

IL-1 Receptor Accessory Protein: A Potential Biomarker for Determination of Disease Activity in Rheumatoid Arthritis

Zeinab Baghernejadan^{1,2}, Nahid Kianmehr³, Anousheh Haghighi³, Mohammad-Ali Assarehzadegan^{1,2}, Ali-Akbar Delbandi^{1,2}, Fatemeh Afrashteh^{1,2}, Zahra Saleh^{1,2}, Milad Karimi^{1,2}, Reza Falak^{1,2}, and Majid Khoshmirsafa^{1,2}

¹ Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran

² Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

³ Department of Rheumatology, Iran University of Medical Sciences, Tehran, Iran

Received: 25 April 2026; Received in revised form: 8 June 2026; Accepted: 11 June 2026

ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by persistent synovitis and synovial hyperplasia. Although interleukin 1 (IL-1) signaling is an important inflammatory pathway in RA, the regulation of IL-1 receptor accessory protein (IL-1RAP) has not been comprehensively characterized.

This cross-sectional study evaluated IL-1RAP alterations in RA at the transcriptional and protein levels, including its membrane-bound and soluble forms. Thirty female RA patients were stratified according to disease activity into low, moderate, and high disease activity subgroups, and 15 healthy controls were included. Surface expression of membrane-bound IL-1RAP (mIL-1RAP) was assessed on peripheral blood leukocyte subsets, including CD4⁺ and CD8⁺ T cells, B cells, monocytes, and granulocytes. Plasma soluble IL-1RAP (sIL-1RAP) levels were measured, and relative mRNA expression of *IL1RAP*, *IL1A*, *IL1B*, *IL1R1*, *IL1R2*, and *IL1RN* was quantified in peripheral blood mononuclear cells (PBMCs).

mIL-1RAP expression was increased in several immune cell subsets, particularly in patients with high disease activity, and was positively associated with disease activity. In contrast, circulating sIL-1RAP levels were reduced and inversely associated with disease activity. *IL1RAP*, *IL1B*, and *IL1RN* transcripts were elevated, with the highest expression in patients with high disease activity. *IL1R1* and *IL1R2* expression remained unchanged, whereas *IL1A* upregulation was limited to high disease activity.

These findings suggest disease activity-dependent alterations in IL-1RAP-related pathways in RA. IL-1RAP may represent a candidate biomarker of disease activity, but larger longitudinal studies are needed to validate its diagnostic, monitoring, and therapeutic relevance.

Keywords: Arthritis; Biomarkers; Interleukin-1; Interleukin-1 receptor accessory protein; Leukocytes; Mononuclear; Rheumatoid

Corresponding Authors: Reza Falak, PhD;
Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran. Tel: (+98 21) 8670 3299, Email: Falak.r@iums.ac.ir

Majid Khoshmirsafa, PhD;
Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran. Tel: (+98 21) 8670 3284, Email: mj.khoshmirsafa@hotmail.com

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by persistent synovitis, synovial hyperplasia, and the development of autoantibodies, particularly rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies.^{1,2} As a heterogeneous disorder, RA manifests with variable clinical phenotypes; however, if left untreated, it may lead to progressive cartilage degradation, bone erosion, and irreversible physical disability.^{1,2} The pathogenesis of RA involves complex crosstalk between innate and adaptive immune responses, and the recruitment of effector leukocytes into the synovial compartment contributes to the formation of a pro-inflammatory microenvironment.^{1,2}

RA affects approximately 0.5% to 1% of adults worldwide and imposes a considerable clinical and economic burden through chronic pain, progressive joint damage, disability, reduced quality of life, impaired work productivity, and long-term treatment costs.³⁻⁵ Although RF, anti-CCP, ESR, and C-reactive protein (CRP) remain valuable biomarkers in RA, they may not fully capture disease activity and inflammatory heterogeneity in all patients.^{6,7} Thus, complementary biomarkers are needed to improve patient stratification, refine disease activity assessment, and support longitudinal monitoring.

The interleukin 1 (IL-1) superfamily plays an important role in RA pathogenesis.⁸ Pro-inflammatory members, particularly IL-1 β , contribute to cartilage damage and bone erosion by stimulating fibroblast-like synoviocytes to produce matrix metalloproteinases and by promoting osteoclastogenesis.^{9,10} The clinical use of IL-1 inhibitors, such as anakinra and canakinumab, further highlights the relevance of this pathway.^{11,12} However, targeting individual cytokines may yield incomplete responses because of the involvement of other IL-1 family members, including IL-33 and IL-36, and the presence of cytokine redundancy.^{13,14} Since many patients still fail to achieve remission, targeting shared signaling components such as IL-1 receptor accessory protein (IL-1RAP) may provide a broader approach to modulating the IL-1 family network.^{15,16}

IL-1RAP functions as a shared co-receptor within the IL-1 receptor family and participates in signaling complexes for IL-1, IL-33, and members of the IL-36 subfamily.¹⁶ Upon cytokine binding to its primary

receptor, IL-1RAP contributes to the formation of a heterotrimeric complex required for downstream signaling pathways.^{16,17} Notably, IL-1RAP exists in distinct isoforms, including a membrane-bound form (mIL-1RAP), which is essential for signal transduction, and a soluble form (sIL-1RAP), which may act as a natural decoy receptor that modulates the activity of IL-1 family cytokines.¹⁷

Despite the key role of IL-1RAP as a signaling hub in inflammatory diseases, its expression and regulatory patterns have not been sufficiently explored in RA. Most previous studies have primarily focused on gene expression, reporting increased *IL1RAP* mRNA levels in the peripheral blood of patients with active RA.^{18,19} However, data at the protein level, particularly regarding the surface expression of mIL-1RAP on specific leukocyte subsets, remain limited. Furthermore, circulating levels of sIL-1RAP and their association with clinical disease activity have not been clearly established in these patients.

Therefore, the present cross-sectional study was designed to characterize IL-1RAP-related alterations in patients with RA across transcriptional, membrane-bound, and soluble compartments. We assessed the surface expression of membrane-bound IL-1RAP (mIL-1RAP) across major peripheral blood leukocyte subsets, including CD4⁺ and CD8⁺ T cells, B cells, monocytes, and granulocytes, while simultaneously measuring plasma soluble IL-1RAP (sIL-1RAP) levels. We also quantified the relative expression of *IL1RAP* and selected IL-1 pathway-related genes in peripheral blood mononuclear cells (PBMCs). Finally, we analyzed the association between IL-1RAP-related parameters and DAS28-ESR disease activity scores to explore the potential relevance of IL-1RAP as a disease activity-associated biomarker candidate in RA.

Study Participants

This cross-sectional study included 30 female patients with RA who were consecutively recruited from the Rheumatology Clinic of Rasool Akram Hospital, Tehran, Iran, between December 2024 and May 2025. Only female patients were included to reduce sex-related immunological variability in this exploratory cohort. The inclusion criteria for RA patients were age 18–65 years, a confirmed diagnosis of RA according to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR)

classification criteria, written informed consent, and availability of the clinical and laboratory data required for disease activity assessment. The diagnosis of RA was confirmed by a rheumatologist.

Patients were excluded if they had concomitant autoimmune or chronic inflammatory diseases, active acute or chronic infection, known malignancy, pregnancy or lactation, recent vaccination, recent surgery or trauma, or use of immunomodulatory medications outside the routine RA treatment regimen that could interfere with the immunological outcomes of the study. The RA patients included in this study were not treatment-naïve and were receiving routine RA treatment at the time of enrollment. To minimize treatment-related variability, patients were included only when their treatment regimen was clinically stable, as far as documented, before sample collection.

Disease activity was assessed using the 28-joint Disease Activity Score with Erythrocyte Sedimentation Rate (DAS28-ESR). The DAS28 score was calculated using the number of tender and swollen joints, ESR levels, and the patient's global health assessment measured on a visual analogue scale (VAS; 0–100 mm).

Based on DAS28-ESR values, patients were stratified into 3 equal subgroups representing distinct disease activity states: low disease activity ($\text{DAS28} \geq 2.6$ and ≤ 3.2 , $n = 10$), moderate disease activity ($\text{DAS28} > 3.2$ and ≤ 5.1 , $n = 10$), and high disease activity ($\text{DAS28} > 5.1$, $n = 10$). Patients with inactive disease or remission ($\text{DAS28} < 2.6$) were excluded to minimize heterogeneity related to remission. The disease activity classification was confirmed by a rheumatologist. The detailed demographic and clinical characteristics are summarized in Table 1.

The control group consisted of 15 age- and sex-matched healthy individuals with no personal or family history (first- or second-degree relatives) of autoimmune or chronic inflammatory diseases. Healthy controls were excluded if they had active or recent infection, recent vaccination, use of immunosuppressive or immunomodulatory medications, known malignancy, pregnancy or lactation, recent surgery or trauma, positive RF or anti-CCP tests, or elevated inflammatory markers such as CRP or ESR.

Sample Collection and PBMC Isolation

Peripheral blood samples (12 mL) were collected from each participant by venipuncture using appropriate laboratory collection tubes. Clot activator tubes were

used for serum isolation and measurement of CRP, RF, and anti-CCP. EDTA-containing tubes were used for complete blood count (CBC) analysis, whole blood flow cytometry, PBMC isolation (5 mL), and plasma separation for sIL-1RAP measurement. Blood for ESR measurement was collected in the appropriate tube according to routine clinical laboratory procedures.

PBMCs were isolated immediately after collection by density gradient centrifugation using Ficoll (Peivand Gene Pazhouh Co., Iran) according to the manufacturer's instructions. Briefly, whole blood was diluted 1:1 with phosphate-buffered saline (PBS) and carefully layered over the gradient medium. After centrifugation at 400g for 20 minutes at room temperature with the brake off, the PBMC interface layer was collected and washed twice with PBS. The isolated PBMCs were stored in RNX-Plus reagent (SinaClon, Iran) at -80°C until RNA extraction.

Flow Cytometry

Immunophenotyping of peripheral blood cells was performed using 100 μL of fresh whole blood. Cells were stained with fluorochrome-conjugated monoclonal antibodies against CD4 (FITC, clone MEM-241), CD8 (PE, clone MEM-31), CD14 (FITC, clone MEM-18), and CD11b (FITC, clone ICRF44), all purchased from EXBIO (Vestec, Czech Republic); CD19 (PerCP, clone 4G7; BD Biosciences, USA); and IL-1RAP (APC, clone REA558, REAfinity™; Miltenyi Biotec, Germany). Antibody titration was performed before the main experiments to determine optimal staining volumes.

After staining, erythrocytes were lysed using $1 \times$ BD FACS™ Lysing Solution (BD Biosciences, USA), and cells were washed and resuspended in wash buffer. Data acquisition was performed immediately using a FACSCalibur flow cytometer (BD Biosciences, USA). After erythrocyte lysis, leukocyte populations were identified using forward scatter/side scatter (FSC/SSC) characteristics and lineage-specific markers. CD4^+ T cells, CD8^+ T cells, B cells, granulocytes, and monocytes were gated as shown in Figure 2. Fluorescence minus one (FMO) controls were used to establish gating boundaries for IL-1RAP positivity and to support consistent gate placement across samples.

A total of 20,000 to 45,000 events were acquired per sample, with an average of approximately 26,700 events per sample. Data were analyzed using FlowJo software (version 10.10.0; Tree Star, Ashland, OR, USA). IL-1RAP surface expression was reported as geometric

mean fluorescence intensity (gMFI) within each gated leukocyte subset.

Quantification of sIL-1RAP with Enzyme-linked Immunosorbent Assay (ELISA)

Plasma concentrations of sIL-1RAP were measured using a commercially available DuoSet® ELISA Development System for human IL-1RAcP/IL-1R3 (DY676, R&D Systems, USA), together with the DuoSet® Ancillary Reagent Kit 2 (DY008, R&D Systems, USA), according to the manufacturer's instructions. Based on a preliminary serial dilution test, plasma samples were diluted 1:100 before analysis. All samples and standards were assayed in duplicate. Optical density was measured at 450 nm with wavelength correction at 570 nm using a microplate reader (Biohit, Finland). Concentrations were calculated from a seven-point standard curve using a four-parameter logistic (4-PL) curve-fitting model and adjusted according to the dilution factor.

RNA Extraction, Complementary DNA (cDNA) Synthesis, and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from PBMCs using RNX-Plus reagent (SinaClon, Iran) according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA). Subsequently, 1.5 µg of total RNA from each sample was reverse-transcribed into cDNA using the Easy cDNA Ultra™ Synthesis Kit (Pars Tous Biotechnology, Iran).

Target gene expression was quantified by quantitative real-time PCR using RealQ Plus 2× Master Mix Green (Ampliqon, Denmark) and gene-specific primers (Metabion, Germany). Reactions were performed in duplicate on a Rotor-Gene Q real-time PCR system (QIAGEN, Hilden, Germany). Thermal cycling conditions consisted of an initial denaturation step at 95 °C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing/extension at 60 °C for 1 minute. Cycle threshold (Ct) values >40 were considered below the limit of quantification and were excluded from the analysis. Relative gene expression levels were calculated using the comparative Ct method ($2^{-\Delta\Delta C_t}$), with β -actin (*ACTB*) as the endogenous reference gene. Primer sequences are listed in Supplementary Table 1.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism software (version 10.6.1; GraphPad Software, San Diego, CA, USA). Data distribution was assessed using the Shapiro–Wilk test. For normally distributed variables, comparisons between two independent groups were performed using the unpaired t test with Welch correction when appropriate, whereas comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test. For non-normally distributed variables, comparisons between two independent groups were performed using the Mann–Whitney U test, whereas comparisons among multiple groups were performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test.

For multiple-group analyses, adjusted *p* values from the corresponding post hoc tests were reported. Correlations between disease activity and immunological parameters were evaluated using Spearman's rank-order correlation coefficient. Data are presented as mean±standard deviation (SD) for normally distributed variables or median (interquartile range, IQR) for non-normally distributed variables. All tests were two-tailed, and *p*<.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics of Study Participants

A total of 30 female patients with RA and 15 age- and sex-matched healthy controls were included in this study. The baseline demographic and clinical characteristics of the participants are summarized in Table 1. There was no statistically significant difference in age between RA patients (50.2±10.35 years) and HCs (50.6±9.70 years; *p*=.60), indicating appropriate age matching between the groups.

IL-1 Receptor Accessory Protein in Rheumatoid Arthritis

Table 1. Demographic and clinical characteristics of participants.

Characteristics	High disease activity RA (n=10)	Moderate disease activity RA (n=10)	Low disease activity RA (n=10)	RA patients (n=30)	Healthy controls (n=15)
Age, mean±SD, y	49.80±9.45	53.50±9.25	47.30±12.19	50.2±10.35	50.60±9.70
DAS28 score, median (IQR)	6.87 (6.24, 7.18)	5.05 (4.99, 5.08)	3.09 (2.99, 3.13)	5.05 (3.11, 6.34)	NA
TJC, median (IQR)	15.5 (13.5, 17)	5.5 (4, 6)	1 (1, 2.25)	5.5 (2, 14)	NA
SJC, median (IQR)	7.5 (6, 10)	2 (1, 3)	0 (0, 1)	2 (0.75, 6.25)	NA
Patient VAS, mean±SD, mm	73.00±11.60	43.00±9.487	17.50±7.546	44.50±24.89	NA
CRP, median (IQR), mg/L	22.08 (16.73, 27.64)	16.15 (12.6, 22.82)	4.82 (3.9, 8.77)	14.75 (8.34, 21.99)	1.63 (1.08, 2.24)
ESR, mean±SD, mm/h	57.80±15.95	47.4±13.36	19.50±6.205	41.57±20.42	13.07±5.561
Anti-CCP, median (IQR), U/mL	198 (122, 355)	118 (27, 185)	142 (50, 251)	138 (55, 247)	3.65 (2.79, 4.41)
Anti-CCP positivity, % (n)	90 (9)	80 (8)	90 (9)	86.7 (26)	0 (0)
RF, median (IQR), IU/mL	195.5 (29.32, 470.5)	144.5 (43.92, 319.5)	54.15 (22.23, 144.4)	98.76 (28.03, 315.6)	8.2 (6.4, 11.03)
RF positivity, % (n)	80 (8)	80 (8)	70 (7)	76.7 (23)	0 (0)
RBC, median (IQR), ×10 ⁶ /μL	4.21 (3.89, 4.54)	4.26 (4.06, 4.71)	4.37 (4.00, 4.70)	4.27 (4.02, 4.62)	4.55 (4.33, 4.79)
WBC, mean±SD, ×10 ³ /μL	6.94±2.13	6.80±1.91	7.22±2.14	6.99±2.00	7.07±1.35
PLT, median (IQR), ×10 ³ /μL	273 (231, 332)	270 (226, 308)	237 (210, 282)	261 (221, 292)	233 (210, 291)

Anti-CCP: anti-cyclic citrullinated peptide; CRP: C-reactive protein; DAS28: disease activity score 28; ESR: erythrocyte sedimentation rate; IQR: interquartile range; NA: not applicable; PLT: platelet count; RA: rheumatoid arthritis; RBC: red blood cell count; RF: rheumatoid factor; SD: standard deviation; SJC: swollen joint count; TJC: tender joint count; VAS: visual analogue scale; WBC: white blood cell count.

Based on DAS28-ESR scores, RA patients were stratified into low disease activity (n=10), moderate disease activity (n=10), and high disease activity (n=10) subgroups. As expected, clinical measures of disease activity, including tender joint count (TJC), swollen joint count (SJC), and patient global assessment measured by VAS, increased progressively from the low disease activity subgroup to the high disease activity

subgroup. Inflammatory markers were also higher in patients with more active disease, with increased CRP and ESR levels observed across disease activity subgroups ($p<0.001$).

Autoantibody levels, including anti-CCP and RF, were higher in the high disease activity subgroup than in the low disease activity subgroup ($p<0.001$ for both comparisons). Anti-CCP positivity was observed in

86.7% of RA patients, while RF positivity was detected in 76.7%. In contrast, hematological indices, including WBC, RBC, and platelet counts, were comparable across the study groups, with no statistically significant differences.

Decreased Plasma Levels of sIL-1RAP in Patients with High Disease Activity

Plasma levels of sIL-1RAP were quantified by ELISA. As shown in Figure 1, the median (IQR) plasma concentration of sIL-1RAP was 127.0 ng/mL (108.8–177.3) in RA patients and 164.0 ng/mL (128.0–290.0) in HCs ($p=0.02$). In the disease activity subgroup analysis, patients with high disease activity showed lower sIL-1RAP levels than HCs (adjusted $p=0.01$) and patients with low disease activity (adjusted $p=0.03$). Pairwise comparisons among RA disease activity subgroups showed no significant difference between low and moderate disease activity (adjusted $p=0.33$) or between moderate and high disease activity (adjusted $p>0.99$). Similarly, sIL-1RAP levels in patients with low or moderate disease activity did not differ significantly from those in HCs (adjusted $p>0.99$ and adjusted $p=0.20$, respectively). These findings suggest that the reduction in circulating sIL-1RAP is most pronounced in patients with high disease activity.

Differential Upregulation of mIL-1RAP on Immune Cells

To characterize cell subset-specific patterns of IL-1RAP expression, surface expression of mIL-1RAP was quantified across major peripheral blood immune cell subsets using flow cytometry (Figures 2 and 3).

Illustrative gating strategies used to identify CD4⁺ T cells, CD8⁺ T cells, B cells, granulocytes, and monocytes are presented in Figure 2A, C, E, G, and I. Representative overlaid histograms showed a rightward shift in fluorescence intensity in RA patients compared with HCs, suggesting increased mIL-1RAP expression across the assessed cell populations (Figure 2B, D, F, H, and J). The most pronounced shift was observed in patients with high disease activity, followed by those with moderate and low disease activity.

Quantitative flow cytometric analyses are summarized in Figure 3. IL-1RAP expression levels were reported as gMFI. Compared with HCs, patients with RA exhibited significantly higher mIL-1RAP expression on CD4⁺ T cells (Figure 3A, $p=0.002$), CD8⁺ T cells (Figure 3B, $p=0.003$), and B cells (Figure 3C, $p<0.05$).

Subgroup analysis indicated that these differences were mainly observed in patients with higher disease activity. Patients with high disease activity showed increased mIL-1RAP expression on CD4⁺ T cells compared with HCs (adjusted $p<0.001$) and patients with low disease activity (adjusted $p=0.02$) (Figure 3A). A similar pattern was observed for CD8⁺ T cells, with the high disease activity subgroup showing significantly higher expression than HCs (adjusted $p<0.001$) (Figure 3B). In B cells, the high disease activity subgroup displayed the highest expression levels, differing significantly from both the low disease activity subgroup (adjusted $p<0.001$) and the moderate disease activity subgroup (adjusted $p<0.001$) (Figure 3C).

In contrast, when all RA patients were analyzed collectively, mIL-1RAP expression on granulocytes and monocytes did not differ significantly from HCs (Figure 3D and E). However, stratification by disease activity revealed subgroup-specific differences. Granulocytes from patients with high disease activity exhibited significantly increased mIL-1RAP expression compared with HCs (adjusted $p<0.001$), patients with low disease activity (adjusted $p=.003$), and patients with moderate disease activity (adjusted $p=0.02$) (Figure 3D). Similarly, monocytes in the high disease activity subgroup showed significantly higher mIL-1RAP expression than HCs (adjusted $p=0.007$) and patients with low disease activity (adjusted $p=0.01$) (Figure 3E).

Transcriptional Upregulation of IL-1 Signaling Components in PBMCs

To further evaluate IL-1RAP-related alterations, the relative gene expression of selected IL-1 family members was analyzed in PBMCs (Figure 4). *IL1RAP* mRNA expression was significantly upregulated in patients with RA compared with HCs (Figure 4E, $p<0.001$). This transcriptional increase was consistent with the elevated surface protein expression observed by flow cytometry. Stratified analysis revealed a disease activity-associated pattern, with the highest *IL1RAP* expression detected in the high disease activity subgroup. Expression levels in this group were significantly elevated compared with HCs (Figure 4E, adjusted $p<0.001$) and were higher than those in patients with low disease activity (adjusted $p=0.04$).

Regarding IL-1 ligands, *IL1B* expression was significantly elevated in the total RA group compared with HCs (Figure 4B, $p<0.001$), with a progressive increase across disease activity subgroups. Patients with

IL-1 Receptor Accessory Protein in Rheumatoid Arthritis

high disease activity exhibited significantly higher *IL1B* expression than those with low disease activity (adjusted $p < 0.001$).

In contrast, *IL1A* expression did not differ significantly between the overall RA group and HCs (Figure 4A, $p = 0.22$). However, stratified analysis showed a selective increase in patients with high disease activity compared with both HCs (Figure 4A, adjusted $p = 0.03$) and patients with low disease activity (adjusted $p = 0.03$).

Although *IL1RAP* was significantly upregulated, the

expression levels of the primary receptor *IL1R1* and the decoy receptor *IL1R2* remained comparable between patients with RA and HCs (Figure 4C and D). In contrast, *IL1RN*, which encodes the natural IL-1 receptor antagonist IL-1Ra, was significantly increased in RA patients (Figure 4F, $p < 0.001$). Elevated *IL1RN* expression was observed across all disease activity subgroups, with the high disease activity subgroup exhibiting significantly higher levels than the low disease activity subgroup (adjusted $p = 0.002$).

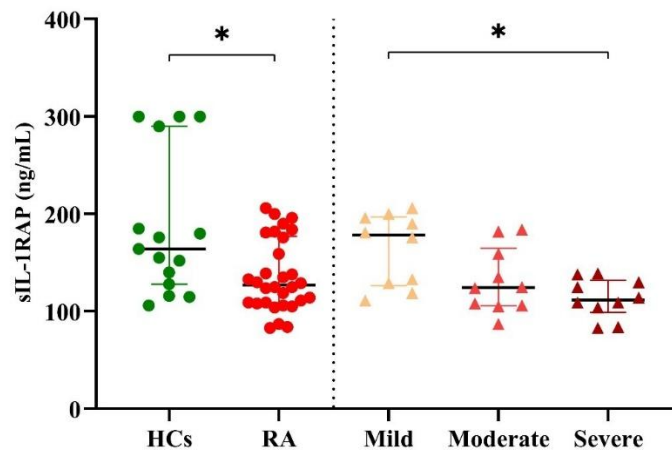
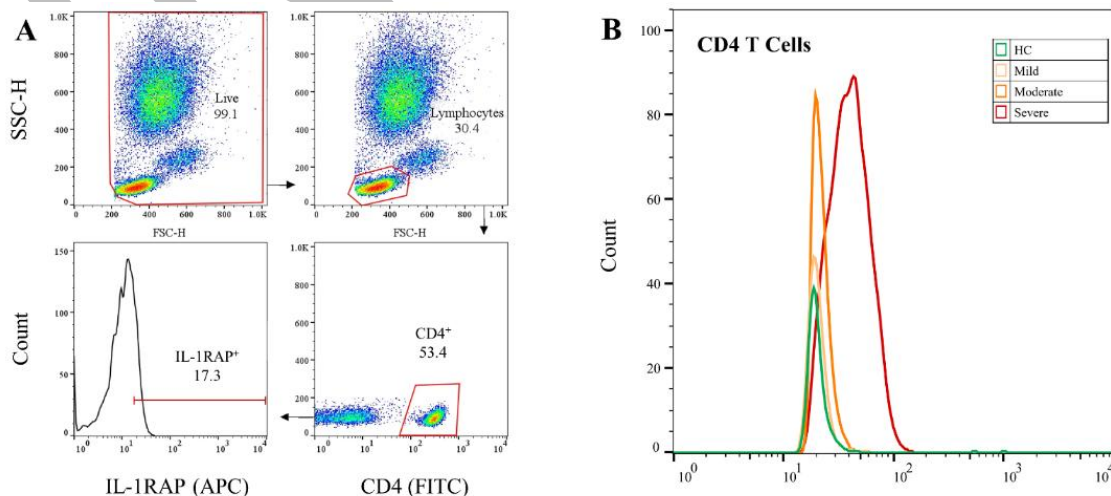
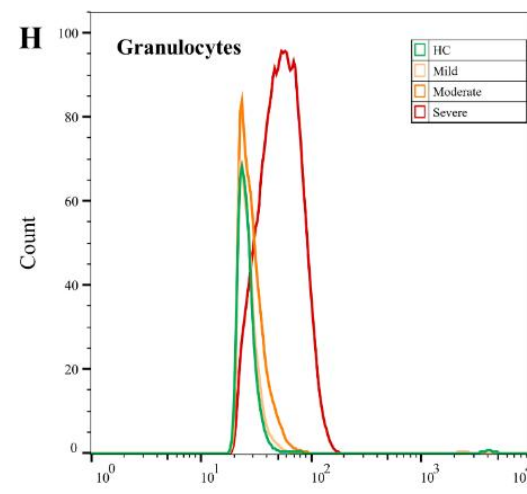
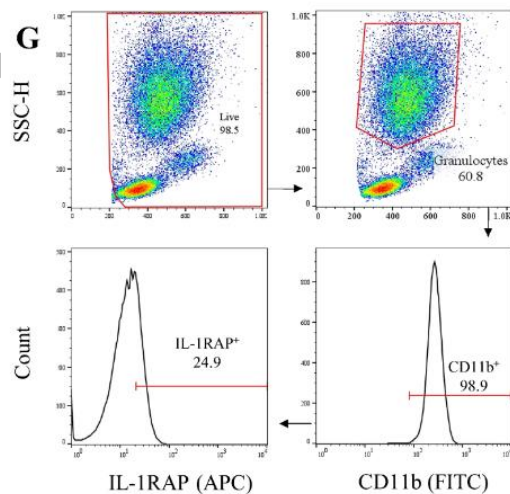
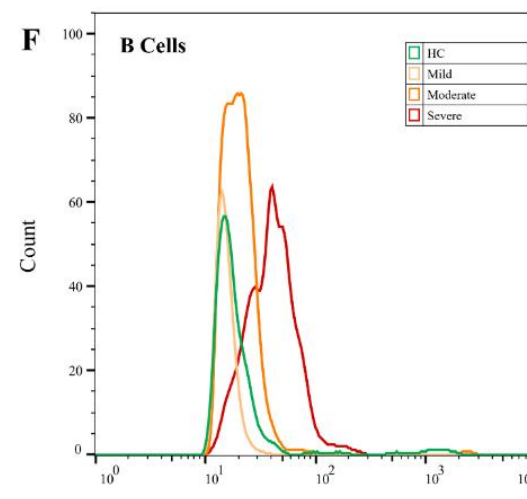
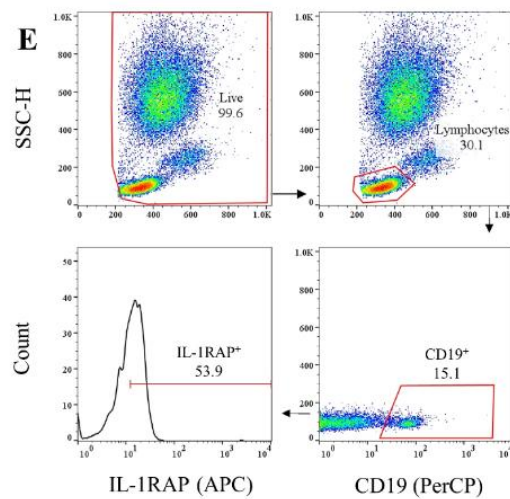
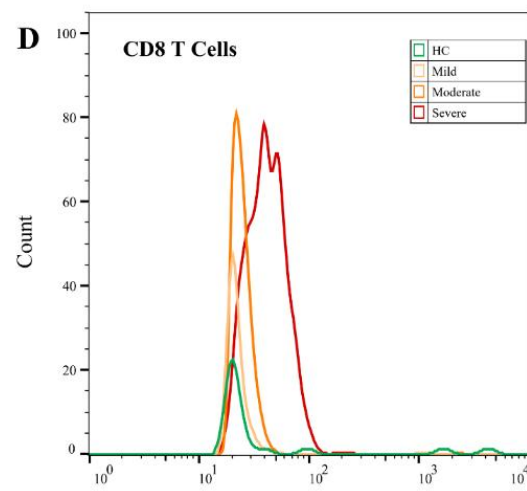
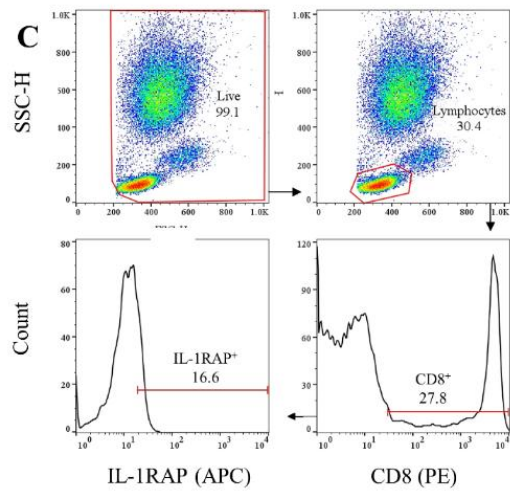


Figure 1. Plasma levels of sIL-1RAP in patients with RA. Plasma sIL-1RAP concentrations were measured by ELISA to compare circulating sIL-1RAP levels between healthy controls (HCs) and patients with RA and to evaluate their distribution across disease activity subgroups. The scatter plot illustrates the distribution of sIL-1RAP levels in HCs ($n = 15$) and RA patients ($n = 30$), as well as in RA patients stratified by DAS28-ESR into low disease activity ($n = 10$), moderate disease activity ($n = 10$), and high disease activity ($n = 10$) subgroups. Data are presented as median (IQR). Comparisons between two groups were performed using the Mann–Whitney U test, and comparisons among multiple groups were performed using the Kruskal–Wallis test followed by Dunn’s multiple comparisons test. * $p < .05$ versus HCs or the indicated groups.





IL-1 Receptor Accessory Protein in Rheumatoid Arthritis

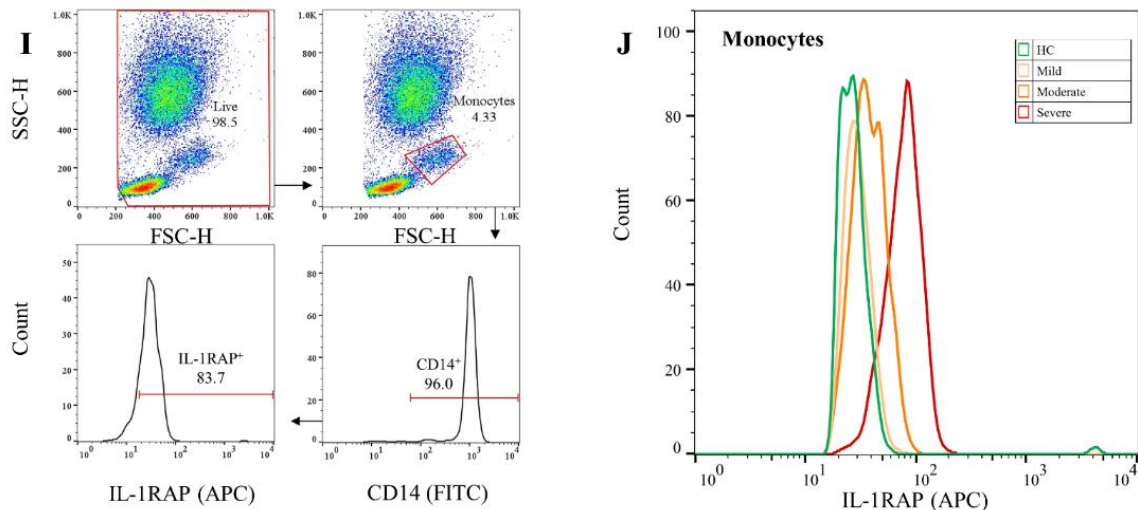
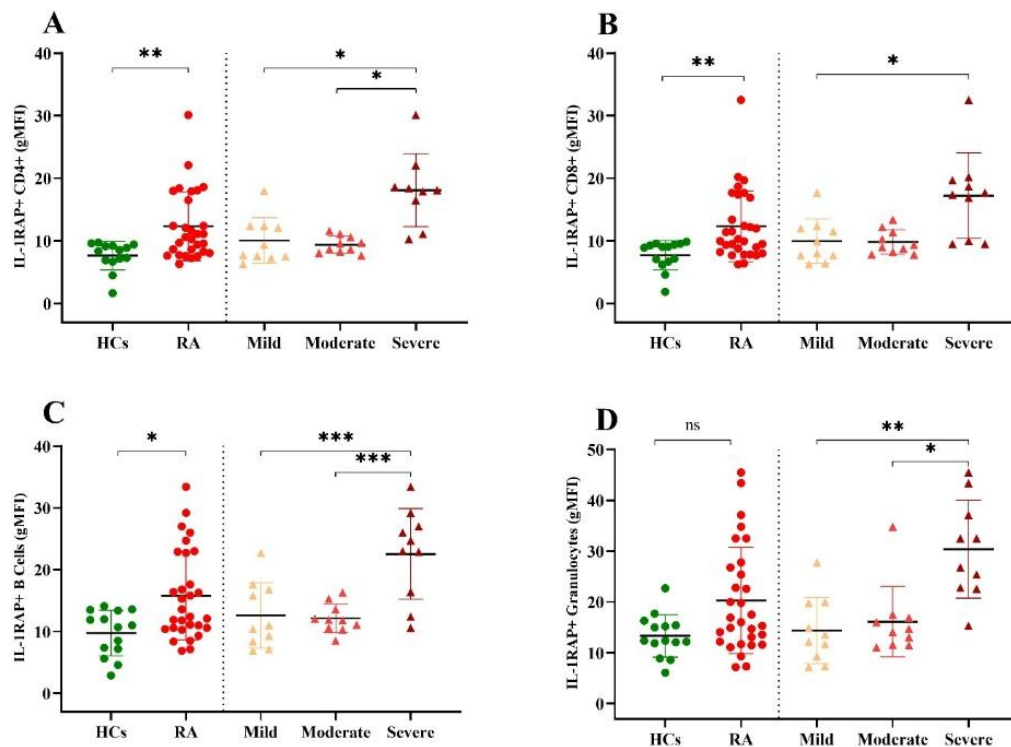


Figure 2. Gating strategy and representative histograms of mIL-1RAP expression. Sequential gating based on forward and side scatter (FSC/SSC) properties and lineage-specific surface markers was used to identify (A) CD4⁺ T cells, (C) CD8⁺ T cells, (E) B cells, (G) granulocytes, and (I) monocytes in peripheral blood samples. Corresponding overlaid histograms illustrate mIL-1RAP expression within each subset (B, D, F, H, and J). Each histogram represents data from four individuals: one healthy control (HC) and three patients with RA representing low, moderate, and high disease activity. APC, allophycocyanin; FITC, fluorescein-5-isothiocyanate; PerCP, peridinin-chlorophyll-protein; PE, phycoerythrin.



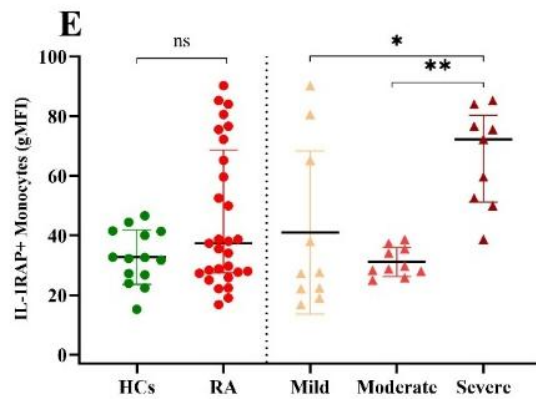
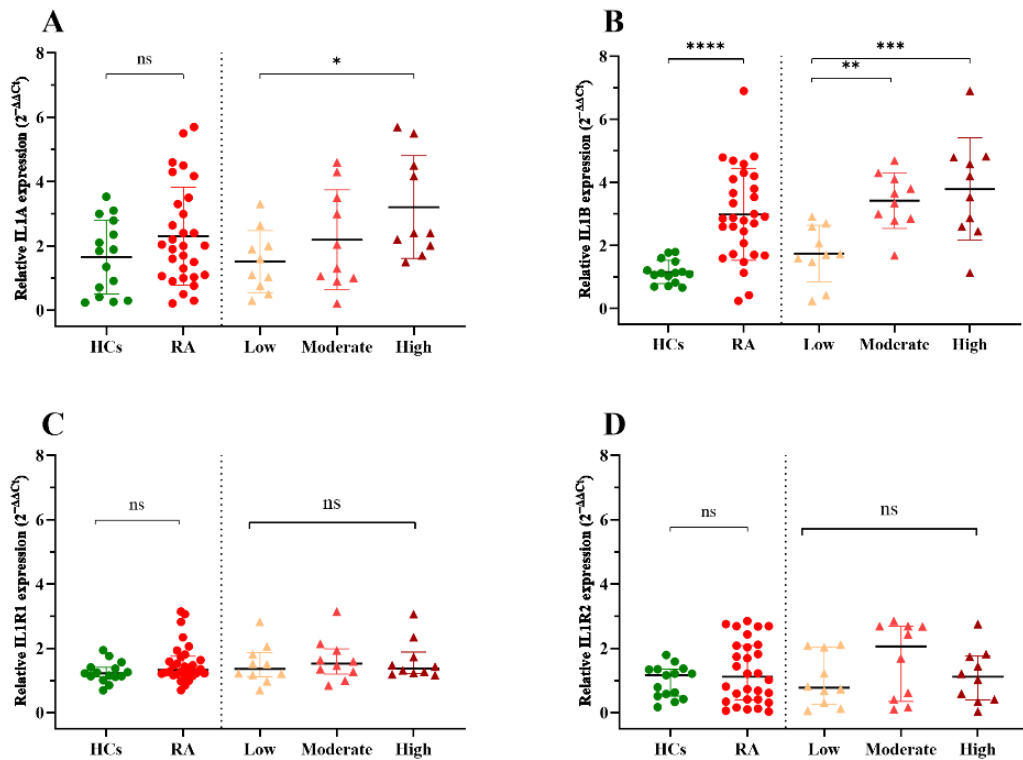


Figure 3. Quantitative analysis of mIL-1RAP expression on leukocyte subsets. Geometric mean fluorescence intensity (gMFI) of mIL-1RAP was quantified on (A) CD4⁺ T cells, (B) CD8⁺ T cells, (C) B cells, (D) granulocytes, and (E) monocytes in healthy controls (HCs) and patients with RA to compare IL-1RAP surface expression across leukocyte subsets and disease activity states. Patients were stratified according to DAS28-ESR into low disease activity (n = 10), moderate disease activity (n = 10), and high disease activity (n = 10) subgroups. Data are presented as median (IQR). Comparisons between HCs and total RA patients were performed using the Mann–Whitney U test. Multiple-group comparisons were performed using the Kruskal–Wallis test followed by Dunn’s multiple comparisons test. **p* < .05; ***p* < .01; ****p* < .001; ns, not significant.



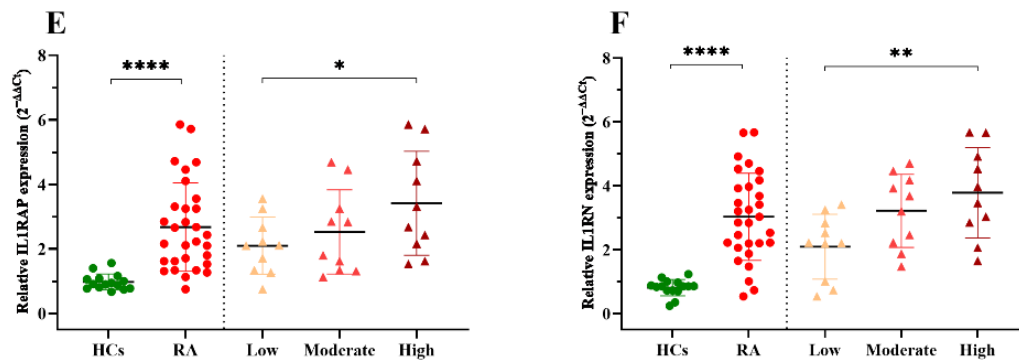


Figure 4. Relative gene expression of IL-1 family members in PBMCs. mRNA expression levels of (A) *IL1A*, (B) *IL1B*, (C) *IL1R1*, (D) *IL1R2*, (E) *IL1RAP*, and (F) *IL1RN* were quantified by real-time quantitative PCR (qPCR) in healthy controls (HCs) and patients with RA to assess transcriptional alterations in IL-1 pathway–related genes. Patients were further stratified according to DAS28-ESR into low, moderate, and high disease activity subgroups. Gene expression levels were normalized to β -actin (*ACTB*) and calculated using the comparative Ct ($2^{-\Delta\Delta C_t}$) method. Data are presented according to their distribution as mean $\pm$ SD or median (IQR), as appropriate for each panel. Comparisons between HCs and total RA patients were performed using the Mann–Whitney U test or unpaired t test with Welch’s correction, as appropriate. Multiple-group comparisons were performed using one-way ANOVA followed by Tukey’s multiple comparisons test or Kruskal–Wallis test followed by Dunn’s multiple comparisons test, as appropriate for each panel. * $p<.05$; ** $p<.01$; *** $p<.001$; **** $p<.001$; ns, not significant.

Correlation between IL-1RAP Expression and Disease Activity

To evaluate the association between IL-1RAP alterations and disease activity in patients with RA, Spearman rank-order correlation analysis was performed between immunological parameters and DAS28 scores (Figure 5).

Consistent with the subgroup analysis, sIL-1RAP levels demonstrated a significant negative correlation with DAS28 (Figure 5A, $r=-0.498$, $p=.005$), indicating that lower circulating concentrations of sIL-1RAP were associated with higher disease activity. In contrast, surface expression of mIL-1RAP across all examined leukocyte subsets showed positive correlations with disease activity. Among innate immune cells, granulocytes exhibited the strongest positive association with DAS28 (Figure 5E, $r=0.549$, $p=.001$), followed by monocytes (Figure 5F, $r=0.503$, $p=.005$). Among adaptive immune cells, CD4⁺ T cells showed a significant positive correlation (Figure 5B, $r=0.515$, $p=.004$), similar to that observed in B cells (Figure 5D, $r=0.495$, $p=.005$). CD8⁺ T cells also showed a positive association with disease activity (Figure 5C, $r=0.447$, $p=0.01$).

Together, these findings suggest that higher disease activity in RA is associated with increased mIL-1RAP expression on peripheral blood immune cells and reduced circulating sIL-1RAP levels.

DISCUSSION

Although IL-1 signaling represents an important inflammatory pathway in RA, the regulation of IL-1RAP, as an essential accessory co-receptor in this pathway, has not been sufficiently characterized. In the present study, we evaluated IL-1RAP at multiple biological levels by analyzing *IL1RAP* mRNA expression in PBMCs, assessing mIL-1RAP expression on peripheral immune cell subsets, and measuring sIL-1RAP levels in plasma. The main finding of this study was that IL-1RAP-related alterations were associated with RA disease activity. Specifically, mIL-1RAP expression was increased across multiple immune cell subsets, particularly in patients with high disease activity, whereas circulating sIL-1RAP levels were reduced and inversely associated with disease activity.

Together, these findings suggest that RA disease activity may be accompanied by a shift in the IL-1RAP profile, characterized by increased cellular IL-1RAP availability and reduced circulating sIL-1RAP. The reduction in sIL-1RAP observed in patients with high disease activity may reflect diminished extracellular regulation of IL-1–mediated inflammation. Under physiological conditions, sIL-1RAP enhances the decoy function of soluble IL-1R2 by markedly increasing its affinity for IL-1 β , thereby strengthening extracellular

cytokine neutralization.²¹ Experimental evidence further supports the functional relevance of this mechanism, as overexpression of sIL-1RAP has been shown to attenuate collagen-induced arthritis in mice through receptor competition and reduced IL-1 responsiveness, particularly in B cells.²² Importantly, although Smith et al did not observe significant differences in sIL-1RAP levels in a mixed group of RA patients, they reported substantial reductions in experimental models of acute inflammation and arthritis, suggesting that this decrease may be more evident under conditions of higher inflammatory burden.²¹ Consistent with this interpretation, in our study, sIL-1RAP levels were lower in RA patients than in controls, while stratified analysis indicated that this reduction was mainly observed in patients with high disease activity. These findings suggest that reduced sIL-1RAP may be more closely related to inflammatory burden than to RA status alone.

Similar reductions in sIL-1RAP have been reported in other IL-1-associated inflammatory conditions, including Schnitzler syndrome,²³ obesity,²⁴ and endometriosis,²⁵ where insufficient counter-regulation of IL-1 signaling has been proposed. In contrast, available data suggest that this pattern may not be uniform across autoimmune diseases. For example, Petrackova et al did not detect *IL1RAP* upregulation in PBMCs of patients with systemic lupus erythematosus (SLE).¹⁸ and Italiani et al reported stable sIL-1RAP levels in SLE despite fluctuations in other IL-1 family inhibitors.²⁶ These comparisons suggest that changes in sIL-1RAP may be context-dependent within the IL-1 regulatory network and may reflect disease-specific inflammatory conditions rather than systemic autoimmunity in general.

In parallel with reduced soluble levels, flow cytometric analysis demonstrated increased mIL-1RAP expression on circulating immune cells. Because IL-1RAP functions as an obligate co-receptor for IL-1, IL-33, and IL-36,¹⁶ increased surface abundance may influence cellular responsiveness across multiple IL-1 family pathways. Although upregulation was detectable in the overall RA group, it was most pronounced in patients with high disease activity. Within the adaptive immune compartment, CD4⁺ T cells, CD8⁺ T cells, and B cells showed increased mIL-1RAP expression, suggesting a possible association with enhanced sensitivity to IL-1-dependent activation signals. Given the established role of IL-1 in promoting Th17 differentiation and B-cell activation,^{27,28} increased

receptor availability may be linked to lower activation thresholds in these subsets, although functional polarization and downstream signaling were not directly assessed in the present study.

The pattern of mIL-1RAP expression also suggested that IL-1RAP alterations may not occur uniformly across all peripheral immune cell lineages. In the overall comparison between RA patients and HCs, significant increases were mainly detected in lymphoid-derived immune cells, whereas monocytes and granulocytes did not differ significantly when all RA patients were analyzed collectively. In contrast, mIL-1RAP upregulation on myeloid-derived cells became evident mainly in patients with high disease activity. This may reflect cell type-specific regulation of IL-1RAP in peripheral blood, as well as differences in activation thresholds, inflammatory exposure, cell turnover, or tissue redistribution of monocytes and granulocytes. Consistent with this interpretation, single-cell analyses of synovial tissue from RA patients have demonstrated expanded *IL1B*⁺ pro-inflammatory monocyte states.²⁹ Our observation that circulating monocytes from patients with high disease activity exhibited elevated IL-1RAP expression raises the possibility that these cells may acquire an IL-1RAP-enriched phenotype in the setting of higher systemic inflammation before or during tissue recruitment. Because neutrophils and monocytes are major mediators of inflammatory amplification and tissue damage in RA,^{30–33} increased IL-1RAP expression on these cells may be associated with enhanced responsiveness not only to IL-1 β but also to other IL-1RAP-dependent cytokines such as IL-33 and IL-36.

Although data on mIL-1RAP expression in RA are limited, evidence from other inflammatory contexts supports the biological plausibility of our findings. In myeloid malignancies, IL-1RAP overexpression enhances NF- κ B-mediated signaling after IL-1 stimulation, whereas IL-1RAP blockade reduces inflammatory cytokine production.^{34,35} Similarly, IL-1RAP inhibition in human endothelial cells reduces IL-1-induced cytokine secretion and adhesion molecule expression.³⁶ However, because our study did not include IL-1RAP blockade, ligand stimulation, or downstream signaling assays, increased mIL-1RAP should be interpreted as a disease activity-associated marker with potential functional relevance rather than direct evidence of causal signaling amplification.

When considered alongside the ELISA findings, the increase in mIL-1RAP and reduction in sIL-1RAP

suggest that high disease activity in RA may be associated with a shift toward greater cellular IL-1RAP availability and reduced soluble regulatory capacity. The positive correlations between mIL-1RAP expression and DAS28 further support an activity-linked IL-1RAP profile. Collectively, these findings indicate that altered IL-1RAP expression may reflect an immunological state associated with higher inflammatory activity in RA.

The transcriptional findings further support the involvement of IL-1RAP-related pathways in RA disease activity. *IL1RAP* mRNA was significantly upregulated in PBMCs and reached its highest levels in patients with high disease activity, broadly consistent with the pattern of increased membrane-bound protein expression observed by flow cytometry. Importantly, *IL1R1* and *IL1R2* expression remained unchanged, suggesting that disease activity-associated alterations in this cohort may be more closely related to accessory receptor regulation than to changes in the primary or decoy IL-1 receptors.

The apparent discrepancy between increased *IL1RAP* mRNA expression in PBMCs and the absence of significant mIL-1RAP upregulation on monocytes and granulocytes in the overall RA group may have several explanations. qPCR was performed on bulk PBMCs, which mainly include lymphocytes and monocytes but not granulocytes; therefore, total PBMC *IL1RAP* expression may largely reflect lymphoid and monocytic changes. In addition, mRNA abundance does not necessarily translate directly into surface protein expression because post-transcriptional regulation, protein trafficking, isoform-specific regulation, and cell turnover may influence membrane IL-1RAP availability. Finally, myeloid mIL-1RAP upregulation became evident mainly after disease activity stratification, suggesting closer association with high inflammatory activity than with RA status alone.

The ligand and antagonist profile also supports an association with IL-1 pathway activation. *IL1B* expression increased progressively with disease activity, whereas *IL1A* was elevated only in patients with high disease activity. In parallel, *IL1RN* was consistently upregulated across disease activity subgroups, with the highest expression in patients with high disease activity. This pattern may reflect concurrent activation of inflammatory and counter-regulatory components of the IL-1 pathway. Similar findings were reported by Edwards et al, supporting the concept that *IL1RN* expression may track inflammatory burden.³⁷ In

addition, our findings are consistent with independent PBMC studies by Petrackova et al, which identified *IL1RAP* among the most discriminatory genes distinguishing RA from controls and other autoimmune diseases.^{18,19}

Mechanistically, IL-1RAP is required for signaling downstream of IL-1, IL-33, and IL-36.¹⁶ Recent studies in systemic sclerosis similarly suggest that IL-1RAP may act as an important inflammatory node.^{18,38} In this context, concurrent upregulation of *IL1B*, *IL1RN*, and *IL1RAP* in RA may reflect remodeling of the IL-1 pathway, characterized by increased IL-1 ligand expression, preserved expression of *IL1R1* and *IL1R2*, increased accessory receptor abundance, and induction of the endogenous antagonist *IL1RN*. However, because this study did not directly assess downstream signaling activity, these transcriptional changes should be interpreted as evidence of IL-1 pathway involvement rather than proof of functional pathway activation.

The divergence between increased *IL1RAP* mRNA expression and reduced circulating sIL-1RAP levels may also reflect compartment-specific regulation of IL-1RAP isoforms. sIL-1RAP is generated predominantly through alternative splicing rather than proteolytic shedding,¹⁶ indicating that increased total *IL1RAP* transcription does not necessarily translate into elevated soluble protein levels. Differential regulation of membrane-bound and sIL-1RAP isoforms may therefore contribute to the opposite patterns observed in patients with high disease activity.

When considered together, increased membrane IL-1RAP availability and reduced circulating sIL-1RAP levels may indicate a shift toward greater cellular responsiveness to IL-1 family cytokines and reduced soluble regulatory capacity in patients with high disease activity. The positive correlations between mIL-1RAP expression and DAS28, together with the inverse correlation between sIL-1RAP and DAS28, further support an activity-associated IL-1RAP profile in RA. Among the evaluated subsets, granulocytes showed the strongest positive correlation between mIL-1RAP expression and DAS28. This suggests that granulocyte-associated mIL-1RAP may be particularly sensitive to disease activity, although lymphoid subsets may also be useful because CD4⁺ T cells and B cells differed significantly between the overall RA group and HCs. Future longitudinal studies should compare the diagnostic and monitoring performance of IL-1RAP across multiple immune cell subsets.

IL-1RAP-based measurements would not be expected to replace established markers such as RF and anti-CCP, which remain valuable for RA diagnosis and classification. Instead, IL-1RAP may provide complementary information on disease activity-associated IL-1 family pathway alterations, particularly because RF and anti-CCP may persist regardless of current inflammatory activity, whereas ESR and CRP may not fully capture cellular immune activation. Thus, IL-1RAP should be explored as a complementary biomarker candidate for patient stratification and longitudinal monitoring rather than as a stand-alone diagnostic test.

Whether IL-1RAP-based measures are treatment-responsive remains unknown and requires longitudinal investigation. The concurrent upregulation of *IL1RAP* at transcriptional and cellular levels also supports further exploration of IL-1RAP as a therapeutic target; however, functional studies using ligand stimulation, IL-1RAP blockade, and downstream signaling assays are needed before causal or therapeutic conclusions can be drawn.

The authors acknowledge several limitations. First, the sample size was modest and participants were recruited from a single center; therefore, the findings require validation in larger, independent cohorts. Second, all RA patients included in this study were female, which may limit the generalizability of the findings to the broader RA population, particularly male patients. Third, the cross-sectional design precludes conclusions regarding temporal relationships, causality, or the predictive value of IL-1RAP-related measures for future disease activity. Fourth, mIL-1RAP expression was assessed on total CD4⁺ T cells without separate analysis of CD4⁺ T-cell subsets. Given the role of IL-1 signaling in Th17 differentiation, increased mIL-1RAP on total CD4⁺ T cells may partly reflect changes in activated subsets, including Th17 cells, rather than uniform changes across the entire CD4⁺ T-cell population. In addition, isoform-specific transcriptional analysis was not performed, although this could provide further insight into the splice-dependent regulation of sIL-1RAP. Another methodological limitation is that qPCR normalization was based on *ACTB* as the sole reference gene; therefore, future studies using multiple validated housekeeping genes would strengthen gene expression analyses. Moreover, sIL-1RAP was measured only in plasma and not in synovial fluid, so the findings may not fully reflect local IL-1RAP regulation

within the joint. Finally, patients were not treatment-naïve, and potential effects of treatment heterogeneity on IL-1RAP expression and IL-1 pathway-related markers cannot be excluded.

In conclusion, IL-1RAP appears to be an altered component of the IL-1 signaling network in RA. We observed a disease activity-associated pattern characterized by increased *IL1RAP* transcription, increased membrane expression across several peripheral immune cell subsets, and reduced circulating sIL-1RAP levels. This pattern occurred alongside elevated *IL1B* and *IL1RN* expression, suggesting an association with IL-1 pathway-related immune alterations in patients with more active disease. These findings indicate that IL-1RAP may represent a disease activity-associated component of IL-1 family signaling and a candidate molecular marker for future biomarker and therapeutic studies. Larger longitudinal and mechanistic investigations are required to validate its clinical utility and functional relevance in RA.

STATEMENT OF ETHICS

The study protocol was approved by the Ethics Committee of Iran University of Medical Sciences (approval code: IR.IUMS.FMD.REC.1402.320). Written informed consent was obtained from all participants prior to enrollment in accordance with the Declaration of Helsinki.

FUNDING

This study was supported by the Vice-Chancellor for Research and Technology of Iran University of Medical Sciences (grant number: 28207).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors would like to thank the patients and healthy volunteers who participated in this study. We also acknowledge the respectful staff of the Rheumatology Clinic of Rasool Akram Hospital for their assistance in patient recruitment.

DATA AVAILABILITY

The data are available from the corresponding author upon request.

AI ASSISTANCE DISCLOSURE

During manuscript preparation, ChatGPT (OpenAI) was used for language editing, grammar correction, wording refinement, and proofreading. ChatGPT was not used to generate or interpret scientific data, perform statistical analyses, or make scientific conclusions. All scientific content, interpretation of the results, and final approval of the manuscript were performed and verified by the authors, who take full responsibility for the content of the manuscript.

REFERENCES

- Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, et al. Rheumatoid arthritis. *Nat Rev Dis Primers*. 2016;12(1):1-23.
- Radu AF, Bungau SG. Management of rheumatoid arthritis: an overview. *Cells*. 2021;10(11):2857.
- Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*. 2016;388(10055):2023-38.
- Di Matteo A, Bathon JM, Emery P. Rheumatoid arthritis. *Lancet*. 2023;402(10416):2019-33.
- Hsieh PH, Wu O, Geue C, McIntosh E, McInnes IB, Siebert S. Economic burden of rheumatoid arthritis: a systematic review of literature in biologic era. *Ann Rheum Dis*. 2020;79(6):771-777.
- Shapiro SC. Biomarkers in rheumatoid arthritis. *Cureus*. 2021;13(5):e15063.
- Abdelhafiz D, Baker T, Glasgow DA, Abdelhafiz A. Biomarkers for the diagnosis and treatment of rheumatoid arthritis: a systematic review. *Postgrad Med*. 2023;135(3):214-23.
- Dinarello CA. The IL-1 family of cytokines and receptors in rheumatic diseases. *Nat Rev Rheumatol*. 2019;15(10):612-32.
- McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med*. 2011;365(23):2205-19.
- Nygaard G, Firestein GS. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. *Nat Rev Rheumatol*. 2020;16(6):316-33.
- Alten R, Gomez-Reino J, Durez P, Beaulieu A, Sebba A, Krammer G, et al. Efficacy and safety of the human anti-IL-1 β monoclonal antibody canakinumab in rheumatoid arthritis: results of a 12-week, phase II, dose-finding study. *BMC Musculoskelet Disord*. 2011;12:153.
- Cohen S, Hurd E, Cush J, Schiff M, Weinblatt ME, Moreland LW, et al. Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum*. 2002;46(3):614-24.
- Wang K, Luo H, Liu L, Gao H, Song Y, Li D. Blockade of IL-1 family cytokines in the treatment of rheumatoid arthritis. *Front Pharmacol*. 2025;16:1577628. doi:10.3389/fphar.2025.1577628.
- Xu Y, Wang JY, Zou Y, Ma XW, Meng T. Role of IL-1 family cytokines IL-36, IL-37, and IL-38 in osteoarthritis and rheumatoid arthritis: a comprehensive review. *J Inflamm Res*. 2024;17:4001-16.
- Liu Q, Wang Y, Jiang Y, Ru Y, Zhao Q, Zhang Z, et al. Anti-IL1RAP antibodies for pan-inhibition of IL-1 family cytokine signaling in inflammatory diseases and oncology. *bioRxiv*. 2026.
- Fields JK, Günther S, Sundberg EJ. Structural basis of IL-1 family cytokine signaling. *Front Immunol*. 2019;10:1412.
- Boraschi D, Italiani P, Weil S, Martin MU. The family of the interleukin-1 receptors. *Immunol Rev*. 2018;281(1):197-232.
- Petrackova A, Horak P, Radvansky M, Skacelova M, Fillerova R, Kudelka M, et al. Cross-disease innate gene signature: emerging diversity and abundance in RA compared to SLE and SSC. *J Immunol Res*. 2019;2019:3575803.
- Petrackova A, Horak P, Radvansky M, Fillerova R, Smotkova Kraiczova V, Kudelka M, et al. Revealed heterogeneity in rheumatoid arthritis based on multivariate innate signature analysis. *Clin Exp Rheumatol*. 2020;38(2):289-298.
- Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis*. 2010;69(9):1580-1588.
- Smith DE, Hanna R, Della F, Moore H, Chen H, Farese AM, et al. The soluble form of IL-1 receptor accessory protein enhances the ability of soluble type II IL-1 receptor to inhibit IL-1 action. *Immunity*. 2003;18(1):87-96.
- Smeets RL, Joosten LA, Arntz OJ, Bennink MB, Takahashi N, Carlsen H, et al. Soluble interleukin-1

- receptor accessory protein ameliorates collagen-induced arthritis by a different mode of action from that of interleukin-1 receptor antagonist. *Arthritis Rheum.* 2005;52(7):2202-11.
23. Migliorini P, Italiani P, Pratesi F, Puxeddu I, Boraschi D. Cytokines and soluble receptors of the interleukin-1 family in Schnitzler syndrome. *Scand J Rheumatol.* 2019;48(3):235-238.
 24. Bozaoglu K, Attard C, Kulkarni H, Cummings N, Diego VP, Carless MA, et al. Plasma levels of soluble interleukin 1 receptor accessory protein are reduced in obesity. *J Clin Endocrinol Metab.* 2014;99(9):3435-43.
 25. Michaud N, Al-Akoum M, Akoum A. Blood soluble interleukin 1 receptor accessory protein levels are consistently low throughout the menstrual cycle of women with endometriosis. *Reprod Biol Endocrinol.* 2014;12:51
 26. Italiani P, Manca ML, Angelotti F, Melillo D, Pratesi F, Puxeddu I, et al. IL-1 family cytokines and soluble receptors in systemic lupus erythematosus. *Arthritis Res Ther.* 2018;20(1):27. doi:10.1186/s13075-018-1525-z.
 27. Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, et al. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity.* 2009;30(4):576-587.
 28. Lipsky PE, Thompson PA, Rosenwasser LJ, Dinarello CA. The role of interleukin 1 in human B cell activation: inhibition of B cell proliferation and the generation of immunoglobulin-secreting cells by an antibody against human leukocytic pyrogen. *J Immunol.* 1983;130(6):2708-2714.
 29. Zhang F, Wei K, Slowikowski K, Fonseka CY, Rao DA, Kelly S, et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat Immunol.* 2019;20(7):928-42.
 30. Rana AK, Li Y, Dang Q, Yang F. Monocytes in rheumatoid arthritis: circulating precursors of macrophages and osteoclasts and their heterogeneity and plasticity role in RA pathogenesis. *Int Immunopharmacol.* 2018;65:348-59.
 31. Salnikova DI, Nikiforov NG, Postnov AY, Orekhov AN. Target role of monocytes as key cells of innate immunity in rheumatoid arthritis. *Diseases.* 2024;12(5):81.
 32. Wright HL, Moots RJ, Edwards SW. The multifactorial role of neutrophils in rheumatoid arthritis. *Nat Rev Rheumatol.* 2014;10(10):593-601.
 33. Deng Y, Li J, Wu R. Neutrophils in rheumatoid arthritis synovium: implications on disease activity and inflammation state. *J Inflamm Res.* 2025;18:4741-4753.
 34. Mitchell K, Barreyro L, Todorova TI, Taylor SJ, Antony-Debré I, Narayanagari SR, et al. IL1RAP potentiates multiple oncogenic signaling pathways in AML. *J Exp Med.* 2018;215(6):1709-27.
 35. De Boer B, Sheveleva S, Apelt K, Vellenga E, Mulder AB, Huls G, et al. The IL1-IL1RAP axis plays an important role in the inflammatory leukemic niche that favors acute myeloid leukemia proliferation over normal hematopoiesis. *Haematologica.* 2021;106(12):3067-78.
 36. Lindkvist M, Göthlin Eremo A, Paramel GV, Anisul Haque S, Rydberg Millrud C, Rattik S, et al. IL1RAP expression in human atherosclerosis: a target of novel antibodies to reduce vascular inflammation and adhesion. *J Am Heart Assoc.* 2025;14(10):e039557.
 37. Edwards CK 3rd, Green JS, Volk HD, Schiff M, Kotzin BL, Mitsuya H, et al. Combined anti-tumor necrosis factor- α therapy and DMARD therapy in rheumatoid arthritis patients reduces inflammatory gene expression in whole blood compared to DMARD therapy alone. *Front Immunol.* 2012;3:366
 38. Grönberg C, Rattik S, Tran-Manh C, Zhou X, Rius Rigau A, Li YN, et al. Combined inhibition of IL-1, IL-33 and IL-36 signalling by targeting IL1RAP ameliorates skin and lung fibrosis in preclinical models of systemic sclerosis. *Ann Rheum Dis.* 2024;83(9):1156-68.