Immunoglobulin G Subclass Antibodies to Measles Virus in Patients with Subacute Sclerosing Panencephalitis or Multiple Sclerosis

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Received 10 August 1988/Accepted 7 October 1988

Mouse monoclonal antibodies specific for human immunoglobulin G (IgG) subclasses and a sensitive immunoassay were used to evaluate the IgG subclass antibody response to measles virus antigens in cerebrospinal fluid and serum samples from 20 patients with subacute sclerosing panencephalitis (SSPE), 12 patients with multiple sclerosis (MS), and 11 controls with high measles virus antibody titers in serum. In patients with SSPE, measles virus-specific antibodies were found mainly in the IgG1 subclass and the IgG subclass distribution remained unchanged, irrespective of the clinical stage or duration of the disease. In patients with MS and in controls, measles virus activity was also associated mainly with IgG1. However, the activity was significantly lower than that found in patients with SSPE. The results suggest that there is no primary abnormality in humoral immune response to measles virus in patients with MS. The disproportionately high levels of the measles virus-specific IgG1 subclass found in patients with SSPE may be due to persistent antigenic stimulation or reflect a defect in immunoregulatory mechanisms in response to viral infection.

Subacute sclerosing panencephalitis (SSPE) is a rare, slowly progressive neurologic disease that occurs most often in children and occasionally young adults. It is caused by a defective type of measles virus (5). The disease is characterized immunologically by increased levels of immunoglobulin G (IgG) and high titers of measles virus antibodies in cerebrospinal fluid (CSF) and serum (3, 9, 10). The major immunologic abnormalities in multiple sclerosis (MS) are elevation of IgG and the presence of oligoclonal bands in CSF. Although the etiologic agent(s) in MS is not known, it has been suggested that the role of neural and viral antigens is important to the apparent defect of immune regulation (25). Serologic studies have shown a greater frequency of measles virus antibodies in the CSF of patients with MS than in the CSF of controls (12, 16, 20).

Four subclasses of human IgG have been identified on the basis of antigenic differences in their heavy chains. On the basis of studies with heterologous antibodies to human IgG subclasses (21–23) and preliminary data on monoclonal antibodies (17), it has been suggested that IgG1 is the predominant IgG present in the CSF of patients with SSPE or MS and that this subclass possesses high measles antibody activity in patients with SSPE. However, systematic quantitation of IgG subclasses to measles virus antibody was not undertaken previously in patients with SSPE or MS because of difficulties in obtaining sufficiently specific antisera to IgG subclasses and the lack of quantitative assays that could measure subclass-specific antibodies.

There are conflicting observations with regard to the distribution of IgG subclasses in certain viral diseases (18). Although these variations may be due to the methods applied for quantitation, it seems more likely that the differences in the specificities of polyclonal and monoclonal subclass antibodies account for these variable results. Since mouse monoclonal antibodies specific for human IgG subclasses have been well characterized (7) and an enzyme-linked immunosorbent assay (ELISA) has recently been developed to determine antiviral IgG subclass reactivity, the object of the present study was to quantitate IgG subclass levels specific to measles virus antibody in matched pairs of CSF and serum samples from patients with SSPE or MS. Such measurements may be helpful in evaluating the immune responses of patients to measles virus infection.

MATERIALS AND METHODS

Collection of samples. Matched pairs of CSF and serum samples from patients with SSPE were collected from the Psychoneurological Institute, Warsaw, Poland. Of 20 patients, 12 were male and 8 were female. The age range of the patients was 6 to 23 years, and the mean age was 13 years. Thirteen patients were in stage II of SSPE, and seven patients were between stages II and III (4). The duration of illness varied from 3 to 38 months. Twelve patients were treated with isopropinosine, and eight were treated with isopropinosine and Propionibacterium granulosum KP-45. Twelve pairs of samples from patients with clinically definite MS were obtained from W. W. Tourtellotte, National Neurological Research Bank, Veterans Administration Wadsworth Medical Center, Los Angeles, Calif. Controls included a group of sera from healthy adults with measles virus antibody titers ranging from 1:256 to 1:1,024 as determined by a virus neutralization test (10).

Measles virus. The Edmonston strain of measles virus was propagated in monolayer cultures of Vero cells (10). At the time of complete cytopathic effect (3 to 5 days), the fluid was discarded and the cells were thoroughly rinsed with phosphate-buffered saline (PBS; pH 7.2) and scraped off the glass. The packed cells were suspended in PBS, sonicated, and centrifuged in a Sorvall centrifuge at 17,000 × g for 1 h. The supernatant was dialyzed and concentrated by lyophilization (11). The concentrated preparation had an infectivity titer of 10^6 50% tissue culture infective doses. An appropriate dilution of measles antigen was used in the ELISA. The

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control antigen was prepared in an identical manner, except that the Vero cells were not infected with measles virus.

Antisera. Mouse monoclonal antibodies to human IgG subclasses were obtained from Unipath Ltd. (Oxoid, U.S.A., Inc., Columbia, Md.) and ICN Inc. (ICN Immunobiologics, Lisle, Ill.) (Table 1). Since investigators have observed variability in the percentage of patients responding with different monoclonal antibodies to the same subclass of IgG (2), two different monoclonal antibodies to each IgG subclass were used.

Quantitation of IgG subclasses by ELISA. The double-antibody sandwich method was used (24). Briefly, wells of a microtiter assay plate (Falcon 3911; Becton Dickinson Labware, Oxnard, Calif.) were coated at 0.1 ml per well with a measles antigen preparation diluted 1:100 with carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4°C. The optimal coating dilution was determined by block titration of the antigen (24). The plate was washed four times with PBS (pH 7.2) containing 0.05 Tween 20 (PBS-Tween), blocked with 0.1 ml of 10% pooled normal lamb serum (Colorado Lamb Serum Co., Denver, Colo.) in PBS, and incubated for 30 min at 37°C before being washed with PBS-Tween. CSF samples from patients with MS or SSPE were serially diluted with 0.5% bovine serum albumin in PBS-Tween from 1:50 to 1:1,600, and sera were diluted from 1:200 to 1:8,000. A 100-μl volume of a diluted sample was added to the appropriate well of the plate. After incubation for 1 h at 37°C, the plate was washed and 0.1 ml of 10% diethanolamine buffer (pH 9.8) was added. The reaction was stopped by adding 25 μl of 3 M NaOH. The optical density at 405 nm (OD405) was measured in a micro-ELISA reader (Dynatech Laboratories, Inc., Chantilly, Va.). The results were expressed as the optical density (OD405) of the diluted samples minus the average value for the wells coated with noninfected Vero cells.

RESULTS

IgG1 antibodies specific to measles virus were detected in all of the specimens tested with monoclonal antibody HP6012. The mean values and standard deviations of measles virus-specific IgG1 in patients with SSPE or MS and controls are shown in Fig. 1. The IgG1 subclass values of patients with SSPE were significantly greater than those of patients with MS (P < 0.001 for CSF and P < 0.001 for sera). The serum values of patients with SSPE were also greater than those of controls without MS (P < 0.05), while there was no significant difference between those of patients with MS and those of controls. When measles virus-specific IgG1 subclass activities in pairs of CSF and serum were determined by using clone SG11, the antibody titers were 5 to 10 times lower than those found with clone HP6012. The IgG1 subclass values showed no significant differences between patients with SSPE in clinical stage II and those with SSPE in stage II-III. Similar results were obtained when specimens from patients with SSPE collected 2 to 10 months after the onset of the disease were compared with those obtained 1 to 3 years after disease onset. The IgG subclass distribution remained relatively unchanged irrespective of the type of treatment.

Measles virus antibody specific to IgG2, IgG3, and IgG4 in most specimens was either low or not detectable (Table 2). However, 2 of 20 specimens from patients with SSPE showed high titers of measles virus antibody specific to the IgG4 subclass (data not shown). Measles virus-specific activity in response to IgG2, IgG3, and IgG4 in patients with SSPE or MS and controls did not differ significantly from one another when two different monoclonal antibodies for each subclass were used.

Since CSF and serum samples from patients with SSPE before treatment with isoprinosine were not available, a comparison of measles virus-specific IgG distribution before and after treatment was not done.
DISCUSSION

We have demonstrated a restriction of measles virus-specific antibody to the IgG1 subclass in CSF and serum samples from patients with SSPE by using monoclonal antibodies and an ELISA method. The subclass distribution of measles virus antibodies in these patients remained unchanged irrespective of the clinical stage, type of treatment, or duration of the disease. Earlier studies (21, 22) with polyclonal antisera to human IgG subclasses and electrophoretic analysis showed restriction of measles virus antibodies to the IgG1 subclass in CSF and serum samples from patients with SSPE. Since their detection method (21, 22) was not sensitive, the possibility of the association of measles virus antibody with IgG2, IgG3, and IgG4 was not completely excluded.

Although measles virus antibody titers were markedly low in sera from the control group, the subclass distribution of the measles virus antibody was similar to that of patients with SSPE. These findings differ from earlier studies (21) in which a similar restriction to the IgG1 subclass was not observed in serum from a child who recovered after measles virus infection or in sera of normal individuals. These discrepancies may be due to different patient populations and the use of polyclonal antisera which may have different specificities for IgG subclasses than the monoclonal antibodies used in the present study.

Measles virus antibodies specific to the IgG2 subclass had very low reactivity in CSF of patients with SSPE compared with that of IgG1. The overall weak measles virus-specific antibody in response to the IgG2 subclass was not surprising since it has been reported that IgG2 possesses major activity in response to bacterial polysaccharides (1). In several other viral infections, including hepatitis B (13) and cytomegalovirus (8), both IgG1 and IgG3 subclass antibody levels were found to be increased. Our study did not show any significant elevation of measles virus antibody titers in the IgG3 subclass. These differences may be due to the nature of the antigens and the clinical manifestations of infection. Although IgG4 subclass-specific antibodies were not commonly elevated in sera from patients with viral infection, our study, as well as a recent report (17), showed a moderate rise in measles virus-specific IgG4 levels in sera from certain patients with SSPE. These findings are consistent with increased levels of the specific IgG4 subclass found in several patients with hepatitis B infection (14) or parasitic disease (6).

The present study also shows that measles virus-specific activity was associated mainly with the IgG1 subclass in CSF and serum samples from patients with MS. Similar results were found in earlier studies (22, 23); however, attempts to quantitate IgG subclass in samples from patients with MS were not made previously. Although measles virus antibody levels in specimens from patients with MS was restricted mainly to IgG1, overall it constitutes only a minor portion of the total IgG (12, 23). In contrast, a major portion of the total IgG in CSF from patients with SSPE was found to be measles virus specific (11). Thus, restriction of measles virus antibody to the IgG1 subclass does not appear to be an abnormal characteristic of the humoral immune response in MS, since similar findings were observed in controls.

Monoclonal antibodies to IgG subclasses are important tools in determining specific antibody responses to viral infections (8, 13, 18) and rheumatic diseases (15). The significance of the predominance of IgG1 subclass antibodies to measles virus is unclear. Although the regulation of antibody subclass expression in humans is not well understood, T cells may provide biologic signals that induce the IgG subclass switch toward a certain isotype during an immune response (19). In general, IgG1 is the predominant subclass with antiviral antibodies. Whether the isotype restriction in SSPE is due to persistent antigenic stimulation or reflects the basic immunoregulatory defects is not clear.

LITERATURE CITED


