Interrelation of T-cell Cytokines and Autoantibodies in Lupus Nephritis: A Cross-sectional Study

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Research Article

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Abstract

Objective: To determine if circulating autoantibodies (autoAbs) and T-helper cell cytokines correlate with a different class of lupus nephritis (LN), including class III/IV, compared to class V and other manifestations of systemic lupus erythematosus (SLE).

Methods: All patients (N=62) met the SLE classification criteria of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) or Systemic Lupus International Collaborating Clinics (SLICC) classification criteria. Demographic, clinical data, and serologic manifestations included anti-DNA, anti-Smith (Anti-Sm), C3, and C4 were included. Plasma levels of interferon gamma (IFN), interleukin 17 (IL-17), interleukin 10 (IL-10) isotype-specific (IgG) anti-DNA, anti-Ro/SSA (SSA), and anti-Sm were measured using enzyme-linked immunosorbent assay (ELISA).

Results: The most common manifestations by history were arthritis in 61% (N=38), photosensitivity rash in 53% (N=33), LN in 44% (N=27), and oral/nasal ulcer in 31% (N=19) SLE patients. AutoAbs (anti-DNA, anti-SSA, and anti-Sm), IFN , and IL-17, but not IL-10, were significantly elevated in SLE patients compared to healthy controls. There were elevated plasma levels of anti-DNA (p = 0.0101) and anti-Sm (p = 0.0499) in patients with a history of LN compared to patients without LN. In contrast, plasma levels of anti-Sm were decreased in patients who had a history of acute mucocutaneous manifestations, including photosensitivity rash and/or malar rash (p = 0.0152). Among the three cytokines that were analyzed, IL-10 was significantly elevated in patients with a history of LN compared to patients without LN (p = 0.0216). IL-17 was positively correlated with anti-SSA (p = 0.0130) and was significantly higher in patients with discoid rash (p = 0.0238) and history of class V LN (p = 0.0055). IFN was positively correlated with anti-DNA (p = 0.0355) and anti-SSA (p = 0.0402).

Conclusion: This cross-sectional study supports the role of different T-helper cell cytokines that may be associated with the development of different autoAbs in influencing the diversity of SLE clinical manifestations. The results suggests that elevated IFN and IL-17 are more generalized features in SLE patients. In contrast, higher levels of IL-10 were observed in patients with a history of LN. This provide insights into the pathogenic mechanisms of LN that can help guide future diagnosis and therapies.

Background

Systemic lupus erythematosus (SLE) is the prototypic systemic autoimmune disease characterized by the presence of autoantibodies (autoAbs) and dysregulation of cytokines (1–4). The etiology and pathogenesis of SLE are not well understood, although it is accepted that both genetic susceptibility and environmental factors contribute to the onset of disease. Just as the factors that lead to onset of disease are highly variable, the systemic manifestations of the disease also exhibit a broad spectrum of the target tissue. The most commonly involved target tissue with clinically significant consequences is the kidney (5–8). The spectrum of lupus nephritis (LN) involvement in SLE can be sub-classified into six categories based on histologic examination (9, 10). Two major autoAbs that are commonly associated with LN

include anti-DNA and anti-Smith (Sm) (11–13). Other autoAbs such as anti-SSA are associated with mucocutaneous manifestations of SLE but not LN (13). Anti-Sm and anti-SSA are antibodies to small nuclear RNP (Sm-RNP) have been observed to be “fixed” and do not vary greatly over time of disease history regardless of disease severity and/or treatment (14–17). Anti-DNA autoantibodies also strongly associated with renal disease and their levels have been associated with disease “flares” and levels rise with disease activity and fall with effective therapies (18).

The three major T-helper cytokines that have been associated with SLE include interferon- (IFN), interleukin 17 (IL-17), and interleukin 10 (IL-10)(19). Other key cytokines in SLE include type I interferon which is produced by many cells types including plasmacytoid dendritic cells and B cells (20, 21), and the finding that type I interferon signature is highly associated with SLE has provided major insights into the etiology and pathogenesis of SLE (20, 22–25). Macrophage cytokines including tumor necrosis factor and interleukin 6 contribute to inflammatory responses and tissue damage in SLE (26, 27). However, it is the T-helper cell cytokines that play a dominant role in providing help to B cells, especially in the follicle or extrafollicular sites, and are thought to play the dominant role in B cell development and differentiation into autoAbs producing plasmablasts and plasma B cells (26, 28). In addition to providing help to B cells, T-helper cytokines can also regulate inflammation (19, 29). IFN and IL-17 act on numerous cell types to promote inflammation, whereas IL-10 exhibits both pro-inflammatory (30) and anti-inflammatory responses (31, 32).

A key question in SLE is whether autoAbs and T-helper cytokines act together to promote LN. When considered separately, it is recognized that autoAbs can form immune complexes (IC) or otherwise bind to the kidney glomerulus to activate complements and promote inflammatory responses in renal damage (33). Cytokines have also been analyzed independently of autoAbs, and the inflammatory actions of IFN and IL-17 have been found to be associated with inflammation in the kidney, whereas IL-10 has been found to reduce inflammation and delay or reduce renal disease in some mouse models (29, 34, 35). These previous studies did not highlight the interrelation of the T-helper cytokines which can both modulate autoAbs production and promote or inhibit inflammation in the kidney. Likewise, autoAbs and IC deposition in tissue can induce chemokines that attract and influence the development of T-helper cytokines (34). Therefore, novel and unique insights into the development of LN in SLE must simultaneously consider the potential interrelation of major T-helper cytokines as well as the levels of the major autoAbs that have been found to contribute to SLE.

Here, we find that T-helper cytokines, IFN and IL-17, but not IL-10, are elevated in SLE patients with the three key autoAbs, anti-DNA, anti-SSA, and anti-Sm. Although IL-10 is not associated with autoAbs production, it is elevated in SLE patients with a history of LN suggesting a proinflammatory activity for IL-10 in the kidney. Although IL-17 is associated with autoAbs and at the renal histological level of analysis, elevated levels of IL-17 are significantly correlated with a history of class V membranous lupus nephritis suggesting a potential role for IL-17 in membranous renal injuries. We have further identified that IL-17 was elevated in patients who developed discoid lupus erythematosus (DLE).

Methods

Study design

This is a cross-section study to examine the correlation between the levels of IFN ，IL-17, and IL-10 in plasma with autoAbs, LN, and other disease manifestations of SLE. All medical records were reviewed at the University of Alabama at Birmingham (UAB). Ethical approval for this study was obtained from the ethics committee at UAB.

Study population

All patients satisfied the following inclusion criteria: age of onset ≥ 18 years and confirmed SLE diagnosis by either the American College of Rheumatology (ACR) 1997 revised criteria (36, 37) or the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria (38). The exclusion criteria were: age < 18years, diagnosis of overlap syndrome, mixed connective tissue disease, or other autoimmune diseases. The studies were conducted in compliance with the Helsinki Declaration and approved by the UAB Institutional Review Board. All data were collected in a manner blinded to enzyme-linked immunosorbent assay (ELISA) data until data collection was completed.

Clinical data collection

The chart review and data collection were carried out at the time of enrollment. Demographic data included age, race, and sex. Clinical data included age at the time of enrollment to the study, mucocutaneous manifestations (malar rash, DLE, photosensitivity, and oral/nasal ulcer), arthritis, serositis, LN, neurological disorder (psychosis, seizure, CNS vasculitis), and hematologic manifestations (leukopenia, hemolytic anemia, and thrombocytopenia). In addition, systemic lupus erythematosus disease activity index (SLEDAI) score, medications used, LN class, and laboratory data (anti-DNA and anti-Sm, C3, and C4) were collected. Acute cutaneous lupus (ACLE) defines as the presence of malar rash or photosensitivity, or both. SLEDAI was used to assess SLE severity at time of enrollment visit based on (1) no activity (score: zero); (2) mild activity (score: 1-5); (3) moderate activity (score: 6-10); (4) high activity (score: 11-19); and (4) very high activity (score: ≥20) (39). All clinical variables were recorded as present or absent.

Renal biopsy

Renal biopsies were reviewed retrospectively. The renal biopsies were classified according to the ISN/RPS (2004) classification for LN, which is based on the extent of glomerular involvement by light microscopy, and whether the injury pattern reflects active lesions (endocapillary hypercellularity, neutrophils/karyorrhexis, fibrinoid necrosis, wire-loop lesions, and cellular/fibrocellular crescents) or chronic lesions (global/segmental glomerulosclerosis and fibrous crescents/broad-based adhesions) (9, 10). Based on the light microscopic findings, LN was classified as follows: minimal mesangial LN (class I), mesangial proliferative LN (class II), focal LN (class III), diffuse LN (class IV), membranous LN (class V), and advanced sclerosing LN (class VI) (9, 10).

Enzyme-linked immunosorbent assay (ELISA) analysis of autoAbs

Plasma levels of IgG anti-Sm and anti-SSA, which detects both Ro52 and Ro60 were determined using commercially available ELISA kits (Alpco, Salem, NH) (20). IgG anti-DNA levels were measured as we previously described (40). The plates were read at 450-650 nm using an Emax Precision Microplate Reader (Molecular Device, Sunnyvale, CA, USA).

ELISA analysis of cytokines

Plasma levels of cytokines including IFN- (Cat#ESS0002, ThermoFisher), IL-17 (Cat#KAC1591, ThermoFisher), and IL-10 (Cat#88-7106-88, ThermoFisher) were analyzed by a sandwich ELISA method according to the manufacturer's protocols. Briefly, for each cytokine, ELISA plates were coated with a capturing anti-cytokine antibody. Following the plasma incubation, ELISAs were developed with a horseradish peroxidase (HRP)–labeled anti-cytokine detection antibody and tetramethylbenzidine substrate (Sigma-Aldrich). OD450–650 nm was measured on an Emax Precision Microplate Reader (Molecular Device, Sunnyvale, CA, USA).

Statistical analysis

Statistical analysis was carried out using IBM© SPSS© Statistics version 27 (IBM© Corp., Armonk, NY, USA), and the figures were created using GraphPad Prism 9 software (La Jolla, CA). Numerical data are expressed as mean and standard deviation (±SD) or standard error of the mean (±SEM), as appropriate. An unpaired one-tailed t-test was used for comparisons of elevated cytokine and autoAbs levels in SLE patients with various clinical features. A linear regression analysis was used to evaluate the correlation between cytokines, autoAbs, and LN. A p-value < 0.05 was considered significant.

Results

Patient characteristics

A total of 62 subjects were included in our cohort (Table 1). The mean age (±SD) at the time of enrollment was 34 years (± 58.17), and 92% (N = 57) were female. Among these, 63% (N = 39) of the patients were African American and 37% (N = 23) Caucasian. The most prevalent clinical features of the SLE patients in our cohort were arthritis in 61% (N = 38), photosensitivity rash in 53% (N = 33), LN in 44% (N = 27), and oral/nasal ulcer in 31% (N = 19). Fifty-nine percent (N = 37) had active disease (SLEDAI score: $\geq$1) at the time of enrollment. At this time, 48% (N = 30) had mild (SLEDAI score: 1-5), 8% (N = 5) had moderate (SLEDAI score: 6-10), and 3% (N = 2) had high disease activity (SLEDAI score: 11-19). Ninety percent of our patients (N = 56) were on Hydroxychloroquine or Quinacrine or both, and 52% (N = 32) were on prednisone at time of enrollment. Eleven healthy donors were included.

Increased plasma levels of cytokines and autoAbs in SLE patients

There were higher levels of IFN (pg/mL) and IL-17 (pg/mL) in SLE patients (N = 62; mean (±SEM) 38.81 (±2.83) and 8.52 (±1.45), respectively) compared to healthy control subjects (N = 11; mean 25.75 (±2.51) and 2.63 (±0.79), respectively) which were statistically significant (p=0.0300 and p=0.0470, respectively) (Fig. 1A). Levels of IL-10 (pg/mL) in SLE 10.52 (±0.71) were not significantly different compared to healthy controls 9.12 (±2.21) (p=0.2346) (Fig. 1A). Also, there were significantly higher levels of circulating anti-DNA-IgG (OD), anti-SSA(unit/ml), and anti-Smith (unit/ml) in SLE patients (N = 62; mean 0.36 (±0.02), 15 (±2.46) and 75 (±11.87) respectively) compared to healthy control subjects (N = 11; mean 0.27 (±0.02), 0.89 (±0.28), and 8 (±3.33), respectively) which were statistically significant (p=0.0280, p=0.0099, and p=0.0105, respectively) (Fig. 1B).

Association of cytokines and autoAbs with LN

Next, we determined if cytokines and autoAbs could be associated with the history of LN and LN classification. Twenty-seven patients were diagnosed with LN among the 62 SLE patients. However, there were no differences in the levels of IFN and IL-17 in patients with or without a history of LN, although IL-10 was significantly elevated in patients with a history of LN compared to patients without LN (p = 0.0216) (Fig. 2A). Interestingly, patients with a history of LN class V exhibited a significantly elevated IL-17 (p = 0.0055) compared to patients with a history of LN class III or IV (Fig. 2B).

Analysis of the levels of circulating IgG autoAbs showed that anti-DNA and anti-Sm were significantly elevated in patients who had a history of LN (p = 0.0101 and p = 0.0499, respectively) (Fig. 3A). Anti-DNA was significantly elevated in patients with class III or IV LN, compared with patients who had developed class V LN (p = 0.0063) (Fig. 3B).

Correlation between cytokines and autoAbs

We determined if there were positive correlations between cytokines and autoAbs**.** IFN was significantly correlated with IL-17 (P=0.0022) (Fig. 4A). Also, IFN was correlated with anti-DNA and anti-SSA (p = 0.0355 and p = 0.0402, respectively) (Fig. 4B) and IL-17 was positively correlated with anti-SSA (p = 0.0130) (Fig. 4C), indicating IFN and IL-17 may play a role in in the generation of autoAbs. IL-10 exhibited no correlation with autoAbs (Fig. 4D).

Association of cytokines with other SLE clinical features

Clinical features of SLE in 62 patients along with IFN , IL-17, and IL-10 were analyzed. While we did not find the association of IFN and IL-10 with the development of arthritis (Fig. 5A), ACLE (Fig. 5B), DLE (Fig. 5C), and serositis (Fig. 5D), patients with DLE exhibited a significantly higher IL-17 compared to patients without DLE (p = 0.0238) (Fig. 5C). By contrast, patients who developed ACLE exhibited significantly lower circulating levels of IL-17, compared to patients who did not develop ACLE (p = 0.0278) (Fig. 5B).

Association of autoAbs with SLE clinical features

Clinical features of SLE in 62 patients along with autoAbs (anti-DNA, anti-SSA, and anti-Sm) were analyzed (Fig. 6A, B, C, and D). Patients who developed ACLE exhibited a significantly lower anti-Sm than patients who did not develop ACLE ($p = 0.0152$) (Fig. 6B). Patients with serositis had significantly higher Anti-DNA and Anti-Sm compared to patients without serositis ($p < 0.0001$ and $p = 0.0065$, respectively) (Fig. 6D).

Discussion

In a single-center, cross-sectional study in 62 patients with SLE, we measured cytokines (IFN , IL-17, and IL-10) and autoAbs (anti-DNA, anti-SSA, and anti-Sm) that have been implicated in the pathogenesis of SLE. Our results suggest that IL-10 and IL-17 were elevated in patients with a history of LN and a history of LN class V, respectively. These results provide insights into the pathogenic mechanisms of LN that can help guide future diagnosis and therapies. Our results are consistent with previous results showing that IFN and IL-17 were significantly elevated in SLE patients compared to healthy control (34, 41–43). This suggests that elevated IFN and IL-17 are more generalized features in SLE patients. In contrast, although IL-10 levels were not significantly higher in SLE compared to healthy controls, higher levels of IL-10 were observed in patients with a history of LN.

The complex effects of IL-10 in LN have been proposed to be due to the opposite effects on the two major effector arms of lupus pathogenesis, inflammation and autoAbs production (44, 45). Blenman KR and Morel showed that in the NZM2410 lupus model, continuous overexpression of low levels of IL-10 significantly delayed antinuclear autoAbs production and decreased clinical nephritis (46). B cell phenotypes were largely unaffected, while T-cell activation was significantly reduced (46). Ishida *et al.* also reported that treatment with anti-IL-10 substantially delayed the onset of autoimmunity in NZB/W F1 mice as monitored either by overall survival or by the development of proteinuria, glomerulonephritis, or autoAbs (47). In contrast to mouse models, IL-10 does not appear to have a protective effect in SLE. Serum IL-10 level was significantly elevated in active SLE patients. Further, IL-10 was positively correlated with SLEDAI and anti-double-stranded DNA titer but was negatively correlated with C3, C4, and lymphocyte counts (48). Higher circulating levels of IL-10 and higher percentages of IL-10 + T cells have been found in patients with was class III or IV LN (35, 49). Our results show that higher levels of IL-10 were observed in patients with a history of LN but IL-10 levels did not correlate with the levels of autoAbs. Together, these results suggest IL-10 is involved in LN pathogenesis- and is a potential therapeutic target in this subgroup (50).

Another notable finding was that plasma levels of IL-17 were significantly higher in patients with a history of class V LN compared to a patient with class III or IV LN. Our results are consistent with previous findings showing that the highest baseline levels of IL-17 were seen in patients with class V LN at repeat biopsy and predicted an unfavorable histopathological response (51). Other than acting directly on podocytes, renal tubular epithelial cells, intrinsic renal cells, and immune cells to promote tissue damage and fibrosis, Th17 cells and IL-17 can also act through IC deposition and recruitment of immune cells to perpetuate kidney injuries (34). As we have identified that circulating IL-17 and IFN exhibited a strong

positive correlation, it is possible that IL-17 and IFN were produced from a subset of T cells that can produce both cytokines. It has been reported that IL-17 and IFN can be produced by a population of $CD4^{-}CD8^{-}$ double-negative T cells in LN patients (52). Surprisingly, anti-DNA, which was elevated in patients with class III or IV LN, was positively correlated with IFN but not IL-17. This suggests that IL-17 may be involved in the development of other autoAbs but not anti-DNA to promote nephritogenic IC formation. In the present study, we have identified that anti-SSA exhibited a strong positive correlation with IL-17.

In the present study, we also identified that IL-17 but not IFN or IL-10 was elevated in patients with mucocutaneous lupus, especially in patients who developed DLE. Consistent with the present results, Georgescu *et al*. reported that serum IL-17F concentrations were higher in DLE, subacute cutaneous lupus erythematosus, and SLE patients than in healthy controls (53). Although we have identified that anti-SSA was strongly correlated with the levels of IL-17, anti-SSA was not elevated in patients with cutaneous lupus. Anti-Sm levels were lower in ACLE + patients compared to ACLE- patients. It is likely that the involvement of cytokines especially IL-17 in DLE is autoAb independent but local tissue inflammation related. Indeed, autoAbs were found to be lower in DLE + SLE patients (54). However, immunohistochemically staining demonstrated elevated IL-17 and/or IL-17-producing cells in several types of SLE skin manifestations, particularly DLE, where high numbers of CD4 + and CD8 + T cells are found in affected areas of tissue (31, 51, 53). Together, these results suggest that specific local blockade of IL-17 and its signaling may be beneficial to patients who developed DLE.

This study's strengths highlight the importance of assessing the association between cytokines for each T-cell subtype and autoAbs in LN patients and other SLE manifestations. Limitations include the cross-sectional nature of the study and specific clinical information were not available for some patients. In addition, a small number of healthy controls were included in the analysis.

Conclusions

This population-based study in middle-aged SLE subjects showed higher levels of IL-10 and IL-17 in patients with a history of LN and a history of LN class V. Additionally, IL-17 levels were elevated in patients who developed DLE. Together, these results suggest that cytokines may play a unique role in contributing to the diverse clinical manifestations of SLE. Further research is warranted to determine the local pathophysiology of these cytokines in patients with LN and mucocutaneous lupus to facilitate the development of targeted therapies for SLE.

Abbreviations

EULAR: European League Against Rheumatism

SLE: Systemic lupus erythematosus

IC: Immune complex

IFN : Interferon gamma

IL-10: Interleukin 10

IL-17: Interleukin 17

Anti-Sm: Anti-Smith

LN: lupus nephritis

autoAbs: Autoantibodies

UAB: The University of Alabama at Birmingham

ACR: American College of Rheumatology

SLICC: Systemic Lupus International Collaborating Clinics

SLEDAI: Systemic lupus erythematosus disease activity index

ACLE: Acute cutaneous lupus

HC: Healthy control

DLE: Discoid lupus erythematosus

SEM: Standard error of the mean

SD: Standard deviation

Declarations

Ethics approval and consent to participate

Ethics approval was granted by the University of Alabama at Birmingham Institutional Review Boards. Informed consent was obtained from each participant, and all samples and data were coded to maintain the anonymity of all patients.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that there is no conflict of interest.

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Authors' contributions

FA, KS, HCH and JDM were responsible for methodology, validation, formal analysis and visualization. FA, KS, HCH and JDM were responsible for the conception of the research idea and writing- Original Draft. HCH, and WWC were responsible for writing – review & editing. WWC, HCH, and JDM were responsible for the supervision. HCH and JDM were responsible for the funding acquisition. The authors read and approved the final manuscript.

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Tables

Table 1. Clinical characteristics of the participants at the time of enrollment

Characteristics	Total patients (N = 62, 100%)
Age at time of enrollment-year, mean (±SD)	34 (±58.17)
Race	
Caucasian	23 (37)
African American	39 (63)
Sex	
Female	57 (92)
Male	5 (8)
Clinical features	
Mucocutaneous disorder	
Malar rash	18 (29)
Discoid rash	10 (16)
Photosensitivity	33 (53)
Oral/nasal ulcers	19 (31)
Arthritis	38 (61)
Serositis (pleuritic, pericarditis)	18 (29)
Lupus nephritis	27 (44)
Autoimmune hepatitis	1 (2)
Pancreatitis	1 (2)
Cardiomyopathy	1 (2)
Neurological disorder (psychosis, seizure, CNS vasculitis)	8 (13)
Hematological disorder	
Hemolytic anemia	4 (7)
Leukopenia	14 (23)
Thrombocytopenia	11 (18)
SLEDAI (score)	
No activity (0)	17 (27)
Mild (1-5)	30 (48)
Moderate (6-10)	5 (8)

High activity (11-19)	2 (3)
Very high activity (≥20)	0 (0)
Missing, (%)	8 (13)
Immunology laboratory results	
Anti-DNA- IgG (OD) mean (±SEM)	0.36 (±0.02)
Anti-SSA- IgG (unit/mL) mean (±SEM)	15 (±2.46)
Anti-Smith (unit/mL), mean (±SEM)	75 (±11.87)
C3 (mg/dL), mean (±SD)	118 (±30.99)
C4 (mg/dL), mean (±SD)	24 (±9.02)
Cytokines	
IFN (pg/mL), mean (±SEM)	38.81 (±2.83)
IL-17 (pg/mL), mean (±SEM)	8.52 (±1.45)
IL-10 (pg/mL), mean (±SEM)	10.52 (±0.71)

CNS: Central nervous system; SLE: systemic lupus erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; IFN ：Interferon gamma; IL: Interleukin; SEM: Standard deviation; SD: Standard deviation

Figures

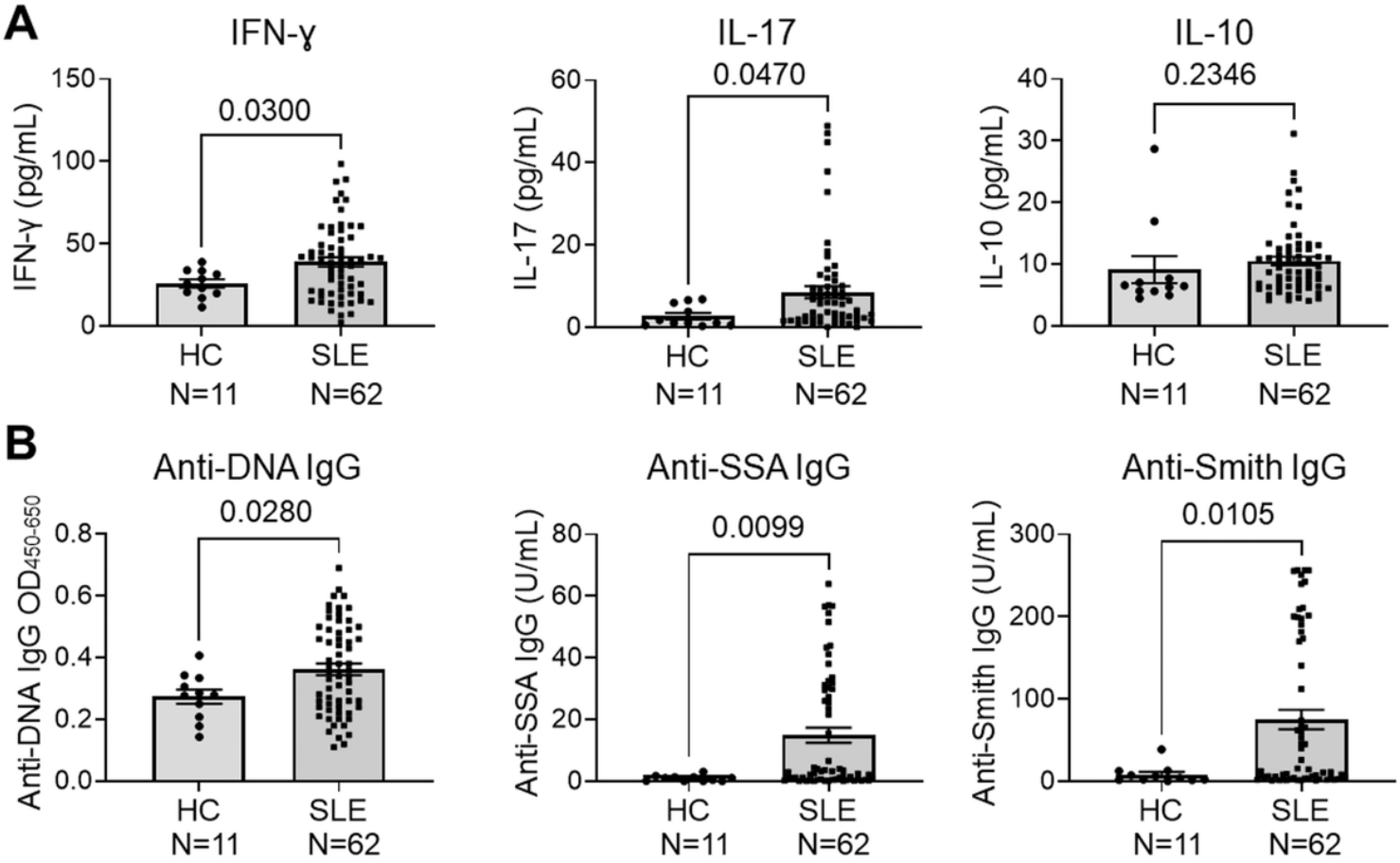

Figure 1

Elevation of cytokines and autoantibodies in SLE patients. Plasma levels of cytokines and autoantibodies in healthy control subjects and SLE patients were measured by ELISA assays. (**A**) Bar graphs showing the levels of IFNγ, IL-17, and IL-10. (**B**) Bar graphs showing the levels of anti-DNA, anti-SSA, and anti-Smith in healthy controls subjects and SLE patients (data are presented as mean ± SEM; unpaired t-test, the p value and the number of subjects in each group are shown in each panel).

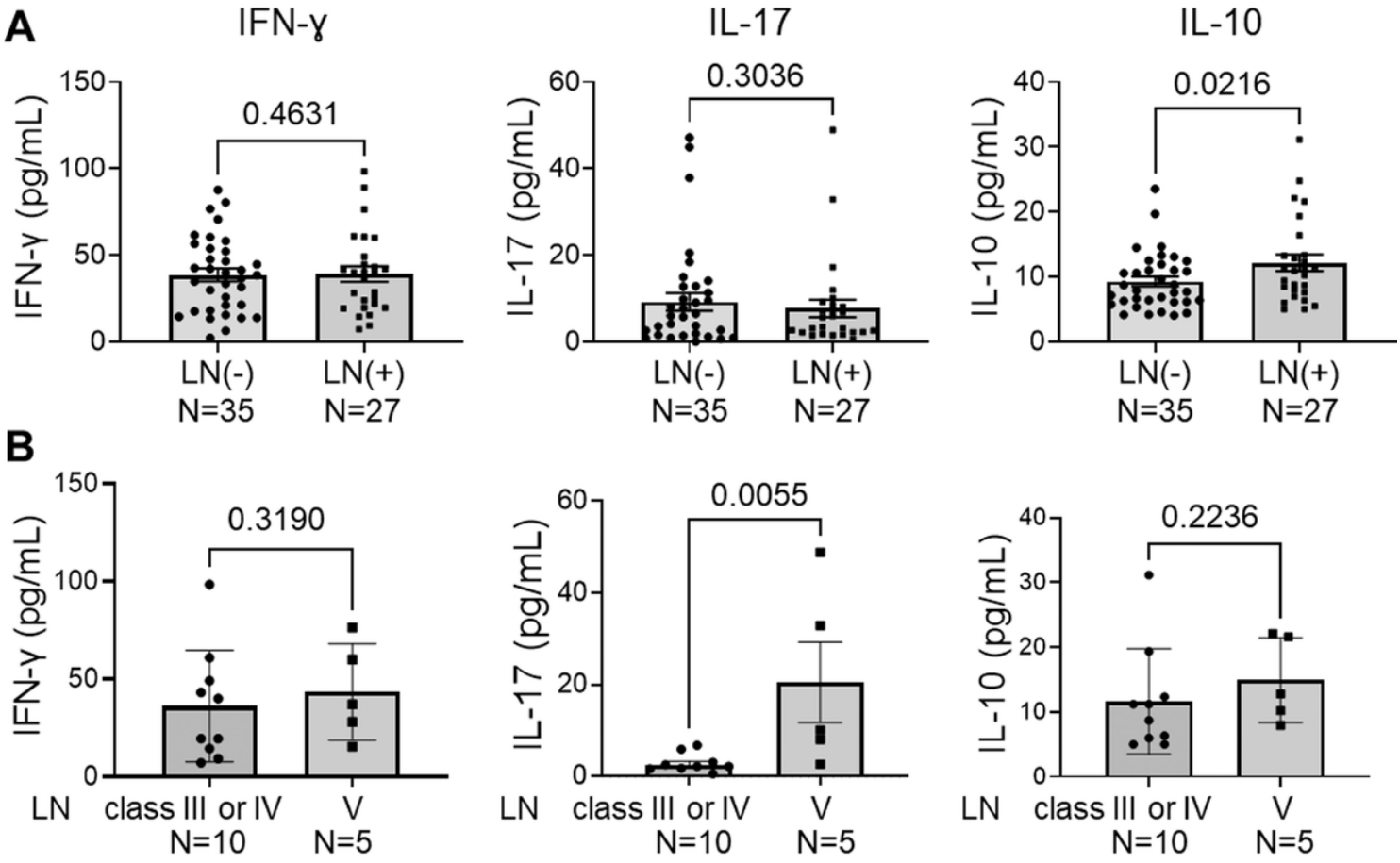

Figure 2

IL-10 was elevated in LN patients and IL-17 was elevated in class V LN patients. Plasma levels of IFN ，IL-17, and IL-10 were measured by ELISA. (**A**) Bar graphs showing the levels of IFNγ, IL-17, and IL-10 in patients with (positive) or without (negative) LN. (**B**) Bar graphs showing the levels of IFNγ, IL-17, and IL-10 in patients who developed class III or IV LN, compared to patients who developed class V LN (All data are presented as mean ± SEM; unpaired t-test, with the p value shown in each panel. The number of patients under each category is shown at the bottom).

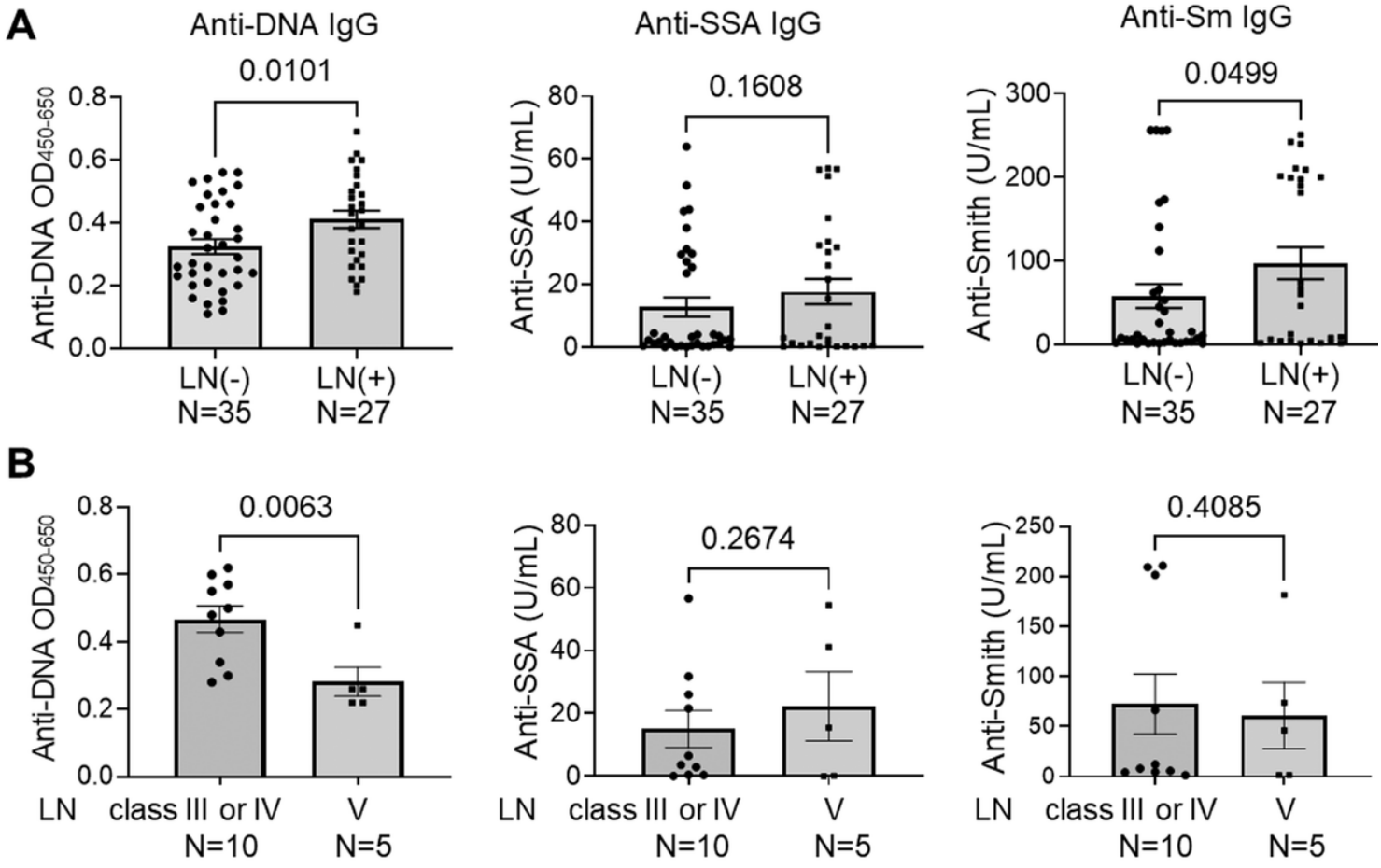

Figure 3

Elevation of anti-DNA and anti-Smith in patients with LN. Plasma levels of IgG anti-DNA, anti-SSA, and anti-Smith were determined by ELISA. (**A**) Bar graphs showing the levels of anti-DNA, anti-SSA, and anti-Smith in patients with (positive) or without (negative) LN. (**B**) Bar graphs showing the levels of anti-DNA, anti-SSA, and anti-Smith in patients who developed class III or IV LN, compared to patients who developed class V LN (All data are presented as mean ± SEM; unpaired t-test, the p value shown in each panel. The number of patients under each category is shown at the bottom).

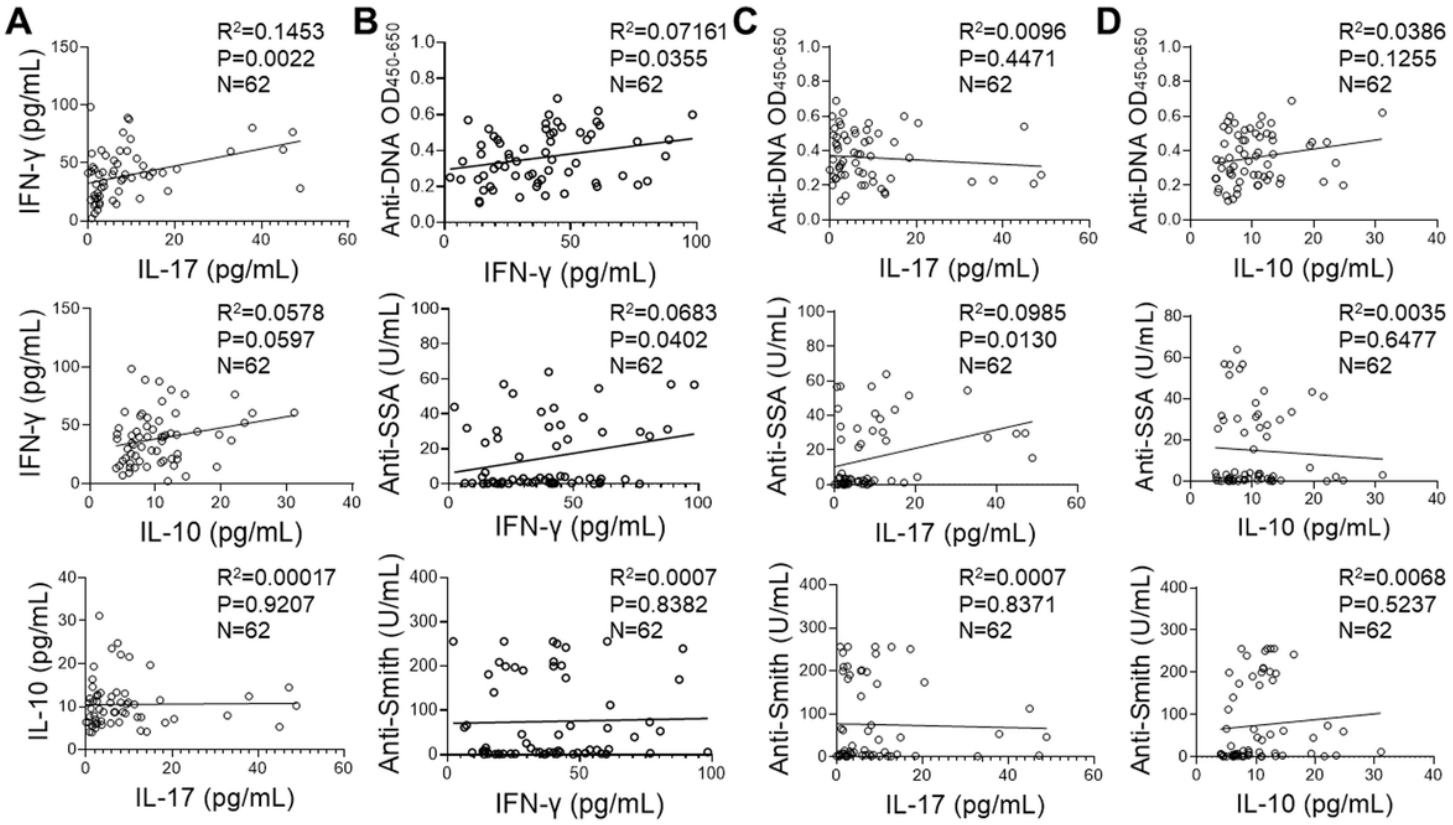

Figure 4

Anti-DNA was positively correlated with IFNγ and anti-SSA was positively correlated with IFNγ and IL-17. (**A**) Regression analysis showing the correlation among circulating levels of IFN , IL-17, and IL-10. (**B-D**) Regression analysis showing the correlation of circulating levels of autoantibodies with IFNγ (**B**), IL-17 (**C**), and IL-10 (**D**). All data were collected at the time of enrollment. All analyses were carried out using a linear regression analysis. The R^2, p value, and the number of subjects are shown on the plot.

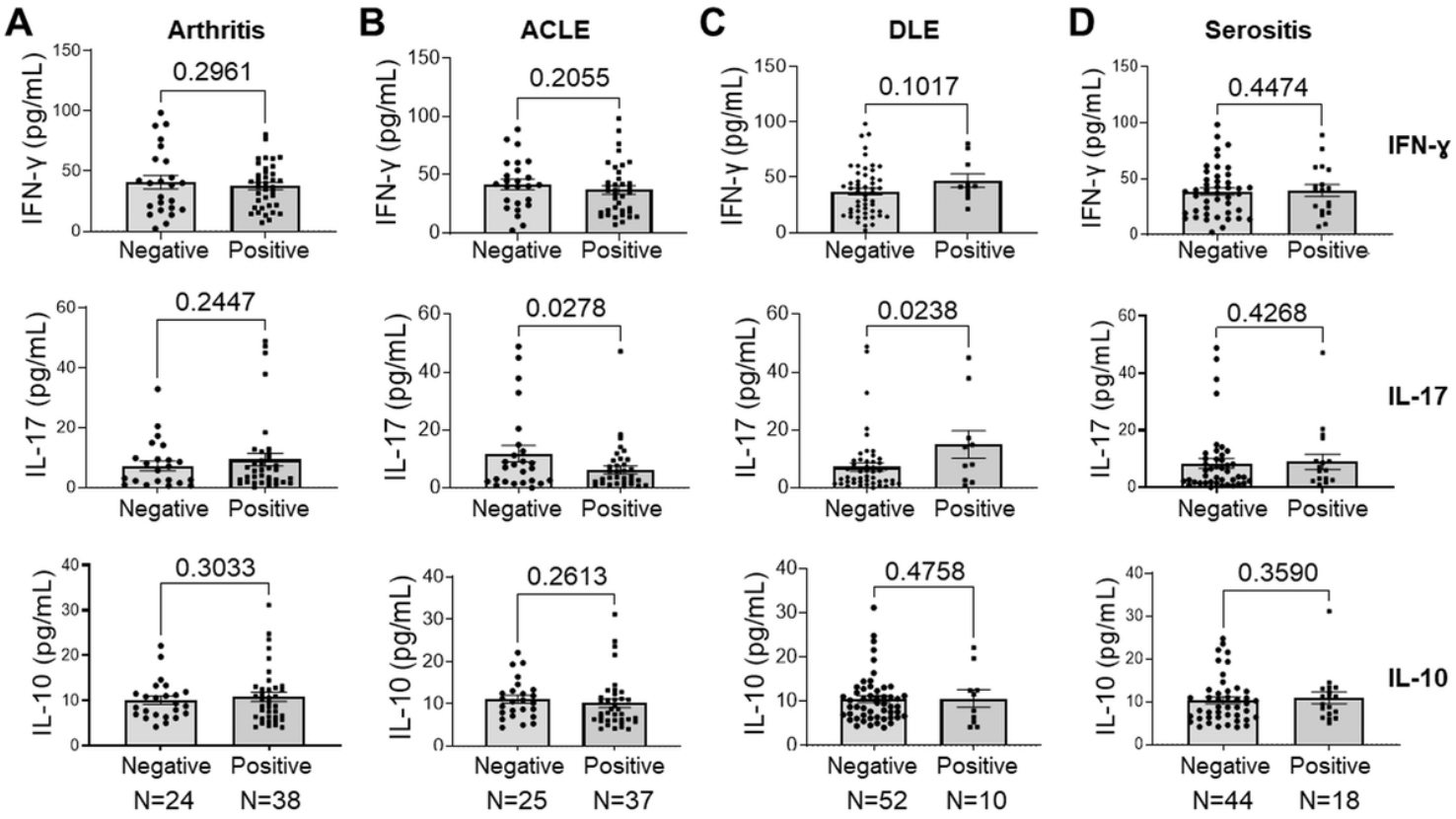

Figure 5

Increased plasma levels of IL-17 in DLE patients. (**A-D**) Bar graphs showing the levels of IFNγ, IL-17, IL-10 in patients with (positive) or without (negative) arthritis (**A**), ACLE (**B**), DLE (**C**), and serositis (**D**) (ACLE; acute cutaneous lupus, DLE; discoid lupus erythematosus, data are presented as mean ± SEM; unpaired t-test, with the p value and the number of patients shown in each panel).

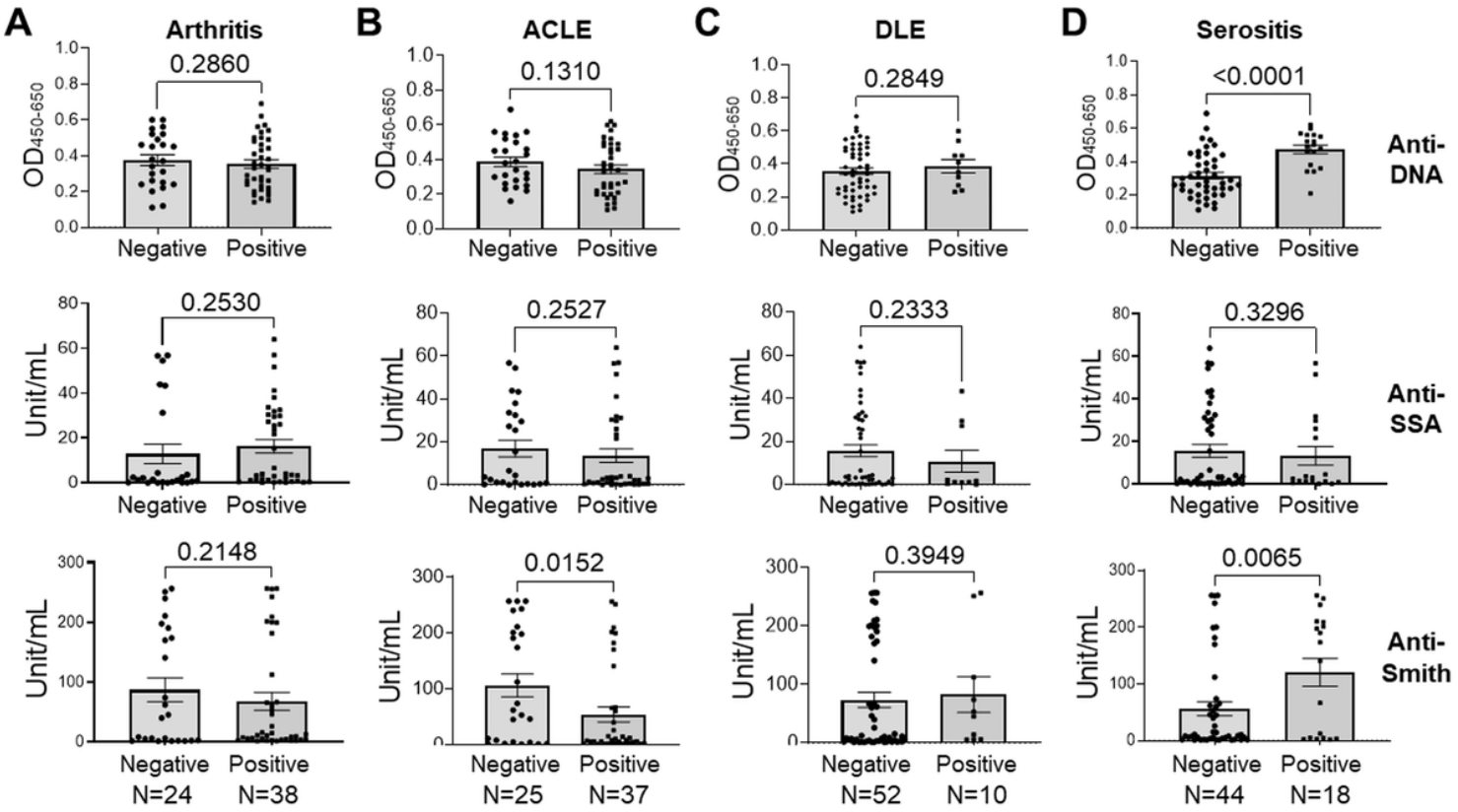

Figure 6

Increased circulating IgG anti-DNA and anti-Smith in patients with serositis. (**A-D**) Bar graphs showing the levels of IgG anti-DNA, anti-SSA, and anti-Smith in patients with (positive) or without (negative) arthritis (**A**), ACLE (**B**), DLE (**C**), and serositis (**D**) (data are presented as mean ± SEM; unpaired t-test, with the p value and the number of patients shown in each panel).