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M2a and M2b macrophages predominate in kidney tissues and M2 subpopulations were associated with the severity of disease of IgAN patients

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ABSTRACT

M2 macrophages play important roles during the injury and repair phases in kidney. Our aims are to investigate the distribution of M2 subpopulations and the correlation with clinicopathological features of IgA nephropathy (IgAN) patients. In this study, renal samples from 49 IgAN patients were detected by immunofluorescence. The markers of M2 macrophages, including M2a (CD206+/CD68+), M2b (CD86+/CD68+) and M2c (CD163+/CD68+) were identified. We found M2a and M2b macrophages were the predominant subpopulations in kidney tissues of IgAN. M2a macrophages were mainly distributed in tubulointerstitium with renal lesions like segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis. However, there were larger numbers of M2c in glomeruli with minor lesions. Moreover, M2a and M2c macrophages were inversely correlated with the clinical and pathologic features, respectively. These results suggest M2 subpopulations were involved in the progression of IgAN, and M2a and M2c macrophages might show different properties to participate in the pathogenesis of IgAN.

1. Introduction

IgA nephropathy (IgAN) is the most common glomerulonephritis worldwide, which is associated with predominantly IgA deposition in the mesangium of the glomeruli [1]. Overall, 30%–40% of the patients develop ESRD within 20–30 years after diagnosis [2] However, the mechanisms underlying IgAN remains unknown. Some studies showed that inflammation participated in the pathogenesis of IgAN [3]. Among various inflammatory cells, macrophages were one of the most important population and play a key role in mediating the glomerular and interstitial injuries in IgAN [4,5].

Macrophages can be broadly divided into two types: the classically activated macrophages (M1), representing a pro-inflammatory type and are produced by exposure to IFN-γ or LPS; and the alternatively activated macrophages (M2), which are associated with a down-regulation of inflammation, elimination of tissue debris and apoptotic bodies, tissue remodeling, as well as fibrosis [6–8]. Recent studies show that M2 macrophages can be further subdivided into at least three subgroups. M2a (wound-healing macrophages) induced by IL-4 and/or IL-13 play an important role in repair and progression of fibrosis and are characterized by cell surface expression of receptors CD206 [8]. M2b macrophages induced by immune complexes with LPS or IL-1β regulate immune response and are characterized by cell surface expression of receptors CD86 [9]. M2c (regulatory macrophages) induced by IL-10, TGF-β, or glucocorticoids exert anti-inflammatory and pro-fibrotic functions and are characterized by cell surface expression of receptors CD163 [10].

Some studies showed that the infiltration of M2 macrophages was correlated with worse renal functions and renal outcomes in IgAN. Li et al. [11] found that M2 macrophages were involved in the pathogenesis of acute renal injury of IgAN with crescents. Another study showed that M2 macrophages are present in new-onset IgAN and mesangial matrix expansion correlated with M2 macrophages [12]. On the other hand, Gutiérrez et al.[13]found that CD163-positive macrophages was negatively correlated with renal function outcome at the end of the follow-up period. However , there are few studies about the renal polarization of M2 subtypes in patients with IgAN. In this study, we analyzed the polarization of different M2 macrophage subpopulations in IgAN and their relationship with clinical and pathological parameters.

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2. Materials and methods

2.1. Patients

Frozen renal samples from 49 patients aged ≥18 years with Immunoglobulin A nephropathy (IgAN) were collected by biopsy in Guangdong Provincial People's Hospital from January 2018 to June 2018. Patients with coexistence glomerular diseases, such as membranous nephropathy, lupus nephritis and diabetic nephropathy, were excluded. The clinical data were collected, including sex, age, proteinuria, serum creatinine (SCr), blood urea nitrogen (BUN), serum albumin (ALB), hypertension and diabetes at the time of biopsy. The study involving human participants was approved by the Ethical Committee of Guangdong Provincial People's Hospital. Written informed consent was obtained from the patients before the enrollment.

According to oxford classification of IgAN [14], M0/M1 was defined as ≤/ > 50% of glomeruli showing mesangial hypercellularity, E0/E1 was defined as absence/presence of endocapillary hypercellularity, S0/S1 was defined as absence/presence of segmental glomerulosclerosis, T0/T1 was defined as tubular atrophy/interstitial fibrosis ≤/ > 25%, and C0/C1 was defined as absence/presence of crescent lesions. We also used G0/G1 to describe those with global glomerulosclerosis < / ≥50%.

2.2. Immunofluorescence and double-staining

Three-micrometer paraffin sections were stained using hematoxylin and eosin, periodic acid–Schiff, and periodic acid methenamine silver-Masson trichrome methods. We tabulated detailed glomerular histologic features from the index biopsies.

In this study, CD68 positive cell was identified as total macrophage. we doubly stained CD206 and CD68, CD86 and CD68, CD163 and CD68 to describe M2a, M2b and M2c like macrophage subtype, respectively. Thin sections (3 μm) of the renal biopsies were fixed with 4% paraformaldehyde (15 min), permeabilized with 0.5% Triton-X 100 (10 min) and blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature. Samples were then incubated with the following primary antibodies diluted in 5% BSA at 4 °C over-night: mouse anti-CD68 monoclonal antibody (1:100, ab955), rabbit anti-CD86 monoclonal antibody (1:200, ab53004), rabbit anti-CD206 polyclonal antibody (1:200, ab64693), and rabbit anti-CD163 polyclonal antibody (1:200, ab87099). The secondary antibody green was Alexa Fluor 488 (1:500, catalog #A-21206, Invitrogen), red was Alexa Fluor 555 (1:500, 4409S, CST) for 1 h. DAPI was used to stain the cell nuclei. The sections were observed under a fluorescence microscope (Nikon 80i; Nikon, Tokyo, Japan).

2.3. Qualitative evaluation of macrophages

CD206+/CD68+, CD86+/CD68+ and CD163+/CD68+ double-positive cells were counted in 6 complete glomerulies and 6 high-power fields of tubulointerstitium in each tissue using a fluorescence microscope at × 40 magnification. Glomerular infiltration was expressed as the number of positive cells per glomerulus. Tubulointerstitial infiltrate is expressed as number of positive cells per high power field (HPF). The results were expressed as the mean ± SD.

2.4. Statistical analysis

Statistical analyses were performed using SPSS (version 20.0; SPSS Inc., Chicago, IL, USA), GraphPad Prism (version 5.0; GraphPad Software, Inc., La Jolla, CA, USA). Fisher's exact test or the χ^2 test was used to compare qualitative data, and the Wilcoxon rank-sum test was used for continuous variables (with calculation of means, standard deviations, and ranges). The Spearman's test was used for the correlation between macrophage subpopulations with clinical and pathologic data. P value < .05 was regarded as significant.

Table 1
Patients' characteristics.

Characteristics	Total, n or mean ± SD
Number of patients	49
Gender	
Male	22
Female	27
Age	38.76 ± 12.89
Hypertensive patients, %	55.10%
Blood pressure (BP)	
Systolic BP, mmHg	131.88 ± 19.22
Diastolic BP, mmHg	81.88 ± 12.70
Diabetic patients, %	2.04%
BUN (mmol/L)	8.96 ± 3.79
SCr (umol/L)	166.19 ± 88.06
eGFR $_{CKD-EPI}$ (ml/min/1.73 m ^{2})	51.91 ± 29.83
Albumin (g/L)	37.62 ± 5.67
Proteinuira (mg/day)	1871.97 ± 1865.54
Therapy	
ACEI/ ARB alone, %	46.94%
Steroid alone, %	8.16%
ACEI/ ARB + steroid, %	12.24%
Steroid + Immunosuppressants, %	8.16%
ACEI/ ARB + steroid + Immunosuppressants, %	14.29%

BUN, blood urea nitrogen; SCr, serum creatinine; eGFR, estimating glomerular filter rate; ACEI/ ARB, angiostensin-converting enzyme inhibitors/ angiostensin receptor blocker.

3. Results

3.1. Characteristics of IgAN patients at the time of renal biopsy

A total of 49 patients were recruited, including 22 (44.89%) males and 27 (55.10%) females. The mean age was 38.76 years ranging from 18 to 65 years. 27 (55.10%) cases were with hypertension, while only one patient (2.04%) was with diabetes. The average level of BUN, SCr, ALB, eGFR and proteinuria were 8.96 ± 3.79 mmol/L, 166.19 ± 88.06 μmol/L, 37.62 ± 5.67 g/L, 51.91 ± 29.83 mL/min/1.73m^2 and 1871.97 ± 1865.54 mg/day, respectively. Treatment data of IgAN after diagnosis were also showed in Table 1.

3.2. Subpopulations of M2 macrophages in glomeruli and tubulointerstitium

Subpopulations of M2 macrophages were identified by immunofluorescence double staining combining the pan-macrophage marker CD68 with CD206 or CD86 or CD163 (Fig. 1A–F). The numbers of glomerular macrophages, M2a, M2b and M2c macrophages were 12.84 ± 7.94, 7.24 ± 4.39, 10.72 ± 6.58 and 3.50 ± 1.98, respectively. In the tubulointerstitium, the numbers of macrophages, M2a, M2b and M2c macrophages were 26.13 ± 17.68, 14.47 ± 8.60, 15.14 ± 12.24 and 6.02 ± 3.68, respectively (Table 2). Regardless of glomerulus or tubulointerstitium, the numbers of M2a and M2b preponderate that of M2c (P < .001).

3.3. The correlation between different M2 macrophage subpopulations with Oxford classification and degree of global glomerulosclerosis

We analyzed macrophages and M2 subpopulations with Oxford classification and global glomerulosclerosis, and found that the numbers of glomerular CD68 macrophages were higher in patients with M1, S1 and C1 (M1 vs M0: 14.73 ± 8.01 vs 9.72 ± 6.97, P = .028; S1 vs S0: 15.83 ± 9.45 vs 9.72 ± 4.33, P = .027; C1 vs C0: 15.67 ± 8.06 vs 9.88 ± 6.79, P = .01; respectively, Fig. 2A, B and D). In the tubulointerstitium, CD68 macrophages counts were larger in S1 and T1 than that of S0 and T0 (S1 vs S0: 31.49 ± 18.88 vs 20.07 ± 14.31, P = .009; T1 vs T0: 33.56 ± 20.05 vs 17.74 ± 9.31, P = .001;

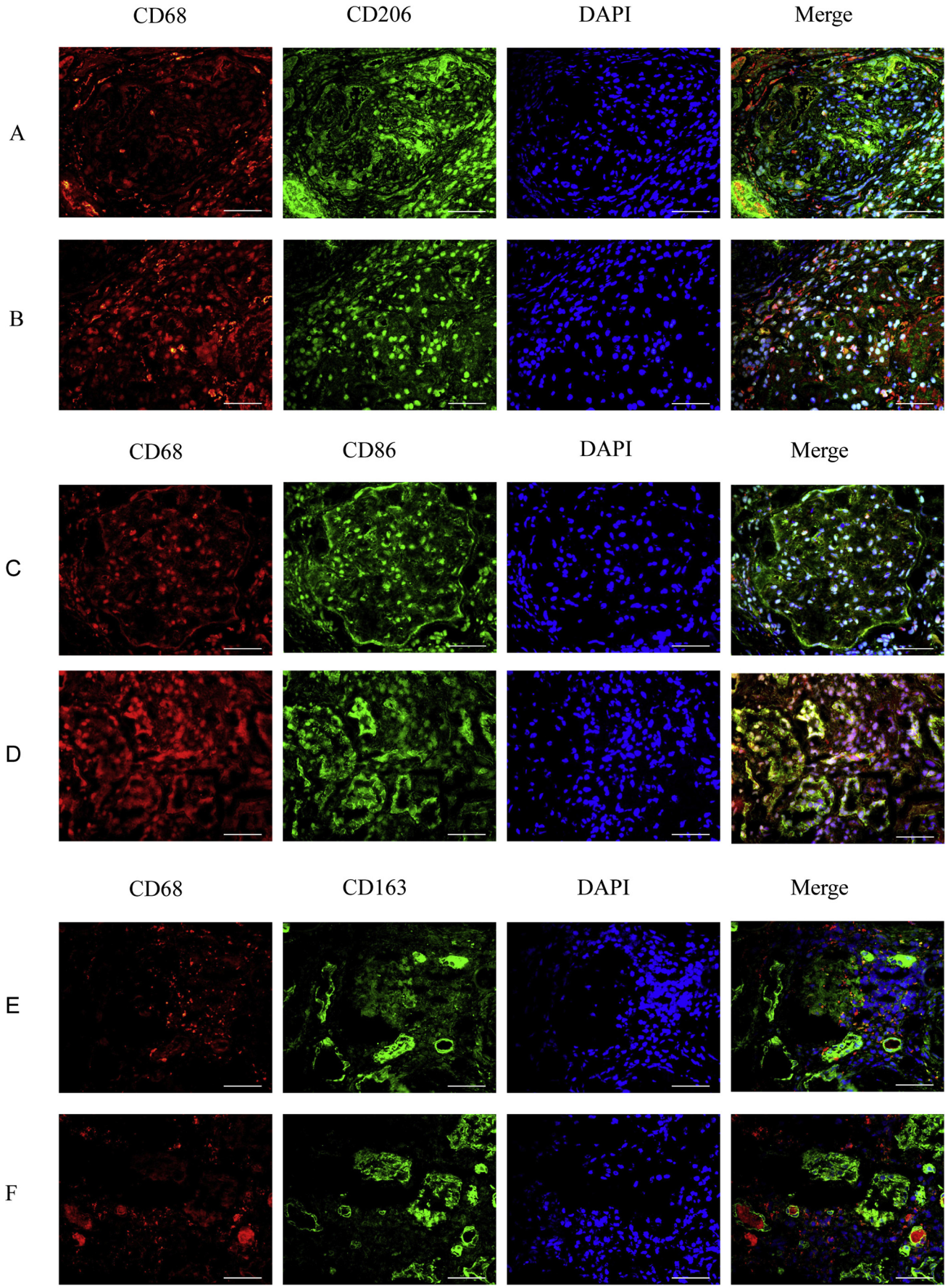

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Fig. 1. Subpopulations of M2 macrophages in Glomeruli and tubulointerstitium of IgAN patients. A-B. M2a macrophages were double-positive for CD68 (red) and CD206 (green) in glomeruli (A) and tubulointerstitium (B) by immunofluorescence. C-D. M2b macrophages were double-positive for CD68 (red) and CD86 (green) in glomeruli (C) and tubulointerstitium (D). *E*-F. M2c macrophages were double-positive for CD68 (red) and CD163 (green) in glomeruli (E) and tubulointerstitium (F) (original magnification in high power field (HPF)). DAPI was used to stain the cell nucles. Scale bars, 50um. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
The pathological data.

Variables	Total, n or mean ± SD
MESCT	
M0	19
M1	30
E0	48
E1	1
S0	23
S1	26
C0	23
C1	26
T0	22
T1	27
G0 (glomerular sclerosis)	32
G1 (glomerular sclerosis)	17
Lee grade	
II	5
III	18
IV	6
V	13
CD68/glomeruli	12.84 ± 7.94
CD68/tubulointerstitium	26.13 ± 17.68
CD206/glomeruli	7.24 ± 4.39
CD206/tubulointerstitium	14.47 ± 8.60
CD86/glomeruli	10.72 ± 6.58
CD86/tubulointerstitium	15.14 ± 12.24
CD163/glomeruli	3.50 ± 1.98
CD163/tubulointerstitium	6.02 ± 3.68

G0, glomerular sclerosis < 50%, G1, glomerular sclerosis ≥ 50%; SD, Standard deviation.

respectively, Fig. 2B and C).

The numbers of M2a macrophages in the tubulointerstitium with M1, S1 and T1 were larger than those of M0 (M1 vs. M0: 15.86 ± 9.03 vs. 10.33 ± 7.11, $P = .053$), S0 (S1 vs. S0: 17.17 ± 9.29 vs. 9.97 ± 6.33, $P = .009$) and T0 (T1 vs. T0: 16.70 ± 9.85 vs. 10.06 ± 5.48, $P = .031$), respectively (Fig. 2A, B and C). There were no differences of numbers of tubulointerstitial M2a macrophages between C1 and C0, G1 and G0. E0 and E1 were excluded because only one patient had endocapillary hypercellularity.

M2b macrophages had an increase trend in glomeruli of G1 (G0 vs. G1: 9.48 ± 8.14 vs. 12.78 ± 1.62, Fig. 2E), but the difference was not significant ($P = .073$). There were no significant differences in glomerular and tubulointerstitial M2b macrophages between M0 and M1, S0 and S1, T0 and T1, C0 and C1, respectively (Fig. 2A, B, C and D, $P > .05$).

There were less M2c macrophages in glomeruli with T1 and G1 (T0 vs. T1: 4.07 ± 1.94 vs. 2.37 ± 1.53, $P = .008$; G0 vs. G1: 4.03 ± 1.96 vs. 2.00 ± 1.17, $P = .02$, respectively, Fig. 2C, E). There were no differences in numbers of glomerular M2c cell between M1 and M0, S1 and S0, C1 and C0. The differences of M2c macrophages in the tubulointerstitium of patients with different classification were not significant ($P > .05$).

3.4. The correlation between different M2 macrophage populations with Lee grade

The numbers of CD68 macrophages in tubulointerstitium were higher in patients with Lee grade IV-V than that of Lee grade II-III (33.82 ± 18.67 vs 20.66 ± 14.49, $P = .003$, Fig. 2F). Tubulointerstitial M2a macrophages were mainly distributed in Lee grade IV-V compared with Lee grade II-III (18.77 ± 9.89 vs. 10.89 ± 6.54, $P = .016$, Fig. 2F), which was not significant in glomeruli between Lee grade II-III and IV-V (6.82 ± 3.87 vs. 8.65 ± 5.58, $P = .303$). On the other hand, compared with patients with Lee grade II-III, there were less glomerular M2c in Lee grade IV-V (4.07 ± 1.88 vs. 2.55 ± 1.84, $P = .048$, Fig. 2F). The differences of M2c macrophages in glomeruli was not significant between Lee grade II-III and IV-V (5.90 ± 4.31 vs. 5.75 ± 2.98, $P = .767$). But there were no significant differences in glomerular and tubulointerstitial M2b between Lee gradeII-III and IV-V ($P > .05$).

3.5. The association of pathological grades and clinical features

Furthermore, the clinical data were noted in Fig. 3, and the differences of SCr levels were significant between T0 and T1 (115.14 ± 47.36 vs. 238.60 ± 167.70 μmol/L, $P < 0.001$), G0 and G1 (137.69 ± 68.64 vs 219.85 ± 97.20 μmol/L, $P = .001$), Lee grade II-III and IV-V (116.36 ± 46.77 vs 212.85 ± 95.63 μmol/L, $P < 0.001$), respectively (Fig. 3A). Moreover, proteinuria was worse in patients with T1, G1 and Lee grade IV-V than that of T0, G0 and Lee grade II-III (T1 vs. T0: 1342.89 ± 2074.62 vs 2499.94 ± 1877.17 mg/d, $P = .002$; G1 vs. G0: 1482.97 ± 1878.71 vs. 2604.19 ± 1652.25 mg/d, $P = .01$; Lee grade IV-V vs. Lee grade II-III: 1202.97 ± 1634.83 vs. 2770.55 ± 1910.64 mg/d, $P < 0.001$, respectively, Fig. 3C). Lastly, patients in Lee grade IV-V had lower serum albumin than that of Lee grade II-III (38.83 ± 6.87 vs. 36.48 ± 4.78 g/L, $P = .03$, Fig. 3B).

3.6. The correlations of pathological and clinical data with M2 macrophage subpopulations

The counts of tubulointerstitial macrophages showed positive correlations with glomerular sclerosis ($r = 0.283$, $P = .049$) and interstitial fibrosis ($r = 0.400$, $P = .004$). Furthermore, tubulointerstitial M2a counts exhibited positive correlations with serum creatinine ($r = 0.363$, $P = .028$), proteinuria ($r = 0.329$, $P = .047$), percent of focal sclerosis ($r = 0.457$, $P = .004$) and interstitial fibrosis ($r = 0.327$, $P = .048$), respectively. In contrast, either the percent of glomerular sclerosis ($r = -0.512$, $P = .006$) or interstitial fibrosis ($r = -0.445$, $P = .002$) showed significant inverse correlations with glomerular M2c counts. We did not find any significant correlations between M2b and any clinical or pathologic characters, including SCr, ALB, proteinuria, percentage of glomerular sclerosis, percentage of focal sclerosis, percentage of glomeruli with crescents and interstitial fibrosis (Table 3).

4. Discussion

In this study, we identified macrophage and M2 macrophage subpopulations (including M2a, M2b and M2c macrophages) in patients with IgAN. We found that infiltration of M2 macrophage subpopulations differed in the kidney tissues and was dominated by M2a and M2b macrophages. Moreover, M2a macrophages were mainly distributed in tubulointerstitium with M1, S1, T1 and Lee Grade IV-V. While there were larger numbers of M2c macrophages in glomeruli of T0, G0 and Lee grade II-III. In addition, M2a macrophages were positively correlated with renal function, focal sclerosis and interstitial fibrosis. However, M2c macrophages were negatively correlated with the severity of glomerular sclerosis and interstitial fibrosis. The differences between M2b macrophages and clinical or pathologic features were not significant.

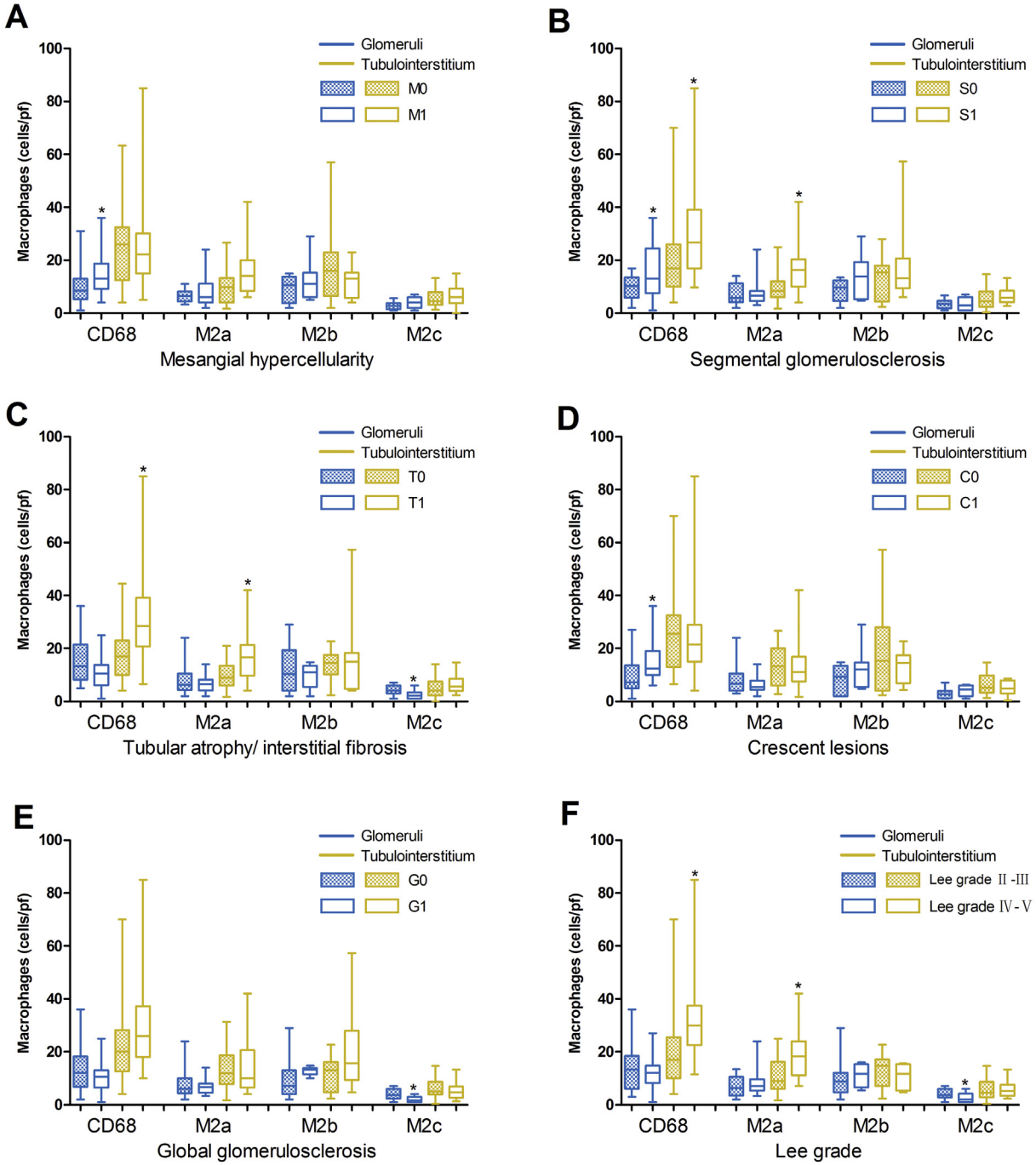

Fig. 2. The correlation between different M2 macrophage subpopulations with Oxford classification, degree of global glomerulosclerosis and Lee grade. Quantitative analysis of CD68 and different M2 macrophages in glomeruli and tubulointerstitium in M0 (mesangial hypercellularity ≤50%) and M1 (mesangial hypercellularity > 50%) (A), S0 (absent of segmental glomerulosclerosis) and S1 (present of segmental glomerulosclerosis) (B), T0 (tubular atrophy/ interstitial fibrosis ≤25%) and T1 (tubular atrophy/ interstitial fibrosis > 25%) (C), C0 (absent of crescent lesions) and C1 (present of crescent lesions) (D), G0 (global glomerulosclerosis < 50%) and G1 (global glomerulosclerosis ≥50%) (E), Lee grade II-III and Lee grade IV-V (F). Significant correlation $^*p < .05$.

Macrophages are one of main cell population mediated inflammation, play an important role in innate and adaptive immune response [15]. Soares et al. [16] analyzed the correlation between macrophages (CD68 positive cells) and the criteria of the Oxford Classification. They reported that a strong correlation between macrophage count and the percentage of glomeruli showing E and tubulointerstitial macrophages correlated moderately with percentage of T and GFR at the time of biopsy. ROC curve analysis demonstrated that a maximum glomerular macrophage count of 6 is the best cut-off for distinguishing E0 from E1. While there was no correlation between macrophages and percentage of M, S, C and T. However, our results showed that tubulointerstitial macrophages were significantly positively correlated with percentage of glomerular sclerosis and tendent to be correlated with percentage of focal sclerosis. There were no data about the correlation of macrophages and E score in our study because of low proportion of patients with E1. The results above indicated that renal macrophages were partially associated the severity of IgAN. Further studies and larger samples are needed to understand the roles and mechanism of macrophages in patients with IgAN.

M2 macrophages comprise a heterogeneous population of cells, with

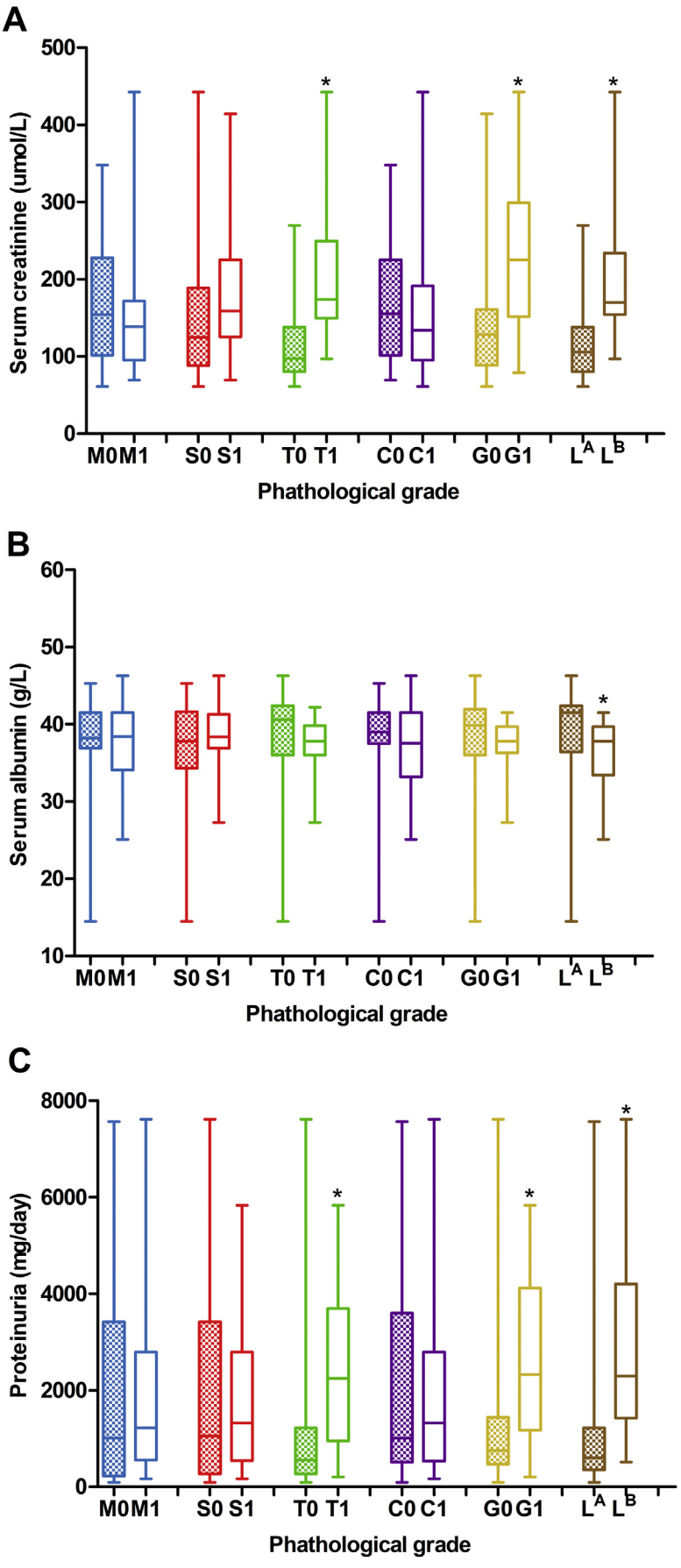

Fig. 3. The association of pathological grades and clinical features. Compare serum creatinine (A), serum albumin (B) and proteinuria (C) between M0 (mesangial hypercellularity ≤50%) and M1 (mesangial hypercellularity > 50%), S0 (absent of segmental glomerulosclerosis) and S1 (present of segmental glomerulosclerosis), T0 (tubular atrophy/ interstitial fibrosis ≤25%) and T1 (tubular atrophy/ interstitial fibrosis > 25%), C0 (absent of crescent lesions) and C1 (present of crescent lesions), G0 (global glomerulosclerosis < 50%) and G1 (global glomerulosclerosis ≥50%), Lee grade II-III (noted as L^A) and Lee grade IV-V (noted as L^B). Significant correlation *p < .05.

Table 3
Correlation between macrophage subpopulations with clinical and pathologic data.

	CD68/ glomeruli	CD68/tubulo-interstitium	M2a/ glomeruli	M2a/tubulo-interstitium	M2b/ glomeruli	M2b/tubulo-interstitium	M2c/ glomeruli	M2c/tubulo-interstitium
SCr (umol/L)	$r = -0.046$	$r = 0.227$	$r = -0.106$	r = 0.363	$r = 0.313$	$r = 0.225$	$r = 0.347$	$r = 0.102$
	$p = .766$	$p = .117$	$p = .551$	p = .028*	$p = .237$	$p = .355$	$p = .077$	$p = .592$
Albumin (g/L)	$r = 0.055$	$r = -0.200$	$r = -0.042$	$r = -0.154$	$r = 0.175$	$r = 0.061$	$r = -0.003$	$r = 0.156$
	$p = .718$	$p = .169$	$p = .814$	$p = .363$	$p = .518$	$p = .803$	$p = .987$	$p = .410$
Proteinuira (mg/ day)	$r = -0.098$	$r = 0.267$	$r = -0.002$	r = 0.329	$r = -0.254$	r = −0.09	$r = -0.116$	$r = -0.075$
	$p = .521$	$p = .064$	$p = .992$	p = .047*	$p = .342$	$p = .713$	$p = .564$	$p = .695$
Glomerular sclerosis (%)	r = −0.154	r = 0.283	$r = 0.052$	$r = 0.095$	$r = 0.103$	$r = 0.278$	r = −0.512	$r = -0.001$
	$p = .311$	p = .049*	$p = .770$	$p = .577$	$p = .705$	$p = .249$	p = .006*	$p = .994$
Focal sclerosis (%)	$r = 0.025$	$r = 0.281$	$r = 0.128$	r = 0.457	$r = -0.144$	$r = 0.024$	$r = -0.028$	r = −0.001
	$p = .868$	$p = .051$	$p = .470$	p = .004*	$p = .593$	$p = .922$	$p = .891$	$p = .996$
Crescents (%)	$r = 0.162$	$r = 0.006$	$r = -0.031$	$r = 0.000$	r = 0.055	$r = -0.279$	$r = 0.206$	$r = -0.194$
	$p = .287$	$p = .967$	$p = .861$	$p = .998$	$p = .841$	$p = .248$	p = .303	$p = .305$
Interstitial fibrosis (%)	$r = -0.118$	r = 0.400	$r = -0.045$	r = 0.327	$r = 0.181$	$r = 0.150$	r = −0.445	$r = 0.097$
	$p = .441$	p = .004*	$p = .799$	p = .048*	$p = .503$	$p = .540$	p = .002*	$p = .661$

Significant correlation *$p < .05$.

diverse functions and phenotypic plasticity. Of which M2a macrophages are important in repair and progression of fibrosis, M2b can regulate immune response and M2c has anti-inflammatory and pro-fibrotic functions. Olmes et al. [17] found that immunosuppressive therapy could increase M2c macrophages in lupus nephritis, as well as kidney allograft transplantation [18]. However, our results showed that larger numbers of M2a and M2b macrophages than M2c macrophages. The likely reason may be associated with different causes of nephritis. What's more, our renal samples were collected from patients before therapy. These data above implied that M2a and M2b macrophages might be more involved in the progression of IgAN.

Mesangial proliferation and gradual progress of tubulointerstitial fibrosis are characteristic of pathological change in IgAN. It has been reported that CD206 positive M2 macrophages (M2a) contribute to pulmonary, hepatic, pancreatic, and peritoneal fibrosis by regulating extracellular matrix deposit [19–22]. We analyzed the distribution of M2a cell in different Oxford classification, degree of global glomerulosclerosis and Lee grade. Our results showed that significantly larger numbers of tubulointerstitial M2a macrophages in kidney tissues of patients with S1, T1 and Lee Grade IV-V, and had increased trend in patients with M1. However, the glomerular M2a macrophages were not associated the pathological features. Which indicated that M2a macrophages were associated with the lesions of the tubulointerstitium. The severity of tubulointerstitial lesions largely reflects the stage of disease at the time of biopsy and those patients with more advanced chronic damage have a shorter time to ESRD [2]. These results showed that M2a macrophages may play roles in the tubulointerstitial injure and fibrosis to aggravate the progression of IgAN.

M2b macrophages induced by immune complexes with LPS or IL-1β regulate immune response and are characterized by cell surface expression of receptors CD86. molecule CD86 may be needed on macrophages when antigens are presented to the immune system. In our study, M2b macrophages showed a tendency towards larger numbers in glomeruli of patients with G1 (global glomerulosclerosis ≥50%). However, the differences were not significant. A study showed that CD80+ and CD86+ cells (Most CD86+ cells) were observed more in IgAN patients with progressive renal injury than in mild cases and controls [23]. Moreover, Wu et al. [24] found that CD86+ macrophages was expressed in the glomeruli and interstitium, especially in the crescent. In kidney of our research, M2b macrophages showed a tendency of larger numbers in glomeruli of patients with G1. These data above indicated that M2b might act as antigen-presenting cells and be associated with global glomerulosclerosis of IgAN. Further studies are needed to conform this conclusion.

M2c macrophages induced by IL-10, TGF-β, or glucocorticoids exert anti-inflammatory and pro-fibrotic functions and are characterized by cell surface expression of receptors CD163. We observed larger numbers of M2c macrophages in glomeruli of patients with T0, G0 and Lee grade II-III. There are evidences showed that immunosuppressive therapy can increase CD163 in macrophages [17,18,25,26]. Lu et al. [27] transferred M2c macrophages into mice on day 5 after adriamycin administration and results showed that M2c macrophages effectively reduced glomerulosclerosis, tubular atrophy, interstitial expansion, and proteinuria in murine adriamycin nephrosis, and they conclude that M2c might protect kidney injure. The results were accordant with ours. However, Ikezumi et al. [12] showed that CD163-positive macrophages were present in new-onset IgAN and may promote the development of fibrotic lesions. Another study showed M2c macrophages were involved in active crescent disease, acute tubular injury and glomerular lesions of IgAN with crescents [28]. Gutiérrez et al. [13] also found that M2c were located in areas surrounding tubules with iron deposits and filled with erythrocyte casts. And M2c macrophages were also involved in the pathogenesis of proliferative glomerular lesions, active crescent and tubular injury in lupus nephritis and vasculitis, but interstitial fibrosis in chronic kidney allograft injury [11,29].These data do not favor our conclusion of M2c might protect chronic kidney injure of IgAN. Therefore, whether M2c macrophages are the actors of disease progression or protection of IgAN remain controversial and further studies are needed.

Previous studies showed there was a positive correlation between the infiltration of CD68+ macrophages and clinical parameters, such as serum creatinine, proteinuria, progression of renal disease and a worse disease outcome in IgAN [4,30,31]. However, the relevance of specific macrophage subpopulations with the clinical features of IgAN remained unclear. In our analysis, we found that the presence of M2a macrophages was positively correlated with serum creatinine, proteinuria. The infiltrations of M2b and M2c macrophages had no correlation with these clinical parameters. Therefore, our data indicated that M2a macrophages more correlated with clinical features than M2b and M2c macrophages, and M2a macrophages might aggravate the progression of IgAN.

However, certain limitations of our study should be noted. Firstly, this study is an observational and small-sample investigation, and we have no follow-up data about prognosis of IgAN. Secondly, only one patient (2.04%) in our cohort presented E1 and there were no data to showed the correlation of M2 subpopulations and E scores. Thirdly, only surface markers of M2 subtypes were analyze and other markers of M2 subtypes (such as functional cytokines) should be analyzed to support our conclusions. We will enlarge the samples, detect more markers and follow up the patients to investigate the roles M2 subpopulations in IgAN.

In conclusion, there were more M2a and M2b macrophages than

M2c in renal tissues of IgAN patients. M2a macrophages were associated with more severely tubulointerstitial pathological features and renal function, but M2c macrophages were negatively correlated glomerular sclerosis and interstitial fibrosis. These results indicated that M2 subpopulations involved in the progression of IgAN, and M2a and M2c macrophages might show different identity tendencies to participate in the pathogenesis of fibrosis of IgAN.

Conflict of interest statement

We declare that the results presented in this paper have not been published previously in whole or part, except in abstract format.

Authors' contributions

Xinling Liang and Wenke Hao designed the research, obtained funding and supervising the work. Wenxue Hu and Jieshan Lin conducted the research and wrote the first draft of the manuscript. Xingji Lian, Feng Yu and Wei Liu contributed to data collection, data interpretation and critical revisions of the manuscript. Yanhua Wu and Xiaowu Fang analyzed the data and contributed to critical revision of the manuscript.

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