RESEARCH

CrossMark

Cytokine and autoantibody clusters interaction in systemic lupus erythematosus

Yovana Pacheco, Julián Barahona-Correa, Diana M. Monsalve, Yeny Acosta-Ampudia, Manuel Rojas,
Yhojan Rodríguez, Juliana Saavedra, Mónica Rodríguez-Jiménez, Rubén D. Mantilla, Carolina Ramírez-Santana,
Nicolás Molano-González and Juan-Manuel Anaya*

Abstract

Background: Evidence supports the existence of different subphenotypes in systemic lupus erythematosus (SLE) and the pivotal role of cytokines and autoantibodies, which interact in a highly complex network. Thus, understanding how these complex nonlinear processes are connected and observed in real-life settings is a major challenge. Cluster approaches may assist in the identification of these subphenotypes, which represent such a phenomenon, and may contribute to the development of personalized medicine. Therefore, the relationship between autoantibody and cytokine clusters in SLE was analyzed.

Methods: This was an exploratory study in which 67 consecutive women with established SLE were assessed. Clinical characteristics including disease activity, a 14-autoantibody profile, and a panel of 15 serum cytokines were measured simultaneously. Mixed-cluster methodology and bivariate analyses were used to define autoantibody and cytokine clusters and to identify associations between them and related variables.

Results: First, three clusters of autoantibodies were defined: (1) neutral, (2) antiphospholipid antibodies (APLA)-dominant, and (3) anti-dsDNA/ENA-dominant. Second, eight cytokines showed levels above the threshold thus making possible to find 4 clusters: (1) neutral, (2) chemotactic, (3) G-CSF dominant, and (4) IFNα/Pro-inflammatory. Furthermore, the disease activity was associated with cytokine clusters, which, in turn, were associated with autoantibody clusters. Finally, when all biomarkers were included, three clusters were found: (1) neutral, (2) chemotactic/APLA, and (3) IFN/dsDNA, which were also associated with disease activity.

Conclusion: These results support the existence of three SLE cytokine-autoantibody driven subphenotypes. They encourage the practice of personalized medicine, and support proof-of-concept studies.

Keywords: Personalized medicine, Autoantibodies, Cytokines, Systemic lupus erythematosus, Subphenotypes, Cluster analysis, Antiphospholipid antibodies, Anti-dsDNA antibodies, Interleukin 8, Interferon alpha, Interleukin 12p40, Taxonomy

Background

Systemic lupus erythematosus (SLE) is a heterogeneous systemic autoimmune disease (AD) characterized by a wide range of clinical and serological manifestations and a high disease burden [1]. SLE pathophysiology encompasses several mechanisms, such as T cell and B-cell abnormalities, impaired apoptotic debris clearance, autoantibody production, and abnormal cytokine secretion [2]. The diversity in clinical expression associated with different autoantibodies among patients supports the existence of different subphenotypes although similar treatment is given to almost all the patients with diverse effectiveness [2].

Autoantibodies are essential biomarkers for the diagnosis and classification of ADs, and several are known to be pivotal in the ADs pathophysiology [3]. In SLE they can form immune complexes, which may be deposited in

*Correspondence: juan.anaya@urosario.edu.co
Center for Autoimmune Diseases Research (CREA) School of Medicine
and Health Sciences, Universidad del Rosario, Carrera 26 # 63B-51, Bogota,
Colombia

Pacheco *et al. J Transl Med (2017) 15:239*

Page 2 of 15

tissues, and activate a direct immune response against a specific organ [4]. Autoantibodies are usually found long before symptom onset [5].

Cluster methodology of autoantibodies in SLE has been used to evaluate several cohorts, both adult and pediatric, worldwide and has given insight into the different subphenotypes due to the correlation among clusters, clinical features, and disease activity [6–17].

Autoimmune diseases evince similar immunopathogenic mechanisms (i.e., the autoimmune tautology) [18]. This explains the fact that one AD may coexist with one or more ADs (i.e., polyautoimmunity) [19], and that one AD may carry several autoantibodies with diverse specificity. Polyautoimmunity has been observed in up to 40% of patients with SLE [20, 21]. In addition, non-lupus autoantibodies are observed frequently in SLE patients. Rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibodies are present in 42 and 5.6% respectively, but only 6.4% of the patients meet the criteria for rhupus [22]. Antiphospholipid antibodies (APLA) may be present in 54% of patients although antiphospholipid syndrome (APS) develops in only 10% of SLE patients [23]. In euthyroid patients with SLE, anti-thyroid peroxidase antibodies (TPOAb) and anti-thyroglobulin antibodies (TgAb) are observed in 21 and 10% of patients respectively, but confirmed autoimmune hypothyroidism is diagnosed in 12% [24].

Cytokines play an essential role in the pathophysiology of SLE. Thus, a number of cytokine-targeted therapies which have shown promising results have been developed, particularly in some subphenotypes of the disease [25]. However, the immune system shows a wide variation at both intra- and inter-individual levels. These differences among individuals, which may explain the differences observed among patients, have been called "immunotypes" [26].

Since human biology is a complex set of interacting components that work together to produce an outcome, a system approach may elucidate these interactions [27]. Systems biology of human disease, also known as systems medicine or network medicine, aims at identifying the main components of a system and at measuring how they change when the system is disturbed [26]. Since understanding the connections of the nonlinear complex processes of cytokines and autoantibodies in real-life settings is a major challenge, we analyzed the simultaneous relationship between them in patients with established SLE.

Methods

Study population

This was a cross-sectional analytical study of 67 consecutive women with SLE. The subjects have been systematically followed at the Center for Autoimmune Diseases Research (CREA) in Bogota, Colombia. All the subjects fulfilled the 1997 update of the American College of Rheumatology (ACR) classification criteria for SLE [28]. The patient socio-demographic and cumulative clinical and laboratory data were obtained by interview, standardized report form, physical examination and chart review as previously reported [23, 29]. The data were collected in an electronic and secure database.

Clinical variables

Clinical and laboratory variables were registered as present or absent at any time during the course of the disease as previously reported [23]. Other manifestations such as polyautoimmunity [19, 30] and current pharmacological treatment were also assessed.

Current disease activity was measured using the Systemic Lupus Activity Questionnaire (SLAQ), a well-known Patient-Reported Outcome (PRO) tool, which presents an adequate performance in large community-based cohorts [31–33]. As SLAQ is unavailable in Spanish, a linguistic validation was done. Working independently, two English proficient physicians (JBC, MR) translated the original US English version into Spanish [32]. Afterwards, they worked together to obtain a single Spanish version. A mother tongue professional translator independently back-translated this version into an English one. Lastly, the physicians compared the two versions to produce a second Spanish version. If there was disagreement, a third English- proficient physician (YR) decided which was the best version. Finally, a definite Spanish form was acquired and used with the patients (see Additional file 1).

Laboratory measurements

Serum samples were obtained during a state of fasting. A total of 14 autoantibodies were evaluated in the sera of patients. Detection of IgM RF, IgG anti-CCP third-generation (CCP3), IgM and IgG anti-cardiolipin antibodies (ACA), IgM and IgG anti-β2glycoprotein-1 (β2GP1) antibodies, IgG anti-double-stranded DNA (dsDNA) antibodies, IgG TgAb, and TPOAb were all quantified by the Enzyme-Linked-Immunosorbent Assay (ELISA) as previously reported [24]. Antinuclear antibodies (ANAs) were evaluated by using an indirect immunofluorescence assay. Positive ANA were considered from dilution 1/80. Negative and positive controls, provided by the manufacturer, were analyzed in parallel. Anti-Ro, anti-La, anti-RNP, and anti-Sm were also evaluated by ELISA. All the assay kits were from Inova Diagnostics, Inc. (San Diego, CA, USA).

Concentration of 15 human cytokines (IL-2, IL-10, IL-6, IL-8, IL-9, IL-13, IL-12/23p40, G-CSF, IFNγ, IFNα,

Pacheco *et al. J Transl Med (2017) 15:239*

Page 3 of 15

IL-4, IL-1β, TNFα, IL-5, IL-17A) in serum samples from patients was assessed by Cytometric Bead Array (CBA, Becton–Dickinson Biosciences, San Diego, CA, USA). The test was done in accordance with the manufacturer's protocols. Briefly, 50 μL of assay beads and 50 μL of the sample under study or standard were added to each sample tube. The samples were incubated at room temperature in the dark for 1 h. Next, the samples were washed with 1 mL of wash buffer, centrifuged, and the resulting pellet was resuspended in 50 μL of PE-labeled antibodies (Detection Reagent). The samples were further incubated for 2 h, washed again, and centrifuged. After discarding the supernatant, the pellet was resuspended in 300 μL of wash buffer and analyzed on the same day in a FACSCanto II™ flow cytometer (BD Bioscience™). Before the analysis, the cytometer was standardized using calibration beads in accordance with the manufacturer's protocol. For each cytokine, a standard curve was assessed, and concentration of each cytokine was calculated as an interpolation of the standard curve using the FCAP Array™ Software (BD Bioscience™). Results were considered positive when the assay results were above a threshold value, which was confirmed in healthy individuals in whom evidence of acute or chronic disease including autoimmune, cardiovascular, or metabolic was not detected (Table 3) [34, 35].

Statistical analyses

The mixed-cluster methodology proposed by Lebart et al. [36] was used to find groups of patients with similar autoantibody and cytokine profiles. In short, cluster analysis seeks groups of individuals with similar values across several variables. The number of groups is algorithmically determined and consolidated in two steps: first, a hierarchical cluster analysis is done based on Ward's distance, for which the number of clusters is determined by means of the between-cluster inertia gain criterion. Second, the cluster membership for each individual is consolidated using a k-means algorithm on the centroids of each cluster. In the end, a categorical variable in which each individual is assigned to one and only one of the clusters derived is obtained [36]. Afterwards, a description of each cluster is developed by studying the distribution of each of the original variables used for clustering in each of the derived groups. This determine the composition and relation of the original variables and the clusters obtained.

This clustering method was used to obtain autoantibody clusters (named profiles from here on) based on the 14 autoantibodies, and cytokine profiles based on the 15 cytokines measured. Cytokines and autoantibodies with frequencies under 5% were excluded from the cluster analysis, since variables with low frequencies

tend to generate clusters of patients with such atypical results exclusively. To assess associations between abovementioned profiles and other variables, we used the Chi square and Kruskall–Wallis tests. Statistical analyses were done using R version 3.3.2.

Ethics

This research was carried out in accordance with Resolution number 008430 of 1993 issued by the Ministry of Health of the Republic of Colombia and was classified as minimal risk research. The Ethics Committee of Universidad del Rosario approved the present project.

Results

Patients

The demographic, clinical, and laboratory characteristics of the patients are shown in Table 1. The median age of patients was 50 (38–57) years with a median age at SLE onset of 29 (22–40) years and a disease duration of 13 (9–21) years. Lupus nephritis was seen in 25 (37%)

Table 1 General characteristics of 67 women with SLE

Age (IQR), years	50 (38–57)
Age at onset disease (IQR), years	29 (22–40)
Disease duration (IQR), years	13 (9–21)
Polyautoimmunity[a] (%)	14 (21)
Educational level (%)	
< 9 years	10 (15)
≥ 9 years	57 (85)
Socioeconomic status[b] (%)	
Low	19 (28)
Intermediate	37 (55)
High	11 (17)
1997 ACR Criteria at diagnosis (%)	
Positive ANAs	58 (87)
Immunologic criteria	54 (81)
Hematologic criteria	48 (72)
Non-erosive arthritis	47 (70)
Photosensitivity	41 (61)
Malar rash	33 (49)
Renal criteria[c]	25 (37)
Oral ulcers	23 (34)
Serositis	19 (28)
Neurologic criteria	12 (18)
Discoid rash	7 (10)

ACR American College of Rheumatology

[a] Polyautoimmunity signifies the presence of more than one autoimmune disease in a single patient

[b] Socioeconomic status was categorized based on Colombian legislation as previously reported [23, 24]

[c] Renal criteria was defined as active urinary sediment, or proteinuria > 500 mg/24 h or positive renal biopsy [23, 24]

Pacheco *et al. J Transl Med (2017) 15:239*

Page 4 of 15

patients at diagnosis. At the time of the study, median disease activity by SLAQ was 16 (10.5–26.5). In addition, patients were receiving medication in the following numbers: 41 (61%) were receiving antimalarials, 39 (58%) corticosteroids, 20 (30%) azathioprine, 10 (15%) methotrexate, 8 (12%) mycophenolate mofetil, 4 (6%) rituximab, and 2 (3%) were receiving belimumab, leflunomide, sulfasalazine, and tacrolimus. No patient was receiving cyclophosphamide. The antibodies that were positive most frequently at the time of the study were ANAs (85%) followed by anti-dsDNA (48%) (Table 2). The cytokines that were positive most frequently were IL-12/23p40 (52%), G-CSF (46%), and IFNα (25%) (Table 3).

Autoantibody clusters

Three clusters of autoantibodies were defined (Fig. 1): (1) neutral, in which the frequency of specific autoantibodies other than ANAs was below 40%; (2) APLA-dominant, which showed a greater than 50% frequency of ACA-IgG/IgM, anti-dsDNA, and anti-RNP; and (3) anti-dsDNA/ENA-dominant, which presented a high frequency of anti-dsDNA, anti-RNP, and anti-Sm antibodies. Table 4 shows the distribution of autoantibodies within clusters. No association between autoantibody clusters and disease activity was found.

Cytokine clusters

Eight cytokines showed levels above the threshold (i.e., > 5%) (Table 3). Four clusters were defined (Fig. 2): (1) neutral, which exhibited a low frequency of cytokines;

Table 2 Autoantibodies in 67 patients with SLE at the time of the study

Autoantibody	N (%)
ANAs	57 (85)
dsDNA	32 (48)
Ro	26 (39)
RNP	25 (37)
RF	24 (36)
ACA-IgG	12 (18)
Sm	12 (18)
ACA-IgM	11 (16)
TPOAb	7 (10)
β2GP1-IgM	7 (10)
β2GP1-IgG	6 (9)
La	5 (7)
TgAb	5 (7)
CCP3	1 (1)

ANAs antinuclear antibodies, *dsDNA* anti-double stranded DNA antibodies, *RF* rheumatoid factor, *ACA* anticardiolipin antibody, *TPOAb* anti-thyroperoxidase antibody, *TgAb* anti-thyroglobulin antibody, *β2GP1* β2 glycoprotein-1, *CCP3* anti-cyclic citrullinated peptide third-generation

Table 3 Cytokine concentration in women with SLE

Cytokine	Healthy controls N = 5[a]	SLE patients N = 67[a]	Number of positive patients (%)[b]
IL-12/23p40	16.13 (18.9)	27.11 (48.9)	35/67 (52)
G-CSF	0 (0)	2.16 (6.19)	31/67 (46)
IFNα	0 (0)	3.72 (12.2)	17/67 (25)
IL-8	11.71 (4.5)	12.67 (25.1)	16/67 (24)
IL-6	0.11 (0.21)	4.99 (28.09)	15/67 (22)
IL-10	0 (0)	0.57 (1.79)	14/67 (21)
IL-1β	0 (0)	0.97 (4.66)	6/67 (9)
IL-17A	0 (0)	7.41 (33.9)	11/67 (16)
TNFα	0 (0)	2.11 (9.34)	9/67 (13)
IL-5	0 (0)	0.17 (0.77)	6/67 (9)
IL-4	0 (0)	0.39 (2.01)	4/67 (6)
IFNγ	0 (0)	0.39 (2.1)	4/67 (6)
IL-2	0 (0)	0.39 (2.23)	2/67 (3)
IL-9	0 (0)	0.13 (0.75)	2/67 (3)
IL-13	0 (0)	0.02 (0.19)	1/67 (1.5)

IL interleukin, *G-CSF* granulocyte colony-stimulating factor, *IFN* interferon, *TNF* tumor necrosis factor

[a] Mean (SD), in pg/mL

[b] Data correspond to those patients with positive values as compared to healthy controls (above the threshold) [34, 35]

(2) chemotactic, characterized by a predominance of IL-8; (3) G-CSF dominant, which presented a high frequency of G-CSF, and IL-12/23p40; and (4) IFNα/Pro-inflammatory, which was dominated by the presence of IFNα, IL-12/23p40, TNFα, IL-17A, G-CSF, and IL-10. Table 5 shows the distribution of cytokines within clusters.

Cytokine clusters and disease activity

There was a significant association between cytokine clusters and disease activity. ($p = 0.022$; Fig. 3a,). The distribution of autoantibody clusters differed between neutral cytokine and IFNα/Pro-inflammatory clusters ($p = 0.031$; Fig. 3b). Tables 6 and 7 show the distribution of cytokines by autoantibody clusters, and autoantibodies by cytokine clusters respectively.

Cytokine and antibody clusters

Finally, when all biomarkers were included (i.e., cytokines and autoantibodies), three clusters were found (Table 8): (1) neutral, (2) chemotactic/APLA, and (3) IFNα/dsDNA (Fig. 4a), which, in turn, evinced an association with SLE activity (p = 0.036; Fig. 4b). Differences among clusters with respect to clinical manifestations were not observed (Table 8).
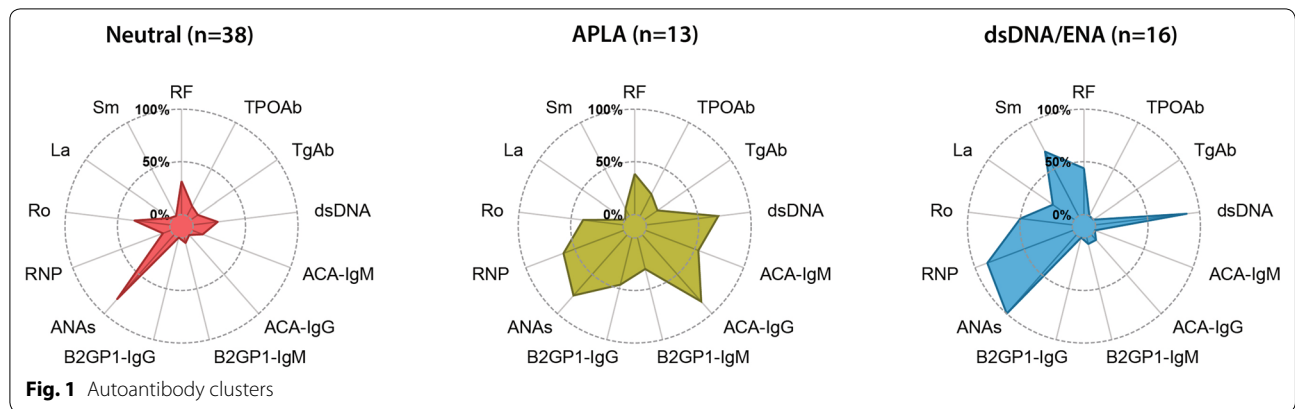
Discussion

The results indicate the presence of three cytokine-autoantibody driven subphenotypes in SLE. First, three autoantibody clusters were identified, namely (1) neutral,

Pacheco *et al. J Transl Med (2017) 15:239*

Page 5 of 15

Fig. 1 Autoantibody clusters

Table 4 Distribution of autoantibodies among autoantibody clusters

Autoantibody	Neutral (n = 38)	APLA-dominant (n = 13)	dsDNA/ENA-dominant (n = 16)	p-value
ANAs	31 (82)	10 (77)	16 (100)	0.145
RF	12 (32)	5 (38)	7 (44)	0.678
CCP3	1 (3)	0 (0)	0 (0)	0.678
TPOAb	4 (10)	3 (23)	0 (0)	0.129
TgAb	3 (8)	2 (15)	0 (0)	0.289
dsDNA	9 (24)	9 (69)	14 (87)	< 0.0001*
ACA-IgM	4 (10)	7 (54)	0 (0)	0.0002*
ACA-IgG	0 (0)	11 (85)	1 (6)	< 0.0001*
β2GP1-IgM	2 (5)	4 (31)	1 (6)	0.028
β2GP1-IgG	0 (0)	6 (46)	0 (0)	< 0.0001*
RNP	3 (8)	8 (61)	14 (87)	< 0.0001*
Ro	13 (34)	5 (38)	8 (50)	0.553
La	1 (3)	0 (0)	4 (25)	0.008
Sm	0 (0)	1 (8)	11 (69)	< 0.0001*

Data correspond to number of patients (%)

ANAs antinuclear antibodies, *RF* rheumatoid factor, *CCP3* anti-cyclic citrullinated peptide third-generation, *TPOAb* anti-thyroperoxidase antibody, *TgAb* anti-thyroglobulin antibody, *dsDNA* anti-double stranded DNA antibodies, *ACA* anticardiolipin antibody, *β2GP1* β2 glycoprotein-1

* Statistically significant after Bonferroni correction

(2) APLA-dominant and (3) anti-dsDNA/ENA-dominant. Cluster analyses in SLE patients have been done previously and tended to show similar results even among different populations and clustering methods (Additional file 2: Table S1). The first report, by Tápanes et al. [16], assessed the relationship between renal outcomes and anti-ENA clusters and proposed 4 clusters based on ENA positivity (no ENA, Ro/La, Sm/RNP, all positive). In the current study, the neutral autoantibody cluster showed a low frequency of autoantibodies, where ANAs stood out as the most abundant. Along the same line, Artim-Esen et al. [8] described a cluster that showed only ANA positivity, a rather unspecific autoantibody that could be similar to our neutral cluster. Furthermore, several cohorts have shown a particular cluster characterized by

anti-dsDNA solely [8, 9, 12, 15]. Although this antibody presents with high frequency in SLE patients [37] it does not allow clusters to be differentiated in other cohorts [7, 11].

A second autoantibody cluster in the current study was dominated by APLA. These autoantibodies were not included in cluster studies until recently. Artim-Esen et al. [8] and To et al. [38] found an APLA dominant cluster which was similar to our results.

A cluster characterized by the presence of anti Sm/RNP antibodies has been consistently reported [7, 10, 14, 15], and in some reports, it has been associated with anti-dsDNA, thus yielding a Sm/RNP/dsDNA cluster [8, 12]. Likewise, a Ro/La cluster has also been reported [9, 10, 15], and in some reports, it has been associated with anti-dsDNA [8, 11, 14].
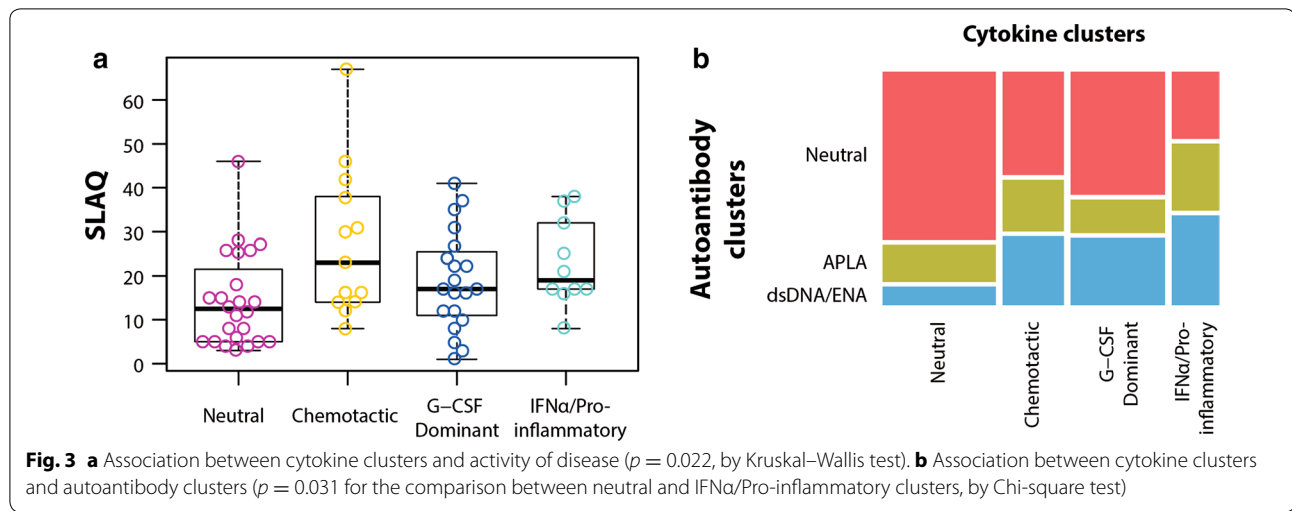
Pacheco *et al. J Transl Med* (2017) 15:239

Page 6 of 15

Fig. 2 Cytokine clusters
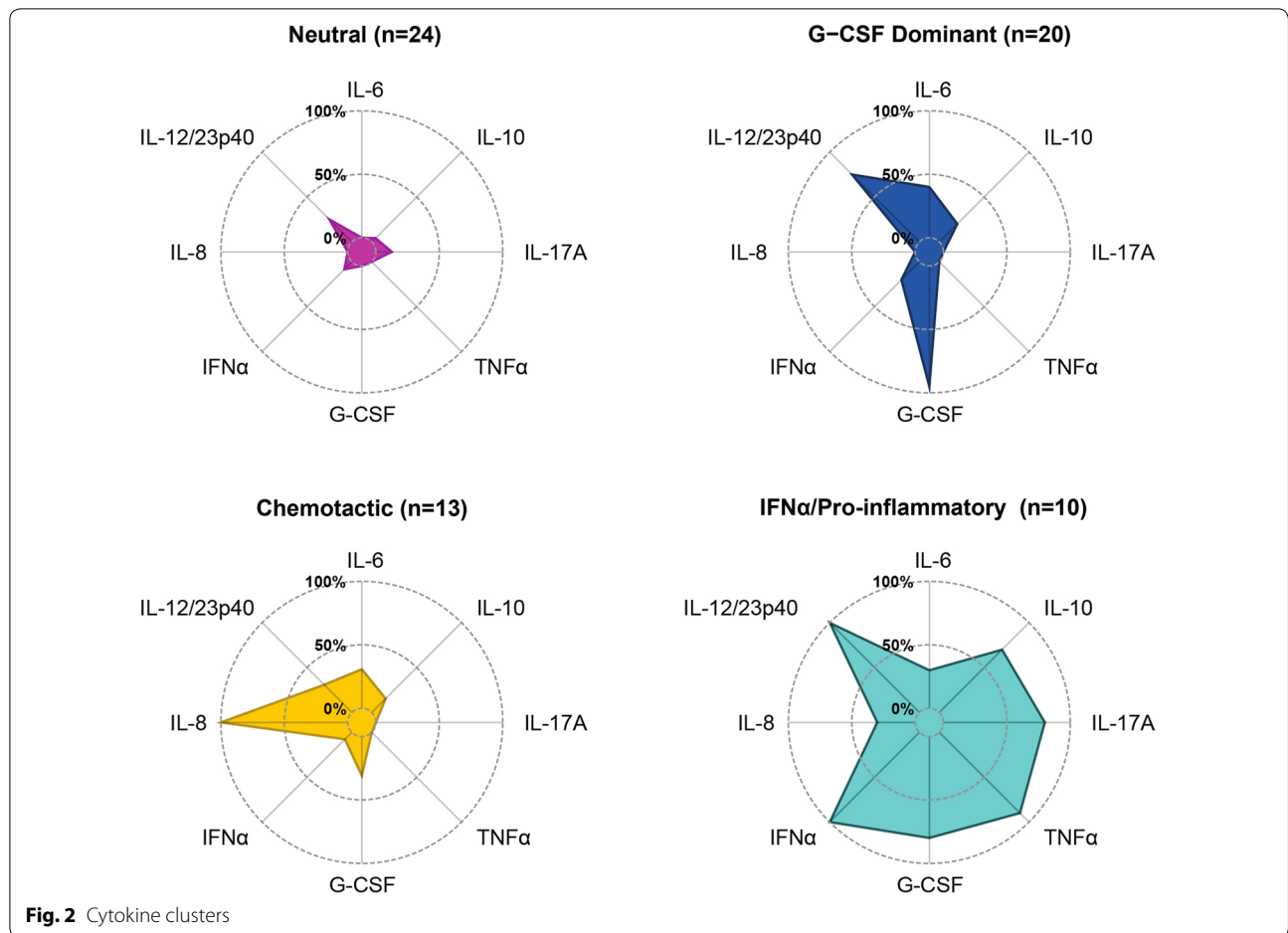
Table 5 Distribution of cytokines among cytokine clusters

Cytokine	Neutral (n = 24)	Chemotactic (n = 13)	G-CSF (n = 20)	IFNα/Pro-inflammatory (n = 10)	p-value
IL-2	1 (4)	0 (0)	0 (0)	1 (10)	0.418
IL-4	0 (0)	0 (0)	1 (5)	3 (30)	0.005
IL-5	1 (4)	0 (0)	1 (5)	4 (40)	0.002*
IL-6	0 (0)	4 (31)	8 (40)	3 (30)	0.009
IL-9	1 (4)	0 (0)	0 (0)	1 (10)	0.418
IL-10	1 (4)	2 (15)	4 (20)	7 (70)	0.0003*
IL-17A	3 (12)	0 (0)	0 (0)	8 (80)	< 0.0001*
TNFα	0 (0)	0 (0)	0 (0)	9 (90)	< 0.0001*
G-CSF	0 (0)	4 (31)	19 (95)	8 (80)	< 0.0001*
IFNα	2 (8)	1 (8)	4 (20)	10 (100)	< 0.0001*
IFNγ	0 (0)	1 (8)	1 (5)	2 (20)	0.162
IL-13	0 (0)	0 (0)	0 (0)	1 (10)	0.122
IL-1β	0 (0)	1 (8)	0 (0)	5 (50)	< 0.0001*
IL-8	0 (0)	13 (100)	0 (0)	3 (30)	< 0.0001*
IL-12/23p40	6 (25)	4 (31)	15 (75)	10 (100)	< 0.0001*

Data correspond to number of positive patients (%)

IL interleukin, *G-CSF* granulocyte colony-stimulating factor, *IFN* interferon, *TNF* tumor necrosis factor

* Statistically significant after Bonferroni correction

Pacheco *et al. J Transl Med (2017) 15:239*

Page 7 of 15

Fig. 3 **a** Association between cytokine clusters and activity of disease ($p = 0.022$, by Kruskal–Wallis test). **b** Association between cytokine clusters and autoantibody clusters ($p = 0.031$ for the comparison between neutral and IFNα/Pro-inflammatory clusters, by Chi-square test)

Table 6 Distribution of cytokines by autoantibody clusters

Cytokine	Neutral (n = 38)	APLA-dominant (n = 13)	dsDNA/ENA-dominant (n = 16)	p-value
IL-2	0 (0)	2 (15)	0 (0)	0.013
IL-4	2 (5)	1 (8)	1 (6)	0.948
IL-5	0 (0)	4 (31)	2 (12)	0.003*
IL-6	6 (16)	5 (38)	4 (25)	0.229
IL-9	1 (3)	1 (8)	0 (0)	0.471
IL-10	2 (5)	7 (54)	5 (31)	0.0005*
IL-17A	6 (16)	3 (23)	2 (12)	0.737
TNFα	2 (5)	3 (23)	4 (25)	0.079
G-CSF	14 (37)	7 (54)	10 (62)	0.186
IFNα	7 (18)	4 (31)	6 (37)	0.299
IFNγ	3 (8)	1 (8)	0 (0)	0.512
IL-13	0 (0)	1 (8)	0 (0)	0.121
IL-1β	3 (8)	2 (15)	1 (6)	0.652
IL-8	7 (18)	4 (31)	5 (31)	0.486
IL-12/23p40	18 (47)	8 (61)	9 (56)	0.632

Data correspond to number of patients (%)

IL interleukin, *G-CSF* granulocyte colony-stimulating factor, *IFN* interferon, *TNF* tumor necrosis factor

* Statistically significant after Bonferroni correction

These findings are supported by the cross-reactivity and similarity of anti-Sm and anti-RNP [39], and the induction of anti-Ro and anti-La by common ribonucleoproteins [40]. It is noteworthy that some authors have found one cluster with positivity for 3 or 4 ENA (with or without anti-dsDNA) [7, 11, 12]. This evidence is similar to our Cluster 3 in which a predominance of anti-dsDNA/ENA was observed. Anti-Sm and anti-La antibodies were virtually absent in neutral and APLA autoantibody clusters (Fig. 1).

Autoantibody clusters did not show an association with disease activity [12, 14]. This could be due to the measurement method, in which a well-known PRO questionnaire (i.e., SLAQ) [41] was used in contrast to physician-based indexes (i.e., SLEDAI) used in other cohorts. Nevertheless, there is no serologic test that reliably measures disease activity in SLE [42].

Second, four cytokine clusters were obtained, namely (1) neutral, (2) chemotactic, (3) G-CSF dominant, and 4) IFNα/Pro-inflammatory. To our knowledge, this is the first report on a serum cytokine cluster analysis in patients with SLE. The composition of the third and fourth cytokine clusters were validated by an external bioinformatic analysis which confirmed biological relationships among cytokines (Additional file 3).

The neutral cytokine cluster displayed a low frequency of cytokines that was below 25% (Fig. 2). The chemotactic

Pacheco *et al. J Transl Med* (2017) 15:239

Page 8 of 15

Table 7 Distribution of autoantibodies by cytokine clusters

Autoantibody	Neutral (n = 24)	Chemotactic (n = 13)	G-CSF (n = 20)	IFNα/Pro-inflammatory (n = 10)	p-value
RF	8 (33)	3 (23)	8 (40)	5 (50)	0.570
CCP3	1 (4)	0 (0)	0 (0)	0 (0)	0.610
TPOAb	2 (8)	1 (8)	4 (20)	0 (0)	0.342
TgAb	1 (4)	1 (8)	2 (10)	1 (10)	0.882
dsDNA	7 (29)	7 (54)	11 (55)	7 (70)	0.115
ACA-IgM	6 (25)	1 (8)	1 (5)	3 (30)	0.154
ACA-IgG	4 (17)	4 (31)	2 (10)	2 (20)	0.499
β2GP1-IgM	2 (8)	4 (31)	0 (0)	1 (10)	0.042
β2GP1-IgG	1 (4)	2 (15)	1 (5)	2 (20)	0.359
ANAs	18 (75)	12 (92)	19 (95)	8 (80)	0.239
RNP	4 (17)	7 (54)	10 (50)	4 (40)	0.062
Ro	9 (37)	4 (31)	7 (35)	6 (60)	0.496
La	1 (4)	1 (8)	2 (10)	1 (10)	0.882
Sm	1 (4)	3 (23)	5 (25)	3 (30)	0.172

Data correspond to number of patients (%)

RF rheumatoid factor, *CCP3* anti-cyclic citrullinated peptide third-generation, *TPOAb* anti-thyroperoxidase antibody, *TgAb* anti-thyroglobulin antibody, *dsDNA* anti-double stranded DNA antibodies, *ACA* anticardiolipin antibody, *β2GP1* β2 glycoprotein-1, *ANAs* antinuclear antibodies

cytokine cluster showed a marked expression of IL-8 followed by lower frequencies of IL-12/23p40, IL-6, and G-CSF. IL-8 is a chemotactic cytokine, particularly involved in recruitment of neutrophils, which induces shape transformation, the 'respiratory burst', and the release of granule contents [43]. Increased levels have been seen in SLE patients and they appear to be influenced by anti-dsDNA (Additional file 4: Table S2). High levels of IL-6 have also been found in SLE [44, 45]. Both IL-6 and IL-8 have been shown to be up-regulated by endothelial cells treated with IgG APLA in vitro [46]. IL-8 has been associated with pregnancy morbidity in patients with SLE [35]. The anti-dsDNA antibody up-regulates IL-8 gene expression and elicits activation-induced cell death of human polymorphonuclear neutrophils [47], and the release of IL-8 [48].

The third cytokine cluster was named G-CSF dominant, since a particularly high frequency of G-CSF was seen although IL-12/23p40 was rather frequent. G-CSF is an essential growth factor for the differentiation of hematopoietic stem cells into granulocytes, particularly neutrophils. Synthetic G-CSF preparations (e.g., filgrastim, pegfilgrastim, lenograstim) are available to treat neutropenia [49]. Data is scarce regarding G-CSF in SLE (Additional file 4: Table S2). Furthermore, IL-12 and IL-23 are mainly pro-inflammatory cytokines that share a common structural unit and receptors. IL-12 consists of two subunits: p35 and p40, whereas IL-23 is comprised of subunits p19 and p40. The two share the p40 subunit, which interacts with the same membrane receptor [50]. Although available assays for p19, p35, and p70 (which

includes subunits IL-12 p35 and p40) exist, we measured IL-12/23p40. IL-12 is pivotal for Th1 differentiation [51] and has been found to be higher in SLE patients [52]; its implication for physiopathology remains under investigation (Additional file 4: Table S2). In addition, IL-23 plays a role in the development of Th17 cells, and promotes IL-17 secretion [53]; clinical trials with anti-IL-12/23 are underway [50].

The last cytokine cluster revealed high levels of diverse cytokines, including G-CSF, IL-12/23p40, IL-17A, and IL-10. Nonetheless, IFNα and TNFα were the most frequent cytokines. Thus, the cluster was named IFNα/Pro-inflammatory. IFNα belongs to the Type I IFN family and is mainly secreted by plasmacytoid dendritic cells (pDC). Type I IFNs promote autoimmunity due to the activation of B-cell responses, maturation of monocytes into DC, and NETosis promotion [4, 54]. One of the SLE hallmarks is its IFN I signature, which is dysregulated when compared to healthy controls (Additional file 4: Table S2). Recent clinical trials in SLE with a Type I IFN blockade (i.e., sifalimumab, anifrolumab) have shown promising results [55, 56].

TNFα is a pro-inflammatory cytokine secreted by monocytes, macrophages, T cells, neutrophils, and mast cells. It promotes lymphocyte recruitment and inflammatory responses. However, it becomes immunosuppressive with chronic exposure [57]. Increased levels, which correlated with disease activity, have been found in SLE patients (Additional file 4: Table S2). A negative feedback loop between Type I IFN and TNFα has been suggested: when Type I IFN prevails, SLE may occur. TNF inhibits

Pacheco *et al. J Transl Med* (2017) 15:239

Page 9 of 15

Table 8 Distribution of autoantibodies and cytokines in integrative clusters

Biomarker	Neutral (n = 41)	Chemotactic/APLA (n = 13)	IFNα/dsDNA (n = 13)	p-value
Autoantibodies				
RF	15 (37)	2 (15)	7 (54)	0.121
CCP3	1 (2)	0 (0)	0 (0)	0.724
TPOAb	4 (10)	2 (15)	1 (8)	0.792
TgAb	3 (7)	0 (0)	2 (15)	0.327
dsDNA	15 (37)	8 (61)	9 (69)	0.065
ACA-IgM	3 (7)	4 (31)	4 (31)	0.041
ACA-IgG	0 (0)	10 (77)	2 (15)	< 0.0001*
β2GP1IgM	0 (0)	6 (46)	1 (8)	< 0.0001*
β2GP1IgG	0 (0)	4 (31)	2 (15)	0.0021
ANAs	35 (85)	11 (85)	11 (85)	0.996
RNP	10 (24)	8 (61)	7 (54)	0.021
Ro	14 (34)	4 (31)	8 (61)	0.168
La	4 (10)	0 (0)	1 (8)	0.506
Sm	5 (12)	3 (23)	4 (31)	0.271
Cytokines				
IL-2	0 (0)	1 (8)	1 (8)	0.196
IL-4	0 (0)	0 (0)	4 (31)	0.0001*
IL-5	0 (0)	1 (8)	5 (38)	0.0001*
IL-6	7 (17)	2 (15)	6 (46)	0.072
IL-9	1 (2)	0 (0)	1 (8)	0.487
IL-10	1 (2)	3 (23)	10 (77)	< 0.0001*
IL-17A	3 (7)	0 (0)	8 (61)	< 0.0001*
TNFα	0 (0)	0 (0)	9 (69)	< 0.0001*
G-CSF	16 (39)	4 (31)	11 (85)	0.007
IFNα	3 (7)	1 (8)	13 (100)	< 0.0001*
IFNγ	2 (5)	0 (0)	2 (15)	0.227
IL-13	0 (0)	0 (0)	1 (8)	0.121
IL-1β	1 (2)	0 (0)	5 (38)	0.0001*
IL-8	6 (15)	7 (54)	3 (23)	0.015
IL-12/23p40	17 (41)	5 (38)	13 (100)	0.0006*
1997 ACR criteria				
Positive ANAs	34 (83)	11 (85)	13 (100)	0.282
Immunologic criteria	32 (78)	10 (77)	12 (92)	0.491
Hematologic criteria	28 (68)	13 (100)	7 (54)	0.024
Non-erosive arthritis	28 (68)	7 (54)	12 (92)	0.092
Photosensitivity	26 (63)	8 (61)	7 (54)	0.826
Malar rash	20 (49)	7 (54)	6 (46)	0.921
Renal criteria[a]	14 (34)	6 (46)	5 (38)	0.734
Oral ulcers	14 (34)	5 (38)	4 (31)	0.917
Serositis	10 (24)	5 (38)	4 (31)	0.604
Neurologic criteria	4 (10)	4 (31)	4 (31)	0.091
Discoid rash	3 (7)	3 (2)	1 (8)	0.252

Data correspond to number of patients (%)

RF rheumatoid factor, *CCP3* anti-cyclic citrullinated peptide third-generation, *TPOAb* anti-thyroperoxidase antibody, *TgAb* anti-thyroglobulin antibody, *dsDNA* anti-double stranded DNA antibodies, *ACA* anticardiolipin antibody, *β2GP1* β2 glycoprotein-1, *ANAs* antinuclear antibodies, *IL* interleukin, *G-CSF* granulocyte colony-stimulating factor, *IFN* interferon, *TNF* tumor necrosis factor, *ACR* American College of Rheumatology

* Statistically significant after Bonferroni correction

[a] Renal criteria was defined as active urinary sediment, or proteinuria > 500 mg/24 h or positive renal biopsy [23, 24]

Pacheco *et al. J Transl Med* (2017) 15:239

Page 10 of 15
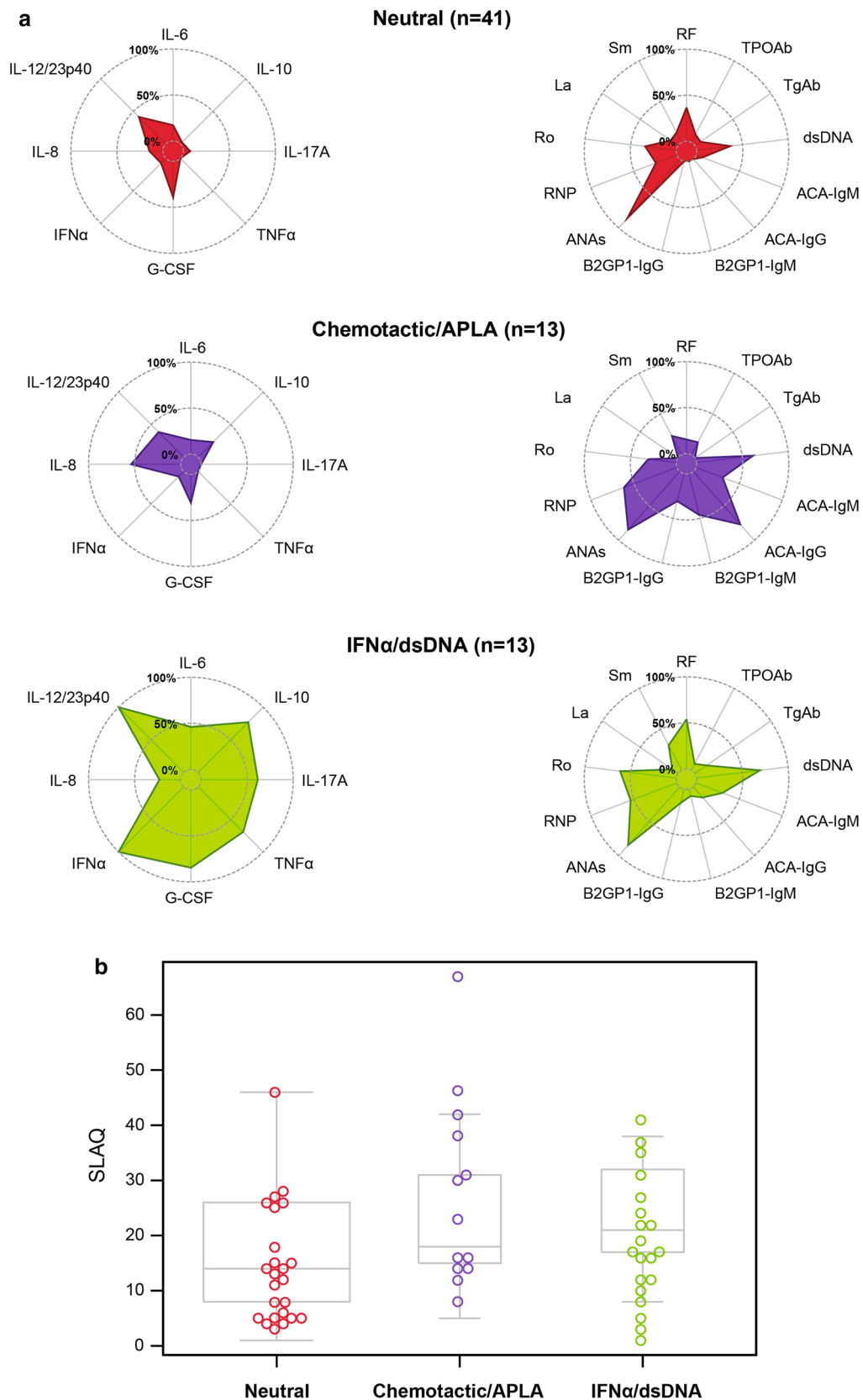
Fig. 4 Integrative analysis. **a** Clusters of cytokines and autoantibodies. **b** Association between clusters and activity of disease ($p = 0.036$)

Pacheco *et al. J Transl Med* (2017) 15:239

Page 11 of 15

the development of pDCs and their production of Type I IFN [58]. IL-17A belongs to the IL-17 family. It is largely produced by Th17 cells although it is secreted by several immune cells [59]. IL-17A has been widely studied in autoimmunity and is thought to play a pivotal role in SLE physiopathology (Additional file 4: Table S2). IL-10 is an immunomodulatory cytokine secreted by several cell populations although it exerts an essential role in B cell processes. Thus, it may promote hyperactivity of the B-cell compartment, thus leading to increased autoantibody production. Due to its dual function (i.e., B-cell stimulation/antibody production, and T-cell inflammatory response reduction), its role in SLE is not fully understood (Additional file 4: Table S2). IL-6 is a multifunctional cytokine secreted by several cells of both the innate and adaptive immune systems as well as by non-immune cells such as fibroblasts [60]. Increased serum levels are found in SLE and appear to be associated with joint involvement [44, 61, 62], and disease activity [52]. A few monoclonal antibodies (e.g., tocilizumab, sarilumab) are current treatment options for different ADs [4]. Nevertheless, randomized clinical trials for SLE are lacking. A summary of the role of IL-6 in SLE is shown in Additional file 4: Table S2.

Some cytokines exhibited a low prevalence (< 5%) (i.e., IL-1β, IL-2, IL-4, IL-5, IL-9, IL-13, IFNγ) in most patients, and thus did not account for any cluster. IL-1β is secreted mainly by innate immune cells. High serum levels are uncommon among SLE patients and appear to lack an association with SLE pathogenesis [63] (Additional file 4: Table S2).

IL-2 is predominantly produced by Tregs. Deficiency in IL-2 secretion is involved in the pathogenesis of SLE through the impairment of Treg growth and survival [64]. Recent evidence has shown that IL-2 secretion is impaired by high levels of IL-23 and IL-23R [65]. This may explain the absence of IL-2 in our clusters due to the high frequency of IL-12/23p40 in patients (Additional file 4: Table S2). IL-4 is secreted by several immune cells, particularly basophils. Since it stimulates B-cells, it may be involved in antibody production and SLE pathophysiology (Additional file 4: Table S2). IL-9 is a pleiotropic cytokine, produced by an ample variety of immune cells including mast cells, NKT cells, Th2, Th17, Treg, and the recently described Th9 [66]. It is considered a proliferative cytokine, which can induce the production of IL-6, mainly by mast cells. It enhances B-cell production of IL-4, IgE, and IgG1 and promotes isotype switching. In contrast, IL-9 secreted by Treg induces tolerance [66]. Some data on the implications of IL-9 for SLE have been described (Additional file 4: Table S2). IFNγ is mainly produced by T and NK cells. It is important for T cell differentiation and B-cell isotype switching [67]. Its role

in SLE is described in Additional file 4: Table S2. Data regarding IL-5 and IL-13 in SLE patients is scarce. IL-5 is pivotal for eosinophil differentiation [68], whereas IL-13 is an immunomodulatory cytokine secreted by Th2 CD4 T-cells (Additional file 4: Table S2).

Regarding the interaction between autoantibodies and cytokines, the absence of autoantibodies was associated with a low frequency of cytokines (Table 6). Disease activity, in turn, was lower in the neutral cytokine cluster (Fig. 3a). The enhancement of antibody production and activation of autoreactive B cells may be favored by a Th2 environment [51, 69]. Some inflammatory cytokines (e.g., TNFα, G-CSF) were less common in the neutral autoantibody cluster although statistical significance was not reached (Table 6). IL-10 was significantly lower in the neutral autoantibody cluster than in the APLA-dominant and dsDNA/ENA dominant clusters (Table 6). Abnormally increased IL-10 synthesis seems contributing to the spontaneous hyperactivity of the B cell compartment, so that it can directly result in autoantibody production by committed plasma cells, circulating immune complexes formation, and eventually in tissue and organ damage [70]. IL-5 was absent in the neutral autoantibody cluster, which may be in line with the reported worse renal outcomes associated with elevated IL-5 urine concentration [68]. Additionally, anti-dsDNA antibodies were less frequent within the neutral cytokine cluster (Table 4), which highlights the involvement of these autoantibodies in SLE pathophysiology. The IFNα/Pro-Inflammatory cluster revealed a significant low frequency of the neutral autoantibody cluster (Fig. 3b). This finding is in line with the capability of IFNs and IL-17 to induce antibody secretion [54, 71]. These results support the fact that SLE disease activity is mediated by cytokine secretion [2] and the potential role of autoantibodies in the enhancement of cytokine production [48, 72].

Our last analysis, which was integrative and in which all the biomarkers were included, showed three clusters which reinforced the results (Fig. 4a). The G-CSF cytokine cluster observed in the second analysis was distributed throughout the three integrative clusters. In addition, these three clusters were associated with the disease activity (Fig. 4b).

Our study supports the importance of individualized treatment of patients since both autoantibody and cytokine clusters were established in a cohort of SLE patients that also showed interaction and association with disease activity. The identification of SLE subphenotypes has been suggested previously [73] and is pivotal for the implementation of personalized medicine [74]. Our results depict the existence of different subphenotypes based on both diverse disease-specific and non-specific autoantibodies and on easy-access molecules

Pacheco *et al. J Transl Med (2017) 15:239*

Page 12 of 15

such as cytokines. A comprehensive assessment of multiple biomarkers, which is feasible with multiplex assay technologies [27], should offer the possibility of a novel taxonomy for SLE and the implementation of targeted therapies based on cytokine patterns (i.e., proof-of-concept studies).

Systems biology approaches have been applied to rheumatic diseases, in order to find novel biomarkers and therapeutic strategies. These efforts have focused on gene-level interactions and their relationship with clinical manifestations. For instance, Chiche et al. [75] found three individual IFN modules through transcriptional repertoire analysis using microarray technology, which showed an association with some clinical variables. Likewise, Bancherau et al. [6] described seven discrete groups of SLE patients based on their gene expression patterns and clinical disease severity. Reclassification of SLE patients based on the results of '*omics*' studies has been proposed [76].

The possible shortcomings of our study must be acknowledged. The main objective was to evaluate simultaneously the relationship among cytokines, autoantibodies, and the disease activity at one point in time. Therefore, the lack of association between clusters and cumulative clinical characteristics was expected. Likewise, the effects of treatment on the modulation of cytokine/autoantibody levels were not taken into account. The main considered outcome was the activity of the disease. However, the results of this exploratory study should stimulate further longitudinal designs using larger groups of patients to fully describe these subtle complexities. Regarding the cytokine measurement method, previous reports on RA patients' sera showed that the correlation of CBA assays with ELISA-based methods for cytokine detection is moderately-high although a lower concentration for some cytokines (i.e., IL-2, TNFα, IL-10) may be detected [77]. Furthermore, CBA is not affected by the presence of RF in contrast to other multiplex technologies (e.g., Luminex) and allows the assessment of multiple biomarkers using relatively small sample volumes [77]. We are aware of the reported low positivity of IFNα levels in serum, which is rarely detectable by ELISA or bioassays, and the suggested assessment of gene expression monitoring [78]. However, our results yielded appropriate IFNα assessment by CBA and even the identification of an IFNα-related cluster. Measurements of IFNγ using CBA appear to be proper which supports a low concentration in our patients [77]. Although other techniques are used in research to measure cytokines, CBA is implemented in many clinical and regular laboratories. This is, therefore, a cost-effective and practical method. Another potential drawback of our study could be the method of disease activity

quantification, SLAQ, a well-known PRO questionnaire [41], which was used under a non-structured validation. Since SLE may exhibit a disconnection between disease activity and patient perceived well-being, PROs may help empower patients on disease management. Numerous regulatory agencies encourage the use of PROs in clinical trials [79]. Note that our results showed an association between SLAQ scores and cytokine clusters. This fact is particularly interesting given that SLAQ does not include autoantibodies (e.g., anti-dsDNA) in its criteria in contrast to clinical indexes (e.g., SLEDAI). Thus, disease activity could be biased by anti-dsDNA. Instead, as shown herein, disease activity as portray by SLAQ may be due to others biomarkers such as cytokines. Another potential limitation of the present study is that the observed results may be due to chance alone or the moderate sample size. However, such a possibility would be unlikely given the highly significant results seen as well as their consistent direction and magnitude within the different analyses.

Conclusions

Systemic lupus erythematosus is a heterogeneous systemic AD with profound cytokine abnormalities. Multiple disease-specific and non-specific biomarkers are present in SLE patients. Clustering methods allow the identification of association among these markers and yield different subphenotypes. Additional systems medicine approaches are warranted in order to reveal the strength of these interactions, which should assist in the implementation of personalized medicine.

Additional files

Additional file 1. SLAQ_SpanishValid. Systemic lupus activity questionnaire (SLAQ) – Spanish linguistic validation. Spanish linguistic validation of SLAQ as described in "Methods" section.

Additional file 2. SLE autoantibody clusters_Summary. SLE autoantibody clusters in the literature – Summary table. Summary table of the available autoantibody clusters in the literature regarding SLE patients. Author, country, clustering method, sample size, and found clusters are included.

Additional file 3. SLE clusters_Bioinformatics analysis. Bioinformatic analysis for G-CSF (CSF3) – Dominant cluster and IFNα/Pro-inflammatory cluster. Bioinformatic analysis for G-CSF (CSF3) – Dominant cluster and IFNα/Pro-inflammatory cluster showing known interactions between cytokines, based on 'STRING: functional protein association networks' (https://string-db.org/).

Additional file 4. Summary of cytokine implicated in SLE. Summary of cytokines implicated in SLE—Summary table. Review of literature on the main cytokines implicated in SLE.

Pacheco *et al. J Transl Med* (2017) 15:239

Page 13 of 15

Research; ACR: America College of Rheumatology; SLAQ: Systemic Lupus Activity Questionnaire; CCP3: anti-CCP third-generation; ACA: anti-cardiolipin antibodies; 2GP1: anti-2 glycoprotein-1; dsDNA: double-stranded DNA; ELISA: enzyme-linked immunosorbent assay; ANAs: antinuclear antibodies; IL: interleukin; G-CSF: granulocyte colony-stimulating factor; IFN: interferon; TNF: tumor necrosis factor; CBA: cytometric bead array; ENA: extractable nuclear antigen antibodies; PRO: patient-reported outcome; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; DC: dendritic cell; pDC: plasmacytoid dendritic cell; NETosis: neutrophil extracellular traps; NK: natural killer; Ab: antibody.

Authors' contributions
JMA conceived the study. YP, JBC, DMM, YAA, MR, YR, JS, MRJ, RM, CRS, NMG and JMA made substantial contributions to acquisition, analysis, and interpretation of clinical and laboratory data. YP, DMM, YAA and CRS did laboratory assays. NMG did statistical analysis. YP, JBC, DMM, YAA, NMG and JMA were major contributors in writing the manuscript. All authors read, critically revised for important intellectual content, and approved the final manuscript. All authors participated sufficiently in the work to take public responsibility for appropriate portions of the content, and agreed to be accountable for all aspects of the work in order to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final mansucript.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References
1. Durcan L, Petri M. Why targeted therapies are necessary for systemic lupus erythematosus. Lupus. 2016;25:1070–9.
2. Tsokos GC, Lo MS, Costa Reis P, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. Nat Rev Rheumatol. 2016;12:716–30.
3. Lleo A, Invernizzi P, Gao B, Podda M, Gershwin ME. Definition of human autoimmunity—autoantibodies versus autoimmune disease. Autoimmun Rev. 2010;9:A259–66.
4. Zharkova O, Celhar T, Cravens PD, Satterthwaite AB, Fairhurst A-M, Davis LS. Pathways leading to an immunological disease: systemic lupus erythematosus. Rheumatology. 2017;56:i55–66.
5. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003;349:1526–33.
6. Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, et al. Personalized immunomonitoring uncovers molecular networks that stratify lupus patients. Cell. 2016;165:551–65.
7. Tan JHT, Hoh SF, Win MTM, Chan YH, Das L, Arkachaisri T. Childhood-onset systemic lupus erythematosus in Singapore: clinical phenotypes, disease activity, damage, and autoantibody profiles. Lupus. 2015;24:998–1005.
8. Artim-Esen B, Çene E, Şahinkaya Y, Ertan S, Pehlivan Ö, Kamali S, et al. Cluster analysis of autoantibodies in 852 patients with systemic lupus erythematosus from a single center. J Rheumatol. 2014;41:1304–10.
9. Li PH, Wong WHS, Lee TL, Lau CS, Chan TM, Leung AMH, et al. Relationship between autoantibody clustering and clinical subsets in SLE: cluster and association analyses in Hong Kong Chinese. Rheumatology. 2013;52:337–45.
10. Ching KH, Burbelo PD, Tipton C, Wei C, Petri M, Sanz I, et al. Two major autoantibody clusters in systemic lupus erythematosus. PLoS ONE. 2012;7:e32001.
11. Tang X, Huang Y, Deng W, Tang L, Weng W, Zhang X. Clinical and serologic correlations and autoantibody clusters in systemic lupus erythematosus: a retrospective review of 917 patients in South China. Medicine (Baltimore). 2010;89:62–7.
12. Jurencák R, Fritzler M, Tyrrell P, Hiraki L, Benseler S, Silverman E. Autoantibodies in pediatric systemic lupus erythematosus: ethnic grouping, cluster analysis, and clinical correlations. J Rheumatol. 2009;36:416–21.
13. Li QZ, Zhen QL, Xie C, Wu T, Mackay M, Aranow C, et al. Identification of autoantibody clusters that best predict lupus disease activity using glomerular proteome arrays. J Clin Invest. 2005;115:3428–39.
14. To CH, Petri M. Is antibody clustering predictive of clinical subsets and damage in systemic lupus erythematosus? Arthritis Rheum. 2005;52:4003–10.
15. Hoffman IEA, Peene I, Meheus L, Huizinga TWJ, Cebecauer L, Isenberg D, et al. Specific antinuclear antibodies are associated with clinical features in systemic lupus erythematosus. Ann Rheum Dis. 2004;63:1155–8.
16. Tápanes FJ, Vásquez M, Ramírez R, Matheus C, Rodríguez MA, Bianco N. Cluster analysis of antinuclear autoantibodies in the prognosis of SLE nephropathy: are anti-extractable nuclear antibodies protective? Lupus. 2000;9:437–44.
17. Park DJ, Kang JH, Kim JE, Lee KE, Lee SS. Comparison of clinical and serological differences according to the autoantibody cluster in women with systemic lupus erythematosus: results from the Korean lupus network (KORNET) registry. Ann Rheum Dis. 2017;76(Suppl 2):SAT0275.
18. Anaya JM. The autoimmune tautology. A summary of evidence. Joint Bone Spine. 2017;84:251–3.
19. Anaya JM. The diagnosis and clinical significance of polyautoimmunity. Autoimmun Rev. 2014;13:423–6.
20. Rojas-Villarraga A, Toro CE, Espinosa G, Rodríguez-Velosa Y, Duarte-Rey C, Mantilla RD, et al. Factors influencing polyautoimmunity in systemic lupus erythematosus. Autoimmun Rev. 2010;9:229–32.
21. Anaya JM, Corena R, Castiblanco J, Rojas-Villarraga A, Shoenfeld Y. The kaleidoscope of autoimmunity: multiple autoimmune syndromes and familial autoimmunity. Expert Rev Clin Immunol. 2007;3:623–35.
22. Amaya-Amaya J, Molano-González N, Franco JS, Rodríguez-Jiménez M, Rojas-Villarraga A, Anaya JM. Anti-CCP antibodies as a marker of rhupus. Lupus. 2015;24:892–4.
23. Franco JS, Molano-González N, Rodríguez-Jiménez M, Acosta-Ampudia Y, Mantilla RD, Amaya-Amaya J, et al. The coexistence of antiphospholipid syndrome and systemic lupus erythematosus in Colombians. PLoS ONE. 2014;9:e110242.
24. Franco JS, Amaya-Amaya J, Molano-González N, Caro-Moreno J, Rodríguez-Jiménez M, Acosta-Ampudia Y, et al. Autoimmune thyroid disease in Colombian patients with systemic lupus erythematosus. Clin Endocrinol (Oxf). 2015;83:943–50.
25. Davis LS, Reimold AM. Research and therapeutics-traditional and emerging therapies in systemic lupus erythematosus. Rheumatology. 2017;56:i100–13.

Pacheco *et al. J Transl Med* (2017) 15:239

Page 14 of 15

26. Brodin P, Davis MM. Human immune system variation. Nat Rev Immunol. 2017;17:21–9.

27. Davis MM, Tato CM, Furman D. Systems immunology: just getting started. Nat Immunol. 2017;18:725–32.

28. American College of Rheumatology Ad Hoc Committee on. Systemic lupus erythematosus guidelines. Guidelines for referral and management of systemic lupus erythematosus in adults. Arthritis Rheum. 1999;42:1785–96.

29. Johar AS, Mastronardi C, Rojas-Villarraga A, Patel HR, Chuah A, Peng K, et al. Novel and rare functional genomic variants in multiple autoimmune syndrome and Sjögren's syndrome. J Transl Med. 2015;13:173.

30. Anaya JM, Castiblanco J, Rojas-Villarraga A, Pineda-Tamayo R, Levy RA, Gómez-Puerta J, et al. The multiple autoimmune syndromes. A clue for the autoimmune tautology. Clin Rev Allergy Immunol. 2012;43:256–64.

31. Yazdany J, Yelin EH, Panopalis P, Trupin L, Julian L, Katz PP. Validation of the systemic lupus erythematosus activity questionnaire in a large observational cohort. Arthritis Rheum. 2008;59:136–43.

32. Karlson EW, Daltroy LH, Rivest C, Ramsey-Goldman R, Wright EA, Partridge AJ, et al. Validation of a Systemic Lupus Activity Questionnaire (SLAQ) for population studies. Lupus. 2003;12:280–6.

33. Romero-Diaz J, Isenberg D, Ramsey-Goldman R. Measures of adult systemic lupus erythematosus: updated version of British Isles Lupus Assessment Group (BILAG 2004), European Consensus Lupus Activity Measurements (ECLAM), Systemic Lupus Activity Measure, Revised (SLAM-R), Systemic Lupus Activity Questi. Arthritis Care Res (Hoboken). 2011;63(Suppl 1):S37–46.

34. Kardinaal AFM, van Erk MJ, Dutman AE, Stroeve JHM, van de Steeg E, Bijlsma S, et al. Quantifying phenotypic flexibility as the response to a high-fat challenge test in different states of metabolic health. FASEB J. 2015;29:4600–13.

35. Willis R, Smikle M, DeCeulaer K, Romay-Penabad Z, Papalardo E, Jajoria P, et al. Clinical associations of proinflammatory cytokines, oxidative biomarkers and vitamin D levels in systemic lupus erythematosus. Lupus. 2017. https://doi.org/10.1177/0961203317706557.

36. Lebart L, Morineau A, Piron M. Statistique exploratoire multidimensionnelle. Paris: Dunod; 1995.

37. Severiche-Maury DM, Restrepo-Escobar M, Naranjo-González LA, Vanegas-García AL, Muñoz-Vahos CH, Carlos María G, Vásquez-Duque G. Ciento quince pacientes con lupus eritematoso sistemico: caracteristicas clínicas e inmunologicas. Rev Colomb Reumatol. 2014;1:183–92.

38. To CH, Mok CC, Tang SSK, Ying SKY, Wong RWS, Lau CS. Prognostically distinct clinical patterns of systemic lupus erythematosus identified by cluster analysis. Lupus. 2009;18:1267–75.

39. Poole BD, Schneider RI, Guthridge JM, Velte CA, Reichlin M, Harley JB, et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. Arthritis Rheum. 2009;60:848–59.

40. Meilof JF, Veldhoven CH, Swaak AJ, Smeenk RJ. Production of anti-Ro/SS-A and anti-La/SS-B autoantibodies is closely coordinated in systemic lupus erythematosus and independent of anti-dsDNA production. J Autoimmun. 1997;10:67–75.

41. Holloway L, Humphrey L, Heron L, Pilling C, Kitchen H, Højbjerre L, et al. Patient-reported outcome measures for systemic lupus erythematosus clinical trials: a review of content validity, face validity and psychometric performance. Health Qual Life Outcomes. 2014;12:116.

42. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. Lupus. 2004;13:290–7.

43. Baggiolini M, Loetscher P, Moser B. Interleukin-8 and the chemokine family. Int J Immunopharmacol. 1995;17:103–8.

44. Eilertsen GØ, Nikolaisen C, Becker-Merok A, Nossent JC. Interleukin-6 promotes arthritis and joint deformation in patients with systemic lupus erythematosus. Lupus. 2011;20:607–13.

45. Wu Y, Cai B, Zhang J, Shen B, Huang Z, Tan C, et al. IL-1β and IL-6 Are Highly Expressed in RF + IgE + Systemic Lupus Erythematous Subtype. J Immunol Res. 2017;2017:5096741.

46. Vega-Ostertag M, Casper K, Swerlick R, Ferrara D, Harris EN, Pierangeli SS. Involvement of p38 MAPK in the up-regulation of tissue factor on endothelial cells by antiphospholipid antibodies. Arthritis Rheum. 2005;52:1545–54.

47. Hsieh SC, Sun KH, Tsai CY, Tsai YY, Tsai ST, Huang DF, et al. Monoclonal anti-double stranded DNA antibody is a leucocyte-binding protein to up-regulate interleukin-8 gene expression and elicit apoptosis of normal human polymorphonuclear neutrophils. Rheumatology. 2001;40:851–8.

48. Sun KH, Yu CL, Tang SJ, Sun GH. Monoclonal anti-double-stranded DNA autoantibody stimulates the expression and release of IL-1beta, IL-6, IL-8, IL-10 and TNF-alpha from normal human mononuclear cells involving in the lupus pathogenesis. Immunology. 2000;99:352–60.

49. Mehta HM, Malandra M, Corey SJ. G-CSF and GM-CSF in Neutropenia. J Immunol. 2015;195:1341–9.

50. Teng MWL, Bowman EP, McElwee JJ, Smyth MJ, Casanova JL, Cooper AM, et al. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. Nat Med. 2015;21:719–29.

51. Croxford AL, Kulig P, Becher B. IL-12-and IL-23 in health and disease. Cytokine Growth Factor Rev. 2014;25:415–21.

52. Talaat RM, Mohamed SF, Bassyouni IH, Raouf AA. Th1/Th2/Th17/Treg cytokine imbalance in systemic lupus erythematosus (SLE) patients: correlation with disease activity. Cytokine. 2015;72:146–53.

53. Leng RX, Pan HF, Chen GM, Wang C, Qin WZ, Chen LL, et al. IL-23: a promising therapeutic target for systemic lupus erythematosus. Arch Med Res. 2010;41:221–5.

54. Rodero MP, Crow YJ. Type I interferon-mediated monogenic autoinflammation: the type I interferonopathies, a conceptual overview. J Exp Med. 2016;213:2527–38.

55. Khamashta M, Merrill JT, Werth VP, Furie R, Kalunian K, Illei GG, et al. Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. Ann Rheum Dis. 2016;75:1909–16.

56. Furie R, Khamashta M, Merrill JT, Werth VP, Kalunian K, Brohawn P, et al. Anifrolumab, an anti-Interferon-α receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus. Arthritis Rheumatol. 2017;69:376–86.

57. Gómez D, Correa PA, Gómez LM, Cadena J, Molina JF, Anaya J-M. Th1/Th2 cytokines in patients with systemic lupus erythematosus: is tumor necrosis factor alpha protective? Semin Arthritis Rheum. 2004;33:404–13.

58. Cantaert T, Baeten D, Tak PP, van Baarsen LGM. Type I IFN and TNFα cross-regulation in immune-mediated inflammatory disease: basic concepts and clinical relevance. Arthritis Res Ther. 2010;12:219.

59. Beringer A, Noack M, Miossec P. IL-17 in chronic inflammation: from discovery to targeting. Trends Mol Med. 2016;22:230–41.

60. Jordan SC, Choi J, Kim I, Wu G, Toyoda M, Shin B, et al. Interleukin-6, A Cytokine Critical to Mediation of Inflammation, Autoimmunity and Allograft Rejection: therapeutic Implications of IL-6 Receptor Blockade. Transplantation. 2017;101:32–44.

61. Umare V, Pradhan V, Nadkar M, Rajadhyaksha A, Patwardhan M, Ghosh KK, et al. Effect of proinflammatory cytokines (IL-6, TNF-α, and IL-1β) on clinical manifestations in Indian SLE patients. Mediators Inflamm. 2014;2014:385297.

62. Cigni A, Pileri PV, Faedda R, Gallo P, Sini A, Satta AE, et al. Interleukin 1, interleukin 6, interleukin 10, and tumor necrosis factor α in active and quiescent systemic lupus erythematosus. J Investig Med. 2014;62:825–9.

63. Kahlenberg JM, Kaplan MJ. The inflammasome and lupus: another innate immune mechanism contributing to disease pathogenesis? Curr Opin Rheumatol. 2014;26:475–81.

64. Humrich JY, Riemekasten G. Restoring regulation—IL-2 therapy in systemic lupus erythematosus. Expert Rev. Clin. Immunol. 2016;12:1153–60.

65. Dai H, He F, Tsokos GC, Kyttaris VC. IL-23 Limits the Production of IL-2 and Promotes Autoimmunity in Lupus. J Immunol. 2017;199:903–10.

66. Rojas-Zuleta WG, Vásquez G. Th9 lymphocytes: a recent history from IL-9 to its potential role in rheumatic diseases. Autoimmun Rev. 2016;15:649–55.

67. Pollard KM, Cauvi DM, Toomey CB, Morris KV, Kono DH. Interferon-γ and systemic autoimmunity. Discov Med. 2013;16:123–31.

68. Brito TNS, Vilar MJ, Almeida JB, Faria ALSB, Medeiros SDV, Medeiros MCC, et al. Measuring eosinophiluria, urinary eosinophil cationic protein and urinary interleukin-5 in patients with Lupus Nephritis. Allergy Asthma Clin Immunol. 2014;10:61.

69. Singh RR. IL-4 and many roads to lupuslike autoimmunity. Clin Immunol. 2003;108:73–9.

70. Peng H, Wang W, Zhou M, Li R, Pan HF, Ye DQ. Role of interleukin-10 and interleukin-10 receptor in systemic lupus erythematosus. Clin Rheumatol. 2013;32:1255–66.

Pacheco *et al. J Transl Med (2017) 15:239*

Page 15 of 15

71. Li D, Guo B, Wu H, Tan L, Chang C, Lu Q. Interleukin-17 in systemic lupus erythematosus: a comprehensive review. Autoimmunity. 2015;48:353–61.

72. Catrina AI, Svensson CI, Malmström V, Schett G, Klareskog L. Mechanisms leading from systemic autoimmunity to joint-specific disease in rheumatoid arthritis. Nat Rev Rheumatol. 2017;13:79–86.

73. Agmon-Levin N, Mosca M, Petri M, Shoenfeld Y. Systemic lupus erythematosus one disease or many? Autoimmun Rev. 2012;11:593–5.

74. Anaya J, Duarte-Rey C, Sarmiento-Monroy JC, Bardey D, Castiblanco J, Rojas-Villarraga A. Personalized medicine. Closing the gap between knowledge and clinical practice. Autoimmun Rev. 2016;15:833–42.

75. Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, et al. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. Arthritis Rheumatol. 2014;66:1583–95.

76. Teruel M, Chamberlain C, Alarcón-Riquelme ME. Omics studies: their use in diagnosis and reclassification of SLE and other systemic autoimmune diseases. Rheumatology. 2017;56:i78–87.

77. Churchman SM, Geiler J, Parmar R, Horner EA, Church LD, Emery P, et al. Multiplexing immunoassays for cytokine detection in the serum of patients with rheumatoid arthritis: lack of sensitivity and interference by rheumatoid factor. Clin Exp Rheumatol. 2012;30:534–42.

78. Mathian A, Hie M, Cohen-Aubart F, Amoura Z. Targeting interferons in systemic lupus erythematosus: current and future prospects. Drugs. 2015;75:835–46.

79. Annapureddy N, Devilliers H, Jolly M. Patient-reported outcomes in lupus clinical trials with biologics. Lupus. 2016;25:1111–21.