Genomic Analysis of Antigenically Related Avian Paramyxoviruses

(Accepted 20 September 1982)

SUMMARY

Six avian paramyxoviruses isolated from wild and domestic ducks in the United States, Hong Kong and Japan were characterized antigenically and genetically. All viruses examined were shown to be antigenically closely related. Oligonucleotide patterns of duck/Miss/334 and duck/Miss/406 were apparently distinguishable from those of duck/Miss/116 and duck/Miss/320 isolated in the same area of the United States. On the other hand, two viruses isolated from a domestic duck in Hong Kong (duck/Hong Kong/D3/75) and from a domestic duck in Japan (duck/Tokyo/41/78) were genetically very similar to that of duck/Miss/116, suggesting that these three viruses represent a genetically homogeneous group and may be of the same origin.

Since the beginning of a surveillance programme for influenza in birds, numerous paramyxoviruses which represent serologically distinct groups have been isolated in many parts of the world and their classification has become a considerable problem (Alexander, 1980; McFerran et al., 1974; Nerome et al., 1978a, b; Collins et al., 1975; Alexander et al., 1979a; Webster et al., 1976; Shortridge et al., 1980; Kida & Yanagawa, 1979; Alexander & Collins, 1981). In addition, a number of avian paramyxoviruses isolated in different countries represented serologically and biochemically homologous groups (Alexander, 1980; Nerome et al., 1978a; Webster et al., 1976; Alexander & Collins, 1981; Fleury & Alexander, 1979; Kessler et al., 1979; Lipkind et al., 1979). In 1975, Webster et al. (1976) isolated seven paramyxoviruses from migrating feral ducks shot on the Mississippi flyway, and five of these isolates were identified as a new serotype (Kessler et al., 1979; Alexander et al., 1979b). Results obtained in previous studies indicate that the above mentioned five viruses have very similar polypeptide patterns and the polypeptide profile of these viruses were different from those of serologically distinct viruses (Alexander & Collins, 1981; Kessler et al., 1979; Alexander et al., 1979b). In addition, two paramyxoviruses which were serologically closely related to the five viruses in the United States, were isolated from a domestic duck (Shortridge & Alexander, 1978) in Hong Kong in 1975 and from a domestic duck in Japan in 1978.

The object of the present study is to describe the results of antigenic and genomic analyses of avian paramyxoviruses that were isolated in different countries between 1975 and 1978.

The following five strains of avian paramyxoviruses isolated in the United States were kindly supplied to us by Dr R. G. Webster: duck/Miss/116, duck/Miss/320/75, duck/Miss/334/75, duck/Miss/406/75 and duck/Miss/604/75. Duck/Hong Kong/D3/75 was also kindly provided by Dr D. J. Alexander. One paramyxovirus isolated in Japan, which we designated as duck/Tokyo/41/78 was used in the test for comparison. All viruses were grown in the allantoic cavity of 11-day-old fertile hens' eggs. For peptide and genetic analysis, representative viruses concentrated by centrifugation at 44,000 g for 1.5 h in an angle-21 rotor were further banded twice in linear 20 to 50% (w/w) sucrose gradients at 64,000 g for 2 h. All of the viruses, with the exception of a virus isolated in Japan, have previously been characterized antigenically and biochemically (Shortridge & Alexander, 1978; Kessler et al., 1979; Alexander et al., 1979b). To obtain more detailed information about the antigenic relationship between the virus isolated in Japan and the other viruses, haemagglutination-inhibition (HI) and immuno-double-diffusion (IDD) tests were undertaken using monospecific antiserum to the haemagglutinin–neuraminidase (HN) glycoprotein of duck/Hong Kong/D3/75 virus. Monospecific antiserum to HN protein was prepared in guinea-pigs by intraperitoneal injection of purified HN protein bound to glutaraldehyde-fixed guinea-pig erythrocytes according to the method described previously (Scheid & Choppin, 1973; Hosaka & Hosokawa, 1977). HI tests were done in microtitre plates...
Fig. 1. Immuno-double-diffusion tests (a, b) with monospecific antiserum to the isolated HN protein of duck/Hong Kong/D3/75, and comparison of the structural polypeptides (c) of duck/Miss/116/75 (abbreviated as 116), duck/Miss/334 (334) and duck/Tokyo/41/78 (41). Centre wells contain antiserum (a-1) to HN protein. (a, b) Outer wells contain different antigens from viruses disrupted with Triton X-100: (1), homologous virus, duck/Hong Kong/D3/75; (2), duck/Miss/116; (3), duck/Miss/320; (4), duck/Miss/334; (5), duck/Miss/406; (6), duck/Miss/604 which was closely related to Newcastle disease virus and (7), duck/Tokyo/41/78. (c) For polypeptide analysis, purified viruses were disrupted with 2% SDS and 2.3% dithiothreitol. The structural polypeptides of representative viruses were separated by electrophoresis on a 13% polyacrylamide gel. Arrows indicate the position of glycopeptides identified by periodate–Schiff (PAS) staining. All structural polypeptides were detected by staining with Coomassie Brilliant Blue (CBB). The numbers represent mol. wt. $\times 10^{-3}$, estimated on the basis of mol. wt. of marker proteins of influenza virus A/PR/8/34, the L and H chains of immunoglobulin, and bovine serum albumin.

using 0.5% chicken erythrocytes. The haemagglutination of duck/Miss/116, duck/Miss/320, duck/Miss/334, duck/Miss/406 and duck/Tokyo/41/78 were inhibited by antiserum to the HN protein of duck/Hong Kong/D3/75 at titres similar to the homologous titres (1:4096) of this monospecific serum (data not shown). To characterize the HN glycoprotein antigens further in the isolate from Japan, IDD tests with monospecific antiserum were performed as described previously (Nerome et al., 1978b). IDD tests indicated that duck/Miss/116, duck/Miss/320, duck/Miss/334, duck/Miss/406 and duck/Tokyo/41/78 viruses gave a single line of precipitin identical to that of the homologous virus (Fig. 1a, b). These tests showed that the HN glycoproteins of five viruses isolated in the United States and Japan are closely related to that of duck/Hong Kong/D3/75.

Recently the structural polypeptides of a large number of avian paramyxoviruses have been examined by SDS–polyacrylamide gel electrophoresis (Alexander & Collins, 1981) and these results have shown a basic similarity in the polypeptides, with viruses possessing between six and nine polypeptides. We have now compared the structural polypeptides of two viruses isolated in the United States, which were well characterized in polypeptide composition, with a
recent isolate from Japan. Polypeptide analysis of the viruses was done by polyacrylamide gel electrophoresis under reducing and non-reducing conditions. The polypeptide patterns of duck/Tokyo/41/78 were essentially indistinguishable from those produced by duck/Miss/116 and duck/Miss/334 viruses (Fig. 1c). These viruses possessed six major polypeptides with mol. wt. 80000, 61000, 57000, 48000, 44000 and 40000 to 41000; two were identified as glycoproteins by carbohydrate staining. These polypeptides appear to be the HN protein and the fusion protein with mol. wts. of 80000 and 57000, respectively. A glycoprotein of mol. wt. 140000 was detected in these viruses under non-reduced conditions and these results (not shown) were similar to those reported by Alexander & Collins (1981), and Alexander et al. (1979b). According to the previous reports (Shortridge & Alexander, 1978; Alexander & Collins, 1981; Alexander et al., 1979b), the migration behaviour of structural polypeptides from the same serological group was much more closely related than that of proteins from serologically distinct viruses. Our results also revealed homology in the migration profile of the structural polypeptides of these six viruses. However, a definite conclusion with respect to their origin can only be obtained by analysis of the genome composition using more suitable techniques. We have, therefore, used oligonucleotide fingerprinting methods in the present study to determine whether the avian paramyxoviruses isolated in different countries were genetically homogeneous. Virus RNAs were extracted from egg-grown viruses as described previously (Palese & Schulman, 1976; Ritchey et al., 1976). Purified RNAs were digested with ribonuclease T1 (Sankyo, Tokyo, Japan) and the 5' terminal of the oligonucleotides was labelled with $[^{32}P]$ATP in the presence of polynucleotide kinase (Boehringer, Mannheim) according to the method described by Billeter et al. (1974) and Nakajima et al. (1978). Two-dimensional separation of labelled oligonucleotides was accomplished by polyacrylamide gel electrophoresis (Billeter et al., 1974; de Wachter & Fiers, 1972). Although serological and peptide analysis had revealed that these viruses were very similar to each other, oligonucleotide mapping showed a marked variability in RNAs of these isolates (Fig. 2a, d, e, f).

In order to estimate the degree of homology among the six viruses used in the tests, about 40 large oligonucleotides of duck/Miss/116 virus were selected as markers and are shown by arrows directed to the left because these oligonucleotides were most likely to represent unique sequence (Fig. 2a). Migration of the oligonucleotides of duck/Miss/116 RNA was distinguishable from that of three other viruses (duck/Miss/320, duck/Miss/334, duck/Miss/406) on the basis of only seven to nine common spots (indicated by arrows directed to the left). However, the oligonucleotide maps showed close similarities between the duck/Miss/406 and duck/Miss/334 (Fig. 2d, e). The results, based on spots unique to each virus, revealed that the minimum differences between both RNAs were six spots (arrows pointing to bottom or top), suggesting genetic derivation from a common origin. Analysis of the oligonucleotide patterns of the remaining duck/Miss/320 virus isolated in the same area and comparison with the RNA patterns of duck/Miss/406 and duck/Miss/334 showed essential similarities among them (Fig. 2d, e, f). However, on close examination, many differences (at least 12 spots) were observed between the RNAs from duck/Miss/406 and duck/Miss/320 as indicated by arrows (upper left to lower right or lower right to upper left).

The oligonucleotide analysis suggests that the avian paramyxoviruses isolated from migrating feral ducks in same area in the United States in 1975 may be divided genetically into three groups despite the close similarities based on antigenic and peptide analysis.

Subsequently, we have compared oligonucleotide maps of two viruses isolated in Hong Kong and Japan with the patterns of above-mentioned four viruses. As shown in Fig. 2, close examination indicated that oligonucleotide maps of duck/Hong Kong/D3/75 and duck/Tokyo/41/78 were very similar to those of duck/Miss/116 according to the considerable number of common spots (35 to 38; Fig. 2a, b, c), and only four to five spots of these two viruses migrated differently from the corresponding spots of the latter virus. Although not all 40 spots selected in oligonucleotide maps of duck/Miss/116 virus could be precisely compared with those of duck/Tokyo/41, due to the light intensity of several spots, comparative analysis of large ribonuclease T1-resistant oligonucleotides showed that 35 out of 40 spots were common to both viruses.
Fig. 2. Oligonucleotide maps of the RNAs of six strains of avian paramyxoviruses which were isolated in the United States, Hong Kong and Japan. The first dimension electrophoresis (left to right) was at pH 3.5 on a 10% polyacrylamide gel, and in the second dimension (bottom to top) it was at pH 8.0 in a 21.8% polyacrylamide gel (Billeter et al., 1974; de Wachter & Fiers, 1972). 'X' and 'B' are the positions of dye markers xylene cyanol FF and bromophenol blue. The arrows directed to the left represent large, ribonuclease T1-resistant oligonucleotides present in duck/Miss/116 which were also detected in oligonucleotide maps of the other five viruses. The spots indicated by arrows pointing to the right were present in duck/Hong Kong/D3/75 but absent in duck/Miss/116 virus. The spots indicated by arrows pointing down (d) were present in duck/Miss/406 but missing in duck/Miss/334. The arrows directed to the top (e) show the spots present in duck/Miss/334 but missing in duck/Miss/406. The spots indicated by arrows pointing to the lower right (d) show those present in duck/Miss/406 but not in duck/Miss/320. The spots shown by arrows pointing to the upper left (f) were present in duck/Miss/320 but absent in duck/Miss/406.
The present studies show that nucleotide mapping can distinguish between antigenically closely related strains. The oligonucleotide maps of duck/Miss/116/75 and duck/Hong Kong/D3/75 were very similar to that of duck/Tokyo/41/78 that was isolated from a domestic duck 3 years after the other virus. One can speculate that the avian paramyxovirus found in a domestic duck in Japan in 1978 may originate from viruses which had been circulating in domestic or feral ducks in about 1975. The above studies point out the value of oligonucleotide maps in comparison of closely related strains of virus from different geographical areas of the world.

The authors are grateful to Dr R. G. Webster for providing the avian paramyxoviruses and for commenting on the manuscript.

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REFERENCES

**Short communications**


*(Received 8 September 1982)*