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Coronavirus Isolates SK and SD from Multiple Sclerosis Patients Are Serologically Related to Murine Coronaviruses A59 and JHM and Human Coronavirus OC43, but Not to Human Coronavirus 229E

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Two coronaviruses (SK and SD), isolated from fresh autopsy brain tissue from two multiple sclerosis patients, were compared with known human and murine coronaviruses. In plaque neutralization assays, antisera prepared against multiple sclerosis isolates SK and SD demonstrated significant cross-reactivity to each other and to murine coronavirus A59, weak cross-reactivity to murine coronavirus JHM, but no cross-reactivity to the human coronavirus 229E. Antiserum to SK or SD failed to inhibit hemagglutination of chicken erythrocytes by the human coronavirus OC43. However, OC43 antiserum neutralized both SD and SK. Specific coronavirus polypeptides were identified and compared by immunoprecipitation and polyacrylamide gel electrophoresis. Infected and mock-infected 17Cl-1 cells were pretreated with actinomycin D and labeled with [^{35}S]methionine. Polypeptides in Nonidet P-40 cytoplasmic extracts were immunoprecipitated with homologous and heterologous antisera. Identical polypeptides were precipitated from A59-, SD-, or SK-infected cell extracts by SD, SK, OC43, or A59 antisera. The polypeptides of human virus 229E were antigenically distinct, with the exception of weak recognition of a polypeptide of 50,000 molecular weight. We conclude that the two multiple sclerosis virus isolates SK and SD are closely related serologically to the murine coronavirus A59 and the human coronavirus OC43.

While working with multiple sclerosis (MS) autopsy tissue (7), workers in our laboratory have isolated two coronaviruses. The SD virus was detected after intracerebral inoculation of fresh, unfrozen MS brain stem homogenate into weanling BALB/c mice. The SK isolate was evident on subculture 12 of mouse 3T3 (17Cl-1) cells inoculated with homogenized fresh MS deep frontal lobe tissue.

Although coronaviruses OC43 and 229E cause respiratory infections in humans (12, 25–28), coronaviruses have not been previously associated with infections of the human central nervous system. In a variety of mammals and birds coronaviruses are associated with hepatitis (35), pneumonitis (32), peritonitis (3, 33), enteritis (17), and encephalitis (2). Chronic or latent infections by murine coronaviruses are frequently encountered in mouse breeding colonies (9, 17, 37). Mouse hepatitis virus infection in mice can be asymptomatic or may be manifested by respiratory or gastrointestinal symptoms. Some mouse hepatis virus strains cause focal or diffuse hepatic necrosis without central nervous system symptoms (A59) (25, 35), whereas neurotrophic strain JHM produces central nervous system lesions, including demyelination and neuronal necrosis (2, 15, 31).

Recognizing the possibility of isolating a contaminating murine virus from the mice or cells used to isolate SD and SK viruses, we evaluated the mice and cell cultures for evidence of latent mouse hepatitis virus infection. Mouse hepatitis virus infection of the mice and cell cultures utilized for our MS virus isolations was not detectable by complement fixation, plaque neutralization, or enzyme-linked immunosorbent assays of sera from uninoculated animals. Electron microscopy and fluorescent antibody studies of the 17Cl-1 uninoculated cell cultures were also negative. Nevertheless, since these MS virus isolates were found by using mouse tissue or cells, further evaluation of their human or murine origin is needed. Therefore, we have compared antigenic properties of human coronaviruses 229E and OC43 and murine coronaviruses

JHM and A59, with MS isolates SK and SD. The viruses are compared by plaque cross-neutralization and immunoprecipitation with homologous and heterologous antisera. The results indicate cross-reactivity of SK and SD with human strain OC43 and murine strains A59 and JHM.

MATERIALS AND METHODS

Cells and virus. MHV strain A59 and BALB/c 3T3 transformed cell line 17Cl-1 (44) were obtained from K. V. Holmes, Uniformed Services University of the Health Sciences, Bethesda, Md. MHV strain JHM and the mouse DBT cell line (20) were obtained from S. Stohlman, University of Southern California, Los Angeles. Human coronavirus 229E was obtained from Ken McIntosh, Children's Hospital Medical Center, Boston, Mass. MS isolates SD and SK were isolated in our laboratory from MS patients (7). Viruses SD and SK were plaque purified three times on 17Cl-1 cells. Human WI38 cells were obtained from the American Type Culture Collection, Rockville, Md. Human rhabdomyosarcoma (RD) and fetal tonsil (FT) cells were obtained from O. Schmidt, University of Washington, Seattle. Other cells listed in Table 1 were provided by the University of Colorado Diagnostic Virology laboratory. All cells were grown in Eagle minimum essential medium supplemented with 10% fetal bovine serum.

Virus production and purification. MHV strains A59 and JHM and MS isolate SK were purified from the supernatant fluids of infected 17Cl-1 cells. Monolayers of 17Cl-1 cells grown on 490-cm^2 plastic roller bottles were infected at a multiplicity of infection of 0.1 to 1.0 at 35°C. At 20 to 24 h after infection, virus in the supernatant fluid was purified through a discontinuous 30 to 50% sucrose gradient as described by Lai and Stohlman (24). MS isolate SD was purified as a cytoplasmic extract (40) since this virus is mainly cell associated. Infected cells (syncytia) were scraped from the surface into cold TMEN buffer (50 mM Tris-maleate, 1 mM EDTA, 100 mM NaCl [pH 6.0]) containing 0.25 M sucrose. Cells were disrupted with a Dounce homogenizer and layered onto an equal volume of TGME (10 mM Tris [pH 7.9], 5 mM $MgCl_2$, 1 mM EDTA, 25% glycerol). The nuclei were pelleted through the TGME by centrifugation at 1,200 × *g* for 15 min at 4°C. The cytoplasmic extract above the TGME interface was collected with a Pasteur pipette and further purified through a 30 to 50% sucrose discontinuous gradient (24). Virus utilized for plaque neutralization studies was isolated as follows from 17Cl-1 cells infected at low multiplicity of infection (less than 0.01) and collected when approximately 50% cytopathic effect was evident. At this time the infected cells were scraped, pelleted at 1,200 × g for 10 min, resuspended in TMEN (pH 6), frozen and thawed three times, and then clarified by centrifuging at 1,200 × g for 15 min. Human virus OC43 was grown in suckling BALB/c mouse brains (26). The virus was collected as a 10% homogenate 48 h after intracerebral inoculation and titrated by hemagglutination (21). Hemagglutination and hemagglutination inhibition assays utilized 0.5% chicken erythrocytes at room temperature (19).

Preparation of antiserum. All animals to be used for antiserum production were shown to be free of neutralizing antibody activity to mouse hepatitis virus and to virus isolates SK and SD. Virus purified on sucrose gradients was diluted to between 10^6 and 10^8 PFU/ml and emulsified 1:1 with complete Freund adjuvant. Rabbits or guinea pigs received two intramuscular injections (0.2 ml in each hip) weekly for 4

TABLE 1. *Host range of human cornonaviruses OC43 and 229E, mouse coronavirus A59, and MS virus isolates SD and SK*[a]

Species	Cell[b]	Virus replication				
		Mouse A59	MS isolates		Human	
			SD	SK	OC43	229E
Human	WI38 (fibroblast)	−	−	−	−	+
	RD (rhabdomyosarcoma)	−	−	−	−[c]	−[c]
	FT (fetal tonsil)	−	−	−	−[c]	−[c]
Mouse	3T3 (17C1-1)	+	+	+	−	−
	DBT	+	+	+	−	−
	NCTC 1469	+	−	−	−	−
	Suckling BALB/c mouse brain	+	+	+	+	ND[d]

[a] +, Virus replication was detectable in supernatant fluid or frozen and thawed cells; no detectable replication. Replication of MS isolates SD and SK, human coronavirus 229E, and mouse virus A59 was detected by plaque titration as described in the text. Human OC43 replication was detected by hemagglutination of chicken erythrocytes.

[b] MS isolates SD and SK also did not grow in the following cells: human HeLa, L132, HEP 2, and primary amnion cells; primate primary Rhesus monkey kidney, primary African green monkey kidney, Vero, and BSC-1 cells; bovine embryonic kidney cells; canine MDCK cells; and rabbit primary kidney cells.

[c] Although Schmidt et al. (University of Washington, Seattle) have reported (38) that OC43 and 229E grow in these cells, apparently extensive adaptation is required. We found no evidence of cytopathic effect or virus growth after weekly reinfection and 12 to 15 blind passages.

[d] ND, Not done.

weeks. At 10 days after the last injection the animals were test bled. Antiserum against SD virus was obtained 10 days after 4 weekly intraperitoneal injections of 10^3 PFU of infectious virus into C57 black mice. Antiserum to human coronavirus 229E was a guinea pig reference antiserum obtained from Harold Kaye, Center for Disease Control, Atlanta, Ga. Antiserum to human strain OC43 included a mouse reference antiserum obtained from Harold Kaye and a mouse immune antiserum (no. 129) provided by Ken McIntosh.

Plaque assays and plaque neutralization. Virus isolates SD and SK and mouse viruses A59 and JHM were titrated by plaque assay on confluent monolayers of DBT cells grown in 60-mm petri dishes. Virus was absorbed in a 0.1-ml volume per dish for 60 min at 35°C and overlaid with 0.6% Seakem agarose, Dulbecco modified Eagle medium supplemented with nonessential amino acids, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, and 1% fetal calf serum. Virus plaques were allowed to develop for 72 h at 35°C in a CO_2 incubator. At this time the plates were overlaid with 2 ml of a 10% neutral buffered Formalin solution and fixed overnight; the agar was removed, and the monolayers were stained with 1% crystal violet. Human virus 229E was titrated by plaque assay on WI38 cells (passages 20 to 30). Overlays consisted of 0.6% Seakem agarose in Dulbecco modified Eagle medium with 2× vitamins (Flow Laboratories), 1× nonessential amino acids (Flow Laboratories), 1% fetal calf serum, and 10 mM HEPES buffer. Plaques developed in 4 to 5 days at 35°C and were fixed and stained as described above. For plaque neutralizations approximately 2,000 PFU of virus were incubated with antiserum (1:1, 0.4-ml total volume) for 1 h at 37°C. Virus was plated in triplicate (0.1 ml) to yield approximately 100 PFU in control or unneutralized plates. Control neutralization assays utilized fetal calf serum or preimmune serum diluted to a level comparable to that of the test antiserum dilution. All antisera were heat inactivated at 56°C for 30 min.

Radiolabeling of infected cell polypeptides. ^{35}S-labeled methionine (1,390 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, Ill. At 4 h before infection, monolayers of 17Cl-1 cells in 100-mm petri dishes were rinsed with phosphate-buffered saline and treated in minimum essential medium containing one-third the normal amount of amino acids (1/3 MEM), 2% dialyzed fetal calf serum, and 1 μg of actinomycin D per ml. After 4 h, these pretreated cells were infected at a multiplicity of infection of 0.1 to 1.0 PFU/cell with 1 ml of virus adsorbed for 1 h at 35°C. After adsorption, monolayers were rinsed once (5 ml of 1/3 MEM) and overlaid with media containing 1 μg of actinomycin D per ml, 1/3 MEM, and 20 μCi of [^{35}S]methionine. Infection was allowed to proceed for 16 to 20 h. Preliminary experiments demonstrated that the same virion polypeptides were made with or without actinomycin D. However, the presence of actinomycin D significantly reduced the level of cellular protein synthesis. Infected and uninfected cells were harvested by removing the labeling media and rinsing each dish with 5 ml of ice-cold phosphate-buffered saline. The cells were transferred to an ice bath, the phosphate-buffered saline was removed, and 500 μl of lysis buffer (0.02 M Tris [pH 7.4], 0.05 M sodium chloride, 0.5% deoxycholate, and 0.5% Nonidet P-40) per dish was added to produce a cytoplasmic extract. After 2 to 3 min at 4°C in lysis buffer, nuclei and cytoplasm were scraped from the dishes, and the nuclei were removed by centrifugation for 3 min (12,800 × *g*) in an Eppendorf microcentrifuge. Supernatants were stored at −20°C until used for immunoprecipitation.

Immunoprecipitation of viral polypeptides. Immunoprecipitation of viral peptides from cytoplasmic extracts was by the method of Kessler (22). Nonspecific precipitates were removed by preclearing 100 μl of infected cell lysate with 30 μl of preimmune antiserum. This preadsorption mixture was incubated at 4°C for 16 h, and the adsorbed antigens were removed by the addition of 200 μl of a 10% Formalin-fixed and washed solution of Cowan 1 strain of *Staphylococcus aureus*. Then 50 μl of immune antiserum was added to 300 μl of adsorbed lysate and incubated at 4°C for 4 h. After the addition of 100 μl of 10% Formalin-fixed and washed *S. aureus*, precipitated antigen antibody-*S. aureus* complexes were washed five times with 1% Triton X-100, 1% deoxycholate, and 0.15 M sodium chloride (pH 7.0). The final pellet was suspended in 50 μl of 1X sample buffer (2% sodium dodecyl sulfate, 10% glycerol, 0.001% bromophenol blue, 62.5 mM Tris-hydrochloride [pH 6.8]) containing 1.2 M urea and 0.1% beta-mercaptoethanol. Proteins were solubilized at 37°C for 60 min. *S. aureus* cells were removed by centrifugation, and the supernatant containing virus-specific polypeptides was stored at −20°C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Radiolabeled polypeptides prepared by immunoprecipitation as described above were thawed and resolubilized at 37°C for 60 min. They were then analyzed on 10 to 20% Tris-glycine polyacrylamide gels cross-linked with *N,N'*-diallyl-tartardiamide (14). Polyacrylamide gradient slab gels 1.5 mm thick and 10 cm long were prepared by the method of Laemmli (23). Gels were run for 18 to 20 h with a constant current of 5 mA. Purified vesicular stomatitis virus radiolabeled with [^{35}S]methionine or ^{14}C-amino acids was utilized for molecular weight standards (46). Gels were impregnated with 2,5-diphenyloxazole by the method of Bonner and Laskey (5), dried with a Hoeffer gel dryer, exposed at −70°C for 3 days to 3 weeks on Kodak XR5 X-Omat film and developed with Kodak reagents.

RESULTS

Biological properties of MS isolates SK and SD. The growth characteristics of coronaviruses SD and SK were very similar to those previously reported for other coronaviruses (25, 36). After infection of 17Cl-1 cells at a multiplicity of infection of 1.0 PFU/cell, newly synthesized virus was first detectable between 4 and 6 h after infection. Although the replication kinetics were similar for isolates SD and SK, the two viruses produced distinct cytopathic effects. At 24 h postinfection virus SD produced large syncytia within which the nuclei migrated to the

center. Under similar conditions, virus SK also produced giant cells, but the nuclei did not aggregate within the syncytia. A maximum concentration of 10^6 to 10^7 PFU/ml of supernatant was obtained between 16 and 20 h after SK infection. Virus concentrations obtainable for SD tended to be 10- to 100-fold below those of SK virus.

The host ranges for coronaviruses SD and SK and for mouse virus A59 and human viruses OC43 and 229E are listed in Table 1. Viruses SD and SK showed similar host ranges and only grew in cells of murine origin. Although this might suggest that the SD and SK virus isolates are of murine origin, it is also true that human virus OC43 also grows very poorly, if at all, in human cells in vitro, but grows in suckling mouse brain. Virus isolates SD and SK did not grow in the mouse line NCTC 1469, whereas A59 did replicate in these cells. WI38 cells that propagated human virus 229E did not propagate virus isolates SD and SK or human virus OC43.

Reciprocal cross-neutralizations. The ability of antisera directed against various coronaviruses to neutralize plaque formation is shown in Table 2. Our isolates were not related to human coronavirus 229E. Viruses SD and SK were very closely related antigenically. SD and SK virus isolates cross-reacted substantially with mouse virus A59 and less strongly with mouse virus JHM. Antiserum prepared against OC43 demonstrated significant cross-reactivity with MS isolates SK and SD, but no cross-reactivity at a 1:20 dilution against A59 or JHM.

Hemagglutination and hemagglutination inhibition. The human coronavirus OC43 is known to hemagglutinate chicken and human "O" erythrocytes (21). Therefore, we investigated the ability of viruses SD and SK to hemagglutinate these cells. OC43 was the only virus capable of hemagglutinating erythrocytes. In addition, only antisera directed against OC43 inhibited this hemagglutination. Therefore, although viruses SK and SD were antigenically related to OC43, they differed from this virus in that they did not hemagglutinate erythrocytes, and they replicated in mouse cell lines.

Immunoprecipitation of radiolabeled viral polypeptides. Virus-specific polypeptides of SK infection were identified by immunoprecipitation of a cytoplasmic extract of infected 17Cl-1 cells labeled with [^{35}S]methionine. Viral polypeptides were identified as polypeptides observed in infected cell lysates immunoprecipitated with immune antiserum and not detectable in infected lysates precipitated with preimmune antiserum or 17Cl-1 control cell lysates precipitated with immune antiserum. Immunoprecipitation of MS isolate SK-infected cells by anti-SK antiserum revealed seven polypeptides (Fig. 1). Antisera to MS isolates SK and SD immunoprecipitated identical proteins from SK infections. In both cases viral proteins with molecular weights of 180,000 (180K), 90K, 50K, 42K, 24K, 23K, and 22K were observed. The 42K polypeptide was observed in amounts varying from barely detectable to very distinct. The polypeptides of 24K and 23K were often not resolved

TABLE 2. *Reciprocal plaque neutralizations*[a]

Antiserum	Plaque neutralization				
	Mouse		MS isolates		Human
	JHM	A59	SK	SD	229E
Anti-A59	40	500	320	320	<20
Anti-SK	500	1,200	10,000	10,000	<20
Anti-SD	40	100	2,000	2,000	<20
Anti-OC43	<20	<20	80	160	<20
Anti-229E	<20	<20	<20	<20	1,000

[a] Numbers given are the highest serum dilution causing greater than 50% neutralization of approximately 100 plaques.

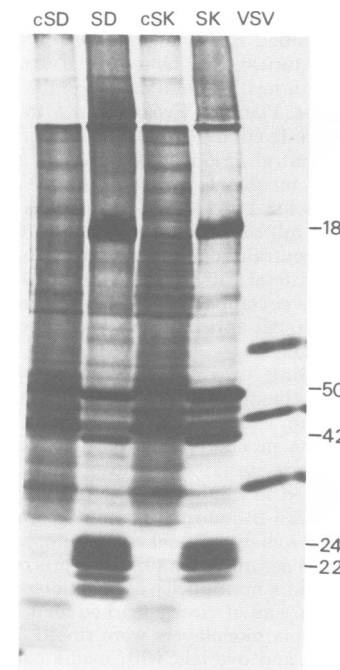

FIG. 1. *Immunoprecipitation of ^{35}S-labeled polypeptides of SK-infected 17Cl-1 Nonidet P-40 cytoplasmic extracts by anti-SK (lane SK) or anti-SD (lane SD) serum. Viral polypeptides (indicated in kilodaltons) are identified as peptides observed in infected cell lysates (lanes SD and SK) immunoprecipitated with immune antiserum and not detectable in uninfected cell lysates (lanes cSD and cSK) precipitated with the same serum. VSV, vesicular stomatitis virus molecular weight markers.*

into two distinct proteins. Infections in which SD-infected cells were labeled and precipitated with anti-SK and anti-SD sera demonstrated an identical polypeptide pattern (data not shown).

Immunoprecipitation of A59 and SK proteins. In an effort to identify cross-reacting polypeptides of MS isolates SK or SD and mouse virus A59, immunoprecipitations of extracts of SK-infected cells were performed with homologous and heterologous antisera (Fig. 2). Polypeptides of 180K, 50K, 24K, 23K, and 22K were precipitated from cytoplasmic extracts of A59 and SK infections by anti-SK serum. However, a 42K polypeptide was observed in anti-SK immunoprecipitated SK infections, but not in A59 infections. Furthermore, an SK polypeptide of 50K seemed to migrate slightly faster than that of the comparable A59 protein.

Reciprocal immunoprecipitations. Immunoprecipitations of A59-infected 17Cl-1 cells with homologous and heterologous antisera are shown in Fig. 3. All of the A59 viral polypeptides were recognized by antisera directed against A59, SD, SK, and OC43 antisera. Antiserum against 229E did not recognize A59 polypeptides, with the exception of the 50K protein. Experiments in which SD or SK infections were immunoprecipitated demonstrates that all of the SD- or SK-specific polypeptides were recognized by anti-A59 or anti-OC43, whereas anti-229E again recognized only the 50K protein (data not shown).

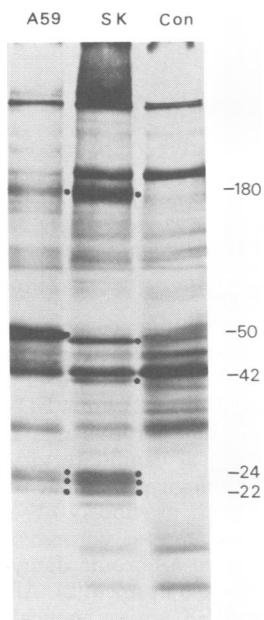

FIG. 2. *Recognition of A59 or SK viral polypeptides after immunoprecipitation by anti-SK guinea pig serum. Viral polypeptides are identified by the black dots, and estimated molecular weights are given in kilodaltons. A59, Polypeptides precipitated from a cytoplasmic extract of virus A59-infected 17Cl-1 cells by anti-SK serum. SK, Polypeptides precipitated from a cytoplasmic extract of virus SK-infected 17Cl-1 cells by anti-SK serum. CON, Polypeptides precipitated from a cytoplasmic extract of uninfected 17Cl-1 cells by anti-SK serum.*

DISCUSSION

Our results revealed extensive serological cross-reactivity between virus isolates SD and SK, murine coronavirus A59, and human virus OC43. Previous investigations have also demonstrated antigenic cross-reactivity between human virus OC43 and various MHV strains, hemagglutinating encephalomyelitis virus 67N of swine, and calf diarrhea coronavirus by fluorescent antibody, serum neutralization, and complement fixation tests (6, 17, 25, 29, 34). Human virus OC43 is antigenically distinct from human virus 229E (25).

The molecular basis of coronavirus cross-reactivity has not previously been reported. We have shown that homologous and heterologous antisera as well as OC43 antiserum precipitated the same polypeptides from A59, SD, or SK infections. These data suggest that all of the component polypeptides of A59, OC43, SD, and SK contain cross-reacting antigenic determinants. An alternative explanation could be that aggregates of the component polypeptides in the cell extracts might be precipitated by antibody to any one component.

This latter explanation is unlikely since purified A59 180K and A59 23K proteins were both recognized by SK antiserum in an enzyme-linked immunosorbent assay performed for us by K. V. Holmes, Uniformed Services University of the Health Sciences, Bethesda, Md. Preimmune and immune rabbit, mouse, and guinea pig antisera prepared against MS isolate SK were sent to K. V. Holmes under code. Virus OC43, A59, and purified A59 El (23K) polypeptide and purified A59 E2 (180K) polypeptide antigens prepared by Sturman et al. (44) were reacted with our antisera by enzyme-linked immunosorbent assay. Whereas none of the preimmune antisera recognized these coronavirus antigens, the anti-SK rabbit, mouse, and guinea pig sera all recognized A59 antigen, E1 and E2 antigens of A59, and OC43 antigen.

Although earlier reports show disparity in the number and size of virus-specific polypeptides of coronaviruses (8, 10, 11, 16, 18), more recent investigations suggest that this apparent diversity in polypeptide patterns may be secondary

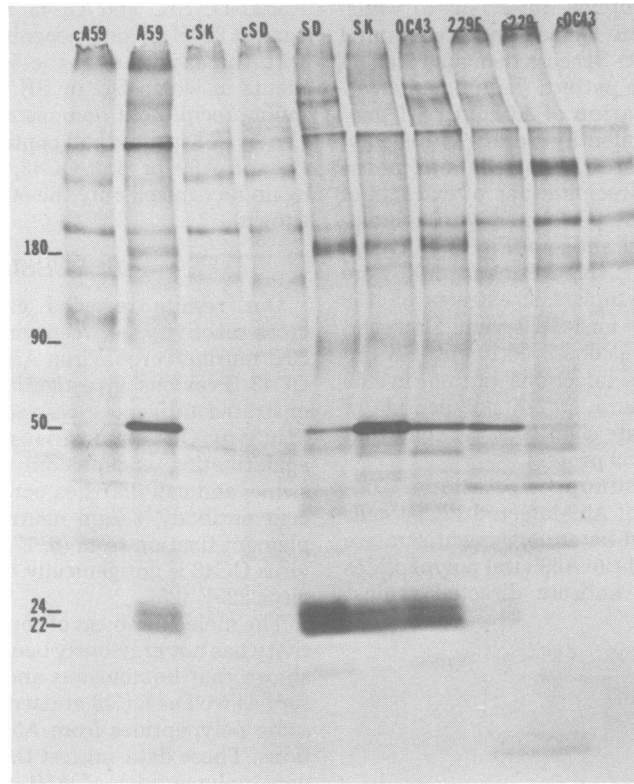

FIG. 3. *Immunoprecipitation of A59-infected 17Cl-1 cytoplasmic extract utilizing homologous and heterologous antisera. Lanes cA59, cSK, cSD, c229E, and cOC43: polypeptides precipitated from uninfected 17Cl-1 cytoplasmic extract by antisera directed against A59, SK, SD, 229E, and OC43, respectively. Lanes A59, SK, SD, OC43, and 229E: polypeptides precipitated from A59 virus-infected 17Cl-1 cytoplasmic extract by antisera directed against A59, SK, SD, OC43, and 229E, respectively.*

to the unusual characteristics of the glycoproteins of these viruses (1, 4, 30, 39, 41–43, 47). When denaturing conditions are identical both murine and human coronaviruses have similar polypeptide components (30). In general, polypeptides of 180K, 90K, 50K, 24K, and 22K, that we observed are in agreement with other recent coronavirus investigations (1, 30, 41, 47). We observe a 42K polypeptide in SD or SK infections that has not been observed in A59 infections. A similar polypeptide has been reported by Anderson et al. (1) in JHM infections. The JHM polypeptide was secondary to a processing event of the 50K protein. Although we have not investigated processing, the fact that antiserum directed against A59 recognizes this protein in SD or SK infections suggests that a similar protein may be transiently produced in A59 infections. The 42K, protein might alternatively be similar to the 38K aggregate of the 24K protein observed in A59 infections under certain preparative conditions (41).

The extensive cross-reaction observed between mouse and human coronaviruses makes it extremely difficult to clearly define the species origin of our MS isolates. However, regardless of the species origin of MS virus SD and SK isolates, we feel that coronviruses represent an additional group of candidate viruses for MS. Particles morphologically similar to the coronaviruses have been previously observed in MS tissue (45), and mouse coronavirus JHM is known to produce a chronic demyelinating-remyelinating disease in mice (2). Although virus isolates SD and SK are antigenically related to both human and mouse viruses, 85% of the general population have cross-reactive serum antibody (Burks et al., unpublished data). This high prevalence of antibody to coronaviruses SD and SK might suggest a human origin. However, antibodies to mouse hepatitis viruses have previously been reported in human sera (13); 69% of the general population have detectable neutralizing antibody at 1:10 dilution to A59 virus and only 33% to mouse hepatitis virus type S (13).

The results of cross-neutralization tests are also compatible with the hypothesis that SD and SK isolates may be human viruses related to the human strain OC43. Since antigenic and serological investigations have not defined the species origin of SD and SK, conclusive demonstration of the etiological association of these coronaviruses and MS will require the direct demonstration of viral antigens or nucleic acids in lesions from human MS tissue, and never in tissue from non-MS patients.

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