Title: IgM anti-phosphorylcoline antibodies associate with senescent and IL-17^{+} T-cells in SLE patients with a pro-inflammatory lipid profile

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ABSTRACT

Objectives: Evaluate whether T-cell subsets and lipid profile could be linked to the cardio-protective effect of IgM-anti-phosphorylcoline (PC) antibodies in SLE.

Methods: Anti-PC antibodies were quantified by ELISA in 197 patients and 99 controls and analyzed in relation to clinical features, treatments and serum lipids. Carotid atheromatosis was evaluated by ultrasonography; Th1, Th17, Treg and $CD4^{+}CD28^{null}$ cells by flow cytometry and cytokine serum levels by immunoassays in a subgroup of 120 SLE patients and 33 controls.

Results: IgM-anti-PC serum levels were reduced in SLE patients compared with controls ($p<0.001$) and associated with age ($\beta=-0.252$; $p=0.002$), HDL ($\beta=0.271$; $p=0.001$), LDL ($\beta=-0.192$; $p=0.017$) and glucocorticoid treatment ($\beta= -0.201$; $p=0.012$), whereas the IgG-to-IgM-anti-PC ratio was increased ($p=0.007$) and associated with age ($\beta=0.194$; $p=0.028$) and SLEDAI ($\beta=0.250$; $p=0.005$). Also, patients with clinical or subclinical cardiovascular (CV) disease exhibited reduced IgM-anti-PC levels than their CV-free counterparts, regardless of glucocorticoid usage ($p=0.001$). $CD4^{+}CD28^{null}$ and Th17 cells were increased in SLE compared with controls ($p<0.01$) and correlated inversely with IgM-anti-PC levels. These associations were observed in patients displaying TG^{high} or HDL^{low} levels, even after adjusting for clinical parameters and treatments ($CD4^{+}CD28^{null}$: $\beta =-0.455$, $p=0.001$; Th17: $\beta=-0.280$, $p=0.035$), but not in those with a normal lipid profile. HDL^{low} and TG^{high} profiles were related to low IgM-anti-PC and Treg levels, respectively, whereas both lipids profiles were associated to inflammatory markers and cytokines.

Conclusions: Present study provides evidence for an association of IgM-anti-PC antibodies with pro-atherogenic T-cell subsets in SLE, TG^{high}/HDL^{low} lipid profile playing a facilitating major role.

Keywords: atherosclerosis; IgM anti-phosphorylcoline antibodies; lipid profile; Th17; Treg; $CD4^{+}CD28^{null}$ cells, systemic lupus erythematosus.

KEY MESSAGES

- Lipid profile and glucocorticoid usage were independent predictors of low IgM-anti-PC levels in SLE.
- SLE patients with clinical or subclinical CVD displayed reduced IgM-anti-PC levels irrespective of treatment.
- Senescent and IL-17^{+}-T-cells were negatively correlated with IgM-anti-PC levels in TG^{high} or HDL^{low} patients.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by an over-activation of the immune system together with an inefficient clearance of apoptotic cells resulting in the production of autoantibodies against cellular components (1). This SLE-related inflammatory environment causes tissue damage in multiple organs, but also triggers the development of premature atherosclerosis and cardiovascular disease (CVD), the leading cause of mortality in these patients (2). Nowadays it is widely accepted that the enhanced CVD in SLE cannot be completely explained by traditional CV-risk factors, thus suggesting that other disease-specific factors must play a role in the development of vascular damage and atheromatosis (3). Therefore, the identification of such pro-atherogenic factors inducing vascular dysfunction could be useful for patient stratification and implementation of preventive therapies in SLE.

Atherosclerosis is initiated by the accumulation in the intima layer of modified lipids, especially low density lipoproteins (LDL) which can activate endothelial cells and promote the recruitment of immune cells (4). The early accumulation of activated monocyte-derived macrophages and dendritic cells in the plaque triggers lesion development and prompts a T-cell subset differentiation (5). In this scenario, excessive responses of T-helper1 (Th1) or Th17 cells may exert a pro-atherogenic effect that could not be properly controlled by regulatory T-cells (Treg) (6), a cellular subset known to have a protective role of CVD development in general population (7). Moreover, several studies associated an imbalanced Th17/Treg response to SLE pathology (8) and lupus-related atherosclerosis (9). In addition, the repeated T-cell stimulation as a consequence of inflammatory environment underlying both atherosclerosis and SLE disease leads to the expansion of immunosenescence-related $CD4^{+}CD28^{null}$ cells, a cytotoxic subset related to endothelial injury and plaque destabilization (10,11).

The inflammatory effects of modified LDL are caused by phospholipids with platelet activating factor (PAF)-like properties generated during oxidation, which have phosphorylcholine (PC) as

a major component (12). Interestingly, naturally occurring auto-antibodies of IgM isotype against PC (anti-PC) are commonly secreted by B1 cells in the general population (13). Since PC is also exposed in apoptotic cells, several studies have demonstrated that the presence of high levels anti-PC antibodies could be a protective marker for atherosclerosis (14) and SLE development (15–17) by inhibiting the pro-inflammatory effects of modified LDL on the endothelium and increasing phagocytosis and clearance of dying cells (18). Therefore, lower levels of IgM-anti-PC predict increased occurrence of atherosclerosis and CVD risk in general population (19,20). Supporting this, both active immunization and passive transfer of anti-PC ameliorates murine atherosclerosis (21). Further, lipid modifications are increased and associated with CVD in SLE (22) and the low IgM anti-PC amounts observed in these patients have been associated with atherosclerosis, disease severity or activity (15,16,23,24). Thus, current evidence points to a role for natural IgM-anti-PC antibodies in atherosclerosis development and the immune dysregulation present in SLE. Therefore, given the role of T-cells both in the endothelial homeostasis and SLE pathogeny, and the involvement of IgM anti-PC levels in the regulation of the inflammatory and pro-atherogenic effects of modified lipids, we aimed to evaluate whether imbalanced T-cell subsets and altered lipid profile in SLE could be linked to the cardio-protective effect attributed to IgM-anti-PC.

METHODS

Patients and controls

One hundred-ninety-seven patients fulfilling the American College of Rheumatology (ACR) revised criteria for SLE classification (25) were sequentially recruited from the outpatient clinic of the Autoimmune Disease Unit (Hospital Universitario Central de Asturias, HUCA) (Table 1). Information on clinical and immunological manifestations, traditional CV-risk factors and CV events along the disease course was obtained after a retrospective review of their clinical records. SLE disease activity index (SLEDAI) (26) and treatments received over the previous 3 months were recorded at the sampling time. Clinical, immunological and demographic characteristics of the SLE cohort (Table 1) are in accordance with data from other Caucasian populations of lupus patients from Europe and North-America, as we previously described (27).

Ninety-nine sex and age-matched volunteers from the same geographic area, without any pathology or treatment, were enrolled as controls. Fresh blood samples from patients and controls were tested for cell count, complete lipid analyses and presence of specific autoantibodies (Supplementary material).

Presence of subclinical carotid atheromatosis and analysis of T-cell subsets and cytokine levels were performed in a subgroup of 120 SLE patients and 33 controls (Supplementary Table 1). Ethics approval for this study was obtained from the Regional Ethics Committee for Clinical Research (Servicio de Salud-Principado de Asturias), according to the Declaration of Helsinki. All individuals signed a written informed consent prior to participation in the study.

Anti-phosphorylcholine antibodies quantification

IgM and IgG antibodies against phosphorylcholine (anti-PC) were quantified in serum samples from patients and controls by an in-house ELISA test. Briefly, microtiter wells (Maxisorp, Nunc) were coated overnight with phosphorylcholine conjugated to bovine serum albumin (PC-BSA) (Biosearch Technologies) in PBS and blocked with PBS-2% BSA for 2 hours at 37°C. Pooled sera from controls serially diluted in Tris Buffered Saline (TBS) were used as anti-PC

standard. Serum samples and anti-PC standard were incubated for 2 hours at room temperature (RT). After washing with TBS/Tween-20 (0.05%), wells were incubated for 2 hours at RT with alkaline phosphatase-conjugated anti-human IgM or IgG (Immunostep). Finally, plates were washed and revealed using p-nitrophenylphosphate as substrate in 1M diethanolamine/1mM $MgCl_2$ buffer (pH 9.8). Absorbance was determined at 405 nm of wavelength. Serum anti-PC arbitrary units were calculated for each sample according to the standard curves. Similarly, total IgM and IgG were quantified by ELISA kits ("Human IgM/IgG Ready-SET-Go!"; eBioscience) following the manufacturer's instructions.

Flow Cytometry

$CD4^{+}CD28^{null}$ T-cells, Th17 and T regulatory (Treg) lymphocytes were quantified in whole peripheral blood samples by flow cytometry (see Supplementary material and Supplementary Figure 1).

Cytokine quantification

Serum samples were maintained at -80ºC until cytokine determinations. IFNα, IL-17A and CCL3 (MIP-1α), IFNγ, IL-10, IL-6, BLyS, CCL2 (MCP-1), ICAM-1 and IP-10 were quantified by immunoassays following the manufacturer's instructions (see Supplementary material).

Doppler ultrasound

Doppler ultrasound was performed in patients and controls in the sonography laboratory of the HUCA. All measures were carried out by the same operator using a Toshiba Aplio XG machine (Toshiba American Medical Systems). The carotid intima-media wall thickness (cIMT) was bilaterally measured, being plaque defined as a distinct area protruding into the vessel lumen at least 0.5 mm, with 50% greater thickness than the cIMT found in surrounding areas or the presence of cIMT>1.5 mm (28). Subclinical atheromatosis was defined as the presence of carotid plaque or cIMT>0.9 mm.

Statistical analysis

Kolmogorov-Smirnov, Mann–Whitney, Kruskal-Wallis or T-tests, Spearman's rank correlations, χ^2-tests and multivariate regression analysis were used as appropriated. Data were expressed as the mean±standard deviation (SD) or median (interquartile range, IQR). A p-value<0.05 was considered statistically significant. Data were analyzed using GraphPad Prism 5 (GraphPad Software) and SPSS 24 statistical software package (IBM) (see Supplementary material).

RESULTS

IgM-anti-PC antibodies are associated with lipid profile in SLE patients

Serum levels of IgM-anti-PC antibodies were diminished in SLE patients compared with controls (Figure 1A), even after adjusting for total IgM levels ($p<0.05$). This reduction was unrelated to disease activity but associated to autoantibody presence and glucocorticoid usage (Figure 1B). Also, IgM-anti-PC antibodies declined with age, disease duration and BMI (Figure 1C). No associations were detected with other clinical features or therapies. Conversely, IgG-anti-PC levels did not differ between patients and controls [211.89 (246.83) vs 175.66 (199.28) AU; p=0.886], SLE patients presenting higher amounts of total IgG ($p<0.001$). However, the IgG-to-IgM-anti-PC ratio was increased in SLE patients [2.90 (5.30) *vs* 1.86 (2.91); p=0.007], and associated to SLEDAI ($\beta=0.250$, p=0.005) and age ($\beta=0.194$, p=0.028) in a multiple backward regression analysis including autoantibodies, disease duration and activity, treatments, BMI and demographic parameters in the initial model.

On the other hand, IgM-anti-PC levels displayed a negative association with triglycerides (TG) and total and LDL-cholesterol in SLE patients and controls (Figure 1D). However, after linear regression analysis adjusting by sex, age and BMI, relevant results were only observed in the patient group, maintaining the negative association with TG but revealing a positive relationship with HDL levels (Table 2). These associations were maintained after excluding SLE patients with dyslipidemia.

Finally, multiple backward regression analysis including serum lipids, autoantibodies, disease duration, treatments, BMI and demographic parameters in the initial model, revealed age ($\beta=-0.252$;p=0.002), HDL ($\beta=0.271$;p=0.001), LDL ($\beta=-0.192$;p=0.017) and glucocorticoid treatment ($\beta=-0.201$;p=0.012) as significant predictors of IgM-anti-PC levels in SLE. No associations were detected between IgG-anti-PC and lipid profile.

Therefore, these results confirm a decrease in IgM-anti-PC antibodies in SLE and suggest a potential role of the lipid profile in IgM-anti-PC occurrence in these patients.

Senescent and IL-17$^+$ T-cells were related to IgM-anti-PC antibodies in SLE patients with a proinflammatory lipid profile

To evaluate the possible association of IgM-anti-PC autoantibodies with T-cell subsets related to endothelial homeostasis and CV-risk, peripheral blood Th1, Th17, Treg and CD4$^+$CD28^{null} cells and subclinical atheromatosis were determined in 120 SLE patients and 33 controls (Supplementary Table 1).

SLE patients with either previous CVD (n=18) or subclinical atheromatosis (n=38) displayed lower IgM-anti-PC levels than those without CV-risk factors (CV-free) (Figure 2A), thus supporting the value of these natural antibodies as CV-risk biomarkers. Similar results were observed after excluding glucocorticoid treated patients from the analysis, IgM-anti-PC being reduced in patients with subclinical atheromatosis or previous CVD compared with those CV-free (p=0.001). No differences between controls and SLE groups were detected with IgG-anti-PC antibodies.

Among the analyzed T-cell subsets, CD4$^+$CD28^{null} and Th17 cells were augmented in all SLE groups, but especially in those with CV involvement (Figure 2B). Accordingly, cIMT was correlated with CD4$^+$CD28^{null} (ρ=0.230;p=0.016) and Th17 (ρ=0.211;p=0.022) cells in SLE patients. Moreover, Foxp3$^+$IL-17$^+$ cells were significantly increased in SLE patients with clinical or subclinical CVD, whereas Treg cells were reduced in all patients groups. Interestingly, Treg-cell frequency was influenced by serum lipids, as it was inversely correlated with TG in controls and SLE patients (ρ=-0.541;p=0.001 and ρ=-0.219;p=0.016, respectively) and directly with HDL in controls (ρ=0.356;p=0.045), hence supporting the role of lipid profile in immune homeostasis. No differences were detected in Th1 frequency between controls and SLE groups.

On the other hand, IgM-anti-PC levels were negatively correlated with CD4$^+$CD28^{null} cells in SLE patients and controls (ρ=-0.237;p=0.013 and ρ=-0.402;p=0.020, respectively), and with Th17 (ρ=-0.258;p=0.004) and Foxp3$^+$IL-17$^+$ (ρ=-0.212;p=0.020) cells in SLE. However, when SLE patients were stratified into tertiles according to serum levels of TG or HDL, these associations only remained in patients with either TG^{high} ($\geq$96 mg/dl, third tertile) or HDL^{low}

(≤53 mg/dl, first tertile) but not in those with a normal lipid profile (TG<third tertile and HDL>first tertile) (Figure 2C). The exclusion of SLE patients with dyslipidemia from the TG^{high}/HDL^{low} group resulted in similar correlations ($CD4^{+}CD28^{null}$: ρ=-0.449,p=0.005; Th17: ρ=-0.419,p=0.008; $Foxp3^{+}IL\text{-}17^{+}$: ρ=-0.313,p=0.053). Moreover, multiple backward regression analysis, entering sex, age, BMI, treatments (including lipid lowering therapies), disease activity and duration in the initial model, revealed IgM-anti-PC antibodies as predictors of both $CD4^{+}CD28^{null}$ (β =-0.455,p=0.001) and Th17 (β=-0.280,p=0.035) cells in SLE patients displaying an altered TG^{high}/HDL^{low} profile, but not in those with normal lipid profile. However, IgM-anti-PC levels were not associated to Treg frequency in any group. Of note, TG^{high} and HDL^{low}-lipid profiles were associated with low Treg cells and IgM-anti-PC levels, respectively, and with inflammatory markers (C-reactive protein, CRP; neutrophils-to-lymphocytes ratio, NLR; red cell distribution width, RDW) but not with disease activity (Table 3).

Finally, serum levels of several cytokines related to endothelial damage were determined in SLE patients and controls (Figure 3). Results showed a positive association of IL-6 and MIP1α with IgM-anti-PC in controls. In SLE, however, IgM-anti-PC levels were directly associated with IFNα, MIP1α and IL-17, and inversely with BLyS, IP-10 and MCP1 levels. Further multiple backward regression analysis including disease duration and activity, autoantibodies, treatments (including lipid-lowering therapies) and demographic parameters in the initial model, revealed IFNα (β=0.265;p=0.002), BLyS (β=-0.193;p=0.031), IP-10 (β=-0.216;p=0.015), MIP1α (β=0.325;p<0.001) and MCP1 (β=-0.225;p=0.010) as significant predictors of IgM-anti-PC levels in SLE. In controls, linear regression analyses adjusted by age and sex revealed an association of IgM-anti-PC with IL-6 (ρ=0.424,p=0.006) and IL-10 serum levels (ρ=-0.449,p=0.005).

Of note, SLE patients with the TG^{high}/HDL^{low} lipid profile presented higher levels of sICAM-1, IP10, MCP1, IL-6 and BLyS than their normal profile counterparts (Table 3).

Taken together, all these results suggest that an association with immune cell subsets may underlie the effect of IgM-anti-PC on CV endpoints, lipid profile playing a facilitating major role.

DISCUSSION

Several studies have demonstrated the relevance of IgM-anti-PC antibodies as cardioprotective factors in general population and SLE. The present work expands the current knowledge on these natural antibodies suggesting a novel atheroprotective mechanism that counteracts the generation of senescent and IL-17^{+} T-cells, and revealing lipid profile as the missing link between IgM-anti-PC levels and the development of inflammatory mediators in SLE patients.

The results herein reported demonstrate the existence of reduced IgM-anti-PC serum levels in SLE, especially in patients with previous CV events or subclinical atheromatosis, thus confirming earlier findings (16,23,24) and supporting their value as biomarkers to identify patients at the pre-clinical stage of CVD. Conversely, IgG-anti-PC levels were unrelated to lipid profile or CV involvement. However, the IgG-to-IgM-anti-PC ratio correlated with disease activity, suggesting that an increase in IgG switching in active patients could reduce IgM-anti-PC protection. Such decrease in circulating IgM-anti-PC amounts was associated with age, BMI, disease duration, presence of autoantibodies and glucocorticoids consumption. However, an unexpected key result of our work was the observation of an association with lipid traits, mainly decreased HDL levels, as crucial factors influencing the low levels of IgM-anti-PC detected in SLE, even in patients without dyslipidemia, and independent of treatments. Of note, the *lupus lipid pattern*, characterized by increased LDL and triglycerides but low HDL levels, is commonly observed in these patients and independently associated to CVD in general population (29). Although pathogenic mechanisms underlying lipid alterations in SLE are yet unknown, some influencing factors seem to exert notable effects on lipoprotein metabolism. On one hand, the activity of lipoprotein lipase (LPL), involved in the hydrolysis of TG to lipoproteins such as chylomicrons and LDL, seems to be impaired in lupus patients, thus resulting in hyperlipoproteinemia and increased TG (30). Furthermore, anti-dsDNA antibodies have been shown to exert anti-LPL activity (31). Particularly, hypertriglyceridemia enhances lipid peroxidation, thus contributing to the generation of pro-atherogenic changes in LDLs (32). On the other hand, chronic inflammation typically present in SLE can also result in an

intensified oxidative stress able to convert anti-atherogenic HDL to a dysfunctional and pro-inflammatory one that fails to inhibit LDL oxidation and may be a cause of increased CV-risk (33). Of note, in spite of the known beneficial effects of antimalarials and statins on SLE dyslipidemia (34,35), the associations between IgM-anti-PC and lipid profiles in our SLE patients were not affected by these treatments. Specific alterations of serum lipids are known to affect the SLE prognosis not only by increasing CV-risk *per se* but also by enhancing chronic inflammation. Although LDL has been classically identified as a circulating biomarker of general CV-risk, oxLDL amount seems to confer an adverse CVD prognosis due to its direct implication in the biology of the atherosclerotic plaque (22). Because oxidized phospholipids such as PC are also present in apoptotic cells, they act as enhancers of the inflammation underlying both atherosclerosis and autoimmunity (36). In this scenario, IgM-anti-PC antibodies recognizing and increasing the clearance of dying cells have been postulated as regulators of the inflammatory pathways driving to atherogenesis in SLE (15,37). Hence, in an environment with increased rate of cell death, reduced levels of IgM-anti-PC could be expected as a consequence of an elevated consumption. Therefore, the *lupus lipid pattern* could influence CV-risk though its effects on IgM-anti-PC.

Our results confirmed the existence of increased levels of the pro-atherogenic $CD4^{+}CD28^{null}$ and Th17 lymphocytes in SLE, especially in patients with CV involvement, in line with previous works (9,11). The quantity of both cellular populations was correlated with the cIMT, supporting their value as markers of premature atherosclerosis in SLE. Moreover, the increased proportion of $Foxp3^{+}IL\text{-}17^{+}$ cells observed in SLE patients with CV alterations evidence the existence of a trans-differentiation of Treg into T-inflammatory cells, previously described in patients suffering atherosclerosis and/or autoimmunity (38). However, our findings go further by pointing out an association between IgM-anti-PC levels and the development of these lymphocyte subsets, able to cause inflammation and endothelial damage. Most importantly, these associations were restricted to SLE patients with a TG^{high} (>96 mg/dl) or HDL^{low} (<53 mg/dl) profiles, even in the absence of dyslipidemia, but not observed in those with normal TG

and HDL levels. SLE patients with TG^{high} or HDL^{low} lipid profiles exhibit higher levels of inflammatory markers (CRP, RDW and NLR) as well as soluble mediators of inflammation or endothelial dysfunction (sICAM-1, IP10, MCP-1, IL-6 and BLyS), supporting TG^{high}/HDL^{low} as a proinflammatory lipid profile (39).

Therefore, our results suggest that in the context of a proinflammatory lipid profile, low levels of IgM-anti-PC allowed the expansion of senescent $CD4^{+}CD28^{null}$ cells and promote Th17 bias and Treg trans-differentiation towards $Foxp3^{+}IL\text{-}17^{+}$ cells. We can speculate that reduced or altered Treg cells, a common feature of SLE patients, could underlay these effects. Actually, present results reveal that Treg cells were influenced by serum lipids. Moreover, our observations are in accordance with experiments of Sun *et al*, demonstrating that purified IgM-anti-PC antibodies promote the *in vitro* polarization of T-cells from SLE patients and controls into Treg cells (40). In fact, natural IgM can switch mature DC to tolerogenic DC by inhibiting the NF-kB pathway (41). In view of the anti-inflammatory and anti-atherosclerotic effects of Treg cells, their expansion has been proposed as a potential therapeutic option in CVD (42), and high levels of IgM-anti-PC antibodies could be a valuable instrument to get that aim.

Among the mechanisms by which serum lipids might influence IgM-anti-PC levels and Treg-Th differentiation, cytokines involved in SLE pathogenesis could play a role. OxLDL are potent inducers of inflammatory mediators by endothelial cells, including MCP-1 and IL-6, both found to be related to higher TG levels and decreased HDL, respectively, in SLE (43). Accordingly, our data confirm the presence of increased levels of both molecules in SLE patients with the TG^{high}/HDL^{low} lipid profile, as well as the cell adhesion molecule ICAM-1, that contributes to the recruitment of inflammatory cells into the vascular endothelium (44). Supporting the role of lipid profile on T-cell responses, IL-6 induced by oxLDL can modify the cytokine pattern of vascular DCs driving $CD4^{+}$ T-cell differentiation toward Th17 instead of Treg cells (45,46). Also, IL-6 stimulates CRP synthesis (47), a classical pro-nflammatory marker that increase coronary artery disease in SLE (48) and also related to this pro-inflammatory lipid profile.

Finally, an unexpected result was the positive association of IgM-anti-PC with IFNα, one of the most relevant etiopathogenic cytokines in SLE. Over-expression of type-I IFNs and interferon-related genes is a common phenomenon in SLE patients and it has been related to a higher prevalence of autoantibodies (49). In fact, IFNα induces the differentiation of B cells into plasmablasts that further differentiate into autoantibody-secreting plasmatic cells (50). Also, the positive correlation of circulating IFNα with IgM-anti-PC levels could reflect the induction of the *IFNα signature* associated to the elimination of dead cells opsonized by complement and IgM-anti-PC (51).

In sum, our findings provide evidence for a novel protective mechanism of IgM-anti-PC antibodies in SLE that involves proinflammatory and pro-atherogenic T-cell subsets and which is dependent on serum lipid profile. Considering our findings, quantification of IgM-anti-PC antibodies may improve CV-risk identification of SLE patients displaying lipid alterations. Also, therapeutic approaches may include strategies for restoring the physiologic balance of natural anti-PC levels as a homeostatic factor of the immune response underlying both SLE progression and atherosclerosis.

Conflict of interest

Authors declare not conflicts of interest.

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FIGURE LEGENDS

Figure 1. Serum levels of IgM-anti-PC antibodies in SLE patients and controls. (A) Serum levels of IgM-anti-PC antibodies were diminished in SLE patients (n=197) compared with controls (n=99) [60.53 (110.10) *vs* 87.70 (98.71) (arbitrary units, AU); p<0.001]. **(B)** IgM-anti-PC serum levels in SLE patients according to the presence (+) or absence (-) of anti-dsDNA (53.01 *vs* 111.40 AU), anti- Sm (31.14 *vs* 62.60 AU), anti-RibP (35.49 *vs* 63.14 AU) or glucocorticoid (GC) treatment (20.86 *vs* 39.26 AU). Also, IgM-anti-PC serum levels from SLE patients were negatively correlated to BMI, disease duration and age. **(C)** Correlation of IgM-anti-PC serum levels and serum lipids (total and LDL-cholesterol, triglycerides) in SLE patients and controls.

Horizontal lines in scatter-plots represent median and interquartile range of IgM-anti-PC detected in each group. Statistical significance was assessed by Mann-Whitney *U* test. Correlation analyses were evaluated by Spearman tests; IgM-anti-PC levels were log-transformed only for the graphical representation.

Figure 2. IgM-anti-PC antibodies and $CD4^+$ T-cell subsets in controls and SLE patients according to the presence of cardiovascular alterations. (A) Serum levels of IgM-anti-PC antibodies in a subgroup of controls (n=33) and SLE patients (n=120) classified by the presence of traditional risk factors (tCVR, n=31), subclinical atheromatosis (sub-CVD, n=30) or cardiovascular disease (CVD, n=18). **(B)** Frequency of $CD4^+CD28^{null}$ cells, IL-17^+Foxp3^+, Th17 and Treg cells in controls and patients grouped by the presence tCVR, sub-CVD or CVD. Representative dot-plots of analyzed T-cell subsets in patients and controls. **(C)** Correlation of IgM-anti-PC serum levels and $CD4^+CD28^{null}$ lymphocytes, IL-17^+Foxp3^+ and Th17 cells frequency in SLE patients displaying a lipid profile with TG^{high} or HDL^{low} levels in comparison to normal values.

Horizontal lines in scatter-plots represent median and interquartile range, and statistical differences among groups were assessed by Kruskal-Wallis and Mann-Whitney-U test

(*p<0.05, **p<0.01, ***p<0.001). Correlation analyses were evaluated by Spearman tests; IgM-anti-PC levels were log-transformed only for the graphical representation.

Figure 3. Circulating levels of cytokines and their association with IgM-anti-PC antibodies in controls and SLE patients. (A) Serum levels of cytokines in controls and SLE patients. Horizontal lines in scatter-plots represent median and interquartile range. Differences between groups were evaluated by Mann-Whitney-U test. Correlation of IgM-anti-PC and cytokine serum levels in controls **(B)** and SLE patients **(C)** (only statistically significant correlations have been represented). Correlation analyses were evaluated by Spearman tests using non-transformed variables (IgM-anti-PC levels were log-transformed only for the graphical representation).

Table 1. Demographic and clinical characteristics of SLE patients and controls.

	SLE patients (n=197)	Controls (n=99)
Demographic features[a]		
Sex (female/male) (n)	186/11	92/7
Age, years (mean ± SD)	49.39 ± 13.44	49.53 ± 13.38
Total cholesterol, mg/dl (mean ± SD)	188.68 ± 35.15	196.02 ± 29.41
HDL cholesterol, mg/dl (mean ± SD)	61.51 ± 17.23	61.60 ± 13.80
LDL cholesterol, mg/dl (mean ± SD)	108.79 ± 31.03	116.98 ± 26.85*
Triglycerides, m/dl (mean ± SD)	92.22 ± 54.51	86.77 ± 37.39
Total/HDL-cholesterol ratio	3.21 ± 1.05	3.32 ±0.84
Atherogenic index of plasma[b]	0.14 ± 0.30	0.12 ± 0.24
Traditional CV risk factors[c], n (%)		
Dyslipidemia	37 (18.78)	
Hypertension	43 (21.83)	
Diabetes (type II)	4 (2.03)	
Obesity (BMI>30)	30 (15.23)	
Smoking habit	50 (23.38)	
Clinical manifestations, n (%)		
Age at diagnosis, years (mean ± SD)	35.63 ± 13.58	
Disease duration, years (mean ± SD)	13.87 ± 10.48	
SLEDAI score (mean ± SD)	2.92 ± 3.22	
ACR criteria		
Malar rash	101 (51.27)	
Discoid lesions	55 (27.92)	
Photosensitivity	118 (59.90)	
Oral ulcers	82 (41.62)	
Arthritis	149 (75.63)	
Serositis	46 (23.35)	
Cytopenia	122 (61.93)	
Renal disorder	58 (29.44)	
Neurological disorder	20 (10.15)	
Autoantibodies, n (%)		
ANAs	197 (100.00)	
Anti-dsDNA / titer, U/ml (mean ± SD)	139 (70.56)/42.13 ± 84.66	
Anti-SSA	100 (50.76)	
Anti-SSB	31 (15.74)	
Anti-Sm	19 (9.64)	
Anti-RNP	36 (18.27)	
Anti-RibP	17 (8.63)	
RF	31 (15.74)	
Anti-cardiolipin IgG	42 (21.32)	
Anti-cardiolipin IgM	35 (17.77)	
Treatment, n (%)		
None or NSAIDs	19 (9.64)	
Antimalarial drugs	161 (81.73)	
Glucocorticoids	70 (35.53)	

Immunosuppressive drugs[d]	44 (22.33)
CV disease[e], n (%)	24 (12.18)

[a] Differences between SLE and control groups were analyzed by T-tests, U-Mann Whitney or χ^2 tests (*$p<0.05$).

[b] Calculated as "Log (Triglycerides/HDL-cholesterol)".

[c] Data exposed of dyslipidemia, hypertension, diabetes type II, obesity and smoking habit are referred to the total number of patients excluding those with CV disease.

[d] Mycophenolate mophetil, azathioprine.

[e] Cerebrovascular disease, heart disease, peripheral vascular disease.

dsDNA: double stranded DNA; RF: rheumatoid factor; NSAID: non-steroidal anti-inflammatory drug.

Table 2. Association of circulating IgM-anti-PC antibodies with lipid profile in controls and SLE patients.

	Controls (N=99)		SLE			
			Total (N=197)		Non-dyslipemic (N=160)	
	Regression coefficient	***p*-value**	**Regression coefficient**	***p*-value**	**Regression coefficient**	***p*-value**
Cholesterol (mg/dl)	-0.220	**0.047**	-0.067	0.402	-0.020	0.825
LDL	-0.210	0.067	-0.130	0.103	-0.104	0.256
HDL	-0.150	0.191	0.272	**0.001**	0.302	**0.001**
Triglycerides	0.014	0.899	-0.228	**0.006**	-0.211	**0.021**
Total/HDL-cholesterol ratio	-0.005	0.970	-0.327	**<0.001**	-0.329	**<0.001**
Atherogenic index of plasma[a]	0.091	0.427	-0.274	**0.001**	-0.273	**0.003**

Linear regression analyses performed with anti-PC amount as the dependent variable and adjusted by age, sex and BMI.

[a] Calculated as "Log (triglycerides/HDL-cholesterol)".

Table 3. IgM-anti-PC levels and disease parameters in SLE patients presenting TG^{high} and/or HDL^{low} profile

	HDL levels			TG levels			TG / HDL profile		
	Low (≤53 mg/dl) (N=42)	**Normal** (>53 mg/dl) (N=78)	*p-value*	**High** (≥96 mg/dl) (N=41)	**Normal** (<96 mg/dl) (N=79)	*p-value*	**TG^{high} / HDL^{low}** (N=52)	**Normal** (N=68)	*p-value*
Age, years (mean ± SD)	48.26 ± 11.46	47.26 ± 11.52	0.644	50.95 ± 12.14	45.87 ± 10.76	**0.031**	49.09 ± 12.07	46.47 ± 10.92	0.298
SLEDAI (mean ± SD)	2.50 ± 2.99	2.37 ± 2.56	0.941	2.66 ± 3.57	2.29 ± 2.14	0.468	2.62 ± 3.26	2.26 ± 2.20	0.800
Anti-dsDNA U/ml (mean ± SD)	65.65 ± 129.95	42.14 ± 78.10	0.648	76.23 ± 157.03	36.94 ± 43.72	0.771	73.02 ± 141.10	33.04 ± 41.37	0.269
CRP, mg/l (mean ± SD)	0.63 ± 1.51	0.21 ± 0.50	**<0.001**	0.59 ± 1.55	0.24 ± 0.51	**0.004**	0.57 ± 1.38	0.20 ± 0.51	**<0.001**
NLR (mean ± SD)	2.42 ± 1.62	1.83 ± 0.93	**0.021**	2.39 ± 1.64	1.86 ± 0.94	0.060	2.38 ± 1.58	1.78 ± 0.84	**0.021**
RDW (mean ± SD)	13.74 ± 1.46	13.42 ± 1.65	0.130	13.94 ± 1.89	13.33 ± 1.37	**0.015**	13.92 ± 1.88	13.24 ± 1.26	**0.013**
IgM-anti-PC (AU) [median (IQR)]	42.32 (85.07)	75.84 (121.02)	**0.014**	53.80 (102.77)	68.56 (130.22)	0.090	51.80 (102.81)	73.21 (126.29)	0.085
T-cell subsets, % [median (IQR)]									
$CD4^{+}CD28^{null}$ cells	10.22 (11.21)	10.09 (9.46)	0.990	10.47 (12.82)	10.08 (9.17)	0.776	10.36 (11.21)	10.05 (8.95)	0.887
Th1 cells	7.98 (13.26)	6.36 (9.34)	0.401	7.97 (13.33)	6.49 (9.14)	0.462	7.66 (13.34)	6.42 (9.17)	0.470
Th17 cells	0.18 (0.34)	0.12 (0.36)	0.193	0.19 (0.39)	0.13 (0.36)	0.277	0.18 (0.35)	0.12 (0.36)	0.335
Treg cells	1.71 (0.90)	1.77 (1.40)	0.507	1.54 (0.99)	1.86 (1.32)	**0.015**	1.65 (0.90)	1.87 (1.40)	**0.044**
$IL17^{+}$ $Foxp3^{+}$ cells	0.07 (0.13)	0.04 (13)	0.226	0.07 (0.17)	0.04 (0.13)	0.250	0.06 (0.14)	0.04 (0.13)	0.329
Cytokines, pg/ml [median (IQR)]									
IFNα	5.01 (19.13)	8.31 (199.10)	0.117	4.99 (38.99)	9.21 (217.88)	0.093	5.69 (52.86)	8.31 (211.63)	0.195
IL-17A	8.48 (16.60)	11.03 (24.39)	0.638	9.32 (16.15)	11.65 (25.00)	0.365	9.45 (21.06)	11.03 (24.48)	0.903
IL-10	1.98 (1.87)	1.88 (2.75)	0.777	2.15 (2.58)	1.81 (1.98)	0.429	2.06 (2.42)	1.73 (2.39)	0.353
IL-6	4.13 (6.84)	1.20 (5.13)	0.070	3.75 (6.43)	0.75 (5.48)	**0.034**	4.27 (6.75)	0.73 (4.65)	**0.005**
IFNγ	29.46 (42.55)	34.82 (54.46)	0.819	25.25 (47.06)	35.24 (53.34)	0.559	33.56 (47.47)	34.40 (54.91)	0.830
BLyS	1178.55 (1078.76)	709.26 (824.14)	**0.031**	967.73 (1114.96)	687.96 (917.62)	**0.029**	1178.55 (1107.09)	623.93 (781.29)	**0.001**
ICAM	497.13 (224.19)	414.66 (156.97)	**0.009**	496.43 (259.07)	422.43 (152.52)	**0.023**	491.60 (228.66)	414.66 (149.35)	**0.017**
IP-10	25.00 (55.55)	11.86 (15.83)	**0.007**	21.78 (42.98)	12.59 (21.83)	0.082	22.11 (51.48)	11.38 (15.43)	**0.009**
MIP1a	2.94 (3.16)	3.36 (13.35)	0.088	2.93 (5.09)	3.33 (11.44)	0.128	2.96 (6.52)	3.36 (11.28)	0.161
MCP1	81.97 (60.72)	46.81 (41.75)	**<0.001**	75.71 (62.94)	48.27 (46.44)	**0.001**	77.25 (53.72)	44.93 (41.32)	**<0.001**

Values represent median (interquartile range, IQR). Differences between pairs of groups were analyzed by U-Mann Whitney tests. U: units; dsDNA: double stranded DNA; CRP: C-reactive protein; NLR: neutrophil to lymphocyte ratio; RDW: red cell distribution width; IQR: interquartile range.

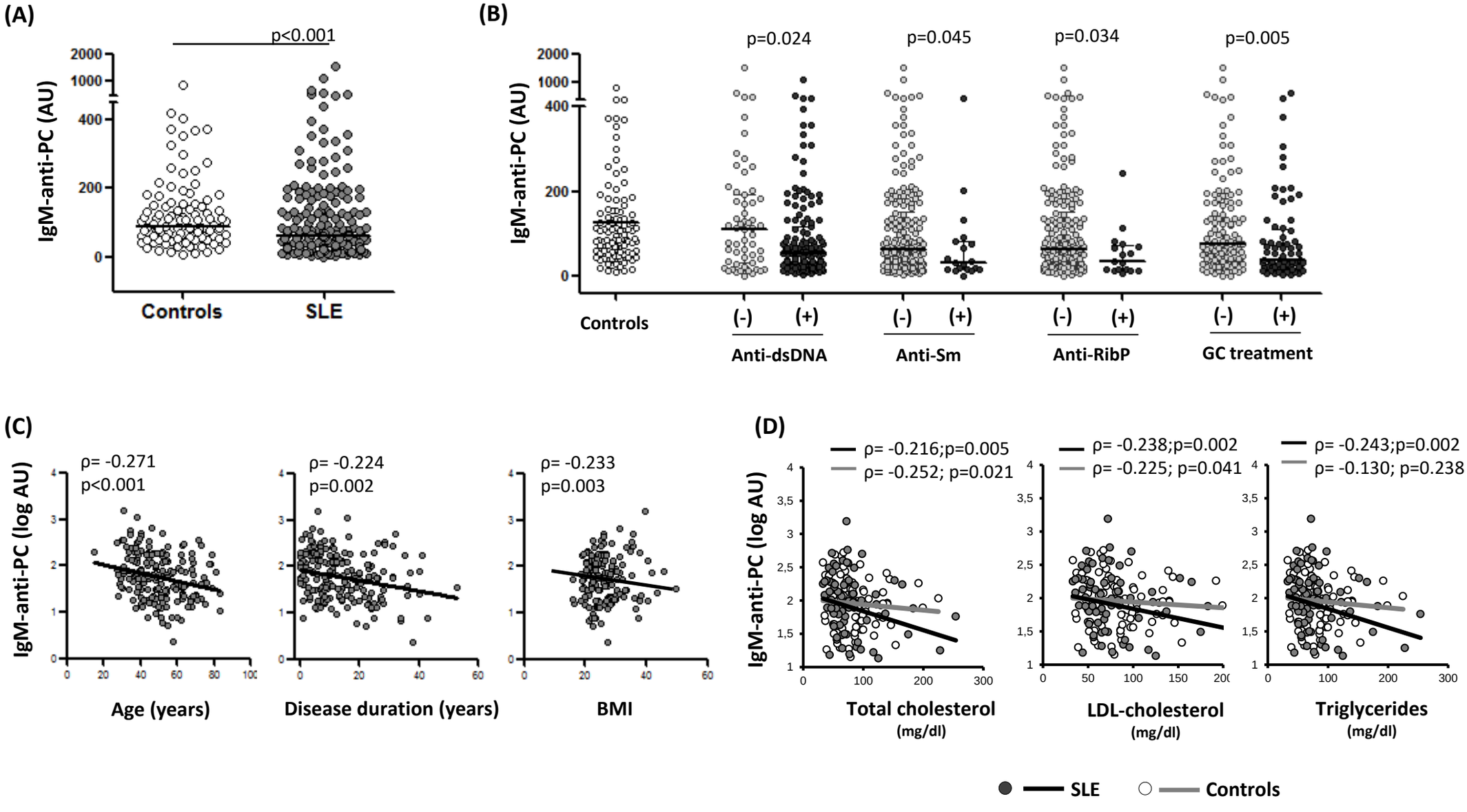

(A)
p<0.001
IgM-anti-PC (AU)
2000
1000
400
200
0
Controls
SLE
(B)
p=0.024
p=0.045
p=0.034
p=0.005
IgM-anti-PC (AU)
Controls
(-)
(+)
Anti-dsDNA
Anti-Sm
Anti-RibP
GC treatment
(C)
IgM-anti-PC (log AU)
ρ= -0.271
p<0.001
ρ= -0.224
p=0.002
ρ= -0.233
p=0.003
Age (years)
Disease duration (years)
BMI
(D)
ρ= -0.216;p=0.005
ρ= -0.252; p=0.021
ρ= -0.238;p=0.002
ρ= -0.225; p=0.041
ρ= -0.243;p=0.002
ρ= -0.130; p=0.238
IgM-anti-PC (log AU)
Total cholesterol
(mg/dl)
LDL-cholesterol
(mg/dl)
Triglycerides
(mg/dl)
SLE
Controls

Figure 1

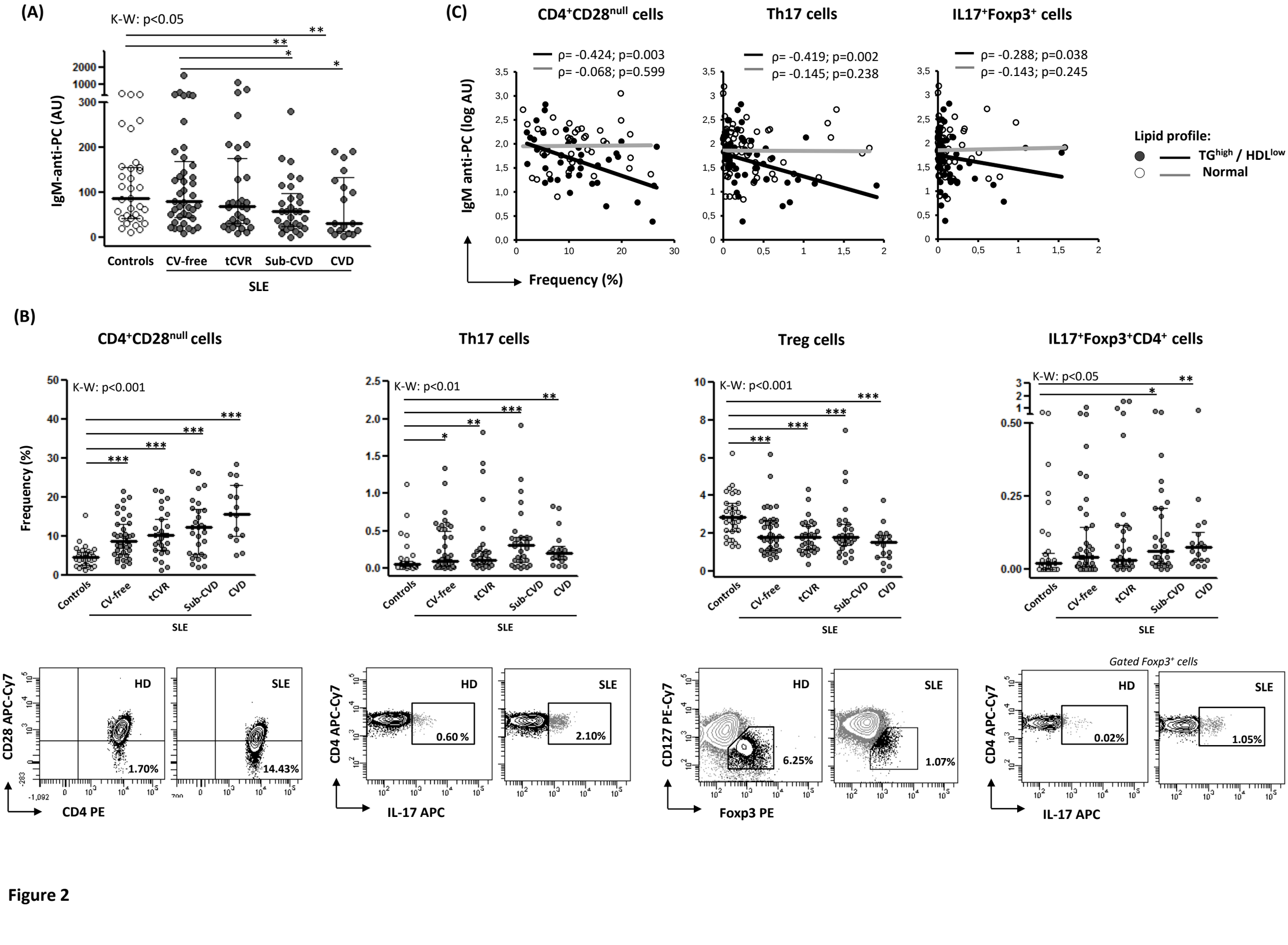

(A)
K-W: p<0.05
IgM-anti-PC (AU)
Controls
CV-free
tCVR
Sub-CVD
CVD
SLE
(C)
CD4+CD28null cells
Th17 cells
IL17+Foxp3+ cells
ρ= -0.424; p=0.003
ρ= -0.068; p=0.599
ρ= -0.419; p=0.002
ρ= -0.145; p=0.238
ρ= -0.288; p=0.038
ρ= -0.143; p=0.245
IgM anti-PC (log AU)
Frequency (%)
Lipid profile:
TGhigh / HDLlow
Normal
(B)
CD4+CD28null cells
Th17 cells
Treg cells
IL17+Foxp3+CD4+ cells
K-W: p<0.001
K-W: p<0.01
K-W: p<0.001
K-W: p<0.05
Frequency (%)
HD
SLE
CD28 APC-Cy7
CD4 PE
1.70%
14.43%
CD4 APC-Cy7
IL-17 APC
0.60 %
2.10%
CD127 PE-Cy7
Foxp3 PE
6.25%
1.07%
Gated Foxp3+ cells
0.02%
1.05%

Figure 2

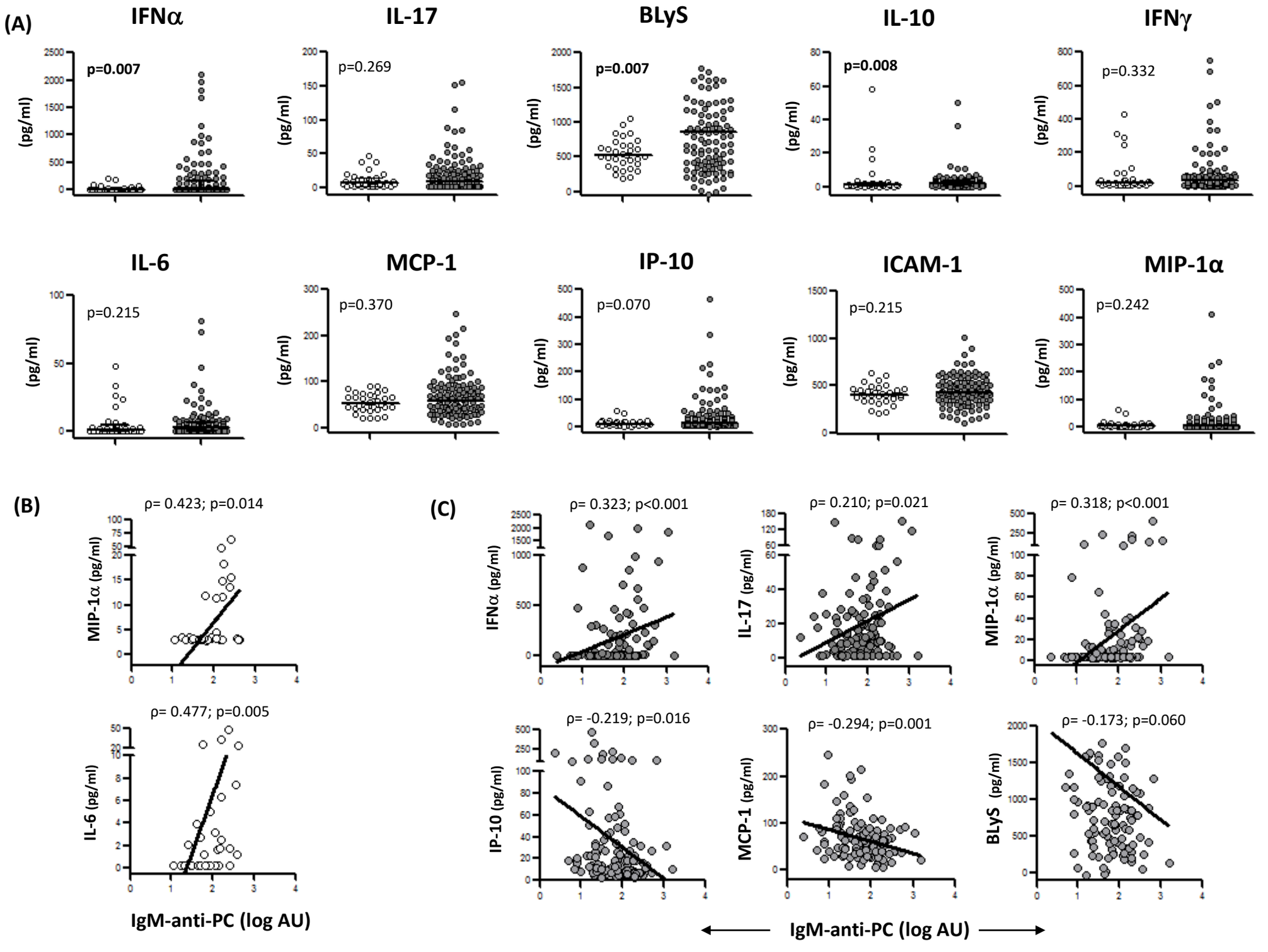
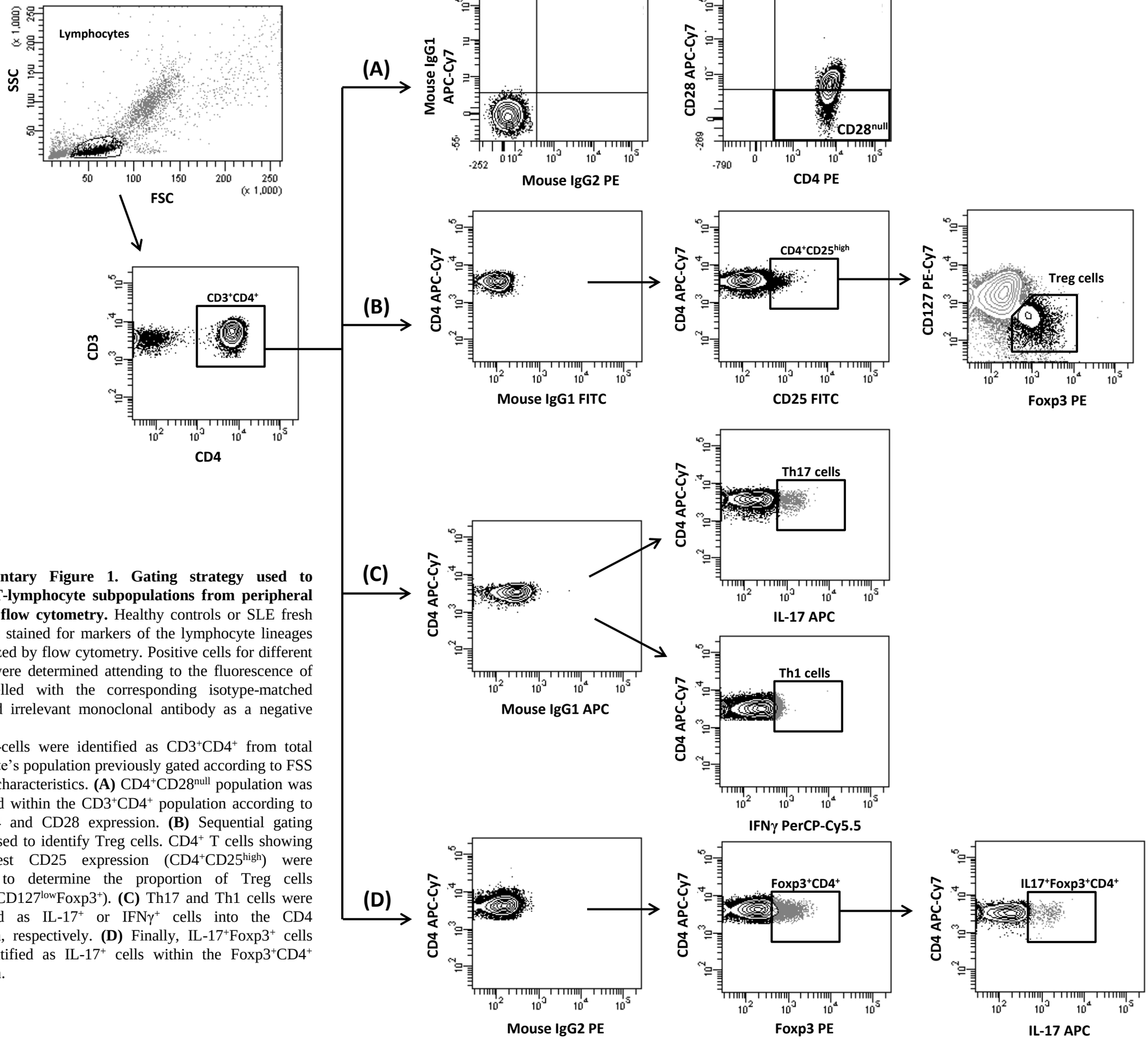

(A)
IFNα
p=0.007
IL-17
p=0.269
BLyS
p=0.007
IL-10
p=0.008
IFNγ
p=0.332
IL-6
p=0.215
MCP-1
p=0.370
IP-10
p=0.070
ICAM-1
p=0.215
MIP-1α
p=0.242
(pg/ml)
(B)
ρ= 0.423; p=0.014
MIP-1α (pg/ml)
ρ= 0.477; p=0.005
IL-6 (pg/ml)
IgM-anti-PC (log AU)
(C)
ρ= 0.323; p<0.001
IFNα (pg/ml)
ρ= 0.210; p=0.021
IL-17 (pg/ml)
ρ= 0.318; p<0.001
MIP-1α (pg/ml)
ρ= -0.219; p=0.016
IP-10 (pg/ml)
ρ= -0.294; p=0.001
MCP-1 (pg/ml)
ρ= -0.173; p=0.060
BLyS (pg/ml)
IgM-anti-PC (log AU)

Figure 3



Supplementary Figure 1. Gating strategy used to identify T-lymphocyte subpopulations from peripheral blood by flow cytometry. Healthy controls or SLE fresh blood was stained for markers of the lymphocyte lineages and analyzed by flow cytometry. Positive cells for different markers were determined attending to the fluorescence of cells labelled with the corresponding isotype-matched conjugated irrelevant monoclonal antibody as a negative control.

Firstly, T-cells were identified as $CD3^+CD4^+$ from total lymphocyte's population previously gated according to FSS and SSC characteristics. **(A)** $CD4^+CD28^{null}$ population was determined within the $CD3^+CD4^+$ population according to their CD4 and CD28 expression. **(B)** Sequential gating strategy used to identify Treg cells. $CD4^+$ T cells showing the highest CD25 expression ($CD4^+CD25^{high}$) were analyzed to determine the proportion of Treg cells ($CD25^{high}CD127^{low}Foxp3^+$). **(C)** Th17 and Th1 cells were determined as IL-17^+ or IFNγ^+ cells into the CD4 population, respectively. **(D)** Finally, IL-17^+Foxp3^+ cells were identified as IL-17^+ cells within the Foxp3^+CD4^+ population.

SUPPLEMENTARY METHODS

Autoantibodies detection and lipid analyses

Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on Hep-2 cells (INOVA Diagnostics). Anti-double strand DNA antibodies (anti-dsDNA) were quantified in a chemiluminescent analyzer (ZENIT RA, Menarini Diagnostics); serum samples presenting >50 IU/ml of IgG anti-dsDNA antibodies were considered positives. ENA specificities (SSA/Ro, SSB/ La, RNP, Sm) were identified by line blot analysis (Euroimmun). Determination of anti-RibP antibodies was performed by fluoro-enzyme immunoassay (Thermo Fisher Scientific-Phadia GmbH) carried out on an automated ImmunoCAP 250 analyzer.

Serum total cholesterol, triglycerides (TG) and high-density lipoprotein cholesterol (HDL) were determined using routine procedures with enzymatic assays from Roche Diagnostics. Low-density lipoprotein (LDL) levels were calculated using the Friedewald formula (1). SLE patients were stratified into tertiles according to serum levels of TG or HDL: patients with TG ≥96 mg/dl (third tertile) were defined as TG^{high}, whereas patients presenting HDL≤53 mg/dl (first tertile) were defined as HDL^{low}.

Flow cytometry

Flow cytometry analyses were performed in whole blood samples collected in tubes with EDTA anticoagulant. Supplementary Figure 1 shows gating strategy followed for the quantification of $CD4^{+}CD28^{null}$ T-cells, Th1, Th17 and T regulatory (Treg) lymphocytes.

Monoclonal antibodies for CD3-PerCP-Cy5.5 (Tonbo biosciences), CD4-PE (Inmunostep) and CD28-APC-Cy7 (eBioscence) were employed to identify $CD4^{+}CD28^{null}$ cells. Treg ($Foxp3^{+}CD127^{-}CD25^{high}$), Th1 ($IFN\gamma^{+}$) and Th17 ($IL\text{-}17^{+}$) cells were marked with monoclonal antibodies specific for CD4-APC-Cy7, CD25-FITC (Immunostep) and anti-CD127-PE-Cy7 (eBioscience). Blood cells were stained extracellularly with the appropriate monoclonal antibody to identify all the subpopulations for 30 min at 4°C. After that, cells were fixed by incubation for 5 min at 4°C with FACS Lysing Solution (BD) and washed twice with PBS. Then, cells were permeabilized ("Fixation/permeabilization buffer set"; eBioscience) and intracellularly stained with anti-Foxp3-PE

and IFNγ-PerCP-Cy5.5 or IL-17A-APC (eBioscience), following the manufacturer's instructions. For all analyzed populations, cells stained with the corresponding isotype-matched irrelevant antibodies were used as negative controls (eBioscence). Adquisition was performed on a BD FACSCanto II flow cytometer. The analysis was based on cells located in a plot-area termed "the living region" which was defined using forward and side-scatter and using FACSDiva Software. Results were expressed as the percentage of positive cells or mean fluorescence intensity (MFI).

Cytokine quantification

Serum samples were maintained at -80ºC until cytokine or chemokine determinations. IFNα, IL-17A and CCL3 (MIP-1α) amounts were quantified by Cytometric Bead Arrays Flex Set, whereas levels of IFNγ, IL-10, IL-6 and BLyS were quantified by LEGENDplex (BioLegend), all of them by using a FACS Canto II flow cytometer (BD) and following the manufacturer's instructions. CCL2 (MCP-1), ICAM-1 and IP-10 (CXCL10) was quantified by ELISA (Mini ELISA Development Kit, PeproTech) following the manufacturer's instructions. The lower limits of detection were (pg/ml): 1.25 for IFNα; 0.30 for IL-17A; 0.20 for MIP-1α; 1.00 for TNFα and IFNγ; 1.10 for IL-10 and IL-6; 10.40 for BLyS; 8.00 for MCP-1; 16.00 for IP-10; and 23.00 for ICAM-1.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normal distribution of the data. Differences between control and patient groups in quantitative variables were examined by T-test, Mann-Whitney U-test or the Kruskal-Wallis test. Correlation analyses of non-parametric variables were evaluated by Spearman tests. However, to an adequate graphical representation of these correlations, anti-PC levels were log-transformed. Additionally, lineal regression adjusted by age, gender and BMI or multivariate backward lineal regression analysis including different patient features, serum determinations or cellular subsets were performed. To this end, variables were previously log-transformed to achieve normal distribution and standardized linear regression coefficients (beta) were used as an estimate of the association. Data were expressed as the mean ± standard deviation (SD) or median (interquartile range, IQR). A p-value<0.05 was considered statistically significant.

Data were analyzed using GraphPad Prism 5 software (GraphPad Software) and SPSS 24 statistical software package (IBM) (see Supplementary material).

REFERENCES

1. Fukuyama N, Homma K, Wakana N, Kudo K, Suyama A, Ohazama H, et al. Validation of the Friedewald Equation for Evaluation of Plasma LDL-Cholesterol. J Clin Biochem Nutr. julio de 2008;43(1):1-5.

SUPPLEMENTARY TABLES

Supplementary Table 1. Demographic, clinical and cardiovascular related parameters of SLE patients.

	SLE patients (n=120)	Controls (n=33)
Demographic features[a]		
Sex (female/male) (n)	113/7	31/2
Age, years (mean ± SD)	47.61 ± 11.46	45.36 ± 11.07
Total cholesterol, mg/dl (mean ± SD)	186.25 ± 34.77	189.12 ± 32.37
HDL cholesterol, mg/dl (mean ± SD)	62.21 ± 17.75	63.94 ± 13.891
LDL cholesterol, mg/dl (mean ± SD)	106.22 ± 30.41	109.00 ± 30.79
Triglycerides, m/dl (mean ± SD)	90.27 ± 46.59	79.42 ± 31.16
Total/HDL-cholesterol ratio	3.20 ± 1.01	3.07 ± 0.87
Atherogenic index of plasma[b]		
Carotid atheromatosis		
Plaque presence, n (%)	10 (8.33)	4 (12.12)
cIMT, mm (mean ± SD)	0.60 ± 0.13	0.59 ± 0.12
cIMT>0.9 mm, n (%)	38 (31.67)	3 (9.10)
Subclinical CVD, n (%)	39 (32.50)	5 (15.15)
Traditional CV risk factors[c], n (%)		
Dyslipidemia	24 (20.00)	
Hypertension	30 (25.00)	
Diabetes (type II)	0 (0.00)	
Obesity (BMI>30)	17 (14.17)	
Smoking habit	29 (24.17)	
Clinical manifestations, n (%)		
Age at diagnosis, years (mean ± SD)	33.23 ± 12.42	
Disease duration, years (mean ± SD)	14.91 ± 11.31	
SLEDAI score (mean ± SD)	2.42 ± 2.71	
ACR criteria		
Malar rash	61 (50.83)	
Discoid lesions	39 (32.50)	
Photosensitivity	69 (57.50)	
Oral ulcers	49 (40.83)	
Arthritis	94 (78.33)	
Serositis	31 (25.83)	
Cytopenia	71 (59.17)	
Renal disorder	39 (32.50)	
Neurological disorder	15 (12.50)	
Autoantibodies, n (%)		
ANAs	120 (100.00)	
Anti-dsDNA/titer, U/ml (mean ± SD)	89 (74.17)/50.37 ± 99.46	
Anti-SSA	69 (57.50)	
Anti-SSB	22 (18.33)	
Anti-Sm	12 (10.00)	
Anti-RNP	28 (23.33)	
Rheumatoid Factor	22 (18.33)	
Anti-cardiolipin IgG	28 (23.33)	
Anti-cardiolipin IgM	23 (19.17)	

Lupus anticoagulant	28 (23.33)
Treatment, n (%)	
None or NSAIDs	5 (4.17)
Antimalarial drugs	107 (89.17)
Glucocorticoids	45 (37.50)
Immunosuppressive drugs[d]	38 (31.67)
Statins	18 (15.00)
CV disease[e], n (%)	18 (15.00)

[a] No significant differences between SLE and control groups (T-tests, U-Mann Whitney or χ^2 tests).
[b] Calculated as "Log (Triglycerides/HDL-cholesterol)".
[c] Data exposed of dyslipidemia, hypertension, diabetes type II, obesity and smoking habit are referred to the total number of patients excluding those with CV disease.
[d] Mycophenolate mophetil, azathioprine.
[e] Cerebrovascular disease, heart disease, peripheral vascular disease.
dsDNA: double stranded DNA; RF: rheumatoid factor; NSAID: non-steroidal anti-inflammatory drug.