Expression of the Lassa virus nucleocapsid protein in insect cells infected with a recombinant baculovirus: application to diagnostic assays for Lassa virus infection

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The coding region of the gene for the nucleocapsid protein of Lassa virus has been inserted into the genome of Autographa californica nuclear polyhedrosis virus (AcNPV) using the transfer vector pAcYM1, so that expression of the foreign DNA is under the control of the promoter of the AcNPV polyhedrin gene. Infection of cultured Spodoptera frugiperda cells with recombinant virus resulted in the synthesis of high levels of a protein that was indistinguishable from the authentic Lassa virus protein by SDS gel electrophoresis and immunoblotting with a variety of specific immune sera and monoclonal antibodies (MAbs). The kinetics of appearance of the protein were comparable to those of polyhedrin production in wild-type AcNPV-infected cells. The recombinant material was antigenic when used in ELISA for Lassa virus-specific antibodies, reacting well with MAbs specific for the nucleocapsid protein and with sera from experimentally infected guinea-pigs. The recombinant ELISA was able to clearly distinguish sera from human cases of Lassa fever against a panel of known negative sera of African origin. Recombinant-infected insect cells were an effective substitute for mammalian cells infected with Lassa virus itself in the immunofluorescence assay for Lassa virus-specific antibodies. This system offers attractive alternatives to the use of Lassa virus-infected materials as reagents in diagnostic procedures.

Introduction

Lassa virus is the aetiological agent of Lassa fever, an acute infectious disease in man that was first recognized in 1969 (Frame et al., 1970). The virus is endemic in many regions of west Africa (Troupet et al., 1970; Keane & Gilles, 1977; Frame et al., 1984; McCormick et al., 1987a), being transmitted to man from the peridomestic rodent host Mastomys natalensis, in which it sets up a life-long chronic infection (Wulff et al., 1975). Seroepidemiological studies suggest that 10 to 50% of the population in endemic areas have antibodies to the virus; it has been estimated that up to 500000 infections may occur per year, with about 5000 deaths (McCormick et al., 1987a, b). Serological, biochemical and morphological studies have established the virus to be a member of the arenaviridae, of which lymphocytic choriomeningitis virus is the prototype. They are enveloped viruses containing two pieces of single-stranded RNA, designated L (large) and S (small), with lengths of approximately 7 kb and 3.4 kb, respectively. The arenavirus S RNA segment codes for the structural proteins of the virion using an ambisense strategy (Auperin et al., 1984). They consist of an internal nucleocapsid protein (N) of Mr 60000 and two envelope glycoproteins (G1 and G2) of Mr 45000 and 38000, respectively.

The considerable morbidity and mortality that Lassa fever imposes upon the local population makes its rapid and simple identification desirable, in order to implement isolation procedures and for the administration of appropriate therapy. It would also be highly advantageous if suspected cases of Lassa fever in travellers returning from west Africa could be diagnosed rapidly in the local diagnostic laboratory, without the necessity to send possibly infected specimens to a suitably equipped containment laboratory. Current methods of laboratory diagnosis include isolation of virus in culture and identification by indirect immunofluorescence, and/or the detection of antiviral antibodies, which also involves immunofluorescence using cells infected with reference virus as antigenic substrate. Both procedures necessitate the use of maximum containment facilities suitable for handling highly pathogenic infectious viruses. This severely restricts the number of laboratories able to carry...
out these tests. If antigenic material is inactivated by physical or chemical means for distribution outside the containment laboratory, exhaustive safety testing is necessary.

The genes coding for the Lassa virus structural proteins have been cloned and sequenced (Clegg & Oram, 1985; Auperin et al., 1986; Auperin & McCormick, 1989) and high level expression of segments of the N protein was obtained in bacteria (Barber et al., 1987). Vaccinia virus recombinants that express the proteins have also been constructed and are able to protect vaccinated guinea-pigs (Clegg & Lloyd, 1987; Auperin et al., 1988; Morrison et al., 1989) against subsequent challenge by Lassa virus. However, it should be noted that primates immunized with recombinants expressing the N protein are apparently not protected (G. Lloyd & J. C. S. Clegg, unpublished).

Here, we describe the insertion of the gene into the baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV) and report high level expression of the complete viral protein in infected insect cells. We present data showing that the expressed N protein functions as an efficient reagent in the detection of Lassa virus-specific antibodies for research and diagnostic purposes.

Methods

Virus and cells. Wild-type AcNPV (from D. H. L. Bishop, NERC Institute of Virology, Oxford, U.K.) and recombinant virus stocks were grown and assayed in semi-confluent layers of Spodoptera frugiperda cells in TC100 medium containing 10% foetal calf serum, kanamycin and streptomycin/penicillin, according to procedures described by Brown & Faulkner (1977), as modified by Overton et al. (1987).

Plasmid constructions. DNA manipulations were carried out essentially as described by Maniatis et al. (1982) and by Crouse et al. (1983). The transfer vector pAcYM1 (Matsuura et al., 1987) was provided by D. H. L. Bishop. The plasmid pLS109, containing a 1830 bp insert coding for the Lassa virus N protein, was used as the source DNA (Clegg & Oram, 1985). Terminal homopolymeric sequences resulting from the cDNA cloning were removed prior to insertion of the N coding region into the BamHI site of the transfer vector pAcYM1, as shown in Fig. 1. Recombinant transfer plasmids were picked after screening by colony hybridization and those with the correct orientation of the N gene, relative to the polyhedrin promoter, were identified by restriction mapping with EcoRV. The structures of both vector-insert junctions were verified by supercoiled plasmid DNA sequencing (Chen & Seeburg, 1985). An EcoRV–HindIII fragment containing the upstream junction was subcloned into pUC18 for sequencing, whereas the downstream junction was sequenced using the pAcN1 transfer plasmid DNA and a specific oligodeoxynucleotide primer complementary to nucleotides 1750 to 1766 of the Lassa virus N gene sequence (Clegg & Oram, 1985).

Transfection and isolation of recombinant viruses. S. frugiperda cells were transfected with mixtures of purified AcNPV DNA and the recombinant AcNPV transfer vector (pAcLN1) DNA according to procedures described by Smith et al. (1983), with the modifications of Matsuura et al. (1987). After 4 days of incubation at 28 °C, the supernatant fluids were harvested and titrated in semi-confluent layers of S. frugiperda cells. Plaques exhibiting no evidence of occlusion bodies, as determined by transmission light microscopy, after 3 to 4 days incubation were recovered into 100 μl of medium and re-titrated as above. Following four rounds of plaque purification, stocks of putative recombinant viruses were made by infecting 25 cm² tissue culture flasks containing semi-confluent layers of insect cells. In order to identify recombinants positively, virus was allowed to infect the cultures of S. frugiperda for 4 days before the cells were harvested. After freezing and thawing twice various dilutions of the cells in phosphate-buffered saline (PBS) were dot-blotted onto Hybond-N (Amersham). The blots were hybridized according to the manufacturer’s protocol, with the 1800 bp Lassa virus N gene fragment excised from pLS156 by BamHI digestion and 32P-labelled by the procedure of Feinberg & Vogelstein (1984).

Purification and characterization of virus DNA. Wild-type or recombinant AcNPV was prepared from culture medium of infected cells by the procedure of Overton et al. (1987). It was purified by isopycnic centrifugation for 18 h at 45000 r.p.m. at 18 °C in a caesium chloride gradient in the Sorvall TV856B vertical rotor, and both nicked and closed circular DNA were pooled together. Recombinant virus DNA was characterized by Southern blot analysis after digestion with BamHI or HindIII and separation of the resultant fragments by electrophoresis in 12% agarose gels. DNA was transferred to Hybond-N before being hybridized with labelled N gene, as above.

Protein analysis. Monolayers of S. frugiperda were infected with AcNPV or recombinant virus at an m.o.i. of 10 p.f.u./cell in 25 cm² tissue culture flasks containing 3 x 10⁶ cells. For time course analysis cells were harvested at various intervals over 4 days and resuspended in PBS. Samples (5 μl) of the cells were mixed with 5 μl 50 mM-Tris–HCl pH 6.8, 1% SDS, 1% mercaptoethanol, 0.1% bromophenol blue, heated to 100 °C for 10 min and electrophoresed in 12% polyacrylamide gels (Laemmli, 1970). Gels were stained with 0.1% Coomassie blue in 10% (v/v) acetic acid containing 20% ethanol. For Western blot analysis, cell lysates were electrophoresed as above and the proteins transferred to nitrocellulose membranes (Hybond C, Amersham) according to Dunn (1986). Blots were blocked by immersion in PBS containing 0.3% Tween 20 for 5 min and immunological detection of Lassa virus-specific proteins was carried out by incubation with Lassa virus N protein-specific monoclonal antibody (MAb) for 4 h. After washing three times in PBS containing 0.05% Tween 20 (PBST), blots were developed using the Vectastain ABC system (Vector Laboratories). They were incubated for 1 h with biotinylated anti-mouse IgG antibodies, washed and further incubated with avidin–peroxidase complex, formed according to the manufacturers’ instructions. Blots were washed again before incubation with 4-chloronaphthol substrate (Hawkes et al., 1982).

Partial purification of recombinant Lassa virus N protein. Insect cells infected with recombinant baculoviruses were harvested 3 days post-infection by centrifugation at 250 g for 5 min at 4 °C. Cell pellets were resuspended in PBS and frozen and thawed 3 times. The disrupted cells were then re-centrifuged at 250 g for 5 min, the supernatants decanted and pellets resuspended in PBS. Pellet and supernatant fractions were analysed by PAGE and protein concentrations determined using the bichromonic acid protein assay (Pierce Chemicals) (Smith et al., 1985).

ELISA. Pelleted fractions of frozen and thawed, recombinant and S. frugiperda cells were diluted to a final protein concentration of 5 μg/ml with carbonate buffer (50 mM-NaHCO₃, pH 9.6) and 100 μl was pipetted into ELISA plate wells (Titertek, Flow Laboratories), before being left overnight at 4 °C. The plates were washed three times in PBST buffer, and test sera, diluted 1:100 in PBST containing 5% calf serum were added to the wells (100 μl/well). After 1 h incubation at room temperature, plates were washed four times with PBST before the
addition of peroxidase-conjugated secondary antibody (Seralab) diluted 1:4000 in PBST containing 5% calf serum. After a further incubation period of 1 h at room temperature, plates were washed again and peroxidase substrate was added (0.4 mg o-phenylenediamine per ml, 0.02% H2O2 in 50 mM-sodium acetate, pH 5.0). Reactions were stopped after 30 min by the addition of 25 µl of 4 M-H2SO4. Plates were read using a TiterTek Multiskan spectrophotometer at 492 nm. The same procedures were used with other antigens, except that plates were coated at protein concentrations of 0.3 µg/well for a bacterial recombinant antigen, which had been purified by ion-exchange chromatography and contained amino acid residues 119 to 570 of the Lassa virus N protein (Barber et al., 1987), and 0.25 µg/well for purified Lassa virus (Clegg & Oram, 1985) inactivated by treatment with β-propiolactone.

Electron microscopy. Infected S. frugiperda cells were fixed in 2.5% glutaraldehyde, embedded in Araldite, cut into 80 nm sections and stained in uranyl acetate and lead citrate using standard techniques. The sections were examined in a Philips EM400T microscope operating at 80 kV.

Immunofluorescence assays. S. frugiperda cells in 25 cm² flasks were infected at an m.o.i. of 10 p.f.u./cell and incubated at 28 °C for 48 h. The cells were harvested by centrifugation, washed twice in PBS, once in PBS containing 0.001% bovine serum albumin, mixed with an approximately equal number of uninfected cells and spotted onto glass microscope slides. After drying in air, the cells were fixed in acetone at 4 °C for 10 min. Samples of human sera appropriately diluted in PBS were applied to each spot. The slides were then incubated for 30 min at room temperature, washed three times with PBS, and a fluorescein-conjugated secondary antibody of appropriate specificity, diluted in PBS, was applied. After a further 30 min incubation the slides were washed three times in PBS, mounted in PBS-glycerol and viewed under ultraviolet illumination.

Results

Recombinant virus construction

The strategy for the construction of the baculovirus transfer vector containing the entire coding region for the Lassa virus N gene is shown in Fig. 1. The procedure involved the removal of the terminal homopolymeric sequences, which might adversely affect transcription of the exogenous gene, and insertion of the DNA into the baculovirus transfer vector pAcYM1. The orientation of the Lassa N gene in candidate clones was determined by restriction mapping and the structures of both vector–insert junctions in the selected plasmid were determined by sequencing (Fig. 2). The sequence upstream of the inserted Lassa virus gene was exactly as expected from published data (Hooft van Iddekinge et al., 1983; Howard et al., 1986; Matsuura et al., 1987). Immediately downstream of the BamHI insertion site there appeared to be an extra G residue, compared with the pAcYM1 sequence given by Matsuura et al. (1987) (nucleotide 2936 in Fig. 2), and three contiguous nucleotides had been deleted (nucleotides 2950 to 2952), compared with the published sequence.

After transfection of S. frugiperda cells with mixtures of AcNPV and recombinant transfer vector, DNA recombinant viruses were identified by their occlusion-negative phenotype in plaque assays. Recombinant viruses were subsequently plaque-purified four times before growth of virus stocks.

Genomic structure of recombinant virus

To verify that the N gene was present and in the correct position (downstream from the polyhedrin promoter)
Fig. 2. Nucleotide sequences around the vector-insert junctions of transfer plasmid pAcLNI+. The origin of the sequences (from the transfer vector pAcYM1, the Lassa virus N gene and pUC18) is indicated above the sequence. The numbering of AcNPV-derived nucleotides is that given by Matsuura et al. (1987) and that of Lassa virus sequence is from Clegg & Oram (1985). The transcriptional start site and the translation start and stop sites are indicated below the sequence.

the recombinant baculovirus, purified viral DNA was digested with the restriction enzymes BamHI or HindIII and Southern blots were performed. DNA bands were hybridized with a radiolabelled complete N gene fragment, excised from pLS156 with BamHI. Digestion of both recombinant transfer vector pAcLNI and recombinant viral DNA with BamHI generated a DNA fragment of 1.8 kb, identifiable by hybridization as the full-length Lassa virus N gene insert (Fig. 3). The replacement of an approximately 1.9 kb band in the wild-type AcNPV DNA digest by a 1.3 kb fragment in that of the recombinant virus DNA is consistent with the BamHI restriction site map (Adang & Miller, 1982; Smith et al., 1983) in the region of the polyhedrin gene and the insertion of the transfer vector-derived BamHI site. Digestion of the recombinant viral DNA with HindIII would be expected to generate a 1553 bp fragment, consisting of a 1122 bp sequence from the 3' end of the Lassa virus N gene together with 431 bp of the AcNPV DNA 930 bp HindIII V fragment, running downstream from the insertion site to the HindIII site at the V fragment-T fragment boundary. The 5' region of the exogenous gene would remain attached to the 8.4 kb HindIII F fragment of AcNPV DNA. The Southern blot showed that this was in fact the case (Fig. 3). It is thus concluded that the N gene is located as expected in the genome of the recombinant virus.

**Expression of exogenous protein in infected insect cells**

In order to determine whether N protein was synthesized in the recombinant virus-infected cells, *S. frugiperda* cells were infected at a high m.o.i. with parental AcNPV or recombinant virus and the cells were harvested at various times post-infection. Infected cell lysates were analysed by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and incubated with a MAb specific for the N protein. Coomassie blue-stained gels of recombinant virus-infected cells (Fig. 4a) reveal a major band, evident 2 to 4 days post-infection, corresponding in size to the 60000 Mr Lassa virus N protein. Immunoblots using MAbs specific for the N protein (Fig. 4b) confirm the identity of this band. Densitometry of the stained gel indicates that the amount of N protein in the cells is approximately

![Fig. 3. Southern blot analysis of purified genomic DNA from recombinant AcNPV. DNA from plasmid pAcLNI (lanes 1 and 5), recombinant virus (lanes 2 and 6), or wild-type virus (lanes 3 and 7) was digested with BamHI (lanes 1 to 3) or HindIII (lanes 5 to 7), analysed on a 0.8% agarose gel and stained with ethidium bromide (a). The DNA was then transferred to Hybond-N and hybridized with 32p-labelled probe for the Lassa virus N gene (b). Fragment sizes (in bp) of components of the 1 kb ladder (Gibco-BRL; lane 4) are indicated.](attachment:image)
Fig. 4. Protein synthesis during the course of infection of cells with recombinant and wild-type baculoviruses. *S. frugiperda* cells were infected at an m.o.i. of 10 with recombinant (lanes 2 to 6) or wild-type virus (lanes 10 to 14). Cells were harvested and prepared for analysis on SDS–polyacrylamide gels after 24 h (lanes 2 and 10), 48 h (3 and 11), 72 h (4 and 12), 96 h (5 and 13) and 120 h (6 and 14). Marker lanes (1 and 9) contained *M.* standards and control lanes contained lysates of uninfected *S. frugiperda* cells (lane 7), uninfected CV1 cells (lane 8), or CV1 cells infected with Lassa virus at an m.o.i. of 0.1 48 h previously (lane 15). One set of samples was stained with Coomassie blue (panel a). The proteins from a replica gel were transferred to nitrocellulose and probed with a MAb (1.52) specific for the Lassa virus N protein (b). Positions of size marker proteins (*M.* × 10^-3), Lassa virus N protein and AcNPV polyhedrin protein (P) are indicated.

20 to 30% of the total protein that can be stained with Coomassie blue. The kinetics of production of the 60000 *M.* protein appear to be similar to those of the polyhedrin protein produced in AcNPV-infected cells. These data are consistent with expression of the Lassa virus N gene under the control of the AcNPV polyhedrin promoter.

Subcellular localization of exogenous protein

At 3 days post-infection infected cells were harvested and disrupted by freezing and thawing. Cell lysates were fractionated by low-speed centrifugation and the pellet and supernatant fractions analysed by PAGE (Fig. 5). The majority of the Lassa virus N protein was found to be associated with the pellet fraction of the centrifuged lysate. Nearly 70% of the protein stained with Coomassie blue in this fraction was found to be of recombinant origin, as determined by densitometric scanning. This sedimentation behaviour of the recombinant protein was consistent with electron microscopic observations of thin sections through infected cells (Fig. 6), which show the accumulation in the cytoplasm of aggregates of material not found in uninfected cells or in cells infected with wild-type virus. This is presumed to be the recombinant protein, apparently segregated into inclusion bodies in the cytoplasm during or after synthesis.

Recombinant N protein as an ELISA antigen

Recombinant N protein was partially purified from infected insect cells by low-speed centrifugation, as described above. Its antigenic behaviour was initially examined by comparing its efficacy as a solid-phase antigen with that of purified Lassa virus that had been inactivated with β-propiolactone. This treatment of the virus results in the formation of a precipitate containing the N and the G2 proteins of the virus, which can be recovered by low-speed centrifugation (J. C. S. Clegg, unpublished observation). Titration of a human anti-Lassa virus convalescent serum on ELISA plates coated with these two antigen preparations resulted in closely
Fig. 6. Electron microscopy of recombinant virus-infected cells. S. frugiperda cells were infected at an m.o.i. of 10 (or mock-infected) and fixed, stained and sectioned for transmission electron microscopy 48 h later. (a) Uninfected cell; (b) wild-type virus-infected cell; (c) recombinant virus-infected cell. A putative recombinant protein-containing body is indicated by an arrow. The bar indicates a distance of 2 μm.

comparable profiles and suggests that the material produced in insect cells is antigenically similar to its authentic counterpart (Fig. 7a). Similar results were obtained with sera from four other Lassa fever patients and from guinea-pigs that had been experimentally infected with Lassa virus (not shown).

The performance of the insect cell-derived recombinant protein as an antigen in ELISA was also compared with that of segments of the protein expressed in Escherichia coli and subsequently purified by ion-exchange chromatography in 8 M-urea (Barber et al., 1987). The bacterial recombinant fragment used here contained amino acids 119 to 570 of the authentic Lassa virus N protein. The behaviour of the antigens was first compared using N protein-specific MAbs raised in mice infected with Lassa virus (G. Lloyd & N. Jones, unpublished; Clegg & Lloyd, 1984). In titrations of a MAb specific for the Lassa virus N protein, the bacterial and the insect cell-derived recombinant protein gave comparable results (Fig. 7b). However, with polyclonal sera the different antigens have divergent titration curves. As shown in Fig. 7(c) for serum from a guinea-pig convalescent from a Lassa virus infection, when the recombinant baculovirus-derived antigen was used, dilution resulted in a classical sigmoid curve with a clear...
Lassa virus recombinant N protein expression

1.00
0.75
0.50
0.25
0.00

0
0.25
0.50
0.75
1.00

50 GS GA JW

Negative sera Positive sera

Fig. 8. Differentiation of human sera positive and negative for Lassa virus antibodies. A panel of 50 human sera from Uganda, known to be negative for Lassa virus-specific antibodies by the conventional immunofluorescence test, was tested by ELISA with protein from recombinant baculovirus-infected S. frugiperda cells as antigen. Three human sera known to be positive for such antibodies were also tested. Absorbance values at a dilution of 1:100 are shown. The open bar shows the mean and standard deviation for the negative sera. The hatched bars show results for each of the known positive sera.

plateau of absorbance, followed by a fall to background levels. The bacterial recombinant antigen gave no initial plateau, but an immediate fall in absorbance. Similar observations have been made using other polyclonal sera of both guinea-pig and human origin. A slightly shorter bacterial recombinant N protein segment, containing amino acid residues 201 to 570 also gave similar results (not shown). The baculovirus-derived antigen thus appears to be considerably more sensitive than the bacterial recombinant for the detection of Lassa virus-specific antibodies in polyclonal sera.

To test the ability of the recombinant baculovirus-derived N protein to differentiate clearly between sera negative and positive for anti-Lassa virus antibodies, 50 human sera obtained from Uganda and known to be negative for anti-Lassa virus antibody (as determined by conventional immunofluorescence assay) were titrated by ELISA using the recombinant antigen (Fig. 8). All sera gave low responses (mean $A_{492} = 0.07 \pm 0.05$). With a cutoff value arbitrarily set at twice the standard deviation of the mean negative response, even a convalescent serum (JW) which had an immunofluorescence titre as low as 1:32 gave an absorbance reading of 0.62 and was thus clearly distinguishable from the negative sera.

Immunofluorescence assay using infected insect cells

Current methods of diagnosis of cases of Lassa fever rely on the detection of virus-specific antibodies, using virus-infected cells as targets in an immunofluorescence assay (Wulff & Lange, 1975). The performance of insect cells infected with the recombinant baculovirus in such an assay was therefore examined. Fig. 9 shows that such cells fluoresce brightly with MAb specific for the Lassa virus N protein and also with human sera known to be positive in the conventional immunofluorescence test using Lassa virus-infected cells. Uninfected S. frugiperda cells included as controls show only weak background fluorescence, easily distinguishable from that of recombinant-infected cells. These results suggest that the recombinant baculovirus-infected insect cells can function as a direct replacement for Lassa virus-infected cells in this type of assay.

Discussion

The transfer vector pAcYM1 contains the entire upstream region of the AcPNV polyhedrin gene, including the A of the translation-initiating ATG codon (Matsuura et al., 1987). Their data showed that this feature was important for high level expression of foreign genes (including the nucleocapsid protein and glycoprotein genes of another arenavirus, lymphocytic chorio-
meningitis virus) in insect cells infected with recombinant AcNPV. Insertion of foreign genes into the untranslated region upstream of the polyhedrin ATG start codon, or into vectors from which sequences from this region had been deleted, resulted in lower levels of expression. We have shown that our recombinant AcNPV, which was also derived using the pAcyT1 transfer plasmid, gives rise to high level expression of the exogenous gene, although we have not compared expression levels obtained using different vectors. The 90 bp untranslated region between the upstream vector-insert junction and the translation start codon, which is somewhat longer than is the case with the lymphocytic choriomeningitis virus N protein gene, does not appear to affect adversely the expression level obtained. The electron microscopic appearance of thin sections of cells infected with the Lassa virus gene-carrying recombinants is also very similar to that of lymphocytic choriomeningitis virus N protein-expressing cells (Matsuura et al., 1987).

The behaviour of the recombinant Lassa virus N protein on SDS-polyacrylamide gels and in Western blots suggests, at least to a first approximation, that it is very similar to the authentic product expressed in Lassa virus-infected cells. As a major objective of this work is to acquire the material necessary to set up hazard-free immunological assays to detect Lassa virus infection we paid special attention to the antigenicity of the recombinant material. In particular we compared the performance of the recombinant protein synthesized in baculovirus-infected insect cells with that of antigen prepared by β-propiolactone inactivation of purified Lassa virus and with recombinant fragments of the nucleocapsid protein expressed in E. coli (Barber et al., 1987). It was clear from the results that the insect cell-derived product had antibody binding characteristics similar to those of the β-propiolactone-inactivated virus. It appears that the presence of glycoprotein G2 in the latter preparation (detectable in immunoblots) does not materially alter the ability of the preparation to detect virus-specific antibodies in animal or human sera. The results of assays using the insect cell-derived antigen, in either ELISA or immunofluorescence format, are also entirely consistent with those obtained using the conventional immunofluorescence test using Lassa virus-infected cells (Wulff et al., 1975). We conclude that the conformation of the insect cell-produced antigen is sufficiently close to that of the authentic protein to allow efficient detection of antiviral antibodies.

The performance of fragments of the Lassa virus N protein, which have previously been produced in this laboratory by synthesis in bacteria (Barber et al., 1987), in immunoassays with polyclonal sera was significantly worse than that of the recombinant baculovirus-encoded protein. Although the bacterial products were able to detect virus-specific antibodies at low dilutions of animal or human convalescent sera, serial dilution resulted in a rapid reduction of signal and low apparent titres. In contrast, the bacterial recombinant antigens gave good results with MAbs specific for the Lassa virus N protein. These MAbs were raised in mice inoculated with infectious Lassa virus and all react well on Western blots (Clegg & Lloyd, 1984 and unpublished work). This suggests that they do not require a native conformation of the antigen for binding. The data imply that the bacterial recombinant antigens exist in a non-native conformation, due either to synthesis in a bacterial environment, absence of the amino-terminal region of the authentic protein, or solubilization and purification of 8 M-urea. The inadequacy of these bacterial recombinant N antigens in diagnostic antibody assays contrasts with the situation found using other recombinant viral proteins produced in bacteria, notably in diagnostic assays for antibodies specific for human immunodeficiency virus. Such assays have been found to be at least as efficient as those using conventionally produced antigens (Maskill et al., 1988; Ng et al., 1989).

The insect cell-produced recombinant antigen has several advantages over alternative materials, which may be equally effective in terms of ability to detect Lassa virus-specific antibodies. It is inherently safe to produce and use and appears to have a low frequency of false positive reactions. Growth of Lassa virus itself requires specialized containment facilities and the inactivation process required so that antigen can be used elsewhere must be stringently monitored. The use of Lassa virus antigens produced in recombinant vaccinia virus-infected cells (Clegg & Lloyd, 1987; Auperin et al., 1988; Morrison et al., 1989) is questionable because of the potentially confusing side reactions to be expected with vaccinia virus-specific antibodies and also because infectious recombinant vaccinia virus is present in the material.

The availability of effective, hazard-free Lassa virus recombinant antigen produced in insect cells [and the recent publication by the Advisory Committee for Dangerous Pathogens of a code of practice for the inactivation of infectious virus in serum specimens from haemorrhagic fever patients by treatment with β-propiolactone (Anonymous, 1988)] has important implications for the handling of suspect Lassa fever cases in the U.K. Such treatment has little effect on the observed titre of virus-specific antibodies (Freeman et al., 1982; G. Lloyd & J. C. S. Clegg, unpublished observations), but efficiently inactivates infectious virus (Lloyd et al., 1982). Instead of transporting possibly infectious specimens from the isolation hospital to a maximum security laboratory located elsewhere, as is the current practice, it
will be possible for a simple serological test to be performed locally. In circumstances where antibody detection constitutes a reliable guide to the existence of Lassa virus infection, i.e. the period from 7 days after onset of symptoms, this would allow a more rapid decision on the necessity for patient isolation and special treatment of specimens, thus expediting proper clinical care of the patient.

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