Identification of a Major Non-structural Protein in the Nuclei of Rift Valley Fever Virus-infected Cells

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SUMMARY

A non-structural protein of mol. wt. 34 x 10^3 was demonstrated in the nuclei of Rift Valley fever virus-infected Vero cells by SDS–polyacrylamide gel electrophoresis. The protein appears to correspond to the virus-induced antigen demonstrated by indirect immunofluorescence in intranuclear inclusions.

Rift Valley fever virus (RVFV) is a member of the Phlebovirus genus of the family Bunyaviridae (Bishop et al., 1980) and has similar structural properties to other members of the family: three RNA segments, designated L, M and S, with mol. wt. of 2·7 x 10^6, 1·6 x 10^6 and 0·6 x 10^6 respectively, two major glycoproteins, G1 and G2, with mol. wt. of 65 x 10^3 and 56 x 10^3 respectively and a non-glycosylated nucleocapsid protein with a mol. wt. of 25 x 10^3 (Rice et al., 1980). Information on replication of the virus is limited and non-structural proteins are unknown (Bishop et al., 1980). However, eosinophilic intranuclear inclusions described previously in RVFV-infected cells were shown to fluoresce specifically in an indirect technique with immune sheep serum (Swanepoel & Blackburn, 1977). The present paper reports identification of a major non-structural protein in the nuclei of RVFV-infected cells.

The studies were performed in Vero cells (Yasamura & Kawakita, 1963) grown in Hanks’-based minimal essential amino acid medium (Eagle, 1959) with 10% foetal calf serum. Eight strains of RVFV were selected at random from 135 isolated from the organs of cattle and sheep during the 1978 epizootic in Zimbabwe (Swanepoel, 1981) and these were twice cloned by plaque technique. RVFV was plaqued as described by Swanepoel et al. (1978) except that Vero cells were used in place of calf testis cells. The plaque-purified strains were used to infect cell culture monolayer spots grown on microscope slides with Bellco micro-slide culture chambers (Bellco Glass Inc., Vineland, N.J., U.S.A.), at an m.o.i. of 10. Chamber wells were seeded with 2 x 10^4 Vero cells in 0·1 ml growth medium and incubated at 37 °C for 24 h before the medium was replaced with 0·1 ml of virus. Inocula were adsorbed for 1 h at 37 °C and replaced with fresh medium. The chambers were incubated at 37 °C in a 5% CO_2 atmosphere and at 24 h post-infection the cultures were washed in phosphate-buffered saline (PBS) pH 7·2, fixed in cold acetone and stained by the indirect immunofluorescence technique with the same reagents as used by Swanepoel & Blackburn (1977). It was found that all eight viruses induced formation of the characteristic intranuclear filaments described by Swanepoel & Blackburn (1977). One strain, 1678/78, was chosen for further investigation and slide cultures infected as above with this virus were processed for examination at various intervals up to 24 h post-infection. At each interval, 100 cells were scored for presence or absence of cytoplasmic and nuclear fluorescence. Cytoplasmic fluorescence could be detected from 8 h post-infection, and by 24 h, 96% of cells showed cytoplasmic fluorescence, while intranuclear fluorescence could be detected at 10 h post-infection, and by 24 h, 84% of cells showed intranuclear fluorescence (Fig. 1). Appearance of extracellular virus was monitored at 3-h intervals from 3 to 24 h post-infection in flask cultures infected at the same m.o.i. as the slide cultures and the growth curve (p.f.u./ml) is shown in relation to the appearance of cytoplasmic and intranuclear fluorescence in Fig. 1.
In labelling studies, monolayers of $10^7$ cells in 75 cm$^2$ flasks were infected as above with virus 1678/78 and at 6 h post-infection the medium was replaced with medium lacking methionine but containing 10 $\mu$Ci/ml $[^{35}\text{S}]$methionine (Amersham International) and 10% dialysed serum. Mock-infected cultures were pulsed in the same way. At 24 h post-infection the monolayers were washed with PBS, the cells scraped into PBS containing 0.05% EDTA and collected by centrifugation. Whole cell lysates were prepared with dissociation buffer (Laemmli, 1970). Nucleus–cytoplasm partitioning was performed with Nonidet P40 (NP40) [0.5% (w/v) in NET-BSA (150 mM-NaCl, 5 mM-EDTA, 50 mM-tris-HCl pH 7.4, 1 mg/ml bovine serum albumin)] according to the method of Wechsler & Fields (1978). The fractions were separated by centrifugation and the nuclei washed in NP40 NET-BSA and resuspended in dissociation buffer. Cytoplasmic fractions were treated with 10 vol. acetone at $-20$ °C, the precipitated proteins collected by centrifugation and the pellets resuspended in dissociation buffer. Virus-induced proteins were also immunoprecipitated from cytoplasmic and nuclear fractions. Following nucleus–cytoplasm partitioning, both fractions in NP40 NET-BSA were made 0.1% (w/v) with respect to SDS and complete disruption of nuclei was obtained by ultrasonic treatment. Precipitation with immune sheep serum was performed essentially as described by Clerx & Bishop (1981), the difference being that a 10% (w/v) suspension of protein A–Sepharose beads (Pharmacia) was substituted for *Staphylococcus aureus* cells. Polypeptides were analysed on linear 12% polyacrylamide slab gels (Laemmli, 1970) and visualized by fluorography (Bonner & Laskey, 1974). Molecular weights of polypeptides were determined in Coomassie Brilliant Blue-stained gels using a range of eight protein standards obtained from Bio-Rad Laboratories.

Two major induced polypeptides were readily detected in infected cells by fluorography and Coomassie Blue staining: the nucleocapsid protein of mol. wt. $25 \times 10^3$ and a non-structural protein (NS1) of mol. wt. $34 \times 10^3$ (Fig. 2a, b). The virus glycoproteins, G1 and G2, either labelled poorly or were poorly immunoprecipitated by the antiserum used here and could only be detected in cytoplasmic fractions after extended exposure of the X-ray film. The nucleocapsid protein occurred primarily in the cytoplasm and its presence in variable amounts in nuclear fractions was probably due to cytoplasmic contamination of nuclear preparations since NP40 treatment does not completely remove cytoplasmic material (Breidis et al., 1981). Contamination of nuclear fractions with the nucleocapsid protein was reduced when partitioned nuclei were subjected to vortex mixing during the washing process. The non-
structural protein occurred in high concentration in nuclear preparations, accounting for most of the incorporation of label in nuclei, while negligible amounts of this protein occurred in cytoplasmic fractions. Immune serum precipitated only a low mol. wt. protein, $<10 \times 10^3$, from the nuclei of mock-infected cells (Fig. 2b). In a further experiment, it was found that the non-structural protein from whole cells, disrupted ultrasonically in NP40 NET-BSA, was taken up readily by nuclei partitioned from unlabelled non-infected cells, while nucleocapsid protein failed to enter such nuclei.

RVFV is the only bunyavirus for which distinct intranuclear inclusions have been described and it seems probable that the non-structural protein demonstrated here corresponds to the inclusion material demonstrated by indirect immunofluorescence. A non-structural protein of similar mol. wt., 'about $30 \times 10^3$', has recently been demonstrated by Ulmanen et al. (1981) in cells infected with Uukuniemi virus, a bunyavirus which belongs to a genus distinct from the phleboviruses, but the authors apparently did not examine nuclear fractions. Studies are in progress to further characterize the $34 \times 10^3$ mol. wt. protein, and to determine whether this protein is related to any of the virus structural proteins.

Special Pathogens Unit
National Institute for Virology
Private Bag X4, Sandringham 2131
Republic of South Africa

REFERENCES
Short communications


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