The Expression and Effect of Helper IL-10-Producing B Cells in Human Minimal Change Nephrotic Syndrome

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Abstract

This study is aimed at determining the putative contribution of B cell types in patients with newly diagnosed minimal change nephrotic syndrome (MCNS) before treatment and 8-12 weeks post-treatment. Significantly higher percentages of CD19+CD27+, CD19+CD38+, and CD19+CD86+ B cells were observed compared to healthy controls. Importantly, lower levels of Breg cells and intracellular expression of IL-10 protein were detected in MCNS patients. 24-h urinary proteins were positively correlated with the percentage of CD19+CD27+ B cells and negatively correlated with the percentage of Breg cells. A positive correlation between the percentage of CD19+CD5+CD1d+ cells and the levels of serum albumin was observed in these patients. Treatment with prednisone (PDN) significantly reduced the frequency of CD19+CD27+, CD19+CD38+, and CD19+CD86+ B cells, and elevated the levels of Breg cells and Breg cell-derived IL-10. This data indicates that several of the CD19+ B cell subtypes and IL-10+ Breg cells have a potentially crucial role in the MCNS patients.

Keywords: B cell, Breg cell, MCNS, Prednisone

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Introduction

Minimal Change Nephrotic Syndrome (MCNS) is the major cause of the nephrotic syndrome (NS) in children, yet comprises only 10-15% of adult NS cases [1-3]. MCNS usually responds to glucocorticoids (steroids), and the long-term prognosis is generally good [4]. On kidney biopsy glomeruli appear normal by light microscopy and without immune deposits by immunofluorescence microscopy. The characteristic disease lesion, diffuse and generalized epithelial foot process effacement affecting glomerular albumin permeability, is only found by electron microscopy [5].

The pathophysiologic process of MCNS remains poorly understood but is frequently triggered by immunogenic stimuli such as infection or allergic reaction [6]. The release of cytokines by T cells has long been considered a key in the pathogenesis of MCNS [3]. However, the beneficial effects of Rituximab as a rescue therapy in children with steroid-dependent MCNS [7,8] suggests that B-lymphocytes may play an important role in this process.

Immunoglobulin production is the result of a complex interaction between B cells, T cells, and antigen-presenting cells such as dendritic cells. Naive B cells are IgM/IgD-positive and must undergo a program of affinity maturation, isotype switch, and plasma cell differentiation before high affinity IgG or IgA antibodies may be produced [9,10]. In this process, B cells express different subsets of receptors conferring differential biological activities. Initial, immature B cells appear as CD27-CD19+. CD38+CD19+ B cells act

as antibody-secreting cells, while CD27+CD19+ B cells serve as memory B cells, CD95+CD19+ B cells signal apoptotic induction, CD38+CD19+ B cells act as antibody-secreting cells following B cell activation, and CD1d^{hi}CD5+CD19^{hi} serve as regulatory B cells (Breg) [11]. Importantly, these B-cell subsets may be involved at different stages of MCNS pathogenesis.

A recent study described a subset of regulatory B cells that do not participate in the pathogenesis of autoimmune diseases and act through the production of regulatory cytokines IL-10 and TGF-ß [10]. The potential of Bregs was first described in the regulation of autoimmunity, such as Experimental Autoimmune Encephalomyelitis (EAE), in which disease severity was increased in B-cell-deficient mice [12]. Later, antigen-induced IL-10 production from B cells was shown to play a critical role in the remission phase of EAE [13]. Similarly, B cell IL-10 production was shown to increase the severity of chronic colitis observed in TCR a-/-mice [14]. It has been demonstrated that transitional two marginal zone Bregs prevent collagen-induced arthritis in mice, but are fully dependent on CD40 and partially dependent on antigen [15]. Recently, a human counter-part of such Bregs has been characterized in peripheral blood. These Bregs are defective for CD40-dependent IL-10 production in lupus patients, indicating that this impaired function might contribute to the disease [16].

Currently, MCNS patients are treated with corticosteroids and other immunosuppressants such as cyclosporine (CyA), cyclophosphamide (CPA) and mycophenolate mofetil (MMF), in addition to anti-coagulation drugs, such as aspirin or dipyridamole. However, it is unclear how these therapeutic strategies affect B cells and immune measures in MCNS patients. Therefore we set out to examine the effects of various treatment strategies on B cell subpopulation ratios and IL-10 production in MCNS patients.

Materials and Methods

Patients

A total of 23 patients with MCNS who underwent renal biopsy were recruited for study from the Nephrology Department at the First Hospital of Jilin University (Jilin University, Changchun, China). The ethnicity of these subjects was Chinese. No patient had received corticosteroids or other immunosuppressive agents within 6 months before this study. The plasma laboratory tests of antinuclear antibody (ANA), anti-Sm antibody, anti-SSA, anti-SSB, anti-neutrophil cytoplasmic antibody (ANCA), antiphospholipid antibody, plasma complement C3 and C4, and rheumatoid factors were performed for all patients. Together with the renal histological and immunofluorescence results, the patients with potential autoimmune diseases were ruled out. None of the patients suffered from diabetes mellitus, neoplasia, active peptic ulcer disease, or viral hepatitis. At the time of blood collection and renal biopsy, no patients had fever or urinary, respiratory, or gastro-intestinal tract infections.

In addition, 12 sex- and age-matched healthy subjects were recruited from the medical examination center of the First Hospital of Jilin University. The study conformed to the guidelines of the Declaration of Helsinki and was approved by the Human Ethics Committee of Jilin University. Written informed consent was obtained from each participant.

Treatment and follow up

Nine patients of 23 MCNS patients were treated with prednisone (PDN, Tianyao Pharmaceuticals, Tianjin, China) at 1 mg/kg/d for the first two months, and gradually decreased to a maintenance dose of 10 mg/d over the next 6 months. In addition, individual patients were treated with aspirin (100 mg/d, Bayer, Germany) or dipyridamole (100 mg/d, Yunpeng Pharmaceutical, Shanxi, China) if the patient had a high risk of a hypercoagulable state. The patients visited the clinical office monthly and were followed up for at least 8-12 weeks after beginning treatment. Blood samples were collected at the time of kidney biopsy and after 8-12 weeks of treatment.

Blood sampling and analyses

The study participants were instructed to fast overnight and venous blood (10 mL) was collected into heparinized tubes the following morning. All samples were subjected to lymphocyte profiling and routine laboratory analyses within 24 h. The ADVIA 1650 biochemical analyzer (Bayer, Pittsburg, PA, USA) was used to measure triglycerides, cholesterol, proteinuria, serum uric acid, and serum albumin. The eGFR was calculated using the revised eGFR formula developed for Japanese subjects [17].

Detection of B cell subpopulations

Peripheral blood mononuclear cells (PBMCs) were harvested from each participant's heparinized blood sample by density-gradient centrifugation using Ficoll-Paque Plus (Amersham Biosciences, Buckinghamshire, UK). The isolated PBMCs were stimulated with 1 µg/mL CpG-B DNA plus 50 ng/mL IL-21 (all from Sigma-Aldrich, St Louis, MO, USA) in complete RPMI-1640 medium (*In vitrogen*, Carlsbad, CA, USA) for 72 h at 37°C in a 5% CO_2 atmosphere. The stimulated PBMCs were harvested, washed with ice-cold phosphate-buffered saline (PBS), and stained with fluorescein-labeled antibodies against various B cell markers (20 µL of PerCy5-anti-CD19, PE-anti-CD38, APC-anti-CD86, PE-anti-CD27, or APC-anti- CD95; BD Pharmingen, San Diego, CA, USA) or isotype-matched control (20 µL of PE-anti-IgG1, APC-anti- IgG1, or PerCy5-anti-IgG1; BD Pharmingen) for 30 min at room temperature in the dark. After a final PBS wash, the immunostained cells were subjected to flow cytometry analysis using a FACSCalibur instrument (Beckton Dickinson, Franklin Lakes, NJ, USA). At least 80,000 events per sample were analyzed by FlowJo software (v7.6.1) [18].

Detection of Breg cells and intracellular IL-10 protein expression

Isolated PBMCs were plated in 24-well plates (10^6 cells/well) and stimulated with 50 ng/mL phorbol myristate acetate (PMA), 500 ng/ml ionomycin, and 10 µg/ml lipopolysaccharide (LPS) (all from Sigma-Aldrich) in complete RPMI-1640 medium for 2 h at 37°C in 5% CO_2. Brefeldin A (GolgiPlug; BD Biosciences, San Jose, CA, USA) was then added to each well and the cells were

incubated for an additional 4 h. The cells were then harvested, washed with ice-cold PBS, and stained with fluorescein-labeled antibodies against various Breg markers (5 μL of APC-anti-CD19, PerCP-anti-CD5, or PE-anti-CD1d; eBiosciences, San Diego, CA, USA) or isotype-matched control (20 μL of APC-anti-IgG1, PerCP-anti- IgG2a, or PE-anti- Ig2b; Beckton Dickinson, San Jose, CA, USA). The immunostained cells were fixed by incubating with 4% paraformaldehyde for 30 min at room temperature and permeabilized by incubating with 0.5% saponin in 10% fetal bovine serum in PBS for 30 min at room temperature. After PBS washes, the cells were stained with 5 μL FITC-anti-IL-10 (eBiosciences) and analyzed by flow cytometry as described above. At least 50,000 events per sample were analyzed.

Statistical analysis

All data are expressed as mean, median, and range. Multiple comparisons between the two groups were analyzed by the Kruskal-Wallis H non-parametric test, using the Bonferonni method to correct P value. Correlations between variables were evaluated by the Spearman's rank correlation test. All statistical analyses were performed by the SPSS software suite (v19.0 for Windows; SPSS, Inc., Chicago, IL, USA). A two-sided P-value of <0.05 was considered statistically significant.

Results

A higher frequency of circulating CD19+CD27+ and CD19+CD38+ B cells in MCNS patients

There was no significant difference in the distribution of age and gender between MCNS patients and healthy controls (HC). Furthermore, there was no significant difference in the concentrations of uric acid, triglycerides, cholesterol and eGFR between the two groups. As expected, the 24-h urinary proteins were significantly higher in MCNS patients, yet albumin levels were lower compared to HC **(Table 1)**.

Flow cytometry analysis indicated that the percentage of peripheral blood CD19+CD27+ in CD19+ B cells and lymphocytes was significantly higher in MCNS than in the HC (25(9.91-49.80) vs. 15.7 (9.46-22.30), *P*=0.0102; 1.61 (0.63-3.37) vs. 0.94(0.67-1.45), *P*=0.0030) **(Figure 1)**. In contrast, the frequencies of CD19+CD95+, CD19+CD95+CD27+ cells were similar between groups **(Table 2)**.

MCNS patients also showed a significantly higher percentage of

Table 1 Demographic and clinical characteristics of participants

	MCNS (n=23)	Healthy Controls (n=12)
Age, year	40 (21-61)	33 (19-40)
Female/Male	12/11	5/7
Urinary proteins, g/24 h	5.67 (3.61-15.10)*	0.05 (0-0.10)
Serum uric acid, μmol/L	395 (265-503)	365.0 (230-400)
Triglycerides, mmol/L	2.04 (1.04-6.33)	1.21 (0.50-1.50)
Cholesterol, mmol/L	4.77 (2.12-8.76)	4.17 (2.80-5.35)
eGFR, mL/min/1.73m²	79.27 (48.90-116.4)	98.25 (90.00-110.00)
serum albumin, g/L	23.8 (12.1-29.3)*	44.10 (40.6-49.1)
Data shown are median and range unless specified. *P<0.05 vs. HC		

CD19+CD38+ cells in CD19+ cells and lymphocytes than in HC (5.58(2.79-8.59) vs. 3.15(2.04-5.32), *P*=0.0008; 0.76(0.54-1.36) vs. 0.51(0.29-0.83), *P*=0.0068) **(Figure 2A and 2C)**, and increased levels of CD19+CD86+ cells in CD19+ B cells and lymphocytes compared to HC (3.7(1.67-6.26) vs. 2.45(1.31-3.84), *P*=0.0348; 0.58(0.31-1.18) vs. 0.38(0.25-0.69), *P*=0.0280) **(Figure 2B and 2D)**. The frequency of CD19+CD38+CD86+ in CD19+ B cells was not significantly different between MCNS patients and controls **(Table 2)**.

MCNS patients have a lower frequency of CD5+CD1d+CD19+ cells and lower expression of Breg cell-derived IL-10

As shown in **Figure 3**, the percentage of CD19+CD5+CD1d+ Breg cells was significantly lower in MCNS patients than in controls (2.12(1.03-4.27) vs. 3.97(1.6-7.21), *P*=0.0184). However, the percentages of CD19+ cells, and the percentages of CD19+CD5+, CD19+CD1d+ in lymphocytes and CD19+ cells were not different between the MCNS patients and HC **(Table 2)**.

The percentage of IL-10 detected in the CD19+CD5+CD1d+ subpopulation was significantly lower in the MCNS patients than in the HC (2.54(1.41-5.69) vs. 4.58(2.45-7.31), *P*=0.0074). Alternatively, the percentage of IL-10 detected in the CD19+ B cells, CD19+CD1d+ B cells, and CD19+CD5+ B cells was similar between MCNS patients and HC **(Table 2)**.

Influence of PDN treatment on B cells in MCNS patients

Nine of 23 MCNS patients in the study were followed up 8-12 weeks after initiation of PDN treatment. Treatment not only reduced the concentration of 24-h urinary proteins but also increased the level of serum albumin in these patients, although the treatment did not significantly alter the values of other measures **(Table 3)**. The percentages of CD19+CD27+, CD19+CD38+, and CD19+CD86+ cells was similar between HC and PDN, although they were significantly lower than that of MCNS patients (*P*<0.05) **(Table 4, Figure 1 and 2)**. The percentages of circulating CD19+CD27+ in CD19+ cells and lymphocytes in patients post-PDN treatment were significantly reduced in relation to pre-PDN treatment levels (*P*=0.0252, *P*=0.0328, respectively, Fig.4a-b). Similarly, the percentage of CD19+CD38+ and CD19+CD86+ in CD19+ cells and lymphocytes in patients post-PDN treatment were significantly reduced compared to levels before PDN treatment (*P*=0.0320, *P*=0.0448, *P*=0.0426, *P*=0.0310, respectively, **Figure 4C-4F**). However, the percentage of CD19+CD5+CD1d+ in CD19+ cells, and IL-10 in CD19+CD5+CD1d+ cells in individual patients became elevated following PDN treatment (*P*=0.0152, *P*=0.0224, respectively, **Figure 4G-4H**).
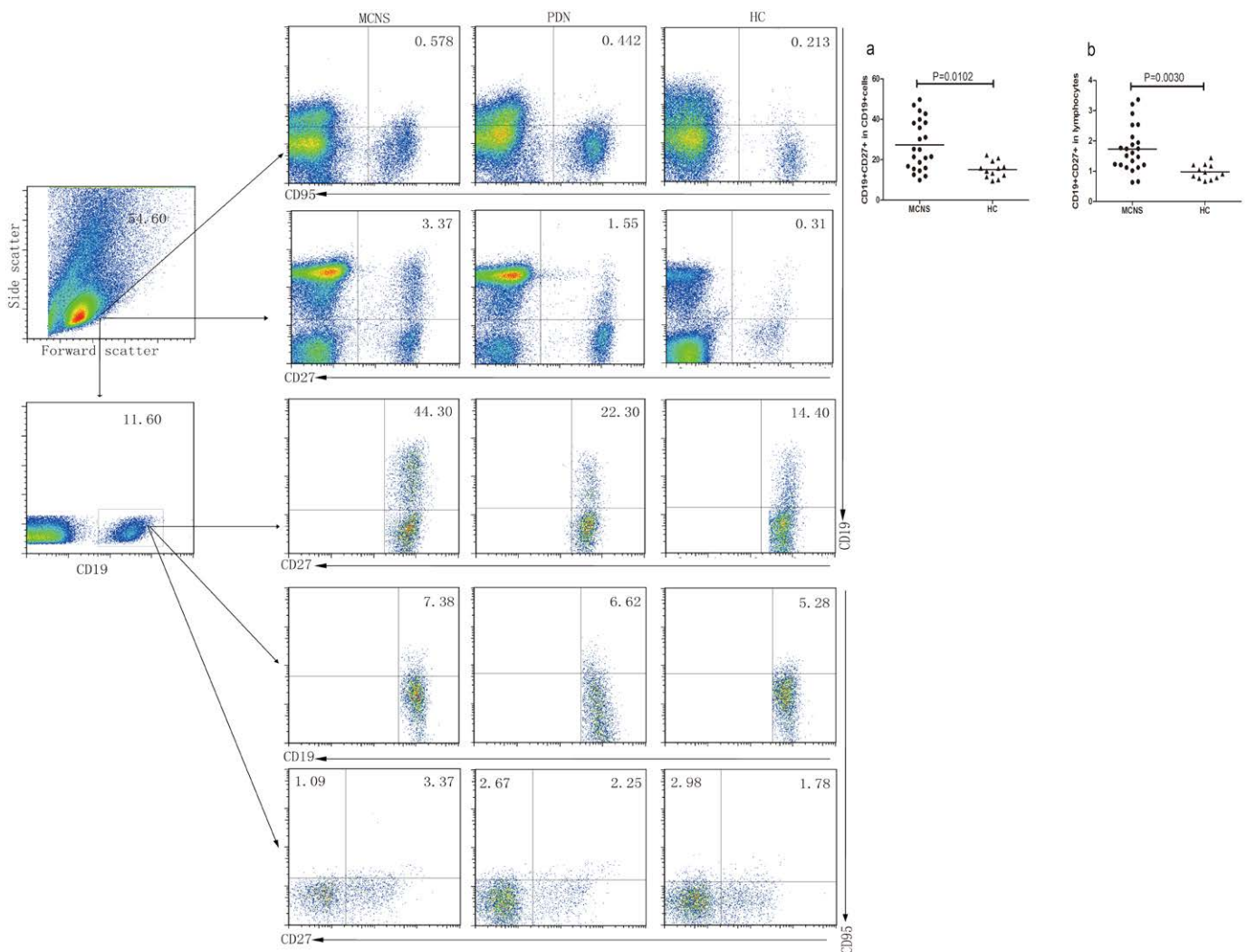
Correlation between CD19+ B cell subtypes and the IL-10+ Breg cells with clinical parameters

24-h urinary proteins were shown to be positively correlated with the percentage of CD19+CD27+ B cells and negatively correlated with the percentage of CD19+CD5+CD1d+ cells (*P*=0.0134 and

Figure 1 FACS analysis of CD19+ B cell subtypes in isolated PBMCs from MCNS patients and healthy controls. (**A**) The percentage of CD19+CD27+ in CD19+ cells. (**B**) The percentage of CD19+CD27+ in lymphocytes. Results are presented as mean percent of individual samples and representative of five independent experiments. Horizontal lines represent median values.

Table 2 The effect of PDN treatment on clinical measurements in MCNS patients.

|	Before PDN treatment	After PDN treatment
Age, year	29 (21-50)	29 (21-50)
Female/Male	4/5	4/5
Urinary proteins, g/24 h	6.02 (4.21-15.10)*	0.81 (0.20-1.26)
Serum uric acid, μmol/L	390 (265-498)	368.0 (224-412)
Triglycerides, mmol/L	2.20 (1.23-6.33)	1.62 (0.81-4.97)
Cholesterol, mmol/L	4.54 (2.12-7.98)	3.04 (1.14-5.98)
eGFR, mL/min/1.73m^2	76.91 (48.90-110.21)	82.23 (59.73-118.65)
serum albumin, g/L	21.63 (12.1-26.87)*	37.14 (31.48-43.59)
Data shown are median and range unless specified; *P<0.05 vs. values before PDN treatment.		

P=0.0393, respectively, **Figure 4A and 4C**). In addition, a positive correlation between the percentage of CD19+CD5+CD1d+ cells and the levels of serum albumin was observed in these patients (P=0.0115, **Figures 4B and Figure 5**).

Discussion

In this study, we examined distinct B cell subtype numbers in serum. Our study showed that the proportions of CD19+CD27+, CD19+CD38+, and CD19+CD86+ in peripheral blood of MCNS patients were elevated compared to normal subjects, and that all above cell numbers dramatically reduced after receiving PDN treatment. The percentage of CD19+CD5+CD1d+ Breg cells and the percentage of intracellular IL-10 were significantly lower in MCNS patients than in healthy controls. In addition, treatment with PDN for 8-12 weeks significantly reduced the frequency of CD19+CD27+, CD19+CD38+, and CD19+CD86+ B cells, and elevated the levels of Breg cells and Breg cell-derived IL-10. These findings support the hypothesis that B cell activation may contribute to the onset and/or progression of MCNS, and furthermore that the various B cell phenotypes may mediate disease pathogenesis.

Accumulating data points toward a strong contribution of B-cell immunity in patients with steroid-sensitive MCNS. Corticosteroids
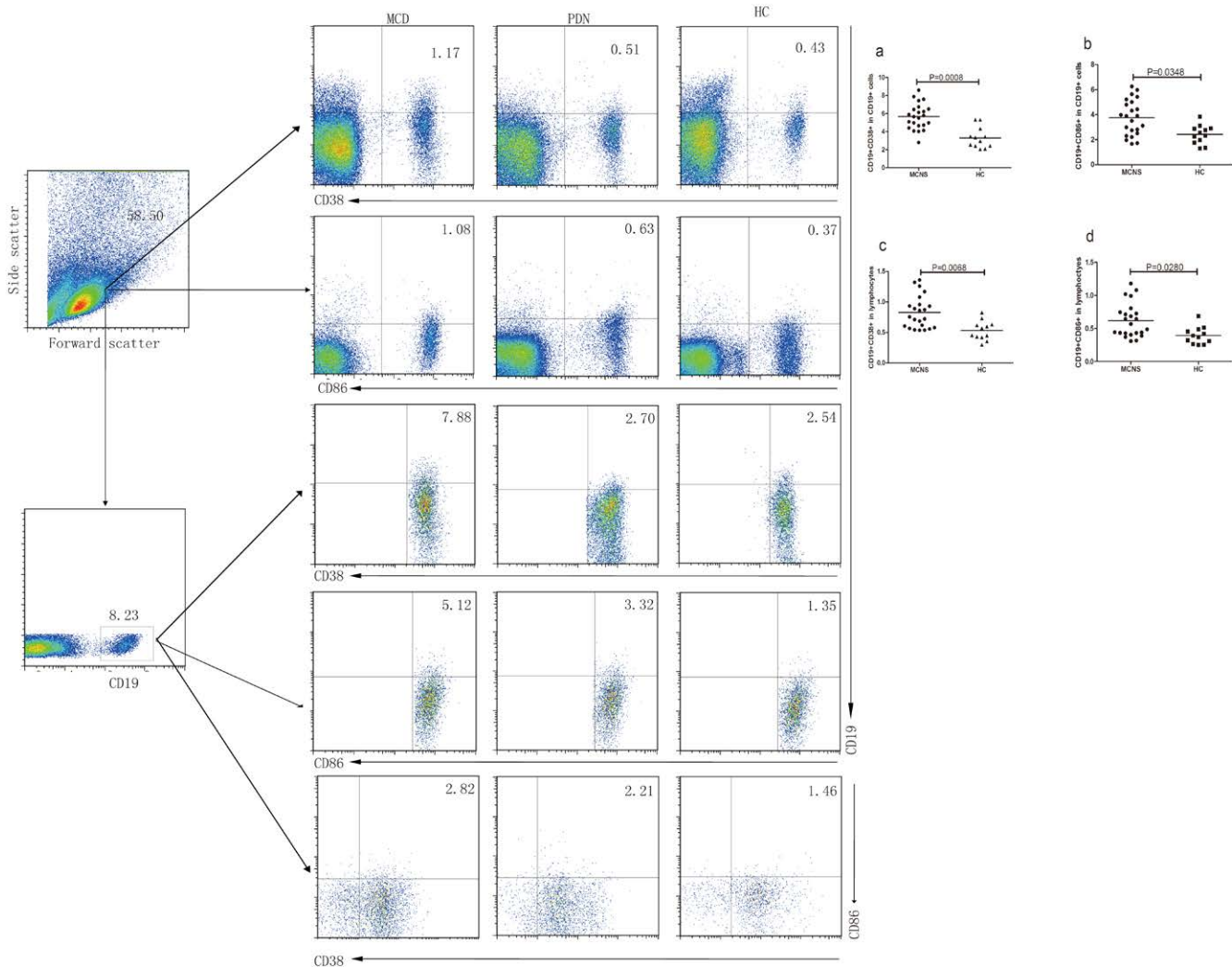
Figure 2 FACS analysis of CD19+ B cell subtypes in isolated PBMCs from MCNS patients and healthy controls represented as the percentage of: (**A**) CD19+CD38+ in CD19+ cells, (**B**) CD19+CD86+ in CD19+ cells, (**C**) CD19+CD38+ in lymphocytes, and (**D**) CD19+CD86+ in lymphocytes. Results are presented as mean percent of individual samples and representative of five independent experiments. Horizontal lines represent median values.

Table 3 Altered frequency of CD19+ B cell subtypes and IL-10+ B cells.

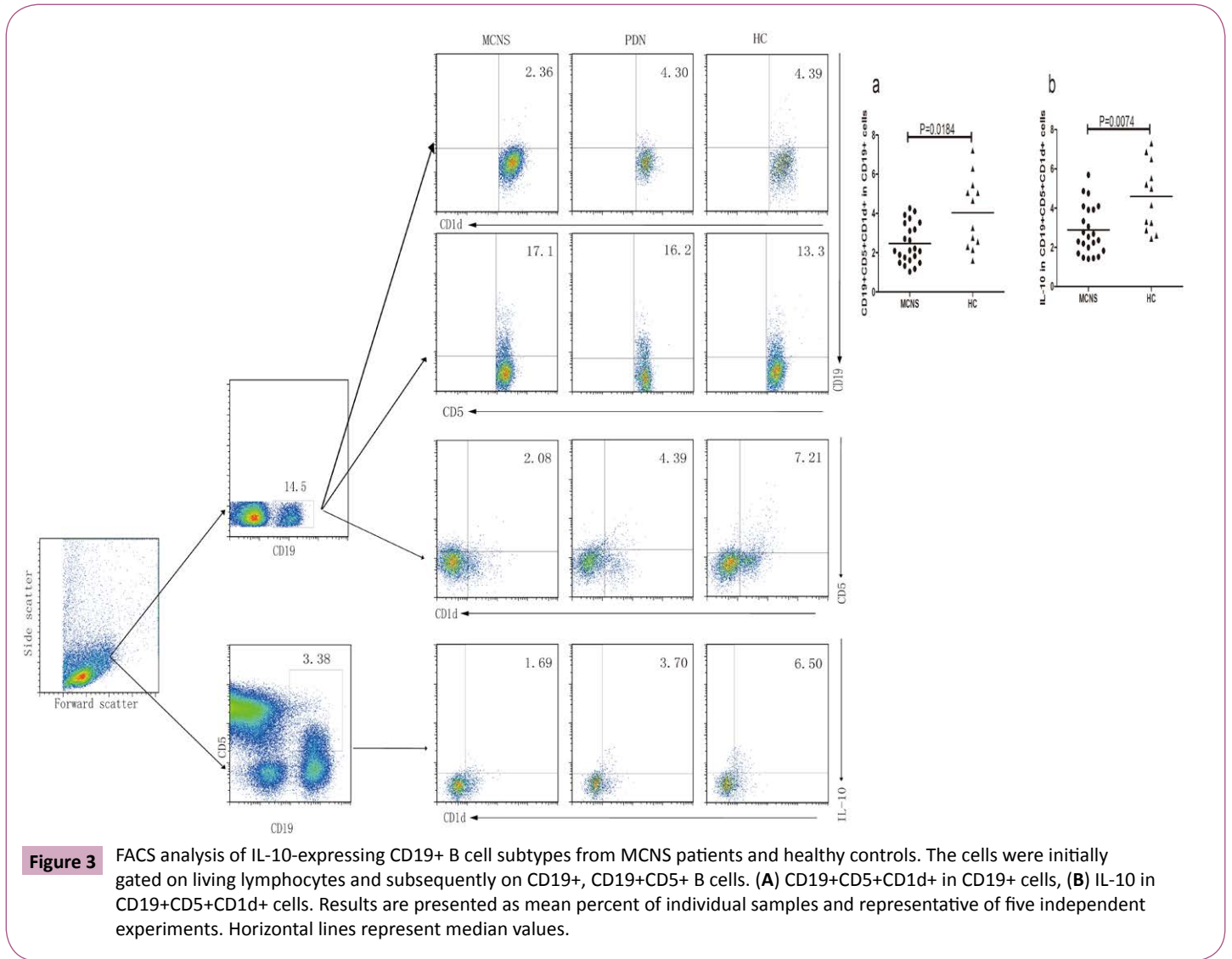| Percentage (%)	MCNS	HC	*P*
CD19+CD95+ in CD19+ B cells	7.35(3.45-13.40)	6.18(2.58-9.52)	0.1829
CD19+CD95+in lymphocytes	0.44(0.27-1.16)	0.41(0.14-0.63)	0.1417
CD19+CD95+CD27+ in CD19+B cells	2.67(1.15-4.34)	1.88(1.10-3.73)	0.1218
CD19+CD38+CD86+ in CD19+B cells	1.84(1.01-3.76)	1.62(0.95-2.82)	0.6385
CD19+CD5+in lymphocytes	1.31(0.73-3.96)	1.28(0.49-1.92)	0.4140
CD19+CD5+in CD19+cells	11(7.11-17.10)	10.6(6.35-14.00)	0.9580
CD19+CD1d+lymphocytes	0.46(0.23-0.79)	0.40(0.18-0.57)	0.1545
CD19+CD1d+in CD19+cells	2.30(1.28-5.62)	1.89(1.04-4.87)	0.2790
IL-10 in CD19+ B cells	1.26(0.51-1.98)	1.71(0.72-2.38)	0.4446
IL-10 in CD19+CD1d+ B cells	2.15(1.13-4.12)	3.29(1.66-5.38)	0.1472
IL-10 in CD19+CD5+ B cells	2.77(1.02-5.48)	3.55(1.62-6.13)	0.3040

have been widely used for the treatment of MCNS patients, and corticosteroids can inhibit inflammation by down-regulating T and B cell function and reducing cytokine production [19,20]. Our study showed that the percentage of CD19+CD27+, CD19+CD38+, CD19+CD86+B cells dramatically reduced after patients received PDN treatment.

Anolik et al. reported that rituximab improved abnormalities in B cell homeostasis with a decreased proportion of autoreactive

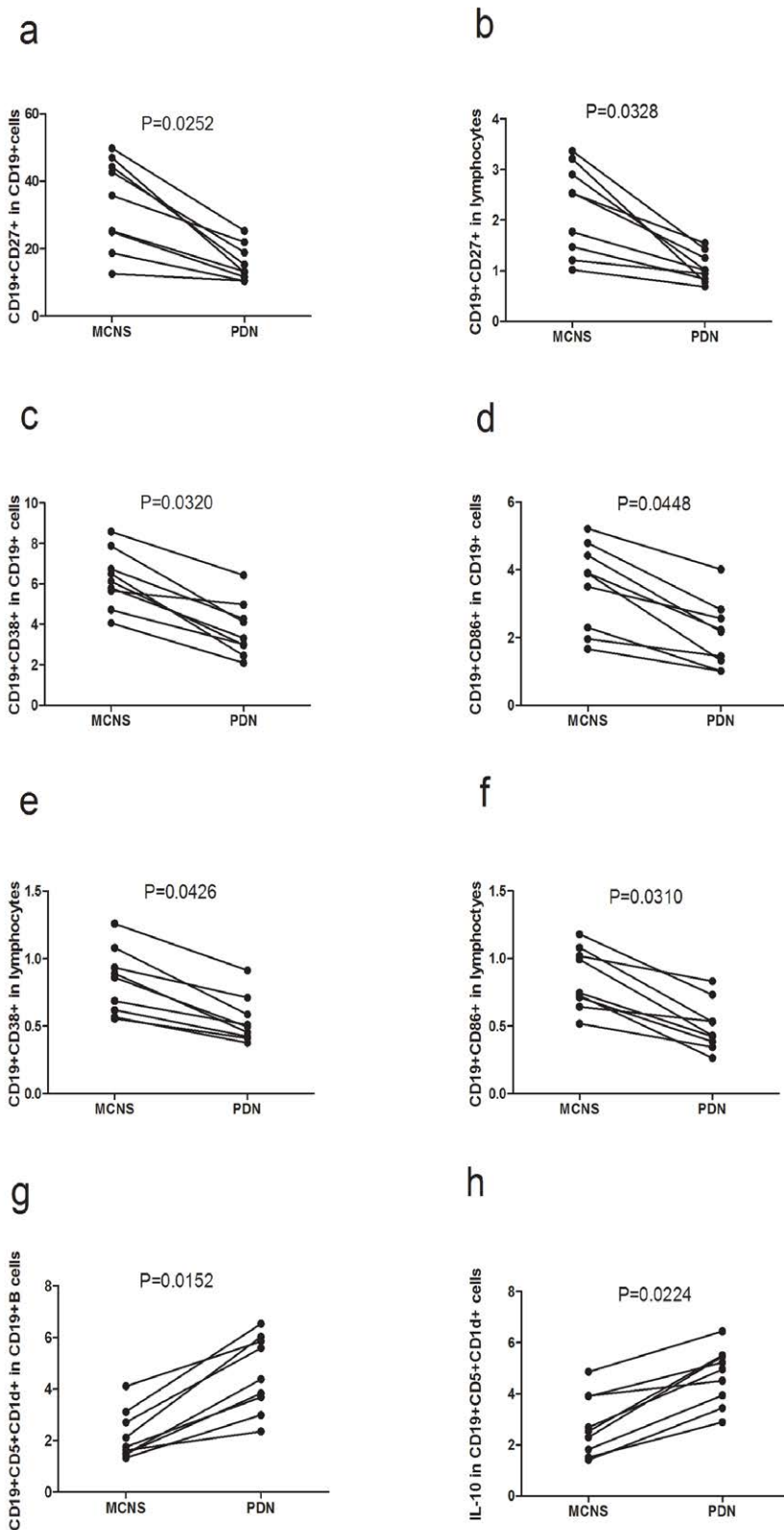**Table 4** Altered frequency of CD19+ B cell subtypes.

Percentage (%)	PDN	HC	P
CD19+CD27+ in CD19+ B cells	16.41 (10.46-25.30)	15.70(9.46-22.30)	0.8592
CD19+CD27+in lymphocytes	1.01(0.69-1.55)	0.94(0.31-1.45)	0.5940
CD19+CD38+in CD19+ B cells	3.32(2.10-6.43)	3.15(2.04-5.32)	0.4996
CD19+CD38+in lymphocytes	0.62(0.37-0.91)	0.51(0.29-0.83)	0.6374
CD19+CD86+ in CD19+ B cells	2.63(1.01-4.02)	2.45(1.31-3.84)	0.8311
CD19+CD86+in lymphocytes	0.43(0.26-0.83)	0.38(0.25-0.69)	0.2020

Figure 3 FACS analysis of IL-10-expressing CD19+ B cell subtypes from MCNS patients and healthy controls. The cells were initially gated on living lymphocytes and subsequently on CD19+, CD19+CD5+ B cells. (**A**) CD19+CD5+CD1d+ in CD19+ cells, (**B**) IL-10 in CD19+CD5+CD1d+ cells. Results are presented as mean percent of individual samples and representative of five independent experiments. Horizontal lines represent median values.

memory B cells, and reconstitution of the B cell lineage after treatment in SLE patients [21]. Even once the total B cell count returns to normal a change in phenotype appeared to occur, with the B cells present being relatively deficient in the expression of CD27, a surface marker of memory B cells [22]. A study in adult Chinese patients also found elevated serum IgE levels in relapsing patients [23] explained by the fact that serum IgE levels reflected immune dysfunction as well as B and T cell activation [24,25]. Our study reveals that MCNS patients carry a higher frequency of memory B cells in peripheral blood, and that urinary proteins are positively correlated with the frequency of CD19+CD27+ B cells in

these patients. Therefore, we speculate that CD19+CD27+ B cells may be involved in the pathogenesis of MCNS.

Extensive studies in mice have demonstrated that Breg cells play important roles in the suppression of experimentally-induced autoimmune diseases, including encephalomyelitis, inflammatory bowel disease, type 1 diabetes, SLE, and collagen-induced arthritis [12,13,16,26-29]. Liang et al. reported that the numbers of CD19+TIM1+IL10+ and CD19+CD5+CD1d+IL10+ B cells were positively correlated with the numbers of CD4+CD25+Foxp3+ T cells in RA patients [30]. A previous study has suggested that the MCNS patients exhibit a significant increase in Th17 number,

Figure 4 Altered frequency of CD19+ B cell subtypes and IL-10+ B cells in MCNS patients before and after PDN treatment. **(A-B)** The percentages of CD19+CD27+ in CD19+ cells and lymphocytes in individual patients before and after PDN treatment. **(C-F)** The percentage of CD19+CD38+, CD19+CD86+ in CD19+ cells and lymphocytes in individual patients before and after PDN treatment. **(G,H)** The percentage of CD19+CD5+CD1d+ in CD19+ cells, IL-10 in CD19+CD5+CD1d+ cells in individual patients before and after PDN treatment.
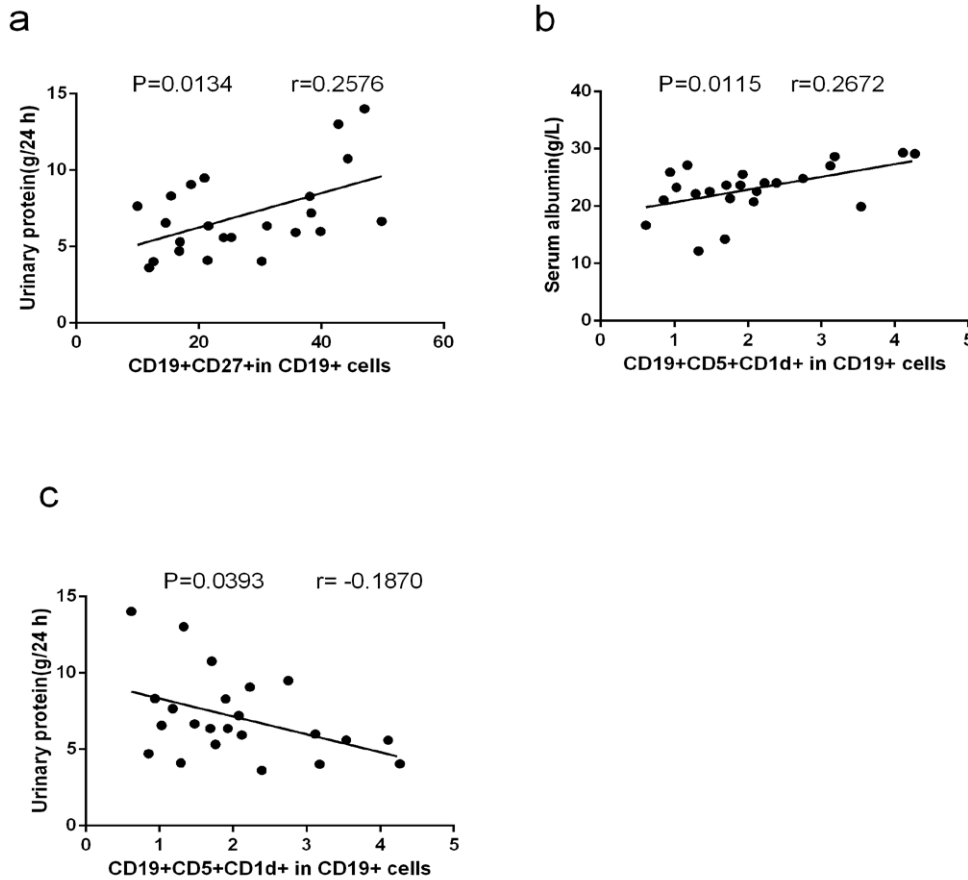
Figure 5 Correlation analysis of clinic pathological features of MCNS with CD19+ B cell subtypes and IL-10+ B cells. (**A**) The values of urinary protein are positively correlated with the percentages of CD19+CD27+ in CD19+ cells. (**B**) The level of serum albumin are positively correlated with the percentage of CD19+CD5+CD1d+ in CD19+ cells. (**C**) The concentrations of 24-h urinary proteins are negatively correlated with the percentage of CD19+CD5+CD1d+ cells.

Th17-related cytokines (IL-17 and IL-23) and transcription factor (RORγt) levels, in addition to an obvious decrease in Treg number, Treg-related cytokines (TGF-β1 and IL-10) and transcription factor (Foxp3) levels [31]. In our current study we showed that MCNS patients have a lower frequency of CD5+CD1d+CD19+ Breg cells. These finding support the notion that Breg cells may promote the activation of Treg cells in MCNS patients.

Breg cells are characterized by the CD1d^{hi}CD5+CD19^{hi} phenotype and the production of regulatory cytokines IL-10 and TGF-β [11,12], which could influence T cell activation and inflammatory responses through IL-10 activity [32]. Our MCNS patient cohort showed significantly down-regulated Breg-derived IL-10, which was in accordance with these previous results. In addition, we found that treatment with PDN for 8-12 weeks not only significantly increased the frequency of circulating Breg cells but also elevated the levels of Breg cell-derived IL-10. Daien et al. demonstrated that the ability of B cells to produce IL-10 was altered in rheumatoid arthritis (RA), an impairment which influenced disease activity, biologic inflammation, and autoantibody levels, strongly suggesting a role of B10 cells in RA initiation [33]. Fillatreau et al. previously reported that B cell-deficient mice developed EAE, which did not enter remission after immunization with myelin oligodendrocyte glycoprotein (MOG). However, CD40-stimulated B cells expression of IL-10 from the control group was able to relieve the EAE [13]. Hussai et al. reported that transfusion of BCR-stimulated IL-10-producing B cells protects NOD mice from type 1 diabetes in part by attenuating the severity of islet inflammation, suggested that this mechanism may play a greater role in protecting the animal from type 1 diabetes [27]. The human study presented here demonstrates Breg cell participation in MCNS pathogenesis, and suggests a potential mechanism related to IL-10 activity.

Conclusion

Our data indicates higher frequencies of CD19+CD27+, CD19+CD38+, and CD19+CD86+ B cells, but lower frequencies of Breg cells along with decreased expression of Breg cell-derived IL-10 in peripheral blood of MCNS patients. In addition, PDN treatment significantly reduced the frequency of CD19+CD27+, CD19+CD38+, CD19+CD86+ B cells, and elevated the levels of Breg cells and Breg cell-derived IL-10. Furthermore, 24-h urinary proteins was positively correlated with the percentage of CD19+CD27+ B cells and negatively correlated with the percentage of Breg cells, while a positive correlation between the percentage

of CD19+CD5+CD1d+ cells and the levels of serum albumin was observed in these patients. Collectively, these findings suggest that B cells may participate in the pathogenic process of MCNS. Despite limitations of small sample size, IL-10-specific testing, one time measurements and a lack of longitudinal follow-up, our findings provide valuable new insights into understanding the pathogenesis of MCNS which may aid in developing new measures for prognosis. Future studies will further investigate the role of Breg cells in the pathogenic process of MCNS and the potential prognostic values.

Authors' contribution

Conceived and designed the experiments: Yanfang Jiang and Jinpeng Qiu

Performed the experiments: Li Zhang and Songchen Zhao
Analyzed the data: Shengguo Zhou and Mingyuan Liu
Contributed reagents/materials/analysis tools: Li Zhang, Rebecca Crew and YanfangJiang
manuscript written by : Li Zhang and Yanfang Jiang

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