Structural Polypeptides of Machupo Virus

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SUMMARY

The structural proteins of an arenavirus pathogen, Machupo virus, were compared to the structural proteins of two previously characterized non-pathogenic arenaviruses, Pichinde and Tacaribe, in SDS-polyacrylamide gels. Similarities in mol. wt. of the major structural proteins from both pathogenic and non-pathogenic viruses were apparent; however, some differences in the number of glycosylation properties of minor proteins were observed.

Machupo virions contain two major protein species. The most prominent is a non-glycosylated protein with a mol. wt. of 68,000, while the other was a glycosylated protein with a mol. wt. of 41,000. Minor amounts of other proteins (mol. wt. 84,000, 74,000, 50,000 and 15,000) and a glycolipid were also observed.

The arenaviruses are grouped taxonomically on the basis of their morphological and serological characteristics (Murphy et al. 1970; Rowe et al. 1970a). Members of this group show varying degrees of cross-reactivity in both complement fixation and indirect immunofluorescent assays (Buckley & Casals, 1970; Rowe et al. 1970a; Peters et al. 1973); however, with one reported exception (Weissenbacher et al. 1976), neutralizing antibodies are highly specific and do not have significant activity against other members of the group (Webb et al. 1968; Casals et al. 1975). Such serological characteristics suggest the presence of at least one common, as well as one distinct, antigenic component among viruses in this group. Polyacrylamide gel electrophoresis of the structural proteins of purified Pichinde (Ramos et al. 1972; Vezza et al. 1977), lymphocytic choriomeningitis, LCM (Pedersen, 1973; Buchmeier et al. 1977), Tacaribe and Tamiami (Gard et al. 1977), and Junin (de Martinez Segovia & De Mitri, 1977) viruses has revealed some heterogeneity with respect to both the molecular weight and number of protein subunits present in these viruses. However, the relationship between detectable protein differences and the observed antigenic variations has not yet been determined. Our purpose in this study is to compare the structural polypeptides of an arenavirus pathogen, Machupo virus, with those of two non-pathogens, one of which is antigenically more similar (Tacaribe virus) than the other (Pichinde virus; Casals et al. 1975), and to look for polypeptide species which may be characteristic of arenavirus pathogens.

Pichinde [strain 3739, American Type Culture Collection (ATCC)], Tacaribe (strain 11573, ATCC) and Machupo (Malale strain) viruses were propagated in baby hamster kidney cells (BHK-21). Virus was inoculated on to cell monolayers in half-gallon glass roller bottles at a multiplicity of 1 plaque forming unit (p.f.u.) per cell. Virus was allowed to adsorb for 1 h, at 37 °C and 50 ml of medium E-199 (Grand Island Biological Company) containing 2% foetal calf serum and antibiotics (100 units penicillin plus 100 μg streptomycin per ml) were then added to each bottle. Pichinde and Machupo virus supernatants were harvested at 72 h post-inoculation, while Tacaribe virus supernatants were harvested at 96 h. Pichinde supernatants were clarified by low speed centrifugation (3000 g for 15 min, in a Sorvall GSA rotor) and then precipitated with 6%, w/v, polyethylene glycol (PEG)
6000 and 0.4 M-NaCl as previously described (Ramos et al. 1972). After holding overnight at 10 °C, the PEG precipitates were pelleted by centrifugation at 8000 g for 45 min in a Sorvall GSA rotor. Supernatants were rapidly poured off and the pellets suspended in medium E-199 to 1/25 of their original volume. These suspensions were then pooled and sonicated in an ice bath at 20 kHz for 2 min before gradient centrifugation. Machupo and Tacaribe virus supernatants were clarified by low speed centrifugation as described above, filtered through two Millipore pre-filter pads and concentrated by molecular filtration in a high volume Pellicon Cassette System (Millipore Corporation). The procedures for the concentration of arenaviruses by this method have been described elsewhere (Gangemi et al. 1977).

Virus concentrates obtained by the PEG and molecular filtration procedures contained approx. 9.5 log_{10} p.f.u. and 10.5 log_{10} of physical particles/ml as determined by plaque assays on Vero cells and quantitative electron microscopic techniques (Sharp, 1965; Monroe & Brandt, 1970). These concentrates were purified by a modification of the techniques described by Gschwender et al. (1975a) and Ramos et al. (1972). More specifically, virus was layered on a discontinuous gradient consisting of 20% and 45%, v/v, Renografin (an Amidotrizoate gradient solution possessing low osmotic potential; Gschwender et al. 1975b) and centrifuged for 2 h at 25000 rev/min in a Beckman SW 27 rotor. Following centrifugation, the diffuse band at the discontinuous interphase was harvested, diluted 1:3 with TNE buffer (0.05 M-tris HCl, pH 7.2, 0.1 M-NaCl, and 0.001 M-EDTA), layered on to 15 to 45%, v/v, continuous Renografin gradients, and centrifuged for 4.5 h in a Beckman SW 27 rotor at 25000 rev/min. Opalescent diffuse bands containing structurally intact virus (determined by negative-staining electron microscopy) which plaqued at an efficiency of 1 p.f.u./50 virus particles, were located 1/3 of the way down each gradient (ρ 1.14 to 1.15 g/ml). Two other diffuse bands, one at the top of the gradient, which contained ruptured virus and unidentifiable debris, and one two-thirds of the way down the gradient (ρ 1.18 to 1.19 g/ml) in which some recognizable virus aggregated with coarse granular-like debris were also observed. Virus banding at a density of 1.14 to 1.15 g/ml was harvested, diluted in TNE and pelleted as described above. These pellets were gently suspended in 2 ml of TNE buffer and centrifuged once more through another 15 to 45%, v/v, continuous Renografin gradient to remove the remaining granular debris still visible by electron microscopy. While the p.f.u.: particle ratio decreased to approx. 1/100 during this second gradient centrifugation, most of the particles retained their structural integrity and were free of contaminating debris. This twice banded virus was pelleted and then suspended in a small volume of TNE so that the final stock suspension contained approx. 12 log_{10} of physical particles and 1 mg/ml of protein. All Machupo virus concentrates were kept in P-4 containment prior to inactivation with 1% sodium dodecyl sulphate (SDS) and 1% 2-mercaptoethanol (2-ME) for gel electrophoresis.

Fig. 1. (a) Virion and ribosomal proteins following electrophoresis in 15% polyacrylamide gels containing SDS (Ramos et al. 1972; Low & Wool, 1967). Purified virions and ribosomes were disrupted with 1% SDS and 2-ME, boiled for 5 min and then electrophoresed at 3 mA/gel. Gels were stained overnight with 0.1% w/v Coomassie brilliant blue in 7.5% v/v acetic acid and then diffusion destained in acetic acid and methanol. The addition of 1% urea and phenylmethane sulphonyl fluoride (a protease inhibitor) during disruption with SDS and 2-ME did not change these electrophoretic profiles. Mol. wt. markers: 92000 (α-phosphorylase), 68000 (bovine albumin), 44000 (ovalbumin), 29000 (carbonic anhydrase), 12500 (cytochrome c). (b) Densitometric tracings of virus structural components following electrophoresis as described in (a) and staining with (1) the periodic acid Schiff reagent (...), (2) Nile blue A or Sudan black (cross-hatched area), or (3) Coomassie brilliant blue (——). Staining procedures were the same as those described by Crowle (1973). The amplitude of some peaks has been reduced for illustrative purposes.
## Short communications

### (a) BHK ribosomes

<table>
<thead>
<tr>
<th>Protein</th>
<th>BHK Ribosomes</th>
<th>Pichinde</th>
<th>Tacaribe</th>
<th>Machupo</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁</td>
<td>m77⁻, N</td>
<td>m77⁻, N</td>
<td>m84⁻, N</td>
<td>m84⁻, N</td>
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<tr>
<td>R₆</td>
<td>m77⁻, G1</td>
<td>m50⁻, G</td>
<td>m74⁻, G</td>
<td>m74⁻, G</td>
</tr>
<tr>
<td>R₁₉</td>
<td>m15⁻</td>
<td>m15⁻</td>
<td>m15⁻</td>
<td>m15⁻</td>
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<tr>
<td>R₂₀</td>
<td>m15⁻</td>
<td>m15⁻</td>
<td>m15⁻</td>
<td>m15⁻</td>
</tr>
</tbody>
</table>

### (b) Molecular weight markers

- 92000
- 68000
- 44000
- 29000
- 12500

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**Fig. 1(a) and (b). For legend see opposite.**
Electrophoresis of purified Pichinde, Tacaribe and Machupo virions in polyacrylamide gels containing sodium dodecyl sulphate resulted in the separation of distinct polypeptide species (Fig. 1a). As described previously (Ramos et al. 1972; Vezza et al. 1977; Gard et al. 1977), Pichinde virions were resolved into three major protein species (N, G1, and G2) and Tacaribe virions into two (N and G). Two prominent proteins were observed in Machupo virions (N and G2) along with minor amounts of other proteins (G1, m84, m74, m15), some of which were also observed in Pichinde and Tacaribe virions. Some variations in the number and amounts of minor structural proteins were observed following prolonged passage (more than ten passages in BHK cells) of each of these three arenaviruses. The protein profiles illustrated in Fig. 1 are from virions which were passaged three times in BHK cells at a multiplicity of 1 p.f.u./cell.

Since a characteristic feature of arenaviruses is their ability to incorporate host ribosomes during maturation, we also looked for the presence of ribosomal proteins in each of the three viruses examined. Ribosomes were extracted from uninfected BHK cells according to the procedure of Farber & Rawls (1975) and electrophoresed in a separate gel (Fig. 1a). The ribosomal protein profiles were very similar to those observed by Low & Wool (1967) for different species of mammalian ribosomes; however, the number of proteins resolved represent only a fraction of the total which can be resolved by more sophisticated electrophoresis techniques. As best illustrated in the Pichinde and Machupo virus gels (Fig. 1a), only a few of the more prominent ribosomal proteins (R1 to R6) were detected and appeared as faint but distinct bands. These ribosomal proteins rapidly disappeared as the number of passages of the viruses increased. Likewise, electron microscopic examination of virus populations passaged eight times or more in BHK cells revealed particles which no longer contained ribosomes. In support of these observations, Vezza et al. (1977) were unable to show the presence of 18S ribosomal RNA when examining the RNA content of cell culture: passaged Pichinde virus, suggesting to them that host ribosomes were not incorporated into their virus particles. Using similar RNA extraction and polyacrylamide gel techniques, Chnault & Thompson (1978, personal communications) were also unable to verify the presence of 18S ribosomal RNA in Pichinde virions which had been continuously passaged in BHK cells.

Since it was previously reported that polypeptides G1 and G2 of Pichinde virus and G of Tacaribe virus were glycosylated and that a small molecular weight lipid was detected in Junin virus (de Martinez Segovia & De Mitri, 1977), we examined the electrophoretic components of Machupo virus following selective staining with (1) the periodic acid Schiff reagent (PAS) which stains only glycosylated components, and (2) Nile blue A or Sudan black which stains only lipids. Fig. 1(b) compares the densitometric tracings of the electrophoretic components from Pichinde, Tacaribe and Machupo viruses following each of these selective staining procedures. As shown, G1 and G2 of Pichinde virus and G of Tacaribe virus were glycosylated while G1 and G2 of Machupo virus were also glycosylated. In addition, a small glycosylated component (GL) which incorporated lipid but not protein-specific stains (glycolipid) was present in each of the three viruses. Molecular weight determinations on each of the virus proteins are presented in Table 1.

Using similar polyacrylamide gel techniques, Killington et al. (1977) recently examined the relationship between the structural proteins and antigens of five different herpesviruses. Results from their study indicated that the degree of antigenic relatedness between herpesviruses could be correlated with the number of shared polypeptides visualized by polyacrylamide gel electrophoresis of purified virions. The data presented in our study and in the other arenavirus structural and serological studies mentioned above indicate that a similar
Table 1. Structural proteins of pathogenic and non-pathogenic arenaviruses

<table>
<thead>
<tr>
<th>Polypeptide type</th>
<th>Pichinde</th>
<th>Tacaribe</th>
<th>Machupo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major non-glycosylated (N)</td>
<td>68,000 (58)</td>
<td>68,000 (56)</td>
<td>68,000 (58)</td>
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<tr>
<td>Glycosylated (G)</td>
<td>65,000 (13)</td>
<td>--</td>
<td>50,000 (8)</td>
</tr>
<tr>
<td></td>
<td>38,000 (22)</td>
<td>38,000 (31)</td>
<td>41,000 (25)</td>
</tr>
<tr>
<td>Other non-glycosylated (m)</td>
<td>--</td>
<td>--</td>
<td>84,000 (3)</td>
</tr>
<tr>
<td></td>
<td>77,000 (1)</td>
<td>77,000 (2)</td>
<td>74,000 (4)</td>
</tr>
<tr>
<td></td>
<td>--</td>
<td>50,000 (6)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>15,000 (6)</td>
<td>15,000 (5)</td>
<td>15,000 (2)</td>
</tr>
</tbody>
</table>

* Calculated from densitometric tracings of subunit components and mass versus migration plots standardized with the mol. wt. markers shown in Fig. 1. In some cases glycoproteins E1 and E2 from Venezuelan equine encephalomyelitis virus were added as additional mol. wt. markers. Molecular weights are the average of three separate PAGE runs.

† Determined by calculating the areas under each of the peaks representing subunit components in densitometric tracings.

correlation between antigenic similarity and the number of shared polypeptides (e.g. Machupo and Tacaribe viruses) also exist among arenaviruses. The structural protein species which we have described for both Pichinde and Tacaribe viruses are very similar in both number and mol. wt. to those previously described (Ramos et al. 1972; Gard et al. 1977; Vezza et al. 1977). It is interesting that an apparently common non-glycosylated polypeptide (mol. wt. 60,000 to 70,000), which represents most of the virion mass, has been observed in each of the arenaviruses so far examined and has in most instances been identified as the major component of virus nucleocapsids. The possibility that this polypeptide may represent a group-specific antigen is indicated by the fact that antibody produced to nucleocapsid protein of Pichinde virus cross-reacts with Tacaribe, Parana, Machupo and LCM infected cells by indirect immunofluorescent techniques (J. D. Gangemi, unpublished observations). While it is apparent that the number and molecular weights of the other virion-associated proteins may vary, striking similarities exist among the polypeptides and especially the glycopeptides, of arenavirus pathogens such as LCM (Pedersen, 1973; Buchmeier et al. 1977), Junin (de Martinez Segoviz & De Mitri, 1977) and Machupo viruses. Future studies designed to analyse the virion-associated proteins of Lassa virus may reveal additional similarities among these pathogens.

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REFERENCES


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