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A Novel μ-Capture Enzyme-Linked Immunosorbent Assay Based on Recombinant Proteins for Sensitive and Specific Diagnosis of Hemorrhagic Fever with Renal Syndrome

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Hantavirus nucleocapsid protein has recently been identified as a major antigen inducing an early and long-lasting humoral immune response in patients with hemorrhagic fever with renal syndrome. A μ-capture enzyme-linked immunosorbent assay utilizing recombinant nucleocapsid proteins of *Hantavirus* strains Hantaan 76-118 (Hantaan serotype) and CG 18-20 (Puumala serotype) as diagnostic antigens and specific monoclonal antibodies as the detection system has been developed. Histidine-tailed recombinant proteins were expressed in *Escherichia coli* and purified in a single step by affinity chromatography on a nickel-chelate resin. The assay was evaluated with a panel of sera from patients with hemorrhagic fever with renal syndrome originating from various geographic regions. The overall sensitivity of the μ-capture enzyme-linked immunosorbent assay (both recombinant antigens) was 100%, and its specificity was also found to be 100%. Immunoglobulin M antibodies were detected as early as on day 3, and maximum titers were obtained between days 8 and 25 after onset of the disease. The assay was regularly found to be positive within 3 to 4 months but in some cases up to 2 years after the acute phase of the disease.

Hemorrhagic fever with renal syndrome (HFRS) is a complex of diseases occurring worldwide and characterized mainly by fever, hemorrhage, and acute interstitial nephritis (3, 11). HFRS is a serious health threat in some countries, with up to 150,000 clinical cases occurring annually in the People's Republic of China (2, 27) and thousands of cases being observed, for instance, in Scandinavia, southern Europe, and Russia (2, 17, 26, 31). HFRS is caused by hantaviruses, a genus of the *Bunyaviridae* family (13). Their natural reservoirs are persistently infected rodents, and transmission to humans is believed to occur via aerosols (32).

Hantaviruses possess a tripartite negative-stranded RNA genome (22) coding for the nucleocapsid protein (S segment); two envelope glycoproteins, G1 and G2 (M segment); and the viral polymerase (L segment) (21, 23, 24). The viruses are antigenically variable and have been attributed to distinct serogroups which are associated with different natural hosts and clinically different severities (14, 30). The *Apodemus*-associated Hantaan serotype is the causative agent of Korean hemorrhagic fever, which is the most severe form of HFRS (13). The Puumala serotype is associated with the bank vole (*Clethrionomys glareolus*) and causes a mild variant of the disease which is known as nephropathia epidemica (11). The Seoul serotype (rat associated) induces a disease that is like Korean hemorrhagic fever but is milder (12). Serotypes Prospect Hill (*Microtus* associated) and Leaky (*Mus* associated) have not yet been related to human disease (1, 33). There are, moreover, a large number of *Hantavirus* isolates which have not yet been attributed to a serogroup.

Since the clinical picture is not diagnostic, the diagnosis of HFRS is established by the detection of antibodies against hantaviruses in patient sera. Several assays for serodiagnosis of HFRS, such as the indirect immunofluorescence assay (IFA), hemagglutination inhibition assays, and various enzyme immunoassays (8, 18, 25, 31), have been described. It is a major disadvantage of currently available tests that hantaviruses are highly pathogenic and their handling requires P3 laboratory facilities. Moreover, antigen preparations are difficult to standardize. The S-segment-encoded nucleocapsid antigen has been shown to be an immunodominant antigen which induces an early and long-lasting humoral immune response during hantavirus infection (7, 34, 35). We recently reported the favorable properties of *Escherichia coli*-expressed recombinant nucleocapsid protein as a diagnostic antigen in solid-phase enzyme-linked immunosorbent assays (ELISAs) (5, 35). We were able to demonstrate that the nucleocapsid proteins of two strains, one belonging to the Hantaan the other belonging to the Puumala serotype, are apparently sufficient to detect antibodies in all patients with HFRS originating from different geographic regions in which various *Hantavirus* strains are presumed to be endemic. The assays were found to be superior to the IFA in terms of sensitivity. The data revealed that recombinant nucleocapsid proteins are probably the antigens of choice for use in future serodiagnostic assays.

Niklasson and Kjellson reported the diagnostic value of immunoglobulin M (IgM) antibody detection for the early diagnosis of HFRS (16). They obtained a diagnostic sensitivity of 100% by means of a μ-capture ELISA. Crude

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preparations of tissue culture-grown viruses were used as antigens in this study. We now report the development of a novel μ-capture ELISA which combines the advantages of recombinant antigens in terms of simplicity of preparation and standardization with high sensitivity and specificity.

MATERIALS AND METHODS

Sera. Acute-phase and/or convalescent-phase sera were collected from patients with HFRS. Twenty-four patients originated from Germany, 8 were from Yugoslavia (sera kindly provided by T. Avsic-Zupanc), 13 were from the People's Republic of China (sera kindly supplied by Tongji Medical University, Wuhan, China), 17 were from Greece (sera kindly provided by A. Antoniadis), and 6 were from Scandinavia (sera kindly provided by B. Niklasson and B. Settergren). Information on the time elapsed between the onset of the disease and the recovery of serum was available for 33 patients. Serial serum samples from eight patients from the People's Republic of China were available.

Expression plasmids. A detailed description of the nucleocapsid protein expression plasmids is provided elsewhere (4). In brief, plasmids were constructed by ligation of inserts into vector pDS56/RBSII-(0)-6His (29) (kindly provided by H. Bujard), which was cut with restriction enzymes *Bam*HI and *Bgl*II and dephosphorylated by treatment with calf intestine alkaline phosphatase (Boehringer, Mannheim, Germany). Inserts were derived from polymerase chain reaction amplification of the HTV (Hantaan strain 76-118) and NEV (Puumala serotype strain CG 18-20) cDNA clones of the S RNA segment (28). Inserted cDNA contained the entire coding region together with adjacent *Bgl*II restriction sites at both the 3′ and 5′ ends. Amplification was done with corresponding specific oligonucleotide primer pairs by using *Taq* DNA polymerase (Perkin-Elmer) according to the manufacturer's protocol. The junction areas of the constructs were verified by DNA sequencing of the expression plasmids (Pharmacia T7 sequencing kit).

Expression and purification of recombinant nucleocapsid proteins. Expression of nucleocapsid proteins was performed in *E. coli* SG 13009 (kindly provided by H. Bujard) harboring *lac* repressor plasmid pDMI,1 and the respective expression plasmid. After preincubation overnight in Luria-Bertani medium containing kanamycin (30 mg/liter) and ampicillin (100 mg/liter), cells were diluted in 0.5 liters of Luria-Bertani medium, supplemented with sodium phosphate at a final concentration of 50 mM (pH 7.3), 0.6% (vol/vol) glycerol, 100 mg of ampicillin per liter, and 30 mg of kanamycin per liter, and further incubated at 37°C. For induction of expression, isopropyl-β-D-thiogalactopyranoside was added at a final concentration of 2 mM at an optical density (OD) (600 nm) of 0.8 and cells were shaken for another period of 5 h. Cells were harvested by centrifugation at 4,000 × *g* for 20 min and stored at −70°C until further use. Frozen cells were resuspended in lysis buffer (5 ml of buffer per g of cells; 6 M guanidine hydrochloride, 0.1 M NaH_2PO_4, 0.001 M Tris, pH 8.0) and stirred (magnetic bar, 100 rpm) for 1 h. The lysate was centrifuged at 10,000 × *g* for 15 min at 4°C. The clear supernatant was collected and processed on a nickel-nitrilotriacetic acid resin. The chromatography procedure was performed according to the manufacturer's protocol (Qiaexpress; Diagen, Düsseldorf, Germany). In brief, the lysate was adsorbed to a nickel-nitrilotriacetic acid column. Washing and elution of desired histidine-tailed proteins were achieved by application of a pH step gradient ranging from pH 8.0 to 4.5 in the presence of 10 mM β-mercaptoethanol. The gradient was prepared in a denaturing buffer containing 8 M urea. Two-milliliter fractions were sampled and analyzed for the presence of the expression product on 12% sodium dodecyl sulfate (SDS)–polyacrylamide gels. Fractions containing the recombinant protein were pooled and dialyzed against 1 M urea in 0.9% NaCl. After dilution to the optimal concentration, the antigen was directly used in the μ-capture ELISA as described below.

Production of monoclonal antibodies. Eight- to 10-week-old female BALB/c mice were immunized by intraperitoneal injection of recombinant nucleocapsid proteins of strains Hantaan 76-118 and CG 18-20, which were obtained by means of the *E. coli* pIN-III-*omp*A expression plasmids and purified by sequential urea extraction and gel elution as described previously (5). Ten micrograms of protein dissolved in 0.5 ml of physiological saline and emulsified with the same volume of complete Freund's adjuvant was applied, followed by booster injections (without adjuvant) on days 21, 35, 49, 50, 51, and 52. Mice were sacrificed at day 53, and spleen cells were fused with SP2/0-Ag14 cells (ATCC CRL 8287) as originally described by Köhler and Milstein (9), following a modified procedure (19). After cultivation for 10 to 14 days, cell culture supernatants were screened for antibodies by means of a solid-phase ELISA with pIN-III-*omp*A-expressed nucleocapsid proteins as antigens (35). Antibody-positive clones were picked, subcloned, and then recloned three times by limiting dilution. For mass production of antibodies, cells were propagated in roller flasks and cultivated for about 6 weeks. The FCS content was continually reduced by the addition of fresh medium as described by Kuhlmann et al. (10). Immunoglobulin classes and light chain types were characterized with a commercially available typing kit (Boehringer). Monoclonal antibodies were purified by affinity chromatography with a protein A-Sepharose column (protein A-Sepharose CL 4B; Pharmacia, Munich, Germany). For use in the μ-capture ELISA, antibodies were coupled to horseradish peroxidase by the method of Nakane and Kawaoi (15).

μ-capture ELISA. Highly activated polystyrene microtiter plates (Greiner, Nürtingen, Germany) were coated with rabbit anti-human IgM antibodies (μ chain specific; Dakopatts, Hamburg, Germany) at a dilution of 1:1,000 in coating buffer (0.05 M sodium carbonate, pH 9.6) and then coated with a proteolytic digest of gelatin in Tris-NaCl buffer (blocking reagent, purchased from Boehringer).

For the assay, the following reagents were successively used: patient sera at a reciprocal dilution of 500, recombinant antigens (CG 18-20 nucleocapsid antigen [NEV N] and Hantaan 76-118 nucleocapsid antigen [HTV N]) at a concentration of 2 μg/ml, and horseradish peroxidase-labelled monoclonal antibodies (A1C5, which is NEV specific, and B5D9, which is HTV specific) at a predetermined optimum dilution. All dilutions were made in postcoating buffer. Incubation steps were done for 1 h at 37°C, each followed by three cycles of washing with washing buffer (0.9% NaCl, 0.1% Tween 20). Color was developed by adding ABTS substrate solution {4 mM 2,2′-azino-di[3-ethyl-benzthiazolinsulfonic acid (6)]} (Boehringer). The assay was stopped and read according to standard procedures. The cutoff extinction of the μ-capture ELISA was determined as the mean extinction of 100 negative serum samples plus 3 standard deviations. The cutoff OD was regularly between 0.08 and 0.100 for both antigens. One positive and 10 negative control serum samples were included on each plate. The cutoff extinction of the individual plate was adjusted according to the variation of the mean extinction of the negative control sera. Assay

TABLE 1. Characterization of nucleocapsid protein expression products

Strain	No. of amino acid residues	Linker amino acids	Molecular mass (kDa)	Polymerase chain reaction primers used for construction
CG 18-20	433 (+ 12 linker amino acids)	MRGS–RSH6	53.0	5′-CCAGATCTATGAGTGACTTGACAGAC-3′ 5′-CCAGATCTTATCTTTAAGAATTCTTGG-3′
Hantaan 76-118	429 (+ 12 linker amino acids)	MRGS–RSH6	50.0	5′-CCAGATCTATGGCAACTATGGAGGAA-3′ 5′-CCAGATCTGAGTTTGAAAGGCTCTTGG-3′

results were quantified in terms of the OD (sample)/OD (cutoff) ratio. Values above 1.0 were considered positive.

The specificity of the assays was reevaluated with an independent negative control panel of 200 serum samples.

Solid-phase ELISA. The solid-phase ELISA was performed as previously described. Recombinant nucleocapsid antigens utilized in this assay were expressed in *E. coli* by means of secretion vector pIN-III-*omp*A and purified by sequential urea extraction and final elution from SDS-polyacrylamide gels (5, 35).

Competitive ELISA. In order to determine whether the selected monoclonal antibodies compete with patient sera for identical epitopes, a competitive ELISA was performed using for each antigen 10 serum samples from patients with HFRS, reacting predominantly with the HTV or NEV nucleocapsid protein. The basic protocol followed the procedure of the solid-phase ELISA (see above). Reactive sites of solid-phase bound antigens were blocked by incubation with the sera, which were appropriately diluted to allow maximum binding. Horseradish peroxidase-labelled monoclonal antibodies A1C5 and B5D9 were reacted with blocked and unblocked antigens, and the OD ratios of the respective wells were taken as a measure of competition. Values between 0.9 and 1.0 were interpreted to be an indication of noncompetition.

IFA. The IFA was performed according to standard procedures. For antigen preparation, freshly grown monolayers of Vero E6 cells were inoculated with stock virus (strain Hantaan 76-118 or CG 18-20) and incubated for 7 to 10 days in maintenance medium. Afterwards, they were trypsinized and seeded to Multitest slides (Flow, Meckenheim, Germany) on which they were grown for another period of 24 h, washed, and fixed. Patient sera were preabsorbed with a rheumatoid factor sorbent (Mastsorb; Mast, Reinfeld, Germany) according to the manufacturer's instructions and serially diluted starting from a reciprocal dilution of 8.

RESULTS

Recombinant antigens. Recombinant antigens are briefly characterized in Table 1, and purified expression products are shown in Fig. 1. The purity of recombinant proteins was about 95% after nickel-chelate chromatography. The proteins were directly used in the μ-capture ELISA without further purification steps. The expression rate in SG 13009 cells was about 10% of total protein. The final recovery of recombinant proteins was about 1 to 2 mg/liter of culture. This amount was sufficient for about 5,000 to 10,000 tests.

Monoclonal antibodies. Seven monoclonal antibody-producing hybridoma cell lines could be established. Six cell lines produced IgG1 antibodies, and one produced IgM antibodies. Two monoclonal antibodies were directed against the NEV nucleocapsid protein, and five were directed against the HTV nucleocapsid protein. All antibodies were immunoblot reactive, indicating that they recognize linear epitopes. HTV-directed antibodies did not cross-react with the NEV nucleocapsid protein. Both NEV-specific monoclonal antibodies, however, were found to be slightly cross-reactive with the HTV antigen, as was detectable by means of the solid-phase ELISA but not by IFA or immunoblot. Monoclonal antibodies A1C5 (NEV specific) and B5D9 (HTV specific) were found to be suitable as a detection system in the μ-capture ELISA. They did not compete with patient sera for the same antigenic sites as revealed by the competitive ELISA (OD ratio of wells with blocked versus wells with unblocked antigens, between 0.9 and 1.0), and they kept their activity after being coupled to horseradish peroxidase. Antibody conjugates were found to be stable after long-term storage at 4°C.

Specificity of the μ-capture ELISA. The specificity of both μ-capture ELISAs (HTV N μ-capture ELISA and NEV-N μ-capture ELISA) was found to be 100% by means of a negative control group of 200 serum samples. The inclusion of a control antigen as described for μ-capture ELISAs based on infected cells (16) as antigens was therefore considered to be unnecessary.

Validity of the μ-capture ELISA. The reactivities of HFRS patient sera in the HTV N and NEV N μ-capture ELISAs are summarized in Table 2. The sera comprise 54 serum samples which were reactive with at least one of the two antigens. The same data are quantitatively plotted in Fig. 2. Results were differentiated according to geographic regions from which the patients originated. Almost all patients who had acquired HFRS in Germany were positive in the NEV N μ-capture ELISA. There was only one serum sample from a German patient that was solely reactive in the HTV-specific ELISA. Most of the NEV-reactive sera were also positive in the HTV N μ-capture ELISA, but the OD (sample)/OD (cutoff) ratios were in most cases lower in the latter test. Two serum samples from the German panel revealed equal

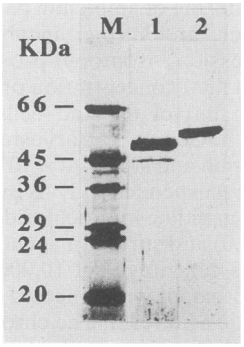

FIG. 1. One-step purification of recombinant nucleocapsid proteins of *Hantavirus* strains Hantaan 76-118 (HTV, lane 1) and CG 18-20 (NEV, lane 2) by nickel-chelate chromatography. Aliquots of 2-ml fractions eluted at a pH of 5.4 were analyzed by 12% SDS–polyacrylamide gel electrophoresis and Coomassie stained. M, molecular mass markers.

TABLE 2. Reactivity of sera from patients from different geographic regions in the NEV N and HTV N μ-capture ELISAs

Origin of patients (no. of serum samples reactive in one of the μ-capture ELISAs)	No. of positive serum samples in:		No. of serum samples reactive in both assays
	HTV N μ-capture ELISA (monoclonal antibody B5D9)	NEV μ-capture ELISA (monoclonal antibody A1C5)	
Germany (19)	16[a]	18	15
China (12)	12	2	2
Greece (16)	16	11	11
Scandinavia (3)	0	3	0
Yugoslavia (4)	1	3	0

[a] Three of these were borderline positive.

OD (sample)/OD (cutoff) ratios in both ELISAs, and one serum sample gave a higher value in the HTV-specific μ-capture ELISA but was also positive in the NEV-specific test. In contrast, all sera from Greek patients were reactive in the HTV N μ-capture ELISA, and only 11 of them were also detected by the NEV-specific test. Similar results were obtained for the Chinese patients whose samples predominantly reacted in the HTV N μ-capture ELISA. Sera from Scandinavian patients with HFRS were reactive only in the NEV N μ-capture ELISA, whereas sera from Yugoslavian patients were either positive in the HTV-specific (n = 1) or in the NEV-specific (n = 3) μ-capture ELISA without revealing cross-reactivity with the heterologous antigen. For 33 patients (corresponding to 56 serum samples if serial samples are included), data on the time elapsed since onset of the disease were available. Thirty-nine of the serum samples were recovered within 40 days since the onset of the disease. All sera were reactive in at least one of the assays, with the exception of the initial samples of two Chinese patients, which were recovered on day 4 or 5 (Fig. 3). Both patients had seroconverted in successive samples obtained on days 13 and 16. The earliest time a patient was bled was on day 3 after the onset of clinical manifestations. The serum was also found to be positive. In general, maximum titers were obtained between days 8 and 25. Sera taken more than 3 to 4 months after the onset of the disease were generally found to be negative (data not shown). On the other hand, we received a serum sample from a German patient which was positive even 2 years after the acute phase of HFRS. Figure 4 compares the OD (sample)/OD (cutoff) values of the μ-capture ELISA and the recombinant antigen-based IgM-

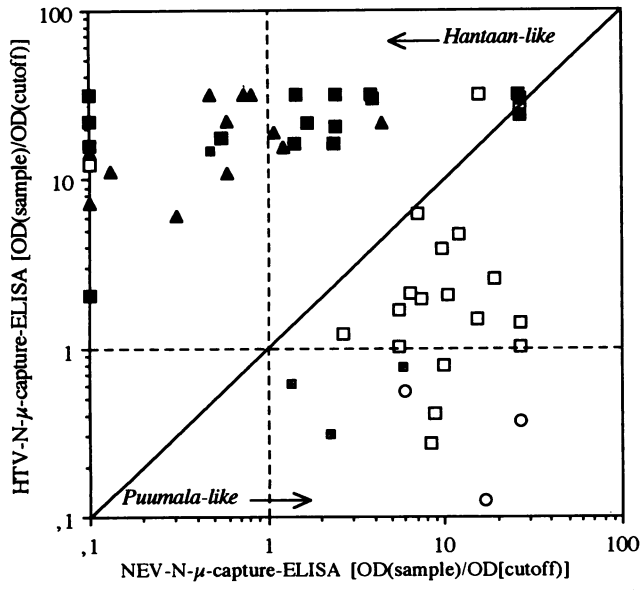

FIG. 2. OD (sample)/OD (cutoff) ratios of sera of patients with HFRS from various geographic regions as obtained by the NEV N and HTV N μ-capture ELISAs. If serial samples were available, the serum with highest extinction was plotted. Symbols: □, German patients; ▲, Greek patients; ⊞, Yugoslavian patients; ○, Scandinavian patients; ■, Chinese patients.

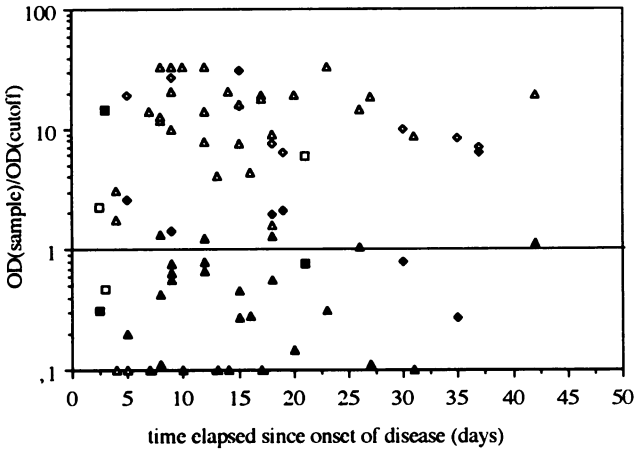

FIG. 3. Reactivity of sera of patients with HFRS from various geographic regions in the N μ-capture ELISA. OD (sample)/OD (cutoff) ratios are correlated to the time of recovery of the serum. Reactivity with the homologous antigen (strain which is thought to be primarily endemic in the respective geographic region) is represented by open symbols, and reactivity with the heterologous antigen is represented by solid symbols. Generally, the reaction with the heterologous antigen is weaker or negative. Serial samples of patients are included. Symbols: ◇, NEV; ◆, HTV (German patients); ▲, NEV; △, HTV (Chinese patients); ■, NEV; □, HTV (Yugoslavian patients).

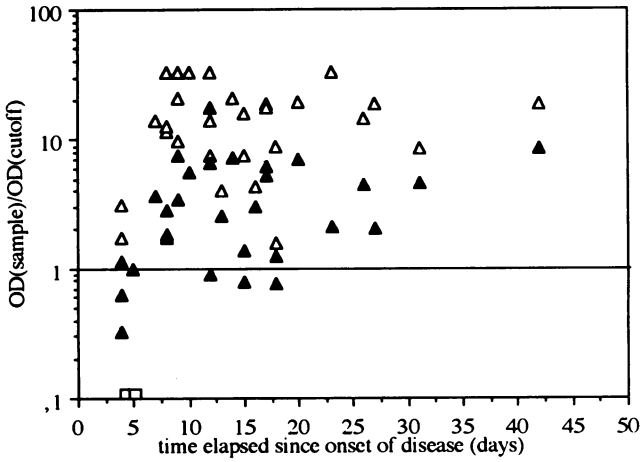

FIG. 4. OD (sample)/OD (cutoff) ratios of sera of Chinese patients with HFRS in the HTV N μ-capture ELISA compared with the IgM-specific HTV N solid-phase ELISA. Serial samples are included. Symbols: □, HTV N μ-capture ELISA; ▲, IgM-specific HTV N ELISA.

specific solid-phase ELISA (35) with the serum panel from Chinese patients. Serial serum samples from eight patients are included (n = 29). The data show that the HTV N μ-capture ELISA recognizes more sera than the IgM-specific solid-phase ELISA and that in some patients antibodies are detected earlier. However, each patient became positive with both assays within 40 days since the onset of the disease (overall sensitivity = 100%). Comparing the results of the μ-capture ELISA and the IFA in consideration of the NEV- and HTV-specific tests, the recombinant antigen ELISA was found to be superior to the IFA in terms of overall sensitivity. If serial serum samples were available, a patient was considered positive if one of the sera was positive in the respective assay. With the samples recovered from German patients within 40 days since the onset of disease manifestations (n = 17) the overall sensitivity of the μ-capture assay was 100 versus 76.4% obtained by the IFA (χ^2 test; $P < 0.05$). If all available sera from patients with HFRS are considered independently from the times of their recoveries, the μ-capture ELISA also detected more sera than the IFA in the panels of Greek (16 versus 14; n = 17) and Yugoslavian (3 versus 1; n = 9) patients, but the difference was not statistically significant (χ^2 test; $P < 0.1$).

DISCUSSION

HFRS is a relevant public health problem in many countries of the world which consequently has stimulated efforts to develop a vaccine. In spite of the importance of HFRS and in spite of the development of various assays for serodiagnosis of the disease, the need for a generally available sensitive and specific antibody detection system has remained unchanged. This is mainly due to the fact that the handling of hantaviruses requires P3 laboratory facilities. Infectious viruses must be safely inactivated and the stability and reproducibility of antigen preparations must be guaranteed before tests are ready for distribution (18, 20). These preconditions are difficult to meet with tissue culture-grown antigens. Moreover, the yield of hantavirus antigens from cell cultures is relatively low. Sensitive IgM detection would allow differentiation between active or past infection, which is of particular importance in areas in which hantaviruses are highly endemic, in which the prevalence of IgG antibodies to hantaviruses is up to 30% (e.g., certain areas in Sweden) (17).

We could recently demonstrate that recombinant viral nucleocapsid protein may be the antigen of choice for a future generation of immunodiagnostic assays (5, 35). We found that sera from patients with HFRS reacted well with the nucleocapsid proteins of two *Hantavirus* strains belonging to the Puumala and Hantaan serotypes, which were expressed in *E. coli* by means of secretion vector pIN-III-*ompA*. Our data have meanwhile been supported by the findings of Groen et al. (6), who found by means of a selective ELISA using virus preparations as antigens that the nucleocapsid protein stimulates an early immune response and that antibodies against it are detectable for years. However, quantitative purification of the pIN-III-*ompA* expression product was laborious because of its poor solubility and the specificity of the assays was affected by residual *E. coli* proteins. The purification procedure was therefore not suitable for large-scale production of diagnostic tests. We now present data on a novel μ-capture ELISA, which utilizes recombinant nucleocapsid proteins of two *Hantavirus* strains as antigens and specific monoclonal antibodies as a detection system. This test design allows a specificity of 100% without the need for highly purified recombinant antigen. Thus, we were able to benefit from a new and simple expression and affinity chromatography system, which allows single-step purification of histidine-tailed recombinant proteins by running them over a nickel-chelate resin (29). The resulting purity of the recovered protein was about 95%. The yield of recombinant protein from 1 liter of culture was sufficient to perform 5,000 to 10,000 tests.

The μ-capture ELISA was found to detect more sera than the IgM-specific solid-phase ELISA and the IFA, although only differences observed with the German serum panel were statistically significant. However, the possibility that the sensitivity of the IFA probably has been roughly overestimated in this evaluation has to be considered, since originally it was part of the case definitions. Therefore, the advantages of the μ-capture ELISA may actually be even more marked. The μ-capture ELISA was found to be positive as early as on day 3 and as late as 2 years after the onset of the disease, but maximum titers were usually obtained between days 8 and 20 and sera reverted to negative 3 to 4 months after the acute phase. These data correlate well with the data of Niklasson and Kjellson, who had stressed the favorable sensitivity of their viral antigen-based μ-capture ELISA (16). Their tests were positive from the second day of the disease, and, in some cases, antibodies remained detectable over a period of up to 9 months. However, those authors used only the Puumala antigen in their assay, and consequently sera from Korean or Asian USSR patients were not or were only weakly reactive.

Regarding the reactivities of sera in the NEV N and HTV N μ-capture ELISAs, our data recently presented for the solid-phase ELISA (35) were fully confirmed. An overall sensitivity of 100% was obtained if both antigens were used. This was particularly useful for the German and Yugoslavian serum panel, whereas for Chinese, Greek, and Scandinavian patients, a single antigen (HTV or NEV nucleocapsid protein) would have been sufficient to recognize all patients. The assay was able to differentiate the etiologic virus type as Hantaan-like or NEV (Puumala)-like by revealing a predominant reactivity against one of the two antigens.

In summary, the recombinant protein- and monoclonal antibody-based μ-capture ELISA proved to be a sensitive and specific diagnostic assay which is easy to handle and to produce. Common sources of false-positive or -negative results in IgM-specific assays, such as the presence of rheumatoid factor or high levels of IgG antibodies, are avoided by the capture technique. The N μ-capture ELISA fulfills the above-mentioned requirements for mass production and may therefore be the serodiagnostic assay of choice for worldwide recognition of acute-phase HFRS.

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