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Title
Responses to rituximab suggest B cell-independent inflammation in cutaneous systemic lupus erythematosus

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Keywords
B cell Targets, Systemic Lupus Erythematosus, Biologic Response Modifiers, Autoimmune Diseases

Running Title
Rituximab for cutaneous lupus

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Abstract

Objective

The immunopathogenesis of SLE is heterogeneous. Responses to rituximab in skin are variable. We performed a detailed assessment of cutaneous responses to determine the phenotype of rituximab-responsive disease.

Methods

82 SLE patients receiving rituximab were prospectively studied. 32 had significant skin involvement before or after treatment. Disease activity was assessed using BILAG-2004. Cutaneous lupus subtype was classified by a dermatologist as acute, subacute or chronic cutaneous lupus erythematosus (ACLE, SCLE, CCLE) or other skin diseases, with supportive photographs or biopsies where necessary.

Results

10/26 (39%) patients with baseline skin disease had a beneficial cutaneous response to rituximab at 6 months with good response in ACLE (6/14, 43%), and poor responses in CCLE (0/8, p=0.034). Clinical response was associated with negative anti-RNP (p=0.024) and anti-Ro (p=0.035) serology. Flares of SCLE and CCLE occurred in 12 patients who either had no skin disease or ACLE at baseline (i.e. a switch in subtype). Concomitant antimalarials or conventional immunosuppressive were not associated with response or flare rate. Post-treatment biopsies confirmed typical active SLE histology in lesions occurring during B cell depletion.

Conclusion

Clinical response to rituximab in cutaneous manifestations of SLE depends on subtype. No CCLE patient responded and new CCLE lesions were observed during B cell depletion, suggesting that initiation and activity of these lesions is not B cell-dependent. Flares of a range of skin diseases after B cell depletion may indicate a change in immune regulation following B cell-targeted therapy.

Disease activity in systemic lupus erythematosus is (SLE) is inadequately controlled by conventional therapies(1-3). Targeted therapies may improve control of inflammation and several different molecules are under investigation or in clinical use. SLE is heterogeneous in immunopathogenesis as well as clinical phenotype and the range of potential targets under investigation reflect this. Many new agents target B cell function. Others target Type 1 interferons, T cell co-stimulation, cytokines or plasma cells(1). Of these agents, experience is greatest for B cell depletion using rituximab. However, this agent also has the most diverse outcomes with positive case series and negative clinical trials. The latter have been attributed to defects in trial design and/or outcome measures(2). Belimumab is licensed and proven to be effective. However, effect size is difficult to judge with a novel composite endpoint and small improvement in response rate compared to placebo. It is currently not clear whether the low response rates for these agents are due to an inappropriate mechanism of action, or pharmacodynamic factor (e.g. patients with non-B cell-mediated disease, or failure of drugs to adequately block pathogenic B cell function), or due to failure of clinical trial designs and outcome measures to accurately represent their efficacy(2).

Given the heterogeneity of SLE, a single therapeutic target may not be appropriate for all patients and disease manifestations. Cutaneous lupus is particularly heterogeneous. Although immune complex deposition is a common feature, non-B cell mechanisms are important with keratinocyte activation and apoptosis, inflammatory cytokine and chemokine production and a plasmacytoid dendritic cell and T cell infiltrate(4). The common occurrence of some subtypes of cutaneous LE in autoantibody-negative patients without SLE in other organs also suggests that the contribution of autoimmune B cells may not be essential to all subtypes.

In our previous study of rituximab in SLE we reported less consistent clinical response in the mucocutaneous domain of the BILAG(5). We therefore undertook detailed assessment of cutaneous SLE in a study of 32 patients to identify features associated with response and flare after rituximab.

Patients and Methods

Patients and therapy

82 patients receiving rituximab therapy for SLE in a single centre were prospectively

studied. All met ACR/SLICC criteria for SLE. Treatment protocol and overall clinical outcomes of the first 41 patients have been described in previous reports, which included 16 of the patients in the present study(5, 6). Usual criteria for rituximab therapy were: active SLE rating 1 x BILAG A or 2 x BILAG B in any domain(s), failure of previous immunosuppressive therapy including cyclophosphamide due to inefficacy or toxicity, or contraindication to cyclophosphamide. Patients received 2 x 1000mg infusions of rituximab on days 1 and 15, each preceded by 100mg methylprednisolone. Concomitant antimalarial or immunosuppressive therapy used at baseline was continued. All patients were followed up for at least 6 months.

32 patients with significant mucocutaneous manifestations of SLE before or after rituximab therapy were analysed. 26 of these patients had active mucocutaneous disease at baseline as a primary or co-primary indication for treatment, and were analysed for characteristics and predictors of response. 6 of these patients, as well as a further 6 patients who had not had skin disease prior to rituximab, had flares of new or different skin disease after rituximab. The clinical characteristics of these 12 patients with flares of cutaneous SLE during B cell depletion were compared to the 20 patients with cutaneous SLE who did not flare.

Classification of skin morphology

Patients were assessed in a combined rheumatology-dermatology clinic. The Gilliam classification terminology was used by a consultant dermatologist to categorise cutaneous lupus erythematosus as acute (ACLE), subacute (SCLE), chronic cutaneous (CCLE), lupus erythematosus non-specific(LENS, e.g. vasculitis) or non-lupus erythematosus skin diseases (NONLE, e.g. psoriasis) for summary statistics and evaluation of the association with response and flare(7). A detailed description of each lesion was documented and is given in the supplementary information (Table S1).

Classification of response

Clinical assessment was performed at baseline and 6 months using BILAG-2004(8). Additional flare visits were performed if clinically indicated and documented. In order to differentiate cutaneous response from overall response, BILAG score has been expressed as a cutaneous response based on the mucocutaneous domain and other dermatological features, and non-cutaneous BILAG response category based on the

other 9 domains of BILAG-2004. Confirmatory photographs or biopsies were obtained where necessary. Consent to publication of photographs was obtained using Leeds Teaching Hospitals NHS Trust consent.

Cutaneous Response

Cutaneous response and flare were classified according to BILAG mucocutaneous domain score, change in morphology and change in therapy. Mucocutaneous response was defined as reduction of BILAG A to B, C or D; reduction of BILAG B to C or D; reduction of BILAG C to D (the last definition applied to mild mucosal ulceration, mild alopecia or chilblains in this study) with no new topical or immunosuppressive therapy for skin disease. Flare was defined as either (i) new BILAG mucocutaneous A-C disease in a patient with no cutaneous involvement at the time of RTX, or (ii) a new cutaneous lupus morphology in a patient with or without other mucocutaneous LE at baseline.

Non Cutaneous Response

Data were presented for the most frequent non-cutaneous domains (musculoskeletal, renal and neurological) for patients with domains rated A or B at baseline. Overall disease activity was presented as number and percentage of patients with a BILAG A, B or C score before or 6 months after rituximab. In order to demonstrate association between cutaneous and non-cutaneous response, this was converted into a total numerical score for the whole group of patients (A=12, B=8, C=1, D/E=0)(9).

B cell depletion

B cell subsets were measured using a highly sensitive B cell assay that can reproducibly enumerate B cells at 0.0001 x 10^9 cells/liter, which we previously demonstrated to predict clinical response(5).

Statistical Analysis

Summary statistics were presented as proportions of patients. Association between clinical and immunological features and response or flare was tested using Chi Square tests.

Histological Analysis

Haematoxylin and eosin stained biopsies were scored (by Dr Edward) for presence of vacuolar degeneration of keratinocytes, basement membrane thickening, interface dermatitis, perivascular and perifollicular infiltration of lymphocytes, neutrophil infiltration and follicular plugging. Staining for dermal mucin and immunofluorescence for IgM, IgG and C3c was performed where necessary to establish the diagnosis.

Results

Overall BILAG responses

Overall, 26 patients had mucocutaneous disease before rituximab and of these, 10 (38.5%) had mucocutaneous response.

Scores have been summarised in Figure 1a for the 4 most commonly involved BILAG domains. Musculoskeletal domain score improved by at least 1 grade in 14/14 patients rated A or B at baseline, and by 2 grades in 5/14. Renal domain score improved by at least 1 grade in 7/7 patients rated A or B at baseline and by 2 grades in 5/7. Neurological domain score improved from B to C in 5/6 patients rated B at baseline, and remained rated B in 1/6.

Other than these 4 domains, BILAG A or B disease was also present in Haematology (4 cases), General (3 cases) and Cardiorespiratory (3 cases) of which all improved by at least 1 grade.

Mucocutaneous non-response was not associated with lack of response in other domains. There was a substantive reduction in total numerical domain score for the musculoskeletal, renal and neurological domains regardless of mucocutaneous response (Figure 1b).

Prediction of mucocutaneous response

Clinical features

Clinical and immunological features of patients with or without cutaneous response were compared. Details are shown in Table 1. Most patients had either ACLE (malar rash or more widespread photoaggravated maculopapular rash, usually in the

context of active disease in other organs) or CCLE (cutaneous or mucosal discoid LE of chilblains). Response rates in these subtypes were significantly different (p=0.034). Notably, none of the patients with CCLE had mucocutaneous response to rituximab. Details of individual patient responses are shown in Supplement 1 (Table S1). LE non-specific lesions were seen in 2 patients with vasculitis and skin disease and resolved completely in both. 2 patients had subacute LE, with widespread papulosquamous LE, and 1 responded completely.

Biomarkers

Unlike disease in other organs, no association between degree of initial B cell depletion and clinical response was observed for cutaneous disease. There were trends for association between anti-dsDNA antibodies and mucocutaneous response (p=0.075), and anti-Ro and mucocutaneous non-response (p=0.031). These relationships may be explained by these features being associated with ACLE and CCLE subtypes respectively (9/14 (64%) of ACLE patients were anti-dsDNA positive, 6/8 (75%) of CCLE patients were anti-Ro positive). There was a significant association between anti-RNP and mucocutaneous non-response (0/7 RNP positive patients responded, p=0.028). This could not clearly be explained by association with other clinical features at baseline (4/14 patients with ACLE (29%) and 2/8 patients with CCLE (25%) were anti-RNP positive).

Antimalarial or concomitant immunosuppressant use was not associated with cutaneous subtype at baseline and was not associated with better clinical response. Indeed, there was a trend to better response in patients not using antimalarials.

Prediction of mucocutaneous flare

Flares were observed in 12 patients. Of these, 9 were clinically typical cutaneous lupus lesions (4 annular SCLE, 5 localised or disseminated discoid LE). 3 patients had atypical LE lesions or diseases not usually associated with SLE (1 patient with biopsy-proven plaque psoriasis; 1 patient with biopsy-proven pemphigus; 1 patient with psoriasiform lesions with histological features of lupus).

Flares occurred in patients with ACLE or no skin disease at baseline. Hence, of the patients with ACLE who initially responded, a proportion developed SCLE or CCLE following B cell depletion. Other than cutaneous lupus morphology there was only a weak trend for association between flare and incomplete B cell depletion and a trend

to association with negative anti-Sm antibodies. There was no evidence that concomitant antimalarials or conventional immunosuppressives agents prevented flares.

Histological Studies

Details of histological studies are shown in Supplement 1 (Table S2). Pre-treatment biopsies confirmed that the cases treated had typical lupus appearances histologically. We did not identify specific features predictive of response. Post-treatment biopsies confirmed that patients with continuing skin activity or flares of LE or other diseases had histological features to support diagnosis. Variable immunofluorescence for IgM, IgG and C3c was observed in patients with active CLE before and after rituximab.

Discussion

In this study we explain variability in efficacy of rituximab in SLE by linking clinical response to cutaneous morphology and antibody status. This is the first evidence that clinical subgroups of SLE patients may require different targeted therapies. Our key findings were that response was better in patients with ACLE (often receiving treatment in the context of systemic multi-organ disease) than in SCLE or chronic cutaneous forms. Further underlining the resistance of non-ACLE skin manifestations to rituximab we noted that, in several patients, flares of SCLE and CCLE were observed after rituximab at a time of near-complete or complete B cell depletion. Despite these negative outcomes in cutaneous disease, these patients simultaneously responded well in other organ systems. We could not explain these results in terms of concomitant therapies used or depth of B cell depletion. Hence our results suggest that the role of B cells in CCLE differs from other cutaneous or systemic manifestations of SLE. This conclusion is consistent with the well-known occurrence of discoid LE in autoantibody-negative individuals who never develop manifestations of SLE in other organs.

Innate and T cell mechanisms have been described that may predominate in these lesions and account for non-response to B cell depletion (reviewed in (10)). Cutaneous LE is initiated by keratinocyte apoptosis, commonly in response to UV light exposure. This leads to surface expression of typical lupus antigens, such as Ro and other danger signals. The expression of these antigens may be exacerbated by defects of apoptotic clearance in some patients. Sensing of nuclear antigen by plasmacytoid dendritic cells (via Toll-like receptors) leads to the production of Type 1 interferons, which have numerous effects in triggering local inflammation and autoimmunity. Keratinocytes activated by UV light or danger signals generate inflammatory cytokines as well as chemokines that lead to tissue inflammation as well as a CD4 and CD8 T cell infiltrate.

B cells and plasma cells may exacerbate tissue inflammation. Generation and deposition of autoantibody-containing immune complexes may lead to local complement activation, as well as increasing IFN production by plasmacytoid dendritic cells(11). However, even if B-plasma cell functions assist in the initiation of cutaneous lesions, the above model would suggest they may not always be essential to their perpetuation.

An additional finding in our study was a relationship between the presence of specific autoantibodies and response to rituximab. Anti-dsDNA and anti-Ro were associated with better and worse clinical response respectively. These antibodies were also associated with clinical subtypes of SLE, and it is therefore unclear from our results whether a patient with ACLE and anti-dsDNA will respond better than a patient with ACLE and Ro. RNP appeared to be predictive of outcome independent of CLE lesional morphology. We note that anti-RNP-containing immune complexes stimulate plasmacytoid dendritic cells to produce type I interferons via TLR7 (in contrast to the TLR9 mediated effects of anti-dsDNA antibodies), and give rise to a particular pattern of Toll-Like Receptor activation(12, 13). Hence this observation may also be consistent with the notion that, once initiated, TLR-IFN innate immune mechanisms may lead to disease that is resistant to B cell targeting.

Whilst B cells may not be required for the persistence of cutaneous lupus, the frequency and characteristics of the flares of CLE or even other skin diseases after rituximab also require explanation. We postulate two additional processes that might account for these cases. First, B cells may have regulatory effects in some circumstances via their secretion of IL-10. IL-10 counter-regulates IL-12, which in turn regulates expression of T cell cutaneous lymphocyte antigen (CLA) and therefore T cell homing to the skin (implicated in all the diseases observed here). Second, B cell lysis may itself have pro-inflammatory effects – suggested by the transient nature of some lesions we observed early after treatment.

These results indicate the importance of detailed phenotyping of patients in assessment of outcomes of targeted therapies in clinical trials. The BILAG index allows differentiation of better responses in different organ systems(8). The Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) allows a more detailed assessment of skin involvement, including differentiation of activity and scarring(14). However, neither of these indices capture clinical morphology or histological features. Whilst further validation of our results is required, we suggest that this information may be essential for the appropriate treatment and monitoring of SLE patients for biologic therapy both in trials and clinical practice.

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Tables

Table 1: Factors associated with cutaneous response to rituximab

Characteristic	Total (n=26)	Cutaneous Response (n=10)	Cutaneous non-response or worsening (n=16)	p
Subtype of skin disease				
ACLE	14	6/14 (42.9%)	8/14 (57.1%)	0.034
SCLE	2	1/2 (100%)	1/2 (0%)	
CCLE	8	0/8 (0%)	8/8 (100%)	
LENS	2	2/2 (100%)	0/2 (0%)	
Serology				
dsDNA	14	7/9 (77.8%)	6/17 (35.3%)	0.075
Ro	16	3/9 (33.3%)	13/15 (76.5%)	0.031
La	8	2/9 (22.2%)	6/17 (35.3%)	0.492
Sm	4	1/9 (11.1%)	3/17 (17.6%)	0.660
RNP	7	0/9 (0.0%)	7/17 (41.2%)	0.024
Low C3	9	3/9 (33.3%)	6/17 (35.3%)	0.920
Low C4	11	4/9 (44.4%)	7/17 (41.2%)	0.873
B cell depletion				
Complete	10	4/10 (40.0%)	6/16 (37.5%)	0.831
Concomitant Therapies				
HCQ	11	2/9 (22%)	10/17 (58.8%)	0.075
IS	15	7/9 (78%)	11/17 (64.7%)	0.492

ACLE = Acute Cutaneous Lupus Erythematosus
SCLE = Subacute Cutaneous Lupus Erythematosus
CCLE = Chronic Cutaneous Lupus Erythematosus
LENS = Lupus Erythematosus non-specific lesions
dsDNA = anti-double stranded DNA antibodies
Ro = anti-Ro/SSA antibodies
La = anti-La/SSB antibodies
RNP = anti-ribonuclear protein antibodies
C3, C4 = complement components C3 and C4
HCQ = hydroxychloroquine
IS = conventional immunosuppressant

Table 2: Factors association with cutaneous flare after rituximab

Characteristic	Total (n=32)	Flare (n=12)	No Flare (n=18)	p
Subtype of skin disease at baseline				
ACLE	14	6/12 (50.0%)	8/20 (40.0%)	
SCLE	2	0/12 (0.0%)	2/20 (100.0%)	
CCLE	8	0/12 (0.0%)	8/20 (40.0%)	0.002
LENS	2	0/12 (0.0%)	2/20 (10.0%)	
No skin disease	6	6/12 (100.0%)	0/20 (0.0%)	
Subtype of skin disease at flare				
ACLE	0	0	N/A	
SCLE	4	4	N/A	N/A
CCLE	5	5	N/A	
LENS/NONLE	3	3	N/A	
Serology at baseline				
dsDNA	17	7/12 (58.3%)	11/20 (55.0%)	0.854
Ro	17	8/12 (66.7%)	11/20 (55.0%)	0.515
La	9	4/12 (33.3%)	5/20 (25.0%)	0.612
Sm	4	0/12 (0.0%)	4/20 (22.2%)	0.098
RNP	7	4/12 (33.3%)	4/20 (20.0%)	0.399
Low C3	12	6/12 (50.0%)	6/20 (30.0%)	0.258
Low C4	14	6/12 (50.0%)	8/20 (40.0%)	0.581
B cell depletion				

Complete	12	3/12 (25.0%)	9/20 (45.0%)	0.258
Concomitant Therapies				
HCQ	12	4/12 (33.3%)	9/20 (45.0%)	0.515
IS	24	10/12 (83.3%)	14/20 (70.0%)	0.399

ACLE = Acute Cutaneous Lupus Erythematosus

SCLE = Subacute Cutaneous Lupus Erythematosus

CCLE = Chronic Cutaneous Lupus Erythematosus

LENS = Lupus Erythematosus non-specific lesions

NONLE = skin disease not typically seen in patients with cutaneous or systemic lupus

dsDNA = anti-double stranded DNA antibodies

Ro = anti-Ro/SSA antibodies

La = anti-La/SSB antibodies

RNP = anti-ribonuclear protein antibodies

C3, C4 = complement components C3 and C4

HCQ = hydroxychloroquine

IS = conventional immunosuppressant

Figure Legends

Figure 1a: Comparative response in individual BILAG domains

Response in the four most common domains rated BILAG A or B at baseline. Bars show percentage of patients with scores A, B or C before (Pre) and 6 months after (Post) rituximab.

Figure 1b: BILAG response in non-cutaneous domains according to cutaneous response.

Total numerical BILAG domain score in the four most common domains rated BILAG A or B at baseline for patients with cutaneous response (n=10) or cutaneous non-response (n=16).

List of Supplementary Data

Supplement 1

Table S1: Details of responses in individual patients

Table S2: Details of histological studies

Supplement 2

Figure S1: Photographs before and after rituximab in patient with SCLE and good response

Figure S2: Histology from same patient as Figure S1 before rituximab

Figure S3: Photographs of active discoid LE in B cell depleted patient with good haematological response

Figure S4: Biopsy of active discoid lesion in B cell depleted patient with good neurological response

Figure S5: Biopsy demonstrating active pemphigus in B cell depleted patient with no prior cutaneous involvement of SLE

Figure S6: Photographs showing patient with persistent active disseminated discoid LE after 2 cycles of rituximab

Figure S7: Biopsy with H&E and mucin staining from a patient who developed SCLE after rituximab

Figure S8: DIF images demonstrating IgM and C3c deposition during B cell depletion

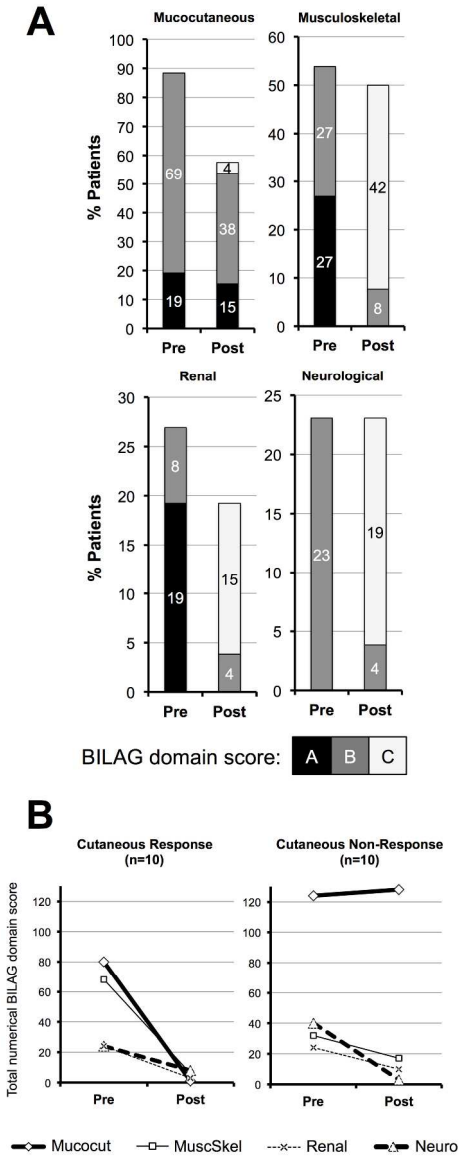

Figure 1a: Comparative response in individual BILAG domains: Response in the four most common domains rated BILAG A or B at baseline. Bars show percentage of patients with scores A, B or C before (Pre) and 6 months after (Post) rituximab.

Figure 1b: BILAG response in non-cutaneous domains according to cutaneous response.
Total numerical BILAG domain score in the four most common domains rated BILAG A or B at baseline for patients with cutaneous response (n=10) or cutaneous non-response (n=16).
214x549mm (300 x 300 DPI)

Supplement 1
Table S1: Details of responses in individual patients

Case	Before Rituximab		After Rituximab		Mucocutaneous BILAG		Overall Mucocutaneous Response
	Description	Classification	Description	Flare Classification	Pre	Post	
1*	Malar ACLE	ACLE	Persistent malar ACLE		B	B	Non Response
2*	Malar ACLE	ACLE	Resolved		B	D	Improved
3*	Malar ACLE	ACLE	Resolved		B	D	Improved
4	Malar ACLE	ACLE	Resolved		B	D	Improved
5	Malar, chest and upper limb ACLE	ACLE	Resolved		B	D	Improved
6	Malar ACLE	ACLE	Resolved		B	D	Improved
7	Malar ACLE	ACLE	Resolved		B	D	Improved
8	Malar ACLE	ACLE	- change		B	B	Non Response
9*	Widespread typical acute LE	ACLE	Transient improvement, developed disseminated discoid LE at 6 months	CCLE	B	B	Switch / Flare
10*	Mild malar ACLE	ACLE	Initial improvement, developed facial discoid LE at 10 months (Figure S4)	CCLE	B	B	Switch / Flare
11*	Widespread, strongly photoaggravated acute LE unresponsive to HCQ	ACLE	Developed disseminated discoid LE at 6 months (usually uncommon in male -n-smokers). Became responsive to HCQ.	CCLE	B	B	Switch / Flare
12*	Acute facial LE with diffuse -n-scarring alopecia	ACLE	Developed disseminated, scarring discoid LE with acral LE (a variant of CCLE) at 3-4 months	CCLE	B	B	Switch / Flare
13*	Widespread ACLE	ACLE	Initial improvement, developed biopsy-proven SCLE with neutrophilia at 7 months	SCLE	B	B	Switch / Flare
14	ACLE with typical biopsy, positive IF (Figure S9).	ACLE	Developed psoriasiform lesions with interface dermatitis but negative IF (Figure S9)	LENS	B	A	Switch / Flare
15	Papulosquamous SCLE (Figures S1 and S2)	SCLE	Complete resolution (Figure S1)		A	D	Improved
16*	Papulosquamous SCLE	SCLE	Papulosquamous SCLE		A	A	Non Response
17*	Numerous discoid lesions on face only	CCLE	Persistently active discoid LE during B cell depletion. Discoid lesions resolved 3 years later when thrombocytopenia relapsed. (Figure S3)		B	B	Non Response