Autoimmune Bullous Diseases

Clinical and Molecular Characterization of Autoimmune Bullous Diseases

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Tremendous advances have been made in the past decades in characterizing the autoantigens of the major autoimmune bullous skin diseases, which provide a direct relationship among distinct clinical phenotypes, the nature and distribution of the targeted autoantigens, and the molecular mechanisms of the immune pathogenesis of these disorders.

Pemphigus encompasses a group of life-threatening autoimmune diseases with extensive blistering of the mucous membranes and the skin, that is characterized by intraepidermal loss of adhesion caused by autoantibodies against components of desmosomes that confer adhesion between epidermal keratinocytes. Anhalt *et al.* (1982) were the first to provide direct evidence that immunoglobulin G (IgG) autoantibodies from pemphigus sera cause epidermal loss of adhesion when injected into neonatal mice. Amagai*et al.* (1991) successfully cloned the cDNA encoding a 130 kDa protein that was recognized specifically by pemphigus sera and was identified as a novel desmosomal adhesion protein, named desmoglein 3 (Dsg 3). Stanley *et al.* (1986) identified another desmosomal cadherin, Dsg 1, which had many structural homologies with Dsg 3 and turned out to be the autoantigen of pemphigus foliaceus, a related disorder characterized by extensive superficial erosions of the skin. Moreover, Stanley and co-workers (Mahoney *et al.*, 1999) provided the pathogenic explanation as to why distinct autoantibody profiles cause a distinct clinical phenotype in pemphigus. The so-called compensation hypothesis is based on the concept that Dsg 1 and Dsg 3 compensate each other's loss of function upon binding of pemphigus autoantibodies in tissues where they are equally expressed. In pemphigus foliaceus, IgG against Dsg 1 causes superficial cutaneous blisters because Dsg 1 is exclusively expressed in the stratum granulosum of stratified squamous epithelia and is thus not substituted by Dsg 3. In pemphigus vulgaris, anti-Dsg 3 IgG autoantibodies cause suprabasilar loss of adhesion of noncornified squamous epithelia (that is mucous membranes) where Dsg 3 is exclusively expressed and cannot be substituted by Dsg1.

The relevance of the adhesive function of desmosomal cadherins for epidermal integrity was demonstrated by Koch *et al.* (1997), who generated Dsg3-deficient mice, which showed blisters of the oral mucosa and snout due to the loss of adhesion between epidermal keratinocytes. Finally, a most elegant study by Amagai *et al.* (2000) showed that blistering due to a loss of adhesive function of Dsg 1 is also an inherent feature of bullous impetigo and staphylococcal scalded skin syndrome, two disorders that are caused by *Staphylococcus aureus* infection of the skin. Staphylococcal exfoliative toxin is a serine protease that specifically cleaves the extracellular portion of Dsg 1 that harbors the major antigenic sites targeted by pathogenic autoantibodies in pemphigus.

Lymphatic tumors, drugs containing thiols and nonthiol components, and various infectious agents have all been implicated as triggering factors in the loss of immunological tolerance against Dsg, leading to the etiopathogenesis of pemphigus. Diaz and colleagues suggested that an endemic variant of pemphigus foliaceus, fogo selvagem, which occurs in restricted areas of central South America, may be triggered by local factors such as infections (Warren *et al.*, 2000). They reported a high prevalence of individuals with IgG antibodies against Dsg 1 in these restricted areas who later developed full-blown fogo selvagem. Immunologically, the patients' autoantibodies targeted epitopes of Dsg 1 (namely its N terminus) that were different from the nonpathogenic epitopes (located in the C terminus of Dsg1) found in the healthy residents of this endemic area (Li *et al.*, 2003). These findings support the concept that a shifted epitope recognition of human autoantigens precedes the clinical outcome of an overt autoimmune disease.

Ongoing research strongly suggests that the production of autoantibodies is tightly controlled by Dsg-specific autoaggressive T cells, which are critical for the induction, perpetuation, and immune surveillance of the autoimmune response against Dsg 3 and Dsg 1 (reviewed in Hertl *et al.*, 2006).

Immunobullous subepidermal blistering diseases are characterized by autoantibodies to cell–matrix adhesion molecules of the dermal–epidermal junction (DEJ). Stanley *et al.* (1981) were the first to characterize a constituent of the DEJ as the target of autoantibodies. They identified a

230 kDa protein (BPAG1) labeled by antibodies in serum of patients with bullous pemphigoid (BP). Labib *et al.* (1986) characterized BP180 (BPAG2) as another autoantigen targeted by BP autoantibodies. Cloning of the corresponding cDNAs demonstrated BP230 to be a constituent of the hemidesmosomal plaque, whereas BP180 was found to be a transmembrane hemidesmosomal glycoprotein and a member of the collagen protein family, also referred to as type XVII collagen (Stanley *et al.*, 1988; Diaz *et al.*, 1990; Giudice *et al.*, 1992). Subsequently, BP180 was shown to be cleaved from the keratinocyte cell surface (Pas *et al.*, 1997; Hirako *et al.*, 1998; Schäcke *et al.*, 1998). This shedding is mediated by disintegrin and metalloprotease proteins (Franzke *et al.*, 2002).

Zone *et al.* (1990) were the first to report that immunoglobulin A (IgA) autoantibodies from patients with linear IgA disease react with a 97 kDa protein (LABD97) of the DEJ. Subsequently, Marinkovich *et al.* (1996) identified a 120 kDa protein (LAD-1), synthesized by keratinocytes as the target of these IgA autoantibodies. On the basis of biochemical studies and peptide sequence analysis, it was demonstrated that LABD97 and LAD-1 represent portions of the BP180 ectodomain resulting from cleavage within the membrane-proximal NC16A domain (Zone *et al.*, 1998; Hirako *et al.*, 2003). In contrast to the majority of BP sera, most LAD sera do not react with this domain of BP180 (Zillikens *et al.* 1997, 2003).

In addition to BP and LAD, pemphigoid gestationis and lichen planus pemphigoides are further diseases associated with an autoimmune response to the immunodominant NC16A domain of BP180. Although in pemphigoid gestationis autoantibodies almost exclusively react with the N-terminal 45 amino acids of this domain, the immune response in lichen planus pemphigoides focuses on the C-terminal portion of NC16A (Zillikens 2003).

The fifth disease that may be associated with autoantibodies to BP180 NC16A is mucous membrane pemphigoid. This is a heterogenous disease with respect to clinical manifestation, the isotype of the associated autoantibody, the side of skin split by 1 M NaCl that is bound by the autoantibody, and the specific target antigen(s) within the DEJ (Chan *et al.*, 2002). The best-characterized autoantigens in mucous membrane pemphigoid include BP180, laminin 332, type VII collagen, and the subunits of $\alpha_6\beta_4$ integrin. Most mucous membrane pemphigoid patients show reactivity with BP180 (Bernard *et al.*, 1990). Domloge-Hultsch *et al.* (1992) described a group of patients with IgG autoantibodies against laminin 332 (previously referred to as laminin 5 or epiligin).

More recently, a previously unknown pemphigoid disease was reported that is associated with autoantibodies to a 200 kDa protein of the lower lamina lucida (Zillikens *et al.*, 1996). By indirect IF microscopy on 1 M NaCl-split skin, the autoantibodies bind exclusively to the dermal side. By immunoblotting, they label a 200 kDa protein extracted from dermis, but do not react with epidermal constituents. The characterization of this autoantigen, which is different from other known autoantigens of the DEJ, awaits elucidation at the molecular level (Dilling *et al.*, 2007).

In 1984, Woodley *et al.* (1988) characterized a 290 kDa dermal protein as the target of autoantibodies in patients with epidermolysis bullosa acquisita Subsequently, this antigen was identified as type VII collagen. Gammon *et al.* (1984) described NaCl splitting of the DEJ leading to cleavage within the lamina lucida. When this 1 M NaCl-split skin is used for indirect IF microscopy, it facilitates rapid differentiation between BP and epidermolysis bullosa acquisita. In addition, autoantibodies to BP230, plectin, BP180, and $\alpha_6\beta_4$ integrin bind to the epidermal side of the split, whereas those to laminin 332, p200, and both type IV and type VII collagen label the dermal side. Therefore, indirect IF microscopy on 1 M NaCl-split skin is used as an initial analysis before further characterizing the specificity of patients' autoantibodies by immunoblotting, immunoprecipitation, or ELISA.

Dermatitis herpetiformis (DH) is a chronic autoimmune bullous disease characterized by chronic itchy lesions of various morphology. Immunologically, granular IgA deposits in dermal papillae are a hallmark of this disorder. DH is tightly associated with gluten-sensitive enteropathy (Katz *et al.*, 1980) and differs from linear IgA disease on the basis of the different pattern of tissue-bound IgA antibodies and the different specificity of autoantibodies. Dieterich *et al.* (1999) detected IgA autoantibodies against tissue transglutaminase in the sera of patients with DH. More recently, Sárdy *et al.* (2002) showed that epidermal transglutaminase, which is highly homologous to tissue transglutaminase, is the nominal autoantigen of DH. This finding explains why the majority of—if not all—patients with DH have cross-reactive IgA autoantibodies against tissue transglutaminase (which provide a reliable serological marker for establishing the diagnosis of DH).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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