ANTIBODY RESPONSE TO STRUCTURAL PROTEINS OF MEASLES VIRUS IN PATIENTS WITH NATURAL MEASLES AND SUBACUTE SCLEROSING PANENCEPHALITIS

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SUMMARY: By immunoprecipitation and SDS-polyacrylamide gel electrophoresis, antibody responses to the structural proteins of measles virus were examined on patients with various forms of natural measles, atypical measles, and subacute sclerosing panencephalitis (SSPE). The serum of atypical measles most strongly reacted with four structural proteins, i.e., hemagglutinin (H), nucleocapsid (NC), fusion (F), and matrix (M) proteins. In natural measles, antibodies to the four structural proteins were detected at such an early convalescent stage as one month after the onset of disease. In late convalescent serums taken 9 years after natural measles, antibodies to the four structural proteins were detected at such an early convalescent stage as one month after the onset of disease. In late convalescent serums taken 9 years after natural measles, however, only low level antibody to M protein was present, whereas antibodies to H, NC and F proteins persisted. The serums and cerebrospinal fluid of SSPE patients showed patterns similar with those of the late convalescent serums. In Vero cells infected with cell-associated SSPE viruses (Niigata-1, ZH, and SI strains), M protein was not clearly demonstrated with serum of either atypical measles or SSPE patients, whereas H protein was demonstrated.

INTRODUCTION

Measles virus infection produces different forms of clinical course such as natural measles, atypical measles and SSPE (Yamanouchi, 1980). Atypical measles has developed in children immunized by the vaccination program of combined killed and live measles vaccines. Although the pathogenesis is not fully understood, atypical measles is assumed to be caused by natural measles virus infection in children previously sensitized with killed measles vaccine and can be considered an unusual form of hyperimmunization (Fulginiti et al., 1967).

SSPE is caused by long persistence in the central nervous system of measles virus, and considered to be a typical slow virus infection (ter Meulen, Katz and Muller, 1973; Morgan and Rapp, 1977; Yamanouchi, 1980). Recent demonstration of lack of antibody response to M protein of measles virus in SSPE patients raised a possibility that functional defect of M protein might be in-

However, little information is available on the antibody responses to structural proteins of measles virus in the subjects who showed normal course of measles virus infection. Therefore, we compared in the present study antibody responses to the four major structural proteins, H, NC, F and M, in natural measles, atypical measles and SSPE.

**Materials and Methods**

**Viruses:** Edmonston strain of measles virus was grown in Vero cells as previously described (Sato, Hayami and Yamanouchi, 1981). SSPE virus, such as cell-associated Niigata-1 (Doi et al., 1972), ZH and SI (Mirchamsy et al., 1978) strains were grown by cocultivation with Vero cells. SSPE virus-infected Vero cells were maintained in Eagle's minimum essential medium (EMEM) supplemented with 5% calf serum, 0.11% sodium bicarbonate, 100 units of penicillin and 100 µg per ml of streptomycin.

**Serums:** The serum obtained from a 20 year-old female (M.I.) who had recovered from natural measles one month before was used as an early convalescent anti measles serum. The serum obtained from a 12 year-old female (T.K.) who had natural measles 9 years ago was used as a late convalescent anti measles serum.

The serum obtained from a female (M.N.) who suffered from atypical measles after combined vaccination with killed and live measles vaccines was used as hyperimmune anti measles serum. The serums of SSPE patients were obtained from three cases (A.K., T.T., S.S.) that were diagnosed as SSPE from clinical symptoms and serological examinations.

**Serological tests:** Virus neutralization (VN) and hemagglutination-inhibition (HI) tests were described previously (Sato et al., 1981). Complement fixation (CF) tests were carried out on microplates with the cell extract of measles virus (Toyoshima strain)-infected KB cells as antigen.

**Labeling virus-infected cells with isotope:** Labeling measles virus-infected Vero cells with isotope was described in a preceding paper (Sato et al., 1981). SSPE virus-infected Vero cells were labeled when more than 50% of the cells formed syncytium (24 hr post inoculation (pi) for Niigata-1, 72 hr pi for ZH, and 90 hr pi for SI strain). After washing twice with EMEM depleted of valine, leucine and tyrosine for 1 hr at 37 C, the cells were fed with labeling medium consisting of EMEM and 100 µCi/ml each of 3H-valine, 3H-leucine and 3H-tyrosine and incubated overnight at 37 C. The methods of preparation of cell extracts and immunoprecipitation were described previously (Sato et al., 1981).

**Polyacrylamide gel electrophoresis (PAGE):** The method of electrophoresis was previously described (Sato et al., 1981); 18% polyacrylamide gel with an acrylamide-bisacrylamide ratio of 30:0.8 was used.

**Infectivity titration of SSPE viruses:** Vero cells infected with Niigata-1,
TABLE I
Serological tests of various anti measles serum

<table>
<thead>
<tr>
<th>Type of antiserum</th>
<th>Sample</th>
<th>Code</th>
<th>HI(1)</th>
<th>VN(2)</th>
<th>CF(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early convalescent</td>
<td>Serum</td>
<td>M.I.</td>
<td>64</td>
<td>200</td>
<td>32</td>
</tr>
<tr>
<td>Late convalescent</td>
<td>Serum</td>
<td>T.K.</td>
<td>64</td>
<td>NT(4)</td>
<td>16</td>
</tr>
<tr>
<td>Atypical measles</td>
<td>Serum</td>
<td>M.N.</td>
<td>2,048</td>
<td>5,700</td>
<td>256</td>
</tr>
<tr>
<td>SSPE</td>
<td>Serum</td>
<td>A.K.</td>
<td>2,048</td>
<td>NT</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>A.K.</td>
<td>256</td>
<td>NT</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>T.T.</td>
<td>1,024</td>
<td>2,800</td>
<td>1,024</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>S.S.</td>
<td>256</td>
<td>2,800</td>
<td>512</td>
</tr>
</tbody>
</table>

1) HI: Hemagglutination inhibition test.
2) VN: Virus neutralization test.
3) CF: Complement fixation test.
4) NT: Not tested.

ZH or SI strain were examined for both cell-associated and virion-associated infectivities. The virus-infected cells were dispersed by adding trypsin-EDTA and inoculated onto normal Vero cell cultures in semimicro-plates (Linbro FB-16-24-TC) for cell-associated infectivity. For virion-associated infectivity, the cells were subjected to freeze-thawing three times. Both the cell homogenate and supernatant after centrifugation at 2,000 rpm for 10 min were inoculated onto normal Vero cell cultures. The cultures were overlaid with medium containing 0.8% Noble-agar. The plaques were counted after incubation for 12 days at 37°C.

Hemadsorption and salt-dependent hemadsorption: The SSPE virus-infected cell monolayers were washed twice with phosphate buffered saline (PBS), pH 7.2, and flooded with 1% African green monkey red blood cells in PBS for hemadsorption or PBS containing 0.4 M ammonium sulfate for salt-dependent hemadsorption (Shirodaria, Dermott and Gould, 1976). After incubation for one hour at 37°C, the cells were washed three times with PBS or PBS containing 0.4 M ammonium sulfate and observed under a microscope.

RESULTS

Antibody Titers in Serological Tests

Serum and cerebrospinal fluid (CSF) samples obtained from natural measles, atypical measles and SSPE were examined by HI, virus neutralization, and CF tests. As summarized in Table I, both HI and CF antibodies persisted for 9 years after natural measles. Significantly high antibody titers in patients with atypical measles and SSPE were confirmed by the three serological tests. Moreover, a high titer of antibody was demonstrated in CSF of an SSPE patient by HI and CF tests.
Antibody Response to Structural Proteins in Natural Measles and Atypical Measles

Serums of early and late convalescence from natural measles and of atypical measles were analyzed for the reaction with structural proteins of measles virus by immunoprecipitation and SDS-PAGE. The results are shown in Fig. 1. The early convalescent serum reacted strongly with H, F, and M proteins and weakly with NC protein, whereas the late convalescent serum showed a markedly decreased reaction with M protein. The decrease in antibody to M protein in contrast to the persistence of antibodies to H, NC, and F proteins at a late convalescent stage was also observed for five paired serums obtained at an acute stage and 7-9 years after natural measles (data not shown). The serum of atypical measles showed stronger reaction with all the four structural proteins than the early convalescent serum. These results indicate long persistence of the antibodies to H and F proteins as well as relatively rapid decrease of the antibody to M protein after natural measles.

Antibody Response to Structural Proteins in SSPE

Four samples of SSPE patients including three serums and one CSF were examined for the antibodies to structural proteins. As shown in Fig. 2, all the serums from different patients showed essentially similar patterns of reaction; stronger reaction was observed with H and F proteins and moderate reaction with NC protein, whereas only minimal or almost negative reaction with M protein. In one patient (A.K.), whose paired serum samples and CSF were examined, similar patterns of reaction were observed with both serum and CSF.
Analysis of Structural Proteins in SSPE Virus-infected Cells

Three strains of cell-associated SSPE viruses were examined first for the production of cell-free virus as well as for hemadsorption. As summarized in Table II, cell-free virus was found in neither homogenate nor supernatant fluid for Niigata-1 strain. The other two strains, ZH and SI strains, were found to release a small amount of cell-free virus and to show hemadsorption under both standard and high salt conditions. None of the three virus strains produced any detectable amount of hemagglutinin in the culture fluid. Analyses for structural proteins in these three SSPE virus-infected Vero cells were carried out with the serums of atypical measles and SSPE. Essentially similar results
TABLE II
Characterization of SSPE virus-infected cells

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Homogenate Infectivity(^1)</th>
<th>Supernatant Infectivity</th>
<th>HA(^3)</th>
<th>Infectious center(^3)</th>
<th>HAD(^4)</th>
<th>SDA(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niigata-1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>480</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ZH</td>
<td>18</td>
<td>12</td>
<td>&lt;1</td>
<td>380</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SI</td>
<td>420</td>
<td>118</td>
<td>&lt;1</td>
<td>NT(^6)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1) Infectivity: PFU/ml; Assayed 2 days after cell transfer.
2) HA: Hemagglutination titers.
3) Infectious center: Number of infected cells/10^5 cells; Assayed 1 week after cell transfer.
4) HAD: Hemadsorption.
5) SDA: Salt-dependent hemadsorption.
6) NT: Not tested.

were obtained with the two serums as shown in Fig. 3. The three virus strains showed similar patterns; NC and F proteins were clearly demonstrated but M protein was either absent or present only in a trace amount. Hemadsorption-positive ZH and SI strains were found to produce H protein. Production of H protein by hemadsorption-negative Niigata-1 strain was also confirmed but in much a smaller amount than ZH and SI strains.

**DISCUSSION**

By the immunoprecipitation method, antibody responses to four major structural proteins i.e., H, NC, F, and M proteins, of measles virus, were analyzed. In agreement with the reports by several investigators (Hall et al., 1979; Wechsler et al., 1979; Machamer et al., 1981), all the serum and CSF samples of SSPE patients lacked detectable antibody to M protein of measles virus in contrast to high levels of antibodies to H and F proteins.

The antibody response to NC appeared not so high as that to H and F proteins. However, this apparently low antibody response was probably due to either inefficient labeling of NC protein with isotope or breakdown of labeled NC protein, since the serum of atypical measles patient, which was considered hyperimmune serum to the all viral polypeptides, reacted with NC similarly at low levels.

The presence of M protein in SSPE virus-infected cells was not clearly demonstrated with the serum of an atypical measles patient; the serum was shown to react with M protein in measles virus-infected cells (Fig. 1).

The serums of SSPE patients were examined with SSPE virus-infected cells for the possible presence of M protein with antigenicity different from that of measles virus. However, the reaction patterns in immunoprecipitation similar
Antibody response to M protein in SSPE patients has been controversial. Hall et al. (1979) suggested that defect of functional M protein in SSPE virus as indicated by the absence of M protein in SSPE virus-infected cells is responsible for the lack of antibody response to M protein. In contrast, Trudgett et al. (1980) demonstrated the antibody to M protein in all the sera of SSPE patients tested by the indirect immunoprecipitation method. In spite of much attention paid on antibody response at structural protein levels in SSPE, antibody response in natural measles has not been investigated in this respect. The present study clearly demonstrated that the antibodies to H, NC, and F proteins persist for such a long period as 9 years after natural measles, while the antibody to M protein disappears within the same period. Such time-dependent decrease in anti M antibody after natural measles was recently suggested by Machamer et al. (1980). Since most SSPE patients were examined to those with the serum of an atypical measles patient may cause argument against this possibility.
for the antibody to M protein later than 6 years, which is an average latent period after natural measles, the lack of the antibody to M protein may not be characteristic to only SSPE. More detailed examinations on antibody responses at structural protein levels in natural measles is required.

All the three strains of SSPE virus used in the present study are highly cell-associated and show high neurovirulence in rodents and monkeys (Yamanouchi et al., 1976; Mirchamsy et al., 1978). The present study disclosed a significant difference in biological properties; Niigata-1 strain did not release cell-free virus and was negative in hemadsorption under either standard or high-salt condition, whereas ZH and SI strains were found to release a small amount of cell-free virus and to be hemadsorption-positive under both the conditions (Table II). On the other hand, all the three strains were found to produce H protein by immunoprecipitation (Fig. 3); the amount appeared to be larger for ZH and SI strains than for Niigata-1 strain, which may reflect the difference in production of cell-free virus and hemadsorption. Salt-dependent hemadsorption was suggested being caused by the precursor of mature hemagglutinin. In this respect, hemagglutinin production in Niigata-1 strain-infected cells can be considered to be blocked at the stage of acquiring antigenicity of hemagglutinin, and not to proceed to express functional hemagglutinin on the cell surface. In contrast, functional hemagglutinin may be expressed on the cell surface in the case of ZH and SI strains.

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