AUTOIMMUNE BULLOUS DISEASES

Duplicating Autoimmune Bullous Diseases by Passively Transferring Autoantibodies into Animals

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doi:10.1038/skinbio.2008.5

In general, autoimmune diseases comprise a group of diseases of unknown etiology in which it is thought that by some unknown mechanism the patient's own immune system fails to recognize ''self'' cells or proteins and mounts an immune response against one or more of the host's own tissues. In many putative autoimmune diseases such as systemic lupus erythematosus, dermatomyositis, scleroderma, mixed connective tissue disease, and rheumatoid arthritis, the autoantibodies and their autoantigenic targets have not been well defined. This has made it difficult to prove in animal models that the autoantibodies binding to their respective target proteins or cells in the host are responsible for the disease. In contrast, investigative dermatology has been fortunate to define and characterize the autoantibodies and autoantigenic targets in a number of autoimmune bullous diseases of the skin, including pemphigus vulgaris (PV), pemphigus foliaceous, cicatricial pemphigoid (CP), epidermolysis bullosa acquisita (EBA), and bullous pemphigoid (BP). In the domain of infectious diseases, it has been said that the proof that an infectious agent causes a disease lies in inoculating an animal with the infectious agent and duplicating the disease: so-called Koch's postulate. In the realm of autoimmunity, the ultimate proof that autoantibodies are pathogenic and responsible for an autoimmune disease lies in injecting the autoantibody into an animal (usually a mouse) and creating the disease in that animal. This has been termed a ''passive transfer'' experiment; it definitively proves that the autoantibody is ''pathogenic'' and causes the disease.

For dermatological diseases, the passive transfer of an autoimmune disease was first accomplished by Anhalt *et al*. (1982) who took IgG autoantibodies from the sera of patients with PV, injected them into newborn mice and created a pemphigus-like disease in the mice that included acantholytic intraepidermal bullae and animals with positive direct and indirect immunofluorescence findings akin to human patients with PV. We now know that the human IgG autoantibodies passively transferred into these mice contained antibodies against desmoglein 3 (Dsg3), a major component of the desmosomal junctions that hold keratinocytes together in the epidermis. The human PV anti-Dsg3 autoantibodies bound to the Dsg3 in mouse skin and induced acantholysis and blister formation. Later, a similar set of experiments by Rock *et al*. (1989) demonstrated that one could also create pemphigus foliaceous in a mouse by passively transferring IgG_4 antibodies against desmoglein 1 from patients with endemic pemphigus foliaceous and very precisely create pemphigus foliaceous in the animal, with acantholytic bullae developing high in the epidermis.

In addition to the pemphigus passive transfer murine models, Amagai *et al*., 2000 also established an ''active'' murine model of pemphigus. This was a creative and elegant experimental strategy, in which they injected Dsg3 null knockout mice with exogenous Dsg3, and the mice generated anti-Dsg3 antibodies. The authors then took the splenic lymphocytes from these mice and transplanted them into immunodeficient mice that natively expressed Dsg3 in the epidermal desmosomes of their skin. These transplanted mice then generated anti-Dsg3 antibodies for many months, and these ''autoantibodies'' bound to the murine Dsg3 in their skin and created intraepidermal acantholytic bullae. Therefore, these mice, just like patients with PV, had intercellular cement substance pemphigus-like indirect immunofluorescence, direct immunofluorescence, and intraepidermal acantholytic blisters on their skin and in their oral mucosa (Amagai *et al*., 2000).

One might assume that all autoimmune antibodies would likely be ''pathogenic'' and induce the blistering disease. This is not the case. Many autoantibodies are only associated with the disease and ''mark'' the disease, but do not cause a disease phenotype such as skin blisters. One example of this is pemphigus paraneoplastica, first identified and characterized by Anhalt *et al*. (1990). These patients have an acantholytic intraepidermal blistering disorder similar to pemphigus in association with an underlying malignancy. In the patient's serum one finds high-titer antibodies to the ''plakin proteins'' such as desmoplakin I, desmoplakin II, the 230 kDa BP antigen 1, envoplakin, and periplakin. These patients also have IgG autoantibodies to Dsg3, just like PV patients. It was shown by Amagai *et al*. (1998) that it is the IgG

autoantibodies to Dsg3 that actually cause the bullous lesions in these patients whereas the antibodies to the plakin proteins are nonpathogenic and only markers of the disease. By indirect immunofluorescence staining, the anti-plakin antibodies in pemphigus paraneoplastica patients label rodent substrates that are rich in plakin proteins such as mouse bladder and mouse heart, whereas sera from PV patients do not. Therefore, the pathogenic antibodies to Dsg3 are the same in both PV and pemphigus paraneoplastica patients, whereas the latter disease has many anti-plakin ''marker'' autoantibodies that are not involved with the phenotype of the disease, but are useful to the clinician for diagnosing it.

In terms of subepidermal autoimmune bullous diseases, both EBA and anti-epiligrin (aka anti-laminin-5) CP have been passively transferred into animals (Lazarova *et al.*, 1996, 2000; Sitaru *et al.*, 2005; Woodley *et al.*, 2005, 2006; Chen *et al.*, 2007). IgG fractions of sera from patients with EBA were injected into hairless mice, and the mice developed subepidermal blisters, human IgG deposits at the dermal–epidermal junction, and nail loss—all features of human EBA (Woodley *et al.*, 2006). In further experiments, affinity-purified IgG antibodies against a small, 20 kDa, subdomain of the amino terminus of type VII (anchoring fibril) collagen from EBA patient sera, were injected into mice, causing them to develop blisters and nail loss as they did in experiments with whole EBA patient sera, showing that EBA patient antibodies against a small domain of the autoantigenic target ($M_r = 290{,}000$ Da) could be highly pathogenic (Chen *et al.*, 2007). In concordance with these studies of human EBA patient sera, rabbits can be immunized against mouse or human type VII collagen (C-7) and high-titer anti-C-7 antibodies generated. When these rabbit IgG C-7 antibodies are injected into mice, the mice develop an EBA-like disease (Sitaru *et al.*, 2005; Woodley *et al.*, 2005). In all of these murine EBA models, the blister phenotype appears to depend upon the fixation of complement. More recently, Sitaru *et al.* (2006) in the Zillikens laboratory developed an active EBA model, which appears to be highly dependent upon the genetics of the strain of mouse used. They injected murine C-7 into several strains of mice and certain strains, such as SJL-1 mice, were highly susceptible to developing anti-C-7 antibodies and an EBA-like disease, whereas EBA could not be induced in SKH-1 mice. This murine work is interesting in light of the observations of Gammon *et al.* (1988) that certain human HLA types such as HLA DR2 predispose individuals to develop EBA.

Patients who have CP, a mucosal-centered, subepidermal, bullous disorder, have been described with IgG autoantibodies against a number of dermal–epidermal junction proteins in the skin including collagen types VII and XVII, laminin-5 (aka: ''epiligrin'', ''kalanin'', ''nicein'', ''BM 600''), the 230 kDa BP antigen 1, and other less well-defined proteins. Nevertheless, one subset of CP patients that has been well defined is those with anti-laminin-5 CP. These elderly patients have IgG autoantibodies against an anchoring filament protein within the lamina lucida called laminin-5 and a host of other previous names (*vide supra*). This is a cruciate glycoprotein consisting of a central α-3 chain flanked by β-3 and γ-2 chains. Most anti-laminin-5 patients have autoantibodies to the central α-chain. Lazarova *et al.* (1996, 2000) injected anti-laminin-5 IgG antibodies into mice, causing them to develop subepidermal bullae and erosions of their skin and mucous membranes. Noncomplement fixing IgG4 antibodies and the Fab fragment of anti-laminin-5 can both passively transfer the disease in mice, demonstrating that complement is not required for the generation of murine anti-laminin-5 CP (Lazarova *et al.*, 2000). Although anti-laminin-5 antibodies can clearly induce one of the main features of the disease, namely subepidermal bullae and erosions, it is not clear why the murine disease is not scarring and mucosal-limited like human CP.

Hall *et al.* (1993) were unable to passively transfer BP into mice by injecting antibodies against a domain of the 230 kDa BP antigen 1 unless the mice were subjected to an inflammatory stimulus first, such as UV light irradiation. It was thought that the skin first needed to be perturbed such that the basal keratinocyte plasma membrane was compromised and subsequently exposed the BP antigen 1 within the hemidesmosome. In contrast to these studies with the 230 kDa BP antigen 1, Liu *et al.* (1993) showed that the amino-acid sequence of the autoantibody-binding domain, so-called NC16A, of the 180 kDa BP antigen 2 (type XVII collagen) was different between the human and murine forms of type XVII collagen. Therefore, one would not expect human BP sera to have anti-type XVII antibodies that could bind to the mouse NC16A pathogenic epitope and invoke BP in mice. To overcome this problem, Liu immunized rabbits with the murine corollary of the antibody-binding site on the human molecule, and by injecting these anti-mouse type XVII collagen rabbit antibodies into mice was able to consistently generate a BP-like disease. In further studies, Liu *et al.* (1995, 2000a, b) showed that the induction of murine BP required complement, required neutrophils, and was mediated through neutrophil elastase. In a more recent study, Nishie *et al.* (2007) generated transgenic mice expressing the human BP 180. Using this mouse model, they injected human BP autoantibody into these mice and induced BP-like skin lesions reminiscent of human BP.

Although the murine form of BP was similar to human BP in many ways, it lacked some hallmarks of the human disease such as the lack of inflammation, edema, and eosinophils which are almost always noted in human BP. Recently, Fairley *et al.* (2005, 2007) observed that human BP patients often have IgE autoantibodies that label both the dermal–epidermal junction of human skin as well as dermal mast cells that are almost always seen in the high papillary dermis of BP patients. These BP IgE autoantibodies bind to the same epitopes of type XVII collagen as BP IgG autoantibodies (Fairley *et al.*, 2005). In addition, these IgE autoantibodies on the mast cells become cross-linked and dimerized by the presence

of dermal "bridging" fragments of type XVII collagen, which then induce mast cell degranulation and the recruitment of eosinophils into the area. Using human skin grafted onto mice, the injection of BP IgE autoantibodies invoked the attraction of eosinophils into the skin with subsequent erythema, urticaria, and edema. In the case of BP, therefore, it appears that both IgG and IgE autoantibodies directed against type XVII collagen are "pathogenic" and responsible for different aspects of the disease phenotype.

In summary, animal models of autoimmune bullous diseases have clearly shown that there are "marker" antibodies that herald the disease and "pathogenic" antibodies that cause the disease. They have shown that various subdomains on the target autoantigen can generate highly pathogenic autoantibodies whereas other subdomains cannot. These models have also been useful for determining the mechanisms and parameters of the disease pathogenesis such as the role of complement, the role of various inflammatory cells, and the role of specific proteinases. These advances in our understanding of these diseases will help clinician scientists in the future tailor treatment strategies based on the mechanistic parameters of each disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by grants RO1 AR47981 (Dr Chen) and RO1 AR33625 (Dr Woodley) from the National Institutes of Health.

TO CITE THIS ARTICLE

Chen M, Woodley DT (2008) Duplicating autoimmune bullous diseases by passively transferring autoantibodies into animals. *J Invest Dermatol* 128: E25–E27

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