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DC-SIGN modulates DC maturation and function in rat renal tubulointerstitial lesions

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1. ABSTRACT

The role of DC-SIGN in tubulointerstitial lesions (TILs) and the effect of anti-P-selectin lectin-EGF domain monoclonal antibody (PsL-EGFmAb) were investigated in rat nephrotoxic nephritis (NTN). On Day 4, immature DC-SIGN$^+$DCs infiltrated into renal tubulointerstitium and matured by Day 14, showing increased migratory capacity and ability to induce T cell proliferation. The distribution of DC-SIGN$^+$ DC significantly correlated with crescent formation, TIL severity, and changes in renal function. RANTES and TNF-alpha mRNA were continuously up-regulated from Day 4, while IL-10 mRNA was down-regulated after a marked increase on Day 4. Expression of IFN-gamma and IL-4 mRNA increased on Day 14 due to DC maturation. PsL-EGFmAb suppressed DC maturation, migration and ability to activate T cells. It also down-regulated TNF-alpha and up-regulated IL-10, resulting in a Th1/Th2 bias. The number of crescents decreased and TILs and renal function improved. These results suggest that DC-SIGN mediates DC tubulointerstitial infiltration and is an important regulator of local immune reactions and TILs. PsL-EGFmAb inhibited DC migration, maturation and function by targeting DC-SIGN, and may therefore be a potential treatment for NTN.

2. INTRODUCTION

Most glomerular diseases, regardless of etiology, are associated with tubulointerstitial lesions (TILs), which include tubular epithelial cell damage and tubulointerstitial fibrosis. Inflammatory cell infiltration into the interstitium results in immune reactions, which are important pathological and physiological processes involved in TIL development, particularly during the development of progressive glomerular disease (1-2). Therefore, investigating the immunological mechanisms underlying TIL development may help to clarify the progression of glomerular diseases.

Our previous work suggested that the adhesion molecule, P-selectin, which mediates the adhesion and infiltration of dendritic cell (DCs), contributes to the initiation of renal tubulointerstitial inflammation and injury. Anti-P-selectin lectin-EGF domain monoclonal antibody (PsL-EGFmAb) inhibits DC infiltration and abrogates the formation of interstitial lesions (3-4). Furthermore, PsL-EGFmAb suppresses DC maturation and function *in vitro*, which may be associated with suppression of DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN); a molecule belonging to the C-

type lectin family, which includes P-selectin (3-5). Recent studies report that macrophages are mainly distributed throughout the glomeruli, while DCs (mainly DC-SIGN$^+$ myeloid DCs) predominantly accumulate in the tubulointerstitium (6-7). However, whether DCs are involved in the development of interstitial lesions, or have protective effects, remains controversial (8-10). One report speculated that interstitial lesions are associated with DC maturation, and function under steady state or disease conditions (11). Further studies on DC maturation and function and the immune molecules that have a regulatory effect on DCs are necessary. DC-SIGN is a pattern recognition receptor (PRR) and an adhesion receptor, and as such plays a critical role in regulating both innate and adaptive immune responses (12). Therefore, on the basis of our previous findings, we treated rats that had immune-mediated nephrotoxic nephritis (NTN) with PsL-EGFmAb to investigate the role of DCs and DC-SIGN in TIL formation, and explored the mechanisms underlying TIL formation in glomerular diseases.

3. MATERIALS AND METHODS

3.1. Animals and reagents

Male Wistar-Kyoto (WKY) rats and male New Zealand white rabbits were purchased from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China). Freund's complete adjuvant (CFA) and Freund's incomplete adjuvant (FIA) were purchased from Sigma (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI) 1640 and fetal bovine serum (FBS) were purchased from Gibco BRL (Crewe, Cheshire, UK). Collagenase D was purchased from Roche Applied Science (Mannheim, Germany). Goat anti-rat DC-SIGN polyclonal antibody, phycoerythrin (PE)-conjugated donkey anti-goat IgG, and rabbit anti-rat factor VIII monoclonal antibody (mAb) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-rat MHC II mAb and PE-conjugated anti-rat CD80 mAb were purchased from eBioscience (Camarillo, CA, USA). TRIzol was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). The RevertAid First Strand cDNA Synthesis Kit was purchased from Fermentas (Burlington, ON, Canada). SYBR Green PCR Master Mix was purchased from Applied Biosystems (Foster City, CA, USA). Anti-rat OX62, CD4 and CD31 microbeads were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Stromal cell-derived factor-1 (SDF-1) was purchased from Peprotech (Rocky Hill, NJ, USA). Cytokine enzyme-linked immunosorbent assay (ELISA) kits for rat interferon-gamma (IFN-gamma) and interleukin-4 (IL-4) were purchased from Biosource (Camarillo, CA, USA). Nycodenz was purchased from Axis Shield (Oslo, Norway). Transwell 24-well plates (8-μm pores) were purchased from Corning (Corning, NY, USA). CFSE dye was purchased from Molecular Probes (Carlsbad, CA, USA). Rabbit anti-rat nephrotoxic serum and PsL-EGFmAb were prepared by our laboratory.

3.2. Animal models

Nephrotoxic rabbit serum was generated as previously described (13). Briefly, rabbits were immunized with homogenized rat renal cortex in CFA, followed by monthly boosting doses in FIA. NTN was induced by injecting 1 μl of nephrotoxic rabbit serum per gram of rat body weight as previously described (13). Fifty-four rats were randomly assigned into three groups: the control group was injected with 0.9% saline (n = 18), the NTN group was injected with nephrotoxic rabbit serum (n = 18), and the PsL-EGFmAb-treated group was injected with nephrotoxic rabbit serum plus PsL-EGFmAb (2 μg per gram rat body weight) and, 2 hours later, injected with PsL-EGF mAb again (2 μg per gram rat body weight) (n = 18). Four, seven and 14 days later, rats from each group were anesthetized with ether and euthanized. Blood samples were obtained to measure renal function, and the kidneys were harvested. Urine was collected the day before euthanasia by housing the animals in metabolic cages for 24-hour urine protein (UP) measurement. Harvested kidneys were quickly fixed in 10% buffered formaldehyde, or minced and digested for DC isolation. All animal studies were approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.

3.3. Biochemical analysis and histopathological measurements in rats

The serum obtained from the WKY rats was tested for blood urea nitrogen (BUN) and serum creatinine (Scr) using a Beckman Automatic Biochemical Analysis Instrument (Fullerton, CA, USA) and the creatinine clearance (Ccr) was calculated. Paraffin-embedded kidney sections were prepared (2 μm thick) using routine procedures. Sections were stained with hematoxylin/eosin or periodic acid- Schiff (PAS) and the gross histology was observed under a light microscope. Injury was evaluated in at least 50 glomeruli per section. Tubulointerstitial damage was evaluated as previously described (8).

3.4. Immunofluorescence staining to determine DC-SIGN$^+$ DC distribution in renal tissues

The distribution of DC-SIGN$^+$ DCs within renal tissues was assessed by immunofluorescence staining using the microscopic image method. Renal tissue sections from rats were blocked with 0.3% bovine serum album (BSA) for 20 min and then incubated with goat anti-rat DC-SIGN polyclonal antibody (1:100) overnight at 4°C. Subsequently, the sections were exposed to PE-conjugated donkey anti-goat IgG (1:200) for 1 h at 37°C, washed with PBS, and mounted. The primary antibody was replaced by PBS for the negative control. The sections were observed under a fluorescence microscope. The area (mm^2) of DC-SIGN$^+$ DCs was analyzed using the KS400 imaging analysis system (Zeiss, Germany).

3.5. Detection of P-selectin, RANTES, TNF-alpha, IL-10, IFN-gamma and IL-4 mRNA levels by real-time PCR

Total RNA was extracted from kidney tissues using TRIzol. cDNA was quantified by quantitative real-time PCR, using beta-actin as a control. Quantitative real-time PCR was performed using a SYBR Green PCR mix in an ABI Prism 7900HT thermocycler (Applied Biosystems). Thermocycler conditions included an initial holding step at 50°C for 2 min, followed by 95°C for 10 min; this was

Table 1. Analysis of UP, BUN, Scr and Ccr in rats at Day 4 post-induction of NTN

Group	UP (mg/24 h)	BUN (mmol/L)	Scr (μmol/L)	Ccr (ml·min^{-1})
Control	9.91±1.56	4.67±0.67	32.23±3.24	4.01±1.02
NTN	54.80±3.43[1]	10.40±0.77[1]	86.45±4.00[1]	1.21±0.09[1]
PsL-EGFmAb	32.68±2.91[2]	6.83±0.83[2]	50.69±4.49[2]	2.58±0.48[2]

Abbreviations: UP, urine protein; BUN, blood urea nitrogen; Scr, serum creatinine; Ccr, creatinine clearance. [1]$P < 0.01$, compared with the control; [2]$P < 0.01$, compared with NTN, n = 6

followed by a two-step PCR program: 95°C for 15 s and 60°C for 60 s for 40 cycles. Data were collected and quantitatively analyzed using an ABI PRISM 7900 sequence detection system (Applied Biosystems). The primer sequences were designed using the Primer Express Software version 2.0 provided with the ABI Prism 7900HT thermocycler (P-selectin, forward, 5'-AGT GTA GTC CTG GGA GCA G-3' and reverse, 5'-TGA GCG ATT TCA TTC TTG T-3'; RANTES, forward, 5'-ATC CCT CAC CGT CAT CCT-3' and reverse, 5'-CTT GCT GCT GGT GTA AAA-3'; TNF-alpha, forward, 5'- GAC CCT CAC ACT CAG ATC ATC-3' and reverse, 5'-ACG CTG GCT CAG CCA CTC-3'; IL-10, forward, 5'- CCC TCT GGA TAC AGC TGC G-3' and reverse, 5'-GCT CCA CTG CCT TGC TTT TAT T-3'; IFN-gamma, forward, 5'- CTA CAC GCC GCG TCT TGG T-3' and reverse, 5'-GAG GCT CTT TCC TTC CAT-3'; IL-4, forward, 5'-CAG CGG TCT GAA CTC ACT-3' and reverse, 5'-GCA AGT ATT TCC CTC GTAG-3'; beta-actin, forward, 5'-CGT GAA AAG ATG ACC CAG ATC A-3' and reverse, 5'-AGA GGC ATA CAG GGA CAA CAC A-3'). The mRNA levels of the samples were quantitatively analyzed using the $2^{-\Delta CT}$ method.

3.6. Phenotypic analysis of MHC II, DC-SIGN and CD80 expressed in renal DCs

DCs were isolated from rat kidneys as previously described (9,13). Briefly, kidneys were finely minced and digested for 45 min at 37°C with 2 mg/ml collagenase D in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum. Cell suspensions were filtered through a 30 μm nylon mesh, and washed with HBSS without Ca^{2+} or Mg^{2+} supplemented with 10 mmol/L EDTA, 0.1% BSA, and 10 mM Hepes. Density centrifugation was performed at 1700 g for 20 min at 4°C using 1.080 g/ml Nycodenz. The cells at the interphase were harvested and isolated using anti-rat OX62 microbeads. Briefly, 0.5×10^6 isolated cells were stained with FITC- and PE-labeled mAbs specific for MHC II and CD80. In addition, 0.5×10^6 DCs were indirectly stained for DC-SIGN using a goat anti-rat DC-SIGN polyclonal antibody and a PE-conjugated donkey anti-goat IgG mAb. Phenotypic analysis was performed by flow cytometry using a FACSCalibur (BD FACSAria™ Cell Sorter).

3.7. DC transendothelial migration and mixed lymphocyte reaction

Vascular endothelial cells were isolated using anti-rat CD31 microbeads, confirmed using anti-rat factor VIII, and then seeded onto 24-well transwell plates (8-μm pores) coated with fibronectin (20 μg/ml) for 1 h at 37°C. DCs (200,000; stained with CFSE) were added to the vascular endothelial cell monolayer. The lower chamber contained SDF-1 (10 ng/ml). After 24 h at 37°C, the number of transmigrated DCs (lower chamber) was determined by flow cytometry.

The ability of the DC to stimulate $CD4^+$ T cells was assayed in a mixed lymphocyte reaction. Allogeneic $CD4^+$ T cells, isolated from peripheral blood mononuclear cells using magnetic bead-labeled anti-rat CD4 mAb, were incubated with irradiated (30 Gy) DCs at a ratio of 10:1 in a 96-well U-bottomed plate at 37°C for 5 days. After 5 days, (^{3}H)TdR (1 μCi/well) was added for 12–16 hours before the end of culture. Triplicate wells were cultured for each group.

3.8. Quantitation of IFN-gamma and IL-4 levels by ELISA

The supernatants from the MLR were collected. The concentration of IFN-gamma and IL-4 was determined using ELISA kits according to the manufacturer's instructions.

3.9. Statistical analysis

SPSS software, version 11.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data are presented as the mean ± SD and evaluated by one-way ANOVA and Spearman's correlation as indicated. A P value < 0.05 was considered significant.

4. RESULTS

4.1. Changes in renal function and histopathology in rats

On Day 4 post-induction of NTN the 24-hour UP level in the NTN group increased ($P < 0.01$), BUN and Scr increased slightly, and there was no clear change in Ccr. From Day 7 to Day 14, 24-hour UP, BUN and Scr levels progressively increased ($P < 0.01$), while Ccr levels progressively decreased ($P < 0.01$). After treatment with PsL-EGFmAb, BUN and Scr levels both decreased ($P < 0.01$), and the Ccr level increased ($P < 0.01$), suggesting that renal function had improved (Table 1).

On Day 4 post-NTN induction, glomerular focal segmental proliferation, tubular mild vacuolation degeneration and interstitial inflammatory cell infiltration were observed in rats in the NTN group. Atypical cellular crescents were observed on Day 7. On Day 14, the size of the glomeruli increased, and the proportion of crescents was < 50% (59.41 ± 2.22%, $P < 0.01$). Destruction of the Bowman's capsule wall, denudation of the glomerular basement membrane (GBM) and tubular basement membrane (TBM), focal and diffuse infiltration by inflammatory cells, and edema were also observed. Pathological changes at Day 14 resembled typical crescentic glomerulonephritis (Figure 1A). PsL-EGFmAb decreased the formation of crescents, especially by Day 14,

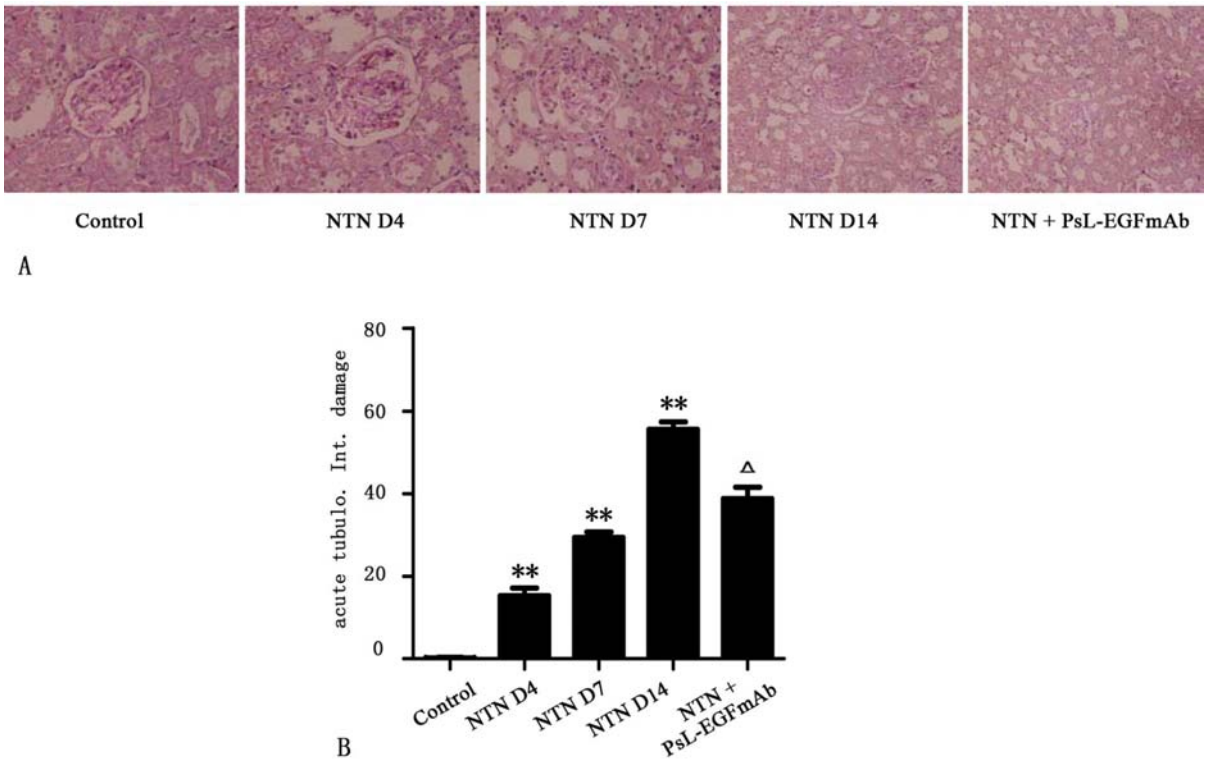

Figure 1. Pathology in rat renal tissues. (A) Sections of rat renal tissue at Days 4, 7 and 14 post-induction of NTN and Day 14 after treatment with PsL-EGFmAb were stained with PAS. Original magnification, ×200. (B) Acute tubulointerstitial damage in rat renal tissues in the indicated groups. $^{**}P < 0.01$, compared with the control; $^{\Delta}P < 0.01$, compared with NTN D14. One representative experiment from three is shown.

when the proportion of crescents was 33.90 ± 6.46%. mAb treatment also alleviated TILs (Figure 1A-B).

4.2. DC-SIGN$^+$ DC distribution and P-selectin expression in rat renal tissues

DC-SIGN$^+$ DCs are usually scattered throughout the normal renal tubulointerstitium; however, their numbers progressively increased from Day 4 post-induction of NTN ($P < 0.01$). DCs mainly accumulated in the tubulointerstitium, or clustered around injured glomeruli, but were not observed within the glomeruli (Figure 2A-B). The distribution of DC-SIGN$^+$ DCs significantly correlated with the number of crescentic glomeruli, the severity of interstitial lesions, and Scr and Ccr levels (r = 0.678, 0.701, 0.673, –0.772, respectively; $P < 0.01$). In addition, expression of P-selectin mRNA was virtually undetectable in normal renal tissues, whereas expression increased continuously from Day 4 post-induction of NTN ($P < 0.01$; Figure 2C) and correlated with DC-SIGN$^+$ DC distribution (r = 0.994, $P < 0.01$). PsL-EGFmAb treatment markedly down-regulated P-selectin mRNA expression, and the number of DC-SIGN$^+$ DCs in kidneys was also reduced, especially on Day 14 ($P < 0.01$; Figure 2A-C).

4.3. Expression of chemokines and cytokines in rat renal tissues

Compared with the low expression of chemokines and cytokines observed in normal rat renal tissues, the expression of RANTES and TNF-alpha mRNA progressively increased from Day 4 post-induction of NTN ($P < 0.01$; Figure 3A-B), whereas IL-10 mRNA levels increased significantly at Day 4 and decreased again at Day 14 ($P < 0.01$; Figure 3C). In addition, levels of IFN-gamma and IL-4 mRNA were both markedly up-regulated on Day 14 after only a mild increase on Day 4 ($P < 0.01$; Figure 3D-E). Both IFN-gamma and IL-4 mRNA levels were enhanced on both Day 7 and Day 14 ($P < 0.01$; Figure 3F). PsL-EGFmAb treatment down-regulated the expression of RANTES and TNF-alpha mRNA and the IFN-gamma/IL-4 mRNA ratio ($P < 0.01$; Figure 3A-B, F), and up-regulated IL-10 mRNA expression ($P < 0.01$; Figure 3C).

4.4. DC maturation and function in renal tissues

DCs isolated from normal rat kidneys were immature. DCs were also immature in rat kidneys at Day 4 post-induction of NTN. However, the expression of MHC II and CD80 was mildly up-regulated and was accompanied by a down-regulation of DC-SIGN expression (Figure 4A). The ability of DCs to migrate and stimulate CD4$^+$ T cells was also weakened (Figure 4B), and the concentration of IFN-gamma and IL-4 in the MLR supernatants was low (Figure 4C). On Day 7, MHC II and CD80 expression was progressively up-regulated and accompanied by a down-regulation of DC-SIGN expression. The ability of DCs to migrate and stimulate CD4$^+$ T cells also improved ($P < 0.01$; Figure 4) and the expression of IFN-gamma, IL-4,

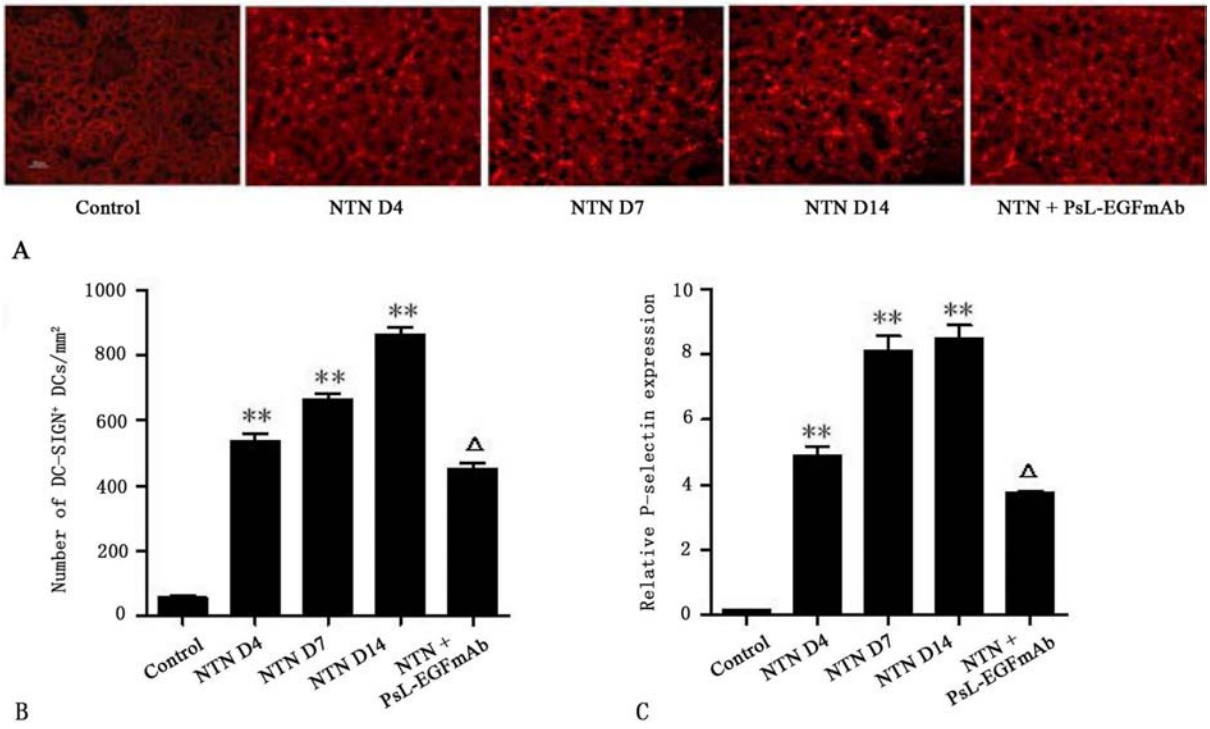

Figure 2. DC-SIGN$^+$ DC distribution and P-selectin expression in rat renal tissues. (A) Sections of rat renal tissue at Days 4, 7 and 14 post-induction of NTN and at Day 14 after treatment with PsL-EGFmAb were stained with DC-SIGN polyclonal antibody. Original magnification, ×200. (B) Number of DC-SIGN$^+$ DC in rat renal tissues from the indicated groups. (C) Relative expression levels of P-selectin in rat renal tissues was detected by real-time PCR. $^{**}P < 0.01$, compared with the control; $^{\Delta}P < 0.01$, compared with NTN D14. One representative experiment from three is shown.

and the IFN-gamma/IL-4 ratio increased ($P < 0.01$; Figure 5A-C). The changes observed on Day 7 indicated that the DCs were semi-mature. On Day 14, expression of MHC II and CD80 markedly increased (Figure 4A), the ability of DCs to migrate and stimulate T cells improved significantly (Figure 4B-C), and the concentration of IFN-gamma and IL-4 and IFN-gamma/IL-4 increased (Figure 5A-C). The changes observed on Day 14 indicated that DCs were mature and that the induced T cells had differentiated into Th1 cells. Furthermore, the ability of DCs to stimulate T cells was clearly related to the ratio of IFN-gamma/IL-4 mRNA expression ($r = 0.900$, $P < 0.01$). Treatment with PsL-EGFmAb suppressed DC maturation, migration and the ability to stimulate T cells ($P < 0.01$; Figure 4A), and was accompanied by a down-regulation of DC-SIGN and CD80 expression. The IFN-gamma/IL-4 ratio also decreased significantly ($P < 0.01$; Figure 5C). These results suggest that PsL-EGFmAb suppresses IFN-gamma expression, but does not affect IL-4 (Figure 5B-C).

5. DISCUSSION

The adaptive immune response mediated by cellular immunity plays a critical role in the development of glomerular diseases, especially immune-related crescentic nephritis (14-15). CD4$^+$ T cell differentiation into Th1 cells plays an important role in initiating these immune reactions. Th1 cells induce delayed type hyper-sensitivity (DTH)-like reactions and crescentic renal lesions (14-16). The number and function of local regulatory T cells (Tregs) and the balance between Th17 cells and Tregs are closely associated with disease progression (15,17,18). In addition, damage to resident renal cells, especially renal tubulointerstitial cells, can also play an important role, in regulating local microenvironmental immune reactions or lesions by secreting chemokines and cytokines that recruit inflammatory cells (19).

An increasing number of studies have focused on the mechanisms underlying the development of renal TILs in glomerular disease. The recently proposed hypothesis of renal immune compartmentalization may help to address these questions (20). Detailed mechanistic information on the role of T cells in immune-mediated kidney diseases was first obtained from murine models of interstitial nephritis and, later, from models of glomerular nephritis, including NTN, which is a model of human crescentic nephritis (15,21). DCs also play a potent regulatory role in T cell activation and the initiation of immune reactions (11,14-15). DC maturation and function, in particular their interaction with T cells, directly impacts on T cell activation, immune responses, and the ensuing Th1/Th2 balance (22-24). It is known that immune regulation of DCs is related to molecular pattern recognition by innate immune molecules such as C-type lectins and Toll-like receptors (TLRs) (12). However, the mechanisms underlying immune regulation by DCs within renal interstitial lesions remain to be fully elucidated (11).

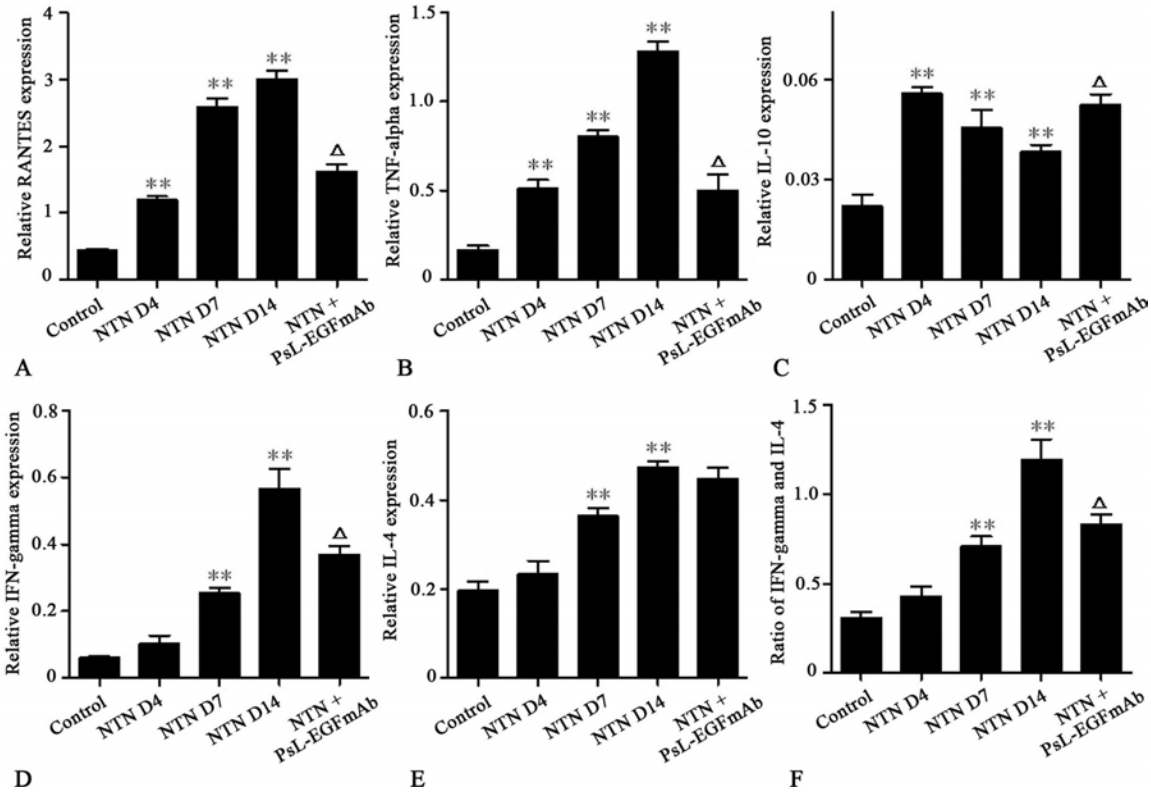

Figure 3. Relative expression of chemokines and cytokines in rat renal tissues. (A–E) Relative expression of RANTES, TNF-alpha, IL-10, IFN-gamma and IL-4 at Days 4, 7 and 14 post-induction of NTN and on Day 14 after treatment with PsL-EGFmAb measured by real-time PCR. (F) Ratio of IFN-gamma to IL-4 in the indicated groups. $^{**}P < 0.01$, compared with the control; $^{\Delta}P < 0.01$, compared with NTN D14. One representative experiment from three is shown.

In this study, we used a rat model of NTN and found that, during the early stages of the disease, DC-SIGN^{+} DCs infiltrated into the renal tubulointerstitium. Chemokines and pro-inflammatory factors were up-regulated, whereas anti-inflammatory factors were slightly down-regulated (after an initial increase). It was therefore clear that the Th1 response was generated after the induction of NTN. PsL-EGFmAb inhibited the infiltration of DC-SIGN^{+} DCs, regulated the production of pro- and anti-inflammatory factors at the mRNA level, (resulting in a bias in the Th1/Th2 balance), and improved rat renal lesions and function. Thus, renal infiltration by DCs is associated with both P-selectin and DC-SIGN. PsL-EGFmAb suppressed the expression of both P-selectin and DC-SIGN. DC-SIGN, a member of the C-type lectin family, is mainly expressed on immature DCs and is down-regulated as they mature (12,25). DC-SIGN also functions as a PRR and an adhesion molecule. DC-SIGN has both positive and negative regulatory effects on the immune regulation of DCs. It mediates the contribution of DCs to pathogen and tumor escape by recognizing molecular patterns, such as the Lewis^{X} antigen and intercellular adhesion molecule (ICAM)-2 or ICAM-3, on pathogens and cells, and by cross-talk with TLRs (25-27). DC-SIGN can also mediate the interactions between DCs and vascular endothelial cells, naive T cells and neutrophils. It also regulates DC migration and initiation of immune responses, and coordinates with adjacent cells (28-29). This study indicates that DC-SIGN is also involved in DC renal infiltration mediated by P-selectin, which regulates the contribution of DCs to renal defense or immune lesions by initiating renal tubulointerstitial inflammatory reactions. Therefore, DC-SIGN may be an important factor in determining the local Th1/Th2 imbalance, leading to crescentic renal lesions and disease progression.

We also found that DCs infiltrating the kidneys undergo a process of maturation, and play a role in renal inflammation and disease progression. This process was identical in all the phases of NTN, and was similar to a DTH reaction (9,22-24). During the early stages of NTN, DC infiltration and initiation of renal tubular interstitial inflammation was observed. The levels of pro- and anti-inflammatory factors also increased, and were related to the involvement of immature DCs in the initiation of local immune defenses. As NTN progressed, DCs matured and, acting as antigen-presenting cells, induced T cells to differentiate into Th1 cells. This resulted in the development of TILs. Thus, our results may shed light on the controversy surrounding whether DCs are involved in TIL formation, or in defense (8–10). PsL-EGFmAb treatment suppressed DC maturation and function by interacting with DC-SIGN (which has a similar carbohydrate recognition domain to that of P-selectin) and inducing Tregs (data not shown). These results indicate that DC-SIGN plays an important role in DC migration, maturation and function.

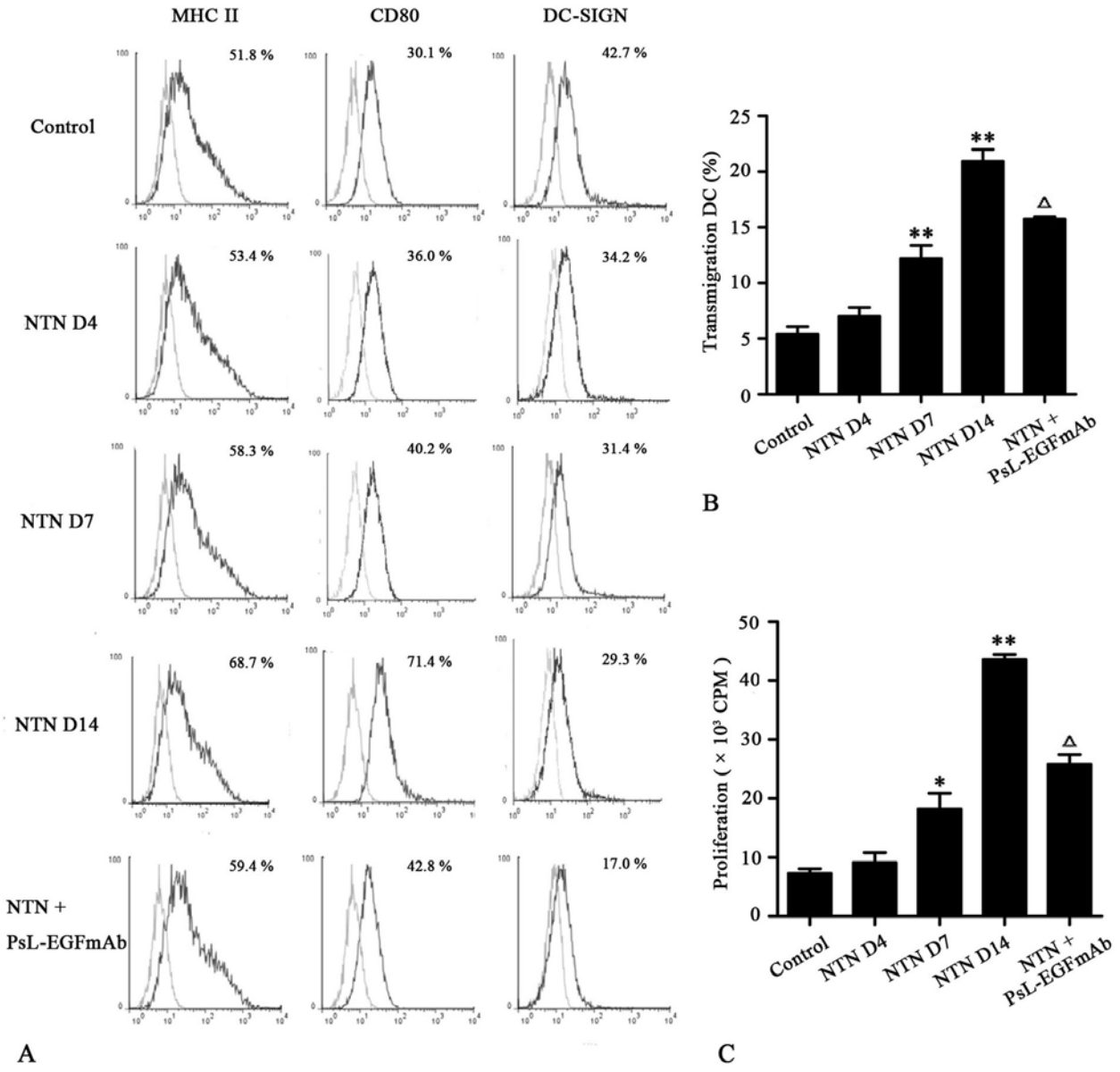

Figure 4. Phenotype and function of isolated DCs. (A) MHC II, CD80 and DC-SIGN expression on DCs isolated from rat renal tissues at Days 4, 7 and 14 post-induction of NTN and Day 14 after treatment with PsL-EGFmAb. (B) Transmigration of isolated DCs in the indicated groups. (C) Ability of isolated DCs to stimulate CD4+ T cells. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the control; $^{\Delta}P < 0.01$, compared with NTN D14. One representative experiment from three is shown.

DCs predominantly accumulate in the renal tubulointerstitium in most renal diseases, and this may be a common characteristic of all types of nephritis. TILs are critical for the progression of glomerular diseases (3,5-7). According to the hypothesis of renal immune compartmentalization, this may be related to the fact that the glomerulus lacks lymph nodes, and therefore T cells do not accumulate. This results in a predominant innate response. However, the tubulointerstitium, with its resident population of DCs, is the site of an acquired immune response (20), which may explain why chronic renal diseases progress to renal failure.

It is thought that TILs occur secondary to glomerular lesions (1,2). Our results indicate that lesions in the glomerular and tubulointerstitium occur simultaneously, and may be associated with anti-GBM antibodies in the nephrotoxic serum interacting with both the GBM and TBM, since they express similar antigens. They might also explain why macrophages mainly infiltrated the glomeruli, while DCs prominently accumulated in the tubulointestitium in the early stage of NTN (6-7). This finding is also consistent with the hypothesis of renal immune compartmentalization (20). Furthermore, DC clustering around injured glomeruli and the presence of

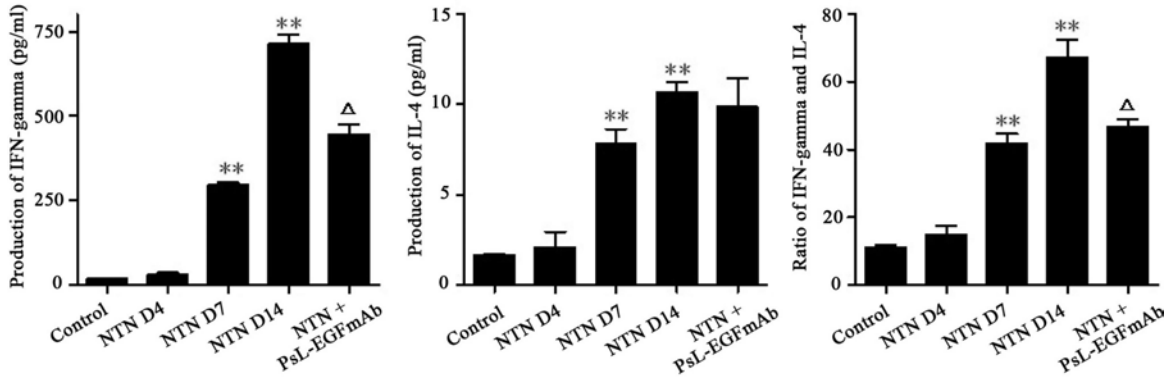

Figure 5. Ability of isolated DCs to produce Th1 and Th2 cytokines. (A,B) Expression of IFN-gamma and IL-4 in the MLR supernatants. (C) Ratio of IFN-gamma to IL-4. $^{**}P < 0.01$, compared with the control; $^{\Delta}P < 0.01$, compared with NTN D14. One representative experiment from three is shown.

inflammatory cells, such as macrophages, may contribute to damage of the Bowman's capsule, promoting glomerular inflammation. After DC-SIGN mediates DC migration into the tubulointestitium, it functions as a PRR, interacting with molecular pattern groups, such as Lewis^X antigen, in the hidden antigens of the TBM or tubular epithelial cells (30); thus, regulating DC maturation and inducing inflammatory immune reactions or TIL formation.

6. ACKNOWLEDGMENTS

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Abbreviations: TIL: tubulointerstitial lesions; DC: dendritic cell; PsL-EGFmAb: Anti-P-selectin lectin-EGF domain monoclonal antibody; DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; PRR: pattern recognition receptor; NTN: nephrotoxic nephritis; WKY: Wistar-Kyoto; CFA: Freund's complete adjuvant; FIA: Freund's incomplete adjuvant; mAb: monoclonal antibody; PE: phycoerythrin; FITC: Fluorescein isothiocyanate; BSA: bovine serum albumin; SDF-1: Stromal cell-derived factor-1; ELISA: enzyme-linked immunosorbent assay; IFN-gamma: interferon-gamma; IL-4: interleukin-4; CFSE: carboxyfluorescein diacetate, succinimidyl ester; UP: urine protein; BUN: blood urea nitrogen; Scr: serum creatinine; Ccr: creatinine clearance; HE: hematoxylin/eosin; PAS: Schiff periodic acid shiff; MLR: mixed lymphocyte reaction; TLR: toll-like receptor; GBM: glomerular basement membrane; TBM: tubular basement membrane; DTH: delayed type hypersensitivity; Tregs: regulatory T cells; ICAM: intercellular adhesion molecule.

Key Words: Glomerulonephritis, Renal tubulointerstitial lesions, Dendritic cell, DC-SIGN, Anti-P-selectin, Domain monoclonal antibody

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