Complement-Dependent Cytotoxicity of Sera Obtained from Subacute Sclerosing Panencephalitis Patients

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Abstract Human lymphoid cells (NC-37) persistently infected with either measles virus (Schwarz and TYCSA strains) or subacute sclerosing panencephalitis (SSPE) virus (Halle and Mantooth strains) were destroyed in the presence of complement by anti-measles sera as well as by sera from SSPE patients. The cytotoxic activity was demonstrated in both IgG and IgM fractions of measles convalescent sera, but only in IgG fraction of SSPE sera. Measles convalescent sera completely lost the cytotoxic activity to all the cell lines, when absorbed with any one of the cell lines, indicating that the viral surface antigens of these cell lines infected with measles or SSPE virus are identical. On the other hand, the cytotoxic activity of SSPE sera could not be readily absorbed with these cells. Thus, the affinity of SSPE sera for the viral surface antigens might be lower than that of measles convalescent sera.

Antisera to measles and sera from subacute sclerosing panencephalitis (SSPE) patients were shown to exert cytotoxic activity in the presence of complement on cells persistently infected with measles virus (2, 4, 5, 7). Steele et al (9), however, reported that SSPE sera were cytotoxic to cells infected with measles virus, but not to cells infected with SSPE virus. The carrier cell lines which they used varied as regards the virus strain as well as the host cell used for establishment of persistence. Accordingly it was difficult to decide whether the virus strain or the host cell, or both, was responsible for the difference in susceptibility to the cytotoxic effect of SSPE sera. Recently we established carrier cell lines by infecting a human lymphoid cell line, NC-37, with measles virus and with SSPE virus (6). In the present study SSPE sera as well as measles convalescent sera were found to exert cytotoxic effect on these carrier cell lines infected with either measles or SSPE virus.

MATERIALS AND METHODS

Cells. An uninfected human lymphoid cell line (NC-37), and four carrier cell lines (NC-Schwarz, NC-TYCSA, NC-Mantooth and NC-Halle) of NC-37 cells persistently infected with Schwarz and TYCSA strains of measles virus and Mantooth
and Halle strains of SSPE virus, respectively, were used. The establishment and maintenance of these persistently infected cell lines were described in a previous paper (6). Almost all cells of each carrier cell line were infected, as demonstrated by hemadsorption and immunofluorescent staining.

**Antisera.** Sera were collected from 8 children diagnosed as having SSPE, from 4 children at a convalescent stage of measles infection and from an adult with measles history. Control sera were collected from 2 children having no history of measles. These sera were kindly supplied by Dr. T. Togashi, Department of Pediatrics, Hokkaido University School of Medicine, Dr. T. Yamanaka, Department of Pediatrics, Sapporo Medical College, and Dr. T. Nunoue, Department of Pediatrics, Kyushu University School of Medicine. Hyperimmune serum was prepared in rabbits by 8 weekly intramuscular injections of Toyoshima strain of measles virus. All sera were heated at 56 C for 30 min before use.

**Cytotoxicity test.** Carrier cell lines, grown as described previously (6) in RPMI-1640 medium supplemented with 10% fetal calf serum, were washed twice with RPMI-1640 medium and suspended in RPMI-1640 medium supplemented with 5% fetal calf serum. One-tenth milliliter of the cell suspension containing 3 X 10^4 cells was mixed with a 0.1-ml volume of each of serial 2-fold dilutions of each test serum, incubated at 37 C for 15 min in a CO2 incubator, and 0.1 ml of complement (commercial lyophilized guinea pig serum) was added to each mixture. The mixtures were further incubated at 37 C for 45 min in the CO2 incubator, and the cells were observed microscopically after addition of 2 drops of isotonic trypan blue solution. As controls, untreated, serum-treated and complement-treated cells were included. These control cells were not stained at all. The cytotoxic titer of each serum was expressed by the reciprocal of the serum dilution which showed 50% of the maximum percentage of stained cells.

**Absorption of sera with carrier cells.** A carrier cell suspension containing approximately 2 X 10^8 cells was frozen and thawed, cell debris was collected by centrifugation, and washed twice with 0.1 M phosphate-buffered saline (PBS). The pellet was mixed with 0.1 ml of antiserum and incubated at 37 C for 1 hr. The mixture was then kept at 4 C overnight and centrifuged at 3,000 rpm for 15 min to remove cell debris.

**Fractionation of IgG and IgM of sera.** Sera were fractionated on a Sephadex G-200 column. One ml of the serum was layered on the top of the column. The effluent was collected in 5-ml fractions, and absorbance of each fraction was measured at OD_{280}. After these fractions were assayed for cytotoxic activity, IgG and IgM fractions were pooled and concentrated with 20% polyethylene glycol.

**RESULTS**

**Cytotoxicity of Sera of Measles and SSPE Patients**

All sera obtained from 4 measles patients and a healthy person with measles history were demonstrated to have the cytotoxic activity against cells of the four carrier cell lines (Fig. 1). The pattern of cytolysis by these sera differed depending
upon the cell line used. More than 90% of NC-Mantooth cells were lysed by all
the sera and no prozone was observed at low serum dilutions, while the maximum
percentage of lysed cells obtained with the other carrier cultures was about 70 to 40%
and a prozone was observed. However, cytotoxic titers of each serum with the different
cell lines resembled one another (Table 1). SSPE sera showed patterns of cytolysis
similar to those caused by sera of measles patients and lysed most effectively NC-
Mantooth cells (Fig. 2). A prozone was observed in NC-Halle and NC-Schwarz
cells. Titers of cytotoxicity, hemagglutination-inhibition (HI) and neutralization
(NT) of sera of SSPE patients were higher than those of measles patient sera (Table
1). The cytotoxic effect of rabbit hyperimmune serum was similar to that of measles

![Cytotoxicity on four carrier cells of measles patients sera. Procedures of this experiment are described in MATERIALS AND METHODS. Guinea pig sera as a source of complement were diluted 4-fold with RPMI-1640 medium. Convalescent sera of No. 1 (○), No. 2 (●), No. 3 (△), No. 4 (▲), and No. 5 (■) were used in this experiment. NC-Schwarz (a), NC-Mantooth (b), NC-Halle (c), and NC-TYCSA (d) were used as target cells.](image-url)
convalescent sera. Sera from children having no history of measles infection did not show any cytotoxic activity.

**Cytotoxicity of Sera of Measles and SSPE Patients Absorbed with Carrier Cells**

Measles convalescent sera, when absorbed with any one of the carrier cell lines, completely lost the cytotoxic activity against all of the four target cells (Fig. 3), whereas SSPE sera similarly absorbed showed only a partial decrease in the cytotoxic activity (Fig. 4). As summarized in Table 2, measles convalescent sera lost HI, NT, and cytotoxic activity after absorption with the carrier cells.

**Cytotoxic Effect of IgG and IgM Fractions of Sera**

IgG and IgM of these sera were fractionated by a Sephadex G-200 column. As shown in Fig. 5, cytotoxic effect against NC-Mantooth cells was demonstrated in IgG fraction but not in IgM fraction of an adult serum and 2 SSPE sera. Both IgG and IgM fraction of a measles convalescent serum and a rabbit hyperimmune serum showed cytotoxic effect (Fig. 6).
IgG obtained by gel filtration using a Sephadex G-200 column was concentrated by dialysis against 20% polyethylene glycol. The IgG diluted at 1:8 to show the maximum cytolysis was used in this experiment. Cytotoxic tests were carried out using serial twofold dilutions of complement (Fig. 7). Uninfected NC-37 cells were not lysed by IgG of SSPE or measles convalescent sera. The concentration of complement required to induce 50% cytolysis (CL50) was determined. When SSPE IgG was used, the activity of complement for NC-Mantooth cells was about 32
CL50/ml, but was less than 16 CL50/ml for the other carrier cells. In the case of measles convalescent IgG, the activity of complement was about 50 CL50/ml for NC-Mantooth cells, but about 16 CL50/ml for the other carrier cells. Thus, the requirement of complement was different between NC-Mantooth cells and the other carrier cells. The cytotoxic activity of these IgGs was highest for NC-Mantooth, followed by NC-TYCSA, NC-Halle and NC-Schwarz.
DISCUSSION

The data described here indicate that sera of SSPE and measles patients induced cytolysis of cells persistently infected with either measles or SSPE virus in the presence of complement. A prozone phenomenon was observed in the cytolysis of NC-Halle and NC-Schwarz cells by SSPE sera, but not in that of NC-Mantooth and NC-TYCSA cells. More than 90% of NC-Mantooth cells were lysed by these sera but 40 to 70% of the other cells were lysed. Sera of SSPE patients contained generally...
higher titers of cytotoxicity, HI and NT than convalescent sera of measles patients.

The cytotoxic activity was detected in IgG fraction of SSPE sera and in IgM and IgG fractions of measles convalescent and rabbit hyperimmune sera. As both IgM and IgG antibodies were found in sera and cerebrospinal fluids of SSPE patient, it was suggested that a continuous antigenic stimulus leads to the persistent existence of

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<th>Table 2. Activities of convalescent and SSPE sera absorbed with carrier cells</th>
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<td>Convalescent 1.</td>
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<td>Convalescent 2.</td>
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<tr>
<td>SSPE 1.</td>
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Cytotoxic tests of unabsorbed sera and sera absorbed with carrier cells were done at the same time, and the cytotoxic titer of each unabsorbed serum differed slightly from that expressed in Table 1.

<sup>a</sup> NC-Halle.
<sup>b</sup> NC-Mantooh.
<sup>c</sup> NC-Schwarz.
<sup>d</sup> NC-TYCSA.

Fig. 5. Gel filtration of sera from an adult (a) and SSPE patients (b, c). Procedures of the cytotoxicity test are described in MATERIALS AND METHODS. One-tenth milliliter of each fraction was used as the source of antibody. Absorbance at 280 nm (---) and cytolytic activity of each fraction (---) are plotted. Convalescent serum No. 1 (a), SSPE sera No. 1 (b), and No. 2 (c) were used.

Fig. 6. Gel filtration of sera from a hyperimmunized rabbit (a) and a measles patient (b). Procedures of the cytotoxic test are described in MATERIALS AND METHODS. A 0.1-ml aliquot of each fraction was used as the source of antibody. Absorbance at 280 nm (---) and cytolytic activity of each fraction (---) are plotted. Convalescent serum No. 2 (b) was used.
CYTOTOXICITY OF SSPE SERA

Fig. 5

Fig. 6
IgM antibody (1). However, IgM fractions of SSPE sera did not show any cytotoxic effect, HI, or NT activities (unpublished data). IgG of SSPE and measles sera showed similar patterns of cytolysis. CL50 for NC-Mantooth cells differed from that for the other cell lines. Perrin et al (8) proposed that complement-dependent cytolysis of measles virus-infected cells was mediated by the alternative complement pathway. The results of complement requirement in this study suggest that the complement pathway related to the complement-dependent cytolysis might be dependent on the virus strain persistently infecting NC-37 cells. Kibler et al (4) suggested that antibodies against hemagglutinin and some other antigens of measles virus were capable of causing cytotoxicity. Ehrnst et al (3) demonstrated that antibodies against hemagglutinin activated complement only via the alternative pathway, whereas antibodies against hemolysin activated the classical pathway. Their data
suggested that the activation of complement via the classical pathway by antibodies against hemolysin was the main pathway in cytolysis of SSPE sera. In the present study, however, there was no difference in the pattern of cytolysis between SSPE sera and measles sera. The degree of cytolysis caused by sera from measles and SSPE patients was markedly different depending upon the cell line used for the test, suggesting that the quantity of viral surface antigens might differ among these carrier cell lines. The prozone phenomenon and the complement activation pathway might be related to such a quantitative difference of viral surface antigens of the cells.

The cytotoxic activity of measles patient sera was removed completely by absorption with carrier cells, but that of SSPE patient sera was only partially absorbed by the carrier cells. It is conceivable that the viral surface antigens of the cells persistently infected with measles virus and SSPE virus were identical qualitatively since the cytotoxic effect of measles patient sera was lost completely after absorption with the carrier cell lines. Furthermore, SSPE sera seemed to bind less effectively with the viral surface antigens than did measles sera because the cytotoxic activity of SSPE sera was less effectively absorbed than measles convalescent sera by the carrier cells.  $^{125}$I-labeled IgG of SSPE sera and rabbit hyperimmune serum were demonstrated to bind with all the carrier cell lines as well as with uninfected NC-37 cells (unpublished data). Therefore, it may be possible that Fc-receptor existing in the surface of NC-37 cells binds with the Fc portion of IgG. We are now testing Fab fragment of IgG for the capacity to bind with the carrier cell lines.

REFERENCES


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