

Effects of DC-SIGN expression on renal tubulointerstitial fibrosis in nephritis

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1. ABSTRACT

Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) is important for dendritic cell (DC) in migrating, recognizing, capturing, presenting antigens and in initiating T cell responses. In the present study, we investigated the role of DC-SIGN in renal tubulointerstitial inflammation and fibrosis. DC-SIGN was mainly expressed in tubular epithelial cells and DC-SIGN⁺ DCs were primarily distributed in renal tubulointerstitial areas during the early stage of nephritis, which was correlated with the degree of renal tubular interstitial lesions and fibrosis. *In vitro*, DC-SIGN expression in cultured human renal tubular epithelial cells was elevated when treated by tumor necrosis factor-alpha, and was inhibited by anti-P-selectin lectin-EGF domain monoclonal antibody (PsL-EGFmAb). In a rat model of chronic renal interstitial fibrosis, there was a significant correlation of DC-SIGN expression with DC-SIGN⁺ DC distribution and the degree of tubulointerstitial lesion. PsL-EGFmAb reduced DC-SIGN expression and DC-SIGN⁺ DC accumulation in renal tissues in this rat model. These results suggest that DC-SIGN plays an important role in DC-mediated renal tubular interstitial lesions induced by immuno-inflammatory responses.

2. INTRODUCTION

Chronic kidney diseases induced by different causes can progressively develop into end stage renal disease (1-2). The deterioration in renal function is highly correlated with tubulointerstitial damage, rather than glomerular lesions (3-4). It is known that tubulointerstitial lesions (TIL) are caused by inflammatory cell infiltration in which adhesion molecules and the cellular adhesion mechanism are involved (5-7). Some studies also suggested that renal tubular epithelium may contribute to the pathogenesis of TIL and renal fibrosis (3-4,8).

Our previous studies have shown that dendritic cells (DCs) play a role in renal infiltration and the initiation of inflammation, which is associated with the migration of DCs mediated by the adhesion molecule P-selectin in the early stage of TIL and renal fibrosis. Blockade with anti-P-selectin lectin-EGF domain monoclonal antibody (PsL-EGFmAb) that targets the carbohydrate-recognition domain (CRD) of P-selectin inhibited the adhesion and migration of DCs in the kidney (9-11). DC-specific ICAM-grabbing non-integrin (DC-SIGN), a molecule designated as CD209, is a member of the C-type lectin

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superfamily, and has a similar CRD as P-selectin (12). DC-SIGN is not only functioning as a pattern recognition receptor (PRR) and adhesion receptor, but also is implicated in immunoregulation of DCs (12-14). We have found that PsL-EGFmAb can inhibit DC-SIGN expression under conditions of inflammation, and inhibit the stimulation of T cells by DCs (15). However, it remains unclear whether DC-SIGN is expressed in renal tissue, whether it mediates DC migration and participation in renal inflammatory infiltration, and what the relationship is with TIL.

In this study, we investigated the expression and distribution of DC-SIGN and DC-SIGN⁺ DCs in the renal tissues of patients with nephritis, the association with renal TIL, and the regulatory effect of PsL-EGFmAb, as well as the expression of DC-SIGN on cultured human renal tubular epithelial cells under inflammatory conditions. Moreover, we established a rat model of chronic renal interstitial fibrosis to determine if PsL-EGFmAb interfered with DC-SIGN expression and DC-SIGN⁺ DC infiltration, and to explore its effect in preventing tubulointerstitial fibrosis (TIF).

3. MATERIALS AND METHODS

3.1. Animals and reagents

Seventy-two male Sprague-Dawley rats (body weight, 200-250 g) were purchased from Shanghai Experimental Animal Centers of the Chinese Academy of Sciences (Shanghai, China). HK-2 cells, a proximal tubular epithelial cell line, were presented from Tenon Hospital, Paris, France. Roswell Park Memorial Institute 1640, fetal bovine serum, and trypsinase were purchased from Gibco BRL (Crewe, Cheshire, UK). Tumor necrosis factor (TNF)-alpha was purchased from Biosource (Camarillo, CA, USA). Mouse anti-human DC-SIGN monoclonal antibody (mAb) was purchased from BD Biosource (San Diego, CA, USA). Mouse anti-rat OX-62 mAb, rabbit anti-rat DC-SIGN, goat anti-human CD1a, and rabbit anti-rat transforming growth factor (TGF)-beta1, alpha-smooth muscle actin (SMA), collagen III (Col III) and fibronectin (FN) polyclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG, FITC-conjugated anti-rabbit IgG, and R-Phycoerythrin (RPE)-conjugated anti-goat IgG were from Jackson (West Grove, PA, USA). Immunohistochemistry (IHC) kits were purchased from Dako (Glostrup, Denmark). The reverse transcription-polymerase chain reaction (RT-PCR) kit was purchased from Promega (Madison, WI, USA). PsL-EGFmAb was prepared in our laboratory (11).

3.2. Patients

Seventy-six patients with glomerulonephritis (GN) who were hospitalized in the Department of Nephrology of Ruijin Hospital between 2005 and 2006 were enrolled in this study. All patients (44 males and 32 females, with a mean age of 35.17 ± 5.84 years) were diagnosed with GN by renal biopsy using light microscopy (stained with hematoxylin/eosin [HE], PAS, or Masson and Jones), immunofluorescence, electron microscopy, and clinical data. The specific diagnoses included IgA nephropathy (n = 28),

focal and segmental sclerosis GN (n = 7), minor change GN (n = 5), focal or diffuse proliferative GN (n = 5), minimal change GN (n = 4), membranous nephropathy (n = 3), and lupus nephritis (n = 24). The patients were divided into three groups according to the degree of severity of TIL (i.e., tubular atrophy, interstitial fibrosis, and inflammatory cell infiltration) as follows: 1) mild group (involved area < 20%, n = 46); 2) moderate group (involved area 20-50%, n = 21); and 3) severe group (involved area > 50%, n = 9). The mean level of blood urea nitrogen (BUN), serum creatinine (Scr) and the creatinine clearance (Ccr) was 10.67 ± 7.05 mmol/L, 175.14 ± 22.35 μ mol/L and 65.25 ± 6.34 ml/min, respectively. Ten renal tissues obtained from unmatched donor kidneys for transplantation served as controls.

3.3. Animal models

Thirty-six rats were randomly assigned into the normal group without any surgical treatment (n = 18) and a sham-operated group anesthetized with ether inhalation and renal decapsulation alone (n = 18). The other 36 rats underwent resection of the left kidney after administration of anesthesia and one week later resection of the upper 1/3 and lower 1/3 of the right kidney to establish a model of chronic renal fibrosis. After the model was established for one week, the 36 rats were randomly assigned into an operated group that drank and ate *ad libitum* after the operation without drug intervention (n = 18) and a PsL-EGFmAb-treated group injected with 2 mg/kg PsL-EGFmAb via the caudal vein during and after the operation (n = 18). One, four and twelve weeks after surgery, 6 rats from each group were anesthetized with ether and sacrificed by decapitation, blood samples were obtained, and the kidneys were harvested. Urine collection was performed the day before euthanasia by housing the animals in metabolic cages. The kidneys were quickly removed and fixed in 10% buffered formaldehyde.

3.4. Biochemical analysis and histopathologic measurements in rats

The serum separated from the blood samples of rats were tested for BUN and Scr by a Beckman Automatic Biochemical Analysis Instrument (Fullerton, CA, USA) and the Ccr was calculated. Paraffin-embedded kidney sections from the rats were prepared in 2 μ m thicknesses by a routine procedure. Sections were stained with HE or Masson to assess histological changes by light microscope. Four grades were ranked according to the degree of renal TIF: 0 (normal); 1 (mild interstitial expansion with an involved area of < 25%); 2 (moderate interstitial expansion with an involved area of 26-50%); and 3 (severe interstitial expansion with an involved area of > 50%). The En Vision method was used to detect the expression of TGF-beta1, alpha-SMA, Col III and FN in renal tissues. A semi-quantitative scoring scale from 0-3, based on the stained area and degree, was used as follows: 0 = no stain; 1 = focal staining; 2 = mild or moderate diffuse staining; and 3 = severe diffuse staining. The number of alpha-SMA positive cells was counted using a microscope in 20 fields ($\times 400$), corresponding to 1 mm² of tissue on renal cortical sections.

3.5. Detection of DC-SIGN expression in renal tissues

An IHC assay was used to determine DC-SIGN

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expression in human and rat renal tissues. The 3 µm thick sections were treated with 3% hydrogen peroxide to block the activity of endogenous peroxidase. Non-specific protein staining was blocked by incubation with normal rabbit serum. The sections were incubated with 1:100 mouse anti-human DC-SIGN mAb at 4 °C overnight, then incubated with 1:400 biotinylated rabbit anti-mouse IgG for 30 min at room temperature, and subsequently incubated with 1:400 streptavidin-peroxidase for 30 min at room temperature. DAB was used to develop the diffuse color staining. The sections were counterstained with hematoxylin and mounted. The primary antibody was replaced by PBS in the negative control. A semi-quantitative scoring scale from 0-3, based on the stained area and degree, was used as follows: 0 = no stain; 1 = occasional staining; 2 = focal staining; and 3 = diffuse staining.

3.6. Immunofluorescent staining for DC-SIGN⁺ DC distribution in renal tissues

The distribution of DC-SIGN⁺ DCs in renal tissues was assayed by dual-label immunofluorescence staining for the microscopic image method. Renal tissue sections from human or rats were blocked with 0.3% bovine serum albumin for 20 min, and then incubated with 1:100 goat anti-human CD1a polyclonal antibody (or mouse anti-rat OX-62 mAb) and mouse anti-human DC-SIGN mAb (or rabbit anti-rat DC-SIGN polyclonal antibody) at 4 °C overnight and followed by a subsequent incubation with 1:200 anti-mouse IgG-FITC and anti-goat IgG-RPE antibodies, respectively. The sections were incubated for 1 h at 37 °C, washed with PBS, and then mounted. The primary antibody was replaced by PBS as a negative control.

The sections were observed by multifunctional automatic microscopy (Axioplan 2 imaging; Carl Zeiss, Jena, Germany) equipped with a digital camera (AxioCam, picture element 3900*3090). CD1a or OX-62 was positively stained by red fluorescence and DC-SIGN was positively stained by green fluorescence. Double stained cells, as shown by yellow fluorescence, represented DC-SIGN⁺ DC (CD1a⁺ DC-SIGN⁺) population. All data were processed with the KS400 imaging process system and software (version 3.0; Carl Zeiss Vision, Hallbergmoos, Germany) and the area (mm²), number, and density of DC-SIGN⁺ DC yellow fluorescence were analyzed.

3.7. Analysis of DC-SIGN on HK-2 cells

The expression of DC-SIGN on HK-2 cells was determined. HK-2 cells were cultured according to conventional methods and placed in a 24-well plate (1×10⁵ cells/well), stimulated with TNF-alpha (final concentration of 50 ng/ml) and PsL-EGFmAb (final concentration of 0.1 mg/ml) when sliding into the homogeneous monolayer, and incubated at 37 °C in 5% CO₂ for 24 h. There were two control groups as follows: one group using PBS instead of PsL-EGFmAb and the other group without TNF-alpha stimulation. The cells were then incubated at 37 °C for 30 min with primary antibody (1:100 mouse anti-human DC-SIGN mAb). After the addition of secondary antibody (1:200 anti-mouse IgG-FITC) had been added, the cells were incubated at 37 °C for 30 min, diluted in PBS, and

observed by fluorescence microscopy.

3.8. Analysis of DC-SIGN mRNA and CD80 mRNA on HK-2 cells

RT-PCR was performed to detect DC-SIGN mRNA and CD80 mRNA expression in HK-2 cells. Total RNA was isolated from HK-2 cells stimulated by TNF-alpha and treated with PsL-EGFmAb using a Trizol kit according to the manufacturer's instructions. cDNA was synthesized from 3.0 µg of total RNA using oligo (dT) primers and a RT-PCR kit. The total volume was 30 µl. The PCR reaction system included 10 × RT buffer (2.5 µl), MgCl₂ (25 mmol/L; 2.0 µl), dNTP (25 mmol/L; 0.2 µl), CD80 primer (50 pmol/L; 0.3 µl), beta-actin primer (50 pmol/L; 0.3 µl), cDNA (3 µl), and TaqE (1 U/µl; 1 µl); the total volume was 25 µl. With the gene sequences of human DC-SIGN, CD80, and GAPDH as references, we used Primer Designer 3.0 (Scientific and Educational Software, Durham, NC, USA) to design primers. DC-SIGN primer sequences, sense 5'-GGCGGCCCTGTACTTTTGG-3', antisense 5'-AAGGCAGCCAAGCAAGGAC-3', and the length of amplified products was 502 bp; CD80 primer sequences, sense 5'-TAGTATATCTGACTTTGA-3', antisense 5'-TTATACAGGGCGTACACT-3', and the length of amplified product was 383 bp; and GAPDH primer sequences, sense 5'-CATCACCATCTTCCAGGAGCG-3', antisense 5'-GAGGGCCATCCACAGTCTC-3', and the length of amplified products was 357 bp. The primers were synthesized by Shanghai Biological Engineering Company (Shanghai, China). The PCR reaction was as follows: an initial melting procedure at 94 °C for 5 min, melting at 94 °C for 40 s, renaturing at 55 °C for 40 s, extension at 72 °C for 90 s, 35 cycles, and then extension at 72 °C for 10 min. The PCR products were visualized by ethidium bromide staining following resolution on a 2% agarose gel. The products were observed under an ultraviolet lamp, scanned and analyzed by a computer image analysis system.

3.9. Statistical analysis

SPSS software, version 11.0, was used for statistical analyses. Data are presented as the mean ± SD, and evaluated by one-way ANOVA and Pearson bivariate correlation tests, as indicated. A difference was considered significant when the *P* value was < 0.05.

4. RESULTS

4.1. DC-SIGN expression in renal tissues

DC-SIGN was rarely observed in normal kidneys, but significantly increased in renal tissues of patients with nephritis, especially in the tubulointerstitium. The expression of DC-SIGN on tubular epithelial cells (97.36% [74/76]) was significantly higher than in the interstitium (71.05% [54/76]) and glomeruli (51.13% [39/76]; *P* < 0.01). Furthermore, the expression of DC-SIGN in the tubulointerstitium was up-regulated when TIL became severe. The expression of DC-SIGN was significantly higher in the severe group (2.37 ± 0.21) than in the mild (0.75 ± 0.14) and moderate groups (1.38 ± 0.16; *P* < 0.01), indicating that the expression of DC-SIGN was correlated with the degree of TIL (*r* = 0.436, *P* < 0.01; Figure 1)

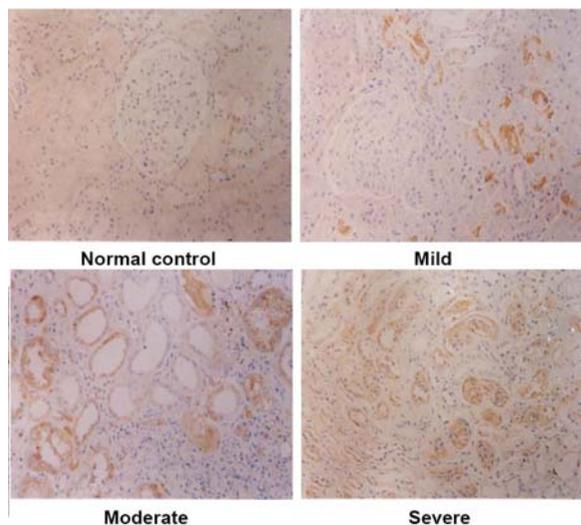


Figure 1. DC-SIGN expression in renal tissue of patients with nephritis. Immunohistochemistry (IHC) assay for DC-SIGN was performed in kidney of patients with nephritis. Original magnification, $\times 200$.

4.2. DC-SIGN⁺ DC distribution in renal tissues

DC-SIGN⁺ DCs were minimally detected in normal renal tissues (336.7 ± 72.8 cells/mm²), while the number of DC-SIGN⁺ DCs significantly increased in the renal tissues with nephritis (1862.5 ± 416.3 cells/mm², $P < 0.01$). There were significantly more DC-SIGN⁺ DC in the renal tissue of patients with severe TIL (2347.3 ± 1103.5 cells/mm²) than that of patients with mild (584.6 ± 247.7 cells/mm²) and moderate TIL (1695.3 ± 904.6 cells/mm²; $P < 0.001$), which was correlated with the degree of TIL ($r = 0.496$, $P < 0.01$; Figure 2). The number of DC-SIGN⁺ DC was also associated with the expression of DC-SIGN in the tubulointerstitium ($r = 0.478$, $P < 0.01$), and with the change of BUN, Scr, and Ccr ($r = 0.298$, $P < 0.05$; 0.367 , $P < 0.05$; and -0.432 , $P < 0.01$; respectively).

4.3. Expression of DC-SIGN, DC-SIGN and CD80 mRNA on HK-2 cells

DC-SIGN was not expressed on HK-2 cells in normal culture condition, but was substantially expressed on HK-2 cells treated with TNF-alpha. PsL-EGFmAb not only inhibited the expression of DC-SIGN on HK-2 cells treated with TNF-alpha (Figure 3A), but also down-regulated the expression of DC-SIGN mRNA and co-stimulatory molecule CD80 mRNA in these cells (Figure 3B).

4.4. Renal histopathology and renal function changes in rats with chronic renal interstitial fibrosis

In the operated group, renal tubules were focally dilated or atrophied, and the basement membrane of some tubules was thickened, comparing with those in the non-operation and the sham-operation groups. Interstitial expansion, inflammatory cell infiltration, and interstitial fibrosis were also evident. In addition, the levels of proteinuria, BUN, and Scr were elevated, and the Ccr was decreased in the operated group (Table 1). After the

operated rats were treated with PsL-EGFmAb, histopathologic changes in the tubulointerstitium were ameliorated (Figure 4), while proteinuria decreased and renal function was improved (Table 1).

4.5. Fibrogenic factors and extracellular matrix (ECM) changes in renal tissues of rats with chronic renal interstitial fibrosis

Comparing with the non-operation and the sham-operation groups, TGF-beta1 expression increased mainly on the renal tubular epithelial cells, interstitial fibroblasts, and glomerular mesangium in the operated rats, while alpha-SMA expression was remarkable in interstitial myofibroblasts and tubular epithelial cells, which corresponded with TIL. In addition, within the tubulointerstitial injury area and glomeruli, more Col III and FN were deposited ($P < 0.05$ - $P < 0.01$; Table 2). There was a significant correlation between the expression of TGF-beta1, alpha-SMA, Col III, and FN and the degree of TIL ($P < 0.05$ - $P < 0.001$; Table 3). The expression of these fibrogenic factors was significantly down-regulated when the operated rats were treated with PsL-EGFmAb (Table 2).

4.6. DC-SIGN expression and DC-SIGN⁺ DC distribution in renal tissues of rat with chronic renal interstitial fibrosis

In the operated group, with the development of histopathologic changes in renal tissues, especially in the tubulointerstitium, DC-SIGN expression increased mainly on the renal tubular epithelial cells, and became especially remarkable 12 weeks after operation (Figure 5A). There was a strong correlation between the number of DC-SIGN⁺ DCs and the degree of TIL ($r = 0.497$, $P < 0.01$). The number of DC-SIGN⁺ DCs was correlated with the degree of TIF and the expression of renal fibrosis-related molecules, including TGF-beta1, alpha-SMA, Col III, and FN. Furthermore, the infiltration of DC-SIGN⁺ DCs was also significantly correlated with the levels of BUN, Scr, and Ccr (Table 4). Treatment with PsL-EGFmAb decreased the expression of DC-SIGN and the accumulation of DC-SIGN⁺ DCs in renal tissues of operated rats (Figure 5B-C), ameliorated the histopathologic injury in the tubulointerstitium (Figure 4), and improved the renal function (Table 1).

5. DISCUSSION

Recent research has demonstrated that the degree of renal tubular injury and interstitial fibrosis correlates more closely to renal failure than glomerular sclerosis does (1-4). Interstitial infiltration of inflammatory cells and immunologic responses contributes to the early stage injury of the renal tubulointerstitium, and tubular epithelial cell activation under conditions of inflammation is also pathogenic to renal interstitial fibrosis (3-6). The inflammatory cells not only secrete various proinflammatory cytokines and profibrotic cytokines, but also transdifferentiates or induces the proliferation of fibroblasts, and enhances the synthesis of ECM as well, contributing to renal fibrosis and the progression of nephritis (6,16-17). Recent studies by us and others have suggested that P-selectin mediates the rapid accumulation of

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Table 1. Examinations of UP, BUN, Scr and Ccr in rats 12 weeks after operation

Group	UP (mg/24h)	BUN (mmol/L)	Scr ($\mu\text{mol/L}$)	Ccr ($\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$)
Normal control	9.40 \pm 3.16	6.63 \pm 1.05	31.67 \pm 5.39	4.01 \pm 1.02
Sham-operation	10.39 \pm 4.84	6.87 \pm 1.11	34.0 \pm 5.63	5.08 \pm 1.71
Operation	101.95 \pm 47.45 ¹	26.46 \pm 12.74 ¹	97.0 \pm 34.05	1.59 \pm 0.41
Operation + PsL-EGFmAb	51.34 \pm 24.75 ²	13.62 \pm 4.98 ²	52.36 \pm 20.73 ²	3.35 \pm 1.09 ²

Abbreviations: UP, urine protein; BUN, blood urea nitrogen; Scr, serum creatinine; Ccr, creatinine clearance. ¹ $P < 0.01$, compared with the sham-operation; ² $P < 0.05$, compared with the operation, $n = 6$.

Table 2. Fibrogenic factors and ECM analysis of rat renal tissues 12 weeks after operation

Group	TGF-beta1	alpha-SMA	Col III	FN
Normal control	0.24 \pm 0.10	0.05 \pm 0.02	0.83 \pm 0.41	0.67 \pm 0.41
Sham-operation	0.27 \pm 0.15	0.07 \pm 0.04	0.95 \pm 0.62	0.83 \pm 0.52
Operation	1.62 \pm 0.45 ¹	132.57 \pm 26.74 ²	2.14 \pm 0.56 ¹	1.79 \pm 0.49 ¹
Operation + PsL-EGFmAb	0.87 \pm 0.17 ³	76.45 \pm 15.24 ³	1.18 \pm 0.34 ³	0.89 \pm 0.48 ³

Abbreviations: TGF-beta1, transforming growth factor-beta1; alpha-SMA, alpha-smooth muscle actin; Col III, collagen III; FN, fibronectin. ¹ $P < 0.05$ and ² $P < 0.01$, compared with the sham-operation; ³ $P < 0.05$, compared with the operation, $n = 6$.

Table 3. Correlation between the expression of fibrogenic factors and the degree of TIL

	TGF-beta1	alpha-SMA	Col III	FN
r value	0.824 ¹	0.364 ²	0.636 ³	0.516 ³

Abbreviations: TGF-beta1, transforming growth factor-beta1; alpha-SMA, alpha-smooth muscle actin; Col III, collagen III; FN, fibronectin. ¹ $P < 0.001$; ² $P < 0.05$; ³ $P < 0.01$.

Table 4. Relationship between the number DC-SIGN⁺ DCs and BUN, Scr, Ccr, TGF-beta1, alpha-SMA, Col III and FN

	BUN	Scr	Ccr	TGF-beta1	alpha-SMA	Col III	FN	TIF
r value	0.254 ¹	0.263 ¹	-0.367 ²	0.477 ²	0.288 ¹	0.408 ²	0.495 ²	0.823 ³

Abbreviations: BUN, blood urea nitrogen; Scr, serum creatinine; Ccr, creatinine clearance; TGF-beta1, transforming growth factor-beta1; alpha-SMA, alpha-smooth muscle actin; Col III, collagen III; FN, fibronectin; TIF, tubulointerstitial fibrosis. ¹ $P < 0.05$; ² $P < 0.01$; ³ $P < 0.001$.

DCs in the renal interstitium, and also plays an important role in the early stage injury of the renal tubulointerstitium (9-10,18).

DCs are highly specialized antigen-presenting cells that can not only initiate but also negatively regulate the immune responses (19-20), which is associated with inflammatory diseases, autoimmune diseases, graft rejection, and tumors (21). Recent studies show that DC-SIGN is a PRR and adhesion receptor of DCs (12-14). On one hand, DC-SIGN could mediate DC migration and adhesion, antigen internalization, and T cell activation; on the other hand, DC-SIGN could be the target of certain pathogens, such as HIV or tumor cells, which may lead to escape from immune surveillance or immune suppression (14). As a multi-function molecule, DC-SIGN has been thought to serve as a new way to further investigate the immune regulation of DCs. However, it remains unclear whether DC-SIGN takes part in immune inflammatory diseases, such as kidney diseases.

In the current study, we found that DC-SIGN was expressed mainly on renal tubular epithelial cells in the early stage of nephritis, and its expression was significantly correlated with the degree of TIL. DC-SIGN was also expressed on HK-2 cells treated with TNF-alpha *in vitro*. PsL-EGFmAb exerted an inhibitory effect on the expression of DC-SIGN mRNA and co-stimulatory molecule CD80 mRNA in HK-2 cells. These findings indicate that

DC-SIGN can also be expressed on tubular epithelial cells and is highly correlated with TIL. Furthermore, the migration and distribution of DC-SIGN⁺ DCs in the renal tubulointerstitium in the early stage of nephritis were correlated not only with the expression of DC-SIGN, but also with the degree of TIL and renal function changes. PsL-EGFmAb inhibited DC-SIGN expression and DC-SIGN⁺ DC accumulation and had a protective effect on renal function. The results demonstrated that DCs take part in the inflammatory infiltration within the kidney in the early stage of nephritis, which is important in TIL and renal disease progression. The chronic renal interstitial fibrosis model further verified that DC-SIGN expression and DC-SIGN⁺ DC accumulation within the renal tubulointerstitium was associated with renal fibrosis and renal function deterioration, which can be inhibited by PsL-EGFmAb. Thus, DC-SIGN might also participate in the initiation of renal tubulointerstitial inflammation, and mediate DC taking part in renal TIL and fibrosis, which is correlated closely with the progression of renal disease.

It is known that the migration of immature DCs from the circulation to inflammatory tissues is mediated by the interaction of DC-SIGN and intercellular adhesion molecule (ICAM)-2 on endothelial cells together with selectins (22). The DC-SIGN-ICAM-3 interaction mediates mature DCs to contact and activate resting T cells and might contribute to initiation of the immune response and two-way immune regulation of a microenvironment for DCs under

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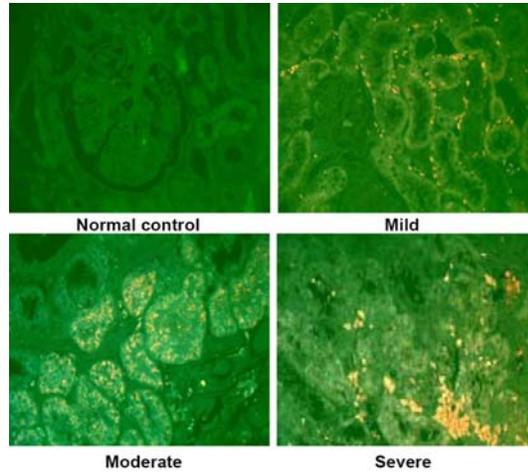


Figure 2. DC-SIGN⁺ DC distribution in renal tissue of patients with nephritis. Sections of renal tissue of patients with nephritis were stained with CD1a and DC-SIGN monoclonal antibodies (mAb) for immunofluorescent assay. Original magnification, $\times 1200$.

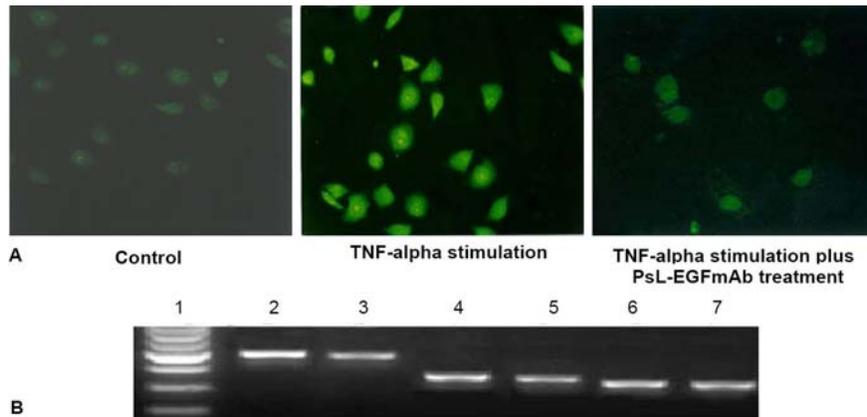


Figure 3. DC-SIGN and CD80 expression on HK-2 cells. (A) Proximal tubular epithelial cell line HK-2 was stimulated with TNF-alpha in the presence or absence of PsL-EGFmAb, with PBS as control. DC-SIGN expression was observed using fluorescence microscopy. Original magnification, $\times 200$. (B) Total RNA was extracted from TNF-alpha-stimulated HK-2 cells with or without treatment of PsL-EGFmAb. Expression of DC-SIGN mRNA and CD80 mRNA were detected by RT-PCR. (1) Marker. (2, 3) DC-SIGN mRNA expression in HK-2 cells treated with TNF-alpha or with TNF-alpha plus PsL-EGFmAb. (4, 5) Expression of CD80 mRNA on HK-2 cells treated with TNF-alpha or with TNF-alpha plus PsL-EGFmAb (6, 7) Both mRNA expressions were normalized to GAPDH.

normal or pathologic conditions (12-13,23). Recent studies have suggested that DC-SIGN is not only an important marker of DCs with both inflammatory and immunologic-regulating characteristics, but is also a molecular basis of DC initiating or mediating innate immune and acquired immune responses (14,24). Our previous study showed the intervention of DC-SIGN could inhibit human DC maturation and stimulation on T cells *in vitro*, and reduce T cell proliferation induced by DCs (15), indicating that inhibition of DC-SIGN can intervene in DCs initiating inflammatory immune responses. The results suggested that DC-SIGN might mediate DC induction of renal tubulointerstitial inflammation and immune responses, taking part in renal inflammatory defense and immune injury, which is related to the disturbance of immune regulation in the renal microenvironment, resulting in

interstitial fibrosis and the progression of the disease.

Renal tubular epithelial cells, with abundant biologic functions, play an important role in renal tubulointerstitial inflammation and immune response (3). We have found that DC-SIGN is expressed on human renal tubular epithelial cells in the early stage of nephritis and on HK-2 cells stimulated by inflammatory cytokines, and PsL-EGFmAb has an inhibitory effect on DC-SIGN and co-stimulatory molecules, indicating that the injured tubular epithelial cells might transdifferentiate into DC-like cells and then participate in the repair of kidney injuries and inflammatory reactions, but induce interstitial injury when they are persistently stimulated by inflammatory factors (25). Having been stimulated, tubular epithelial cells up-regulate adhesion molecule expression, secrete

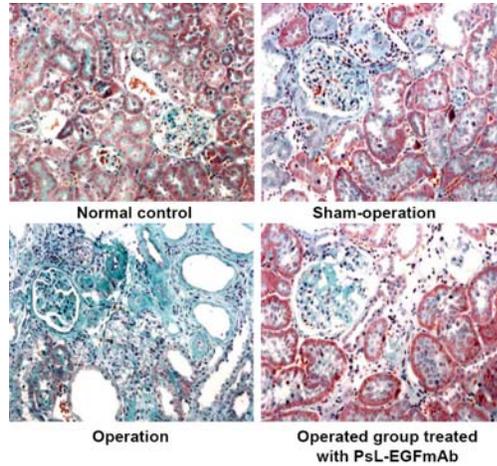


Figure 4. Histopathologic changes in renal tissues of rats 12 weeks after operation. Rats were surgically operated to set up animal model with nephritis. Renal tissues were obtained and sectioned for histopathologic examination 12 weeks after operation. The sections were stained and analyzed with Masson for tissue damage. Original magnification, $\times 200$.

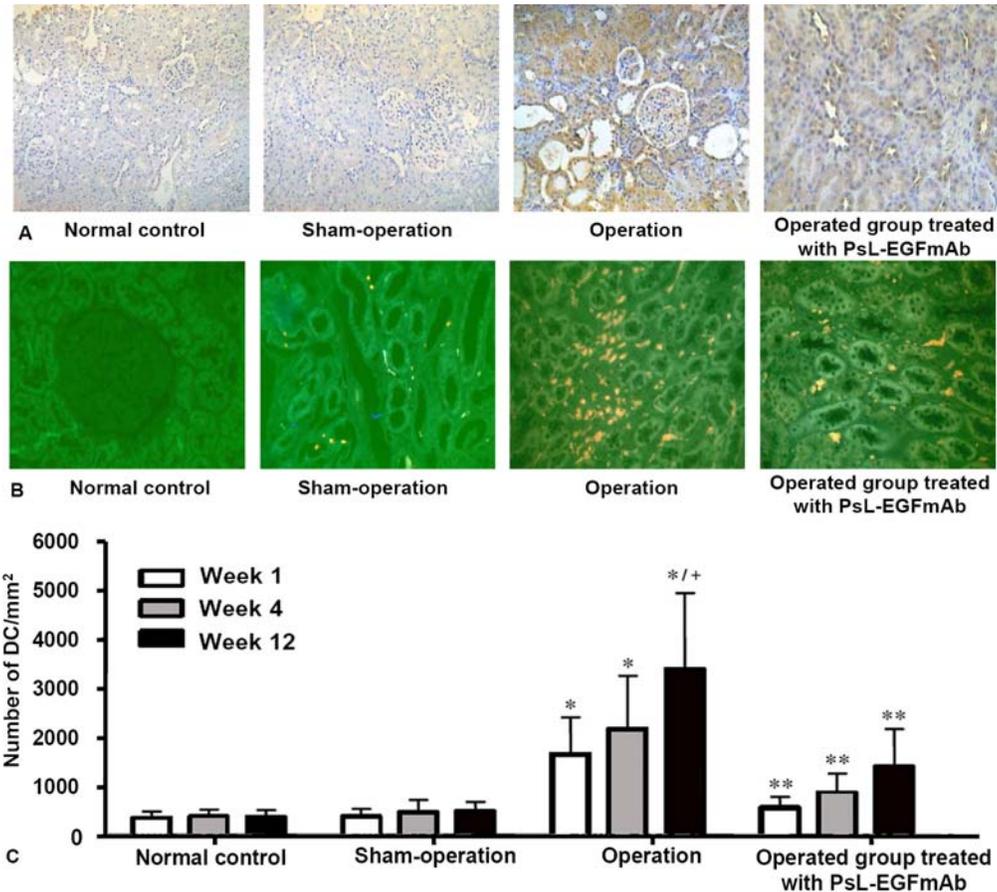


Figure 5. DC-SIGN expression and DC-SIGN⁺ DC distribution were assayed in renal tissues of rat with nephritis by 12 weeks. (A) The sections were stained and analyzed with DC-SIGN mAb. Original magnification, $\times 200$. (B) To assay the distribution of DC-SIGN⁺ DC, the sections were stained with DC-SIGN and OX62 mAbs. Original magnification, $\times 1200$. (C) DC-SIGN⁺ DC number in the indicated groups 1, 4, and 12 weeks after operation. To compare with the sham-operated group, $^*P < 0.01$; To compare with the operated group by 1 or 4 weeks, $^+P < 0.01$; To compare with the operated group, $^{**}P < 0.01$.

chemokines, attract circulating inflammatory cells for recruitment into injured areas in the tubulointerstitium, and initiate local inflammation. Thus, renal tubular epithelial cells might be a critical factor in tubulointerstitial damage (26-27). As important components of renal interstitium, tubular epithelial cells do not express or have a low level of expression of adhesion molecules under normal conditions. When they are stimulated in pathologic conditions, tubular epithelial cells might transdifferentiate to cells which can express high levels of co-stimulated molecules, conferring an antigen presenting function to the cells, and take part in renal tubulointerstitial inflammatory immune responses (28-29). It has aroused great concern that tubular epithelial cell transdifferentiation plays a role in the repair of tubular injuries and interstitial fibrosis (16-17). There is double biological significance that cell transdifferentiation can repair tissue injuries and induce tissue proliferation or fibrosis (30-31). This study also suggested that tubular epithelial cells play an important role in renal tubulointerstitial inflammation and immune responses. Further research is needed to investigate the interaction of tubular epithelial cells and DCs in a micro-inflammatory state, and the role of DC-SIGN in regulating tubular endothelial cell transdifferentiation. DC-SIGN might regulate local inflammation in the tubulointerstitium through modulation of the function of DC-like cells, which were transdifferentiated from renal tubular epithelial cells, as it does with DCs. However, the underlying mechanism remains to be further studied.

There is mounting evidence has been shown by recent studies that DCs play a key role in many diseases, such as nephritis and the inhibition of DCs has become a new strategy in the treatment of these diseases (10,32). In this study, we demonstrated that PsL-EGFmAb not only inhibits the expression of DC-SIGN, but also attenuates renal injuries by modulating DC adhesion and migration in nephritis. This monoclonal antibody can also intervene in DC maturation and function in inflammatory conditions (15). In accordance with the accumulating data in the literature, our results suggest that PsL-EGFmAb might be used as a new anti-adhesion and anti-inflammation approach in preventing and treating DC-related inflammatory immune diseases, such as nephritis.

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Abbreviations: DC: dendritic cell; DC-SIGN: dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin; PsL-EGFmAb: anti-P-selectin lectin-EGF domain monoclonal antibody; CRD: carbohydrate-recognition domain; PRR: pattern recognition receptor; mAb: monoclonal antibody; ICAM: intercellular adhesion molecule; IHC: immunohistochemistry; HE: hematoxylin/eosin; TGF-beta1: transforming growth factor-beta1; TNF-alpha: tumor necrosis factor-alpha; FITC: fluorescein isothiocyanate; RPE: R-Phycoerythrin; RT-PCR: reverse transcription-polymerase chain reaction; ECM: extracellular matrix; FN: fibronectin; Col III: collagen III; alpha-SMA: alpha-smooth muscle actin; TIL: tubulointerstitial lesion; TIF: tubulointerstitial fibrosis; BUN: blood urea nitrogen; Scr: serum creatinine; Ccr: creatinine clearance; UP, urine protein

Key Words: DC-SIGN, Dendritic Cells, Renal Tubular Epithelial Cells, Renal Tubulointerstitial Fibrosis, Chronic Kidney Diseases

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