Immunoglobulin G4 is prevailing over immunoglobulin G1 in autoimmunity of pemphigus and bullous pemphigoid: analysis of tissue-bound antibodies in active diseases

JUSTYNA GORNOWICZ-POROWSKA, PAWEŁ PIETKIEWICZ, MONIKA BOWSZYC-DMOCHOWSKA, MARIAN DMOCHOWSKI

Cutaneous Histopathology and Immunopathology Section, Department of Dermatology, Poznan University of Medical Sciences, Poland

Abstract

Introduction: Pemphigus and bullous pemphigoid (BP) are immune-mediated blistering diseases. Autoimmunity in these dermatoses is associated with stimulation of Th1/Th2 cells responsible for pathogenic autoantibodies production. Still, there is no consensus on the role of immunoglobulin G (IgG) subclasses in pemphigus/BP pathogenesis.

Aim of the study: To statistically analyze the IgG, IgG1- and IgG4-positive results of direct immunofluorescence (DIF) test performed in patients with pemphigus and BP.

Material and methods: Altogether, 117 specimens (pemphigus + BP) were included in this study. Frozen sections of skin/mucosa were subjected to DIF. The IgG/IgG1/IgG4 FITC-labeled polyclonal antibodies were used to analyze the subclass restriction.

Results: Immunoglobulin G deposits were detected in 44 of 71, IgG1 in 34, IgG4 in 60 pemphigus biopsies. Immunoglobulin G deposits were detected in 8 of 46, IgG1 in 15, IgG4 in 36 BP biopsies. There are significant differences between number of positive vs. negative results regarding (i) IgG4 vs. IgG deposits, IgG4 vs. IgG1 deposits in both pemphigus and BP, (ii) IgG deposits in pemphigus vs. IgG deposits in BP. There are no significant differences between (i) IgG1 vs. IgG deposits in both pemphigus and BP, (ii) IgG1 deposits in pemphigus vs. IgG1 deposits in BP, (iii) IgG4 deposits in pemphigus vs. IgG4 deposits in BP. There are also significant differences between IgG1 strong/weak vs. IgG4 strong/weak in both pemphigus and BP.

Conclusions: The fluorescence intensity of tissue-bound IgG4 is significantly higher than fluorescence intensity of IgG and IgG1 in both pemphigus and BP, what may suggest that IgG4 is the initial and predominant tissue-bound antibody subclass detected in these diseases.

Key words: autoimmunity, pemphigus, pemphigoid, bullous, immunoglobulin G.

Introduction

Autoimmune bullous diseases (ABDs) are group of relatively rare organ-specific disorders associated with an immune response to molecular components of the desmosome/elements of dermal-epidermal junction (DEJ) or enzymes involved in maintaining tissue integrity [1, 2].

Clinically, ABDs are characterized by skin blistering and its evolutionary lesions (Fig. 1A, B, Fig. 2A) resulting from development of an autoimmune response caused by prolonged inflammatory process and subsequent tissue destruction. According to the histological sites of blistering, they can be classified into: epidermal (intraepithelial), includ-
Fig. 1. A middle-aged man with PF relapse – serum anti-DSG1 IgG > 200 RU/ml and anti-DSG3 IgG 0.221 RU/ml in ELISAs (cut-off 20 RU/ml in both tests). A) Discolored residue macules and numerous crust-covered erosions. B) Impetiginization. C) Subcorneal blister with acantholytic cells (H+E). D) Positive pemphigus IgG4 deposits in outer root sheath (plucked scalp hair DIF). E) Lack of unequivocal IgG deposits (perilesional skin DIF). F) Positive pemphigus IgG4 deposits in lower epidermis (perilesional skin DIF)
ing pemphigus vulgaris (PV) and pemphigus foliaceus (PF), and subepidermal subgroups varying on level of split [2] (Fig. 1C, Fig. 2B), including bullous pemphigoid (BP). T-lymphocytes are critical in the induction and regulation of both cell-mediated and humoral immune response in ABDs [3]. A pathogenic role of autoantibodies (abs) for blister formation in ABDs is reported. Pemphigus and BP, being the two most frequent and severe types of ABDs, are characterized by the ab-driven pathogenesis [3-5] and T-cell involvement [6]. However, the abs response against proteins in ABDs is heterogeneous [7] (in case of pemphigus, it is most commonly directed against desmoglein 1 and 3 – DSG1/3, while in case of BP – against two hemidesmosomal proteins – BP180/BP230), but shows some inclination to subclass distribution. In light of this, autoimmunity in both pemphigus and BP is predominantly of the IgG isotype, but studies of the subclass’ distribution within the IgG class present a rather confusing picture. It is known, that immunoglobulin G (IgG) is the most abundant isotype in human serum, constituting about 80% of the total serum immunoglobulin [8]. There are four IgG subclasses in human, listed in accordance with their decreasing serum concentrations: IgG1, IgG2, IgG3, and IgG4. Literature data showed that the different IgG subclasses are characterized by variable ability to fix complement [9]. The ability of abs to fix complement enables the activation of the classical pathway, followed by chemotaxis of the inflammatory cells, such as leukocytes, and release of proteolytic enzymes [10]. Therefore, IgG3 is the most effective complement activator, followed by IgG1. On the other hand, IgG2 is relatively inefficient at complement activation, whereas IgG4 is not able to activate the complement cascade in a classical pathway at all [8, 11]. Because of its inability to classically fix complement, IgG4 has been considered rather a non-inflam-

Fig. 2. An elderly woman with urticarial BP – serum anti-BP180 IgG > 200 RU/ml and anti-BP230 IgG 0 RU/ml; in blister fluid anti-BP180 IgG > 200 RU/ml and anti-BP230 IgG 0.210 RU/ml in ELISAs (cut-off 20 RU/ml in both tests). A) Small, tense blisters on urticarial skin of the medial surface of thigh. B) Subepidermal blister with eosinophils in inflammatory infiltrate (H + E, original objective magnification 40×). C) Lack of IgG antibodies to epithelial basement membrane (IIF on monkey esophagus, original magnification 40×). D) IgG4 antibodies reacting along epithelial basement membrane (IIF on monkey esophagus, original magnification 40×)
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matory “protective” ab [12]. In vivo data showed that blister formation in animal model of BP required the activation of complement [10, 13, 14], thus the complement-binding IgG1 autoantibody may contribute to the pathogenesis of BP. On the other hand, IgG4 abs may predominate in pemphigus group, suggesting that tissue damage does not depend on complement activation [12, 15]. Interestingly, IgG subclasses show >95% sequence homology of heavy chains, but each of them express a unique profile of effector activities [16]. Each IgG subclass may be associated with different functional property determining the pathogenic potential of IgG abs. An interesting property of IgG4 subclass is the “Fab-arm exchange”-capacity. In that process half-molecules are continuously exchanged among IgG4. The phenomenon is thought to be related to amino-acid sequence that differs IgG4 and IgG1 and via the lack of interchain disulphide bonds [17], makes IgG4 a heterobivalent ab [18]. A unique profile of effector functions was ascribed to each IgG subclass. Still, probably multiple factors may affect the effector ligand activation and subclass functional capabilities (e.g. epitope density, antibody/antigen ratio) [8]. Therefore, the results of further studies may identify the link between the abs and complement in their pathogenic role in ABDs. However, the possible sequence of events may involve complement activation by complement-fixing abs, mast cell degranulation and subsequent inflammatory cells (e.g. neutrophil/eosinophil) recruitment [2, 19].

Immune responses observed in ABDs are subdivided on the basis of cytokine production patterns. The type 1 response promotes cellular immunity through the production of type 1 cytokines (e.g. IFN-γ, IL-2) by T-helper type 1 (Th1-cells), whereas the type 2 response enhances humoral immunity through the secretion of type 2 cytokines (IL-4, IL-5, IL-13) by Th2-cells [20]. The imbalance between these responses may play a pathogenic role in several autoimmune diseases, including pemphigus and BP. It has been considered that Th2-type cells play a key role in the development of ABDs. However, recent studies have revealed that autoreactive T-cells show the features of Th1, as well as Th2, in patients with PV [21-23], PF [24] and BP [25, 26]. Thus, probably both autoreactive Th1- and Th2-cells may be involved in the regulation of the production of pathogenic abs by B-cells in pemphigus and BP in these patients.autoimmunity against DSG-/DEJ-components may be Th1-regulated for IgG1 and Th2-regulated for IgG4 [27, 28]. It is suggested that DSG3- and BP180-reactive T-cells, respectively in PV and BP, presumably foster the production of abs of the Th2-dependent IgG4 subtype, that are preferentially seen in active stages of these disorders [6]. In light of above, during the active phases of pemphigus and BP, abs appear to be Th2-regulated IgG4 and IgE class [27, 29, 30]. Consistently, Th2-activation and enhanced IgE production has been reported in ABDs patients [31]. Nevertheless, recent studies showed that Th1-cytokines play a role in the development of the ABDs lesions in addition to Th2-cytokines [21, 25].

In the context of this report, it should be noted that a hyper-IgG4 state is uncommon in humans, having only been described in people receiving repetitive cutaneous immunization with mono- and oligoclonal antigens [32]. In light of this, increased amounts of total and antigen-specific IgG4 occur in atopic diseases. Thus, it may suggest that IgG4 is a blocking ab for anaphylactic sensitization responses [33]. Funakoshi et al. [32] postulated that skin blisters could act as a form of chronic autovaccination to antigens, leading to IgG4-mediated response that could potentially elevate the total serum IgG4, in relation to other IgG subclasses. It is possible that chronic immunization with cutaneous antigens may generally skew the immune response toward a hyper-IgG4 state. Due to DNA rearrangement (recombination of variable regions of abs) immunoglobulin class may undergo class-switch recombination to a new isotype. However, repetitive antigenic exposure can encourage subsequent isotype switching. Interleukin 4 and IL-13 promote isotype switching, priorly to IgG4 and subsequently to IgE [34].

Previously, it was demonstrated that the main isotypes of tissue-bound and circulating abs are IgG4 and IgG1 in both pemphigus and BP [8, 35-37], but the role of Th1/Th2-cells as target for specific modulation of T-cell-dependent production of pathogenic auto-abs in these disorders still remains a matter of debate. It is reported that intraepidermal blister formation in pemphigus group is caused by binding of IgG to keratinocyte cells without engaging innate immune effectors, and IgG4 abs seem to mainly mediate IgE-mediated responsethat could potentially elevate the total serum IgG4, in relation to other IgG subclasses. It is possible that chronic immunization with cutaneous antigens may generally skew the immune response toward a hyper-IgG4 state. Due to DNA rearrangement (recombination of variable regions of abs) immunoglobulin class may undergo class-switch recombination to a new isotope. However, repetitive antigenic exposure can encourage subsequent isotype switching. Interleukin 4 and IL-13 promote isotype switching, priorly to IgG4 and subsequently to IgE [34].
Specimens collected from January 2011 to December 2012, and 46 BP patients. Altogether 117 frozen sections from patients with ABD, including 71 pemphigus specimens (61 PV and 10 PF) were studied. None patient had been treated for ABD or with treatment outcome and eventual remissions. Specific disease variants are associated with unique ab profiles, thus suggesting new avenues of research into the pathogenesis of ABDs. Nevertheless, better understanding of the pathophysiology of ABDs requires prospective studies of both cellular and humoral response in various disease stages that may provide the basis for study on the immunoregulatory mechanisms.

**Aim of the study**

The aim of the study was to statistically analyze the IgG-positive, IgG1-positive (Th2-dependent in mice, but Th1-dependent in humans) and IgG4-positive (Th2-dependent in humans) results of DIF tests performed in patients with pemphigus (PV/PF) and BP.

**Material and methods**

**Specimens and patients**

The study was carried out at the Cutaneous Histopathology and Immunopathology Section, Department of Dermatology, Poznan University of Medical Sciences, Poland. Altogether 117 frozen sections from patients with ABD, including 71 pemphigus specimens (61 PV and 10 PF) collected from August 2005 to December 2012 and 46 BP specimens collected from January 2011 to December 2012, were studied. None patient had been treated for ABD beforehand. The clinical suspicion of pemphigus/BP was confirmed, and thus diagnosis established, with DIF of perilesional skin/mucosa demonstrating pemphigus-specific/pemphigoid-specific deposits of IgG/IgG1/IgG4 and/or 3rd component of complement (C3) (intercellular deposits throughout the epidermis/linear deposits at the DEJ, respectively), and corroborated with histology (conventional hematoxylin and eosin staining, H + E, was performed in all cases). ELISA against DSG1 and DSG3 was used to distinguish PV and PF, and ELISA against BP180 and BP230 was done to corroborate the diagnosis of BP. None of the female patients included were pregnant.

**Immunofluorescence procedure**

For DIF staining 4 µm cryostat sections of perilesional skin/mucosa were cut. The tissue sections were incubated in a humid chamber for 30 minutes at room temperature (RT) with commercially available fluorescein isothiocyanate (FITC)-conjugated rabbit polyclonal abs against the human IgG (Dako, Denmark) and FITC-conjugated mouse monoclonal abs against human IgG subclasses: IgG1 and IgG4 (Sigma, USA). The abs were used at a working dilution of 1 : 100 in phosphate buffer saline (PBS). The samples were then washed in PBS (pH 7.2) at RT for 15 min with gentle agitation. Then, slides were coverslipped and examined by microscopy with fluorescent startar (BX40, Olympus, Japan). The intensity of the immunoglobulin fluorescence staining was evaluated by an arbitrarily assigned semiquantitative five-point scale (from “–” to “+++”). The fluorescence intensity of immunoglobulin deposition was divided into two groups for the purpose of statistical analysis: (i) strong – including “++” moderately positive staining and “+++” strongly positive staining, (ii) weak – including “–” no staining, “+/–” doubtful staining and “+” weakly positive staining.

**Statistical analysis**

Fluorescence staining intensity of immunoglobulin subtype, as well as number of positive/negative results (IgG/IgG1/IgG4) in pemphigus and BP was tested by McNemar’s test and Liddell’s exact test (to analyze the intensity of immunoglobulins deposition in the one examined group – pemphigus or BP). Results were analyzed using Fisher’s exact test and χ² test with Yates’ continuity correction data to detect differences of the intensity of DIF results between IgG, IgG1 and IgG4 deposition in pemphigus and in BP (to compare the proportion of IgG/IgG1/IgG4 staining in two different group – pemphigus versus BP). A p < 0.05 was considered statistically significant. Statistical analysis was performed using StatsDirect statistical software (www.statsdirect.com, USA).

**Results**

A summary of the isotype distribution of IgG/IgG1/IgG4 in biopsies from pemphigus/BP perilesional skin/mucosa is shown in Table 1. Deposits of IgG were present in 44 pemphigus cases (61.97%) and 8 BP cases (17.39%). There were 60 IgG4-positive (84.51%), 11 IgG4-negative (15.49%), 34 IgG1-positive (47.89%), 37 IgG1-negative (52.11%) results in pemphigus samples. In BP samples, we reported 36 IgG4-

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Positive (78.26%), 10 IgG4-negative (21.74%), 15 IgG1-positive (32.61%), 31 IgG1-negative (67.39%) results. In 30 of 71 (42.25%) pemphigus biopsies IgG4 was the only of the examined IgG subclass detected, whereas IgG1 was the only subclass observed in 4 (5.63%) of pemphigus specimens. In case of BP, IgG4 was the only of the examined IgG subclass detected in 23 of 46 biopsies (50.00%), whereas IgG1 was the only subclass observed in 2 of 46 specimens (4.35%). There were 37 IgG4-positive, 7 IgG4-negative, 25 IgG1-positive and 19 IgG1-negative in IgG-positive pemphigus samples, whereas 7 IgG4-positive, 1 IgG4-negative, 5 IgG1-positive, 3 IgG1-negative results were observed in IgG-positive BP samples. C3 deposition was noted almost in all BP cases (only one samples was negative), whereas 23 pemphigus cases were C3-negative.

The DIF test assessing IgG4 deposition has statistically significant greater autoimmunity detection in both pemphigus and BP (Fig. 1E, F), in comparison to IgG and IgG1 evaluation, what was shown in Table 1. Using McNemar’s test and Liddell’s exact test, analysis of tissue-bound antibodies in pemphigus/BP specimens

### Table 1. IgG, IgG1 and IgG4 of tissue-bound antibodies in pemphigus/BP specimens

<table>
<thead>
<tr>
<th>Study group</th>
<th>Positive results, n (%)</th>
<th>Statistical significance</th>
<th>Pemphigus vs. BP statistical significance</th>
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<tbody>
<tr>
<td>Pemphigus (n = 71)</td>
<td>IgG 44 (61.97%)</td>
<td>IgG vs. IgG1 p = 0.089a IS, p = 0.0872b IS</td>
<td>IgG in pemphigus vs. IgG in BP p &lt; 0.0001c, p &lt; 0.0001d</td>
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<td>IgG1 34 (47.89%)</td>
<td>IgG vs. IgG4 p = 0.0062a, p = 0.0052b</td>
<td>IgG1 in pemphigus vs. IgG1 in BP p = 0.126a IS, p = 0.1486b IS</td>
</tr>
<tr>
<td></td>
<td>IgG4 60 (84.51%)</td>
<td>IgG1 vs. IgG4 p &lt; 0.0001a, p &lt; 0.0001b</td>
<td>IgG4 in pemphigus vs. IgG4 in BP p = 0.4625a IS, p = 0.5397b IS</td>
</tr>
<tr>
<td>BP (n = 46)</td>
<td>IgG 8 (17.39%)</td>
<td>IgG vs. IgG1 p = 0.0961a IS, p = 0.0923b IS</td>
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<tr>
<td></td>
<td>IgG1 15 (32.61%)</td>
<td>IgG vs. IgG4 p &lt; 0.0001a IS, p &lt; 0.0001b</td>
<td>IgG4 in pemphigus vs. IgG4 in BP p = 0.4625a IS, p = 0.5397b IS</td>
</tr>
<tr>
<td></td>
<td>IgG4 36 (78.26%)</td>
<td>IgG1 vs. IgG4 p &lt; 0.0001a, p &lt; 0.0001b</td>
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*aMcNemar’s test, bLiddell’s exact test, cFisher’s exact test, dχ² test with Yates’ continuity correction data, n – number of cases, BP – bullous pemphigoid, IS – insignificant

positive (78.26%), 10 IgG4-negative (21.74%), 15 IgG1-positive (32.61%), 31 IgG1-negative (67.39%) results. In 30 of 71 (42.25%) pemphigus biopsies IgG4 was the only of the examined IgG subclass detected, whereas IgG1 was the only subclass observed in 4 (5.63%) of pemphigus specimens. In case of BP, IgG4 was the only of the examined IgG subclass detected in 23 of 46 biopsies (50.00%), whereas IgG1 was the only subclass observed in 2 of 46 specimens (4.35%). There were 37 IgG4-positive, 7 IgG4-negative, 25 IgG1-positive and 19 IgG1-negative in IgG-positive pemphigus samples, whereas 7 IgG4-positive, 1 IgG4-negative, 5 IgG1-positive, 3 IgG1-negative results were observed in IgG-positive BP samples. C3 deposition was noted almost in all BP cases (only one samples was negative), whereas 23 pemphigus cases were C3-negative.

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### Discussion

There are strong data about the essential role of autoreactive T-cells in the regulation of the production of pathogenic abs in pemphigus and BP [6]. However, the findings regarding the imbalance of immune responses in ABDs are complicated and studies on IgG and distribution of its subclasses in pemphigus/BP have painted a controversial picture. Results obtained by various authors depended on the applied antigen recombinants, patients’ selection procedure and test systems applied, that reflected the advances in laboratory techniques. Together with previous findings, this study suggested that Th2-responses play a dominant role in the development of ABDs [20]. The proportion of IgG4-positive pemphigus/BP patients was higher than IgG- and IgG1-positive pemphigus/BP patients in DIF. The predominance of IgG4 subclass observed in this work is similar to previously reported findings. It should be noted that our investigation was carried out during active stage of diseases, so the findings may be in line with thesis that auto-abs of the Th2-dependent IgG4 subtype are preferentially seen in the active stages of ABDs, while auto-abs of the Th1-dependent IgG1 subclass are predominant during the chronic course of these disorders [3].

Studies on the IgG subclass distribution in BP, using various biochemical and molecular methods, regarded
autoimmune response to DEJ components or specific response to BP180/BP230 and their domains. Thus, findings based on these investigations may be divergent. Previous data reported that in BP sera, IgG4 and IgG1 circulating abs were detected with a similar frequency (100% and 83%, respectively) [41]. In contrast, another report demonstrated that sera of patients with BP contain predominantly auto- abs of the IgG4 subclass directed against the DEJ [29, 42], what was consistent with our observation of tissue-bound abs. Cited works, in accordance with presented results, showed that abs of the IgG1 subclass are also present in BP patients, although at lower levels than IgG4 [29, 42]. In addition, BP patients with severe disease exhibit IgE autoantibodies against the major BP abs [43]. It is known that IgG4 and IgE production is regulated concordantly and requires the stimulation of Th2-cells [31]. Particularly, IL-4 producing Th2-cells were increased in BP [44] and these T-cells seem to induce B-cell proliferation and differentiation into IgE-secreting cells in BP. Al-Karawi [9], using indirect immunofluorescence (IIF) and DIF with monoclonal abs specific for 4 human IgG subclasses, investigated their distribution in BP. This study reported 58.8% IgG1, 5.9% IgG2, 17.6% IgG3, 88.2% IgG4 and 94.1% C3 of tissue-bound abs in BP and 56% IgG1, 0% IgG2, 16% IgG3 and 96% IgG4 of circulating abs [9]. In addition, Bowszyc-Dmochowska and Dmochowski [39] detected IgG1 deposits in 63% and IgG4 deposits in 79% of BP cases showing IgG deposition. It seems that quite similar findings on IgG4 are presented in our work – we discovered 78.26% IgG4 and 32.61% IgG1 of tissue-bound abs in BP. However, analyzing only IgG-positive samples, there are only 15% IgG4-positive samples and 11% IgG1-positive results. Also earlier experiments with IIF and immunoblotting (IB) revealed that the subclass of BP abs is of IgG4 isotype [27, 43–47]. Our previous data, obtained with IIF, confirm that the circulating abs in subepidermal IgG-mediated ABDs belong predominantly to IgG4 isotypes [48] (Fig. 2C, D). Although the cause of the prominent IgG4 production in BP has not been established, it is possible that it may be a result of specific genetic factors [49]. Moreover, it has also been speculated that continued antigenic stimulation affects the normal distribution of IgG subclasses and leads to IgG4-restricted response [50]. Interestingly, as was revealed by Al-Karawi study [9], the distribution of IgG subclass in BP sera did not correlate with their complement activating capacity. Conversely, other report indicated that IgG1 appeared to be the only subclass capable of complement fixation in BP [51]. Thus, perhaps in some cases complement activation, that requires at least 2 closely spaced IgG molecules to bind antigen, did not occur due to too few antigenic sites available [9]. Findings that sera containing only IgG4 do not activate complement have also been noted by Kelly et al. [52]. There is a hypothesis that although IgG4 does not activate complement by the classical pathway, it is possible that C3 deposits could occur via the activation of alternative pathway [53–55] or that small amount of IgG1 to IgG3 subclasses abs activated the classical pathway [35]. This discrepancy may suggest that the inflammatory response in BP may occur via the different mechanism, which involves mast cells [56]. Thus, the interaction of IgG4 abs with mast cells in the skin may be an alternative/additional mechanism leading to inflammation and blister formation in BP [9].

<p>| Table 2. Differences of the antibody prevalence between the IgG1 strong/weak and IgG4 strong/weak in pemphigus and BP |
|---------------------------------|-----------------|---------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Study group</strong></th>
<th><strong>Intensity of DIF</strong></th>
<th><strong>Statistical significance</strong></th>
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<tbody>
<tr>
<td>Pemphigus ($n = 71$)</td>
<td></td>
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<tr>
<td>IgG1</td>
<td>Strong, $n$ (%)</td>
<td>Weak, $n$ (%)</td>
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<tr>
<td>15 (21.13%)</td>
<td>56 (78.87%)</td>
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<tr>
<td>Pemphigus ($n = 71$)</td>
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<tr>
<td>IgG4</td>
<td></td>
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<tr>
<td>46 (64.79%)</td>
<td>25 (35.21%)</td>
<td></td>
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<tr>
<td>BP ($n = 46$)</td>
<td></td>
<td></td>
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<tr>
<td>IgG1</td>
<td></td>
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</tr>
<tr>
<td>4 (8.70%)</td>
<td>42 (91.30%)</td>
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<tr>
<td>BP ($n = 46$)</td>
<td></td>
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<tr>
<td>IgG4</td>
<td></td>
<td></td>
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<tr>
<td>24 (52.17%)</td>
<td>22 (47.83%)</td>
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</table>

a McNemar’s test, b Liddell’s exact test, $n$ – number of cases, BP – bullous pemphigoid, s/w: strong/weak
IgG4 is prevailing over immunoglobulin G1 in autoimmunity of pemphigus and bullous pemphigoid: analysis of tissue-bound antibodies in active diseases

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The literature data suggesting the pathogenic role of IgG4, as well as some experimental models, show that anti-DSG3 IgG4 may induce acantholysis. However, the function of IgG1 in acantholysis is also considered. In active form of PV the IgG4 is predominant [16]. Probably, as mentioned above, in IgG4-way the complement activation is not required for blister formation in pemphigus [16]. However, the possible pathogenic role of other IgG subclasses should not be excluded. Indeed, in some PF cases, only IgG1 were found [71]. Previous studies showed that abs in pemphigus (PV/PF) are predominantly of the IgG4 subclass during active disease, but how much they comprise of total IgG4, and how much IgG4 concentration is increased, in relation to other IgG subclasses, is unknown. Dmochowski et al. [32] examined pemphigus patients’ sera using IIF and IB. Using IIF, IgG4 and IgG2 dominated, while using IB, only IgG4 dominated [69]. Thus, our results on tissue-bound abs agree with previous reports on circulating abs depicting that the IgG4 are detected more frequently than IgG1 in pemphigus patients. Funakoshi et al. [32], with the use of subclass ELISA, tried to estimate total and DSG-specific IgG subclasses in pemphigus. This experiment indicated that DSG-specific abs comprised a median of 7.1% and 4.2% of total IgG4 in PV and PF, with 8-fold and 4-fold enrichment in IgG4 vs. IgG1 [32]. Thus, it may suggested that DSG-specific abs are significantly enriched in IgG4, which may explain the enrichment of total serum IgG4 in some pemphigus patients [32], what is compatible with presented here results on tissue-bound abs and our personal experience with DIF of plucked scalp hair (Fig. 1D, E, F). In both PV and PF, patients with active disease demonstrate DSG-reactive IgG4 and IgG1, while patients in remission and some healthy relatives of pemphigus patients can demonstrate only anti-DSG IgG1 [27, 28, 37, 72, 73]. Probably IgG2 and IgG3 anti-DSG abs have not been associated with pemphigus [7, 74]. Moreover, an IgG4-specific ELISA was shown to have greater sensitivity and specificity than a total IgG anti-DSG ELISA in detecting active stage of some kind of PF, suggesting a more significant clinical association of pathogenic abs with IgG4 rather than with other IgG subclass in this patient. Dańczak-Pazdrowska et al. [75], with the use of modified ELISA and IIF, detected significant higher anti-DSG3 IgG4 in PV and anti-DSG1 IgG4 in PF in comparison to IgG1, as well as a higher titer of anti-DSG3 IgG1 in PF compared to IgG4. In the cited studies, Funakoshi et al. [32] and Dańczak-Pazdrowska [75] presented that the acquisition of an anti-DSG IgG4-response is a characteristic serologic finding marker in pemphigus patients with active disease. Upregulation of Th2-cytokines (IL-4, IL-10, IL-13) is observed in pemphigus and may promote an IgG4> IgG1 serum ab profile [32]. There are findings indicating that IgG4-depletion reduces the pathogenic activity of pemphigus sera [32] and reduces keratinocytes dissociation by PV-IgG by a mean 81%, indicating that pathogenic abs are preferentially enriched in the serum IgG4 fraction. However, the issue to resolve remains whether DSG-specific IgG1 could perpetuate active disease in patient who are depleted of IgG4 [32]. There are studies suggesting that IgG4 is the major pathogenic IgG subclass in relation to other subclasses in PV patients, since PV-IgG depleted of IgG4 (which would contain IgG1, IgG2, IgG3 subclasses) demonstrated pathogenicity similar to negative control. Prior studies have shown that patients in clinical remission and even unaffected relatives of pemphigus patients can express DSG-specific IgG1 without evidence of clinical disease [27, 37, 72, 73]. Additionally, if IgG1 abs subsequently switch to IgG4 with chronic active disease, IgG4 depletion strategies will ultimately capture these pathogenic ab populations [32]. Additional study on pemphigus [67] presented conflicting observation and indicated that IgG4 was the most common subclass in patients in remission, whereas the IgG1 was found in 100% of patients with active disease and only 50% of those in a state of clinical remission. However, there is also a hypothesis that IgG4 was the predominant subclass and IgG1 is only present at an early stage of the disease. Thus, it seems that IgG4 has a protective role in pemphigus as well [10]. Kricheli et al. [37], with IIF and western blot (WB), detected PV-IgG4 in 62% of the patients, but in only 1.8% relative, and was absent in the controls. Moreover, PV-IgG1, IgG2 and IgG4 were found to react mainly with DSG3 and PV-IgG3 mainly with DSG1 and DSG3 [37]. The non-complement fixing PV-IgG4 and at least one complement-fixing PV-IgG subclass appear to be involved in the pathogenesis of the disease. The absence of PV-IgG4 among relatives being PV-IgG carriers seems to be linked to the fact, that they do not develop pemphigus. Examination with IIF revealed circulating PV-IgG in 64% of the patients, in 15% of relatives and in none of the controls [37]. With WB, the results were 91%, 49% and 12%, respectively [37]. The IgG4 anti-DSG1 and anti-DSG3 abs appear to be associated with the onset and activity of the diseases, while the IgG1 is thought to correlate with the remission of the diseases [27, 37, 73]. Interestingly, Eming et al. [76], with the use of ELISPOT, found that DSG3-specific autoreactive Th1- and Th2-cells occur at similar frequencies in acute onset PV. However, the work with magnetic cell sorting cytokine secretion assay (MACS), DSG-3-reactive Th2-cells are detected at similar level in acute onset, chronic active and remittent PV, while the number of autoreactive Th1-cells exceeded that of Th2-cells in chronic active PV [77]. Conversely, Bjol et al. [36], using modified IB, reported that sera of patients with active pemphigus contained abs of the IgG1 and IgG4 subclass. Moreover, the sera of patients in remission, those of healthy unaffected relatives and normal controls contained only the IgG1 subclass. The sera of healthy relatives and normal controls that contain an ab binding pemphigus antigens is of the IgG1 subclass only and is considered to be nonpathogenic or natural ab [38]. Furthermore, using the animal model [72], it was shown that IgG4, but not IgG1, abs from PF
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Conclusions

The fluorescence intensity of tissue-bound IgG4 is significantly higher than fluorescence intensity of IgG and IgG1 in both pemphigus and BP, what may suggest that IgG4 is the initial and predominant tissue-bound ab subclass detected in these diseases. Therefore, demonstration of IgG4 with DIF enhances the early diagnosis of pemphigus/BP. The demonstration of IgG4 predominance on skin/mucosa lesions, as well as the presence of sole IgG4 subclass ab in some cases may indicate that IgG4 could be the initial immunopathologic event found in patients with pemphigus/BP.

References


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