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Downregulation of type I interferon signalling pathway by urate in primary human PBMCs

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Abstract

Type I interferons (IFN1s) mediate innate responses to microbial stimuli and regulate interleukin (IL)-1 and IL-1 receptor antagonist (Ra) production in human cells. This study explores interferon-stimulated gene (ISG) alterations in the transcriptome of patients with gout and stimulated human primary cells in vitro in relation to serum urate concentrations. Peripheral blood mononuclear cells (PBMCs) and monocytes of patients with gout were primed in vitro with soluble urate, followed by lipopolysaccharide (LPS) stimulation. Separately, PBMCs were stimulated with various toll-like receptor (TLR) ligands. RNA sequencing and IL-1Ra cytokine measurement were performed. STAT1 phosphorylation was assessed in urate-treated monocytes. Cytokine responses to IFN-β were evaluated in PBMCs cultured with or without urate and restimulated with LPS and monosodium urate (MSU) crystals. Transcriptomics revealed suppressed IFN-related signalling pathways in urate-exposed PBMCs or monocytes which was supported by diminishment of phosphorylated STAT1. The stimulation of PBMCs with IFN-β did not modify the urate-induced inflammation. Interestingly, in vivo, serum urate concentrations were inversely correlated to in vitro ISG expression upon stimulations with TLR ligands. These findings support a deficient IFN1 signalling in the presence of elevated serum urate concentrations, which could translate to increased susceptibility to infections.

Medeea Badii and Valentin Nica share first authorship.

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KEYWORDS
gout, hyperuricemia, IL-1, soluble urate, type I interferons

INTRODUCTION

Hyperuricemia, defined as elevated serum urate concentration above the saturation threshold, is mostly an asymptomatic condition but constitutes a prerequisite for gouty arthritis [1]. Normal serum urate concentrations in humans have been reported to range from 2 [2] and 6.8 mg/dL [3]. Individuals with hyperuricemia are also prone to develop other comorbidities such as hypertension, acute and chronic kidney disease, metabolic syndrome and diabetes mellitus [4], and most studies indicate that hyperuricemia precedes the onset of these diseases [5–7]. Priming human monocytes with soluble urate increased the responsiveness of the immune cells to a second stimulation with TLR2/4 ligands [8]. Urate priming increases IL-6 and IL-1β production, whereas IL-1Ra is decreased [8], and the process is mediated by the activation of the AKT-PRAS40 pathway and suppression of autophagy [9].

Several studies link hyperuricemia or gout to type I interferon (IFN1) pathway modulation. Serum urate concentrations are influenced by a combination of genetic traits and environmental factors, such as increased production or reduced excretion [10, 11]. DNA methylation changes, especially in *SLC2A9*, directly influence serum urate concentrations and are associated with urate transport and regulation of IFN1 [12]. In individuals with hyperuricemia, genome-wide DNA methylation analysis shows variations at loci such as *HLA-G, PRKAB2* and *IFITM3*, indicating that urate might impact AMPK and IFN1 pathways [13]. In patients with gout, DNA methylation alterations were identified in transcription factors (TFs) involved in interferon signal transduction [14]. In vitro, monosodium urate (MSU) crystals transcriptionally reprogram macrophages, influencing SCL transporters and AP-1 activation, without affecting interferon regulatory factors (IRFs) or interferon genes, unlike LPS stimulation [15]. Meanwhile, transcriptome sequencing of human monocytes treated in vitro with the soluble form of urate shows that genes involved in IFN1-related signalling pathways are suppressed [9]. In support of this, a clinical study assessing the inflammatory effects of soluble urate found that genes belonging to the type I IFN signalling pathway were upregulated in patients receiving rasburicase to lower uric acid levels [16].

The induction of IFN1 and ISGs is essential for the host's immune response to microbial pathogens, particularly viruses [17], but also bacteria, parasites and fungi [18]. IFN1, such as IFN-α and IFN-β, are induced by the engagement of Toll-like receptors such as TLR3 [19], TLR4 [20], TLR7/8 and TLR9 [21, 22], acting locally in an autocrine and paracrine manner. They induce immune responses by binding the IFNAR1/IFNAR2 receptor and activating the JAK–STAT pathway. Downstream IFNAR, canonical signalling leads to the formation of STAT1 dimers, STAT3 dimers and the ISGF3 complex, which translocate to the nucleus to regulate the expression of ISGs [23, 24]. Interestingly, INF1 also antagonize inflammatory responses, especially through the regulation of IL-1 bioactivity. IFN1 can induce the expression of the IL-1 receptor antagonist (IL-1Ra) in monocytes [25] and PBMCs [26]. Furthermore, IFN1 have been documented to dampen IL-1β production in vitro [27] and in vivo [28], indicating their ability to suppress IL-1-mediated inflammation.

The primary objective of this study is to assess the interaction between elevated urate concentrations and IFN1-related pathways. We propose that IFN1 downregulation might serve as a mechanism that explains the altered IL-1β and IL-1Ra production observed in urate priming in vitro. We analyse the transcriptomic changes following urate exposure in human primary cells of patients with gout and healthy donors. Additionally, we examine ISG expression in peripheral blood mononuclear cells (PBMCs) of patients with gout and hyperuricemia upon TLR ligand stimulation.

MATERIALS AND METHODS

Due to main text space limitation, a detailed version of this section is provided in Additional File 1.

Participants

The study included patients with diagnosed gout ($n = 52$, median serum urate 6.60 mg/dL), along with healthy volunteers for in vitro validation experiments.

This study was approved by the Ethical Committees of the "Iuliu Haţieganu" University of Medicine and Pharmacy or Radboud University Medical Center.

Cell preparation and culture conditions

PBMCs were isolated using Ficoll-Paque (GE Healthcare) from fresh whole blood and used immediately for

in vitro experiments. The urate priming experiments were conducted using RPMI culture medium supplemented with 10% human pooled serum. 5×10^5 PBMCs were primed for 24 h with medium RPMI, urate (50 mg/dL), IFN-β (100 IU/mL), or urate + IFN-β, followed by stimulation with LPS (10 ng/mL) or LPS (10 ng/mL) + MSU (300 μg/mL). The priming experiment for RNA-Seq involved 24 h priming with urate 12.5 and 50 mg/dL followed by 24 h restimulation with LPS 10 ng/mL. In separate experiments, PBMCs were stimulated for 24 h with Poly: IC (10 μg/mL), CpG (1 μg/mL) and heat-killed *Candida albicans* (10^6 col/mL) without serum supplementation.

Cytokine measurements

IL-1β, IL-1Ra and IL-6 were measured using ELISA (R&D Systems, Minneapolis). The lowest range of detection was 39 pg/mL for IL-1β, 390 pg/mL for IL-1Ra and 94 pg/mL for IL-6.

Intracellular phospho-STAT1 expression

PBMCs from healthy volunteers were primed for 24 h with urate 50 mg/dL and restimulated for 4 h with LPS 10 ng/mL + MSU (300 μg/mL). Cells were stained intracellularly with pSTAT1 antibody (PE anti-STAT1 Phospho [Ser727] Antibody) and measured using an FC500 flow cytometer (Beckman Coulter). The identification and selection of monocytic cell populations were carried out using a series of gating steps as described in Additional File 1.

Transcriptomic profiling

RNA-sequencing of stimulated PBMCs was performed with the DNBseq technology (Beijing Genomics Institute, Beijing, China). Publicly available data [9] were used for urate-exposed monocytes.

Statistical analysis

The statistical analysis was performed using GraphPad version 9.0 (GraphPad Software, La Jolla, California, USA) and R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

The schematic representations were created using Biorender.

RESULTS

IFN1 signalling pathway is down-regulated in PBMCs of patients with gout upon urate pre-treatment and LPS restimulation

To investigate alterations in gene transcription due to urate priming, PBMCs ($n = 52$) isolated from patients with gout were primed with RPMI medium, a high concentration (50 mg/dL), or a low concentration (12.5 mg/dL) of soluble urate for 24 h. Following the removal and washing of urate, the cells were restimulated for 24 h with LPS 10 ng/mL. Cells were supplemented with 10% serum in all experiments (Figure 1a). Following differential expression analysis, up- and down-regulated genes (Table S1) were analysed separately. Pathway enrichment analysis of the differentially expressed genes (DEGs) with decreased transcription level (adjusted p-value <0.05 and log2 FoldChange < −1) reveals significant Gene Ontology Biological Processes (GOBP) terms associated with the type I interferon signalling pathways, immune responses to virus and bacteria and pathways that involve complement activation (Table S2, Figure 1b). Upregulated DEGs were enriched for GOBP terms such as cell response to bacteria, cell chemotaxis and migration (Table S3). The enrichment map of GOBP visually represents clusters of enriched terms to show close relationships and associations between terms related to type I interferons and viral processes, indicating that they contain common genes (Figure 1c). GSEA analysis revealed a significant enrichment of IFN1 gene sets in the down-regulated genes, with a p-adjusted value of 2.42E−05 and a negative enrichment score (NES) of −2.128 for the "type I interferon signalling pathway." (Figure 1d, Table S4). Several of these terms, such as "response to virus," "response to type I interferon," or "type I interferon signalling pathway" were associated with downregulated gene transcription (e.g. *IFI27, IFIT1, IFIT2, IFITM3, ISG15, MX1, OAS1, OAS2, OAS3, USP18*) (Figure 1e) upon urate 50 mg/dL priming in PBMCs. TF over-representation analysis of down-regulated DEGs in urate-primed PBMC identified a predicted TF co-expression network of the top 10 TFs (STAT1, STAT2, NR1H3, BATF3, TFEC, IRF9, ETV3L, IRF5, IRF7 and EGR2) (Figure 1f, Table S5).

DEG analysis of PBMCs primed with urate (12.5 mg/dL) and restimulated with LPS reveals decreased expression of genes belonging to IFN1, albeit to a lesser extent than the higher dose of urate (Table S6). GSEA using Reactome (Table S7) and GO Biological Processes (Table S8) helped uncover biologically relevant pathway alterations, even in the presence of minimal or no significant changes in individual gene expression. Notably,

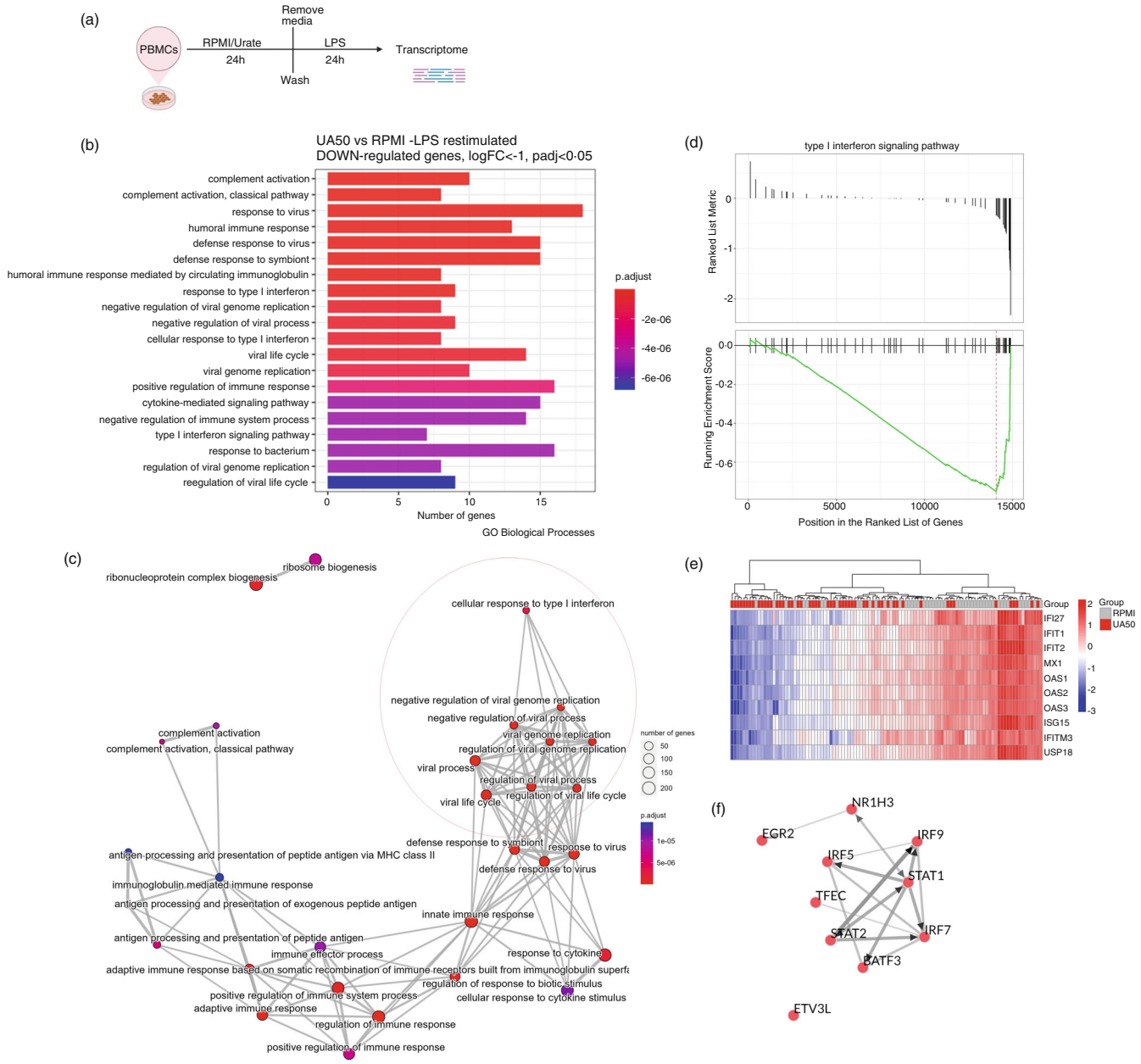

FIGURE 1 RNA-Seq reveals downregulation of type I interferon signalling pathways in human PBMCs upon urate priming. Schematic representation of in vitro experiments used for transcriptomic analysis ($n = 52$) (a); Pathway enrichment analysis of down-regulated genes at *p*adj <0.05 and log2FC < -1 using Gene Ontology—Biological processes (top 20 terms) (b). Enrichment map of GO Biological Processes terms of down-regulated genes at *p*adj <0.05 and log2FC < -1 (c). Curve of Gene Set Enrichment Analysis (GSEA) enrichment scores for type I interferon signalling pathways (*p*adj $= 242022E - 05$; NES $= -2128$) (d). Heatmap of differentially expressed genes (down-regulated) that overlap the GO BP terms, response to type I interferon, cellular response to type I interferon and type I interferon signalling pathway (variance stabilized normalized counts corrected for batch effect); The relative value for each gene is shown by the colour intensity of the Z score, with red indicating up-regulated and blue indicating downregulated genes (e). Network Visualizations with ChEA3 of transcription factors (TFs) according to the similarity in their co-expression patterns using differentially expressed gene set of urate 50 mg/dL + LPS 10 ng/mL at *p*adj <0.05; logFC < -1 (f).

terms related to "interferon responses" and "viral regulation" were associated with down-regulated gene expression, even at a lower dose of urate (Figure S1). The effect size differs dose-dependently between urate 50 and 12.5 mg/dL, with consistent changes in gene expression in response to both conditions (Figure S2).

Downregulation of the IFN1 pathway in human monocytes treated with urate

To investigate the specific regulation of IFN1-related pathways by urate alone, we analysed the previously published dataset described by Crisan et al. [9] in which

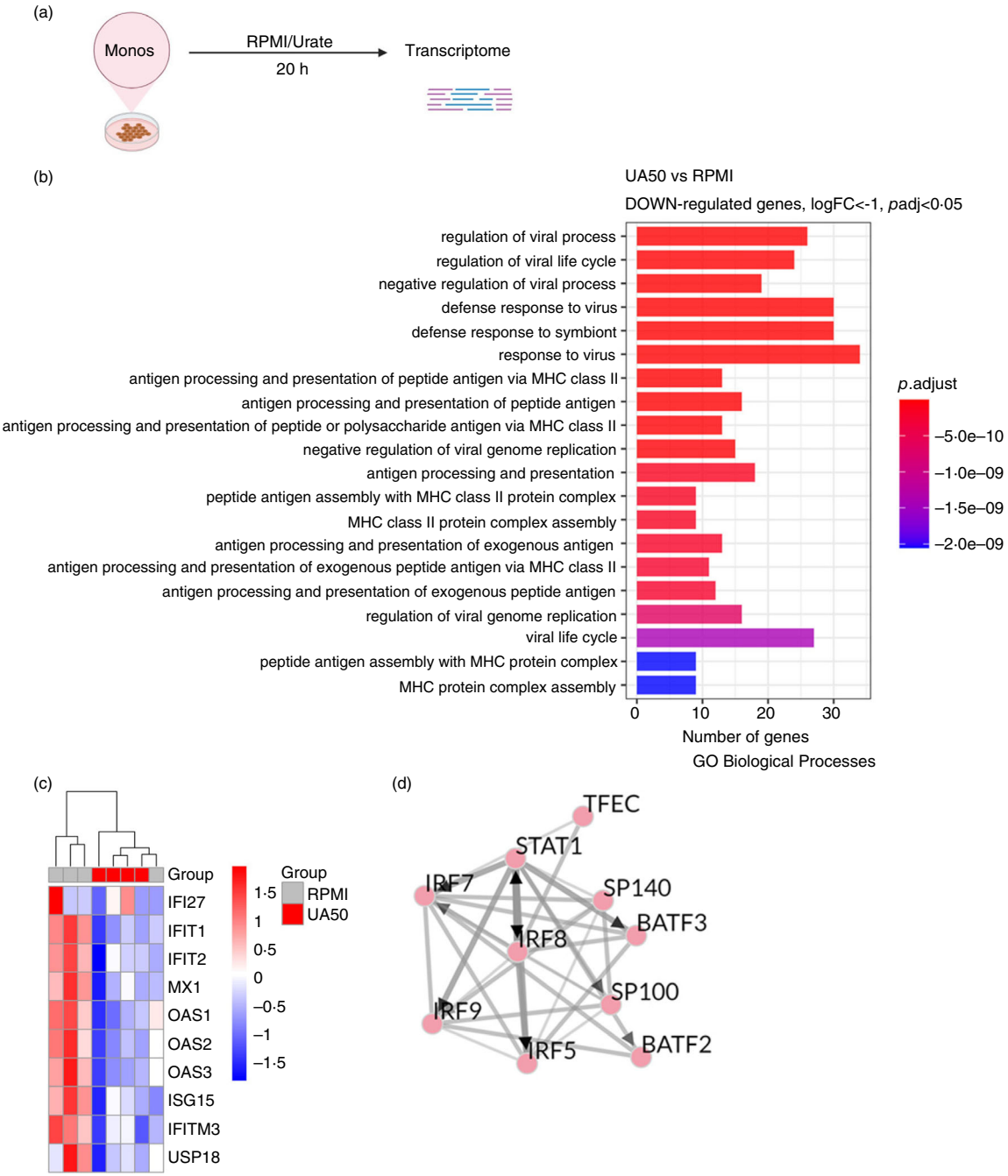

FIGURE 2 RNA-Seq confirms downregulation of type I interferon in primary human monocytes. Schematic representation of in vitro experiments used for transcriptomic analysis ($n = 4$) (a); Pathway analysis of down-regulated differentially-expressed genes at *p*adj <0.05 and log2FC < −1 for GO Biological processes (top 20 terms) (b). Heatmap of Interferon Stimulated Genes treated with urate 50 mg/dL and RMPI medium for control. The relative value for each gene is shown by the colour intensity of the Z score, with red indicating up-regulated and blue indicating downregulated genes (c). Network visualizations with ChEA3 of transcription factors according to the similarity in their co-expression patterns using differentially expressed gene set of urate 50 mg/dL at *p*adj <0.05; logFC < −1 (d).

monocytes isolated from healthy volunteers were primed with RPMI or a high dose of urate (50 mg/dL) for 20 h (Figure 2a). GOBP gene enrichment analysis of urate down-regulated genes identified enrichment for virus-regulated pathways (Figure 2b, Table S9). Among the genes associated with these terms are *IFIT1, MX1, OAS1, OAS2, OAS, ISG15, IFITM3, RSAD2* and *USP18*. These genes also overlapped with the terms from the pathway analysis conducted on PBMCs. When examining the same set of genes depicted in Figure 1f, we observe a stronger grouping on the samples that received urate treatment only (Figure 2c). Within the down-regulated

gene set, we identified significant enrichment for key TFs of IFN1, such as STAT1, IRF5 and IRF9 (Figure 2d, Table S10).

STAT1 phosphorylation is decreased upon urate treatment

To assess at the protein level whether the signal transduction pathway associated with ISGs is altered in response to urate, PBMCs from healthy volunteers ($n = 5$, two independent experiments) were primed for 24 h with urate 50 mg/dL or RPMI medium and were restimulated with LPS 10 ng/mL + MSU 300 μg/mL for an additional 4 h (Figure 3a). At the end of the experiment, the cells were stained for intracellular phosphorylated STAT1 and extracellular markers to differentiate monocytic cell populations (Figure S3, Table S11). Urate treatment alone decreased STAT1 phosphorylation in HLA-DR+ or classical monocytes (left subpanel Figure 3b,c). Although not statistically significant, there is a noticeable difference in reduced phosphorylated STAT1 levels in urate 50 mg/dL treated and LPS + MSU restimulated HLA-DR+ (Figure 3b,c) and classical (Figure 3c) monocytes

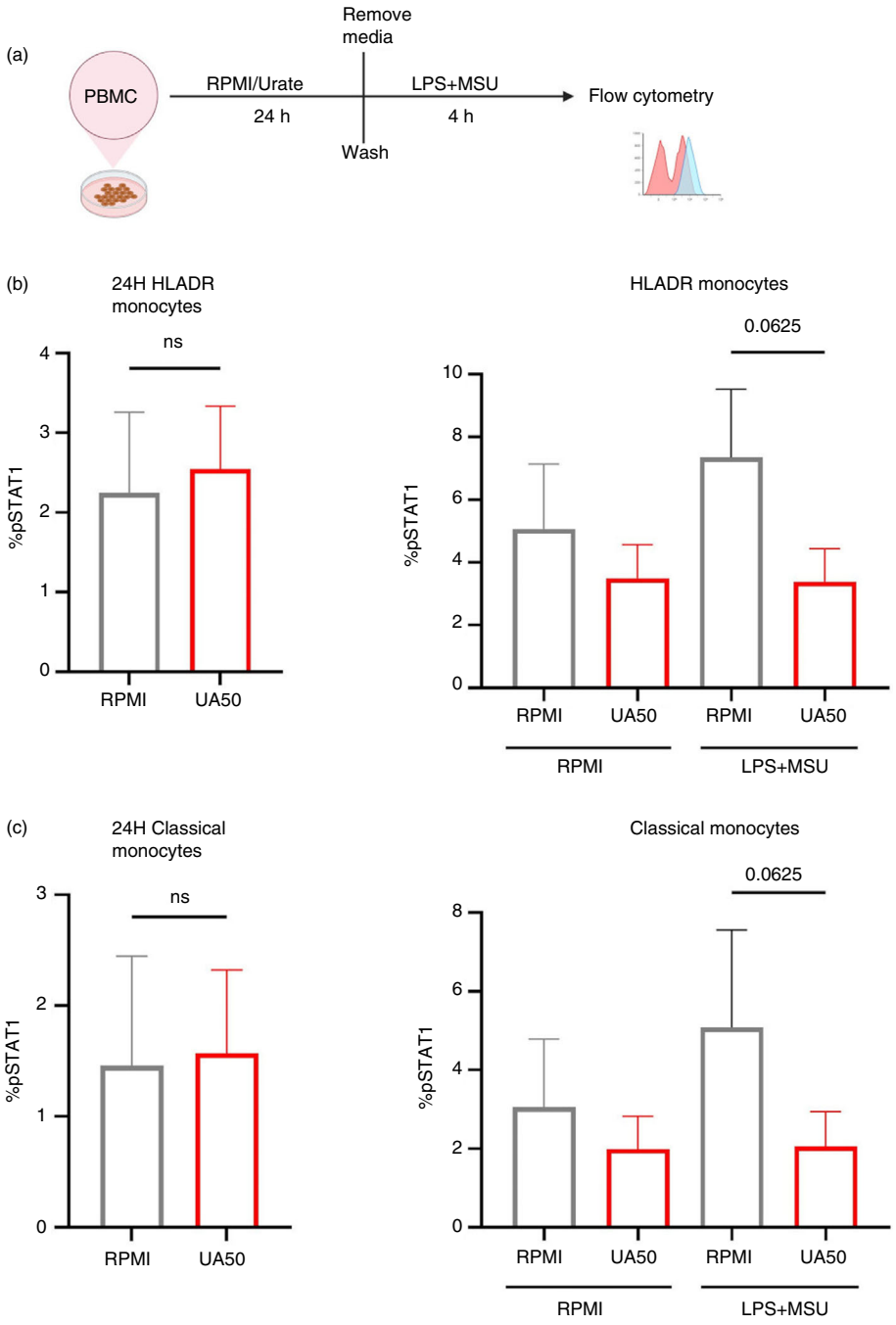

FIGURE 3 Phosphorylated STAT1 is decreased upon urate treatment in monocytes. Schematic representation of in vitro experiments used for flow cytometry experiment; PBMCs primed in vitro with urate 50 mg/dL for 24 h and restimulated with LPS 10 ng/mL + MSU crystals 300 μg/mL for 4 h (a). Phosphorylated STAT1 in gated HLA-DR+ monocytes (b) and classical monocytes (c) ($n = 5$); Wilcoxon test; Data presented as mean with SEM and representative of two independent experiments.

($p = 0.0625$). These observations were not possible for intermediate and non-classical monocytes, most likely because the population numbers were too low (Figure S3). The classic pathway for IFN1 involves the formation of the STAT1/STAT2/IRF9 complex, known as the ISGF3 complex, which translocates to the nucleus to bind to ISRE and induce the expression of antiviral ISGs [18]. We examined the transcriptome data for PBMCs primed with 50 and 12 mg/dL urate and observed a dose-dependent decrease in the expression of genes integral to the ISGF3 complex (Figure S4). These results complement the TF enrichment analysis shown in Figures 1 and 2.

Induction of the IFN1 pathway with IFN-β did not reverse the pro-inflammatory effect in urate

Given that urate treatment leads to the transcriptional suppression of ISGs, we subsequently aimed to explore whether we could reverse the priming effect induced by urate by stimulating the IFN1 pathway in vitro using human recombinant interferons. To accomplish this, PBMCs were cultured for 24 h with urate 50 mg/dL in the presence or absence of IFN-β 100 IU/mL followed by 24 h restimulation with LPS 10 ng/mL + MSU 300 μg/mL (Figure 4a). Treatment with urate alone resulted in increased IL-1β and IL-6 concentrations, along with a decrease in IL-1Ra, characteristic of the urate priming phenotype as described previously [9]. Although treatment with IFN-β alone significantly induced IL-1Ra production in the first 24 h, the production of IL-1Ra, IL-1β and IL-6 after restimulation was not affected by the addition of IFN-β recombinant protein in the presence of urate (Figure 4b).

ISGs in stimulated PBMCs are inversely correlated with the circulating serum urate levels

We next sought to expand upon these observations by investigating the potential impact of urate on infection

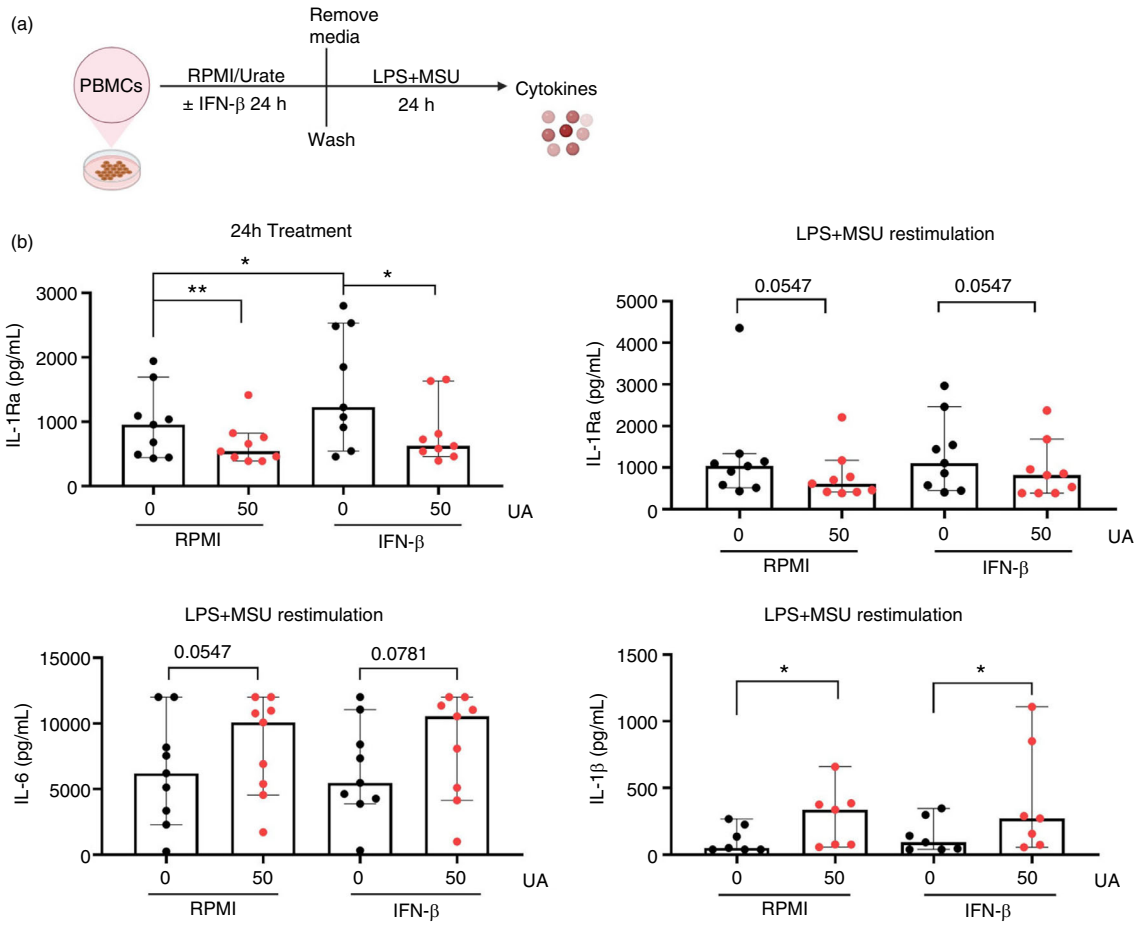

FIGURE 4 Cytokine production given by IFN-β stimulation in the presence of urate. Schematic representation of in vitro experiments used for cytokine measurements ($n = 9$) (a). Coincubation of IFN-β (100 IU/mL) with urate (50 mg/dL) followed by restimulation with LPS (10 ng/mL) + MSU (300 μg/mL) in PBMCs of healthy volunteers (b). IL-1Ra, IL-1β and IL-6 cytokine production in supernatants from at least three independent experiments; Wilcoxon test; Data presented as median with 95% CI.

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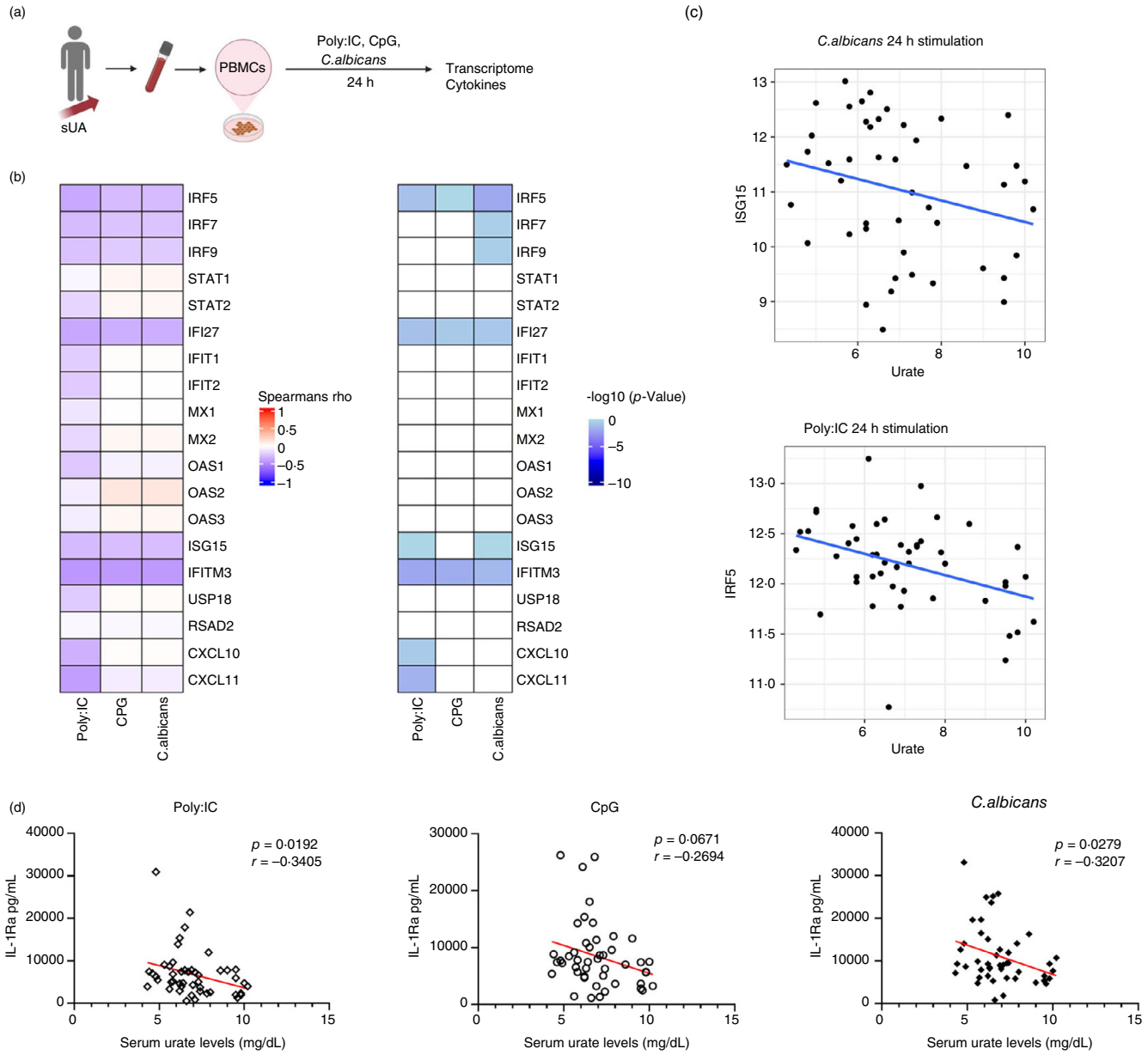

FIGURE 5 Serum urate levels in patients are inversely correlated with gene expression of ISGs. Schematic representation of in vitro experiments used for transcriptomic analysis (a). ComplexHeatmap for Spearman rho and *p*-values of serum urate levels in correlation with gene expression (variance stabilized normalized counts) of 24 h treated PBMCs with Poly: IC ($n = 47$), CpG ($n = 47$) and heat-killed *Candida albicans* ($n = 46$) (b). Scatter plots of genes that inversely correlate with serum urate concentrations in 24 h stimulation of PBMCs with *C. albicans* ($r = -0.3755$, $p = 0.0093$) and Poly: IC ($r = -0.3715$, $p = 0.0101$)—gene expression on the *y*-axis as variance stabilized normalized counts and on the *x*-axis serum urate levels in mg/dL along with the fitted linear regression line in blue (c). Correlation of serum urate levels with IL-1Ra cytokine production in 24 h treated PBMCs with PBMCs Poly: IC, CpG and heat-killed *C. albicans* (d).

responses in individuals with hyperuricemia. For this purpose, PBMCs isolated from patients with gout (serum urate range: min (3.8 mg/dL), median (6.60 mg/dL), max (11.2 mg/dL)) were treated in vitro for 24 h with various TLR ligands to mimic a context of microbial infection. RNA sequencing and cytokine measurement were performed (Figure 5a).

The expression of ISGs in PBMCs stimulated with viral-like ligands such as Poly: IC and CpG were negatively correlated with circulating serum urate concentrations. A similar effect was observed in PBMCs stimulated with *C. albicans* (Figure 5b,c; Table S12), indicating that the downregulation of ISGs in the presence of urate is exerted on the broad antimicrobial ISG response and not

restricted only to viral ligand stimulation. IL-1Ra cytokine production in PBMCs stimulated with Poly: IC, CpG and *C. albicans* was likewise observed to have an inverse correlation with serum urate concentrations (Figure 5d).

DISCUSSION

This study evaluated the expression of ISGs by assessing the transcriptomes of human primary cells following exposure to soluble urate. The downregulation of IFN1-related pathways was observed in PBMCs of patients with gout treated in vitro with soluble urate at concentrations of 12.5 and 50 mg/dL and restimulated with LPS. The analysis of differentially expressed genes and TFs in urate-treated monocytes from healthy volunteers confirmed inhibition of the IFN1 signalling pathways. Treatment with urate resulted in lower levels of phosphorylated STAT1 upon subsequent restimulation with LPS + MSU Previous studies described that in vitro exposure of human monocytes with urate results in elevated IL-1β and reduced IL-1Ra [9]. However, the addition of IFN-β in vitro did not rescue the pro-inflammatory effects of urate pre-treatment. These results suggest that the priming effect of urate is probably not driven by IFN1 signalling. Alternatively, it could account for another potential harmful consequence of cell exposure to urate. Interestingly, we identified an inverse relationship between the concentrations of circulating serum urate in patients and the expression of ISGs following in vitro stimulation of human PBMCs with fungal and viral ligands. The observed IFN1 downregulation at in vivo-relevant serum urate concentrations carries noteworthy implications for our understanding of how urate may influence the immune response to microbial infections.

In response to infection with various pathogens, cells like macrophages or dendritic cells produce IFN1, a key component of innate immune responses that bridge the transition to adaptive immunity [29]. In monocytes pre-treated with urate, previous transcriptomic studies identified downregulated genes enriched in pathways related to infection and autoimmunity, including IFN1 signalling-related genes associated with terms like "Influenza A" or "Systemic lupus erythematosus" [9]. In our study, we conducted transcriptomic analysis on human peripheral blood mononuclear cells (PBMCs) exposed initially to soluble urate and subsequently LPS-restimulated in vitro. The analysis of differentially expressed genes using GO Biological Processes revealed a reduction in the expression of genes annotated to the IFN1-related Gene Ontology (GO) (Figure 1b). Similarly, other top terms included genes involved in pathways related to complement activation, aligning with previously reported findings [9]. While the exposure of cells to a lower concentration of urate (12.5 mg/dL) did not greatly affect the overall differential gene expression (DEG) analysis, many genes within the interferon signalling pathway still show significant down-regulation, although the effect of lower dose urate priming is smaller and implies more subtle changes (Figure S2). Nevertheless, GSEA identifies terms related to "interferon responses" and "viral regulation" that are associated with down-regulated gene expression similar to that observed with urate 50 mg/dL exposure (Figure S1). It is intriguing to note that the genes downregulated by urate priming, such as interferon-induced proteins with tetratricopeptide repeats (*IFIT1, IFIT2*), 2′-5′-oligoadenylate synthetases (*OAS1, OAS2, OAS3*) and interferon-induced transmembrane proteins (*IFITM3*) (Figure 1e), were upregulated in patients undergoing treatment with rasburicase, a urate-lowering drug [16].

Our findings were endorsed in publicly available transcriptomic data of monocytes treated with urate only (Figure 2), suggesting that urate might modulate interferon-related immune responses under these conditions. A recent epigenome-wide association study (EWAS) focusing on serum urate found changes in DNA methylation to causally influence serum urate concentrations. The analysis of GO Biological processes for CpG sites linked to urate reveals enrichment of pathways related to urate transport and IFN1 signalling and regulation pathways [12]. We previously showed an inverse relationship, that hyperuricemia results in genome-wide differentially methylated sites (e.g., *IFITM3*) [13]. Ultimately, further investigation is needed to understand whether urate affects DNA methylation or if alterations in DNA methylation have consequences for urate concentrations, particularly in relation to IFN1 regulation.

Urate may influence signalling pathways driven by interferons, possibly through mechanisms that inhibit STAT1 activation, as urate treatment potentially reduces STAT1 phosphorylation, which is particularly visible after stimulation with LPS + MSU (Figure 3). These results align with a previous report from our group showing a decrease in phosphorylated STAT3 and IL-1Ra levels upon pre-exposure to urate [30]. STAT3 homodimers can also be involved in the IFN1 signalling pathway to counterbalance the pro-inflammatory responses under certain conditions [24]. Still, activated STAT1 homodimers are predominantly associated with the IFN-γ (type II interferon) signalling pathway [31], but IFN1 can also promote IFN-γ production, which in turn activates STAT1 [31]. Additionally, the activation of STAT1 can be influenced by other cytokines such as IL-10 [32]. The observed pattern of suppressed pSTAT1 following in vitro urate priming is consistent with reduced activation of

other TFs like *IRF5*, *IRF9, STAT1* and *STAT2* (Figure 1f, Figure 2d), responsible for inducing the transcription of ISGs [33]. Moreover, several of these TFs are downregulated by urate in a dose-dependent manner (Figure S4). In the PBMCs of patients with gout, Wang et al. identified differentially methylated TF motifs, such as *STAT2* and *IRF1*, that may impact gene regulation [14].

Inhibition of IFN1 promotes pro-inflammatory activation in atherosclerosis. The uptake of acetylated LDL (acLDL) by macrophages and the subsequent foam cell formation leads to a reduced type I interferon response, which is recovered after administration of exogenous IFN-β treatment [34]. Here, the stimulation of PBMCs with IFN-β in the presence of urate at 50 mg/dL (Figure 4) did not alter the pro-inflammatory effects of urate pre-treatment. We can still observe a reduction in IL-1Ra and induction of IL-6 and IL-1β on LPS + MSU restimulation. This implies that the downregulation of genes in the IFN1 pathway is unlikely to be the mechanistic factor contributing to the observed proinflammatory effects of urate (Figure 4). Alternatively, it might represent a potentially detrimental consequence of prior exposure to elevated urate concentrations that could impact susceptibility to infections.

This potential link between elevated urate levels and increased infection risk is underscored by the presence of a pro-inflammatory state related to hyperuricemia, leading to the hypothesis that patients with gout may have an enhanced susceptibility to infections. Several studies show an association between hyperuricemia or gout and poor responses to Sars-CoV-2 infection [35, 36] or pneumonia [37]. High serum urate concentrations were associated with an increased risk of composite outcome and mechanical ventilation in COVID-19 [35]. On the other hand, other studies propose that low urate concentrations can independently predict a worsened outcome of COVID-19 in patients with severe and critical forms of the disease [38]. In patients hospitalized with SARS-CoV-2 infection, there is a drastic decrease in serum urate concentrations, especially in severe cases requiring mechanical ventilation, which is restored upon discharge [39]. Oxidative stress during the resolution of inflammatory processes could explain the urate consumption, given urate's antioxidant effect in scavenging free radicals to mitigate oxidative damage [40]. However, most of these studies do not include serum urate concentrations before infection or admission, and the impact of urate concentration changes in the context of COVID-19 remains unclear. Interestingly, in one study, a history of hyperuricemia was significantly linked to COVID-19 severity in a Japanese cohort [41]. In PBMCs of patients with gout, the expression of ISGs like Interferon Regulatory Factor 5 (*IRF5*), Interferon Alpha Inducible Protein 27 (*IFI27*) and Interferon Induced Transmembrane Protein 3 (*IFITM3*), shows a negative correlation with circulating serum urate concentrations when triggered with Poly: IC, CpG and *C. albicans* (Figure 5b,c), consistent with our in vitro findings. However, this may indicate a wider effect of urate on ISG expression, extending beyond merely viral stimulation responses.

Another aspect of significant relevance is the downregulated IL-1Ra cytokine production at these circulating urate concentrations. The downregulation of IL1Ra in response to urate exposure is supported by in vitro evidence [18]. The induction of IL-1Ra production by IFN-β (Figure 4b) aligns with previous evidence [29]. Still, the long-lasting effect of urate remains unaffected by this short-term induction of IL-1Ra, implying a transient impact of IFN1 but a lasting effect in response to urate. Following TLR stimulation, IL-1Ra also exhibits a negative relationship with serum urate levels (Figure 5d), reinforcing the in vitro findings of IL-1Ra downregulation [9] within the context of circulating urate concentrations. This is an important aspect, since in circulation, a systemic increase of anti-inflammatory IL-1Ra may occur as a result of systemic increase of pro-inflammatory mediators. Plasma levels of IL-1Ra have been reported to positively correlate with urate levels [42]. Based on our observations, we could not establish a clear interaction between these pathways, as they seem to be independent of each other in terms of regulation by urate and this needs further investigation. However, despite not being mechanistically connected, both ISGs and IL-1Ra exhibit a parallel decrease in response to urate.

This study shows a distinct transcriptional shift in human myeloid cells exposed to soluble urate characterized by downregulation of genes involved in IFN1 regulation, viral and bacterial replication-related processes and complement activation. While these observations were particularly pronounced in vitro when exposing immune cells to high urate concentrations, they remain consistent when using lower concentrations. Importantly, these results have been validated in stimulated PBMCs from patients with varying serum urate concentrations. This observed effect seems to be soluble urate-specific since transcriptome analysis of MSU crystals treated macrophages did not impact genes belonging to IFN1 or IRFs [15].

While this study provides valuable insights into soluble urate in vitro, it is critical to acknowledge certain limitations. Our results on urate-exposed PBMCs were further examined in a publicly available transcriptomic dataset on urate-treated monocytes. While these results may not be entirely comparable, there is significant overlap in many of the top terms identified in the pathway analysis. Next, we only investigated the effect of IFN-β,

while the impact of IFN-α and other interferons (IFN-γ) remains unexplored. Protein concentration analysis of pSTAT1 was restricted due to a limited number of available samples. Monitoring infections in a cohort of patients with hyperuricemia and gout was not possible, which could have added valuable insights to our findings. To expand the relevance of this study, further studies should focus on evaluating IFN1 changes before and after urate-lowering therapy in patients to better understand the significance of urate modification not only in infection-related immune responses but also in broader scopes.

The present study found that urate-treated human PBMCs and monocytes show downregulation of the IFN1 signalling pathway, supported by diminished STAT1 phosphorylation. Given that inducing the IFN1 pathway with IFN-β did not reverse the pro-inflammatory effect of urate, this is unlikely to play a role in the inflammatory priming due to urate. However, this suggests that elevated urate pre-exposure could negatively impact immune responses to viral stimuli. Indeed, in patients with hyperuricemia, high serum urate concentrations are inversely correlated with in vitro responses to both viral and fungal stimulations. This correlation supports an impaired IFN1 signalling and, consequently, may render individuals more susceptible to microbial infections.

AUTHOR CONTRIBUTIONS

MB, VN, TOC, LABJ carried out the conceptualization and design of the study. MB, VK, OG, GC, AS, BK, IH and VN performed the acquisition of data. IH, CP, SR provided patient material or data. RAP, HINT Consortium provided administrative support. MB, VN, BN, MGN, TOC and LABJ provided statistical analysis and interpretation of the data. MB, VN, TOC and LABJ critically drafted the manuscript. All authors contributed to data interpretation, revised the manuscript and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

In vitro experiments were approved by the Ethical Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca (approval no. 425/2016) and by the Ethical Committee of the Radboud University Medical Center (no. NL32357.091.10; registration number 2010/104). All participants provided written informed consent before inclusion. All experiments were conducted according to the principles of the Declaration of Helsinki.

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REFERENCES

1. Bardin T, Richette P. Definition of hyperuricemia and gouty conditions. Curr Opin Rheumatol. 2014;26(2):186–91.
2. Kuwabara M, Niwa K, Ohtahara A, Hamada T, Miyazaki S, Mizuta E, et al. Prevalence and complications of hypouricemia

in a general population: a largescale cross-sectional study in Japan. PLoS One. 2017;12(4):1–13.
3. Dalbeth N, Choi HK, Joosten LAB, Khanna PP, Matsuo H, Perez-Ruiz F, et al. Gout. Nat Rev Dis Primers. 2019;5:1.
4. Joosten LAB, Crişan TO, Bjornstad P, Johnson RJ. Asymptomatic hyperuricaemia: a silent activator of the innate immune system. Nat Rev Rheumatol. 2020;16(2):75–86.
5. Grayson PC, Young Kim S, Lavalley M, Choi HK. Hyperuricemia and incident hypertension: a systematic review and meta-analysis. Arthritis Care Res. 2011;63(1):102–10.
6. Johnson RJ, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M, et al. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes. 2013;62(10):3307–15.
7. Lv Q, Meng X-F, He F-F, Chen S, Su H, Xiong J, et al. High serum uric acid and increased risk of type 2 diabetes: a systemic review and meta-analysis of prospective cohort studies. PLoS One. 2013;8(2):e56864.
8. Crişan TO, Cleophas MCP, Oosting M, Lemmers H, Toenhake-Dijkstra H, Netea MG, et al. Soluble uric acid primes TLR-induced proinflammatory cytokine production by human primary cells via inhibition of IL-1Ra. Ann Rheum Dis. 2016;75(4):755–62.
9. Crişan TO, Cleophas MCP, Novakovic B, Erler K, van de Veerdonk FL, Stunnenberg HG, et al. Uric acid priming in human monocytes is driven by the AKT–PRAS40 autophagy pathway. Proc Natl Acad Sci. 2017;114(21):5485–90.
10. Köttgen A, Albrecht E, Teumer A, Vitart V, Krumsiek J, Hundertmark C, et al. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nat Genet. 2013;45(2):145–54.
11. Li L, Zhang Y, Zeng C. Update on the epidemiology, genetics, and therapeutic options of hyperuricemia. Am J Transl Res. 2020;12(7):3167–81.
12. Tin A, Schlosser P, Matias-Garcia PR, Thio CHL, Joehanes R, Liu H, et al. Epigenome-wide association study of serum urate reveals insights into urate co-regulation and the SLC2A9 locus. Nat Commun. 2021;12(1):1–18.
13. Badii M, Gaal OI, Cleophas MC, Klück V, Davar R, Habibi E, et al. Urate-induced epigenetic modifications in myeloid cells. Arthritis Res Ther. 2021;23(1):1–11.
14. Wang Z, Zhao Y, Phipps-Green A, Liu-Bryan R, Ceponis A, Boyle DL, et al. Differential DNA methylation of networked signaling, transcriptional, innate and adaptive immunity, and osteoclastogenesis genes and pathways in gout. Arthritis Rheumatol. 2020;72(5):802–14.
15. Cobo I, Cheng A, Murillo-Saich J, Coras R, Torres A, Abe Y, et al. Monosodium urate crystals regulate a unique JNK-dependent macrophage metabolic and inflammatory response. Cell Rep. 2022;38(10):110489.
16. Tanaka T, Milaneschi Y, Zhang Y, Becker KG, Zukley L, Ferrucci L. A double blind placebo controlled randomized trial of the effect of acute uric acid changes on inflammatory markers in humans: a pilot study. PLoS One. 2017;12(8):e0181100.
17. Stetson DB, Medzhitov R. Type I interferons in host defense. Immunity. 2006;25(3):373–81.
18. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol. 2015;15(2):87–103.
19. Okahira S, Nishikawa F, Nishikawa S, Akazawa T, Seya T, Matsumoto M. Interferon-β induction through toll-like receptor 3 depends on double-stranded RNA structure. DNA Cell Biol. 2005;24(10):614–23.
20. Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, et al. LPS-TLR4 signaling to IRF-3/7 and NF-κB involves the toll adapters TRAM and TRIF. J Exp Med. 2003;198(7):1043–55.
21. Saitoh SI, Abe F, Kanno A, Tanimura N, Mori Saitoh Y, Fukui R, et al. TLR7 mediated viral recognition results in focal type I interferon secretion by dendritic cells. Nat Commun. 2017;8(1):1592.
22. Yang K, Puel A, Zhang S, Eidenschenk C, Ku CL, Casrouge A, et al. Human TLR-7-, -8-, and -9-mediated induction of IFN-α/β and -λ is Irak-4 dependent and redundant for protective immunity to viruses. Immunity. 2005;23(5):465–78.
23. Stark GR, Darnell JE. The JAK-STAT pathway at twenty. Immunity. 2012;36(4):503–14.
24. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. Nat Rev Immunol. 2014;14(1):36–49.
25. Jenkins JK, Arend WP. Interleukin 1 receptor antagonist production in human monocytes is induced by IL-1α, IL-3, IL-4 and GM-CSF. Cytokine. 1993;5(5):407–15.
26. Tilg H, Mier JW, Vogel W, Aulitzky WE, Wiedermann CJ, Vannier E, et al. Induction of circulating IL-1 receptor antagonist by IFN treatment. J Immunol. 1993;150(10):4687–92.
27. Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Förster I, et al. Type I interferon inhibits Interleukin-1 production and inflammasome activation. Immunity. 2011;34(2):213–23.
28. Castiglia V, Piersigilli A, Ebner F, Janos M, Goldmann O, Damböck U, et al. Type I interferon signaling prevents IL-1β-driven lethal systemic hyperinflammation during invasive bacterial infection of soft tissue. Cell Host Microbe. 2016;19(3):375–87.
29. Mayer-Barber KD, Yan B. Clash of the cytokine titans: counter-regulation of interleukin-1 and type i interferon-mediated inflammatory responses. Cell Mol Immunol. 2017;14(1):22–35.
30. Badii M, Klück V, Gaal O, Cabău G, Hotea I, Nica V, et al. Regulation of SOCS3-STAT3 in urate-induced cytokine production in human myeloid cells. Joint Bone Spine. 2024;91(3):105698.
31. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol. 2005;5(5):375–86.
32. Rahimi AAR, Gee K, Mishra S, Lim W, Kumar A. STAT-1 mediates the stimulatory effect of IL-10 on CD14 expression in human monocytic cells. J Immunol. 2005;174(12):7823–32.
33. Yoneyama M, Suhara W, Fukuhara Y, Sato M, Ozato K, Fujita T. Autocrine amplification of type I interferon gene expression mediated by interferon stimulated gene factor 3 (ISGF3). J Biochem. 1996;120(1):160–9.
34. Willemsen L, Chen H-J, van Roomen CPAA, Griffith GR, Siebeler R, Neele AE, et al. Monocyte and macrophage lipid accumulation results in Down-regulated type-I interferon responses. Front Cardiovasc Med. 2022;9:829877.
35. Chen B, Lu C, Gu HQ, Li Y, Zhang G, Lio J, et al. Serum uric acid concentrations and risk of adverse outcomes in patients

with COVID-19. Front Endocrinol (Lausanne). 2021;12: 633767.
36. Topless RK, Gaffo A, Stamp LK, Robinson PC, Dalbeth N, Merriman TR. Gout and the risk of COVID-19 diagnosis and death in the UK biobank: a population-based study. Lancet Rheumatol. 2022;4(4):e274–81.
37. Spaetgens B, De Vries F, Driessen JHM, Leufkens HG, Souverein PC, Boonen A, et al. Risk of infections in patients with gout: a population-based cohort study. Sci Rep. 2017;7(1): 1429.
38. Li G, Wu X, Zhou CL, Ming WY, Song B, Bin CX, et al. Uric acid as a prognostic factor and critical marker of COVID-19. Sci Rep. 2021;11(1):1–9.
39. Dufour I, Werion A, Belkhir L, Wisniewska A, Perrot M, De Greef J, et al. Serum uric acid, disease severity and outcomes in COVID-19. Crit Care. 2021;25(1):212.
40. Harrold LR, Etzel CJ, Gibofsky A, Kremer JM, Pillinger MH, Saag KG, et al. Sex differences in gout characteristics: tailoring care for women and men. BMC Musculoskelet Disord. 2017; 18(1):108.
41. Fukushima T, Chubachi S, Namkoong H, Otake S, Nakagawara K, Tanaka H, et al. U-shaped association between abnormal serum uric acid levels and COVID-19 severity: reports from the Japan COVID-19 task force. Int J Infect Dis. 2022;122:747–54.
42. Ruggiero C, Cherubini A, Ble A, Bos AJG, Maggio M, Dixit VD, et al. Uric acid and inflammatory markers. Eur Heart J. 2006;27(10):1174–81.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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