Demonstration of Nuclear Immunofluorescence in Rift Valley Fever Infected Cells

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SUMMARY

Eosinophilic intranuclear filaments described previously in Rift Valley Fever infected cells, were shown to fluoresce specifically in an indirect technique with antiserum to the virus. Actinomycin D failed to suppress development of the filaments or replication of the virus.

The virus of Rift Valley Fever (RVF) produces eosinophilic intranuclear inclusions in cell cultures and in hepatocytes of susceptible species (Daubney, Hudson & Garnham, 1931; Findlay, 1933; Mims, 1957; Plowright & Ferris, 1957; Coackley, 1963; McGavran & Easterday, 1963). While the nuclear changes described by most authors consist of margination of chromatin and accumulation of rounded eosinophilic masses in the nucleoplasm, Plowright & Ferris (1957) and Coackley (1963) described eosinophilic filaments induced by recent isolates in cultures of lamb testis cells. We observed intranuclear filaments in infected calf testis cultures stained with haematoxylin and eosin and investigated their nature more closely.

Five viruses were used: (1) neurotropic RVF from the Veterinary Research Institute, Onderstepoort, South Africa, following 119 intracerebral (i.c.) passes in mice; (2) RVF 80612A, also from Onderstepoort and isolated from a lamb, following an unrecorded number of intraperitoneal passes in mice, mouse liver being used as a source of virus; (3) RVF 763/70 from an aborted sheep foetus in Rhodesia following 6 i.c. passes in mice; (4) RVF 2269/74 and (5) RVF 2575/74 from cattle foetuses in Rhodesia following 3 i.c. passes in mice.

Antiserum was prepared by infecting a sheep subcutaneously with 10⁶ mouse i.c. LD⁵₀ of virus 763/70 and bleeding it 4 weeks later when its serum had a haemagglutination-inhibition (HAI) titre (Clarke & Casals, 1958) of 1:2560. Guinea pigs were inoculated intramuscularly with 2 ml of HAI antigen, prepared from livers of mice infected with virus 80612A, in Freund's incomplete adjuvant. The guinea pigs were boosted intraperitoneally without adjuvant 3 weeks later and exsanguinanted after a further week. Pooled serum had an HAI titre of 1:2560.

Tubes with 'flying' coverslips were seeded with 3 x 10⁵ primary calf testis (CT), BHK21 CI 13 (Macpherson & Stoker, 1962) or Vero cells (Yasamura & Kawakita, 1963). Medium (Eagle, 1959) with 2% horse serum was used during experiments. Cultures were infected with approx. 10 infectious units (TCD₅₀) of virus per cell and coverslips of each type of cell and virus combination, together with non-infected controls, were harvested at four-hourly intervals from 12 to 24 h post-infection. Coverslip preparations were fixed in acetone at 0 to 4 °C for 10 min, washed in phosphate-buffered saline (PBS), pH 7.2 and allowed to react with 1:2 dilution of sheep anti-RVF serum for 20 min. After thorough washing in PBS the cultures were stained with fluorescein-conjugated anti-sheep immunoglobulin (Wellcome Reagents, Ltd., Beckenham, U.K.), washed again and mounted on slides with
Fig. 1. (a) Specific immunofluorescence of cytoplasm and nuclear filaments of calf testis cells 16 h after infection with RVF virus. Magnification × 475. (b) Intense fluorescence at 20 h. Magnification × 475. (c) Lack of specific fluorescence in non-infected cultures. Magnification × 475. (d) Electron micrograph of intranuclear filament composed of bundles of fibrils. Nuclear membrane in upper left corner. Magnification × 52,000.
glycerol-PBS medium. Replicate infected CT coverslips were allowed to react with a 1:2 dilution of guinea pig anti-RVF serum prior to treatment with the sheep serum and conjugate. Further replicates were stained directly with conjugate without prior treatment with RVF antibody. Stained coverslips were examined on a microscope with incident lighting from an Osram HBO-200 mercury vapour lamp with a BG 12 exciter filter and K 530 suppression filter.

Monolayers of CT cells in 250 ml flasks were infected with 1 to 10 TCD₉₀/cell of virus 763/70 and harvested with a rubber policeman after 24 h when less than 25% of cells showed c.p.e. The cells were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, embedded in Araldite CY212, sectioned on a Reichert UN2 ultramicrotome, stained with uranyl acetate and lead citrate and examined on an Hitachi HU 11E electron-microscope at 50 kV.

Cytoplasmic fluorescence and nuclear filaments were produced in all three types of cell by each of the viruses tested. It was evident in cells examined 12 h after infection and weakly fluorescent filaments were discernible in nuclei. At 16 h the filaments were strongly fluorescent and were plainly visible in all cells with cytoplasmic fluorescence (Fig. 1a). At 20 and 24 h, cells which were becoming rounded and stripping from coverslips, had intensely fluorescent cytoplasms and thickened nuclear filaments (Fig. 1b). Fluorescence did not occur in controls (Fig. 1c).

The failure of previous investigators to observe nuclear fluorescence (Iwasa, 1959; Easterday & Jaeger, 1963; McGavran & Easterday, 1963; Hahon, 1969; Pini, Lund & Davies, 1973) is difficult to explain. It could be that the appearance of intranuclear antigen is not a regular or obligatory feature of virus replication. It was observed that filaments which fluoresced strongly under the microscope used in the present investigation were difficult to detect under a similar microscope which utilized transmitted rather than incident lighting.

Electron-microscopy revealed structures within nuclei of infected CT cells which appear to correspond to the filaments demonstrated by immunofluorescence. The structures are composed of fibrils 5 nm or less in thickness lying more or less parallel to each other in bundles (Fig. 1d). Cytoplasmic changes correspond to those described by Murphy, Harrison & Whitfield (1973) and consisted essentially of Golgi proliferation and accumulation of virus particles in the cisternae of this organ and endoplasmic reticulum. In previous studies, electron-microscopy failed to reveal virus particles or amorphous aggregates in nuclei of mouse hepatocytes (McGavran & Easterday, 1963; Murphy et al. 1973), but granular masses thought to correspond to inclusion bodies were seen in the nuclei of BHK21 cells (Lecatsas & Weiss, 1968).

To determine whether or not cellular DNA participates in production of nuclear filaments or in replication of the virus, sensitivity to actinomycin D was tested in CT coverslip cultures infected with 10 TCD₉₀/cell of virus 763/70. Cultures were treated for 30 min with 1 ml of 1:10 sheep anti-RVF serum following absorption of the inoculum. Antiserum was replaced with medium containing 10 μg/ml of actinomycin D ('Lyovac Cosmegen', Merck, Sharp and Dohme, New Jersey, U.S.A.). The drug was omitted from infected control cultures. Antiserum used to treat infected cultures was free from residual infectivity when tested by i.c. inoculation of day-old mice. Treatment with actinomycin D failed to suppress development of intranuclear filaments or infectivity for mice in cultures tested at 24 h.

The results indicate that intranuclear filaments are produced in diverse mammalian cell cultures by highly-passaged RVF virus as well as by recent isolates, and that the filaments
are associated with virus-specified antigen. Lack of sensitivity to actinomycin D at a concentration which inhibits replication of orthomyxoviruses (Barry, Ives & Cruickshank, 1962) indicates that production of the nuclear antigen and replication of RVF virus are independent of participation of cellular DNA.

The relationship of the intranuclear filaments to the events of virus replication has not been established. Following infection of cells in culture with high multiplicities of RVF virus there is a lag of 4 to 6 h following which there is an exponential increase in infectivity until a peak is reached in 16 to 30 h; the exact timing depending on the type of cell and whether monolayer or suspension cultures are used (Johnson & Orlando, 1968; Johnson, Orlando & Patrick, 1969). We observed that intranuclear filaments were present 12 to 16 h after infection but did not examine cell cultures earlier; nor did Coackley (1963) and Lecatsas & Weiss (1968). Nuclear filaments have not been seen to occur in vivo, but Mims (1957) and McGavran & Easterday (1963) observed eosinophilic intranuclear inclusions in hepatocytes of mice 3 and 8 h after the administration of saturating doses of virus. Mims noted that nuclear changes involving margination of chromatin were the earliest histological signs of infection to develop within 1 h of the administration of virus. Even accepting that eosinophilic inclusions seen in tissues correspond to the filaments seen in cell cultures, there is insufficient evidence available to indicate whether virus-associated antigen in the nucleus is likely to be an early non-incorporated product or a late and possibly incorporated product. Clearly, there is need for further investigation.

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References


Short communications


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