



HAL
open science

Polarization of circulating follicular helper T cells correlates with bullous pemphigoid severity

Corine Pérals, Sébastien Le Jan, Céline Muller, Richard Le Naour, Philippe Bernard, Manuelle Viguier, Nicolas Fazilleau

► **To cite this version:**

Corine Pérals, Sébastien Le Jan, Céline Muller, Richard Le Naour, Philippe Bernard, et al.. Polarization of circulating follicular helper T cells correlates with bullous pemphigoid severity. *British Journal of Dermatology*, 2025, 192 (2), pp.283-292. 10.1093/bjd/ljae355 . hal-04927583

HAL Id: hal-04927583

<https://hal.science/hal-04927583v1>

Submitted on 5 Feb 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Polarization of circulating follicular helper T cells correlates with bullous pemphigoid severity

Corine Pérals,¹ Sébastien le Jan,² Céline Muller,^{2,3} Richard Le Naour,² Philippe Bernard,^{2,4} Manuelle Viguié^{2,3} and Nicolas Fazilleau¹

¹Toulouse Institute for Infectious and Inflammatory Diseases (Infinity), INSERM U1291, CNRS U5051, University Toulouse III, Toulouse, France

²EA 7509, IRMAIC, Reims-Champagne Ardenne University, Reims, France

³Department of Dermatology, Centre de Référence Maladies Rares des Maladies Bulleuses Auto-immunes (MALIBUL), Reims, France

⁴Department of Dermatology, Limoges University Hospital, Limoges, France

M.V. and N.F. contributed equally to this work.

Correspondence: Nicolas Fazilleau. Email: nicolas.fazilleau@inserm.fr

Linked Article: Solimani and Möbs *Br J Dermatol* 2025; 192:184.

Abstract

Background Follicular helper T (Tfh) cells form a distinct population of T-helper cells with different polarizations (type 1, type 2 and type 17) that regulates humoral responses and may participate in the pathophysiology of B-cell-mediated autoimmune diseases such as bullous pemphigoid (BP), a dermatosis mediated by autoantibodies specific for hemi-desmosomal proteins.

Objectives To evaluate the impact on circulating Tfh cells of super potent topical corticosteroid (TCS) treatment, which is more effective and safer than high doses of oral corticosteroids, and is the recommended first-line treatment of BP.

Methods Using flow cytometry, we compared the frequency, polarization and activation of Tfh cells in the blood of patients with BP with age- and sex-matched control participants without BP at baseline and longitudinally, after the initiation of TCS treatment.

Results We found that, at baseline, circulating Tfh cells were more frequent in patients with BP than in participants without BP and exhibited an activated phenotype. We further showed a decrease in type 1 and an increase in type 17 Tfh cells in the blood of patients with BP, which resulted in a higher type 2+type 17 to type 1 Tfh cell ratio. This ratio correlated positively with disease severity, as measured by the Bullous Pemphigoid Disease Area Index. Remarkably, with TCS treatment, although the frequency of Tfh cells in patients with BP returned to a level similar to that of control participants, the activated phenotype persisted. Interestingly, serum interleukin-21 levels and the Tfh cell subset ratio, similarly to disease activity and serum anti-BP180 and anti-BP230 autoantibodies, decreased with TCS treatment.

Conclusions Overall, our findings suggest the involvement Tfh cell polarization in the pathophysiology of BP and open the door to modulation of Tfh cell activity for treatment purposes.

Lay summary

Bullous pemphigoid is an autoimmune disease that affects people's skin. It appears as blisters on the skin. Treatment with drugs called 'topical corticosteroids' (or 'TCS' for short), applied directly to the skin, is effective and safe. 'Tfh' cells, a type of white blood cell, are involved in regulating the body's immune system. They may also be involved in the development bullous pemphigoid.

We looked at the effect of TCS treatment on Tfh cells in French patients with bullous pemphigoid. We found that there are more Tfh cells in people with bullous pemphigoid than in people without it. There was more of a specific subset of these Tfh cells in those with more severe bullous pemphigoid. Treatment with TCS reduces the number of these specific Tfh cells and decreases the amount of autoantibody. TCS treatment also reduces the severity of bullous pemphigoid.

Our findings suggest that tracking Tfh cells in people with bullous pemphigoid could help predict disease relapses before symptoms are experienced. Targeting Tfh cells could be an interesting treatment for bullous pemphigoid and other specific autoimmune diseases.

What is already known about this topic?

- Bullous pemphigoid (BP) is the most common B-cell-mediated cutaneous autoimmune bullous disease.
- BP manifests as cutaneous blisters without mucosal involvement and is characterized by the presence of autoantibodies directed against two proteins of the dermoepidermal junction (BP180 and BP230).
- Super potent topical corticosteroids (TCS) are more effective and safer than high-dose oral corticosteroids in treating BP and are the recommended first-line treatment.

Accepted: 5 September 2024

© The Author(s) 2024. Published by Oxford University Press on behalf of British Association of Dermatologists. All rights reserved. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

What does this study add?

- Circulating follicular helper T (Tfh) cells – the cognate regulators of B cells – are more frequent and biased toward type 17 Tfh cells in people with BP than in people without the disease.
- This results in a higher type 2 + type 17 to type 1 ratio, which correlates positively with BP severity.
- TCS treatment induces a decrease in disease severity, a decrease in serum autoantibodies against BP180 and BP230, and a decrease in the type 2 + type 17 to type 1 cell ratio.

What is the translational message?

- Tracking the Tfh cell subset ratio in people with BP treated with TCS may be useful in predicting BP relapses in advance of an increase in autoantibodies and disease symptoms.
- Targeting Tfh cells could be of interest for treatment purposes in BP and also in other B-cell-mediated diseases, in order to limit (or even annihilate) relapse risk.

Bullous pemphigoid (BP) is the most common autoimmune skin-blistering disease; it occurs most commonly in older people. BP is characterized clinically by urticarial erythema with tense blisters. It is mediated by the presence of autoantibodies (autoAbs) directed against two structural proteins of the dermoepidermal junction: BP180 (collagen XVII) and BP230. Super potent topical corticosteroids (TCS) are more effective and safer for the treatment of BP than high-dose oral corticosteroids.¹ In France, TCS is the recommended first-line treatment for BP, regardless of disease severity.^{1–3}

Interactions between autoreactive B cells and T cells promote the production of autoAbs specific for autoantigens that contribute to several pathologies. More precisely, follicular helper T (Tfh) cells form a subset of CD4⁺ T lymphocytes specialized in supporting B-cell maturation and regulating antibody (Ab) responses in secondary lymphoid organs.^{4,5} There, Tfh cells are required for the generation of germinal centre reactions, in which the selection of high-affinity Ab-producing B cells and the development of memory B cells occur. Tfh cells express the master regulator B-cell lymphoma 6 and the chemokine receptor CXCR5, the expression of which enables Tfh cells to migrate into CXCL13-rich germinal centres. Tfh cells also express the surface receptor inducible T-cell co-stimulator (ICOS), which promotes Tfh cell differentiation and maintenance (enhancing B-cell help and Ab production), and programmed cell death protein 1 (PD-1), which provides inhibitory signals to regulate Tfh cell activity and prevent excessive B-cell responses.⁶ Tfh cells secrete interleukin (IL)-21 to promote B-cell survival and maturation.

Levels of circulating Tfh cells are increased in a number of autoimmune diseases,⁷ including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and Sjögren syndrome.^{8–15} In BP, Li *et al.* found that an increased frequency of ICOS⁺ and PD-1⁺ circulating Tfh cells, and an increased level of IL-21 positively correlate with levels of serum anti-BP180 Abs, which have been recognized as a serum marker of disease severity.¹⁶ They also found that serum levels of IL-21 and the frequency of Tfh cells were significantly reduced after efficient treatment with systemic corticosteroid (methylprednisolone). In the context of TCS treatment, it is questionable whether Tfh cells are also affected and whether these cells play a crucial role in BP severity. In this study, we evaluated the frequency, activation and the

polarization status of the circulating Tfh cell pool in the blood of people with BP receiving TCS treatment and compared them with immunological (autoAb serum levels) and biologic parameters (IL-21 levels in serum and blister fluid), and with disease activity as measured by the Bullous Pemphigoid Disease Area Index (BPDAI).

Materials and methods**Patients**

This ancillary study was part of a larger prospective, single-centre study carried out in the Autoimmune Bullous Diseases Reference Centre at the Department of Dermatology of Reims University Hospital (Reims, France) between September 2013 and July 2017. Patients with newly diagnosed BP were included according to the following criteria: clinical features typical of BP with the presence of at least three of four well-established criteria;¹⁷ subepidermal blister on skin biopsy; and deposits of IgG and/or C3 in a linear pattern along the epidermal basement membrane zone on direct immunofluorescence. Patients with BP were initially treated according to the investigators' decision (see Table 2).² Patients were followed-up with visits planned for days 30, 150 and 270. At each visit, clinical data and BP treatments were recorded. Blood samples were collected at each visit (from baseline to day 270) and blister fluids only at baseline. Peripheral blood mononuclear cells (PBMCs) were isolated and stored in liquid nitrogen for <5 years. Biologic samples for the current study were available from a subgroup of 20 patients, with additional samples obtained from 18 of these patients during follow-up after treatment initiation (with TCS alone or with combined TCS and doxycycline). Blood samples from 20 age- and sex-matched patients were used as controls [13 women, 7 men; mean (SD) age 77.5 (9.2) years].

Clinical and immunological evaluations

BP disease activity and severity were evaluated at baseline and at the different follow-up visits using the BPDAI score. The BPDAI score ranges from 0 to 360 points; it evaluates notable skin and mucous membrane disease activity, and

considers the presence and the number of lesions with erosions, blisters, urticaria or erythema, as well as disease extent. Complete remission of BP was defined as the absence of new or established BP skin or mucosal lesions or pruritus; and flares as the appearance of at least three new BP lesions (blisters, eczematous lesions or urticarial plaques), or at least one large (> 10 cm diameter) eczematous lesion or urticarial plaque that did not heal within 1 week.¹⁸ Serum anti-BP180 and anti-BP230 IgG Abs titres were measured at baseline and at the follow-up visits using an enzyme-linked immunosorbent assay (ELISA) kit (EUROIMMUN, Lübeck, Germany), following the manufacturer's instructions.

Cytokine levels

IL-21 levels in serum and in blister fluid were measured by ELISA (BioLegend, San Diego, CA, USA), according to the manufacturer's protocol.

Phenotyping analysis

PBMCs were isolated by centrifugation over Ficoll-Paque gradients (GE Healthcare Lifescience, Chicago, IL, USA) to assess lymphocyte markers. All PBMCs were preserved through biobanking and frozen for future use. Whole circulating Tfh cells were characterized using a combination of different monoclonal Abs against CD3 (clone BW264/56), CD4 (SK3), PD-1 (EH12.1) and HLA-DR (AC122) (all BD Biosciences, San Jose, CA, USA), and CD45RA (REA1047), CXCR5 (REA103), CXCR3 (REA232), CCR6 (REA190) and ICOS (REA192) (Miltenyi Biotec, Bergisch Gladbach, Germany). Stained cells were acquired using a LSRII flow

cytometer (BD Biosciences). The percentages of each sub-population were determined using FlowJo™ 10 software (BD Biosciences). Doublets and dead cells were excluded using appropriate forward and side scatter gates.

Statistical analysis

Data are presented as median with a 95% confidence interval. Prism version 10 (GraphPad Inc., La Jolla, CA, USA) was used for statistical analysis. A *P*-value < 0.05 was considered to indicate statistical significance.

Results

Characteristics of patients with bullous pemphigoid

The study was conducted in 20 people with BP [13 women, 7 men; mean (SD) age 78.8 (8.7) years] (Table 1). The most common comorbidities were cardiovascular (high blood pressure in 16 patients) and neurological disorders (dementia and/or stroke in 5 patients) (Table 1). Before treatment, the mean (SD) BPDAI score was 45.4 (31.9) and the mean (SD) levels of anti-BP230 and anti-BP180 Abs were 28.9 (39.2) U mL⁻¹ and 64.1 (53.7) U mL⁻¹, respectively. In the majority of patients (*n* = 18/20; 90%), the initial treatment was TCS (clobetasol; dosage range 10–30 g daily) alone or TCS + doxycycline in one patient (Table 2). Systemic corticosteroids were initially started in one patient and, owing to mild disease, no treatment was given to another patient (Table 2). Therefore, these two patients were not analysed in the follow-up study, which was restricted to patients treated with TCS. To evaluate the efficiency of TCS treatment in these 18

Table 1 Baseline clinical and immunological characteristics in patients recently diagnosed with bullous pemphigoid (BP)

Patient no.	Sex/age (years)	Main comorbidities	BPDAI score	Anti-BP230 Abs (U mL ⁻¹)	Anti-BP180 Abs (U mL ⁻¹)
1	M/78	HBP, dementia, obliterative arteriopathy, hypertrophic cardiopathy	30	9	7
2	M/78	HBP, dyslipidaemia, coronaropathy, diffuse atherosclerosis, haemochromatosis	110	5	150
3	F/77	HBP, obesity	52	54	85
4	F/79	HBP, phlebitis, pulmonary embolism	5	8	26
5	F/84	HBP, dementia, stroke	63	107	111
6	F/87	HBP, obesity	24	5	7
7	F/80	HBP	51	10	126
8	F/90	HBP, diabetes, dementia	66	10	150
9	F/59	HBP	90	0	78
10	F/76	HBP, cirrhosis, CRF	35	72	11
11	M/85	HBP, diabetes, prostate cancer, stroke, MI	14	0	14
12	F/94	HBP, breast cancer, dementia	24	130	130
13	F/84	Breast cancer, NMSC	16	79	22
14	F/77	Diabetes, MI, dyslipidaemia	8	5	7
15	F/72	Diabetes, COPD, dyslipidaemia, arrhythmia	24	0	75
16	M/63	HBP, diabetes, TB, dyslipidaemia	28	47	68
17	F/74	HBP, RA	52	5	150
18	M/91	HBP, MGUS	114	21	7
19	M/72	Osteoporosis	70	10	74
20	M/75	HBP, diabetes	33	0	84

Diagnosis of BP in patients negative for or with a low level of anti-BP180 Abs was confirmed with positive direct immunofluorescence. Abs, antibodies; BPDAI, Bullous Pemphigoid Disease Area Index; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure; F, female; HBP, high blood pressure; M, male; MGUS, monoclonal gammopathy of undetermined significance; MI, myocardial infarction; NMSC, nonmelanoma skin cancer; RA, rheumatoid arthritis; TB, tuberculosis.

Table 2 Treatments and clinical status of studied patients at each visit

Patient no.	Baseline Initiated treatment	Day 30		Day 150		Day 270	
		Treatment	Clinical status	Treatment	Clinical status	Treatment	Clinical status
1	TCS 20 g daily	TCS 20 g EOD	CR	None	CR	None	CR
2	TCS 30 g daily	TCS 30 g daily	CR	TCS 30 g weekly	CR	None	Flares
3	TCS 30 g daily	TCS 10 g daily+MTX 12.5 mg weekly	CR	MTX 12.5 mg weekly	CR	MTX 7.5 mg weekly	CR
4	TCS 10 g daily	TCS 10 g daily	CR	TCS 10 g weekly	CR	None	CR
5	TCS 30 g daily	TCS 30 g daily+MTX 12.5 mg weekly	CR	MTX 12.5 mg weekly	CR	MTX 12.5 mg weekly	CR
6	TCS 10 g daily	TCS 10 g daily	CR	TCS 10 g weekly	CR	None	CR
7	TCS 30 g daily	TCS 30 g daily	CR	ND	ND	ND	ND
8	TCS 30 g daily	TCS 10 g daily+prednisone 0.5 mg kg ⁻¹ daily+MTX 10 mg weekly	CR	ND	ND	ND	ND
9	TCS 30 g daily	TCS 30 g EOD+MTX 15 mg weekly	CR	MTX 15 mg weekly	CR	MTX 15 mg weekly	CR
10	TCS 30 g daily	TCS 30 g daily	CR	TCS 30 g weekly	CR	TCS 20 g weekly	CR
11	TCS 20 g daily	TCS 20 g EOD	CR	None	CR	None	CR
12	TCS 20 g daily	TCS 20 g daily	Flares	TCS 20 g twice weekly	Flares	TCS 20 g weekly + doxycycline 200 mg daily	CR
13	TCS 20 g daily	TCS 20 g daily	CR	TCS 10 g weekly	CR	TCS 10 g weekly	CR
14	None	None	CR	TCS 10 g weekly	CR	None	CR
15	TCS 30 g daily	TCS 30 g daily	CR	TCS 30 g weekly	CR	None	CR
16	TCS 20 g daily	TCS 20 g daily	CR	TCS 20 g twice weekly	CR	TCS 20 g weekly	CR
17	TCS 30 g daily + doxycycline 200 mg daily	TCS 30 g daily + doxycycline 200 mg daily	CR	TCS 20 g EOD	CR	TCS 10 g daily + MTX 15 mg weekly	CR
18	TCS 30 g daily	TCS 30 g daily	CR	TCS 20 g weekly + MTX 7.5 mg weekly	CR	TCS 20 g weekly + MTX 7.5 mg weekly	CR
19	Prednisone 0.5 mg kg ⁻¹ daily	Prednisone 0.5 mg kg ⁻¹ daily	Flares	ND	ND	ND	ND
20	TCS 30 g daily	TCS 30 g daily	CR	TCS 30 g weekly	CR	TCS 30 g weekly	CR

CR, complete remission; EOD, every other day; MTX, methotrexate; ND, not done (patient lost to follow-up or died); TCS, topical corticosteroids (i.e. clobetasol).

patients, we monitored the BPDAl score and serum levels of anti-BP230 and anti-BP180 Abs 30, 150 and 270 days after the TCS boost, and found that they all decreased significantly and dramatically in all patients (Figure 1), highlighting the efficacy of TCS treatment in this population of patients with BP.

Follicular helper T-cell frequency and activation status in patients with bullous pemphigoid after topical corticosteroid treatment

To evaluate the impact of TCS treatment on circulating Tfh cells, we studied the PBMCs of patients with BP by flow cytometry and compared them with PBMCs from age- and sex-matched donors without BP. To focus on circulating Tfh cells, we gated on CD3⁺ CD4⁺ CD45RA⁻ CXCR5⁺ cells (Figure 2a; Figure S1 (see Supporting Information)). The frequency of circulating Tfh cells among the total number of CD4⁺ T cells was higher at baseline in people with BP (6%) vs. controls without BP [3%; $n=20$, $P=0.005$ (Figure 2b)]. No significant correlations were found between the frequency of circulating Tfh cells at baseline in people with

BP and their BPDAl score or the levels of anti-BP180 and anti-BP230 Abs (data not shown). Notably, 30, 150 and 270 days after starting TCS treatment, the frequency of circulating Tfh cells in people with BP returned to a similar level as seen in control participants without BP, with no significant differences seen at these timepoints. Moreover, we found that circulating Tfh cells at baseline in patients with BP were more activated than those in control participants without BP, as illustrated by the higher expression of HLA-DR (a molecule typically expressed by antigen-presenting cells that is also expressed on T cells and has been associated with T-cell activation) [19% in people with BP vs. 6% in control participants without BP; $n=20$, $P=0.006$ (Figure 2c)], of ICOS [15% in people with BP vs. 9% in control participants without BP; $P=0.02$ (Figure 2d)] and of PD-1 [14% in people with BP vs. 2% in control participants without BP; $n=20$, $P=0.03$ (Figure 2e)]. Strikingly, the higher expression of HLA-DR, ICOS and PD-1 at the surface of Tfh cells remained stable in people with BP after TCS treatment, while the frequency of Tfh cells decreased. Overall, we comprehensively demonstrated that TCS treatment in patients with BP led to

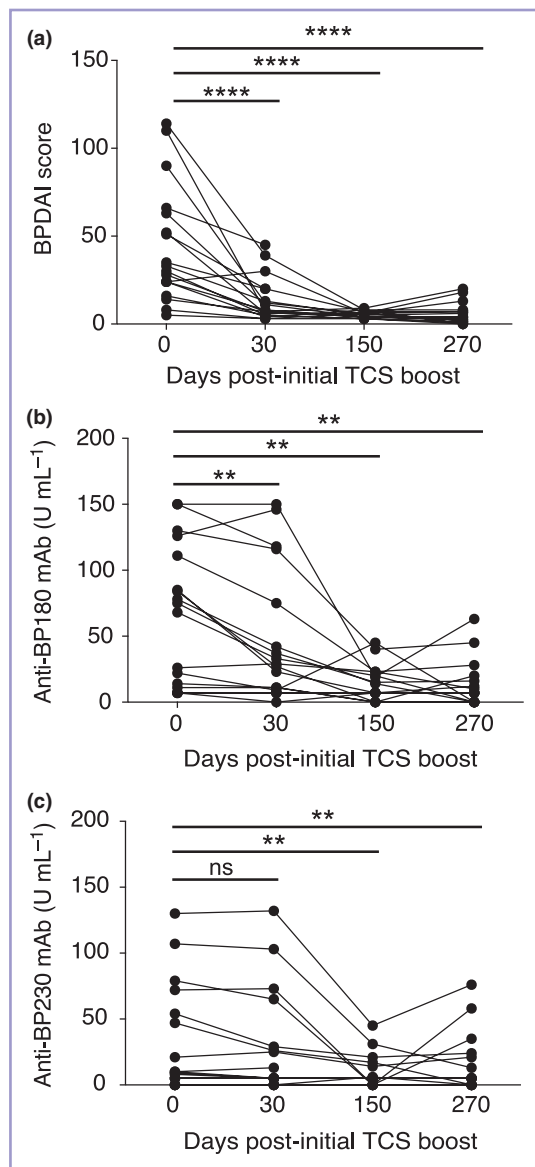


Figure 1 Clinical and serological evolution over time in patients with bullous pemphigoid (BP) after the initiation of super potent topical corticosteroid (TCS) treatment. (a) Clinical evolution, as measured by Bullous Pemphigoid Disease Area Index (BPDAI) score. (b) Serological evolution of anti-BP180 antibody concentration. (c) Serological evolution of anti-BP230 antibody concentration. The number of patients evaluated on days 0 and 30 after the initial TCS boost was 18; on days 150 and 207, the number of patients evaluated was 16. ** $P < 0.01$, **** $P < 0.0001$ (Wilcoxon matched-pairs signed-rank test). mAb, monoclonal antibody.

a decrease in circulating Tfh cells without grossly modifying their activation status.

Peripheral and local interleukin-21 production in patients with bullous pemphigoid after topical corticosteroid treatment

As Tfh cells produce and secrete high levels of IL-21 to perform their B-cell helper functions, we assessed serum IL-21 levels during BP pathogenesis. At baseline, we found that serum IL-21 levels from people with BP (34.57 ng mL⁻¹) were

higher than in control participants without BP (5.41 ng mL⁻¹) [$n = 18$, $P = 0.009$ (Figure 3a)]. No significant correlations were observed between these serum IL-21 levels and the frequencies of circulating Tfh cells or with the frequencies of the measured activation markers (HLA-DR, ICOS and PD-1) at the surface of circulating Tfh cells at baseline in the people with BP (data not shown). Notably, TCS treatment induced a significant decrease in serum IL-21 levels as soon as 30 days after initiating treatment (Figure 3a). Remarkably, we also detected IL-21 in all blister fluids obtained from eight patients at a much higher concentration than in their serum samples (422.5 ng mL⁻¹; Figure 3b). Taken together, at baseline, patients with BP have excessive levels of IL-21 – the main cytokine secreted by Tfh cells – in blister fluid and serum. IL-21 levels decrease along with clinical improvement following TCS treatment.

Polarization of circulating follicular helper T cells in patients with bullous pemphigoid after topical corticosteroid treatment

In autoAb-mediated diseases, Tfh cells play a critical role in guiding B cells to produce these autoAbs; the balance between circulating Tfh cell subsets can influence autoAb production.^{19,20} In this context, we studied the expression of the chemokine receptors CXCR3 and CCR6 at the surface of circulating Tfh cells, which allowed us to subdivide the Tfh cell population into Tfh1 (CXCR3⁺ CCR6⁻), Tfh2 (CXCR3⁻ CCR6⁻) and Tfh17 (CXCR3⁻ CCR6⁺) cell subsets (Figure 4a). We found that the frequency of circulating Tfh2 cells was similar between patients with BP at onset (43%) and control participants without BP [45%; $n = 20$, $P = 0.44$ (Figure 4a)]. In contrast, we found a decrease in circulating Tfh1 cells [34% in people with BP vs. 40% in control participants without BP; $n = 20$, $P = 0.04$ (Figure 4a)], which was related to an increase in circulating Tfh17 cells in people with BP at disease onset [21% in people with BP vs. 8% in control participants without BP; $n = 20$, $P = 0.02$ (Figure 4a)]. Notably, no significant correlations between circulating Tfh1, Tfh2 or Tfh17 cells with baseline BPDAI in people with BP were found (Figure S2a; see Supporting Information). Previous research has found that only blood Tfh2 and Tfh17 cells effectively induce naïve B cells to produce immunoglobulins (Tfh1 cells lack this ability).¹⁹ This skewing toward Tfh2 and Tfh17 cells is a characteristic of autoimmune diseases evaluated by calculating the Tfh2 + Tfh17 to Tfh1 cell ratio. Herein, we found that, at baseline, the Tfh2 + Tfh17 to Tfh1 cell ratio was around 1 in control participants without BP and significantly higher in people with BP [1.99 in people with BP vs. 1.39 in control participants without BP; $n = 20$, $P = 0.02$ (Figure 4b)]. Notably, while TCS treatment did not significantly affect the proportion of distinct Tfh cell subsets compared with baseline (Figure S2b), the Tfh2 + Tfh17 to Tfh1 cell ratio decreased and returned to the ratios found in control participants without BP (Figure 4c). Interestingly, the higher the baseline BPDAI in patients with BP was, the higher the Tfh2 + Tfh17 to Tfh1 cell ratio [$r^2 = 0.328$, $P = 0.008$ (Figure 4d)]. Thus, at baseline, patients with BP had a different circulating Tfh cell pool than control participants without BP, with more Tfh17 cells and fewer Tfh1 cells – a feature that positively correlates with disease severity and is modulated by effective TCS treatment.

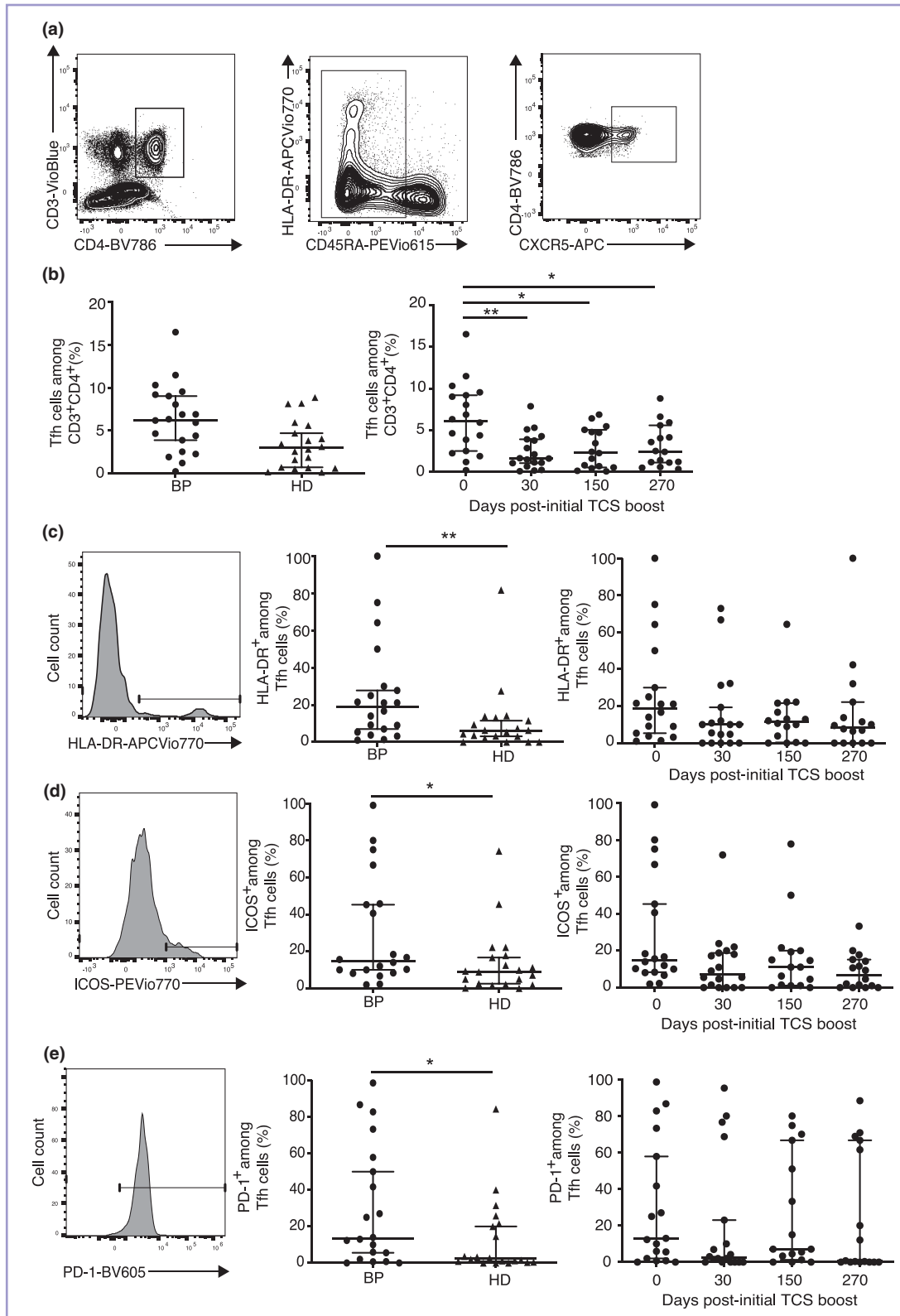


Figure 2 Circulating follicular helper (Tfh) cell frequencies in patients with bullous pemphigoid (BP) decreased during topical corticosteroid (TCS) treatment and clinical remission. (a) Gating strategy of circulating Tfh cells. (b) Tfh cell frequency among total CD3⁺ CD4⁺ T cells in patients with BP at baseline (circles) and in control participants without BP (triangles, left; *n*=20) and in patients with BP after initiating TCS treatment (right; *n*=18 at days 0 and 30 and *n*=16 at days 150 and 270). (c–e) HLA-DR, inducible T-cell co-stimulator (ICOS) and programmed cell death protein 1 (PD-1) expression at the surface of circulating Tfh cells at baseline in patients with BP (circles) and control participants without BP (triangles, middle; *n*=20) and in patients with BP after initiating TCS treatment (right; *n*=18 at days 0 and 30 and *n*=16 at days 150 and 270). **P*<0.05; ***P*<0.01 (Mann–Whitney *U* test). HD, healthy donors (i.e. control participants without BP).

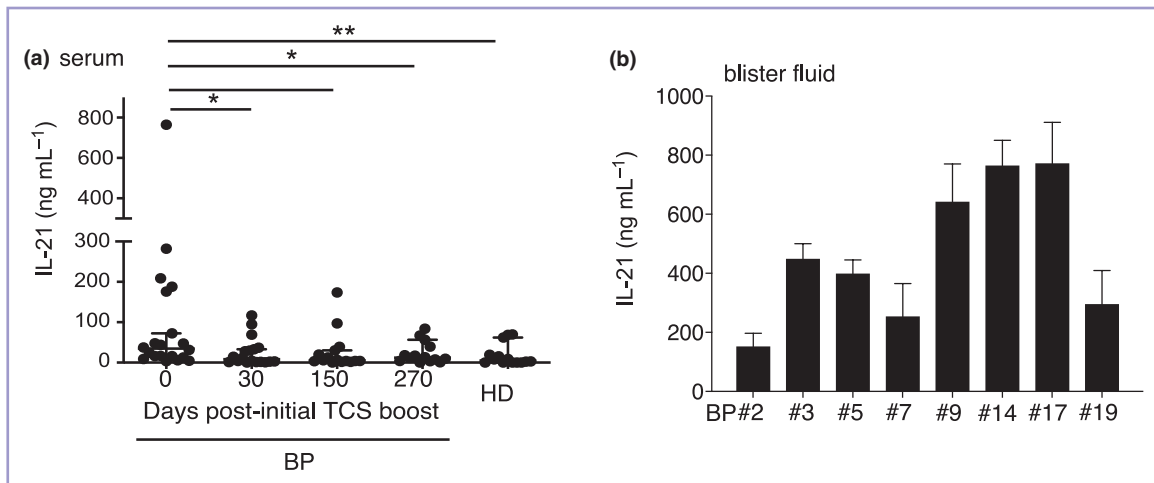


Figure 3 Serum interleukin (IL)-21 levels in patients with bullous pemphigoid (BP) decreased during topical corticosteroid (TCS) treatment. (a) Serum IL-21 levels in patients with BP at different timepoints ($n=18$ at days 0 and 30; $n=16$ at days 150 and 270) and for control participants without BP ($n=18$). (b) IL-21 levels from the blister fluid of eight patients with BP at baseline. * $P<0.05$, ** $P<0.01$ (Mann–Whitney U test). HD, healthy donors (i.e. control participants without BP).

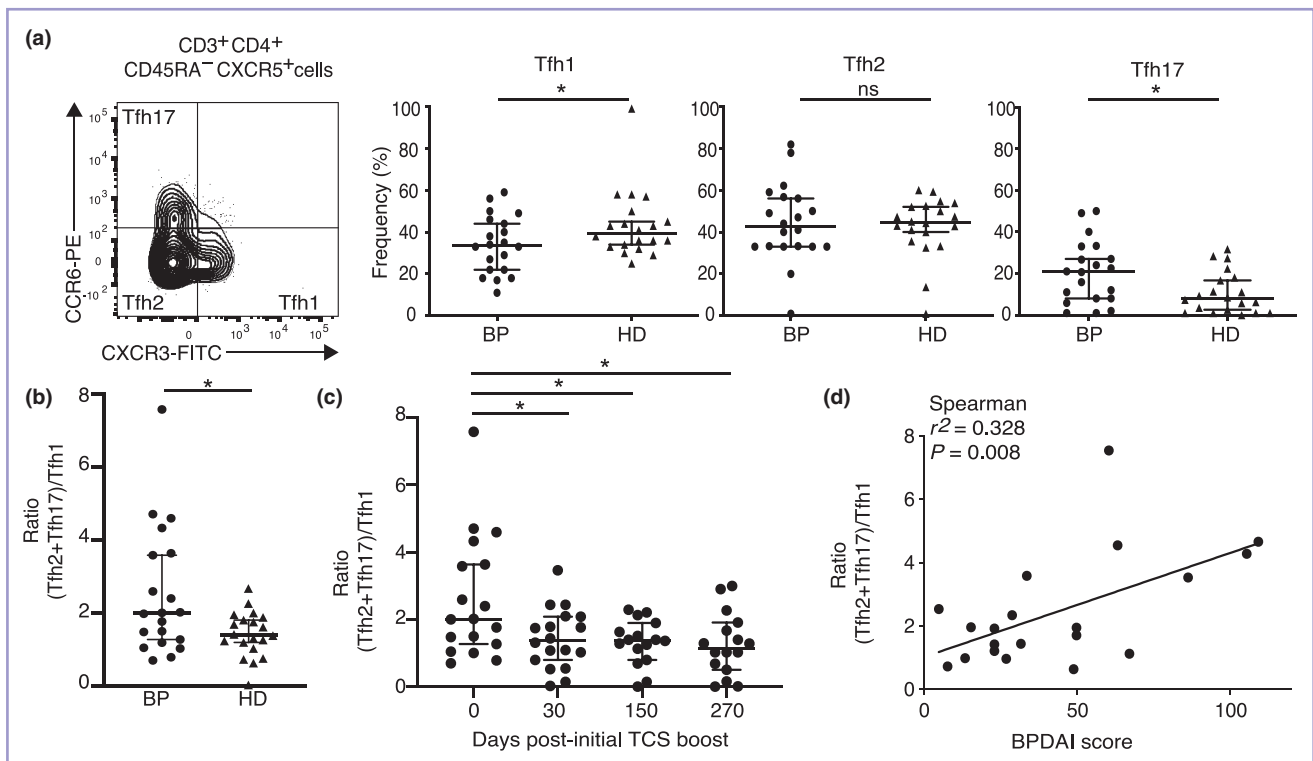


Figure 4 Baseline bullous pemphigoid (BP) severity is positively correlated with the Tfh2 + Tfh17 to Tfh1 ratio. (a) Gating strategy to divide circulating follicular helper T (Tfh) cells in Tfh1, Tfh2 and Tfh17 cell subsets (left). Tfh cell subset frequencies among circulating Tfh cells at baseline for patients with BP (circles) and for control participants without BP (triangles; $n=20$). Estimated Tfh2 + Tfh17 to Tfh1 ratio at baseline in (b) patients with BP and control participants without BP ($n=20$) and in (c) patients with BP after starting topical corticosteroid (TCS) treatment ($n=18$ at days 0 and 30 and $n=16$ at days 150 and 270). (d) Correlation between Bullous Pemphigoid Disease Area Index (BPDAI) score and Tfh2 + Tfh17 to Tfh1 ratio at baseline in patients with BP ($n=20$). ns, not significant. * $P<0.05$ (Mann–Whitney U test). FITC, fluorescein isothiocyanate; HD, healthy donors (i.e. control participants without BP).

Discussion

Super potent TCS treatment is now routinely used to treat patients with BP, with higher efficacy and better safety than systemic corticosteroids.¹ This is due to local and systemic effects, as systemic absorption of TCS may result in

prolonged, although potentially lower, systemic steroid levels.²⁰ However, whether the medical effectiveness of TCS relies on modifications of Tfh cell frequency, phenotype or polarization has not yet been elucidated. In this context, we studied a group of people with BP treated with TCS and tracked their circulating Tfh cell pools from baseline to

270 days after TCS treatment was initiated and compared them with the circulating Tfh cell pools of age- and sex-matched controls without BP. At disease onset, we found that more circulating Tfh cells were present in people with BP than in controls without BP, with a bias toward type 17 Tfh cells, resulting in a higher type 2+type 17 to type 1 Tfh cell subset ratio. Notably, this ratio was positively correlated with disease severity and decreased with effective TCS treatment. Thus, we comprehensively demonstrated that the polarization of Tfh cells correlates with BP severity, suggesting that Tfh cells contribute to the pathogenesis of BP.

It has previously been shown that the frequency and activation status of circulating Tfh cells is higher at baseline in patients with BP than in controls without BP.¹⁶ Moreover, in the study by Li *et al.*,¹⁶ IL-21 serum levels in patients with BP at disease onset were higher than those of control participants without BP, and – similarly to what we observed with topical steroids – serum IL-21 levels were reduced under effective oral methylprednisolone treatment in these patients with BP. Finally, Li *et al.* elegantly demonstrated *in vitro* that the absence of CD4⁺ T cells (thus Tfh cells) and IL-21 neutralization reduced anti-BP180 Ab production by B cells.¹⁶ In our study, in response to effective TCS treatment, we found a significant reduction in the frequency of circulating Tfh cells, along with a decrease in IL-21 serum levels, as early as 30 days after initiation of local treatment. However, while circulating Tfh cells were less abundant after TCS treatment, their phenotype remained activated, with high expression of HLA-DR, ICOS and PD-1, suggesting that these cells could be rapidly effective and favour disease relapses, as often seen in people with BP.²¹ Interestingly, we have provided evidence for high levels of IL-21 in the blister fluid of patients with BP at disease onset, suggesting a potential role for this cytokine and therefore for one of its main producers – Tfh cells – in the local skin environment. Notably, immunohistochemical staining has previously shown an increase of Tfh cells in the skin lesions of patients with BP.²² We hypothesize that these Tfh cells may support the autoimmune response that targets components of the skin basement membrane by facilitating local B cells to produce anti-BP180 and anti-BP230 autoAbs, contributing to the blistering and skin damage seen in BP.

Tfh cells can be divided into different subsets, including circulating Tfh1, Tfh2 and Tfh17 cells, based on the cytokines they produce, the chemokine receptor they express and the type of immune responses they help orchestrate.^{19,23,24} We found that the increase in circulating Tfh cells in patients with BP at disease onset largely relied on the increase in Tfh17 cells and the decrease in Tfh1 cells. In addition, it has been shown that Tfh17 cells could be pathogenic in pemphigus vulgaris, another B-cell-mediated blistering dermatosis.²⁵ Tfh1 cells are also known to be less efficient B-cell helpers than their Tfh2 and Tfh17 counterparts.¹⁹ Altogether, these results suggest the involvement of pathogenic Tfh17 cells in BP pathophysiology through their potential role in the exacerbation of B-cell help.

An imbalance in Tfh cell subsets – leading to changes in the Tfh2+Tfh17 to Tfh1 ratio – has been found in other autoimmune diseases. For example, Tfh17 cells have been implicated in the pathogenesis of SLE.^{26–28} Similarly, increased

numbers of Tfh17 cells and associated IL-17 production have been seen in the synovial tissues of people with RA, contributing to joint inflammation and destruction.²⁹ In Sjögren syndrome, studies have suggested that an altered Tfh subset balance – particularly the Tfh2+Tfh17 to Tfh1 ratio – might contribute to the immune dysfunction found in this disease.¹⁵ Overall, an elevated Tfh2+Tfh17 to Tfh1 cell ratio has been suggested to indicate a bias toward excessive humoral immune responses, possibly more focused on autoAb production.^{7,30} Here, we highlighted an elevated Tfh2+Tfh17 to Tfh1 cell ratio in people with BP at onset vs. controls without BP, and showed a decrease in this ratio along with effective TCS treatment in people with BP. Furthermore, we have demonstrated a positive correlation between this Tfh2+Tfh17 to Tfh1 ratio and the activity and the severity of BP. Besides clinical activity, it would also be valuable to track the Tfh cell subset ratio in the context of clinical outcomes of patients with BP undergoing treatment. Potentially, these data could serve as a foundation for developing a predictive tool for clinicians, allowing them to anticipate BP relapses well in advance, even before the appearance of autoAbs and clinical symptoms.

Specifically targeting Tfh cells could be interesting for treatment purposes not only in BP, but also in other B-cell-mediated diseases to limit (or even annihilate) the risk of relapse. The use of steroids – systemic or topical – leads to a global reduction in circulating Tfh cells in BP, SLE and graft-versus-host disease (but not in multiple sclerosis).^{31–33} Notably, the decrease in Tfh cells seen in patients with BP did not decrease the activation status of these cells. In SLE, paradoxically, this decrease correlates with an increase in Tfh17 cells,³¹ suggesting that in some diseases Tfh cells could be hyporesponsive to steroids. Thus, a more targeted approach could be obtained with dupilumab, a monoclonal Ab that blocks IL-4R α and is currently a promising drug for the treatment of BP.^{34–36} Another possibility – owing to the high expression level of ICOS at the surface of Tfh cells – could be to target ICOS to control disease activity in BP, as has been demonstrated in skin fibrosis and in a mouse model of pemphigus vulgaris.^{37,38}

Acknowledgements

We are indebted to Professor Frank Antonicelli (EA 7509, Reims-Champagne Ardenne University) for organizing the cohort of patients with bullous pemphigoid investigated in this study. We also thank the Infinity flow cytometry core facility (A.L. Iscache, V. Duplan-Eche and F. L'Faqihi).

Funding sources

This study was supported by the French Department of Health's Programme Hospitalier de Recherche Clinique Interrégional 2012, INSERM; The Inspire Program from the Region Occitanie/Pyrénées-Méditerranée (#1901175); and the European Regional Development Fund (MP0022856).

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

All data are available in the main text.

Ethics statement

The study was approved by the institutional ethics committee (Commission Nationale de l'Informatique et des Libertés, DR-2013-320) and funded by the French Department of Health's Programme Hospitalier de Recherche Clinique (PHRC) Interrégional 2012.

Patient consent

Written informed consent was obtained from all participants prior to participation.

Supporting Information

Additional [Supporting Information](#) may be found in the online version of this article at the publisher's website.

References

- Joly P, Roujeau JC, Benichou J *et al.* A comparison of oral and topical corticosteroids in patients with bullous pemphigoid. *N Engl J Med* 2002; **346**:321–7.
- Clape A, Muller C, Plee J *et al.* Feasibility and healthcare costs of superpotent topical corticosteroid therapy in bullous pemphigoid: a prospective, observational study in an academic centre in France. *Br J Dermatol* 2020; **183**:775–6.
- Castel M, Alexandre M, Jelti L *et al.* Updated French guidelines for the therapeutic management of bullous pemphigoid. *Ann Dermatol Venereol* 2022; **149**:81–91.
- Fazilleau N, Mark L, McHeyzer-Williams LJ *et al.* Follicular helper T cells: lineage and location. *Immunity* 2009; **30**:324–35.
- Crotty S. T follicular helper cell differentiation, function, and roles in disease. *Immunity* 2014; **41**:529–42.
- Song W, Craft J. T follicular helper cell heterogeneity. *Annu Rev Immunol* 2024; **42**:127–52.
- Ueno H, Banachereau J, Vinuesa CG. Pathophysiology of T follicular helper cells in humans and mice. *Nat Immunol* 2015; **16**:142–52.
- Simpson N, Gatenby PA, Wilson A *et al.* Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum* 2010; **62**:234–44.
- Le Coz C, Joubin A, Pasquali JL *et al.* Circulating TFH subset distribution is strongly affected in lupus patients with an active disease. *PLOS ONE* 2013; **8**:e75319.
- Choi JY, Ho JH, Pasoto SG *et al.* Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. *Arthritis Rheumatol* 2015; **67**:988–99.
- Wang J, Shan Y, Jiang Z *et al.* High frequencies of activated B cells and T follicular helper cells are correlated with disease activity in patients with new-onset rheumatoid arthritis. *Clin Exp Immunol* 2013; **174**:212–20.
- Liu R, Wu Q, Su D *et al.* A regulatory effect of IL-21 on T follicular helper-like cell and B cell in rheumatoid arthritis. *Arthritis Res Ther* 2012; **14**:R255.
- Arroyo-Villa I, Bautista-Caro MB, Balsa A *et al.* Constitutively altered frequencies of circulating follicular helper T cell counterparts and their subsets in rheumatoid arthritis. *Arthritis Res Ther* 2014; **16**:500.
- Dupre A, Pascaud J, Riviere E *et al.* Association between T follicular helper cells and T peripheral helper cells with B-cell biomarkers and disease activity in primary Sjögren syndrome. *RMD Open* 2021; **7**:e001442.
- Li XY, Wu ZB, Ding J *et al.* Role of the frequency of blood CD4⁺ CXCR5⁺ CCR6⁺ T cells in autoimmunity in patients with Sjögren's syndrome. *Biochem Biophys Res Commun* 2012; **422**:238–44.
- Li Q, Liu Z, Dang E *et al.* Follicular helper T cells (Tfh) and IL-21 involvement in the pathogenesis of bullous pemphigoid. *PLOS ONE* 2013; **8**:e68145.
- Vaillant L, Bernard P, Joly P *et al.* Evaluation of clinical criteria for diagnosis of bullous pemphigoid. French Bullous Study Group. *Arch Dermatol* 1998; **134**:1075–80.
- Murrell DF, Daniel BS, Joly P *et al.* Definitions and outcome measures for bullous pemphigoid: recommendations by an international panel of experts. *J Am Acad Dermatol* 2012; **66**:479–85.
- Morita R, Schmitt N, Bentebibel SE *et al.* Human blood CXCR5⁺CD4⁺ T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* 2011; **34**:108–21.
- Joly P, Roujeau JC, Benichou J *et al.* A comparison of two regimens of topical corticosteroids in the treatment of patients with bullous pemphigoid: a multicenter randomized study. *J Invest Dermatol* 2009; **129**:1681–7.
- Wang Y, Mao X, Wang Y *et al.* Relapse of bullous pemphigoid: an update on this stubborn clinical problem. *Ann Med* 2018; **50**:234–9.
- Ohuchi K, Fujimura T, Lyu C *et al.* Possible roles of CXCL13/CXCR5 axis in the development of bullous pemphigoid. *J Dermatol* 2021; **48**:353–9.
- Eisenbarth SC, Baumjohann D, Craft J *et al.* CD4⁺ T cells that help B cells – a proposal for uniform nomenclature. *Trends Immunol* 2021; **42**:658–69.
- Olatunde AC, Hale JS, Lamb TJ. Cytokine-skewed Tfh cells: functional consequences for B cell help. *Trends Immunol* 2021; **42**:536–50.
- Holstein J, Solimani F, Baum C *et al.* Immunophenotyping in pemphigus reveals a T_H17/T_{FH}17 cell-dominated immune response promoting desmoglein1/3-specific autoantibody production. *J Allergy Clin Immunol* 2021; **147**:2358–69.
- Kim V, Lee K, Tian H *et al.* IL-17-producing follicular Th cells enhance plasma cell differentiation in lupus-prone mice. *JCI Insight* 2022; **7**:e157332.
- Hsu HC, Yang P, Wang J *et al.* Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol* 2008; **9**:166–75.
- Blanco P, Ueno H, Schmitt N. T follicular helper (Tfh) cells in lupus: activation and involvement in SLE pathogenesis. *Eur J Immunol* 2016; **46**:281–90.
- Yang P, Qian FY, Zhang MF *et al.* Th17 cell pathogenicity and plasticity in rheumatoid arthritis. *J Leukoc Biol* 2019; **106**:1233–40.
- Schmitt N, Bentebibel SE, Ueno H. Phenotype and functions of memory Tfh cells in human blood. *Trends Immunol* 2014; **35**:436–42.
- Mao M, Xu S, Lin L *et al.* Impact of corticosteroids on the proportions of circulating Tfh cell subsets in patients with systemic lupus erythematosus. *Front Med (Lausanne)* 2022; **9**:949334.
- Knorr DA, Wang H, Aurora M *et al.* Loss of T follicular helper cells in the peripheral blood of patients with chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 2016; **22**:825–33.
- Ratzer R, Romme Christensen J, Romme Nielsen B *et al.* Immunological effects of methylprednisolone pulse treatment in progressive multiple sclerosis. *J Neuroimmunol* 2014; **276**:195–201.
- Yan T, Zhang Z. Adaptive and innate immune pathogenesis of bullous pemphigoid: a review. *Front Immunol* 2023; **14**:1144429.

- 35 Zhao L, Wang Q, Liang G *et al.* Evaluation of dupilumab in patients with bullous pemphigoid. *JAMA Dermatol* 2023; **159**:953–60.
- 36 Moghadam P, Tancrede E, Bouaziz JD *et al.* Efficacy and safety of dupilumab in bullous pemphigoid: a retrospective multicentric study of 36 patients. *Br J Dermatol* 2023; **189**:244–6.
- 37 Taylor DK, Mittereder N, Kuta E *et al.* T follicular helper-like cells contribute to skin fibrosis. *Sci Transl Med* 2018; **10**:eaaf5307.
- 38 Kim AR, Han D, Choi JY *et al.* Targeting inducible costimulator expressed on CXCR5+PD-1+ TH cells suppresses the progression of pemphigus vulgaris. *J Allergy Clin Immunol* 2020; **146**:1070–9.