Isolation and Characterization of Monoclonal Antibodies to Simian Virus 5 and Their Use in Revealing Antigenic Differences between Human, Canine and Simian Isolates

By R. E. RANDALL,* D. F. YOUNG, K. K. A. GOSWAMI AND W. C. RUSSELL

Department of Biochemistry and Microbiology, University of St Andrews, St Andrews, Fife KY16 9AL, U.K.

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SUMMARY

Hybridomas secreting monoclonal antibodies to simian virus 5 (SV5) were obtained following immunization of mice with purified preparations of a human isolate (LN) of SV5. Immune precipitation studies showed that these monoclonal antibodies had specificities for the haemagglutinin-neuraminidase (HN), fusion (F), nucleo-, matrix and phospho- (P) proteins of SV5. By use of a radioimmune competition assay the monoclonal antibodies to the HN protein were assigned to four groups, members of which recognized different antigenic sites on the protein. All the anti-HN antibodies and the anti-F antibody neutralized virus infectivity. The 54 monoclonal antibodies obtained were used to determine whether there were antigenic differences between five human, two canine and one simian isolate of SV5. Although most of the monoclonal antibodies reacted with all isolates, a few did reveal antigenic differences in the HN, F and P proteins. Furthermore, analysis by SDS-PAGE showed that while the electrophoretic mobilities of most of the virus polypeptides of these isolates were similar some differences could be detected. In particular the P protein showed the most marked mobility differences between the human, canine and simian isolates. Slight differences in the mobility of the F1 glycoprotein could also be visualized.

INTRODUCTION

Simian virus 5 (SV5) has been isolated from the tissues of a number of animals including dogs, monkeys and humans. In dogs, SV5 commonly causes minor respiratory tract infections, although the virus has also been recovered from the cerebrospinal fluid of a dog with temporary posterior paralysis (Baumgartner et al., 1981). This particular isolate has subsequently been shown to cause experimentally induced encephalitis when inoculated into gnotobiotic dogs (Baumgartner et al., 1982). Because SV5 commonly infects dogs the virus is usually referred to in veterinary circles as canine parainfluenza virus. However, SV5 was first isolated from rhesus monkey kidney cell cultures (Hull et al., 1956) and contamination of primary monkey cell lines with SV5 has been commonly encountered, presumably because monkeys may be persistently infected with the virus (Hsiung, 1972). The origin of SV5 infections in monkeys is open to question (Hsiung, 1972) and it has been proposed that monkeys acquire infection via contact with humans after being brought into captivity (Tribe, 1966). On a number of occasions the isolation of SV5 from human tissue has been reported (Hsiung, 1972; Robbins et al., 1981), including on five separate occasions from the bone marrows of multiple sclerosis patients (Goswami et al., 1984; Mitchell et al., 1978). Limited seroepidemiological studies have indicated that a reasonably high proportion (20 to 40%) of people have antibodies that react with SV5 (Hsiung, 1972; Goswami et al., 1984). Although SV5 has not been associated with any acute human disease, recent evidence suggests that it may be involved in the aetiology of at least some cases of multiple sclerosis. The basis of this evidence is that a number of multiple sclerosis...
patients have antibody to SV5 in their cerebrospinal fluid and that these antibodies appear to form specific oligoclonal bands since the bands can be absorbed out with purified preparations of SV5 (Goswami et al., 1987).

SV5 is a member of the paramyxovirus family (Kingsbury et al., 1978) and is antigenically related to parainfluenza virus type 2 (Goswami & Russell, 1982). The virion contains six structural proteins, the haemagglutinin–neuraminidase (HN), matrix (M), nucleo- (NP), phospho- (P), large (L) and fusion (F) proteins (Mountcastle et al., 1971; McSharry et al., 1975; Peluso et al., 1977). The HN and F proteins are glycosylated and form spikes which protrude from the virus envelope. The HN protein has haemagglutinin and neuraminidase activity and the F protein is involved in cell to cell fusion (see Choppin & Scheid, 1980). The M protein is located on the inner surface of the virion envelope and the NP, P and L proteins associate with the single-stranded genomic RNA to form a ribonucleoprotein complex termed the nucleocapsid (McSharry et al., 1975; Buetti & Choppin, 1977). SV5 also encodes two non-structural proteins, V and SH (small hydrophobic); polypeptide V appears to be a truncated form of the P protein (Paterson et al., 1984; Hiebert et al., 1985).

A limited number of monoclonal antibodies (MAbs) to SV5 has been previously isolated: two to the HN glycoprotein and two to the virus NP protein (Goswami & Russell, 1983). In this paper we report on the isolation and characterization of a more comprehensive bank of 54 MAbs to SV5 and their use in detecting antigenic differences between human, canine and simian isolates of SV5.

METHODS

Cells and viruses. Vero and BHK cells (Flow Laboratories) were grown as monolayers in 75 cm² tissue culture flasks or in rotating 80 oz Winchester bottles, in Dulbecco’s modification of Earle’s tissue culture medium containing 10% newborn calf serum. Five human isolates of SV5 (designated LN, RQ, DN, NR, MT; Goswami et al., 1984), two canine isolates (M7 and CPI-78-238; Cornwell et al., 1976 and Baumgartner et al., 1981, respectively) and a prototype simian isolate (Pr; obtained from Central Public Health Laboratory, Colindale, London, U.K.) were grown and titrated under appropriate conditions in Vero cells using medium containing 2% calf serum. All the virus isolates were plaque-purified and the virus stocks obtained were free from mycoplasma contamination. Furthermore, special emphasis was placed on obtaining virus stocks free from high levels of defective interfering particles. This was achieved by infecting the monolayers with approximately 0-1 p.f.u./cell and harvesting the virus present in the culture medium when the cells were showing between 70 and 80% c.p.e. All virus stocks were harvested before 30 h post-infection.

Purification of virus. Monolayers of Vero cells in rotating Winchester bottles were infected with SV5 strain LN at an m.o.i. of 0-1 p.f.u./cell. When the cells showed 10 to 20% c.p.e., the medium was decanted, the monolayer washed twice with warm phosphate-buffered saline (PBS) and the infected cells were reincubated at 37°C in medium without calf serum until 80% of the cells showed a c.p.e. (6 to 10 h later). The medium was then harvested and the small amount of cell debris present was pelleted by low speed centrifugation (10000 g for 15 min), followed by filtration through Whatman No. 1 filter paper. The virus was then pelleted by high speed centrifugation (70000 g for 3 h).

Immunization of mice, production and selection of antibody-secreting hybridomas and nomenclature for MAbs. Inbred BALB/c mice were immunized intraperitoneally with purified preparations of the SV5 isolate LN (250 μg of protein per immunization) precipitated on alum (Chase, 1967). At the same time mice were immunized with a killed and fixed suspension of Bordetella pertussis (2 x 10⁸ cells per mouse) to enhance the immune response (Köhler, 1981). After 28 days, mice were reimmunized intraperitoneally with virus only, and after a further 21 days they were boosted by both an intraperitoneal and a tail vein immunization. Four days later their spleens were removed, the splenocytes were fused with SP2/0-Ag-14 myeloma cells (Shulman et al., 1978) and the hybrid cells were plated in 96-well microtitre plates so that approximately one colony of cells grew in every other well under selective growth conditions. Cell culture conditions were based on those previously described (Fazekas de St. Groth & Scheidegger, 1980; Köhler & Milstein, 1975). Hybridomas secreting antibodies specific for virus antigens were differentiated from those secreting antibody to uninfected cell antigens using a method in which infected or uninfected cell antigens were bound to sheets of nitrocellulose, media from hybridoma cells were then incubated with the nitrocellulose sheets, and bound antibody was identified using ¹²⁵-I-labelled Protein A or with peroxidase-conjugated anti-mouse immunoglobulin. Full details of the technique have been published elsewhere, as have the methods for subcloning hybridomas secreting specific antibody to virus antigens and for the production of ascitic fluids (Randall et al., 1984). Each MAb was originally given a laboratory number corresponding to the hybridoma colony tested. Subsequently when the reactivity of the MAbs had been established, they were given a capital letter
corresponding to the virus protein recognized, followed by a trivial small letter as identifying labels for individual antibodies, e.g. HNα. Monoclonal antibodies that reacted with the HN protein were tested for their ability to compete with the binding of other MAbs with reactivities to the same protein. If any competition between two antibodies was noted these antibodies were placed in a group which was given a number that was incorporated into the name of a particular antibody e.g. HN-4a, HN-4b, HN-5a, HN-5b etc. The full name of the antibody was prefixed with SV5 to denote the antibody was isolated from mice immunized with SV5 e.g. SV5-HN-4a. It should be noted that the nomenclature for the two MAbs to the HN glycoprotein previously isolated by Goswami & Russell (1983) has not been altered significantly i.e. MAb HN-1 in their nomenclature is now referred to as MAb HN-1a.

Preparation of radiolabelled antigen extracts, immune precipitation and SDS-PAGE. Vero or BHK cell monolayers in 75 cm² tissue culture flasks or in rotating 80 oz Winchester bottles were infected with 0·1 p.f.u. of SV5 per cell. After an adsorption period of 2 h at 37 °C, the inoculum was removed and replaced with tissue culture medium containing 2% newborn calf serum. When the cells showed a 10 to 20% c.p.e. they were radioactively labelled for 4 to 6 h with L-35S]methionine (500 Ci/mmol; Amersham) in tissue culture medium containing one-tenth the normal concentration of methionine (i.e. 1·5 mg/l). At the end of the labelling intervals, the cells were washed in ice-cold PBS and lysed into immune precipitation buffer [10 mm-Tris-HCl pH 7·2, 5 mm-EDTA, 0·5% NP40, 0·65 M-NaCl and in some experiments 0·1% SDS (see text)]; 4 × 10⁶ to 10 × 10⁶ cells per ml of buffer] by sonication with an ultrasonic probe. Soluble antigen extracts were obtained after pelleting the particulate material from these total cell antigen extracts by centrifugation at 400000 g for 30 min. Immune complexes were formed by incubating 0·2 ml samples of the soluble antigen fraction with an excess of antibody (1 µl of undiluted ascitic fluid) for 2 h at 4 °C. If the antibody involved bound directly to Protein A the immune complexes were isolated on an excess of a fixed suspension (Kessler, 1975) of the Cowan A strain of Staphylococcus aureus (20 µl of a 10% w/v suspension per µl of ascitic fluid for 30 min at 4 °C). If the MAbs did not bind Protein A directly, these immune complexes were isolated in an identical manner, except preparations of fixed S. aureus that had been previously saturated with sheep anti-mouse immunoglobulin (SAFU, Carluke, U.K.) were used (50 µl per µl of ascitic fluid). This latter method is an extremely simple method for precipitating MAbs that do not bind Protein A directly. The immune complexes on S. aureus were washed three times by suspension and sedimentation (2500 g for 3 min) from immune precipitation buffer containing 10% sucrose. The proteins in the immune complexes were dissociated by heating (80 °C for 5 min) in gel electrophoresis sample buffer (0·05 M-Tris-HCl pH 7·0, 0·2% SDS, 5% 2-mercaptoethanol and 5% glycerol), and analysed by SDS-PAGE. After electrophoresis, gels were either stained with Coomassie Brilliant Blue and destained in acetic acid–methanol or were stained using the Bio-Rad silver staining kit (total protein stain) and dried. Labelled polypeptides were visualized by autoradiography with Fuji X-ray film.

Radioimmune competition assay. Monoclonal antibodies were purified on a Protein A-Sepharose column (Goswami & Russell, 1983) and radioactively labelled with 125I using the chloramine-T method (Hunter, 1978). Labeled MAbs were titrated against infected cell antigen bound to nitrocellulose using an assay that has been described elsewhere (Randall et al., 1984) with the modification that the nitrocellulose sheet was sandwiched between 84-well Teraski plates (10 µl of diluted antibody per well). Unlabelled MAbs were then tested for their ability to compete with the binding of the 125I-labelled antibodies by making fourfold dilutions of ascitic fluids (starting at a 1/20 dilution) in an appropriate dilution of the 125I-labelled antibody in PBS containing 1% bovine serum albumin. The antibodies were then reacted with virus antigens bound to nitrocellulose sheets as described.

Assay to ascertain the reactivity of MAbs to various isolates of SV5. Vero cells, grown as monolayers in 96-well microtitre plates, were infected with the different isolates of SV5. When the cells showed an 80% c.p.e. (24 to 30 h post-infection) the cells were fixed in 5% formal–saline, 2% sucrose in PBS for 10 min at 20 °C. The monolayers were then washed three times with PBS, permeabilized with 0·5% NP40, 10% sucrose in PBS for 5 min at 20 °C and washed three times in PBS containing 1% calf serum. The monolayers were reacted for 1 h at 20 °C with MAbs (as ascitic fluid diluted 1/500 in PBS). The monolayers were washed four times with PBS containing 1% calf serum and incubated for 1 h with 125I-labelled Protein A (Amersham). If the MAb did not bind Protein A directly the washed monolayer was first treated for 1 h at 20 °C with rabbit anti-mouse immunoglobulin (diluted 1/10000 in PBS; Nordic Immunological Laboratories, Maidenhead, U.K.) and washed four times with PBS containing 1% calf serum before the addition of 125I-labelled Protein A. The monolayers were then washed once with PBS containing 0·1% NP40 and three times with PBS, dried and exposed to Fuji X-ray film with an intensifying screen at −70 °C.

Neutralization test. One-hundred µl amounts of twofold dilutions of MAbs (as ascitic fluids diluted in tissue culture medium containing 2% calf serum) were incubated at 37 °C for 2 h with 100 µl of the SV5 isolate LN (1 × 10⁵ p.f.u./ml). The antibody–virus mixtures were then used to infect Vero cells growing as monolayers in 96-well microtitre plates. After a 2 to 4 h adsorption period the virus–antibody inoculum was replaced with tissue culture medium containing 2% calf serum and the cells were reincubated at 37 °C for a further 3 to 4 days, when they were fixed with formal–saline, stained with crystal violet and the c.p.e. endpoint was determined.
Fig. 1. Analysis of the total polypeptide content of purified preparations of the SV5 isolate LN used to immunize mice for the production of hybridomas. The polypeptides were separated by electrophoresis through a 10% SDS-polyacrylamide slab gel and were visualized by silver staining the gel. The mol. wt. of the virus polypeptides were estimated by comparison with the electrophoretic mobilities of proteins with known mol. wt. (Bethesda Research Laboratories protein mol. wt. standards; data not shown).

Fig. 2. Analysis of [³⁵S]methionine-labelled polypeptides, separated by electrophoresis through a 15% polyacrylamide slab gel, from the immune precipitates formed by the reaction of soluble antigen extracts of SV5 (Pr)-infected BHK cells with either a mouse polyclonal anti-SV5 serum (lane 1), or with MAbs to the HN (lane 2), F (lane 3), NP (lane 4), P (lanes 5 and 7) and M (lane 6) polypeptides. The buffer used to prepare the soluble antigen extract for the immune precipitates shown in lanes 1 to 6 contained both 0.1% SDS and 0.5% NP40 as detergents, whereas that used for lane 7 contained 0.1% NP40 as the sole detergent.

RESULTS

Isolation and characterization of MAbs

Purified preparations of an SV5 derived from a human isolate (LN) were used to immunize BALB/c mice for the production of hybridoma cells. The virus was purified as described in Methods. SDS–PAGE analysis revealed that more than 70% of the proteins present in these virus preparations were of virus origin (Fig. 1). Fifty-four hybridoma cell lines were established that secreted MAbs to SV5. The specificities of these MAbs were determined by immune precipitation (Fig. 2). Of these MAbs, 17 reacted with the HN glycoprotein, one with the F glycoprotein and 22, 11 and three with the M, P and NP proteins respectively. The MAb to the F protein precipitated both the precursor F₀ polypeptide and its processed form, which dissociates into two polypeptides F₁ and F₂ under the reducing conditions used for SDS–PAGE.
Antigenic differences in isolates of SV5

Monoclonal antibodies to the P protein immune-precipitated complexes of the P, N and L proteins from the standard soluble antigen extracts of SV5-infected cells (i.e., in which 0.5% NP40 was the only detergent). However, the addition of 0.1% SDS to the soluble antigen extract resulted in the NP protein being released from these complexes (Fig. 2; compare lanes 5 and 7). To dissociate the P and L proteins a higher concentration of SDS (0.5%) was required (Fig. 3). If the soluble antigen extracts were made from SV5-infected BHK cells a number of extra bands were specifically precipitated by MAbs to the P protein in the absence of noticeable proteolysis of other virus proteins. However, if the soluble antigen extract was made from SV5-infected Vero cells, these antibodies precipitated the P protein and only one extra polypeptide, with an estimated mol. wt. of 24000 (compare Fig. 2 and 3).

Evidence that MAbs bind to different antigenic sites on the HN protein

The MAbs that reacted with the HN protein were examined for their ability to neutralize virus infectivity. The results, summarized in Table 1, show that all the antibodies to the HN protein neutralized virus infectivity, as did the MAb to the F protein. We then determined...
### Table 1. Summary of properties of MAbs

<table>
<thead>
<tr>
<th>MAb</th>
<th>Ability to bind to Protein A</th>
<th>Neutralization of virus infectivity* (LN, RQ, DN, NR, MT)</th>
<th>Human isolates (LN, RQ, DN, NR, MT)</th>
<th>Canine isolates (M7, CPI)</th>
<th>Simian isolate (Pr)</th>
<th>Ability to bind to Protein A</th>
<th>Neutralization of virus infectivity* (LN, RQ, DN, NR, MT)</th>
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<th>Canine isolates (M7, CPI)</th>
<th>Simian isolate (Pr)</th>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>Pb</td>
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<td>+</td>
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<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Pg</td>
<td>+ ND</td>
<td>+ 1/200</td>
<td>+</td>
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<td>+</td>
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* Final dilution of ascitic fluid that still neutralizes virus infectivity.
† HN-1a and HN-2a are MAbs HN-1 and HN-2 respectively previously isolated by Goswami & Russell (1983). HN-2a was not available for these tests but has been included to take into account that nomenclature.
‡ Weak binding to Protein A.
§ Not determined.
|| Anti-HN MAbs that do not prevent binding of any other MAb but have not been labelled with 125I themselves for use in competition tests.
Antigenic differences in isolates of SV5

whether anti-HN antibodies bound to different regions of the HN protein by a radioimmune competition assay (Fig. 4). By this method the anti-HN antibodies were placed into different groups, antibodies within a group showing some competitive binding with at least one other member of the group, but failing to compete with the binding of any antibody in another group. The MAbs were given specific names (see Methods), instead of their original trivial laboratory ones, once their reactions in these competitive assays had been established (Fig. 4). The region of the HN protein recognized by one group of antibodies we defined as an antigenic site. These antigenic sites could be divided into smaller overlapping immunogenic regions called epitopes that were defined as the minimum region of the HN protein required for the binding of an antibody. Thus, MAbs which bound to the same antigenic site may not recognize the same epitope within that site. For example, the MAbs HN-4c and HN-4a competed poorly with the binding of 125I-labelled HN-4b while in the same test the unlabelled HN-4b was an efficient competitor (Fig. 4). In contrast, HN-4a was the most efficient antibody in competing with the binding of 125I-labelled HN-4a, while HN-4b was an extremely poor competitor (data not shown). Furthermore, HN-4d, HN-4e and HN-4f all successfully competed with the binding of HN-4a (data not shown) but not HN-4b (Fig. 4). However, because HN-4a and HN-4b showed some competitive binding they have been placed in the same group of antibodies. Not all the MAbs were purified and labelled with 125I for use in the competition assay. If an unlabelled antibody competed with the binding of a 125I-labelled antibody then both antibodies were placed in the same group and a reciprocal analysis was not usually carried out. A summary of the competition results is given in Table 1. However, it should be noted that as well as competition between the binding of antibodies to the HN protein it also appeared that the binding of one antibody could enhance the binding of another antibody; for instance compare the intensity of binding of 125I-labelled HN-4b in the presence of HN-5a to that in the presence of HN-3a (Fig. 4).

Studies on the differences between human, monkey and canine isolates of SV5

Five human isolates (LN, RQ, NR, MT and DN), a simian isolate (Pr) and two canine isolates (M7 and CPI) of SV5 were analysed by SDS-PAGE for differences in the electrophoretic mobilities of their proteins. Fig. 5(a) shows an analysis of the immune precipitates formed following the reaction of a mouse polyclonal anti-SV5 serum with labelled soluble antigen extracts of BHK cells infected with the different isolates of SV5. While the electrophoretic patterns of most of the virus polypeptides of the different isolates were similar, clear differences in the electrophoretic migrations of some polypeptides could be seen. In particular, the P polypeptide of M7 migrated slightly slower than that of the human isolates and these in turn migrated slower than the P polypeptide of the simian isolate (Fig. 5b). Slight differences in the migration of the F1 polypeptide could also be seen. For example, the F1 protein of SV5 human isolate NR migrated faster than the F1 protein from all the other isolates (Fig. 5a, see also Fig. 8).

The various SV5 isolates were also tested for their ability to react with the MAbs to SV5. Several screening procedures were used including immunofluorescence, radioimmune assay using SV5 antigens bound to nitrocellulose (as used for screening hybridomas secreting antibody to SV5; see Methods) and a radioimmune assay in which the MAbs were reacted with virus-infected cells grown in 96-well microtitre plates that had been fixed and permeabilized as for immunofluorescence. It was found that this latter test was the most convenient and an example of such a test is shown in Fig. 6. The majority of MAbs reacted with all the isolates, but a number of antibodies revealed antigenic differences between the human, simian and canine isolates examined. Thus, for example HN-3a reacted with all the human and canine isolates examined but failed to react with the simian isolate (see below); HN-4b and HN-z failed to react with either of the canine isolates but did react with the human and simian isolates (Fig. 6, rows a, wells 7 and 8); F-la reacted with the human and simian isolates but failed to react with the canine isolates (Fig. 6, rows d, well 4) and three MAbs, Pa, Pd and Ph, to the P protein could distinguish between the human, canine and simian isolates of SV5 (e.g. Fig. 6, rows c, wells 1,4 and 6). However using this assay no differences in the reactivities of any of the MAbs with the human
Fig. 5. Analysis of the $[^{35}S]$methionine-labelled polypeptides present in immune precipitates formed by the reaction of soluble antigen extracts of BHK cells infected with various isolates of SV5 with either a mouse polyclonal anti-SV5 serum (a) or with a MAb to the P protein (b). The polypeptides present in the immune precipitates were separated by electrophoresis through a 15% SDS-polyacrylamide gel. The soluble antigen extracts used in the immune precipitation reactions contained both 0.5% NP40 and 0.1% SDS as detergents. Lanes 1, canine isolate M7; lanes 2, simian isolate Pr; lanes 3 to 7, human isolates DN, LN, NR, MT, RQ, respectively.

isolates of SV5 were observed. Furthermore, no MAb was identified that could distinguish between the two canine isolates.

Confirmation that certain antibodies reacted with some of the SV5 isolates but not others was obtained by immune precipitation and two such results are shown in Fig. 7 and 8. It can be seen from Fig. 7 that HN-3a failed to react with the HN protein of the simian isolate whereas HN-4a reacted with the HN protein of all the isolates examined. In contrast, it can be seen from Fig. 8 that F-1a reacted with the human and simian isolates but not with the canine (M7) isolate. A summary of the characteristics of the MAbs and their reactivities with the different SV5 isolates is given in Table 1.

DISCUSSION

In this paper we report the isolation and characterization of MAbs to SV5. Virus for the immunization of mice was purified by the rapid and simple technique described in Methods. Although the addition of medium without calf serum and its time of harvesting is critical, the yield and purity of the preparations we obtained using this method (Fig. 1) were routinely better than when we followed the method described previously (Goswami & Russell, 1982; data not shown).

Monoclonal antibodies were isolated which had specificities to the HN, F, P, M and NP proteins of SV5. The MAbs to the P protein were of interest in that they immune-precipitated nucleoprotein complexes, consisting of the P, NP and L proteins, from soluble antigen extracts of SV5-infected cells in which NP40 was the only detergent. Antibodies to the NP also precipitated some P and L proteins from similar soluble antigen preparations although the ratio of NP to P protein precipitated was approximately 10 to 1 (data not shown) compared to 1:2 that was precipitated by anti-P MAbs. This was presumably because, of the uncomplexed proteins in the soluble antigen extract, there was more NP than P protein. The NP protein was released from the complexes by treatment with 0.1% SDS but higher concentrations of SDS were required to dissociate the P and L proteins (Fig. 3). The origin of the minor bands precipitated
Fig. 6. Illustration of the assay used to screen for antigenic differences between different isolates of SV5. In the test shown, monolayers of Vero cells in 96-well microtitre plates were infected with either human (LN) (a), simian (Pr) (b) or canine (M7) (c) isolates of SV5 and reacted with MAbs that bound Protein A directly; bound antibodies were thus detected with 125I-labelled Protein A and autoradiography. Rows a, wells 1 to 11 show the reactivity of anti-HN antibodies; rows b, wells 1 to 11 anti-M antibodies; rows c, wells 1 to 9 anti-P antibodies and rows d, wells 1 and 2 and well 4 anti-NP and anti-F antibodies respectively. Arrows indicate differences between the binding of antibodies to cells infected with M7 or Pr isolates compared to cells infected with the LN isolate. w indicates weak binding of the antibody. The antibodies used in this experiment that did not react with all the isolates shown are HN-4b and HN-z (rows a, wells 7 and 8 respectively), Pa, Pd and Ph (rows c, wells 1, 4 and 6 respectively) and F-la (rows d, well 4). A summary of all the results is given in Table 1.

Fig. 7. Analysis of the immune precipitates formed by the reaction of MAbs HN-3a (a) and HN-4a (b) with soluble antigen extracts of Vero cells infected with human (LN, RQ, MT, NR, DN) (lanes 3 to 7 respectively), canine (M7) (lanes 1) or simian (Pr) (lanes 2) isolates of SV5. The infected cell polypeptides were labelled with [35S]methionine and the polypeptides present in the immune complexes separated by electrophoresis through a 10% polyacrylamide slab gel and visualized by autoradiography.

by anti-P antibodies from soluble antigen preparations of BHK cells is unclear. Differences in the electrophoretic mobilities of these minor polypeptides between the human and monkey isolates were observed, and these reflected similar differences in the electrophoretic mobilities of the P protein of these isolates, suggesting that these minor polypeptides are related to the P protein (data not shown). It is unlikely that they represent breakdown products of the P protein as no proteolysis of the other virus proteins was seen. Furthermore, virus proteins, in particular the NP and M proteins, in Vero cell lysates are much more sensitive to proteolysis than in BHK cell lysates (R. E. Randall et al., unpublished observations) and a similar array of P-related bands in Vero cell lysates have not been seen. Paterson et al. (1984) and R. A. Lamb (personal communication) came to the conclusion that polypeptide V (Fig. 3) is synthesized by translation beginning at a second downstream initiation site on the P mRNA in the same reading frame as P. It might therefore be that the multiple bands precipitated by anti-P antibodies from BHK lysates reflect the use of multiple internal initiation sites on the P mRNA some of which are not utilized in Vero or CV-1 cells.

Anti-HN antibodies were used to define at least four different antigenic sites on the HN glycoprotein; antibodies to all these sites neutralized virus infectivity. It is clear that not all the MAbs that react with one antigenic site recognize the same epitopes within that site. For example, although HN-4a and HN-4b showed some competitive binding with one another and thus were placed in the same group of antibodies, homologous competitive binding of these antibodies was much stronger than heterologous competitive binding. Furthermore, HN-4a reacted with all the SV5 isolates examined, whereas HN-4b failed to react with the canine
isolates. There is, however, another possible explanation for these results. It may be that HN-4a and HN-4b recognize distinct antigenic sites but that the binding of an antibody to one site alters the conformation of the HN protein so as to weaken the binding of an antibody at another site. The reverse may also be true. In the competition assays it was noted that some MAbs appeared to enhance the binding of $^{125}$I-labelled antibodies (Fig. 4). This positive cooperative binding may be significant with regard to how polyclonal antisera interact with proteins.

We have used these MAbs to examine antigenic differences between human, canine and simian isolates of SV5. The radioimmune assay we found to be most convenient relied upon the interaction of the MAbs with infected monolayers in 96-well microtitre plates (Fig. 6). It is interesting to note that although this assay was not employed to screen for hybridomas secreting antibodies to SV5, no antibody was isolated that did not react in this assay. Using it we failed to detect any antigenic differences between the human isolates of SV5, although we did note small variations in the electrophoretic mobility of the $F_1$ polypeptide (Fig. 5 and 8). However, minor differences between human isolates have been reported previously, based mainly on the efficiency of neutralization of the RQ and LN isolates by specific antisera, including HN-1a (Goswami et al., 1984). An analysis of more human isolates and from different geographical regions (all the human isolates of SV5 used in this study were isolated in London) needs to be carried out in order to determine whether the degree of antigenic variation among human isolates of SV5 is less than that reported for other paramyxoviruses such as mumps, parainfluenza type 3, respiratory syncytial and measles viruses (Norrby, 1985; Rydbeck et al., 1986; Van Wyke Coelingh et al., 1985; Mufson et al., 1985; Sheshberadaran et al., 1983). In this context however, it should be noted that in contrast to comparisons between human and bovine

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Fig. 8. Analysis of the $^{35}$S-labelled polypeptides present in the immune precipitates formed by the reaction of the anti-F MAb, F-1a, with soluble antigen extracts of BHK cells infected with human (RQ, MT, NR, LN, DN) (lanes 2 to 6 respectively), canine (M7) (lane 1) or simian (Pr) (lane 7) isolates of SV5. Polypeptides were separated by electrophoresis through a 15% polyacrylamide slab gel and visualized by autoradiography.
parainfluenza virus type 3 (Ray & Comphans, 1986) there appears to be a very high degree of antigenic conservation between the human, canine and simian isolates of SV5 with the majority of MAbs reacting with all of the isolates examined. We did identify small antigenic differences between the HN, F and P proteins of the human, canine and simian isolates, although none of the MAbs to the M protein distinguished these isolates. Thus, as has been reported for parainfluenza virus type 3 (Galinski et al., 1987), the M protein may be the most evolutionarily conserved virus protein. The electrophoretic mobility of the P protein varied between the human, canine and simian isolates of SV5 as determined by SDS–PAGE. While in this study we examined only a few SV5 isolates, it is of interest to note that the reactivity of the MAbs to the two canine isolates M7 and CPI were the same even though these viruses were isolated originally in the U.K. and U.S.A. respectively. Other researchers have also noted small antigenic differences between canine isolates and human and simian isolates (Lazar et al., 1970). Whether the differences between the human, canine and simian isolates of SV5 are sufficient to restrict virus transmission between species or whether different SV5 isolates regularly cross species barriers remains to be established. However, such studies will be of obvious importance if the observation that SV5 plays a role in the aetiology of some cases of multiple sclerosis (Goswami et al., 1987) can be substantiated.

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REFERENCES


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