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CHARACTERISATION OF ORBIVIRUSES OF THE KEMEROVO SEROGROUP: COMPARISON OF PROTEIN AND RNA PROFILES

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Summary. — Nine viruses of the Kemerovo serogroup (orbivirus genus; family, *Reoviridae*) isolated from seabird ticks (*Ixodes uriae* and *Ornithodoros maritimus*) from eight different geographical locations (four from Scotland, two from Morocco, one from Eire, one from England, one from the Faeroes Islands) were examined. All nine viruses produced unique RNA electropherotypes but showed a 2 : 4 : 3 : 1 grouping of the ten double-stranded RNA segments. The virus-induced proteins were labelled with ^{35}S--methionine in a Vero cell line. Seven of the viruses isolated from *Ixodes uriae* produced similar profiles of nine major polypeptides. The remaining two viruses, isolated from *Ornithodoros maritimus* from Morocco, produced profiles in which only five major viral proteins could be readily detected, four of which had similar molecular weights to proteins apparent in the other isolates.

Key words: Ixodes uriae; Ornithodoros maritimus; Kemerovo; orbivirus

Introduction

The genus orbivirus belongs to the family *Reoviridae* and is composed of 12 serogroups. The Kemerovo serogroup has 4 subgroups containing at least 20 viruses all isolated from ticks (Gorman *et al.*, 1983). Many of these viruses have been isolated from ticks collected in seabird colonies: those isolated from hard tick species (*Ixodes uriae* and *I. signatus*) belong to the Great Island subgroup, and from soft tick species (*Ornithodoros capensis* and *Argas* species complex), the Chenuda subgroup. Members of the Kemerovo serogroup have been characterized on the basis of serological grouping (Casals, 1971; Libíková and Casals, 1971; Main *et al.*, 1976). Studies on the RNA and protein profiles of a few Kemerovo group viruses have demonstrated 10 segments of double stranded RNA (dsRNA) and at least 9 virus induced intracellular proteins (Eley *et al.*, 1984, 1985; Gorman *et al.*, 1984; Slávik *et al.*, 1984; Spence *et al.*, 1984; 1985; Spence *et al.*, 1986). Seabird colonies represent a confined ecosystem, therefore, Kemerovo group viruses isolated from seabird ticks provide a unique system for studying factors that influence virus evolution, such as geographical and temporal

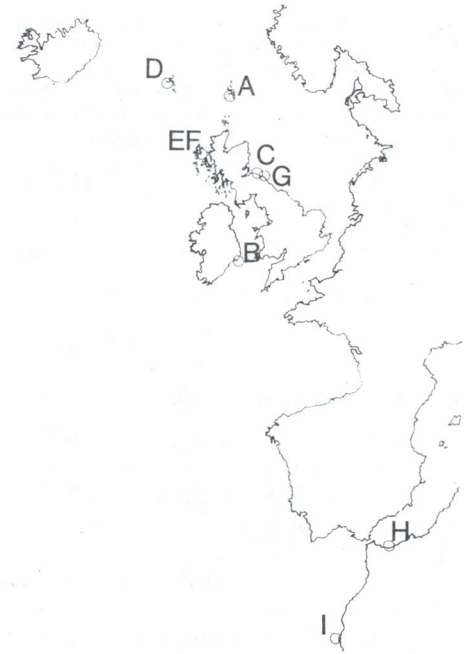

Fig. 1.
Geographic distribution of the 9 Kemerovo group viruses. Letters refer to different isolates which are described in the text.

distribution, and vector species. This communication reports the comparison of the dsRNA segments and induced proteins of 9 Kemerovo serogroup viruses from 2 species of seabird ticks collected from 8 geographical locations.

Materials and Methods

The geographical locations of seabird colonies from which ticks were collected are shown in Fig. 1. The following viruses were isolated from *I. uriae* Foula F80-2(A) from 10 unfed females collected on 21. 7. 80, Great Saltee GS80-7 (B) from 5 males (Nuttall *et al.*, 1984), St. Abb's FT363 (C) from 10 engorged nymphs (Nuttall *et al.*, 1981), Mykines M82-3 (D) from one engorged female collected on 17. 7. 82, Shiants M325 (E) from 3 engorged females and Shiants M330 (F) from 9 males collected on 1. 7. 79 (Nuttall *et al.*, 1982), and Inner Farne 1F-1 (G) from 5 males collected on 1. 7. 79. Kala Iris (H) and Essaouria (I) viruses were isolated by Professor C. Chastel (University of Brest) from *O. maritimus* collected from seabird colonies in Morocco.

The methods of isolation, production and assay of stock virus have been described previously (Nuttall *et al.*, 1981). All viruses were grown in Vero cell cultures. Double stranded RNA was extracted as described by Eley *et al.* (1984). Briefly, virus infected monolayers of Vero cells were lysed at 24—48 hr post infection (p.i.) with SDS in a solution containing 10 mmol/l EDTA. Cellular DNA was precipitated with NaCl at a final concentration of 1 mol/l. The supernatant was digested with 200 μg/ml of proteinase K (Boehringer Mannheim) at 37 °C and extracted twice with phenol equilibrated in 10 mmol/l Tris-HCl pH 7,4, before being washed twice in ether. The dsRNA was precipitated with 2.2 vol of ethanol, pelleted, dried, resuspended in distilled water, then digested with 1 μg/ml DNase 1 (Sigma) at 37 °C. Following a further phenol extraction and ether wash, the solution was made 2 mol/l with respect to LiCl to precipitate remaining single stranded RNA and DNA fragments. The dsRNA was precipitated in ethanol, pelleted, dried, resuspended in distilled water and stored at —50 °C.

Virus induced proteins were radioactively labelled. Vero cell monolayers in 25 cm^2 culture flasks were infected with between 1—10 PFU/cell of virus for 1 hr at 37 °C, then overlaid with

5 ml of Eagle's Minimal Essential Medium (EMEM) supplemented with 3% foetal calf serum. At 8 hr p.i. the cells were washed with methionine deficient EMEM and incubated in the same medium for 30 min. The cells were then pulsed with 1.86 MBq of ^{35}S-methionine (Amersham International) for 1 hr at 37 °C. The cells were harvested into ice-cold PBS, pelleted at 2000 × g and boiled for 2 mins in solubilisation buffer (Moore *et al.*, 1980).

The dsRNA segments were separated in a SDS-free system on a 15 cm long 10% polyacrylamide resolving gel with a 3% stacking gel using a discontinuous SDS-free Tris-glycine buffer system. Electrophoresis was performed for 18 hr at 18 mA. Gels were silver stained by the method of Herring *et al.* (1982).

The proteins were separated on 15 cm long 15% SDS-polyacrylamide resolving gel with a 5% stacking gel using a discontinuous Tris-glycine buffer system (Laemmli, 1970). Electrophoresis was performed for 18 hr at 10 mA. The gel was fixed in 40% methanol; 10% acetic acid before being dried and exposed to Fuji X-ray film at —20 °C.

Results

Virus induced polypeptides

Examination of the virus induced polypeptides separated by electrophoresis showed that 7 isolates (Foula F80-2, Great Saltee GS80-7, St. Abbs FT363, Mykines M82-3, Shiants M325, Shiants M330 and Inner Farne IF-1) produced 9 major proteins with similar profiles (Fig. 2). In particular viral proteins (VPs) 1, 2 and 6 had similar molecular weights whereas the remaining proteins showed small differences. Of these isolates only Inner Farne IF-1 (Fig. 1, track G) failed to shut-off host cell synthesis after 8 hr p.i., a feature shared with the remaining isolates, Kala Iris and Essaouria (Fig. 2, tracks H and I respectively). Host cell protein synthesis was still apparent at 30 hr p.i. with these 3 isolates (data not shown). The profiles of Kala Iris and Essaouria were similar to each other but different from the other isolates. Only 5 virus-induced polypeptides were identified for Kala Iris and Essaouria (see Fig. 1, tracks H and I). Four of these approximately corresponded in molecular weight to VPs 1, 3, 6 and 7 of the other isolates, the fifth having an unique position. Due to poor shut-off of host protein synthesis it was difficult to distinguish the viral polypeptides of these 2 isolates.

Viral RNA

The RNA electropherotypes demonstrated that each isolate had an unique pattern although each had 10 segments migrating in a 2 : 4 : 3 : 1 grouping, that is, 2 large, 4 medium, 3 small and 1 very small. Segments 1, 2 and 10 of Kala Iris and Essaouria (Fig. 3 tracks H and I) appeared appreciably larger than those of the other isolates.

Discussion

The RNA and protein profiles of 9 orbiviruses of the Kemerovo serogroup were examined. The viruses were isolated from 2 species of ticks collected from seabird colonies in various geographical locations. All except Kala Iris and Essaouria serotypes are members of the Great Island subgroup of the Kemerovo serogroup (Gorman *et al.*, 1983; Jacobs *et al.*, in preparation). Each virus contained 10 segments of dsRNA which produced unique profiles

when separated by polyacrylamide gel electrophoresis. Within this variation a pattern of 2 large, 4 medium, 3 small and 1 very small molecular weight segments were found. Other Kemerovo serogroup viruses have also shown the 2 : 4 : 3 : 1 pattern: Nugget virus isolated in Australia (Gorman *et al.*, 1984); RS-10 strain identified in Czechoslovakia (Libíková *et al.*, 1963;Slávik *et al.*, 1984) M349/1 and M349/2 isolated from St. Abb's Head, Scotland (Eley *et al.*, 1984), and Mill Door/79, Mill Door/81 and North Clett/81 from the Isle of May, Scotland (Spence *et al.*, 1985). In contrast, serotypes of bluetongue virus, the type species of the orbivirus genus, were shown to produce 3 : 3 : 3 : 1 distribution of their RNA segments (Gorman 1979; Knudson *et al.*, 1982).

The extensive variation in genome profiles of the Kemerovo serogroup viruses was not reflected in the virus-induced polypeptides. Seven of the viruses (Foula F80-2, Great Saltee GS 80-7, St. Abbs FT363, Mykines M82-3, Shiants M325, Shiants M330, Inner Farne IF-1), all of which were isolated from *I. uriae* collected in Great Britain and Eire, produced patterns of 9 major polypeptides that showed only minor variations in size. In contrast, the 2 viruses (Kala Iris and Essaouria) isolated from *O. maritimus* collected in Morocco, produced profiles similar to each other but distinct from the rest. In these viruses 5 major polypeptides could be identified. Nine major polypeptides have been identified in Vero cells infected with another Kemerovo group virus (Eley *et al.*, 1985), although more than 10 have also been reported for Kemerovo serogroup viruses (Spence *et al.*, 1985, Gorman *et al.*, 1984). Ten virus specified proteins have been detected in cells infected with orbiviruses from serogroups Bluetongue, Eubenangee and Wallal (Gorman *et al.*, 1981).

It has been proposed that members of the Kemerovo serogroup can be separated into 4 sub-groups on the basis of virus origin (Casals, 1971; Main *et al.*, 1976; Gorman *et al.*, 1983). Our data would support the inclusion of the viruses isolated from *I. uriae* in one of these sub-groups (Great Island), distinct from the viruses isolated from *O. maritimus* (Essaouria and Kala Iris). This corroborates the data from serological comparisons of these isolates (Jacobs *et al.*, in preparation).

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Explanation of Figures (Plates XXXIV—XXXV):

Fig. 2. Autoradiograph of the virus induced proteins labelled in Vero cells with ^{35}S-methionine and separated on a 15% polyacrylamide gel. Electrophoresis was performed for 18 hr at 10 mA. (A) Foula F80-2, (B) Great Saltee GS80-7, (C) St. Abbs FT363, (D) Mykines M82-3, (E) Shiants M325, (F) Shiants M330, (G) Inner Farne IF-1, (H) Kala Iris, (I) Essaouria, (J) Mock-infected. The major polypeptides of tracks A to G labelled VP1 to VP9. Those of tracks H and I have been indicated by (●).

Fig. 3. 10% polyacrylamide gel of extracted intracellular dsRNA of the nine virus isolates. The gel was electrophoresed for 18 hr at 18 mA and the bands visualised by silver staining. (A) Foula F80-2, (B) Great Saltee GS80-7, (C) St. Abbs FT363, (D) Mykines M82-3, (E) Shiants M325, (F) Shaints M330, (G) Inner Farne IF-1, (H) Kala Iris, (I) Essaouria.