

Original Article

Hemolytic streptococcus exacerbates tubulointerstitial lesions in IgA nephropathy through Th22 cells chemotaxis and proliferation

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Abstract: Background: Tonsillitis and the immunopathologic response induced by it are essential for IgA nephropathy (IgAN) progression. In this study, we investigated the possible mechanism underlying Th22 cells overrepresentation in tonsillitis related IgAN. Methods: The distribution of Th22 cells, and the expressions of CCR10 and CCL27 in IgAN patients were detected. Relationship between Th22 cell chemotaxis and renal pathology lesions was determined. Transwell assay was performed to investigate the contribution of human tubular epithelial cell (human kidney 2 cell, HK2) to Th22 cell chemokines underlying Hemolytic streptococcus (HS) infection. Additionally, the impacts of tubular inflammatory on Th22 cell proliferation were investigated. Results: Th22 cells were significantly increased in IgAN patients. Accordingly, the expressions of CCR10 and CCL27 were increased in IgAN patients. Higher Th22 cells, CCR10 and CCL27 correlated with severer tubulointerstitial lesions. It was observed that CCR10 and CCL27 were predominantly expressed on tubular epithelial cells, and supernatants of HK2 were chemotactic for Th22 cells. This activity of HK2 was partly blocked by anti-CCL27. Tonsillitis promoted the over expression of Th22 cells, CCR10 and CCL27 in IgAN patients, and exacerbated their renal lesions. Inactivated HS aggravated Th22 cell chemotaxis by promoting CCL27 secretion. Additionally, inactivated HS could accelerate Th22 cell proliferation by promoting the secretion of IL-1, IL-6 and TNF- α . Conclusion: Th22 cell chemotaxis was involved in the pathogenesis of IgAN. Tubular epithelial cells are vulnerable to Th22 cell chemotaxis in IgAN. Tonsillitis may exacerbate the progression of IgAN by promoting Th22 cell chemotaxis and cell proliferation.

Keywords: IgAN, Th22 cells, CCR10, CCL27, tonsillitis

Introduction

IgA nephropathy (IgAN) is the most common cause of end stage renal diseases [1]. It is noted that upper respiratory tract infection, especially *Hemolytic streptococcus* (HS) infection induced tonsillitis, can exacerbate the progression of IgAN. The immunopathological response induced by this infection is essential for IgAN renal damage [2]. It was recently confirmed that T-help type 22 (Th22) cell disorder is involved in IgAN, and higher percentages of Th22 cells correlated with severer proteinuria [3, 4]. These suggest that Th22 cell accumulation plays an important role in the progression of IgAN.

Lymphocytes accumulation may attribute to either cell proliferation or cell infiltration. CC

chemokines are the keys to lymphocytes infiltration. Th22 cell can preferentially express CC chemokine receptor CCR10, which is the highly specific receptor for CCL27. The expression of chemokines and their corresponding receptors may change depending on different disease status. For example, the expressions of CCL20 and CCR6 increased in murine crescentic glomerulonephritis [5]. The expressions of CCR4 and CCL22 increased in anti-glomerular basement membrane glomerulonephritis, and involved in disease progression from acute glomerular injury to irreversible tissue damage [6]. It can be argued that the cell infiltration induced by these chemokines plays a critical role in nephritis development.

In this study, we investigated the possible mechanism underlying Th22 cell overrepresenten-

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Table 1. Demographic, clinical, and biochemical characteristics and pathology of patients with IgAN (n = 60)

	Control group (N = 16)	IgAN group (N = 30)	IgAN+ tonsillitis group (N = 14)
Male (n, n%)	5 (31.25%)	10 (33.33%)	5 (35.71%)
Age (years)	26.69±1.35	30.33±9.69	30.79±8.47
SBP (mmHg)	119.75±9.09	121.53±19.35	119.14±13.52
DBP (mmHg)	77.75±2.62	80.13±13.10	76.36±11.28
Proteinuria (g/24 hours)	Negative	1.03±1.23*	0.81±0.95*
Hematuria (n (n%))	Negative	20 (66.67%)*#	13 (92.86%)*
Serum albumin (g/l)	43.26±3.21	38.70±4.69*	39.09±7.11*
Blood urea nitrogen (mmol/l)	4.78±0.84	6.37±6.21	6.78±5.79
Serum creatinine (umol/l)	81.38±13.74	93.49±26.75	81.27±21.53
Uric acid (umol/l)	312.56±55.82	355.48±126.44	319.16±62.34
Peripheral blood lymphocytes (10 ⁹ /l)	1.42±0.44	1.99±0.65*	2.03±0.71*
Pathological Lesions (n (n%))			
Mesangial hypercellularity (M1)		30 (100%)	14 (100%)
Endocapillary hypercellularity (E1)		15 (50%)*#	8 (57.14%)
Segmental glomerulosclerosis (S1)		13 (43.33%)*#	13 (92.86%)*
Tubular atrophy/interstitial fibrosis (T1-2)		1 (3.33%)*#	3 (21.43%)

*P < 0.05 compared with control group; #P < 0.05 when IgAN group compared with IgAN+ tonsillitis group.

tation in IgAN. Furthermore, the negative effects of tonsillitis on IgAN underlying Th22 cell disorder were explore.

Materials and methods

Reagents

Fluorochrome-labeled antibodies specific for various markers were purchased. These markers include CD3 (PerCP-Cyanine5.5; eBioscience), CD4 (FITC; eBioscience), INF- γ (APC, eBioscience), IL-17 (PE-Cyanine7, eBioscience), IL-22 (PE, eBioscience) and CCR10 (eFluor450, BioLegend). Antibodies for immunohistochemistry were purchased. These markers include anti-human CCL27 mAb and anti-human CCR10 mAb (Abcam, USA). Antibodies for differentiation assay were purchased from PeroTech. These markers include IL-1, IL-2, IL-6, TNF- α , anti-CD3 mAb, and anti-CD28 mAb. Anti-CCL27 antibody for blockage experiment of transwell assay was purchased from Abcam. The ELISA kits for IL-1, IL-6, TNF- α , and CCL27 were purchased from R&D. CD4⁺ T cell isolation kit was purchased from Miltenyi Biotec. Transwell system were purchased from Corning-Costar, USA.

HS was isolated from tonsils of IgAN patients by throat swab, and ensured by using hematoxy-

lin-eosin staining, than purified, and diluted to 1×10^{10} CFU/mL in sterile phosphate-buffered saline. All bacteria were formalin-inactivated. The vaccine did not contain any viable microorganisms, as confirmed by sterility test [1].

Study population

60 adults, ages 21-40 years old, with biopsy-confirmed IgAN within 1 year were included. The demographic, clinical, and biochemical characteristics and pathology of all objects are shown in **Table 1**.

The inclusion criteria for IgAN patients without tonsillitis (IgAN group) was without repeated history of tonsil infection or current antiadoncus; the exclusion criteria were 1) having acute infection; 2) having tonsil infection; 3) underwent glucocorticoid or immunosuppressant treatments; or 4) having other immune related diseases or complications. The inclusion criteria for IgAN patient with tonsillitis (IgAN+ tonsillitis group) is throat swab culture proved tonsil infection; the exclusion criteria are those for IgAN group, except having tonsil infection. The exclusion criteria for healthy controls (HC groups) are having any perceptible diseases including immune disease, renal disease, infection.

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All patients were recruited and examined in the Xiangya Hospital, Central South University. This study was carried out according to the Declaration of Helsinki and approved by the Medical Ethics Committee of the Xiangya Hospital of Central South University for Human Studies (approval number 201403270), and all subjects provided signed informed consent.

Flow cytometry

Peripheral blood mononuclear cells from subjects were isolated using ficoll purchased from GE Healthcare Life Sciences. The cells were then stained for CD3, CD4, INF- γ , IL-17, IL-22, and CCR10. Th22 cells (CD3⁺CD4⁺INF- γ IL-17⁺Th22⁺) [7] and the CCR10 expressed on them were isolated and quantified by flow cytometry. Flow cytometry was conducted, using Becton Dickinson FACS calibur system in Central Lab of Xiangya Hospital.

Enzyme-linked immunosorbent assay (ELISA)

All the serum was isolated immediately from the fresh blood and preserved at -80°C before use. The serum IL-6, IL-1 β , TNF- α , and CCL27 of subjects were quantified by ELISA according to the manufacturer's recommendations.

Renal histopathology and immunohistochemistry

The renal tissues were stained with hematoxylin and eosin (HE) and periodic acid-Schiff reagents, then analyzed by a renal pathologist under a light microscope according to Oxford Classification of IgA nephropathy 2016 [8]. The expressions of CCR10 and CCL27 in kidneys were analyzed by immunohistochemistry. Image-Pro Plus 6.0 was used for quantitative calculation.

Isolation of CD4⁺ T lymphocytes

Peripheral blood mononuclear cells from healthy controls and IgAN patients with or without tonsillitis were prepared using a Ficoll purchased from GE Healthcare Life Sciences. CD4⁺ T lymphocytes were isolated using a CD4⁺ lymphocyte isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). With this kit, non-CD4⁺ cells such as neutrophils were tagged. Subsequently, non-target cells are magnetically labeled with the CD4⁺ T Cell MicroBead Cocktail. These cells were then magnetic separated by LD columns with manual separators.

Th22 cells chemotaxis assay

Experiments were independent biological repeats. CD4⁺ T lymphocytes of IgAN patients were isolated and purified using a CD4⁺ T cell isolation kit. Purified CD4⁺ T lymphocytes drawn from one patient with IgAN at a single time point were divided into different treatment groups in one repeat experiment.

Before transwell assay, HK2 was first incubated and treated with/without inactivated HS (1×10^8 CFU/mL) for 96 hours. CD4⁺ T lymphocytes (1×10^7) were added to the upper chambers of a 24-well transwell plate in RPMI-1640 supernatant with 0.5% FBS in a final volume of 100 μ l, and the lower chambers were filled with 600 μ l of the supernatant of cultured HK2. Blocking experiments were performed by mixing HK2 culture supernatant with 100 ng/mL anti-CCL27 mAbs. The transwell chambers were incubated at 37°C in 5% CO₂ for 5 hours. Blank group represent the migration of T lymphocytes that responded to only supernatant. The cells in the lower chamber were assigned a chemotaxis index (chemotaxis index = number of migrated Th22 cells in each experiment group/number of migrated Th22 cells in Blank group) and analyzed by flow cytometry.

Th22 cells differentiation

Purified IgAN CD4⁺ T cells were cultured in RPMI-1640 medium containing IL-2 (2 ng/mL) in 24-well plate and stimulated with CD3 and CD28 antibodies (1 μ g/mL each) for 168 hours. The exogenous cytokines used to modulate cell differentiation were IL-1 β (20 ng/mL), IL-6 (100 ng/mL), and TNF- α (50 ng/mL).

Statistical analysis

Data were expressed as the mean \pm standard deviation or median with minimum and maximum values. Comparison between groups were analyzed by using one-way analysis of variance, Kruskal-Wallis H test, or the Mann-Whitney U-test. Correlations among variables were determined by calculating the Spearman or Pearson rank correlation coefficients. $P < 0.05$ was defined as statistically significant. Statistical analyses were performed using SPSS 19.0 software. The exact P values were expressed as $P < 0.001$ if the P value was less than 1×10^{-3} .

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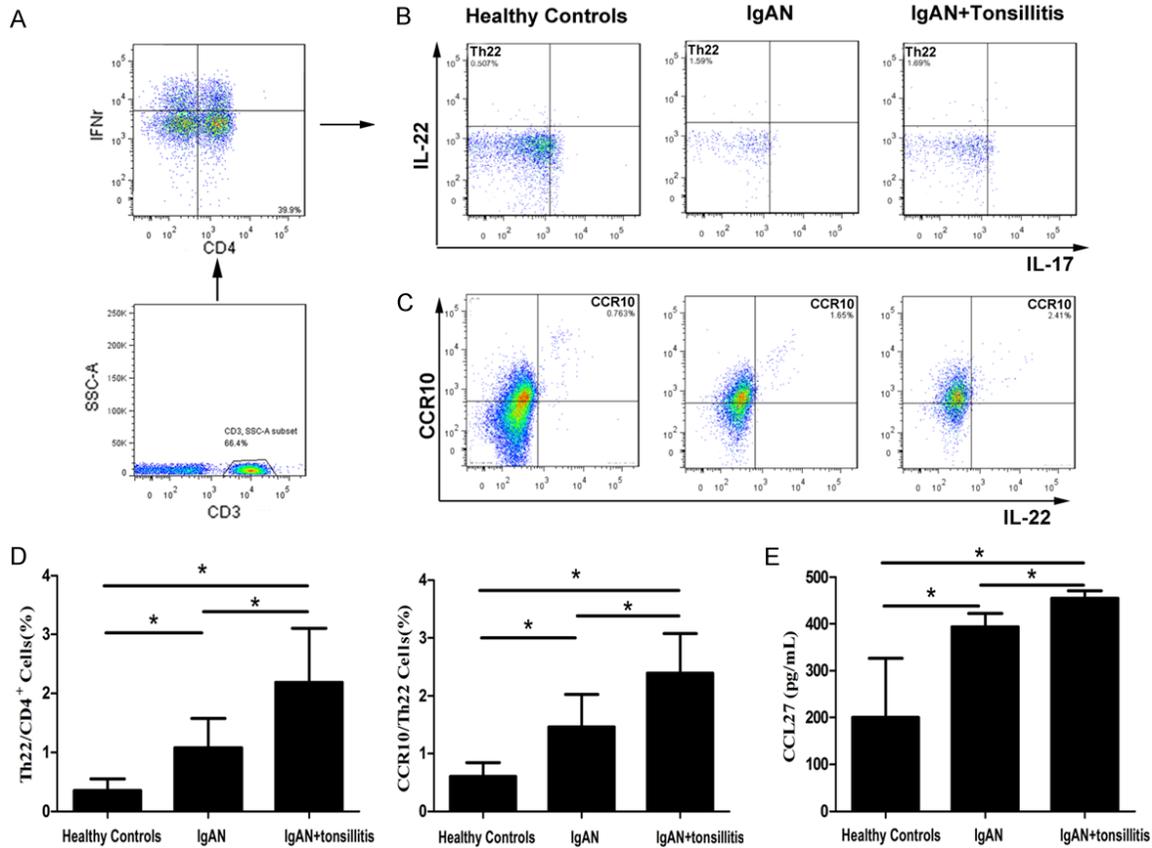


Figure 1. The Th22 cells distribution, CCR10 expression and CCL27 concentration increases in peripheral blood of IgAN patients. A: Th22 cells within CD4⁺ T cells were identified based on their expression of CD3⁺, CD4⁺ IL-17⁺, and INF- γ . B: Representative flow chart of Th22 cells in the peripheral blood of IgAN patients. C: Representative flow chart of CCR10⁺ expressed on IL-22⁺ T cells. D: Percentages of Th22 cells and CCR10 presented on them increased in IgAN patients. Higher percentages of Th22 cells and CCR10 presented on them were observed in IgAN patients with tonsillitis. E: Concentrations of serum CCL27 were analyzed using ELISA. CCL27 level in creased in IgAN patients. Higher level of CCL27 was observed in IgAN patients with tonsillitis. *represents $P < 0.05$.

Results

Tonsillitis promotes the overrepresentation of Th22 cells, CCR10, and CCL27 in IgAN patients

Th22 cell chemotaxis is involved in the progression of murine IgAN [3], but it is unclear whether IgAN patients share the same pathogenesis. To explore the possible mechanism of Th22 cell accumulation in IgAN patients, we first explored the distribution of Th22 cells in IgAN patients, and found that percentages of Th22 cells in the peripheral blood of IgAN patients ($1.08 \pm 0.50\%$) increased compared with healthy controls ($0.35 \pm 0.19\%$, $P < 0.001$) (Figure 1). To characterize these Th22 cells in more detail, the expressions of cell surface receptor CCR4, CCR6, and CCR10 on Th22 cells were further

detected. Data showed that the expression of CCR10 on Th22 cells increased in IgAN patients ($1.46 \pm 0.56\%$), compared with healthy controls ($0.60 \pm 0.24\%$, $P < 0.001$) (Figure 1C). Whereas, the expressions of CCR4 and CCR6 on Th22 cells failed to be up-regulated (Data not shown). It is well known that CCL27 can induce Th22 cell chemotaxis by interacting with CCR10. Thus we further analyzed the serum CCL27 levels in subjects. Data showed that serum CCL27 increased in IgAN patients (394.34 ± 28.25 pg/ml) compared with controls (200.68 ± 25.94 pg/ml, $P < 0.001$) (Figure 1E). This overrepresentation of Th22 cells, CCR10, and CCL27 indicated an up-regulation of Th22 cell chemotaxis in IgAN.

We further noticed that tonsillitis aggravated the overrepresentation of Th22 cells, CCR10,

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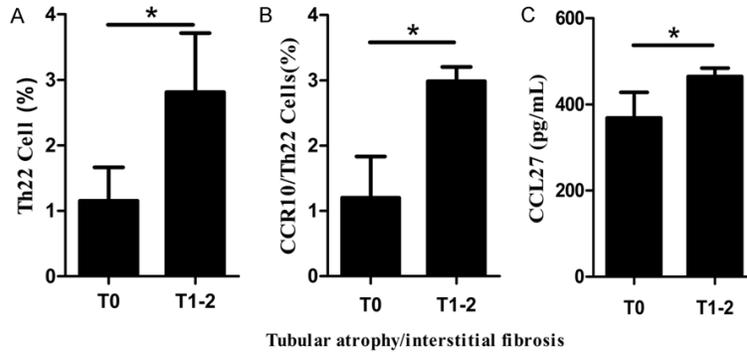


Figure 2. Severer tubular atrophy/interstitial fibrosis relates to higher Th22 cells proportion. Severity of tubulointerstitial injury in IgAN patients was analyzed by renal histopathology according to Oxford Classification of IgA nephropathy. A: Higher Th22 cell percentage was observed in IgAN patients with tubular atrophy/interstitial fibrosis. B: Higher expression of CCR10 was observed in IgAN patients with tubular atrophy/interstitial fibrosis. C: Higher levels of serum CCL27 was observed in IgAN patients with tubular atrophy/interstitial fibrosis. *represents $P < 0.05$.

Overexpressions of Th22 cells, CCR10, and CCL27 correlate with tubulointerstitial injury

To illuminate the impact of Th22 cell accumulation on IgAN, we examined renal pathology lesions of patients according to MEST scores. Data suggested that Th22 cell chemotaxis response was involved in tubulointerstitial lesions. As shown in **Figure 2**, higher Th22 cell percentages were observed in IgAN patients with severer tubular atrophy/interstitial fibrosis ($2.81 \pm 0.90\%$ vs. $1.15 \pm 0.50\%$; $P < 0.001$). Similarly, higher CCR10 ($2.99 \pm 0.22\%$ vs. $1.19 \pm 0.63\%$, $P < 0.001$) expression and serum CCL27 (464.67 ± 19.89 pg/ml vs. 368.58 ± 59.20 pg/ml, $P < 0.001$) levels were observed in IgAN patients with severer tubular atrophy/interstitial fibrosis. This indicated that the up-regulated Th22 cells chemotaxis response may have a negative effect on tubulointerstitial injury in IgAN.

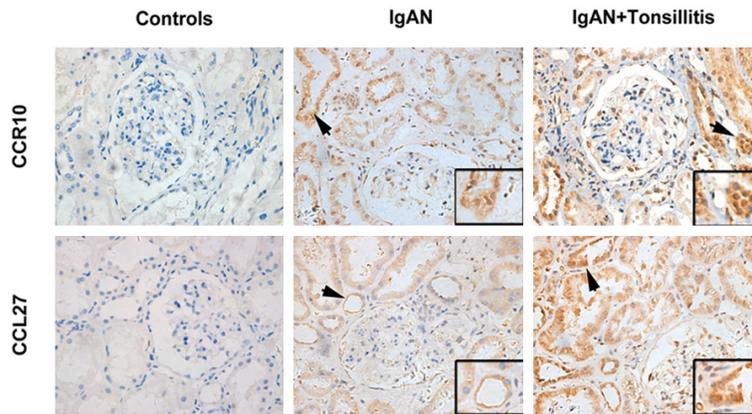


Figure 3. CCR10 and CCL27 expression in the kidneys (400 \times). Representative images of immunohistochemistry staining (brown) of anti-CCR10 and anti-CCL27 in renal tissue from the healthy renal tissue from patients with renal carcinoma, IgAN patients, and IgAN patients with tonsillitis. CCL27 and CCR10 were predominantly expressed in the renal tubules. Compared with controls, the expressions of CCL27 and CCR10 were much higher in IgAN patients, especially those with tonsillitis.

Renal tissues of IgAN patients over-express CCR10 and CCL27

and CCL27 in IgAN. Percentages of Th22 cells were significantly higher in IgAN+ tonsillitis patients ($2.19 \pm 0.92\%$) compared with patients without tonsillitis ($P < 0.001$). We also found that the expression of CCR10 ($2.39 \pm 0.68\%$) on Th22 cells and the serum CCL27 level (455.53 ± 16.12 pg/ml) increased in IgAN+ tonsillitis patients compared with patients without tonsillitis (**Figure 1**). This suggested that tonsillitis may promote Th22 cell chemotaxis response in IgAN.

To investigate the interaction between Th22 cell chemotaxis and tubulointerstitial lesions in IgAN, the renal expressions of CCR10 and CCL27 were determined by immunohistochemistry. As visualized by the yellow areas in **Figure 3** indicating immunohistochemical reactants, abundant renal expression of CCR10 was observed in IgAN patients (0.039 ± 0.012), while control groups barely expressed CCR10 (0.007 ± 0.001 , $P < 0.001$). A significantly increased renal expression of CCL27 was showed in IgAN patients (0.028 ± 0.010 , $P < 0.001$). Specifically, CCR10 and CCL27 were predominantly expressed in the renal tubules. We further noticed that ton-

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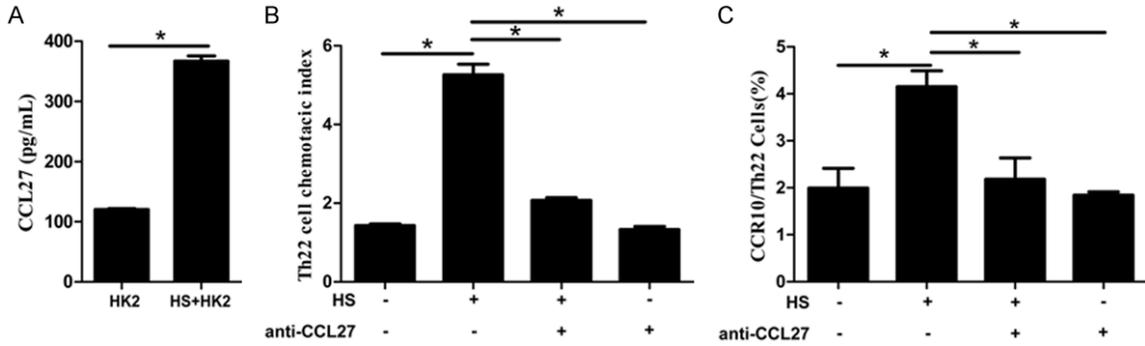


Figure 4. Tubular epithelial cells were chemotactic for Th22 cells partly through secreting CCL27 in vitro. A: Human tubular epithelial cells (human kidney 2 cells, HK2) were incubated and treated with/without inactivated Hemolytic streptococcus (HS) for 96 hours. Concentration of CCL27 in the supernatant was analyzed by ELISA. B: Supernatant of cultured HK2 treated with/without inactivated HS was used to induce the chemotaxis of Th22 cells in the absence or presence of anti-CCL27. The Th22 cell chemotaxis index = number of migrated Th22 cells in each experiment group ÷ number of migrated Th22 cells in blank group (n = 4). C: Bars represent the expressions of CCR10 on recruited Th22 cells in each groups of transwell assay (n = 4). *represents P < 0.05.

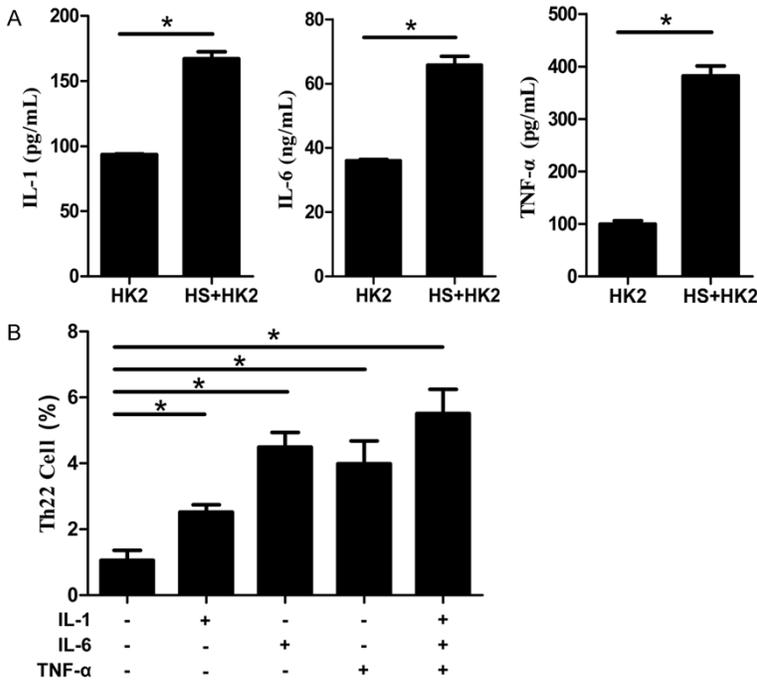


Figure 5. Inflammatory response of tubular epithelial cells promotes Th22 cells differentiation. A: Human tubular epithelial cells (human kidney 2 cells, HK2) were incubated and treated with/without inactivated Hemolytic streptococcus (HS) for 96 hour. Concentration of proinflammatory cytokines, i.e. IL-1, IL-6, and TNF-α, in the supernatant was analyzed by ELISA. B: Purified CD4⁺ T cells isolated from peripheral blood of IgAN patients were stimulated with different combination of IL-1, IL-6, and TNF-α for 168 hours. Plate-bound anti-CD3 and anti-CD28 mAbs were used to maintain the activity of lymphocytes. Comparisons of Th22 cell percentages between different groups were determined by performing Kruskal-Wallis one-way analysis of variance for ranking (n = 4). *represents P < 0.05.

densities of CCR10 (0.060±0.015) and CCL27 (0.051±0.012) in IgAN patients with tonsillitis were much higher than patients without tonsillitis.

Tubular epithelial cells induce Th22 cells chemotaxis by secreting CCL27 in vitro

It is noted that Th22 cell can be recruited through CCR10-CCL27 axis. Previously, we had demonstrated that CCR10 and CCL27 were predominantly expressed in the renal tubules, and tonsillitis exacerbated this expression. Taken together, we hypothesized that HK2 could induce Th22 cell chemotaxis through CCR10-CCL27 axis at the presence of HS infection. To verify this theory, we first analyzed the capability of HK2 to secrete CCL27 at the presence or absence of inactivated HS stimulation. Data suggested that HK2 was capable to secrete CCL27 (120.43±1.20 pg/ml) (Figure 4A). Whereas inactivated HS significantly stimulate the secretion of CCL27 produced by HK2 (367.27±8.25 pg/ml, P < 0.001).

illitis aggravated the renal expressions of CCR10 and CCL27. Additionally, the average optical

tion of CCL27 produced by HK2 (367.27±8.25 pg/ml, P < 0.001).

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Next, we performed transwell assay to testify the chemotactic effect of HK2 on Th22 cells. Results showed that the supernatant of HK2 was capable to induce Th22 cell chemotaxis (1.43 ± 0.04), while inactivated HS significantly promote Th22 cell recruitment of HK2 (5.26 ± 0.27). Whereas these stimulatory effects were blocked by anti-CCL27 significantly (2.07 ± 0.07 , $P < 0.001$) (**Figure 4B**). Correspondingly, the CCR10 expressed on the recruited Th22 cells increased in HS treated HK2 groups (4.15 ± 0.33), while anti-CCL27 significantly inhibited the CCR10 expression (2.18 ± 0.45 , $P < 0.001$) (**Figure 4C**).

Th22 cells differentiation in vitro

As is known that lymphocyte overrepresentation may attribute to either cell infiltration or cell proliferation. We had confirmed that Th22 cell infiltration increased in IgAN. Here, we further explored the changes of Th22 cell proliferation in IgAN. Previous studies have demonstrated that IL-1, IL-6 and TNF- α [9-11]. In this study, we examined the impacts of these cytokines on Th22 cell proliferation by stimulating CD4⁺ T lymphocytes from IgAN patients with IL-1, IL-6, and TNF- α . As shown in **Figure 5**, the proportion of Th22 cells in groups treated with IL-1 and TNF- α increased ($2.52 \pm 0.23\%$ and $3.99 \pm 0.69\%$). The highest Th22 cell proportion was observed in IL-6 treated group ($4.49 \pm 0.45\%$). This suggested that inflammatory response in IgAN can aggravate Th22 cell overrepresentation.

Discussion

Immune system disorder and inflammatory response are common pathogenesis of glomerular nephritis. Th cell disorder have been reported involved in the progression of IgAN. Yang L *et al.* found a T lymphocyte subgroup drifting (increases in Th2 and Th17 along with decreases in Treg) in IgAN, which worsened the aberrant glycosylation of IgA1 [12]. Peng *et al.* confirmed a relationship between Th22 cell overrepresentation and proteinuria in IgAN [4]. Here, we demonstrated that Th22 increased in IgAN.

This Th22 cell overrepresentation in IgAN was partly attribute to chemotaxis response induced by CCL27-CCR10 axe. CCL27 is the specific ligand for CCR10. CCR10 is a cell surface recep-

tor which is expressed on Th22 cell. The interaction between CCL27 and CCR10 can induce Th22 cell chemotaxis and infiltration. Numerous studies have demonstrated that renal tissue can secrete CC chemokines, inducing the cell infiltration and local inflammation. Meng *et al.* demonstrated an upregulation of Th17 cells in murine IgAN underlying the effects of CCL20 [1]. Xiao *et al.* demonstrated overexpressions of CCL20, CCL22, and CCL27 showed in murine IgAN, and led to an up-regulation of Th22 cells chemotaxis [3]. We found that renal tissue can induce Th22 cell chemotaxis and infiltration in IgAN by secret CCL27.

Chemotaxis can induced local infiltration and accumulation of T lymphocyte, leading to the progression tissue inflammation. Numerous studies have demonstrated that renal infiltration and accumulation of T cells participate in the pathogenesis and progression of nephritis, including IgAN. Klocke J *et al.* confirmed that renal infiltration of CD4⁺ and CD8⁺ T cell contributes to the pathogenesis of lupus nephritis [13]. Lin FJ *et al.* reported that renal interstitial infiltration of Treg and Th17 cells promote tubular atrophy/interstitial fibrosis in IgAN [14]. van Es LA *et al.* demonstrated that GMP-17-positive T lymphocytes in renal tubules predicted disease progression in early stages of IgAN. These suggest that local renal infiltration of lymphocyte contribute to kidney damage, especially tubulointerstitial lesions in IgAN.

Our data demonstrated that tubular epithelial cells were vulnerable to Th22 cell chemotaxis response in IgAN. We found that CCL27 and CCR10, the key issues for Th22 cell chemotaxis, were predominantly expressed on renal tubules. Meanwhile, this overexpression of CCL27 and CCR10 were positively correlated with tubulointerstitial injury in IgAN. *In vitro* experiments further confirmed that renal tubular epithelial cells were chemotactic for Th22 cells by secreting CCL27. Numerous studies have demonstrated that renal epithelial cell can induce inflammatory cell infiltration by secreting CC chemokines. Wu HJ *et al.* demonstrated that proximal tubular epithelial cells can overproduce CCL2 and CCL5 in mouse model of protein overload renal injury [15]. Lin M *et al.* found that proximal tubular epithelial cell can overproduce IL-6 and CCL2 in diabetic nephropathy, inducing macrophage infiltration and local inflammatory response [16]. This activity of

renal epithelial cell to overproduce chemokines make it vulnerable to inflammatory infiltration.

Additionally, we found that tonsillitis promoted the Th22 cell chemotaxis in IgAN. This activity was partly rely on the up-regulation of CCL27 and CCR10 expression induced by HS infection. *In vitro* experiment confirmed that HS infection facilitated Th22 chemotaxis response by promoting CCL27 secretion. Besides, HS infection could promote the secretion of proinflammatory cytokines, such as IL-1, IL-6, and TNF- α . These proinflammatory cytokines can promote Th22 cell differentiation, thus leading to an aggravation of Th22 cells accumulation in kidneys. These results are consistent with Chen X *et al.* research, which confirmed that tonsillitis can promote the secretion of Th cytokines, thus participated in the pathogenesis of IgAN [17]. He L *et al.* demonstrated that IgAN was associated with Th1/Th2 imbalance, and HS infection aggravate this imbalance [18]. Taken together, these suggested that HS infection may promote the progression of IgAN through exacerbating Th lymphocytes disorder.

In conclusion, our study demonstrated that up-regulated Th22 cell chemotaxis was involved in the pathogenesis of IgAN. This Th22 chemotaxis was partly induced by the overexpression of CCL27 and CCR10. Tubular epithelial cells in IgAN can induce Th22 cell chemotaxis by overproducing CCL27. Tonsillitis may exacerbate the progression of IgAN by promoting Th22 cell overrepresentation. It will be necessary to evaluate the potential therapeutic approaches for IgAN on Th22 cell overrepresentation, which may proved a basis to develop novel strategy for IgAN therapy.

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Disclosure of conflict of interest

None.

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