J. gen. Virol. (1986), **67**, 281–287. *Printed in Great Britain*

Key words: *mumps virus/antigenic subtypes/monoclonal antibodies*

Antigenic Variation of Envelope and Internal Proteins of Mumps Virus Strains Detected with Monoclonal Antibodies

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(*Accepted 9 October 1985*)

SUMMARY

Antigenic characteristics of nine mumps virus strains were determined by immunofluorescence and radioimmunoprecipitation assay (RIPA) using a collection of 44 monoclonal antibodies. These antibodies were directed against five different structural components of mumps virus, the haemagglutinin–neuraminidase (HN), fusion (F), matrix (M), phospho- (P) and nucleocapsid (NP) proteins. The nine mumps virus strains could be divided into two groups according to their antigenic characteristics. One group included two strains isolated more than a decade ago and the Jeryl Lynn vaccine strain. These three strains reacted with a wider range of monoclonal antibodies than the second group of six recently isolated strains of different geographical origin. In the F, M and P proteins variations were only found in single antigenic determinants. In the HN and NP components, RIPA revealed variations in three and seven determinants respectively. The Jeryl Lynn vaccine strain showed a unique lack of reaction with one anti-HN antibody clone in the RIPA.

INTRODUCTION

Mumps virus is monotypic as determined with polyclonal sera in neutralization assays (Cantell, 1961; Kingsbury *et al.*, 1978; Norrby, 1985). Re-infections with monotypic viruses are not considered possible because of the solid life-long immunity induced by the primary infection. This contrasts with antigenically unstable viruses such as influenza where rapid changes of antigens may occur and immunity is not life-long. The reason for this difference in stability between monotypic and antigenically unstable viruses remains to be elucidated. With the advent of monoclonal antibodies it is possible to analyse antigen characteristics of viral proteins with greater accuracy (Norrby, 1985; Örvell & Norrby, 1985; Yewdell & Gerhard, 1981). This has led to the detection of differences between strains of monotypic viruses (e.g. measles; Sheshberadaran *et al.*, 1983).

Although re-infection with mumps virus is assumed not to occur, many patients and many physicians have observed more than one 'mumps' infection with swollen parotid glands. Whether these are due to re-infections by mumps virus or the result of infections with other viruses such as the parainfluenza viruses (Zollar & Mufson, 1970) which also can cause parotitis, remains unclear. Analysis of the surface characteristics of different isolates showing wide variation in viral proteins, especially the surface proteins, can indicate but not prove whether re-infection is possible. Previous studies on mumps virus have revealed some differences between laboratory strains. The molecular weight of the haemagglutinin–neuraminidase (HN) proteins was found to differ slightly (McCarthy & Johnson, 1980), and the antigenic characteristics of laboratory strains were found to vary in the HN (Server *et al.*, 1982; Örvell, 1984; Wolinsky *et al.*, 1985), fusion (F) (Wolinsky *et al.*, 1985), nucleocapsid (NP) and phospho- (P) proteins (Örvell, 1984). Biological tests have also shown differences in neuropathogenicity (McCarthy *et al.*, 1980) and haemagglutinin antigen (Cantell, 1961).

In this study, several mumps virus strains, mostly recent isolates from different parts of the world, were compared with the aid of a large collection of monoclonal antibodies in order to assess the antigenic stability of envelope and internal components of mumps virus.

METHODS

Viruses and cells. Nine mumps virus strains were used for this study. Six were recent isolates from different parts of the world. One was from Reykjavik, Iceland (no. 107), three from Stockholm, Sweden (no. 321, 761 and 859) and two from Buenos Aires, Argentina (designated CJO and CRS). All six strains were within four passages from initial isolation when subjected to analysis. None of the isolates was more than 4 years old. Attempts were made to select strains from patients with different symptoms and from various sources of isolation. Strains 107, 761, 859 and CJO were from patients with meningitis symptoms. The 107 strain was isolated from a throat wash but strains 761, 859 and CJO were from cerebrospinal fluid. The CRS strain was isolated from throat washings from a patient with swollen parotid glands. No. 321 was from abortion material after a subclinical infection.

Three older strains were also analysed. The S69 and SBL-1 strains were isolated in Stockholm in the years 1969 and 1971 respectively. Their source of isolation and patient symptoms they caused are unknown. The last strain included was the Jeryl Lynn (JL) vaccine strain obtained as lyophilized vaccine from Merck, Sharp & Dohme. It had been attenuated by several passages in chicken embryo fibroblasts (Buynak & Hilleman, 1966).

All virus strains were grown in Vero cells, which were used for all experiments. The cell culture medium was Eagle's MEM (Flow Laboratories) supplemented with penicillin (60 μg/l), streptomycin (50 μg/l) and 5% inactivated newborn calf serum (Flow Laboratories). Culture bottles were purchased from Nunc (Roskilde, Denmark).

Monoclonal antibodies. A large number of monoclonal antibodies directed against the proteins of the SBL-1 strain of mumps virus have been characterized and extensively described in a recent publication (Örvell, 1984). The numbers of monoclonal antibodies used in this study were seven, five, 14, nine and nine directed against the HN, F, NP, P and matrix (M) proteins respectively.

Most of the 44 antibody clones were directed against separate antigenic sites. However, several were related giving similar blocking patterns in ELISA competition experiments (Örvell, 1984). Thus, anti-HN (αHN) clones 2034, 2082 and 5342 were identical and so were αF clones 2109 and 5418. Similar, but not identical, were αHN clones 1992 and 5374, αP clones 689, 1997 and 2069 and αM clones 657, 1909 and 2119.

Antigenic characterization of viral proteins

Radioimmunoprecipitation assay (RIPA). This was performed with previously described methods (Örvell & Norrby, 1980; Sheshberadaran *et al.*, 1983). The virus strains were inoculated and adsorbed onto Vero cells in 175 cm^2 culture bottles and at the time when 10 to 30% of the cell layer had started to show cytopathic effects, 600 μCi [^{35}S]methionine was added in 12 ml MEM without unlabelled methionine. Subsequent steps were carried out as described previously (Örvell & Norrby, 1980; Sheshberadaran *et al.*, 1983).

Immunofluorescence studies (IF). Confluent Vero cell monolayers grown on microscope slides in Leighton tubes were infected with the different virus strains. When cytopathic effects were extensive the cells were fixed in cold (−20 °C) acetone and stored at −20 °C until used. The monoclonal antibodies used were a 1 : 50 dilution of the original ascites. After incubation with the antibodies for 20 min, the slides were washed with phosphate-buffered saline (PBS) after which goat anti-mouse fluorescein-labelled antibodies (Nordic Immunological Laboratories, Tilburg, The Netherlands) were added and incubated for 20 min. After washing and mounting with a glycerol : water (4 : 1) solution, the slides were examined with an epifluorescence microscope (Kristensson *et al.*, 1983).

Haemagglutination inhibition (HI). The virus material used was obtained from infected Vero cells showing extensive cytopathic effect. After harvesting, it was spun down in a Sorvall GSA rotor at 5875 *g* for 20 min. After resuspension in PBS the virus material was sonicated and then treated with Tween 80 and ether (Norrby, 1962). The assay was performed using the microtitre technique as previously described (Örvell, 1976) using 4 haemagglutinating units of virus material. Guinea-pig blood diluted to 0·5% in PBS was used. All assays were performed at 4 °C. Only one αHN clone, 5500, was used. Other αHN clones had no HI activity against the SBL-1 strain which had been used for their production.

RESULTS

Comparative analysis of envelope protein antigens of mumps virus strains

The two older strains, SBL-1 and S69, reacted with all seven HN monoclonal antibodies; however, the S69 strain reacted only weakly with clones 5374 and 5500 in IF tests (Table 1). The Jeryl Lynn strain showed a unique lack of reaction with antibody clone 5374 in both IF and RIPA, but reacted with all other monoclonal antibodies. In contrast, the recently isolated strains

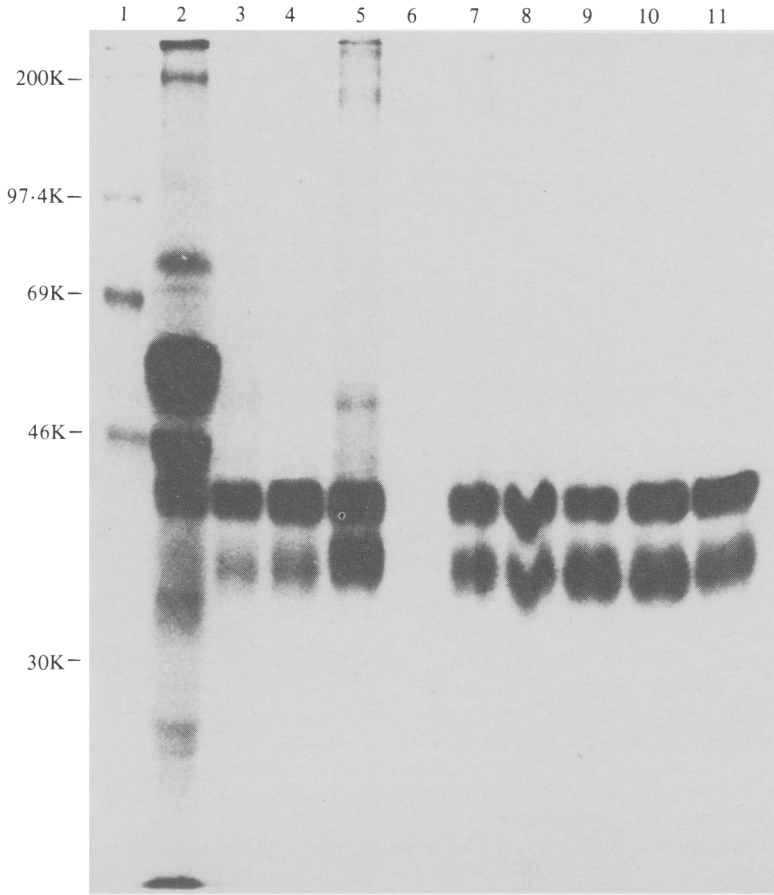

Fig. 1. RIPA picture of strain 107 reacted with αM clones. Lane 1, molecular weight markers; lane 2, anti-mumps virus whole serum; lanes 3 to 11, clones 654, 657, 693, 745, 778, 1909, 2107, 2119, 2124 respectively. The double bands are due to breakdown of the proteins during the RIPA procedure.

Table 1. *Reaction pattern of mumps virus strains with monoclonal antibodies against the envelope proteins HN and F*

Monoclonal antibody	SBL-1 IF/R*	HI†	S69 IF/R	HI	JL IF/R	HI	107 IF/R	HI	321 IF/R	HI	761 IF/R	HI	859 IF/R	HI	CJO IF/R	HI	CRS IF/R	HI
αHN 1933	+/+		+/+		+/+		−/w		−/w		−/−		−/−		−/−		w/−	
1992‡	+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+	
5374‡	+/+		w/+		−/−		+/+		−/+		+/+		+/+		+/+		+/+	
2034§	+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+	
2082§	+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+	
5342§	+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+	
5500	+/+	2048	w/+	64	+/+	1024	−/w	32	−/w	16	−/−	32	−/−	8	−/−	16	−/−	16
αF 2109‖	+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+	
5418‖	+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+	
2159	+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+	
5439	+/+		w/w		−/−		w/−		−/−		−/−		−/−		−/−		−/−	
5525	+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+		+/+	

* IF, Immunofluorescence; R, RIPA. Results are expressed as: +, positive reaction; −, negative reaction; w, weak and variable reaction.

† HI, Haemagglutination inhibition titre.

‡ Bind to similar antigenic sites (Örvell, 1984).

§ Bind to the same antigenic site.

‖ Bind to the same antigenic site.

Table 2. *Reaction pattern of mumps virus strains with monoclonal antibodies against the P protein*

Monoclonal antibody	SBL-1 IF/R*	S69 IF/R	JL IF/R	107 IF/R	321 IF/R	761 IF/R	859 IF/R	CJO IF/R	CRS IF/R
632	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
680	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
689†	+/+	−/+	+/+	−/−	−/−	−/−	−/w	−/−	−/w
1997†	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2005	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2037	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2060	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2067	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2069†	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+

* See Table 1 (*).
† Bind to similar antigenic sites (Örvell, 1984).

Table 3. *Reaction pattern of mumps virus strains with monoclonal antibodies against the NP protein*

Monoclonal antibody	SBL-1 IF/R*	S69 IF/R	JL IF/R	107 IF/R	321 IF/R	761 IF/R	859 IF/R	CJO IF/R	CRS IF/R
652	+/+	+/w	+/+	w/−	w/−	+/−	w/−	w/−	+/−
667	+/+	+/+	−/w	w/−	w/−	−/−	w/−	−/−	−/−
728	+/w	+/−	+/w	+/−	+/−	+/−	+/−	+/−	+/−
781	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2002	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2004	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2045	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2050	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2054	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2099	+/+	+/+	+/+	+/+	+/w	+/w	+/w	+/w	+/w
2111	+/+	−/+	w/+	+/−	w/w	w/−	+/−	+/−	−/−
2132	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
2142	+/+	+/+	+/+	+/w	+/−	+/−	+/−	+/−	+/w
5495	+/w	+/+	w/w	+/−	+/w	+/−	+/−	+/−	+/−

* See Table 1 (*).

did not react or reacted only weakly with antibody clones 1933 and 5500. Strain 321 did not react with clone 5374 in IF whereas the other recent isolates did.

The five monoclonal antibodies against the F protein identified four antigenic sites. One of the antibody clones, 5439, reacted strongly only with the SBL-1 strain, whereas reactions with other strains were weak or in most cases absent.

Comparative analysis of the internal proteins of the mumps virus strains

The M protein of all nine strains reacted identically with eight out of nine monoclonal antibodies tested. The only variation in reaction was found with clone 745 which reacted weakly or not at all with strains 107, 321 and 761 in both IF and RIPA (Fig. 1). Other strains reacted well with this clone.

The P protein of all strains also reacted identically with eight out of nine antibody clones (Table 2). One clone, 689, reacted with the Jeryl Lynn and S69 strains in addition to SBL-1, but not at all or only poorly with the other strains.

The greatest differences between strains were found in the NP (Table 3). Of the 14 clones, 12 or 13 reacted with the S69 and Jeryl Lynn strains in both IF and RIPA tests, although some reactions were weak. On the other hand, the six recently isolated strains reacted with eight to ten clones in both IF and RIPA. In general the IF test revealed more positive reactions than the RIPA test.

DISCUSSION

Monoclonal antibodies are very useful for investigating surface characteristics of viral proteins (Norrby, 1985; Örvell & Norrby, 1985; Yewdell & Gerhard, 1981). Relationships between different viruses have been substantiated (Norrby *et al.*, 1985) and intratypic variations of viruses have been demonstrated with the use of monoclonal antibodies (Sheshberadaran *et al.*, 1983).

The paramyxoviruses are monotypic viruses (Cantell, 1961; Kingsbury *et al.*, 1978; Norrby, 1985) because of their stability compared to some other viruses such as influenza virus which exhibits rapid changes in its surface proteins (Webster *et al.*, 1982). However, monoclonal antibodies have detected differences in strains of measles and respiratory syncytial (RS) viruses in addition to this present study of mumps. Measles virus shows slight variations between strains, mostly in the M protein but also to some extent in the haemagglutinin protein (Sheshberadaran *et al.*, 1983). RS virus is composed of two variants both of which can circulate in the population at the same time. These variants exhibit extensive differences in the G envelope glycoprotein and some differences in other proteins (Mufson *et al.*, 1985).

The results of this study show that antigenic variations between mumps strains occur. The two older strains, SBL-1 and S69, showed only minor variations in antigenic sites and the six recently isolated strains also proved to be very similar. However, differences between the two older strains, SBL-1 and S69, and the six recently isolated ones were found in four viral proteins. This indicates that evolution has occurred in the mumps genome within the span of 10 to 15 years.

The HN and NP viral proteins are the proteins exhibiting greatest variability. The HN shows differences in three out of five antigenic sites tested. The seven αHN clones used in this study were directed against at least five antigenic sites, clones 2034, 2082 and 5342 being similar. These three clones did not detect any variability in their site of recognition, indicating its stability. The same can be said for clone 1992, but clone 5374, which has similar features in competition experiments (Örvell, 1984), showed some variation in the mumps strains. This indicates that subtle changes have occurred in the HN protein structure, leading to altered reactivity with clone 5374 but leaving the presumably overlapping site detected by clone 1992 intact. The two clones 5500 and 1933 reacted with unique antigenic sites. Both demonstrated differences among the nine viral strains. Both clones 5500 and 1933 reacted better with the older strains SBL-1 and S69 and the Jeryl Lynn strain than with the six recently isolated strains. This indicates that loss or alteration of specific antigenic sites has occurred over time. Clone 5500 gave very high HI activity with strains SBL-1 and Jeryl Lynn indicating a reaction with the attachment site, or one very close, in these viral strains. Loss of reaction with this clone, as has happened totally or partially with other strains, indicates vulnerability of this viral attachment site.

The NP exhibited some variations among the strains. Several monoclonal antibodies (no. 781, 2002, 2004, 2045, 2050, 2054 and 2132) to the NP showed strong reactions with all nine strains, demonstrating stability of their corresponding antigenic site. Other antibodies generally reacted better with the older strains SBL-1 and S69 as well as the Jeryl Lynn strain than with the six recently isolated strains. Most antibodies showed positive or weak reactions with all nine strains in IF, indicating destruction of the antigenic sites during processing for RIPA (Norrby, 1985). This could either be because of detergent lability of the antigenic site itself or due to conformational changes which lead to non-reactivity with one or more monoclonal antibodies. One mutational change leading to greater sensitivity to detergents could thus cause several monoclonal antibodies not to react, although directed to different antigenic sites. Because of this, the degree of difference between the strains is difficult to assess. It might be considerably less than the number of different antigenic sites that RIPA indicates. The acetone treatment used for fixation before IF staining does not seem to cause extensive denaturation of the proteins.

Only one clone, 745, detected differences among strains in the M protein. Here the differences are found among the six recently isolated strains, three reacting well with clone 745 and three reacting poorly. One interpretation of this phenomenon is that an evolutionarily favourable change has occurred and viral strains harbouring this change are gradually taking over in the

population. This finding is in contrast to most other variations in the mumps strains where the six recently isolated strains represent one group showing similar antigenic properties and the older strains another group.

The similarities within the two groups of strains, the six recently isolated ones and the older two, SBL-1 and S69, support the view that only one mumps virus strain is circulating in the population at a given time. This points to greater evolutionary fitness of the newly appearing strains, possible because of a more effective immune response against the older strains.

It is of interest that the Jeryl Lynn vaccine was found to be similar to the SBL-1 and S69 strains. The Jeryl Lynn strain was passaged several times in chicken embryo fibroblasts (Buynak & Hilleman, 1966) with consequent reduction of its pathogenic capacity. The similarities between the Jeryl Lynn strain and the SBL-1 and S69 strains indicate that only subtle changes in the protein structure are needed for attenuation. The Jeryl Lynn strains did not react with αHN monoclonal antibody 5374 with which all other strains reacted except for two: S69 which reacted weakly, and 321 which failed to react in IF. No other markers were unique to the Jeryl Lynn strain. Small structural changes with functional consequences can be highly important in pathogenicity and virulence and are possibly not detectable by the methods used. This could be because (i) too few antibody clones were used and therefore did not cover all important epitopes, or (ii) small quantitative changes in antigen–antibody binding are not detectable with the largely qualitative methods used, or (iii) the experimental procedure might destroy important epitopes.

The strains were isolated from three different sources of infection, i.e. cerebrospinal fluid, mouth wash and abortion material. The organ in which the virus had grown does not seem to have affected the pattern of antigenic sites of the viral strains.

This study demonstrates that many antigenic sites on mumps virus proteins have been stable over a period of 10 to 15 years. Other sites seem, however, to be more vulnerable to alterations. This should be taken into consideration when monoclonal antibodies are used for diagnostic purposes. Whether the extent of change is sufficient to enable re-infection with mumps virus is doubtful.

The authors wish to thank Dr Margret Gudnadottir, Reykjavik, Iceland, Dr Monica Grandien, Stockholm, Sweden, Dr Marianne Forsgren, Stockholm, Sweden and Dr Sueko Takimoto, Buenos Aires, Argentina, for kindly donating the viral strains, Mrs Marianne Jellne and Miss Marie Mannerlöf for excellent technical assistance and Mrs Kathrin Häger and Mrs Lena Björklund for typing the manuscript. This work was supported by grant no. B85-16X-0016-21B from the Swedish Medical Research Council.

REFERENCES

BUYNAK, E. B. & HILLEMAN, M. R. (1966). Live attenuated mumps virus vaccine. 1. Vaccine development. *Proceedings of the Society for Experimental Biology and Medicine* **123**, 768–775.

CANTELL, K. (1961). Mumps virus. *Advances in Virus Research* **8**, 123–164.

KINGSBURY, D. W., BRATT, M. A., CHOPPIN, P. W., HANSON, R. P., HOSAKA, Y., TER MEULEN, V., NORRBY, E., PLOWRIGHT, W., ROTT, R. & WUNNER, W. H. (1978). Paramyxoviridae. *Intervirology* **10**, 137–152.

KRISTENSSON, K., ÖRVELL, C., LEESTMA, J. & NORRBY, E. (1983). Sendai virus infection in the brains of mice: distribution of viral antigens studied with monoclonal antibodies. *Journal of Infectious Diseases* **147**, 297–301.

McCARTHY, M. & JOHNSON, R. T. (1980). A comparison of the structural polypeptides of five strains of mumps virus. *Journal of General Virology* **46**, 15–27.

McCARTHY, M., JUBELT, B., FAY, D. B. & JOHNSON, R. T. (1980). Comparative studies of five strains of mumps virus *in vitro* and in neonatal hamsters: evaluation of growth, cytopathogenicity and neurovirulence. *Journal of Medical Virology* **5**, 1–15.

MUFSON, M. A., ÖRVELL, C., RAFNAR, B. & NORRBY, E. (1985). Two distinct sybtypes of human respiratory syncytial virus. *Journal of General Virology* **66**, 2111–2124.

NORRBY, E. (1962). Hemagglutination by measles virus. IV. A simple procedure for production of high potency antigen for hemagglutination inhibition (HI) tests. *Proceedings of the Society for Experimental Biology and Medicine* **111**, 814–818.

NORRBY, E. (1985). Variability of antigen epitopes of monotypic viruses. In *Proceedings of the Fourth International Symposium on Rapid Methods and Automation in Microbiology and Immunology*. Edited by K. O. Habermehl. Heidelberg: Springer-Verlag (in press).

NORRBY, E., SHESHBERADARAN, H., McCULLOUGH, K., CARPENTER, W. C. & ÖRVELL, C. (1985). Is rinderpest virus the archevirus of the morbillivirus genus? *Intervirology* **23**, 228–232.

ÖRVELL, C. (1976). Identification of paramyxovirus-specific haemolysis-inhibiting antibodies separate from haemagglutination-inhibiting and neuraminidase-inhibiting antibodies. *Acta pathologica et microbiologica scandinavica* (*Section B, Microbiology*) **84**, 441–450.

ÖRVELL, C. (1984). The reactions of monoclonal antibodies with structural proteins of mumps virus. *Journal of Immunology* **132**, 2622–2629.

ÖRVELL, C. & NORRBY, E. (1980). Immunological relationships between homologous structural polypeptides of measles and canine distemper virus. *Journal of General Virology* **50**, 231–245.

ÖRVELL, C. & NORRBY, E. (1985). Antigenic structure of paramyxoviruses. In *Immunochemistry of Viruses. The Basis for Serodiagnosis and Vaccines*, pp. 241–264. Edited by M. H. V. van Regenmortel & A. R. Neurath. New York: Elsevier.

SERVER, A. C., MERZ, D. C., WAXHAM, M. N. & WOLINSKY, J. S. (1982). Differentiation of mumps virus strains with monoclonal antibody to the HN glycoprotein. *Infection and Immunity* **35**, 179–186.

SHESHBERADARAN, H., CHEN, S. N. & NORRBY, E. (1983). Monoclonal antibodies against five structural components of measles virus. I. Characterization of antigenic determinants on nine strains of measles virus. *Virology* **128**, 341–353.

WEBSTER, R. G., LAVER, W. G., AIR, G. M. & SCHILD, G. C. (1982). Molecular mechanisms of variation in influenza viruses. *Nature, London* **296**, 115–121.

WOLINSKY, J. S., WAXHAM, M. N. & SERVER, A. C. (1985). Protective effects of glycoprotein-specific monoclonal antibodies on the course of experimental mumps virus meningoencephalitis. *Journal of Virology* **53**, 727–734.

YEWDELL, J. W. & GERHARD, W. (1981). Antigenic characterization of viruses by monoclonal antibodies. *Annual Review of Microbiology* **35**, 185–206.

ZOLLAR, L. M. & MUFSON, M. A. (1970). Acute parotitis associated with parainfluenza 3 virus infection. *American Journal of Diseases of Children* **119**, 147–148.

(*Received 31 July 1985*)