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Hepatitis C virus RNA and core protein in kidney glomerular and tubular structures isolated with laser capture microdissection

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Summary

The role of hepatits C virus (HCV) in the production of renal injury has been extensively investigated, though with conflicting results. Laser capture microdissection (LCM) was performed to isolate and collect glomeruli and tubules from 20 consecutive chronically HCV-infected patients, namely 6 with membranoproliferative glomerulonephritis, 4 with membranous glomerulonephritis, 7 with focal segmental glomerulosclerosis and 3 with IgA-nephropathy. RNA for amplification of specific viral sequences was provided by terminal continuation methodology and compared with the expression profile of HCV core protein. For each case two glomeruli and two tubular structures were microdissected and processed. HCV RNA sequences were demonstrated in 26 (65%) of 40 glomeruli, but in only 4 (10%) of the tubules ($P < 0{\cdot}05$). HCV core protein was concomitant with viral sequences in the glomeruli and present in 31 of the 40 tubules. HCV RNA and/or HCV core protein was found in all four disease types. The immunohistochemical picture of HCV core protein was compared with the LCM-based immunoassays of the adjacent tissue sections. Immune deposits were detected in 7 (44%) of 16 biopsy samples shown to be positive by extraction methods. The present study indicates that LCM is a reliable method for measuring both HCV RNA genomic sequences and HCV core protein in kidney functional structures from chronically HCV-infected patients with different glomerulopathies and provides a useful baseline estimate to define the role of HCV in the production of renal injury. The different distribution of HCV RNA and HCV-related proteins may reflect a peculiar 'affinity' of kidney microenvironments for HCV and point to distinct pathways of HCV-related damage in glomeruli and tubules.

Keywords: hepatitis C Virus, laser capture microdissection, terminal continuation RNA amplification, nephropathies

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Introduction

Investigation of the role of hepatitis C virus (HCV) in the pathogenesis of glomerular nephropathies has produced conflicting results [1]. Identification of HCV-related proteins and/or HCV RNA genomic sequences by means of immunohistochemistry [2] and *in situ* hybridization [3] has been attempted.

Viral RNA has been located in the capillary endothelial cells and tubular epithelial cells of HCV-infected patients with a wide variety of renal diseases including membranoproliferative glomerulonephritis (MPGN), membranous glomerulonephritis (MGN), focal segmental glomerulosclerosis (FSGS), and crescentic glomerulonephritis [4], and regardless of the pattern of glomerular injury [5]. These data have highlighted the difficulty of applying *in situ* hybridization to HCV RNA.

Detection of viral proteins in renal tissue was also reported. HCV core protein has been found in both glomerular structures and tubular epithelial cells [6]. Tubulointerstitial vessels display specific immune reactants [7]. No obvious relationship has been established between the type and severity of renal injury and the presence of HCV-related proteins *in situ*.

We have recently shown that HCV core protein participates in the formation of cryoprecipitable immune complexes [8]. Moreover, HCV nucleocapsid protein devoid of enveloped proteins has been detected in the bloodstream of HCV chronic carriers and may be a good indicator of the circulating viral load [9]. The occurrence of nonenveloped nucleocapsid in the serum of chronic HCV carriers with normal transaminase levels and without histological evidence of progressive liver disease suggests that it is overproduced during HCV virogenesis [10].

To better characterize tissue distribution, HCV RNA and HCV core protein were studied in both glomeruli and tubules isolated by laser capture microdissection (LCM).

LCM is a well-established method for the enrichment of structures or cells of interest from histological sections, and overcomes the problem of tissue heterogeneity [11]. Its effect on the nucleic acids has been thoroughly investigated and sample analyses of DNA and RNA extracted by LCM have been described [12].

In addition, proteomics has provided a complementary approach to the study of gene expression by supplying further information regarding the effect of post-translational or post-transcriptional modifications [13].

The present study demonstrates that the combination of extraction techniques with LCM is a useful way of determining specific viral nucleic acids and viral proteins in individual kidney structures from HCV-infected patients with a defined renal injury.

Materials and methods

Patients

Twenty consecutive chronically HCV-infected patients (12 M, 8 F) aged 38–67 years (mean 54·4 years) attending the Section of Internal Medicine and Clinical Oncology of the Department of Internal Medicine and Human Oncology and the Section of Nephrology of the Department of Emergency and Transplantation of the University of Bari Medical School were enrolled. They underwent kidney biopsy for diagnostic purposes. Informed consent was obtained from each patient and the study was approved by the University Ethical Committee. HCV infection was established by ELISA detection of anti-HCV antibodies (HCV 3·0, Ortho Clinical Diagnostics, Raritan, NJ, USA), and by recombinant-based immunoblot assay (RIBA, Ortho Clinical Diagnostics) and HCV-RNA in serum (Amplicor HCV, Roche Diagnostic Systems, Branchburg NJ, USA). All patients were negative for HBsAg and HIV antibodies. None had clinical and/or biochemical evidence of autoimmune disease, and all were negative for antinuclear and antismooth muscle autoantibodies. The source of HCV infection was not known, though 11 patients had a history of blood transfusion 15–20 years prior to enrolment. No patient had received interferon therapy or immunosuppressive drugs at the time of kidney biopsy. Baseline evaluation included disease history, current signs and symptoms and previous medications. Physical examination and laboratory values were recorded.

Liver biopsy was performed in 16 patients. Specimens were evaluated under code by the same pathologist to assess conventional histological diagnosis of chronic active liver disease [14]. Serum cryoglobulins were determined as described elsewhere [8].

Laser capture microdissection (LCM)

Frozen tissue specimens were cut as a series of 6 μm-thick sections with a clean blade and mounted on slides coated with a thermoplastic membrane (PEN foil slides; Leica Microsystems, Wetzlar, Germany). Each section was used for tissue isolation by LCM with the Leica SVS LMD System (Leica Microsystems). Glomerular and tubular structures were selectively dissected by focal melting of the membrane with a UV-laser beam (337 nm) set to pulse at 80 kW. Microdissected fragments dropped by gravity into cap-tubes under microscope inspection. To minimize degradation, slides were fixed with 70% ethanol for 1 min, washed in diethylpyrocarbonate (DEPC)-treated deionized water and stained with the Histogene LCM kit (Arcturus, Mountain View, CA, USA) to preserve the integrity of cellular nucleic acids. A number of dissections were performed to obtain 2 glomerular and 2 tubular structures for each case.

Terminal continuation RNA amplification

Total RNA extracted with the PicoPure RNA Isolation Kit (Arcturus) from LCM-isolated glomeruli and tubules was redissolved in DEPC-treated water and incubated with DNAse I (Promega, Madison, WI, USA) for 2 h at 37°C. Greater amounts of RNA were obtained from the extracted nanograms by amplification with the recently described terminal continuation (TC) method [15], which provides linear amplification with significant representations of transcripts under a wide variety of staining conditions of tissue samples and permits the synthesis of first-strand cDNA complementary to the RNA template. A second-strand cDNA complementary to the first strand cDNA is generated prior to *in vitro* transcription with the double-stranded cDNA as template. Briefly, extracted RNA was reverse transcribed in the presence of 10 ng/μl TC primer selected from 5′-terminus of HCV genome (nt 17–32) to which T7-bacteriophage promoter sequence was attached (5′AAACGACGGCCAG TGAATTGTAATACGACTCACTATAGGCGCGCCAGCCC CCTGAT-3′) and 10 ng/μl polyd(T) primer (3′-TTTT TTTTTTTTTTTTT-5′) in 1 mM dNTPs, 5 mM DTT, 20 U RNase inhibitor and 5 U reverse transcriptase (Invitrogen, Carlsbad, CA, USA) in a final volume of 20 μl. Synthetized single-strand cDNA was converted into double-stranded cDNA by adding 10 mM TRIS (pH 8·3), 50 mM KCl, 1·5 mM $MgCl_2$ and 0·5 U RNAse H (Invitrogen) in 99 μl volume.

Second-strand synthesis proceeded for 10 min at 37°C for RNAse H digestion; 3 min at 95°C for denaturation; 3 min at 50°C for annealing; 30 min at 75°C for elongation. One μl containing 5 U Taq polymerase (Promega) was added at the start of the denaturation step. The reaction was terminated with 5 M ammonium acetate. Samples were phenol-extracted and ethanol-precipitated. The cDNA was resuspended in 20 μl RNase-free distilled water and dialysed against 18 MOhm RNase-free distilled water for two hours. Specific β-actin gene amplification was performed in each sample to check the integrity of the extracted nucleic acids. The101 bp amplicon product was generated by PCR (forward primer 5′-CTTCTTTCTTGGGTATGGAATCC-3′ and reverse primer 5′-CTAAAAGACCTC TATGCCAAC ACA-3′).

To detect HCV-RNA genomic sequences 2 μl of cDNA was used as template with a primer pair selected from the highly conserved 5′-terminus of the HCV genome. The pair consisted of upstream (KY80), 5′-GCAGAAAGCGTCTAGC CATGGCGT-3′ (nt 56–79) and downstream primer (KY78), 5′-CTCGCAAGCACCCTATCAGGCAGT-3′ (nt 276–299) [16]. Ten μl aliquots of the final amplified product were run on agarose gel stained with ethidium bromide and analysed under ultraviolet light. Sensitivity of the PCR protocol was assessed against the WHO First International Standard. A minimum of 250 genome equivalents/ml (genome/ml) was detected. Specificity was performed by sequencing amplified products. Sequence reactions were carried out on an ABI Prism 310 genetic analyser (Perkin Elmer, Faster City, CA, USA).

HCV core protein enzyme immunoassay

Glomerular and tubular structures adjacent to the kidney section area from which HCV-RNA was recovered were microdissected and collected in cap-tubes containing 50 μl of the following extraction buffer: 50 mM Tris-HCl (pH 7·5) with 150 mM NaCl, 1% Nonidet P40, 0·5% sodium deoxycholate, 10 mg/ml leupeptin, 10 mg/ml aprotinin. Samples were held at 4°C for no longer than 2 h before introduction into the immunoassay module.

HCV core protein in the samples solubilized in the buffer was detected with an ELISA kit (Ortho Track-C assay) provided by Ortho Clinical Diagnostics as described previously [17]. Solubilized solution was treated with dissociating buffer containing 0·3% Triton X100, 1·5% 3-[(3-cholamidopropyl)-dimethylammonium]-1-propanesulphonate and 15% sodium dodecylsulphate. Fifty μl dissociating buffer were added to 50 μl recovered cell proteins, followed by incubation at 56°C for 30 min. Duplicate wells coated with monoclonal antibodies directed to different regions of HCV core protein were incubated with pretreated samples at room temperature for 60 min under shaking. Antigen-antibody complexes formed on the microwell surface were detected by anti-HCV core antibodies (Fab fragments) conjugated with horseradish peroxidase (HRP) for 30 min. Colour was then developed with ortho-phenylenediamine and hydrogen peroxide, and the product was read at 492 nm. Quantitative control calibrations at 0, 1·5, 15, 100, 200, and 800 pg/ml were included to generate a calibration curve. Specificity of the reaction was determined by including solubilized cells obtained from 5 HBsAg-positive patients: 4 with MPGN, 1 with MGN. Three patients with FSGS negative for HCV and HBV markers were also included.

Immunohistochemistry

HCV core protein was detected by direct immunostaining. To strengthen the efficiency of immunodetection, tissue sections were subjected to acid electroelution (AEE) as reported elsewhere [7].

Sections were incubated with antic22–3 monoclonal antibody (antic22–3 mAb) produced in mice by using recombinant immunogen (c-22–3, yeast expressed SOD-fused HCV core protein, sequence: 1–120) recognizing amino acids 29–43 of the core protein [18]. It was conjugated with HRP with a peroxidase labelling kit from Roche Diagnostics. This combination (antic22–3/HRP mAb) was diluted in PBS containing 1% BSA and used at a protein concentration of 1 μg/ml. Sections were first incubated with normal rabbit serum (Dakopatts, Copenhagen, Denmark) and then with the antic22–3/HRP mAb for 3 h.

The adjacent tissue sections were tested by the same procedure as the negative controls, for which the mAb was adsorbed by preincubation with the recombinant core protein at 37°C for 12 h. In the absorption experiments, the mAb was also preincubated with either HCV-related antigens (E2, NS3, NS4, NS5) or HBV (HBsAg) and HAV (HAV-Ag) antigens. An irrelevant mouse mAb (antihuman chorionic gonadotropin/HRP mAb) was used as further control. Immunostaining was also performed without the primary antibody. Five or more sections were examined for each case.

Detection of immunoglobulins (IgG, IgM and IgA) and complement fractions in kidney sections was carried out without prior AEE. FITC-conjugated murine mAbs to human immunoglobulins as well as to human C1q, C3 and C4 proteins were obtained from DAKO (Carpinteria, CA, USA).

Results

Clinical and laboratory features

All 20 patients were anti-HCV positive and viremic at the time of kidney biopsy. Average serum HCV RNA was 476 000 IU/ml (range 244 000–860 000). No distinct profile was found for HCV genotype distribution. Type 1 was present in 13 (65%) patients and type 2 in 7 (35%).

As shown in Table 1, nephrotic syndrome or proteinuria were the prime reasons for hospital referral. The mean 24-h urinary protein was 3·5 ± 2·5 g. Urinalysis with microscopic

Table 1. Clinical and laboratory features of chronically HCV-infected patients.

	Age/sex	Serum creatinine (n.v. ≤ 1·2 mg/dl)	Micro haematuria	Proteinuria (n.v. ≤ 0·23 g/24h)	Complement C4 (n.v. 10–40 mg/dl)	Cryoglobulin (type)	Diagnosis
1	40/F	1·0	+	5·0	2·6	+ (II)	MPGN
2	67/M	2·0	+	9·45	29	–	FSGS
3	33/F	0·8	–	2·86	32	–	MGN
4	76/F	0·7	+	3·6	38	–	FSGS
5	74/M	1·02	–	4·5	29	–	FSGS
6	64/F	0·9	+	2·5	6	+ (III)	MGN
7	57/M	1·7	+	2·6	17	–	MGN
8	78/M	2·0	+	4·4	6·9	+ (II)	MPGN
9	62/F	0·8	+	0·6	5	+ (II)	MPGN
10	44/F	1·4	+	4·0	2	+ (II)	MPGN
11	74/M	1·2	+	10·0	16	–	FSGS
12	60/M	0·9	+	1·1	7	+ (II)	MPGN
13	47/M	2·4	+	4·3	19	–	MPGN
14	41/M	2·1	+	5·0	22	–	IgA-N
15	62/F	3·0	+	2·1	24	–	FSGS
16	59/M	1·6	–	2·25	19	–	IgA-N
17	44/M	2·0	+	1·6	21	–	IgA-N
18	64/F	1·4	–	1·5	16	–	FSGS
19	68/M	2·2	+	1·8	18	–	FSGS
20	72/M	2·3	+	0·9	22	–	MGN

MPGN, membranoproliferative glomerulonephritis; MGN, membranous glomerulonephritis; FSGS, focal segmental glomerulosclerosis; IgA-N, IgA nephropathy.

examination of sediment showed microhematuria in 16 (80%) with red cell casts in 4. Mean serum creatinine was 1·57 ± 0·66 mg/dl.

Liver enzymes were elevated in 14 (70%), with aspartate aminotransferase levels of 69 ± 56 IU/l. To clarify the degree of histological injury, liver biopsy was performed in 16 patients. Histological pictures were compatible with chronic inflammation. Minimal to mild interface inflammatory activity was found in most patients. In one patient septal incomplete cirrhosis was demonstrated.

Cryoglobulinemia was found in 6 (30%) patients with a history of palpable purpura. Mean cryocrit level was 5·3 ± 2·6%. Immunochemically, cryoglobulins were characterized as mixed type II in 5 patients and type III in one. All cryoglobulinemic patients showed hypocomplementemia and high levels of rheumathoid factor activity.

MPGN was the major pattern of glomerular disease in the cryoglobulinemic patients. The immunofluorescence pattern in the four MPGN patients, usually consisted of deposits of IgG and/or IgM and/or complement fractions.

The four MGN patients disclosed staining of IgG, occasionally accompanied by C3 and/or C1q deposits. Except for one patient in whom a connective tissue disorder was suspected, no specific comorbid condition was assessed in the other cases.

FSGS was diagnosed in 7 patients devoid of any specific comorbidity. No immunoreactant deposits were demonstrated in this group. IgA-nephropathy was present in the last 3 patients and IgA and C3 immunodeposits were found.

Laser capture microdissection analyses

After microscopic control of staining quality and tissue preservation, sections were used for microdissection. An example of LCM-derived tissues is shown in Fig. 1. To ensure complete extraction, RNA pellets were redissolved in DEPC-treated with sterile water and incubated with DNase. A good-quality RNA was successfully obtained for each sample (OD 260/OD 280 ratio: 2·16 ± 0·44; OD 260/OD 230 ratio: 2·96 ± 89).

We dissected approximately 1000–1600 cells/shot. In preliminary experiments, the total amount of RNA obtained by LCM corresponding to around 1000 cells/shot ranged from 0·5 to 0·9 μg.

Performance of the HCV RNA PCR assay with microdissected glomerular structures demonstrated a linear relationship between the number of isolated structures and TC RNA amplification reaction as detected by agarose intensity signal. Specific HCV RNA product reached the detection limit of almost 0·2 μg total RNA capable of displaying 250 HCV RNA genome/ml (Fig. 2). In each sample, integrity of the template was demonstrated by β-actin gene sequence amplification.

Protein macromolecules from LCM-derived tissue samples were intact and retained their antigenic properties. Previously, it was demonstrated that even in the absence of protease inhibitors HCV core protein retained immunoreactivity for at least 5 h. It was also shown that the average weight of HCV core protein for LCM-derived samples

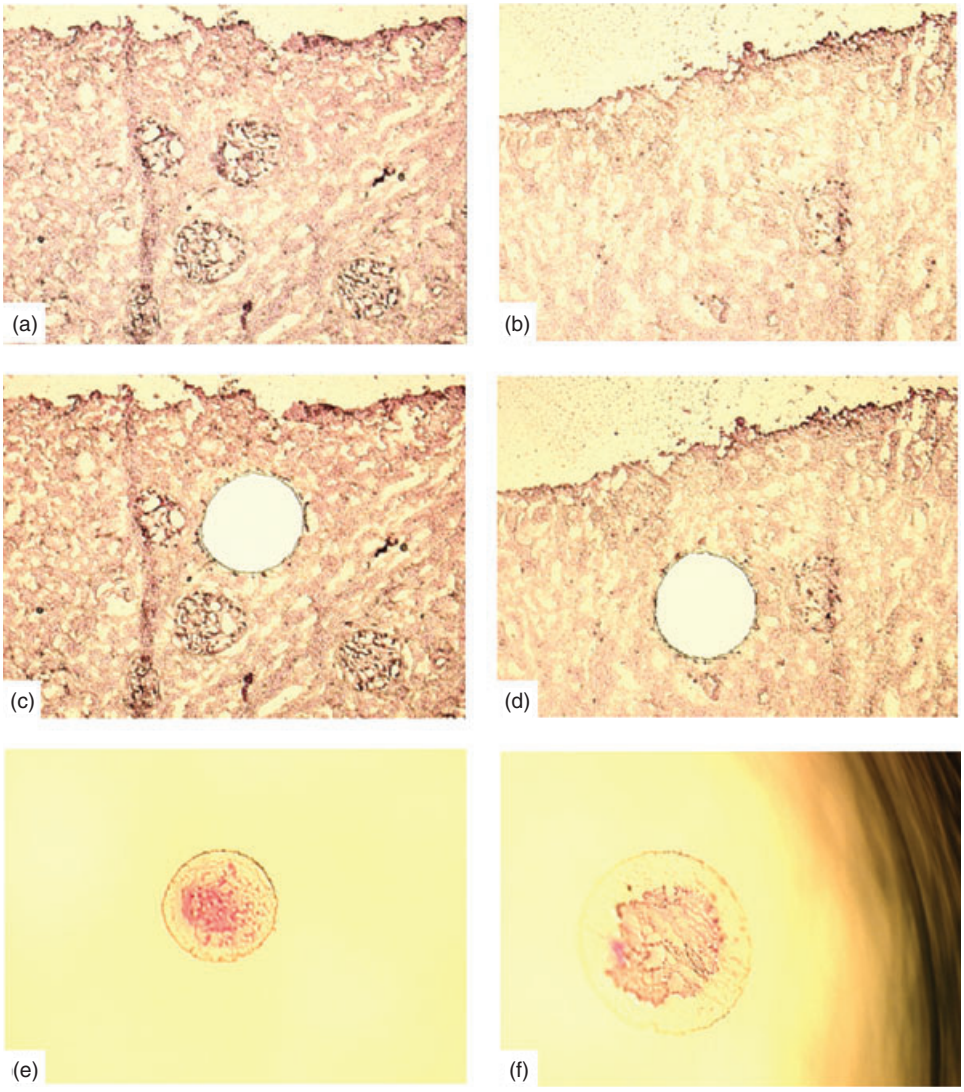

Fig. 1. LCM-derived samples. (a) and (b) kidney sections before LCM; (c) and (d) kidney sections after LCM: note empty space due to removal of renal structures; (e) glomerular and (f) tubular structures, respectively, transferred to the cap surface.

containing approximately 1000 cells/shot ranged from 9 to 21 pg/ml, as defined by a specific calibration curve using spiked known concentrations of recombinant HCV core protein diluted in extraction buffer. Functional sensitivity of the test, defined as the lowest concentration measured with an interassay coefficient of variance of 20%, was 4 pg/ml. Detection limit of HCV core assay with kidney tissue samples was 2 pg/ml. A strong degree of linearity was observed between LCM-procured glomeruli and the HCV core protein-based immunoassay ($R = 0{\cdot}89$).

HCV RNA genomic sequences and nonenveloped core protein were differently distributed in renal functional structures. Results are reported in Table 2. Viral RNA was demonstrated in 26 of 40 (65%) microdissected glomeruli and in 4 tubular structures ($P < 0{\cdot}05$) derived from adjacent areas of the same sections. In 10 (83%) of 12, 6 (75%) of 8, 8 (57%) of 14 and 2 (33%) of 6 from MPGN, MGN, FSGS and IgA-nephropathy, respectively, specific viral sequences were demonstrated in LCM-derived glomeruli. Interestingly, except for 2 of 8 MGN and 2 of 14 FSGS tubular structures, no HCV RNA amplicons were demonstrated in the remaining tubules.

The distribution of HCV core protein varied. It always accompanied HCV RNA sequences in the glomeruli structures, and was also detected in 31 of the 40 tubules (77%). Core protein was demonstrated in 11 of 12 (92%), in 8 of 8 (100%), in 10 of 14 (71%) and in 2 of 6 (33%) MPGN, MGN, FSGS and IgA-nephropathy tubules, respectively.

Immunohistochemical pictures

Morphological distribution of HCV core immune reactants was investigated by immunohistochemistry on kidney sec-

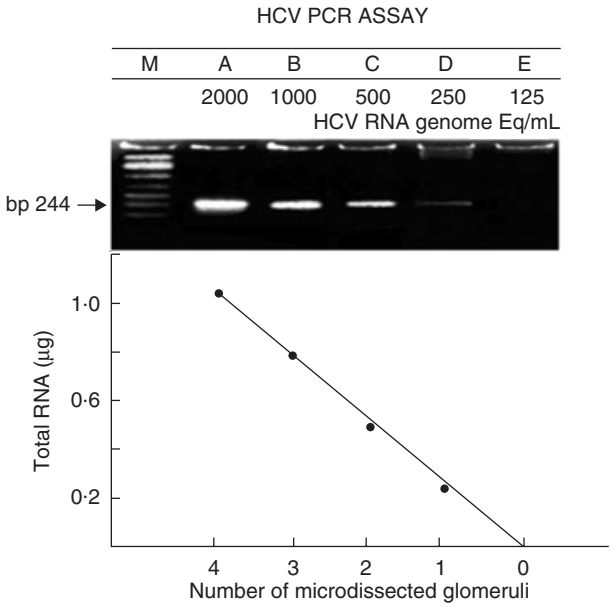

Fig. 2. Relationship between terminal continuation-amplified RNA, number of microdissected glomerular structures and analytical sensitivity of RT PCR-derived HCV RNA products. Lane M: marker. Lanes A to E: a two-fold dilution series of standard HCV RNA.

tions adjacent to those used for LCM. Seven of the 16 kidney biopsy samples positive in the LCM-based immunoassay (44%) gave a positive signal. Immune deposits were detected either in glomeruli, where they colocalized with IgG and/or IgM molecules and/or complement in mesangial or subendothelial areas, or in the epithelial cells of the tubules with cytoplasmic features (Fig. 3).

Discussion

Heterogeneity of histological structures is the major obstacle to the use of tissue extracts. Histological entities of interest are often part of complex morphological structures that can be correctly identified under high-power magnification. LCM-based tissue procurement increases the yield of high-quality RNA and dilutes out contaminating structures. TC amplification generates RNA in microgram quantities, starting with as little as few nanograms of total RNA from microdissected cell populations. Previous [17] and the present data provide evidence that combination of LCM with a sensitive immunoassay detects picograms of HCV core protein. Results also confirm that protein macromolecules obtained by LCM extracts retain their antigenic reactivity.

Kidney biopsies of HCV-infected patients displayed a variety of histological patterns. HCV core protein was detected in 77% of tubules and in 65% of glomeruli. HCV-RNA genomic sequences accompanied HCV core protein in the glomeruli whereas it was found in only 10% of considered tubules. This, indeed, probably reflects methodological bias mainly related to the poor efficiency of the PCR assay used to detect HCV-RNA. PCR efficiency mainly depends on the proportion of HCV in the sample and thus on the amount of specific viral genomic sequences. TC-RNA amplification converted nanograms to micrograms of LCM-derived tissues and comparable levels of extracted RNA occurred in HCV negative ($2{\cdot}6 \pm 02$ μg) and positive ($2{\cdot}2 \pm 1{\cdot}1$ μg) samples.

Given the relevant enrichment of blood vessel structures in glomeruli, discrepancy of HCV RNA detection may reflect peculiar composition of circulating viral particles. It may be assumed that they included immune complexes whose distribution are frequently characterized by conspicuous deposits in glomeruli, but are occasionally seen in tubular cells or interstitium as in lupus nephritis and primary interstitial nephritis [19].

Occurrence of HCV core protein in conjunction with HCV RNA genomic sequences in glomeruli may support the notion that it participates as a component of the intact virion, whereas it may be recruited as nonenveloped protein in renal tubules where viral genomic sequences are virtually absent.

Though we have no direct evidence for virus binding to the surface of cell populations within the complex morphology of the glomerulus, it can be inferred that potential receptor-like activities capable of retaining both intact viral particles and/or nonenveloped core protein are expressed. Indeed, many surface proteins have been described to act as (co)-receptors and accessory molecules for binding of HCV

Table 2. Detection of HCVRNA and HCV nonenveloped core protein in extracts from glomerular and tubular structures isolated by Laser Capture Microdissection.

Histology	No. of patients	No. of microdissected samples	HCV RNA genomic sequences		HCV core protein	
			Glomeruli	Tubules	Glomeruli	Tubules
Membranoproliferative Glomerulonephritis	6	12	10 (83%)	0	10 (83%)	11 (92%)
Membranous Glomerulonephritis	4	8	6 (75%)	2 (25%)	6 (75%)	8 (100%)
Focal segmental Glomerulosclerosis	7	14	8 (57%)	2 (14%)	8 (57%)	10 (71%)
IgA nephropathy	3	6	2 (33%)	0	2 (33%)	2 (33%)

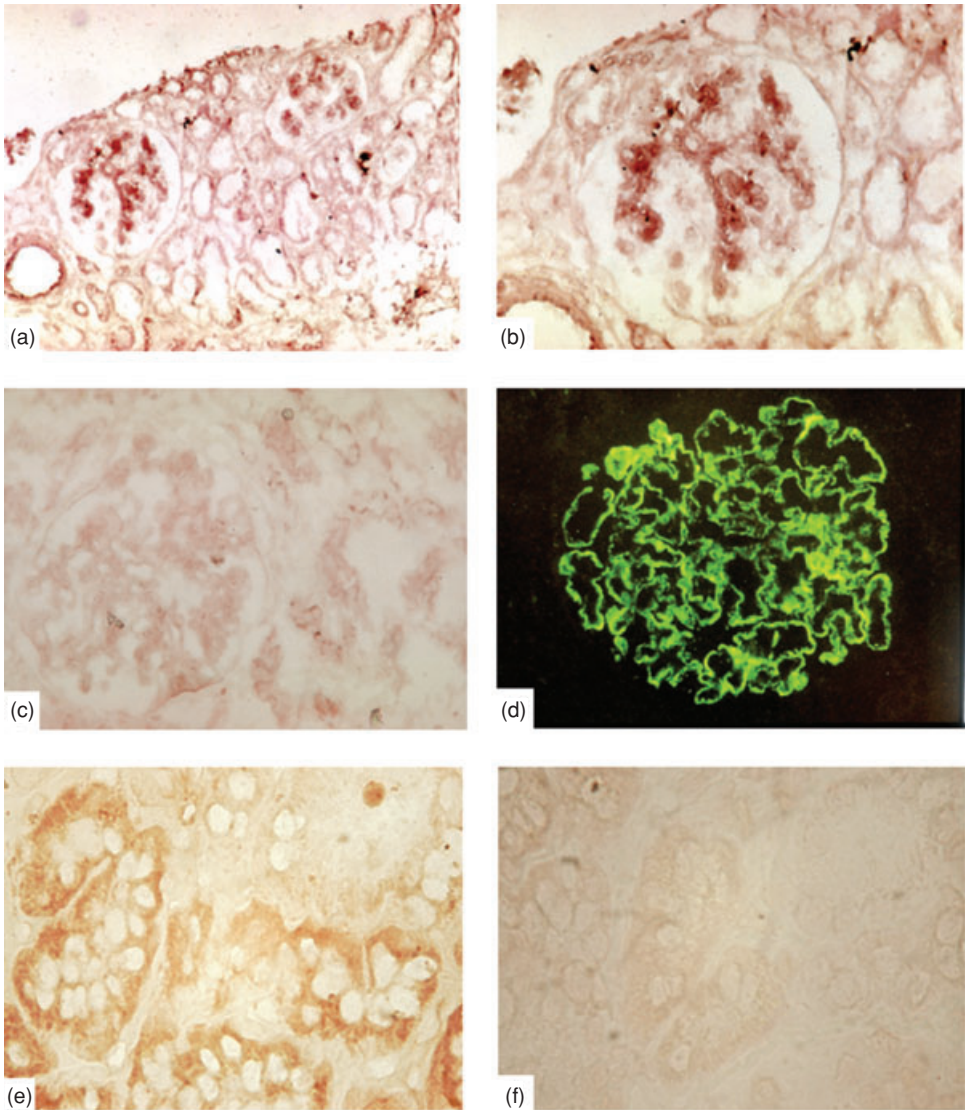

Fig. 3. (a,b) (low and high magnification, respectively) demonstrate homogeneous deposition of HCV core protein in glomerular structures; (c) specific reaction was abolished by preincubation of anti-HCV core antibody with c22–3 protein; (d) immunofluorescence demonstrates the presence of IgM molecules in the glomerular structure of the kidney tissue section; (e) core protein immunoreactants are demonstrated in the cytoplasm of epithelial cells of tubular structures; (f) complete absence of the signal in HCV-unrelated kidney biopsy.

particles. Dendritic cell-specific C-type lectin (DC-SIGN) and its liver-expressed homologue (L-SIGN) [20] capture HCV particles from blood and internalize within dendritic cells and sinusoidal endothelial cells [21]. Implications for virus–host interactions class B1 human scavenger receptor [22] and heparan sulphate [23] other than low density lipoprotein receptor [24] and CD 81 [25] have been proposed.

Non-enveloped nucleocapsid may theoretically reach the cell membrane of tubular epithelium via different mechanisms, including engagement via the C1q receptor [26] or/and through its Fcγ receptor-like activity [27].

The unexpected disproportionate distribution of HCV RNA emphasizes the possible attachment of viral particles, and may reflect different expression of cell surface molecules with putative receptor-like activities.

If confirmed by using direct characterization of receptors for HCV in glomeruli and tubules, these findings may have substantial pathobiological implications because it can be assumed that glomerular and tubular damage are the results of different mechanisms.

Occurrence of HCV in renal tissue regardless of peculiar histological features argues against its direct role in the production of renal damage. By contrast, the relatively low prevalence of renal injury in HCV chronic carriers [28] strongly supports the crucial role of local factors and the distinct biological properties of immune complexes in its pathogenesis.

The findings of IgM, IgG, IgA and complement fractions at the site of glomerular and tubular structures with the same distribution of HCV strongly supports the occurrence of an immune-mediated pathogenetic mechanism, with the virus being the target for circulating immunoglobulins, and possibly complement activation and production of inflammatory factors.

In addition, the lack of demonstration in FSGS patients of immunoglobulins or *in situ* deposition of complement fractions points to the existence of further potential pathogenetic mechanisms of renal damage. Notably, it has been suggested that HCV core protein interacts with several cellular proteins [29] and is capable of inducing a fibrogenic effect by increasing secretion of collagenopoietic molecules (TGF-β1, procollagen alpha 1), whereas HCV nonstructural proteins have distinct biological functions in that they preferentially induce proinflammatory actions [30]. This implies that different distribution of HCV-related proteins in renal functional structures may sustain distinct pathways.

These insights may help in understanding the mechanisms of HCV-related damage in kidney tissue. Most importantly a detailed knowledge of pathogenetic mechanisms may provide the basis for the development of more specific and efficient therapeutic approaches in the foreseeble future [31].

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