The impact of lesional inflammatory cellular infiltrate on the phenotype of bullous pemphigoid

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Abstract

Background The influence of cutaneous cellular infiltration on the phenotype of bullous pemphigoid (BP) remains to be established.

Objectives To evaluate the main histopathological characteristics of patients with BP and to assess the association between the composition of lesional inflammatory infiltrate and the various clinical, immunological and immunopathological aspects of the disease.

Methods Retrospective study encompassing patients diagnosed with BP throughout the years 2009–2020 in a specialized tertiary referral centre.

Results The study encompassed 136 patients with BP, of whom 27 (19.9%) demonstrated a cell-poor inflammatory infiltrate in lesional skin specimens. Overall, 78 (57.4%), 71 (52.2%) and 5 (3.7%) specimens were found to include eosinophil-predominant, lymphocyte-predominant and neutrophil-predominant inflammatory infiltrates, respectively. Relative to the remaining patients with BP, those with an eosinophil-predominant inflammatory infiltrate had higher (90.8% vs. 77.2%; \( P = 0.030 \)) whilst those with a cell-poor inflammatory infiltrate lower (70.3% vs. 88.7%; \( P = 0.017 \)) seropositivity of anti-BP180 NC16A IgG. The latter subgroup presented with higher prevalence of mucosal involvement (25.9% vs. 8.3%; \( P = 0.011 \)) and a non-inflammatory clinical phenotype (50.0% vs. 17.1%; \( P = 0.041 \)). Patients with lymphocyte-predominant inflammatory infiltrate manifested with higher severity BPDAl scores and a lower frequency of the non-inflammatory subtype (11.1% vs. 36.4%; \( P = 0.035 \)), whilst those with a neutrophilic infiltrate presented with lower mean (SD) levels of anti-BP180 NC16A IgG [269.3 (227.6) vs. 722.7 (1499.6) U/mL; \( P = 0.003 \)].

Conclusions Eosinophil-predominance and high cellularity in the lesional inflammatory infiltrate of BP skin are associated with increased seropositivity of anti-BP180 NC16A IgG. Lymphocyte-predominant infiltrates predict a more severe phenotype, pointing towards a pathogenic role of autoreactive lymphocytes.

Conflict of interest

None.

Funding sources

Clinical Research Unit Pemphigoid Diseases (KFO 303) and Cluster of Excellence Precision Medicine in Chronic Inflammation (EXC 2167), both funded by Deutsche Forschungsgemeinschaft.

Introduction

Bullous pemphigoid (BP) is the most prevalent subepidermal autoimmune blistering disease (SAIBDs) worldwide. The disease is characterized by the presence of circulating IgG autoantibodies targeting BP180 and/or BP230, two components of the junctional adhesion complexes, called hemidesmosomes, which promote dermal–epidermal cohesion. The major epitopes on BP180 cluster within the juxtamembranous non-collagenous 16A (NC16A) domain, which has been identified as the immunodominant region, with up to 90% of BP sera reacting to it. BP230 is targeted in about 50% of patients, with the most immunogenic epitopes located in the globular C-terminal portion.

Histologic sections of BP classically demonstrate a subepidermal blister with a variable degree of inflammatory infiltrate comprised of lymphocytes, neutrophils and characteristically

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eosinophils. In the prodromal, non-bullous phase of the disease, urticarial lesions may present with eosinophilic spongiosis, without a frank subepidermal clef

Whilst direct immunofluorescence (DIF) and immunoserological assays embody an indispensable role in establishing the diagnosis of BP, revealing the histological features and determining the types of inflammatory cells infiltrating lesional skin are still of value to differentiate between different SAIBDs.

The influence of different histological features on the morphological and immunological phenotype of BP remains poorly understood. The primary objective of the current study was to evaluate the histopathological characteristics of a relatively large cohort of patients with BP. The secondary objective was to throw light on the association between the compositions of lesional inflammatory infiltrate and the various clinical, immunological and immunopathological aspects of the disease.

Methods

Study population and eligibility criteria

We conducted a retrospective cohort study, including patients diagnosed with BP between January 1st, 2009, and February 28th, 2020, in a referral centre for autoimmune bullous diseases at the Department of Dermatology, University of Lübeck, Lübeck, Germany. The current study was approved by the institutional review board (20-110A).

Diagnosis of BP was established in accordance with the following eligibility criteria: (i) suggestive clinical presentation; (ii) linear deposits of immunoreactants along the dermal–epidermal junction (DEJ) by DIF microscopy of a perilesional skin biopsy; (iii) detection of circulating autoantibodies binding to the epidermal side of 1 mL salt-split normal human skin by indirect immunofluorescence (IIF) microscopy and/or the presence of circulating IgG autoantibodies against BP180 NC16A and/or BP230, as identified by enzyme-linked immunosorbent assay (ELISA).

The current study encompassed only patients who underwent lesional skin biopsies and had thorough access to histopathological specimens.

Definition of covariates

Haematoxylin and eosin-stained lesional skin specimens were independently reviewed and scored by three board-certified dermatologists with compatible training in dermatopathology (KK, CMH, and AV). Eligible patients' skin biopsies were divided into cell-rich, cell-intermediate and cell-poor based on the intensity of the inflammatory infiltrate (Fig. 1). Lesional cutaneous specimens were evaluated for the presence of eosinophilic, neutrophilic and lymphocytic inflammatory infiltration. The density of these inflammatory infiltrates was scored in ascending order from '0' to '3', with '0' representing a complete absence of the inflammatory infiltrate whilst '3' mirroring a highly dense

Figure 1  Lesional histopathological sections of BP patients showing cell-poor (a), cell-intermediate (b), cell-rich (c), eosinophil- (d), lymphocyte- (e) and neutrophil (f)-predominant inflammatory infiltrates. * in (e) represents a subepidermal cleft.
inflammatory infiltrate (Fig. S1). Inflammatory infiltrates scored ‘2’ and ‘3’ were considered as predominant with the respective inflammatory cell infiltrate, that is, eosinophil-, lymphocyte- and neutrophil-predominant (Fig. 1). Hypothetically, a certain specimen may include more than a single predominant inflammatory cell.

Lesional cutaneous specimens were additionally screened for the presence of obvious subepidermal cleft, acanthosis, parakeratosis and eosinophilic spongiosis. All analysed skin specimens were collected prior to the initiation of immunosuppressive/immunomodulatory agents or topical corticosteroid treatment.

The severity of BP was assessed based on the Bullous Pemphigoid Disease Area Index (BPDAI). This score had been documented, including its four subcomponents (cutaneous erosion/blister, cutaneous urticaria/erythema, damage and pruritus). Since the aforementioned scoring system was introduced only in 2012, the BPDAI score was available for only 65 out of 136 (47.8%) patients. The non-inflammatory, or the exclusively erosive, phenotype was defined in patients whose BPDAI urticaria/erythema score was zero.

Previously published protocols were followed for DIF and IIF on 1 M salt-split normal human skin and monkey oesophagus. The levels of serum anti-BP180 NC16A and anti-BP230 IgG autoantibodies were measured by ELISA (Euroimmun, Lübeck, Germany). Seropositivity was determined based on the cut-off values proposed by the manufacturer (i.e. 20 U/mL). Owing to their influence on clinical manifestation and prognosis, circulating eosinophil counts and C-reactive protein (CRP) levels were measured prior to the administration of any systemic management.

Statistical analysis
Baseline characteristics were described by means and standard deviations (SDs) for continuous variables, whereas categorical variables were described by percentages. The comparison of clinical, immunological and immunopathological variables between subgroups was performed using the Chi-square test and t-test for categorical and continuous variables, respectively. SPSS software, version 25 (SPSS, Armonk, NY, USA: IBM Corp.), was utilized to perform all statistical analyses.

Results

Demographic characteristics of the study population
The study population included 136 patients with BP, of whom 54 (39.7%) were males and 82 (60.3%) females. The mean age (SD) at diagnosis was 79.7 (9.6) years, and the median (range) age was 81.1 (49.6–98.2) years.

The main findings of histopathological analysis
Overall, 109 (80.1%) patients demonstrated cell-rich or cell-intermediate inflammatory infiltrate, whereas 27 (19.9%) patients displayed cell-poor inflammatory infiltrate in lesional skin specimens. Lymphocytic infiltration (at different degrees) was observed in all specimens. Whilst eosinophilic infiltrate was observed in the vast majority of patients (n = 122; 89.7%), neutrophilic infiltrate was less frequently encountered (n = 22; 16.2%). When categorizing the specimens according to the predominant cell in the infiltrate, 78 (57.4%) specimens were found to include eosinophil-predominant inflammatory infiltrate, whereas 71 (52.2%) and 5 (3.7%) constituted of lymphocyte-predominant and neutrophil-predominant inflammatory infiltrates, respectively.

Regarding additional histologic items investigated, eosinophilic spongiosis was observed in nine (6.6%) specimens, flame figures were not observed. Acanthosis (n = 24; 17.6%) and parakeratosis (n = 20; 14.7%) were the most frequent epidermal alterations.

Table 1 differentiates between patients with cell-poor inflammatory infiltrate (n = 27) relative to those with cell-rich or cell-intermediate inflammatory infiltrates (n = 109). Patients in the former subgroup demonstrated lower seropositivity of anti-BP180 NC16A IgG (70.3% vs. 88.7%; P = 0.017), higher prevalence of mucosal involvement (25.9% vs. 8.3%; P = 0.011) and non-inflammatory phenotype (50.0% vs. 17.1%; P = 0.041).

Whilst patients with cell-poor inflammatory infiltrate had significantly lower prevalence of subepidermal cleft (37.0% vs. 66.1%; P = 0.006), eosinophil-predominant (14.8% vs. 67.9%; P < 0.001) and lymphocyte-predominant (14.8% vs. 61.5%; P < 0.001) inflammatory infiltrate, they presented higher frequency of parakeratosis (29.6% vs. 11.0%; P = 0.014).

The association between cellularity of the lesional inflammatory infiltrate and the features of BP

Table 2 elucidates differences between patients with eosinophil-predominant inflammatory infiltrate (n = 58) and without (n = 28) eosinophil-predominant inflammatory infiltrate. A higher seropositivity rate of anti-BP180 NC16A IgG (90.8% vs. 77.2%; P = 0.030) but lower seropositivity rate of anti-DEJ IgG by IIF on monkey oesophagus (69.3% vs. 85.5%; P = 0.033) were encountered amongst patients with eosinophil-predominant inflammatory infiltrate.

On the histological level, patients with eosinophil-predominant inflammatory infiltrate exhibited a higher frequency of subepidermal blister (71.8% vs. 44.8%; P = 0.001), and lower frequency of cell-poor inflammatory infiltrate (12.2% vs. 31.5%; P = 0.006). Parakeratosis was less common amongst patients with eosinophil-predominant inflammatory infiltrate (Table 2).

The association of lymphocyte-predominant inflammatory infiltrate with the features of BP

Relative to the remaining study participants (n = 65), patients with lymphocyte-predominant inflammatory infiltrate
(n = 71) presented with a more severe phenotype. The latter was mirrored by higher mean (SD) levels of erosion/blister BPDAI [28.1 (23.7) vs. 19.0 (11.4); P = 0.046], urticaria/erythema BPDAI [21.5 (21.5) vs. 7.6 (9.1); P = 0.007] and pruritus BPDAI [21.3 (8.6) vs. 16.8 (8.6); P = 0.040] scores. The frequency of the non-inflammatory subtype of BP (11.1% vs. 36.4%; P = 0.035) and cell-poor inflammatory lesional infiltrate (5.6% vs. 35.4%; P < 0.001) was lower amongst patients with lymphocyte-predominant inflammatory infiltrate (Table 3).

| Table 1 | Clinical, immunological, histological and immunopathological characteristics of BP patients with cell-poor as compared to cell-rich inflammatory and cell-intermediate infiltrate in lesional skin specimens |
|---------|----------------------------------------------------------------------------------------------------------|
|         | BP with cell-poor inflammatory infiltrate (n = 27) | BP with cell-rich and -intermediate inflammatory infiltrate (n = 109) | P value |
| Age at diagnosis; years | Mean (SD) | 78.8 (10.0) | 80.0 (9.6) | 0.497 |
| Sex, n (%) | | | | |
| Male | 10 (37.0%) | 44 (40.4%) | 0.752 |
| Female | 17 (63.0%) | 65 (59.6%) | |
| DPP4i-associated BP, n (%) | 0 (0.0%) | 11 (10.1%) | 0.085 |
| PD-1-associated BP, n (%) | 0 (0.0%) | 2 (1.8%) | 0.478 |
| Mucosal involvement, n (%) | 7 (25.9%) | 9 (8.3%) | 0.011 |
| Mean BPDAI severity score (SD) | | | |
| Erosion/blister cutaneous activity* | 27.6 (37.4) | 23.4 (14.4) | 0.720 |
| Urticaria/Erythema activityb | 19.1 (35.0) | 14.5 (13.6) | 0.723 |
| Pruritus scoreb | 18.5 (6.0) | 19.8 (9.3) | 0.639 |
| Damage scoreb | 1.3 (2.7) | 2.0 (3.1) | 0.867 |
| Non-inflammatory (exclusively erosive) phenotype, n (%)b | 4 (50.0%) | 7 (17.1%) | 0.041 |
| BP180 NC16A ELISAc | | | |
| Seropositivity, n (%) | 19 (70.3%) | 94 (88.7%) | 0.017 |
| ELISA value, mean (SD); U/mL | 603.2 (1602.3) | 663.3 (1336.1) | 0.842 |
| BP230 ELISAd | | | |
| Seropositivity, n (%) | 4 (50.0%) | 18 (54.5%) | 0.817 |
| ELISA value, mean (SD); U/mL | 116.5 (210.5) | 168.7 (374.8) | 0.708 |
| Indirect immunofluorescence seropositivity, n (%) | | | |
| Salt split human skin* | 26 (96.3%) | 98 (96.3%) | 0.565 |
| Monkey oesophagusf | 23 (85.2%) | 76 (73.8%) | 0.216 |
| Histological findings in lesional skin specimens, n (%) | | | |
| Eosinophil-predominant; infiltrate | 4 (14.8%) | 74 (67.9%) | <0.001 |
| Eosinophilic infiltrate | 21 (77.8%) | 101 (92.7%) | 0.023 |
| Neutrophil-predominant infiltrate | 1 (3.7%) | 4 (3.7%) | 0.993 |
| Neutrophilic infiltrate | 2 (7.4%) | 20 (18.3%) | 0.167 |
| Lymphocyte-predominant infiltrate | 4 (14.8%) | 67 (61.5%) | <0.001 |
| Lymphocytic infiltrate | 27 (100.0%) | 109 (100.0%) | 1.000 |
| Subepidermal blister | 10 (37.0%) | 72 (66.1%) | 0.006 |
| Eosinophilic spongiosis | 1 (3.7%) | 8 (7.3%) | 0.496 |
| Parakeratosis | 8 (29.6%) | 12 (11.0%) | 0.014 |
| Acanthosis | 6 (22.2%) | 18 (16.5%) | 0.486 |
| Linear deposits of immunoreactants by direct immunofluorescence, n (%)g | | | |
| IgG | 20 (83.3%) | 87 (85.3%) | 0.809 |
| IgA | 2 (8.3%) | 8 (7.8%) | 0.936 |
| C3 | 21 (87.5%) | 89 (87.3%) | 0.974 |
| Isolated C3 | 4 (16.7%) | 13 (12.7%) | 0.613 |
| Eosinophil count, mean (SD); cells/µLh | 1157.8 (790.3) | 1408.5 (1107.3) | 0.513 |
| C-reactive protein, mean (SD); mg/Li | 46.6 (45.5) | 30.0 (25.3) | 0.344 |

Was available in in 65a, 49b, 133c, 41d, 132e, 126f, 81g, 58h. Bold: significant values.

BP, bullous pemphigoid; CI, confidence interval; DPP4i, dipeptidyl peptidase 4 inhibitor; n, number; PD-1, programmed death 1; SD, standard deviation.
Table 2 Clinical, immunological, histological and immunopathological characteristics of BP patients with and without eosinophil-predominant inflammatory infiltrate

|                              | BP with eosinophil-predominant inflammatory infiltrate (n = 78) | BP without eosinophil-predominant inflammatory infiltrate (n = 58) | P value |
|------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------|
| Age at diagnosis; years      | Mean (SD) 80.0 (9.6)                                          | 78.8 (10.0)                                                    | 0.497   |
| Sex, n (%)                   | Male 32 (41.0%)                                               | 22 (37.9%)                                                    | 0.715   |
|                              | Female 46 (59.0%)                                             | 36 (62.1%)                                                    |         |
| DPP4i-associated BP, n (%)   | 6 (7.7%)                                                      | 5 (8.6%)                                                      | 0.844   |
| PD-1-associated BP, n (%)    | 1 (1.3%)                                                      | 1 (1.7%)                                                      | 0.832   |
| Mucosal involvement, n (%)   | 9 (11.5%)                                                     | 7 (12.1%)                                                     | 0.924   |
| Mean BPDAI severity score (SD)|                                                             |                                                              |         |
| Erosion/blister cutaneous activity | 24.3 (14.5)                                              | 23.8 (28.3)                                                    | 0.938   |
| Urticaria/Erythema activity  | 14.0 (14.1)                                                   | 17.8 (25.3)                                                    | 0.500   |
| Pruritus score               | 19.6 (8.9)                                                     | 19.6 (8.8)                                                     | 0.974   |
| Damage score                 | 2.0 (3.0)                                                      | 1.6 (3.1)                                                      | 0.636   |
| Non-inflammatory (exclusively erosive) phenotype, n (%) | 6 (18.2%)                                                   | 5 (31.3%)                                                     | 0.304   |
| BP180 NC16A ELISA         | Seropositivity, n (%) 69 (90.8%)                              | 44 (77.2%)                                                    | 0.030   |
|                             | ELISA value, mean (SD); U/mL 794.7 (1657.3)                   | 459.6 (892.7)                                                 | 0.169   |
| BP230 ELISA                | Seropositivity, n (%) 10 (52.6%)                              | 12 (54.5%)                                                    | 0.902   |
|                             | ELISA value, mean (SD); U/mL 131.9 (239.7)                    | 181.5 (423.4)                                                 | 0.654   |
| Indirect immunofluorescence  | Seropositivity, n (%) Slight split human skin                 | 73 (94.8%)                                                    | 0.622   |
|                             | Monkey oesophagus                                             | 52 (69.3%)                                                    | 0.033   |
| Histological findings in lesional skin specimens, n (%) |                                                             |                                                              |         |
| Cell-poor infiltrate         | 4 (5.1%)                                                      | 23 (39.7%)                                                    | <0.001  |
| Eosinophilic infiltrate      | 78 (100.0%)                                                   | 44 (75.9%)                                                    | <0.001  |
| Neutrophil-predominant infiltrate | 1 (1.3%)                                                   | 4 (6.9%)                                                      | 0.085   |
| Neutrophilic infiltrate      | 13 (16.7%)                                                    | 9 (15.5%)                                                     | 0.857   |
| Lymphocyte-predominant infiltrate | 44 (56.4%)                                              | 27 (46.6%)                                                    | 0.255   |
| Lymphocytic infiltrate       | 78 (100.0%)                                                   | 58 (100.0%)                                                   | 1.000   |
| Subepidermal clef             | 56 (71.8%)                                                    | 26 (44.8%)                                                    | 0.001   |
| Eosinophilic spongiosis      | 7 (9.0%)                                                      | 2 (3.4%)                                                      | 0.200   |
| Parakeratosis                 | 3 (3.8%)                                                      | 17 (29.3%)                                                    | <0.001  |
| Acanthosis                   | 12 (15.4%)                                                    | 12 (20.7%)                                                    | 0.422   |
| Linear deposits of immunoreactants by direct |                                                             |                                                              |         |
| immunofluorescence, n (%)    | IgG 64 (86.5%)                                                | 43 (82.7%)                                                    | 0.558   |
|                             | IgA 4 (5.4%)                                                  | 6 (11.5%)                                                     | 0.210   |
|                             | C3 66 (89.2%)                                                 | 44 (84.6%)                                                    | 0.448   |
|                             | Isolated C3 9 (12.2%)                                         | 8 (15.4%)                                                     | 0.602   |
| Eosinophil count, mean (SD); | cells/µLh 1425.6 (835.7)                                      | 1315.2 (1363.4)                                               | 0.653   |
| C-reactive protein, mean (SD); |                                                         |                                                              | 0.900   |
| mg/Li | 32.6 (27.1)                                                  | 31.6 (32.3)                                                    |         |

Was available in in 65a, 49b, 135c, 41d, 132e, 130f, 81g, 58d. Bold: significant values.
BP, bullous pemphigoid; CI, confidence interval; DPP4i, dipeptidyl peptidase 4 inhibitor; n, number; PD-1, programmed death 1; SD, standard deviation.

The influence of neutrophil-predominant inflammatory infiltrate on the features of BP
Patients with neutrophilic infiltration presented with decreased mean (SD) levels of anti-BP180 NC16A IgG [269.3 (227.6) vs. 722.7 (1499.6) U/mL; P = 0.003], lower prevalence of eosinophilic infiltrate (77.3% vs. 92.1%; P = 0.036) and higher mean (SD) levels of CRP [47.3 (33.7) vs. 27.9 (26.3); P = 0.032; Table 4].
Table 3 Clinical, immunological, histological and immunopathological characteristics of BP patients with and without lymphocyte-predominant inflammatory infiltrate

|                                | BP with lymphocyte-predominant inflammatory infiltrate (n = 71) | BP without lymphocyte-predominant inflammatory infiltrate (n = 65) | P value |
|--------------------------------|---------------------------------------------------------------|-----------------------------------------------------------------|---------|
| Age at diagnosis; years        |                                                               |                                                                 | 0.497   |
| Mean (SD)                      | 80.0 (9.6)                                                    | 78.8 (10.0)                                                    |         |
| Sex, n (%)                     |                                                               |                                                                 |         |
| Male                           | 29 (40.8%)                                                    | 25 (38.5%)                                                     | 0.777   |
| Female                         | 42 (59.2%)                                                    | 40 (61.5%)                                                     |         |
| DPP4i-associated BP, n (%)     |                                                               |                                                                 | 0.429   |
| PD-1-associated BP, n (%)      |                                                               |                                                                 | 0.950   |
| Mucosal involvement, n (%)     | 9 (12.7%)                                                     | 7 (10.8%)                                                     | 0.730   |
| Mean BPDAI severity score (SD) |                                                               |                                                                 |         |
| Erosion/blister cutaneous activity | 28.1 (23.7)                                                | 19.0 (11.4)                                                   | 0.046   |
| Urticaria/Erythema activity    | 21.5 (21.5)                                                   | 7.6 (9.1)                                                     | 0.007   |
| Pruritus score                 | 21.3 (8.6)                                                    | 16.8 (8.6)                                                     | 0.040   |
| Damage score                   | 2.2 (3.0)                                                     | 2.2 (3.4)                                                     | 0.056   |
| Non-inflammatory (exclusively erosive) phenotype, n (%) | 3 (11.1%)                                                    | 8 (36.4%)                                                     | 0.035   |
| BP180 NC16A ELISA              |                                                               |                                                                 |         |
| Seropositivity, n (%)          | 59 (84.3%)                                                    | 54 (85.7%)                                                    | 0.818   |
| ELISA value, mean (SD); U/mL   | 736.2 (1692.9)                                                | 556.5 (945.6)                                                | 0.458   |
| BP230 ELISA                   |                                                               |                                                                 |         |
| Seropositivity, n (%)          | 14 (56.0%)                                                    | 8 (50.0%)                                                     | 0.707   |
| ELISA value, mean (SD); U/mL   | 165.4 (401.5)                                                | 147.8 (251.7)                                                | 0.877   |
| Indirect immunofluorescence seropositivity, n (%) | 65 (82.9%)                                                    | 59 (85.9%)                                                    | 0.580   |
| Salt split human skin          |                                                               |                                                                 |         |
| Monkey oesophagus              | 53 (75.7%)                                                    | 46 (76.7%)                                                    | 0.899   |
| Histological findings in lesional skin specimens, n (%) | 4 (5.6%)                                                     | 23 (35.4%)                                                    | <0.001  |
| Cell-poor infiltrate           |                                                               |                                                                 |         |
| Eosinophilic infiltrate        | 63 (88.7%)                                                    | 59 (90.8%)                                                    | 0.696   |
| Eosinophil-predominant infiltrate | 44 (62.0%)                                                | 34 (52.3%)                                                    | 0.255   |
| Neutrophil-predominant infiltrate | 2 (2.8%)                                                   | 3 (4.6%)                                                     | 0.578   |
| Neutrophilic infiltrate        | 11 (15.5%)                                                    | 11 (16.9%)                                                    | 0.821   |
| Lymphocytic infiltrate         | 71 (100.0%)                                                   | 65 (100.0%)                                                  | 1.000   |
| Subepidermal cleft             | 42 (59.2%)                                                    | 40 (61.5%)                                                    | 0.777   |
| Eosinophilic spongiosis        | 7 (9.9%)                                                      | 2 (3.1%)                                                     | 0.112   |
| Parakeratosis                  | 8 (11.3%)                                                     | 12 (18.5%)                                                    | 0.237   |
| Acanthosis                     | 12 (16.9%)                                                    | 12 (18.5%)                                                    | 0.812   |
| Linear deposits of immunoreactants by direct immunofluorescence, n (%) | 55 (83.3%)                                                    | 52 (86.7%)                                                    | 0.602   |
| IgG                            | 3 (4.5%)                                                      | 7 (11.7%)                                                     | 0.140   |
| IgA                            | 57 (86.4%)                                                    | 53 (88.3%)                                                    | 0.740   |
| C3                             | 11 (16.7%)                                                    | 6 (10.0%)                                                    | 0.274   |
| Isolated C3                    | 1182.9 (781.1)                                                | 1627.8 (1328.2)                                             | 0.081   |
| Eosinophil count, mean (SD); cells/µL | 28.3 (29.8)                                                | 35.7 (28.2)                                                    | 0.339   |

Was available in in 65s, 49s, 133s, 41d, 132s, 130s, 126s, 81s, 58s. Bold: significant values.
BP, bullous pemphigoid; CI, confidence interval; DPP4i, dipeptidyl peptidase 4 inhibitor; n, number; PD-1, programmed death 1; SD, standard deviation.

The influence of subepidermal blister on the features of BP
Table S1 lists the various characteristics of patients with a histological finding of subepidermal blister (n = 82) as compared to those lacking this finding (n = 54). Patients in the former group demonstrated lower mean (SD) values of the following severity scores: erosion/blister BPDAI [19.4 (10.7) vs. 34.1 (29.3); P = 0.036], urticaria/erythema BPDAI [9.2 (9.7) vs. 26.7 (24.7); P = 0.012], and pruritus BPADI [17.4 (9.2) vs. 23.0 (7.2); P = 0.001].
Whilst patients with subepidermal split had more frequent eosinophil-predominant inflammatory infiltrate (68.3% vs. 40.7%; \(P = 0.001\)), they had lower prevalence of cell-poor inflammatory infiltrate (12.2 vs. 31.5%; \(P = 0.006\)), parakeratosis (7.3% vs. 25.9%; \(P = 0.003\)) and acanthosis (8.5% vs. 31.5%; \(P = 0.001\)).

**Discussion**

The current study revealed that the majority of patients with BP presented with eosinophil-predominant or lymphocyte-predominant cutaneous inflammatory infiltrates, whereas cell-poor and neutrophilic inflammatory infiltration was confined to

| Table 4 Clinical, immunological, histological and immunopathological characteristics of BP patients with and without neutrophilic inflammatory infiltrate |
|---------------------------------|-----------------|-----------------|
| BP with neutrophilic inflammatory infiltrate (n = 22) | BP without neutrophilic inflammatory infiltrate (n = 114) | \(P\) value |
| Age at diagnosis; years | | |
| Mean (SD) | 80.0 (9.6) | 78.8 (10.0) | 0.497 |
| Sex, n (%) | | |
| Male | 7 (31.8%) | 47 (41.2%) | 0.409 |
| Female | 15 (68.2%) | 67 (58.8%) | 0.506 |
| DPP4i-associated BP, n (%) | | |
| 1 (4.5%) | 10 (8.8%) | 0.506 |
| PD-1-associated BP, n (%) | | |
| 0 (0.0%) | 2 (1.8%) | 0.531 |
| Mucosal involvement, n (%) | | |
| 2 (9.1%) | 14 (12.3%) | 0.671 |
| Mean BPDAI severity score (SD) | | |
| Erosion/blister cutaneous activity\(a\) | 20.0 (9.0) | 25.2 (21.6) | 0.404 |
| Urticaria/Erythema activity\(b\) | 7.9 (7.0) | 16.9 (19.7) | 0.185 |
| Pruritus score\(b\) | 19.8 (7.4) | 19.6 (9.2) | 0.922 |
| Damage score\(b\) | 2.8 (4.0) | 1.7 (2.8) | 0.336 |
| Non-inflammatory (exclusively erosive) phenotype, n (%)\(b\) | | |
| 1 (11.1%) | 10 (25.0%) | 0.367 |
| BP180 NC16A ELISA\(c\) | | |
| Seropositivity, n (%) | 20 (95.2%) | 93 (83.0%) | 0.151 |
| ELISA value, mean (SD); U/mL | 269.3 (227.6) | 722.7 (1499.6) | 0.003 |
| BP230 ELISA\(d\) | | |
| Seropositivity, n (%) | 5 (71.4%) | 17 (50.0%) | 0.301 |
| ELISA value, mean (SD); U/mL | 430.1 (681.3) | 102.6 (207.1) | 0.253 |
| Indirect immunofluorescence seropositivity, n (%) | | |
| Salt split human skin\(a\) | 19 (95.0%) | 105 (93.8%) | 0.829 |
| Monkey oesophagus\(d\) | 15 (75.0%) | 84 (76.4%) | 0.895 |
| Histological findings in lesional skin specimens, n (%) | | |
| Cell-rich infiltrate | 2 (9.1%) | 25 (21.9%) | 0.167 |
| Eosinophilic infiltrate | 17 (77.3%) | 105 (92.1%) | 0.036 |
| Eosinophil-predominant infiltrate | 13 (59.1%) | 65 (57.0%) | 0.857 |
| Lymphocyte-predominant infiltrate | 11 (50.0%) | 60 (52.6%) | 0.821 |
| Lymphocytic infiltrate | 22 (100.0%) | 114 (100.0%) | 1.000 |
| Subepidermal cleft | 17 (77.3%) | 65 (57.0%) | 0.075 |
| Eosinophilic spongiosis | 1 (4.5%) | 8 (7.0%) | 0.669 |
| Parakeratosis | 3 (13.6%) | 17 (14.9%) | 0.877 |
| Acanthosis | 3 (13.6%) | 21 (18.4%) | 0.590 |
| Linear deposits of immunoreactants by direct immunofluorescence, n (%)\(d\) | | |
| IgG | 20 (95.2%) | 87 (82.9%) | 0.148 |
| IgA | 3 (14.3%) | 7 (6.7%) | 0.238 |
| C3 | 17 (81.0%) | 93 (88.6%) | 0.338 |
| Isolated C3 | 1 (4.8%) | 16 (15.2%) | 0.200 |
| Peripheral Eosinophil count, mean (SD); cells/µL\(h\) | 1466.7 (1082.0) | 1365.7 (1082.0) | 0.766 |
| C-reactive protein, mean (SD); mg/L\(i\) | 47.3 (33.7) | 27.9 (26.3) | 0.032 |

Was available in in 65\(a\), 49\(b\), 133\(c\), 41\(d\), 132\(f\), 126\(g\), 81\(h\). Bold: significant values.

BP, bullous pemphigoid; CI, confidence interval; DPP4i, dipeptidyl peptidase 4 inhibitor; n, number; PD-1, programmed death 1; SD, standard deviation.

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the minority of patients. Whilst eosinophil-predominant inflammatory infiltrate was associated with elevated seropositivity of BP180 NC16A IgG, cell-poor inflammatory infiltrate predicted lower seropositivity of BP180 NC16A IgG, increased the prevalence of mucosal involvement and a non-inflammatory clinical phenotype. Patients with lymphocyte-predominant inflammatory infiltrate manifested with increased clinical disease activity and lower frequency of the non-inflammatory phenotype of BP, whilst those with neutrophilic infiltrate presented with lower levels of BP180 NC16A IgG and higher levels of CRP. Interestingly, patients with apparent subepidermal splitting showed more frequent eosinophil-predominant, less frequent cell-poor inflammatory infiltrate and lower BPDAI scores.

The impact of diverse histological findings on the phenotype of BP is yet to be decisively established. Izumi et al.19 attested that a morphological phenotype of erythema and urticarial plaques in BP was associated with an increased number of infiltrating eosinophils in lesional skin sections. Interestingly, these patients demonstrated reactivity against the full-length BP180 as well as to its immunodominant domain (NC16A). In the same study, a subset of BP patients with reduced erythema and scant lesional infiltration of eosinophils was identified (coined as ‘the non-inflammatory phenotype’). The latter group reacted against the full-length BP180, but not against its NC16A domain.19 Our findings, therefore, align with the aforementioned study as our patients with an eosinophil-predominant infiltrate proved significantly more seropositive to anti-BP180 NC16A IgG, whereas those with cell-poor infiltrates had a lower positivity rate.

The pathomechanism underlying the association between anti-BP180 NC16A IgG and lesional eosinophilic infiltration remains to be fully elucidated. However, binding of autoantibodies to BP180 NC16A was shown to induce complement activation and mast cell degranulation, resulting in activation of an inflammatory pathway,20 in which eosinophils and neutrophils are vital in degrading DEJ components by secreting proteases.21 Degradation fragments of BP180 were found to function as chemoreactants and further recruit inflammatory cells and amplify inflammation.22

Of note, Izumi et al.19 depicted that 50.0% (7/14) of their patients with the non-inflammatory phenotype (typified by absent to scant eosinophilic infiltrate and sparse urticarial plaques) had been exposed to dipeptidyl peptidase 4 inhibitor (DPP4i) agents.19 Congruently, two additional Japanese studies attested that patients with DPP4i-associated BP had a significantly decreased number of eosinophils in lesional histologic sections.23,24 In the current study, however, we did not detect a differential exposure to these drugs amongst patients with and without eosinophilic infiltrate. Correspondingly, two European studies originating from Finland and Greece revealed a comparable number of infiltrating eosinophils in lesional histological specimens of patients with DPP4i-associated BP and non-DPP4i-associated BP.25,26 Further research, utilizing larger sample sizes, is necessary to assess whether DPP4i drugs exert an effect on the recruitment of eosinophils in lesional skin.

It is a held belief that apart from a subepidermal cleft, the key histologic feature of BP is a predominantly eosinophilic dermal cellular infiltrate.27 The ultrastructural and biological properties of eosinophils imply that they may play a significant role in the initiation and/or progression of BP.28 Several observations in BP patients’ skin and in vitro studies suggested a pathogenic role of eosinophils in BP29,30 (reviewed in Ref. 31). In a recent study using a humanized IgE receptor mouse model of BP, eosinophils were shown to be a prerequisite for anti-BP180 IgE-mediated tissue injury and blister formation.52 Of note, 12/15 lipoygenase-expressing eosinophils appeared to be essential for the resolution of skin inflammation in a BP-like mouse model of BP.33,34

Whilst the substantial pathogenic role exerted by autoreactive B cells and T cells has clearly been shown,35 the influence of lymphocyte-predominant inflammatory infiltrate on the phenotype of BP has been poorly investigated. Our study indicates that lymphocytes infiltrated lesional skin specimens of all study participants, thus outreaching the observation of a recent small-scale histological study that reported dermal perivascular lymphocytic infiltrate in 32.0% of patients with BP.8 The same study, though, disclosed that all patients with pemphigoid gestation showed lymphocytic infiltration.6 Our study revealed that patients with lymphocyte-predominant inflammatory infiltrate were typified by a more severe phenotype, both on the erosive and erythematous level. Whilst this association has not been reported as yet, it can be conceived in light that BP is an antibody-mediated autoimmune disease that relies on autoreactive T-helper (Th) cells which, in turn, promote the activation of autoreactive B cells.35–37 Further research is warranted to isolate the direct effect of infiltrating BP180 NC16A-autoreactive B and T cells on the clinical and immunological characteristics of the disease. Correspondingly, a pathogenic role of BP180- and desmoglein-3-autoreactive T cells was demonstrated in models of other inflammatory dermatoses.38,39

Neutrophil-predominant BP was recognized as a rare histological variant of BP.40,41 However, the real prevalence of this variant and its associated morphological and immunological features were not systematically assessed. Investigating a relatively large cohort of patients with BP, we found that 12.3% of our patients had neutrophil-predominant infiltrations. Intriguingly, these patients exhibited lower levels of anti-BP180 NC16A IgG and a higher frequency of cell-poor inflammatory infiltration. When the scattered case reports of neutrophil-predominant BP were reviewed, the levels of anti-BP180 NC16A IgG were found to be low and to range between 31.01 and 85.0 U/mL,42 thus lending weight to our observation. Different from a recent case-series reporting two cases of neutrophil-predominant BP arising after the administration of programmed death (PD)-1 antagonists,42 none of our two patients with PD-1-associated BP had a
neutrophilic-predominant infiltration. Prominent neutrophilic infiltration was recently found to unduly the association of BP with comorbid psoriasis.\textsuperscript{43,44} Although neutrophils emerged as a key player in the pathogenesis of BP in experimental murine models,\textsuperscript{45} their role in human BP is less established as neutrophil-predominant infiltration is rarely encountered in lesional skin of BP patients. A recent study in a humanized IgE receptor mouse model depicted that eosinophils, independent of neutrophils, were required for inducing blister formation by anti-human BP180 NC16A IgE.\textsuperscript{32} These findings indicate the existence of distinct eosinophil- and neutrophil-driven mechanisms of blister formation in BP. Of note, in an ex vivo model using crossections of normal human skin, separation of the DEJ by BP patients’ IgG was dependent on immune-complex activated neutrophils.\textsuperscript{46,47}

We found that skin specimen including subepidermal blisters were accompanied by a higher cellularity and an eosinophil predominance. This finding stems from the pivotal contribution of Fc receptor-mediated mechanisms in tissue destruction and blister formation in BP. The latter is strongly implicated with extravasation of inflammatory effector cells and infiltration of lesional skin.\textsuperscript{21} The elevated cellularity of erosive lesions was additionally corroborated in a histological study reporting that transition from normal skin to skin with subepidermal bullae was associated with a significant increase in the density of inflammatory cell infiltrates.\textsuperscript{48} Unexpectedly, our study specified that patients with histological subepidermal blisters were associated with decreased severity scores (including even the erosion/blister BPDAI). Putative mechanistic interpretations of this observation are far from being compelling.

The current study throws light on a topic that has not been sufficiently investigated in the past. The utilization of a relatively large cohort managed in a specialized centre in which patients routinely undergo comprehensive work-up enables to investigate the association of histological properties of BP with a wide array of clinical, immunoserological and immunopathological variables. A selection bias stemming from the referral-centre setting could not be thoroughly refuted.

In conclusion, the current study provided some novel insights regarding the influence of histological features on the clinical and immunological phenotype of BP. Whilst eosinophil-(57%) and lymphocyte-predominant-(52%) inflammatory infiltrates were present in the majority of patients with BP, cell-poor (20%) and neutrophilic infiltrates (19%) emerged in the minority of patients. Whilst the seropositivity of anti-BP180 NC16A IgG was positively associated with eosinophil-predominant infiltrates, it was inversely associated with cell-poor infiltrates. The latter additionally projected a higher frequency of mucosal involvement and a non-inflammatory clinical phenotype. The previously unexplored subgroup of patients with lymphocyte-predominant inflammatory infiltrates displayed increased severity scores and lower frequency of the non-inflammatory phenotype of BP. The presence of neutrophilic infiltrates was associated with lower levels of anti-BP180 NC16A IgG and higher levels of CRP, whilst patients with subepidermal blisters more frequently showed an eosinophil-predominant, less frequently a cell-poor inflammatory infiltrates, and lower BPIVAI scores. Our findings substantiate the role of anti-BP180 NC16A IgG in the recruitment and aggregation of eosinophils in lesional skin and introduce a potential influence of lymphocytic infiltrate on the phenotype of BP.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Lesional histopathological sections of BP patients skin exemplifying the scores measuring density of eosinophilic infiltrate. The subfigures A, B, C and D stand for the scores 0, 1, 2 and 3, respectively.

Table S1. Clinical, immunological, histological, and immunopathological characteristics of BP patients with and without subepidermal cleft in lesional histological specimen.