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REVIEW ARTICLE

The relevance of the IgG subclass of autoantibodies for blister induction in autoimmune bullous skin diseases

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Abstract Autoimmune bullous skin diseases are characterized by autoantibodies and T cells specific to structural proteins maintaining cell–cell and cell–matrix adhesion in the skin. Existing clinical and experimental evidence generally supports a pathogenic role of autoantibodies for blister formation. These autoantibodies belong to several IgG subclasses, which associate with different functional properties and may thus determine the pathogenic potential of IgG antibodies. In pemphigus diseases, binding of IgG to keratinocytes is sufficient to cause intraepidermal blisters without engaging innate immune effectors and IgG4 autoantibodies seem to mainly mediate acantholysis. In contrast, in most subepidermal autoimmune blistering diseases, complement activation and recruitment and activation of leukocytes by autoantibodies are required for blister induction. In these conditions, tissue damage is thought to be mainly mediated by IgG1, but not IgG4 autoantibodies. This review summarizes the current knowledge on the pathogenic relevance of the IgG subclass of autoantibodies for blister formation. Characterization of the pathogenically relevant subclass(es) of autoantibodies not only provides mechanistic insights, but should greatly facilitate the development of improved therapeutic modalities of autoimmune blistering diseases.

Keywords Autoimmune bullous diseases · IgG subclasses · Complement

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Introduction

Autoimmune blistering diseases are associated with an autoimmune response directed to structural proteins mediating cell–cell and cell–matrix adhesion in the skin [62, 66]. Both autoantibodies and autoreactive T cells have been found in patients with these organ-specific autoimmune diseases. However, blister induction is mainly mediated by autoantibodies. Autoimmune blistering diseases are classified based on the ultrastructural site of deposition of immunoreactants and on the molecular target of autoantibodies. Diseases of the pemphigus group are associated with autoantibodies to epidermal components mediating cell–cell adhesion and are characterized by acantholytic blisters within the epidermis [39, 71]. Tissue-bound and circulating autoantibodies to the dermal–epidermal junction are characteristic immunopathological features of subepidermal autoimmune bullous diseases [62, 85]. Target antigens of autoantibodies have been identified for the majority of autoimmune blistering diseases. In most of these diseases, the pathogenicity of autoantibodies is supported by clinical observations and extensive experimental evidence [62].

Antibodies are effector molecules of the adaptive immune system secreted by plasmablasts and long-lived plasma cells. Antibody responses are physiologically mounted following an infection or vaccination and protect against various pathogens. Occasionally, in the setting of an autoimmune disease, antibodies to autologous structures may develop and cause different forms of tissue damage. The immunopathology induced by autoantibodies, similar to the immunity mediated by antibodies to pathogens, relies on several mechanisms of action of antibodies, including direct

mechanisms, which are mediated by the antibody's variable regions (e.g., by steric hindrance and signal transduction), and indirect mechanisms, which are triggered by the constant regions of antibodies. For the latter, (auto)antibodies typically interact through their Fc portions with other factors of the innate immune system, including the complement system and inflammatory cells [62].

Antibodies of the IgG isotype predominate in the systemic immune response, as reflected in serum immunoglobulin concentration, and activate a wide range of effector functions. Four subclasses of IgG are defined, originally from the antigenic uniqueness of their heavy chains, which are products of distinct genes [20, 27, 77]. The subclasses are designated as IgG1, IgG2, IgG3 and IgG4 in order of their serum concentration ~60, 25, 10 and 5%, respectively. Although the heavy chains show >95% sequence homology, each IgG subclass expresses a unique profile of effector activities [35, 56, 59, 76, 80, 82]. Protein antigens characteristically provoke IgG1 and IgG3 responses and these isotypes are able to activate all types of Fc receptors and the C1 component of complement. The IgG4 subclass may be characteristic of chronic antigen stimulation, as in autoimmune disease; it has restricted Fc receptor activating abilities and does not activate C1q. The IgG2 subclass often predominates in responses to carbohydrate antigens; it has restricted Fc receptor and C1 activating abilities [35, 56, 80, 82].

The pathogenic potential unfolded by autoantibodies is determined not only by their specificity and affinity, but also by their isotype. Autoantibodies against cutaneous proteins in autoimmune blistering diseases belong to different IgG subclasses. This paper summarizes the current knowledge on the relevance of IgG subclasses for tissue injury in autoimmune bullous diseases.

Pemphigus diseases

Pemphigus designates a group of life-threatening-autoimmune blistering diseases characterized by intraepithelial blister formation caused by loss of cell–cell adhesion [39, 54, 71]. IgG autoantibodies in patients with pemphigus seem to mediate their pathogenic functions independently of their Fc portions [62]. Patients' IgG autoantibodies are pathogenic in C5-deficient mice and F(ab')2, Fab, and scFv fragments of autoantibodies induce acantholysis by passive transfer in wild type mice showing that complement activation or other Fc-mediated effects are not required for pathogenicity [4, 21, 45, 55].

Numerous studies clearly demonstrated that tissue bound and circulating autoantibodies in pemphigus patients mainly belong to the IgG1 and IgG4 subclasses [2, 5, 8, 9, 16–18, 23, 28, 36, 38, 40, 49, 61, 79]. The IgG subclass distribution of autoantibodies in a representative pemphigus patient is shown in Fig. 1. While it is generally agreed upon the subclass distribution of IgG autoantibodies, the relevance of autoantibodies of different IgG isotypes for acantholytic blistering in pemphigus is still a matter of debate. IgG4 autoantibodies, known to have poor complement- and leucocyte-activating properties, predominate in pemphigus vulgaris and foliaceus. While several studies suggest a pathogenic role of IgG4 in pemphigus, the capacity of IgG1 autoantibodies to induce acantholysis has not yet been ruled out. In patients with active pemphigus vulgaris, IgG4 autoantibodies against desmogleins were found to predominate [5, 17, 23, 40]. The transplacental transfer of these autoantibodies in mothers with pemphigus induces acantholytic skin disease in neonates [52]. In endemic pemphigus foliaceus the early antibody response in normal subjects living in the endemic area and in patients before the onset of clinical disease is mainly IgG1. Acquisition of an IgG4 response seems to be a key step in the development of clinical disease [78]. Clinical and experimental data suggests that IgG1 differ from IgG4 autoantibodies in terms of both their epitope specificity and pathogenic potential [9, 42]. A monoclonal IgG4 antibody against desmoglein 3 generated from a patient with active pemphigus vulgaris induces acantholysis in cultured skin and when injected in neonatal mice [86]. The fact that IgG4 antibodies purified from patients with fogo selvagem, an endemic form of pemphigus foliaceus, are pathogenic in mice further supports the notion that IgG4 is pathogenic and complement activation is not required for blister formation [57], but does not exclude a pathogenic potential of IgG autoantibodies belonging to other subclasses. Indeed, in several pemphigus foliaceus patients, only IgG1 autoantibodies were found that caused intraepidermal blisters by passive transfer into neonatal mice [29]. This observation clearly shows that autoantibodies of different subclasses may display blister-inducing activity. In addition, in paraneoplastic pemphigus, autoantibodies against desmoglein 3 mainly belong to the IgG1 and IgG2 subclasses suggesting that the IgG subclass per se is not a direct determinant of the antibodies' pathogenic potential in pemphigus [24].

Experimental evidence using a murine model of pemphigus vulgaris shows that, similar to human pemphigus, the autoimmune response in mice is biased toward non-complement fixing autoantibodies. In this

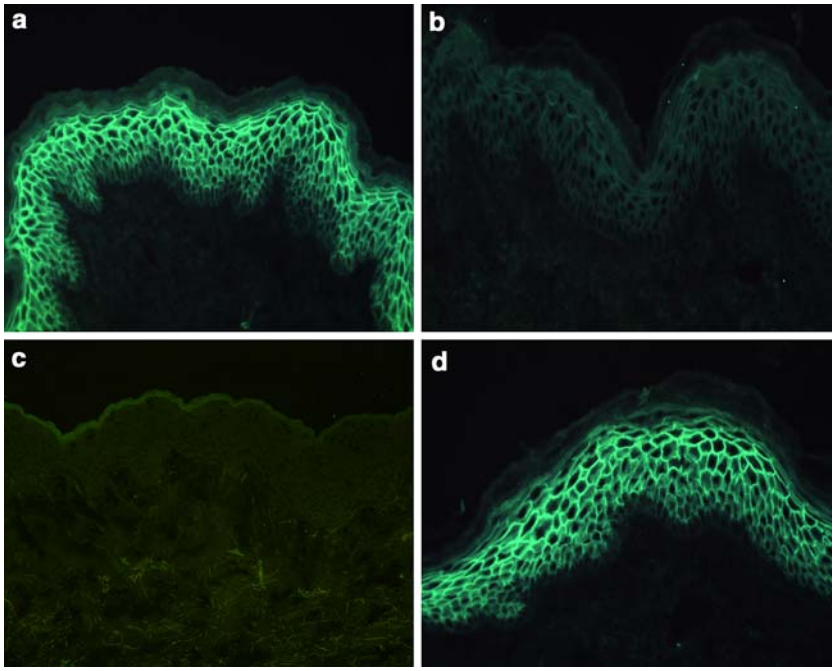

Fig. 1 IgG subclass distribution of circulating pemphigus autoantibodies. A 1:10 dilution of serum from a patient with pemphigus foliaceus was incubated with 6 μm-thick cryostat sections of normal human skin for 30 min at room temperature. Bound antibodies, visualized using an FITC-labeled antibody specific to human IgG, were of **a** IgG1 and **d** IgG4 subclasses. In contrast, no binding of **b** IgG2 and **c** IgG3 autoantibodies was evidenced

model, in immunodeficient mice infused with splenocytes from desmoglein-deficient mice immunized against this antigen, IgG autoantibodies are produced by homeostatically expanded antigen-specific B cells under T cell control and mice develop a phenotype reminiscent of pemphigus vulgaris. These IgG autoantibodies predominantly belong to the IgG1 subclass, which is a non-complement fixing antibody in the mouse [3, 50]. These results demonstrate that non-complement-fixing autoantibodies can induce acantholysis and suggest a similar mechanism in patients, but they do not exclude a pathogenic potential of patients' IgG1 autoantibodies in pemphigus.

In conclusion, in pemphigus diseases the autoantibodies mainly belong to the IgG4 and IgG1 subclasses. Extensive experimental evidence demonstrates the blister-inducing potential of IgG4 autoantibodies. The pathogenic activity of autoantibodies of other subclasses seems likely, but needs further investigation.

Subepidermal autoimmune blistering diseases

Bullous pemphigoid and pemphigoid gestationis

Bullous pemphigoid is an autoimmune blistering disease characterized by subepidermal blisters and associated with linear deposits of C3 and IgG at the epidermal basement membrane zone. Autoantibodies in bullous pemphigoid are directed against two hemidesmosomal antigens, BP230 and BP180/type XVII collagen [87]. Pemphigoid gestationis, also referred to as herpes gestationis, is a subepidermal blistering disease associated with pregnancy and characterized by linear deposition of C3 and, to a lesser extent of IgG at the dermal–epidermal junction, as detected by immunofluorescence microscopy [62, 66]. The autoimmune response in bullous pemphigoid and pemphigoid gestationis is mainly directed against epitopes clustered within the immunodominant 16th non-collagenous (NC16) A region of type XVII collagen [48, 65, 88].

Experimental evidence generally supports the pathogenic role of autoantibodies against type XVII collagen for blister formation. Data from passive transfer animal models strongly suggest that antibodies to type XVII collagen are directly involved in the pathogenesis of bullous pemphigoid [44, 84]. In addition, in an ex vivo model utilizing cryosections of human skin, it has been demonstrated that binding of autoantibodies to the immunodominant NC16A domain of type XVII collagen, is the first critical step in subepidermal blister formation [31, 64].

Analysis of the subclass distribution of IgG autoantibodies in the skin of patients with bullous pemphigoid by immunofluorescence microscopy, revealed IgG4 as being the predominant subclass of autoantibodies in bullous pemphigoid, followed by IgG1 autoantibodies, while IgG2 and IgG3 autoantibodies were found only occasionally [1, 10–12, 22, 61, 83]. In addition, serum autoantibodies binding to the dermal–epidermal junction by immunofluorescence microscopy also mainly belong to the IgG4 and IgG1 subclasses [10, 11, 61, 83].

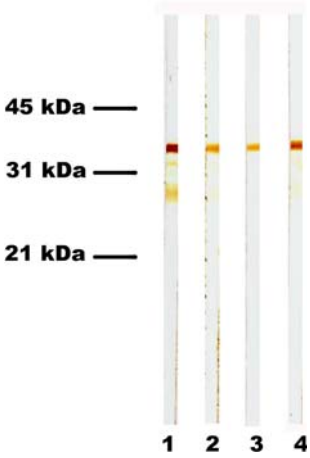

Fig. 2 IgG1 and IgG4 autoantibodies mainly target type XVII collagen in bullous pemphigoid. Immunoblot analysis of serum from a bullous pemphigoid patient with recombinant type XVII collagen revealed that reactivity against its immunodominant domain consists mainly of IgG1 (*lane 1*) and IgG4 (*lane 4*), and less IgG2 (*lane 2*) and IgG3 (*lane 3*) autoantibodies

Subsequent molecular analysis of IgG autoantibodies by immunoblotting and ELISA generally confirmed the predominance of IgG1 and IgG4 autoantibodies reactive with type XVII collagen and BP230 (Fig. 2) [6, 19, 32, 41, 69].

In contrast to bullous pemphigoid, in pemphigoid gestationis tissue-bound and circulating autoantibodies seem to mainly belong to the IgG1 and IgG3 subclasses [14, 37]. However, a recent study challenged these reports revealing IgG4 as the predominant IgG subclass of tissue-bound autoantibodies in pemphigoid gestationis patients [53], a pattern similar to the one found in bullous pemphigoid. Further studies should solve this contradiction.

Data from several studies in patients suggested a pathogenic role of IgG1 autoantibodies for blister formation (briefly reviewed in [43]). In a recent study, ELISA analysis showed that autoantibodies against the N-terminus of the extracellular domain of type XVII collagen predominantly belong to the IgG1 subclass. More importantly, a NC16A-specific IgG1 response was predominant in the acute phase of bullous pemphigoid, while IgG4 was predominantly detected in bullous pemphigoid patients in remission [32]. Using immunoaffinity purified IgG subclasses, it has been shown that IgG1, but not IgG4 autoantibodies from bullous pemphigoid patients activate the complement system in vitro (Fig. 3) [46, 72]. This observation is in line with the currently accepted view that IgG4 is unable to activate the classical pathway of complement. However, until recently it was unclear which IgG subclass is actually pathogenic in bullous pemphigoid. Using the ex vivo cryosection model, we demonstrated that, in addition to IgG1, IgG4 autoantibodies are also able to activate leukocytes and to induce leukocyte-dependent tissue damage (Fig. 4) [46]. Our results are in line with recent studies demonstrating that both polyclonal human IgG1 and IgG4 from patients with Wegener's granulomatosis and chronic urticaria can activate leukocytes [33, 70]. Although the pathogenic potential of IgG4 autoantibodies was significantly lower compared to IgG1, IgG4 autoantibodies, which generally predominate, may activate the inflammatory cells already recruited into the upper dermis by complement-fixing IgG1 autoantibodies and thus amplify the recruitment of additional leukocytes and the extent of blister formation. Therefore, when associated with IgG1 and/or IgG3 autoantibodies, IgG4 may significantly contribute to the pathology induced by autoantibodies in antibody-induced granulocyte-mediated autoimmune blistering diseases [46].

Several reports suggested that binding of bullous pemphigoid antibodies to keratinocytes triggers a signal transduction [58, 73–75]. Bullous pemphigoid autoantibodies trigger a signal-transducing event that

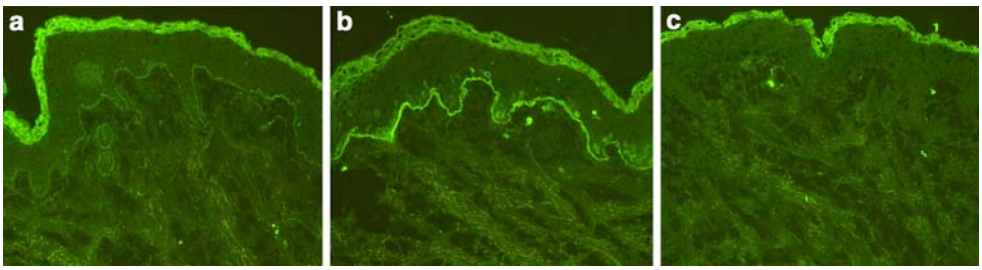

Fig. 3 IgG4 autoantibodies, in contrast to IgG1, do not fix complement to the dermal–epidermal junction in bullous pemphigoid. Cryosections of normal human skin were incubated with serum and immunoaffinity purified IgG1 and IgG4 antibody preparations from a bullous pemphigoid patient and, subsequently, treated with normal human serum as a source of complement. Both **a** serum and **b** purified IgG1 autoantibodies fixed complement C3 at the dermal–epidermal junction in a linear fashion. **c** In contrast, incubation of cryosections with IgG4 specific for the dermal–epidermal junction does not result in C3 deposition (all magnifications, ×200)

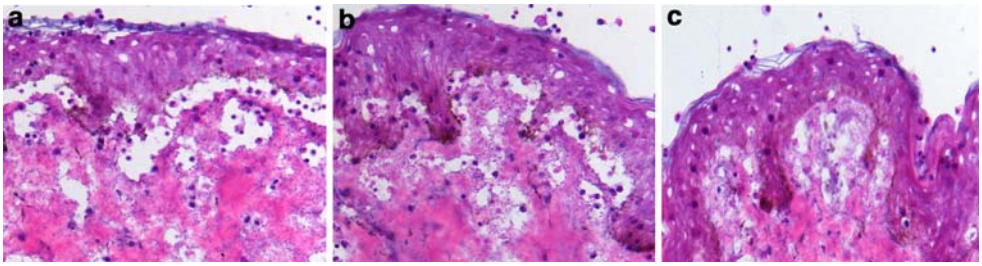

Fig. 4 IgG4 autoantibodies from bullous pemphigoid patients induce dermal–epidermal separation in sections of human skin. Dermal–epidermal separation in sections of normal human skin is induced by **a** IgG1 and **b** IgG4 autoantibodies from a bullous pemphigoid patient. **c** IgG antibodies from a healthy control (NHS) do not induce subepidermal splits (all magnifications, ×200)

leads to expression and secretion of interleukin-6 and interleukin-8 from human cultured keratinocytes [58]. A series of studies from another group demonstrated that IgG1 autoantibodies from bullous pemphigoid patients and rabbit IgG against type XVII collagen induces Ca^{2+} release from intracellular storage sites [73–75]. Interestingly, complement activation by these IgG1 autoantibodies did not result in lysis of keratinocytes [73]. While the relevance of these findings is not yet fully understood, IgG2 and IgG4 patients' autoantibodies were found to inhibit the transient increase of intracellular Ca^{2+} induced by bullous pemphigoid IgG1 antibody [74].

Mucous membrane pemphigoid

Mucous membrane pemphigoid is a heterogeneous disease with regard to the clinical phenotype and the target antigens. Different target antigens have been identified in mucous membrane pemphigoid, including BP180, laminins 5 (epiligrin) and 6, β4 integrin [62, 66]. In general, autoantibodies in mucous membrane pemphigoid mainly belong to the IgG4 and IgG1 subclasses [7, 34]. Interestingly, in anti-epiligrin cicatricial pemphigoid, autoantibodies against laminin 5 almost exclusively belong to the IgG4 subclass [34]. Consistent with these findings, sera from patients with anti-laminin 5 IgG autoantibodies do not fix C3 to the epidermal basement membranes and do not induce leukocyte-dependent dermal–epidermal separation in vitro [34, 60]. These data suggest that complement activation does not play a major role in this disease and subepidermal blisters in these patients may develop via a direct effect of anti-laminin 5 IgG itself [34, 60].

Diseases associated with autoimmunity against type VII collagen

Epidermolysis bullosa acquisita is a chronic subepidermal blistering disease characterized by circulating and tissue-bound antibodies targeting the non-collagenous domain 1 (NC1) of type VII collagen. The pathogenic relevance of antibodies against type VII collagen is supported by compelling evidence: (1) autoantibodies from patients with epidermolysis bullosa acquisita were shown to recruit and activate leukocytes ex vivo resulting in dermal–epidermal separation in cryosections of human skin [60, 63], (2) antibodies against type VII collagen induce subepidermal blisters when passively transferred into mice [67, 81], (3) immunization with recombinant autologous type VII collagen induces an autoimmune response to this protein resulting in a blistering phenotype closely resembling human epidermolysis bullosa acquisita [68].

Tissue-bound and circulating antibodies in epidermolysis bullosa acquisita patients mainly belong to the IgG1 and IgG4 subclasses [7, 15, 26, 47]. A similar distribution of IgG subclasses of autoantibodies is found also in SJL mice immunized against murine type VII collagen [68]. In these mice, while both non-complement-fixing IgG1 and complement-fixing IgG2a and IgG2b autoantibodies are produced after immunization, IgG2a/b autoantibodies seem to induce blistering [68].

Systemic lupus erythematosus and inflammatory bowel diseases may be also associated with autoantibodies against type VII collagen [13, 25, 30, 51]. However, in contrast to epidermolysis bullosa acquisita, autoantibodies from patients with bullous systemic lupus erythematosus and inflammatory bowel diseases mainly belong to IgG2 and IgG3, respectively [30, 51]. The pathogenic relevance of autoantibodies against type VII collagen in inflammatory bowel diseases has not yet been addressed [51]. IgG autoantibodies from patients with bullous systemic lupus erythematosus were shown to induce leukocyte-dependent dermal–epidermal separation in cryosections of human skin ex vivo [30]. This findings suggest that the presence of complement-fixing autoantibodies is not a strict requirement for blistering in patients.

Conclusion and perspectives

The polyclonal antibody response against structural skin proteins in autoimmune bullous diseases is heterogeneous, but shows a skewing in subclass distribution of autoantibodies. The strong bias toward production of IgG4 autoantibodies in these organ-specific autoimmune diseases suggests chronic antigenic stimulation. In pemphigus, IgG4 autoantibodies that dominate the autoimmune response are clearly pathogenic. However, IgG1 autoantibodies also likely possess blister-inducing potential that requires further investigation. In subepidermal autoimmune blistering diseases, the effector functions of autoantibodies are important for blistering. Thus, in bullous pemphigoid and epidermolysis bullosa acquisita, complement-fixing IgG1 autoantibodies may show a significantly higher pathogenic potential when compared with IgG4 autoantibodies. Characterization of the blister-inducing capacity of different subclasses of autoantibodies in autoimmune bullous diseases will not only provide relevant mechanistic insights, but should also greatly facilitate the development of improved therapeutic modalities of autoimmune blistering diseases. Detailed knowledge on the pathogenic IgG isotype(s) will serve as a basis for the development of IgG subclass-specific immunoapheresis, skewing autoantibody production toward non-pathogenic subclasses by immunotherapy or blocking of complement or leukocytes activation by targeting specific IgG subclasses. A promising approach is represented by interventions aimed at inhibiting the production of autoantibodies in general or skewing the production of autoantibodies toward non-pathogenic subclasses. The molecular targets of these approaches may include different cytokines (e.g., IL-12 and IL-17) and their activity could be modulated using inhibitory antibodies, small peptide inhibitors or peptidomimetics as well as immunization with the autoantigen together with adjuvants known to induce a Th2 immune response.

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