Rituximab and Corticosteroid Effect on Desmoglein-Specific B Cells and Desmoglein-Specific T Follicular Helper Cells in Pemphigus

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Pemphigus is an autoimmune blistering disease mediated by autoantibodies directed against desmogleins (DSGs). We recently showed that first-line treatment with rituximab (RTX) enables more patients to achieve long-lasting remission off therapy than corticosteroids alone. To understand the immunological mechanisms that mediate long-lasting clinical remission after RTX treatment, we analyzed the phenotype of DSG-specific memory B cells and DSG-specific T follicular helper cells by flow cytometry and measured antibody-secreting cells by enzyme-linked immune absorbent spot in patients treated with corticosteroids alone or RTX. This post hoc analysis of the RITUX3 trial showed that RTX induced a significant decrease of IgG-switched DSG-specific memory B cells. Accordingly, anti-DSG antibody-secreting cells were no longer detected in patients in complete remission after RTX. In contrast, corticosteroids did not modify the frequency or the phenotype of DSG-specific memory B cells, and anti-DSG antibody-secreting cells were still detected after treatment, even in patients in remission. Using peptide-HLADRBI*0402 tetramer staining, we identified DSG-3-specific T follicular helper cells, which dramatically decreased after RTX, while remaining stable after corticosteroid treatment. Our findings suggest that long-lasting response to RTX in pemphigus relies on the decrease of DSG-specific circulating T follicular helper cells, which correlates with a sustained depletion of IgG-switched memory autoreactive B cells, leading to the disappearance of anti-DSG antibody-secreting cells.

INTRODUCTION

Pemphigus is a life-threatening autoimmune disease of the skin and mucosa due to the production of antibodies (Abs) directed against two desmosomal proteins: desmoglein (DSG)-1 and DSG-3, which are adhesion molecules of the epidermis (Schmidt et al., 2019). We recently reported the long-lasting efficacy of rituximab (RTX), the anti-CD20 mAb, in patients with pemphigus (Joly et al., 2017). Relative to a standard regimen of corticosteroids (CSs) alone, RTX associated with a short-term regimen of prednisone allowed 89% of patients to achieve complete remission off therapy at month (M) 24. RTX is thought to act mainly by B-cell depletion in pemphigus, as in other autoimmune diseases (Alberici et al., 2015; Albers et al., 2017). Relapses have been reported to be associated with B-cell reconstitution. Up to now, the effect of RTX has mainly been assessed on total B cells, which does not necessarily reflect its effect on autoreactive DSG-specific B cells. In particular, we previously reported in patients with pemphigus treated with RTX as second-line treatment that long-term remission was associated with prolonged failure of anti-DSG B-cell response (Colliou et al., 2013).

Most of the anti-DSG Abs belong to the IgG4 subclass (Ellebrecht et al., 2018), which suggests the involvement of T follicular helper (TFH) cells in the selection process within the germinal center. TFH cells are characterized by the expression of CXCR5 chemokine receptor and the production of IL-21, a cytokine that is essential for the generation of memory B cells and plasma cells (Zotos et al., 2010). A higher frequency of circulating TFH cells and increased serum IL-21 levels have been recently reported in patients with pemphigus relative to healthy controls (Hennerici et al., 2016). This latter study suggested the existence of DSG-3–specific autoreactive T helper (Th) cells producing IL-21 on ex vivo stimulation with DSG-3. The aim of this postanalysis of the RITUX3 trial was to investigate the effect of RTX compared with a standard CS
regimen alone on the frequency and phenotype of DSG-specific B cells and DSG-specific TFH cells in patients with pemphigus. In particular, we studied the relationships between autoreactive B-cell and T-cell populations and long-lasting clinical remission or the onset of relapses.

RESULTS

Clinical course of patients
A total of 90 patients were randomly assigned to standard CS regimen or RTX plus short-term prednisone groups (Figure 1). At the final evaluation at M36, 44 of 46 patients (96%) from the RTX group were in complete remission off therapy, including two patients who were secondarily treated with RTX between M24 and M36, and two patients had persistent disease activity. A total of 15 patients from the CS group were in complete remission off therapy or still on minimal therapy at M24, and 29 patients had persistent disease activity. According to the protocol of the RITUX3 trial, 20 of 44 patients (45%) from the CS group were secondarily treated with RTX between M24 and M36 (after assessment of the primary endpoint at M24) owing to persistent disease activity or CS side effects. Because the treatments of patients from the CS group were no longer homogeneous during the third year of the trial, biological analyses were not performed after the M24 visit in these patients from the CS group.

Serum autoantibodies against DSG-1 and DSG-3
Relative to the RITUX3 trial (Joly et al., 2017), which reported the monitoring of biological parameters until M24, we have shown in Figure 2 the evolution of mean serum anti-DSG-1 and anti-DSG-3 Ab ELISA values of patients from the RTX group until M36. In both treatment groups, autoantibody titers decreased from baseline to M9. They then increased mainly in patients from the CS group, whereas they remained negative or at very low levels in most patients from the RTX group. At M24 evaluation, 5% of patients from the RTX group had positive serum anti-DSG-1 versus 29% of patients from the CS group (P < 0.01). Anti-DSG-3 Abs were still detected at M24 in 14% of patients from the RTX group versus 45% of those from the CS group (P < 0.01). In patients from the RTX group, positive anti-DSG-1 and anti-DSG-3 Abs were detected at the M36 evaluation in 2% and 27% of patients’ sera, respectively.

Evolution of circulating B cells and TFH cells and IL-21 serum level
The evolution of peripheral whole blood B cells, TFH cells, and serum IL-21 level during the course of treatment with RTX or CS alone is shown in Figure 3. The TFH-cell subpopulation was defined by the phenotype CD3+CD4+CD45RA−CXCR5+. This analysis was performed longitudinally in 11 patients from the RTX group and in nine...
patients from the standard CS group. Serum IL-21 levels were measured by ELISA in all patients. As expected, a dramatic decrease in peripheral whole blood B cells was observed after the initial infusion of RTX, followed by a transient increase from M9 to M12 and a further progressive increase after the last infusion of RTX at M18, whereas no significant evolution was observed in patients treated with CS alone (Figure 3a). In patients from the RTX group, a 50% decrease in TFH-cell frequency, which paralleled B-cell depletion, was observed from M3 to M24, followed by an increase of TFH cells up to baseline values, which occurred after B-cell recovery (Figure 3b). In contrast, no variation in TFH-cell frequency was observed in patients treated with a standard CS regimen. A dramatic and persistent decrease of serum IL-21 level was observed from baseline to M36 in patients treated with RTX. Conversely, whereas only a transient decrease of IL-21 level was observed from baseline to M3 in patients from the standard CS group, IL-21 levels increased after M3 in these patients once CS doses were tapered under 20 mg/day (Figure 3c).

To assess the potential prognostic value of serum level of IL-21 and TFH-cell frequency, we compared these parameters between patients with long-lasting clinical remission and those who relapsed during the follow-up. Because too few patients in the RTX group had persistent disease activity, we performed these experiments in patients treated with CS alone. We did not observe a statistically significant difference in IL-21 serum level (P = 0.53) or frequency of whole TFH cells (P = 0.80) between patients with prolonged remission and those who further relapsed.

**Immunophenotyping of DSG-1— and DSG-3—specific B cells**

DSG-1— and DSG-3—specific circulating B cells were detected in blood samples collected at baseline from 30 patients with pemphigus at frequencies ranging from 0.11% to 0.54% (mean ± SEM: 0.23 ± 0.01%) for DSG-1—specific B cells and from 0.1% to 0.6% (mean: 0.21 ± 0.01%) for DSG-3—specific B cells of whole CD19+ B cells (baseline frequencies in Supplementary Figure S1a and b). DSG-specific B cells from patients with pemphigus were more frequently CD27+ and class-switched IgG+ CD27+ than whole B cells (CD27+: 51 ± 2% of DSG-specific B cells vs. 31 ± 3% of whole CD19+ B cells, P < 0.0001; IgG+ CD27+: 15.5 ± 1.4% of DSG-specific B cells vs. 8.4 ± 1.2% of whole CD19+ B cells, P < 0.0001) (Supplementary Figure S1b and c).

To assess the differential effects of CS alone and CS combined with RTX, we analyzed the evolution of
DSG-1− and DSG-3−specific B cells at M36 (corresponding to 18 Ms after the last infusion of RTX) in patients from the RTX group and between M9 and M12 (when prednisone doses were tapered < 20 mg/day) in patients from the CS group. Surprisingly, DSG-1− and DSG-3−specific B cells were still detected at M36 evaluation in patients in complete remission after RTX treatment at mean frequencies of 0.23 ± 0.01% and 0.23 ± 0.02% of B cells, respectively, which were close to the frequencies measured at baseline (Figure 4a and b). The mean frequency of DSG-1− and DSG-3−specific CD27+ memory B cells decreased from baseline to M36 after RTX treatment (DSG-1+ B cells: 55 ± 4% vs. 36 ± 4%, P < 0.001; DSG-3+ B cells: 48 ± 4% vs. 36 ± 5%, P < 0.05) (Figure 4c). Additionally, the frequency of class-switched DSG-specific CD27+ IgG+ B cells dramatically decreased from baseline to M36 after RTX (DSG-1+ B cells: 13.5 ± 1.6% vs. 3.8 ± 1.0%, P < 0.001; DSG-3+ B cells: 13.0 ± 2.4% vs. 4.5 ± 0.9%, P < 0.01) (Figure 4d). The one patient whose blood sample was available out of two patients with persistent disease activity after RTX had the highest frequency of class-switched DSG-3−specific CD27+ IgG+ B cells (25.0%, Figure 4d, black arrow). In contrast with the changes observed in the RTX group, we did not observe...
any modification in the frequency of DSG-specific class-switched CD27+IgG+ B cells from baseline to M12 in patients treated with CS alone (DSG-1+ B cells: 20.9 ± 2.7% vs. 15.9 ± 3.9; P = 0.17; DSG-3+ B cells: 18.7 ± 4.3% vs. 20.0 ± 5.3; P = 0.83) (Figure 4e–h).

**Assessment of circulating anti–DSG-1 and anti–DSG-3 IgG-secreting B cells using enzyme-linked immune absorbent spot assay**

DSG-1– and DSG-3–specific Ab-secreting-cells (ASCs) were measured in 11 patients from the RTX group (including 10 patients in complete remission off treatment and one patient with persistent disease activity) and in 11 patients from the standard CS group. At baseline, anti–DSG-1 and/or anti–DSG-3 IgG-ASCs were detected both in patients from the RTX group (0.10 ± 0.07% and 0.43 ± 0.16%, respectively) and in those from the standard CS group (0.10 ± 0.05% and 0.32 ± 0.10%, respectively) (Figure 5a and b). After B-cell recovery at M36 evaluation, anti–DSG-1 and anti–DSG-3 IgG-ASCs were no longer detectable in 10 patients from the RTX group who were in clinical remission (P < 0.01 versus baseline samples), whereas 0.17% anti–DSG-3 IgG-ASCs were still detected in the one patient with persistent mucosal lesions (Figure 5a, black arrow and Figure 5c). In contrast, anti–DSG-1 and/or anti–DSG-3 IgG-ASCs were still detected after treatment in patients from the CS group, even in those in clinical remission, although at a lower frequency than at baseline (DSG-1 ASC: 0.10 ± 0.05 at baseline vs. 0.01 ± 0.01 at M12, P = 0.5: DSG-3 ASC: 0.32 ± 0.10 at baseline vs. 0.089 ± 0.050 % at M12; P < 0.05) (Figure 5b and c).

**Effect of treatments on frequencies and phenotype of circulating DSG-3–specific Th cells and TFH cells**

Because anti–DSG-1 and anti–DSG-3 IgG-ASCs were no longer detected in patients in complete remission after RTX treatment, whereas non switched DSG-1– and DSG-3–specific naive and, to a lesser degree, memory B cells were still detected after RTX treatment, we further investigated DSG-3–specific Th cells and TFH cells.

Using immunodominant peptide of DSG-3 (190-204)/HLA-DRB1*0402 tetramer staining, we studied the frequency of DSG-3–specific Th cells and TFH cells at baseline and at M36 in seven patients treated with RTX and at baseline and at M9–12 in six and nine patients from the CS group respectively, who all carried the HLA-DRB1*0402 PV susceptibility allele. DSG-3–specific circulating Th cells and TFH cells were detected in blood samples collected at baseline at frequencies ranging from 0.8–4.5% (mean: 3.0 ± 0.27%) for Th cells and from 0.07–0.68% (mean: 0.24 ± 0.05%) for TFH cells of whole CD4+ T cells. The frequency of DSG-3–specific circulating Th cells and TFH cells dramatically decreased after RTX treatment (Th: 3.0 ± 0.31% at baseline vs. 0.4 ± 0.12% at M36; P < 0.0001; TFH: 0.25 ± 0.08% at baseline vs. 0.05 ± 0.009% at M36; P = 0.02) (Figure 6a and b). In contrast, although DSG-3–specific Th cells from patients treated with CS alone decreased after CS treatment (2.94 ± 0.51% at baseline vs. 0.97 ± 0.17% at M9–12, P = 0.0009), DSG-3–specific...
TFH cells were still detected after CS treatment at similar levels as at baseline, even in patients in clinical remission (0.22 ± 0.06 at baseline vs. 0.35 ± 0.10% at M9–12, P = 0.34) (Figure 6c and d).

**DISCUSSION**

This study shows that long-lasting efficacy of RTX in patients with pemphigus is associated with prolonged disappearance of circulating anti–DSG-1 and anti–DSG-3 IgG-secreting B cells and, consequently, the dramatic decrease of serum anti–DSG-1 and anti–DSG-3 IgG+ Abs, as demonstrated by enzyme-linked immune absorbent spot (ELISPOT) and ELISA, respectively. These findings contrast with the presence of circulating DSG-1– and DSG-3–specific B cells in patients in complete remission after RTX therapy at frequencies that were in fact very close to those observed at baseline before treatment, corresponding to around 0.2% of total B cells. This discrepancy was likely related to the phenotype of the reappearing B cells after the initial RTX-induced B-cell depletion, among which most of the DSG-3–specific B cells were naive non switched CD27− B cells. The decrease in CD27 expression by DSG-specific B cells is in accordance with our previous findings, which also reported a decrease in the frequency of DSG-specific memory B cells after RTX treatment (Hébert et al., 2019; Pollmann et al., 2019). It is also in accordance with the decrease in transcription of the CD27 gene that we recently reported in patients treated with RTX. The only patient who still had active disease after RTX treatment had a high frequency of memory class-switch IgG B cells among DSG-3–specific B cells (25%) and, accordingly, a high frequency of circulating anti–DSG-3 IgG-secreting B cells (0.17%) and high titer of serum anti–DSG-3 IgG+ Abs (1,500 U/ml).

Because of their capacity to regulate the maturation of B cells into memory B cells and IgG-secreting plasma cells, TFH cells have been suspected to be involved in several autoimmune disorders, such as systemic lupus erythematosus (Choi et al., 2015), rheumatoid arthritis (Arroyo-Villa et al., 2014), and IgG4-related diseases (Chen et al., 2018). A decrease of circulating TFH cells has been described in patients with Sjögren syndrome or type 1 diabetes mellitus after RTX treatment (Verstappen et al., 2017; Xu et al., 2013). An effect of RTX on DSG-specific T cells from patients with pemphigus was previously reported by the team of Hertl by using ELISPOT assay (Eming et al., 2008), who observed a decrease in the number of DSG-3–specific CD4+ Th1 and Th2 cells in patients treated with RTX. Using tetramer technology, we demonstrated the in vivo presence of DSG-3–specific Th cells and TFH cells in patients with pemphigus. Strikingly, we observed here a dramatic decrease in DSG-3–specific TFH cells in the blood of patients after RTX treatment, which remained at very low levels up to M36 after RTX treatment, whereas the whole circulating TFH-cell compartment returned to baseline values.

In addition, we observed a dramatic decrease of serum IL-21 levels, which paralleled B-cell depletion after RTX treatment. IL-21, which is highly produced by TFH cells, plays a crucial role in B-cell maturation, in particular, the production of memory B cells. It has been recently reported that T cells produced IL-21 when cultured with recombinant DSG, suggesting that the decrease of serum IL-21 levels observed after RTX treatment in this study might be at least partly explained by the decrease of circulating DSG-3–specific TFH cells (Hennerici et al., 2016).

The disappearance of DSG-1– and DSG-3–specific IgG-switched CD27+ B cells perfectly correlated with the disappearance of DSG-3–specific TFH cells and the decrease of serum IL-21 levels, which are both known to play a major role in B-cell maturation (Zotos et al., 2010).
In mice, TFH differentiation is initiated early when dendritic cells prime naive CD4\(^+\) T cells (Kitano et al., 2011). However, this priming is not sufficient to complete full TFH-cell differentiation but instead drives the production of pre-TFH cells, a partially differentiated intermediate that expresses CXCR5 and the transcriptional repressor Bcl6 (Goenka et al., 2011). Thus, the absence of B cells or the presence of B cells with irrelevant antigen-specificity impairs TFH-cell generation (Johnston et al., 2009). Moreover, maintenance of the TFH-cell phenotype requires sustained antigenic stimulation by B cells (Baumjohann et al., 2013). In a mouse model of pemphigus, adoptive transfer of DSG-3–specific B cells was not sufficient to induce the disease in recipient mice deficient for both T and B cells, whereas the cotransfer of similar B cells with activated ICOS\(^+\) TFH cells was pathogenic (Kim et al., 2020).

Our observations in patients with pemphigus are in accordance with this model in which the absence of DSG-specific B cells following RTX treatment impacts the maturation and/or maintenance of DSG-specific TFH cells, which in turn blocks the isotype switch of DSG-specific B cells.

Thus, our results collectively suggest that the blockage of reappearing B cells at the naive stage and the blockage of the class switch from IgM to IgG might play a major role in the long-lasting effect of RTX in patients with pemphigus. These findings seem likely related to a specific effect of RTX, because we did not observe such variations in the number of whole and DSG-specific TFH cells or any reversal in the balance between memory and naive DSG-specific B-cell subpopulations in patients treated with CS alone. Indeed, the persistence of DSG-specific TFH cells in these latter patients might have favored the persistence of circulating DSG-specific CD27\(^+\)IgG\(^+\) B cells and anti-DSG IgG-ASCs, which were responsible for the increase of serum anti-DSG Abs observed once CS doses had been tapered.

Overall, this study shows that the therapeutic effect of RTX in pemphigus is not only because of a long-lasting depletion of DSG-specific B cells, but rather relates to an inefficient maturation and class-switching process of DSG-specific B cells, which likely involves the long-lasting disappearance of DSG-specific TFH cells, a population that is closely associated with the anti–DSG-3 Ab response in pemphigus (Kim et al., 2020).

**MATERIALS AND METHODS**

**Patients**

A total of 90 patients, 74 with pemphigus vulgaris and 16 with pemphigus foliaceus, were included in a randomized clinical trial (RITUX3; ClinicalTrials.gov number, NCT00784589). The Ethics Committee (CPP Nord-Ouest) approved the study. Patients with newly diagnosed pemphigus were assigned to receive either 1 or 1.5 mg/kg/day of oral prednisone, with a progressive tapering of prednisone doses over M12–18, or two infusions of 1 g of RTX on day 0 and day 14, and 500 mg at M12 and M18, combined with a short-term prednisone regimen, 0.5 mg/kg/day for moderate pemphigus and 1 mg/kg/day for severe pemphigus. The initial prednisone dose was gradually reduced after achievement of disease control, with the aim to stop prednisone after M3 in patients with moderate pemphigus and after M6 in patients with severe pemphigus. Patients were followed for up to 3 years.

**Clinical and immunological evaluations**

Complete remission and relapse were defined according to the consensus statement definition for pemphigus end points (Murrell et al., 2008). Blood samples of healthy donors were from The French Blood Donor Service. Participants gave their written informed consent. Blood samples of patients were collected at baseline and at each follow-up visit until the end of the study at M36.

**Serum autoantibody titers**

Titers of serum anti–DSG-1 and anti–DSG-3 IgG Abs were measured using an ELISA (EUROIMMUN, Lübeck, Germany).

**Cytokine levels**

Serum levels of IL-21 were measured by ELISA (BioLegend, San Diego, CA), according to the manufacturer’s protocol.

**Phenotyping analysis**

PBMCs were isolated by centrifugation over Ficoll-Paque gradients (GE Healthcare Lifesciences, Chicago, IL) to assess B- and T-lymphocyte markers. The phenotype of PBMCs was determined by flow cytometry with mAbs against CD19, CD24, CD27, CD38, IgD, IgM, and IgG (Beckman Coulter, Brea, CA and BD Biosciences, Franklin Lakes, NJ). Whole circulating TFH cells were further characterized by using a combination of different mAbs against CD3, CD4, CD45RA, and CXCR5 (Miltenyi Biotec, Bergisch Gladbach, Germany and BD Biosciences). The percentages of each subpopulation were determined using FlowJo software (BD Biosciences).

**Anti-DSG B-cell analysis**

To analyze the phenotype of B cells specific for DSG-1 and DSG-3, B cells were isolated and labeled as described by Hébert et al. (2019) with recombinant DSG-1 or DSG-3 kindly provided by M. Herlt (Eming et al., 2008). The percentage and phenotype of DSG-1– and DSG-3–specific B cells were determined using FlowJo software.

**Anti–DSG-3 TFH-cell analysis**

To verify that our patients with pemphigus had the HLADRB1*0402 genotype of pemphigus vulgaris susceptibility, peripheral blood from patients was collected after obtaining informed consent. Genomic DNA from PBMCs was isolated using the Nucleospin tissue kit (Macherey-Nagel, Düren, Germany). The specific analysis of HLADRB1*04 was performed using the Olerup SSP DRB1*04 PCR Typing Kit (Bionobis, Guyancourt, France) according to the manufacturer’s protocol. Autoreactive T cells were only characterized in patients with pemphigus vulgaris with the HLADRB1*0402 genotype. Frequency and phenotype of DSG-3–specific Th cells and TFH cells were determined by flow cytometry using mAbs against CD3, CD4, CD45RA, and CXCR5 (Miltenyi Biotec and BD Biosciences) and a tetramer-based detection system using HLADRB1*0402-tetramers loaded with the DSG-3 190-204 immunodominant peptide (LNSKIAFKIVSQEPA, obtained from NIH Tetramer core facility, Emory University, Atlanta, GA). The percentages of DSG-3–specific Th cells and TFH cells were determined using FlowJo software.

**DSG-specific B-cell detection using ELISPOT assay**

The frequencies of circulating total IgG and DSG-specific IgG-ASCs were determined by human IgG ELISPOT Basic (Mabtech, Nacka Strand, Sweden). PBMCs from patients with pemphigus and healthy donors were prestimulated with RB48 (1 µg/ml) and recombinant human IL-2 (10 ng/ml) in complete medium (RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml
penicillin, and 100 µg/ml streptomycin; Thermo Fisher Scientific, Waltham, MA, USA) in separate plates for 72 hours. Plates of ELISpot MAIPS-4510 (Merck Millipore, Darmstadt, Germany) were coated overnight at 4 °C with anti-IgG human Abs. Plates were washed and blocked with complete medium before use. Prestimulated PBMCs were washed, resuspended in complete medium, transferred to plate, and incubated for 24 hours with 1 × 10^5–4 × 10^5 PBMCs per well to detect anti-DSG-1 and anti-DSG-3 IgG-secreting ASCs and with 2.5 × 10^3–1 × 10^4 PBMCs per well to detect total IgG-ASCs. IgG-ASCs were detected by addition of biotinylated mouse IgG anti-human IgG. Frequency of anti-DSG-1 or anti-DSG-3 IgG-secreting ASCs was calculated after incubation for 2 hours with histidine-tagged recombinant DSG-1 or DSG-3 proteins (1 µg/ml) in PBS with calcium (Eurobio, Les Ulis, France). Biotinylated anti-histidine (0.5 µg/ml) (Abcam, Cambridge, United Kingdom) was then added. Streptavidin enzyme and substrate tetramethylbenzidine were used to detect spots. The number of spots was determined with ELISpot Plate Readers and ImmunoSpot software (CTL Europe GmbH, Bonn, Germany). Results were expressed as frequencies of DSG-specific IgG-ASCs among total IgG-ASCs. The sensitivity and specificity of our ELISpot assay were estimated at 1 DSG-1-specific ASC/10^5 total ASCs and 100%, respectively.

**Statistical analysis**

Data are presented as mean ± SD. Prism software (Graph Pad, San Diego, CA) was used for statistical analysis. Statistical analysis was performed by Fisher’s exact test to compare the frequencies of patients with DGS Abs between RTX and CS groups. The paired t-test was performed to compare patients before and after treatment. For multiple analyses, one-way ANOVA with Dunnett post-test or two-way ANOVA with Sidak’s multiple comparisons test were used. A two-way ANOVA with Sidak’s multiple comparisons test was used. A P < 0.05 was considered significant for all analyses.

**CONFLICT OF INTEREST**

MH has received unrestricted grant funds from Topas Therapeutics, Hamburg, Germany. PJ is a consultant for Roche, GlaxoSmithKline, Eli Lilly, PrincipiaBio, Sanofi Aventis, Argenx. The remaining authors state no conflict of interest.

**ACKNOWLEDGMENTS**

This study was supported by INSERM, Normandie University, the French Programme Hospitalier de Recherche Clinique, French Ministry of Health (No 2008-003266-31), the French Society of Dermatology, The Inspire Program from the Region Occitanie/Pyrénées-Méditerranée (#1901175), and the European Regional Development Fund (MP0022856). We thank the French Study Group on Autoimmune Bullous Skin Diseases (Catherine Prost-Squarcioni, Bruno Labéille, Catherine Picard-Dahan, Maria Polina Kontanistou, Guillaume Chaby, Marie-Alex Richard, Jean David Bouazzz, Sophie Duvert-Lehembre, Philippe Bernard, Frederic Caux, Marina Alexandre, Saska Ingen-Housz-Oro, Pierre Valbre, Emmanuel Delaporte, Gaelle Quereux, Alain Dupuy, Sebastien Debabarieux, Martine Avenel-Audran, Michel D’Incan, Christophe Bedane, Nathalie Bénétton, Denis Jullien, Nicolas Dupin, Laurent Misery, Laurent Machet, Marie Belylot-Bary, Olivier Dereure, and Bruno Sassolas). We thank Françoise Hau, Fabienne Jouen, and Vincent Ferranti for technical assistance. We are grateful to Nikki Sabourin-Gibbs, Rouen University Hospital, for editing the manuscript.

**AUTHOR CONTRIBUTIONS**

Conceptualization: MMV, SC, MV, NF, PJ; Formal Analysis: MMV; Funding Acquisition: NF, PJ; Investigation: MMV, CP, MLG, VH, FC, CM, GR, MP; Methodology: MMV, SC, NF; Project Administration: NF, PJ; Resources: NH, OB, NF, PJ; Supervision: SC, NF, PJ; Validation: MMV, CP; Visualization: MMV, SC; Writing - Original Draft Preparation: MMV, SC, NF, PJ; Writing - Review and Editing: MMV, CP, MLG, VH, FC, CM, GR, MP, MV, NH, OB, SC, NF, PJ

**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.01.031.

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Supplementary Figure S1. Frequency and phenotype of peripheral blood DSG-1— and DSG-3—specific B cells evaluated by flow cytometry in patients with pemphigus at D0 and in HDs. Frequency of (a) DSG-1— and (b) DSG-3—specific B cells among purified CD19+ B cells in patients with pemphigus (gray squares) and in HDs (white circles). Phenotype of B-cell subpopulations: (c) Memory B cells (CD19+CD27+), and (d) class-switched memory B cells (CD19+CD27+IgG+) among whole B cells and DSG-specific B cells. *P < 0.05; **P < 0.01; ***P < 0.001. D0, baseline; DSG, desmoglein; HD, healthy donor.