see commentary on page 5

Compartment specific expression of dendritic cell markers in human glomerulonephritis

S Segerer[1], F Heller[1], MT Lindenmeyer[1], H Schmid[1], CD Cohen[1], D Draganovici[1], J Mandelbaum[1], PJ Nelson[1], H-J Gröne[2], EF Gröne[2], A-M Figel[3], E Nössner[3] and D Schlöndorff[1,4]

[1]Medizinische Poliklinik—Innenstadt, University of Munich, Munich, Germany; [2]Department of Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany; [3]GSF—Institute of Molecular Immunology, Munich, Germany and [4]Mount Sinai School of Medicine, New York, New York, USA

Macrophages and dendritic cells are heterogenous and highly plastic bone marrow-derived cells that play major roles in renal diseases. We characterized these cells using immunohistochemistry in 55 renal biopsies from control patients or patients with glomerulonephritis as an initial step towards postulating specific roles for these cells in kidney disease. In proliferative glomerulonephritis numerous CD68 positive (pan monocyte, macrophage and dendritic marker) cells were found in both glomeruli and the tubulointerstitial space, however, a myeloid dendritic cell marker (DC-SIGN) was identified only in the tubulointerstitium. A significant number of plasmacytoid dendritic cells (identified as BDCA-2 positive cells) were seen at sites of interstitial inflammation, including follicular aggregates of inflammatory cells. Langerin positive cells (a marker of Langerhans' cells) were detectable but rare. The area of either CD68 or DC-SIGN positive interstitial cells correlated with serum creatinine. Low levels of DC-SIGN, DC-LAMP and MHC class II mRNA were present in the tubulointerstitial space in controls and increased only in that region in proliferative glomerulonephritis. We demonstrate that the CD68 positive cells infiltrating the glomerulus lack dendritic cell markers (reflecting macrophages), whereas in the tubulointerstitial space the majority of CD68 positive cells are also DC-SIGN positive (reflecting myeloid dendritic cells). Their number correlated with serum creatinine, which further emphasizes the significance of interstitial DCs in progressive glomerular diseases.

Kidney International (2008) **74,** 37–46; doi:10.1038/ki.2008.99; published online 26 March 2008

KEYWORDS: dendritic cells; glomerulonephritis; macrophages; interstitial inflammation

Correspondence: *S Segerer, Medizinische Poliklinik—Innenstadt, Klinikum Innenstadt der Universität, Pettenkoferstr. 8a, Munich 80336, Germany. E-mail: Stephan.segerer@lrz.uni-muenchen.de*

Received 28 May 2007; revised 7 January 2008; accepted 8 January 2008; published online 26 March 2008

The mononuclear phagocyte system represents a family of cells, including bone marrow progenitors, monocytes, tissue macrophages, and specialized antigen-presenting cells, that is, myeloid or conventional dendritic cells (mDCs).[1,2] A substantial heterogeneity and plasticity exists both within the Mac and DC lineage, which develop from bone marrow-derived progenitor populations.[1,3–6]

DCs reside in tissues of most organs under normal conditions and also function as 'immunological sensors' for danger signals.[7,8] Immature DCs capture antigen, change the phenotype, and migrate to lymphoid organs, where the mature DCs optimize clonal selection of rare T and B cells, and initiate immune responses.[9] A major difference between Macs and DCs is that only DCs can activate naive T cells.[10]

For both Macs and DCs, various markers have been used that differ substantially between humans and mice.[11–15] Cells with dendritic morphology and major histocompatibility complex (MHC) class II expression were described in normal rat and mouse kidney, and they were able to stimulate T-cell responses *in vitro*.[12,16,17] Fractalkine receptor-positive cells formed a network within the normal mouse kidney, coexpressed CD11c, F4/80, and MHC class II, and demonstrated immature costimulatory competence, but phagocytic ability.[13]

In the human kidney, data on DCs are scarce. CD68 (macrosialin) is commonly used as a pan monocyte–macrophage marker, but it was also present on DCs.[18] CD68-positive cells infiltrate the glomerular tuft and the tubulointerstitium during glomerulonephritis (GN).[19–21] A number of surface markers have been used to characterize human DCs. DC-SIGN (DC-specific intercellular adhesion molecule-3 (ICAM-3) grabbing, non-integrin; CD209) is a C-type lectin that binds to ICAM-3 on T cells.[22,23] Differentiation of monocytes into immature DCs *in vitro* coincides with a strong induction of DC-SIGN.[22] DC-SIGN-positive cells (most likely reflecting mDCs) have been found in tissue sections from the lungs, intestine, cervix, placenta, lymph nodes, and renal allografts.[15] S100 has been described to be expressed by Langerhans cells in the human skin and lymph nodes,[24] by DCs in human renal allografts,[14] and by DCs in rat lymph nodes.[14,25–27] Langerin is a C-type lectin that is expressed by Langerhans cells from the skin and small

intestine.[28,29] Langerin-positive cells are involved in immunological surface surveillance. Blood dendritic cell antigen-2 (BDCA-2) is a type II C-type lectin that is expressed on human CD11$^-$, CD123^{bright} blood cells reflecting plasmacytoid DCs (pDCs).[30,31] HLA-DR-positive DCs, which coexpressed BDCA-1 (CD1c), a marker of mDCs and BDCA-2-positive pDCs, were described in human renal allografts and in IgA nephropathy.[15]

The aim of this study was to further characterize the different populations of monocyte–macrophage–DCs and their respective intrarenal location in biopsies from patients with glomerular diseases as an initial step toward postulating specific roles for these cells. Infiltrates in the glomerulus lacked the classical markers for DCs, whereas cells with mDC and pDC markers are commonly found in the renal tubulointerstitium. CD68- and DC-SIGN-positive cells correlated with renal function at the time of biopsy.

RESULTS

Distribution of cells with DC/Mac markers in normal renal tissue

In normal renal tissue, CD68 stained round circulating cells present in both glomerular and tubulointerstitial capillaries, as well as in veins and arteries. Outside of the vascular lumen, the tubulointerstitium contains a scattered population of spindle-shaped CD68-positive cells between the tubuli and the peritubular capillaries (Figure 1d).

In normal renal tissue from tumor nephrectomies or biopsies without histopathological findings, DC-SIGN was expressed by cells scattered throughout the tubulointerstitium (Figure 1a and b). The DC-SIGN-positive cells were predominantly localized between tubules and peritubular capillaries (Figure 1a). DC-SIGN-positive cells were not detectable in glomeruli. Intrinsic renal cells, including cells of the glomerular tuft, tubular epithelial cells, endothelial cells, and cells of arterial vessel walls, were DC-SIGN negative. The population of interstitial DC-SIGN-positive cells was smaller than the population of CD68-positive cells on consecutive sections. Because of the low numbers of these two cell types in normal renal tissue, it was not possible to determine the percentage of double-positive cells using consecutive sections.

As expected, S100 was expressed in renal nerves, particularly those accompanying larger vessels (Figure 1c).[32] In addition, a very small population of S100-positive cells with dendritic morphology was found in the tubulointerstitium (Figure 1g).

Langerin was not found on intrinsic renal cells (Figure 1e and f). A very small population of Langerin-positive cells was localized around tubules. Additionally, some Langerin-positive cells were detected between epithelial cells with morphological characteristics of collecting tubular cells (columnar epithelium without brush border; Figures 1f and 2i).

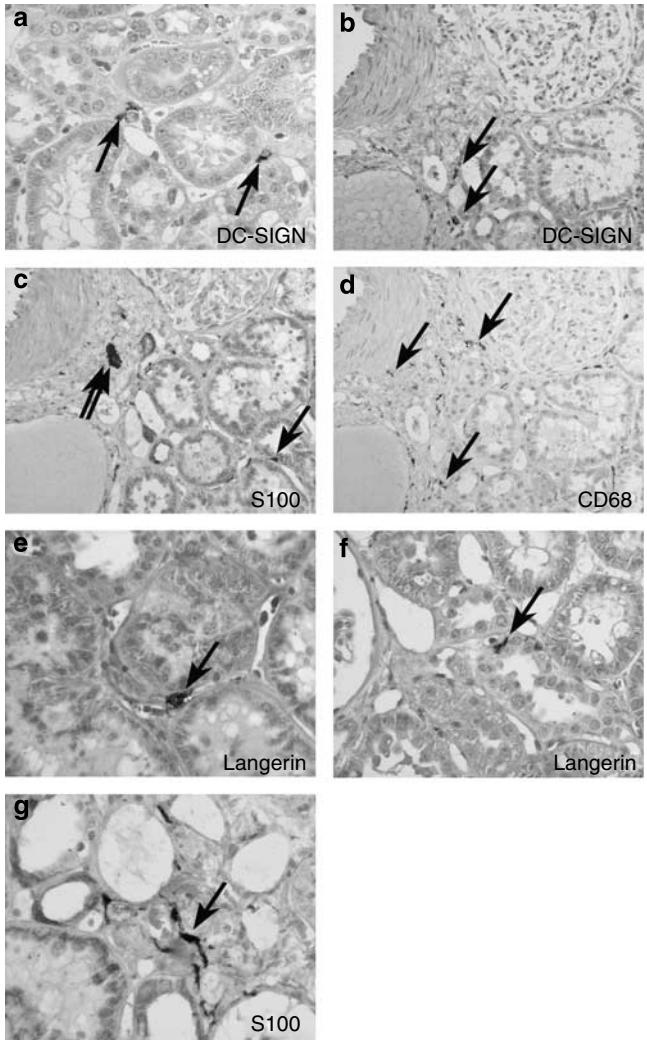

Figure 1 | DC/Mac markers in normal renal tissue. Immunohistochemistry was performed on tissue sections from histologically normal areas of tumor nephrectomies for (**a**, **b**) DC-SIGN, (**c**, **g**) S100, (**d**) CD68, and (**e**, **f**) Langerin (original magnification, $\times$ 200 (**b**–**d**); $\times$ 400 (**a**, **e**–**g**)). Note that there are scattered positive cells in the tubulointerstitium with the highest numbers for CD68 (arrows in panel d). S100 was expressed by nerves (double arrow in panel c) and interstitial cells with dendritic morphology (arrows in panels c and g). Examples of positive cells are labeled with arrows.

Distribution of cells with DC/Mac markers in GN

The basic morphological and clinical information available on the study population is summarized in Table 1. In contrast to the low number of DC-SIGN-positive cells in normal renal tissue (Figure 1), a prominent accumulation of DC-SIGN-positive cells was present in biopsies with lupus nephritis (Figure 2b) or necrotizing GN (Figure 3b). The accumulation of DC-SIGN-positive cells was restricted to the tubulointerstitial compartment (Figures 2b, e and 3b). DC-SIGN-positive cells were associated with sites of interstitial injury and at times clustered around injured glomeruli (Figure 2b). There was considerable overlap between the pattern of CD68- and DC-SIGN-positive cells in the tubulointerstitium, indicating a large population of double-positive cells (Figures 2a, b and 3a, b, f, and g). To confirm this double-positive population, we performed confocal double immunofluorescence for DC-SIGN and CD68 on selected biopsies with lupus

nephritis. By this method, the vast majority of DC-SIGN-positive infiltrating cells in the tubulointerstitium were found to be CD68 positive (Figure 2e–g). Owing to some tubular background, a small DC-SIGN single-positive population cannot be excluded. Additionally, there was a small population of CD68-positive cells that were DC-SIGN negative.

In contrast to the prominent interstitial DC-SIGN-positive cell population, DC-SIGN-positive cells were not found within glomerular tufts (Figures 2b and 3b), even though a prominent accumulation of CD68-positive cells was present in the glomeruli (Figures 2a and 3a).

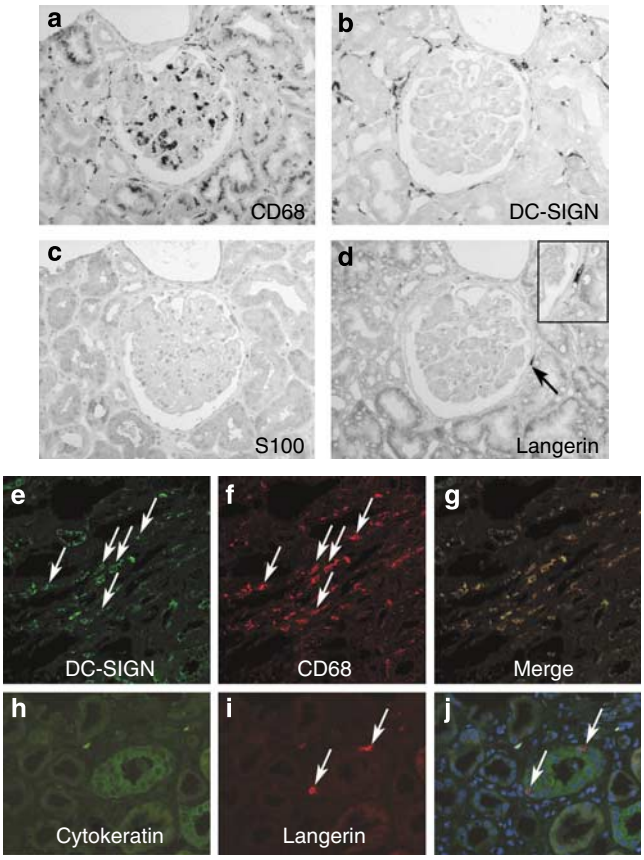

Figure 2 | DC/Mac markers in lupus nephritis. Immunohistochemistry was performed on sections from renal biopsies with lupus nephritis for (**a**) CD68, (**b**) DC-SIGN, (**c**) S100, and (**d**) Langerin. Original magnification × 200. (**a**, **b**) There is a prominent number of CD68-positive cells infiltrating the glomerular tuft, which are DC-SIGN negative. In the tubulointerstitium, a significant overlap between CD68 and DC-SIGN staining exists. S100- and Langerin-positive cells were rare (arrow in panel d, illustrated in higher magnification; original magnification × 1000). Double immunofluorescence was performed on a section from a renal biopsy with lupus nephritis for (**e**) DC-SIGN and (**f**) CD68. (**g**) Merged image of panels e and f (original magnification × 630). There is almost a complete overlap between the interstitial DC-SIGN and CD68 (some of the double-positive cells are illustrated with arrows). Double immunofluorescence was performed on a section from a renal biopsy with membranous nephropathy for (**h**) cytokeratin and (**i**) Langerin. Two Langerin-positive cells (arrows) were clearly localized within the epithelial cell layer ((**j**) triple filter with nuclei stained in blue with DAPI).

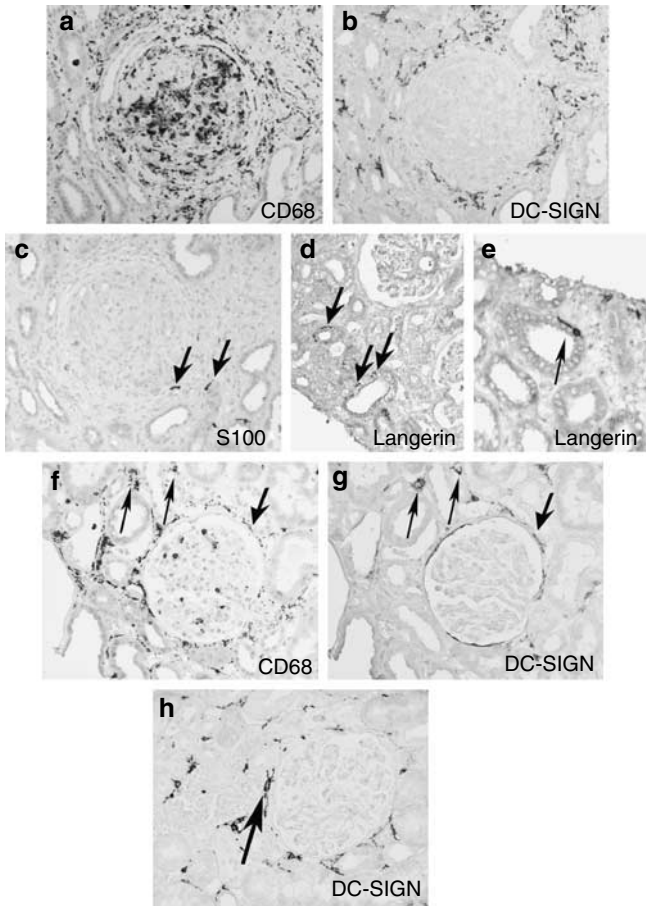

Figure 3 | DC/Mac markers in necrotizing GN and membranous nephropathy. Immunohistochemistry was performed on sections from renal biopsies (**a–e**) with necrotizing GN and (**f–h**) with membranous nephropathy for (**a**, **f**) CD68, (**b**, **g**, **h**) DC-SIGN, (**c**) S100, and (**d**, **e**) Langerin. Original magnification × 200. (**d** and **e**) Langerin-positive intraepithelial cells are illustrated (arrows). Note the overlap between DC-SIGN- and CD68-positive cells (arrows in panels f and g). (**g**) DC-SIGN signal was rarely found on parietal epithelial cells. (**g**) A typical dendritic morphology is shown by DC-SIGN-positive cells (arrow).

Table 1 | Clinical and morphological information on the study population

Disease	*n*	Gender (female)	Age (years, range)	Mean creatinine (mg per 100 ml)	Proteinuria (g per 24 h)
Necrotizing GN	8	5	54 (38–77)	3.1 (1–4.7)	0.5 (0.3–0.6)
Lupus nephritis	8	8	35 (17–65)	2.9 (0.9–10)	3.9 (0.9–8)
MCD	8	1	56 (26–68)	1.6 (1–3)	7.2 (3.9–15)
FSGS	8	4	43 (15–80)	1.1 (0.6–1.7)	9.4 (0.8–15)
Membranous nephropathy	8	2	54 (16–79)	1.5 (0.7–2)	5.3 (2.5–8)
Minimal lesions	10	5	33 (17–55)	1.1 (0.8–1.9)	0.3 (0.1–0.6)

FSGS, focal-segmental glomerulosclerosis; GN, glomerulonephritis; MCD, minimal change disease.

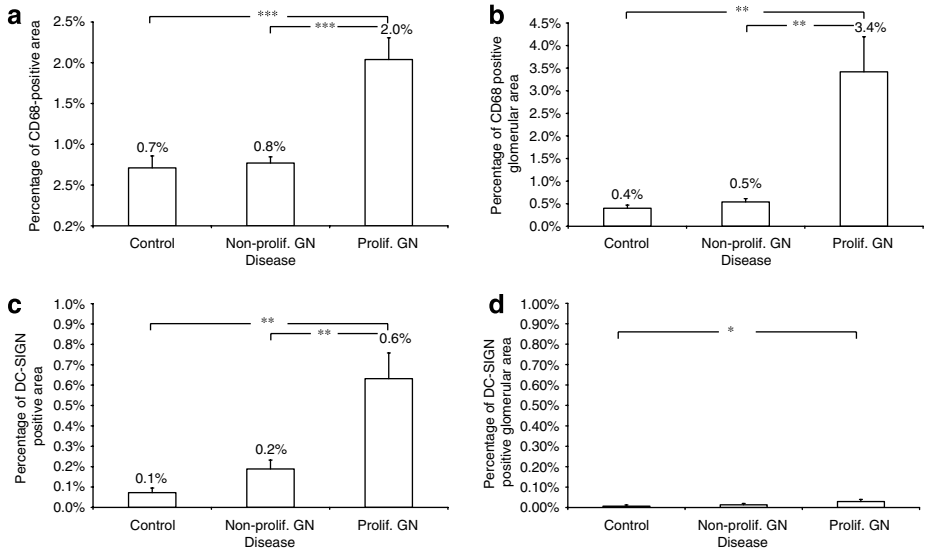

Figure 4 | Morphometric quantification of DC/Mac markers. Morphometric analysis of the area of color product of (**a**, **b**) CD68 and (**c**, **d**) DC-SIGN expressed as (**a**, **c**) a percentage of high-power field or (**b**, **d**) as per glomerular cross-section. Note that CD68 increases both in the glomerulus and the tubulointerstitium, whereas DC-SIGN staining was almost exclusively restricted to the tubulointerstitium (**c**, **d**; $*P<0,05$, $**P<0,01$, $***P<0,001$).

CD68- and DC-SIGN-positive cells are difficult to quantify because of their 'dendritic' morphology with multiple long cell extensions and the clustering within the tubulointerstitium. We therefore performed a morphometric analysis quantifying the area of positive color product and expressing it as a percentage of the measured area (either per area of the high-power field or per glomerular tuft area). A significant increase in the CD68-positive area was found in renal biopsies with proliferative GN (Figure 4a and b), with the most prominent accumulation in the glomeruli (Figure 4b). In the tubulointerstitium, the relative percentage of DC-SIGN-positive staining area was smaller than the area positive for CD68 (Figure 4a and c), but a significant increase of DC-SIGN-positive area was found (Figure 4c).

Scattered S100- and Langerin-positive cells were rarely seen in the tubulointerstitium and were essentially absent from glomerular tufts. Interestingly, Langerin-positive cells were commonly found within epithelia with collecting-tubular morphology (Figure 3d and e).

As examples of non-proliferative glomerular diseases, we studied biopsies with focal–segmental glomerulosclerosis, minimal change disease, and membranous GN (Figure 3). In these diseases, the numbers of glomerular CD68-positive cells were not significantly different compared to normal controls. Again, the DC-SIGN-positive cells were restricted to the tubulointerstitium. Occasionally, glomerular parietal epithelial cells were found to be DC-SIGN positive, predominantly in biopsies with non-proliferative GNs.

The area of positive staining for CD68 as well as for DC-SIGN correlated significantly with the serum creatinine at the time of biopsy (CD68: Spearman $r=0.41$, $P=0.017$; DC-SIGN: Spearman $r=0.37$, $P=0.035$) but not with proteinuria.

BDCA-2-positive cells in GN

In search of further markers to define subsets of DCs in formalin-fixed, paraffin-embedded tissue, an antibody against BDCA-2 (reflecting pDCs) was established.[15,31,33] BDCA-2 has been used to identify pDCs in frozen tissue from renal allografts.[15] The cells were smaller than DC-SIGN-positive cells, consistent with characteristics of pDCs in human tonsils (Figure 5a).[30,31,33]

In normal renal tissue, only a minimal number of BDCA-2-positive cells were seen within the tubulointerstitium. BDCA-2 was not present on renal parenchymal cells (Figure 5c–f). Biopsies from patients with either proliferative or non-proliferative GN demonstrated a higher number of BDCA-2-positive cells in diseased interstitial areas (Figure 5). In particular, areas of nodular inflammatory cell accumulations, resembling tertiary lymphoid nodules, contained a prominent number of BDCA-2-positive cells (Figure 5d). The accumulation of BDCA-2-positive cells was restricted to the tubulointerstitium. As DC-SIGN is considered a marker for mDCs and BDCA-2 for pDCs, the interstitium contains both cell types.

Expression of CD68, DC-SIGN, DC-LAMP, Langerin, and HLA-DRα mRNA in GN

To further characterize the expression of markers of Macs and DCs, we used real-time reverse transcriptase-PCR on micro-dissected renal biopsies with proliferative GN (including lupus nephritis ($n=8$) and crescentic GN ($n=8$)). Six biopsies taken from living donors before transplantation served as normal controls (Figure 6). Expression of CD68 mRNA was found in both the glomeruli and the tubulointerstitium (Figure 6a and b). There was a trend toward higher expression in proliferative GN, which, in contrast to the immunohistochemistry data, did not reach the level of significance.

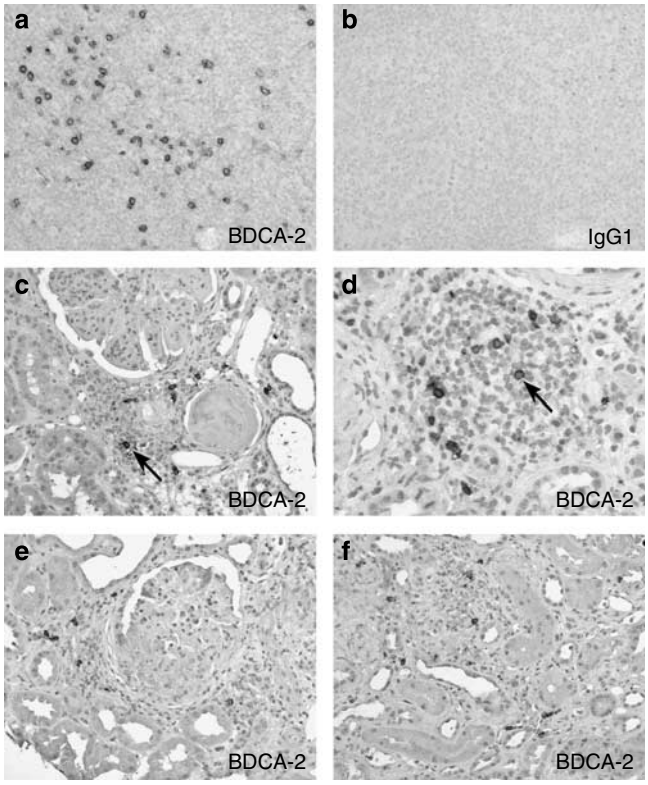

Figure 5 | pDCs in renal biopsies. Immunohistochemistry performed with a monoclonal antibody against (**a**, **c**–**f**) BDCA-2 or (**b**) isotype control immunoglobulin on (**a**, **b**) tissue sections from the human tonsil or (**c**, **d**) renal biopsies from patients with lupus nephritis, (**e**) necrotizing GN, and (**f**) membranous nephropathy (original magnification, × 200 (**a**–**c**, **e**, **f**); × 400 (**d**)). (**c**, **d**) Note the accumulation of BDCA-2-positive cells in areas of nodular infiltrates (arrow). (**e**) BDCA-2-positive cells were restricted to the tubulointerstitium, even in areas of severe glomerular injury. (**f**) In membranous nephropathy, BDCA-2-positive cells were present in areas of interstitial injury.

In the glomeruli, the levels of mRNA for DC-SIGN were too low to be quantified. In contrast, expression was detectable within the tubulointerstitial compartment, with a significant increase in biopsies with proliferative GN (Figure 6c). Thus, the DC-SIGN mRNA expression data support the results of the immunostaining. The expression of mRNA for DC-LAMP as a marker of mature DCs was only significantly increased in the tubulointerstitium of proliferative GN (Figure 6d). Consistent with our immunohistological findings, Langerin mRNA was not detectable in the glomeruli from normal or diseased kidneys, but Langerin mRNA was present in the tubulointerstitium of both normal and diseased kidneys (Figure 6e). Finally, we quantified the expression of MHC class II (reflected by HLA-DRα mRNA), which was also upregulated only in the tubulointerstitial compartment, but not in the glomeruli (Figure 6f and g). In summary, a significant induction of mRNAs for markers of DCs (DC-SIGN and DC-LAMP) and of MHC class II (for antigen-presenting cells) was restricted to the tubulointerstitium.

DISCUSSION

Macs and DCs play important roles in renal inflammation. Both can show dynamic changes in their function and phenotype.[34,35] Generally, DCs are divided into mDCs and pDCs. Human mDCs express BDCA-1 and DC-SIGN (at least in most cases), whereas pDCs are positive for BDCA-2.[36] Furthermore, DCs can be manipulated to either activate or suppress T-cell responses.[10] In this regard, pDC may play a role in generating regulatory T cells and contributing to peripheral tolerance.[37]

Macs can be activated in a classical (interferon-γ dependent) and alternative manner (by T helper type 2 cytokines), resulting in proinflammatory versus anti-inflammatory phenotypes.[10] These differences in phenotypes likely reflect adaptation to different microenvironments.[38] Therefore, both DCs and Macs could not only be involved in the progression of the renal disease but may also promote tolerance or healing processes.[39] In human tissues, including the kidney, different forms of Macs and DCs remain poorly defined because of the lack of markers that correlate with the functional phenotype. CD68 has been used extensively as a marker for Macs. For example, in chronic GN, the accumulation of CD68-positive cells in the tubulointerstitium has been considered to represent macrophage infiltration.[35,40,41] Our results demonstrate that CD68-positive cells infiltrating the glomeruli and the tubulointerstitium represent different cell types as determined by their surface markers. In the glomeruli, the CD68-positive cells were negative for DC markers such as DC-SIGN, Langerin, BDCA-2, and S100, indicating that they were Macs.

By contrast, in the tubulointerstitium of biopsies with GN, most CD68-positive cells were also DC-SIGN positive, indicating that they were mDCs. The CD68/DC-SIGN double positivity was confirmed by double fluorescence confocal microscopy. In renal allografts and IgA nephropathy, it was recently reported that DC-SIGN-positive cells also express BDCA-1, another marker for mDCs.[15] DC-SIGN facilitates interaction with resting T cells (via ICAM-3) and allows for the T-cell receptor to scan the DC surface to find the small amounts of MHC–peptide ligands.[22,23] DC-SIGN also binds to Lewis X epitopes present on neutrophils and to a lesser extent on monocytes.[42,43] Thus, DC-SIGN combines the function of a pattern recognition receptor and an adhesion molecule, allowing interactions between immune cells present in the injured tubulointerstitium.

In progressive kidney diseases, including proliferative GN, T cells and CD68-positive cells accumulate in the tubulointerstitium in parallel and with a similar distribution.[19] Both cell types correlate with renal function at the time of biopsy.[44] In addition, we now demonstrate that the degree of tubulointerstitial positivity for DC-SIGN correlates with renal function at the time of biopsy in glomerular diseases. Furthermore, the majority of CD68-positive cells, previously considered to represent Macs, are also DC-SIGN positive. Our findings in the tubulointerstitial involvement of primary

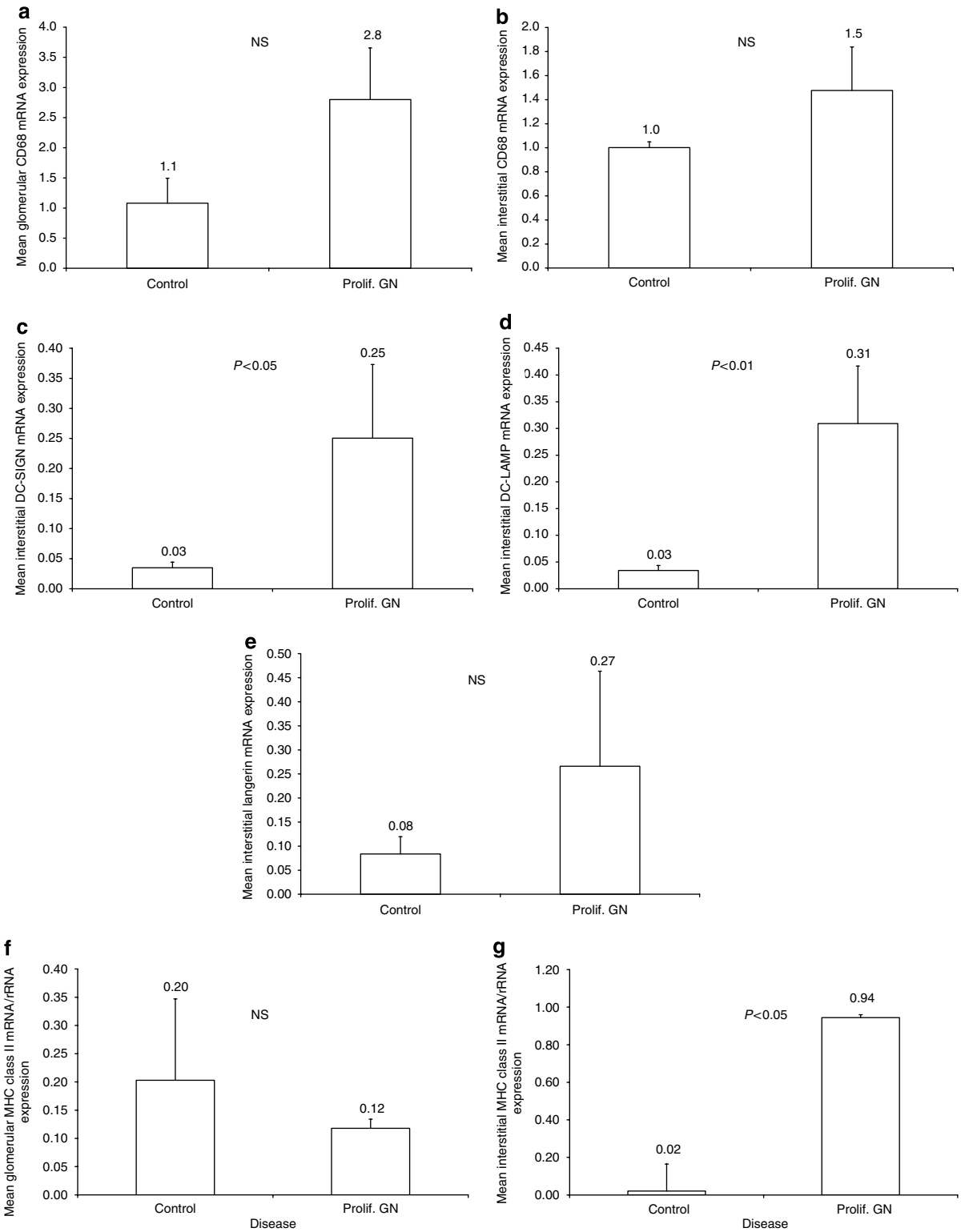

Figure 6 | mRNA Expression of DC/Mac markers in renal biopsies. Quantification of mRNAs by real-time reverse transcriptase-PCR for CD68 ((**a**) glomerular, (**b**) tubulointerstitium) and in the tubulointerstitium for (**c**) DC-SIGN, (**d**) DC-LAMP, and (**e**) Langerin. The expression level of DC-SIGN, DC-LAMP, and Langerin in the glomeruli was too low to be quantified. MHC class II expression was significantly upregulated (**g**) in the tubulointerstitium, but not (**f**) in the glomeruli.

glomerular diseases and those of Woltman *et al.*[15] in renal biopsies from transplants and IgA nephropathy underline the significance of human renal DCs for the interstitial component of kidney diseases.[36] They also indicate that CD68 positivity should no longer be equated with Macs in the tubulointerstitium.

BDCA-2 is a marker for pDCs with the morphological appearance of lymphocytes and the functionality of immature DCs.[30,31,33] Consistent with the data in biopsies from renal transplants and IgA nephropathy, we found a considerable number of BDCA-2-positive cells in the tubulointerstitium of both proliferating and non-proliferating forms of GN. The BDCA-2-positive pDCs were clearly associated with sites of interstitial inflammation and were frequently found in nodular mononuclear cell accumulations, that is, tertiary lymphoid follicles.[15] This may be of particular interest, as pDCs are considered to play a role in the peripheral generation of regulatory T cells and hence peripheral tolerance.[37]

Our data on the differential infiltration of Macs and DCs in the glomeruli and tubulointerstitium, respectively, may indicate different pathophysiologies in these renal compartments. Similar considerations appear to apply to DCs and Macs in the murine kidney, as recent studies have demonstrated that a significant percentage of F4/80-positive cells are actually immature or resident DCs.[12,13] Furthermore, in mouse models of renal disease, the distribution of F4/80-positive cells is predominantly restricted to the tubulointerstitium, whereas CD68-positive macrophages infiltrating the glomeruli were essentially F4/80 negative.[45] In human renal allografts and in IgA nephropathy, a very low number of DC-SIGN-positive cells were described in the glomeruli (1/50 and 1/12, respectively).[15] In contrast, Morelli *et al.*[46] described an accumulation of DCs in the glomeruli and the tubulointerstitium in non-human primates after mobilization with Fms-like tyrosine kinase 3 ligand. Our results are applicable to only those antigens used here, particularly DC-SIGN, and we cannot rule out a DC-SIGN-negative population of intraglomerular DCs.

The rare appearance of DCs in the glomerulus is noteworthy in the context that the glomerular compartment is also rarely infiltrated by lymphocytes, either T or B cells, in inflammatory renal diseases.[47,48] In contrast, macrophages and neutrophils are the predominant cell types recruited to the glomerulus. A hypothetical, teleological explanation for this finding might be that the glomerulus has to be 'protected' from antigen-processing DCs, as many circulating molecules reach the mesangial area of the glomerulus because of the high intraglomerular capillary pressure and its leaky, fenestrated endothelium. DCs would be exposed to a plethora of antigens, resulting in potentially massive local immunological responses. The removal of locally accumulating antigens is accomplished by mesangial cells serving as housekeepers and, under marked immune complex deposition, by additional help from neutrophils and macrophages. Thereby, an excessive acquired immune response would be avoided, as it could endanger the delicate structure necessary for glomerular function. The absence of DCs from the glomerulus is also in keeping with the lack of lymphatics draining the glomerulus. DCs leave the site of antigen processing via lymphatic vessels to reach the draining lymph nodes, and propagate an acquired immune response.[49,50] Therefore, the absence of DCs from the glomerulus, the lack of glomerular lymphatics, and the very rare glomerular T-cell infiltrates may indicate that the glomerulus represents a special immunological niche. The glomerulus might therefore react predominantly by an innate response, whereas the tubulointerstitium with its resident DCs would, in addition, be the site of an acquired immune response. This might be one of the potential mechanisms whereby renal DCs could contribute to progression of renal disease, a hypothesis that should be further explored.

Our results show, for the first time, the presence of Langerin-positive cells and Langerin mRNA in the tubulointerstitial compartment of the human kidney. Langerin is a glycoprotein expressed by Langerhans cells, which reside in the epithelium of the skin and most mucosal surfaces.[51] It is a type II transmembrane C type lectin receptor, particularly for mannose-containing structures, and has binding specificities similar to DC-SIGN.[52,53] Langerhans cells are a population of immature DCs involved in antigen processing in the skin and intestinal mucosa, but Langerin-positive DCs can be found in diseased tissues, for example, in breast cancer.[51,53,54] Langerin mRNA was found to be expressed in the spleen, lymph nodes, thymus, liver, lung, and heart in mice.[55] By immunohistochemistry, scattered Langerin-positive cells were present adjacent to or even within tubules. Of particular interest may be the close association of Langerin-positive cells with tubular epithelia (commonly showing morphological characteristics of collecting tubules). In this location, Langerin-positive cells could perform a special immune surveillance against ascending pathogens similar to the function of Langerhans cells in the epidermis and intestinal epithelium.

In summary, our data suggest that in the kidney, CD68-positive cells comprise not only Macs, but also, and in the tubulointerstitium to a major extent, DC-SIGN-positive mDCs. Therefore, CD68 should no longer be used as a marker for Macs, especially not in the renal tubulointerstitium. In biopsies from a variety of primary glomerular diseases, we could show a significant correlation between interstitial mDCs and serum creatinine at the time of biopsy, potentially indicating that renal DCs may play a role in interstitial disease progression. Furthermore, we described BDCA-2-positive pDCs in the diseased tubulointerstitium and, finally, a small population of Langerin-positive cells surrounding or even incorporated in the tubular epithelial layer. There they might perform a role in the local surveillance against ascending pathogens, a hypothesis that can now be tested.

MATERIALS AND METHODS

Study population

Biopsies with proliferative forms of GN included lupus nephritis ($n=8$) and necrotizing GN ($n=8$). Non-proliferating glomerular diseases were focal–segmental glomerulosclerosis ($n=8$), membranous nephropathy ($n=8$), and minimal change disease ($n=8$). Biopsies without lesions and hence no identifiable disease ($n=10$),

and non-involved areas from tumor nephrectomies ($n = 5$), served as controls. Ethical approval for the use of archival renal biopsy material was given by the local committee for human subjects.

Immunohistochemistry

Immunohistochemistry was performed on 3 µm sections, as previously described.[44,56] As primary antibodies, the monoclonal mouse anti-CD68 (Clone PG-M1; Dako, Germany, Hamburg), mouse anti-DC-SIGN (CD209, clone DCN46; BD Pharmingen, Heidelberg, Germany), mouse anti-Langerin (clone 12D6; Vector, Burlingame, CA, USA), mouse anti-human BDCA-2 (Acris, Hiddenhausen, Germany), and the polyclonal rabbit anti-S100 (Dako) were used on consecutive sections. The antibodies were tested in various dilutions, and with different antigen retrievals on tissue from human tonsils (Figure 7).[19,21] In addition to perifollicular cells, CD68 stains a population of intrafollicular cells (Figure 7a and b). DC-SIGN stains perifollicular cells with a stellate shape (Figure 7d and e). Langerin stains intraepithelial cells as expected and a smaller population of perifollicular cells (Figure 7g and h). The smallest cell population is detected by S100 predominantly in the perifollicular area (Figure 7j). The corresponding controls did not result in a black reaction product (Figure 7c, f, i and k).

Immunofluorescence

Double-labeling immunofluorescence was performed on selected biopsies for CD68/DC-SIGN and Langerin/cytokeratin, as previously described.[44]

Real-time reverse transcriptase-PCR

The ERCB–KFB (the European Renal cDNA Bank–Kroener Fresenius Biopsy Bank) is an international multicenter study of European nephrology centers for the analysis of renal gene expression. Biopsy specimens were manually microdissected in RNase inhibitor (for details, see Cohen *et al.*[57]). Proper microdissection of glomerular samples was confirmed after reverse transcription, by high expression of Wilms tumor antigen 1 mRNA, as a marker for glomerular epithelial cells. Informed consent was obtained before renal biopsies were performed. Microdissected tubulointerstitial or glomerular compartments were from biopsies with proliferative GNs (lupus nephritis ($n = 8$) and crescentic GN ($n = 8$)) and compared to pretransplantation kidney biopsies from living donors (pre-Tx; $n = 6$). Real-time reverse transcriptase-PCR was performed on a TaqMan ABI 7700 Sequence Detection System (Applied Biosystems, Darmstadt, Germany) using heat-activated *Taq*DNA polymerase (Amplitaq Gold; Applied Biosystems). Quantification of the given templates was performed according to the standard curve method.

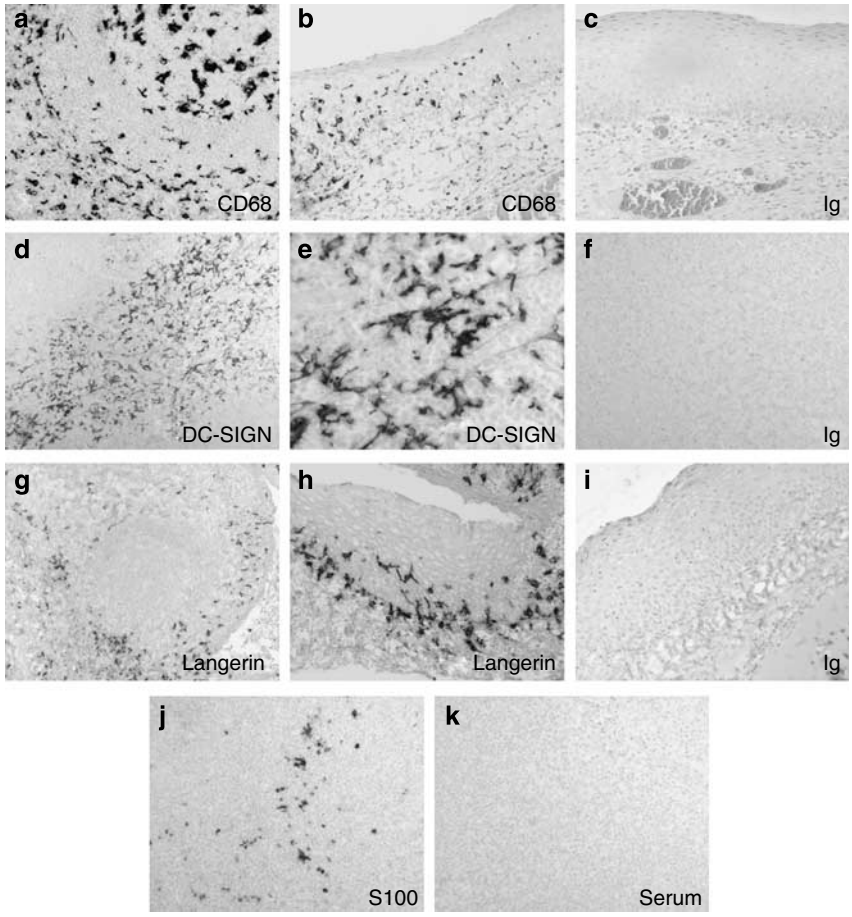

Figure 7 | Establishment of the antibodies against DC/Mac markers on human tonsils. Immunohistochemistry was performed on sections from human tonsils using monoclonal antibodies against (**a**, **b**) CD68, (**d**, **e**) DC-SIGN, (**g**, **h**) Langerin, and (**j**) a polyclonal antiserum against S100, or (**c**, **f**, **i**, **k**) the corresponding isotype immunoglobulin controls (original magnification, × 100 (**d**, **f**, **g**); × 200 (**a**–**c**, **h**–**k**); × 400 (**e**)). Note the differences in antigen expression by cells in the follicles and the surrounding T-cell areas. Isotype controls did not result in a positive color product.

Commercially available predeveloped TaqMan reagents were used for the target genes CD68, DC-SIGN, DC-LAMP, HLA-DRα, Langerin (all from Applied Biosystems), and three endogenous control genes (18S rRNA, cyclophilin A, GAPDH; Applied Biosystems). The data shown in the text and figures are normalized to 18S rRNA. All measurements were performed in duplicates. Controls consisting of bidistilled H_2O were negative in all runs. The biopsy samples used for the mRNA analysis were from a different cohort than the morphological study.

Confocal microscopy

Fluorescence images were captured with a Leica TCS SP2 Confocal System, equipped with lasers exciting at 488 and 543 nm (Ar/Kr), 633 nm (HeNe), and 405 nm (diode laser) on a Leica DM IRBE microscope stand with HCX PL APO 63×1.40 NA oil immersion objective lens (Leica Microsystems, Heidelberg, Germany). To avoid possible cross talk of the fluorochromes, the width of the detection channels and filter settings were carefully controlled and images were acquired using the sequential image recording method. For evaluation of colocalization, single *z*-planes were analyzed with Leica confocal software LCSLite (Leica Microsystems) and ImageJ 1.37 (Wright Cell Imaging Facility, Toronto, ON, Canada).

Digital image analysis

Morphometric analysis was performed on 15 consecutive high-power fields (original magnification $\times 400$) or on 15 glomeruli by the use of Qwin software (Leica, Bensheim, Germany).[47] The area was measured by an observer blinded to the diagnosis, and expressed as a fraction of the area of the high-power field or area of the glomerular tuft.

Statistical analyses

For the comparison of means, the non-parametric Dunn's multiple comparison test and Spearman rank correlation were used for the correlations between expression data (InStat Software, Version 3.05; Intuitive Software for Science, San Diego, CA, USA). $P < 0.05$ was considered to be statistically significant.

DISCLOSURE

The authors have no relationships with companies resulting in financial interests associated with data presented in this paper.

ACKNOWLEDGMENTS

We thank members of the European Renal cDNA Bank–Kroener-Fresenius Biopsy Bank at the time of the study: CD Cohen, M Fischereder, H Schmid, L Weber, M Kretzler, D Schlöndorff, Munich; JD Sraer, P Ronco, Paris; MP Rastaldi, G D'Amico, Milano; P Doran, H Brady, Dublin; D Mönks, C Wanner, Würzburg; AJ Rees, Aberdeen; F Strutz, G Müller, Göttingen; P Mertens, J Floege, Aachen; N Braun, T Risler, Tübingen; L Gesualdo, FP Schena, Bari; J Gerth, G Wolf, Jena; R Oberbauer, D Kerjaschki, Vienna; B Banas, BK Krämer, Regensburg; M Saleem, Bristol; RP Wüthrich, Zürich; W Samtleben, Munich; H Peters, HH Neumayer, Berlin; M Daha, Leiden; K Ivens, B Grabensee, Düsseldorf; F Mampaso(✠), Madrid; J Oh, F Schaefer, M Zeier, H-J Gröne, Heidelberg; P Gross, Dresden; G Tonolo, Sassari; V Tesar, Prague; H Rupprecht, Bayreuth. Parts of the study have been presented in abstract form at the meeting of the International Society of Nephrology (Rio, 2007) and the meeting of the German Society of Nephrology (Munich, 2007). SS and CDC are supported by the Else Kröner-Fresenius Stiftung, Germany; SS and EN by the Deutsche Forschungsgemeinschaft (SE 888/4-1), SFB455, SFBTR36, and the EU Network of Excellence 'MAIN' (FP6-502935). A-MF is supported by the Universität Bayern e.V., according to the Bayerische Eliteförderungsgesetz.

REFERENCES

1. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005; **5**: 953–964.
2. Hume DA. The mononuclear phagocyte system. *Curr Opin Immunol* 2006; **18**: 49–53.
3. Naik SH, Sathe P, Park HY *et al.* Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived *in vitro* and *in vivo*. *Nat Immunol* 2007; **8**: 1217–1226.
4. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol* 2007; **7**: 19–30.
5. Onai N, Obata-Onai A, Schmid MA *et al.* Identification of clonogenic common Flt3(+)M-CSFR(+) plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol* 2007; **8**: 1207–1216.
6. Merad M, Ginhoux F. Dendritic cell genealogy: a new stem or just another branch? *Nat Immunol* 2007; **8**: 1199–1201.
7. Quah BJ, O'Neill HC. Maturation of function in dendritic cells for tolerance and immunity. *J Cell Mol Med* 2005; **9**: 643–654.
8. Steinman RM, Hemmi H. Dendritic cells: translating innate to adaptive immunity. *Curr Top Microbiol Immunol* 2006; **311**: 17–58.
9. Banchereau J, Briere F, Caux C *et al.* Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; **18**: 767–811.
10. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003; **3**: 23–35.
11. Hart DN, Fabre JW. Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. *J Exp Med* 1981; **154**: 347–361.
12. Kruger T, Benke D, Eitner F *et al.* Identification and functional characterization of dendritic cells in the healthy murine kidney and in experimental glomerulonephritis. *J Am Soc Nephrol* 2004; **15**: 613–621.
13. Soos TJ, Sims TN, Barisoni L *et al.* CX3CR1+ interstitial dendritic cells form a contiguous network throughout the entire kidney. *Kidney Int* 2006; **70**: 591–596.
14. Kerjaschki D, Regele HM, Moosberger I *et al.* Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. *J Am Soc Nephrol* 2004; **15**: 603–612.
15. Woltman AM, de Fijter JW, Zuidwijk K *et al.* Quantification of dendritic cell subsets in human renal tissue under normal and pathological conditions. *Kidney Int* 2007; **71**: 1001–1008.
16. Kaissling B, LeHir M. Characterization and distribution of interstitial cell types in the renal cortex of rats. *Kidney Int* 1994; **45**: 709–720.
17. Austyn JM, Hankins DF, Larsen CP *et al.* Isolation and characterization of dendritic cells from mouse heart and kidney. *J Immunol* 1994; **152**: 2401–2410.
18. Falini B, Flenghi L, Pileri S *et al.* PG-M1: a new monoclonal antibody directed against a fixative-resistant epitope on the macrophage-restricted form of the CD68 molecule. *Am J Pathol* 1993; **142**: 1359–1372.
19. Segerer S, Cui Y, Hudkins KL *et al.* Expression of the chemokine monocyte chemoattractant protein-1 and its receptor chemokine receptor 2 in human crescentic glomerulonephritis. *J Am Soc Nephrol* 2000; **11**: 2231–2242.
20. Segerer S, Henger A, Schmid H *et al.* Expression of the chemokine receptor CXCR1 in human glomerular diseases. *Kidney Int* 2006; **69**: 1765–1773.
21. Segerer S, Hughes E, Hudkins KL *et al.* Expression of the fractalkine receptor (CX3CR1) in human kidney diseases. *Kidney Int* 2002; **62**: 488–495.
22. Geijtenbeek TB, Torensma R, van Vliet SJ *et al.* Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 2000; **100**: 575–585.
23. Steinman RM. DC-SIGN: a guide to some mysteries of dendritic cells. *Cell* 2000; **100**: 491–494.
24. Nakajima T, Kodama T, Tsumuraya M *et al.* S-100 protein-positive Langerhans cells in various human lung cancers, especially in peripheral adenocarcinomas. *Virchows Arch A Pathol Anat Histopathol* 1985; **407**: 177–189.
25. Cocchia D, Tiberio G, Santarelli R *et al.* S-100 protein in 'follicular dendritic' cells or rat lymphoid organs. An immunochemical and immunocytochemical study. *Cell Tissue Res* 1983; **230**: 95–103.
26. Xu W, Chen S, Huang J *et al.* The expression and distribution of S-100 protein and CD 83 in thyroid tissues of autoimmune thyroid diseases. *Cell Mol Immunol* 2004; **1**: 378–382.
27. Perez L, Shurin MR, Collins B *et al.* Comparative analysis of CD1a, S-100, CD83, and CD11c human dendritic cells in normal, premalignant, and malignant tissues. *Histol Histopathol* 2005; **20**: 1165–1172.
28. Asahina A, Tamaki K. Role of Langerhans cells in cutaneous protective immunity: is the reappraisal necessary? *J Dermatol Sci* 2006; **44**: 1–9.

29. de Witte L, Nabatov A, Pion M *et al.* Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. *Nat Med* 2007; **13**: 367–371.
30. Dzionek A, Fuchs A, Schmidt P *et al.* BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol* 2000; **165**: 6037–6046.
31. Dzionek A, Sohma Y, Nagafune J *et al.* BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J Exp Med* 2001; **194**: 1823–1834.
32. Zimmer DB, Cornwall EH, Landar A *et al.* The S100 protein family: history, function, and expression. *Brain Res Bull* 1995; **37**: 417–429.
33. Dzionek A, Inagaki Y, Okawa K *et al.* Plasmacytoid dendritic cells: from specific surface markers to specific cellular functions. *Hum Immunol* 2002; **63**: 1133–1148.
34. Kluth DC, Erwig LP, Rees AJ. Multiple facets of macrophages in renal injury. *Kidney Int* 2004; **66**: 542–557.
35. Sean Eardley K, Cockwell P. Macrophages and progressive tubulointerstitial disease. *Kidney Int* 2005; **68**: 437–455.
36. John R, Nelson PJ. Dendritic cells in the kidney. *J Am Soc Nephrol* 2007; **18**: 2628–2635.
37. Merad M, Collin M, Bromberg J. Dendritic cell homeostasis and trafficking in transplantation. *Trends Immunol* 2007; **28**: 353–359.
38. Stout RD, Suttles J. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. *J Leukoc Biol* 2004; **76**: 509–513.
39. Wang Y, Wang YP, Zheng G *et al. Ex vivo* programmed macrophages ameliorate experimental chronic inflammatory renal disease. *Kidney Int* 2007; **72**: 290–299.
40. Eardley KS, Zehnder D, Quinkler M *et al.* The relationship between albuminuria, MCP-1/CCL2, and interstitial macrophages in chronic kidney disease. *Kidney Int* 2006; **69**: 1189–1197.
41. Yoshimoto K, Wada T, Furuichi K *et al.* CD68 and MCP-1/CCR2 expression of initial biopsies reflect the outcomes of membranous nephropathy. *Nephron Clin Pract* 2004; **98**: c25–c34.
42. van Gisbergen KP, Geijtenbeek TB, van Kooyk Y. Close encounters of neutrophils and DCs. *Trends Immunol* 2005; **26**: 626–631.
43. Gijzen K, Broers KM, Beeren IM *et al.* Binding of the adhesion and pathogen receptor DC-SIGN by monocytes is regulated by the density of Lewis X molecules. *Mol Immunol* 2007; **44**: 2481–2486.
44. Segerer S, Banas B, Wornle M *et al.* CXCR3 is involved in tubulointerstitial injury in human glomerulonephritis. *Am J Pathol* 2004; **164**: 635–649.
45. Masaki T, Chow F, Nikolic-Paterson DJ *et al.* Heterogeneity of antigen expression explains controversy over glomerular macrophage accumulation in mouse glomerulonephritis. *Nephrol Dial Transplant* 2003; **18**: 178–181.
46. Morelli AE, Coates PT, Shufesky WJ *et al.* Growth factor-induced mobilization of dendritic cells in kidney and liver of rhesus macaques: implications for transplantation. *Transplantation* 2007; **83**: 656–662.
47. Heller F, Lindenmeyer MT, Cohen CD *et al.* The contribution of B cells to renal interstitial inflammation. *Am J Pathol* 2007; **170**: 457–468.
48. Segerer S, Mack M, Regele H *et al.* Expression of the C–C chemokine receptor 5 in human kidney diseases. *Kidney Int* 1999; **56**: 52–64.
49. Stuht S, Gwinner W, Franz I *et al.* Lymphatic neoangiogenesis in human renal allografts: results from sequential protocol biopsies. *Am J Transplant* 2007; **7**: 377–384.
50. Kerjaschki D, Huttary N, Raab I *et al.* Lymphatic endothelial progenitor cells contribute to *de novo* lymphangiogenesis in human renal transplants. *Nat Med* 2006; **12**: 230–234.
51. Valladeau J, Duvert-Frances V, Pin JJ *et al.* The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells, and is rapidly internalized from the cell surface. *Eur J Immunol* 1999; **29**: 2695–2704.
52. Stambach NS, Taylor ME. Characterization of carbohydrate recognition by Langerin, a C-type lectin of Langerhans cells. *Glycobiology* 2003; **13**: 401–410.
53. McGreal EP, Martinez-Pomares L, Gordon S. Divergent roles for C-type lectins expressed by cells of the innate immune system. *Mol Immunol* 2004; **41**: 1109–1121.
54. Treilleux I, Blay JY, Bendriss-Vermare N *et al.* Dendritic cell infiltration and prognosis of early stage breast cancer. *Clin Cancer Res* 2004; **10**: 7466–7474.
55. Takahara K, Omatsu Y, Yashima Y *et al.* Identification and expression of mouse Langerin (CD207) in dendritic cells. *Int Immunol* 2002; **14**: 433–444.
56. Segerer S, Bohmig GA, Exner M *et al.* Role of CXCR3 in cellular but not humoral renal allograft rejection. *Transpl Int* 2005; **18**: 676–680.
57. Cohen CD, Frach K, Schlondorff D *et al.* Quantitative gene expression analysis in renal biopsies: a novel protocol for a high-throughput multicenter application. *Kidney Int* 2002; **61**: 133–140.