


Mononuclear cells from patients with rhupus are influenced by TNF in their production of gp130/sIL-6Rb and APRIL

Luis M Amezcua-Guerra,^{1,2} Fausto Sánchez-Muñoz,¹ Santiago Guzmán-García,³ Ricardo Márquez-Velasco,¹ Enrique Becerril-Villanueva,⁴ Yadira Vázquez-Panchos,¹ Yaneli Juárez-Vicuña ¹

To cite: Amezcua-Guerra LM, Sánchez-Muñoz F, Guzmán-García S, *et al.* Mononuclear cells from patients with rhupus are influenced by TNF in their production of gp130/sIL-6Rb and APRIL. *Lupus Science & Medicine* 2023;**10**:e000970. doi:10.1136/lupus-2023-000970

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/lupus-2023-000970>).

Received 25 May 2023
Accepted 8 October 2023



© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

¹Department of Immunology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico

²Department of Health Care, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico

³Department of Rheumatology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico

⁴Laboratory of Psychoimmunology, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico City, Mexico

Correspondence to

Dr Yaneli Juárez-Vicuña;
yaneli2608@hotmail.com

ABSTRACT

Objective Rhupus is a rare disease that shares characteristics of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). While several studies have explored the clinical and immunological profiles of patients with rhupus, the underlying cause of the disease remains unknown due to its complex pathogenesis. The objective of this study was to investigate the role of tumour necrosis factor (TNF) in the production of inflammatory molecules by peripheral blood mononuclear cells (PBMCs) from patients with rhupus.

Methods The study involved five healthy controls, seven patients with rhupus and seven patients with SLE. PBMCs were obtained from each participant and stimulated with recombinant human TNF for 24 hours. The levels of various molecules secreted by the cells, such as cytokines and chemokines, were measured using immunobead-based assays on xMAP technology.

Results The production levels of some molecules were higher in TNF-stimulated PBMCs from patients with rhupus and SLE than in unstimulated cells. In addition, the levels of certain molecules, including gp130/sIL-6Rb, a proliferation-inducing ligand (APRIL), interferon- β , matrix metalloproteinase-3 and interleukin (IL)-12, were higher in PBMCs from patients with rhupus even without TNF stimulation. Similarly, the levels of gp130/sIL-6Rb and APRIL were higher in TNF-stimulated PBMCs from patients with rhupus than in healthy controls. These results were further validated against patients with RA using enzyme-linked immunosorbent assay.

Conclusions These findings suggest that the spontaneous production of molecules by cells from patients with rhupus may contribute to the development of the disease, and that TNF may play a role in this process by regulating the secretion of gp130/sIL-6Rb and APRIL.

INTRODUCTION

Tumour necrosis factor (TNF) is a proinflammatory cytokine that influences various signalling pathways and contributes to the development of autoimmune diseases.¹ Inappropriate activation of TNF signalling may lead to the production of chemokines, proteases and other inflammatory mediators.² Additionally, TNF can either stimulate

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Tumour necrosis factor (TNF) is recognised as a pivotal player in the pathophysiology of autoimmune diseases, primarily through its influence on the production of biologically active molecules.

WHAT THIS STUDY ADDS

⇒ This study reveals a novel facet on the regulatory role of TNF by demonstrating its tight control over the production of gp130/sIL-6Rb and a proliferation-inducing ligand in mononuclear cells from patients with rhupus (systemic lupus erythematosus and rheumatoid arthritis overlap).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our research shed light on the central role of TNF in the pathogenesis of rhupus, suggesting that it could be a promising therapeutic target for managing this complex disease.

or suppress the expression of certain genes, such as interleukin (IL)-1 β , IL-23 and various chemokines.^{3–5} In autoimmune diseases, TNF-mediated production of cytokines and chemokines is crucial as it activates synovial fibroblasts and macrophages, leading to overproduction of cathepsins and matrix metalloproteinases (MMPs) that contribute to bone erosions.⁶

Rhupus is a rare condition in which patients exhibit clinical and serological manifestations of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).^{7,8} The underlying cause of rhupus is not yet known, but factors such as hormones, environmental triggers and immune system dysregulation are thought to play a role.^{9,10} Patients with rhupus typically display symmetric erosive polyarthritis, serositis, a malar rash with photosensitivity, oral ulcers, and antibodies to double-stranded DNA (dsDNA) and citrullinated proteins (ACPA).^{11,12}

Given the role of TNF in RA pathogenesis, it is reasonable to suspect that it may also play a regulatory role in the production of relevant molecules in rhupus. This study aimed to investigate whether TNF contributes to the production of inflammatory mediators by peripheral blood mononuclear cells (PBMCs) from patients with rhupus.

PATIENTS AND METHODS

A cross-sectional, observational study was conducted within a single-centre facility. We included all patients diagnosed with rhupus, identified from our SLE outpatient cohort. To be included, patients had to simultaneously meet the American College of Rheumatology classification criteria for both RA and SLE.^{13 14} The study also included patients diagnosed solely with SLE or RA, as well as unrelated healthy individuals, for comparative analysis. Disease activity assessments were undertaken using the SLE Disease Activity Index 2000 (SLEDAI-2K) for SLE manifestations and the Disease Activity Score 28 based on C-reactive protein (DAS28-CRP) index for RA features.

The study was conducted in two distinct phases to investigate the production of soluble molecule and validate significant findings. In the initial phase, quantification of soluble molecules was performed using multiplexed immunobead-based assays (Luminex). This enabled a comparative analysis of various molecules present in cell culture supernatants obtained from patients with rhupus, SLE and healthy controls. Once molecules with significantly altered concentrations were identified, the study proceeded to the validation phase. This was achieved by using ELISA techniques to accurately measure the levels of the specific molecules. Additionally, samples from patients with RA were included in this phase, further enhancing comparative analysis.

Patients consented to the use of blood samples and clinical data for research purposes. Patients and the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

PBMCs were isolated by density gradient centrifugation with Histopaque-1077 (Sigma, St Louis, Missouri, USA) within 2 hours of blood collection. The culture medium consisted of RPMI 1640 (Sigma, Ronkonkoma, New York, USA) supplemented with 2 mM L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin and 10% heat-inactivated fetal bovine serum. Cells were grown in 24-well, flat-bottom plates (1×10^6 in 1 mL) on one of two conditions: with culture medium alone or with culture medium plus 100 µg/mL recombinant human TNF (R&D Systems, Minneapolis, Minnesota, USA). After 24 hours, cell culture supernatants were harvested, aliquoted and stored at -70°C until use.

Levels of various cytokines and inflammatory mediators were measured using multiplexed immunobead-based assays on xMAP technology (Luminex MAGPIX System, San Francisco, California, USA). Levels of IL-27, gp130/sIL-6Rb, IL-22, sIL-6Ra, interferon (IFN)- α 2, IL-26, IL-19,

IL-20, IL-29, IL-35, B cell-activating factor (BAFF), a proliferation-inducing ligand (APRIL), MMP1, IFN- β , MMP3, sCD163, pentraxin-3, TSLP, sCD30, IL-8, TWEAK, IL-28A, sTNF-R2, chitinase-3-like protein-1, sTNF-R1, IL-12 and osteopontin were measured using the Bio-Plex Pro 37-Plex Human Inflammation Panel 1 (Bio-Rad Laboratories, Hercules, California, USA). Additionally, levels of APRIL and gp130/sIL-6Rb were quantified in cell culture supernatants using ELISA (Fine Test, Wuhan Fine Biotech, Wuhan, China; range 15.625–1000 pg/mL and 0.25–16 ng/mL, respectively).

Statistics

Statistical analysis was performed using non-parametric tests, with results presented as proportions and percentages for discrete variables and medians and IQRs for continuous variables. Comparisons between groups were made using the Wilcoxon paired t-test and Kruskal-Wallis tests with Dunn's post-test, and Spearman's correlation test was used to assess associations between variables. A p value less than 0.05 was considered statistically significant. The statistical analysis was performed using SPSS V.24 software (IBM).

RESULTS

The study comprised a total of 29 participants: 7 patients diagnosed with rhupus, 7 patients with SLE, 10 patients with RA and 5 healthy controls. Among the patients with rhupus, all were female with a median age of 48 years (43–50); patients with SLE were also female with a median age of 33 years (27–40). Among patients with RA, seven were female and three were male, with a median age of 47 years (42–60). Healthy controls were female with a median age of 30 years (27–37). Both patients with rhupus and SLE presented with active disease based on respective clinimetric indices (table 1).

The study primarily examined the impact of TNF stimulation on the release of molecules from cultured PBMCs. As shown in table 2, TNF-stimulated PBMCs from patients with rhupus and SLE had higher levels of most studied molecules in comparison with unstimulated cells. Exceptions existed, where TNF stimulation failed to induce the release of gp130/sIL-6Rb, sTNF-R2 and osteopontin in PBMCs from patients with rhupus, and gp130/sIL-6Rb, IL-26, sTNF-R2 and osteopontin in patients with SLE. Control cells, when stimulated with TNF, demonstrated increased production of sIL-6Ra, IL-19, BAFF, APRIL, sCD163, sTNF-R2 and sTNF-R1.

An assessment of inflammatory molecule production among the study groups was performed using the Kruskal-Wallis test. Variations were observed in the levels of gp130/sIL-6Rb, IL-19, IL-29, BAFF, APRIL, IFN- β , MMP3 and IL-12. Subsequent Dunn's multiple comparison tests indicated significant differences exclusively in the comparison of unstimulated PBMCs. Figure 1 shows that the median levels of certain molecules, including gp130/sIL-6Rb, APRIL, IFN- β , MMP3 and IL-12, were

Table 1 Clinical and laboratory data of study participants

	SLE (n=7)	Rhupus (n=7)	RA (n=10)
Age, years	33 (27–40)	48 (43–50)	47 (42–60)
Female, n (%)	7 (100)	7 (100)	7 (70)
Disease duration, years	6 (3–15)	2 (1–8)	3 (1–13)
Comorbidities			
Antiphospholipid syndrome, n (%)	2 (28)	1 (14)	0
Diabetes, n (%)	0	1 (14)	5 (50)
Systemic hypertension, n (%)	0	0	2 (20)
Main clinical features			
Mucocutaneous involvement, n (%)	7 (100)	7 (100)	0
Arthritis, n (%)	7 (100)	7 (100)	10 (100)
Renal involvement, n (%)	4 (57)	1 (14)	0
Neurological involvement, n (%)	0	0	0
Haematological, n (%)	4 (57)	5 (71)	0
Antirheumatic therapy			
Glucocorticoids, n (%)	5 (71)	3 (43)	4 (40)
Azathioprine, n (%)	3 (43)	1 (14)	0
Mofetil mycophenolate, n (%)	2 (28)	1 (14)	0
Methotrexate, n (%)	3 (43)	5 (71)	10 (100)
Leflunomide, n (%)	0	0	3 (30)
Antimalarials, n (%)	5 (71)	6 (85)	6 (60)
Laboratory values			
Leucocytes, 10 ⁹ /L	5.3 (3.9–5.7)	5.0 (4.1–5.7)	7.9 (7.0–10.4)
Haemoglobin, g/L	138 (111–141)	129 (119–143)	137 (130–151)
Platelets, 10 ⁹ /L	255 (235–269)	271 (143–352)	282 (233–365)
Serum creatinine, mg/dL	0.6 (0.5–0.7)	0.6 (0.5–0.8)	0.7 (0.6–0.9)
C-reactive protein, mg/L	5.1 (0.5–7.7)	8.9 (6.2–29.5)	5.9 (1.4–11.2)
C3, mg/L	80.3 (75.4–120.9)	121.8 (103.6–147.9)	–
C4, mg/L	15.3 (12.0–39.2)	22.0 (15.9–28.3)	–
ESR (mm/hour)	19.0 (5.0–23.0)	23.0 (13.0–32.0)	13.5 (11.2–25.7)
Rheumatoid factor ≥20 IU/mL, + (%)	1 (14)	7 (100)	10 (100)
Antinuclear antibodies ≥1:160, + (%)	7 (100)	7 (100)	–
Anti-Ro/SSA antibodies, + (%)	1 (14)	4 (57)	–
Anti-La/SSB antibodies, + (%)	0	4 (57)	–
SLEDAI-2K	4 (2–8)	4 (0–4)	–
DAS28-CRP	–	2.8 (1.5–3.0)	2.3 (1.5–3.1)

Data are expressed as median (IQR) unless otherwise specified.

DAS28-CRP, Disease Activity Score 28-joint count C-reactive protein; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; SLEDAI-2K, SLE Disease Activity Index 2000.

higher in the PBMCs of patients with rhupus compared with controls. Meanwhile, [figure 2](#) illustrates that TNF-stimulated PBMCs displayed similar levels of gp130/sIL-6Rb and APRIL across the study groups, despite their elevated levels in patients with rhupus compared with controls.

The connection between inflammatory molecule production and lupus activity was examined. For patients

with SLE, a positive correlation was observed between the production of APRIL and SLEDAI-2K, both in unstimulated cells ($r=0.991$; $p=0.000015$) and TNF-stimulated cells ($r=0.773$; $p=0.042$) (online supplemental table 1). Conversely, TNF-stimulated PBMCs from patients with SLE exhibited a negative correlation between disease activity and the production of IL-27 ($r=-0.901$; $p=0.006$), IL-20 ($r=-0.755$; $p=0.050$), sCD30 ($r=-0.847$; $p=0.016$)

Table 2 Production levels of molecules in unstimulated and TNF-stimulated PBMC of controls, patients with SLE and patients with rhusus

	SLE		Rhusus		Controls	
	RPMI	TNF	RPMI	TNF	RPMI	TNF
IL-27 (p28)	9.0 (8.3–25.2)	51.64 (21.6–60.7) *	21.6 (2.2–41.0)	54.4 (33.8–66.4)*	2.2 (2.2–14.0)	24.5 (8.9–63.2)
gp130/sIL-6Rb	133.5 (114.9–180.9)	161.8 (107.1–194.4)	157.9 (76.5–178.4)	156.8 (110.2–212.3)	71.8 (55.1–78.8)†	83.3 (61.8–89.6)‡
IL-22	5.3 (2.0–30.8)	67.4 (31.4–79.4)*	14.6 (1.2–34.2)	78.0 (55.3–92.6)*	0.1 (0.07–2.3)	54.0 (16.9–88.0)
sIL-6Ra	61.5 (25.1–86.5)	91.7 (31.0–112.3)*	53.6 (39.5–88.7)	78.6 (44.1–144.1)*	40.3 (38.1–47.6)	70.4 (56.8–75.7)*
IFN- α 2	5.5 (2.5–9.6)	15.8 (8.6–17.8)*	9.3 (1.9–12.8)	17.2 (13.2–24.0)*	1.9 (0.9–3.0)	11.9 (4.4–19.4)
IL-26	492.3 (408.2–665.1)	1171.8 (417.8–1476.3)	682.3 (398.7–912.2)	1082.7 (625.8–1321.0)*	117.7 (80.5–548.5)	295.1 (179.9–1147.4)
IL-19	21.7 (10.6–25.3)	29.9 (21.0–30.4)*	20.4 (9.0–24.5)	33.7 (23.6–35.3)*	4.1 (3.4–13.5)†	21.0 (9.3–35.0)*
IL-20	16.0 (2.0–187.2)	471.0 (191.1–476.7) *	68.5 (7.8–195.0)	558.2 (422.7–605.7) *	2.0 (2.0–9.2)	421.3 (110.3–567.4)
IL-29	11.9 (6.6–26.8)	36.4 (21.1–52.1)*	13.7 (3.3–21.1)	44.2 (30.6–64.2)*	0.2 (0.2–5.2)†	32.5 (8.8–42.3)
IL-35	11.8 (4.7–153.9)	239.4 (117.6–265.5)*	47.3 (9.4–125.9)	255.3 (216.4–329.9)*	4.7 (1.4–8.2)	186.4 (50.7–283.1)
BAFF	2116.8 (914.53–3724.4)	5429.9 (3963.3–6113.2)*	3559.2 (1740.8–4224.0)	5871.6 (5388.4–5915.2) *	1016.3 (860.1–1507.4)†	4379.5 (2535.3–5381.0)*
APRIL	10 300.4 (6176.6–11 800.5)	11 067.4 (9902.2–11 979.0)*	9071.3 (6176.6–12 155.8)	11 067.4 (10 495.6–14 787.1)	4305.5 (3331.1–4953.6)†	8636.1 (5130.6–9879.9)*‡
MMP1	126.4 (126.4–350.0)	932.5 (695.5–1137.3)*	126.4 (126.4–666.2)	956.5 (779.3–1302.1)*	126.4 (126.4–190.1)	832.3 (466.5–1195.3)
IFN- β	4.5 (1.2–5.6)	8.8 (6.1–10.9)*	5.0 (1.0–5.6)	9.54 (8.5–15.0)*	0.2 (0.2–1.2)†	10.7 (2.4–10.9)
MMP3	260.7 (121.0–883.3)	1200.1 (923.6–1421.5)*	567.0 (228.8–913.6)	1334.5 (1227.4–1523.4)*	20.5 (20.5–174.9)†	1227.4 (451.9–1471.9)
sCD163	484.0 (197.8–2736.5)	4317.6 (2081.7–4423.5)*	1129.9 (197.8–1956.7)	4462.0 (3592.3–5562.1)*	251.2 (112.4–305.2)	3728.2 (1228.9–4848.3)*
Pentraxin 3	19.3 (4.4–31.6)	156.9 (25.8–199.7)*	27.9 (12.7–79.4)	119.5 (55.4–171.6)*	8.0 (5.5–22.3)	104.6 (53.6–169.0)
TSLP	7.5 (2.7–12.9)	26.2 (16.0–28.2)*	11.8 (1.3–19.1)	30.2 (22.1–41.8)*	1.3 (1.3–2.0)	21.1 (7.6–30.0)
sCD30	0.1 (0.08–1.3)	3.1 (2.0–6.1)*	2.1 (0.1–5.9)	3.5 (1.6–7.2)*	0.08 (0.08–1.16)	2.28 (1.18–7.29)
IL-8	721.6 (156.5–2854.4)	14 626.6 (3995.8–18 447.0)*	1998.7 (289.8–4687.7)	13 521.2 (10 180.0–19 006.5)*	72.8 (24.2–539.9)	9372.9 (2028.0–14 032.5)
TWEAK	231.2 (159.7–478.5)	406.7 (356.3–776.8)*	169.6 (124.5–245.3)	418.5 (288.7–503.0)*	71.0 (68.2–166.3)	296.1 (133.2–475.3)
IL-28A	2.1 (0.9–14.3)	23.0 (11.9–28.0)*	5.7 (0.9–13.7)	28.0 (24.3–31.1)*	0.9 (0.9–1.5)	23.0 (7.6–29.9)
sTNF-R2	75.7 (17.3–706.0)	196.9 (21.6–527.8)	566.9 (29.7–721.6)	486.9 (46.6–1346.4)	99.8 (54.0–134.3)	269.6 (113.0–338.3)*

Continued

Table 2 Continued

	SLE		Rhusus		Controls	
	RPMI	TNF	RPMI	TNF	RPMI	TNF
Chitinase 3-like protein-1	17.8 (13.2–33.6)	31.8 (22.1–58.1)*	10.6 (10.0–26.0)	36.3 (23.5–51.5)*	13.2 (4.3–35.6)	31.8 (17.3–57.3)
sTNF-R1	17.2 (5.6–42.7)	67.0 (24.4–79.0)*	46.4 (6.6–50.3)	69.1 (41.6–81.0)*	9.6 (7.1–13.8)	57.2 (25.1–66.5)*
IL-12	0.3 (0.2–0.8)	1.8 (0.9–1.8)*	0.8 (0.2–1.1)	1.8 (1.5–2.4)*	0.07 (0.07–0.2)†	1.6 (0.4–1.9)
Osteopontin	311.3 (87.8–4723.8)	3367.9 (152.3–4248.4)	1385.3 (505.6–4410.6)	7513.7 (2641.0–11499.0)	257.0 (83.4–1872.7)	4848.0 (424.1–11030.2)

*The Wilcoxon paired t-test was used to assess differences in the levels of molecules between unstimulated and TNF-stimulated cells. Data are expressed as median (IQR).

†The Kruskal-Wallis test was used for comparisons of unstimulated PBMCs among groups, $p < 0.05$.

‡The Kruskal-Wallis test was used for comparisons of TNF-stimulated PBMCs among groups, $p < 0.05$.

APRIL, a proliferation-inducing ligand; BAFF, B cell-activating factor; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; PBMC, peripheral blood mononuclear cell; TNF, tumour necrosis factor.

and chitinase-3-like protein-1 ($r = -0.793$; $p = 0.033$). In patients with rhusus, unstimulated production of gp130/sIL-6Rb ($r = 0.926$; $p = 0.003$) and MMP3 ($r = 0.759$; $p = 0.048$) correlated positively with SLEDAI-2K, along with TNF-stimulated production of gp130/sIL-6Rb ($r = 0.926$; $p = 0.003$) (online supplemental table 1).

The relationship between inflammatory molecule production and RA activity was investigated in patients with rhusus. Positive correlations were found between DAS28-CRP index and unstimulated levels of gp130/sIL-6Rb ($r = 0.786$; $p = 0.036$), IFN- $\alpha 2$ ($r = 0.757$; $p = 0.049$), IL-29 ($r = 0.786$; $p = 0.036$) and IFN- β ($r = 0.821$; $p = 0.023$). However, no correlations were observed with TNF-stimulated production of the studied molecules (online supplemental table 2).

In the second phase of the study, quantification of gp130/sIL-6Rb and APRIL production by PBMCs was achieved through ELISA. The results indicated an upward trend, with TNF stimulation leading to increased secretion of gp130/sIL-6Rb across the study groups. Comparison of unstimulated gp130/sIL-6Rb production using the Kruskal-Wallis test revealed differences among the study groups, and Dunn's multiple comparison tests demonstrated higher median levels in unstimulated PBMCs of patients with rhusus compared with patients with RA. Furthermore, a tendency towards higher gp130/sIL-6Rb production in unstimulated PBMCs of patients with rhusus compared with controls was observed. Similarly, TNF-stimulated PBMCs from patients with rhusus displayed a tendency towards higher production compared with patients with RA (online supplemental figure 1A).

Finally, the analysis of APRIL production levels revealed a tendency towards higher levels following TNF stimulation. Notably, unstimulated APRIL production in PBMCs appeared similar between patients with rhusus and SLE, with a tendency towards higher levels in patients with

rhusus compared with patients with RA. While levels of APRIL in TNF-stimulated PBMCs remained similar across the study groups, higher production was observed in patients with rhusus compared with patients with RA (online supplemental figure 1B).

DISCUSSION

Rhusus, a complex condition co-occurring with both SLE and RA features, presents with elevated levels of various biomarkers, including anti-dsDNA antibodies, ACPA, C-reactive protein (CRP) and erythrocyte sedimentation rate.^{15–17} However, no studies have yet investigated the cytokine and chemokine profiles in rhusus. This study found that PBMCs from patients with rhusus spontaneously produce different molecules, such as gp130/sIL-6Rb, APRIL, IFN- β , MMP3 and IL-12.

Previous studies have reported elevated levels of cytokines and chemokines in both patients with RA and SLE compared with healthy individuals. In patients with RA, unstimulated cells have higher levels of IL-1, IL-6, IL-10 and TNF, while in patients with SLE, unstimulated cells produce higher levels of IFN- γ , IL-4 and IP-10.^{18,19} Although the molecules upregulated in patients with rhusus, such as gp130/sIL-6Rb, IFN- β and IL-12, may belong to different cytokine families, they all activate the JAK/STAT signalling pathway, which can promote inflammation in patients with rhusus.²⁰ For instance, activation of the JAK/STAT pathway by gp130/sIL-6Rb can promote synovial tissue proliferation and osteoclast differentiation.²¹ In patients with SLE, surface expression of gp130/sIL-6Rb on B and T CD4⁺ cells is higher than in healthy controls, and its expression on B cells correlates with disease activity.²² Meanwhile, IFN- β expression is increased in the synovial tissue in patients with RA, and some immune cells, such as macrophages and dendritic cells, express IFN- β .²³ Likewise, patients with SLE have

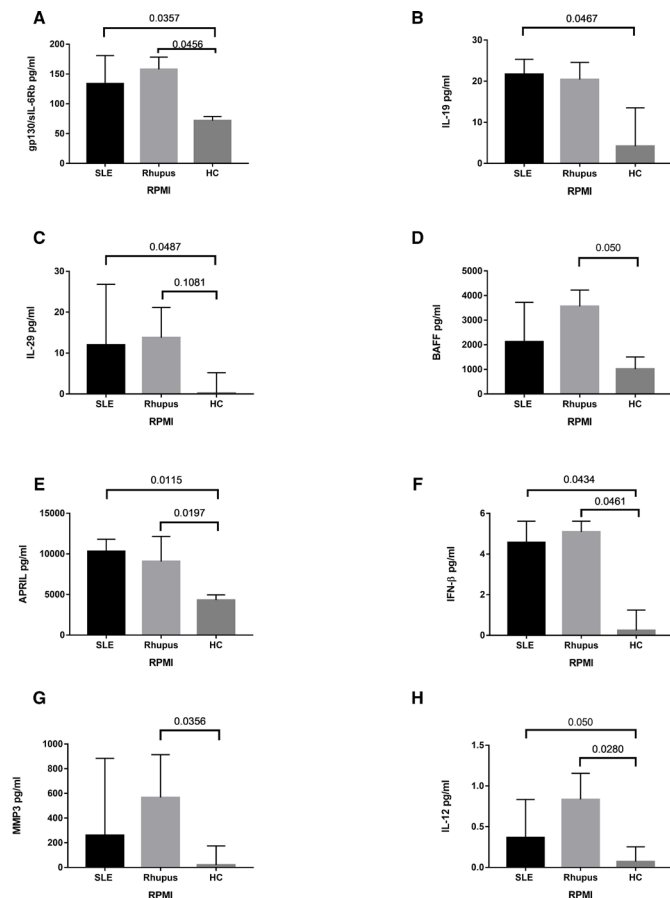


Figure 1 Increased production levels of molecules in unstimulated PBMCs from patients with rhupus. We observed that production of gp130/sIL-6Rb (A), IL-19 (B), IL-29 (C), BAFF (D), APRIL (E), IFN- β (F), MMP3 (G) and IL-12 (H) was different between groups (SLE, rhupus and healthy controls (HC)). Analyses were performed using Kruskal-Wallis test followed by Dunn's multiple comparison tests. Data are expressed as median (IQR). APRIL, a proliferation-inducing ligand; BAFF, B cell-activating factor; IFN, interferon; IL, interleukin; MMP3, matrix metalloproteinase-3; PBMCs, peripheral blood mononuclear cells.

higher percentages of IFN- β ⁺ naïve B cells, which is associated with a history of lupus nephritis.²⁴

Notably, the production of gp130/sIL-6Rb was elevated in stimulated PBMCs from patients with rhupus. This transmembrane protein is expressed widely in haematopoietic and non-haematopoietic cells, and the cytokines that signal through the gp130/sIL-6Rb pathway regulate the differentiation and activity of osteoblasts, osteoclasts and chondrocytes.^{25 26} Although it remains unclear how TNF regulates gp130/sIL-6Rb expression in rhupus, studies have demonstrated that TNF can regulate gp130/sIL-6Rb expression in PC12 cells and sympathetic neurons. Additionally, treating human umbilical vein endothelial cells with TNF leads to an increase in gp130/sIL-6Rb levels in cell lysates, indicating that TNF stimuli in endothelial cells may promote a proinflammatory response.^{27 28} Recent studies have emphasised the crucial role of TNF as a cytokine that governs the production

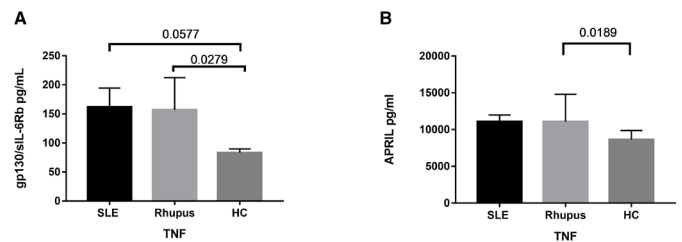


Figure 2 Increased production of gp130/sIL-6Rb and APRIL by PBMCs stimulated with TNF from patients with rhupus. We observed that production of gp130/sIL-6Rb (A) and APRIL (B) was different between groups (SLE, rhupus and healthy controls (HC)). Analyses were performed using Kruskal-Wallis test followed by Dunn's multiple comparison test. Data are expressed as median (IQR). APRIL, a proliferation-inducing ligand; PBMCs, peripheral blood mononuclear cells; TNF, tumour necrosis factor.

of proinflammatory cytokines and chemokines. TNF also plays a significant role in the synthesis of chemokines by eosinophils, such as CCL17, CCL22, CXCL9 and CXCL10.^{1 29}

Our study also highlighted the heightened production of APRIL by unstimulated PBMCs in patients with rhupus. The role of APRIL in sustaining autoreactive cell survival, immunoglobulin-class switching and cell maturation is pivotal in both SLE and RA pathogenesis.^{30 31} Increased levels of APRIL are present in inflamed joint synovial fluid and its association with arthritis in patients with SLE accentuates its role.³² Interestingly, APRIL production is increased in PBMCs from patients with rhupus following TNF stimulation. Recent studies have shown that the expression of APRIL is upregulated in dendritic cells upon stimulation with IFN- α and IFN- γ .³³ Both patients with SLE and RA have higher serum levels of APRIL, which contributes to autoimmune diseases by sustaining B cell activation.³⁴ In patients with RA, the presence of cells producing and secreting APRIL is a common feature, and the synovium of these patients retains soluble APRIL, generating APRIL-rich niches.³⁵

Our findings also revealed significant differences in the production levels of gp130/sIL-6Rb and APRIL between patients with rhupus and RA, strengthening the notion that the two conditions have distinct cytokine profiles. Although the Luminex and ELISA are different, our results again show that PBMCs from patients with rhupus exhibit spontaneous production of gp130/sIL-6Rb. The observed elevation of gp130/sIL-6Rb may activate the IL-6/IL-6 receptor system, central to various autoimmune and inflammatory diseases, including RA.^{36 37} Furthermore, the cytokine profiles of PBMCs from patients with rhupus and SLE were notably similar, aligning with previous studies linking IL-6 to clinical and ultrasound arthritis measures in SLE.³⁸ On the other hand, our results suggest a TNF-regulated production of gp130/sIL-6Rb in patients with rhupus, even though the mechanisms underlying this regulation remain unclear.

A noteworthy finding of this study is the elevated production of APRIL in unstimulated PBMCs derived

from patients with rhus. Curiously, this production of APRIL exhibited similarity between unstimulated PBMCs from patients with rhus and RA. APRIL, a molecule recognised for its role in promoting the proliferation and differentiation of B cells, as well as the upregulation of B cell-effector molecules, holds significance within this context.³⁰ Notably, various cell types participate in the production and secretion of APRIL. Neutrophils and macrophages have been identified as sources of APRIL in the lesions of patients with RA, while certain patients with SLE display increased serum levels of APRIL.^{35–39}

In conclusion, our study sheds light on the cytokine and chemokine profiles of patients with rhus, revealing the potential impact of TNF on the expression of key molecules like gp130/sIL-6Rb and APRIL. Despite limitations due to the rare nature of rhus and the absence of functional assays, our results highlight TNF as a potential therapeutic target for managing rhus. As we navigate the complex landscape of rhus, further investigations are essential to corroborate our findings and unravel the intricacies of its underlying mechanisms.

Contributors YJ-V had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis and serves as guarantor. Conceptualisation—LMA-G, FS-M, SG-G, YV-P and YJ-V. Methodology, formal analysis and investigation—LMA-G and YJ-V. Resources—SG-G, YV-P and YJ-V. Writing (original draft)—LMA-G, YV-P and YJ-V. Funding acquisition—FS-M, SG-G, RM-V and EB-V. Supervision and project administration—LMA-G, RM-V and EB-V. All authors have read and agreed to the published version of the manuscript.

Funding The Open Access funding for this article was supported by the Instituto Nacional de Cardiología Ignacio Chávez.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by the local ethics committee of Instituto Nacional de Cardiología Ignacio Chávez (no. 10–662). Procedures were conducted according to ethical standards outlined in the 2013 Declaration of Helsinki and local regulations. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplemental information.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

Yaneli Juárez-Vicuña <http://orcid.org/0000-0002-6467-6638>

REFERENCES

- Jang D-I, Lee A-H, Shin H-Y, *et al.* The role of tumor necrosis factor alpha (TNF- α) in autoimmune disease and current TNF- α inhibitors in therapeutics. *Int J Mol Sci* 2021;22:2719.
- Moelants EAV, Mortier A, Van Damme J, *et al.* Regulation of TNF- α with a focus on rheumatoid arthritis. *Immunol Cell Biol* 2013;91:393–401.
- Wysocki T, Paradowska-Gorycka A. Pharmacogenomics of anti-TNF treatment response marks a new era of tailored rheumatoid arthritis therapy. *Int J Mol Sci* 2022;23:2366.
- Yarilina A, Park-Min K-H, Antoniv T, *et al.* TNF activates an IRF1-dependent autocrine loop leading to sustained expression of chemokines and Stat1-dependent type I interferon-response genes. *Nat Immunol* 2008;9:378–87.
- Notley CA, Inglis JJ, Alzabin S, *et al.* Blockade of tumor necrosis factor in collagen-induced arthritis reveals a novel immunoregulatory pathway for Th1 and Th17 cells. *J Exp Med* 2008;205:2491–7.
- Goldring SR. Pathogenesis of bone erosions in rheumatoid arthritis. *Curr Opin Rheumatol* 2002;14:406–10.
- Amezcu-Guerra LM. Overlap between systemic lupus erythematosus and rheumatoid arthritis: is it real or just an illusion? *J Rheumatol* 2009;36:4–6.
- Panush RS, Edwards NL, Longley S, *et al.* Rhus' syndrome. *Arch Intern Med* 1988;148:1633–6.
- Ahsan H. Rhus: dual rheumatic disease. *J Immunoassay Immunochem* 2022;43:119–28.
- Lozada-Navarro AC, Castillo-Martínez D, Moreno-Ramírez M, *et al.* An imbalance in the T-helper phenotypes displayed by senescent CD4(+)CD28(Null) T cells is associated with erosive arthritis (Rhus syndrome) in systemic lupus erythematosus. *Lupus* 2018;27:2155–60.
- Li J, Wu H, Huang X, *et al.* Clinical analysis of 56 patients with Rhus syndrome: manifestations and comparisons with systemic lupus erythematosus: a retrospective case-control study. *Medicine (Baltimore)* 2014;93:e49.
- Amezcu-Guerra LM, Springall R, Marquez-Velasco R, *et al.* "Presence of antibodies against cyclic Citrullinated peptides in patients with 'Rhus': a cross-sectional study". *Arthritis Res Ther* 2006;8:R144.
- Aletaha D, Neogi T, Silman AJ, *et al.* Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
- Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
- Tani C, D'Aniello D, Delle Sedie A, *et al.* Rhus syndrome: assessment of its prevalence and its clinical and instrumental characteristics in a prospective cohort of 103 SLE patients. *Autoimmun Rev* 2013;12:537–41.
- Frade-Sosa B, Narváez J, Salman-Monte TC, *et al.* A comparative study on clinical and serological characteristics between patients with Rhus and those with systemic lupus erythematosus and rheumatoid arthritis. *Lupus* 2020;29:1216–26.
- Amezcu-Guerra LM, Márquez-Velasco R, Bojalil R. Erosive arthritis in systemic lupus erythematosus is associated with high serum C-reactive protein and anti-cyclic citrullinated peptide antibodies. *Inflamm Res* 2008;57:555–7.
- Havemose-Poulsen A, Sørensen LK, Stoltze K, *et al.* Cytokine profiles in peripheral blood and whole blood cell cultures associated with aggressive periodontitis, juvenile idiopathic arthritis, and rheumatoid arthritis. *J Periodontol* 2005;76:2276–85.
- Lit LCW, Wong CK, Tam LS, *et al.* Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus. *Ann Rheum Dis* 2006;65:209–15.
- Hu X, Li J, Fu M, *et al.* The JAK/STAT signaling pathway: from bench to clinic. *Signal Transduct Target Ther* 2021;6:402.
- Miao P, Zhou XW, Wang P, *et al.* Regulatory effect of anti-Gp130 functional mAb on IL-6 mediated RANKL and WNT5A expression through JAK-STAT3 signaling pathway in FLS. *Oncotarget* 2018;9:20366–76.
- De La Torre M, Urra JM, Blanco J. Raised expression of cytokine receptor Gp130 subunit on peripheral lymphocytes of patients with active lupus. a useful tool for monitoring the disease activity *Lupus* 2009;18:216–22.
- van Holten J, Smeets TJM, Blankert P, *et al.* Expression of interferon beta in Synovial tissue from patients with rheumatoid arthritis: comparison with patients with osteoarthritis and reactive arthritis. *Ann Rheum Dis* 2005;64:1780–2.

- 24 Alduraibi F, Fatima H, Hamilton JA, *et al.* Lupus nephritis correlates with B cell interferon- β , anti-Smith, and anti-DNA: a retrospective study. *Arthritis Res Ther* 2022;24:87.
- 25 Betz UA, Müller W. Regulated expression of GP130 and IL-6 receptor alpha chain in T cell maturation and activation. *Int Immunol* 1998;10:1175–84.
- 26 Sims NA, Walsh NC. GP130 cytokines and bone remodelling in health and disease. *BMB Rep* 2010;43:513–23.
- 27 März P, Gadiant RA, Otten U. Expression of Interleukin-6 receptor (IL-6R) and Gp130 mRNA in Pc12 cells and sympathetic neurons: modulation by tumor necrosis factor alpha (TNF-alpha). *Brain Res* 1996;706:71–9.
- 28 Zegeye MM, Lindkvist M, Fälker K, *et al.* Activation of the JAK/Stat3 and PI3K/AKT pathways are crucial for IL-6 Trans-signaling-mediated pro-inflammatory response in human vascular endothelial cells. *Cell Commun Signal* 2018;16:55.
- 29 Liu LY, Bates ME, Jarjour NN, *et al.* Generation of Th1 and Th2 Chemokines by human Eosinophils: evidence for a critical role of TNF-alpha. *J Immunol* 2007;179:4840–8.
- 30 Shabgah AG, Shariati-Sarabi Z, Tavakkol-Afshari J, *et al.* The role of BAFF and APRIL in rheumatoid arthritis. *J Cell Physiol* 2019;234:17050–63.
- 31 Salazar-Camarena DC, Ortiz-Lazareno P, Marín-Rosales M, *et al.* BAFF-R and TACI expression on CD3+ T cells: interplay among BAFF, APRIL and T helper cytokines profile in systemic lupus erythematosus. *Cytokine* 2019;114:115–27.
- 32 Tan S-M, Xu D, Roschke V, *et al.* Local production of B lymphocyte stimulator protein and APRIL in arthritic joints of patients with inflammatory arthritis. *Arthritis Rheum* 2003;48:982–92.
- 33 Litinskiy MB, Nardelli B, Hilbert DM, *et al.* Dcs induce CD40-independent immunoglobulin class switching through BLYS and APRIL. *Nat Immunol* 2002;3:822–9.
- 34 Seyler TM, Park YW, Takemura S, *et al.* BLYS and APRIL in rheumatoid arthritis. *J Clin Invest* 2005;115:3083–92.
- 35 Gabay C, Krenn V, Bosshard C, *et al.* Synovial tissues concentrate secreted APRIL. *Arthritis Res Ther* 2009;11:R144.
- 36 Mihara M, Hashizume M, Yoshida H, *et al.* IL-6/IL-6 receptor system and its role in physiological and pathological conditions. clinical science. *Clin Sci (Lond)* 2012;122:143–59.
- 37 Zou R, Huang X, Xu P. The study of GP130/the inflammatory factors regulating osteoclast differentiation in rheumatoid arthritis. *Biochem Biophys Rep* 2021;26:100934.
- 38 Ball EMA, Gibson DS, Bell AL, *et al.* Plasma IL-6 levels correlate with clinical and ultrasound measures of arthritis in patients with systemic lupus erythematosus. *Lupus* 2014;23:46–56.
- 39 Morel J, Roubille C, Planelles L, *et al.* Serum levels of tumour necrosis factor family members a proliferation-inducing ligand (APRIL) and B lymphocyte Stimulator (BLYS) are inversely correlated in systemic lupus erythematosus. *Ann Rheum Dis* 2009;68:997–1002.

Supplementary Table 1. Correlation between SELDAI-2K index and production of molecules in unstimulated and TNF-stimulated PBMCs from patients with SLE and Rhupus.

	SLE				Rhupus			
	Unstimulated PBMC		TNF-stimulated PBMC		Unstimulated PBMC		TNF-stimulated PBMC	
	Correlation coefficient (r)	P	Correlation coefficient (r)	P	Correlation coefficient (r)	P	Correlation coefficient (r)	P
IL-27	0.027	0.954	-0.901	0.006	0.058	0.901	0.193	0.679
gp130/sIL-6Rb	-0.090	0.848	-0.450	0.310	0.926	0.003	0.926	0.003
IL-22	-0.126	0.788	-0.667	0.102	0.463	0.296	0.463	0.296
sIL6-Ra	-0.432	0.333	-0.432	0.333	-0.31	0.618	-0.309	0.501
IFN-α2	-0.018	0.969	-0.697	0.082	0.272	0.554	0.193	0.679
IL-26	-0.162	0.728	-0.342	0.452	0.540	0.211	-0.077	0.869
IL-19	-0.126	0.788	-0.577	0.175	0.374	0.445	0.386	0.393
IL-20	-0.109	0.816	-0.755	0.050	0.463	0.296	0.347	0.445
IL-29	0.109	0.816	-0.631	0.129	0.579	0.174	0.077	0.869
IL-35	-0.009	0.985	-0.721	0.068	0.642	0.120	0.347	0.445
BAFF	-0.18	0.969	-0.523	0.229	0.579	0.174	-0.154	0.741
APRIL	0.991	0.000015	0.773	0.042	0.270	0.558	0.463	0.296
MMP1	0.060	0.899	-0.685	0.090	0.383	0.396	-0.039	0.935
IFN-β	0.036	0.939	-0.685	0.090	0.309	0.501	0.136	0.771

MMP3	-0.018	0.969	-0.523	0.229	0.759	0.048	0.386	0.393
sCD163	-0.396	0.379	-0.667	0.102	0.463	0.296	0.347	0.445
Pentraxin 3	-0.473	0.284	-0.396	0.379	0.347	0.445	0.039	0.935
TSLP	-0.126	0.788	-0.739	0.058	0.280	0.543	-0.039	0.935
sCD30	-0.056	0.905	-0.847	0.016	0.193	0.679	0.077	0.869
IL-8	-0.198	0.670	-0.667	0.102	0.463	0.296	-0.270	0.558
TWEAK	0.324	0.478	-0.360	0.427	0.617	0.140	0.463	0.296
IL-28A	0.000	1.000	-0.667	0.102	0.701	0.080	0.154	0.741
sTNF-R2	-0.306	0.504	-0.342	0.452	-0.424	0.343	-0.424	0.343
Chitinase 3-like 1	-0.541	0.210	-0.793	0.033	0.641	0.121	0.501	0.252
sTNF-R1	-0.342	0.452	-0.667	0.102	0.347	0.445	-0.116	0.805
IL12	0.164	0.726	-0.748	0.053	0.331	0.469	-0.039	0.935
Osteopontin	0.342	0.452	0.252	0.585	0.386	0.393	-0.154	0.741

Spearman correlation test was used to assess the association between SLEDAI-2K index and the production of molecules in unstimulated and TNF-stimulated

PBMCs from SLE and Rhupus patients. Significant p values are in bold.

Supplementary Table 2. Correlation between the DAS28-CRP index and production of molecules in unstimulated and TNF-stimulated PBMCs from patients with Rhupus.

Molecule	Rhupus			
	Unstimulated PBMC		TNF-stimulated PBMC	
	Correlation coefficient (r)	P	Correlation coefficient (r)	P
IL-27	0.559	0.192	0.679	0.094
gp130/ sIL-6Rb	0.786	0.036	0.643	0.119
IL-22	0.536	0.215	0.750	0.052
sIL6-Ra	0.214	0.645	-0.107	0.819
IFN- α 2	0.757	0.049	0.643	0.119
IL-26	0.429	0.337	0.000	1.000
IL-19	0.571	0.180	0.536	0.215
IL-20	0.536	0.215	0.536	0.215
IL-29	0.786	0.036	0.643	0.119
IL-35	0.667	0.102	0.679	0.094
BAFF	0.571	0.180	0.500	0.253
APRIL	0.500	0.253	0.714	0.071
MMP1	0.571	0.180	0.607	0.148

IFN-β	0.821	0.023	0.667	0.102
MMP3	0.703	0.078	0.714	0.071
sCD163	0.536	0.215	0.679	0.094
Pentraxin 3	0.179	0.702	-0.071	0.879
TSLP	0.667	0.102	0.536	0.215
sCD30	0.679	0.094	0.643	0.119
IL-8	0.536	0.215	0.179	0.702
TWEAK	0.429	0.337	0.750	0.052
IL-28A	0.739	0.058	0.643	0.119
sTNF-R2	0.000	1.000	-0.143	0.760
Chitinase 3-like 1	0.556	0.195	0.286	0.535
sTNF-R1	0.536	0.215	0.357	0.432
IL12	0.667	0.102	0.607	0.148
Osteopontin	0.000	1.000	-0.071	0.879

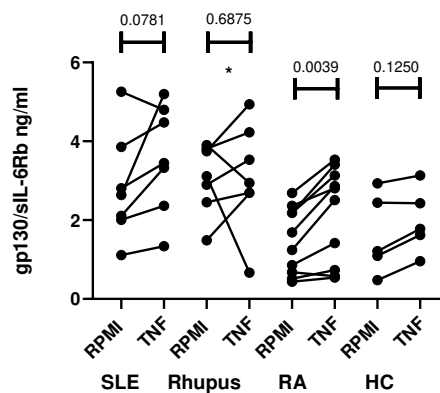
Spearman correlation test was used to assess the association between DAS28-CRP index and the production of molecules in unstimulated and TNF-stimulated PBMCs from Rhupus patients. Significant p values are in bold.

(a)

Kruskal-Wallis test	
P value	0.0161

* RPMI Rhupus vs RPMI AR
p= 0.0243

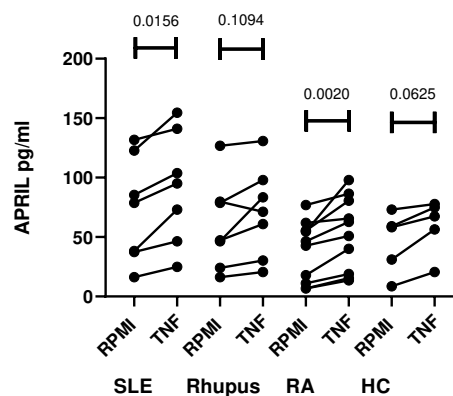
Kruskal-Wallis test	
P value	0.1188



(b)

Kruskal-Wallis test	
P value	0.3072

Kruskal-Wallis test	
P value	0.2774



Supplementary Figure 1. Increased production levels of gp130/sIL-6Rb and APRIL in PBMCs from patients with Lupus erythematosus systemic, Rhupus, Arthritis Rheumatoid and controls.

We observed that production of gp130/sIL-6Rb (a) and APRIL (b) were significantly upregulated after TNF treatment in AR patients. Furthermore, the production levels of APRIL (b) were significantly upregulated after TNF treatment in SLE

patients. APRIL: a proliferation-inducing ligand; RA: rheumatoid arthritis; HC: healthy controls; SLE: systemic lupus erythematosus. Analyses were performed using Wilcoxon paired t-test.