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Abstract: **BACKGROUND:** Nodular inflammatory cell infiltrates with defined microarchitecture, i.e. tertiary lymphoid organs, develop in the tubulointerstitium during chronic renal inflammation. CCR6 and the corresponding ligand CCL20 are involved in the formation of gut-associated lymphatic tissue. We hypothesized that CCR6 might be involved in the formation of nodular infiltrates in the kidney. **METHODS:** CCR6- and CD20-positive B cells were localized in renal biopsies with IgA nephropathy (n = 13), membranous nephropathy (n = 12), crescentic glomerulonephritis (cGN, n = 11) and chronic interstitial nephritis (n = 13), and in pre-implantation biopsies as controls (n = 8). The mRNA expression of CCR6 and the ligand CCL20 was quantified by real-time RT-PCR in 51 renal biopsies of the same disease entities. **RESULTS:** In the pre-transplant biopsies, CCR6 was expressed by endothelial cells of peritubular and glomerular capillaries. In patients with glomerulonephritis, infiltrating cells were positive particularly in areas of nodular inflammatory cell accumulations. A major part of the CCR6-positive cells were CD20-positive B cells, but a part of the CD3-positive T cells were also found to be positive. The constitutive expression of CCR6 on the endothelium of glomerular capillaries was lost in biopsies with progressive injury. Tubular epithelial cells expressed CCR6 in inflamed kidneys, most commonly on the basolateral side. **CONCLUSIONS:** CCR6 and the corresponding ligand CCL20 might therefore be involved in the recruitment of T and B cells to organized nodular infiltrates in chronic renal inflammation. The functional role of endothelial CCR6 needs to be evaluated in further studies.

DOI: <https://doi.org/10.1093/ndt/gfq560>

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ZORA URL: <https://doi.org/10.5167/uzh-41068>

Journal Article

Published Version

Originally published at:

Welsh-Bacic, D; Lindenmeyer, M; Cohen, C D; Draganovici, D; Mandelbaum, J; Edenhofer, I; Ziegler, U; Regele, H; Wüthrich, R P; Segerer, S (2011). Expression of the chemokine receptor CCR6 in human renal inflammation. *Nephrology, Dialysis, Transplantation*, 26(4):1211-1220.

DOI: <https://doi.org/10.1093/ndt/gfq560>

Expression of the chemokine receptor CCR6 in human renal inflammation

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Abstract

Background. Nodular inflammatory cell infiltrates with defined microarchitecture, i.e. tertiary lymphoid organs, develop in the tubulointerstitium during chronic renal inflammation. CCR6 and the corresponding ligand CCL20 are involved in the formation of gut-associated lymphatic tissue. We hypothesized that CCR6 might be involved in the formation of nodular infiltrates in the kidney.

Methods. CCR6- and CD20-positive B cells were localized in renal biopsies with IgA nephropathy ($n = 13$), membranous nephropathy ($n = 12$), crescentic glomerulonephritis (cGN, $n = 11$) and chronic interstitial nephritis ($n = 13$), and in pre-implantation biopsies as controls ($n = 8$). The mRNA expression of CCR6 and the ligand CCL20 was quantified by real-time RT-PCR in 51 renal biopsies of the same disease entities.

Results. In the pre-transplant biopsies, CCR6 was expressed by endothelial cells of peritubular and glomerular capillaries. In patients with glomerulonephritis, infiltrating cells were positive particularly in areas of nodular inflammatory cell accumulations. A major part of the CCR6-positive cells were CD20-positive B cells, but a part of the CD3-positive T cells were also found to be positive. The constitutive expression of CCR6 on the endothelium of glomerular capillaries was lost in biopsies with progressive injury. Tubular epithelial cells expressed CCR6 in inflamed kidneys, most commonly on the basolateral side.

Conclusions. CCR6 and the corresponding ligand CCL20 might therefore be involved in the recruitment of T and B cells to organized nodular infiltrates in chronic renal inflammation. The functional role of endothelial CCR6 needs to be evaluated in further studies.

Keywords: B cells; CCL20; CCR6; chemokine receptor; glomerulonephritis

Introduction

Lymphocytes, macrophages and dendritic cells accumulate in the tubulointerstitium of the human kidney during progressive diseases [1–3]. The numbers of interstitial infiltrating T cells and macrophages correlate with renal function at the time of biopsy [2,4]. In addition, to diffuse infiltrates, leucocytes form nodular structures in about one-third of the biopsies with chronic diseases [3,5]. These structures are called tertiary lymphoid organs (TLOs), and the functional role of TLOs in the kidney is under intense investigation [6–8].

Chemokines are members of a large family of chemotactic cytokines, which orchestrate the recruitment of inflammatory cell subsets under homeostatic and pathological condition [9]. Expression and presentation of chemokines in particular microenvironments are involved in the formation of lymphoid tissue as well as in chronic renal inflammation [10–12]. The chemokine receptor CCR6 signals after binding the chemokine CCL20, which is the only known ligand for this receptor so far [13,14]. CCR6 is acquired during B-cell maturation and is expressed by all bone marrow, and peripheral blood-derived naive and memory B cells [15]. CCR6 expression has been shown in memory T cells, regulatory T cells, and IL-17-producing CD4-positive T cells (a distinct subset of cells now referred to as Th17 cells) [16–19]. CCR6 is also expressed on a subset of dendritic cells [17,20,21].

CCR6-positive B cells play an important role in the formation of gut-associated lymphatic tissue, i.e. Peyer's patches and isolated lymphoid follicles [22]. The formation of TLOs during chronic inflammation might in part be a recapitulation of the embryonic formation of secondary lymphoid organs [5]. Therefore, we hypothesized that CCR6 might be involved in the formation of nodular infiltrates in chronic renal inflammation. Therefore, we studied

the expression of CCR6 and the ligand CCL20 in the most common forms of human glomerulonephritis, as a prerequisite for further functional studies.

Materials and methods

Study population

Renal biopsies were formalin-fixed, and paraffin-embedded following routine protocols. The diagnosis was based on light microscopy, immunohistochemistry and electron microscopy. Archival sections were used for the current study from patients with crescentic glomerulonephritis (cGN, $n = 11$), IgA nephropathy ($n = 13$), membranous nephropathy ($n = 12$) and chronic interstitial nephritis ($n = 13$). Allograft biopsies taken before implantation served as controls ($n = 8$).

Immunohistochemistry

Immunohistochemistry was performed as previously described [3,23]. In brief, sections were dewaxed in xylene, rehydrated in a graded series of ethanol, and incubated in 3% hydrogen peroxide. The Avidin/Biotin blocking Kit (Vector, Burlingame, CA, USA) was used to block endogenous biotin. An autoclave oven (or microwave treatment) was used for heat-based antigen retrieval in Antigen retrieval solution (Vector). Incubation with the primary antibody was performed for 1 h. Incubation with biotinylated secondary antibodies (Vector) for 30 min was followed by the ABC reagent (Vector). 3,3'-Diaminobenzidine (DAB, Sigma, Taufkirchen, Germany) with metal enhancement was used as a detection system. Two polyclonal antibodies against CCR6 raised in two different rabbits were tested and resulted in the same pattern (anti-human CCR6 by MBL International Corporation, Woburn, MA, USA and Chemicon, Temecula, CA, USA). The primary polyclonal antibody against CCR6 (Chemicon) was used in a dilution of 1:1000 in 10% non-fat dry milk on renal biopsies. Other antibodies used were a monoclonal antibody against CD20 (clone L26, DakoCytomation, Dako Deutschland, Hamburg, Germany), an anti-CD3 antibody (clone: CD3-12, rat anti-human, Serotec, Oxford, UK), and an anti-CD34 antibody (Accurate Chemical and Scientific Corporation, Westbury, NY, USA).

CD34 was stained on a total of nine biopsies on consecutive sections with CCR6 (four biopsies with chronic interstitial nephritis and five controls).

The numbers of CD20- and CCR6-positive cells were scored semi-quantitatively: 0, no infiltrating cells; 1, scattered positive infiltrating cells between tubules; 2, accumulations of several infiltrating cells between tubules; and 3, nodular appearance of the infiltrates by an observer blinded to the disease entities.

Immunofluorescence

Double-labelling immunofluorescence was performed as described previously [3]. As secondary reagents, biotinylated antibodies (Vector), Streptavidin/FITC complex (Vector), Cy3-labelled anti-rat antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA), and Texas red- and FITC-labelled anti-mouse IgG (Vector) were used.

Real-time RT-PCR

To quantify the mRNA expression of CCL20 and CCR6, we used real-time RT-PCR as described [24]. The biopsies were from patients with cGN ($n = 8$), IgA nephropathy ($n = 14$), membranous nephropathy ($n = 20$) and allograft biopsies taken before implantation served as controls ($n = 9$). The renal biopsies were obtained from a multicentre renal biopsy bank (the European Renal cDNA Bank, ERCB). Informed consent was obtained before renal biopsies were performed. The microdissected tubulointerstitial compartments were used.

Real-time RT-PCR was performed on a TaqMan ABI 7700 Sequence Detection System (Applied Biosystems, Darmstadt, Germany) using heat-activated TaqDNA polymerase (Amplitaq Gold, Applied Biosystems). Quantification of the given templates was performed according to the standard curve method. Commercially available pre-developed TaqMan reagents were used for the target genes CCR6 (NM_031409) and CCL20 (NM_004591) (all from Applied Biosystems), and two endogenous control genes (18S rRNA, GAPDH; Applied Biosystems). The normalization to the two reference genes (housekeeper genes) gave comparable results. The data shown in the text and figures are normalized to GAPDH. All measurements were performed in duplicates. Controls consisting of bidistilled H₂O were negative in all runs. The samples used for the

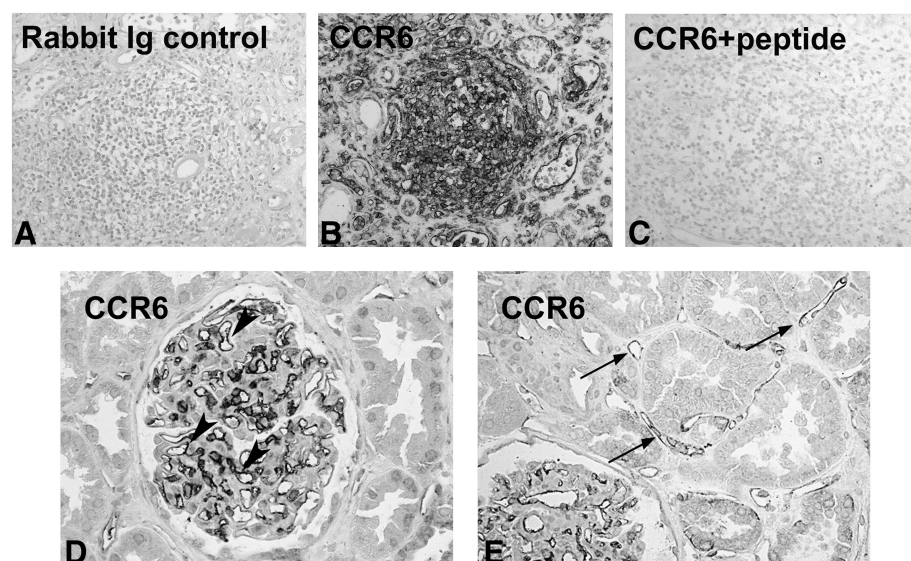


Fig. 1. Establishment of the anti-CCR6 antibody and expression in normal human kidney. Immunohistochemistry was performed on an allograft nephrectomy (A–C), and a pre-transplant biopsy (D and E) with a polyclonal antibody against CCR6 (B, D and E, Chemicon), a non-immune rabbit serum (A), and after pre-absorption of the antiserum with the peptide (C, original $\times 250$ D, E, original $\times 200$ in A–C). The negative controls performed with non-immune rabbit serum (A) and pre-absorption with the peptide used for the induction of the antiserum did not demonstrate a positive colour product (C). Prominent signal of a nodular infiltrate in the allograft nephrectomy (B). In the well-preserved pre-transplant biopsies, a positive signal was present on endothelial cells of glomerular capillaries (arrowheads, D) and peritubular capillaries (E, arrows).

mRNA analysis were from a different cohort than the biopsies used for immunohistochemistry.

Quantification and statistical analysis

The semi-quantitative scores for the interstitial CCR6-positive cells and the percentage of CCR6 staining per glomerular area were evaluated by observers blinded to the diagnosis. For the comparison of medians, the non-parametric Kruskal–Wallis test and Dunn's multiple comparisons test were used. Spearman rank correlations were performed for the correlations with clinical and morphological data (InStat® software, version 3.05, Intuitive Software for Science, San Diego, CA, USA). A $P < 0.05$ was considered to be significant. Error bars illustrate standard error of the mean (SEM).

Results

Description of the antibody and CCR6 expression in pre-transplantation biopsies

For the establishment of the polyclonal anti-CCR6 antiserum, we used tissue sections from allograft nephrectomies

(Figure 1A–C). A reliable staining pattern was achieved using a heat-based antigen retrieval. Expression of CCR6 was present on inflammatory cells and on endothelial cells in these positive controls (Figure 1B). A second antiserum against CCR6 resulted in the same pattern (not illustrated). A non-immune rabbit serum did not result in a black colour product (Figure 1A). Additionally, the pre-incubation with the peptide used for the generation of the antibody completely abolished the signal (Figure 1C).

Double labelling of an allograft nephrectomy confirmed that CD20-positive B cells formed a major part of the CCR6-positive infiltrating cells (Figure 2A–C). CD3-positive T cells represent additional CCR6-positive cells (Figure 2D and E). Positivity of endothelial cells was confirmed in double-labelling studies with CD34, an endothelial marker (Figure 2F–H).

Eight allograft biopsies taken before implantation were used as controls (Figure 1D and E). These pre-transplantation biopsies demonstrated well-preserved renal tissue, with variable degrees of global glomerulosclerosis. Mild interstitial

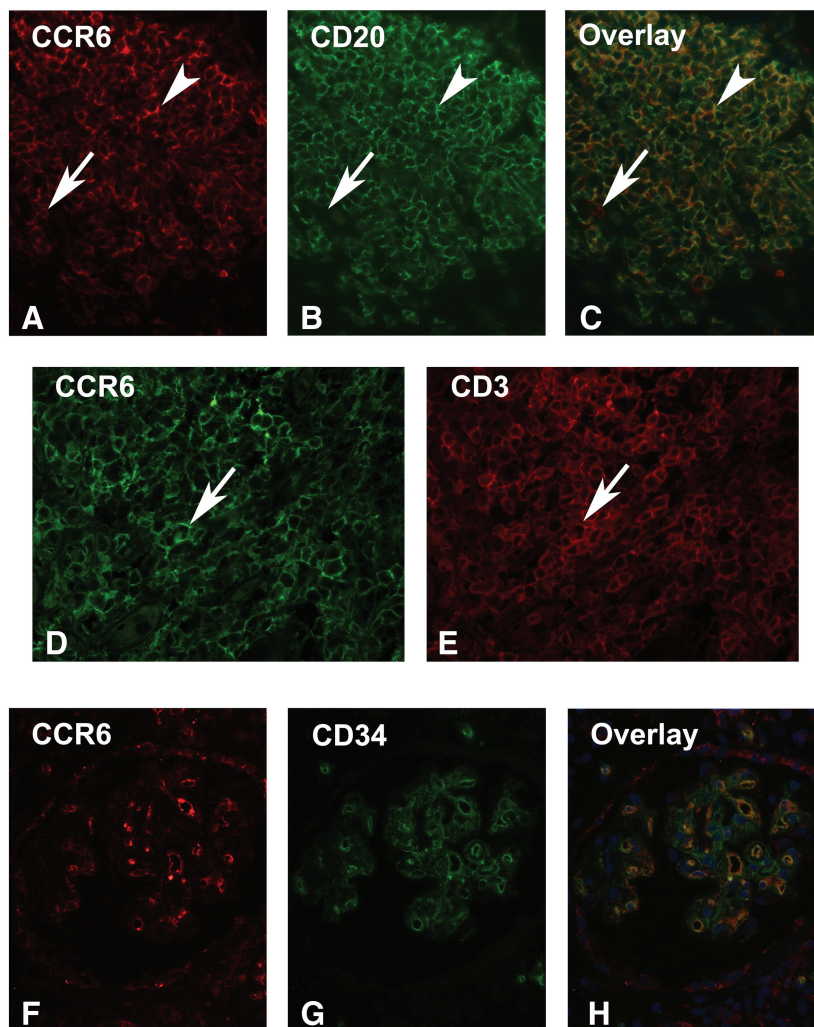


Fig. 2. Double immunofluorescence for CCR6. Double Immunofluorescence was performed on tissue sections from an allograft nephrectomy. A–C. Double labelling for CCR6 (A), CD20 (B) and the overlay in C illustrates the majority of this nodular aggregate being CCR6-positive B cells (arrows). D and E illustrate an area with a high number of CD3/CCR6 double-positive cells (arrowhead). F–H illustrate CCR6-positive endothelial cells in a glomerulus (F: CCR6, G: CD34, H: overlay, original $\times 400$).

Table 1. Basic clinical parameters of the patients included in the immunohistochemistry cohort

Disease	<i>n</i>	Age (years)	Creatinine (mg/dL)	Proteinuria (g/24 h)
IgA N	13	37 (14–59)	1.5 (0.8–8.3)	1.5 (0–7)
Membranous N	12	45 (13–65)	1.1 (0.6–6.4)	7 (2.4–22)
cGN	11	63 (35–84)	3.8 (1.4–8.4)	0.4 (0.3–8)
c Int	13	44 (3–81)	4.8 (1.8–13)	0.6 (0.08–25)
Controls	8	27 (0–67)	n.a.	n.a.

Data are presented as median (range) except *n*. n.a., not available; N, nephropathy; cGN, crescentic glomerulonephritis; c Int, chronic interstitial nephritis.

infiltration was present surrounding sclerosed glomeruli, a feature commonly found in ageing kidneys. These biopsies therefore cannot be regarded as normal, as these were exposed to cold ischaemia and the circumstances of brain-dead of the deceased donors, but the morphology demonstrated well-preserved tissue architecture.

In these biopsies, we found CCR6 to be expressed by endothelial cells of glomerular and peritubular capillaries without prominent variation between the biopsies (Figure 1D and E). Some circulating cells in the lumen of open glomerular capillaries or peritubular capillaries were found to be CCR6 positive, but these cells were rare. Expression of CCR6 by tubular epithelial cells was only occasionally found.

CCR6 expression in IgA nephropathy

Thirteen biopsies from patients with IgA nephropathy were studied (Table 1). Expression of CCR6 was found on endothelial cells of glomerular and peritubular capillaries. The staining was less intense as compared with controls. Furthermore, segmental to global loss of staining was present in glomeruli (Figure 3A). Parietal epithelial cells were found to be positive in a few glomeruli (Figure 3D). Tubular epithelial cells showed weak basolateral staining with focal distribution, particularly in tubules adjacent to sites of interstitial inflammation (Figure 3C). Infiltrating CCR6-positive

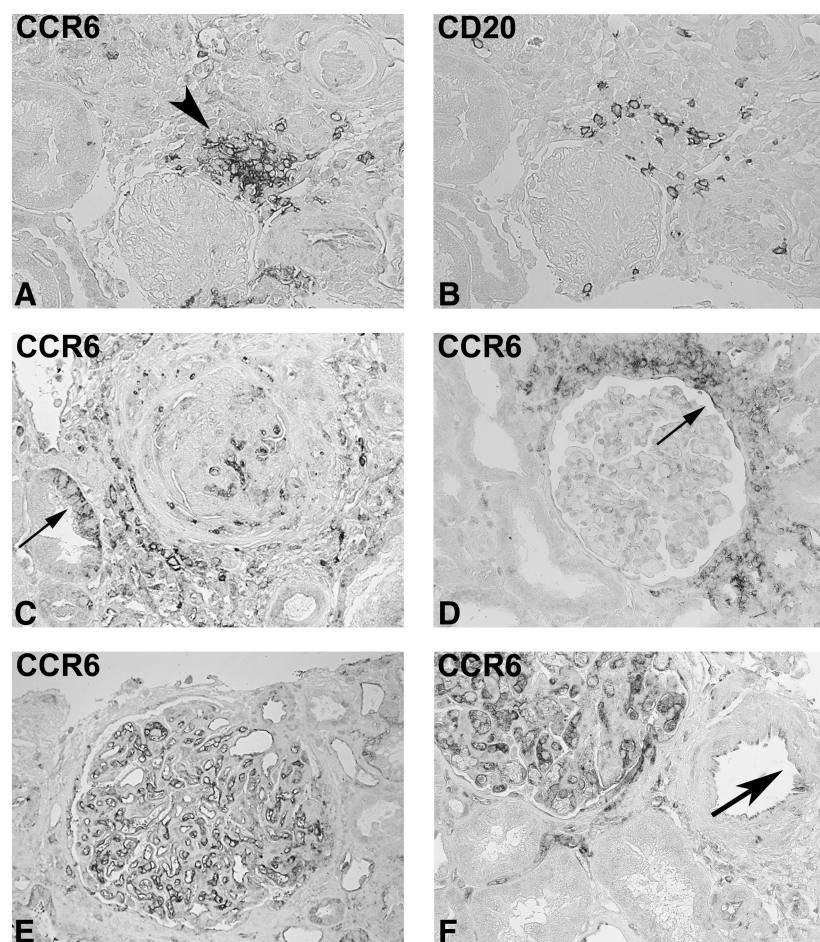


Fig. 3. CCR6 expression in renal biopsies from patients with IgA nephropathy and membranous nephropathy. Immunohistochemistry was performed on renal biopsies from patients with IgA nephropathy (A–D) and with membranous nephropathy (E–F) with a polyclonal antibody against CCR6 (A, C–F) and a monoclonal antibody against CD20 (B; original $\times 100$ in C–F, $\times 250$ in A and B). Consecutive section in A and B demonstrates that only the periphery of the CCR6-positive small nodular infiltrate consists of CD20-positive B cells. In C and D, the glomerular endothelial staining is lost. Tubular CCR6 staining is illustrated in C (arrow). D demonstrates prominent CCR6-positive peri-glomerular infiltration and CCR6 expression by parietal epithelial cells (arrow). The staining pattern was similar in membranous nephropathy (E and F). Endothelial cells of arteries were rarely found to be CCR6 positive.

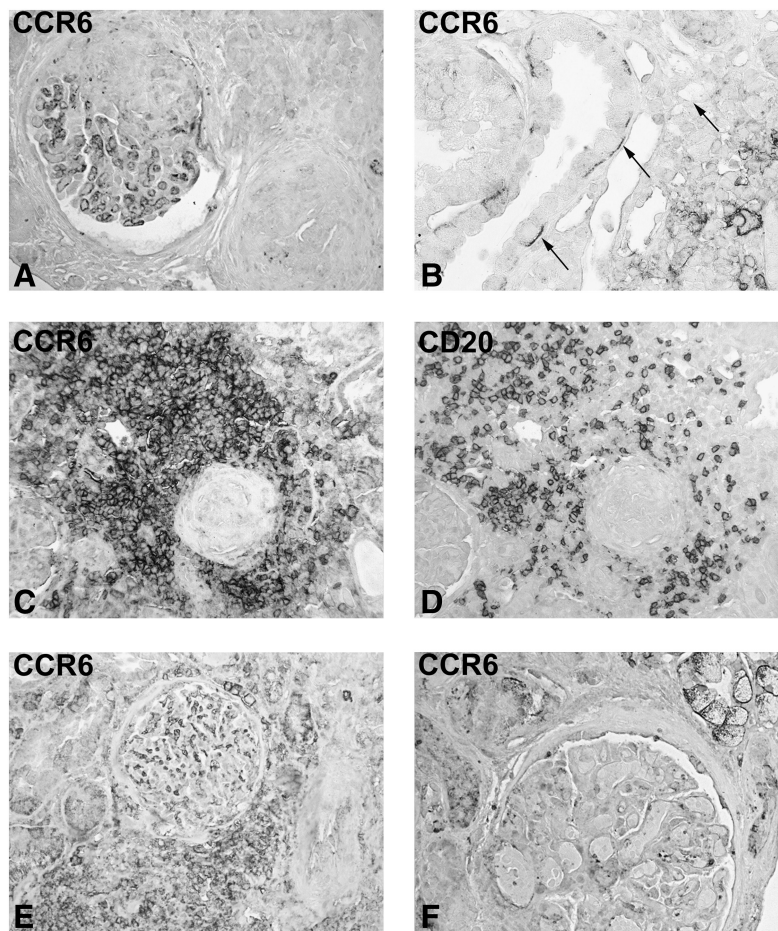


Fig. 4. CCR6 expression in biopsies with crescentic GN and chronic interstitial nephritis. Immunohistochemistry was performed on renal biopsies from patients with cGN (A–D) and patients with chronic interstitial nephritis (E and F) with a polyclonal antibody against CCR6 (A–C, E and F) and with a monoclonal antibody against CD20 (D, original $\times 250$ in A–E, and $\times 400$ in F). A segmental loss of the endothelial staining in the upper part of the glomerulus is illustrated in A. Basal tubular expression of CCR6 is shown in B. A prominent accumulation of CCR6-positive cells was found around globally sclerosed glomeruli, with CD20-positive cells being a major part of the infiltrate (C and D). In chronic interstitial nephritis, both interstitial accumulation of CCR6-positive cells (E) and loss of endothelial CCR6 were present (F).

cells were found scattered throughout the cortex and at times accumulated around the glomeruli. Nodular infiltrates were present in 5 out of 13 biopsies. In small accumulations, CD20-positive cells were outnumbered by CCR6-positive cells not expressing CD20 (Figure 3A and B).

CCR6 in membranous nephropathy

Included were 12 biopsies from patients with membranous nephropathy (Table 1). This was the group of patients with the best preserved renal function. On intrinsic renal cells, CCR6 was found to be expressed as in IgA nephropathy (Figure 3E and F). Infiltrating CCR6-positive cells were only found with diffuse distribution, and no formation of nodular structures was present both in the CCR6 and CD20 staining (Figure 3E and F).

CCR6 expression in cGN

Eleven biopsies from patients with crescentic glomerulonephritis were included. All of these were pauci-immune by immunohistochemistry. Therefore, no crescentic IgA

nephritis or anti-glomerular basement membrane diseases were included in this group.

CCR6 was found to be expressed on glomerular endothelial cells in preserved glomeruli, as described for normal controls, but segmental loss of positive staining was common (Figure 4A). Tubular epithelial cells were CCR6-positive in 8 out of 10 biopsies (Figure 4B). Occasionally, intercalating cells of collecting ducts were found to be positive (Figure 4B). An intensive accumulation of CCR6-positive infiltrating cells was present in the tubulointerstitium, but not in the glomeruli (Figure 4C and E). Nodular infiltrates were almost uniformly CCR6 positive (Figure 4C and D). These structures were commonly found around the completely sclerotic glomeruli (Figure 4C and D). On the other hand, scattered infiltrating cells were only occasionally CCR6 positive. The distribution of CCR6-positive cells matched only about half of the CD20-positive cells (Figure 4C and D). In cGN, a high number of macrophages are usually present in the glomerular tuft and in crescents [25]. The absence of CCR6 staining on infiltrating cells in glomerular tufts and crescents indicates that CCR6 is mainly expressed by lymphocytes (loca-

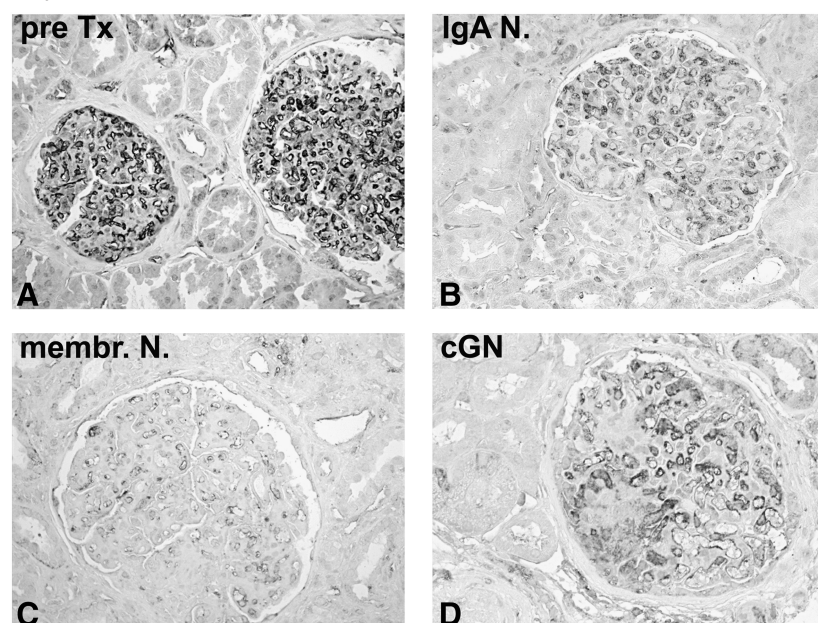


Fig. 5. Comparison glomerular CCR6 expression between the glomerular diseases. Immunohistochemistry was performed with a polyclonal antibody against CCR6 on renal biopsies before implantation (pre Tx control, **A**), with IgA nephropathy (**B**, IgA N.), with membranous nephropathy (membr. N., **C**) and with cGN (**D**, all original $\times 250$). A weaker endothelial staining was found in all three glomerulopathies with segmental to global loss of the staining pattern, even in preserved glomerular capillaries.

lized in the tubulointerstitium), but not macrophages recruited to glomeruli.

CCR6 in chronic interstitial nephritis

Included were 13 biopsies with chronic interstitial nephritis (Table 1). The pattern of CCR6 expression was comparable to the glomerular diseases presented above. Glomerular endothelial cells stained positive for CCR6, but the staining intensity was weaker than in controls. Occasionally, parietal epithelial cells demonstrated a positive signal. In 10 out of 13 biopsies, tubular epithelial

cells were found to be focally CCR6-positive on basolateral membranes of atrophic tubules of cortex and medulla (Figure 4F). Inflammatory cells positive for CCR6 were mainly scattered diffusely throughout the renal cortex (Figure 4E). Additionally, in 9 out of 13 biopsies, nodular structures were present, where the majority of these cells were CCR6 positive (not illustrated).

In four biopsies, CD34 was stained in parallel with CCR6. Complete loss of CD34 and CCR6 was present in globally sclerosed glomeruli. Furthermore, some glomeruli demonstrated a less prominent CCR6 staining, and also, CD34 was preserved.

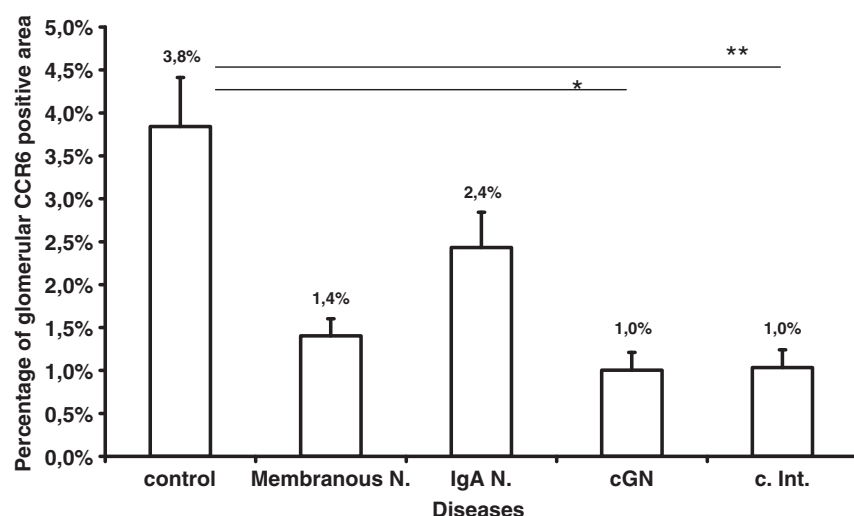


Fig. 6. Morphometric quantification of glomerular CCR6 expression. Mean percentage of CCR6-positive colour product per glomerular area is illustrated (* $P < 0.05$, ** $P < 0.01$).

Table 2. Morphological results of the study population

Disease	<i>n</i>	Interstitial CD20 scores	Interstitial CCR6 scores
IgA N	13	1 (0–3)	1 (0–3)
Membranous N	12	1 (0–2)* vs cGN	1 (0–3)
cGN	11	2 (1–3)*** vs control	2 (1–3)* vs control
c Int	13	2 (1–3)** vs control	2 (1–3)** vs control
Controls	8	0 (0–1)	0 (0–1)

Data are presented as median (range) except *n*. n.a., not available; N, nephropathy; cGN, crescentic glomerulonephritis; c Int, chronic interstitial nephritis.

**P* < 0.05.

***P* < 0.01.

****P* < 0.001.

Common pattern of CCR6 staining in different forms of renal inflammation

The staining pattern for CCR6 was not associated with particular renal disease entities (Figure 5). The constitutive expression of CCR6 on endothelial cells on peritubular and glomerular capillaries decreases in biopsies with chronic glomerular injury as well as in chronic interstitial nephritis. Particularly, in cGN, a prominent segmental to global loss of CCR6 expression was present (Figure 6). As could be expected, globally sclerosed glomeruli demonstrated complete loss of CCR6 staining. The loss of glomerular staining in patients with chronic interstitial nephritis indicates that this is not specific for the glomerular injury process, but rather due to nephrosclerosis which is also present in chronic interstitial nephritis (with globally sclerosed glomeruli). The glomerular area of CCR6 expression did not correlate with serum creatinine, proteinuria, or the overall percentage of sclerosed glomeruli (not significant).

A semi-quantitative score was used to characterize the tubulointerstitial infiltration by CD20- and CCR6-positive cells (Table 2). There was an extremely significant correlation between the CD20 and CCR6 scores (Spearman *r* = 0.69, 95% confidence interval 0.51–0.81, *P* < 0.0001). Furthermore, CCR6 scores were associated with serum creatinine at the time of biopsy (Spearman *r* = 0.47, 95% confidence interval 0.17–0.69, *P* = 0.0027), but not with proteinuria (not significant). The median creatinine was significantly higher in the interstitial scores 1–3 vs 0, with a progressive increase from scores 1–3 (not illustrated). The interstitial CD20 scores were not associated with serum creatinine and proteinuria (not significant), consistent with previously published data [3].

A negative correlation was found between the percentage of CCR6 expression in glomeruli and the interstitial CCR6 scores (Spearman *r* = −0.42, 95% confidence interval from −0.64 to −0.13, *P* = 0.0049).

CCR6 and CCL20 mRNA expression in patients with glomerulonephritis

The mRNA expression of CCL20 and the corresponding receptor CCR6 was quantified by real-time RT-PCR in the microdissected tubulointerstitium in renal biopsies with IgA nephropathy, membranous nephropathy and cGN (Table 3). All biopsies demonstrated a prominent ex-

Table 3. Basic clinical parameters of the patients included in the mRNA expression cohort

Disease	<i>n</i>	Age (years)	Creatinine (mg/dL)	Proteinuria (g/24 h)
IgA N	14	52 (17–72)	2.3 (1.5–6.2)	3.0 (0.5–6.3)
Membranous N	20	55 (17–85)	1.0 (0.7–2)	4.7 (0.6–9.8)
cGN	8	53 (27–69)	1.3 (0.9–6.4)	0.7 (0.1–1.8)
Controls	9	56 (27–70)	n.a.	n.a.

Data are presented as median (range) except *n*. n.a., not available; N, nephropathy; cGN, crescentic glomerulonephritis; c Int, chronic interstitial nephritis.

pression of CCL20 mRNA (Figure 7A). Expression of CCR6 was most prominently induced in biopsies with IgA nephropathy and cGN (Figure 7B). A corresponding expression was found for CCL20 and CCR6 mRNA, but only the induction of the receptor reached the level of significance (Figure 7A and B). The expression of CCL20 mRNA correlated significantly with the expression of the corresponding receptor CCR6 mRNA (*P* < 0.05), but not with serum creatinine or proteinuria at the time of biopsy.

Discussion

The list of human diseases in which CCR6 plays a significant role is rapidly growing and contains common ones like colorectal carcinoma [26], Crohn's disease, and rheumatoid arthritis [27]. Particularly, the description of CCR6 expression on Th17 cells sparked the recent interest in this chemokine receptor [28]. The ligand receptor pair CCL20/CCR6 is involved in the normal development of gut-associated lymphatic tissue, as well as in the mentioned common human diseases [29].

In the mouse gut, the deficiency of CCR6 leads to reduced sizes of Peyer's patches with loss of B cells and T cells, and particularly a reduction of regulatory CD4-positive T cells [30]. M cells, a specialized cell type within Peyer's patches, are also reduced in number [31]. CCR6-deficient mice are protected from oral *Yersinia enterocolitica* infection, which exploits M cells for infection [31]. Isolated lymphoid follicles are organized lymphoid structures in the gut in which B cells are the major CCR6-positive population. The formation of these structures was significantly disturbed due to a reduction of B-cell influx in CCR6-deficient mice [22]. These studies highlight the role of CCR6 in lymphogenesis of the gut. CCR6 has also recently been documented on mouse regulatory T cells and in IL-17-producing CD4-positive T cells. CCR6 deficiency aggravated renal injury in mouse nephrotoxic nephritis and increased mortality illustrating the recruitment of regulatory T cells through CCR6 [32]. Future studies need to address the expression of CCR6 by inflammatory subsets in the human kidney.

CCL20 can also be induced under inflammatory conditions [29]. Therefore, we hypothesized that the formation of tertiary lymphoid organs might in part be a recapitulation of embryogenesis. We localized CCR6 in human kid-

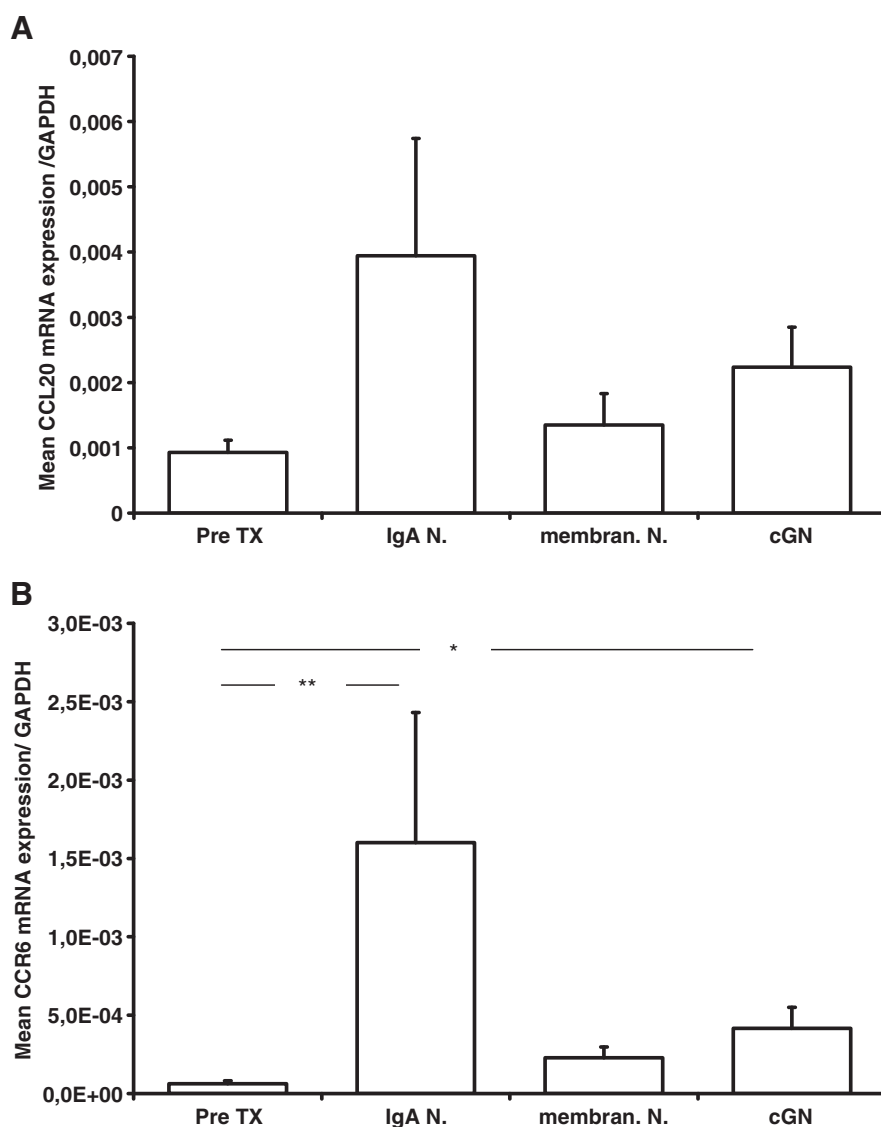


Fig. 7. Real-time expression of CCL20 (A) and CCR6 (B) in the tubulointerstitium of renal biopsies from patients with IgA nephropathy (IgA N.), membranous nephropathy (membran. N.) and cGN. Allograft biopsies taken before implantation were used as controls (Pre Tx, * $P < 0.05$, ** $P < 0.01$).

ney diseases in which the formation of tertiary lymphoid organs has previously been described [3,33]. This is the first detailed analysis on the expression of CCR6 in human renal biopsies from patients with glomerular and tubulointerstitial diseases. Consistent with our hypothesis, we found that a prominent number of B cells and T cells were CCR6-positive in the tubulointerstitium. The distribution of CCR6-positive cells was interesting as these were mainly found at sites of larger, nodular lymphocyte accumulations. Staining for CD20-positive cells demonstrated that a major part (about half) of the CD20-positive cells were also CCR6-positive. The majority of the remaining cells are most likely T cells (lymphocytes according to the morphology).

In contrast to the loss of CCR6 on glomerular endothelial cells, we found an increase of interstitial CCL20 and CCR6 mRNA expression particularly in IgA nephropathy. There was an inverse correlation between the glomerular expression of CCR6 and the tubulointerstitial scores. Therefore,

the interstitial expression is consistent with the immunohistochemistry finding.

As previously described, T and B cells are present within the tubulointerstitium in high numbers during chronic renal inflammation, but not within the glomerular tuft [3,34]. This mirrors the distribution of CCR6 infiltrating cells in renal tissue. Furthermore, glomerular macrophages do not seem to express CCR6 because CCR6-positive cells were not present in this tissue compartment. In contrast to the high number of CCR6-positive cells in TLOs, the diffuse interstitial infiltrates were rarely CCR6 positive. This could be explained in two ways, either a particular recruitment of CCR6-positive cells to tertiary lymphoid organs or an induction of CCR6 in nodular infiltrates. This is the first chemokine receptor described in the human kidney which is expressed both on B cells and T cells. Other chemokine receptors have been shown to be expressed on T cells (e.g. CCR5 [34]) or on B cells (e.g. CXCR5 [3]). CCR6 might

therefore be involved in orchestrating a rendezvous between T cells and B cells, particularly at sites of nodular infiltrates.

The only study on CCL20 and CCR6 in human kidneys has been published by Woltman *et al.* [35]. In this study, the authors described the expression of CCL20 by tubular epithelial cells, and CCR6 by a subset of inflammatory cells in rejecting allografts [35]. Particularly, CCR6 was localized to dendritic cells in rejecting renal allografts [35]. Our study is the first addressing the expression in endogenous kidney diseases. We could confirm the expression of CCR6 by dendritic cells in allograft nephrectomies (not illustrated).

The constitutive expression of CCR6 on endothelial cells of glomerular and peritubular capillaries was an unexpected finding. This expression was reduced during inflammation. Evidence for CCR6 expression on endothelial cells has been provided for human umbilical vein endothelial cells, saphenous vein endothelial cells, and dermal and lung microvascular endothelial cells [36,37]. Expression of CCR6 has been found to be induced on human umbilical vein endothelial cells after infection with human herpes virus 8 [38]. Induction of CCR6 on human umbilical vein endothelial cells was also described after combined stimulation with hepatocyte growth factor and vascular endothelial growth factor (VEGF) [36]. Loss of CCR6 was present in focal and global glomerulosclerosis. Due to limitations in materials, we cannot at the moment exclude that there is a regulation of CCR6 independent of endothelial cell loss. This and the functional role of a constitutive expression of CCR6 on renal endothelial cells remain to be evaluated. Another ligand receptor pair, i.e. CXCL12/CXCR4, has been shown to be involved in angiogenesis of the kidney during development but also in neoangiogenesis in malignant tumours [39,40]. Mechanisms involved in endothelial biology might be the recruitment of endothelial stem cells or a direct induction of migration/proliferation of endothelial cells. Interactions between CXCR4 and CCL20 have been shown to play a role in malignant diseases; therefore, an interaction of these systems might be envisioned in the renal vasculature [41].

In summary, we found CCR6 to be expressed by infiltrating lymphocytes, both B cells and T cells, predominantly in nodular accumulation and, to a lesser extent, in diffuse interstitial infiltrates. Furthermore, there might be a role for CCR6 on intrinsic renal cells particularly endothelial cells. The functional evaluation will have to await further *in vitro* studies and studies on CCR6 mutant mice. These studies are currently hampered as useful mouse models of tertiary lymphoid organ formation in the kidney associated with chronic inflammation wait to be established.

Acknowledgements. S.S. is supported by a grant by the Novartis Stiftung (No. 09B26), a grant by the University of Zurich (Forschungskredit) and a grant by the Swiss National Foundation (32003B_129710/1), and C.D.C. is supported by the Else Kröner-Fresenius Foundation. We thank all participating centres of the European Renal cDNA Bank-Kroener-Fresenius biopsy bank (ERCB-KFB) and their patients for their cooperation. Active members at the time of the study: Clemens David Cohen, Holger Schmid, Michael Fischereder, Lutz Weber, Matthias Kretzler, Detlef Schlöndorff, Munich/Zurich/Ann Arbor/New York; Jean Daniel Sraer, Pierre Ronco, Paris; Maria Pia Rastaldi, Giuseppe D'Amico, Milano; Peter Doran, Hugh Brady, Dublin; Detlev Mönks, Christoph Wanner, Würzburg; Andy Rees, Aberdeen; Frank Strutz, Gerhard Anton Müller, Göttingen; Peter Mertens, Jürgen Floege, Aachen; Norbert Braun, Teut Rislér, Tübingen; Loreto

Gesualdo, Francesco Paolo Schena, Bari; Jens Gerth, Gunter Wolf, Jena; Rainer Oberbauer, Dotscho Kerjaschki, Vienna; Bernhard Banas, Bernhard Krämer, Regensburg; Moin Saleem, Bristol; Rudolf Wüthrich, Zurich; Walter Samtleben, Munich; Harm Peters, Hans-Hellmut Neumayer, Berlin; Mohamed Daha, Leiden; Katrin Ivens, Bernd Grabensee, Düsseldorf; Francisco Mampaso (†), Madrid; Jun Oh, Franz Schaefer, Martin Zeier, Hermann-Joseph Gröne, Heidelberg; Peter Gross, Dresden; Giancarlo Tonolo, Sassari; Vladimir Tesar, Prague; Harald Rupprecht, Bayreuth; Hans-Peter Marti, Bern.

Conflict of interest statement. None declared.

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Received for publication: 20.12.09; Accepted in revised form: 19.8.10

Nephrol Dial Transplant (2011) 26: 1220–1228

doi: 10.1093/ndt/gfq558

Advance Access publication 14 September 2010

Anti-C1q autoantibodies do not correlate with the occurrence or severity of experimental lupus nephritis

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Abstract

Background. In systemic lupus erythematosus patients, a strong association between the occurrence of antibodies against complement C1q (anti-C1q) and lupus nephritis can be observed. However, the predictive value of anti-C1q titres for a renal flare remains to be determined. Increasing titres of anti-C1q before the occurrence of clinical apparent nephritis might not only serve as a clinical parameter but also indicate a direct pathogenic mechanism of anti-C1q.

Methods. The aim of this study was to analyse the occurrence of anti-C1q before the onset of experimental lupus nephritis in MRL/MpJ $+/+$ mice and to correlate anti-C1q titres with the type and severity of glomerulonephritis (GN) developing at advanced age.

Results. As judged by a number of morphological and immunological analyses, GN in MRL/MpJ $+/+$ mice resembled human lupus nephritis and occurred in variable degrees of severity. We also observed an abundant and early presence of anti-C1q. However, anti-C1q neither correlated