 DOI: 10.5114/ceji.2013.34362

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 poly-/monoclonal 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 IgG1strong/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.*

(Centr Eur J Immunol 2013; 38 (1): 80-91)

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-

Correspondence: Justyna Gornowicz-Porowska, MSc Eng/PhD, Cutaneous Histopathology and Immunopathology Section, Department of Dermatology, Poznan University of Medical Sciences, Przybyszewskiego 49, 60-355 Poznań, Poland, tel. +48 61 869 13 67, e-mail: justynagornowicz1@poczta.onet.pl

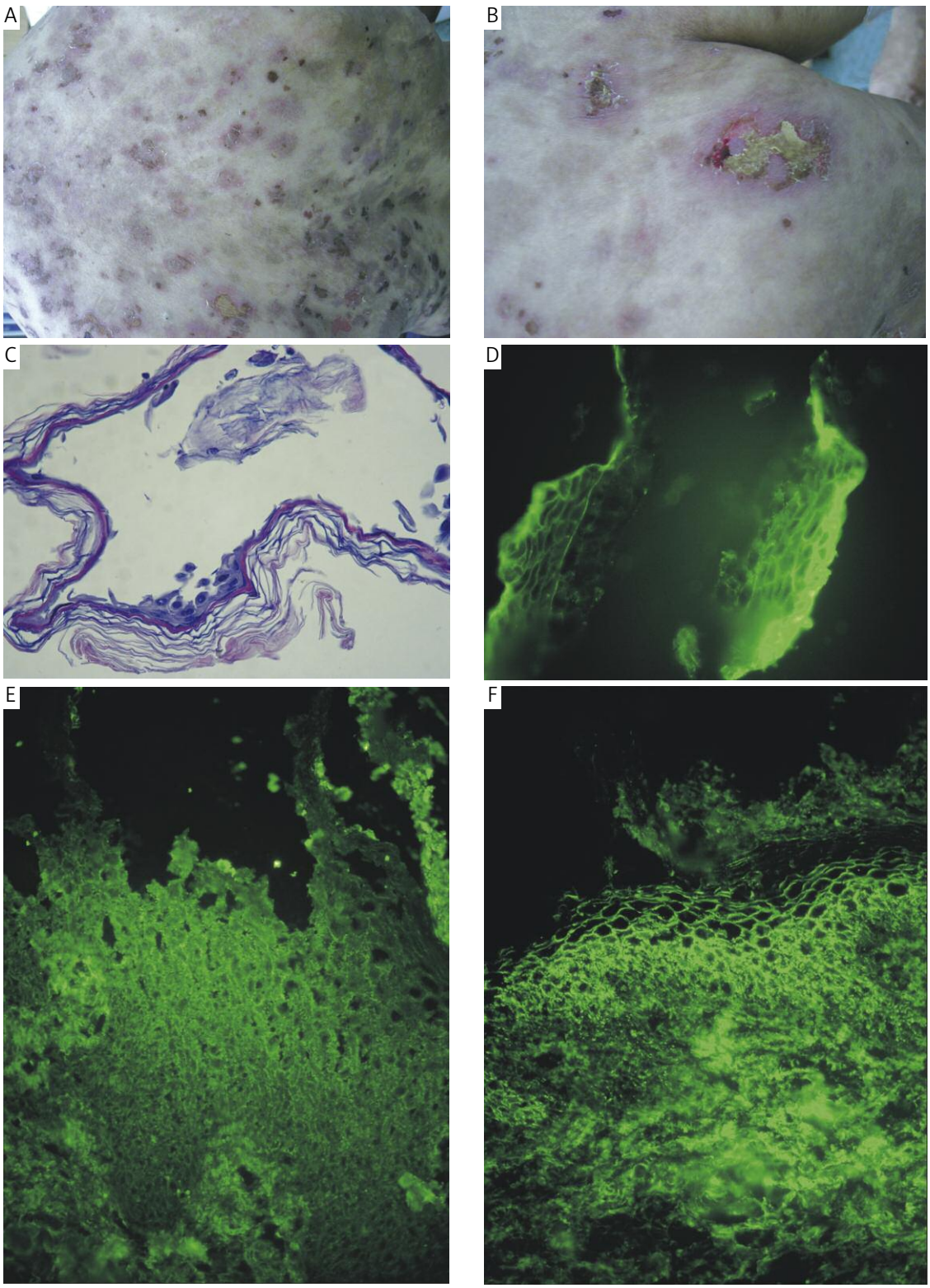

Fig. 1. A middle-aged man with PF relapse – serum anti-DSG1 IgG > 200 RU/ml and anti-DSG3 IgG 0.221 RU/ml in ELISAs (cut-off 20 RU/ml in both tests). A) Discolored residue macules and numerous crust-covered erosions. B) Impetiginization. C) Subcorneal blister with acantholytic cells (H + E). D) Positive 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)

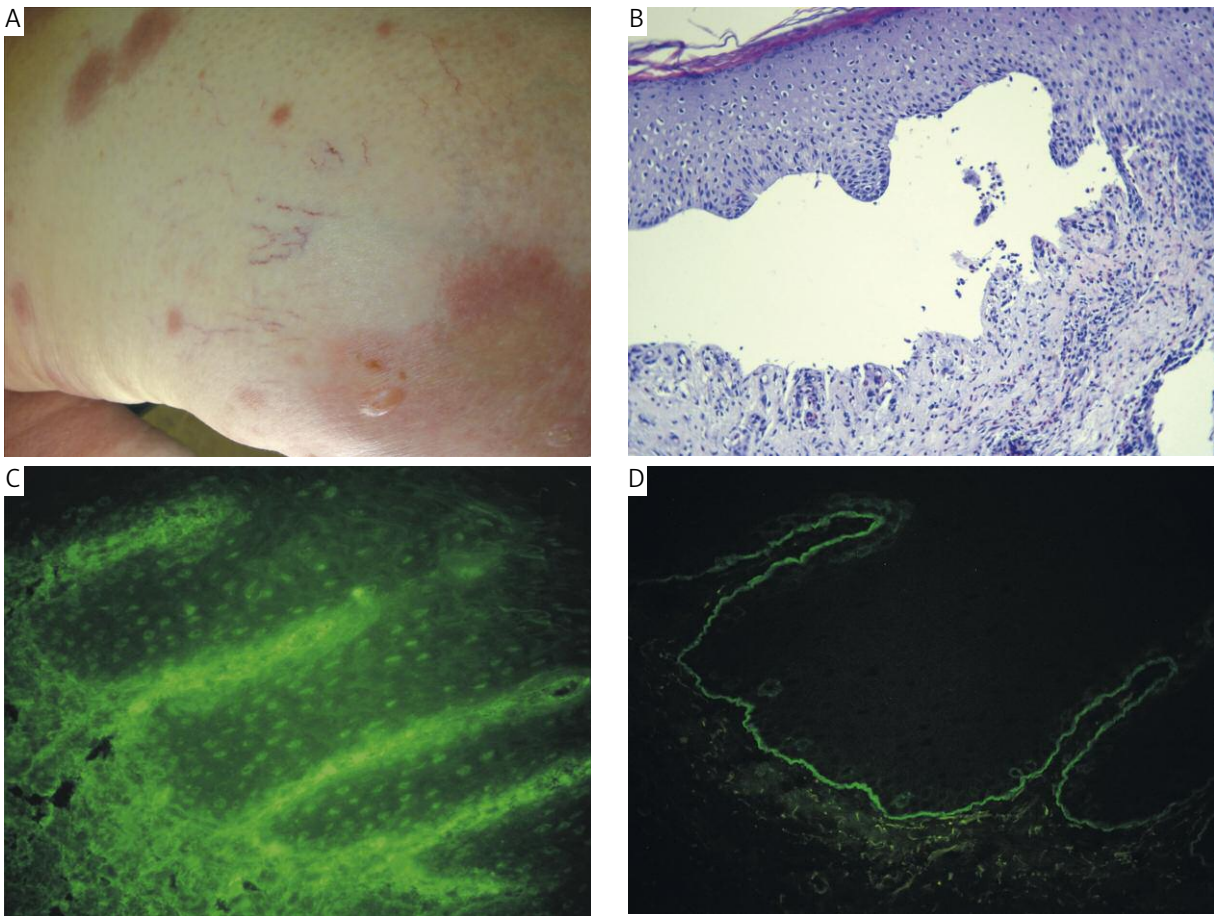

Fig. 2. An elderly woman with urticarial BP – serum anti-BP180 IgG > 200 RU/ml and anti-BP230 IgG 0 RU/ml; in blister fluid anti-BP180 IgG > 200 RU/ml and anti-BP230 IgG 0.210 RU/ml in ELISAs (cut-off 20 RU/ml in both tests). A) Small, tense blisters on urticarial skin of the medial surface of thigh. 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×)

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-

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/BP and 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-/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 acantholysis [16]. In contrast, blister induction requires the complement activation/recruitment and ab-driven leukocyte activation in most subepidermal ABDs. Thus, IgG1, but not IgG4, is thought to mediate tissue damage [16]. Disease-specific subclass distribution may also be responsible for false-negative results in diagnostic tests of ABDs (subthreshold IgG in skin specimens) [38]. Accordingly, Bowszyc-Dmochowska and Dmochowski [39] proposed to use IgG4-conjugate for diagnostic purposes.

It should be noted, that the IgG subclass distribution in ABDs is clinically/therapeutically relevant. The manipulation of ab isotype distribution is promising and could contribute significantly to a more effective treatment in ABDs [10]. In the clinical practice, a global immunosuppressive treatment with glucocorticosteroids and immunosuppressive drugs were used [40]. However, in light of the serious side effects of such therapy, the attempts have been made to modulate the autoimmune response in a more specific manner, both *in vitro* and *in vivo* [3]. Since the production of pathogenic auto-abs presumably requires the participation of Th-cells that regulate immunoglobulin isotype switching, autoreactive T-cells may serve as an ideal target for specific modulation of the production of pathogenic auto-abs in these disorders [3]. Therefore, IgG4 is an attractive prospective therapeutic target, as it is the least abun-

dant IgG subclass, and unlike other subclasses, does not activate complement and therefore is thought to be relatively unimportant for fighting infection [32]. Hence, IgG4 could serve as a therapeutic target in ABDs, for example through subclass-specific immunoadsorption or by selective depletion of surface-IgG4 positive memory B-cells [32].

In light of abovementioned information, the question about the pathogenic role of auto-ab classes in ABDs should be raised. It is still unclear which class initiates the autoimmune response and which only represents a secondary event in the context of an epitope spreading phenomenon. Thus, we analyzed the presence and intensity of IgG/IgG1/IgG4 deposition with direct immunofluorescence (DIF) in pemphigus and BP patients. This report adds to an increasing literature, in which IgG/IgG1/IgG4 abs have been shown to exhibit crucial activities in ABDs pathomechanism. In recent years, several studies investigated the isotype profile of circulating abs in human individuals affected with ABDs. Literature data often have established correlations between ab-isotype profiles and duration or severity of the disorders, 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 starter (BX40, Olympus, Japan). The intensity of the immunoglobulin fluorescence staining was evaluated by an arbitrarily assigned semi-quantitative 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 χ^2 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-

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

Study group	Positive results, *n* (%)	Statistical significance	Pemphigus vs. BP statistical significance
Pemphigus (*n* = 71)	IgG 44 (61.97%)	IgG vs. IgG1 $p = 0.089^a$ IS, $p = 0.0872^b$ IS	IgG in pemphigus vs. IgG in BP $p < 0.0001^c$, $p < 0.0001^d$
	IgG1 34 (47.89%)	IgG vs. IgG4 $p = 0.0062^a$, $p = 0.0052^b$	
	IgG4 60 (84.51%)	IgG1 vs. IgG4 $p < 0.0001^a$, $p < 0.0001^b$	IgG1 in pemphigus vs. IgG1 in BP $p = 0.126^c$ IS, $p = 0.1486^d$ IS
BP (*n* = 46)	IgG 8 (17.39%)	IgG vs. IgG1 $p = 0.0961^a$ IS, $p = 0.0923^b$ IS	
	IgG1 15 (32.61%)	IgG vs. IgG4 $p < 0.0001^a$, $p < 0.0001^b$	IgG4 in pemphigus vs. IgG4 in BP $p = 0.4625^c$ IS, $p = 0.5397^d$ IS
	IgG4 36 (78.26%)	IgG1 vs. IgG4 $p < 0.0001^a$, $p < 0.0001^b$	

[a]McNemar's test, [b]Liddell's exact test, [c]Fisher's exact test, [d]χ^2 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 biopsy 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, the performance was significantly different between these assays: (i) in case of pemphigus – IgG4 vs. IgG Liddell's exact test, $p = 0.0052$; McNemar's test, $p = 0.0062$; IgG4 vs. IgG1 Liddell's exact test, $p < 0.0001$; McNemar's test, $p < 0.0001$), (ii) in case of BP – IgG4 vs. IgG Liddell's exact test, $p < 0.0001$; McNemar's test, $p < 0.0001$; IgG4 vs. IgG1 Liddell's exact test, $p < 0.0001$; McNemar's test, $p < 0.0001$). There was no statistically significant difference between IgG1 vs. IgG deposits in both pemphigus and BP.

The analysis of IgG/IgG1/IgG4 isotype distribution between pemphigus and BP indicated that there were no significantly differences between IgG1/IgG4 distributions in these groups, but there was a statistically significant difference between IgG distribution (IgG deposits in pemphigus vs. IgG deposits in BP – Fisher's exact test, $p < 0.0001$; χ^2 test with Yates' continuity correction data, $p < 0.0001$), what was shown in Table 1.

The examination of fluorescence intensity staining between IgG1strong/weak and IgG4strong/weak in pemphigus and BP was presented in Table 2. Obtained results indicated that there were statistically significant differences between these parameters (Liddell's exact test, $p < 0.0001$; McNemar's test, $p < 0.0001$ in both analyses).

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

Table 2. Differences of the antibody prevalence between the IgG1 strong/weak and IgG4 strong/weak in pemphigus and BP

Study group		Intensity of DIF		Statistical significance
		Strong, *n* (%)	**Weak, *n* (%)**	
Pemphigus ($n = 71$)	IgG1	15 (21.13%)	56 (78.87%)	IgG1-s/w vs. IgG4-s/w in pemphigus $p < 0.0001^a$, $p < 0.0001^b$
	IgG4	46 (64.79%)	25 (35.21%)	
BP ($n = 46$)	IgG1	4 (8.70%)	42 (91.30%)	IgG1-s/w vs. IgG4-s/w in BP $p < 0.0001^a$, $p < 0.0001^b$
	IgG4	24 (52.17%)	22 (47.83%)	

[a]McNemar's test, [b]Liddell's exact test, n – number of cases, BP – bullous pemphigoid, s/w: strong/weak

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 reports 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 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].

Literature data also presented studies on specific immune response to BP180/BP230. In light of above, Döpp *et al.* [57] analyzed the IgG subclass distribution of abs to full-length BP180 NC16A and recombinant fragments of this domain with the use ELISA and IB. This group of researchers showed that IgG4 and IgE are the major isotypes of immunoglobulin targeting BP180 NC16A before initiation of treatment in patients with BP [57]. Using IB, this group of researchers demonstrated that 66% of BP sera contained IgG4, 50% IgG1, 44% IgG2, and 44% IgG3 abs. These observation is in contrast to IIF, that found IgG2 and IgG3 abs only in a small minority of BP sera [57].

On the other hand, there are data significantly different from the abovelisted works and results presented here. There is a hypothesis that in BP, IgG1 and IgG3 isotypes, but not the IgG4 subclass, are thought to trigger inflammatory pathways resulting in tissue damage [58]. Mihai *et al.* [58] isolated IgG1 and IgG4 abs from BP serum and analyzed their blister-inducing potential. They reported that complement-

fixing IgG1 abs induced subepidermal splits. Moreover, IgG4 did not fix complement, but, like IgG1, activated leukocytes and induced dermal-epidermal separation [58]. However, it was demonstrated that the potential of IgG4 to induce Fc-dependent dermal-epidermal separation was significantly lower in comparison to IgG1 [58]. Probably abs to BP180 recognize multiple epitopes on both extracellular (ECD) and intracellular domain (ICD) of BP180. Nevertheless, the sera of most patients bind the NC16A subdomain, an immunodominant region on the ECD close to transmembrane domain [59-62]. In contrast, BP230-specific abs predominantly recognize sequences contained within the COOH-terminal region of the protein [12]. Laffitte *et al.* [12], with IB, detected that 100% and 77% of BP sera, respectively, contained IgG1 and IgG4 abs binding the ECD of BP180. Furthermore, they found that 82% and 35% of BP sera reacting with the ICD of BP180 has IgG1 and IgG4 abs, respectively [12]. Findings obtained by Laffitte *et al.* [12] also suggested that IgG1 and, to a lesser extent, IgG4 are the predominant subclasses in BP sera. In line with these results are the data of Bernard *et al.* [29], which indicated that 90% and 80% of BP180-reactive sera, respectively, contained abs of the IgG1 and IgG4 isotype. Moreover, IgG4 abs to BP230 were found more frequently than IgG1 suggesting a predominance of IgG4 to BP230 [29]. Laffitte *et al.* [12] detected 57% specific IgG1-response and 71% IgG4-response to BP230, whereas abs of the IgG2 and IgG3 subclasses were detected less frequently. In BP patients with serum IgG to BP230-C and BP230-N, IgG4 and IgG1 abs to these domains were found in 86% and 50% of sera, respectively [31]. Moreover, it is suggested that isotype restriction of the ab response to BP180 shows significant changes in the course of the disease [12]. It is possible that the apparently more frequent detection of specific IgG4 in patient with longer disease duration reflects an isotype-switch due to a shift in the Th1/Th2 balance in the chronic phase of BP [12]. As the profile of IgG subclasses is similar, it is difficult to raise any speculation about the possibility that different T-cells cytokine subsets regulate the humoral response to putatively pathogenic and secondary, "non-pathogenic" IgG isotypes [12].

Interestingly, study of Hofmann *et al.* [63], taking into account the distinction between responses to BP180-N and BP180-C, showed predominant role of IgG1 in BP. Using newly developed ELISA, they [63] found that in BP180-N-reactive sera from active BP, 85% were IgG1-positive (including both IgG1- and IgG4-positive), with 45% being IgG1 exclusively, while 46% IgG4-positive (including both IgG1- and IgG4-positive), with only 6% being IgG4-positive only. Moreover, anti-BP180-N IgG1 was predominant in the acute phase of BP (52% IgG1 vs. 4% IgG4) [63]. They provide convincing findings, strongly suggesting that BP IgG1 binds to the pathogenic epitope within the NC16A domain and activates the complement system, thus initiating BP [19]. In contrast, the majority of BP sera reactive with the BP180-C showed a dual IgG1 and IgG4 response: 76% were IgG1-positive (including both IgG1- and IgG4-positive) with 19% being IgG1 exclusively, as well as 69% IgG4-positive (including both IgG1- and IgG4-positive), with 13% being IgG4-positive only [63]. Strikingly, Hofmann *et al.* [63] uncover a dual IgG1 and IgG4 response to BP180-N more frequently in BP patients with extensive skin involvement than patients with localized BP. Thus, it may suggest that anti-BP180-N IgG4 abs can sustain the disease phenotype. Hofmann *et al.* [63] reported decreased IgG1-reactivity during the course of disease, whereas IgG4-reactivity showed a tendency to increase with a longer disease duration. IgG1- and IgG4-reactivity to the COOH-terminus of BP180 was independent of the clinical activity of BP [63].

Interestingly, Modre *et al.* [10] postulate that disease activity depends on the IgG ab isotype rather than on the total IgG ab, and isotype-switching from "inflammatory" IgG1 to "blocking" IgG4 subclass abs might contribute to disease remission (in light of this IgG4 has been regarded as the less inflammatory "blocking" ab) [64-66]. It is possible that the degree of inflammation is dependent on the IgG-isotype rather than on the total IgG level [10]. In the early immune response in BP, the IgG1 level is usually higher than the IgG4, but through a sequential switching process the IgG4 may slowly increase [10]. Modre *et al.* [10] hypothesize that isotype-switching from highly pathogenic IgG1 to non-complement-activating IgG4 is favorable for the host in chronic infectious and inflammatory states by reduction in the inflammatory response. The finding that IgG4 is the predominant class contrasts with Modre *et al.* [10], who observed this ab in remission, but not early disease. Probably all BP patients showed a similar IgG subclass shift with remission independently of the disease duration [10]. Revealed discrepancies may result from several causes: (i) the different monoclonal abs to a specific IgG subclass exhibit variable sensitivity and specificity, (ii) detection of different antigenic regions of examined protein, (iii) the disease stage and the immunosuppressive treatment might influence the subclass profile [12].

In consideration of these data, the pathogenetic link between BP and atopy may be observed. However, it is still widely accepted that immune response in atopic diseases is related to exogenous allergens and BP is related to hemidesmosomal autoantigens. Nonetheless, analyzing this question one should be aware of two issues: (i) processing of exogenous allergens (perhaps afterward they are not recognized as foreign molecule), (ii) hidden or sequestered antigens may not be recognized as self-antigens. Thus, it can be concluded that BP seems to be an age-related atopy [1].

Also analysis of IgG-mediated autoimmunity and IgG subclass distribution in pemphigus provides ambiguous data. Numerous studies demonstrated that abs in pemphigus mainly belong to the IgG1 and IgG4 subclass [7, 27, 37, 67-70]. However, the relevance of IgG subclasses for acantholysis in pemphigus still remains a matter of debate [16].

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.* [69] 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 [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, Bhol *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

patients are able to induce experimental PF in mice. This suggests that the IgG4 isotype may play a direct role in the tissue damage in pemphigus, what was confirmed by our results. Currently the role of IgG1 anti-DSG1 and anti-DSG3 in the development and progression of pemphigus is still unknown. Some authors are tempted to extrapolate the data obtained on pemphigus/BP investigation on mouse model. However, IgG subclass naming in mouse and human differs substantially, thus confusing both investigators and readers. Human subclasses do not have close structural homologues in mice. While in human, IgG subclasses were numbered in accordance to sequence of discovery (and concurrently to decreasing concentration in overall IgG class), in mouse their number assignment reflected electrophoretic mobility. Thus, mice IgG1 is unrelated to human IgG1, but is rather Th2-dependent like human IgG4 [18], and these basic facts were sometimes confusingly described even in top-level literature [78]. Regarding the mechanisms of pemphigus/BP-immunity, it seems that a dog model might prove to be more useful. In summary, these data support the hypothesis that immunoglobulin isotype-switching may play an important role in the development and progression of PF and PV. Regarding the findings of various authors, it appears that IgG1 and IgG4 anti-DSG abs exhibit different tissue and antigenic specificity [70].

Moreover, it is suggested that pemphigus may be associated with the imbalance between DSG3-responsive Th2-cells and regulatory T-cells (Tregs) specialized in counter-regulating the devastating T-cell autoimmune response. Thereby, autoreactive Tregs may represent an ideal tool to specifically restore immune tolerance in ABDs [6]. It is known that there is strong immunogenetic background of pemphigus. In light of this, literature data identified DSG3-reactive Th1-cells [21] and Th2-cells [22, 79], which recognized portions of the extracellular domain of DSG3 in the context of PV-associated HLA class II alleles.

Shirakata *et al.* [35], using IIF, detected IgG4, IgG1, and IgG2 respectively in 86%, 33%, 40% of BP patients, whereas IgG3 was not detected. Analyzing pemphigus patients, they reported IgG4, IgG1, IgG2 and IgG3 in 100%, 70%, 10% and 10%, respectively [35].

The positive IgG4 result is a stark message that the patient suffers from Th2-mediated disease. What is also worth depicting, DIF IgG4 studies seem to be characterized by noticeably less intense background fluorescence. Therefore, we recommend staining for IgG4 subclass abs as a more precise method of autoimmunity detection in pemphigus/BP. Collectively, the data obtained in our study, along with these presented above and our ten-year clinical observations, indicate that in both BP and pemphigus, the most prominent subclass was IgG4, that suggests that IgG4 may be considered to be a pathogenic ab [37]. Thence, we believe that demonstrated detailed analysis will be the support in understanding the humoral autoimmune response in patients with pemphigus/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 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

1. Dmochowski M (2006): Autoimmunizacyjne dermatozy pęcherzowe. Wydawnictwo Naukowe Akademii Medycznej im. Karola Marcinkowskiego, Poznań; 116-199.
2. Gornowicz-Porowska J, Bowszyc-Dmochowska M, Dmochowski M (2012): Autoimmunity-driven enzymatic remodeling of the dermal-epidermal junction in bullous pemphigoid and dermatitis herpetiformis. Autoimmunity 45: 71-80.
3. Hertl M (2000): Humoral and cellular autoimmunity in autoimmune bullous skin disorders. Int Arch Allergy Immunol 122: 91-100.
4. Lever WF (1953): Pemphigus. Medicine (Baltimore) 32: 1-123.
5. Stanley JR, Hawley-Nelson P, Yuspa SH, et al. (1981): Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. Cell 24: 897-903.
6. Hertl M, Eming R, Veldman C (2006): T cell control in autoimmune bullous skin disorders. J Clin Invest 116: 1159-1166.
7. Dmochowski M, Nie Z, Kiyokawa C, Hashimoto T (1999). Human desmocollin 1a transiently expressed in COS-7 cells and NIH 3T3-3 cells is reacted by IgG4 antibodies in a pemphigus foliaceus serum. J Dermatol Sci 21: 42-48.
8. Futei Y, Amagai M, Ishii K, et al. (2001): Predominant IgG4 subclass in autoantibodies of pemphigus vulgaris and foliaceus. J Dermatol Sci 26: 55-61.
9. Al-Karawi KS (2002): Immunoglobulin G subclass distribution of bullous pemphigoid autoantibodies and complement fixation studies. Saudi Med J 23: 1492-1495.
10. Modre B, Allen J, Wojnarowska F (1999). Does class switching contribute to remission in bullous pemphigoid? Acta Derm Venereol 79: 127-131.
11. McConell I, Munro A, Waldman H (1981): Immunoglobulins as proteins. In: The immune system. 2nd ed. McConell I, Munro A, Waldman H (eds.). Blackwell Scientific Publications, Oxford, UK; 3-21.
12. Laffitte E, Skaria M, Jaunin F, et al. (2001): Autoantibodies to the extracellular and intracellular domain of bullous pemphigoid 180, the putative key autoantigen in bullous pemphigoid, belong predominantly to the IgG1 and IgG4 subclasses. Br J Dermatol 144: 760-768.
13. Liu Z, Giudice GJ, Swartz SJ, et al. (1995): The role of complement in experimental bullous pemphigoid. J Clin Invest 95: 1539-1544.
14. Kotnik V (2011): Complement in skin diseases. Acta Dermatovenerol Alp Panonica Adriat 20: 3-11.

15. Jones CC, Hamilton RG, Jordon RE (1988): Subclass distribution of human IgG autoantibodies in pemphigus. J Clin Immunol 8: 43-49.
16. Sitaru C, Mihai S, Zillikens D (2007): The relevance of the IgG subclass of autoantibodies for blister induction in autoimmune bullous skin diseases. Arch Dermatol Res 299: 1-8.
17. King DJ, Adair JR, Angal S, et al. (1992): Expression, purification and characterization of a mouse-human chimeric antibody and chimeric Fab' fragment. Biochem J 281 (Pt 2): 317-323.
18. Aalberse RC, Stapel SO, Schuurman J, Rispens T (2009): Immunoglobulin G4: an odd antibody. Clin Exp Allergy 39: 469-477.
19. Liu Z (2002): Are anti-BP180 IgG1 or IgG4 autoantibodies pathogenic? J Invest Dermatol 119: 989-990.
20. Echigo T, Hasegawa M, Shimada Y, et al. (2006): Both Th1 and Th2 chemokines are elevated in sera of patients with autoimmune blistering diseases. Arch Dermatol Res 298: 38-45.
21. Hertl M, Amagai M, Sundaram H, et al. (1998): Recognition of desmoglein 3 by autoreactive T cells in pemphigus vulgaris patients and normals. J Invest Dermatol 110: 62-66.
22. Lin MS, Swartz SJ, Lopez A, et al. (1997): Development and characterization of desmoglein-3 specific T cells from patients with pemphigus vulgaris. J Clin Invest 99: 31-40.
23. Riechers R, Grötzinger J, Hertl M (1999): HLA class II restriction of autoreactive T cell responses in pemphigus vulgaris: review of the literature and potential applications for the development of a specific immunotherapy. Autoimmunity 30: 183-196.
24. Lin MS, Fu CL, Aoki V, et al. (2000): Desmoglein-1-specific T lymphocytes from patients with endemic pemphigus foliaceus (fogo selvagem). J Clin Invest 105: 207-213.
25. Büdinger L, Borradori L, Yee C, et al. (1998): Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. J Clin Invest 102: 2082-2089.
26. Lin MS, Fu CL, Giudice GJ, et al. (2000): Epitopes targeted by bullous pemphigoid T lymphocytes and autoantibodies map to the same sites on the bullous pemphigoid 180 ectodomain. J Invest Dermatol 115: 955-961.
27. Bhol K, Natarajan K, Nagarwalla N, et al. (1995): Correlation of peptide specificity and IgG subclass with pathogenic and nonpathogenic autoantibodies in pemphigus vulgaris: a model for autoimmunity. Proc Natl Acad Sci U S A 92: 5239-5243.
28. Spaeth S, Riechers R, Borradori L, et al. (2001): IgG, IgA and IgE autoantibodies against the ectodomain of desmoglein 3 in active pemphigus vulgaris. Br J Dermatol 144: 1183-1188.
29. Bernard P, Aucouturier P, Denis F, Bonnetblanc JM (1990): Immunoblot analysis of IgG subclasses of circulating antibodies in bullous pemphigoid. Clin Immunol Immunopathol 54: 484-494.
30. Christophoridis S, Büdinger L, Borradori L, et al. (2000): IgG, IgA and IgE autoantibodies against the ectodomain of BP180 in patients with bullous and cicatricial pemphigoid and linear IgA bullous dermatosis. Br J Dermatol 143: 349-355.
31. Bowszyc-Dmochowska M (2001): Studies on pathogenesis of bullous pemphigoid. Poznan, Poland: Ph.D. Doctoral thesis, Poznan University of Medical Sciences.
32. Funakoshi T, Lunardon L, Ellebrecht CT, et al. (2012): Enrichment of total serum IgG4 in patients with pemphigus. Br J Dermatol 167: 1245-1253.
33. Gondo A, Saeki N, Tokuda Y (1987): IgG4 antibodies in patients with atopic dermatitis. Br J Dermatol 117: 301-310.
34. Zhang K, Mills FC, Saxon A (1994): Switch circles from IL-4-directed epsilon class switching from human B lymphocytes. Evidence for direct, sequential, and multiple step sequential switch from mu to epsilon Ig heavy chain gene. J Immunol 152: 3427-3435.
35. Shirakata Y, Shiraishi S, Sayama K, Miki Y (1990): Subclass characteristics of IgG autoantibodies in bullous pemphigoid and pemphigus. J Dermatol 17: 661-666.
36. Bhol K, Mohimen A, Ahmed AR (1994): Correlation of subclasses of IgG with disease activity in pemphigus vulgaris. Dermatology (Basel) 189 Suppl 1: 85-89.
37. Kricheli D, David M, Frusic-Zlotkin M, et al (2000): The distribution of pemphigus vulgaris-IgG subclasses and their reactivity with desmoglein 3 and 1 in pemphigus patients and their first-degree relatives. Br J Dermatol 143: 337-342.
38. Buschman KE, Seraly M, Thong HY, et al. (2002): A predominant IgG4 subclass may be responsible for false-negative direct immunofluorescence in bullous pemphigoid. J Cutan Pathol 29: 282-286.
39. Bowszyc-Dmochowska M, Dmochowski M (2002): Immediate hypersensitivity phenomena in bullous pemphigoid: critical concepts. J Med 33: 189-198.
40. Pietkiewicz P, Torz M, Gornowicz-Porowska J, et al. (2012): Contemporary methods of treatment of pemphigus vulgaris from the point of view of a junior physician. Dermatol Klin 14: 75-81.
41. Bernard P, Prost C, Aucouturier P, et al. (1991): The subclass distribution of IgG autoantibodies in cicatricial pemphigoid and epidermolysis bullosa acquisita. J Invest Dermatol 97: 259-263.
42. Bird P, Friedmann PS, Ling N, et al. (1986): Subclass distribution of IgG autoantibodies in bullous pemphigoid. J Invest Dermatol 86: 21-25.
43. Delaporte E, Dubost-Brama A, Ghohestani R, et al. (1996): IgE autoantibodies directed against the major bullous pemphigoid antigen in patients with a severe form of pemphigoid. J Immunol 157: 3642-3647.
44. Teraki Y, Hotta T, Shiohara T (2001): Skin-homing interleukin-4 and -13-producing cells contribute to bullous pemphigoid: remission of disease is associated with increased frequency of interleukin-10-producing cells. J Invest Dermatol 117: 1097-1102.
45. Huilgol SC, Black MM (1995): Management of the immunobullous disorders. II. Pemphigus. Clin Exp Dermatol 20: 283-293.
46. Brooks WS, Lee YY, Abell E, Deng JS (1989): Comparison of IgG subclasses and complement binding activity of autoantibodies from patients with bullous pemphigoid and pemphigus. J Clin Lab Anal 3: 307-311.
47. Zhou S, Wakelin SH, Allen J, Wojnarowska F (1998): Blister fluid for the diagnosis of subepidermal immunobullous diseases: a comparative study of basement membrane zone autoantibodies detected in blister fluid and serum. Br J Dermatol 139: 27-32.
48. Pietkiewicz P, Gornowicz-Porowska J, Dmochowski M, Bowszyc-Dmochowska M (2012): Serum IgG4 antibody examination in patients with IgG-mediated autoimmune subepidermal blistering dermatoses increases autoimmunity detection. Przegl Dermatol 99: 520.
49. Schur PH (1972): Human gamma-g subclasses. Prog Clin Immunol 1: 71-104.
50. Aalberse RC, van der Gaag R, van Leeuwen J (1983): Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. J Immunol 130: 722-726.

51. Suzuki M, Harada S, Yaoita H (1992): Purification of bullous pemphigoid IgG subclasses and their capability for complement fixation. Acta Derm Venereol 72: 245-249.
52. Kelly SE, Cerio R, Bhogal BS, Black MM (1989): The distribution of IgG subclasses in pemphigoid gestationis: PG factor is an IgG1 autoantibody. J Invest Dermatol 92: 695-698.
53. McConell I, Munro A, Waldman H (1981): Complement. In: The Immune System. 2nd ed. McConell I, Munro A, Waldman H (eds.). Blackwell Scientific Publications, Oxford, UK; 40-54.
54. Lucisano Valim YM, Lachmann PJ (1991): The effect of antibody isotype and antigenic epitope density on the complement-fixing activity of immune complexes: a systematic study using chimaeric anti-NIP antibodies with human Fc regions. Clin Exp Immunol 84: 1-8.
55. Perelmutter L (1983): IgG4 and the immune system. Clin Rev Allergy 1: 267-287.
56. Nakagawa T, De Weck AL (1983): Membrane receptors for the IgG4 subclass on human basophils and mast cells. Clin Rev Allergy 1: 197-206.
57. Döpp R, Schmidt E, Chimanovitch I, et al. (2000): IgG4 and IgE are the major immunoglobulins targeting the NC16A domain of BP180 in Bullous pemphigoid: serum levels of these immunoglobulins reflect disease activity. J Am Acad Dermatol 42: 577-583.
58. Mihai S, Chiriac MT, Herrero-González JE, et al. (2007): IgG4 autoantibodies induce dermal-epidermal separation. J Cell Mol Med 11: 1117-1128.
59. Giudice GJ, Emery DJ, Zelickson BD, et al. (1993): Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. J Immunol 151: 5742-5750.
60. Matsumura K, Amagai M, Nishikawa T, Hashimoto T (1996): The majority of bullous pemphigoid and herpes gestationis serum samples react with the NC16a domain of the 180-kDa bullous pemphigoid antigen. Arch Dermatol Res 288: 507-509.
61. Zillikens D, Rose PA, Balding SD, et al. (1997): Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. J Invest Dermatol 109: 573-579.
62. Perriard J, Jaunin F, Favre B, et al. (1999): IgG autoantibodies from bullous pemphigoid (BP) patients bind antigenic sites on both the extracellular and the intracellular domains of the BP antigen 180. J Invest Dermatol 112: 141-147.
63. Hofmann S, Thoma-Uszynski S, Hunziker T, et al. (2002): Severity and phenotype of bullous pemphigoid relate to autoantibody profile against the NH2- and COOH-terminal regions of the BP180 ectodomain. J Invest Dermatol 119: 1065-1073.
64. van der Zee JS, van Swieten P, Aalberse RC (1986): Serologic aspects of IgG4 antibodies. II. IgG4 antibodies form small, nonprecipitating immune complexes due to functional monovalency. J Immunol 137: 3566-3571.
65. García BE, Sanz ML, Gato JJ, et al. (1993): IgG4 blocking effect on the release of antigen-specific histamine. J Investig Allergol Clin Immunol 3: 26-33.
66. Yamada H, Hashimoto T, Nishikawa T (1989): IgG subclasses of intercellular and basement membrane zone antibodies: the relationship to the capability of complement fixation. J Invest Dermatol 92: 585-587.
67. David M, Katzenelson V, Hazaz B, et al. (1989): Determination of IgG subclasses in patients with pemphigus with active disease and in remission. Arch Dermatol 125: 787-790.
68. David M, Katzenelson V, Mimouni D, Milner Y (2006): The distribution of pemphigus vulgaris-IgG subclasses in patients with active disease. J Eur Acad Dermatol Venereol 20: 232.
69. Dmochowski M, Hashimoto T, Nishikawa T (1992): The analysis of IgG subclasses of anti-intercellular antibodies in pemphigus by an immunoblot technique. Arch Dermatol Res 284: 309-311.
70. Hacker MK, Janson M, Fairley JA, Lin MS (2002): Isotypes and antigenic profiles of pemphigus foliaceus and pemphigus vulgaris autoantibodies. Clin Immunol 105: 64-74.
71. Hacker-Foegen MK, Janson M, Amagai M, et al. (2003): Pathogenicity and epitope characteristics of anti-desmoglein-1 from pemphigus foliaceus patients expressing only IgG1 autoantibodies. J Invest Dermatol 121: 1373-1378.
72. Rock B, Martins CR, Theofilopoulos AN, et al. (1989): The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). N Engl J Med 320: 1463-1469.
73. Warren SJ, Arteaga LA, Rivitti EA, et al. (2003): The role of subclass switching in the pathogenesis of endemic pemphigus foliaceus. J Invest Dermatol 120: 104-108.
74. Torzecka JD, Woźniak K, Kowalewski C, et al. (2007): Circulating pemphigus autoantibodies in healthy relatives of pemphigus patients: coincidental phenomenon with a risk of disease development? Arch Dermatol Res 299: 239-243.
75. Dańczak-Pazdrowska A (2005): IgG, IgG 1 and IgG 4 antibodies to desmoglein 1 in pemphigus diseases. Ph.D. Doctoral dissertation, Poznan University of Medical Sciences, Poznań.
76. Eming R, Büdinger L, Riechers R, et al. (2000): Frequency analysis of autoreactive T-helper 1 and 2 cells in bullous pemphigoid and pemphigus vulgaris by enzyme-linked immunospot assay. Br J Dermatol 143: 1279-1282.
77. Veldman C, Stauber A, Wassmuth R, et al. (2003): Dichotomy of autoreactive Th1 and Th2 cell responses to desmoglein 3 in patients with pemphigus vulgaris (PV) and healthy carriers of PV-associated HLA class II alleles. J Immunol 170: 635-642.
78. Corry DB, Kheradmand F (1999): Induction and regulation of the IgE response. Nature 402 (6760 Suppl): B18-23.
79. Rizzo C, Fotino M, Zhang Y, et al. (2005): Direct characterization of human T cells in pemphigus vulgaris reveals elevated autoantigen-specific Th2 activity in association with active disease. Clin Exp Dermatol 30: 535-540.