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SUMMARY

Lassa virus was purified from culture fluids of infected CV-1 monkey kidney cells and its structural proteins analysed by polyacrylamide gel electrophoresis. Stained gels showed a typical arenavirus profile, with a prominent protein of molecular weight 60,000, corresponding to the nucleocapsid protein N, and two faint broad bands with molecular weights of 45,000 and 38,000, the envelope glycoproteins G1 and G2. G1 and G2 were shown to be glycosylated by their ability to bind concanavalin A to nitrocellulose transfers of the separated proteins ('Western blots'). N and G2 bound antibody from guinea-pig or human convalescent sera but G1 was inactive, presumably as a result of denaturation. This technique also revealed other apparently virus-specific minor bands with molecular weights of 76,000 and 68,000. When Western blots of proteins of infected cells which had been lysed in SDS were probed with anti-Lassa virus serum or stained for glycoproteins, four virus-specific bands were apparent: the N, G1 and G2 proteins seen in purified virus, and a glycoprotein of molecular weight 72,000 which probably corresponds to the envelope protein precursor (GPC) seen in other arenavirus systems. Immunoprecipitates from infected CV-1 cells labelled with [35S]methionine contained three major virus-specific proteins: the nucleocapsid protein N and proteins of 36,000 and 24,000 molecular weight (designated fN1 and fN2). Similar immunoprecipitates from Vero cells contained fN1 and fN2 and only very low levels of N. The polypeptides fN1 and fN2 are most probably fragments of N, since Western blots probed with anti-Lassa virus serum showed that lysis of cells in non-ionic detergent rather than SDS results in the appearance of fN2 with concomitant reduction or disappearance of N. These fragments do not exist in the intact cell, but are found as a consequence of rather specific proteolysis upon disruption under non-denaturing conditions. The proteolytic activity responsible was refractory to inhibition by phenylmethylsulphonyl fluoride, aprotinin, pepstatin A or sodium bisulphite, and was more active in Vero than in CV-1 cells.

INTRODUCTION

The arenaviruses are primarily agents causing acute or persistent infections in rodents, although some of them can cause severe haemorrhagic disease in man. The prototype, lymphocytic choriomeningitis (LCM) virus, is distributed almost world-wide, but of the remainder the Tacaribe complex is confined to the New World (mainly South America), Lassa virus is found in West Africa and viruses closely related to it have been isolated in Mozambique and Zimbabwe (Wulff et al., 1977; Johnson et al., 1981).

The enveloped viruses contain a single-stranded RNA genome of total mol. wt. about 4 x 10^6 (Ramsingh et al., 1980) in two segments of negative polarity. The major structural proteins of the viruses comprise a nucleocapsid protein (N) and, in most cases, two glycoproteins (G1 and G2) which are components of the virus envelope, although in some viruses only a single glycoprotein has been found (for reviews, see Rawls & Leung, 1979; Pedersen, 1979; Howard & Simpson, 1980). Other minor proteins have been found in purified arenavirus preparations, but their status as true components of the virion is at present unclear. In addition, an RNA polymerase
activity, which would be expected in a negative-strand RNA virus, has been found in purified preparations of Pichinde virus (Carter et al., 1974; Leung et al., 1979), as well as a protein kinase activity in purified LCM virus (Howard & Buchmeier, 1982). These enzyme activities have not yet been identified with polypeptides observable by polyacrylamide gel electrophoresis.

A further virus-specific protein (GPC) has been found in immunoprecipitates from cells infected with LCM, Tacaribe or Pichinde viruses (Buchmeier et al., 1978; Buchmeier & Oldstone, 1979; Saleh et al., 1979; Harnish et al., 1981). This is a glycoprotein of mol. wt. 70000 to 79000 which appears to be processed by proteolytic cleavage and carbohydrate modification to give the mature envelope glycoproteins.

Here we show that purified Lassa virus has a major protein complement very similar to that of other arenaviruses, confirming the results of Kiley et al. (1981). These proteins can also be demonstrated in extracts of infected cells, together with a large glycoprotein which is analogous to the GPC of other arenavirus systems. The N protein of Lassa virus is shown to be highly susceptible to limited proteolytic cleavage in cell extracts made under non-denaturing conditions, giving rise to two specific fragments which can constitute the majority of the N-specific material in lysates made with non-ionic detergents.

METHODS

Virus and cells. The virus used in these studies originated from the serum of a patient who contracted Lassa fever in Zaria, Northern Nigeria (Grundy et al., 1980). The virus (designated GA 391) was isolated in tissue culture (Vero cells) in this laboratory in 1974 and stored at -70 °C. The working stock had been passaged in Vero cells three times and had a titre of 10^7.5 TCID_50/ml. Infectious virus assays were carried out using Vero cell monolayers prepared in microtitre plates (Sterilin) as described by Lloyd et al. (1982).

Virus for purification was grown in CV-1 monkey kidney cells which were propagated in Leibowitz L-15 medium supplemented with 5% foetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml). All operations with infectious virus were conducted in Category A (equivalent to P4) containment facilities.

Virus growth and purification. Confluent monolayers of CV-1 cells in 1750 cm² plastic roller bottles (Falcon; Becton-Dickinson) were infected at a multiplicity of 0.1 in 10 ml of Plaisner’s modified Eagle’s medium (Plaisner et al., 1974) supplemented with 2% FCS and antibiotics as above (maintenance medium). After 60 min adsorption, 150 ml maintenance medium was added and incubation continued at 37 °C. Medium was harvested and replaced on day 2 post-infection and finally harvested on day 3. Virus was purified by a method similar to that of Kiley et al. (1981). The pooled medium was centrifuged at 12000 g for 10 min at 4 °C to remove cells and debris and the supernatant brought to 5% NaCl and 7% polyethylene glycol 6000 (BDH) by addition of solids with gentle stirring at 4 °C. It was allowed to stand at 4 °C for 2-3 h and then centrifuged at 12000 g for 40 min at 4 °C. The resulting pellets were resuspended in 1/100 the original volume of 200 mM-glycine, 100 mM-NaCl, 1 mM-EDTA, 50 mM-Tris-HCI pH 7.5 (GTNE) and clarified by centrifugation at 12000 g for 10 min at 4 °C. The supernatant was layered on 30 ml glycerol-tartrate gradients (Obijeski et al., 1974) in heat-sealable tubes for the Beckman 60 Ti ultracentrifuge. The visible virus band about halfway down the tube was recovered, diluted in maintenance medium. Two days after infection, cells were labelled (if appropriate) with [35S]methionine. The monolayers were washed twice in cold phosphate-buffered saline (PBS). For direct lysis in SDS the cells were dissolved in SDS-electrophoresis sample buffer (1% SDS, 1% 2-mercaptoethanol, 15% glycerol, 0.01% bromophenol blue, 50 mM-Tris–HCl pH 6-8; ESB) and heated at 60 °C for 2 h to denature the proteins and inactivate virus. The viscosity of the extracts was reduced before electrophoresis by repeated rapid pipetting. For lysis under non-denaturing conditions the cell monolayer was exposed to PBS containing 1% Triton X-100 and 0-1% SDS (PTS) for 5 min at room temperature. Debris was removed by centrifugation at 12000 g for 3 min and the extracts stored at -70 °C until required.

Antisera. Hyperimmune antisera to GA 391 were prepared in Dunkin and Hartley strain guinea-pigs (350 g). Stock virus was diluted in PBS containing 0.75% bovine serum albumin (BSA) and 0.1 ml containing 10^5 TCID_50/ml was injected intraperitoneally (i.p.). On day 28 post-infection some animals received a further 0.1 ml (10^5 TCID_50/ml) sample i.p. Two months after the first dose the guinea-pigs were anaesthetized with ether and
bled by cardiac puncture. The blood was placed at 4°C overnight and serum recovered, clarified by centrifugation (1000 g) and stored at −20°C. The antibody titres of both one-dose and two-dose sera were 1/2048, as estimated by indirect immunofluorescence on acetone-fixed Lassa-infected Vero cells. Human serum was obtained from a Lassa fever patient 68 days after the onset of symptoms (Emond et al., 1982). It had an immunofluorescence titre of 1/16000. Sterilization of antisera was carried out using 0-2% f-propiolactone (Lloyd et al., 1982).

Radioimmune precipitation. Cell lysates labelled with [35S]methionine were centrifuged at 12000 g for 3 min immediately before use. Five-hundred ml aliquots were mixed with 5 ml normal guinea-pig serum or anti-Lassa immune serum and left at room temperature for 2 h. Protein A-Sepharose CL4B beads (Pharmacia; 40 ml of a 50% (v/v) suspension in PBS) were then added and the mixture intermittently agitated for a further 30 min. The beads were pelleted by centrifugation at 12000 g for 2 min and washed three times by suspension in PBS containing 1% Triton X-100. Finally the beads were resuspended in 50 ml ESB and heated at 60°C for 2 h to release and denature bound proteins and to inactivate any residual virus.

Polyacrylamide gel electrophoresis. Samples were made 1% SDS, 1% 2-mercaptoethanol, 100 mM-Tris-HCl pH 6-8, 15% glycerol, 0-01% bromophenol blue and heated at 100°C for 2 min immediately before analysis on 8 to 15% polyacrylamide concave exponential gels using the buffer system of Laemmli (1970), except that the lower electrode buffer was one-third the usual concentration in all components. Gels were run at 75 mA for 1-5 mm-thick gels or 40 mA for 0-75 mm-thick gels. They were stained for protein when required in 0-05% Kenacid blue R (BDH; a substitute for Coomassie Brilliant Blue) in 25% isopropanol, 10% acetic acid and destained in 10% isopropanol, 10% acetic acid. Fluorography was carried out as described by Laskey (1980). Molecular weights were estimated by comparison of electrophoretic mobilities with those of mixtures of standard marker proteins, either unlabelled (Sigma) or 14C-methylated (Amersham International). Proteins separated on gels were transferred to nitrocellulose sheet (BA85; Schleicher & Schüll) essentially as described by Towbin et al. (1979), except that the transfer buffer was 96 mm-glycine, 12.5 mm-Tris, 20% isopropanol and current was passed at 200 to 300 mA for 14 to 16 h. The transfer was monitored by staining the nitrocellulose sheet with amido black (Towbin et al., 1979) and destaining in 10% acetic acid, 10% isopropanol. Protein-binding sites on the transfers were blocked before further analysis by soaking in PBS, 2-5% BSA for 1 h at room temperature with constant agitation.

Glycoprotein detection. Nitrocellulose transfers were incubated at room temperature for 1 h in PBS, 0·5% Triton X-100 containing 50 μM-Mg++, Mn++ and Ca++ ions (GS buffer) and 10 μg concanavalin A (Pharmacia) per ml with constant agitation. The transfers were washed five times in GS buffer, incubated in GS buffer containing horseradish peroxidase (Sigma, 50 μg/ml) for 1 h as before and subjected to another washing cycle. The positions of glycoprotein bands (located by successive binding of concanavalin A and peroxidase) were revealed by incubation with aminoethylcarbazole (200 μg/ml), H2O2 (0·03%) in 50 mm-sodium acetate buffer pH 5·0. This technique is the subject of a separate communication (Clegg, 1982).

Immunological detection of protein on nitrocellulose transfers. Transfers were incubated at room temperature with constant agitation in PBS containing 2·5% BSA, 5% FCS, 1% Triton X-100 and 0·1% SDS (AB buffer) in which antisera had been suitably diluted. After 2 to 4 h, the transfers were washed three times in PBS, 0·05% Triton X-100, incubated as above with AB buffer containing a 1/100 dilution of horseradish peroxidase-conjugated antiglobulin of the appropriate species (Miles Laboratories) for 1 h and subjected to another washing cycle. Antibody-binding bands were revealed by incubation with aminoethylcarbazole as for the glycoprotein detection method.

RESULTS

Protein components of purified Lassa virus

Infection of CV-1 cells with Lassa virus at multiplicities of 0·1 to 0·01 resulted in growth to a maximum titre of 10^8-5 TCID50/ml within 2 or 3 days without noticeable cytopathic effect. Virus was purified using a polyethylene glycol concentration step followed by isopycnic banding on glycerol–tartrate gradients (Obijeski et al., 1974). The virus formed a sharp opalescent band about halfway down the gradient, well separated from the small amount of cellular debris that was present at the top. The overall recovery of infectivity after pelleting and resuspension of the virus band was 5 to 10% of that in the starting tissue culture fluid.

Samples of purified virus were analysed on 8 to 15% polyacrylamide gradient gels in the buffer system of Laemmli (1970). In gels stained for protein the pattern was dominated by a polypeptide of mol. wt. 60000 (Fig. 1, lane a), and two other components were visible. These have apparent mol. wt. of 45000 and 38000 and were identified as glycoproteins after transfer to nitrocellulose by their ability to bind concanavalin A (Fig. 1, lane b). Low levels of higher molecular weight glycoproteins were also visible. All binding of the lectins to the transfer was abolished in the presence of 100 mM-α-methyl-D-mannoside (not shown). In view of the striking
analogy between these findings and the protein composition of previously analysed arenaviruses (Pedersen, 1979), the prominent 60000 mol. wt. protein was denoted N, the nucleocapsid protein, and the two major glycoproteins G1 and G2, the virus envelope proteins. A minor component of mol. wt. about 76000 was also visible in Kenacid blue-stained gels of purified Lassa virus (Fig. 1, lane a).

The ability of the separated proteins from purified virus to bind antibodies present in sera from experimentally infected guinea-pigs given 1 or 2 doses of Lassa virus (Fig. 1, lanes d to f) or from a human Lassa fever patient (Fig. 1, lane g) was also investigated. The profile of antibody binding activity was very similar for all three sera tested. N and G2 were the major antigenically active species, but other minor antibody binding bands were also visible, notably those with mol. wt. of 76000 and 68000 migrating just behind N, 46000 between N and G2 and 25000 and 20000, which have been seen only with the lower dilution of guinea-pig antibody and with the human serum. There is some indication that the level of antibody binding to G2 is preferentially increased in serum from a guinea-pig which has received a second Lassa virus injection (compare lanes d and e). No antibody binding was observed using normal guinea-pig serum (Fig. 1, lane c) or human serum (not shown). These findings are additional evidence of the virus-
Lassa virus proteins

Fig. 2. SDS–gel electrophoresis of proteins of Lassa virus-infected cells. Two days after infection at a multiplicity of 0.1, cell lysates were made in electrophoresis sample buffer and heated at 60 °C for 2 h, and at 100 °C for 2 min immediately before electrophoresis on an 8 to 15% polyacrylamide gradient gel. Samples containing the equivalent of about 5 × 10⁴ cells (lanes b to e) or 2.5 × 10⁴ cells (lanes f to j) were analysed and transferred to nitrocellulose. Lanes (a) and (j) contained 0.5 μg purified virus as marker and were similarly analysed and transferred. Lanes (a to e) were incubated with guinea-pig anti-Lassa virus serum (two injections) at a dilution of 1/100 for 4 h; lanes (f to j) with concanavalin A. Subsequent steps were described in Methods. (a, j) Purified virus marker; (b, f) uninfected Vero cells; (c, g) Lassa virus-infected Vero cells; (d, h) uninfected CV-1 cells; (e, i) Lassa virus-infected CV-1 cells.

specific nature of the N and G2 proteins and suggest that other protein components, whose function and possible relationship to the major structural proteins are unknown, are associated with the purified virus preparation. It is striking that the major glycoprotein G1 is devoid of antibody binding activity under these circumstances, presumably as a result of irreversible denaturation during preparation of the transfers.

Virus-specific proteins in infected cells

Lysates of cells infected with Lassa virus were surveyed for virus-specific proteins by binding antibodies or concanavalin A to nitrocellulose transfers of electrophoretically separated proteins. Vero or CV-1 cell monolayers were infected with Lassa virus at a multiplicity of 0.1, and the cells lysed in electrophoresis sample buffer 2 days after infection. For the antibody binding assay, samples equivalent to about 5 × 10⁴ cells were subjected to SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose and incubated with Lassa virus-specific guinea-pig antiserum. Control uninfected Vero and CV-1 cells (Fig. 2, lanes b and d) contain a number of bands capable of non-specifically binding guinea-pig antibodies and those bands are also present in the infected cell lysates (Fig. 2, lanes c and e). In addition, three bands found only in infected cells can be seen. Two of these can be identified by their electrophoretic mobility compared with a purified virus reference (lane a) as virus N and G2 proteins. The third apparently virus-specific protein is not found in purified virus and migrates as a broad band with a mol. wt. of about 72000 and is considerably more prominent in Vero cells than in CV-1 cells. Where similar transfers were incubated with concanavalin A and peroxidase, a complex
Fig. 3. Radioimmune precipitation from Lassa virus-infected cell extracts. Two days after infection at a multiplicity of 0.1, cells were labelled with $[^{35}S]$methionine (20 µCi/ml) for 60 min and lysed in PBS containing 1% Triton X-100 and 0.1% SDS. Cellular debris was removed by centrifugation and aliquots incubated with appropriate serum. Immune complexes were recovered on Protein A-Sepharose beads, solubilized in electrophoresis sample buffer, analysed on 8 to 15% polyacrylamide gradient gels and radioactive proteins located by fluorography. (a) Mock-infected Vero cell extract precipitated with guinea-pig anti-Lassa virus serum (two doses); (b to e) Lassa virus-infected Vero cell extracts containing (b) no protease inhibitor, (c) aprotinin (500 kIU/ml), (d) pepstatin A (1 µM), (e) sodium bisulphite (10 mM) precipitated with anti-Lassa virus serum; (f) mock-infected CV-1 cell extracts precipitated with anti-Lassa virus serum; (g) Lassa virus-infected CV-1 cell extract precipitated with anti-Lassa virus serum.

Fig. 4. Effects of lysis buffer on antibody-binding proteins in infected cells. Cells infected as for Fig. 1 were lysed directly in ESB or in PTS. After removal of debris by centrifugation the latter extracts were incubated at room temperature for 3 h, then made 1% SDS, 1% 2-mercaptoethanol and heated at 60 °C for 2 h. All extracts were heated at 100 °C for 2 min before electrophoresis of samples equivalent to 5 × 10⁴ cells on 8 to 15% polyacrylamide gradient gels and transfer to nitrocellulose. The transfer was incubated with guinea-pig anti-Lassa virus serum (two doses) at a dilution of 1/100 for 4 h, and subsequently treated as described in Methods. (a) Mock-infected Vero cells lysed in ESB; (b) infected Vero cells lysed in ESB; (c) infected Vero cells lysed in PTS; (d) mock-infected CV-1 cells lysed in ESB; (e) infected CV-1 cells lysed in ESB; (f) infected CV-1 cells lysed in PTS.

pattern of host cell glycoproteins was revealed (Fig. 2, lanes f and h) in uninfected cells. In infected cells virus-specific glycoproteins could be seen superimposed on the host cell pattern (Fig. 2, lanes g and i). These corresponded in mobility to the non-structural 72000 mol. wt. protein detected by antibody binding, and to the envelope glycoproteins G1 and G2 present in purified virus (Fig. 2, lane f). The 72000 mol. wt. protein is thus identified as a glycoprotein and,
Lassa virus proteins

by analogy with other arenavirus systems where a cell-associated precursor of the virion structural proteins has been found (Buchmeier & Oldstone, 1979; Saleh et al., 1979; Harnish et al., 1981), it has been denoted GPC. GPC appears more prominent in Vero cells than in CV-1 cells as revealed by concanavalin A binding, but this may be at least partly due to differences in the background pattern of host cell glycoproteins. Conversely, the mature glycoproteins G1 and G2 are much more prominent in CV-1 cells and, indeed, are barely visible in Vero cells.

Radioimmune precipitation from infected cell lysates

As an alternative approach to the investigation of the synthesis of virus-specific proteins, immunoprecipitations of labelled cell lysates made under non-denaturing conditions were performed, with results which were somewhat unexpected when compared with those from Western blot analysis. Vero or CV-1 cells were infected at a multiplicity of 0-1 with Lassa virus, labelled 2 days after infection for 60 min with $^{35}$S methionine, and lysed in PBS containing 1% Triton X-100 and 0.1% SDS. Virus-specific labelled proteins were precipitated with guinea-pig anti-Lassa virus serum and Protein A-Sepharose beads and analysed by polyacrylamide gel electrophoresis. Little labelled protein was precipitated from uninfected cells (Fig. 3, lanes a and f), but infected cells (lane b) contained a number of virus-specific products precipitated by guinea-pig anti-Lassa virus serum, most prominent among which were two bands of apparent mol. wt. 36000 and 24000 which were denoted fN1 and fN2 respectively. There were also weaker bands at the expected positions of N and GPC. Although the 36000 mol. wt. protein migrated near the position expected for G2, its high level relative to N and the sharpness of the band made this assignment unlikely. The unexpectedly low level of N and the appearance of two new polypeptides the sum of whose molecular weights approximated to the molecular weight of N suggested that N was undergoing a rather specific proteolytic event to yield the two smaller proteins. However, lysates made in the presence of the protease inhibitors aprotinin (500 kIU/ml), pepstatin A (1 μM), sodium bisulphite (10 mM) (Hubscher et al., 1981) (Fig. 3, lanes c to e) or phenylmethylsulphonyl fluoride (1 mM, not shown) did not contain significantly higher levels of N or reduced levels of the smaller polypeptides, indicating that any proteolytic event involved was refractory to inhibition by these materials. Lysates made from similarly labelled infected CV-1 cells in the absence of protease inhibitors did contain higher levels of N but also significant quantities of the 36000 and 24000 mol. wt. proteins (Fig. 3, lane g). This suggested that the degradation of N was at least partly host cell-dependent. The weak bands migrating between N and fN1 in immunoprecipitates from CV-1 cells may be related to the larger glycoprotein G1.

The contrast between infected Vero cell lysates made in electrophoresis sample buffer, which contained N protein easily detectable by antibody binding to Western blots, and lysates for immune precipitation, which contained barely detectable levels, suggested that degradation of N occurred after cell lysis in the absence of effective protein denaturation. To test this, cells were lysed 2 days after infection either in ESB and heated immediately at 60 °C for 2 h to achieve rapid denaturation, or in PBS containing 1% Triton X-100, 0.1% SDS and incubated at room temperature for 3 h to mimic the conditions under which immunoprecipitation takes place. The proteins were then denatured by addition of concentrated solutions of SDS and 2-mercaptoethanol and heating at 60 °C for 2 h. Both the resulting preparations were heated at 100 °C for 2 min immediately before analysis by polyacrylamide gel electrophoresis and transfer to nitrocellulose. As expected, infected Vero and CV-1 cell lysates made under immediately denaturing conditions contained easily detectable N protein (Fig. 4, lanes b and e) as well as GPC and G2. However, cell extracts made under non-denaturing conditions contained no detectable N protein (Vero cells, Fig. 4, lane c) or considerably less N protein (CV-1 cells, Fig. 4, lane f), but did contain an additional protein of mol. wt. 24000 which co-migrated with fN2 found in radioimmune precipitates (not shown). These data are consistent with the possibility that fN2 originates from the cleavage of N which occurs in infected cell lysates made under non-denaturing conditions. Antibody binding to fN1 on Western blots could not be readily demonstrated, possibly because of the obscuring effect of G2-bound antibody.
The major protein components of the Lassa virus particle are the nucleocapsid protein and two envelope glycoproteins as found in most other members of the Arenaviridae (Pedersen, 1979; Howard & Simpson, 1980) although Tacaribe and Tamiami viruses are reported to have only a single envelope glycoprotein (Gard et al., 1977). There are also some similarities between the minor virion components of Lassa virus reported here and those found in other arenaviruses. Thus, the 76000 and 68000 mol. wt. polypeptides may correspond to components in a similar size range found in Pichinde, Junin, Tacaribe, Tamiami and Machupo viruses (Howard & Simpson, 1980), which have frequently been denoted 'P', to stand for the RNA polymerase possessed by arenaviruses (Carter et al., 1974), although there is little evidence to support this assignment. The antibody-binding properties of the Lassa polypeptides indicate that, although not abundant in the purified virion, they are virus-specific, as would be expected of a virus-coded enzyme. They do not bind concanavalin A, so they are unlikely to be related to the glycoprotein precursor GPC found in infected cells. Low levels of small proteins have been found in various arenavirus preparations (Pedersen, 1979; Howard & Simpson, 1980) and may be related to the antibody-binding 20000 and 25000 mol. wt. components found here in Lassa virus.

There is good agreement between the present data and those of Kiley et al. (1981), who have presented the only previous analysis of Lassa virus proteins. They also found a nucleocapsid protein and two envelope glycoproteins, although the molecular weight values are somewhat different, probably due to the different gel electrophoretic systems employed, and possibly the different host cells in the case of the glycoproteins.

In Lassa virus-infected Vero cells the glycoprotein precursor GPC is more prominent than it is in CV-1 cells as judged by antibody binding, and possibly also by concanavalin A binding to nitrocellulose transfers. Antibody binding shows the levels of G2 are about the same in both cell lines, although concanavalin A binding to G1 and G2 is much greater in CV-1 cells than in Vero cells. However, quantitative interpretation of the concanavalin A data is complicated by possible differences in glycosylation between proteins synthesized in different cell lines and modifications to the carbohydrate structures during maturation, which might lead to variations in the affinity of the glycoproteins for the lectin. Differences in sugar composition have been observed between GPC and the mature glycoproteins in LCM or Tacaribe virus-infected BHK cells (Buchmeier & Oldstone, 1979; Saleh et al., 1979) and may well underlie the changes in efficiency of concanavalin A binding during the processing of GPC to G1 and G2 which are so apparent in Vero cells. It is thus likely that the antibody binding data are a more accurate reflection of the steady-state levels of the glycoproteins and we can conclude that the glycoprotein precursor GPC is present in infected Vero cells at levels comparable to the mature envelope proteins, but at much lower levels in infected CV-1 cells, presumably as a result of differing processing efficiencies in the two cell lines. The Western blot antibody binding assay indicates clearly that the nucleocapsid protein exists inside infected cells as the 60000 mol. wt. polypeptide found in mature virus particles. When the cells are lysed under conditions which do not result in rapid denaturation of proteins, as for immunoprecipitation, N is to a large extent proteolytically cleaved at a rather specific point to give the polypeptides here denoted fN1 and fN2 (f for fragment). This interpretation is supported by data (to be published elsewhere) indicating that fN1 can bind monoclonal antibodies directed against the N protein. The cleavage of N is apparently host cell-dependent since it was somewhat less marked in CV-1 cells than in Vero cells. This may account for the greater stability of the N protein of other arenaviruses, which have so far been analysed by radioimmunoprecipitation only in BHK cells (Buchmeier et al., 1978; Saleh et al., 1979; Harnish et al., 1981), unless Lassa virus N protein is intrinsically more susceptible to degradation. It is likely that this proteolysis of N is the source of the soluble complement-fixing antigens which are characteristic of cells infected with arenaviruses (see Pedersen, 1979; Howard & Simpson, 1980). These antigens consist of polypeptides with mol. wt. in the range 15000 to 25000, carry serological determinants also found on virus structural proteins, and are often stable to heat and digestion with proteolytic enzymes (Bro-Jørgensen, 1971; Buchmeier et al., 1977; de Mitri & de Martinez Segovia, 1980). The soluble antigen of Junin virus-infected cells has previously been
suggested to be a fragment of the nucleocapsid protein (de Mitri & de Martinez Segovia, 1980).

One surprising aspect of the present results is the failure of G1 to bind antibody after SDS-gel electrophoresis and transfer to nitrocellulose. While it is likely that, of the total antibody repertoire, only the subset directed against denaturation-resistant (probably primary structure) determinants is active in this assay, it would be expected that polyvalent sera generally contain antibodies of this type. However, it is clear that the several guinea-pig and human sera from Lassa virus infections tested did not contain such G1-specific antibodies. This may be the result of absence of primary structure determinants on G1 as it is presented to the immune system of the infected animal, or the result of complete non-immunogenicity of the protein, although this latter explanation cannot be considered likely unless corroborated by information from some other system, e.g. radioimmune precipitation.

The results described here provide biochemical evidence supporting the inclusion of Lassa virus in the Arenaviridae, which previously rested on electron microscopic and serological grounds. They also demonstrate that at the molecular level Lassa virus appears to be a very typical member of the family, and thus allow analogies with data on the structure and multiplicity of its less hazardous relatives to be drawn with more confidence than was previously the case.

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