A Comparison of Paramyxoviruses by Immunoprecipitation

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SUMMARY

Using the technique of immune precipitation of [35S]methionine-labelled infected cell polypeptides followed by SDS-polyacrylamide gel electrophoresis and autoradiography it has been shown that SV5 and a closely related isolate are both antigenically related to human parainfluenza virus type 2. Limited cross-reactions were also demonstrated between parainfluenza virus types 1 and 3 by this method and the apparent molecular weights of the major structural components of human parainfluenza virus types 2 and 3 have been deduced.

The parainfluenza (PF) viruses are members of the Paramyxoviridae and most of the information on their properties has been obtained from studying viruses derived from non-human hosts, i.e. Newcastle disease virus (avian), Sendai virus (murine) and SV5 virus (simian). Paramyxoviruses associated with human diseases have been divided into five different species, namely mumps and parainfluenza types 1, 2, 3 and 4 (Kingsbury et al., 1978) but they are relatively poorly characterized since they do not readily give good virus yields in the standard tissue culture systems. The human viruses cause parotitis and a wide range of respiratory illnesses, particularly in children (McIntosh & Clark, 1980) and they have also been associated with persistent infections in adults (Gross et al., 1973; Parkinson et al., 1980). There have also been a number of reports of isolation of paramyxoviruses from tissues derived from multiple sclerosis (MS) patients (for review, see Cook & Dowling, 1980) although these isolations have not been substantiated by other workers. Attempts have recently been made to isolate agents from the bone marrow of MS patients (Mitchell et al., 1978) and although the results have been complicated by the presence of mycoplasmas (Mitchell et al., 1979), a paramyxo-like virus was isolated after passage in human fibroblasts and in Vero cells. By neutralization and fluorescent antibody techniques this isolate (designated RQ) was shown to be closely related to SV5 virus (K. K. A. Goswami & W. C. Russell, unpublished results) but since the original patient had no demonstrable antibodies to this virus and since SV5 virus is known to be a common contaminant of some tissue culture cell lines it seemed very likely that the RQ isolate was a laboratory contaminant. On the other hand, although the SV5 virus was originally isolated (Hull et al., 1956) from rhesus and cynomolgus monkey kidney cell cultures there is now evidence that the virus (or a closely related virus) is widespread in man and domestic animals (for review, see Hsiung, 1972). There is also considerable confusion regarding the classification of SV5 virus and its relatives and some workers have grouped it with parainfluenza type 2 (Isaacson & Koch, 1965) and others in a new grouping, parainfluenza type 5 (Chang & Hsiung, 1965).

In view of this paucity of information on the properties of the human paramyxoviruses and the possible relationship to persistent disease, we have begun to characterize some of the human paramyxoviruses by examining their antigenic interrelationships using the technique of immunoprecipitation of radiolabelled infected cell polypeptides. This procedure is particularly appropriate as there is very little, if any, inhibition of cellular polypeptide synthesis on infection and selection of the virus-coded polypeptides with specific antisera in the presence of staphylococcal protein A (Kessler, 1975) can give sufficient sensitivity to make meaningful comparisons.
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In the initial studies it was found that the RQ isolate propagated readily in Vero cells grown in H21 medium (Gibco) with 10% calf serum, releasing virus into the medium to a titre of approx. 10^6 p.f.u./ml. Concentration of the virus was achieved by adding solid polyethylene glycol (PEG 6000; Hopkin & Williams, Chadwell Heath, Essex, U.K.) to a final concentration of 7.5% and leaving overnight at 4 °C before pelleting by centrifugation (5000 g, 3 h) and then resuspending in dilute buffer (20 mM-tris–HCl pH 7.8, 1 mM-EDTA). The virus was then partially purified by carefully layering the suspension on top of 5 ml of a 15% sucrose solution in dilute buffer over a 2 ml 50% sucrose cushion in a 15 ml centrifuge tube. After centrifugation (100000 g, 3 h) the material at the interface of the sucrose cushion was collected and subjected to centrifugation in a 30 to 100% ‘Urografin’ gradient (Russell et al., 1971) (100000 g, 15 h). A number of discrete bands were obtained and the appropriate fractions containing haemagglutinin (using chick erythrocytes) and infectivity were pooled and analysed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) (Russell & Blair, 1977). Polypeptides of apparent mol. wt. of approx. 70K, 60K, 55K, 38K and 22K were noted (data not shown). This purified virus was then alum-precipitated (Mautner & Willcox, 1974) and inoculated intraperitoneally into rabbits. Boosting some 3 weeks later followed by an intravenous inoculation (without alum) a few days before bleeding produced antisera which reacted specifically in neutralization tests (Cook et al., 1979) against RQ virus and against a prototype SV5 virus obtained from the Central Public Health Laboratories, Colindale. Other prototype human parainfluenza viruses obtained from the same source were not neutralized by these sera, although they were neutralized specifically by standard sera obtained from the Department of Microbiological Reagents and Quality Control, Central Public Health Laboratories, Colindale (namely parainfluenza types 1, 2, 3, 4a, 4b, and mumps). The latter sera had been obtained by inoculation of rabbits with tissue culture fluids from infected cells.
The RQ and SV5 viruses were also found to be indistinguishable by haemagglutination inhibition using the RQ and the standard SV5 antisera. The relationship between these two viruses was further explored by comparing the polypeptide patterns produced by labelling infected Vero cells with [35S]methionine and analysing by SDS–PAGE and autoradiography (Russell & Blair, 1977). A number of labelled polypeptides not detected in uninfected cells could be recognized in cells infected by both SV5 and RQ isolates. The major labelled polypeptide in each infected cell extract had an apparent mol. wt. of about 60K and another, more diffuse band of slower electrophoretic mobility presumably corresponding respectively to the nucleoprotein (NP) and major glycoprotein (HN) polypeptides of SV5 virus as described by Peluso et al. (1977). Closer analysis of the autoradiograms suggested that the NP and HN polypeptides of the RQ isolate had slightly different electrophoretic mobilities from the corresponding SV5 polypeptides. Immune precipitation of labelled cell extracts from BHK21 and Vero cells, infected with both SV5 and RQ viruses using antisera against the two viruses (Fig. 1a, b), confirmed this difference, the HN and NP polypeptides not being so well-separated in the SV5-infected labelled extract as in the case of the RQ-infected cell extract. Labelled uninfected cell extracts gave no significant precipitations in these experiments. Assignations of polypeptides M, P and F1 were made on the basis of previous studies on SV5 (Peluso et al., 1977; Merz et al., 1981).
Fig. 1 (a, b) also shows that immunoprecipitation of both SV5 and RQ nucleoprotein (NP) was obtained with a standard antiserum against parainfluenza type 2, there being no similar cross-reaction with antisera against parainfluenza types 1, 3, 4 and mumps and Sendai antisera (not all data shown). The reciprocal reaction of $^{35}$S-labelled parainfluenza type 2-infected cell extracts with the various antisera is shown in Fig. 1 (c). In this case it is notable that both the HN and NP polypeptides of parainfluenza type 2 as shown in the homologous interaction had significantly slower electrophoretic mobilities than the corresponding polypeptides of SV5 and RQ, i.e. HN had an apparent mol. wt. of about 80K and NP about 74K. The provisional assignments of F1 and M are based on the results of similar studies with other paramyxoviruses. It was also noted that the nucleoprotein polypeptide of parainfluenza type 2 was precipitated by the SV5 and RQ antiserum, indicating a distinct antigenic relationship between the SV5/RQ viruses and type 2 parainfluenza virus. It is not clear without further study if the 55K polypeptide which was also precipitated is a proteolytic breakdown product of NP or is indicative of cross-reaction with another NP-associated protein such as the P protein (the presence of proteolytic enzymes appears to be quite an important factor in these studies and it was only when proteolytic inhibitors, aprotinin, TPCK and TLCK, were included in the extraction buffers that more consistent results were obtained; this was particularly apparent with cells infected by the human parainfluenza viruses). No significant labelled polypeptides were precipitated by reacting parainfluenza type 2-infected cell extracts with antisera against the other parainfluenza viruses (not all data shown).

To ascertain if this lack of cross-reaction between the parainfluenza type 2/SV5/RQ group and the other parainfluenza types was also apparent in the reciprocal reactions, i.e. by precipitation of type 1- and type 3-infected labelled cells with the heterologous antisera, Vero cells infected with human parainfluenza type 3 and chick embryo fibroblast cells infected with Sendai virus (a type 1 murine paramyxovirus) were also labelled with $^{35}$S-methionine and cross-reactions determined by immunoprecipitation. (We were not able to label cells infected with human paramyxovirus type 1 sufficiently well for good immunoprecipitation.) Fig. 2 (a) shows that Sendai virus polypeptides precipitated with a homologous serum had apparent mol. wt. of about 80K, 72K and 62K consistent with previously published values of P, HN and NP polypeptides respectively (Lamb et al., 1976). The Sendai nucleoprotein (NP) polypeptide was precipitated by parainfluenza type 1 antiserum but not by parainfluenza type 3 or other antisera. A serum against parainfluenza type 3 precipitated a number of polypeptides from cells labelled after infection with the type 3 virus (Fig. 2 b). The major band of apparent mol. wt. about 72K, presumably corresponding to the nucleoprotein, was also precipitated with the Sendai antiserum. A polypeptide of apparent mol. wt. 95K is also apparent and by analogy with Sendai virus this could be designated as the P polypeptide. A diffuse band of slightly faster electrophoretic mobility would be consistent with the HN polypeptide. Other polypeptides of approx. mol. wt. 40K and 50K could be assigned to the F1 and M polypeptides by analogy with Sendai virus. The NP polypeptide is precipitated by antisera against PF1 but antisera against the other parainfluenza viruses showed no similar cross-reactions (not all data shown). A rabbit antiserum against purified parainfluenza type 3 virus was prepared as described above for the RQ antiserum and this gave identical results to the standard typing serum. From these studies it is evident then that there is a one-way cross-reaction between the type 1 and type 3 parainfluenza viruses mediated primarily via the nucleoprotein polypeptide.

These studies have therefore demonstrated that a virus which was found in association with an MS bone marrow aspirate after fusion and passage in tissue culture has properties almost identical to a prototype SV5 virus. These findings do not, of course, throw any further light on the possible relationship of SV5 virus to the aetiology of MS but they do make a further.
addition to the list of paramyxoviruses found to be associated with the disease (see Cook & Dowling, 1980). It may be simply that the isolations reflect the ubiquity of the paramyxoviruses or more interestingly that they reflect their propensity to persist in tissues. Whether persistence per se is related to the disease processes is another question and in the light of these conjectures it seemed opportune, as a necessary preliminary, to characterize more definitively the human paramyxoviruses and to examine their interrelationships with paramyxoviruses derived from other species.

The human paramyxoviruses are relatively poorly characterized mainly because they do not grow readily in standard tissue cultures. In the studies described here we have shown, however, that reasonable levels of radioactively labelled polypeptides can be obtained in cells infected with parainfluenza viruses types 2 and 3. The virus polypeptides have been selected by immune precipitation with specific antisera and comparative analyses have been carried out to study the antigenic interrelationships with some other paramyxoviruses. Attempts have also been made to label cells infected with the types 1 and 4 human paramyxoviruses but these have not been so successful even when utilizing other cell systems such as primary baboon kidney cells.

The investigations described in this paper have clearly shown that there is a relationship between the SV5/RQ virus and human paramyxovirus type 2 primarily in terms of common antigenic sites on the nucleoprotein. Indeed, this relationship is similar to that noted between the nucleoproteins of Sendai and human paramyxovirus type 1. Since Sendai virus is accepted as a type 1 paramyxovirus (Kingsbury et al., 1978) it seems reasonable to place the SV5 virus group in a similar relationship to human parainfluenza type 2. Thus, there seems no justification for creating another type 5 grouping as suggested by Hsiung (1972). It will be interesting to ascertain if there is a similar relationship between the canine paramyxovirus (CP-1), which is closely related to SV5 (Evermann et al., 1981), and human paramyxovirus type 2. There is also an antigenic cross-reaction between types 1 and 3 human paramyxoviruses as indicated by precipitation of nucleoprotein of type 3 by Sendai and type 1 antisera. This cross-reaction, however, is not reciprocated when analysing Sendai virus-infected cells. Thus, although the Sendai nucleoprotein was precipitated by both the Sendai and type 1 antisera, no significant precipitation was obtained with the type 3 antisera. These results suggest that there is a relationship between types 1 and 3 which is not apparent with the other types and with mumps virus. It should be considered, however, that it is possible that antisera derived from animals other than rabbits could demonstrate different degrees of cross-reaction in these tests.

There have been a number of reports mainly from Choppin and his colleagues on the properties of SV5 polypeptides (Peluso et al., 1977; Etkind et al., 1980) and the results described here are very similar to their findings. Sendai virus polypeptides have been characterized by a number of different investigators (e.g. Lamb et al., 1976) and our results are in general agreement. The human paramyxoviruses have not been analysed in terms of polypeptides in any detail although there has been a recent report on those of parainfluenza type 3 (Guskey & Bergstrom, 1981) and a few on mumps virus polypeptides (Naruse et al., 1981; McCarthy & Johnson, 1980; Rima et al., 1980). It is noteworthy that in these experiments the nucleoprotein polypeptides of PF3 and mumps, of apparent approx. mol. wt. 69K and 68K respectively, were significantly larger than those from the paramyxoviruses of the avian and murine species so far described. Our own studies, indicating mol. wt. of approx. 74K and 72K for the NP polypeptides of parainfluenza virus types 2 and 3 respectively, are consistent with this trend towards larger nucleoproteins in the human paramyxoviruses.

These limited investigations have shown the utility of the immunoprecipitation technique in analysing antigenic relationships among the paramyxoviruses although the results, to a large extent, will be governed by the quality of the antisera. The development of the monoclonal
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techniques of Kohler & Milstein (1975) and their application to the complex antigenic structures present in the paramyxoviruses should allow a more definitive characterization of these relationships in the future.

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