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Antibodies Against Measles Virus Polypeptides in Different Disease Conditions

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The occurrence of antibodies to the nucleoprotein and matrix (M) antigens of measles virus was determined in early and late measles convalescent sera and in sera from patients with multiple sclerosis, subacute sclerosing panencephalitis, chronic active hepatitis, and atypical measles. Antibodies to the two components were identified separately in serially diluted samples both by radioimmune precipitation assays and by complement fixation tests employing purified nucleoprotein and M components as antigens. The antibody response to M antigen in connection with acute infections was weak, and with time titers of antibodies to M antigen were reduced below detectable levels in most cases. A different situation was seen in patients with atypical measles. A pronounced antibody response to M antigen was shown to be a part of the generally accentuated immune response in these patients. Confirming results of others, it was shown that in spite of the increased antibody titers against most measles components in sera from patients with subacute sclerosing panencephalitis, no or only low titers of antibodies to M antigen were present. However, a similar representation of antibodies to measles virus components was also seen in sera from patients with active chronic hepatitis. The significance of this finding for the interpretation of a weak antibody response to M antigen in the presence of a pronounced antibody response to other components is discussed.

Measles virus can cause both acute and persistent infections. The most extensively studied form of persistent infection is the rare disease subacute sclerosing panencephalitis (SSPE). One important feature of this disease is the occurrence of an accentuated humoral immune response (14). Serum antibody titers are 10 to 100 times higher than in normal late convalescent sera, and in cerebrospinal fluid, measles virus-specific oligoclonal immunoglobulin G, which is locally produced in the central nervous system, can be demonstrated.

An accentuated humoral immune response has further been found in studies of sera from patients with atypical measles. This form of measles occurs in individuals who have received inactivated measles vaccine. Due to an antigen defect of the vaccine, it does not give long time protection but instead provides conditions for development of immune pathological reactions (10).

Increased measles virus antibody titers have also been encountered in certain other disease conditions. Patients with systemic lupus erythematosus and chronic active hepatitis frequently have high serum antibody levels (6). However, this phenomenon concerns not only measles virus antibodies but also antibodies to some other viruses, e.g., rubella virus. An increase in measles virus serum antibody titers, although less pronounced, is also seen in patients with multiple sclerosis (9). About 60% of these patients in addition produce oligoclonal measles virus-specific immunoglobulin G in their central nervous system. Also, in patients with multiple sclerosis it was found that the changes in antibody production are not unique to measles virus antibodies, and it has therefore been proposed that the phenomena observed reflect a more general disturbance of immune regulation.

Antibodies to viral antigens can be studied by nonselective techniques such as complement fixation (CF) tests with whole virus antigen or by techniques demonstrating antibodies to a certain structural component, as for example in neutralization or hemagglutination-inhibition (HI) tests. During recent years the radioimmune precipitation assay (RIPA) has been employed to selectively demonstrate the occurrence of antibodies reacting with different structural com-

ponents. In several studies the RIPA test was used to characterize the antibody response to measles virus polypeptides in different groups of patients. A special interest has been focused on the antibody response to the matrix (M) antigen since it was found that in patients with SSPE the antibody response to this polypeptide is very weak or undetectable in spite of an accentuated antibody response to other polypeptides (3, 18). It has been proposed that the poor antibody response is due to a defect in production of M antigen or a rapid degradation of this antigen in infected cells. However, later studies (13) have shown that the antibody response to M antigen in connection with a regular infection is weak and that the titers of antibodies rapidly decline to undetectable levels. Thus antibodies to the M antigen were found only in sera from patients with symptoms of measles, either in connection with natural disease or atypical measles (8), whereas sera from patients with multiple sclerosis did not contain antibodies to the matrix antigen (5).

The purpose of the present study was to compare the antibody response to the nucleoprotein (NP) antigen, which dominates the antibody response in connection with a regular measles infection (11), and the M antigen in different disease conditions. Antibodies to the two components were determined not only in endpoint dilution experiments by the RIPA test, but also by CF tests in which purified preparations of NP and M components (16) were used as antigens. The groups of sera studied were collected about 2 weeks and more than 10 years after regular measles and from patients with atypical measles, SSPE, multiple sclerosis, and chronic active hepatitis. To improve possibilities for detection of antibodies to the M antigen, both late convalescent sera and sera from patients with chronic active hepatitis were screened for HI antibodies, and the 25% of samples with the highest titers in each group were selected for further analysis.

MATERIALS AND METHODS

Sera. Early convalescent sera from 10 cases of regular measles were collected 10 to 20 days after the appearance of rash. The cases were defined by clinical characteristics and by the occurrence of increased measles virus CF antibody titers in early convalescent sera as compared to matching acute sera. A total of 56 late convalescent sera obtained from individuals who 10 to 20 years earlier had had regular measles were obtained from the Department of Virology, National Bacteriological Laboratory, Stockholm. The measles virus HI antibody titers of these samples were determined, and the 14 sera with the highest titers were selected for further studies. Sera from six patients with definite multiple sclerosis analyzed in a separate study (17) and six patients with clinically and serologically defined SSPE were also used in this study. Sera from 24 patients with chronic active hepatitis (verified clinically and by liver biopsy) were obtained from the Department of Immunology, National Bacteriological Laboratory, Stockholm. After screening for measles virus HI antibodies, the six samples with the highest titers were selected for further studies. Finally, sera were collected 10 to 20 days after development of symptoms of atypical measles from five patients. All patients had a prior history of vaccination with inactivated vaccine and showed a pronounced measles antibody response in connection with development of symptoms.

Purification of NP and M antigens. The technique described recently (16) was used to purify NP and M antigens. Antigens were prepared by sedimenting material from a 1% Triton X-100 (Cutscum; Fischer Scientific Co., Fair Law, N.J.) extract of LEC measles virus-infected cells and further fractionation of the interphase material in a linear CsCl gradient, density range 1.20 to 1.33 g/ml. NP components contaminated with a small amount of M antigen banded at 1.30 to 1.32 g/ml, and M antigen free of NP material banded at 1.20 to 1.22 g/ml. Each material was recovered and used as antigen in CF tests.

Serological tests. HI tests were performed with a Tween 80- and ether-treated virus material, using a microtiter assay as described previously (11). CF tests were also performed in a microtiter assay. Two CF units of NP or M antigen were used per serum dilution. The specificity of the antigen preparations was determined by use of rabbit hyperimmune sera. The degree of contamination of the NP preparations with M antigen was less than 5% and therefore did not influence the specificity of the test.

RIPA. The RIPA technique used was recently described in detail (12, 15, 16). LEC measles virus-infected Vero cells were labeled with 20 μCi of [^{35}S]-methionine per ml for 3 days and then scraped off. After two washings the cells were disrupted in an RIPA buffer containing 0.15 M NaCl, 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.4), 1% sodium deoxycholate, 1% Triton X-100, 0.5% sodium dodecyl sulfate, and, as protease inhibitors, 1 mM phenylmethylsulfonyl fluoride and 100 kU of Aprotinin (Sigma) per ml. The lysate was centrifuged at 35,000 rpm for 60 min in an SW50 rotor (Spinco Division, Beckman Instruments Inc.), and the supernatant was collected and stored at −20°C. A 0.1-ml volume of the lysate was mixed with 20 μl of serial fivefold dilutions of samples, and the final volume was adjusted to 0.5 ml with RIPA buffer. After incubation at 4°C for 1 h, protein A bound to Sepharose CL-4E (Pharmacia Fine Chemicals, Uppsala, Sweden) was added. After further incubation for 1 h, the Sepharose beads were washed four times in RIPA buffer, and the immune complexes were disrupted with a lysis buffer for sodium dodecyl sulfate-polyacrylamide electrophoresis, boiled at 100°C for 2 min and electrophoresed in 15% acrylamide slab gels with sodium dodecyl sulfate-phosphate buffer. The slab gels were fixed, dried, and autoradiographed against Kodak X-ray film by the

use of conventional procedures. The highest dilution of serum which gave a visible band of precipitated polypeptide was taken as the endpoint.

RESULTS

Characterization by RIPA tests of the occurrence of antibodies to NP and M antigens. Under the conditions of performance of RIPA tests, antibodies to the hemagglutinin (H), NP, fusion (F1), and M components were readily determined. Due to the prolonged time of labeling no P antigen was detectable (4), and some degradation products of the NP polypeptide (predominantly about 45,000 molecular weight [45K]) occurred in the antigen material.

Table 1 gives a summary of the range of ratios of NP over M antibody endpoint titers in RIPA tests. This form of relative expression is used since due to inherent technical qualities RIPA tests do not allow absolute titer determinations. Factors such as varying degree of labeling of different antigen preparations and time of autoradiography exposure influence antibody titers. Antibodies to the NP antigen always dominated over antibodies to other structural proteins. However, whereas antibodies to NP antigen occurred in all samples there was a markedly varying presence of antibodies to the M antigen. In many cases it therefore was necessary to describe the ratio of antibodies to NP and M antigens as larger than a certain value instead of giving an absolute value.

Eight of 10 early convalescent sera contained detectable antibodies to M antigen. In one case the ratio of NP to M antibodies was 25, but in the other cases it was 125 or higher. The antibody response to M antigen appeared to be both weak and transient (Fig. 1). Only 3 of the 14 selected high-titer late convalescent sera contained detectable antibodies to M antigen. Similarly, antibodies to M antigen were only occasionally seen in sera from patients with multiple sclerosis (two of six samples positive). SSPE sera contained high titers of HI antibodies and antibodies to NP antigen determined by RIPA tests. However, confirming observations by others (3, 18), only one of six samples contained antibodies to M antigen (Fig. 2A). As a consequence the ratios of antibodies to NP and M antigen were high. A similar finding was somewhat unexpectedly made in tests with selected high-titer sera from patients with chronic active hepatitis (Fig. 2B). Only one of six samples contained demonstrable antibodies to M antigen, whereas antibodies to NP antigen occurred in high titers.

Sera from cases of atypical measles contained high titers of HI antibodies, corresponding to titers found in the sera selected from patients

TABLE 1. *Antibody response to measles virus NP and M antigens in different sets of sera*[a]

Serum	No. of sera	Range of HI serum titers	NP/M endpoint antibody titers[b]
Measles early convalescent	10	160–2,560	25–>125 (8)
Measles late convalescent	14	320–2,560	125–>625 (3)
Multiple sclerosis	6	20–640	125–3,125 (2)
SSPE	6	160–5,120	>125–>3,125 (1)
Chronic active hepatitis	6	1,280–20,480	>125–>625 (1)
Atypical measles	5	2,560–10,240	25–125 (5)

[a] As determined by RIPA tests. The number of sera in each group with detectable antibodies to M antigen and the ratio of NP to M endpoint antibody titers are given. The range of HI serum titers in each set of serum samples is also presented.

[b] Range of ratios in RIPA tests. Parentheses give number of sera with detectable antibodies to M antigen.

with chronic active hepatitis. As a further similarity, sera of both categories contained high titers of antibodies to NP antigen as determined by RIPA tests. However, whereas antibodies to M antigen were rarely seen in sera from patients with SSPE or chronic active hepatitis, they occurred in readily measurable titers in sera from all patients with atypical measles (Fig. 2C). In the latter cases ratios of antibodies against NP and M antigens corresponded to, or in many cases were even lower than, those found in early measles convalescent sera.

Characterization, by CF tests with purified virus components, of the occurrence of antibodies to NP and M antigens. There was a good agreement between occurrence of antibodies to NP and M antigens as determined by RIPA tests and by CF tests with purified antigens. However, antibodies to M antigen were more readily identified by the CF test than by the RIPA test, and in a few cases, where no antibodies to M antigen were detected in the RIPA test, the M antigen CF test showed a moderate titer of antibodies. This was interpreted to mean that the CF test allowed detection of antibodies to a larger spectrum of antigenic sites than the RIPA test (see Discussion). Table 2 gives a summary of data obtained in NP and M antigen CF tests.

After regular measles the antibody response to M antigen was weak, and in about half of the cases no antibodies were detectable in late convalescent sera or in sera from patients with multiple sclerosis. Sera from five of six SSPE

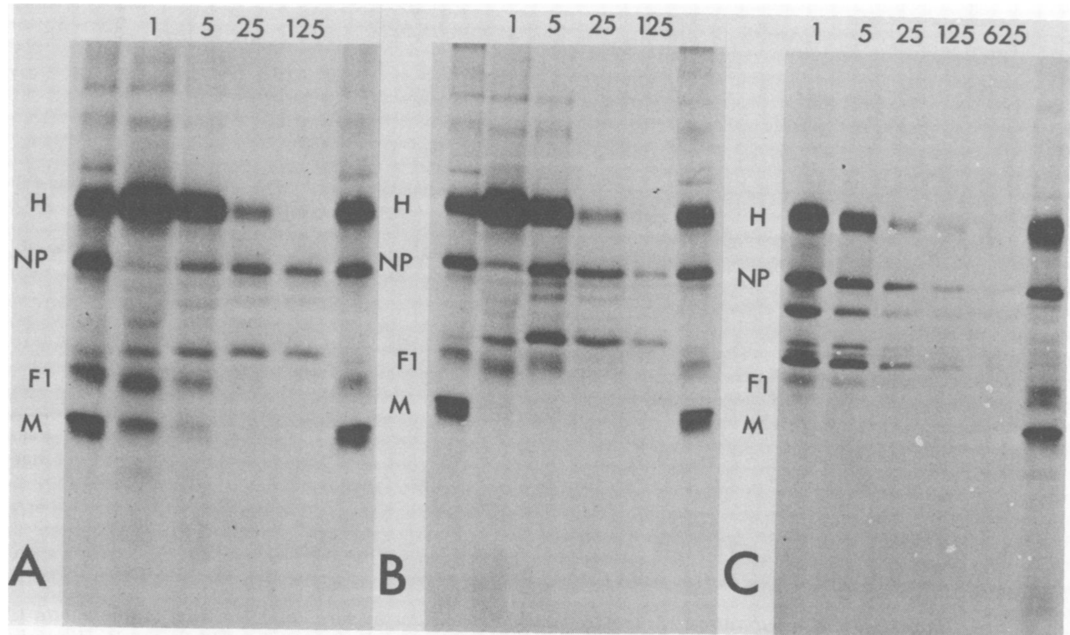

FIG. 1. *Radioimmune precipitation of measles virus polypeptides by sera collected 10 days after regular measles (A), more than 10 years after regular measles from a healthy individual (B), and from a patient with definite multiple sclerosis (C). The following polypeptides can be identified: hemagglutinin (H, 79K), nucleoprotein (NP, 60K), hemolysin-fusion factor (F1, 41K), and matrix protein (M, 36K). Serial fivefold dilutions of 20 μl of serum in a final reagent volume of 0.5 ml were tested. Lanes not labeled with dilution factors contain reference virus preparations.*

patients contained detectable antibodies to M antigen, but the titer was 10 times lower than the matching CF antibody titer against NP antigen. Only two of six sera from patients with chronic active hepatitis contained detectable CF antibodies against M antigen, and the titers were 20 and 160. Relatively high titers of CF antibodies against M antigen were found in patients with atypical measles. The ratios of NP over M antigen CF antibody titers, as in the case of measles early convalescent sera, were about 4.

DISCUSSION

The two methods used for determination of antibodies against NP and M antigens gave results that were in good agreement, but the CF test appeared to be relatively more efficient for demonstration of antibodies to the M antigen. Since the CF test in general has a low sensitivity compared to the RIPA test, this difference in efficacy for demonstration of antibodies to M antigen most likely is due to inherent limitations of the RIPA test. Several factors can be of importance in this context.

(i) The relative labeling of different polypeptides with [^{35}S]methionine may vary due both to the relative amount of individual components produced and to their relative content of methionine. The degree of labeling influences their detectability by autoradiography of the electrophoretically separated polypeptides. In the present experiments antigen preparations labeled for a relatively long time (3 days) were used to allow maximal labeling of individual polypeptides. The effective labeling of M antigen was shown by the precipitation of this component by antibodies in sera from cases from acute and atypical measles.

(ii) The sensitivity to proteolytic breakdown varies for different structural polypeptides. The P component shows the highest sensitivity to proteolytic breakdown (4), and because of the long time used for labeling of antigens no antibodies to P antigen were detected in this study. Also the NP antigen is degraded to a certain extent by proteolytic enzymes, but antibodies to this component are readily detected by identification of uncleaved polypeptides and degradation products in the autoradiogram. The M component appears relatively resistant to proteolytic cleavage.

(iii) The overall number of antigenic sites in different components varies, and furthermore, there may be a variation in sensitivity of differ-

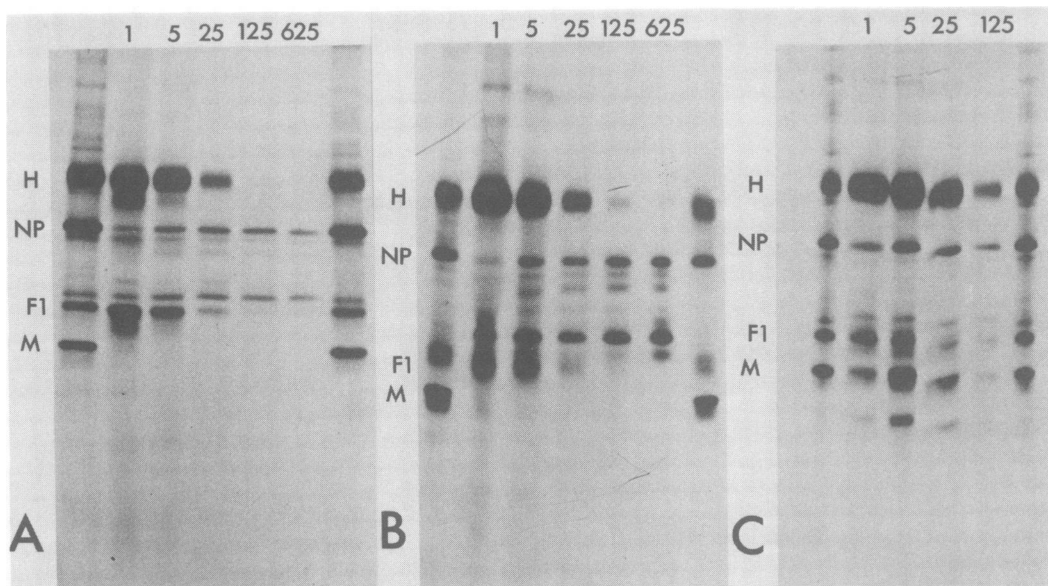

FIG. 2. *Radioimmune precipitation of measles virus polypeptides by sera collected from patients with (A) SSPE, (B) chronic active hepatitis, and (C) atypical measles. For explanation of markings see the legend to Fig. 1.*

TABLE 2. *Antibody response to measles virus NP and M antigen as determined by CF tests with purified antigens*

Serum sample	No. of sera	CF antibody titer in test with: NP antigen, range (mean value)		CF antibody titer in test with: M antigen, range (no. of positive samples; mean value)		Range of NP/M CF antibody titer ratios
Measles early convalescent	10	40–640	(260)	<10–160	(9;68)	1–>32
Measles late convalescent	14	<10–640	(160)[a]	<10–320	(8;—)[b]	1–>32
Multiple sclerosis	6	20–320	(110)	<10–320	(1;—)[b]	1–>32
SSPE	6	80–5,120	(450)	<10–640	(5;46)	8–>32
Chronic active hepatitis	6	1,280–20,480	(3,800)	<10–160	(2;—)[b]	128–>2,048
Atypical measles	5	640–2,560	(2,100)	80–2,560	(5;500)	2–32

[a] Twelve of 14 sera contained detectable antibodies.
[b] Mean value not calculated.

ent components to denaturing effects of the treatment used to dissociate virus products in the cell lysate.

(iv) Antibodies belonging to different immunoglobulin classes react differently in the RIPA and CF tests. Thus, human immunoglobulins M and G3 can fix complement, but would not be expected to be precipitated by *Staphylococcus* protein A.

It seems likely that the phenomena discussed under (iii) above may be of major importance in explaining the difference in capacity of RIPA and CF tests to detect antibodies against M antigen. Thus M antigen may have both a relatively low number of antigenic sites, which could partly explain the limited and transient response to M antigen after a regular infection, and also a relatively higher susceptibility to denaturing effects of the lysis buffer than, e.g., the NP antigen.

In parallel tests antibodies to structural components of another paramyxovirus, Sendai virus, were studied (Örvell, unpublished data). In contrast to the situation after a measles infection, antibodies to Sendai virus M protein were clearly demonstrable even a long time after the primary immunization. It is not known which qualitative difference between the M components of measles and Sendai virus may explain the observed difference in antigenicity.

Whereas the RIPA test, in confirmation of results obtained by others (3, 18), in most cases of SSPE does not detect antibodies to M antigen, the CF test detected antibodies to M antigen in

five of six patients. However, the relative titer of antibodies to M antigen in relationship to antibodies to NP antigen is low compared with the relative occurrence of these antibodies in sera from patients with acute or atypical measles, emphasizing that there may be some defect in the production of M antigen in SSPE patients. This defect may be quantitative, although a complete absence of M production appears uncommon, and also qualitative with a change of certain antigenic sites on which reactivity in the RIPA test is dependent. It should be mentioned that analysis of virus-specific polypeptides in cell cultures infected with defective SSPE measles virus strains has demonstrated a limitation in the capacity of the virus to synthesize M protein (2, 7).

In the present study a very limited antibody response to M antigen was found not only in serum samples from patients with SSPE but also in sera from patients with chronic active hepatitis. This was somewhat unexpected since increased titers of antibodies against measles virus as well as certain other viruses, e.g., rubella virus, has been interpreted to signify that there is a general activation of immunoglobulin production in these patients. One would therefore anticipate that there should be a certain parallel between the rise in titers of antibodies against NP and M antigens. The fact that this is not the case could have either of two explanations. One possibility is that, similar to what has been proposed for SSPE, a defective measles virus infection is involved in chronic active hepatitis. This explanation appears less likely in view of the fact that the accentuated production of immunoglobulin concerns not only antibodies to measles virus. However, there are examples of a nonspecific activation of antibody production to one virus in connection with an intense immune response to another virus in the central nervous system (B. Vandvik, R. E. Nilsen, F. Vartdal, and E. Norrby, submitted for publication), and furthermore, a transient production of autoantibodies is observed in connection with a regular measles infection (see reference 1). The other possibility is that the primary antibody response to the M antigen is weak and poorly memorized and that therefore a polyclonal activation of B cells a long time after the primary infection might be less likely to include an antibody response to M antigen.

Recently the concentration of antibodies to NP and M antigens was determined in sera from five cases of systemic lupus erythematosus selected to contain high titers of measles antibodies (Norrby and Örvell, unpublished data). Antibodies to M antigen were detectable in three patients, but the ratio of antibodies to NP and M antigen was high (625 or higher). Thus measles antibody characteristics of sera from this kind of patients are similar to those of sera from patients with active chronic hepatitis.

The high-titer antibody response to M antigen in patients with atypical measles is of interest. It shows that the M antigen produced during the acute infection in these patients can give an intense immunization. This immunization probably infers that the preceding immunization with inactivated measles vaccine also included a sensitization with M antigen, but it can also be that the conditions of immune pathological reactions in patients with atypical measles enhance the immune response, including also antibodies to the M antigen.

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