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Cyclosporine and fedratinib combination therapy via modulating Th17/Treg balance in Rat model of membranous glomerulonephritis

Ali Ghassabi [a,b,1], Maryam Hosseini [c,1], Hemayat Abdoli Goungormaz [d], Mohammad Sadegh Soltani-Zangbar [d,e], Mahsa Beomidehagh [d,f], Davoud Rostamzadeh [g], Mohammadbagher Pirouzpanah [b], Arshad Ghaffari-Nasab [h], Arash Khaki [i], Leili Aghebati-Maleki [d], Elham Badihi [b], Farshid Afandideh [b], Reihane Shahabirad [b], Ali Akbar Shekarchi [j], Javad Ahmadian Heris [k], Leila Roshangar [b], Jalal Etemadi [l], Mehdi Yousefi [b,e,*]

[a] Faculty of Veterinary, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[b] Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Trauma Research Center, Shahid Rajaee (Emtiaz) Trauma Hospital, Shiraz University of Medical Sciences, Shiraz, Iran
[d] Immunology Research Center, Tabriz University of Medical Science, Tabriz, Iran
[e] Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[f] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[g] Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[h] Department of Clinical Sciences, Maragheh University of Medical Sciences, Maragheh, Iran
[i] Department of Pathobiology, Faculty of Veterinary, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[j] Department of Pathology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[k] Department of Allergy and Clinical Immunology, Pediatric Hospital, Tabriz University of Medical Sciences, Tabriz, Iran
[l] Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

A progressive kidney disease associated with inflammation and the immune system is called membrane glomerulonephritis (MGN). The present study investigatedthe combination of cyclosporine and fedratinib on Th17/regulatory T cells (Tregs) in rat models of MGN. Rats were given several doses of anti-Fx1A to induce MGN, and the resultant five groups of rats were fedratinib-cyclosporin receiving PHN rats, fedratinib, cyclosporin, and healthy rats. Following that, the blood's biochemistry was ascertained, and splenocytes were separated to use flow cytometry to look into the proportion of Th17 and Treg cells in the blood. A real-time PCR test was used to assess the corresponding Tregs and Th17 cell transcription factors andtheir related cytokine gene expressions. Finally, serum analysis was employed to indicate serum cytokines signatures of Th17 cells and Tregs through ELISA. The combination of cyclosporine-fedratinib induced noticeably diminished levels of serum total protein, albumin, and urea in rats versus the PHN group. Th17 cell frequency and its related transcription factors and cytokines genes showed increased expression in the PHN model compared to the control group and PHN groups with different treatments.

In contrast, Tregs frequency and its related transcription factors and cytokines genes showed decreased expression in the PHN model compared to the control group and PHN groups with different treatments. Serum cytokine assay confirmed gene expression results. The combination of cyclosporine and fedratinib was capable of reducing Th17 cells in favor of Tregs enhancement in PHN rats, suggesting a novel combination therapy in the treatment of MGN.

* Corresponding author. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail address: yousefime@tbzmed.ac.ir (M. Yousefi).
[1] Ali Ghassabi and Maryam Hosseini equally contributed to this study.

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Abbreviations:

ABMR	Antibody-mediated rejection
cDNA	Complementary DNA
ELISA	Enzyme-linked immunosorbent assay
FoxP3	Forkhead box P3
GVHD	Graft versus host disease
I.L.s	Interleukins
JAK	Janus kinase
MGN	Membranous glomerulonephritis
PCR	Polymerase chain reaction
PHN	Passive Heymann nephritis
RORγT	Retinoic acid-related orphan receptor γ
STAT3	Signal transducer and activator of transcription 3
TGF-β	Tumor growth factor beta
Th17	T helper 17
Tregs	Regulatory T cells

1. Introduction

One of the most prevalent forms of chronic glomerulonephritis, membraneous glomerulonephritis (MGN), can have multiple etiological causes, including primary autoimmune renal disease or secondary manifestations from other systemic illnesses [1]. The sickness can gradually develop under disturbed immunological responses and overload inflammation processes [2]. Chronic inflammation is one of the leading players in MGN pathogenesis [3]. In this case, glomerulonephritis (G.N.) is mediated mainly by Th17-producing $CD4^+$ T cells, which are essential inflammatory mediators [4]. Several studies have documented a high frequency of Th17 cells in the nephritic kidneys of individuals suffering from ANCA-associated vasculitis, primarily in animal models of exacerbated crescentic nephritis [5]. The primary transcription factors of Th17 cell activation that induce the production of multiple cytokines, such as interleukin (IL)-17, IL-21, and IL-23, are retinoic acid-related orphan receptor γ (RORγT) expression and signal transducer and activator of transcription 3 (STAT3). Th17 commitment is primarily driven from naïve T cells [6–8]. Contrarily, regulatory T cells (Treg) can modulate over-shooting immune responses to protect from a wide range of autoimmune illnesses, including G.N [9,10]. The factor forkhead box P3 (FoxP3) is responsible for developing Tregs in rodents and humans. Tregs secrete two central anti-inflammatory cytokines, IL-10 and tumor necrosis factor (TGF)-β, to protect against experimental G.N [11–13]. Thus, in healthy bodies, the activation of Th17 and Tregs is balanced, whereas in organ-specific defense and renal illnesses, there is an imbalance. Numerous investigations have revealed a changed ratio of Th17 to Treg cells [14].

Currently, there is no standardized approach to treat MGN in adults, and treatment options range from no treatment to corticosteroids, alkylating agents, and cyclosporine [15]. Cyclosporine is a calcineurin inhibitor that is used as an immunosuppressive drug. It is sometimes spelled cyclosporine and cyclosporine [16]. It is administered intravenously or orally to treat eczema, psoriasis, Crohn's disease, nephrotic syndrome, rheumatoid arthritis, and organ transplant rejection [17]. Additionally, it is used as eye drops for dry eyes or keratoconjunctivitis sicca. It is thought that cyclosporin functions by impairing lymphocyte function [18]. It accomplishes this by combining with cyclophilin to inhibit calcineurin's phosphatase activity, which in turn reduces T-lymphocytes' generation of inflammatory steroids [19]. High blood pressure, headaches, kidney issues, excessive hair growth, and vomiting are typical adverse effects [20]. An elevated chance of infection, liver issues, and lymphoma are among the other serious side effects. To reduce the possibility of adverse effects, blood levels of the medicine should be monitored [21]. Over 70 percent of MGN patients obtained total or partial disappearance of proteinuria when using calcineurin inhibitors, such as cyclosporine, according to randomized prospective clinical trials. However, most calcineurin inhibitor-treated patients are thought to relapse or become treatment dependent, requiring long-term therapy to stay in remission. This exposes them to the nephrotoxic adverse effects of the medications and the ensuing renal inflammation associated with calcineurin inhibitor nephrotoxicity [22,23]. Therefore, new treatment strategies are needed for these patients to reduce the risk of chronic nephrotoxicity.

The importance of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway for signaling growth hormone receptors and cytokines is widely acknowledged. Most research has been done on renal illnesses using the JAK2 signaling pathway through STAT1 and STAT3 out of all the JAK-STAT cascades [24]. For the first time, a JAK2-selective inhibitor called fedratinib was authorized to treat myelofibrosis [25]. Fedratinib is an anti-cancer drug used to treat myeloproliferative disorders, such as myelofibrosis. It is marketed under the trade name Inrebic [26]. The medication is administered orally, such as fedratinib hydrochloride pills. It inhibits Janus kinase 2 (JAK-2) in a semi-selective manner [27]. On August 16, 2019, the FDA gave it its approval. Fedratinib is approved in the European Union to treat disease-related splenomegaly or symptoms in people with primary myelofibrosis who have had essential thrombocythaemia or polycythemia vera and are either ruxolitinib-treated or JAK inhibitor naïve [28]. The protein kinase JAK-2 is competitively inhibited by fedratinib; FLT3 and RET, two related kinases, are also susceptible [29]. The activity against JAK3 and other tyrosine kinases was noticeably lower. The inhibitor suppresses proliferation and induces apoptosis in treated cells by blocking downstream cellular signaling (JAK-STAT) [8]. Accordantly, in this research, we aimed to analyze the efficacy of cyclosporine and fedratinib combination on Th17/Tregs functions in MGN rat models. Furthermore, JAK2 allows both IL-6 and IL-23 to activate STAT3, which in turn encourages the production of IL-17 [30].

2. Materials and methods

2.1. Animals

The study included male Sprague Dawley rats (Pasteur, Karaj, Iran), 200–250 g in weight, and 7–9 weeks of age. The animals were kept in housing with a 12-h light and 12-h dark cycle, a temperature range of 20–26 °C, and a relative humidity of 30–70 %. This study was completed in line with the guidelines of the ARRIVE declarations and carried out per the following Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and associated guidelines. The study was conducted using protocols authorized by Tabriz Medical University's Institutional Animal Care Committee (ethical code: IR.TBZMED.VCR.REC.1399.053).

2.2. The experimental timetable and model for passive Heymann nephritis (PHN)

Anti-Fx1A antibody (commercial sheep antibody against Rat Fx1A serum, PTX-002S, Probetex, Inc., San Antonio, TX, USA) was injected intravenously several times a day in escalating doses to produce MGN in the rat population according to the PHN model. In line with this, the rats were given 1 mg/kg on days 1–3; on days 4–5 and 6–7, the dose was raised to 1.5 mg/kg and 2 mg/kg, respectively. After three weeks, PHN rat groups additionally received an injection of 2.5 mg/kg of the antiserum [31]. As the vehicle control, 0.9 % sodium chloride, or regular saline, was utilized. A computerized procedure was used to randomly divide 50 rats into five groups, each to achieve body weight balance with respect to group assignment. The groups included PHN (normal saline), Fedra receiving (2.27 mg/kg fedratinib intra-peritoneally over 28 days), C.P. receiving (15 mg/kg cyclosporinee orally over 28 days), Fedra-CP receiving (both fedratinib and cyclosporine), and healthy control

(normal saline). Graphical timetable of the study is provided in Fig. 1.

2.3. Analysis of biochemical parameters

To collection of serums, blood samples from treated animals were allowed to stand at four °C for 1 h, centrifuged for 15 min at 150×*g* at four °C, and stored at −80 °C for biochemical analysis. Then, the total protein, albumin, creatinine, urea, triglyceride, and cholesterol were evaluated by a biochemical autoanalyzer (Bayer Express plus Clinical Chemistry autoanalyzer, Bayer® Germany).

2.4. Cell isolation from spleen

The PHN rats were sedated by intraperitoneal injection of 10 % chloral hydrate at a rate of 0.5 mL per 100 g of body weight to assess the immunological reactions in the spleens of the rats after treatment. To isolate splenic cells, the rat's spleen was aseptically harvested and minced into smaller particles. Then, the crushed mass passed through a 70 μm cell filter to prepare the single-cell suspension in RPMI 1640 medium. The suspension was centrifuged at 450×*g* for 5 min, and the pellet was then resuspended in lysis solution for red blood cells (Seoul, Korea) and allowed to sit at room temperature for five more minutes. The cells were then rinsed with PBS for 10 min at 300×*g*. The cells were counted using the Trypan Blue Exclusion method, and their viability was verified before being used for phenotypic analysis, mRNA expression analysis, and cytokine secretion detections.

2.5. Flow cytometric analysis

To determine the Treg percentage in the treated PHN rats, 10^6 splenocytes were resuspended in 100 μL of cold FACS buffer (PBS+2% FBS) and stained using anti-rat CD4 antibodies conjugated with FITC (Miltenyi Biotec, Gladbach, Germany). Then, the samples were fixed, permeabilized, and intracellularly stained with APC-conjugated anti-rat Foxp3 antibody (Miltenyi Biotec, Gladbach, Germany). To determine the phenotype of Th17 cells in splenocytes of rats, cytokine expression of Th17 cells was stimulated using PMA (10 ng/ml), ionomycin (500 ng/ml), and brefeldin A (5 μg/ml) for 5 h. Subsequently, 1 × 106 cells were stained with FITC-conjugated anti-rat CD4, followed by intracellular cytokine staining with PE-conjugated anti-rat IL-17A antibody (Miltenyi Biotec, Gladbach, Germany). Isotype controls were used for correct compensation and antibody specificity confirmation. Flow cytometry analysis was performed using FACSCanto, BD, USA, and the results were presented as a percentage of cytokine-producing cells in CD4 + T cells using BD FACScanto software (version 3.0).

2.6. Gene expression assay

Real-time polymerase chain reaction (PCR) was used to measure the levels of gene expression of cytokines, such as IL-10, IL-21, IL-17, and TGF-β, as well as the relative transcription factors such as FoxP3, RORγt, and STAT3. The rats' splenocytes were used for the assessment. In short, the manufacturer's recommendations were followed while extracting total RNA from the cells utilizing an RNA extraction kit (Hamburg, Germany). Next, cDNA synthesis was carried out in accordance with the manufacturer's instructions utilizing a cDNA synthesis kit (Exiqon,

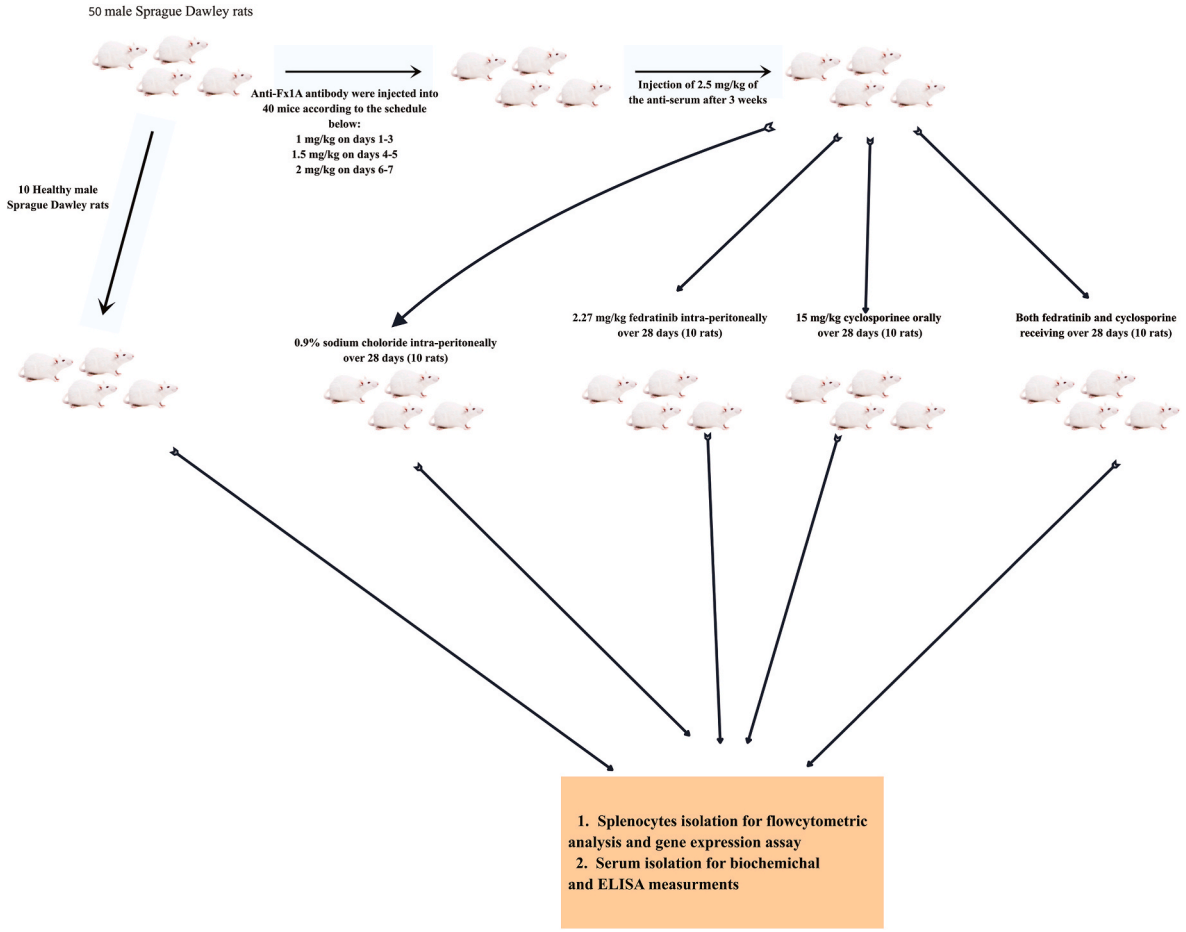

Fig. 1. Timetable of the study.

Denmark). Based on the manufacturer's instructions, real-time PCR was used to quantify each mRNA's expression level using an Ampliqon SYBR Green Real-Time PCR kit. Finally, the mRNA expression was normalized by detecting the β-actin housekeeping gene. The relative gene expression was calculated using the $2^{-\Delta\Delta.T.C.T.}$ formula. Supplementary Table 1S lists the primer sequences utilized in the qPCR assay that were created using the OLIGO v. 7.56 program (MBI, Inc., CA, USA).

2.7. Serum cytokine evaluation

Following the manufacturer's instructions, ELISA kits (Mybiosource, San Diego, USA) were used to measure the serum quantities of anti-inflammatory and inflammatory cytokines, such as TGF-β, IL-10, IL-17, and IL-21. In short, antibodies against IL-10, IL-17, IL-21, and TGF-β were coated in the wells of microtiter ELISA plate (Maxisorp, Denmark). Next, the respective wells were filled with the serums from each animal group nd biotinylated mouse anti-rIL-10, anti-rIL-17, anti-rIL-21, and anti-rTGF-β mAb. The presence of an anti-cytokine antibody was next examined by including an anti-mouse IgG Ab (Sigma) coupled with streptavidin alkaline phosphatase. Lastly, a reader for ELISA plates (LabSystems, Finland) was used to measure the absorbance at 405 nm after adding 4 mg/ml of *p*-nitrophenyl phosphate as the substrate.

2.8. Statistical analysis

GraphPad Prism version 8 (GraphPad, CA) was used to analyze all the data, and the results are shown as mean ± standard deviation (S.D.). *A one-way ANOVA was performed to compare the means between groups, and then Dunnett's T3 multiple comparisons test was performed. A p-value of less than 0.05 was deemed statistically significant.*

3. Results

3.1. The positive effects of fedratinib and cyclosporine in laboratory findings of PHN rats

As illustrated in Fig. 2, PHN rats showed significantly elevated levels of protein, albumin, creatinine, and urea compared to the control group. Moreover, the Fedra receiving group and C.P. receiving rats had remarkably enhanced albumin and creatinine compared to the normal

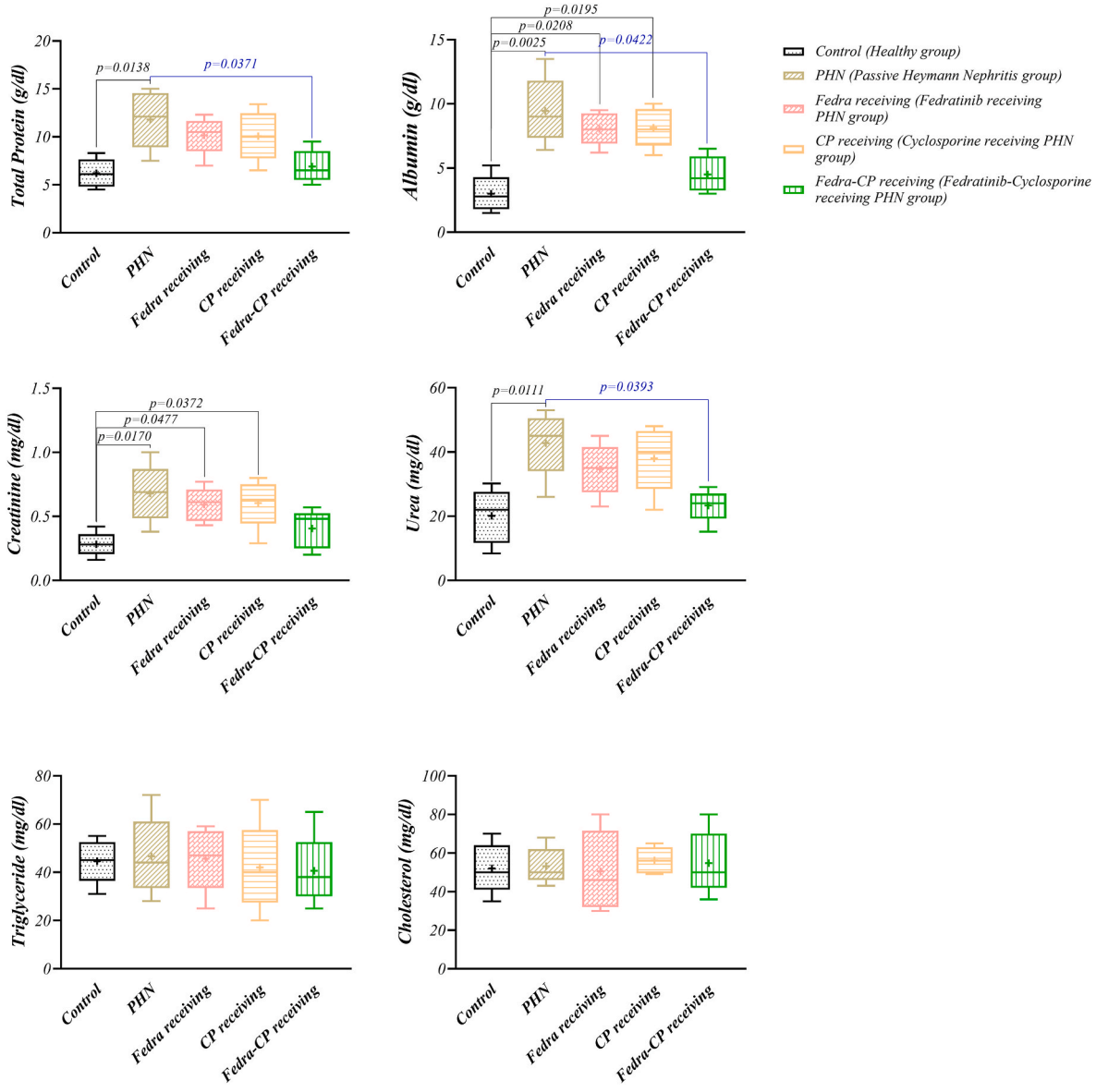

Fig. 2. Laboratory analysis of PHN rats. The serums of PHN rats receiving cyclosporine and fedratinib were assessed to examine total protein, albumin, creatinine, and urea ($P < 0.05$).

rats. However, Fedra-CP-receiving rats presented noticeably diminished amounts in total protein, albumin, and urea compared with the PHN group. Notably, the difference in triglyceride and cholesterol levelswas not statistically meaningful among the studied groups (Supplementary Table 2S).

3.2. The lower percentage of Th17 cells and higher incidence of Tregs in Fedra-CP group

The frequency of two types of T cells, Th17 and Tregs, were assessed in the splenocytes of treated rats. As depicted in Fig. 3, in groups of PHN, Fedra-receiving, and C.P. receiving, the percentage of Tregs significantly declined when compared to healthy rats, while in rats received fedratinib and cyclosporine, the incidence of Tregs noticeably augmented over the PHN group. On the other hand, the percentage of Th17 cells as remarkably higher in three groups, including PHN, Fedra receiving, and C.P. receiving rats over the control. In contrast, the incidence of Th17 cells in splenocytes of the Fedra-CP receiving group significantly diminished in comparison to the PHN group (Supplementary Table 2S).

3.3. Up-regulated expressions of relative anti-inflammatory genes in the Fedra-CP receiving group

The expression levels of transcription factors, including FoxP3, STAT3, RORγt, and inflammatory and anti-inflammatory cytokines, were examined using real-time PCR. Our results demonstrated that the fold change of Foxp3 gene expression reduced in PHN, Fedra receiving, and C.P. receiving rats while the expression of STAT3 and RORγt genes significantly enhanced in these groups compared with the control. However, in the Fedra receiving group, the expression of the FoxP3 gene notably increased, but the gene expressions of STAT3 and RORγt remarkably decreased when compared with MGN model rats (Fig. 4, Supplementary Table 2S).

Moreover, we evaluated the gene expression of inflammatory and anti-inflammatory. As illustrated in Fig. 5, the fold changes of IL-10 and TGF-β gene expressions significantly deceased in PHN, Fedra receiving, and C.P. receiving rats when compared to healthy control while the expression of IL-10 not TGF-β gene remarkably elevated in Fedra-CP receiving rats over the PHN group. Furthermore, the splenocytes of PHN, Fedra receiving, and C.P. receiving groups showed notably higher gene expressions of IL-17 and IL-21 versus the control group. Fedra-CP receiving rats showed decreased fold change of IL-17 and IL-21 genes compared to the PHN group (Supplementary Table 2S).

3.4. The reduced inflammatory but elevated anti-inflammatory cytokines in fedra-CP-receiving rats

According to Fig. 6, the evaluation of serum cytokines of treated rats presented that PHN animals and PHN groups treated with neratinib or cyclosporine had significantly decreased levels of IL-10 and TGF-β over the control. However, in PHN rats ing both of drugs, fedratinib and cyclosporinee, the anti-inflammatory cytokines IL-10 and TGF-β were notably increased compared with the PHN group. Furthermore, in comparison to normal rats, the levels of IL-17 and IL-21 were significantly higher in PHN animals, and the groups received fedratinib and cyclosporine, while in the fedra-CP group, the serum concentrations of these cytokines were remarkably diminished when compared with PHN rats (Supplementary Table 2S).

4. Discussions

Membranous glomerulonephritis is an inflammatory and immune-mediated kidney disease that mainly affects adult populations [1,2]. Notwithstanding the lack of a standardized treatment approach for MGN [32], we planned to employ a combination of cyclosporine and fedratinib to evaluate the Th17 cells/Tregs affection through induction of PHN in experimental rats.

Previous studies have revealed that chronic inflammation is one of the main players of autoimmune diseases, particularly systemic lupus erythematosus and membranous glomerulonephritis, driven mainly by Th17 activation [3]. Paust et al. found that renal injury in experimental glomerulonephritis is significantly caused by the IL-23/IL-17 pathway in

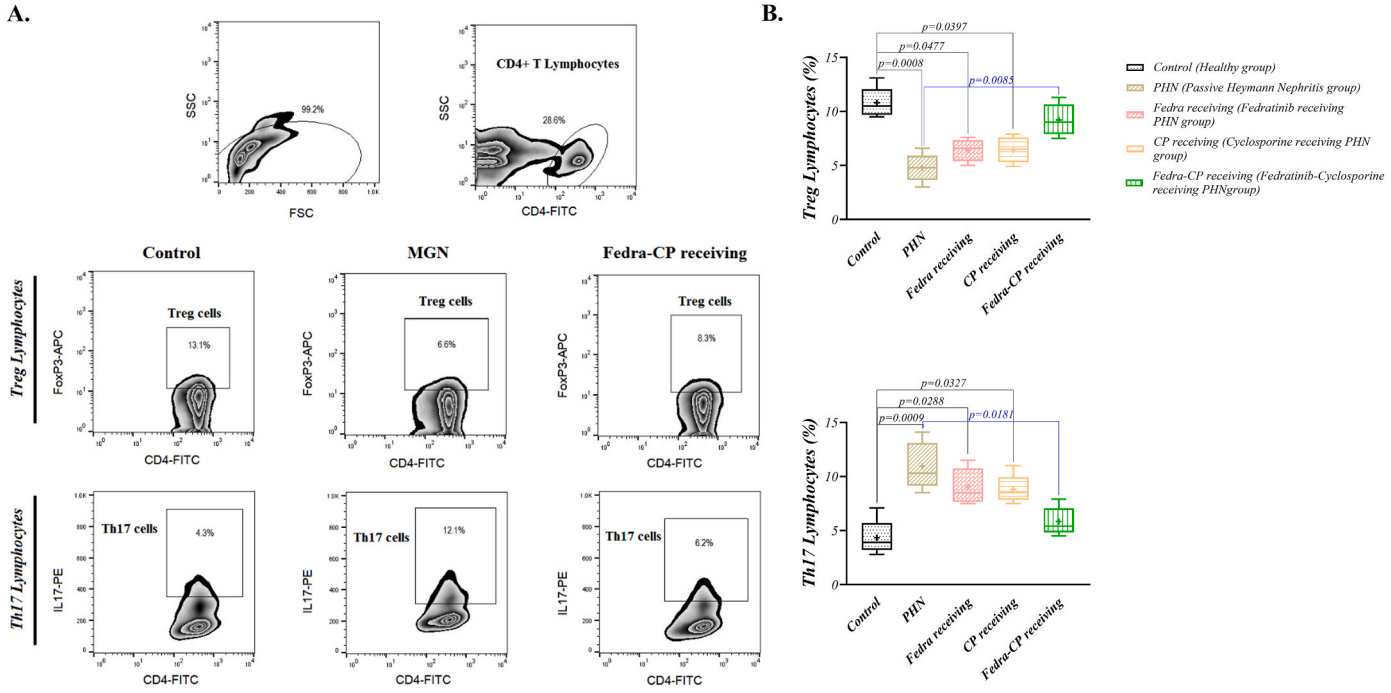

Fig. 3. The incidence of Th17 cells and Tregs. A) The dot plot analysis of splenocytes isolated from PHN rats treated with cyclosporine and fedratinib was employed to evaluate the percentage of Th17 lymphocytes and Tregs. B) The percentage of Th17 cells and Tregs were depicted in different studied groups using flow cytometry ($P < 0.05$). Th17, T helper 17. Tregs, regulatory T cells.

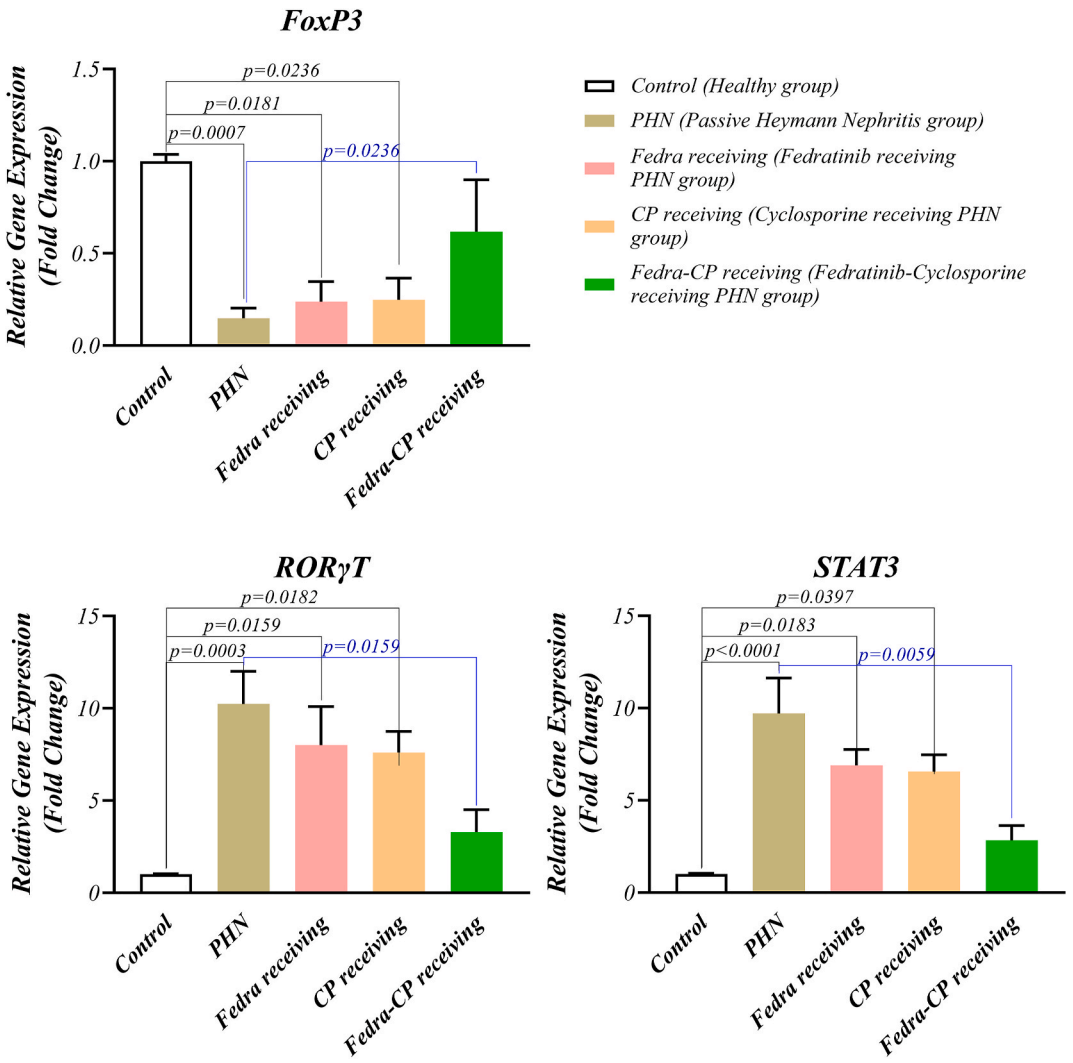

Fig. 4. The gene expressions of FoxP3, STAT3, and RORγt. The fold change of FoxP3, STAT3 and RORγt genes were evaluated in splenocytes of PHN rats treated with cyclosporine and fedratinib using real-time PCR ($P < 0.05$). FoxP3, forkhead box P3. RORγt, retinoic acid-related orphan receptor γ. STAT3, signal transducer and activator of transcription 3.

Th17 cells [33]. In two cohort studies of M.N. conducted by Rosenzwajg et al. and Roccatello et al., low levels of Treg cells were reported. In contrast, the cells' contentwas restored following remission [34,35]. Consistently, Ostmann et al. identified IL-10 as a critical cytokine produced by Tregs to protect from experimental crescentic G.N. [12], and injection of FoxP3+ Tregs into mice with acute G.N. can notably improve the course of nephritis [36]. In parallel with these findings, our flowcytometric analysis showed that the percentage of Th17 significantly enhanced in PHN rats. At the same time, Tregs incidence reduced notably compared with normal animals, which were subsequently supported by gene expression levels of transcription factors of STAT3 and RORγt for Th17 cells and FoxP3 for Tregs activation. The gene and protein expressions of IL-21 and IL-17, signature cytokines of Th17, were also enhanced remarkably whereas IL-10 and TGF-β, signatures cytokines of Tregs, were significantly diminished in PHN rats over the healthy animals verifying Th17-skewed inflammation in this rat model.

Cyclosporin is an immunosuppressive drug that serves as a calcineurin inhibitor that binds to cyclophilin receptors, causing the inhibition of the calcium-dependent IL-2 pathway in cells [37]. Thus, cyclosporine can interfere with T cell proliferation and attenuate T cell activation, particularly Th17, and inhibition of IL-17A production [38]. Despite rare studies on cyclosporine's impact on T cell responses in MGN, we found that in contrast to Th17 cells, cyclosporine had a positive effect on Tregs activation. However, these data were not statistically meaningful. Interestingly, the combination of cyclosporine with fedratinib was capable of profound enhancement in T cells skewing to Tregs versus Th17 cells meanwhile monotherapy with fedratinib were also not able to induce significant changes in Th17/Treg cells in comparison to PHN rats. This evidence displayed that none of the drugs, cyclosporine and fedratinib, were enough to elicit an anti-inflammatory impression in PHN rats, while the combination was significantly effective. To justify this observation, we can refer to prior studies describing the inflammatory agonistic effect of cyclosporine in kidney illnesses [39]. Matyjek and colleagues [40] investigate the efficacy of cyclosporine administration in nephrotic syndrome (N.S.) in adults. Their funding indicated that the moderate doses of cyclosporine (2.3–3.1 mg/kg/day) were sufficient to elicit the initial remission of.N.S. In another study by Yenigun et al. [41], they showed that cyclosporine is not inferior to cyclophosphamide in the treatment of idiopathic membranous glomerulonephritis. Their findings indicated that seventy-six percent of the cyclophosphamide group versus 82.4 % of the cyclosporine group had a partial or complete remission of proteinuria at the end of 12 months ($P = 0.89$). Serum albumin levels increased and proteinuria was significantly reduced in all groups ($P = 0.001$). A transcriptomics analysis conducted by González-Guerrero et al. have shown that cyclosporine can induce a proinflammatory response in renal

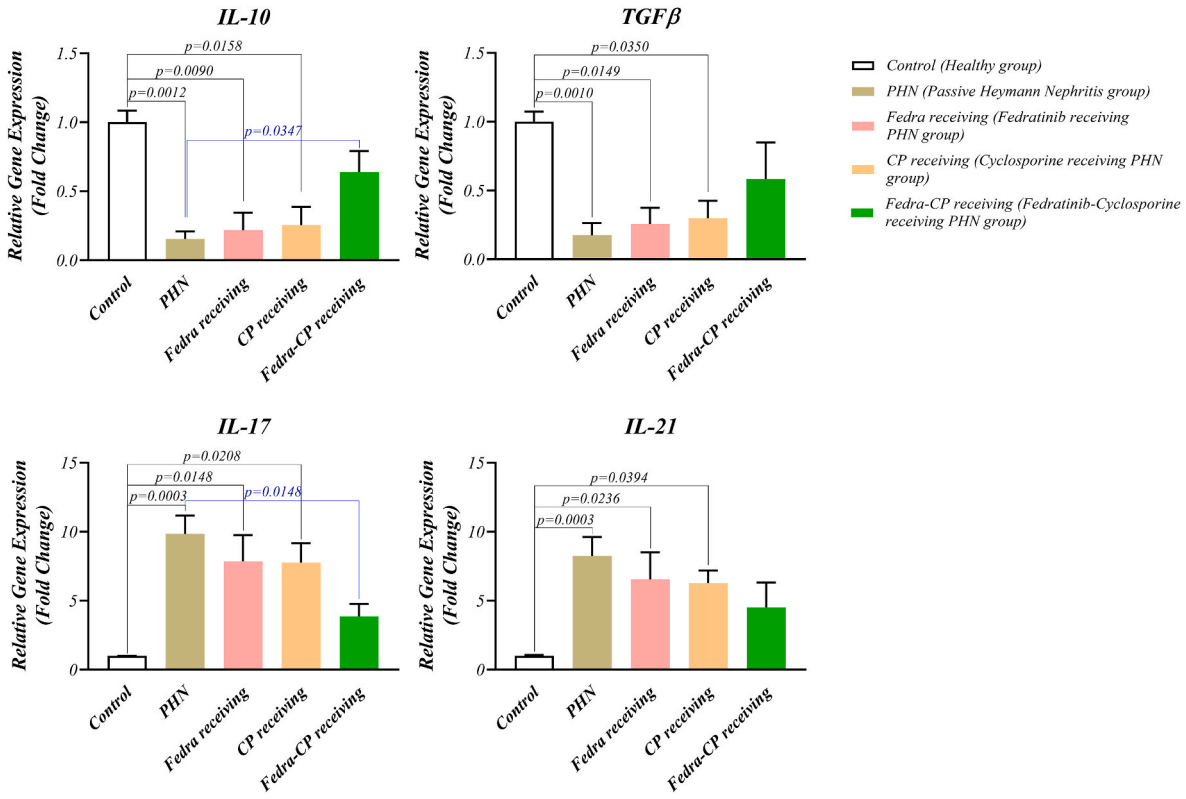

Fig. 5. The expressions of inflammatory and anti-inflammatory cytokine genes. The fold change of anti-inflammatory cytokine genes, including IL-10 and TGF-β, and inflammatory cytokine genes, including IL-17 and IL-21, were assessed in the splenocytes of PHN animals treated with cyclosporine and fedratinib using real-time PCR ($P < 0.05$). I.L., interleukin. TGF-β, tumor necrosis factor-β.

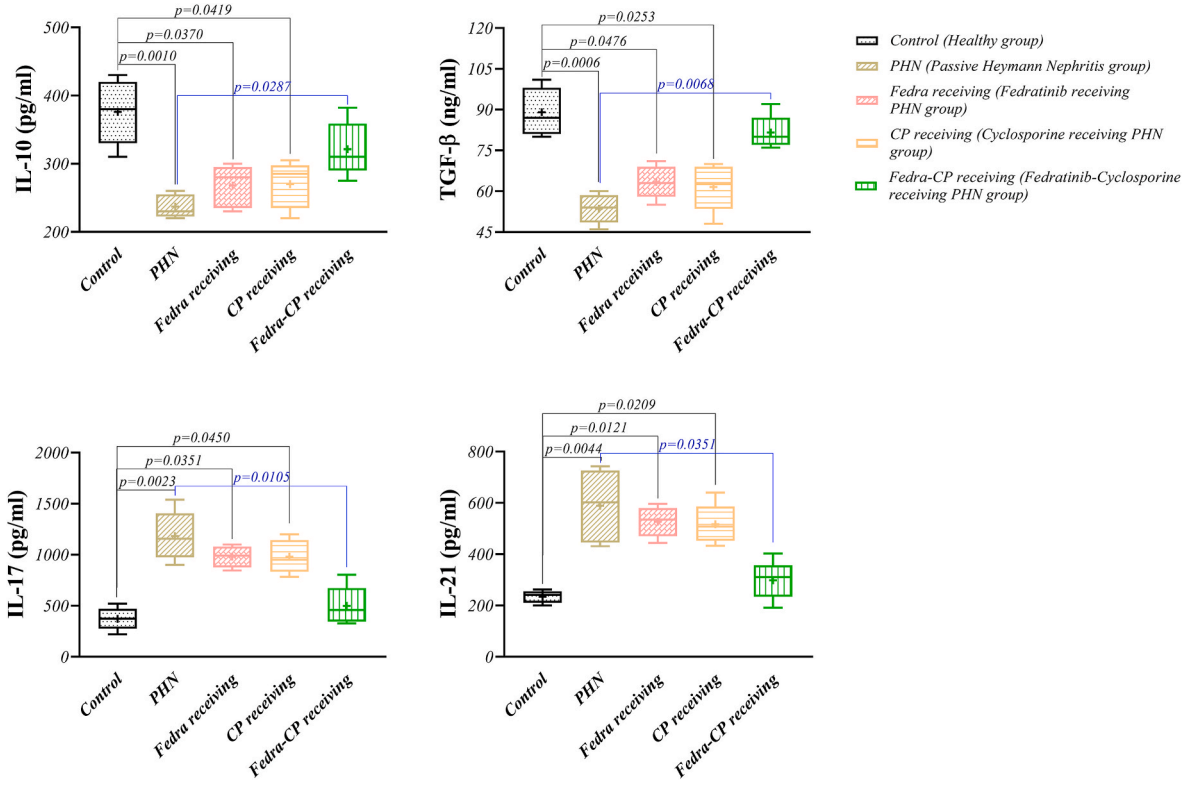

Fig. 6. The protein expressions of inflammatory and anti-inflammatory cytokines. The anti-inflammatory cytokines, IL-10 and TGF-β, and inflammatory cytokines, including IL-17 and IL-21, were measured in the serums of PHN animals treated with cyclosporine and fedratinib using ELISA ($P < 0.05$). I.L., interleukin. TGF-β, tumor necrosis factor-β.

tubular cells through protein kinases such as the JAK2/STAT3 pathway to promote proinflammatory gene expressions that is why cyclosporine causes nephrotoxicity. This result was then supported by a murine model of nephrotoxicity occurred following cyclosporine administration and activation of proinflammatory pathways in renal tubular cells, suggesting the limited benefits of cyclosporine in MGN therapy [22]. On the other side, JAK2 inhibition has been proposed as a novel therapy for inflammatory diseases. It has been shown that JAK2 inhibitors can diminish the Th17-specific immune response in a murine arthritis model [42]. In parallel, Loverre et al. recognized the activation of JAK2 as the potential signaling molecule associated with tubular IL-17 induction in acute antibody-mediated rejection (ABMR) [43]. Fedratinib, a new JAK2 inhibitor, suppresses the expression of several Th17 signature cytokines, particularly IL-17, and subsequently alleviates cytokine storm-mediated signs in acute COVID-19 patients infections [44].

Meanwhile, these findings suggested that the monotherapy of fedratinib was ineffective in COVID-19 patients due to the reversibility of JAK2 inhibition. Still, it was promising with other antiviral drugs [44, 45]. Betts et al. demonstrated that JAK2-deficient T lymphocytes shifted towards Treg polarization, reducing graft versus host disease (GvHD). As such, it has been declared that selective JAK2 inhibition can induce beneficial Tregs [46]. Convinced with thisis evidence, we observed that the combination of fedratinib and cyclosporine remarkably diminished Th17 cells activation in favor of Tregs functions in PHN rats alongside the significantly decreased levels of total protein, albumin, and urea in the serum of the animals. Nevertheless, the signaling pathways involved in the synergistic effects of fedratinib and cyclosporine on T cells deviation is blurred.

This research offers promising insights into the potential benefits of combining cyclosporine and fedratinib to modulate the Th17/Treg balance in a rat model of membranous glomerulonephritis (MGN). However, it is essential to consider several limitations. One point of this study's limitation is the reduction of the number of markers in immunophenotyping, which was done due to financial constraints. Perhaps using more markers would have resulted in more accurate immunophenotyping results. The other limitation is that the study uses a rat model of MGN induced by anti-Fx1A, which may not fully replicate the pathophysiology of human MGN. The dosages of cyclosporine and fedratinib used in the study may not accurately reflect the therapeutic dosages suitable for human patients. Additionally, the route of administration, treatment duration, and pharmacokinetics in rats can differ significantly from those in humans. Addressing these limitations in future research would help validate the findings and improve their potential for translation to human clinical practice.

5. Conclusion

Membranous glomerulonephritis is an inflammatory disorder of the kidney caused by the overactivation of Th17 cells. PHN rats experienced the same immune response by limited Treg functions in favor of Th17 lymphocytes. The administration of cyclosporine and fedratinib was capable of reducing Th17 functions while elevating Tregs activation in PHN rats. It is ambiguous how and by which mechanism the combination of these drugs worked to elicit anti-inflammatory impacts tending toward Tregs actions. Ongoing research potentially uncover and answer the questions.

CRediT authorship contribution statement

Ali Ghassabi: Writing – original draft, Methodology, Investigation, Conceptualization. **Maryam Hosseini:** Writing – original draft, Conceptualization. **Hemayat Abdoli Goungormaz:** Methodology, Investigation. **Mohammad Sadegh Soltani-Zangbar:** Methodology, Investigation, Formal analysis. **Mahsa Beomidehagh:** Methodology, Investigation. **Davoud Rostamzadeh:** Methodology. **Mohammadbagher Pirouzpanah:** Methodology. **Arshad Ghaffari-Nasab:** Methodology. **Arash Khaki:** Project administration, Conceptualization. **Leili Aghebati-Maleki:** Software, Methodology. **Elham Badihi:** Methodology. **Farshid Afandideh:** Methodology. **Reihane Shahabirad:** Methodology. **Ali Akbar Shekarchi:** Methodology, Investigation. **Javad Ahmadian Heris:** Writing – review & editing, Investigation. **Leila Roshangar:** Writing – review & editing, Validation. **Jalal Etemadi:** Validation, Project administration, Conceptualization. **Mehdi Yousefi:** Supervision, Project administration, Conceptualization.

Ethics approval and consent to participate

This study was completed in accordance with the guidelines of the ARRIVE declarations and carried out in accordance with Guidance on the operation of the Animals (Scientific Procedures) Act 1986 and associated guidelines. It was conducted using protocols authorized by Tabriz Medical University's Institutional Animal Care Committee (ethical code: IR.TBZMED.VCR.REC.1399.053).

Consent for publication

All authors read and approved the final manuscript for publication.

Availability of data

The data cannot be publicly shared but are limitedly available by contacting the corresponding author of this study privately.

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Declaration of Competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101874.

Data availability

The data cannot be publicly shared but are limitedly available by contacting the corresponding author of this study privately.

References

[1] A. Taherkhani, S. Kalantari, A. Arefi Oskouie, M. Nafar, M. Taghizadeh, K. Tabar, Network analysis of membranous glomerulonephritis based on metabolomics data, Mol. Med. Rep. 18 (5) (2018) 4197–4212.
[2] A. Barbari, Pre-and posttransplant refractory idiopathic membranous glomerulonephritis: the Forgotten potential Culprit, Exp. Clin. Transplant. Off. J. Middle East Soc. Organ Transplant. 15 (5) (2017) 483–489.
[3] J. Wu, B. Liu, C. Liang, H. Ouyang, J. Lin, Y. Zhong, Y. He, J. Zhou, Y. Zhou, J. Zhou, Zhen-Wu-tang attenuates cationic bovine serum albumin-induced inflammatory response in membranous glomerulonephritis rat through inhibiting AGEs/RAGE/NF-κB pathway activation, Int. Immunopharm. 33 (2016) 33–41.
[4] C.F. Krebs, T. Schmidt, J.-H. Riedel, U. Panzer, T helper type 17 cells in immune-mediated glomerular disease, Nat. Rev. Nephrol. 13 (10) (2017) 647–659.
[5] C.F. Krebs, H.-J. Paust, S. Krohn, T. Koyro, S.R. Brix, J.-H. Riedel, P. Bartsch, T. Wiech, C. Meyer-Schwesinger, J. Huang, Autoimmune renal disease is exacerbated by S1P-receptor-1-dependent intestinal Th17 cell migration to the kidney, Immunity 45 (5) (2016) 1078–1092.

[6] H. Mortazavi, M.S. Soltani-Zangbar, S. Eghbal-Fard, A. Mehdizadeh, A. Kamrani, F. Chakeri-Khiavi, H.S. Kafil, F. Jadidi-Niaragh, S. Rahimifar, H.T. Khosroshahi, Cytokine profile, Treg/Th17 cell frequency changes during different posttransplantational time points in patients undergoing renal transplantation, J. Cell. Physiol. 234 (11) (2019) 20935–20943.
[7] H. Cui, N. Wang, H. Li, Y. Bian, W. Wen, X. Kong, F. Wang, The dynamic shifts of IL-10-producing Th17 and IL-17-producing Treg in health and disease: a crosstalk between ancient" Yin-Yang" theory and modern immunology, Cell Commun. Signal. 22 (1) (2024) 99.
[8] Y. Liu, W. Wang, J. Zhang, S. Gao, T. Xu, Y. Yin, JAK/STAT signaling in diabetic kidney disease, Front. Cell Dev. Biol. 11 (2023) 1233259.
[9] C. Kurts, U. Panzer, H.-J. Anders, A.J. Rees, The immune system and kidney disease: basic concepts and clinical implications, Nat. Rev. Immunol. 13 (10) (2013) 738–753.
[10] M. Abdeladhim, J.L. Karnell, S.A. Rieder, In or out of control: Modulating regulatory T cell homeostasis and function with immune checkpoint pathways, Front. Immunol. 13 (2022) 1033705.
[11] M.A. Koch, G.s. Tucker-Heard, N.R. Perdue, J.R. Killebrew, K.B. Urdahl, D. J. Campbell, The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation, Nat. Immunol. 10 (6) (2009) 595–602.
[12] A. Ostmann, H.-J. Paust, U. Panzer, C. Wegscheid, S. Kapffer, S. Huber, R. A. Flavell, A. Erhardt, G. Tiegs, Regulatory T cell–derived IL-10 ameliorates crescentic GN, J. Am. Soc. Nephrol. 24 (6) (2013) 930–942.
[13] Y. Zong, K. Deng, W.P. Chong, Regulation of Treg cells by cytokine signaling and co-stimulatory molecules, Front. Immunol. 15 (2024) 1387975.
[14] R. Motavalli, J. Etemadi, M.S. Soltani-Zangbar, M.-R. Ardalan, H. Kahroba, L. Roshangar, M. Nouri, L. Aghebati-Maleki, F.M. Khiavi, S. Abediazar, Altered Th17/Treg ratio as a possible mechanism in pathogenesis of idiopathic membranous nephropathy, Cytokine 141 (2021) 155452.
[15] J.L. Daza, M.V. Cabrera, M. de Rosa, I. Roca, V. Remache, J.S.R. Bello, Primary membranous nephropathy in Latin America: a multicentre study, Rev. Colomb. Reumatol. (2024).
[16] M. Simonenko, D. Hansen, J. Niebauer, M. Volterrani, S. Adamopoulos, C. Amarelli, M. Ambrosetti, S.D. Anker, A. Bayes-Genis, T.B. Gal, Prevention and rehabilitation after heart transplantation: a clinical consensus statement of the European association of Preventive Cardiology, Heart Failure association of the ESC, and the European Cardio Thoracic transplant association, a section of ESOT, European journal of preventive cardiology 31 (11) (2024) 1385–1399.
[17] F.F. Konen, N. Möhn, T. Witte, M. Schefzyk, M. Wiestler, S. Lovric, K. Hufendiek, P. Schwenkenbecher, K.-W. Sühs, M.A. Friese, Treatment of autoimmunity: the impact of disease-modifying therapies in multiple sclerosis and comorbid autoimmune disorders, Autoimmun. Rev. 22 (5) (2023) 103312.
[18] L. Gouvea, M. Mimouni, S. Alshaker, N. Din, D.D. Deangelis, N. Tucker, H. Gill, A. R. Slomovic, C.C. Chan, Clinical features and management of keratoconjunctivitis associated with inadequate tear drainage, Can. J. Ophthalmol. 59 (3) (2024) e206–e212.
[19] A. Mandal, P. Das, R. Bhowmik, H. Mazumdar, M.A. Shaharyar, R. Kumari, S. Jana, S. Patra, P.K. Haldar, S. Karmakar, An insight into the agents used for immunomodulation and their mechanism of action, in: How Synthetic Drugs Work, Elsevier, 2023, pp. 503–528.
[20] I. Karunarathna, K. Kusumarathna, P. Jayathilaka, C. Withanage, Comprehensive management of Hypertension: strategies, guidelines, and emerging therapies. Uva Clinical Lab. Retrieved from Comprehensive Management of Hypertension: Strategies, Guidelines, and Emerging Therapies, 2024.
[21] N. Méndez-Sánchez, C.E. Coronel-Castillo, M.M. Ramírez-Mejía, Chronic Hepatitis C Virus infection, Extrahepatic disease and the impact of new Direct-Acting antivirals, Pathogens 13 (4) (2024) 339.
[22] C. González-Guerrero, C. Ocaña-Salceda, S. Berzal, S. Carrasco, B. Fernández-Fernández, P. Cannata-Ortiz, J. Egido, A. Ortiz, A.M. Ramos, Calcineurin inhibitors recruit protein kinases JAK2 and JNK, TLR signaling and the UPR to activate NF-κB-mediated inflammatory responses in kidney tubular cells, Toxicol. Appl. Pharmacol. 272 (3) (2013) 825–841.
[23] C. Zhang, L. Leng, Z. Li, Y. Zhao, J. Jiao, Identification of biomarkers and drug repurposing candidates based on an immune-, inflammation-and membranous glomerulonephritis-associated triplets network for membranous glomerulonephritis, BMC Med. Genom. 13 (1) (2020) 1–11.
[24] Y. Nozaki, Renal disorders: Involvement of JAK-STAT pathway, in: JAK-STAT Signaling in Diseases, CRC Press, 2020, pp. 159–176.
[25] H.A. Blair, Fedratinib: first approval, Drugs 79 (15) (2019) 1719–1725.
[26] S. Rinella, Combination Fedratinib and Venetoclax Treatment Have Activity against Human B Cell Acute Lymphoblastic Leukemia with High FLT3 Expression, The University of Wisconsin-Madison, 2023.
[27] Q. Qiu, F. Chi, D. Zhou, Z. Xie, Y. Liu, H. Wu, Z. Yin, W. Shi, H. Qian, Exploration of Janus kinase (JAK) and histone deacetylase (HDAC) bispecific inhibitors based on the moiety of fedratinib for treatment of both hematologic malignancies and solid cancers, J. Med. Chem. 66 (8) (2023) 5753–5773.
[28] M. Martino, M. Pitea, A. Sgarlata, I.M. Delfino, F. Cogliandro, A. Scopelliti, V. Marafioti, S. Polimeni, G. Porto, G. Policastro, Treatment strategies used in treating myelofibrosis: State of the Art, Hematol. Rep. 16 (4) (2024) 698–713.
[29] J. Narayanan, T. Tamilanban, P.S. Kumar, A. Guru, S. Muthupandian, M. Kathiravan, J. Arockiaraj, Role and mechanistic actions of protein kinase inhibitors as an effective drug target for cancer and COVID, Arch. Microbiol. 205 (6) (2023) 238.
[30] R. Chilamakuri, S. Agarwal, COVID-19: characteristics and therapeutics, Cells 10 (2) (2021) 206.
[31] M.J. Coyne, A.E. Schultze, D.J. McCrann III, R.E. Murphy, J. Cross, M. Strong-Townsend, C. Drake, R. Mack, Evaluation of renal injury and function biomarkers, including symmetric dimethylarginine (SDMA), in the rat passive Heymann nephritis (PHN) model, PLoS One 17 (5) (2022) e0269085.
[32] R.P. Valentini, T.K. Mattoo, G. Kapur, A. Imam, Membranous glomerulonephritis: treatment response and outcome in children, Pediatr. Nephrol. 24 (2) (2009) 301–308.
[33] H.-J. Paust, J.-E. Turner, O.M. Steinmetz, A. Peters, F. Heymann, C. Hölscher, G. Wolf, C. Kurts, H.-W. Mittrücker, R.A. Stahl, The IL-23/Th17 axis contributes to renal injury in experimental glomerulonephritis, J. Am. Soc. Nephrol. 20 (5) (2009) 969–979.
[34] D. Roccatello, S. Sciascia, D. Di Simone, L. Solfietti, C. Naretto, R. Fenoglio, S. Baldovino, E. Menegatti, New insights into immune mechanisms underlying response to Rituximab in patients with membranous nephropathy: a prospective study and a review of the literature, Autoimmun. Rev. 15 (6) (2016) 529–538.
[35] M. Rosenzwajg, E. Languille, H. Debiec, J. Hygino, K. Dahan, T. Simon, D. Klatzmann, P. Ronco, B-and T-cell subpopulations in patients with severe idiopathic membranous nephropathy may predict an early response to rituximab, Kidney Int. 92 (1) (2017) 227–237.
[36] M.A. Kluger, S. Melderis, A. Nosko, B. Goerke, M. Luig, M.C. Meyer, J.-E. Turner, C. Meyer-Schwesinger, C. Wegscheid, G. Tiegs, Treg17 cells are programmed by Stat3 to suppress Th17 responses in systemic lupus, Kidney Int. 89 (1) (2016) 158–166.
[37] T. Amber, S. Tabassum, Cyclosporin in dermatology: a practical compendium, Dermatol. Ther. 33 (6) (2020) e13934.
[38] H. Guo, Y. Ju, M. Choi, M.C. Edman, S.G. Louie, S.F. Hamm-Alvarez, J.A. MacKay, Supra-lacrimal protein-based carriers for cyclosporine A reduce Th17-mediated autoimmunity in murine model of Sjögren's syndrome, Biomaterials 283 (2022) 121441.
[39] C. Blume, G. Heise, A. Hess, C. Waldner, B. Grabensee, K. Schroer, P. Heering, Different effect of cyclosporine A and mycophenolate mofetil on passive Heymann nephritis in the rat, Nephron Exp. Nephrol. 100 (2) (2005) e104–e112.
[40] A. Matyjek, A. Rymarz, M. Sobol, S. Niemczyk, The efficacy OF CYCLOSPORINE ADMINISTRATION IN nephrotic syndrome IN adults, in: NEPHROLOGY DIALYSIS TRANSPLANTATION, OXFORD UNIV PRESS GREAT, 2017. CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
[41] E.C. Yenigun, D. Turgut, S. Piskinpasa, R. Ozturk, F. Dede, A.R. Odabas, Cyclosporine is not inferior to cyclophosphamide in the treatment of idiopathic membranous glomerulonephritis: single centre experience, Int. J. Clin. Exp. Med. 1 (2016) 316–322.
[42] F. Sun, W. Gu, Baicalin attenuates collagen-induced arthritis via inhibition of JAK2-STAT3 signaling and regulation of Th17 cells in mice, Journal of Cell Communication and Signaling 13 (1) (2019) 65–73.
[43] A. Loverre, T. Tataranni, G. Castellano, C. Divella, M. Battaglia, P. Ditonno, M. Corcelli, M. Mangino, L. Gesualdo, F. Schena, IL-17 expression by tubular epithelial cells in renal transplant recipients with acute antibody-mediated rejection, Am. J. Transplant. 11 (6) (2011) 1248–1259.
[44] D. Wu, X.O. Yang, TH17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor Fedratinib, J. Microbiol. Immunol. Infect. 53 (3) (2020) 368–370.
[45] J.P. Bewersdorf, S.M. Jaszczur, S. Afifi, J.C. Zhao, A.M. Zeidan, Beyond ruxolitinib: fedratinib and other emergent treatment options for myelofibrosis, Cancer Manag. Res. 11 (2019) 10777.
[46] B.C. Betts, D. Bastian, S. Iamsawat, H. Nguyen, J.L. Heinrichs, Y. Wu, A. Daenthanasanmak, A. Veerapathran, A. O'Mahony, K. Walton, Targeting JAK2 reduces GVHD and xenograft rejection through regulation of T cell differentiation, Proc. Natl. Acad. Sci. USA 115 (7) (2018) 1582–1587.