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RESEARCH ARTICLE

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Successful pharmacological intervention at different levels of the complement system in an in vitro complement fixation model for bullous pemphigoid

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Abstract

Bullous pemphigoid (BP) is characterized by deposition of immunoglobulins and complement along the epidermal basement membrane (BM). In humans, there is a lack of functional studies targeting the complement system (CS). This study investigates activation of all complement pathways in BP skin biopsies. Moreover, pharmacological inhibition at different levels of the CS was investigated using anti-complement compounds in a complement fixation BP assay. In this retrospective study, 21 frozen biopsies from BP patients were stained by direct immunofluorescence for C1q, MBL, ficolin-2, C4d, properdin, C3c and C5b-9. Sera from 10 patients were analysed in a complement fixation assay in the presence of C1 inhibitor, anti-factor B monoclonal antibody (mAb), anti-C3 mAb and anti-C5 mAb and compared with dexamethasone. The two readouts were the quantity of complement deposited along the BM and the release of sC5b-9 in the supernatant. Our results show classical and alternative complement pathway activation in BP skin biopsies, but could not demonstrate significant lectin pathway activation. In contrast to dexamethasone, complement deposition along the BM could be selectively inhibited by anti-C1 and anti- factor B. More downstream, selective intervention at the level of C3 and C5 could effectively reduce complement deposition along the BM and the release of sC5b-9 in the supernatant. This study shows that selective intervention in either the classical, alternative or terminal pathway prevented deposition of complement along the BM in an in vitro BP model. The results of our study greatly encourage the clinical development of complement inhibitors for the treatment of BP.

KEYWORDS
bullous pemphigoid, complement, immunofluorescence, in vitro, skin

Abbreviations: BM, basement membrane; BP, bullous pemphigoid; C5aR1, C5a receptor 1; CS, complement system; DIF, direct immunofluorescence; FFPE, formalin-fixed paraffin-embedded tissue; IIF, indirect immunofluorescence.

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1 | INTRODUCTION

Bullous pemphigoid (BP) is the most prevalent autoimmune blistering disease, which commonly affects the elderly. Clinically, BP is characterized by intense itching and the development of urticarial plaques with tense dome-shaped fluid-filled blisters on the skin and less often mucosae. The development of these skin lesions is caused by circulating autoantibodies directed against basement membrane (BM) proteins BP180 and/or BP230.[1,2] Histopathology reveals a subepidermal blister accompanied by an infiltrate of lymphocytes, eosinophils, neutrophils, mast cells and monocytes/macrophages. Deposition of immunoglobulins and/or complement are the gold standard for a definite diagnosis of BP. These immune deposits are demonstrated by direct immunofluorescence (DIF) on skin biopsies that reveal a specific linear n-serrated pattern of immunoglobulins and/or complement along the BM. Deposition of complement fragments such as C3c and C1q is routinely used for diagnostic purposes.[3,4] Moreover, deposition of complement of different pathways has been demonstrated by DIF and immunoperoxidase staining on formalin-fixed paraffin-embedded tissue.[5,6] In a large cohort ($n = 300$) of patients, C3c deposition could be demonstrated in approximately 85% of skin biopsies from BP patients.[7] Experimental mouse knockout models have also demonstrated a crucial role for complement in the pathogenesis of BP, mainly via the classical and alternative pathways.[8,9] More recent experimental work also indicated an important role for C5a-C5a Receptor 1 (C5aR1)-axis activation in the development of BP and could be an interesting target for intervention.[10–12] However, these findings in mice cannot be directly translated into clinical practice, let alone clinical trials targeting the CS, until functional studies with human materials are performed. Insight in complement-mediated mechanisms and the routes of complement activation in BP are crucial, considering the emerging therapeutic treatment options of drugs targeting the complement system at various levels. Currently, corticosteroids are the mainstay of therapy for BP but are associated with many and sometimes severe adverse effects. When resistance to corticosteroids develops, patients show high relapse rate and managing the disease can be challenging for clinicians.[13] For these reasons, there is a need for more personalized therapy, targeting crucial components of the CS in the pathogenesis of BP.

In this study, we investigated all three pathways of complement activation in skin biopsies from BP patients by DIF, including the lectin pathway. Moreover, pharmacological intervention in different routes of complement by several clinical (registered for the use in other diseases than dermatological diseases) and non-clinical anti-complement agents was evaluated in a complement fixation BP assay.

2 | MATERIALS AND METHODS

2.1 | Direct immunofluorescence study on skin biopsies from BP patients (cohort I)

In this single-centre retrospective study, 21 patients were included who underwent a diagnostic skin biopsy for suspicion of BP between 2018 and 2021 in the Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands (cohort I). The study protocol was approved by the local Medical Ethical Committee of the Erasmus Medical Center (MEC-2019–0207). A final diagnosis of BP was made based on clinical features compatible with BP and DIF showing linear IgG and/or C3c along the BM in an n-serrated pattern. From all patients, also serological data were available, for example serum ELISA results (Dermatology Profile ELISA (IgG), Euroimmun AG) and indirect immunofluorescence (IIF) on monkey oesophagus (Inova Diagnostics, San Diego, CA) (Table 1). Biopsies were snap-frozen in liquid nitrogen and stored at −80°C. Frozen sections were stained with haematoxylin and eosin for light microscopic evaluation. For detection of IgG and C1q, directly FITC-labelled antibodies were used and detected on Ventana Benchmark Ultra (Ventana Medical Systems Inc.). Intensity scores for IgA and IgM were retrieved from the pathology records. For complement factors C1q, MBL, ficolin-2, C4d, properdin, C3c and C5b-9, direct immunofluorescence was performed by automated immunofluorescent staining using the Ventana Benchmark Discovery (Ventana Medical Systems Inc.). In brief, wet slides were loaded and incubated with primary antibody of interest for 32 min (Table 2) at 37°C followed by detection with either omnimap-anti rabbit or mouse, labelled with HRP (#760–4310 or #760–4311, Ventana) and visualized with FAM (#760–243, Ventana). Slides were covered with anti-fading medium (DAKO, S3023). Direct immunofluorescence intensity was scored by two independent pathologists (J.G. and J.D.) on a nominal scale of 0–3: none (0), weak,[1] moderate[2] and strong.[3]

2.2 | Complement fixation assay using BP patient serum (cohort II)

In this separate single-centre retrospective study (cohort II), ten patients with BP were included using the same criteria for BP diagnosis as described previously. The study protocol was approved by the local Medical Ethical Committee of the Erasmus Medical Center (MEC-2021-0313). Leftover serum from patients diagnosed with BP was previously collected and stored at −80°C. In 18/21 DIF-positive patients, either ELISA or IIF on monkey oesophagus was also positive. Leftover serum was available for additional analysis from 14/21 patients. Complement fixation assay was found positive in 7/14 (50%) patients. A total of ten patients were included in the analysis: seven patients from cohort I and three additional patients of which no more frozen material was available (and thus excluded from cohort I). The last three patients had circulating autoantibodies confirmed by positive ELISA tests (all positive for BP180, one also for BP230) and were able to induce complement fixation in vitro. Five μm thick skin cryosections were prepared from normal human skin from reduction mammoplasty. Complement activation assay was performed as described before: Cryosections of healthy human skin from mammoplasty were incubated with 1:8 diluted sera of BP patients in the presence of 10 mM EDTA to prevent unwanted complement activation, for 45 min at 37°C.[14,15] PBS was used for washing away the residual serum. Subsequently,

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TABLE 1 Cohort I.

Pt nr.	Fix test nr	Fix	ELISA	IIF	IgG	IgM	IgA	C1q	C3c	C4d	Properdin	FCN2	MBL2	C5b-9
1	9	+	BP180 ~ BP230	+	2	0	0	0	1	1	0	0	0	0
2		NA	Bp180	–	2	0	+/–	0	2	3	2	2	0	2
3	10	+	BP180 BP230 ~	+	1	0	0	0	1	3	0	0	0	3
4		NA	BP180	–	1	0	0	0	1	2	0	0	0	0
5	11	–	BP180	–	1	0	0	1	3	3	3	0	0	2
6	12	–	BP180	+	3	0	0	0	2	3	3	0	0	2
7	13	–	BP180	+	2	0	0	0	2	3	3	0	0	1
8		NA	–	–	3	0	+/–	0	1	0	2	0	0	2
9		NA	BP180	–	1	0	0	0	2	3	1	0	0	2
10	5	–	BP180	–	1	0	0	0	3	3	2	0	0	3
11		NA	–	–	1	0	+/–	0	2	1	3	0	0	2
12	7	+	BP180	+	1	0	0	0	2	2	0	0	0	1
13	3	–	–	–	1	0	0	0	0	0	0	0	0	2
14		NA	NA	NA	1	0	0	2	3	3	2	0	0	2
15	8	–	–	+	1	0	0	1	2	2	3	0	0	2
16	4	+	BP180	+	2	0	0	0	2	3	2	0	0	2
17	6	+	BP180	+	2	0	0	0	2	3	2	0	0	3
18	1	+ NA	BP180 BP230	+	3	0	0	0	3	3	3	0	0	1
19	2	+	BP180 BP230	+	2	0	0	0	1	3	2	0	0	0
20	14	–	–	+	1	0	+/–	1	3	3	3	0	0	0
21	15	+	BP180	–	1	0	0	0	2	3	1	0	0	0

Note: No serum left for all analysis; IgM and IgA according to earlier pathology report.
Abbreviation: NA, not available.

TABLE 2 Antibodies and dilutions used in the study.

Antibody	Host and targets species	Supplier	Catalogue#	Concentration/ dilution
IgG	Polyclonal-anti-human, FITC-labelled	Roche	760–2680	163.4 µg/mL
C1q	Polyclonal-anti-human, FITC-labelled	Roche	760–2688	66.6 µg/mL
C3c	Polyclonal rabbit-anti-human C3c	Dako	F0201	1:200
C4d	polyclonal rabbit-anti-human C4d	Biomedica	BI-RC4D	1:600
Properdin	Polyclonal rabbi-anti-human properdin	Kindly provided by prof. M.R. Daha, Leiden.		1:400
MBL	monoclonal mouse-anti-human MBL, clone 3E7	Hycult, Uden, The Netherlands	HM2061	1:200
Ficolin-2	Monoclonal mouse-anti-human ficolin-2, clone GN5	Hycult, Uden, The Netherlands	HM2091	1:150
C5b-9	Polyclonal rabbit-anti-human	Abcam	Ab55811	1:800

slides were incubated with 1:10 diluted normal human complement preserved serum (NHS, Quidel #A113) in HEPES buffer (0.01 M HEPES, 0.15 M NaCl, 135 nM CaCl2, 1 mM MgCl2; pH 7.4) to induce complement activation.[16] All analyses were performed in duplicate and incubated with NHS alone (negative control), EDTA (positive control) or in a dose-dependent fashion in the presence of either dexamethasone (Monofree Dexamethason eyedrops, Théa Pharma, Haarlem, The Netherlands), anti-C5 monoclonal antibody (mAb)

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(Eculizumab (Soliris ©), Alexion Pharmaceuticals), purified C1 esterase inhibitor (Berinert ©, CSL Behring), anti-factor B mAb (28.4.2, kindly provided by prof. S. de Cordoba) or anti-C3 mAb (12.17.3, kindly provided by prof. S. de Cordoba).[17,18] Isotype controls were used as negative controls and EDTA as positive control. Assay supernatants were collected and stored at −80°C for further sC5b-9 ELISA analysis. After washing with PBS, sections were stained for deposition of complement component C3c (Dako F0201) or C5b-9 (Abcam ab55811, for evaluation of eculizumab intervention downstream C3). Direct immunofluorescence intensity was scored by two independent pathologists (J.G. and J.D.) on a modified nominal scale of 0–6: 0 = no deposition, 1 = mild to no deposition, 2 = mild deposition, 3 = mild to moderate deposition, 4 = moderate deposition, 5 = moderate to strong deposition, 6 = strong deposition. Supernatants (duplicates) of the complement activation assay were collected and levels of sC5b-9 were measured in duplicate by ELISA as previously described, to determine fluid-phase complement activation.[19]

2.3 | Statistical analyses

Differences in the nominally scored intensity of staining between treated and untreated patients in the complement fixation tests were assessed using chi-squared tests. The non-parametric Kruskal–Wallis test, followed by Mann–Whitney *U*-tests were performed to compare sC5b-9 concentrations in the supernatant between treated and untreated patients in the complement fixation tests. Statistical analyses were performed using SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 28.0.1.0 Armonk, NY.), and two-sided *p*-values ≤0.05 were considered statistically significant.

3 | RESULTS

3.1 | Complement activation in skin biopsies from patient with BP (cohort I)

First, we evaluated the activation of different complement pathways as well as terminal pathway activation in $n = 21$ lesional skin biopsies from patients with BP by DIF. All patients showed linear depositions of immunoglobulins and/or complement component in a n-serrated pattern along the dermal-epidermal junction. IgG was found positive in 100% of cases (57% 1+, 29% 2+, 14% 3+), and weak co-deposition of IgA was found in four cases (19%) and IgM was always negative. Complement C3c was found in 95% (24% 1+, 48% 2+, 24% 3+) and C5b-9 in 90% (10% 1+, 67% 2+, 14% 3+). Classical pathway analyses revealed that C1q was positive in 19% of cases (14% 1+, 5% 2+) and C4d in 90% of cases (10% 1+, 14% 2+, 67% 3+). Alternative pathway analyses demonstrated properdin positivity in 76% of cases (10% 1+, 33% 2+, 33% 2+). Lectin pathway was only found in one case by means of ficolin-2 deposition 2+ (5%), while all other cases were ficolin-2 and MBL negative.

3.2 | Complement deposition and intervention in an in vitro complement fixation assay (cohort II)

Next, we evaluated different clinical and non-clinical complement inhibitors in an in vitro complement fixation assay to dissect the role(s) of the different complement pathway(s). As a comparison, a dexamethasone group was added since corticosteroids are still the mainstay of therapy nowadays. The clinically approved C1 inhibitor as well as the anti-C3 and anti-factor B mAbs, which are not approved for clinical use, prevented linear C3c BM deposition in a dose-dependent fashion. In addition, the anti-C5 mAb (the first complement inhibitor approved for clinical use) prevented C5b-9 deposition along the dermo-epidermal junction. By contrast, high-dose dexamethasone could not prevent linear C3c deposition (Figures 1 and 2). In summary, all four complement inhibitors were successful in preventing solid phase complement activation in our in vitro assay. Subsequently, we also tested the inhibiting capacity of all complement inhibitors on the release of sC5b-9 in the supernatant after incubation on the sections, termed fluid-phase complement activation. In parallel with local complement deposition, C1 inhibitor, the anti-C5 mAb and the anti-C3 mAb (partly) prevented the formation of sC5b-9 in supernatant after incubation on the sections. By contrast, however, the anti-factor B mAb did not prevent fluid-phase complement activation, reflected by increased sC5b-9 levels (Figure 3).

4 | DISCUSSION

Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by the formation of autoantibodies against BP180 and/or BP230. Corticosteroids are the mainstay of therapy but are associated with many, and sometimes severe adverse effects. Therefore, there is a high demand for novel therapeutic drugs, targeting crucial components in the pathogenesis of BP with a better safety profile. Complement is critically involved in the pathogenesis of BP and hence a potential target for therapy. Furthermore, the approval of multiple complement therapeutics for clinical use in the past two decades has sparked further interest in the potential of complement inhibitors for the treatment of other autoimmune diseases. The current study shows that, in contrast to high-dose dexamethasone, selective intervention in either the classical, alternative or terminal pathway prevented deposition of complement along the BM in a complement fixation BP assay. The results of our study greatly encourage the clinical development of complement inhibitors for the treatment of BP.

To our knowledge, this is the first study that investigated all three complement pathways in BP by means of DIF, including the lectin pathway. Our results confirm earlier reports of classical and alternative complement pathway activation, but could not demonstrate significant lectin pathway activation.[20,21] Therefore, our results discourage the clinical development of lectin pathway inhibitors for BP. Importantly, classical and alternative pathway activation

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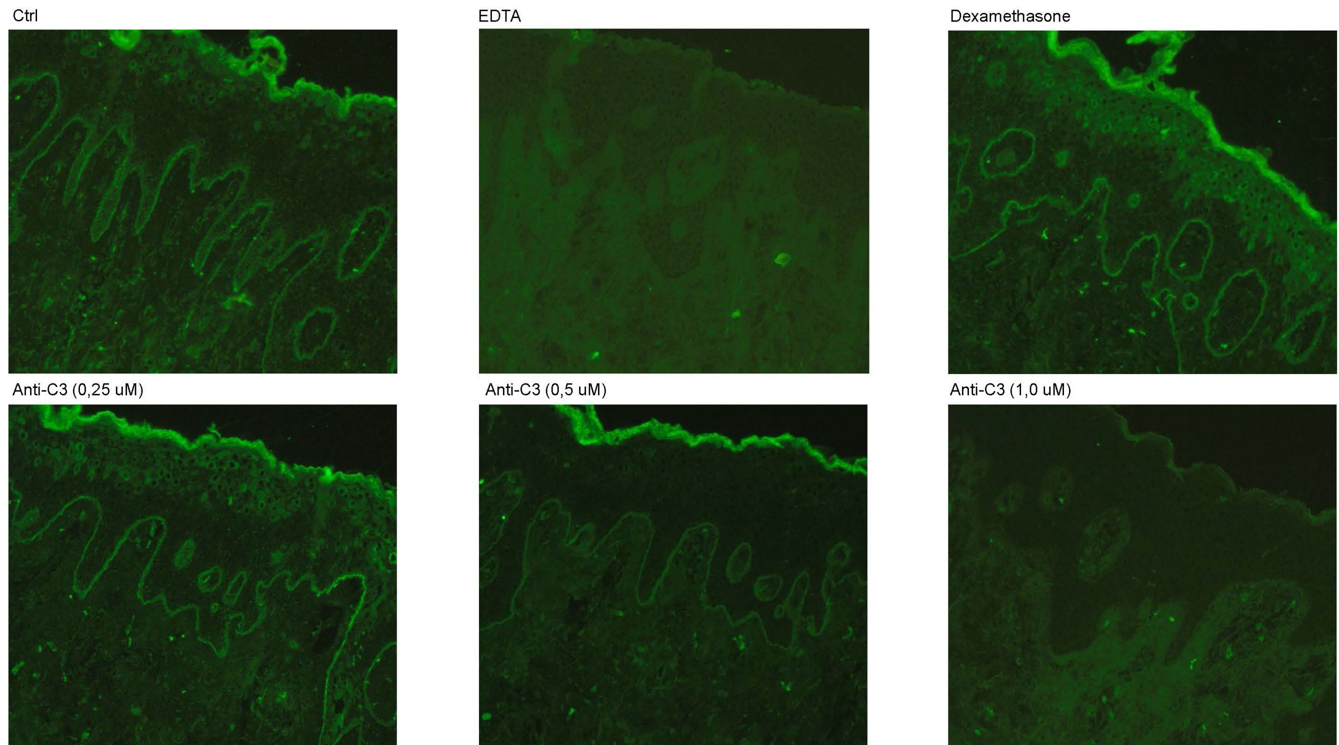

FIGURE 1 Complement fixation test indirect immunofluorescence C3c deposition. Representative photographs for analysis with anti-C3 in a dose-dependent fashion. Panel A, negative control (ctrl) with PBS (score 2); panel B, positive control EDTA (score 0); panel C, dexamethasone (score 2); panel D, anti-C3 0.25 μM (score 2); panel E, anti-C3 0.5 μM (score 1); panel F, anti-C3 0.25 μM (score 0).

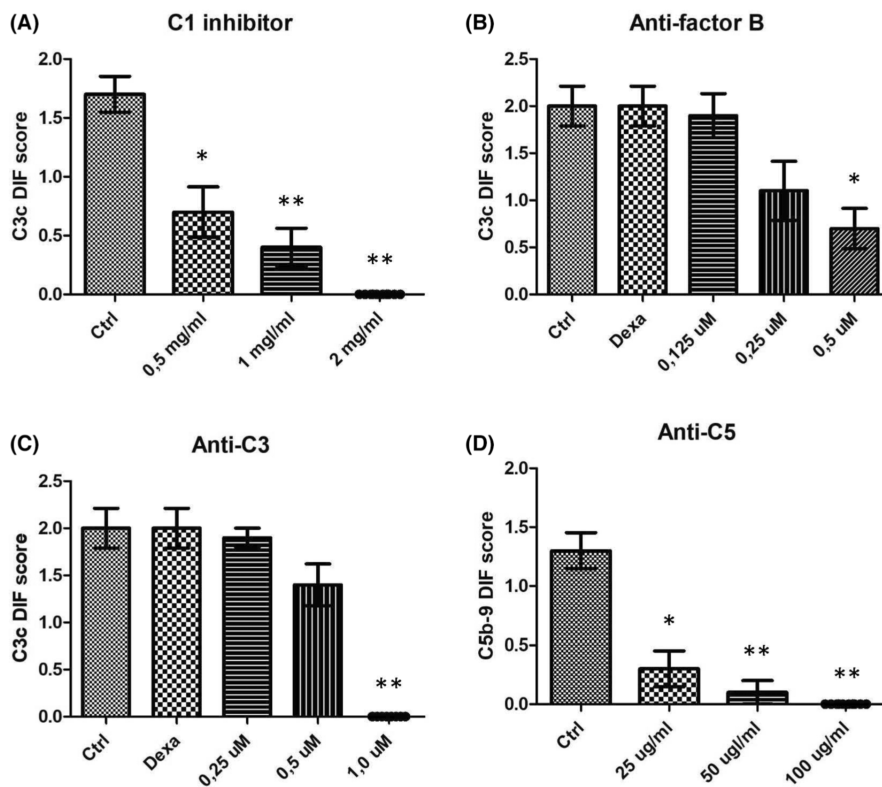

FIGURE 2 Complement fixation test indirect immunofluorescence C3c or C5b-9 scores in the presence or absence of C1 inhibitor (panel A), anti-factor B mAb (panel B), anti-C3 mAb (panel C) and anti-C5 mAb (panel D) in a dose-dependent fashion.

could be selectively inhibited by the clinically approved complement inhibitor Berinert (plasma-derived C1 inhibitor) or the anti-factor B mAb, which is not approved for clinical use. Although both inhibitors reduced local C3c along the BM, only C1 inhibition could also significantly prevent the release of sC5b-9 in the supernatant. There is, however, a discrepancy between the low amount of local BM C1q

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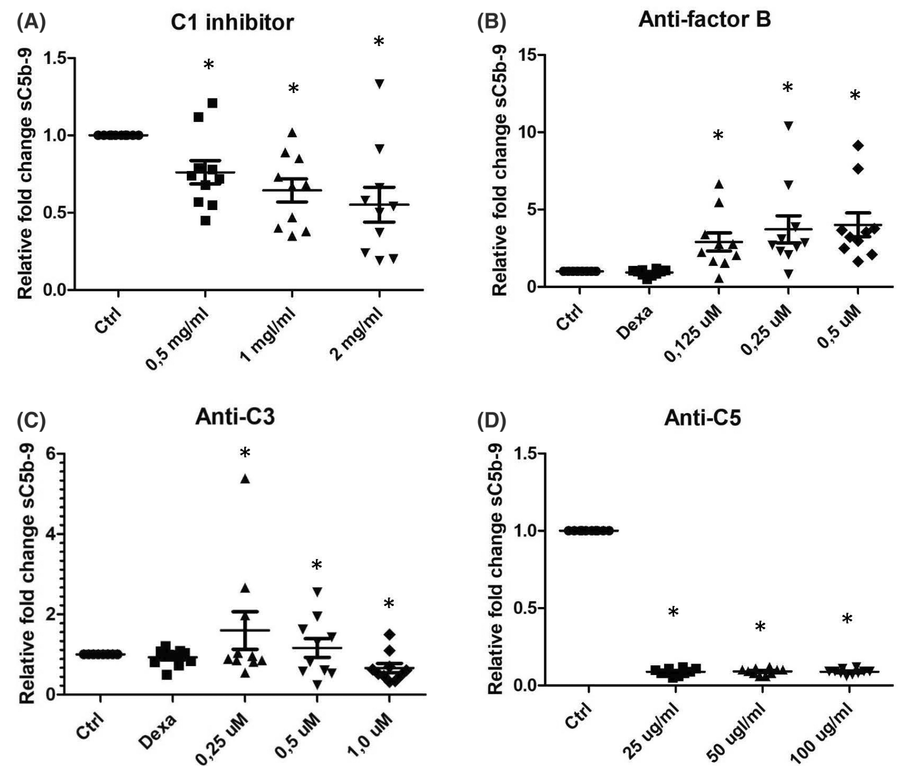

FIGURE 3 Complement fixation test release of sC5b-9 in the supernatant (on the section) in the presence of C1 inhibitor (panel A), anti-factor B mAb (panel B), anti-C3 mAb (panel C) and ani-C5 mAb (panel D) in a dose-dependent fashion. The relative release of sC5b-9 is compared with a negative control treated with PBS.

(19%) detected by DIF in BP skin biopsies and the strong inhibitory capacity of C1 inhibitor on local C3c BM deposition and the release of sC5b-9 in supernatant. It is well known from renal pathology that C1q has a low tissue residency time, an historic problematic issue in proving antibody-mediated rejection (ABMR) until the "discovery" of C4d as a magic marker of ABMR. This is most likely explained by the fact that C1q produces a *stronger opsonization* response than C4d.[22,23] We found strong C4d deposition by DIF as a results of classical pathway activation in our study. Although C4d deposition could hypothetically also be explained by lectin pathway activation, no significant deposition of lectin pathway initiators (MBL and Ficolin-2) was found, although we did not examine collectin-11 and ficolin-3. Therefore, we reason that (comparable to the renal transplant setting) C1q is initially highly deposited in vivo in BP, but is difficult to detect due to a low tissue residency time. By contrast, C4d is a much more sensitive marker for BP, which we already published in an earlier study and is comparable to other clinical settings such as ABMR.[24] We also found stronger inhibition by C1-inhibitor on local C3c deposition compared with inhibition on the release of sC5b-9 in the supernatant. This could theoretically be explained by strong (parallel) alternative complement pathway activation. However, based on our results using anti-FB this is not likely. Therefore, we hypothesize that inhibition of local C3c was sufficient to prevent local C3c deposition but not enough to inhibit fluid-phase complement activation in the supernatant in the presence of 12.5% serum (1:8 dilution). In contrast to C1-inhibitor, anti-factor B allowed the release of sC5b-9 in the supernatant. We hypothesize that BP is mainly a classical pathway-driven disease, in which the alternative pathway mainly functions as an amplification loop. Therefore, inhibition of factor B was probably sufficient to prevent solid phase C3c deposition along the BM, but fluid-phase activation was still possible through strong classical pathway activation in the supernatant.

Our findings are in line with previous experimental animal studies in which C4 knockout mice, and mice treated with anti-C1q mAb, showed protection against blistering in an experimental BP model.[8,9] In these animals, however, it is always questionable whether these findings can be translated into humans, since there are significant differences in the CS between rodents and humans, especially in the lectin pathway.[25,26] A major finding of our study is that classical pathway inhibition can prevent C3 deposition and release of sC5b-9 in supernatant in a *human* in vitro BP model. The successful intervention at the level of C1 supports recent positive findings by an ongoing phase 1 trial with a monoclonal antibody against C1s in healthy volunteers and patients with complement-mediated disorders (NCT02502903).[14,27] Another promising therapeutic intervention might be the use of a novel CD55/CD46 fusion protein that inhibits the C3-convertase of both the classical and alternative pathways as well as the C5-convertase. A recent study showed prevention of BM C3b deposition using a similar in vitro model as described in the current study.[28]

More downstream, selective intervention at the level of C3 or C5 (Eculizumab) could also successfully reduce complement deposition along the BM and the release of sC5b-9 in the supernatant. The rationale for inhibition of C5 is supported by the recent experimental publication on the detrimental effects of C5a-C5aR1 activation in BP. Absence of C5 or C5aR1 in mouse knockout models demonstrated protection against the development of BP.[10,12,29] Moreover, very recent data showed that dual inhibition

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of C5 and leukotriene B4 (Nomacopan) protected against the development of BP in humans.[30,31] Although C5-blocking drugs are interesting, it will also interfere with C5a-C5aR2-axis activation, which has recently shown to be protective for the development of BP.[10] Therefore, selective inhibition of C5a-C5aR1 axis, for example by avacopan or avdoralimab, might be more interesting thereby leaving the potentially beneficial C5a-C5aR2 interaction intact.[32] In fact, avdoralimab is currently evaluated in a phase 2 clinical trial (NCT04563923) with an enrolment target of 40 BP patients. Future studies should be aimed to investigate the efficacy of both drugs in inhibiting complement activation in BP. In vitro studies could potentially help in investigating this issue, although full-blood assays have to be developed in which immune cells also play a role. This is because C5a-C5aR1 interaction in BP has been mainly attributed to the recruitment of neutrophils via mast cells.[12]

Although we used concentrations in the therapeutic range of all compounds, it is not possible to adequately compare or predict the clinical efficacy of these drugs in this study. However, taking the foregoing into account, overall, it appears that both Berinert and Eculizumab are promising candidates for clinical development in BP. Considering our findings, an oral C3 inhibitor (Pegcetacoplan) could be another interesting candidate. This drug has recently been FDA-approved for the treatment of paroxysmal nocturnal haemoglobinuria.[33] By contrast, high-dose dexamethasone could not prevent either local skin deposition or release of complement in our in vitro model. These results encourage the clinical development of complement inhibitors for BP, which may lead to more effective and personalized treatments, with a favourable safety profile.

Although our results are encouraging, there are several limitations to our study. Firstly, the complement fixation model only investigates deposition of complement fragments in an isolated in vitro system, independent of circulating immune cells. It is well known that many immune cells, among which mast cells, eosinophils and neutrophils, are involved in the pathogenesis of BP. In favour of our findings though, it is known that many of these cells are recruited consequently/secondary to complement activation. Secondly, although we analysed the release of sC5b-9 in the supernatant, the model does not allow the analysis of functional consequences of complement inhibition on blister formation. Future experiments targeting complement could therefore focus on whole biopsy in vitro culture BP models or multilayer skin tissue models. On the contrary, major strengths of the current study are the extensive immunofluorescence studies with panels of antibodies against all three complement pathways, the combination of solid phase and fluid-phase complement activation in our in vitro model with various complement inhibitors.

Noteworthy, besides complement activation, also complement-independent mechanisms play a role in the pathogenesis of BP. In vitro studies that cultured keratinocytes in the presence of anti-human BP180, demonstrated binding, internalization, ubiquitination and degradation of BP180-IgG complex. Eventually, these pathogenic event lead to impairment of haemidesmosomal formation and weakening to the lamina densa.[1,34–39] These complement-independent mechanisms were supported by further in vivo studies in mice demonstrating that F(ab') 2 fragments of anti-BP180 IgG antibodies from BP patients or rabbit IgG against humanized NC16A induced (in part) skin fragility in neonatal humanized mice ([40]). Furthermore, Ujiie et al. found that passive transfer of human BP autoantibodies induced blister formation in neonatal C3-deficient COL17 humanized mice. The same study also showed that IgG4, a non-complement fixing subclass, could induce subepidermal blistering.[41] For a more extensive review on this topic, we refer to a recent publication of Papara et al.,[39] Altogether, these data support the role of complement-independent processes in the pathogenesis of BP, which are in part IgG4 mediated. Most probably, bot complement-dependent and independent processes act simultaneously in the same patient. In many cases, complement might be a driver of BP-disease, while in other cases, it may act more as an amplifier of the disease. In view of this, a combined therapy of complement targeting compound together with Fc-receptor blocking agents would be most optimal in targeting BP.

In conclusion, at this moment no complement-targeting drugs have been approved for clinical use in BP. The current study compared various clinically approved and non-approved complement inhibitors for the treatment of BP in an in vitro BP model. We found that different complement inhibitors could prevent the deposition complement along the BM and the release of sC5b-9 in the supernatant. According to our in vitro data, compounds targeting the classical pathway or terminal pathway are likely the most potent complement inhibitors and are therefore promising candidates for clinical development in BP.

AUTHOR CONTRIBUTIONS

Jenny Giang, Martijn B. A. van Doorn, Thierry P.P. van den Bosch and Jeffrey Damman designed and performed experiments, analysed data and wrote the manuscript. Gilles F.H. Diercks, Santiago Rodriguez de Cordoba, Marco W.J. Schreurs and Felix Poppelaars edited and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

F.P. owns stock in Chemocentryx, Omeros Corporation and Apellis Pharmaceuticals and has been involved as a consultant for Invizius. The other authors declare that there is no conflict of interest regarding the publication of this article.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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