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A COMPARISON OF THE INDUCED POLYPEPTIDES AND RNAs OF THREE ORBIVIRUSES ISOLATED FROM TICKS *(IXODES URIAE)* COLLECTED IN SEABIRD COLONIES ON THE ISLE OF MAY, SCOTLAND

R. P. SPENCE[1], P. A. NUTTALL, N. F. MOORE

Natural Environment Research Council, Institute of Virology, Mansfield Road, Oxford, OX1 3SR, U.K.

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Summary. — Three orbiviruses of the Kemerovo serogroup, isolated from ticks from two sites on the Isle of May in Scotland, were plaque purified and the proteins and RNA induced in Vero cells compared. Two viruses (Mill Door/79 and Mill Door/81) from the same site differed in the migration of at least 7 segments of dsRNA. The third virus (North Clett/81) was clearly distinguished from Mill Door/79 and Mill Door/81 viruses in the migration of 6 dsRNA segments. Analysis of virus-induced polypeptides demonstrated minor molecular weight differences, but partial proteolysis failed to show significant variations in the proteins. Precipitation of radiolabelled virus-induced proteins by hyperimmune ascitic fluids did not distinguish between the isolates.

Key words: Orbivirus; Kemerovo serogroup; Great Island Complex

Introduction

Viruses belonging to the Kemerovo serogroup of the orbivirus genus are tick-borne, birds being the predominant vertebrate host (Gorman *et al.*, 1983). The serogroup is divided into four antigenic complexes: Kemerovo, Great Island, Chenuda and Wad Medani. Viruses within each complex have been isolated usually from the same or similar tick species (Gorman *et al.*, 1983; Libíková and Casals, 1971; Main *et al.*, 1976). Viruses belonging to the Great Island complex are maintained in seabird colonies by the hard tick, *Ixodes uriae*, in scattered areas of both the northern and southern hemispheres. They are closely related to one another by complement fixation tests, and to a lesser degree with viruses of the other antigenic complexes (Main *et al.*, 1976).

Mill Door/79 virus was isolated from ticks collected on the Isle of May in Scotland in 1979 and was shown to be closely related to Cape Wrath virus, a member of the Great Island complex, by complement fixation tests (Spence *et al.*, 1985*a*). It produced at least 13 viral polypeptides when grown in Vero

[1] Present address: Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX

cell culture and possessed a genome of 10 segments of double-stranded RNA (Spence *et al.*, 1985*b*). The electrophoretic pattern of the RNA was distinct from orbiviruses belonging to other serogroups (Spence *et al.*, 1984).

Following the isolation of Mill Door/79, two other orbiviruses of the Great Island complex were isolated from ticks collected on the Isle of May during 1981. One, Mill Door/81, was collected from the same colony as Mill Door/79, and the other, North Clett/81, from a separate colony. The three isolates were distinguished from each other by plaque reduction neutralization tests (Spence *et al.*, 1985*a*): Mill Door/79 and North Clett/81 did not cross-react, North Clett/81 and Mill Door/81 show a reduced level of cross-reaction compared with the homologous titres, and Mill Door/79 and Mill Door/81 showed one-way cross-neutralisation. This paper reports preliminary studies to determine whether these isolates represented variants selected from a single gene pool circulating either within an individual colony, or within the seabird-tick (*Ixodes uriae*) ecosystem as a whole. The viral RNA and polypeptides of Mill Door/79 virus were compared with the two other isolates.

Materials and Methods

Viruses and cell culture. Viruses were isolated from ticks by intracerebral inoculation of tick homogenates into 2 day old suckling mouse. Mouse brain from the first passage was homogenised and then passed in suckling mice followed by a single passage in Vero cells before plaque purification (Spence *et al.*, 1985*a*). Stock virus was produced and assayed in Vero cell cultures (Nuttall *et al.*, 1981).

Radiolabelling of intracellular proteins. Vero cells, inoculated at a multiplicity of 20–30 PFU per cell, were labelled at 7 hr post-infection (p.i.) for 15 min with 3.7 MBq /ml [^{35}S] methionine (Amersham International plc., England).

Limited or partial proteolysis. Essentially the method of Crump and Moore (1981) was used. Polypeptides were digested with 175 μg/ml *Staphylococcus aureus* V8 protease (Miles Laboratories Ltd., Stoke Poges, England). Digestion products were separated on 17.5% polyacrylamide gels for 20 hr at 5 mA.

Immune-precipitation of intracellular virus products. Cells inoculated and labelled essentially as above were solubilized in sterile NTEP buffer (150 m mol/l NaCl, 50 mmol/l Tris, pH 7.9, 5 mmol/l EDTA, 0.05% NP40). Cell membranes were disrupted by 6 cycles of freeze-thawing and the remaining cell debris removed by centrifugation. The supernatant was adsorbed under constant agitation with a 1% (w/v) suspension of *S. aureus* protein A (Sigma, London) for 1 hr, and then pelleted. The supernatant was then agitated for 1 hr periods after the sequential addition of hyperimmune mouse ascitic fluid raised against the isolates, rabbit anti-mouse IgG and protein A (1% w/v). The protein A was pelleted and washed 6 times before solubilization by heating at 100 °C for 5 min in solubilization buffer (Moore *et al.*, 1980). Insoluble material was removed by centrifugation and the supernatant stored at 4 °C until required for electrophoresis.

Extraction of double-stranded RNA from infected cells. dsRNA was extracted by a modification of the method of Travassos da Rosa *et al.* (1984). After proteinase K digestion the samples were phenol extracted, ethanol precipitated and treated with 1 μg/ml DNase I (Boehringer Mannheim, W. Germany). The samples were then extracted with phenol: chloroform (1 : 1) before lithium chloride fractionation. The dsRNA segments were separated on a 10% polyacrylamide gel by electrophoresis at 15 mA for 15 hr and visualised by silver stainig using the method of Herring *et al.* (1982).

SDS-polyacrylamide gel electrophoresis. Proteins were normally separated on 16 cm long 12.5% polyacrylamide slab resolving gels with 5% stacking gels using the discontinuous Tris-glycine buffer system of Laemmli (1970). Other polyacrylamide concentrations used are described in the text. Electrophoresis was performed for 15–18 hr at a constant 10 mA. The gels were fixed with 40% (v/v) methanol and 10% (v/v) glacial acetic acid in water. After drying the gels were exposed to Fuji X-ray film at −20 °C.

Results

Comparisons of viral RNAs

The electrophoretic profiles of the dsRNA genomes of the three isolates were examined by running them either singly or together on the same polyacrylamide gel (Fig. 1). The overall patterns of migration were the same, showing a 2 : 4 : 3 : 1 arrangement, but variation was shown in the distance migrated by individual segments. The 3 viruses differed from each other in the migration of at least 6 dsRNA segments.

Comparison of virus-induced proteins

Fig. 2 compares the profiles of virus-induced proteins of the 3 isolates. Minor differences in the molecular weights of 5 proteins were detected corresponding to p69, p53/51, p37 and p21 of Mill Door/79 virus. When labelled with [^{14}C] amino acids and then compared using a Joyce Loebl Chromoscan, no significant differences were detected between the isolates in the amounts of each polypeptide (data not shown).

Further comparisons of the isolates were made by carrying out partial proteolysis on the induced proteins. Fig. 3a shows a proteolysis gel of Mill Door/79 (1) and North Clett/81 (3) viruses; polypeptides that varied in molecular weight are indicated. No differences in the digestion patterns were detected indicating that the positions of cleavage sites were similar for the proteins of these isolates. Fig 3b demonstrates a similar gel of Mill Door/79 (1) and Mill Door/81 (2) viruses; only one obvious size variation was detected between these two isolates but no peptide variation was visible in the proteolysis patterns. Three proteins induced by North Clett/81 and Mill Door/81 (Fig. 3c, 3 and 2 respectively) varied in molecular weight. The digestion did not produce distinct oligopeptides for these proteins, but oligopeptides were detected from a protein of North Clett/81 that were not visible with Mill Door/81 virus (e.g. arrow).

The 3 isolates were also compared by immune-precipitation. Polypeptides p93, p53/51 and p37 were precipitated by both heterologous and homologous hyperimmune ascitic fluids (Fig. 4). Polypeptide p53/51 was also precipitated by non-immune ascitic fluid.

Discussion

The circulation, within seabird colonies, of orbiviruses of the Great Island complex provides a unique system for the study of virus "evolution". The high degree of antigenic variation shown between virus isolates from ticks collected within a single colony and in different colonies can be studied on a time scale, and with respect to geographical location of the colony, and the relative roles of ticks and the immune status of the seabird population in selecting new antigenic variants.

Mill Door/79, North Clett/81 and Mill Door/81 viruses were previously compared by neutralization tests and found to be serologically distinct

(Spence *et al.*, 1985*a*). The purpose of this investigation was to determine whether the RNA and proteins of these viruses also differed.

The RNA migration profiles of the 3 isolates were similar, showing the 2 : 4 : 3 : 1 pattern previously described for Nugget virus (Gorman *et al.*, 1984), a member of the Kemerovo serogroup (Doherty *et al.*, 1975). However, when the dsRNA profiles of the 3 isolates were compared each was readily distinguished. Similar heterogeneity in the migration of RNA segments has been reported for orbiviruses in other serogroups (Gorman *et al.*, 1977; 1981; Gorman and Taylor, 1978; Squire *et al.*, 1983).

Analysis of virus-induced polypeptides of the Isle of May isolates by direct polyacrylamide gel electrophoresis failed to demonstrate variations in structural polypeptides other than small differences in molecular weight. Partial proteolysis only distinguished between one polypeptide of North Clett/81 and Mill Door/81 viruses. These results demonstrate that differences between dsRNA segments, as detected by polyacrylamide gel electrophoresis, do not necessarily correspond with differences in the migration patterns of viral proteins. If differences in migration of dsRNA segments reflect differences in molecular weight, variation in nontranslated regions of the dsRNA may account for the variation between isolates. Alternatively, secondary or tertiary structure of dsRNA segments may account for the variations in mobility.

Hyperimmune ascitic fluids distinguished between the 3 isolates in plaque--reduction neutralization tests (Spence *et al.*, 1985*a*) but failed to distinguish between them in cross-immune precipitation experiments. This observation has been made previously by Huismans and Bremer (1981) in cross-immune precipitation experiments between bluetongue virus serotypes. They suggested that cross-neutralization between serotypes may require recognition of more than one antigenic determinant, whereas cross-immune precipitation may require recognition of only one determinant.

Analysis of orbivirus genomes appears to be a more sensitive method of distinguishing between viruses than analysis of proteins. However, whether the differences in the mobilities of genome segments has any functional significance is questionable because their products do not display the same differences. The data presented here demonstrates that significant genetic variation exists among viruses both between and within neighbouring seabird colonies. Hence the genetic pool of viruses within these colonies is possibly large, but as only three isolates were examined during this study any conclusions drawn from these results are necessarily limited. Further studies are necessary using larger numbers of isolates before the significance of the genetic variation can be understood.

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Explanations to Figures (Plates III–VI):

Fig. 1. 10% polyacrylamide gel of the extracted intracellular dsRNA of Mill Door/79, North Clett/81 and Mill Door/81 viruses. Track A, Mill Door/79; Track B, Mill Door/79 and North Clett/81; Track C, North Clett/81; Track D, North Clett/81 and Mill Door/81; Track E, Mill Door/81; Track F, Mill Door/81 and Mill Door/79; Track G, Mill Door/79 viruses. 1 μg of each dsRNA sample was loaded per track and dsRNA visualized by silver staining. dsRNA segments 3 and 6 of Mill Door/79 and North Clett/81 viruses comigrated. dsRNA segments 7 and 8 of Mill Door/79 and Mill Door/81 viruses comigrated, and dsRNA segment 10 of Mill Door/81 and North Clett/81 viruses comigrated. The segment designations are for Mill Door/79 virus. With Mill Door/81 and North Clett/81 virus segments 3 and 4 have similar mobilities.

Fig. 2. Virus-induced proteins of the Isle of May isolates. 12.5% polyacrylamide gel of proteins labelled for 15 min from 7 hr post-infection with [^{35}S]-methionine. Track A, mock infected cells; B, Mill Door/79; C, North Clett/81; D, Mill Door/81. Arrows indicate major viral polypeptides; p141, p93, p69, p65, p53/51, p44, p37, p27, p21 and p20 as determined for Mill Door/79.

Fig. 3. Co-proteolysis of virus-induced proteins. The proteins were separated in the first dimension on a 17-5% polyacrylamide gel. A longitudinal strip containing all the virus proteins was layered onto a 5% stacking gel above a 17.5% polyacrylamide gel. The proteins were digested with *Staphylococcus* V8 protease (175 μg/ml). Two viruses were compared in each gel (a, b, c), and labelled: 1 = Mill Door/79, 2 = Mill Door/81, 3 = North Clett/81. Arrows indicate virus induced proteins, triangles indicate proteins with differing migration rates. On gel c the solid arrow in the body of the gel indicates one of the products of p44 from North Clett/81 not identified in Mill Door/81.

Fig. 4. Autoradiograph of a 12.5% polyacrylamide gel of immune precipitates of [^{35}S]-methonine--labelled virus induced proteins. Tracks A—D show proteins from mock infected cells treated with either control ascitic fluid (A) or hyperimmune ascitic fluid against Mill Door/79, (B) North Clett/81 (C), and Mill Door/81 (D) viruses. Tracks E—H: Mill Door/79 infected cells treated with either control ascitic fluid (E) or hyperimmune ascitic fluid against Mill Door/79 (F), North Clett/81 (G), and Mill Door/81 (H) viruses. Tracks I—L: North Clett/81 infected cells treated with either control ascitic fluid (I) or hyperimmune ascitic fluid against Mill Door/79 (J), North Clett/81 (K) and Mill Door/81 (L) viruses. Tracks M—P: Mill Door/81 infected cells treated with control ascitic fluid (M), hyperimmune ascitic fluid against Mill Door/79 (N), North Clett/81 (O), and Mill Door/81 (P) viruses. Track Q: intracellular polypeptides induced by Mill Door/79 virus.