OPEN

Going Further: Comprehensive Disease Control of Rheumatoid Arthritis, Targeting Cytokines and Chemokines

Efrain Chavarria-Avila, PhD,†‡ Monica Vazquez-Del Mercado, MD, PhD,†‡§‖*
Oscar Pizano-Martínez, PhD,†¶ German Roman-Lugo, MD,† Karla Arrona-Rios, MD,‡
Felipe Perez-Vazquez, MD, PhD,†‡# Jeniffer P. De-La-Cruz, MD,‡ Ivette Calderon-Espinoza, MD,‡*
*Andrea Aguilar-Vazquez, BSc,†** Marisol Esesarte-Rodriguez, MD,†*
Edy D. Rubio-Arrellano, MD, PhD,# and Sergio Duran-Barragan, MD†§¶††

Objectives: Mechanism of action of biological and synthetic disease-modifying antirheumatic drugs (DMARDs) includes the inhibition of specific proinflammatory cytokines. This study aimed to elucidate the cytokines and chemokines inhibited by different treatments (conventional synthetic DMARD [csDMARD], biological and targeted synthetic DMARD) in rheumatoid arthritis (RA).

Methods: Fifty-nine RA patients with low disease activity or remission included in a cross-sectional study were classified by treatment in groups: abatacept, certolizumab, rituximab (RTX), tocilizumab, tofacitinib (TOF), baricitinib (BAR), and csDMARD. Cytokine and chemokine serum levels were measured by LEGENDplex Human Inflammation panel. Quantitative variables were compared using Student *t* or Mann-Whitney *U* test as appropriate, whereas qualitative variables were compared using χ^2 or Fisher exact test. $p < 0.05$ was considered significant.

Results: Certolizumab, RTX, tocilizumab, and TOF showed that most cytokine pathways inhibited: tumor necrosis factor α, interferon γ, interleukin 1β (IL-1β), IL-12, IL-18, and IL-23; in addition, csDMARDs showed a similar inhibition patron except for IL-23. Serum level of tumor necrosis factor α pathway was one of the most inhibited being undetectable in RTX, TOF, and BAR groups. Interleukin 6 was shown to be inhibited by abatacept, RTX, and TOF; however, higher levels were observed in 3 patients treated with tocilizumab. Abatacept, certolizumab, RTX, and TOF downregulated IL-10 in this group of patients but remained detectable in almost half of the subjects, with the highest levels in the BAR group. The active pathways that remained the most were CC chemokine ligand 2, IL-8, IL-17, and IL-33.

Conclusions: Understanding the cytokine chemokine pathways inhibition could help rheumatologists to prescribe a tailored therapy using the arsenal of DMARDs for individualized RA treatment in an evidence-based decision manner.

Key Words: antirheumatic agents, chemokines, cytokines, rheumatoid arthritis, therapy

(*J Clin Rheumatol* 2021;27: e432–e439)

From the *Departamento de Disciplinas Filosófico, Metodológicas e Instrumentales, and †Instituto de Investigación en Reumatología y del Sistema Músculo Esquelético (IIRSME), Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara; ‡Servicio de Reumatología 004086, PNPC CONACyT, División de Medicina Interna, Hospital Civil Dr. Juan I. Menchaca; §UDG-CA 703 inmunología y Reumatología, ‖Instituto de Biología Molecular y Genómica, ¶Departamento de Clínicas Médicas, #Departamento de Fisiología, Instituto de Terapia Experimental y Clínica, and **Doctorado en Ciencias Biomédicas Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara; and ††Clínica de Investigación en Reumatología y Obesidad, Guadalajara, Jalisco, México.

Correspondence: Sergio Duran-Barragan, MD, Sierra Mojada 950, Guadalajara, Jalisco, México 4430. E-mail: duranbarragan@yahoo.com.mx.

The authors declare no conflict of interest.

Author Contributions: E.C.-A.: contributed to the conception and the design of the study, verified the analytical methods, carried out the experiments, drafting the article and approval of the final version of article. M.V.–.M.: contributed to the conception and the design, execution, analysis and interpretation of data, revising critically the draft of article and approval of the final version of article. O.P.-M.: contributed to the execution, analysis and interpretation of data, drafting the article and approval of the final version of article. G.R.-L.: contributed to the conception, verified the analytical methods, carried out the experiments, drafting the article and approval of the final version of article. K.A.-R.: contributed to the execution, revising critically the draft of article and approval of the final version of article. F.P.-V.: contributed to the conception, analysis and interpretation of data, drafting the article and approval of the final version of article. J.P.C.: contributed to the execution, analysis and interpretation of data, drafting the article and approval of the final version of article. I.C.-E.: contributed to the execution, revising critically the draft of article and approval of the final version of article. A.A.-V.: contributed to the execution, analysis and interpretation of data, revising critically the draft of article and approval of the final version of article. M.E.-R.: contributed to the execution, revising critically the draft of article and approval of the final version of article. E.D.R.-A.: contributed to the execution, revising critically the draft of article and approval of the final version of article. S.D.-B.: contributed to the conception and the design of the study, execution, verified the analytical methods, revising critically the draft of article and approval of the final version of article.

Supplemental digital content is available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.jclinrheum.com).

ISSN: 1076-1608
DOI: 10.1097/RHU.0000000000001515

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease that primarily affects the joints and is associated with autoantibodies that target various molecules including modified self-epitopes.[1]

Rheumatoid arthritis affects nearly 1% of the adult population worldwide, but its prevalence and incidence vary across geographic areas and countries.[1,2] Differences may stem from a combination of genetics influence and environmental, socioeconomic, and cultural factors.[2] The overall estimated prevalence of RA in Mexico is 1.6%, but there are major regional variations in the country.[2]

The environment, including smoking, diet, obesity, infections, and microbiota, has been proposed to induce the development of RA in genetically predisposed individuals.[3] Modern genetic technologies combined with large, well-characterized clinical cohorts have improved our understanding of the genetics of the disease.[3] Genome-wide association studies using single-nucleotide polymorphisms have characterized more than a hundred loci associated with RA risk. Most of them are implicated in the pathogenesis and immune mechanisms shared with other chronic inflammatory diseases.[4]

In most patients, the pathogenesis of RA begins years before clinical disease is evident, although acute onset reflecting

immediate immune disturbance is also possible.[1,4] Early-stage RA patients show synovial inflammation based on mononuclear cell infiltration, mainly CD4 T cells and macrophages, together with early stromal cell activation that contributes to undifferentiated arthritis.[1,5] The role of macrophages and fibroblasts is more prominent when the disease has established itself, and DNA methylation patterns in fibroblast-like synoviocytes, isolated from individuals with early RA, differ from those of individuals with the established disease.[1,5] The pathway analysis showed that the main differences are found in cell differentiation, adhesion, and proliferation, all these regulated by chemokines and cytokines involved in autocrine, paracrine, and endocrine signaling.[1,4,5]

Immunogenetics of RA suggests aberrant pathways of T-cell activation have a key role during the onset and/or manifestation of the disease.[6] Naive CD4 T cells may differentiate into one of several lineages of T helper (T_H) cells, including T_H1, T_H2, and T_H17, and inducible regulatory T cells, as defined by their pattern of cytokine production and function.[7] These cells play a central role in the local generation of specific autoantibodies along the cytokine and chemokine network. The identification of key cells, mediators, and mechanisms implicated in the pathogenesis of RA could provide the basis for the development of new and double- or multiple-targeted disease-modifying antirheumatic drugs (DMARDs).[5,8]

The American College of Rheumatology/European League Against Rheumatism RA criteria (2010) enabled the classification of early onset RA patients showing similar disease activity (by clinical assessment and ultrasound) and less damage, compared to the previous standard assessment.[9] Therefore, these new classification criteria should ensure an earlier intervention, a shorter disease, less joint damage, and improvements in the patients' outcome.[9]

There are multiple drugs for RA treatment, some of them used as first-line therapy and some others as second line. In recent years, the disease progression and outcome of patients with RA have improved because of the development of biological DMARDs (bDMARDs) and targeted synthetic DMARDs (tsDMARDs). Biological treatments for RA target different signaling pathways: CD28/CTLA4 system (abatacept [ABT]), tumor necrosis factor α (TNF-α) inhibitors (certolizumab [CZP]), CD20 B cell–depleting (rituximab [RTX]), interleukin 6 (IL-6) receptor inhibition (tocilizumab [TOC]), and Janus kinase (JAK) inhibitors (tofacitinib [TOF], baricitinib [BAR]).

The mechanism of action of biological and synthetic DMARD includes the inhibition of specific proinflammatory cytokines; however, it is unknown if these drugs exert their action on the specific target for which they were developed or have an alternative mechanism inhibiting multiple cytokines.

The importance of this research lies on improving the understanding of the biological impact in lymphocyte T-cell modulation, which in turn modifies the signaling pathways in RA patients under DMARD treatment. Our aim was to elucidate the cytokines and chemokines inhibited by different treatments (conventional synthetic DMARDs [csDMARDs], b/tsDMARDs) in RA patients with low disease activity or in clinical remission. This information could help the clinician select the most appropriate treatment in accordance to the cytokine and chemokine profile.

METHODS

Study Design

We conducted a cross-sectional study where RA patients (classified by the American College of Rheumatology/European League Against Rheumatism 2010 criteria) of the outpatient rheumatology clinic at Hospital Civil Dr. Juan I. Menchaca and Clinica de Investigacion en Reumatologia y Obesidad with low disease activity or remission according to the Disease Activity Score for 28-joint count (DAS28) were tested for cytokine and chemokine serum levels.

Patients

Subjects with RA 18 years or older were included. Inclusion criteria were as follows: patients in treatment with b/tsDMARD or csDMARD and low disease activity or remission for at least 3 months. Exclusion criteria included pregnancy, recent clinical infection, patients with evidence of latent tuberculosis, any other autoimmune disease, and a history of malignant neoplasm in the last 5 years. Because of the lack of recommendations for a specific DMARD according to a cytokine and chemokine profile baseline, patients were classified by their current treatment in the following groups: ABT, CZP, RTX, TOC, TOF, BAR, and csDMARD. Seventeen patients outwent monotherapy, 21 were in dual therapy, and 21 were treated with a third or fourth of one of the following drugs: methotrexate (MTX), chloroquine/hydroxychloroquine (CLQ/HCLQ), sulfasalazine (SSZ), leflunomide (LFN), or prednisone (PDN).

Procedures

Patients were examined with a structured questionnaire, general physical examination (blood pressure, heart and respiratory rate, and body temperature), disease activity assessment, and laboratory tests. Evaluation of RA disease activity was done with Clinical Disease Activity Index (CDAI), physical disability with the Health Assessment Questionnaire (HAQ), and quality of life with the European Quality of Life 5-Dimension. Laboratory tests included erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and anti–cyclic citrullinated peptide (anti-CCP) antibodies.

Serum levels of cytokines (IL-1β, IL-6, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33, interferon α [IFN-α], IFN-γ, and TNF-α) and chemokines (CC chemokine ligand 2 [CCL2], CXCL8) were measured with the LEGENDplex Multi-Analyte Flow Assay kit by BioLegend (BioLegend, San Diego, CA) multiplex assay using a flow cytometer.

The kit consists of a multiplex analysis that quantifies 13 analytes in the same sample where all molecules lay in the assay detection range of 2.4 to 10,000 pg/mL except for IL-33 that has a detection range of 12.2 to 50,000 pg/mL. In this assay, specific monoclonal antibodies for each cytokine or chemokine are on the bead surface.

The standard curve and the samples were analyzed with an NxT Attune flow cytometer Applied Biosystems (Thermo Fisher Scientific, Waltham, MA). Once the flow cytometer is set up properly, it is able to differentiate specific beads for each analyte by their size and fluorescence intensity; at least 300 events must be acquired per analyte.

The results were analyzed with the LEGENDplex v8 software (BioLegend). The serum concentration of each analyte was quantified in picograms per milliliter.

Ethics

The present study was approved by the Institutional Review Board Committee (registered no. 0266/18) of Hospital Civil Dr. Juan I. Menchaca of the Universidad de Guadalajara and Review Board Committee (14-CEI-008-20170526) of Clinica de Investigacion en Reumatologia y Obesidad. In compliance with the Regulations of the General Health Law on Health Research (1987) and the Declaration of Helsinki, all individuals selected for this study were asked to sign an informed consent form to give their

authorization for clinical and anthropometric evaluations and blood sampling.

Statistical Analysis

Central tendency measures were used to describe the characteristics of the groups; for the quantitative variables, we used mean, median, or average range as central tendency measurements and SD, interquartile range, or sum of ranges as data dispersion quantification, and these were compared with the Student *t* test, Mann-Whitney *U* test, 1-way analysis of variance, or Kruskal-Wallis test as appropriate. The qualitative ones were represented as frequency with percentage, and they were compared with χ^2 or Fisher exact test. Sample size calculation was not possible because of the high variation in serum level of cytokines and chemokines. $p < 0.05$ was considered statistically significant.

RESULTS

Fifty-nine RA patients who fulfilled the criteria were recruited. Demographic and clinical characteristics are shown in Table 1.

Patients were classified according to treatment. The csDMARD group had 11 patients: 8 (72.7%) with monotherapy, 2 (18.2%) in dual therapy, and 1 (9.1%) with 2 or more csDMARD. The ABT group had 11 patients: 1 (9.1%) with monotherapy, 9 (81.8%) with dual therapy, and 1 (9.1%) with 2 or more csDMARD. The CZP group had 9 patients, 1 (11.1%) with monotherapy, 5 (55.6%) with dual therapy, and 3 (33.3%) with 2 or more csDMARDs. The RTX group had 11 patients: 4 (36.4%) with monotherapy, 1 (9.1%) with dual therapy, and 6 (54.5%) with 2 or more csDMARD. The TOC group had 9 patients: 2 (22.2%) with monotherapy, 5 (55.6%) with dual therapy, and 2 (22.2%) with 2 or more csDMARD. The TOF group had 4 patients: 2 (50%) with monotherapy and 2 (50%) with 2 or more csDMARD. The BAR group had 4 patients: 1 (25%) with monotherapy and 3 (75%) with dual therapy (Table 1).

A comparison was made between the characteristics of the patients with csDMARD versus b/tsDMARD as shown in Table 2. Difference was found between the anti-CCP titers, with a higher value of 360.67 IU in the csDMARD group versus 72.76 IU in the b/tsDMARD group ($p = 0.003$).

The b/tsDMARD group had a tendency to show higher values of disease activity than the csDMARD group by DAS28-ESR (3.33 vs. 2.78, $p = 0.066$) and CDAI (7.98 vs. 3.68, $p = 0.088$).

Cytokines and Chemokines Levels Between Groups

Proinflammatory Cytokines

Interleukin 1β levels were most detected in the BAR group compared with the rest of the groups, being undetectable in the TOF group. Interleukin 6 levels were higher ($p = 0.03$) in the TOC group (493.23 pg/mL) and undetectable in the TOF group (Fig. 1A). Interleukin 12 serum levels were detected in only 4 subjects: 2 in ABT, 1 in CZP, and 1 in TOC. Interleukin 17 serum levels were detectable in 86% of the subjects; however, there was no difference between treatment groups. We only found a tendency toward higher levels in the csDMARD and BAR groups and a tendency toward lower levels in the ABT group. Interleukin 18 was poorly detected in the study group. It was undetectable in the RTX and TOF groups; however, in the ABT group, 55% of subjects showed identifiable levels. Interleukin 23 serum levels were measured in 73% of the csDMARD group, but undetectable in 61% of all subjects, especially in the TOF group. Interleukin 33 levels were higher ($p < 0.001$) in the BAR group (252.38 pg/mL)

TABLE 1. Clinic and Demographic Characteristics of the Study Group

Variable	
Age, mean ± SD, y	55.1 ± 12.97
Disease duration, mean ± SD, y	12.87 ± 10.10
BMI, kg/m^2	27.153 ± 4.53
HAQ-DI	0.70 ± 0.70
EuroQol 5-D, median (IQR)	6 (5–8)
Female/male, n	52/7
Smokers, n	19
Alcoholism, n	1
Comorbidities	
DM2, n	5
HBP, n	14
CV disease, n	3
Osteoporosis, n	10
Cancer, n	0
Autoantibodies	
RF positive, n (%)	56 (94.9)
Anti-CCP positive, n (%)	28 (47.5)
Treatment groups	
csDMARD, n	11
MTX[a] monotherapy, n (%)	8 (72.7)
MTX plus ≥ 1 csDMARD,[b] n (%)	3 (27.3)
ABT, n	11
ABT monotherapy, n (%)	1 (9.1)
ABT plus ≥ 1 csDMARD,[c] n (%)	10 (90.9)
CZP pegol, n	9
CZP[a] monotherapy, n (%)	1 (11.1)
CZP plus ≥ 1 csDMARD,[d] n (%)	8 (88.9)
RTX, n	11
RTX monotherapy, n (%)	4 (36.4)
RTX plus ≥ 1 csDMARD,[e] n (%)	7 (63.6)
TOC, n	9
TOC monotherapy, n (%)	2 (22.2)
TOC plus ≥ 1 csDMARD,[f] n (%)	7 (77.8)
TOF, n	4
TOF monotherapy, n (%)	2 (50)
TOF plus ≥ 2 csDMARD,[g] n (%)	2 (50)
Baricitinib, n	4
BAR monotherapy, n (%)	1 (25.0)
BAR plus MTX,[a] n (%)	3 (75.0)
Additional treatment	
PDN, n (%)	10 (16.9)
Dose (mg), median (IQR)	3.75 (2.50–6.25)

[a]One patient with PDN.

[b]SSZ = 1; CLQ = 1, SSZ + CLQ = 1.

[c]MTX = 5 LFN = 2; CLQ = 1; SSZ = 1, MTX + CLQ = 1.

[d]MTX = 4; SSZ = 1, SSZ + LFN = 1; MTX + CLQ = 1; MTX + SSZ = 1.

[e]MTX = 1, MTX + SSZ = 3; MTX + AZA = 1; MTX + CLQ = 1; MTX + CLQ + SSZ = 1.

[f]MTX = 4; SSZ = 1, MTX + CLQ = 2.

[g]MTX + CLQ = 1; MTX + SSZ = 1.

BMI, body mass index; CV, cardiovascular disease; EuroQol 5-D, European Quality of Life 5-Dimension; DM2, type 2 diabetes mellitus; HBP, high blood pressure; IQR, interquartile range.

TABLE 2. Clinical and Demographic Characteristics of Patients With RA in Treatment With MTX Respect to b/tsDMARD

Variable	Study Group		*p* value
	csDMARD (n = 11)	b/tsDMARD (n = 48)	
Age, mean ± SD, y	52.6 ± 10.97	55.6 ± 13.43	0.489[a]
BMI, kg/m^2	26.18 ± 3.03	27.37 ± 4.81	0.437[a]
Male/female, n	0/11	7/41	0.177[b]
Disease duration, mean ± SD, y	9.36 ± 6.41	13.68 ± 10.66	0.204[a]
Smokers, n	4	15	0.743[b]
Alcoholism, n	0	1	0.629[b]
DM2, n	2	3	0.200[b]
HBP, n	1	13	0.206[b]
CV disease, n	1	2	0.503[b]
Osteoporosis, n	1	9	0.441[b]
RF positive, n	11	45	0.622[b]
Anti-CCP positive, n	3	25	0.626[b]
RF at diagnosis, mg/dL	431.04 ± 623.89	133.28 ± 168.04	0.147[a]
Anti-CCP at diagnosis, IU	360.67 ± 77.53	72.76 ± 148.36	0.003[a]
ESR, mm/h	37.45 ± 23.38	30.1 ± 23.76	0.357[a]
DAS28-ESR	2.78 ± 0.67	3.33 ± 1.45	0.066[a]
CDAI	3.68 ± 2.83	7.98 ± 8.05	0.088[a]
HAQ-DI	0.47 ± 0.48	0.75 ± 0.74	0.126[a]
EuroQol 5-D, median (IQR)	6 (5–8)	6 (5–7.75)	0.496[a]

[a]Student *t* test. This *p* value is very similar to its nonparametric counterpart; variable values are presented as mean ± SD for comparative purposes with literature available.

[b]χ^2 test.

CV, cardiovascular; DM2, type 2 diabetes mellitus; EuroQol 5-D, European Quality of Life 5-Dimension; HBP, high blood pressure; IQR, interquartile range; IU, international unit; n, subjects amount.

and lower in the TOF group, with an average of (23.87 pg/mL) (Fig. 1B).

Serum levels of the TNF-α pathway was one of the most inhibited, being undetectable in the RTX, TOF, and BAR groups. Interferon α2 showed a tendency toward higher levels in the ABT group (500.05 pg/mL) and a lower level tendency in the RTX group (4.74 pg/mL). Interferon γ was found to be inhibited in 88% of the subjects, remaining undetectable for the CZP, RTX, and TOF groups (Table 3).

Anti-inflammatory Cytokines

A tendency of elevated levels of IL-10 was obtained in the BAR (25.47 pg/mL) and csDMARD groups (15.95 pg/mL).

Chemokines

Interleukin 8 levels were detectable in 80% of the subjects, showing a tendency toward higher levels ($p = 0.05$) in the TOC group (96.27 pg/mL) and a tendency toward lower levels in the TOF group (3.67 pg/mL) (Fig. 1C). CC chemokine ligand 2 was the most detected chemokine among the study groups, showing higher levels in the ABT group (3717.03 pg/mL) and difference between the RTX (810.27 ± 472.85), CZP (931.87 ± 451.91), and csDMARD groups (926.21 ± 432.30) (Fig. 1D).

Concentrations of cytokines and chemokines expressed in medians and interquartile range by treatment are reported in Supplementary Digital Content 1a and 1b, http://links.lww.com/RHU/A201, showing no difference inside the group as monotherapy or adding a csDMARD.

Correlation Between Cytokines, Chemokines, and Disease Activity

Higher correlations were found between IL-1β with IFN-α2, IL-23, and IL-33. All the same, CCL2 positively correlated with IFN-α2 and IL-8; IL-10 also positively correlated with IL-33 (shown in Fig. 2).

Also, low correlations between IL-33 with CDAI ($r = 0.370$, $p = 0.007$) and DAS28-ESR ($r = 0.283$, $p = 0.043$) were found.

On the other hand, IL-8 displayed a medium to low correlation with age ($r = 0.478$, $p < 0.001$), HAQ Disability Index (HAQ-DI) ($r = 0.398$, $p = 0.006$), and disease duration ($r = 0.304$, $p = 0.016$), which indicate this chemokine could be implicated in disease activity and progression, considering that IL-8 also correlated with IFN-α2 ($r = 0.362$, $p = 0.042$).

One of the least inhibited cytokines by the cs/b/tsDMARD in our study group was IL-17, which correlated with RF level at diagnosis ($r = 0.395$, $p = 0.005$) and IL-33 ($r = 0.480$, $p < 0.001$).

Other correlations are shown in Figure 2 and Supplementary Digital Content 2, http://links.lww.com/RHU/A202.

DISCUSSION

We included in a cross-sectional study 59 RA patients characterized by low disease clinical activity or clinical remission. As a relevant finding, we observed low levels of cytokines and chemokines independently of the DMARD treatment: cs/b/tsDMARD.

It has been demonstrated that serum cytokine profiles of RA patients are different from those of healthy individuals; RA patients had increased levels of IL-1β, IL-4, IL-5, IL-6, IL-8 (CXCL8), IL-10, IL-13, IL-17, IL-35, IFN-γ, TNF-α,

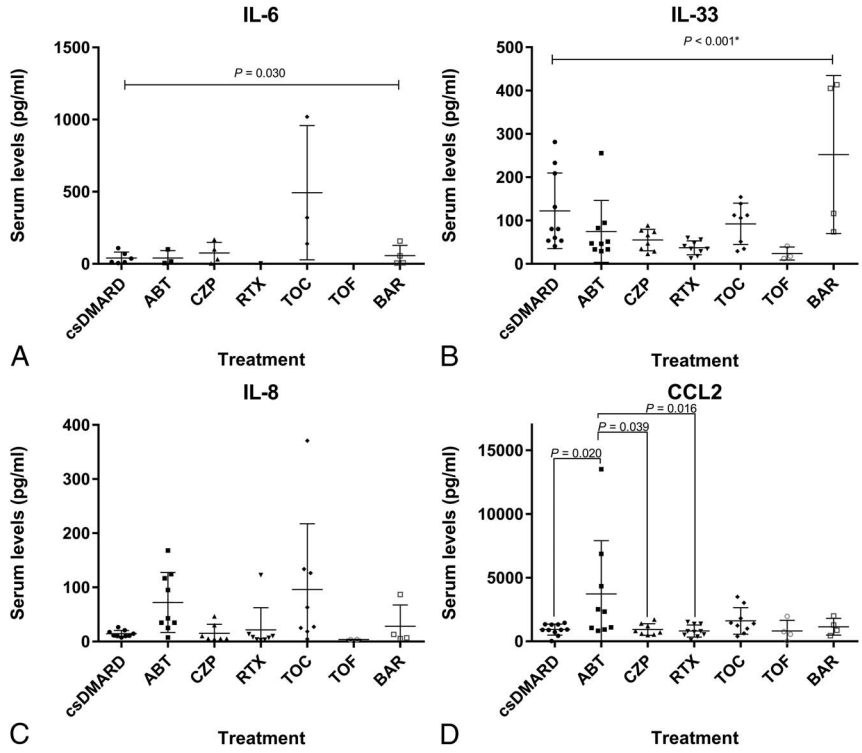

FIGURE 1. Serum cytokine level comparison between different groups. A, Interleukin 6, 38 samples (64%) were not detectable; (B) IL-8, 12 samples (20%) were not detectable; (C) IL-33, 8 samples (14%) were not detectable; (D) CCL2, 4 samples, (7%) were not detectable. *Abatacept versus BAR ($p = 0.003$), CZP versus BAR ($p = 0.001$), RTX versus BAR ($p < 0.001$), TOC versus BAR ($p = 0.013$), TOF versus BAR ($p = 0.003$).

granulocyte-macrophage colony-stimulating factor, and CCL2 (MCP-1),[10,11] supporting the hypothesis that RA is a complex immune-inflammatory condition, which involves dysregulation of cellular, humoral, and innate immunity, with a high systemic impact.

A dysregulation in the T_H1/T_H2 balance, where the proinflammatory cytokine TNF-α and the rest of T_H1 cytokines play a key role, has been considered as a cause for RA pathogenesis. However, the involvement of regulatory T cells and T_H17 cells with the T_H17 cytokine profile has challenged this classic paradigm.

It has been shown that the administration of IL-33 exacerbates collagen-induced arthritis in experimental models. A positive correlation between with RA disease activity and cytokine levels in serum and/or synovial fluid was found.[12] The study of Xiangyang et al.[13] showed correlation between serum levels of IL-33 with DAS28 in RA patients. The number of painful and swollen joints was higher in the group positive for IL-33. Meanwhile, C-reactive protein, IL-1β, IL-6, and TNF-α did not differ between groups. However, in our study, patients showed weak positive correlation between IL-33, CDAI, and DAS28-ESR; thus, our patients had low disease activity or remission.

Different treatments have been tested throughout the decades with different mechanisms of action albeit with the same goal: the inhibition or downregulation of proinflammatory cytokines such as IL-1β and TNF-α.

According to our results, the b/tsDMARDs including CZP, RTX, TOC, and TOF were the most effective for pathway inhibition: TNF-α, IFN-γ, IL-1β, IL-12, IL-18, and IL-23, just as csDMARD so too showed a similar inhibition pattern with the exception of IL-23. Lina et al[14] demonstrated that patients with RA who were given a combined treatment of etanercept and MTX showed significantly decreased levels of IL-1β, TNF-α, IL-6, IL-17, and IL-23, whereas transforming growth factor β was significantly elevated versus MTX alone.

In our study, IL-6 was shown to be inhibited by ABT, RTX, and TOF; however, higher levels were detected in 3 patients treated with TOC, probably as a result of a positive feedback loop induced by blocking its receptors. Yarilina et al.[15] used JAK inhibitors to suppress activation of blood and RA synovial macrophages, including a subset of proinflammatory responses by TNF-α. It was also reported that the IL-6 receptor was blocked by TOF, which indicates that signaling inhibition by other cytokines contributes to the clinical efficacy of JAK inhibitors on the effector phase of immune response. These results raise the possibility that inhibition of TNF-α and IFN signaling help explain the therapeutic efficacy of JAK inhibitors.[15]

Differences observed between TOF and BAR in the inhibition of cytokines and chemokines in our results may be explained by the affinity of TOF toward JAK1/3 compared with the smaller JAK3 effect by BAR as has been shown by Dowty et al.[16] Li et al.[11] suggest that TOF increases the levels of IL-35 as a possible mechanism of action occurring in parallel. Indeed, they found a negative correlation between this cytokine and disease activity.

In this study, ABT, CZP, RTX, and TOF downregulated IL-10, contrasting with BAR and csDMARD, which showed the highest levels of this cytokine. According to Herman et al.,[17] MTX induces IL-10 secretion and significantly reduces T_H1 profile in peripheral mononuclear cells derived from active RA patients. Equally important, the reduction of IL-10 achieved with the use of ABT, CZP, or RTX may suggest that the mechanism of downregulation is mediated by IFN signaling pathways. The

TABLE 3. Serum Cytokine Level Comparison Between Groups

	csDMARD (n^a = 11)	ABT (n^a = 11)	CZP (n^a = 9)	RTX (n^a = 11)	TOC (n^a = 9)	TOF (n^a = 4)	BAR (n^a = 4)		
Molecule	Serum Level, Mean ± SD, pg/mL (n^b)	Serum Level, Mean ± SD, pg/mL (n^b)	Serum Level, Mean ± SD, pg/mL (n^b)	Serum Level, Mean ± SD, pg/mL (n^b)	Serum Level, Mean ± SD, pg/mL (n^b)	Serum Level, Mean ± SD, pg/mL (n^b)	Serum Level, **Mean ± SD**, pg/mL (n^b)	*p* value[a]	n^c (%) of Undetectable Serum Level
IL-1β	15.97 ± 10.95 (3)	37.27 (1)	11.96 ± 14.15 (3)	3.15 ± 0.55 (3)	8.53 (1)	—[d]	17.46 ± 17.50 (3)	0.37	45 (76%)
IL-10	15.95 ± 22.03 (9)	7.45 ± 9.12 (3)	10.53 ± 10.53 (2)	2.49 ± 0.52 (3)	12.79 ± 10.61 (5)	3.09 (1)	25.47 ± 31.70 (4)	0.77	32 (54%)
IL-12	—	43.20 ± 5.03 (2)	39.64 (1)	—	69.89 (1)	—	—	0.20	55 (93%)
IL-17	582.03 ± 515.99 (10)	257.88 ± 311.39 (9)	286.05 ± 271.25 (8)	397.55 ± 289.40 (9)	344.14 ± 317.91 (9)	267.38 ± 31.25 (2)	513.43 ± 475.19 (4)	0.50	8 (14%)
IL-18	515.43 ± 194.01 (3)	401.21 ± 373.66 (6)	569.42 ± 215.75 (2)	—	1027.82 ± 468.44 (2)	—	877.46 ± 155.38 (2)	0.19	44 (75%)
IL-23	22.78 ± 27.01 (8)	166.45 ± 280.14 (3)	76.46 ± 67.42 (5)	4.63 (1)	11.89 ± 1.60 (2)	—	45.19 ± 32.52 (4)	0.44	36 (61%)
IFN-α2	36.34 ± 36. 76 (10)	500.05 ± 785.49 (7)	111.93 ± 80.94 (6)	4.74 ± 3.85 (3)	113.08 ± 155.56 (7)	117.66	107.27 ± 92.33 (4)	0.24	21 (36%)
IFN-γ	642.57 ± 50.32 (2)	134.22 (1)	—	—	721.31 (1)	—	552.65 ± 241.72 (3)	0.31	52 (88%)
TNF-α	4.5 (1)	9.48 (1)	11.48 (1)	—	11.55 ± 2.93 (2)	—	—	0.53	54 (91%)

[a]Subjects in treatment group.

[b]Subjects with detectable serum levels.

[c]Subjects with undetectable serum levels among all treatment groups.

[d]Undetectable serum levels.

findings of McInnes et al.[18] placed TOF as the most potent inhibitor of IL-6, IFN-γ, and IL-10. Excessive inhibition of IL-10 is not desirable because of its global anti-inflammatory effects in RA. However, the IL-10 pathway remains unclear across varying treatments, and there are contradictory data among different studies.[19]

Interferon α2 plays a key role in RA pathogenesis, inducing cellular proliferation and T_H1 polarization through JAK1. Baricitinib is selective against JAK1/JAK2 and TOF to JAK1/JAK3. Our results demonstrated that only RTX and TOF were able to inhibit IFN-α2 in these patients. Indeed, the most active pathways were CCL2 (93.2%), IL-8 (79.6%), IL-17 (86.4%), and IL-33 (86.4%).

The interaction between cytokines and their signaling pathways are the basis for the development of new strategies with small molecules or bispecific antibodies.[20] Rheumatologists now have a large variety of highly efficient drugs with different mechanisms of action in which the molecular target of the drug is known, which is not the case for csDMARDs.[21]

The serum levels of cytokines/chemokines found in our report suggest that additional mechanisms beyond the redundancy of their pathways are underlying the targeted one. This is a window of opportunity to look for double- or triple-targeted therapies, despite the high cost that this approach represents for the industry. One short phrase: we do not know how many applications will be approved by the Food and Drug Administration in the future for the b/tDMARD or csDMARD, one example being the COVID-19 (coronavirus disease 2019) pandemic, where TOF, HCLQ, and CLQ have been proven to be apparently effective.[22] This effect could be more important in the long term than their present therapeutic indications.

Existing treatment options have been shown to reduce disease activity, slow joint damage, and improve patients' related outcomes and disability. Comprehensive disease control of RA includes a clinical disease remission (DAS28–C-reactive protein <2.6), inhibition of radiological progression (increment of total Sharp score ≤0.5), and normal functionality (HAQ-DI <0.5), but no normalization in the cytokine-chemokine profile is considered. A drug that achieves a comprehensive disease control and better immune-regulation would be desirable. In order to follow the efficacy of targeted therapies, we recommend measuring the cytokine-chemokine profiles in addition to the standard RA laboratories.

A great number of published articles related to treatment outcome using DMARD monotherapy or in combination are lacking in endpoints supporting suppression of the targeted cytokine/chemokine profiles in patients. To date, there are a limited number of ongoing head-to-head superiority clinical trials in RA that have been correctly designed to directly compare the efficacy of targeted therapies. The paradox, however, is that rheumatologists have little or no idea how to approach tailored therapy in order to optimize the use of the DMARD arsenal following the cytokine and chemokine inhibition profiles. This could contribute to an individualized RA therapy.[23]

Limitations

The levels of chemokines and cytokines reported in this article do not guarantee a persistently low or high cytokines levels by a particular DMARD through the course of treatment. The small sample and high variation in cytokine and chemokine serum levels make the extrapolation of our results difficult. Osiri et al.[24] conducted a similar cross-sectional study in which no association was found with the treatment because of the variability of cytokine and chemokine serum levels. We expect our article to add to a field with scarce information and contribute as a basis for future

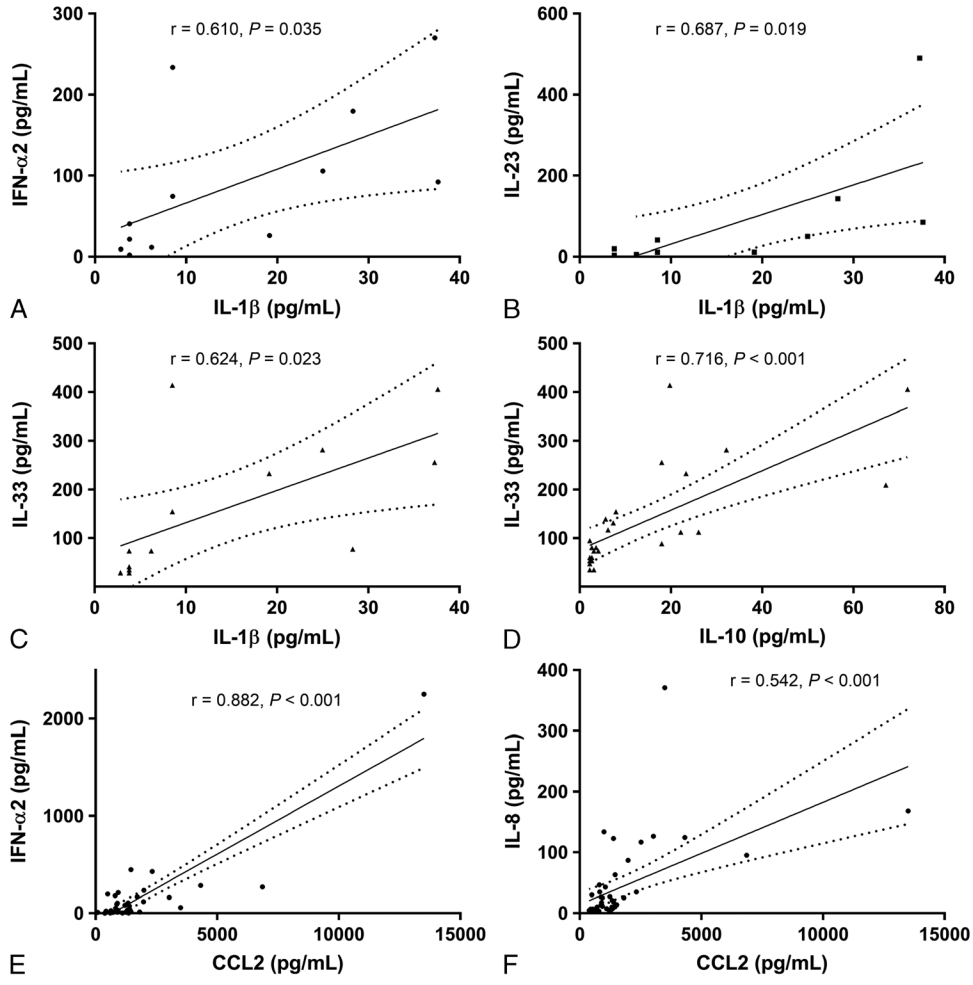

FIGURE 2. Correlation between serum cytokines levels. A, Interleukin-1β versus IFN-α2, (B) IL-1β versus IL-23, (C) IL-1β versus IL-33, (D) IL-10 versus IL-33, (E) CCL2 versus IFN-α2, (F) CCL2 versus IL-8.

analysis on the DMARD effects over the cytokine and chemokine network, utilizing another methodological approach (cases and controls, clinical assays, or cohort studies), focusing on the molecules that are shown to be inhibited by a specific treatment.

ACKNOWLEDGMENTS

The authors thank Juan P. Batiz-Andrade and Erick A. Wolfskill-Cox for their contribution to the final version of the article.

REFERENCES

1. Smolen JS, Aletaha D, Barton A, et al. Rheumatoid arthritis. *Nat Rev Dis Primers*. 2018;4:18001.
2. Moreno-Montoya J, Alvarez-Nemegyei J, Sanin LH, et al. Association of regional and cultural factors with the prevalence of rheumatoid arthritis in the Mexican population: a multilevel analysis. *J Clin Rheumatol*. 2015;21: 57–62.
3. Takeno M, Kitagawa S, Yamanaka J, et al. 5-Hydroxy-2-methylpyridine isolated from cigarette smoke condensate aggravates collagen-induced arthritis in mice. *Biol Pharm Bull*. 2018;41:877–884.
4. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2016; 388:2023–2038.
5. Calabresi E, Petrelli F, Bonifacio AF, et al. One year in review 2018: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol*. 2018;36: 175–184.
6. Cope AP, Schulze-Koops H, Aringer M. The central role of T cells in rheumatoid arthritis. *Clin Exp Rheumatol*. 2007;25(5 Suppl 46):S4–S11.
7. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). *Annu Rev Immunol*. 2010;28:445–489.
8. Croia C, Bursi R, Sutera D, et al. One year in review 2019: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol*. 2019;37:347–357.
9. Aydin SZ, Castillo-Gallego C, Nam J, et al. The new ACR/EULAR criteria for rheumatoid arthritis can identify patients with same disease activity but less damage by ultrasound. *Eur J Rheumatol*. 2017;4:118–121.
10. Alex P, Szodoray P, Knowlton N, et al. Multiplex serum cytokine monitoring as a prognostic tool in rheumatoid arthritis. *Clin Exp Rheumatol*. 2007;25:584–592.
11. Li Y, Yuan L, Yang J, et al. Changes in serum cytokines may predict therapeutic efficacy of tofacitinib in rheumatoid arthritis. *Mediators Inflamm*. 2019;2019:5617431.
12. Macedo RB, Kakehasi AM, Melo de Andrade MV. IL33 in rheumatoid arthritis: potential contribution to pathogenesis. *Rev Bras Reumatol Engl Ed*. 2016;56:451–457.
13. Xiangyang Z, Lutian Y, Lin Z, et al. Increased levels of interleukin-33 associated with bone erosion and interstitial lung diseases in patients with rheumatoid arthritis. *Cytokine*. 2012;58:6–9.
14. Lina C, Conghua W, Nan L, et al. Combined treatment of etanercept and MTX reverses T_H1/T_H2, T_H17/Treg imbalance in patients with rheumatoid arthritis. *J Clin Immunol*. 2011;31:596–605.

15. Yarilina A, Xu K, Chan C, et al. Regulation of inflammatory responses in tumor necrosis factor–activated and rheumatoid arthritis synovial macrophages by JAK inhibitors. *Arthritis Rheum*. 2012;64:3856–3866.
16. Dowty ME, Lin TH, Jesson MI, et al. Janus kinase inhibitors for the treatment of rheumatoid arthritis demonstrate similar profiles of in vitro cytokine receptor inhibition. *Pharmacol Res Perspect*. 2019;7:e00537.
17. Herman S, Zurgil N, Langevitz P, et al. Methotrexate selectively modulates T_H1/T_H2 balance in active rheumatoid arthritis patients. *Clin Exp Rheumatol*. 2008;26:317–323.
18. McInnes IB, Byers NL, Higgs RE, et al. Comparison of baricitinib, upadacitinib, and tofacitinib mediated regulation of cytokine signaling in human leukocyte subpopulations. *Arthritis Res Ther*. 2019;21:183.
19. Hodge JA, Kawabata TT, Krishnaswami S, et al. The mechanism of action of tofacitinib—an oral Janus kinase inhibitor for the treatment of rheumatoid arthritis. *Clin Exp Rheumatol*. 2016;34:318–328.
20. Noack M, Miossec P. Selected cytokine pathways in rheumatoid arthritis. *Semin Immunopathol*. 2017;39:365–383.
21. Bluml S. Biologicals and small molecules for rheumatoid arthritis [in German]. *Z Rheumatol*. 2020;79:223–231.
22. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. 2020;105949.
23. Chen W, Foo SS, Taylor A, et al. Correction for Chen et al., Bindarit, an inhibitor of monocyte chemotactic protein synthesis, protects against bone loss induced by chikungunya virus infection. *J Virol*. 2015;89:12232.
24. Osiri M, Wongpiyabovorn J, Sattayasomboon Y, et al. Inflammatory cytokine levels, disease activity, and function of patients with rheumatoid arthritis treated with combined conventional disease-modifying antirheumatic drugs or biologics. *Clin Rheumatol*. 2016;35:1673–1681.