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Antibodies Against the Measles Matrix Polypeptide After Clinical Infection and Vaccination

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Sera from patients exposed to measles virus were investigated for the presence of antibodies against each of the viral antigens. All sera with measurable neutralizing titers contained antibodies against the two surface proteins (the glycoprotein and fusion protein), the nucleocapsid protein, and one of the internal proteins (P_2). However, only sera from individuals with clinical symptoms of measles infection (natural measles and atypical measles) contained antibodies against the measles virus matrix protein. Levels of matrix-specific antibodies were highest in patients with atypical measles infection.

Glycoproteins that are present on the surfaces of membrane-containing virions are also expressed at the surfaces of infected cells. It has been shown repeatedly that these antigens are involved in virus neutralization and immune-mediated lysis of infected cells. For example, the glycoprotein of vesicular stomatitis virus induces neutralizing antibodies (15), and it is also recognized by cytotoxic T cells (9, 26). Similarly, antibodies against the major glycoprotein (gp71) of Friend leukemia virus neutralize the virus, and Friend leukemia virus-transformed cells are lysed in the presence of these antibodies and complement (2, 20). However, viral glycoproteins on infected cells do not seem to be the exclusive targets for cell lysis. Recently, it has been shown in several laboratories that cytotoxic T cells in mice can exhibit extensive cross-reactivity between type A influenza virus strains with serologically distinct hemagglutinins and neuraminidases (4, 6, 7, 27, 28). It has been suggested that this cross-reactivity is mediated by the matrix protein, a shared antigen which was previously presumed to be internal. Indeed, antibodies against the matrix protein lyse influenza virus-infected cells in the presence of complement (1, 4). It has also been reported that the matrix protein may play a role (either directly or indirectly) in T-cell lysis of vesicular stomatitis virus-infected cells, although the glycoprotein is the major target antigen (26). Thus, viral proteins not found on the surfaces of virions may be expressed at the surfaces of infected cells and be recognized by antibodies or cytotoxic cells. The expression of these antigens, which do not elicit neutralizing antibodies, may vary with cell type and virus strain and may play an important role in maintaining viral infections that persist in vivo despite high titers of neutralizing antibodies.

There is compelling evidence to suggest that subacute sclerosing panencephalitis is caused by a persistent infection of measles virus or a closely related agent (14). With this in mind, we analyzed the expression of measles virus antigens on the surfaces of lytically and persistently infected cells (12, 13; manuscript in preparation). As an alternate approach, the specificity of antibodies against all measles virus structural antigens in sera and cerebrospinal fluids (CSF) of patients with subacute sclerosing panencephalitis was determined. Measles-specific antibodies were shown to be produced locally in the CSF of patients with subacute sclerosing panencephalitis (23) and thus serve as indicators for the presence of viral antigens in the central nervous system. Antibody specificity in sera and CSF from subacute sclerosing panencephalitis patients was compared with that in sera from individuals with various clinical symptoms of natural measles infection and from vaccinated individuals. During the course of this work it was found that persons with various manifestations of measles infection differed in their antibody responses. This paper shows that antibodies against the matrix protein are found only in early convalescent sera after natural measles infection and in sera from patients with atypical measles. Results concerning measles-specific antibodies in sera and CSF from subacute sclerosing panencephalitis patients will be reported in a future publication.

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MATERIALS AND METHODS

Buffers and solutions. BN buffer contained 0.01 M barbitol (pH 7.4) and 0.15 M NaCl. NET buffer contained 0.05 M tris(hydroxymethyl)aminomethane (pH 7.2), 0.005 M ethylenediaminetetraacetate, 0.15 M NaCl, and 0.1 mM phenylmethyl sulfonyl fluoride, and NET-T was NET buffer containing 0.05% Triton X-100. Phosphate-buffered saline (PBS) contained 0.02 M sodium phosphate (pH 7.2) and 0.15 M NaCl, and PBS-KCl was PBS with 0.005 M KCl. SP buffer contained 0.1 M tris(hydroxymethyl)aminomethane (pH 6.8), 2% sodium dodecyl sulfate (SDS), 2% β-mercaptoethanol, 20% glycerol, and 0.002% bromophenol blue.

Labeling of cell cultures with [^{35}S]methionine. HEp-2 cells and HEp-2 cells persistently infected with measles virus (HEp-2-PI) (11, 12) were in monolayer cultures in Joklik minimal essential media supplemented with 5% fetal calf serum. They were labeled as follows. The growth medium of nearly confluent cultures in 10-cm petri dishes was replaced with 5 ml of methionine-free minimal essential medium containing nonessential amino acids, 3% dialyzed fetal calf serum, 0.015 M phosphate, and 0.01 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.2). After 1 h [^{35}S]methionine (400 Ci/mmol; Amersham/Searle) was added to 50 μCi/ml. After 6 h, the cells were washed twice, incubated for 1 h in complete medium, and removed from the surfaces of the dishes by incubation at 37°C in PBS-KCl containing 0.02% ethylenediaminetetraacetate. They were washed once in PBS and stored at −70°C as pellets containing 10^6 cells. Unlabeled HEp-2 cells were harvested with ethylenediaminetetraacetate and stored as pellets containing 3×10^6 cells.

Lactoperoxidase-catalyzed iodination. Nearly confluent monolayers of HEp-2 and HEp-2-PI cells were harvested with 0.02% ethylenediaminetetraacetate in PBS-KCl. They were washed twice with BN buffer containing 2% fetal calf serum and twice with BN buffer containing 10^{-5} M KI. Next, 5×10^6 cells were suspended in a solution containing 0.5 ml of Eagle basal salts and 10^{-5} M KI. At room temperature, 10 μl of lactoperoxidase (10 mg/ml; Calbiochem) and 0.5 mCi of Na^{125}I (15 mCi of I per μg; Amersham/Searle) were added; this was followed by four additions of 10 μl of 5×10^{-3} M H_2O_2 at 5-min intervals. The cells were washed five times with Eagle basal salts containing 10^{-4} KI. Pellets containing 10^6 cells per sample were stored at −70°C.

Cell extracts. Frozen cell pellets (10^6 ^{35}S- or ^{125}I-labeled cells or 3×10^6 unlabeled cells) were suspended in 200 μl of NET buffer. Triton X-100 was added to 1%, and the suspensions were kept at 4°C for 15 min. Nuclei and large particulate material were removed by centrifugation at $700 \times g$ for 6 min. A 10-μl amount of cytoplasmic extracts from [^{35}S]methionine-labeled HEp-2-PI cells contained approximately 10^6 cpm, of which 70 to 80% was precipitable with trichloroacetic acid.

Immune precipitations. Protein A-bearing *Staphylococcus* immunoadsorbant (Staph A) was prepared from *Staphylococcus aureus* type A (Cowan I strain), as described previously (21). Immune precipitation has also been described previously (11). Briefly, extracts from 10^6 labeled HEp-2 or HEp-2-PI cells in 200 μl were preadsorbed for 15 min at 22°C with 50 μl of Staph A suspension, followed by the removal of Staph A by centrifugation at $9,000 \times g$ for 1 min. Samples (20 μl) of diluted serum or CSF were preadsorbed for 15 min at 22°C with 20 μl of extract prepared from uninfected, unlabeled HEp-2 cells. A 20-μl amount of Staph A-adsorbed labeled extract was added to the 40-μl mixture of antiserum and unlabeled extract, and the mixture was incubated for 60 min at 22°C. Antigen-antibody complexes were precipitated by the addition of 50 μl of Staph A suspension. After an incubation of 15 min at 22°C, complexes were pelleted at $9,000 \times g$ for 1 min and washed four times with 1.0 ml of NET-T buffer. (In some cases, the first supernatant from the Staph A pellet was removed and further incubated for 60 min with 10 μl of rabbit or goat antiserum directed against the heavy chain of human immunoglobulin G [IgG], IgM, IgA, or IgD. After a 15-min incubation with 50 μl of Staph A, complexes were pelleted and washed in the same manner.) The final pellet was resuspended in 70 μl of SP buffer and placed in boiling water for 3 min to dissociate the antigen-antibody complexes, and the Staph A was removed by centrifugation. The radioactivity in small samples of the supernatants was determined, the remainder was applied to SDS-polyacrylamide gels. Human convalescent serum precipitated 7 to 8% of the total trichloroacetic acid-insoluble counts from extracts of [^{35}S]methionine-labeled HEp-2-PI cells and less than 2% from HEp-2 cells.

SDS-polyacrylamide gel electrophoresis. Protein samples were electrophoresed in 10% discontinuous slab gels (19) for 4 h at 110 V. Gels were fixed for 30 min in a mixture of methanol, acetic acid, and water (5:1:5). After equilibration with 7% acetic acid, the gels were impregnated with PPO (2,5-diphenyloxazole), rinsed with water, dried, and exposed to Kodak Royal X-Omat film (3). For quantitation of viral antigens, films were preexposed to a hypersensitizing flash of light (18), fluorograms were scanned with a Quick Scan scanner (Helena Laboratories, Beaumont, Tex.), and the areas under the peaks were integrated with a PDP-11 computer.

Virus neutralization. A 100-μl amount of serially diluted serum or CSF was mixed with a 100-μl suspension of measles virus containing 10^4 plaque-forming units (both in serum-free medium) and incubated for 1 h at 22°C; 100 μl of this mixture was added to confluent monolayers of CV-1 cells in Linbro trays (2×10^5 cells per 16-mm well), the trays were incubated for 2 h at 37°C, and then 0.9 ml of complete medium was added. After 3 days at 37°C, plates were read for cytopathic effects, and each neutralizing titer was expressed as the inverse of the highest serum dilution that prevented cytopathic effects.

Sera and CSF. The clinical material used in this work is described in Table 1. It was obtained from Dale E. McFarlin (National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Md.), Kenneth L. Herrmann (Center for Disease Control, Atlanta, Ga.), Saul Krugman (Department of Pediatrics, New York University Medical Center), and volunteers at the Duke University Medical Center.

TABLE 1. *Sera and CSF investigated*

Compound	Description	Neutralizing titer
Sera		
MC	Serum from 13-year-old child vaccinated before 1 year of age	<5
M1	Acute serum from patient with natural measles infection	<10
M2	Acute serum from patient with natural measles infection	10
M3 through M9	Early convalescent sera from patients with natural measles infections (2 to 3 weeks after clinical symptoms)	800
M10 through M12	Late convalescent sera from individuals after natural measles infections (more than 5 years after clinical symptoms)	40–160
M13 through M17	Acute sera from individuals vaccinated with attenuated measles virus	<10
M18 through M22	Early convalescent sera from individuals vaccinated with measles virus	200–800
M23 through M26	Late convalescent sera from individuals vaccinated with measles virus (more than 5 years after vaccination)	20–160
M27 through M31	Sera from patients with atypical measles	1,600–3,200
M32	Serum from patient with clinical measles encephalitis	200
M33 through M35	Sera from patients with suspected measles encephalitis (low neutralizing titers)	20
CSF		
CSF 1 and 2	CSF from normal individuals	<5
CSF 3 and 4	CSF from clinical cases of measles encephalitis	<10

Serum from a seronegative 13-year-old child who had been vaccinated before 1 year of age was used as a control in all experiments described. Rabbit anti-human IgG and IgM and goat anti-human IgD and IgA (all heavy chain specific) were purchased from Cappel Laboratories.

RESULTS

Detection of measles virus-specific antibodies in human serum. Measles-specific antibodies were analyzed as follows. Sera (or CSF) were incubated with [^{35}S]methionine-labeled extracts from HEP-2-PI cells. Complexes of labeled antigens and antibodies were precipitated with Staph A, dissociated with SDS, and electrophoresed in SDS-polyacrylamide gels. Antigens were identified by fluorography, and their presence served to identify specific antibodies.

Figures 1A and B show the electrophoretic patterns of the polypeptides that were precipitated by early convalescent serum (M3) (Table 1) from HEp-2 and HEp-2-PI extracts. (HEp-2-PI cells were used as the most convenient source of infected cell extracts. These were HEp-2 cells that were persistently infected with measles virus, and they expressed viral antigens in a manner identical to HEp-2 cells lytically infected with measles virus [12; manuscript in preparation].) Convalescent measles serum precipitated several major and minor polypeptides from HEp-2-PI extracts that were not precipitated from uninfected HEp-2 cell extracts. They migrated in the positions of the viral structural polypeptides (10, 22, 25). P_1 corresponds to the surface glycoprotein, P_2 is located internally in the virion, P_3 is the nucleocapsid polypeptide, P_5

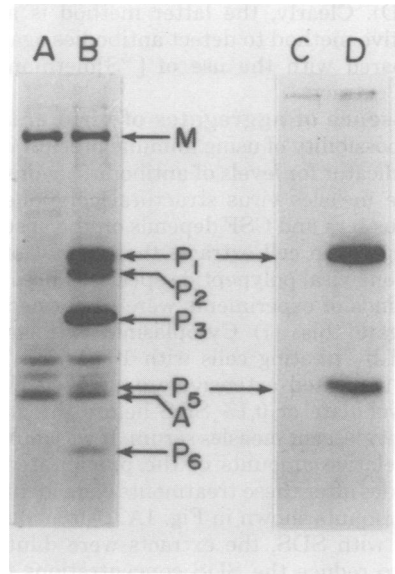

FIG. 1. *Polypeptides precipitated from uninfected and persistently infected cells with early convalescent measles serum M3. Fluorogram of immune precipitates from [^{35}S]methionine-labeled uninfected HEp-2 cells (lane A) and HEp-2-PI cells (lane B). Autoradiogram of immune precipitates from ^{125}I-labeled uninfected HEp-2 cells (lane C) and HEp-2-PI cells (lane D). M, Myosin; A, actin.*

is the large nonglycosylated polypeptide in the fusion protein, and P_6 corresponds to the matrix protein. A large virus-specific polypeptide (molecular weight, ~200,000) (8) and polypeptide P_4 were not observed. P_4 was precipitated in some

experiment but always poorly and not reproducibly. Since P_4 is probably a degradation product of the viral nucleocapsid (22; manuscript in preparation), it is not considered below. Two major polypeptides were also precipitated from uninfected cell extracts, which corresponded closely in molecular weight to myosin and actin. With the occasional exception of small amounts of polypeptides P_2 and P_3, no viral antigens were precipitated by control serum MC (data not shown).

The fusion polypeptide P_5 was not labeled extensively, and it migrated close to cellular actin. This often made it difficult to determine levels of antibodies against this antigen. Therefore, an alternate method was used, which took advantage of the fact that lactoperoxidase-catalyzed iodination of intact HEp-2-PI cells labeled the surface proteins P_1 and P_5 very efficiently and in large excess over host surface antigens. P_1 and P_5 were precipitated exclusively by early convalescent measles serum and could be easily identified in SDS-polyacrylamide gels (Fig. 1C and D). Clearly, the latter method is a more sensitive method to detect antibodies against P_5 compared with the use of [^{35}S]methionine-labeled extracts.

Absence of aggregates of viral antigens. The possibility of using immune precipitation as an indicator for levels of antibodies against each of the measles virus structural components in various sera and CSF depends on the absence of aggregates in cell extracts that consist of more than one viral polypeptide species. Three different kinds of experiments were performed to investigate this. (1) Cytoplasmic extracts (prepared by treating cells with 1% Triton X-100) were sonicated extensively or treated with 1% deoxycholate or 0.1% SDS before the addition of convalescent measles serum. It was found that the relative amounts of the precipitated polypeptides after these treatments were identical to the amounts shown in Fig. 1A. (After the treatment with SDS, the extracts were diluted 10-fold to reduce the SDS concentrations for immune precipitations.) (ii) Cytoplasmic extracts were fractionated into soluble and particulate fractions by centrifugation at 85,000 × *g* for 90 min. Convalescent serum precipitated viral polypeptides P_1 and P_5 from the soluble fraction in the same relative amounts shown in Fig. 1A. The nucleocapsid polypeptide P_3, polypeptide P_2, and the matrix polypeptide P_6 were present in reduced amounts in the soluble fraction, and the only viral polypeptides that were precipitated from the pelleted material were P_2 and P_3 (Fig. 2B and C). Nucleocapsids are large structures that would be expected to pellet under the centrifugation conditions employed, and apparently the matrix polypeptide aggregates under the conditions used to prepare cytoplasmic extracts. The failure to detect polypeptide P_6 in the pelleted material by immunoprecipitation was probably due to the fact that it could not be resuspended well and that it was removed from the reaction mixture during adsorption with Staph A. (iii) To rule out possible aggregates of polypeptides P_2, P_3, and P_6, cytoplasmic extracts were reacted with specific rabbit serum raised against purified nucleocapsids (21). This precipitated polypeptides P_2 and P_3, but it did not precipitate the aggregated P_6 (Fig. 2D).

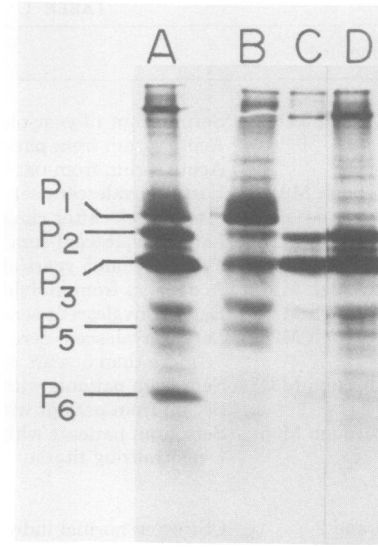

FIG. 2. *Polypeptides precipitated from fractionated HEp-2-PI extracts. Lane A, Total cytoplasmic extract plus human serum M27; lane B, supernatant of cytoplasmic extract after centrifugation at 85,000 × g for 90 min plus serum M27; lane C, pelleted cytoplasmic material resuspended by sonication in the original volume with NET-T plus serum M27; lane D, total cytoplasmic extract plus rabbit serum against purified nucleocapsids.*

Thus, the only aggregates with more than one antigen contained nucleocapsids (consisting of polypeptides P_3 and P_2). We found that the only satisfactory method to obtain nucleocapsids free of P_2 involved extraction with Triton X-100 and deoxycholate and equilibrium centrifugation in Renografin and CsCl (21). However, this method was too laborious to be of use in the experiments described in this paper. These experiments did not rule out the possibility of mixed miscelles with surface proteins P_1 and P_5. However, this seems unlikely since we have observed in similar assays that dog convalescent serum against canine distemper virus precipitated P_5 but not P_1

from HEp-2-PI extracts (manuscript in preparation).

Antibodies in sera and CSF in individuals after exposure to measles virus. Table 1 lists the sera and CSF that were analyzed for measles-specific antibodies. Included were sera from individuals at three stages of natural measles infection: acute, early convalescent (2 to 3 weeks after clinical symptoms), and late convalescent (10 to 20 years after clinical symptoms). Also included were acute, early, and late convalescent sera from individuals vaccinated with attenuated measles virus, sera from individuals with atypical measles infection, sera and CSF from individuals with measles encephalitis, and appropriate controls.

Immune precipitation patterns of the various sera and CSF listed in Table 1 were identical within each group, and therefore only representative results are shown in the fluorogram in Fig. 3. Acute sera with low neutralizing titers (M1 and M13) gave the same negative precipitation pattern as the control serum (MC). All other sera tested contained antibodies against the viral glycoprotein (P_1), the nucleocapsid protein (P_3), the fusion protein (P_5), and the internal viral protein (P_2). At the dilutions used there were differences in the amounts of the precipitated surface antigens P_1 and P_5. Of the three early convalescent sera from vaccinated individuals, two (sera M18 and M20) precipitated less viral antigen than serum M19. These differences correlated with differences in neutralizing titers (neutralizing titers were 200 for M18 and M20 and 800 for M19). Similarly, later convalescent serum M10 (neutralizing titer, 40) precipitated less P_1 and P_5 than M11 (neutralizing titer, 80). However, the correlation between neutralizing titer and precipitation of viral antigens did not always hold. This is most strikingly demonstrated if late convalescent sera after natural infection are compared with sera after vaccination. Neutralizing titers were similar (80 for both M11 and M23), but sera from vaccinated individuals were much less efficient in precipitating viral antigens than sera from individuals with natural infections. Also, two sera from measles encephalitis cases (M32 and M33) precipitated viral antigens with equal efficiency, although one (M33) had a low neutralizing titer.

These sera were also reacted with iodinated HEp-2-PI cells for a more sensitive test of P_5-

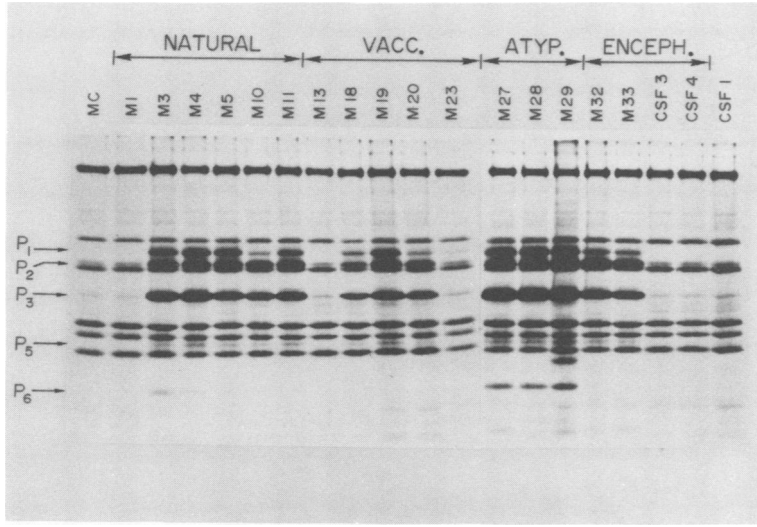

FIG. 3. *Fluorogram of polypeptides precipitated from [^{35}S]methionine-labeled HEp-2-PI cells by various sera and CSF. All serum samples were diluted 1:20, and CSF were diluted 1:10. The following sera were used: seronegative control (MC; neutralizing titer, <5); acute serum during natural measles infection (M1; neutralizing titer, <10); early convalescent serum after natural measles (M3, M4, M5; neutralizing titers, 800); late convalescent serum after natural measles (M10, M11; neutralizing titers, 40 and 80, respectively); acute serum after vaccination (M13; neutralizing titer, <10); early convalescent serum after vaccination (M18, M19, M20; neutralizing titers, 200, 800, and 200, respectively); late convalescent serum after vaccination (M23; neutralizing titer, 80); sera from cases of atypical measles (M27, M28, M29; neutralizing titers, 1,600, 3,200, and 3,200, respectively); sera from cases of measles encephalitis (M32, M33; neutralizing titers, 200 and 20 respectively). CSF were CSF 1 from a normal individual and CSF 3 and 4 from cases of measles encephalitis (all with neutralizing titers of <10).*

specific antibodies. Antibodies against the fusion protein were indeed present (data not shown).

The various sera differed in ability to precipitate the matrix polypeptide. Significant precipitation was achieved only by sera from patients with atypical measles (sera M27 through M29). Most early convalescent sera from individuals with natural measles (six of eight tested) also precipitated the matrix protein, but not as efficiently (see data for sera M3 and M4). All other sera (early and late convalescent sera after vaccination, later convalescent sera after natural measles, and sera from patients with measles encephalitis) did not precipitate the matrix protein. Atypical serum M29 also precipitated a polypeptide that migrated slightly faster than actin. This is possibly a degradation product of the nucleocapsid polypeptide that could have been produced by proteolytic activity in this particular serum.

Neither CSF from individuals with measles encephalitis (CSF 3 and 4) nor CSF from a normal individual (CSF 1) precipitated any viral polypeptides. (All had undetectable viral neutralizing titers.)

Quantitation of specific antibodies. We attempted to quantitate the relative amounts of virus-specific antibodies in the various sera by reacting [^{35}S]methionine-labeled HEp-2-PI extracts with twofold serum dilutions. The following sera were used: M3 (early convalescent after natural measles; neutralizing titer, 800), M11 (late convalescent after natural measles; neutralizing titer, 80), M19 (early convalescent after vaccination; neutralizing titer, 800), and M27 (atypical measles serum; neutralizing titer, 3,200). Fluorograms of precipitated antigens are shown in Fig. 4.

These fluorograms were also scanned, and areas under the various polypeptide peaks were integrated for each titration. The relative amount of each precipitated viral antigen was determined, and these values were plotted against serum concentrations. Antibodies

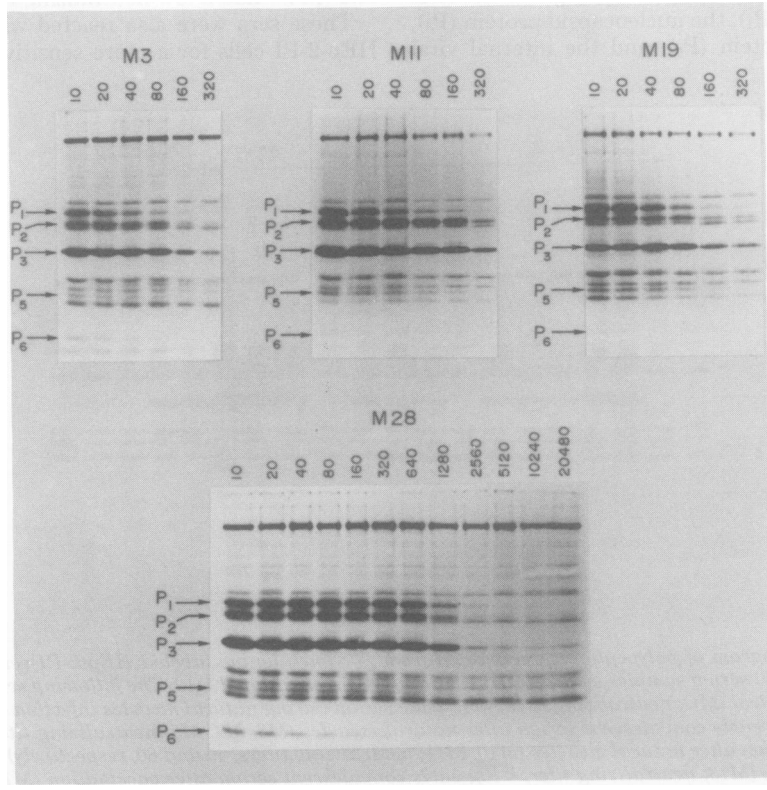

FIG. 4. *Fluorograms of polypeptides precipitated from [^{35}S]methionine-labeled HEp-2-PI cells with serial dilutions of four different sera. The sera used were early and late convalescent sera after natural measles (M3, and M11; neutralizing titers, 800 and 80, respectively), early convalescent serum after vaccination (M19; neutralizing titer, 800), and serum from a case of atypical measles (M28; neutralizing titer, 3,200). Reciprocal serum dilutions are shown.*

against nucleocapsid polypeptide P_3 and polypeptide P_2 seemed to be most abundant in all sera. However, these apparent high titers probably reflect the fact that P_2 and P_3 are present in very large nucleocapsid structures which require relatively few antibody molecules for precipitation. Polypeptide P_5 migrated too close to contaminating host polypeptides to permit accurate analysis. Table 2 shows the serum concentrations required for 50% precipitation of P_1 and P_6, when the amount precipitated by atypical serum M28 was used as the maximum. From these experiments the following conclusions were drawn. (i) The glycopolypeptide P_1 reacted with atypical serum with a 50% precipitation point of approximately 1:820; with early convalescent serum this point was 1:40, and for the other two sera it was 1:15. (The precipitation of the fusion polypeptide P_5 seemed to follow a similar pattern [data not shown].) (ii) Precipitation of the matrix polypeptide P_6 could be measured only for early convalescent serum after natural infection and atypical serum. Matrix-specific antibody titers in the atypical serum were clearly the highest; the 50% precipitation point was approximately 1:75, whereas that for early convalescent serum was 1:25.

Measles-specific immunoglobulins that do not bind to Staph A. It has been reported that Staph A does not bind all classes of human immunoglobulins (5, 16). The possible presence of such measles-specific immunoglobulins was investigated as follows. Serum samples representative of the various groups discussed (sera M1, M3, M12, M13, M18, M28, and M33) were incubated with extracts from [^{35}S]methionine-labeled HEp-2-PI cells, as described above. The supernatant from Staph A-precipitated antigen-antibody complexes was removed and incubated for 1 h with a secondary antibody directed against human IgG, IgM, IgD, or IgA. Staph A was added to precipitate any secondary complexes, which were analyzed on gels as described above. No significant amounts of non-Staph A-binding antibodies directed against any of the measles virus antigens were present in the serum samples investigated (data not shown). The absence of measles-specific IgM in the acute sera suggests that these samples were not collected at peak IgM response.

TABLE 2. *Serum concentrations required for 50% precipitation of viral antigens P_1 and P_6*[a]

Serum	Neutralizing links	Precipitated antigens:	
		P_1	P_6
M3	800	40	25
M11	80	15	ND[b]
M19	800	15	ND
M28	3,200	820	75

[a] Serum concentrations are given as the reciprocal dilutions at which 50% of maximal antigen precipitation occurred. Values were determined after scanning the fluorograms shown in Fig. 4 and plotting the relative areas under the peaks versus serum concentration for each antigen. Maximum antigen precipitation occurred with serum M28 in all cases.

[b] ND, Not determined. Quantitation was not possible because too little polypeptide precipitated.

DISCUSSION

In this paper, we describe a convenient and sensitive immunoprecipitation test for antibodies against all structural components of measles virus. This method was applied to determine antibody specificity in sera from individuals with different manifestations of measles infection and in CSF from two patients with measles encephalitis. The latter had undetectable neutralizing titers and did not precipitate any viral antigens. Acute sera with low viral neutralizing titers also failed to precipitate viral antigens. The other sera tested contained antibodies against all viral antigens but one, the matrix protein. High matrix-specific antibody titers were present only in atypical measles sera, lower titers were detected in early convalescent sera from patients with natural measles, and none could be detected in the other sera. All five atypical sera tested gave identical results; two of either early convalescent sera were negative for matrix antibodies, although they did precipitate P_1, P_2, P_3, and P_5. It is possible that these sera were collected too early or too late after the appearance of clinical symptoms. Increased antibody levels against the matrix polypeptide in atypical sera relative to early convalescent sera reflected an overall increase in virus-specific antibodies. Polypeptides P_1 and P_5 were also precipitated with greater efficiency by atypical sera (Fig. 3 and Table 2).

Four sera were chosen to determine whether this type of analysis can be used for the quantitation of virus-specific antibodies. The results (Fig. 4 and Table 2) show that this requires antigens, such as the surface glycopolypeptide P_1, that incorporate radioactive precursor material readily, and/or sera with relatively high levels of antibodies. The matrix polypeptide was weakly labeled, and, with the exception of atypical sera, antibody concentrations were low. Consequently, quantitation of this polypeptide was very difficult. Thus, the absence of detectable matrix-specific antibodies in late convalescent and vaccination sera may reflect a lower titer to all viral antigens, with the level of anti-matrix antibodies below the level of detection in the assay.

It should be noted that we found no clear correlation between neutralizing titers of the sera and their ability to precipitate the surface antigens P_1 and P_5. Within each group there was a positive correlation (Fig. 3); i.e., late convalescent serum M10 (neutralizing titer, 40) precipitated less P_1 and P_5 than M11 (neutralizing titer, 80), and early convalescent serum M19 (neutralizing titer, 800) precipitated more of these antigens than M18 and M20 (neutralizing titers, 200). However, several discrepancies were noted. Measles encephalitis sera M32 (neutralizing titer, 200) and M33 (neutralizing titer, 20) precipitated nearly equivalent amounts of P_1 and P_5. Also, serum M11 (late convalescent after natural infection) showed much greater viral surface antigen precipitation than serum M23 (late convalescent after vaccination), although both sera had neutralizing titers of 80. The titration experiment in Fig. 3 showed that late convalescent serum M11 (neutralizing titer, 80) was as efficient as vaccination serum M19 (neutralizing titer, 800) with regard to precipitation of the viral surface antigens.

However, a positive correlation between neutralizing titers and viral surface antigen precipitation would not necessarily be expected when one considers that the two assays detect virus-specific antibodies by different mechanisms. Neutralization of viral infectivity may involve only a minor population of antibodies directed against one or a few antigenic determinants on a presumed viral surface antigen. On the other hand, the immunoprecipitation assay described here most likely detects a broad spectrum of antibodies directed against many antigenic determinants on the viral surface polypeptides. It is also possible that the configurations of the viral surface antigens on the intact virion (used in the neutralization test) and in the cytoplasmic extracts (used for immunoprecipitation) differ.

Clearly antimatrix activity does not correlate with neutralizing titers, which were high in many of the matrix-negative sera. Instead, there seems to be a correlation between the expression of clinically apparent symptoms of measles and the presence of antimatrix antibodies. It has been suggested that a cellular immune reaction may contribute to the rash during natural measles infection and to the complications during atypical measles (17). Whether such reactions are mediated through the matrix protein at the surfaces of infected cells remains to be determined. It has been shown for influenza virus that the matrix protein, although internal in the virion, can be expressed at the surfaces of infected cells (1, 4). Also, the presence of antibodies against the matrix protein results in more rapid clearance of influenza virus from the lungs of mice, without preventing the development of pneumonia (24). These results suggest a possible role of the matrix polypeptide in the pathogenesis of influenza.

However, it is also possible that exposure of the measles virus matrix protein to the immune system of a host is more pronounced during severe clinical symptoms, either through increased expression at the cell surface or through increased cytopathology of infected cells. In this case, increased matrix-specific antibody titers would be a result of, rather than the cause of, the more severe clinical manifestations.

For further studies, it will be necessary to follow several patients closely and measure their antibody responses at regular intervals during various manifestations of measles infection. Furthermore, cellular immune responses involving the matrix protein should be determined.

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