Proc. Natl. Acad. Sci. USA
Vol. 76, No. 12, pp. 6601–6605, December 1979
Medical Sciences

Antigenic relationships between measles and canine distemper viruses: Comparison of immune response in animals and humans to individual virus-specific polypeptides

(morbillivirus polypeptides/subacute sclerosing panencephalitis)

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Communicated by Werner Henle, July 27, 1979

ABSTRACT **Precipitation with hyperimmune rabbit sera, sera from patients convalescing from measles, and sera from patients with subacute sclerosing panencephalitis, followed by electrophoresis, enabled antigenic relationships between the individual polypeptides of measles and canine distemper viruses to be examined. Virus isolates from patients with acute measles or subacute sclerosing panencephalitis showed no antigenic differences. With rabbit hyperimmune sera, antigenic cross-reactivity was present between all polypeptides of measles and canine distemper viruses except H. The N polypeptides showed the highest degree of crossreactivity and were interpreted as group-specific antigens. Both convalescent measles sera and sera from subacute sclerosing panencephalitis showed high antibody titers to all measles polypeptides except L and M. However, these sera contained only low activities to the N and F_1 polypeptides from canine distemper virus.**

The morbilliviruses are highly contagious agents that cause diseases of clinical importance. Many studies have been carried out to characterize antigenic relationships among these viruses (1). These studies produced apparently equivocal findings which seem to result from many factors, such as animal host, origin of virus strain, and immunization procedures as well as the different serological assays used. These serological assays measured immune responses to either total viral protein or to single biologically active antigens such as hemagglutinin and hemolysin (1–6). With the development of an immunoprecipitation assay in combination with electrophoresis, antibodies against individual proteins from different viruses can now be simultaneously compared. The present study evaluates antigenic relationships among individual polypeptides from measles virus, virus isolated from patients with subacute sclerosing panencephalitis (SSPE), and canine distemper virus (CDV). It also examines the humoral immune responses in diseases associated with these viruses.

MATERIALS AND METHODS

Cells and Virus. VERO cells and CV1 cells were obtained from Flow Laboratories (McLean, VA) and maintained in Eagle's minimal essential medium with 5% fetal calf serum and 50 mM Hepes. Stock measles virus (Edmonston), SSPE virus (LEC), and CDV (Onderstepoort) were grown in VERO cells at a multiplicity of infection of 10^{-2} and harvested 12 hr after complete cell fusion (48 hr after infection for Edmonston and LEC; 60 hr after infection for CDV).

Preparation of Radiolabeled Antigens. Cells (2×10^6) on a 3-cm plastic petri dish were infected with 1 ml of virus at a multiplicity of infection of 1 and incubated at 37°C. When 20–30% of the monolayer had formed syncytia (11–12 hr after infection for Edmonston and LEC; 15–16 hr for CDV), the cells were washed three times in Eagle's minimal essential medium without methionine and incubated for 1 hr with the depleted medium. Fresh methionine-free medium was added and the cells were incubated for a further 3 hr with 200 μCi of [^{35}S]-methionine (1 Ci = 3.7×10^{10} becquerels) (i.e., until 90% of the cells had formed syncytia). The monolayer was washed three times in buffer A (0.15 M NaCl/10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.01% sodium azide/1% Nonidet P-40/500 units of aprotinin per ml/0.2 mg of phenylmethylsulfonyl fluoride per ml) and stored overnight at −20°C in 0.5 ml of buffer A. The monolayers were thawed and a further 0.5 ml of buffer was added. The mixture was mixed vigorously on a Vortex for 1 min at room temperature and diluted to 12 ml. The lysate was sonicated for 5 min and centrifuged at $1500 \times g_{av}$ for 5 min at 4°C. The supernatent was centrifuged at 4°C for 30 min at $100{,}000 \times g_{av}$ and the pellet was discarded.

Immune Precipitation. Two hundred microliters of the antigen and 5 μl of the appropriate sera dilution in buffer A were incubated overnight at 4°C, then for 30 min at 4°C with 20 μl of a 10% suspension of fixed *Staphylococcus aureus* (Cowen I strain) (7). The mixture was pelleted at $10{,}000 \times g$ for 2 min at room temperature and washed twice in buffer A. The pellet was immediately boiled in 20 μl of sample buffer [8 M urea/2% sodium dodecyl sulfate ($NaDodSO_4$)/2.5% 2-mercaptoethanol]. Initial experiments were performed with other detergents (Triton X-100, Sarkosyl, and $NaDodSO_4$), but either caused high levels of nonspecific precipitation or reduction in the antigenicity of some proteins.

Polyacrylamide Gel Electrophoresis. Samples were run for 6 hr at 150 V on 15% discontinuous $NaDodSO_4$ gels as described (8).

Reagents. Aprotinin and phenylmethylsulfonyl fluoride were from Sigma. L-[^{35}S]Methionine (>600 Ci/mmol) was obtained from Amersham. ^{35}S-Labeled influenza-infected CEF cells were kindly supplied by F. X. Bosch (Institute for Veterinary Virology, Giessen).

Sera. R1–3: serum from rabbits hyperimmunized with measles virus, SSPE virus, and CDV, respectively. R4: serum from a rabbit hyperimmunized with rinderpest virus, kindly supplied by F. Brown (Pirbright, Surrey, U.K.). R5 + R6: pre-immune serum corresponding to R1 + R2, respectively. D1: canine serum from a distemper convalescent, kindly obtained from M. Appel (Cornell University). D2: pre-immune serum corresponding to D1. S1–12: sera from classical SSPE patients

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Abbreviations: SSPE, subacute sclerosing panencephalitis; CDV, canine distemper virus; $NaDodSO_4$, sodium dodecyl sulfate.

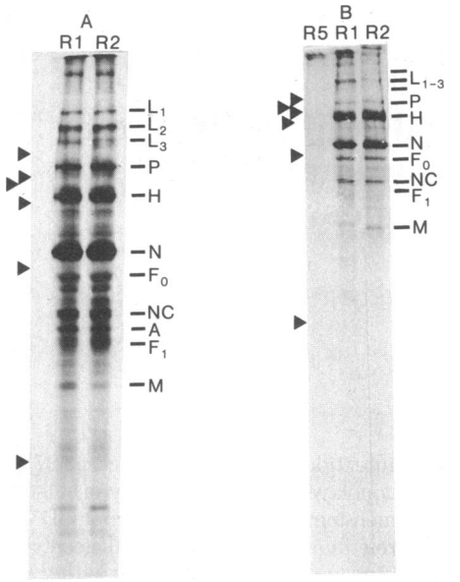

FIG. 1. Immune precipitation with rabbit sera of SSPE and measles viral polypeptides. (*A*) Immune precipitated polypeptides from cells infected with SSPE virus. (*B*) Immune precipitated polypeptides from cells infected with measles virus. Each track is designated by the code of the sera used (1:10 dilution) as described in *Materials and Methods*. Arrowheads designate positions of coelectrophoresed polypeptides from influenza-virus-infected cells; M_r are taken as 95,000 (P_1), 87,000 (P_2), 84,000 (P_3), 78,000 (HA), 53,000 (NP), 25,000 (M), and 23,000 (NS 1) (top to bottom). A, position of cellular actin.

3 to 12 years old. M1: convalescent sera taken 2 months after measles encephalitis. M2: convalescent measles serum taken 3 years after the initial infection. M3: convalescent measles serum taken 5 months after appearance of rash. M4–6: sera from adults who had measles in early childhood and had no known recent contact with measles patients (i.e., 20–25 years after infection). V1–4: sera from children taken 2–5 months after vaccination with Schwarz measles vaccine. N1 + 2: serum from 7-month-old infants with no detectable measles antibody and no known contact with measles patients.

RESULTS

Antigenic Relationship Between Measles and SSPE Viruses. Sera from rabbits hyperimmunized with either SSPE virus or measles virus precipitated the same polypeptides whether antigens from cells infected with measles virus or SSPE virus were used (Fig. 1). These sera precipitated the following polypeptides designated by Graves *et al.* (9) as L, H, N, F_0, F_1, and M and also those designated P and NC. However, we saw three polypeptides (M_r 120,000, 108,000, and 103,000) in the L region. NC is thought to be a cleavage product of N, by analogy to Sendai virus (10, 11). Polypeptide 'P' is thought to be analogous to that designated 2 or P by other workers even though it migrates in a different position relative to H. These differences in the relative migration of polypeptides can be explained by variation in electrophoretic conditions, as reported for reovirus (12).

Antigenic Relationship Between Measles Virus and CDV. Sera from rabbits immunized with CDV and serum from a dog convalescing from distemper had titers against the N, F_0, NC, and F_1 polypeptides of measles virus equivalent to those in the measles virus-specific serum (Fig. 2). The activities of both CDV sera to the P and M polypeptides are low, with the activity to M in the CDV-specific rabbit serum being lower than that of the dog serum. No activity against the H polypeptide of measles virus was found in the distemper-specific rabbit sera, although low levels were observed in the convalescent canine serum. Activity against the L polypeptides was found only in the measles and CDV-specific rabbit sera.

When antigens from CDV-infected cells reacted against homologous rabbit sera (Fig. 3, lanes R3), 10 polypeptides were consistently precipitated. By analogy with measles virus, these polypeptides have been designated L_1, L_2, P, H, N, F_0, NC, M, and F_2 with M_r of 120,000, 108,000, 81,000, 76,000, 66,000, 47,000, 44,000, 40,000, 35,000, and 9000, respectively. A similar profile was obtained when canine convalescent sera was used except that L_1 and L_2 were not precipitated. When rabbit hyperimmune sera raised against either Edmonston or LEC virus were used (Fig. 3*B*), their activity against L_1 and L_2 was equal to that of canine distemper-specific rabbit serum. However, activities against other polypeptides were either low (N, F_0, NC, F_1, and M) or not detectable (P, H, and F_2).

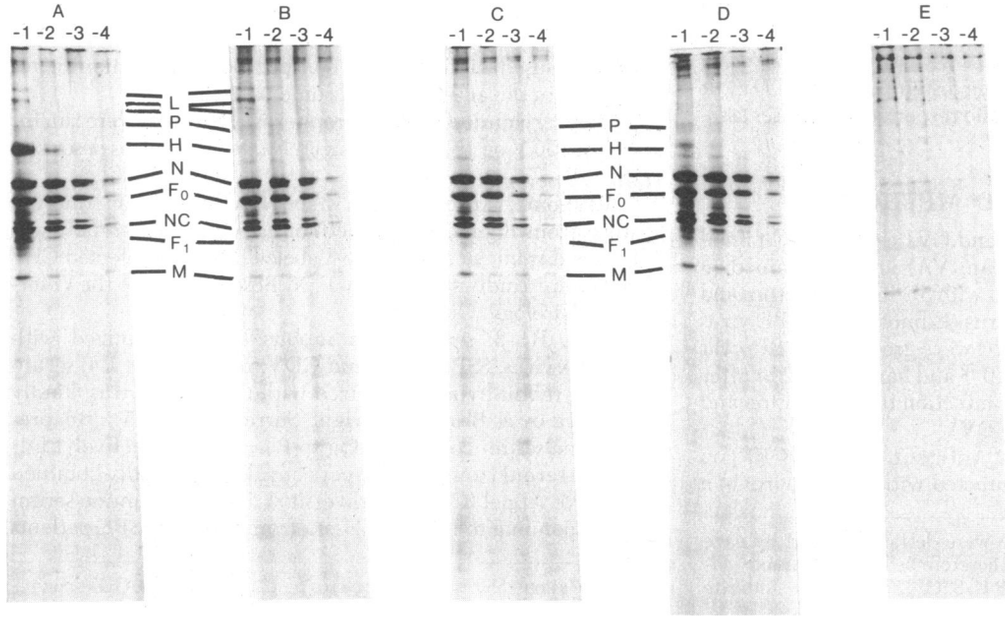

FIG. 2. Immune precipitation of measles virus-specific polypeptides with serial dilutions of various animal sera. (*A*) Measles-specific rabbit serum R1; (*B*) CDV-specific rabbit serum R3; (*C*) rinderpest-specific rabbit serum R4; (*D*) convalescent dog serum D1; (*E*) pre-immune serum (R5). Each track is designated by the index of the serum concentration used.

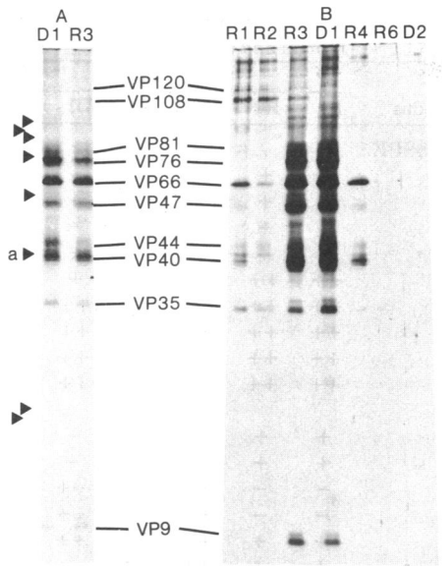

FIG. 3. Immune precipitation of polypeptides from cells infected with CDV. Autoradiograph from a gel exposed for 4 days (*A*) and from a gel exposed for 3 weeks (*B*). Tracks are indicated as for Fig. 1. All sera were diluted 1:10. Arrowheads indicate positions of influenza virus M_r markers. a, Position of cellular actin.

The activity of rinderpest-specific rabbit sera to antigens from measles-infected cells was similar to that observed for the CDV-specific rabbit sera, except that no activity against the L polypeptides could be detected (Fig. 2*C*). However, the rinderpest antibodies crossreacted only with the N and F_1 polypeptides of CDV (Fig. 3*B*).

Virus-Specific Antibodies in Human Sera. Analysis of convalescent measles sera revealed high antibody titers against polypeptides P, H, N, F_0, NC, and F_1 of measles virus (Figs. 4 and 5). However, no activity against the L or the M protein was detected even though undiluted sera were tested (Fig. 5), it was

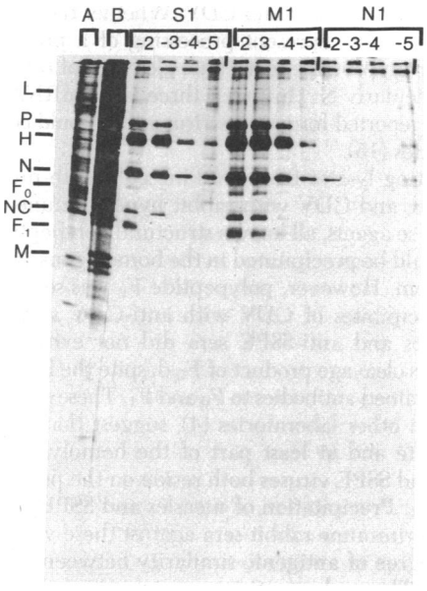

FIG. 4. Immune precipitation by human sera of polypeptides from cells infected with measles virus. Total lysate from uninfected cells (*A*) and from infected cells (*B*). S1, SSPE serum; M1, measles convalescent serum; N1, human negative sera. Each track is designated by the index of the serum concentration used for immune precipitation.

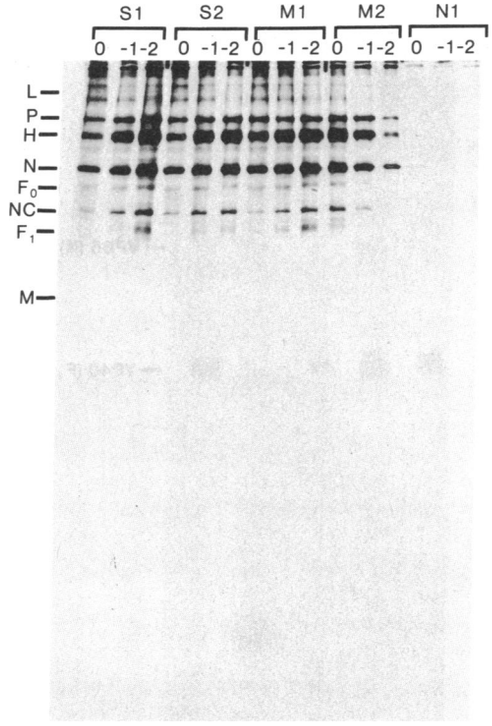

FIG. 5. Immune precipitation of measles-specific polypeptides by use of concentrated human sera. S1 and S2, SSPE sera; M1 and M2, measles convalescent sera. Each track is designated by the index of the serum concentration used for immune precipitation.

a major virus-specific protein in the lysate (Fig. 4*B*), and it could be precipitated by rabbit hyperimmune sera (Fig. 2*A*). Because this protein can be easily resolved from cellular polypeptides, not only could it be shown to be present in the supernatant from an immune precipitation with human sera, but also it could subsequently be precipitated by rabbit hyperimmune sera. These observations demonstrate that M protein from measles-infected cells remains antigenically intact throughout the in-

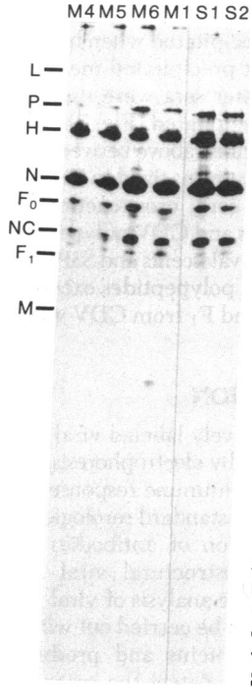

FIG. 6. Immune precipitation by human sera of polypeptides from cells infected with SSPE virus. Each track is designated as for Fig. 1. M4–6 were diluted 1:10. M2, S1, and S2 were diluted 1:100.

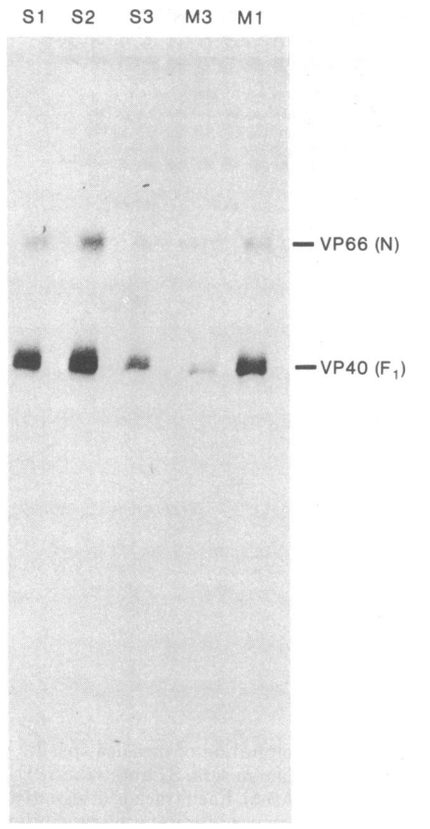

FIG. 7. Immune precipitation by human sera (1:10 dilution) of polypeptides of CDV-infected cells. Each track is designated as for Fig. 1.

cubation with human sera. In addition, no activity could be detected against the L polypeptides. A similar absence of activity against M and L proteins has been observed in SSPE sera (Figs. 4 and 5), convalescent sera from other patients after acute measles, adults who have had measles during early childhood, and vaccinated children (sera M3–6, S1–14, and V1–4). Identical results were obtained when human sera were tested against the LEC isolate of SSPE virus (Fig. 6).

No CDV polypeptides were precipitated when human sera were used at a concentration that precipitated measles polypeptides. However, when high-titer sera were used at high concentration, N and F_1 were precipitated (Fig. 7).

The antigenic relationships described above between measles, SSPE, and CDV polypeptides are summarized in Table 1; i.e., by use of rabbit hyperimmune sera, crossreaction can be demonstrated between all measles and CDV polypeptides except H. Sera from both measles convalescents and SSPE patients efficiently precipitate all measles polypeptides except L and M and are able to precipitate N and F_1 from CDV when used at high concentrations.

DISCUSSION

Immunoprecipitation of radioactively labeled viral polypeptides from infected cells, followed by electrophoresis, provides a basis for analyzing in detail the immune response of a host against a viral agent. In contrast to standard serological assays, this technique allows identification of antibodies directed against both structural and nonstructural viral antigens. Moreover, a comparative antigenic analysis of viral polypeptides from related virus strains can be carried out without purification of individual viral proteins and production of monospecific antisera. In order to detect the crossreactivity

Table 1. Immune response to morbillivirus polypeptides in human and animal sera

Viral polypeptides	Sera						
	R1	R2	R3	R4	D1	S	M
Measles or SSPE							
L_1	+	+	+	−	−	−	−
L_2	+	+	+	−	−	−	−
L_3	+	+	+	−	−	−	−
P	+	+	+	+	+	++	++
H	++	++	−	−	+	++	++
N	++	++	++	++	++	++	++
F_0	++	++	++	++	++	++	++
F_1	++	++	++	++	++	++	++
M	++	++	+	+	++	−	−
CDV							
L_1	+	+	+	−	−	−	−
L_2	+	+	+	−	−	−	−
P	−	−	++	−	++	−	−
H	−	−	++	−	++	−	−
N	+	+	++	+	++	+	+
F_0	+	+	++	+	++	−	−
F_1	+	+	++	+	++	+	+
M	+	+	++	−	++	−	−

++, Strong immune reaction; +, weak immune reaction; −, no detectable immune reaction.

between measles virus, SSPE virus and CDV, we used hyperimmune rabbit sera and sera from SSPE patients and from dogs and humans convalescing after natural infection. The hyperimmune sera from experimentally immunized rabbits and SSPE patients were included in order to detect minor antigenic relationships among the viruses tested.

The measles virus-specific polypeptides described in these studies are broadly similar to those reported by other workers (9, 10, 13). By analogy with the electrophoretic profile from purified virus proteins (14) and the measles polypeptides described above, a preliminary assignment of the ten CDV polypeptides was made. However, we found three polypeptides in the L region of the gel with both viruses, although L_3 could not be consistently shown for CDV. Whether these are primary gene products or represent processing of a precursor is not known; serum proteases can cause cleavage of other polypeptides, particularly N. However, three large mRNA molecules have been reported for measles virus which could code for such polypeptides (15).

By treating lysates from cells infected with measles virus, SSPE virus, and CDV with rabbit hyperimmune sera raised against these agents, all known structural polypeptides of these viruses could be precipitated in the homologous antigen–antibody system. However, polypeptide F_2 was seen only in immune precipitates of CDV with anti-CDV serum, whereas antimeasles and anti-SSPE sera did not exhibit antibodies against this cleavage product of F_0 despite the fact that all sera tested contained antibodies to F_0 and F_1. These data, along with those from other laboratories (4), suggest that the antibody-binding site and at least part of the hemolysin activity for measles and SSPE viruses both reside on the polypeptide designated F_1. Precipitation of measles and SSPE viral antigens with hyperimmune rabbit sera against these viruses revealed a high degree of antigenic similarity between all their polypeptides. These observations are in apparent conflict with earlier studies which reported antigenic differences between the M proteins. However, the sera used in the earlier experiments were derived from a short-term immunization with purified protein, whereas sera used in our current studies came from a long-term immunization protocol with whole virus.

When such a protocol is used with other similar viruses, it leads to the recognition of an increased number of antigenic sites (16). Rabbit hyperimmune sera against CDV and rinderpest viruses showed antigenic crossreactivity with measles or SSPE virus polypeptides P, N, F_0, F_1, and M but not with H. However, the canine convalescent sera did show a weak immune reaction of H. When CDV was used as antigen, measles, SSPE, and rinderpest sera had only low activity against N, F_0, F_1, and M and none against P or H. Therefore, the heterologous neutralizing activity in sera raised against these viruses, as previously reported (reviewed in ref. 1), probably depends upon inactivation of F_1. This is supported by the observation that the integrity of the fusion proteins of paramyxoviruses is an important determining factor in their infectivity (17). In contrast to H protein, the N polypeptides are antigenically similar and can therefore be considered as a group-specific antigen for the morbilliviruses. These data explain why previous serological studies using infected tissue culture cells as source of antigens (2, 3, 5) showed a higher antigenic relationship among the morbilliviruses than assays depending on virus neutralization.

The comparative analyses of the L polypeptides of measles virus, SSPE virus, and CDV showed identical electrophoretic mobilities and displayed a high degree of antigenic crossreactivity. However, an immune reaction was observed only in the rabbit hyperimmune sera and not in serum specimens after natural infection with these viruses, suggesting that the L polypeptides are poor immunogens during normal infections.

Precipitation of measles or SSPE viral antigens with sera from patients convalescing after measles infection or vaccination and sera and cerebrospinal fluid from patients with SSPE revealed high antibody activity against all structural polypeptides of these viruses, except L and M. Failure of human serum samples to react with measles or SSPE M proteins is not due to an antigenically inactive viral M protein, as the control experiments have shown. It has to be assumed either that a measles infection in its natural host induces an immune response against M protein of short duration which cannot be detected in IgG fractions of convalescent serum samples or that the M protein is not normally accessible to the immune system. It is conceivable, therefore, that during viral infection with clinical complications accompanied by massive cell destruction sufficient M protein is available to mount an immune response. This appears to be the case in atypical measles (18) and in severe influenza infections (19). It is surprising that in SSPE sera no antibody activity against measles M protein was detectable despite a strong hyperimmune reaction against other proteins of measles virus. These observations would support the hypothesis that an abnormality in measles virus M polypeptide may play a pathogenetic role in this disease (5).

In the human sera tested, the only CDV-specific antibody activities observed were those against the N and the F_1 polypeptides, indicating that the neutralizing activity of these sera to CDV depends on the crossreactivity of F_1.

The techniques used here have demonstrated antigenic crossreactivity to individual virus polypeptides in the IgG populations of animal sera raised against three members of the morbillivirus group. Such activities of the IgG molecules have also been determined in sera from patients convalescing from natural infection. It is possible by modification of these techniques to include IgM and IgA antibody activities, to examine the humoral response of the host throughout the course of disease, and to examine the role of CDV in human diseases of the central nervous system such as multiple sclerosis.

We thank Magdalene Pföhler for excellent technical assistance and Helga Schneider for typing this manuscript. This work was supported by the Deutsche Forschungsgemeinschaft.

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