained if the locally injected serum caused inflammation and edema which damaged keratinocytes and permitted entry of antibodies. Of course, in erythema multiforme the hallmark of disease is necrotic keratinocytes which are presumably permeable to antibodies. In this respect, it is significant that only lesional skin of patients had positive direct immunofluorescence findings. Again, these findings suggest that only when they are damaged do the keratinocytes become permeable to these antibodies. These observations suggest that something other than the antibodies cause the initial damage to the keratinocyte. However, once that damage occurs then the anti-desmoplakin antibodies might play a role in amplification of the pathologic process.

The future directions of research related to autoantibodies and blistering diseases will try to answer several of the questions outlined above. Do these antibodies directly interfere with the function of adhesion molecules or is their effect more indirect, for example, through signal transduction? Can antibodies against cytoplasmic components of adhesion structures cause disease or are these autoantibodies epiphenomena? Are there other anti-cell adhesion autoantibody diseases not yet defined? And, perhaps most importantly for the patients with these sometimes devasting diseases, can we translate this basic knowledge into new and effective therapies?

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