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Antigenic Properties and Diagnostic Potential of Puumala Virus Nucleocapsid Protein Expressed in Insect Cells

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Puumala virus (PUU) is a member of the genus *Hantavirus* in the family *Bunyaviridae* and the causative agent of nephropathia epidemica, a European form of hemorrhagic fever with renal syndrome. Sera of nephropathia epidemica patients react specifically with PUU nucleocapsid (N) protein. In order to safely provide large quantities of antigen for diagnostic purposes, PUU Sotkamo strain N protein was expressed by using the baculovirus system in Sf9 insect cells to up to 30 to 50% of the total cellular protein. The recombinant N protein (bac-PUU-N) was solubilized with 6 M urea, dialyzed, and purified by anion-exchange liquid chromatography. In an immunoglobulin M μ-capture assay purified and unpurified bac-PUU-N antigen showed identical results compared with the results of a similar assay based on native PUU antigen grown in Vero E6 cells. An immunoglobulin G monoclonal antibody-capture assay based on unpurified bac-PUU-N also showed results identical to those of an assay with native PUU-N antigen. Moreover, a panel of monoclonal antibodies reactive with eight different epitopes showed identical reactivity patterns with both natural and bac-PUU-N antigen, while two epitopes in PUU-N expressed as a fusion protein in *Escherichia coli* were not recognized. Puumala hantavirus N protein expressed by the baculovirus system offers a safe and inexpensive source of specific antigen for large-scale diagnostic and seroepidemiological purposes.

Puumala virus (PUU) is a member of the genus *Hantavirus* in the family *Bunyaviridae* (17, 43). PUU is carried by chronically infected bank voles (*Clethrionomys glareolus*) (3, 58) and is the causative agent of nephropathia epidemica (NE), a form of hemorrhagic fever with renal syndrome. NE is characterized by high fever, headache, nausea, vomiting, abdominal pains, renal failure, and, occasionally, visual disturbances, respiratory symptoms, encephalitis, or hepatitis (19, 31), and it can rarely be fatal (mortality of less than 0.2%) (53). PUU, as well as its natural host, is found in most of Europe and Siberia and also in Japan (20), causing thousands of NE cases annually.

Several serotypes or genotypes of hantaviruses, each carried primarily by a different rodent or insectivore host, have been defined. They are as follows: Hantaan virus (HTN) (21), Seoul virus (20), PUU, Prospect Hill virus (23), Thailand virus, Thottapalayam virus (4), Dobrava virus or Belgrade virus (2, 9), Sin Nombre virus (32), and Tula virus (36). PUU, HTN, and Seoul virus are known to cause the different forms of hemorrhagic fever with renal syndrome, with approximately 150,000 cases occurring worldwide annually, the most severe form caused by HTN having a mortality level of 3 to 10% (22). In the Americas, Sin Nombre virus causes hantavirus pulmonary syndrome with 50% mortality (5), and recently several other novel American hantaviruses have been discovered, e.g., Shelter Island virus (47), Bayou virus (30), Black Creek Canal virus (39), and a virus from *Reithrodontomys megalotis* (14).

Hantaviruses are enveloped negative-strand RNA viruses with a tripartite genome (1, 6). The 6.5-kb L segment of PUU codes for the polymerase (35, 50); the 3.7-kb M segment codes for two glycoproteins, G1 (68 kDa) and G2 (54 kDa); and the 1.8-kb S segment encodes, from nucleotide 43 to 1343, a 50-kDa (433-amino-acid [433-aa]) nucleocapsid protein (N) (8, 49, 55). N is the major antigenic protein, and a strong antibody response to N can be detected at an early phase of the disease in NE patient sera. Antibodies to the glycoproteins can also be detected, but the immunoglobulin G (IgG) antibody response appears more slowly than the response to the N protein (24, 27, 54). Antibodies against hantaviruses persist for decades after infection (45), with up to 20% seroprevalence in areas of endemicity in Finland and Sweden (our unpublished observation and reference 34).

N is more conserved between different hantaviruses than are the glycoproteins. Serologically and genetically, HTN, Seoul virus, and Dobrava virus, carried by *Murinae* rodents, are related to each other, while PUU, Tula virus, Prospect Hill virus, Sin Nombre virus, and the other American hantaviruses, carried by *Arvicolinae* and *Sigmodontinae* rodents, form another related group (1, 4, 12, 30, 36, 46, 48, 57). PUU strains vary by up to 5% in their N amino acid sequences with few effects on their antigenic properties (37, 38).

Hantaviruses, and especially PUU, grow slowly, produce low yields of viral antigen in cell culture, and are potentially hazardous, thus requiring Biosafety level 3 facilities. Specific tests for diagnosis of acute hantavirus infection using both native viral antigen and recombinant N expressed in *Escherichia coli* (7, 10, 55, 56) for detection of IgM-class antibodies (33, 59) or low-avidity IgG antibodies (11, 15) have been described.

PUU infection is common in many European countries, e.g., it is the most frequently (over 1,000 cases per year) serologically diagnosed acute viral disease in Finland. We describe here the expression of PUU Sotkamo strain N protein in insect cells and show that it is antigenically indistinguishable from native PUU N protein and provides an easy and reliable source of specific antigen for detection of hantavirus antibodies.

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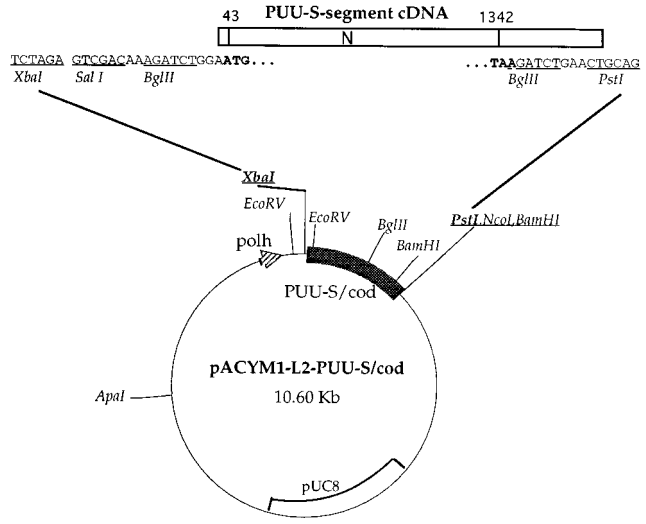

FIG. 1. Construction of pACYML2-PUU-S/cod. The region of PUU S coding for the N protein was amplified, subcloned into the pGEM 3Z vector, and ligated into the *Xba*I and *Pst*I sites of pACYML2 as described in Materials and Methods.

MATERIALS AND METHODS

Construction of recombinant baculoviruses. The region of the PUU S segment coding for N was amplified by PCR as described before (55), cut with *Sal*I and *Pst*I, and cloned into pGEM 3Zf+ vector (Promega, Madison, Wis.), from which the insert was cut with *Xba*I and *Pst*I and subcloned into a baculovirus expression vector, pAcYML2 (18) (kindly provided by Johan Peränen, Institute of Biotechnology, University of Helsinki), which was modified from pAcYM1 (29) by insertion of a polylinker into the *Bam*HI site of pAcYM1 as depicted in Fig. 1. The resulting plasmid construct, pAcYML2-PUU-S/cod, was purified with a CsCl gradient (41) and cotransfected with wild-type baculovirus (sE2 strain) (51) DNA into Sf9 cells by using Lipofectin reagent (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) according to the manufacturer's recommendations. Recombinant baculoviruses were obtained by visual screening of plaques not expressing polyhedrin as described previously (51). Metabolic labeling (as shown in Fig. 2) was done at 40 h postinfection for 4 h in Grace's medium (51) supplemented with 50 μCi of [^{35}S]methionine (Amersham, Buckinghamshire, England) per ml.

Previously described PUU cDNA clones expressing the control constructs coding for aa 1 to 267 of N (N-del) (55) and G2 (aa 649 to 1148 beginning with an extra methionine) (54) with or without an extra signal sequence (bac-PUU-G2+ss and bac-PUU-G2−ss, respectively) were cloned into the *Bam*HI site of baculovirus expression vector pACYM1 (pVT-Bac [52] for G2+ss) as described above. PUU-N was expressed in *E. coli* as a β-galactosidase fusion protein (β-gal-PUU-N) as described previously (15, 55).

Expression and purification of recombinant PUU-N. Sf9 cells infected with bac-PUU-N recombinant baculovirus were grown in 500-ml suspension cultures with TNM-FH medium (51) supplemented with 5% fetal calf serum and antibiotics for 72 h. The cells were pelleted in a GSA Sorvall rotor at 4,000 rpm for 5 min and washed with 0.2 volumes of phosphate-buffered saline (PBS). To certain preparations a mixture of proteinase inhibitors, EDTA (0.5 mg/ml)–leupeptin (10 μg/ml)–pepstatin A (10 μg/ml)–aprotinin (1 μg/ml) (Boehringer, Mannheim, Germany), was added, and the cell suspensions were processed further immediately or stored at −70°C. Cellular proteins were extracted by denaturation with 6 M urea during 30 min on ice. Cell debris was removed by centrifugation, and extracted proteins were either dialyzed overnight against 50 mM Tris (pH 7.5)–10% glycerol–5 mM dithiothreitol or passed through Sephadex PD-10 columns (Pharmacia) in the above-described buffer. The antigen was further purified by anion-exchange chromatography (high-performance liquid chromatography [HPLC]; Mono-Q column) and elution with a KCl gradient in 50 mM Tris (pH 7.5)–10% glycerol–5 mM dithiothreitol. bac-PUU-N was eluted at 0.25 mM KCl.

Testing of stability of bac-PUU-N. The stability of the bac-PUU-N antigen in solution was studied by incubation of the unpurified protein for 1 h to overnight at 37°C, for 1 to 3 h at 56°C, and for 3 weeks at 4°C. To study the effect of repeated freezing and thawing, the antigen was frozen at −70°C, thawed, and then subjected to 5 or 10 cycles of freezing on dry ice. In addition, the antigen was immobilized and dried on enzyme immunoassay (EIA) microtiter plates and studied by IgG EIA after storage for up to 4 weeks at 4°C or −70°C.

Antigenic characteristics of recombinant PUU proteins determined with MAbs. The production and characterization of bank vole and human monoclonal antibodies (MAbs) to PUU have been described previously (26, 28). Recombinant bac-PUU-N, bac-PUU-G2+ss, and β-gal-PUU-N were adsorbed to microtiter well plates overnight at 4°C. Unsaturated protein-binding sites were blocked by addition of 3% bovine serum albumin (BSA) in PBS for 1 h. MAbs (hybridoma supernatants diluted 1:10 in EIA buffer [PBS with 0.5% BSA and 0.05% Tween-20]) were incubated for 1 h. Specific antibody binding was detected with alkaline phosphatase-conjugated goat anti-mouse IgG followed by *p*-nitrophenylphosphate substrate according to the manufacturer's instructions (Sigma). Optical density (OD) at 405 nm was measured after 30 min. All incubations (100 μl/well) were performed at 37°C, and plates were washed five times with washing buffer (0.9% NaCl, 0.05% Tween) between each step.

PUU IgM μ-capture assay. Human sera were analyzed for PUU-specific IgM by a modification of a procedure described previously (33). Optimal conditions for the assay were determined by box titrations of all included reagents. Goat anti-human IgM serum (Cappel), diluted 1:500 in 0.05 M carbonate buffer (pH 9.6), was incubated in microtiter wells at room temperature overnight and stored at 4°C until use. Patient and control sera, diluted 1:200 in EIA buffer, were incubated for 1 h in duplicate wells. bac-PUU-N antigen (purified antigen, 1.0 μg/ml; unpurified antigen, 1:500 dilution of solubilized insect cell extract) and negative control antigen (diluent only) were incubated alone for 1 h and then with horseradish peroxidase-conjugated bank vole anti-PUU MAb 1C12, diluted 1:2,000, for 1.5 h. All incubations and washings were performed as described above. Specific antibody binding was detected by using tetramethylbenzidine substrate according to the manufacturer's instructions (ICN Biochemicals, Cleveland, Ohio). The enzyme reaction was stopped after 15 min with 2 M H_2SO_4, and OD at 450 nm was measured. In all plates, one acute-phase NE patient serum sample was used as an internal standard. The mean OD value of the sample was given a value of 1.000 for each plate, and the mean values of the

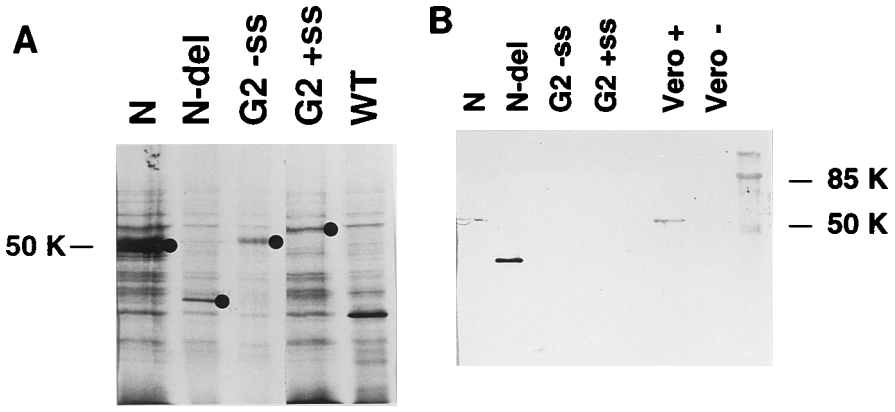

FIG. 2. Metabolic labeling (A) and immunoblotting (B) using a pool of NE patient sera with PUU baculovirus expression constructs. The proteins expressed (as explained in Materials and Methods; bands shown by dots) were as follows: N, the whole N reading frame (i.e., bac-PUU-N; the sample was diluted for the immunoblot); N-del, aa 1 to 267 of N; G2 −ss, aa 649 to 1148 without a signal sequence; and G2 +ss, aa 649 to 1148 with an additional signal sequence. WT, wild-type baculovirus; Vero +, Vero E6 cells infected for 14 days with PUU; Vero −, uninfected Vero E6 cells.

sample duplicates were adjusted correspondingly. The results were calculated by reducing the mean OD value of the sample duplicates with the background mean OD (obtained with diluent only).

Five groups of PUU antibody negative control sera, with 10 serum samples in each group, were used to estimate the cutoff levels of the assay. Four patient groups consisted of 10 patients suffering from measles virus, varicella-zoster virus, cytomegalovirus, and influenza A virus infection, respectively, and 10 serum samples were obtained from apparently healthy individuals.

PUU IgG MAb-capture assay. The assay for PUU-specific IgG was modified from a previously described assay (24). Optimal conditions for the assay were determined by box titrations of all included reagents. Microtiter plates were coated with bank vole MAb 1C12 (10 μg/ml) as described above. Unpurified bac-PUU-N antigen (1:1,000 dilution of solubilized insect cell extract in EIA buffer) and negative control antigen (diluent only) were incubated alone for 1 h and then with duplicates of patient and control sera, diluted 1:400 in EIA buffer, for 1 h. Specific antibody binding was detected with alkaline phosphatase-conjugated goat anti-human IgG (Sigma), diluted 1:2,000, and *p*-nitrophenylphosphate substrate as described above. An NE convalescent serum sample was used as an internal standard as described above. Serum samples from 40 patients suffering other viral diseases (those mentioned above) were used to calculate the cutoff level of the assay.

The antigen was serially titrated 1:10 to 1:40, 960 (approximately 100 μg to 25 ng of total protein per ml) by using one PUU IgG-positive serum sample and one negative control serum sample. A panel of 42 serum samples examined in the assay was collected from apparently healthy individuals in northern Sweden; of these samples, 22 had previously been found positive for PUU-specific IgG antibodies by EIA with native N antigen and neutralization tests (13, 27).

RESULTS

Baculovirus expression of PUU proteins. The region of the PUU Sotkamo strain S segment coding for N was cloned into a baculovirus transfer vector, and the resulting construct (pAcYML2-PUU-S/cod; Fig. 1) was recombined with wild-type baculovirus for expression of recombinant PUU-N under the control of the polyhedrin promoter. Sf9 insect cells infected with recombinant baculoviruses overexpressed a 50-kDa protein (Fig. 2A) recognized by NE patient sera as determined by immunoblotting (Fig. 2B). The recombinant protein, bac-PUU-N, was expressed at levels of up to 30 to 50% of the total cellular protein, as estimated from band densities determined with a Millipore BioImage scanner. The protein was, however, found to be largely insoluble under physiological conditions. Therefore, the protein was solubilized with 6 M urea and then subjected either to dialysis or to chromatography on Sephadex (PD-10) columns to remove the urea (Fig. 3). The protein, before and after the treatment, was recognized specifically by sera from NE patients and PUU-infected bank voles (Fig. 4) in immunoblotting. An Sf9 cell suspension culture (2×10^6 cells/ml) infected with bac-PUU-N and treated as described above yielded approximately 40 mg of total protein per liter.

A recombinant protein consisting of aa 1 to 267 of PUU-N (N-del) was also readily recognized by NE sera as determined by immunoblotting (Fig. 2). The constructs coding for G2 with or without signal sequence were expressed at lower levels, and although they were recognized by polyclonal protein-specific rabbit antisera (54) and vaguely by NE patient sera in EIA (data not shown), they could not be detected by any PUU-G2-specific MAbs (26, 28) (Table 1). The bac-PUU-N antigen was further purified by anion-exchange liquid chromatography (Fig. 3B).

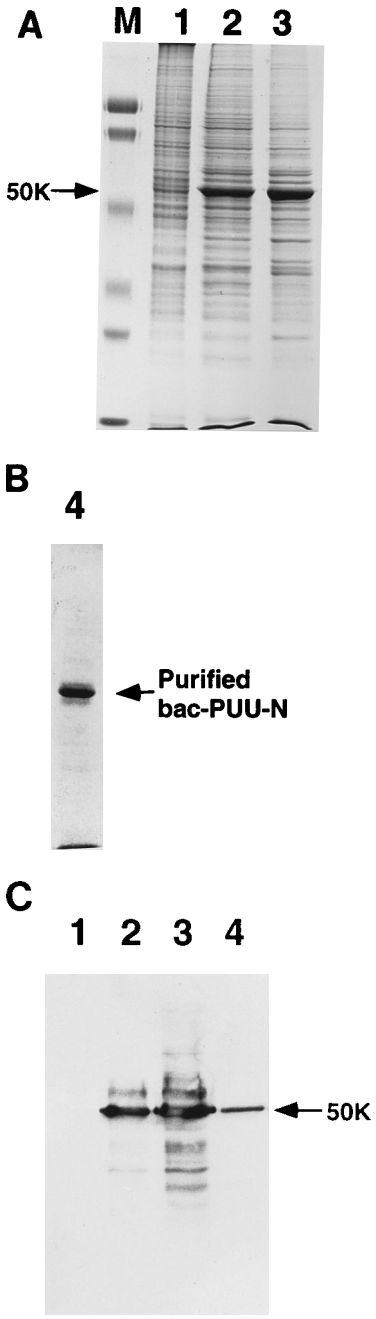

FIG. 3. Expression, solubilization, and purification of bac-PUU-N. (A and B) sodium dodecyl sulfate-polyacrylamide gel electrophoresis Coomassie blue stain; (C) immunoblotting with MAb 1C12. Lanes 1, uninfected Sf9 cells; lanes 2, cell extract of bac-PUU-N-infected Sf9 cells; lanes 3, bac-PUU-N antigen after solubilization (supernatant of centrifuged extract); lanes 4, bac-PUU-N after purification by HPLC.

Characterization of antigenic properties of bac-PUU-N. The authenticity of the antigenic properties of bac-PUU-N was analyzed with a panel of MAbs. All 11 bank vole MAbs, selected against native PUU Sotkamo strain N protein and reactive with eight different N epitopes (28), also recognized bac-PUU-N (Table 1). In contrast, when the same cDNA was expressed as a β-galactosidase fusion protein in *E. coli* (15, 55), the protein was not recognized by two MAbs, 3H9 and 5F4.

The unpurified antigen, stored with protease inhibitors, proved to be stable after repeated freezing and thawing and under short-term heat treatment. The antigenicity also proved to be unchanged after storage for several weeks at 4°C or −70°C either in solution or applied to microtiter well plates. However, after 7 weeks two batches of antigen stored without protease inhibitors showed variable degrees of proteolysis. In immunoblotting using PUU-N-specific rabbit antiserum these batches were seen to be degraded (Fig. 5), the major degradation products having the mobility of 38- to 40-kDa proteins. MAb 1C12, which reacted with a glutathione *S*-transferase–

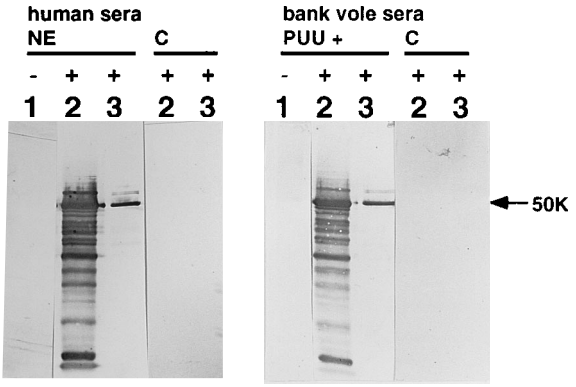

FIG. 4. Immunoblotting using serum pools of NE patients (NE) and PUU-infected bank voles (PUU+) and PUU-negative human and bank vole control sera (C) with a suspension of control Sf9 cells (lanes 1), a suspension of bac-PUU-N-infected Sf9 cells (lanes 2), and bac-PUU-N antigen after solubilization treatment (supernatant of centrifuged extract) (lanes 3).

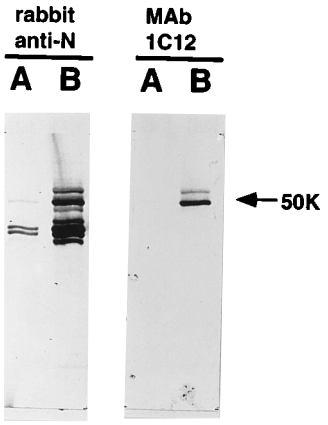

FIG. 5. Solubilized bac-PUU-N antigen stored without proteinase inhibitors for 7 weeks at 4°C. Results of immunoblotting of two different batches, A and B, with polyclonal rabbit anti-β-gal-PUU-N serum (55) and anti-PUU-N bank vole MAb 1C12 are shown.

PUU-N fusion protein containing the N-terminal 79 aa of PUU-N (our unpublished results), did not recognize the degraded forms of N protein, suggesting that the degradation was N terminal.

Evaluation of diagnostic applicability. The properties of the bac-PUU-N antigen were further studied in μ-capture IgM and MAb-capture IgG assays. In the μ-capture IgM assay bac-PUU-N and a previously characterized strongly reactive, PUU-N-specific, horseradish peroxidase-conjugated bank vole MAb, 1C12 (28), were used for detection of bac-PUU-N captured by patient IgM antibodies. Fifty PUU antibody negative control sera, of which 40 were from patients suffering from other viral infections, were used for determination of the cutoff level of the assay. The absorbance for the negative controls with the purified antigen was 0.027 ± 0.029 (mean ± standard deviation [SD]) (mean + 3 × SD = 0.114); with the unpurified antigen it was somewhat higher, i.e., 0.048 ± 0.023 (mean + 3 × SD = 0.117). For both antigens a cutoff level was set at 0.150. Forty-two sera previously found positive in a PUU μ-capture IgM assay using native PUU antigen were examined in the assay. All were found positive, with OD values ranging from 0.314 to 1.004 for the purified antigen and from 0.261 to 1.267 for the nonpurified antigen (Fig. 6).

To study the applicability of the bac-PUU-N antigen for detection of human IgG antibodies against PUU, a panel of sera was studied in a MAb-capture assay. A specific reaction was seen even at a 1:40,960 dilution of the insect cell extract (equivalent to approximately 10 ng of bac-PUU-N per ml) when unpurified bac-PUU-N was serially titrated in the assay. With a selected working dilution of 1:1,000 (approximately 1 μg of total protein per ml) a panel of negative sera gave a mean OD of 0.004 (± 0.009). A cutoff OD level of 0.1 was chosen, and a panel of 42 serum samples from healthy individuals was studied. Twenty-two of these samples had been earlier found to have PUU-specific IgG antibodies as a sign of past hantavirus infection in a similar assay using native PUU antigen grown in Vero E6 cells. This result had also been verified by a neutralization test (13). As shown in Fig. 7, the

TABLE 1. Reactivities of a panel of MAbs with bac-PUU-N, β-gal-PUU-N, and bac-PUU-G2+ss

MAb	Epitope	Reactivity with[a]:		
		β-gal-PUU-N	bac-PUU-N	bac-PUU-G2+ss
N-specific MAbs				
3H9	N-a	−	+	−
5E1	N-b	+	++	−
5B5	N-c	+++	+++	−
3G5	N-d	+	++	−
5F4	N-e	−	+++	−
1C12	N-f	+++	+++	−
3E11	N-f	+++	+++	−
5A3	N-f	+	+	−
2E12	N-g	+	++	−
4C3	N-h	+++	+++	−
4E5	N-h	+++	+++	−
G2-specific MAbs				
4G2	G2-a1	−	−	−
1C9	G2-a2	−	−	−
5B7	G2-b	−	−	−

[a] −, OD of <0.150; +, OD of 0.151 to 0.500; ++, OD of 0.501 to 1.000; +++, OD of >1.000.

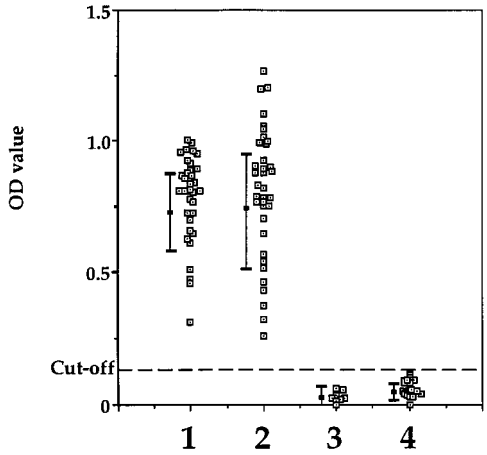

FIG. 6. IgM μ-capture results obtained by using bac-PUU-N antigen with sera previously determined to be IgM positive or IgM negative by the same assay based on native viral antigen grown in Vero E6 cells. OD values for the positive sera with HPLC-purified (column 1) and unpurified (column 2) bac-PUU-N antigen and for the negative sera with HPLC-purified (column 3) and unpurified (column 4) antigen are shown. The mean values and SDs are depicted with bars.

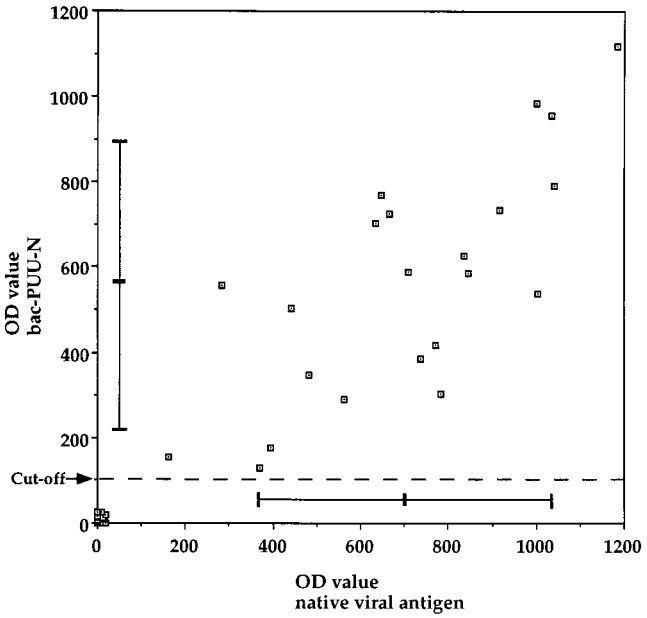

FIG. 7. IgG MAb-capture results for a panel of serum samples from healthy blood donors ($n = 42$) with unpurified bac-PUU-N antigen. *x* axis, OD values of assay based on native viral antigen grown in Vero E6 cells; *y* axis, absorbance values for the same samples with bac-PUU-N antigen. The mean values and SDs for the positive samples are depicted with bars (correlation coefficient [*r*] = 0.928).

same 22 samples were found positive by the bac-PUU-N IgG MAb-capture test (OD range of 0.155 to 1.118), and all negative sera gave ODs lower than the cutoff value (0.000 to 0.026). Pairwise comparison of the OD values of all the serum samples in the two assays gave a highly significant correlation coefficient value of 0.92 ($P < 0.0005$).

DISCUSSION

The PUU nucleocapsid protein has earlier been demonstrated to be the major antigen in the human immune response (24, 27, 54). High levels of N-specific IgM are produced during the acute phase of NE, and IgG antibodies to N, but also IgG antibodies to the glycoproteins, prevail for decades (45). Thus, N is an essential and sufficient antigen both for diagnosis of acute infection and for seroloepidemiological surveys. We and others (15, 59) have earlier demonstrated that PUU N protein expressed in *E. coli* is suitable for diagnostic purposes, and Jenison and coworkers (14) demonstrated that it reacts well also with sera from American hantavirus pulmonary syndrome patients. Expression of HTN N in insect cells has been described previously (42, 44); in most cases this antigen could not detect antibodies to PUU (40).

The expression level of bac-PUU-N was extremely high, and the antigenic properties of bac-PUU-N were identical to those of the native viral antigen as judged by the reactivity of the anti-PUU-N MAbs. In contrast, when the same cDNA construct covering the N protein coding region was expressed in *E. coli* as a β-galactosidase fusion protein, two of the MAbs, 3H9 and 5F4, did not recognize the bacterial fusion protein in an EIA. The recognition site of MAb 3H9 is in the middle part of the protein, aa 251 to 260 (25), which also serves as a major B-cell epitope for many NE patients (25, 54) and is highly variable among the hantaviruses. The loss of these two epitopes in PUU N expressed in *E. coli* may be secondary to antigen preparation procedures or differential refolding of the bacterial product compared with the native protein, or the fusion part of the protein may hide an epitope or affect its structure. Bac-PUU-N lacks any extra amino acid sequences, such as poly-His, which could affect the antigenicity or folding of the protein. Another disadvantage of recombinant antigens expressed in *E. coli* is the extensive need for purification because of the presence of antibodies reactive with *E. coli* proteins in human sera.

Epitope mapping of PUU-N showed that even if the amino terminus of the protein is the most important antigenic part, the epitope pattern may vary considerably from one patient to another and significant antigenic epitopes are found throughout the protein (54)—including in the C terminus (25)—suggesting that the whole protein would be optimal for diagnostic purposes.

The PUU G2 constructs included as controls, although of the expected size and recognized by protein-specific polyclonal antisera, were not detected by anti-G2 MAbs, suggesting that the epitopes are conformational, dependent on correct glycosylation, coexpression of G1, or the suboptimal nature of the constructs.

Because of the high expression level, bac-PUU-N seems to form insoluble aggregates, in which, unlike the case with the nucleocapsid inclusion bodies found in PUU-infected Vero E6 cells, the N protein may not be properly folded. However, after solubilization with 6 M urea and transfer into physiological buffers, the protein seems to be able to fold readily into an antigenically proper conformation.

The yield of antigen was approximately 10 mg of bac-PUU-N antigen per liter of insect cell culture, which is enough for at least 1,000 microtiter well plates or antibody measurements of 15,000 patient samples. Provided that a cocktail of proteinase inhibitors was added, the antigen was stable for months during storage in solution or when adsorbed to microtiter plates. In the IgM and IgG assays, based on previously described PUU-specific MAbs, the bac-PUU-N antigen showed results identical to those obtained with native antigen grown in Vero E6 cells. The approach described here should provide a useful tool for diagnostics and epidemiology of hantaviral infections.

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