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B cell subset composition segments clinically and serologically distinct groups in chronic cutaneous lupus erythematosus

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Abstract

Objective—While the contribution of B-cells to SLE is well established, its role in chronic cutaneous lupus erythematosus (CCLE) remains unclear. Here, we compare B-cell and serum auto-antibody profiles between patients with systemic lupus erythematosus (SLE), CCLE, and overlap conditions.

Methods—B-cells were compared by flow cytometry amongst healthy controls, CCLE without systemic lupus (CCLE+/SLE−) and SLE patients with (SLE+/CCLE+) or without CCLE (SLE+/CCLE−). Serum was analyed for autoreactive 9G4+, anti-double-stranded DNA, anti-chromatin and anti-RNA antibodies by ELISA and for anti-RNA binding proteins (RBP) by luciferase immunoprecipitation.

Results—Patients with CCLE+/SLE− share B-cell abnormalities with SLE including decreased unswitched memory and increased effector B-cells albeit at a lower level than SLE patients. Similarly, both SLE and CCLE+/SLE-patients have elevated 9G4+ IgG autoantibodies despite

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lower levels of anti-nucleic acid and anti-RBP antibodies in CCLE+/SLE−. CCLE+/SLE− patients could be stratified into those with SLE-like B-cell profiles and a separate group with normal B-cell profiles. The former group was more serologically active and more likely to have disseminated skin lesions.

Conclusion—CCLE displays perturbations in B-cell homeostasis and partial B-cell tolerance breakdown. Our study demonstrates that this entity is immunologically heterogeneous and includes a disease segment whose B-cell compartment resembles SLE and is clinically associated with enhanced serological activity and more extensive skin disease. This picture suggests that SLE-like B-cell changes in primary CCLE may help identify patients at risk for subsequent development of SLE.B-cell profiling in CCLE might also indentify candidates who would benefit from B-cell targeted therapies.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by heterogeneous clinical manifestations and the production of diverse autoantibodies resulting from defective B cell tolerance and B cell hyper-responsiveness to stimulation.[1] While skin involvement is common in SLE,[2] it may also be present in patients with ‘primary’ chronic cutaneous lupus erythematosus (CCLE), in the absence of systemic involvement. CCLE includes discoid lupus erythematosus (DLE) and other conditions that often lead to permanent skin scarring,[3] and up to 20% of these patients develop SLE over several years.[3–5] However, the presence of DLE in patients with SLE has been found to reduce the risk of severe systemic manifestations, including lupus nephritis.[6] These findings suggest potential immunopathogenic differences across lupus categories.

In SLE, B cell hyperactivity is illustrated by the diversity and abundance of autoantibodies,[7] the concentration of risk alleles on B cell signalling pathways,[8] and the clinical benefit imparted by anti-B cell therapies.[9 10] In contrast, limited autoantibody production and poor response to B cell depletion in CCLE (relative to the dermatological improvement observed in SLE), have called into question the pathogenic role of B cells in this condition.[11 12]

Multiple B cell abnormalities have been consistently documented in SLE including the expansion of plasmablasts (PB), transitional and pregerminal centre cells[13 14]; increased $IgD^{-}CD27^{-}$ double negative (DN) B cells,[15 16] owing to the preferential expansion of the effector DN2 compartment,[17–19] and the contraction of IgD+CD27+ USM B cells.[19] Moreover, SLE is characterised by profound defects in the censoring of autoreactive B cells both centrally (antinuclear reactivity),[20 21] and peripherally, as illustrated by autoreactive VH4.34 antibodies that are recognised by the rat anti-human idiotypic antibody 9G4 (9G4+),[22] whose expansion is promoted by defective germinal centre censoring.[23] These defects lead to the accumulation of high levels of serum 9G4+ IgG in 45%–70% of patients with SLE with very high disease specificity (>90%).[24] These autoantibodies are associated with higher renal, neurological, haemato-logical and cardiovascular activity, but not skin manifestations.[22] 9G4+ IgG antibodies also correlate with anti-double-stranded DNA (dsDNA) IgG and contribute a substantial proportion of anti-dsDNA antibodies and

a majority of autoantibodies recog-nising apoptotic cells, a major immunogenic source in SLE.[22 25]

In contrast to SLE, little is known about the regulation and potential role of B cells in CCLE. Similarly, little information is available regarding B cell tolerance in this condition. In this study, we compared B cell and autoantibody profiles between patients with primary CCLE and patients with SLE with and without CCLE. Our results demonstrate CCLE heterogeneity with SLE-like abnormalities in a significant fraction of patients. This profile was associated with selective breakdown of B cell tolerance and the expression of autoantibodies. We postulate that B cell profiling may help identify patients with CCLE likely to progress to SLE and more likely to respond to B cell therapies.

PATIENTS AND METHODS

Patient samples

We collected blood samples among participants of the Georgia Organized Against Lupus (GOAL), a population-based cohort of individuals with a validated diagnosis of either SLE or primary CCLE. GOAL recruitment and data collection are described in the online supplemental methods and published elsewhere.[26] Medical records review, physician assessment and picture review were conducted to validate the lupus diagnosis. Cases with a dermatologist-documented diagnosis of either DLE, lupus erythematosus panniculitis (LEP), lupus erythematosus tumidus (LET) or chilblain lupus erythematosus (ChLE) were classified as CCLE. The 1997 Revised American College of Rheumatology Classification Criteria for SLE,[27] and the attending rheumatologist/dermatologist judgement were used to classify cases into three categories: primary CCLE (CCLE+/SLE−), SLE associated with CCLE (SLE+/CCLE+) and SLE without CCLE (SLE+/CCLE−). The Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) scores[28] were assessed within 14 days of the blood draw in a subset of participants. No patients were being treated with B cell depletion therapy. Thirty-nine additional patients with SLE were assessed for serological studies. Additionally, we utilised 46 health control donors for flow cytometry and 69 for serological studies.

Patient and public involvement

We have convened a diverse group of GOAL participants into the Lupus Patient Advisory Research Council (L-PARC). L-PARC members meet at least once a year with researchers to provide feedback on study measures and advice on recruitment, retention and dissemination of findings.

B cell phenotyping by flow cytometry

Blood was collected from patients in BD Vacutainer CPT tubes and peripheral blood mononuclear cells (PBMCs) were isolated and banked in liquid nitrogen until use. For flow cytometry analysis of the B cell subsets, PBMCs were stained at 4°C for 30 min in phopsphate buffered saline (PBS) plus 2% fetal bovine serum with fluorochrome-conjugated antibodies against the markers described in online supplemental table 1 and washed. Cells were then stained with Fixable Viability Dye eFluor506 (eBioscience) and fixed with 0.5%

formaldehyde in PBS followed by washing and acquisition on a LSRII Flow Cytometer (BD Bioscience). Flowjo (BD Bioscience) software was used for analysis. Clustering analysis is described in the online supplemental methods.

Apoptotic cells binding assay

Apoptosis was induced in CD45-negative Jurkat cells (J45.1; American Type Culture Collection), which were then incubated with patient serum as described previously,[25] and in the online supplemental methods.

Serological assays

9G4+ IgG, IgM and IgA antibodies were assayed by ELISA as described in the online supplemental methods. Anti-dsDNA IgG and antichromatin IgG levels were tested by QUANTA Lite dsDNA ELISA and Chromatin ELISA kits (INOVA Diagnostics). Anti-RNA was quantified by ELISA,[19] and anti-RNA-binding protein antibodies by luciferase immunoprecipitation assay as described previously,[29 30] and in the online supplemental methods.

Statistical analysis

Statistical analysis was done using Graphpad Prism V.8. Mann-Whitney U test was used to compare differences between two groups and Kruskal-Wallis test with Dunn's multiple comparison test to compare multiple groups. Fischer's exact test was used for contingency testing of distribution in two categories and χ^2 test for more than two categories. Correlation was determined by Pearson correlation coefficient or Spearman's rank correlation coefficient.

RESULTS

Description of patients

We obtained blood samples of 207 patients: 69 CCLE+/SLE−, 53 CCLE+/SLE+ and 85 SLE+/CCLE. Among the CCLE+/SLE− and CCLE+/SLE+ cases, 65 (94%) and 52 (98%) had a diagnosis of DLE, respectively. Of those, 9/65 and 3/52 had DLE associated with LEP or ChLE. The non-DLE cases were diagnosed with LEP (three in the CCLE+/SLE− and one in the CCLE+/SLE+ groups) and one in the CCLE+/SLE− had LET. Table 1 depicts demographic and disease characteristics by group; patients who were CCLE+/SLE− were significantly older and had shorter disease duration than the two other groups.

B cell homeostasis in CCLE

Canonical human CD19+ B cell subsets were defined by the expression of IgD, CD27 and CD38 as combined naive and transitional (N+T; unswitched memory (USM); isotype switched memory (SWM); DN; and PB (figure 1A)).[31] The expression of CD11c and CD21 further discriminated DN1, DN2 and DN3 among DN cells and resting naive (rNAV) and activated naive (aNAV). CD24 expression identified transitional populations (T1+T2) in the N+T compartment (figure 1B).[32–34]

All three lupus groups shared characteristic perturbations of normal B cell homeostasis consisting of loss of USM cells and expansion of PB (figure 1C). DN cells were expanded in all lupus groups, but with higher values in SLE+/CCLE− than in CCLE+/SLE−. Notably, within patients with SLE, the presence of CCLE correlated with DN expansion of lower magnitude (figure 1C), a feature consistent with both, the association between DN2 and LN in SLE and the decreased incidence of LN in SLE+/CCLE+. Consistent with our previous findings in SLE and severe COVID-19 infections,[35] DN expansions were accounted for in all groups by increases of effectors DN2 and DN3 cells with concomitant reversal of the normal predominance of DN1 cells, a population transcriptionally linked to resting SWM cells (figure 1D,E).[19] Similarly, aNAV, representing DN2 progenitors, were also expanded in SLE+/CCLE− and SLE+/CCLE+, relative to CCLE+/SLE− (figure 1F). Immature T1/T2 were expanded in CCLE relative to HCD and SLE+/CCLE− (figure 1G). As further illustrated below, however, all lupus groups were heterogeneous for these populations and included a significant fraction of patients with values above the upper limit of healthy individuals. This was particularly true for SWM and a substantial fraction of patients with SLE+/CCLE− (17%), SLE+/CCLE+ (19%) and CCLE+/SLE− (16%) had a frequency of SWM more than 2SD over the HCD mean.

B cell fingerprinting and disease heterogeneity

Multivariant analysis of B cell profiles revealed significant heterogeneity within patients with lupus (figure 2A). Overall, the combined cohort of HCD and all lupus subsets could be clustered into five separate groups defined by the relative frequencies of three B cell types: (1) early B cell (rNAV, early T1+T2); (2) memory (USM, SWM and DN1); and effector (aNAV, DN2, DN3 and PB). HCD could be separated into two clusters (III/IV) defined by higher frequencies of USM and rN+T3 subsets (figure 2A). In contrast, patients with SLE were concentrated within three clusters with only small fractions (15%–16%), expressing HCD-like B cell profiles. SLE clusters I/II were characterised by a more activated B cell profiles with high frequencies of effector B cells including aN, DN2, DN3 and PB (figure 2A–C), which were most pronounced in cluster II. In turn, SLE cluster V was characterised by the coordinated expansion of T1/T2 and rN/T3 cells in combination with the largest decrease of USM cells (figure 2C). Patients with CCLE+/SLE− displayed the largest degree of B cell heterogeneity of all groups with significant representation within all clusters (figure 2A,B). Overall, while a small majority (58%), expressed SLE-like profiles (I,II,V), 42% had HCD-like B cells. Within the CCLE+/SLE− that clustered with patients with SLE, 64% belonged in clusters I/II with cluster II contributing 12% of all patients with CCLE.

Serological autoimmunity in CCLE

Given that subsets of patients with CCLE+/SLE− shared SLE B cell abnormalities, we examined whether tolerance was similarly compromised using serological autoreactivity as a readout. We found that class switched 9G4+ IgG (figure 3A) and 9G4+ IgA (figure 3B) antibodies were elevated in all lupus groups, which shared similar frequencies (48%–57%) and median values of 9G4+ IgG and IgA antibodies. Only minor differences were observed in 9G4+ IgM (figure 3C).

The canonical autoreactivity of 9G4 antibodies is imparted by its germline sequence and results in binding to the B220 epitope expressed on B cells which results in the majority of naive B cells becoming 9G4+.[36] In addition, 9G4 antibodies also mediate high reactivity against apoptotic cells in patients with SLE through HCDR3-determined binding.[25 37] Of interest, a much larger fraction of 9G4+ antibodies in patients with CCLE+SLE− were autoreactive against apoptotic cell antigens (48% positive) (figure 3D) with a significantly lower level of anti-B cell autoreactivity (17% of samples with serum 9G4+ antibodies) (figure 3E). Anti-dsDNA and anti-chromatin antibodies were present in patients with CCLE+/SLE− but a lower frequency than in SLE (figure 4A,B; anti-dsDNA: 33% of SLE+/CCLE+ and 44% of SLE+/CCLE− compared with only 13% of CCLE+/SLE−) and anti-chromatin antibodies were similar. Anti-dsDNA and anti-chromatin were positively correlated in both groups of patients with SLE (figure 4C). Consistent with previous findings, 9G4+ IgG positively correlated with anti-dsDNA and anti-chromatin IgG in the SLE+ groups (figure 4D,E). In contrast, no correlation was found in patients with CCLE+/SLE−. Instead, anti-dsDNA and anti-chromatin antibodies were both sharply uncoupled from 9G4+ IgG even in patients with elevated values of both types of autoantibodies.

Anti-RNA antibodies are commonly found in SLE and we have shown that this association is particularly strong in patients with expanded effector B cells.[19] Anti-RNA antibodies were elevated in all three lupus groups including in 46% of patients with CCLE+/SLE− compared with 72% of patients with SLE+/CCLE− and 56% of patients with SLE+/CCLE+. As shown in figure 4F, anti-RNA titers were lower in patients with CCLE+/SLE− than in patients with SLE+/CCLE−.

We used a sensitive and highly quantitative luciferase immune-precipitation assay to quantify serum anti-RNA-binding protein (RBP) antibodies (Sm, RNP, Ro52 and Ro60).[29 30] In all, RBP autoantibodies were more common in SLE groups. Consistent with our previous findings, the frequency of anti-Sm antibodies (71%), was significantly higher than that commonly encountered in cohorts with lower representation of African American patients.[19 38] RBP autoantibodies were also present in patients with CCLE+/SLE− although at a significantly lower frequency. Nonetheless, detectable levels of anti-Sm, were present in 38%, a rate consistent with SLE cohorts with lower representations of African American patients. Anti-RNP (17%), anti-Ro52 (19%) and anti-Ro60 (49%), while substantial, were also lower than observed in SLE+/CCLE− and SLE+/CCLE+ (figure 5A). In patients with CCLE+/SLE−, only anti-Ro52 and anti-Sm titers were higher than HCD and titers for each antigen were higher in patients with SLE+/CCLE− and SLE+/CCLE+ than in patients with CCLE+/SLE− (figure 5B).

Hierarchical clustering demonstrated a strong association between anti-Ro52 and anti-Ro60 reactivity and between anti-Sm and anti-RNP autoantibodies (figure 5C). The vast majority (95%) of HCD did not have reactivity to any of the tested antigens. In contrast, only 33% of patients with CCLE+/SLE− lacked autoreactivity but only 16% had reactivity against multiple autoantigens compared with 44% and 24% of patients with SLE+/CCLE− and SLE+/CCLE+, respectively (figure 5D). Anti-Sm and anti-RNP were highly correlated in patients with SLE but dissociated in patients with CCLE+/SLE− (figure 5E).

Clinical and immunological associations of B cell heterogeneity in CCLE

As previously indicated (figure 2), patients with CCLE+/SLE− displayed a high degree of B cell heterogeneity which can be more precisely evaluated by restricted analysis of this clinical group. Hence, we determined potential clinical and immunological associations of the two major clusters of CCLE through a comparison of patients with SLE-like and HCD-like B cell phenotypes. Patients with CCLE with a SLE-like B cell phenotype were more likely to have generalised lesions (58%) and less likely to be males (8%) than those with a HCD-like B cell phenotype (15% generalised lesions; 31% males) (figure 6A). Disease duration did not differ between the two groups (online supplemental figure 1). CLASI scores were measured in 32 patients with CCLE+/SLE− and skin activity did not differ between the groups. However, there was a trend towards greater skin damage in patients with a SLE-like phenotype (online supplemental figure 2). The group with SLE-like B cells also had more historical serological autoreactivity including ANA, anti-dsDNA, anti-Ro and anti-La (figure 6B,C), as well as more contemporaneous anti-dsDNA and antichromatin reactivity (figure 6D) and higher titers of anti-RNP and anti-Ro52 (figure 6E). Interestingly, patients with CCLE with expanded transitional cells also had more anti-RNP antibodies, particularly anti-Ro60 (data not shown).

DISCUSSION

Our study represents the first systematic B cells description across the spectrum of clinical CCLE phenotypes, spanning from primary CCLE without systemic disease to SLE with or without a CCLE component. Our data establish that a subset of patients with CCLE share B cell abnormalities characteristic of SLE, including contraction of USM B cells and expansion of effector B cell subsets. Why the former population is depleted remains unclear. A similar decrease is observed in Sjögren's syndrome,[39] rheumatoid arthritis,[40] vasculitis[41] and other autoinflammatory diseases including inflammatory bowel disease, in which it may be restored by tumour necrosis factor inhibition.[42] Of note, USM loss is an early feature of Sjögren's syndrome correlated with serological autoimmunity and disease progression.[39] This change may represent the loss of a MZ-equivalent endowed with protective functions such as apoptotic clearance,[43] Interleukin-10-mediated B regulatory activity,[44] and dilution of autoreactivity.[45] Moreover, as in Sjogren's Syndrome,[39] this B cell feature might identify patients with primary CCLE who might be at risk for progression to SLE. In addition to the loss of putatively protective B cells, primary CCLE also shared with other lupus groups an enhancement of effector aN and DN2 B cells and PB, although of lower magnitude. In SLE, effector DN2 and plasma cells localise to the kidney and likely directly contribute to pathology.[18] Similarly, B cells and plasma cells infiltrate the skin in CCLE, particularly in established scarred lesions.[46] Whether the phenotype of skin-infiltrating B cells in CCLE corresponds to that of circulating and kidney-infiltrating B cells remain to be elucidated.

Our findings demonstrate high B cell heterogeneity in lupus, with normal B cell signature in 48% of primary CCLE and 15% of SLE. This data suggest that patients with primary CCLE are more likely to have a B-cell-independent disease, which in turn might identify those with lower risk of SLE progression. Conversely, 38% of patients with primary CCLE exhibit a highly activated SLE-like B cell profile. This group of patients is clinically and serologically

distinct with more generalised skin lesions and higher autoantibody loads; features that have been reported to increase the risk of SLE among those with primary DLE.[47–51] Our findings suggest that the expansion of effector B cells may identify a distinct CCLE group with higher risk of systemic progression. Long-term longitudinal analysis are warranted to demonstrate the predictive value of B cell phenotype in the development of SLE among patients with primary CCLE.

Also of note, patients with SLE without CCLE lesions had higher proportion of DN2 cells and multiple autoantibodies, features that we previously found to be associated with lupus nephritis.[19] Patients with SLE and DLE are less likely to develop renal disease,[6] and we have shown that SLE B cells are driven by disease-associated epigenetic programmes that promote effector DN2 and PB differentiation.[34] It is therefore possible that CCLE-associated B cells may be regulated by distinct differentiation and effector programmes, resulting in a less pathogenic phenotype, whether in primary CCLE or in the context of SLE. Finally, a subset of patients with CCLE+SLE− clustered together with a group of patients with SLE through their shared frequency of transitional B cells. Patients with SLE may display expanded transitional B cells with increased TLR7 and interferon-regulated gene expression.[32] It remains to be determined if patients with primary CCLE and transitional B cell expansion represent a distinct disease group and what are the clinical implications of this phenotype. Similarly, an association between any of the B cell profiles demonstrated in our work in CCLE and the interferon signature reported in a fraction of patients with CCLE remains to be investigated.[52]

This study also provides original information regarding the breakdown of B cell tolerance in primary CCLE. Such a defect is demonstrated by the increase in a fraction of patients with one or more SLE-associated autoantibodies including dsDNA, chromatin, RNA and some anti-RNA-binding protein antibodies. Moreover, a significant fraction of primary CCLE also displayed defective tolerance in the autoreactive 9G4 B cell compartment, a feature characteristic of SLE.[23 53] However, meaningful differences were also observed between CCLE and SLE. Thus, unlike patients with SLE, increased class-switched 9G4+ antibodies were uncoupled in CCLE from anti-DNA/chromatin autoantibodies and the autoreactivity of these antibodies was much more pronounced against self-antigens expressed by apoptotic cells than B cells.

Combined, our observations suggest that primary CCLE is characterised by limited breakdown of tolerance that has not yet disseminated to other antigens. This mechanistic scenario is also supported by the dissociation between anti-Sm and U1RNP reactivity in CCLE but not in SLE. This observation has important implications for the development and progression of these diseases. Indeed, these findings are consistent with the notion that concurrent development of antibodies against multiple autoimmune targets is an important component of disease progression, as demonstrated by the progressive development of different autoantibodies during the preclinical phase of SLE prior to full-blown disease and diagnosis.[54 55] Longitudinal studies of epitope spreading in CCLE B cells could represent an informative approach to understand the nature of the triggering antigens responsible for the initial breakdown of tolerance, and of late-target antigens that might be responsible for disease dissemination and SLE development.

Our study has limitations. First, the cross-sectional design does not allow to infer cause–effect. Second, the wide range of disease duration of our sample might impact the results. However, we did not find significant differences between patients with a HCD-like and SLE-like B cell phenotype by disease duration. Third, skin activity and damage were examined in a subset of participants, and we cannot generalise those results to the full sample. Four, findings of this study are best generalised to individuals in the Southeastern USA, where a large majority of patients with lupus are black.

In summary, we demonstrate that CCLE is a heterogeneous condition clinically, serologically and immunologically. B cell heterogeneity is indicated by both phenotypic and serological diversity with the latter suggesting an early and/or limited breakdown of tolerance. A deeper understanding of these findings should improve our understanding of disease pathogenesis, enhance prognostic power for SLE development, and lead to the development of more precise and effective therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

Data are available upon reasonable request. All data including deidentified patient data,flow cytometry files and R code are available upon reasonable request from Dr Ignacio Sanz, ignacio.sanz@emory.edu.

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Key messages

What is already known about this subject?

While the contribution of B cells to systemic lupus erythematosus (SLE) pathogenesis is apparent, their role in primary chronic cutaneous lupus erythematosus (CCLE) is less clear. Although CCLE is characterised by low serological activity, some patients with this condition can produce autoantibodies and potentially develop systemic phenotypes. However, little is known about B cell phenotype and B cell tolerance in CCLE.

What does this study add?

B cell phenotypes were found to be heterogeneous in primary CCLE, with some patients resembling healthy donors and others showing an expansion in effector B cells resembling that seen in SLE.

Patients with a SLE-like B cell phenotype were more likely to have generalised lesions and were more serologically active, with a high prevalence of nucleic acid and RNA-binding protein-specific antibodies.

Autoreactive VH4.34 9G4+ IgG antibodies were elevated in patients with CCLE; however, these antibodies were not associated with antinucleic acid IgG as typically occurs in SLE.

How might this impact on clinical practice or future developments?

The heterogeneity in B cell phenotype may reflect fundamentally different disease processes in CCLE. B cell phenotype should be examined as a potential prognostic marker for patients with CCLE that may develop systemic disease.

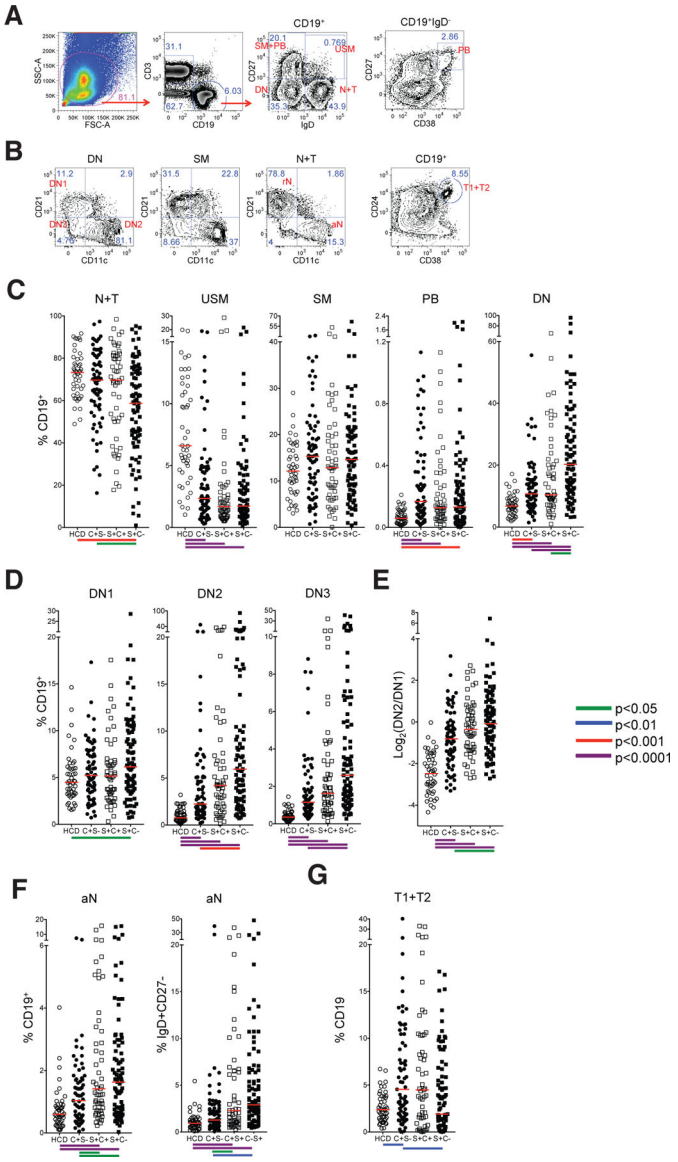

Figure 1.

Perturbations of B cell homeostasis in patients with CCLE and SLE. (A) Gating scheme for the flow cytometer analysis of human B cells for a representative SLE sample. CD19+, CD3− B cells are divided into IgD+CD27− naive plus transitional (N+T), IgD+CD27+ USM, IgD−CD27+ memory plus plasmablasts (PB) SM+PB and IgD−CD27− DN, PB were gated as IgD−CD27++CD38++. (B) Separation of DN into DN1 (CD21+CD11c−), DN2 (CD21−CD11c+) and DN3 (CD21−CD11c−). aN were gated from N+T based on CD21−CD11c+. High CD24 and CD38 expression was used to gate transitional (T1+T2). (C) B cell subset frequencies were compared among healthy controls (HCD, n=46), primary CCLE (C+S−, n=69), SLE overlapped with CCLE (S+C+, n=53) and SLE without CCLE (S+C−, n=85). Short horizontal lines indicate the median. The frequency of PB (IgD−CD27++CD38++) was subtracted from that of the IgD−CD27+ compartment to derive the proportion of SM. (D) The percentage of DN1, DN2 and DN3 as a proportion of CD19+.

(E) Relative frequencies of DN2 to DN1 is expressed as the $\log^2$ transformed ratio of DN2 to DN1. (F) The percentage of aN as proportion of CD19+ and total naive. (G) The percentage of T1+T2 as a proportion of total CD19+. Bars beneath each plot indicate the statistical significance as determined by a Kruskal-Wallis test followed by Dunn's multiple comparisons test with $p<0.05$ (green), $p<0.01$ (blue), $p<0.001$ (red), $p<0.0001$ (dark purple). aN, activated naive; CCLE, chronic cutaneous lupus erythematosus; DN, double negative; PB, plasmablasts; SLE, systemic lupus erythematosus; USM, unswitched memory.

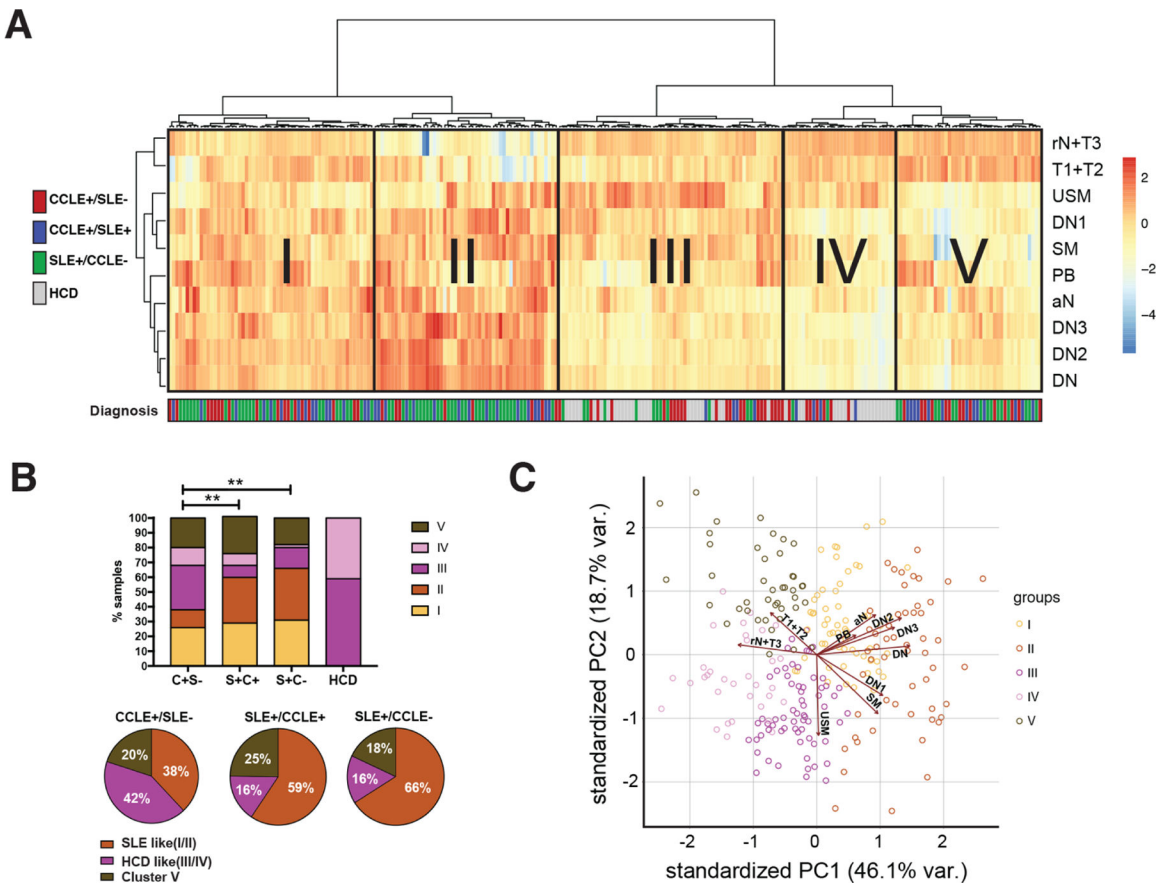

Figure 2.

B cell fingerprint of patients with CCLE+/SLE−, SLE+/CCLE+ and SLE+/CCLE−. (A) Hierarchical clustering of samples by B cell subset frequency. Patients are clustered on the top and diagnosis is indicated by colour underneath. B cell subsets are clustered on the right. Patients were divided into five groups as indicated by Roman numeral (I–V). (B) Group distribution for different diagnostic categories, the distribution of patients with CCLE+/SLE− significantly differed from that of SLE+/CCLE+ and SLE+/CCLE−. The majority of SLE+/CCLE+ and SLE+/CCLE− samples were in groups I and II, while HCD samples were only found in groups III and IV. More patients with CCLE+/SLE− were in the HCD-enriched groups III and IV and fewer in SLE-enriched groups I and II. χ^2 test was used to compare frequencies, because no HCD clustered in I,II and V χ^2 tests comparing HCD to patients with lupus were not performed. (C) Principal component plot with cluster group indicated by colour and loading vectors for each B cell subset indicated. X^2 test: **p<0.001. CCLE, chronic cutaneous lupus erythematosus; HCD, healthy controls; SLE, systemic lupus erythematosus

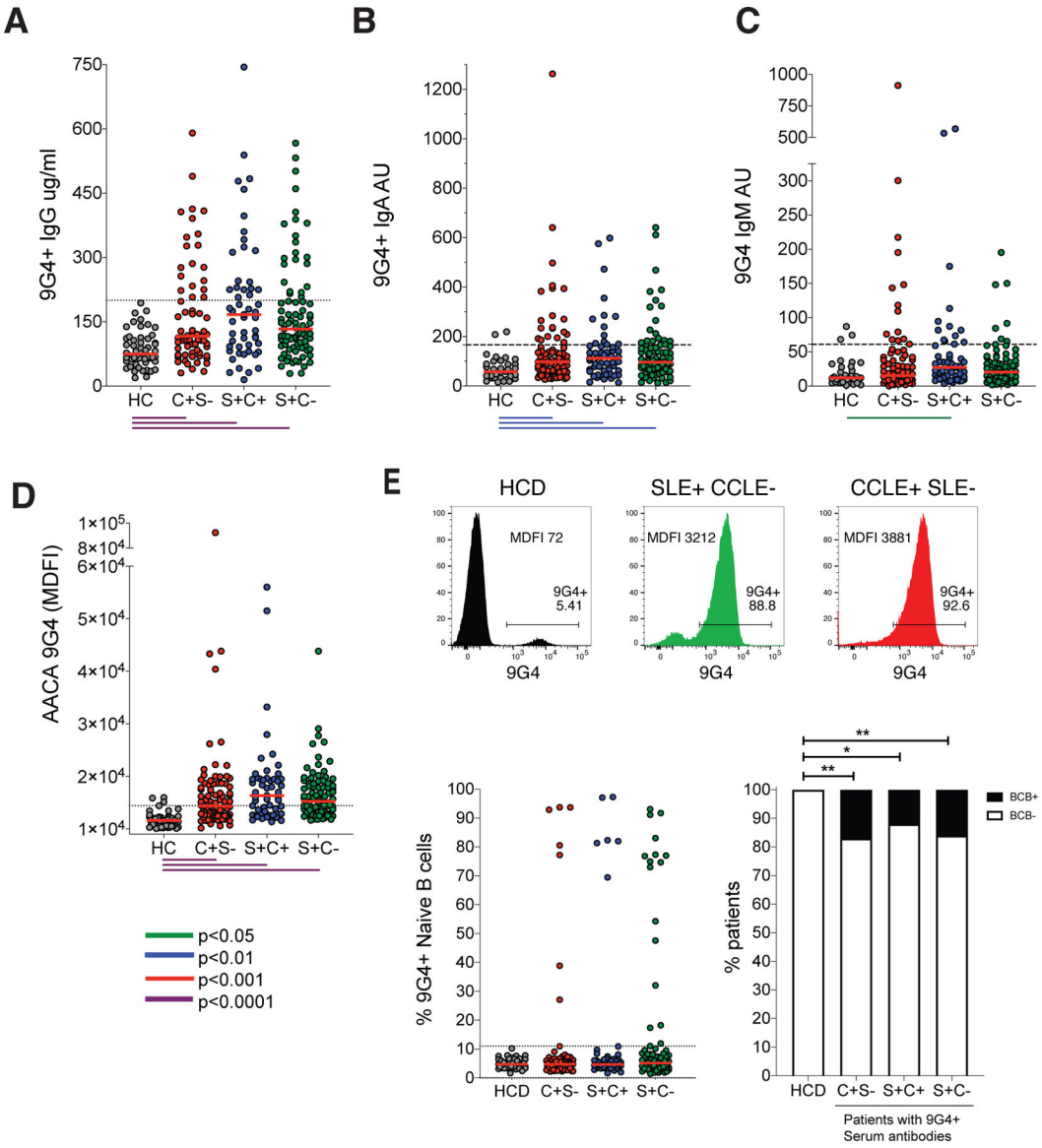

Figure 3.

Like in patients with SLE, autoreactive 9G4+ autoantibodies are increased in the serum of patients with CCLE+/SLE−. (A) 9G4+ IgG is increased in in all three groups relative to HCD. (B) 9G4+ IgA is also increased in patients with lupus. (C) 9G4+ IgM was only significantly elevated in the SLE+/CCLE−. (D) 9G4+ AACA are found in the serum of both patients with SLE and CCLE. 9G4 Median fluorescence intensity (MDFI) of apoptotic Jurkat cells are shown after incubation with patient serum. (E) 9G4+ autoreactive antibodies that bind B cells are present in both patients with SLE and CCLE. On top representative 9G4 staining for naive B cells is shown. In HCD only the minor population of Vh4.34 expressing cells are 9G4+, in some patients with SLE+/CCLE− and CCLE+/SLE− almost all naive B cells are 9G4+ due to surface bound anti-B cell antibodies. Below left the frequency of naive B cells that are 9G4+ is shown. Below right the proportion of patients with elevated 9G4+ IgG, IgA or IgM that have greater than 11% naive 9G4+ B cells is shown. Kruskal-

Wallis test followed by Dunn's multiple comparisons test: $p<0.05$ (green), $p<0.01$ (blue), $p<0.001$ (red), $p<0.0001$ (dark purple); Fischer's exact test: *$p<0.05$, **$p<0.01$. AACA, anti-apoptotic cell antibodies; CCLE, chronic cutaneous lupus erythematosus; HCD, healthy controls; SLE, systemic lupus erythematosus.

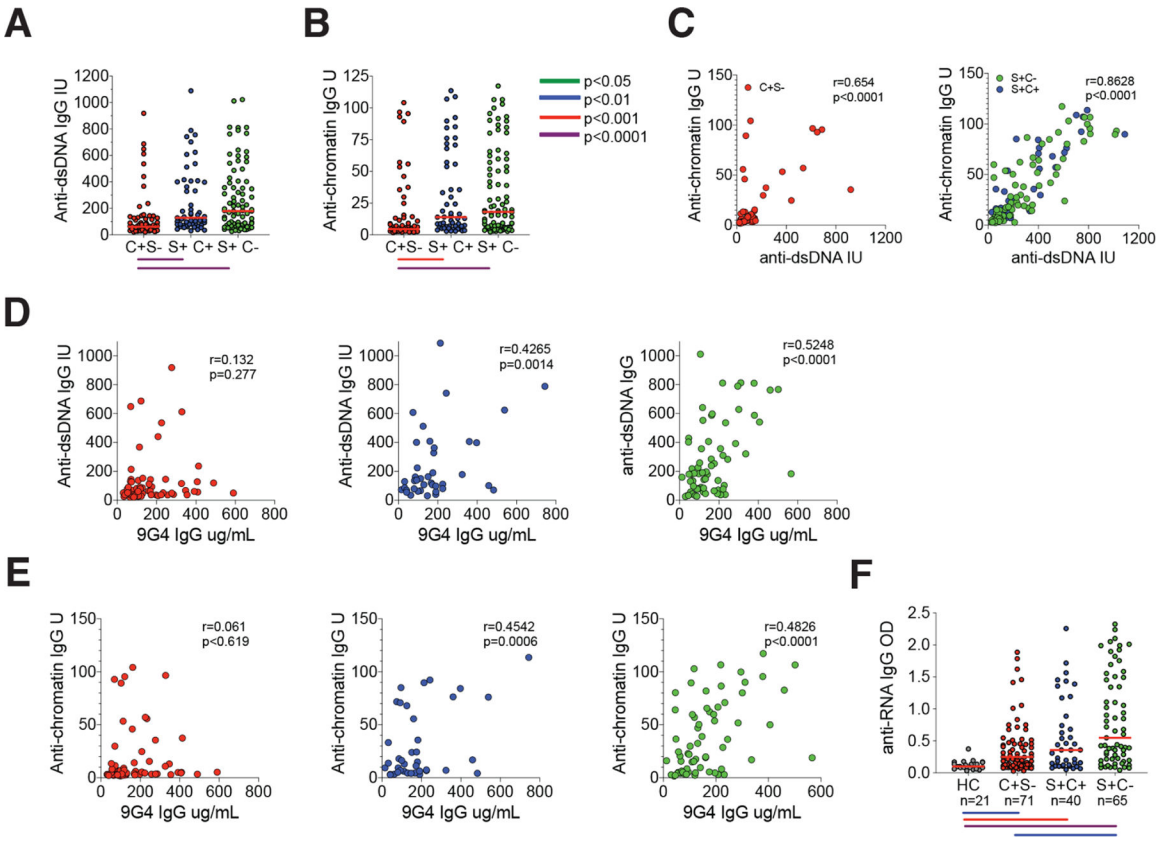

Figure 4.

Antinucleic acid antibodies are decreased in patients with CCLE+SLE− relative to patients with SLE and are not correlated with 9G4+ IgG. (A) Anti-dsDNA IgG is decreased in CCLE+/SLE− relative to SLE+/CCLE− and patients with SLE+/CCLE+. (B) Antichromatin IgG is also decreased in CCLE+/SLE− relative to SLE. (C) Anti-dsDNA IgG and antichromatin IgG are correlated in both patients with CCLE+/SLE− (left) and SLE (right). (D) 9G4+ IgG is highly correlated with anti-dsDNA IgG in patients with SLE+CCLE and SLE+CCLE+ but not patients with CCLE+/SLE−. (E) Similarly, antichromatin IgG is correlated with 9G4+ IgG only in patients with SLE. (F) Anti-RNA IgG is increased in patients with CCLE+/SLE−, SLE+/CCLE− and SLE+/CCLE+ but is significantly higher in SLE+/CCLE− and SLE+/CCLE+ than CCLE+/SLE−. Kruskal-Wallis test followed by Dunn’s multiple comparisons test: $p<0.05$ (green), $p<0.01$ (blue), $p<0.001$ (red), $p<0.0001$ (dark purple); Pearson correlation coefficient r and p are shown. CCLE, chronic cutaneous lupus erythematosus; dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus.

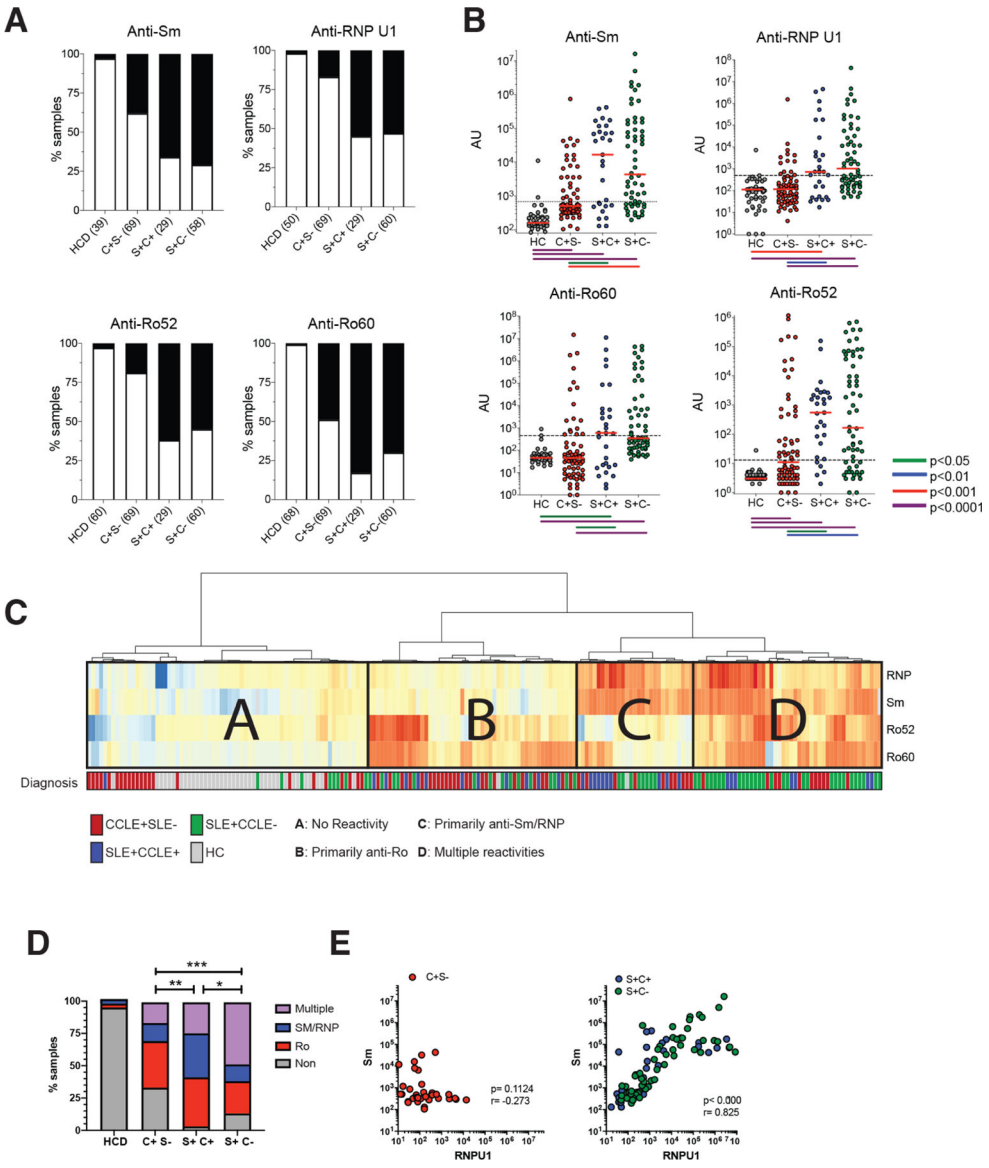

Figure 5.

Anti-RNA-binding protein antibodies are elevated in patients with SLE relative to CCLE+/SLE−. (A) The frequency of positive samples for each of the indicated antigen specificities as determined by LIPS for HCD and each patient group. Samples were considered positive if they were higher than the mean value of HCD+3SD. (B) Anti-Sm, anti-RNP, anti-Ro52 and anti-Ro60 levels as assayed by LIPS and expressed as arbitrary units, median values are indicated by the red line, the Kruskal-Wallis test was used to compare each group, p values>0.05 are indicated by colour lines underneath for each comparison. The dashed line indicates the threshold that was considered positive. (C) Hierarchical clustering of luciferase immunoprecipitation system (LIPS) assay values, samples are clustered by patient on top and antigen on the left, diagnosis is indicated by colour underneath. Samples can be grouped into four patterns of reactivity as indicated by letter. (D) Distribution of patients and HCD across the four groups from C above.

Differences in distribution between the three patient groups were analysed using the χ^2 test. (E) Anti-Sm and anti-RNP plotted for CCLE+/SLE− (left) or SLE+/CCLE+ and SLE+/CCLE− (right). Kruskal-Wallis test followed by Dunn's multiple comparisons test: $p<0.05$ (green), $p<0.01$ (blue), $p<0.001$ (red), $p<0.0001$ (dark purple); Spearman correlation coefficient r and p is shown; X^2 test: *$p<0.05$, **$p<0.001$, ***$p<0.0001$. CCLE, chronic cutaneous lupus erythematosus; HCD, healthy controls; SLE, systemic lupus erythematosus.

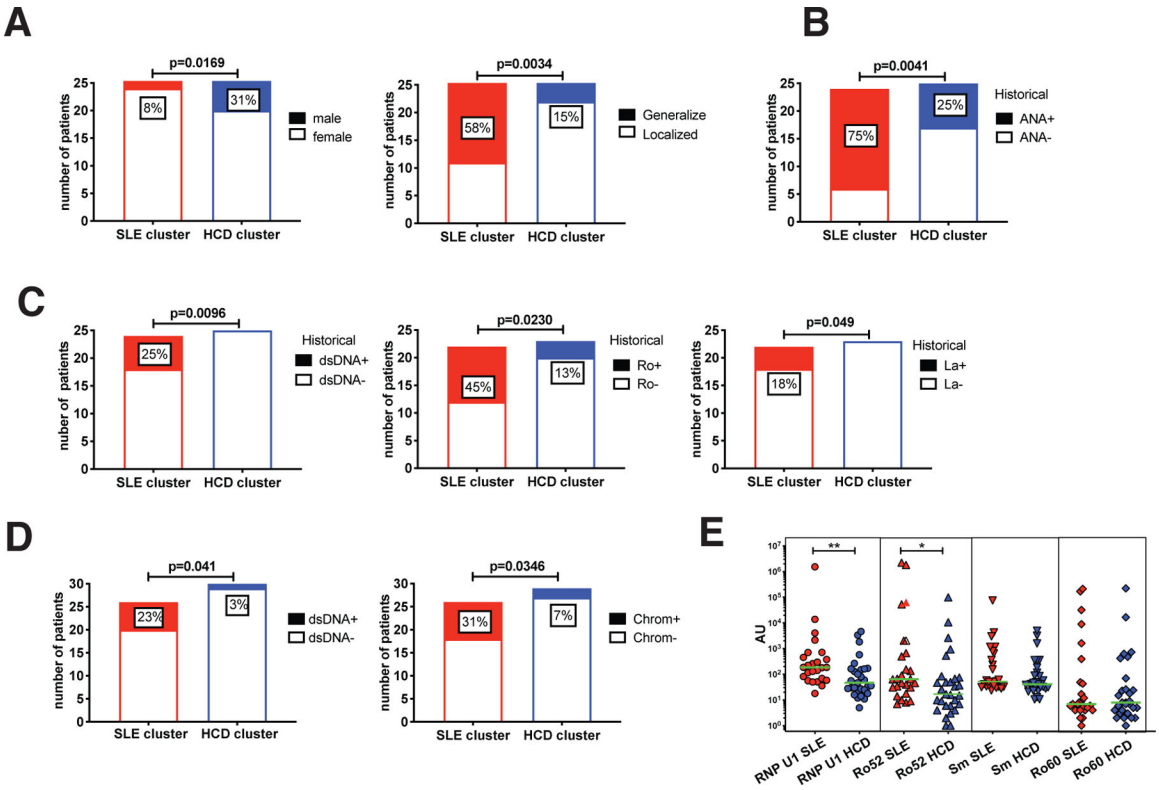

Figure 6.

Patients with primary CCLE with a SLE-like B cell subset composition are serologically and clinically distinct from patients with CCLE that resemble HCD. (A) Primary CCLE from clusters III and IV (figure 2A) that resemble patients with SLE (red) are less likely than those from clusters I and II that resemble HCD (blue) to be male (left) and more likely to have generalised skin lesions above and below the neck (right). (B) Frequency of historical anti-nuclear antibody (ANA) reactivity in patients with primary CCLE with a SLE-like (red) or HCD-like B cell subset composition (blue). (C) Frequency of historical anti-dsDNA, anti-Ro and anti-La reactivity in patients with primary CCLE with a lupus-like (red) or HCD-like B cell subset composition (blue). (D) Frequency of anti-dsDNA and antichromatin reactivity at the time of flow analysis for patients with primary CCLE with a Lupus-like (red) or HCD-like B cell subset composition (blue). (E) Anti-RNA protein-binding reactivity as assayed by LIPS in patients with primary CCLE with a Lupus-like (red) or HCD-like B cell subset composition (blue) at the time of flow analysis. The Mann-Whitney test was used to compare distributions; (*$p<0.05$; **$p<0.005$). Fischer's exact test was used to analyse differences in frequencies as indicated by the p value. CCLE, chronic cutaneous lupus erythematosus; dsDNA, double-stranded DNA; HCD, healthy controls; SLE, systemic lupus erythematosus.

Table 1

Descriptive characteristics of patient samples

Characteristics	CCLE+/SLE− (n=69)	SLE+/CCLE+ (n=53)	SLE+/CCLE− (n=85)	P value
Age, mean±SD	51.2±13.7	43.0±12.7	47.6±13.6	0.0047
Disease duration, mean±SD	9.9±9.5	10.1±9.1	14.0±9.7	0.013
Gender, n (%)				
Male	11 (15.9)	6 (11.3)	6 (7.1)	0.22
Female	58 (84.1)	47 (88.7)	79 (92.9)	
Race*, n (%)				
Black or African American	63 (92.6)	48 (90.6)	80 (94.1)	0.74
White	5 (7.4)	5 (9.4)	5 (5.9)	
Family history of lupus, n (%)				
No	53 (80.3)	37 (71.2)	65 (76.5)	0.51
Yes	13 (19.7)	15 (28.8)	20 (23.5)	
DLE location†, n (%)				0.034
Above the neck	40 (61.5)	20 (38.5)	NA	
Below the neck	1 (1.5)	3 (5.7)	NA	
Above and below the neck	24 (36.9)	29 (55.8)	NA	
SLE outcomes				
Disease activity, SLAQ score mean±SD	NA	19.5±7.8	16.7±8.7	0.077
Organ damage, n (%)				
No damage (SA-BILD score=0)	NA	4 (9.1)	9 (10.7)	0.4
Mild damage (SA-BILD score=1–2)	NA	20 (45.5)	28 (33.3)	
Severe damage (SA-BILD score≥3)	NA	20 (45.5)	47 (56.0)	
Immunosuppressive drugst‡, n(%)	7 (10.6)	10 (27.0)	34 (40.5)	0.0002

*One participant who self-reported ‘other race’ is not listed.

†DLE cases per group are n=65 within CCLE+/SLE− and n=52 within CCLE+/SLE+.

‡Comprise any or a combination of azathioprine, cyclophosphamide, cyclosporine, methotrexate and mofetil mycophenolate; there were 3 missing data for CCLE+/SLE−, 16 for SLE+/CCLE+ and 1 for SLE+/CCLE−.

CCLE, chronic cutaneous lupus erythematosus; DLE, discoid lupus erythematosus; SA-BILD, self-administered Brief Index of Lupus Damage; SLAQ, Systemic Lupus Activity Questionnaire; SLE, systemic lupus erythematosus.