Supplemental material

Immunohistochemical staining:

Immunohistochemical staining was performed for quantification purposes since quantification is easier on immunohistochemical-stained compared to immunofluorescence-stained slides. All immunohistochemical staining was performed on deparaffinized 4 µm sections of formalin-fixed paraffin-embedded (FFPE) material of allograft kidney biopsies. Endogenous peroxidase activity was blocked by incubating the sections in 3% Hydrogen Peroxide and 100% Alcohol 1:1 for 15 minutes.

DC-SIGN (CD209) staining: Heat induced antigen retrieval was performed with Target Retrieval Solution, pH 6.0 (Dako, Carpinteria, CA) in pressure cooker heated 123 ± 2^{o}C for 45 seconds at 15 PSI. Primary anti-DC-SIGN mouse monoclonal antibody (R&D Systems, Minneapolis, MN; clone: 120612) was applied at 1:50 dilution for 45 minutes in room temperature. The sections were washed with TBS/Tween 20 for 10 minutes, incubated with Labeled Polymer-HRP anti-mouse (Dako EnVision+ System) for 30 minutes, visualized with DAB (brown color) for 5 minutes, and counterstained with Harris Hematoxylin.

Double immunohistochemical staining for DC-SIGN and CD3: Antigen retrieval was performed in pressure cooker with citrate buffer (similar to DC-SIGN staining described above). DC-SIGN staining was performed and visualized with DAB (brown color) as above. Primary anti-CD3 rabbit polyclonal antibody (Dako, A0452; dilution 1:300) was then applied for 45 minutes, washed with TBS/Tween 20, incubated with Poly-AP anti-rabbit (Leica Biosystems, Cat# PV6133, Buffalo Grove, IL) for 30 minutes, visualized with PermaRed/AP (red color, DBS; Cat# K049) for 5 minutes, and counterstained with Harris Hematoxylin.

Double immunohistochemical staining for CD68 and CD3: Antigen retrieval was performed in pressure cooker with citrate buffer (similar to DC-SIGN staining described above). Primary anti-CD3 rabbit polyclonal antibody (Dako, A0452; dilution 1:300) was first applied for 45 minutes, washed with TBS/Tween 20, incubated with Labeled Polymer-HRP anti-rabbit (Dako EnVision+ System) for 30 minutes, visualized with DAB (brown color) for 5 minutes, and washed again. Primary Anti-CD68 mouse monoclonal antibody (Dako, clone: KP1; dilution 1:500) was then applied for 40 minutes, washed, incubated with Poly-AP anti-mouse (Leica Biosystems, Cat# PV6110) for 30 minutes, visualized with PermaRed/AP (red color; DBS; Cat# K049) for 5 minutes, and counterstained with Harris Hematoxylin.

Double immunohistochemical staining for CD3 and Ki67: Antigen retrieval was performed in pressure cooker with citrate buffer (similar to DC-SIGN staining). Ki67 antibody was first applied (DakoCytomation, Cat# M7240, mouse monoclonal, clone: mb1, 1:200 dilution) for 40 minutes and the sections were washed in TBS followed by 30 minutes incubation with labeled Polymer-HRP anti-mouse IgG and visualized with DAB (brown color) as above. Primary anti-CD3 rabbit polyclonal antibody (Dako, A0452; dilution 1:300) was then applied for 45 minutes, washed with TBS/Tween 20, incubated with Poly-AP anti-rabbit (Leica Biosystems, Cat# PV6133, Buffalo Grove, IL) for 30 minutes, visualized with PermaRed/AP (red color, DBS; Cat# K049) for 5 minutes, and counterstained with Harris Hematoxylin

Quantification for immunohistochemical staining:

In a subset of 40 allograft biopsies, leukocyte staining was quantified in different anatomic microcompartments as follows: (1) Glomeruli: average number of stained leukocyte per glomerulus (2) Peritubular capillaries, tubules, and arteries: number of stained leukocytes in the most inflamed peritubular capillary, tubule, and artery, respectively.

In all allograft biopsies, densities of interstitial DC-SIGN$^+$, CD68$^+$, and CD3$^+$ cells in the cortex of allograft biopsies were determined as follows: Microscopic images from the complete sampled cortex was obtained under x600 magnification. Positively stained cells were counted in each high power images and cell densities were determined by averaging the number of positively stained cells per high power field (hpf). Because DC-SIGN$^+$ cells often show cytoplasmic projections, only cells with circumferential staining around the nuclei were counted. Concerning ki67/CD3 double immunostaining in biopsies with high total inflammation score, the percentage of Ki-67/CD3 cells was assessed among ≥200 lymphocytes using at least three x600 microscopic fields as recommended[1]. The inflamed areas with most abundant ki67 distribution were chosen

Immunofluorescence staining:

Immunofluorescence staining was performed for illustration purposes since cellular detailes (e.g. cytoplasmic protrusions) are better observed using immunofluorescence microscopy compared to immunohistochemical-stained sections assessed by light microscopy. All immunofluorescence staining were performed on deparaffinized 4 µm sections of FFPE sections of allograft kidney biopsies.

Double immunofluorescence staining for DC-SIGN and CD31: Antigen retrieval was performed in pressure cooker with citrate buffer (similar to DC-SIGN immunohistochemical staining described above). Primary anti-CD31 rabbit polyclonal antibody (Abcam, Cambridge, MA; ab28364; dilution 1:50) was first applied. Sections were incubated at 4°C overnight and washed in PBS twice (5 minutes each). Primary DC-SIGN antibody was applied at 1:40 dilution, incubated 45 minutes in room temperature, and washed in PBS twice (5 minutes each). Alexa

Fluor 568 donkey anti-rabbit IgG (Life technologies, A10042; dilution 1:200) mixed with Alexa Fluor 488 donkey anti-mouse IgG (Life technologies, A11029; dilution 1:200) were then applied. The sections were incubated for 30 minutes at room temperature, washed in PBS twice (5 minutes each), and mounted with Fluorescent Mounting Medium.

Double immunofluorescence staining for DC-SIGN and CD3: Antigen retrieval was performed in pressure cooker with citrate buffer (similar to DC-SIGN staining described above). Primary DC-SIGN was applied at 1:40 dilution mixed with primary anti-CD3 rabbit polyclonal antibody (Dako, A0452; dilution 1:300) for 45 minutes. Secondary antibodies for anti-DC-SIGN (Alexa-488, Life Technologies, A11029; dilution 1:200) and for anti-CD3 (Alexa-R-568, Life Technologies, A10042; dilution 1:200) were then applied for 30 minutes. The sections were then washed in PBS twice (5 minutes each) and mounted with Fluorescent Mounting Medium.

Immunofluorescence-fluorescence *in situ* hybridization staining and quantification:

Combined DC-SIGN immunofluorescence staining and XY fluorescence *in situ* Hybridization (FISH) was performed on 4-μm renal biopsy sections using a modified avidin-biotin immunofluorescence method. Pressure-cooker antigen retrieval was performed by heating tissue sections in Trilogy solution (Cell Marque Corporation, Rocklin, CA) for 3 minutes, followed by cooling sections to room temperature. Slides were washed in PBS for 10 minutes then incubated in avidin D for 20 minutes, biotin for 20 minutes, mouse anti-DC-SIGN (R&D Systems; dilution 1:40) for 2 hours, horse anti-mouse IgG (Vector Labs, Burlingame, CA; dilution 1:100 dilution) for 60 minutes, and Cy5-streptavidin (Invitrogen, Camarillo, CA; dilution 1:100) for 30 minutes. Slides were dehydrated in ethanol and dried in a 65°C oven for 10 minutes.

Hybridization was performed with X and Y chromosome-centromeric region specific probes in a ThermoBrite system (Abbott Molecular Diagnostics, Des Plaines, IL). Two post-hybridization washes were performed in 2x sodium saline citrate (SSC)/0.3% NP-40 at 72°C for 3 minutes, followed by 2x SSC/0.3% NP-40 at room temperature for 1 minute. The sections were counterstained with DAPI. Microscopic assessment was performed with an Olympus BX61 epifluorescence microscope under a 60x and 100x objective lens. Entire cortex of each case was assessed and the presence of the X and Y chromosomes in each cell was identified as spectrum aqua (X) and spectrum orange (Y) spots in DAPI-stained nuclei. DC-SIGN^{+} cell count was performed only in nuclei containing two distinct fluorescent signals.

Supplemental table 1:post-biopsy immunosuppressive therapy[1] in the studied cohort

	IFTA absent (n=24)	**IFTA & low DC-SIGN$^+$ cell density (n=58)[2]**	**IFTA & high DC-SIGN$^+$ cell density (n=23)**	**P value**
# patients receiving immunosuppressive therapy	12/24 (50%)	25/55 (45%)	13/23 (56%)	0.67
- Steroids	6/24 (25%)	19/55 (35%)	10/23 (43%)	0.41
- Thymoglobulin	2/24 (8%)	1/55 (2%)	3/23 (13%)	0.13
- IVIG[3]	3/24 (13%)	2/55 (4%)	5/23 (21%)	**0.04**
- Plasmapheresis	8/24 (33%)	7/55 (13%)	5/23 (21%)	0.10
- Rituximab	2/24 (8%)	1/55 (2%)	1/23 (4%)	0.39

Abbreviations: IFTA, interstitial fibrosis/tubular atrophy; IVIG, Intravenous immunoglobulin

[1] With regard to treatment subgroups, several patients received more than one type of treatment at once

[2] Treatment was not specified in 3 patients

[3] IVIG: When pair comparison was performed, significant difference was only observed when comparing IFTA & high DC-SIGN$^+$ cell density vs. IFTA & low DC-SIGN$^+$ cell density where the former was treated more aggressively (P=0.02)

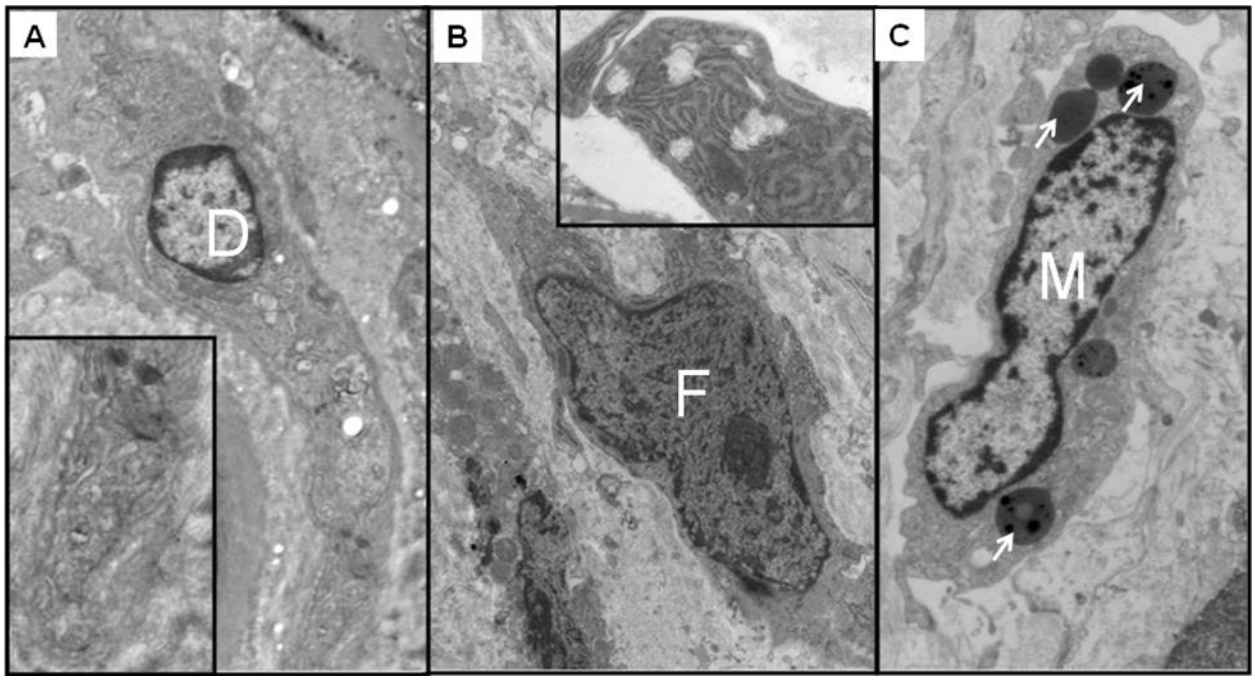
Supplemental table 2: Fluorescence *in situ* hybridization (FISH) in sex-mismatched recipients

Pt #	Donor	Recipient	Dx	Time post-tx (days)	# Donor DC-SIGN$^+$ cells	# Recipient DC-SIGN$^+$ cells	% of Recipient: Donor DC-SIGN$^+$ cells
1	F (XX)	M (XY)	NSA	50	14	3	18%
2	F (XX)	M (XY)	IFTA	1295	1	16	95%
3	F (XX)	M (XY)	IFTA	637	3	19	86%
4	M (XY)	F (XX)	TCMR	3395	0	32	100%
5	F (XX)	M (XY)	ATI	34	5	2	29%
6	F (XX)	M (XY)	IFTA	725	3	4	57%
7	F (XX)	M (XY)	Mixed Rejection	1016	1	11	92%
8	F (XX)	M (XY)	NSA	49	11	1	8%
9	F (XX)	M (XY)	IFTA	510	2	11	85%

Abbreviations: ATI, acute tubular injury; Dx, diagnosis; IFTA, interstitial fibrosis and tubular atrophy; F, female; M, male; NSA, no significant abnormalities; TCMR, T cell mediated rejection; Pt, patient; tx, transplant

In each biopsy, >100 tubular epithelial cells from donor origin and $\geq$ 7 DC-SIGN$^+$ cells that showed two FISH signals (either XX or XY) were identified

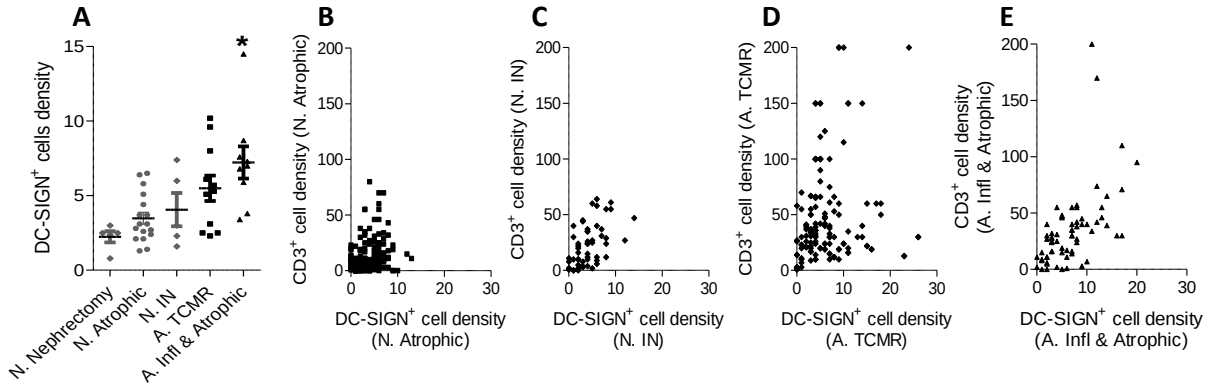
Supplemental figure 1:



Representative transmission electron microscopy images of an interstitial dendritic cell **(A)**, a fibroblast **(B)**, and a macrophage **(C)**. Kidney dendritic cells **(A)** are characterized by relatively round nuclei, fair amount of mitochondria-rich pericaryon, and cytoplasmic protrusions. While endoplasmic reticulum and mitochondria can extend to larger protrusions, of the kidney dendritic cell, such protrusions are characterized by light appearance (inset A) due to the lack of abundant ribosomal components and actin filaments. Fibroblasts **(B)** are characterized by indented nuclei, very small cytoplasmic rim in the pericaryon, and dark-appearing ribosome-rich cytoplasmic protrusions (inset B). Macrophages **(C)** usually lack prominent cytoplasmic protrusions but contain several lysosomes (arrows). Dendritic cells can best be distinguished from fibroblasts by the presence of light vs. dark cytoplasmic protrusions, respectively, and from macrophages by the lack of abundant lysosomes (Electron microscopy, original magnification x10,000, insets: original magnification x25,000).

Abbreviations: D, dendritic cell; F, fibroblast; M, macrophage.

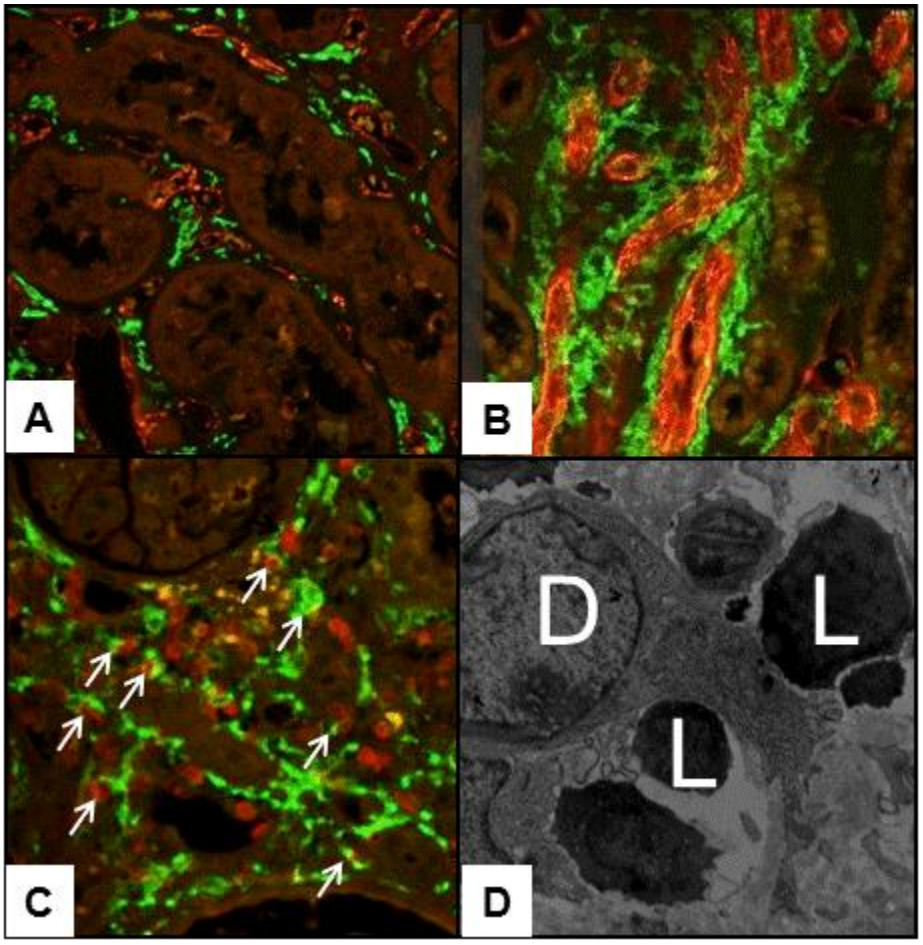
Supplemental figure 2:



(A) The densities of DC-SIGN$^+$ cells as assessed by average number of cells in total high power fields. The density of DC-SIGN$^+$ cell increased from nephrectomies, to native atrophic biopsies, to native biopsies with interstitial nephritis, to inflamed allografts with and without atrophy [ANOVA: P=0.001; Tukey tests: inflamed atrophic allografts vs. nephrectomies (P<0.05) or vs. native atrophic biopsies (P<0.05)]. (**B-D)** Correlation between average number of DC-SIGN$^+$ cells in each hpf and CD3$^+$ cells in the same hpf in native nephrectomies (r=0.29, P=0.08; not shown), **(B)** in native atrophic biopsies (r=0.35, P<0.001), **(C)** in native biopsies with interstitial nephritis (r=0.61, P<0.001), **(D)** in allografts with TCMR (r=0.29, P<0.001)**,** and **(E)** in inflamed atrophic allografts (r=0.64, P<0.001)**.**

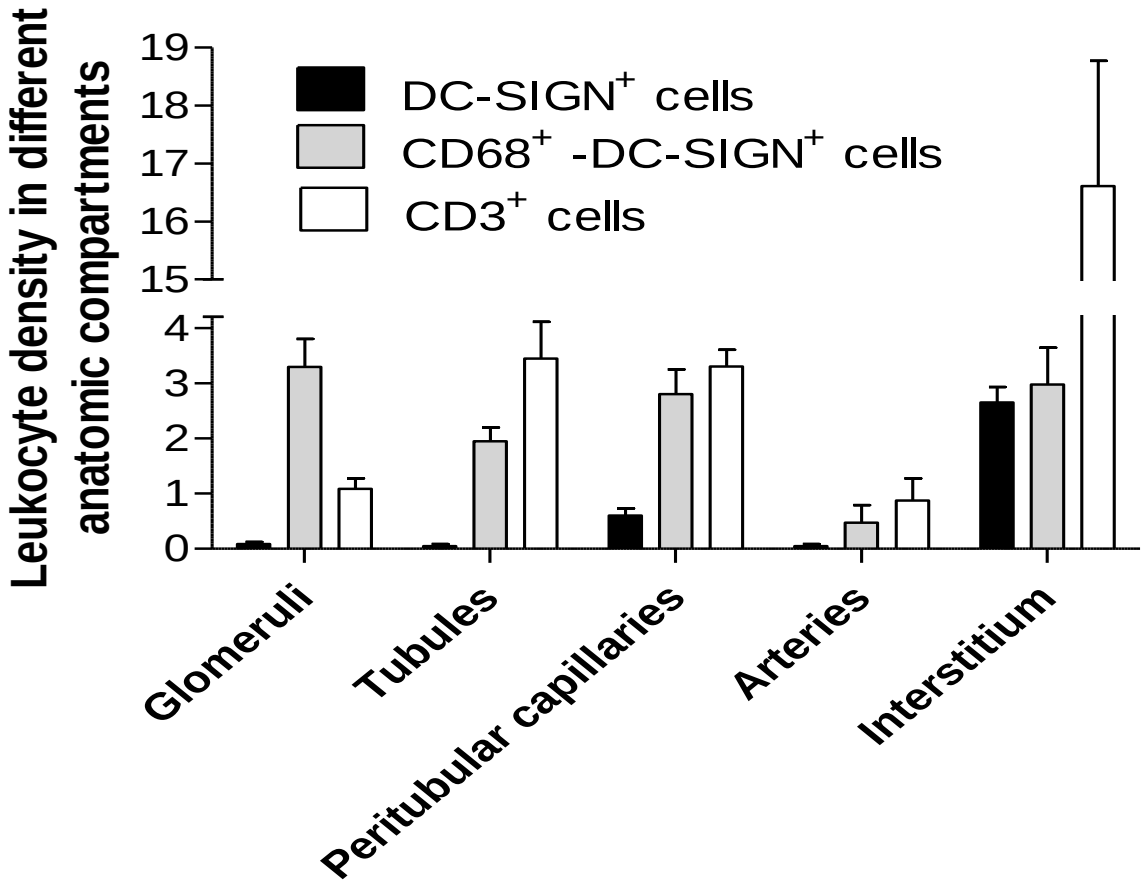
Abbreviations: A, allograft; Infl, inflamed; N, native; TCMR, T cell mediated rejection

Supplemental figure 3:



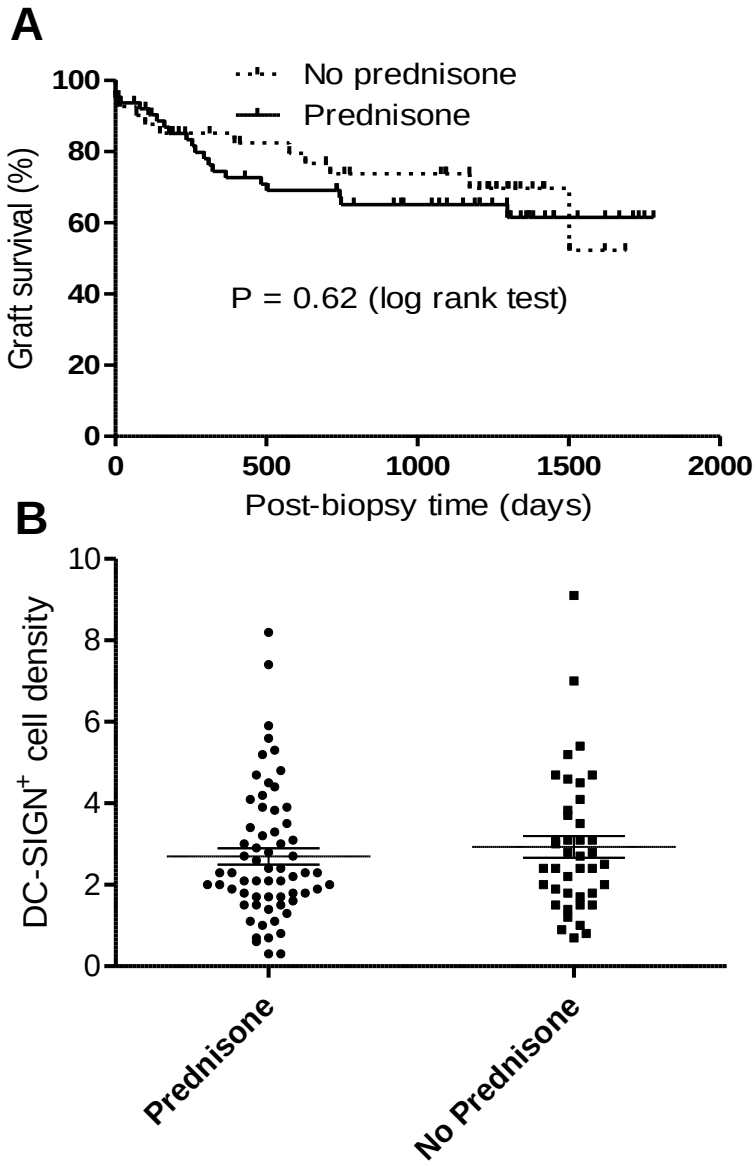
Representative images of the association of DCs with peritubular capillaries and lymphocytic infiltrates; In selected cases, double immunofluorescence staining for DC-SIGN with CD31 or CD3 was performed. **(A-B)** Double immunofluorescence staining for DC-SIGN (green) and CD31 (red). **(A)** In an allograft with no significant abnormalities, scattered interstitial stellate-shaped DC-SIGN^{+} cells are seen in close proximity to, but not in the lumina of, the peritubular capillaries. **(B)** An inflamed allograft biopsy showing aggregations of interstitial DC-SIGN^{+} cells around dilated peritubular capillaries (red) Note that DC-SIGN^{+} cells are located within the interstitial space rather than within the lumina of the peritubular capillaries (**A-B**: original magnification x400). **(C)** Double immunofluorescence staining for DC-SIGN (green) and CD3 (red) in an inflamed allograft biopsy showing that DC-SIGN^{+} cells cluster with infiltrating CD3^{+} cells (original magnification ×200). **(D)** Ultrastructural studies (on the same biopsy in C) revealing a cell with ultrastructural morphologic characteristics of a kidney dendritic cell (D) in direct physical contact with infiltrating lymphocytes (L) (transmission electron microscopy, original magnification x8,000).

Supplemental figure 4:



The distribution of DC-SIGN$^+$, CD68$^+$-DC-SIGN$^+$, and CD3$^+$ cells within different anatomic microcompartments in a subset of 40 kidney allograft biopsies. CD3$^+$ cells were most abundantly seen within the interstitium. CD3$^+$ cells were also the dominant leukocytes in the tubules and arteries. CD68$^+$-DC-SIGN$^+$ cells were dominant within the glomeruli, co-dominant in the pritubular capillaries (together with CD3$^+$ cells), and commonly encountered in the tubules and inflamed arteries. In contrast to CD68$^+$-DC-SIGN$^+$ cells, DC-SIGN$^+$ cells were only frequently encountered in the interstitium, occasionally encountered in the peritubular capillaries, and largely non-existent in the glomeruli, tubules, or arteries.

Supplemental figure 5:



(A) Effects of prednisone maintenance on allograft survival and **(B)** DC-SIGN^{+} cell density [prednisone maintenance [2.3 (1.7, 3.4) (n=64)] vs. no prednisone maintenance 2.5 (1.8, 3.7) (n=41); P=0.45].

References:

1. Nakano, O, Sato, M, Naito, Y, Suzuki, K, Orikasa, S, Aizawa, M, Suzuki, Y, Shintaku, I, Nagura, H, Ohtani, H: Proliferative activity of intratumoral CD8(+) T-lymphocytes as a prognostic factor in human renal cell carcinoma: clinicopathologic demonstration of antitumor immunity. *Cancer Res*, 61**:** 5132-5136, 2001.