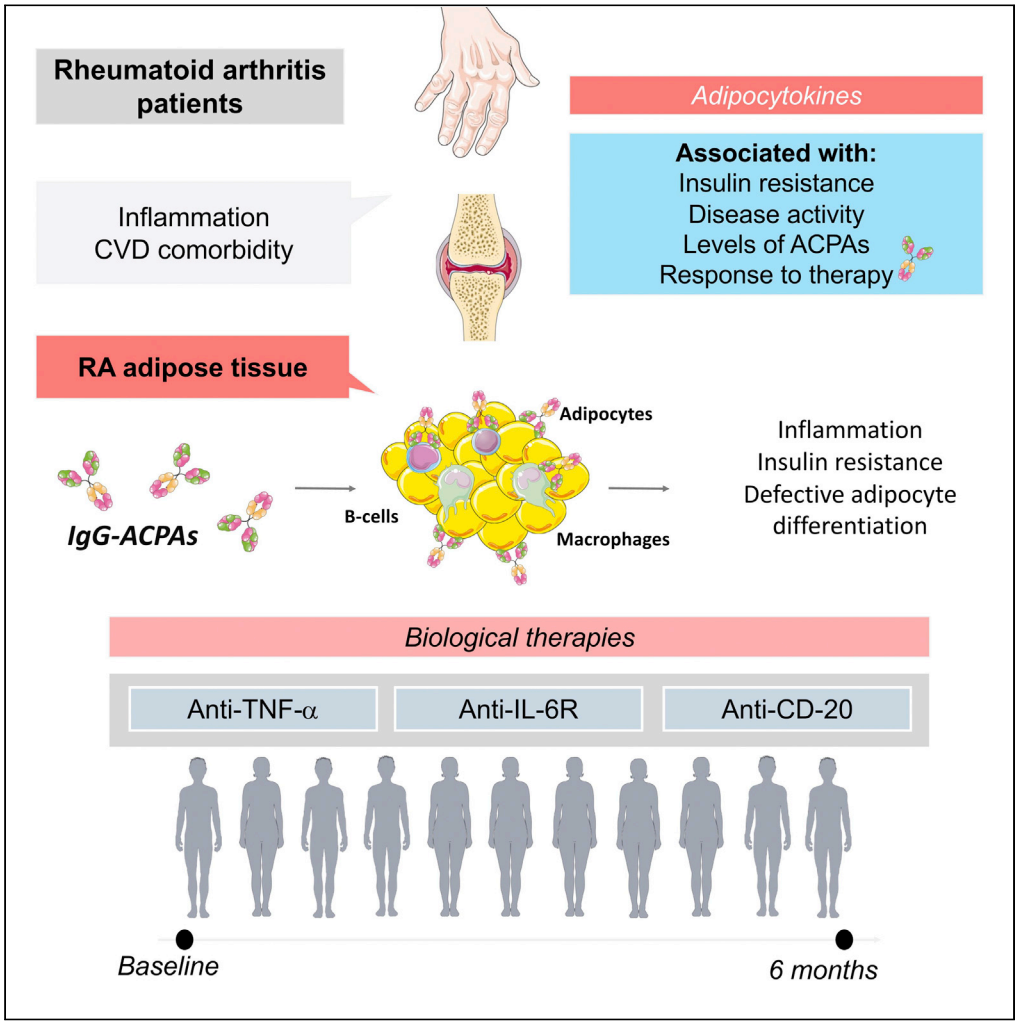
iScience

CellPress
OPEN ACCESS

Article

Pathogenic mechanisms involving the interplay between adipose tissue and auto-antibodies in rheumatoid arthritis



Iván Arias-de la Rosa, Alejandro Escudero-Contreras, Miriam Ruiz-Ponce, ..., Eduardo Collantes-Estévez, Chary López-Pedrera, Nuria Barbarroja

ivan.arias.delarosa@gmail.com (I.A.-d.l.R.)
barbarrojan@gmail.com (N.B.)

Highlights

IR is related to disease activity, inflammation, and autoimmunity in RA patients

IR state and adipocytokines might be associated with a worse response to biologics

Visfatin could be used as a potential biomarker of subclinical atherosclerosis

ACPAs might directly impact adipose tissue

Arias-de la Rosa et al., iScience 25, 104893
September 16, 2022
https://doi.org/10.1016/j.isci.2022.104893

iScience

CellPress
OPEN ACCESS

Article

Pathogenic mechanisms involving the interplay between adipose tissue and auto-antibodies in rheumatoid arthritis

Iván Arias-de la Rosa,[1,10,*] Alejandro Escudero-Contreras,[2,10] Miriam Ruiz-Ponce,[2] Laura Cuesta-López,[2] Cristóbal Román-Rodríguez,[2] Carlos Pérez-Sánchez,[2] Patricia Ruiz-Limón,[3,5] Rocío Guzman- Ruiz,[4,5] Fernando Leiva-Cepas,[6] Juan Alcaide,[3,5] Pedro Segui,[7] Chamaida Plasencia,[8] Ana Martinez-Feito,[9] Pilar Font,[2] María C. Ábalos,[2] Rafaela Ortega,[2] María M. Malagón,[4,5] Francisco J. Tinahones,[3,5] Eduardo Collantes-Estévez,[2,10] Chary López-Pedrera,[2,10] and Nuria Barbarroja[2,10,11,*]

SUMMARY

We aimed to evaluate the association between adipose tissue (AT) dysfunction, autoimmunity, and disease activity in rheumatoid arthritis (RA). A cross-sectional study including 150 RA patients and 50 healthy donors and longitudinal study with 122 RA patients treated with anti-tumor necrosis factor (TNF)-α, anti-interleukin 6 receptor (IL6R) or anti-CD20 therapies for 6 months were carried out. *In vitro* experiments with human AT and adipocyte and macrophage cell lines were performed. A collagen-induced arthritis mouse model was developed. The insulin resistance and the altered adipocytokine profile were associated with disease activity, the presence of anti-citrullinated proteins anti-bodies (ACPAs), and worse response to therapy in RA. AT in the context of arthritis is characterized by an inflammatory state alongside the infiltration of macrophages and B/plasmatic cells, where ACPAs can have a direct impact, inducing inflammation and insulin resistance in macrophages and promoting a defective adipocyte differentiation, partially restored by biologicals.

INTRODUCTION

Rheumatoid arthritis (RA) is closely related to the development of cardiovascular and other metabolic comorbidities, such as insulin resistance (IR), type 2 diabetes mellitus, or obesity (Aviña-Zubieta et al., 2008; Chung et al., 2008). In fact, RA patients might display an altered body composition since the early stages of the disease are often characterized by increased fat mass, pointing out to the adipose tissue (AT) as an important key organ regulator of outcomes in RA (Longo et al., 2019). Our group has recently demonstrated that IR can be significantly higher in RA patients with a normal weight, suggesting that the chronic inflammatory state induced by RA mediates alterations in glucose and lipid metabolism associated with this disease (Arias-de la Rosa et al., 2018).

The dysregulation of the AT in RA has been manifested by the abnormal circulating levels of adipokines, which are molecules mainly produced by this organ. These molecules not only are involved in metabolic processes but also have been associated with the pathogenesis of RA, acting in effector cells and perpetuating inflammation (Del Prete et al., 2014). However, to date, the relationship between adipokines and disease activity or autoimmunity is not completely understood yet.

AT is mainly composed of adipocytes but also can contain other immune cells that influence the regulation of metabolic, endocrine, inflammatory, and immune activities (Del Prete et al., 2014).

The most abundant immune cells in AT are macrophages that, under inflammatory or metabolic alterations, can be polarized to an inflammatory phenotype recognized as the M1 state that has a profound effect on the inflammatory burden in this organ (Caslin et al., 2020). In the subcutaneous AT of RA patients, the proportion of AT macrophages and their crown-like structures is increased and is related to the positivity of auto-antibodies, the systemic inflammatory markers, and the IR state (Giles et al., 2018). More studies are necessary to completely

[1]GENYO (Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government, PTS Granada, Granada, Spain

[2]Rheumatology Service/Department of Medical and Surgical Sciences, Maimonides Institute for Biomedicine Research of Cordoba (IMIBIC)/ /University of Cordoba/ Reina Sofia University Hospital, Córdoba, Spain

[3]Department of Endocrinology and Nutrition, Virgen de la Victoria Hospital, Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga University, Málaga, Spain

[4]Department of Cell Biology, Physiology, and Immunology, Maimonides Institute for Biomedical Research of Cordoba (IMIBIC)/University of Córdoba/Reina Sofía University Hospital, Córdoba, Spain

[5]CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain

[6]Department of Pathology, Reina Sofia University Hospital, Cordoba, Spain

[7]Radiology Service, Maimonides Institute for Biomedical Research of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Córdoba, Cordoba, Spain

[8]Rheumatology Department, La Paz University Hospital, Madrid, Spain

[9]Immunorheumatology Department, IDIPAZ, Madrid, Spain

Continued

understand the role of macrophages in the AT function in RA and to know the effects of auto-antibodies in the polarization state of macrophages.

B cells can be accumulated in distinct fat depots such as visceral, subcutaneous, and brown AT (Srikakulapu et al., 2020). Interestingly, B cells are one of the first immune cells infiltrated in the AT of obese mice and their key role in the obese-induced AT expansion has been suggested (DeFuria et al., 2013; Winer et al., 2011). Moreover, AT inflammation induced by B cells might lead to the release of pro-inflammatory immunoglobulins G (IgG) and cytokines into the circulatory system (Camell et al., 2019; Hao et al., 2011). In humans, it has recently been shown that the secretion of IgGs derived from B cells residents in subcutaneous AT led to the isotype class switching and the differentiation into plasma cells. Finally, the identification of elevated germinal B cells in human subcutaneous AT compared with peripheral blood cells in obesity could indicate that anti-bodies can be produced locally in this tissue (Frasca et al., 2018).

Taking into account the presence of auto-antibodies in RA and the evidence of the possible infiltration of B cells in the AT and the generation of anti-bodies on this tissue, it is feasible to think that autoimmunity can have a profound effect on the AT of RA patients, however, no study has evaluated the presence of B cells in the AT in the context of arthritis and the direct effect of the specific auto-antibodies for RA (anti-citrullinated proteins anti-bodies, ACPAs) on this organ and specifically in the cells that are composing it.

There is a big gap in the knowledge about AT regulation in RA. Consequently, we hypothesized that the presence of autoimmunity and chronic inflammation facilitates the development of metabolic diseases in these patients, mainly targeting AT. Thus, biological therapies aimed to inhibit the inflammatory profile and B-cells might help to ameliorate the metabolic alterations associated with RA.

Moreover, the pathogenic effects of ACPAs and systemic inflammation disturbing AT function in RA patients have been investigated. We explored this pathogenic process using multiple approaches *in vivo*, *ex vivo*, and *in vitro*, combining the characterization of two cohorts of RA patients (cross-sectional and longitudinal studies), a mouse model of collagen-induced arthritis (CIA), and experiments in explants of human AT, human macrophages, and murine 3T3-L1 adipocytes.

RESULTS

Cardiometabolic risk signs are increased in RA: insulin resistance is associated with inflammation and disease activity

Clinical and laboratory parameters of the RA patients and the healthy age- and sex-matched donors included in the cross-sectional study are described in Table 1. RA patients had an average age of 49.78 $\pm$ 8.80 years, with a disease duration of 10.92 $\pm$ 8.75 years and moderate disease activity with a mean Disease activity score 28 (DAS28) of 4.36 $\pm$ 1.36. Overall, patients exhibited significantly elevated levels of insulin, triglycerides, acute phase reactants (erythrocyte sedimentation rate, ESR, and C reactive protein, CRP), and complement component 3 (C3). The prevalence of cardiometabolic comorbidities such as Atherogenic and ApoB/A risks, IR, smoking, and arterial hypertension were significantly increased [Table 1]. Moreover, significant correlations among inflammatory markers (ESR, $r = 0.262$, $p = 0.04$; CRP, $r = 0.414$, $p < 0.01$) and disease activity (DAS28, $r = 0.321$, $p < 0.01$) with IR were found in our RA cohort, confirming previous studies indicating that insulin resistance is associated with systemic inflammation and disease activity in RA.

Altered adipocytokine profile in RA is associated with disease activity, insulin resistance, subclinical atherosclerosis, and autoimmunity RA patients showed elevated serum levels of cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6 [Figure 1A] and adipokines including leptin, resistin, visfatin, vaspin, and omentin compared with healthy donors (HDs). In contrast, serum levels of adiponectin (AdipoQ) were reduced in RA patients compared with HDs [Figure 1B]. In addition, levels of IL-6, leptin, resistin, and omentin were associated with disease activity showing significantly higher levels in patients with moderate-high disease activity compared with remission or low disease activity patients [Figure 1C]. Besides, IR patients based on homeostatic model assessment-insulin resistance (HOMA-IR) showed significantly lower levels of adiponectin and higher levels of TNF-α, leptin, and resistin compared with insulin sensitive (IS) patients [Figure 1D]. Correlation studies revealed that ACPAs titers positively correlated with levels of resistin, visfatin, IL-1β, TNF-α, IL-6 and negatively with omentin levels [Figure 1E]. On the other hand, 37.6% of RA patients showed a pathological carotid intima media thickness (CIMT) that was associated with increased serum levels of visfatin. In fact, Receiver Operating Characteristic (ROC) curve analysis revealed that visfatin

[10]These authors contributed equally

[11]Lead contact

*Correspondence: ivan.arias.delarosa@gmail.com (I.A.-d.l.R.), barbarrojan@gmail.com (N.B.)

https://doi.org/10.1016/j.isci.2022.104893

Table 1. Cross-sectional study

	RA patients	Healthy donors
Clinical parameters		
Female/male (*n*/*n*)	102/48 (150)	30/20 (50)
Age (years)	49.78 ± 8.80	47.10 ± 9.29
Disease duration (years)	10.92 ± 8.75	–
DAS28	4.36 ± 1.36	–
RF positivity (%)	60**	0
ACPAS positivity (%)	72**	0
Cardiometabolic comorbidities		
Atherogenic index	3.85 ± 1.06*	3.56 ± 1.05
ApoB/A index	0.61 ± 0.16*	0.55 ± 0.16
BMI (Kg/m^2)	26.78 ± 5.39*	23.30 ± 4.70
HOMA-IR	2.35 ± 1.27*	1.42 ± 0.87
AT risk (%)	34*	18
ApoB/A risk (%)	40*	25
Insulin resistance (%)	20*	10
Obesity (%)	20	12
Smoking (%)	30*	16
Arterial hypertension (%)	18*	0
Type 2 diabetes mellitus (%)	4	0
Laboratory parameters		
Glucose (mg/dL)	92.11 ± 31.15	85.04 ± 9.77
Insulin (mU/L)	8.49 ± 6.03*	6.86 ± 4.80
Total cholesterol (mg/dL)	200.57 ± 36.60	194.387 ± 34.08
HDL-cholesterol (mg/dL)	55.92 ± 16.41	56.64 ± 17.90
LDL-cholesterol (mg/dL)	122.96 ± 30.73	125.35 ± 27.38
Apolipoprotein A (mg/dL)	147.59 ± 28.64	146.89 ± 30.39
Apolipoprotein B (mg/dL)	86.99 ± 22.84	82.20 ± 24.06
Triglycerides (mg/dL)	110.05 ± 61.12**	87.27 ± 43.66
ESR (mm/h)	24.51 ± 17.80**	8.39 ± 6.54
CRP (mg/dL)	14.31 ± 22.41**	2.92 ± 11.90
C3 (mg/dL)	136.12 ± 34.83*	122.73 ± 31.20
C4 (mg/dL)	25.42 ± 9.05	25.43 ± 7.93
Ongoing treatments		
NSAIDs (*n*)	124	–
Corticosteroids (*n*)	105	–
Methotrexate (*n*)	90	–
Leflunomide (*n*)	60	–
Animalarials (*n*)	39	–

Clinical and laboratory characteristics of RA patients and healthy donors.
Data are represented by mean ± SD. RA, rheumatoid arthritis; HDs, healthy donors; DAS28, disease activity score 28; RF, rheumatoid factor; ACPAs, anti-bodies to citrullinated protein antigens; AT, atherogenic; ApoB/A, apolipoprotein B/A; BMI, body mass index; HOMA-IR, homeostatic model assessment-insulin resistance; HDL, high density lipoproteins; LDL, low density lipoproteins; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; C3, complement component 3; C4, complement component 4; NSAIDs, nonsteroidal anti-inflammatory drugs. *Significant differences vs HDs, $p < 0.05$; **significant differences vs HDs, $p < 0.01$.

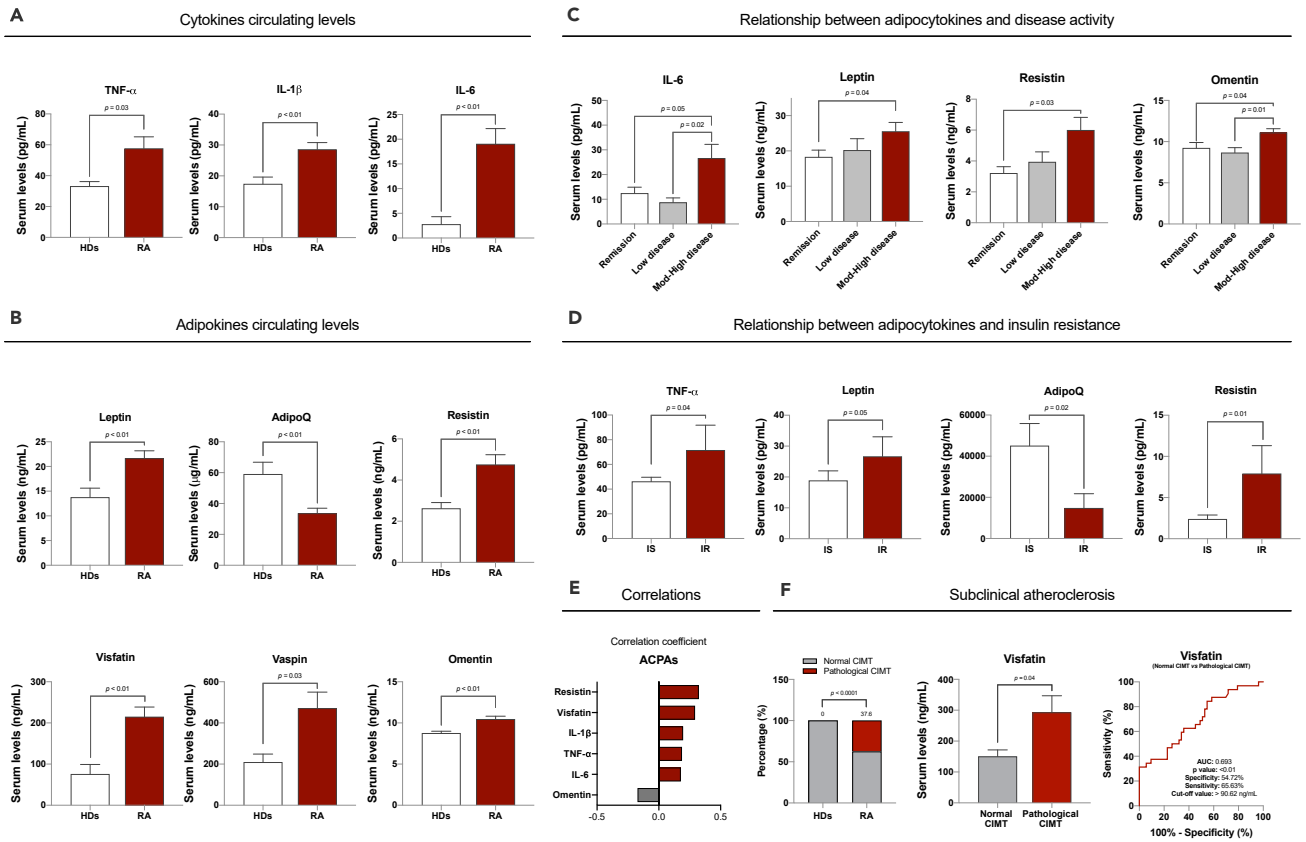


Figure 1. Association between adipocytokine profile and insulin resistance, disease activity, subclinical atherosclerosis, and autoimmunity

(A) Cytokines serum levels of RA patients compared with HDs.

(B) Adipokines serum levels of RA patients compared with HDs.

(C) Association studies of adipocytokines with disease activity.

(D) Association studies of adipocytokines with insulin resistance.

(E) Correlation analysis of adipocytokines with ACPAs titers.

(F) Percentage of RA patients with subclinical atherosclerosis, association with visfatin serum levels and ROC analysis. These analysis were performed in 150 RA patients and 50 HDs. HDs: healthy donors; RA: rheumatoid arthritis; mod: moderate; TNF-α: tumor necrosis factor alpha; IL: interleukin; AdipoQ: adiponectin; ACPAs: anti-bodies to citrullinated protein antigens; CIMT: carotid intima media thickness; AUC: area under the curve. Significance was determined by *t*-test or Mann–Whitney rank-sum test for two independent groups. One-way ANOVA or Kruskall–Wallis tests were performed for multiple comparisons. Pearson correlation coefficients were determined ($p < 0.05$ was considered statistically significant). Data are represented as mean ± SEM.

levels could independently discriminate patients with normal vs pathological CIMT (AUC = 0.693, $p < 0.01$) [Figure 1F]. These results suggest the relation between inflammation, disease activity, and autoimmunity with RA-associated AT alterations and the potential role of visfatin as an important biomarker of cardiovascular disease (CVD) risk in RA patients.

Effect of arthritis development on the AT: presence of macrophages and B cells

To analyze the effect of arthritis on the AT, we performed a CIA mice model with arthritis [Figure 2A]. The development of arthritis in mice promoted a rise in the plasma levels of TNF-α and leptin and a significant reduction of plasma levels of adiponectin, accompanied by a systemic insulin resistance state [Figure 2B] as previously demonstrated by our group (Del Prete et al., 2014). Accordingly, the mRNA expression of genes involved in the insulin and glucose signal was reduced in the gonadal white AT (gWAT) of CIA mice [Figure 2B]. Of note, we identified the presence of macrophages and B cells in the AT of CIA lean mice. Thus, in this tissue the mRNA expression of specific B2 cell markers such as CD-19, CD-20, and CD-138 [Figure 2D]. We also observed significant higher mRNA expression of a marker for macrophages (F4/80) was increased, alongside the upregulation of genes related to M1 polarization state (pro-inflammatory) (TNF-α and Monocyte chemoattractant protein, MCP-1) and the downregulation of genes associated with M2 polarization state (anti-inflammatory) (CD-206 and

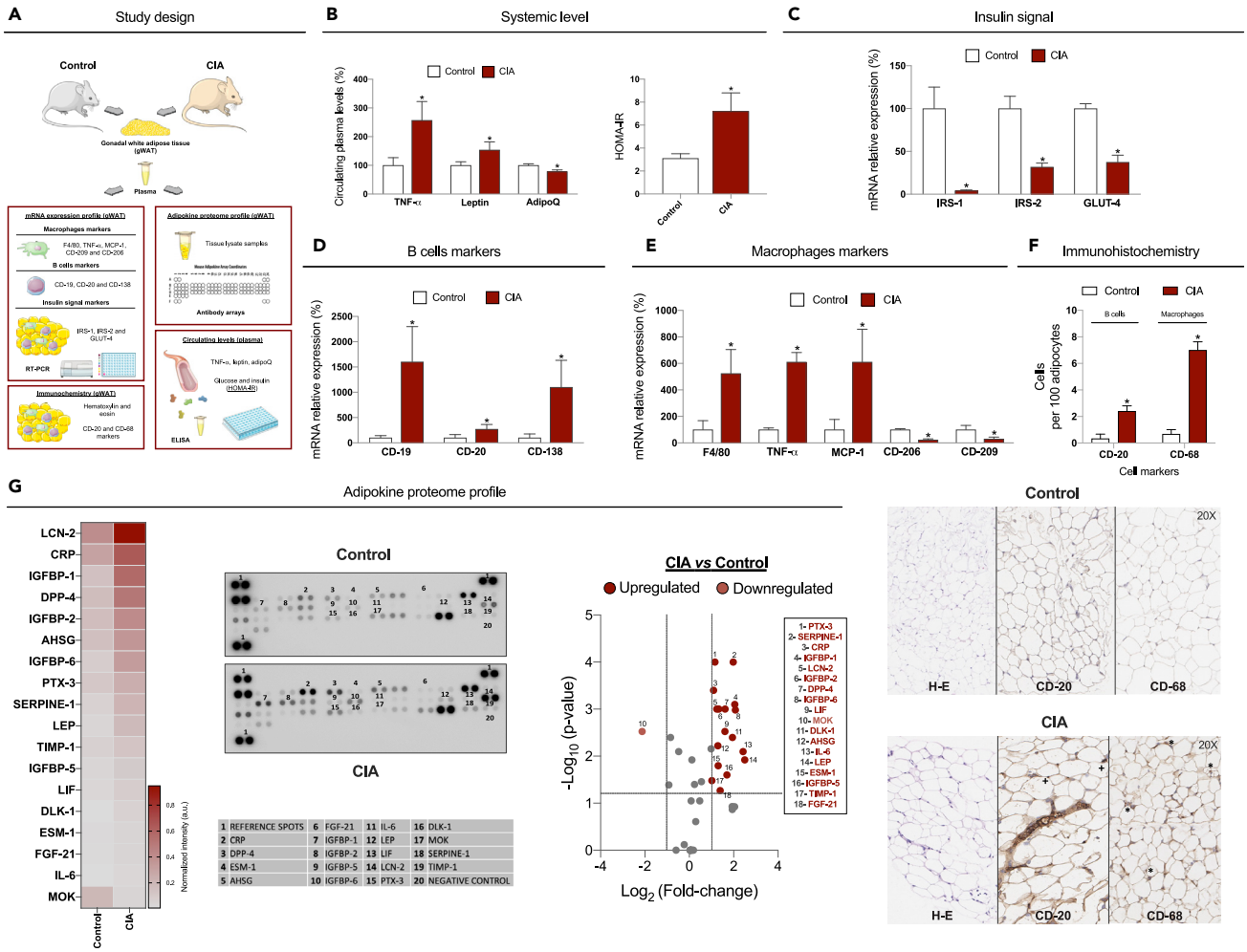


Figure 2. Effects of the CIA development at both systemic and adipose tissue levels

(A) Design of the study: control (n = 5) and CIA mice (n = 20).

(B) Plasma levels of adipocytokines and insulin resistance in CIA mice (n = 20) compared with the control group (n = 5).

(C) mRNA relative expression of genes involved insulin signal in gWAT of CIA (n = 20) and control group mice (n = 5).

(D) mRNA relative expression of B cell markers in gWAT in CIA (n = 20) and control group mice (n = 5).

(E) mRNA relative expression of markers of presence and polarization state of macrophages in gWAT of CIA (n = 20) and control group mice (n = 5).

(F) B cell and macrophage presence by immunohistochemistry of gWAT in CIA (n = 10) and control group mice (n = 5).

(G) Adipokine proteome profile in gWAT of CIA (n = 5) and control group mice (n = 5). CIA: collagen induced arthritis; gWAT: gonadal white adipose tissue; TNF-α: tumor necrosis factor alpha; MCP-1: monocyte chemotactic protein 1; CD: cluster differentiation; IRS: insulin receptor substrate; GLUT-4: glucose transporter type 4; adipoQ: adiponectin; HOMA-IR: homeostatic model assessment-insulin resistance; LCN-2: lipocalin-2; CRP: C-reactive protein; IGFBP: insulin like growth factor binding protein; DPP-4: dipeptidylpeptidase 4; AHSG: alpha 2-HS glycoprotein; PTX-3: pentraxin 3; SERPINE-1: serpin peptidase inhibitor, clade E member 1; LEP: leptin; TIMP-1: tissue inhibitor of metalloproteinase 1; LIF: leukemia inhibitory factor; DLK-1: delta like non-canonical notch ligand 1; ESM-1: endothelial cell specific molecule 1; FGF-21: fibroblast growth factor 21; IL-6: interleukin-6; MOK: MAPK/MAK/MRK overlapping kinase; H-E: hematoxylin-eosin. Significance was determined by t-test or Mann–Whitney rank-sum test for unpaired groups. *Significant differences vs control group, $p < 0.05$. Data are represented as mean ± SEM.

CD-209) in CIA mice compared with the control group [Figure 2E]. The presence of macrophages and B cells was also confirmed by immunohistochemistry (CD-20 and CD-68 markers, respectively), as seen in Figure 2F.

In parallel with the presence of infiltrated macrophages and B cells in the white AT, an altered adipokine proteome profile was found in CIA mice compared with the control group [Figure 2G]. Thus, 18 out of the 36 proteins included in the analysis were altered, most of them upregulated in the gWAT of CIA mice compared with the non-disease group. Most of these proteins play an important role in insulin

resistance. Thus, macrophages and B cells infiltrated in the AT of CIA mice might significantly contribute to the development of insulin resistance in this tissue associated with the systemic inflammation.

Pathogenic effect of ACPAs in the white AT: whole tissue, macrophages, and adipocytes

After observing the alteration in the AT of RA patients and the relationship between adipocytokines and ACPAs, we next aimed to analyze the effects of enriched IgG-ACPAs in the human AT biology and function. In a previous study, we showed that the AT from CIA mice was altered, characterized by increased expression of inflammatory molecules and decreased expression of genes involved in lipid accumulation, lipogenesis, and insulin signal (Arias-de la Rosa et al., 2018).

The treatment of visceral AT explants from obese patients with enriched IgG-ACPAs for 24h promoted a significant upregulation in the expression of genes involved in inflammation (IL-6, MCP-1, IL-8, and TNF-α) and a significant downregulation in the expression of genes related to lipogenesis and insulin signaling compared with the IgG from normal human serum (IgG-NHS) [Figure 3A], similar to what was observed in the AT of CIA mice in our previous study, suggesting a role of ACPAs in the dysfunction of the AT in the context of RA.

Moreover, to understand the impact of ACPAs specifically on the main cells composing the AT, we first analyzed the influence of ACPAs in the differentiation process of fibroblast to adipocyte. After 9 days of differentiation, cells treated with enriched IgG-ACPAs showed a defective differentiation based on the prevention of lipid uploading compared with those non-treated or treated with IgG-NHS, as seen by the quantification of oil red O staining and bodipy 493/503 lipid probe [Figure 3B].

Besides, the treatment with enriched IgG-ACPAs promoted a significant downregulation in the expression of genes related to lipid accumulation (Diacylglycerol *o*-Acyltransferase (DGAT)-1 and DGAT-2), lipogenesis (Sterol Regulatory Element-Binding transcription factor 1(SREBP-1)A and Peroxisome Proliferator Activated Receptor-gamma (PPAR-γ) and insulin signal (Glucose Transporter Type 4, GLUT-4) during the differentiation process, as it can be observed in the analysis of the area under the curve [Figure 3C]. These results suggest that ACPAs might have a deep impact in the normal adipocyte differentiation process.

Next, we performed *in vitro* studies with macrophage derived from THP-1 cell line to evaluate the role of ACPAs in the macrophage activation and polarization. Thus, enriched IgG-ACPAs induced a significant increase in the expression of pro-inflammatory molecules such as interferon (IFN)-γ, IL-1β, IL-12, IL-23, MCP-1, IL-6, and TNF-α classically related to M1 polarized state (inflammatory phenotype) [Figure 3D]. On the other hand, the treatment with enriched IgG-ACPAs reduced the expression of genes related to an M2 polarization state (anti-inflammatory phenotype) such as IL-10, arginase (ARG)-1, and CD206 and genes related to lipid accumulation (perilipin-1, PLIN-1) and insulin signal (Insulin Receptor Substrate, IRS-1, and kinase protein B, AKT) [Figure 3E]. These data indicate that ACPAs profoundly alter the polarization state of macrophages toward a pro-inflammatory M1 phase.

Influence of insulin resistance and adipokines in the therapeutic response to biologics

In this analysis, patients from the longitudinal study under biological therapy (n = 123) were used. RA patients treated with anti-TNF-α (n = 45), anti-IL6R (n = 22), or anti-CD20 (n = 56) were evaluated at baseline and after 6 months of therapy starting accordingly to the clinical routine practice.

Clinical and laboratory parameters of RA patients included in the longitudinal study are shown in Table 2. The mean disease activity significantly improved after the three treatments. Besides, anti-TNF-α and anti-CD-20 therapies were the only ones capable of reducing the levels of rheumatoid factor and ACPAs, respectively.

According to European League Against Rheumatism (EULAR) criteria and within the expected values, 5% of patients responded to anti-TNF-α, 85.5% responded to anti-IL-6R, and 65.5% responded to anti-CD20.

Of note, RA patients with insulin resistance (classified by the levels of HOMA-IR > 2.5) at baseline showed a worse response to the treatment with the three biologics, measured by less change in DAS28 levels [Figure 4A]. Accordingly, those non-responder patients (classified according to EULAR criteria) had higher levels of HOMA-IR and insulin at baseline compared with the responder RA patients [Figures 4B–4D]. Moreover, levels of vaspin and visfatin were significantly increased before the treatment in the

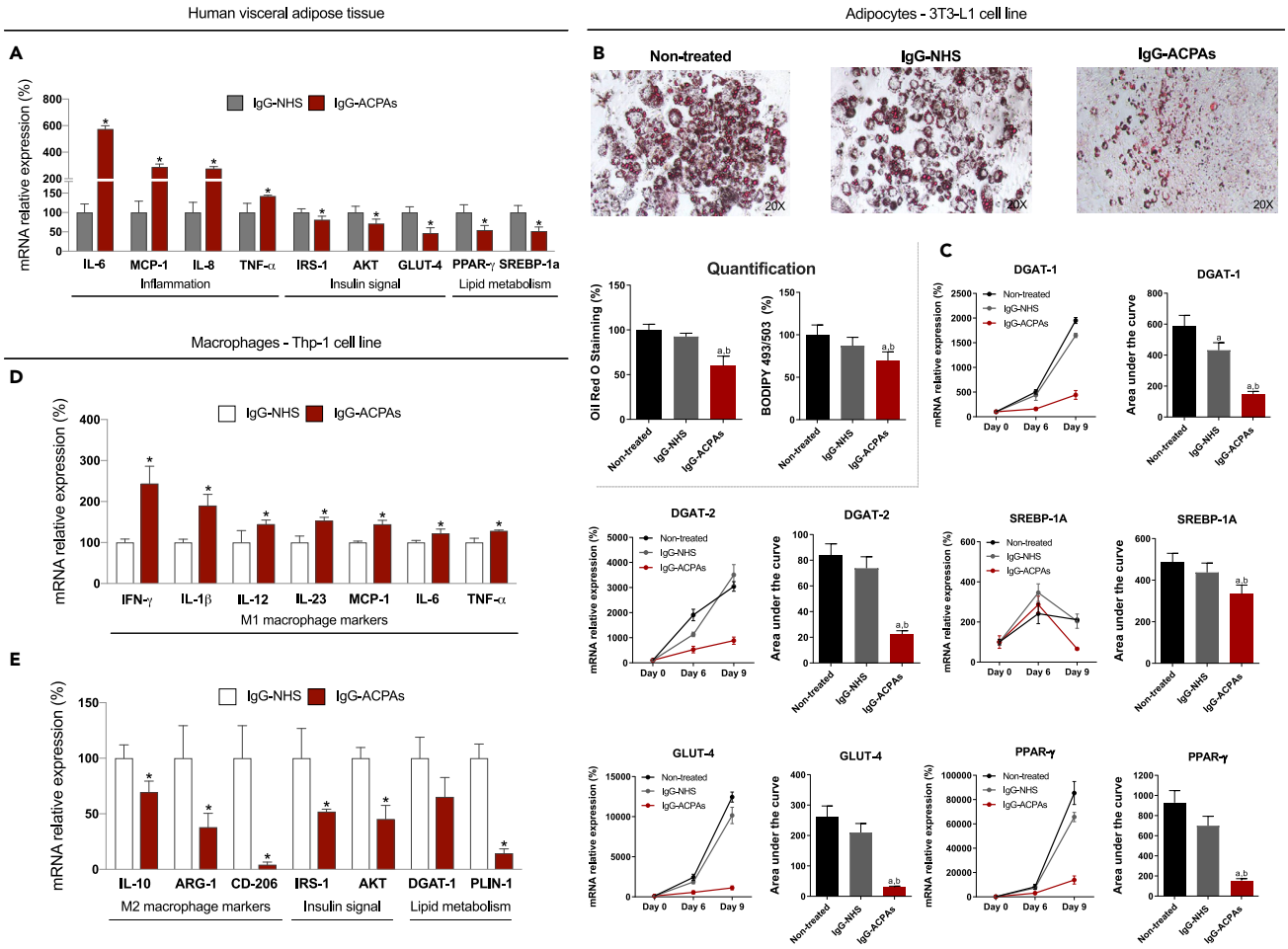


Figure 3. Direct *in vitro* effect of IgG-ACPAs on human visceral adipose tissue, adipocytes, and macrophages

(A) Expression of genes related to inflammation, insulin signal and lipid metabolism in explants of human visceral adipose tissue treated with enriched IgG-ACPAs or IgG-NHS. The experiment was performed in duplicate and repeated 10 times.

(B) Impact of enriched IgG-ACPAs in the differentiation process of fibroblast to adipocyte from day 0 to day 9: quantification of lipid content through oil red O and bodipy staining.

(C) mRNA relative expression of genes involved in lipid accumulation, lipogenesis, and insulin signal in adipocytes treated with enriched IgG-ACPAs, IgG-NHS, or non-treated during the differentiation process.

(D) mRNA relative expression of genes associated with M1 macrophage polarization state in macrophages treated with enriched IgG-ACPAs or IgG-NHS.

IgG: immunoglobulin; NHS: normal human serum; ACPAs: anti-bodies to citrullinated protein antigens; IL: interleukin; MCP-1: monocyte chemotactic protein 1; TNF-α: tumor necrosis factor alpha; IRS: insulin receptor substrate; AKT: kinase protein B; GLUT-4: glucose transporter type 4; PPAR-γ: peroxisome proliferator activated receptor gamma; SREBP-1a: sterol regulatory element-binding transcription factor 1; IFN-γ: interferon gamma; ARG-1: arginase 1; DGAT: diacylglycerol o-acyltransferase 1; PLIN-1: perilipin-1. Significance was determined by *t*-test or Mann–Whitney rank-sum test for two independent groups. One-way ANOVA or Kruskall–Wallis tests were performed for multiple comparisons. The experiments with macrophages and adipocytes were performed in triplicate and repeated three times. *Significant differences vs IgG-NHS, $p < 0.05$; [a]significant differences vs non-treated, $p < 0.05$; [b]significant differences vs IgG-NHS, $p < 0.05$. Data are represented as mean ± SEM.

(E) mRNA relative expression of genes associated with M2 macrophage polarization state, insulin signal and lipid metabolism in macrophages treated with enriched IgG-ACPAs or IgG-NHS.

non-responders group [Figures 4E and 4F] compared with the responder RA patients. These data point out the deep influence of the alteration of the AT in the response to the therapy with biologics and suggest the potential role of these adipokines as biomarkers of a therapeutic response.

In vivo effect of biologics in the adipocytokine profile

We next aimed to know the effects of the three biologics on the levels of ACPAs and the adipocytokine profile. Among all the treatments, anti-CD20 therapy was the only one capable of reducing the levels of

Table 2. Longitudinal study

	Anti-TNF-α		Anti-IL-6R		Anti-CD-20	
Time (months)	0	6	0	6	0	6
Size population (*n*)	45		22		56	
Clinical parameters						
Female/male (*n*/*n*)	34/11		20/2		19/36	
Age (years)	52.2 ± 10.5		48.73 ± 11.46		56.07 ± 11.01	
Disease duration (years)	12.2 ± 10.19		10.21 ± 6.65		15.53 ± 10.31	
DAS28	4.88 ± 0.20	3.144 ± 0.21*	5.27 ± 0.31	2.88 ± 0.29*	5.22 ± 0.19	4.00 ± 0.19*
RF (UI/mL)	123.94 ± 35.76	111.77 ± 35.18	105.44 ± 29.08	51.33 ± 11.75*	83.42 ± 30.37	97.28 ± 50.06
Laboratory parameters						
ESR (mm/h)	21.78 ± 2.22	23.36 ± 3.37	26.83 ± 5.36	5.27 ± 1.10*	29.35 ± 2.35	19.94 ± 1.81*
CRP (mg/dL)	17.35 ± 4.23	7.33 ± 1.59*	13.53 ± 3.82	0.73 ± 0.19*	10.00 ± 3.39	6.37 ± 3.15
Concomitant treatment						
NSAIDs (*n*)	37		18		39	
Corticosteroids (*n*)	36		20		41	
Methotrexate (n)	23		9		29	
Leflunomide (*n*)	18		6		24	
Antimalarials (*n*)	11		0		11	
EULAR criteria						
Responders (%)	75		85.5		65.5	

Clinical details of RA patients treated with anti-TNF-α, anti-IL6R, and anti-CD20 therapy.
Data are represented by mean ± SD DAS28: disease activity score 28; RF: rheumatoid factor; ESR: erythrocyte sedimentation rate; CRP: c-reactive protein; NSAIDs: nonsteroidal anti-inflammatory drugs; EULAR: European alliance of associations for rheumatology. *Significant differences vs T0, $p < 0.05$.

ACPAs in parallel with serum levels of IL-1β, TNF-α, IL-6, and visfatin and increased the levels of omentin and adipoQ after 6 months of treatment [Figure 4G].

Anti-TNF-α therapy for 6 months induced a significant downregulation of IL-1β, visfatin, and resistin [Figure 4H]. Finally, anti-IL6R treatment downregulated the serum levels of IL-1β and IL-6 levels and upregulated serum leptin levels after 6 months of treatment [Figure 4I]. Despite the effects of these therapies on the adipocytokine profile, in our cohort of RA patients, no changes were observed in the levels of HOMA-IR after 6 months of treatment (data not shown), suggesting that a longer time of treatment would be necessary to reduce the insulin resistance state.

These results indicated that biological therapies might impact the AT, acting on macrophages and B cells modulating inflammation and the alteration in the expression of adipokines.

In vitro effect of anti-TNF-α and anti-IL6R therapies on the adipocyte differentiation and macrophage polarization

We next aimed to evaluate the direct effects of the biologics in the adipocytes and macrophages in the context of RA. For this purpose, these cells were induced with IgG-ACPAs and treated with infliximab (anti-TNF-α) or tocilizumab (anti-IL6R). Anti-CD20 treatment was not considered in this set of experiments since its therapeutic target is the depletion of B cells.

As seen in the previous results, the treatment with enriched IgG-ACPAs promoted an impaired differentiation of adipocytes. The addition of anti-IL6R or anti-TNF-α to IgG-ACPAs since day 0 of the differentiation process was able to rescue the adipocyte differentiation as seen at day 9 by both O Red Oil and bodipy stainings [Figure 5A]. This effect was more noticeable after treatment with anti-TNF-α. Moreover, the expression of pro-adipogenic and lipogenic genes (SREBP-1A, PPAR-γ, DGAT-1, DGAT-2) was upregulated in the mature adipocytes that had been treated with IgG-ACPAs combined with anti-IL6R or anti-TNF-α since day 0 of the differentiation induction,

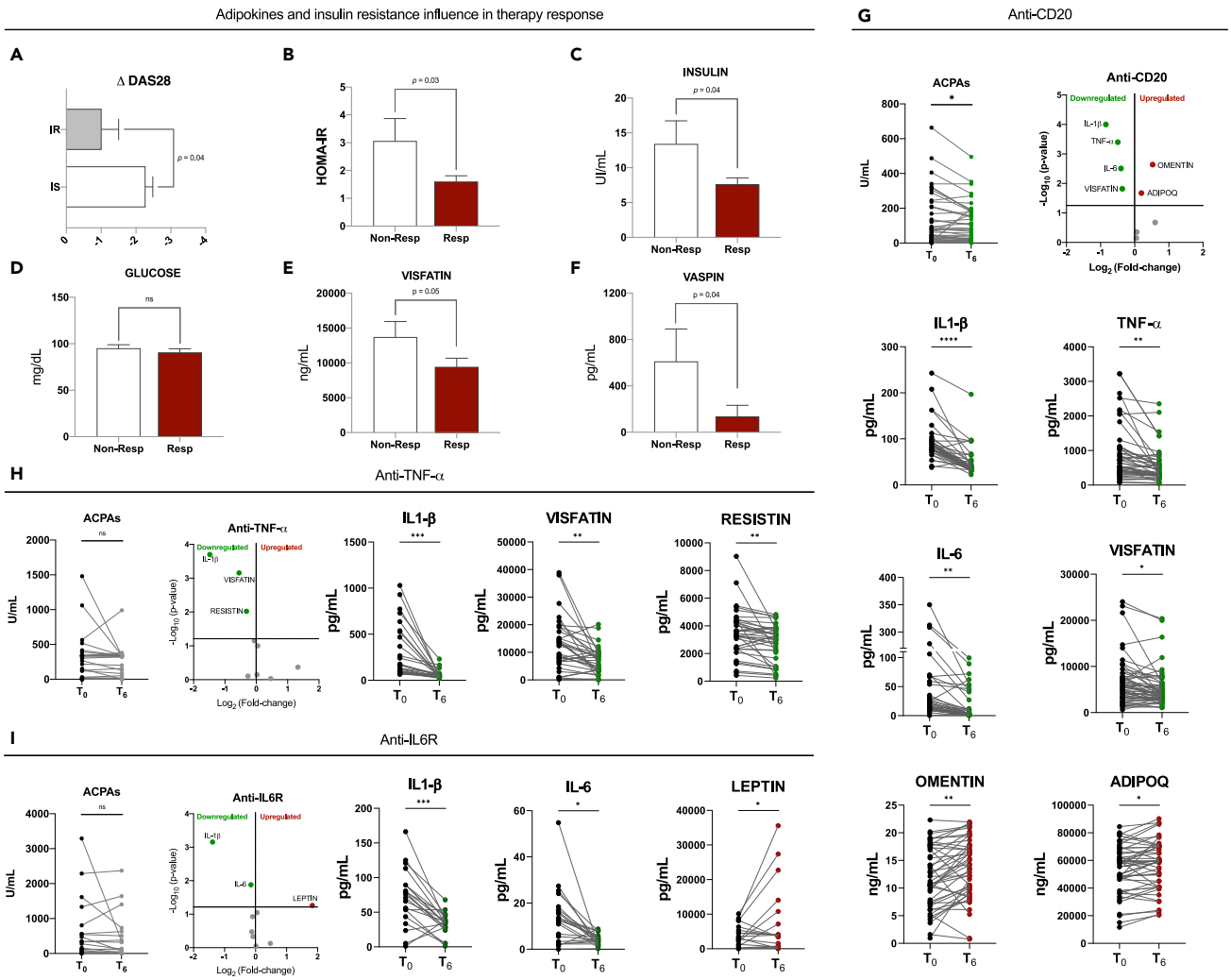


Figure 4. Effect of biological drugs. Insulin resistance influence in the therapy response

(A) Δ DAS28 in insulin resistance RA patients compared with insulin sensitivity RA patients after six months of treatment with biologicals (n = 67).

(B) HOMA-IR levels at baseline in responders and non-responder RA patients (n = 67).

(C) Insulin levels at baseline in responder and non-responder RA patients (n = 67).

(D) Glucose levels at baseline in responder and non-responder RA patients (n = 123).

(E) Visfatin levels at baseline in responder and non-responder RA patients (n = 123).

(F) Vaspin levels at baseline in responder and non-responder RA patients (n = 123).

(G) Effect of anti-CD20 therapy on the ACPAs titers and the adipocytokine profile after six months of treatment (n = 56).

(H) Effect of anti-TNF-α therapy on the ACPAs titers and the adipocytokine profile after six months of treatment (n = 45).

(I) Effect of the anti-IL6R therapy on the ACPAs titers and the adipocytokine profile after six months of treatment (n = 22). DAS28: disease activity score 28; HOMA-IR: homeostatic model assessment-insulin resistance; IR: insulin resistance; IS: insulin sensitivity; ns: non-significant; ACPAs: anti-bodies to citrullinated protein antigens; Resp: responder; TNF-α: tumor necrosis factor alpha: IL: interleukin; IL-6R: interleukin 6 receptor; CD: cluster differentiation; ADIPOQ: adiponectin. Significance was determined by t-test or Mann–Whitney rank-sum test for unpaired groups. t-test or Wilcoxon matched-pairs signed rank test were used for paired groups. *Significant differences vs baseline, $p < 0.05$. **Significant differences vs baseline, $p < 0.01$. ***Significant differences vs baseline, $p < 0.001$. Data are represented as mean ± SEM.

supporting the data found in the lipid content analyses. Besides, the expression of GLUT-4 involved in insulin signal was also increased in adipocytes treated with the combined treatment of IgGs-ACPAs plus anti-IL6R or anti-TNF-α [Figure 5B].

On the other hand, the treatment of M0 macrophages with IgGs-ACPAs plus anti-IL6R therapy reduced the expression of some pro-inflammatory cytokines such as IL-6 and TNF-α [Figure 5C] and increased the expression of CD-206 [Figure 5D] altered by the treatment with enriched IgG-ACPAs alone. The treatment

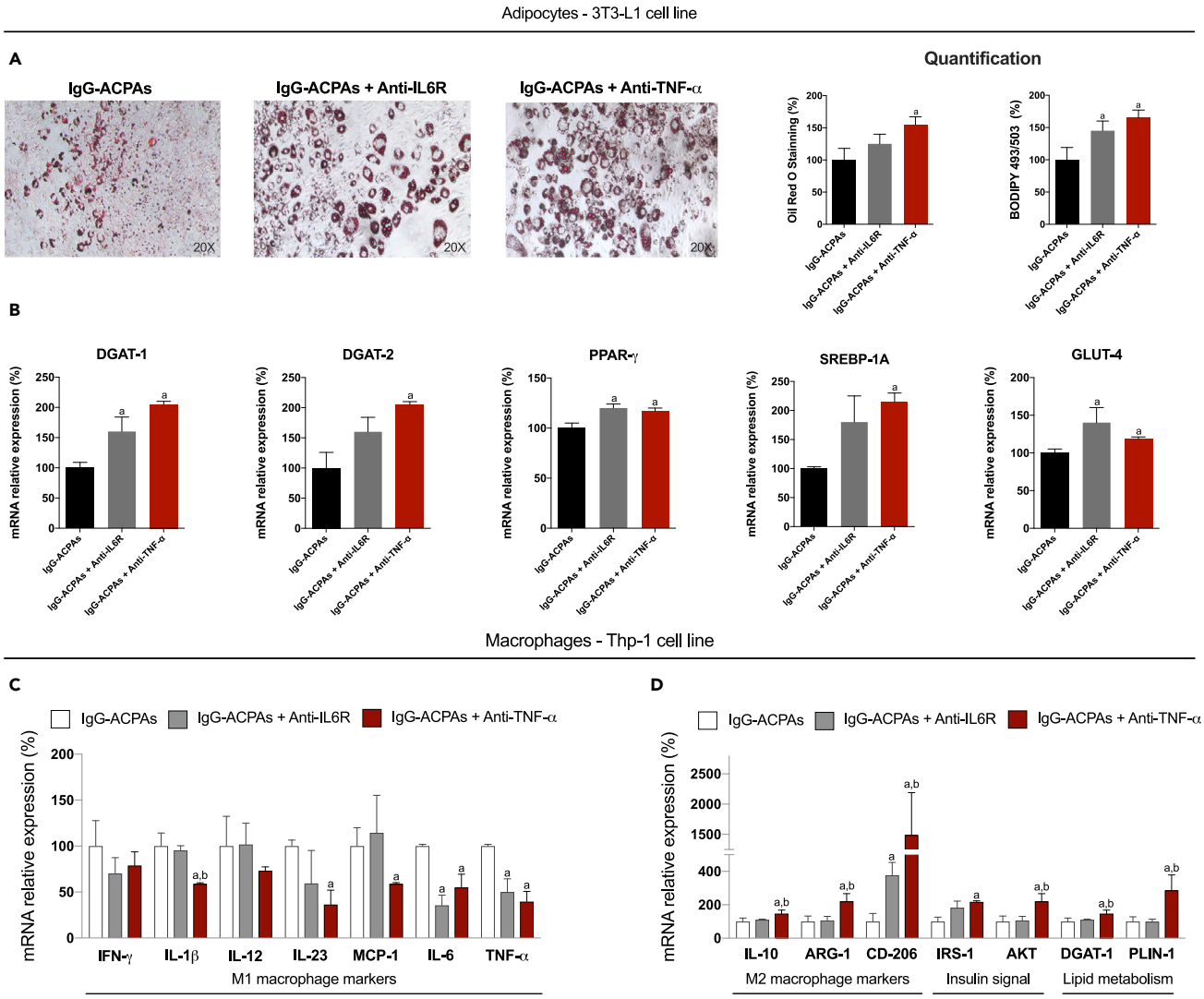


Figure 5. *In vitro* effect of biological treatments in the adipocyte differentiation and macrophage polarization

(A) Impact of anti-TNF-α and anti-IL6R in the adipocyte differentiation blockage induced by Ig G-ACPAs: O Red oil staining and lipid content analyses.

(B) mRNA relative expression of genes related to lipid accumulation, lipogenesis, and insulin signal in adipocytes at day 9 of differentiation treated with IgG-ACPAs combined with anti-TNF-α or anti-IL6R.

(C) mRNA relative expression of genes related to M1 macrophage markers in Thp-1-derived macrophages treated with IgG-ACPAs combined with anti-TNF-α or anti-IL6R for 24 h.

(D) mRNA relative expression of genes related to M2 macrophage markers, insulin signal, and lipid metabolism in Thp-1-derived macrophages treated with IgG-ACPAs combined with anti-TNF-α or anti-IL6R for 24h. IgG: immunoglobulin; ACPAs: anti-bodies to citrullinated protein antigens; DGAT: diacylglycerol *o*-acyltransferase 1; PPAR-γ: peroxisome proliferator activated receptor gamma; SREBP-1a: sterol regulatory element-binding transcription factor 1; GLUT-4: glucose transporter type 4; IFN-γ: interferon gamma; IL: interleukin; MCP-1: monocyte chemotactic protein 1; TNF-α: tumor necrosis factor alpha; IRS: insulin receptor substrate; AKT: kinase protein B; ARG-1: arginase 1; PLIN-1: perilipin-1. One-way ANOVA or Kruskall–Wallis tests were performed for multiple comparisons. Each experiment was performed in triplicate and repeated three times. [a]Significant differences vs IgG-ACPAs, $p < 0.05$; [b]Significant differences vs IgG-ACPAs + anti-IL6R, $p < 0.05$. Data are represented as mean ± SEM.

with anti-IL-6R had no effects on the expression of genes related to insulin signal compared with the treatment with IgG-ACPAs alone [Figure 5D]. The addition of anti-TNF-α to the IgG-ACPAs treatment prevented the upregulation of most of the inflammatory and M1 markers analyzed (IL-1β, IL-23, MCP-1, IL-6, and TNF-α) [Figure 5C]. In addition, this combined treatment induced the expression of IL-10, ARG-1, and CD-206, M2 markers [Figure 5D]. With respect to the expression of genes involved in insulin signaling and lipid accumulation, treatment with anti-TNF-α restored the reduced expression of IRS-1, AKT, DGAT-1, and PLIN-1 promoted by the treatment with IgG-ACPAs alone [Figure 5D].

These data support the concept that biologics can impact the cell components of the AT and that the control of inflammation, especially TNF-α might restore macrophages and adipocyte abnormalities that could be observed in the AT of RA patients.

DISCUSSION

Herein, the molecular mechanisms underlying the relationship between clinical aspects of RA (such as chronic inflammation, auto-immunity. and therapy response) and the alteration of the AT have been studied simultaneously using human (cross-sectional and longitudinal cohorts), CIA mice, explants of human AT and cellular models. Through this approach, we here describe the profound effect of RA in the AT, the pathogenic role of ACPAs in the main cell components, and the role of the AT in the therapeutic response to biologics.

In this study, the link between autoimmunity and alterations in the adipocytokine levels in a large cohort of RA patients is described. A previous study suggested that a well-known adipokine, leptin, could be implicated in the humoral response to citrullinated proteins in obese RA patients (Gómez-Bañuelos et al., 2015). In this sense, obesity has been proposed as a stimulator of auto-antibody production that might stimulate the progression of other autoimmune conditions (Tsigalou et al., 2020). These studies suggest the role of the AT in immunity in obesity; however, we here postulated that the dysfunction of the AT, produced by the chronic inflammation even in non-obese patients can have a profound impact on the pathogenesis of RA. Different cells and the cytokines produced by them have been proposed to have critical roles in the development of RA. Among them, endothelial cells, antigen-presenting cells, neutrophils, synovial fibroblast, B cell, T cell, and macrophage have been involved in the inflammation of the synovial tissue and the bone destruction [reviewed in 16]. Our work points out to the adipocyte as another relevant cell type to take into account in the pathogenesis of RA.

The imbalance in the adipocytokine profile has been described in RA (Ruscitti et al., 2018). Increasing and supporting the evidence, our work shows higher serum levels of cytokines TNFα, IL-1β, IL-6 (as it could be expected) and altered levels of the adipokines: leptin, resistin, omentin, visfatin, vaspin, and adiponectin in RA patients compared with HDs (Abella et al., 2017; Lee and Bae, 2018; Liu et al., 2015; Yoshino et al., 2011; Ozgen et al., 2010; Szumilas et al., 2020). Of note, these alterations were associated with the disease activity and the levels of ACPAs, indicating the impact of inflammation and autoimmunity on the AT.

Particularly, in our cohort of RA patients, leptin levels were associated with disease activity. In this sense, it is well recognized the role of leptin in inflammatory processes, inducing the production of cytokines such as TNF-α, IL1β, and IL6, which play a key role in the pathogenesis of RA (Abella et al., 2017; Ruscitti et al., 2018). This adipokine also has an impact on the immune response, owing to its direct effects on B cells (Liu et al., 2015). In fact, it has been described that RA patients with obesity had an elevated production of leptin according to the presence of ACPAs (Ruscitti et al., 2018). In contrast in our cohort of RA patients, no association was found between ACPAs and leptin levels, which might be owing to the low proportion of patients with obesity in our cohort.

Resistin has been implicated in the production of chemokines in fibroblast-like synoviocytes suggesting its pathogenic role in RA (Sato et al., 2017). Furthermore, higher levels of resistin were observed in synovial fluid samples from RA patients and were correlated with disease activity and joint damage. In contrast to our results, a previous study did not identify statistical differences between the blood levels of resistin in RA and HDs (Sato et al., 2017). We found higher levels of resistin in the serum of RA patients and a correlation between those levels and disease activity and ACPAs levels.

On the other hand, the knowledge about omentin and vaspin in rheumatic diseases is limited, whereas omentin levels were found higher in juvenile idiopathic arthritis (Cantarini et al., 2011) and psoriatic arthritis (Xue et al., 2012) compared with HDs, its levels were reduced in synovial fluid of RA patients compared with osteoarthritis (Senolt et al., 2010). We here provided more information about the presence of omentin in RA serum and its relationship with disease activity and ACPAs, suggesting a role of this adipokine in the pathogenesis of RA.

We further confirm the study of Ozgen M et al. in which higher serum vaspin levels in RA patients were described (Ozgen et al., 2010). Vaspin has been shown to increase in patients with type 2 diabetes mellitus (Li et al., 2011) and obesity (Feng et al., 2014), suggesting its role in IR; however, further studies should be carried out to elucidate the function of this adipokine in RA.

Finally, visfatin has been defined as a pro-inflammatory cytokine involved in TNF-mediated IR in adipocytes (McGee et al., 2011). In our study, serum levels of visfatin were increased in RA patients supporting a previous study by Lee et al. (Lee and Bae, 2018). Of note, we here test visfatin as a biomarker of subclinical atherosclerosis in RA patients. Other authors have described the presence of visfastin within and in secretomes of unstable atherosclerotic plaques and have suggested the increased visfatin levels as a risk factor in patients with T2DM and ischaemic cerebrovascular disease and as a potential therapeutical target in cardiometabolic diseases (Auguet et al., 2016; Dahl et al., 2007; Kong et al., 2014; Zheng et al., 2019). Our results show that visfastin levels were elevated in RA patients with increased CIMT and their levels could discriminate between patients with pathological and normal CIMT, pointing out visfatin as a potential biomarker for subclinical atherosclerosis in RA patients.

We previously reported that the chronic inflammation in RA mediates alterations in glucose and lipid metabolism affecting metabolic tissues including skeletal muscle and specially AT (Arias-de la Rosa et al., 2018). In addition, Giles JT et al. described the presence of AT macrophages and crown-like structures associated with the inflammatory profile, the IR state, and the serum ACPAs positivity in RA patients (Giles et al., 2018). In this regard, our study not only shows the presence of macrophages in AT of mice with arthritis but also B cells in parallel with the reduced expression of genes related to insulin signal and an altered adipokine proteome profile suggesting the role of B cells and macrophages in the dysfunction of AT in a context of arthritis.

ACPAs target different cells, molecular, and clinical mechanisms involved in RA pathogenesis (Yu and Lu, 2019). In this study, we were able to show that ACPAs directly induced alterations in visceral human AT promoting the alteration of genes related to inflammation, impaired insulin signaling, and alteration in the lipid metabolism pointing out a direct effect of ACPAs on the metabolic alterations observed in RA patients. The relevance of these results was further supported after the *in vitro* treatment of adipocytes and macrophages with enriched IgG- ACPAs, exacerbating a pro-inflammatory profile, inducing an M1 polarization state and blocking the adipocyte differentiation capacity. An imbalance between pro-inflammatory M1 macrophage and anti-inflammatory M2 macrophage has been described in the context of inflammatory RA. The dysregulation of this axis has been related to the osteoclastogenesis and the ACPA positivity (Guo et al., 2018). In our work, we found that enriched IgG-ACPAs induced the expression of M1 markers in macrophages, supporting the concept that the imbalance between M1 and M2 macrophages can be associated with the ACPAs action in RA.

The mechanism by which ACPAs stimulate cells is complex and incompletely defined. However, up to date, two main mechanisms of ACPAs pathogenicity have been proposed. Firstly, ACPAs can bind to a citrullinated protein on the cell surface triggering signaling processes. Secondly, ACPAs can be an agonist for a receptor-mediated response, reacting via Fc receptors (Toes and Pisetsky, 2019). In our work, we treated several cell lines including macrophages and 3T3-L1 cells and AT with enriched IgGs-ACPAs. In this sense, it is well known that macrophages express high levels of Fcg receptors (Catrina et al., 2021). In addition, in a recent study, we have demonstrated in *in vitro* experiments that the effects of IgGs-ACPAs on isolated human monocytes, lymphocytes, and neutrophils are mediated by Fc Receptors. For that, these leukocytes were preincubated with FcR Blocking before the treatment with purified IgG-ACPAS, blocking their effects (Ibáñez-Costa et al., 2022).

Regarding the AT and adipocytes, several Fc Receptors are expressed in the AT and have been proposed to influence the metabolism and cytokine production of this tissue (Palming et al., 2006; van Beek et al., 2015). In addition, a recent study has shown that adipocyte-differentiated 3T3-L1 express FcgRIIb and FcγRIII (Nakai et al., 2017).

On the other hand, in a recent study, citrunillation has been analyzed across 30 human tissues, AT was the fifth organ with the highest levels of citrullination, alongside the expression of PAD4 (Lee et al., 2018) (peptidyl arginine deiminase 4), an enzyme initiator of the citrullination process associated with RA (Koushik et al., 2017). In this sense, Lu et al. described how ACPAs can activate the ERK1/2 and JNK kinases through the citrullinated GRP78 (78 kDa glucose-regulated protein) on RA peripheral blood mononuclear cells (PBMCs) (Lu et al., 2013).

Taking these facts into account, the effects of ACPAs in the AT could be probably owing to these two mechanisms, dependent on Fc Receptors and independent of Fc Receptors, through their interaction with citrullinated proteins.

Our data also indicate that therapeutic approaches to inhibit B cells, by reducing the levels of ACPAs anti-bodies, might provide a potential tool to control metabolic alterations induced by RA. In this sense, our longitudinal study

has elucidated the effects of different biological therapies in the AT dysfunction through the modulation of adipocytokine profile.

Previous studies have demonstrated that the adipocytokine profile might be modulated by TNF-α and IL-6R inhibitors, although controversial results have been found (Derdemezis et al., 2009; Gonzalez-Gay et al., 2008, 2010; Hoffman et al., 2019; Wueest et al., 2021).

In vitro studies demonstrated that the pathogenic effects of ACPAs on macrophages and adipocytes might be restored first by the addition of anti-TNF-α therapy reducing inflammation, promoting the expression of genes related to an anti-inflammatory profile of macrophages (M2) and normalized glucose and lipid metabolisms and moderately by the anti-IL6R treatment that partially restored the effect of ACPAs.

Our study highlights that the importance of the AT in the pathogenesis and clinical management of the non-obese RA patients should not be underestimated and how inflammatory mediators and ACPAs can have a deep impact on the AT function in RA.

Following three strategies including human cohorts, arthritic mice, and *ex vivo* and *in vitro* experiments, our results indicate that the AT in non-obese RA patients might be characterized by the increased presence of M1 macrophages and B cells, inducing a local inflammatory state, the release of adipocytokines, and the possible production of ACPAs.

These auto-antibodies, in turn, can have a deep impact on the adipocytes and the macrophages, leading to a dysfunctional AT and affecting the rest of the body.

Thus, biological therapies aimed to reduce inflammation or the levels of ACPAs (anti-TNFα, anti-IL-6R, or anti-CD20) could directly impact the AT, modulating the altered metabolic profile in RA.

Recent biological therapies have been developed for the treatment of RA, being a revolution in the treatment of this disease [reviewed in 26]. However, a significant proportion of RA patients do not completely respond, thus there is an urgent need to identify factors in RA patients that can be markers of non-response. In this sense, an important fact observed in our cohort of RA patients is that insulin-resistant subjects are worse responders to biological therapies, supporting the contention that RA patients with IR should be taken into account in the daily clinical practice.

Finally, specific molecules mainly released by an altered AT, such as vaspin and visfatin in RA, could be monitored to identify patient responders to biological treatments.

Limitations of the study

The major limitation of this study is the lack of AT samples from RA patients' availability. Studies carried out in these samples would give us much more insight into the primary effect of RA in this organ and the pathogenic mechanisms of ACPAs in the AT. In addition, in our study, the *in vitro* treatments were carried out with IgGs isolated from patients with high levels of ACPAs and negative for rheumatoid factor. Thus, cells were treated with enriched IgGs with ACPAs. The authors are aware that the observed effects are mainly owing to the action of IgGs-ACPAs but other IgGs might be present in that sera.

Finally, another limitation was the sample size and the lack of randomization in the longitudinal cohorts. In order to confirm our results about the biomarkers for the therapy response, further studies in larger cohorts of validation should be performed.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104893.

ACKNOWLEDGMENTS

We thank all the patients for their kind participation in this study. This research was funded by grants from the Institute of Health Carlos III, ISCIII (PI17/01316, PI20/00079, PI21/005991, and RICOR-RD21/0002/0033), co-financed by the European Regional Development Fund (ERDF), a way to make Europe, Spain, MINECO (RyC-2017-23437), the Andalusian Foundation of Rheumatology (FAR) and Consejería de Conocimiento, Investigación y Universidad, Junta de Andalucía (P20_01367). C.L.-P. was supported by a contract from the Junta de Andalucia (Nicolas Monardes program). J.A. was supported by a "PFIS" predoctoral contract (FI19/00177). P.R.L. was supported by a "Sara Borrell" postdoctoral contract (CD19/00216) from the ISCIII-Madrid (Spain), co-financed by the Fondo Europeo de Desarrollo Regional-FEDER.

AUTHOR CONTRIBUTIONS

Conceptualization, supervision, writing – original draft preparation, writing – review and editing, project administration, funding acquisition: N.-B., I.A.-R., C.L.-P., A.E.-C., M.M.-M., F.J.-T., and E.C.-E. Methodology, software, validation, formal analysis, data curation: L.C.-P., R.G.-R., M.R.-P., C.P.-S., C.R.-R., P.R.-L., M.C.A.-A., J.-A., F.L.-C. Data curation and followed up with patients: P.S., C.P., A.M.-F., R.O., and P.F.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: March 7, 2022
Revised: July 4, 2022
Accepted: August 3, 2022
Published: September 16, 2022

REFERENCES

Abella, V., Scotece, M., Conde, J., Pino, J., Gonzalez-Gay, M.A., Gómez-Reino, J.J., Mera, A., Lago, F., Gómez, R., and Gualillo, O. (2017). Leptin in the interplay of inflammation, metabolism and immune system disorders. Nat. Rev. Rheumatol. *13*, 100–109. https://doi.org/10.1038/nrrheum.2016.209.

Aletaha, D., Neogi, T., Silman, A.J., Funovits, J., Felson, D.T., Bingham, C.O., 3rd, Birnbaum, N.S., Burmester, G.R., Bykerk, V.P., Cohen, M.D., et al. (2010). 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum. *62*, 2569–2581. https://doi.org/10.1002/art.27584.

Arias-de la Rosa, I., Escudero-Contreras, A., Rodríguez-Cuenca, S., Ruiz-Ponce, M., Jiménez-Gómez, Y., Ruiz-Limón, P., Pérez-Sánchez, C., Ábalos-Aguilera, M.C., Cecchi, I., Ortega, R., et al. (2018). Defective glucose and lipid metabolism in rheumatoid arthritis is determined by chronic inflammation in metabolic tissues. J. Intern. Med. *284*, 61–77. https://doi.org/10.1111/joim.12743.

Aron-Wisnewsky, J., Tordjman, J., Poitou, C., Darakhshan, F., Hugol, D., Basdevant, A., Aissat, A., Guerre-Millo, M., and Clément, K. (2009).

Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J. Clin. Endocrinol. Metab. *94*, 4619–4623. https://doi.org/10.1210/jc.2009-0925.

Auguet, T., Aragonès, G., Guiu-Jurado, E., Berlanga, A., Curriu, M., Martinez, S., Alibalic, A., Aguilar, C., Camara, M.L., Hernández, E., et al. (2016). Adipo/cytokines in atherosclerotic secretomes: increased visfatin levels in unstable carotid plaque. BMC Cardiovasc. Disord. *16*, 149. https://doi.org/10.1186/s12872-016-0320-5.

Aviña-Zubieta, J.A., Choi, H.K., Sadatsafavi, M., Etminan, M., Esdaile, J.M., and Lacaille, D. (2008). Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum. *59*, 1690–1697. https://doi.org/10.1002/art.24092.

Camell, C.D., Günther, P., Lee, A., Goldberg, E.L., Spadaro, O., Youm, Y.H., Bartke, A., Hubbard, G.B., Ikeno, Y., Ruddle, N.H., et al. (2019). Aging induces an Nlrp3 inflammasome-dependent expansion of adipose B cells that impairs metabolic homeostasis. Cell Metab. *30*, 1024–1039.e6. https://doi.org/10.1016/j.cmet.2019.10.006.

Cantarini, L., Simonini, G., Fioravanti, A., Generoso, M., Bacarelli, M.R., Dini, E., Galeazzi, M., and Cimaz, R. (2011). Circulating levels of the adipokines vaspin and omentin in patients with juvenile idiopathic arthritis, and relation to disease activity. Clin. Exp. Rheumatol. *29*, 1044–1048.

Caslin, H.L., Bhanot, M., Bolus, W.R., and Hasty, A.H. (2020). Adipose tissue macrophages: unique polarization and bioenergetics in obesity. Immunol. Rev. *295*, 101–113. https://doi.org/10.1111/imr.12853.

Catrina, A., Krishnamurthy, A., and Rethi, B. (2021). Current View on the Pathogenic Role of Anti-citrullinated Protein Antibodies in Rheumatoid Arthritis. RMD Open *7*, e001228. https://doi.org/10.1136/rmdopen-2020-001228.

Chung, C.P., Oeser, A., Solus, J.F., Avalos, I., Gebretsadik, T., Shintani, A., Raggi, P., Sokka, T., Pincus, T., and Stein, C.M. (2008). Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. Atherosclerosis *196*, 756–763. https://doi.org/10.1016/j.atherosclerosis.2007.01.004.

Dahl, T.B., Yndestad, A., Skjelland, M., Øie, E., Dahl, A., Michelsen, A., Damås, J.K., Tunheim, S.H., Ueland, T., Smith, C., et al. (2007). Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization. Circulation *115*, 972–980. https://doi.org/10.1161/CIRCULATIONAHA.106.665893.

DeFuria, J., Belkina, A.C., Jagannathan-Bogdan, M., Snyder-Cappione, J., Carr, J.D., Nersesova, Y.R., Markham, D., Strissel, K.J., Watkins, A.A., Zhu, M., et al. (2013). B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proc. Natl. Acad. Sci. USA *110*, 5133–5138. https://doi.org/10.1073/pnas.1215840110.

Del Prete, A., Salvi, V., and Sozzani, S. (2014). Adipokines as potential biomarkers in rheumatoid arthritis. Mediat. Inflamm. *2014*, 425068. https://doi.org/10.1155/2014/425068.

Derdemezis, C.S., Filippatos, T.D., Voulgari, P.V., Tselepis, A.D., Drosos, A.A., and Kiortsis, D.N. (2009). Effects of a 6-month infliximab treatment on plasma levels of leptin and adiponectin in patients with rheumatoid arthritis. Fundam. Clin. Pharmacol. *23*, 595–600. https://doi.org/10.1111/j.1472-8206.2009.00717.x.

Feng, R., Li, Y., Wang, C., Luo, C., Liu, L., Chuo, F., Li, Q., and Sun, C. (2014). Higher vaspin levels in subjects with obesity and type 2 diabetes mellitus: a meta -analysis. Diabetes. Res. Clin. Pract. *106*, 88–94. https://doi.org/10.1016/j.diabres.2014.07.026.

Frasca, D., Diaz, A., Romero, M., Thaller, S., and Blomberg, B.B. (2018). Secretion of autoimmune antibodies in the human subcutaneous adipose tissue. PLoS One *13*, e0197472. https://doi.org/10.1371/journal.pone.0197472.

Giles, J.T., Ferrante, A.W., Broderick, R., Zartoshti, A., Rose, J., Downer, K., Zhang, H.Z., and Winchester, R.J. (2018). Adipose tissue macrophages in rheumatoid arthritis: prevalence, disease-related indicators, and associations with cardiometabolic risk factors. Arthritis Care Res. *70*, 175–184. https://doi.org/10.1002/acr.23253.

Gómez-Bañuelos, E., Navarro-Hernández, R.E., Corona-Meraz, F., Madrigal-Ruíz, P.M., Martín-Marquez, B.T., Pizano-Martinez, O.E., Aguilar-Arreola, J., Perez-Cruz, P.J., Macias-Reyes, H., Gonzalez-Lopez, L., et al. (2015). Serum leptin and serum leptin/serum leptin receptor ratio imbalance in obese rheumatoid arthritis patients positive for anti-cyclic citrullinated peptide antibodies. Arthritis Res. Ther. *17*, 335. https://doi.org/10.1186/s13075-015-0850-8.

Gonzalez-Gay, M.A., Garcia-Unzueta, M.T., Gonzalez-Juanatey, C., Miranda- Filloy, J.A., Vazquez-Rodriguez, T.R., De Matias, J.M., Martin, J., Dessein, P.H., and Llorca, J. (2008). Anti-TNF-alpha therapy modulates resistin in patients with rheumatoid arthritis. Clin. Exp. Rheumatol. *26*, 311.

Gonzalez-Gay, M.A., Vazquez-Rodriguez, T.R., Garcia-Unzueta, M.T., Berja, A., Miranda-Filloy, J.A., de Matias, J.M., Gonzalez-Juanatey, C., and Llorca, J. (2010). Visfatin is not associated with inflammation or metabolic syndrome in patients with severe rheumatoid arthritis undergoing anti-TNF-alpha therapy. Clin. Exp. Rheumatol. *28*, 56.

Guo, Q., Wang, Y., Xu, D., Nossent, J., Pavlos, N.J., and Xu, J. (2018). Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res. *6*, 15. https://doi.org/10.1038/s41413-018-0016-9.

Guzmán-Ruiz, R., Ortega, F., Rodríguez, A., Vázquez-Martínez, R., Díaz-Ruiz, A., Garcia-Navarro, S., Giralt, M., Garcia-Rios, A., Cobo-Padilla, D., Tinahones, F.J., et al. (2014). Alarmin high-mobility group B1 (HMGB1) is regulated in human adipocytes in insulin resistance and influences insulin secretion in β-cells. Int. J. Obes. *38*, 1545–1554. https://doi.org/10.1038/ijo.2014.36.

Hao, Y., O'Neill, P., Naradikian, M.S., Scholz, J.L., and Cancro, M.P. (2011). A B cell subset uniquely responsive to innate stimuli accumulates in aged mice. Blood *118*, 1294–1304. https://doi.org/10.1182/blood-2011-01-330530.

Hoffman, E., Rahat, M.A., Feld, J., Elias, M., Rosner, I., Kaly, L., Lavie, I., Gazitt, T., and Zisman, D. (2019). Effects of tocilizumab, an anti-interleukin-6 receptor antibody, on serum lipid and adipokine levels in patients with rheumatoid arthritis. Int. J. Mol. Sci. *20*, E4633. https://doi.org/10.3390/ijms20184633.

Ibáñez-Costa, A., Pérez-Sánchez, C., Patiño-Trives, A.M., Luque-Tevar, M., Font, P., Arias de la Rosa, I., Román-Rodriguez, C., Abalos-Aguilera, M.C., Conde, C., Gonzalez, A., et al. (2022). Splicing machinery is impaired in rheumatoid arthritis, associated with disease activity and modulated by anti-TNF therapy. Ann. Rheum. Dis. *81*, 56–67. https://doi.org/10.1136/annrheumdis-2021-220308.

Kong, Q., Xia, M., Liang, R., Li, L., Cu, X., Sun, Z., and Hu, J. (2014). Increased serum visfatin as a risk factor for atherosclerosis in patients with ischaemic cerebrovascular disease. Singap. Med. J. *55*, 383–387. https://doi.org/10.11622/smedj.2014091.

Koushik, S., Joshi, N., Nagaraju, S., Mahmood, S., Mudeenahally, K., Padmavathy, R., Jegatheesan, S.K., Mullangi, R., and Rajagopal, S. (2017). PAD4: pathophysiology, current therapeutics and future perspective in rheumatoid arthritis. Expert. Opin. Ther. Targets. *21*, 433–447. https://doi.org/10.1080/14728222.2017.1294160.

Lee, Y.H., and Bae, S.C. (2018). Circulating adiponectin and visfatin levels in rheumatoid arthritis and their correlation with disease activity: a meta-analysis. Int. J. Rheum. Dis. *21*, 664–672. https://doi.org/10.1111/1756-185X.13038.

Lee, C.Y., Wang, D., Wilhelm, M., Zolg, D.P., Schmidt, T., Schnatbaum, K., Reimer, U., Pontén, F., Uhlén, M., Hahne, H., and Kuster, B. (2018). Mining the human tissue proteome for protein citrullination. Mol. Cell. Proteomics. *17*, 1378–1391. Epub 2018 Apr 2. https://doi.org/10.1074/mcp.RA118.000696.

Li, K., Li, L., Yang, M., Liu, H., Liu, D., Yang, H., Boden, G., and Yang, G. (2011). Short-term continuous subcutaneous insulin infusion decreases the plasma vaspin levels in patients with type 2 diabetes mellitus concomitant with improvement in insulin sensitivity. Eur. J. Endocrinol. *164*, 905–910. https://doi.org/10.1530/EJE-10-1023.

Lima, L.M., Carvalho, M.d., and Sousa, M.O. (2007). Apo B/apo A-I ratio and cardiovascular risk prediction. Arq. Bras. Cardiol. *88*, e187–e190. https://doi.org/10.1590/s0066-782x2007000600014.

Liu, D., Luo, S., and Li, Z. (2015). Multifaceted roles of adiponectin in rheumatoid arthritis. Int. Immunopharmacol. *28*, 1084–1090. https://doi.org/10.1016/j.intimp.2015.08.013.

Longo, M., Zatterale, F., Naderi, J., Parrillo, L., Formisano, P., Raciti, G.A., Beguinot, F., and Miele, C. (2019). Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. Int. J. Mol. Sci. *20*, E2358. https://doi.org/10.3390/ijms20092358.

Lu, M.C., Lai, N.S., Yin, W.Y., Yu, H.C., Huang, H.B., Tung, C.H., Huang, K.Y., and Yu, C.L. (2013).

Anti-citrullinated protein antibodies activated ERK1/2 and JNK mitogen-activated protein kinases via binding to surface-expressed citrullinated GRP78 on mononuclear cells. J. Clin. Immunol. *33*, 558–566. https://doi.org/10.1007/s10875-012-9841-6.

McGee, K.C., Harte, A.L., da Silva, N.F., Al-Daghri, N., Creely, S.J., Kusminski, C.M., Tripathi, G., Levick, P.L., Khanolkar, M., Evans, M., et al. (2011). Visfatin is regulated by rosiglitazone in type 2 diabetes mellitus and influenced by NFκB and JNK in human abdominal subcutaneous adipocytes. PLoS One *6*, e20287. https://doi.org/10.1371/journal.pone.0020287.

Millán, J., Pintó, X., Muñoz, A., Zúñiga, M., Rubiés-Prat, J., Pallardo, L.F., Masana, L., Mangas, A., Hernández-Mijares, A., González-Santos, P., et al. (2009). Lipoprotein ratios: physiological significance and clinical usefulness in cardiovascular prevention. Vasc. Health Risk Manag. *5*, 757.

Nakai, K., Tanaka, H., Yamanaka, K., Takahashi, Y., Murakami, F., Matsuike, R., Sekino, J., Tanabe, N., Morita, T., Yamazaki, Y., et al. (2017). Effects of C-reactive protein on the expression of matrix metalloproteinases and their inhibitors via Fcγ receptors on 3T3-L1 adipocytes. Int. J. Med. Sci. *14*, 484–493. https://doi.org/10.7150/ijms.18059.

Ozgen, M., Koca, S.S., Dagli, N., Balin, M., Ustundag, B., and Isik, A. (2010). Serum adiponectin, adiponectin and vaspin levels in rheumatoid arthritis. Arch. Med. Res. *41*, 457–463. https://doi.org/10.1016/j.arcmed.2010.08.012.

Palming, J., Gabrielsson, B.G., Jennische, E., Smith, U., Carlsson, B., Lena M S Carlsson, L., and Lönn, M. (2006). Plasma cells and Fc receptors in human adipose tissue–lipogenic and anti-inflammatory effects of immunoglobulins on adipocytes. Biochem. Biophys. Res. Commun. *343*, 43–48. https://doi.org/10.1016/j.bbrc.2006.02.114.

Pfaffl, M.W., Tichopad, A., Prgomet, C., and Neuvians, T.P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity BestKeeper–Excel-based tool using pair-wise correlations. Biotechnol. Lett. *26*, 509–515. https://doi.org/10.1023/b:bile.0000019559.84305.47.

Prevoo, M.L., van 't Hof, M.A., Kuper, H.H., van Leeuwen, M.A., van de Putte, L.B., and van Riel, P.L. (1995). Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. *38*, 44–48. https://doi.org/10.1002/art.1780380107.

Ramírez-Zacarías, J.L., Castro-Muñozledo, F., and Kuri-Harcuch, W. (1992). Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry *97*, 493–497. https://doi.org/10.1007/BF00316069.

Ruscitti, P., Di Benedetto, P., Berardicurti, O., Liakouli, V., Carubbi, F., Cipriani, P., and Giacomelli, R. (2018). Adipocytokines in rheumatoid arthritis: the hidden link between inflammation and cardiometabolic comorbidities. J. Immunol. Res. *2018*, 8410182. https://doi.org/10.1155/2018/8410182.

Sato, H., Muraoka, S., Kusunoki, N., Masuoka, S., Yamada, S., Ogasawara, H., Imai, T., Akasaka, Y., Tochigi, N., Takahashi, H., et al. (2017). Resistin upregulates chemokine production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. Arthritis Res. Ther. *19*, 263. https://doi.org/10.1186/s13075-017-1472-0.

Senolt, L., Polanská, M., Filková, M., Cerezo, L.A., Pavelka, K., Gay, S., Haluzík, M., and Vencovsky, J. (2010). Vaspin and omentin: new adipokines differentially regulated at the site of inflammation in rheumatoid arthritis. Ann. Rheum. Dis. *69*, 1410–1411. https://doi.org/10.1136/ard.2009.119735.

Solomon, D.H., Garg, R., Lu, B., Todd, D.J., Mercer, E., Norton, T., and Massarotti, E. (2014). Effect of hydroxychloroquine on insulin sensitivity and lipid parameters in rheumatoid arthritis patients without diabetes mellitus: a randomized blinded crossover trial. Arthritis Care Res. *66*, 1246–1251. https://doi.org/10.1002/acr.22285.

Srikakulapu, P., and McNamara, C.A. (2020). B lymphocytes and adipose tissue inflammation. Arterioscler. Thromb. Vasc. Biol. *40*, 1110–1122. https://doi.org/10.1161/ATVBAHA.119.312467.

Szumilas, K., Szumilas, P., Słuczanowska-Głąbowska, S., Zgutka, K., and Pawlik, A. (2020). Role of adiponectin in the pathogenesis of rheumatoid arthritis. Int. J. Mol. Sci. *21*, E8265. https://doi.org/10.3390/ijms21218265.

Toes, R., and Pisetsky, D.S. (2019). Pathogenic effector functions of ACPA: where do we stand? Ann. Rheum. Dis. *78*, 716–721. Epub 2019 Apr 20. https://doi.org/10.1136/annrheumdis-2019-215337.

Tsigalou, C., Vallianou, N., and Dalamaga, M. (2020). Autoantibody production in obesity: is there evidence for a link between obesity and autoimmunity? Curr. Obes. Rep. *9*, 245–254. https://doi.org/10.1007/s13679-020-00397-8.

van Beek, L., Vroegrijk, I.O.C.M., Katiraei, S., Heemskerk, M.M., van Dam, A.D., Kooijman, S., Rensen, P.C.N., Koning, F., Verbeek, J.S., Willems van Dijk, K., and van Harmelen, V. (2015). FcRγ-chain deficiency reduces the development of diet-induced obesity. Obesity *23*, 2435–2444. https://doi.org/10.1002/oby.21309.

Walldius, G., and Jungner, I. (2006). The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy–a review of the evidence. J. Intern. Med. *259*, 493–519. https://doi.org/10.1111/j.1365-2796.2006.01643.x.

Winer, D.A., Winer, S., Shen, L., Wadia, P.P., Yantha, J., Paltser, G., Tsui, H., Wu, P., Davidson, M.G., Alonso, M.N., et al. (2011). B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat. Med. *17*, 610–617. https://doi.org/10.1038/nm.2353.

Wueest, S., Seelig, E., Timper, K., Lyngbaek, M.P., Karstoft, K., Donath, M.Y., Ellingsgaard, H., and Konrad, D. (2021). IL-6 receptor blockade increases circulating adiponectin levels in people with obesity: an explanatory analysis. Metabolites *11*, 79. https://doi.org/10.3390/metabo11020079.

Xue, Y., Jiang, L., Cheng, Q., Chen, H., Yu, Y., Lin, Y., Yang, X., Kong, N., Zhu, X., Xu, X., et al. (2012). Adipokines in psoriatic arthritis patients: the correlations with osteoclast precursors and bone erosions. PLoS One *7*, e46740. https://doi.org/10.1371/journal.pone.0046740.

Yoshino, T., Kusunoki, N., Tanaka, N., Kaneko, K., Kusunoki, Y., Endo, H., Hasunuma, T., and Kawai, S. (2011). Elevated serum levels of resistin, leptin, and adiponectin are associated with C-reactive protein and also other clinical condition in rheumatoid arthritis. Intern. Med. *50*, 269–275. https://doi.org/10.2169/internalmedicine.50.4306.

Yu, H.C., and Lu, M.C. (2019). The roles of anti-citrullinated protein antibodies in the immunopathogenesis of rheumatoid arthritis. Ci ji yi xue za zhi = Tzu Chi Med. J. *31*, 5–10. https://doi.org/10.4103/tcmj.tcmj_116_18.

Zheng, L.Y., Xu, X., Wan, R.H., Xia, S., Lu, J., and Huang, Q. (2019). Association between serum visfatin levels and atherosclerotic plaque in patients with type 2 diabetes. Diabetol. Metab. Syndrome *11*, 1–7. https://doi.org/10.1186/s13098-019-0455-5.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Inmunoglobulins (IgGs)		
IgG-ACPAs	RA serum	N/A
IgG-NHS (normal human serum)	HD serum	N/A
Biological samples		
Human serum samples	Reina Sofia Hospital (Cordoba, Spain)	N/A
Human adipose tissue	Virgen de la Victoria Hospital (Malaga, Spain)	N/A
Fetal bovine serum	(Biowest, Nuaillé, France)	S1400-100
Antibodies		
Anti-CD68 monoclonal antibody, unconjugated, Clone 514H12	Leica Biosystems	#PA0286: RRID:AB_10554758
Anti-CD20 monoclonal antibody unconjugated, Clone L26	Leica Biosystems	# RTU-CD20-L26: RRID:AB_563520
Chemicals, peptides, and recombinant proteins		
BODIPY 493/503 lipid probe	Invitrogen (Leiden, Netherlands)	D3922
DMEM medium	(Biowest, Nuaillé, France)	L0060
Radioimmunoprecipitation assay (RIPA) buffer	This paper	
Sodium deoxycholate	Sigma-Aldrich	302-95-4
Nonidet P-40	Sigma-Aldrich	9002-93-1
Tris-HCL	Fisher scientific	15893661
M199 medium	(Biowest, Nuaillé, France)	L0330
Isobutyl methylxanthine	Sigma-Aldrich	I5879
Dexamethasone	Sigma-Aldrich	D4902
Insulin	Sigma-Aldrich	I9278
RPMI 1640 medium	(Biowest, Nuaillé, France)	L0500-500
Phorbol 12-myristate 13-acetate	(PMA, Sigma-Aldrich, St Louis, Missouri, USA)	P8139
TRI Reagent	Sigma-Aldrich	T9424
Critical commercial assays		
TNF-α enzyme-linked immunosorbent assay	Bionova (Madrid, Spain)	950.090.096
IL-6 enzyme-linked immunosorbent assay	Bionova (Madrid, Spain)	950.030.096
IL1-β enzyme-linked immunosorbent assay	Bionova (Madrid, Spain)	850.006.096
Leptin enzyme-linked immunosorbent assay	Cusabio (Houston, USA)	CSB-E04649h
Adiponectin enzyme-linked immunosorbent assay	Cusabio (Houston, USA)	CSB-E07270h
Resistin enzyme-linked immunosorbent assay	Cusabio (Houston, USA)	CSB-E06884h
Omentin enzyme-linked immunosorbent assay	Cusabio (Houston, USA)	CSB-E09745h
Vaspin enzyme-linked immunosorbent assay	Wuhan Fine Biotech (Wuhan, China)	EH3948
Visfatin enzyme-linked immunosorbent assay	RayBiotech, (Norcross, GA, USA)	EIA-VIS-1
Adipokine proteome profile	(R&D Systems, Minneapolis, USA)	ARY013
HiTrap Protein G HP kit	(GE Healthcare, Uppsala, Sweden)	GE17-0404-01
Anti-ACPAs ELISA kit	(DRG Instruments GmbH, Marburg, Germany)	EIA-4848

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
PrimeScript RT Master Mix (Perfect Real Time)	Takara	Cat# RR036A
GoTaq qPCR Master Mix	Promega	Cat# A6001
Experimental models: Cell lines		
Thp1 cell line	ATCC	ATCC TIB-202
3T3L1 cell line	ATCC	ATCC CL-173
Experimental models: Organisms/strains		
Mouse: Collagen induced arthritis mice	Washington Biotechnology Inc. (Baltimore, MD, USA)	N/A
Oligonucleotides		
Primers for mouse samples, see Table S2	This paper	N/A
Primers for human samples, se Table S1	This paper	N/A
Software and algorithms		
ImageJ	Website	https://imagej.nih.gov/ij/download.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Nuria Barbarroja (barbarrojan@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cross-sectional study

A total of 200 subjects, 150 RA patients and 50 healthy donors matched for age and gender were included in this study to analyze the CVD signs, the adipocytokine profile and its association with clinical parameters of the disease. RA patients complied with at least four 1987 American College of Rheumatology (ACR) disease criteria and achieved a total score of $\geq$6 according to 2010 ACR classification (Aletaha et al., 2010). The study was conducted in accordance with the Declaration of Helsinki principles. Patients were receiving the following treatments: corticosteroids (n = 105), hydroxychloroquine (HCQ) (n = 39), nonsteroidal anti-inflammatory drugs (NSAIDs) (n = 124), methotrexate (MTX) (n = 90) and leflunomide (n = 60). All patients were assessed for the presence of antibodies to ACPAs or rheumatoid factor (RF). DAS28 was examined following the guidelines of the ACR. Moderate-high disease activity was established as DAS28 > 3.2, low disease activity 2.6 < DAS28 > 3.2, and remission state DAS28 < 2.6 (Prevoo et al., 1995).

All patients filled the health assessment questionnaire and the visual analogue scale (VAS) to assess the pain. None of the healthy donors had a history of other autoimmune disease.

Ethics, consent and permissions

All participants enrolled were Caucasian, were recruited consecutively in a monographic consulting of Rheumatoid arthritis at the Department of Rheumatology, and gave their written informed consent approved by the Ethical Committee of the Reina Sofia Hospital (Cordoba, Spain).

Longitudinal study

A total of 123 RA patients were included in the longitudinal study to analyze the effect of the treatments in the adipocytokine profile, the impact of insulin resistance in the therapeutical response and to identify potential biomarkers of response to therapy. Fifty-six patients were treated with anti-CD20 therapy, 22 patients were treated with anti-IL6R therapy and 45 patients were treated with anti-TNFα therapy. Those patients were evaluated clinically and analytically at baseline and after 6 months of treatment. After 8h of fasting, blood samples were collected from patients for laboratory tests. Disease activity and concomitant treatment with Disease Modifying Anti-Rheumatic Drugs (DMARDs) and/or glucocorticoids were recorded.

Ethics, consent and permissions

All participants enrolled were Caucasian, recruited at the Departments of Rheumatology from the Reina Sofia Hospital (Cordoba, Spain) and La Paz University Hospital (Madrid, Spain) following the routine clinical practice, and gave their written informed consent approved by the Ethical Committee from both hospitals.

CIA mice model

All animal experiments were carried out in accordance with the ARRIVE guidelines and with the UK Animals (Scientific Procedures) Act, 1986, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). Twenty-five DBA1/J male mice with 7–8 weeks old were used. Five mice were used as healthy controls, and 20 mice were injected subcutaneously with/complete Freund's adjuvant emulsion (100 lg per mouse); on day 21, mice were boosted with a mixture of collagen solution and incomplete Freund's adjuvant emulsion (100 lg per mouse). Between days 22 and 42, macroscopic signs of arthritis were scored three times weekly, where each paw received a score: 0 = no visible effects of arthritis; 1 = oedema and/or erythema of one digit; 2 = oedema and/or erythema of two digits; 3 = oedema and/or erythema of more than two digits; and 4 = severe arthritis of entire paw and digits. The arthritic index (AI) was calculated by addition of individual paw scores (up to maximum of 16). Diseased mice were classified into two groups according to the AI score: low disease, 1–4; and moderate–severe disease, 5–16. Mice were weighed daily. The CIA mouse model was generated by Washington Biotechnology Inc. (Baltimore, MD, USA). After termination, gonadal adipose tissue and plasma were isolated and frozen at −80°C for gene and protein analyses.

Ex vivo experiments in white human adipose tissue

Visceral adipose tissue was obtained from obese patients undergoing bariatric surgery at the Endocrinology department, Virgen de la Victoria Hospital, Malaga, Spain. Patients gave their written informed consent approved by the Ethical Committee of the Virgen de la Victoria Hospital (Málaga, Spain). Adipose tissue was placed in a cell culture petri dish and dissect into 1- to 2 cm pieces. Primary culture of adipose tissue pieces was carried out in M199 medium (10% FBS, 1% penicillin and streptomycin) in 12 wells plate. Adipose tissue was treated with IgG-NHS or IgG-enriched-ACPAs (500 μg/mL) for 24h.

In vitro experiments with adipocytes (3T3-L1)

3T3-L1 cells were obtained from ATCC (Manassas, VA, USA). Cells were cultured, tested for mycoplasma contamination and adipocyte differentiation according to the protocol described by Guzman-Ruiz et al. (Guzmán-Ruiz et al., 2014). Briefly, 100% confluent cells (day 0) were incubated in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS, 0.5 mM isobutyl methylxanthine (IBMX), 0.25 μM dexamethasone and 10 μg/ml insulin for 72 h (day 3). The medium was replaced by DMEM with 10% FBS and 10 μg/ml insulin for an additional 72-h period (day 6) and was then exchanged by DMEM without insulin until day 9.

Firstly, to study the effects of ACPAs in adipocyte differentiation, in addition to the cocktail of differentiation, cells were also treated with IgG-enriched ACPAs (500 μg/mL) at day 0, 3 and 6.

Secondly, to reveal the effects of biologics in adipocytes activated by IgG-ACPAs, 3T3-L1 pre-adipocytes on day 0 of differentiation were treated IgG-enriched-ACPAs (500 μg/mL) alone or in combination with anti-IL6R (Tocilizumab, 20 μg/mL) or anti-TNF-α (infliximab, 100 μg/mL) during all the differentiation process. Subsequently, cells were collected for mRNA analyses at day 9.

In vitro experiments with macrophages (Thp1)

THP-1 cell line obtained from ATCC was cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Biowest, Nuaillé, France) supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France), 4 mM L-glutamine (Biowest, Nuaillé, France) and 100 U/ml penicillin, 100 mg/ml streptomycin and 250 pg/ml fungizone (BioWhittaker/MA Bioproducts, Walkersville, Maryland, USA), at 37°C in a humidified 5% carbon dioxide atmosphere. THP-1 cells ($8x10^5$ cells/ml) were differentiated to macrophages with 100 ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich, St Louis, Missouri, USA) for 72 hours. Differentiated macrophages were treated with IgG-enriched-NHS or IgG-enriched-ACPAs (500μg/mL) alone or in combination with anti-IL6R (Tocilizumab, 20 μg/mL) or anti-TNF-α (infliximab, 100 μg/mL) for 24h.

METHOD DETAILS

Cardiovascular risk assessment

Hypertension were considered in patients with persistent high blood pressure (140/90 mmHg or higher). Diabetes was assessed by Hemoglobin A1c (HbA1c) (>6.5%) and fasting plasma glucose levels (>126 mg/dL). Obesity was defined by body mass index (BMI): m/h2 where m is weight and "h" is height. Obesity was established on > 30 kg/m^2. Smoking habit was registered through a personal questionnaire. Atherogenic risk was calculated by atherogenic index (AI) based on the levels of total cholesterol (mg/dL) and HDL (mg/dL): AI = TC/HDL. The index determined atherogenic risk with higher values of 4.5 in female and 5 in male (Millán et al., 2009). Levels of apolipoproteins A and B were used to calculate ApoB/ApoA ratio that established different CVD risk groups. These groups were: low CVD risk (female: 0.3–0.59; male: 0.4–0.69), moderate CVD risk (female: 0.6–0.79; male: 0.7–0.89) and high CVD risk (female: 0.8–1; male: 0.9–1.1). In this study, subjects were separated in two different groups according ApoB/ApoA ratio: low CVD risk and moderate-high CVD risk (Walldius and Jungner, 2006; Lima et al., 2007).

To assess insulin resistance, we used the HOMA-IR based on insulin concentration (mU/L) and glucose levels (mg/dL) (insulin x glucose / 405) distinguishing two groups (insulin resistant group: HOMA-IR>2.5 and normoglycaemic group: HOMA-IR<2.5) (Solomon et al., 2014).

Carotid intima media thickness assessment

Ninety-three out of RA patients and fifty-eight HDs underwent high-resolution B-mode ultrasonography for CIMT measurements. All ultrasound scanning was performed by a single experienced vascular sonographer on the carotid arteries, using carotid duplex equipment (LOGIC E9). IMT was measured at the distal wall of the carotid artery on a 10-mm segment and defined as the distance from the leading edge of the lumen-intima surface to the leading edge of the media–adventitia interface of the far wall.

Pathological CIMT was defined as a focal structure that encroached into the arterial lumen of at least 50% of the surrounding IMT value or demonstrated a thickness >1.5 mm, as measured from the media-adventitia interface to the intima-lumen interface.

Circulating adipocytokine levels

The serum concentrations of TNF-α, IL-6, IL-1β (BioNova, Madrid, Spain), leptin, adiponectin, resistin, omentin (Cusabio, Houston, USA), visfatin (RayBiotech, Norcross GA, USA) and vaspin (Wuhan Fine Biotech, Wuhan, China) were quantified by enzyme-linked immunosorbent assay (ELISA) kits in both, cross-sectional and longitudinal cohorts according to the manufacturer's instructions.

Immunohistochemistry of adipose tissue

The adipose tissue was fixed for 24 hours in formalin and processed in paraffin by a conventional procedure. Three micron sections were made for immunohistochemistry of CD20 (Leica Biosystems, USA, Clone L26) in order to detect B lymphocytes and CD68 (Leica Biosystems, USA, Clone 514H12) for macrophage detection. Two independent investigators counted the number of adipocytes, B lymphocytes, and macrophages in ten consecutive areas at 40X magnification. The number of macrophages was normalized to 100 adipocytes (Aron-Wisnewsky et al., 2009).

Adipokine proteome profile

Total protein from mice gonadal adipose tissue was extracted using a radioimmunoprecipitation assay (RIPA) buffer (0.1% SDS, 0.5% sodium deoxycholate, 1% nonidet P-40, 150mmol L-1 Tris-HCL; pH 8.0)

supplemented with protease inhibitors. Proteins (300μg) were subjected to mouse adipokine array in nitrocellulose membranes each containing 38 different capture antibodies printed in duplicate following the manufacturer instructions (R&D Systems, Minneapolis, USA).

Isolation of enriched IgGs-ACPAs

IgG-ACPAs antibodies from the pooled sera of 15 RA patients (characterized by high titers of ACPAs >300 U/ml) and IgG-NHS (normal human serum) antibodies from the pooled sera of 15 healthy donors were purified using HiTrap ® Protein G HP kit (GE Healthcare, Uppsala, Sweden). To assess the anti-ACPAs activity of these isolated antibodies, 1:100 diluted samples were tested using anti-ACPAs ELISA kit according to the manufacturer's instructions (DRG Instruments GmbH, Marburg, Germany).

Lipid content measured by oil red O staining and bodipy probe

At day 9 of differentiation, lipid accumulation was assessed by Oil Red O solution, visualized by Moticam 1080p Full HD (Ramírez-Zacarías et al., 1992). Differentiated cells were loaded with 5 μM BODIPY 493/503 lipid probe) (Invitrogen, Leiden, Netherlands) at 4°C for 15 min, the medium was then removed and cells washed three times with DMEM solution. Immediately thereafter, the live cells were analyzed by fluorimetric assay (Infinite 200 PRO, Tecan Austria GmbH).

RT-PCR

Total RNA from adipocytes and macrophages was extracted using TRI Reagent (Sigma) following the manufacturer's recommendations. Gene expression was assessed by real time PCR using a Light Cycler Thermal Cycler System (Roche Diagnostics, Indianapolis, Indiana, USA). Expression of genes of interest was corrected by the geometrical average of β-actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β2 microglobulin and 36B4 using the BestKeeper tool (Pfaffl et al., 2004). The BestKeeper application can be downloaded on http://www.wzw.tum.de/gene-quantification/bestkeeper.html. Oligonucleotides used for mouse and human samples were described in Tables S1 and S2.

QUANTIFICATION AND STATISTICAL ANALYSIS

Normality test were performed. In addition, to compare two independent groups, we used parametric test (Student's *t* test) or non-parametric test (Mann-Whitney rank sum test). For multiple comparisons, one-way ANOVA test or Kruskall-Wallis test were performed. To analyze qualitative data chi-squared test was performed. Correlations were assessed by Pearson correlation coefficients ($p < 0.05$ was considered statistically significant). Graphpad Prism Version 9.0.1 were used for all analyses.