Immunoglobulin Class and Immunoglobulin G Subclass Enzyme-Linked Immunosorbent Assays Compared with Microneutralization Assay for Serodiagnosis of Mumps Infection and Determination of Immunity

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Total immunoglobulin G (IgG) and IgG subclass reactivities with purified mumps glycoproteins (GP) and nucleoprotein (NP), measured in enzyme-linked immunosorbent assays (ELISAs), were compared with titers in a mumps microneutralization assay (NT). For determination of mumps immunity, the sensitivity of both ELISAs was 100% in comparison with the NT and the specificity was 90%. IgG1 was the dominant subclass against the two antigens found in seropositive healthy individuals. In samples from patients with clinical mumps infections and positive mumps IgM, titer rises of total IgG against NP were invariably seen before GP titer rises. Significant but often late titers rises in NT were found in all patients. Changes of IgG1 levels against both antigens followed the changes of total specific IgG. High levels of IgG3 against NP were diagnostic for mumps infection. In parainfluenza infections, titer rises in the mumps ELISAs and NT were found, but mumps IgM, NP IgG3, and the high ratio between the NP and GP titers found in early samples from patients with mumps infection were not observed.

Mumps infection normally causes an acute, self-limiting disease, but because of occasional complicating meningitis and orchitis, vaccination against mumps has been introduced in many countries. Monitoring of vaccination demands accurate methods for measurement of immunity and for diagnosis of current mumps infection. For determination of mumps immunity, different types of neutralization tests (NTs; 5), hemagglutination inhibition tests (1), hemolysis in gel tests (HIG; 5), enzyme-linked immunosorbent assay (ELISA; 19), and measurement of cell-mediated immune response (2) have been used. The serodiagnosis of current mumps infection has often been made with complement fixation, by demonstration of either titer rises between paired samples or differences in antibody levels to virus-bound and soluble antigens in single samples (7). ELISAs for measurement of immunoglobulin G (IgG) and IgM antibodies to mumps virus have also been used (6, 15, 20, 23). Cross-reactivity with other paramyxoviruses is, however, a well-known problem in mumps serology (9, 11). Recent studies with monoclonal antibodies have confirmed that members of the paramyxovirus group have epitopes in common (18). The virus preparations used as antigens also often contain substances such as actin (24), to which autoimmune reactivity may be found.

The specificity of the currently used assays for detection of mumps antibodies thus appears to be a greater problem than sensitivity. In the present study, the serodiagnosis of mumps infection and immunity was, therefore, evaluated in IgG ELISAs with highly purified nucleoprotein (NP) and glycoprotein (GP) antigens. For diagnosis of current mumps infection, an IgM(μ) capture ELISA with labeled mumps virus was used. Examination of virus-specific IgG subclasses has previously proven to be of value in viral serology (12, 13, 22). IgG subclass reactivity with the two purified antigens was, therefore, studied. The different IgG assays and HIG were compared with an NT for evaluation of mumps immunity.

MATERIALS AND METHODS

Sera. Of 220 serum samples examined (Table 1), 70 were chosen from blood donors, of which 40 were positive and 30 were negative in the mumps NT. Samples (n = 102) from 60 patients with clinically suspected mumps infection were studied. From 42 of the patients, paired samples were available. In seven serum sample pairs, the first sample was drawn too late (>1 week after the onset of symptoms) to be regarded as a true acute-phase sample. At least one sample from each mumps patient was positive or gave a borderline absorbance value in the mumps μ capture ELISA. The 60 patients with mumps infection had a mean age of 23 years (range, 3 to 65), and 25 were females and 35 were males. Seven patients had a history of previous clinical mumps, and seven had had a mumps vaccination. Clinical symptoms were swollen parotid glands alone (42 patients) or with meningitis or orchitis (12 patients) or the latter symptoms alone (6 patients). The 48 samples from patients with other serologically verified viral diseases were all paired and drawn at an interval of about 14 days. All samples were from 1984. After initial analysis, sera were stored at −20°C until used for this study.

NT. The NT was a conventional NT (5) in microplates using 75 to 100% tissue culture infective doses of the NBL strain of mumps virus (16) and twofold dilutions of serum from patients, from 1/2 to 1/1,024. A human mumps immunoglobulin preparation included in all nine tests had a titer of 3,200 in four of the tests and a titer of 1,600 in five of them. A postimmunization serum pool from guinea pigs was included in four tests and gave titers of 320 in three tests and
TABLE 1. Serum samples included in the study

<table>
<thead>
<tr>
<th>Serum donors, no. of donors (no. of samples)</th>
<th>No. of samples examined in each test</th>
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<tbody>
<tr>
<td></td>
<td>NT</td>
</tr>
<tr>
<td>Healthy donors, 70 (70)</td>
<td>70</td>
</tr>
<tr>
<td>Patients with suspected mumps infection, 60 (102)</td>
<td>102</td>
</tr>
<tr>
<td>Patients with other viral diseases,a 24 (48)</td>
<td>12</td>
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</table>

a Paired samples from two patients each with varicella-zoster virus, cytomegalovirus, herpes simplex virus, adenovirus, respiratory syncytial virus, morbilli, influenza A, influenza B, or primary Epstein-Barr virus infections and from three patients each with parainfluenza type 2 and 3 infections.

Preparation of uninfected CAM antigen. Uninfected chorioallantoic membranes (CAMs) were rinsed, freeze-thawed three times, homogenized in physiological NaCl, and centrifuged at 700 × g. The supernatant was used as a control antigen.

IgM(μ) capture ELISA. Purified mumps virions from allantoic fluid, labeled with peroxidase by the periodate method (25), were used as the antigen in an IgM(μ) capture ELISA (6). One mumps IgM-positive sample and one negative sample were controls on each plate. The optical density (OD) value for the negative control was always below 0.1. A serum giving an OD twice that of the negative control was regarded as giving a borderline reaction, and an OD three or more times that of the negative control was clearly positive. OD values for the positive control varied between 0.3 and 0.5. In 40 samples examined twice, no discrepant results as to positive, negative, or borderline reactions were found.

NP and GP ELISAs for total virus-specific IgG. The antigen concentrations for coating were determined by chessboard titration with mumps antibody-positive and -negative serum samples. The lowest antigen concentration at the plateau before OD values started to increase with further dilutions of the antigens was chosen. The concentrations were 0.6 μg of protein per well for the NP antigen and 0.2 μg of protein per well for the GP antigen. For determination of serum reactivity with egg proteins, ELISA plates were coated with 1 μg of CAM antigen per well. At a serum dilution of 10-2, more than 40% of the sera examined gave OD values of >0.2 in this assay. Addition of 1% (vol/vol) CAM antigen to the serum dilution buffer abolished this reactivity. The serum samples to be examined were thus titrated 10-fold from 10-2 to 10-5 in ELISA buffer containing 1% control antigen. Five prediluted serum standards were included on each plate. One mumps antibody-positive control sample with an endpoint titer of 10-4 in both NP and GP ELISAs was diluted for each assay, as well as an NT negative control sample. The IgG titer was the inverted value of the serum dilution passing the OD of 0.2. The reproducibility of the ELISAs was studied in 12 sets of assays. Inter assay variation of the endpoint titer of the positive control serum was 15%.

ELISAs for IgG subclasses to mumps NP and GP antigens. Plates and coating and dilution buffers for ELISAs for IgG subclasses to mumps NP and GP antigens were as in...
ELISAs for total IgG. Serum samples were examined in two dilutions for each subclass. In the IgG1 assay, either $10^{-2}$ and $10^{-3}$ or $10^{-4}$ and $10^{-5}$ dilutions were examined, depending on the titers in the ELISAs for total IgG. For the other subclasses, serum dilutions of $10^{-2}$ and $10^{-3}$ were used. Two dilutions of immunoglobulin (gamma globulin; Kabi Vitrum, Stockholm, Sweden) for each subclass were controls on each plate. Monoclonal antibodies to human IgG1 to IgG4 (8, 14) were used to detect IgG subclasses (anti-IgG1 clone NL16, anti-IgG2 clone HP6002, anti-IgG3 clone ZQ4, and anti-IgG4 clone RJ4). The anti-IgG2 clone was a kind gift from C. B. Reimer, Centers for Disease Control, Atlanta, Ga., and the other clones were from Seward Laboratories, London, United Kingdom. ELISAs for IgG subclass analyses have been previously described (12, 13, 22). OD values for a serum dilution of $10^{-2}$ or $10^{-3}$ are presented from the subclass assays. Interassay variation of OD of the positive control was between 8 and 15% in eight sets of assays. Most samples were examined twice. Positive and negative reactions in the subclass assays were always reproducible.

**RESULTS**

**NT and ELISAs for total IgG to mumps virus NP and GP.** Thirty mumps virus NT-negative samples and 40 NT-positive samples from healthy individuals were examined. Of the 30 NT-negative samples, 60% had titers of <100, 20% had titers between 100 and 500, and 10% had titers between 500 and 1,000 in the mumps GP ELISA (Fig. 2). The titers were similar in the NP ELISA, but only 45% had titers of <100, and one of the sera with a GP titer of 600 had a titer of >1,000 in the NP ELISA. Of the 40 NT-positive samples, 65 and 55% had ELISA titers of >1,000 in the GP and NP ELISAs, respectively (Fig. 2). The remaining NT-positive sera gave titers between 500 and 1,000.

In patients with suspected mumps infection, the kinetics of the NP and GP antibody responses differed (Fig. 2). NP antibodies were invariably found before GP antibodies. All samples drawn on day 4 or later after disease had titers of >1,000 in the NP ELISA, whereas such titers were generally observed after day 13 in the GP ELISA. As a consequence, important titer rises were measured more frequently in the GP ELISA than in the NP ELISA (Table 2).

In 17 of the 18 serum pairs from patients with other diseases except mumps and parainfluenza, titer changes in the assays for total IgG were less than twofold (Table 2). In most samples, there was a decrease in mumps titer between acute- and convalescent-phase samples (data not shown). In one serum pair from a patient with mycoplasma infection, titers in the NP and GP ELISAs increased twofold. This was also seen in four of six serum pairs from patients with parainfluenza type 2 or 3 infection (Table 2). Titer increases of fourfold or greater were seen in both ELISAs for one of three serum pairs tested from patients with parainfluenza type 2 infection. In this patient, NT titers rose from 8 to 64. All titer rises measured in other than mumps infections occurred simultaneously with those of the NP and GP antibodies.

The NT titer changes of 31 paired samples are shown in Table 2. The GP ELISA correlated better than the NP ELISA to the NT, but neutralizing antibodies became measurable later than GP antibodies. All patients acquired neutralizing antibodies, but in a few cases not until day 25 after the onset of disease.

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**FIG. 2.** ELISA IgG titers to NP and GP and NT titers in healthy controls and in patients during current mumps infection. Abbreviations: NPE, ELISA for total IgG titers to NP antigen; GPE, ELISA for total IgG titers to GP antigen.
onset of the dominant IgG. Specific IgG2 antigens in the samples; i.e., NT-negative HIG positive. were negative in between ELISA. The NT, and IgG2 was found. None of the samples from healthy donors contained measurable IgG3 to the mumps GPs. Trace amounts of NP IgG3 were found in 20% of NT-positive samples (Fig. 3). No mumps-specific IgG4 was found in the NP ELISA.

IgG4 to the GP antigen was found in 5% of the NT-positive samples, but when sera were examined without addition of CAM to the serum dilution buffer, about 40% of the samples, NT negative as well as NT positive, gave an OD of >0.5 in the GP ELISA for IgG4.

The development of the IgG1 response to the two mumps antigens in current mumps infection was paralleled by that of total IgG. Specific IgG2 was not found. NP IgG3 was higher than in any of the healthy individuals (OD, >0.5) in all but two samples from mumps-infected patients drawn after day 6 of infection (Fig. 3). High levels of IgG3 to the GP antigen were found in 10% of the same convalescent-phase samples. The frequency of specific IgG4 to the two antigens was similar to that found in healthy seropositive donors.

When NP and GP IgG titer rises were found in other than mumps infections, these were confined to IgG1. Detectable mumps IgM or IgG3 antibodies to the NPs were found only in suspected mumps infections.

Comparison of assays for mumps immunity. With a titer of 500 as a limit for mumps immunity, both the NP and GP ELISA had 100% sensitivity with NT-positive samples. The specificity in both assays with this limit was 90% in the NT-negative samples; i.e., 27 of 30 samples gave titers below 500 (Fig. 2).

In the HIG test, 61 sera gave results concordant with the NT, and 9 gave divergent results. Four NT-positive samples were negative in the HIG test, and five NT-negative samples were HIG positive. Three of the five samples that were negative in the NT but positive in the HIG test gave titers between 500 and 1,000 in the GP ELISA. With a limit of more than 8 mm for positive results in the HIG assay, the sensitivity with NT-positive samples was thus 90% (36 of 40) and the specificity with NT-negative samples was 83% (25 of 30).

Diagnostic criteria in mumps infection. Serological markers of current infection found in single samples were a positive or borderline IgM reaction in the μ capture ELISA, a combination of a negative NT and NP ELISA titers of >1,000, NP ELISA titers four times higher than the GP ELISA titers, and OD values of >0.5 in the NP IgG3 ELISA. These criteria were not found in infections other than mumps. The sensitivities of the four criteria at different times after infection are shown in Table 3.

In paired samples, significant titer rises in the two IgG ELISAs and in the NT were three further diagnostic criteria (Table 2). When fourfold or greater rises in paired samples were considered diagnostic, the sensitivities of the GP and NP ELISAs and the NT in comparison with the μ capture assay were 91, 86, and 74%, respectively.

When the seven diagnostic criteria described (Tables 2 and 3) for current mumps infection were applied, 29 (82%) of 35 patients were positive by more than four of them. Three patients with orchitis not preceded by clinical mumps fulfilled fewer than four criteria because of high IgG and NT titers already in their acute-phase samples. A subclinical mumps infection probably preceded their orchitis, thereby obscuring the evaluation of titers in relation to the onset of disease. Slow or defective reactivity to the NP antigen was found in two patients with clinically typical mumps infections and significant NT and GP IgG titer rises. These patients had only borderline IgM values, low NP IgG3

NP and GP ELISAs for IgG subclasses. IgG1 was the dominant IgG subclass to both NP and GP antigens in sera from healthy donors. Ninety percent of the NT-negative samples gave an OD of <0.5 in the IgG1 assay. NT-negative sera with total IgG titers of >500 also gave ODs of >0.5 in the IgG1 subclass assay for NP and GP. No mumps-specific IgG2 was found. None of the samples from healthy donors contained measurable IgG3 to the mumps GPs. Trace amounts of NP IgG3 were found in 20% of NT-positive samples (Fig. 3). No mumps-specific IgG4 was found in the NP ELISA.

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TABLE 3. Single samples positive for mumps virus infection by different criteria

<table>
<thead>
<tr>
<th>Criterion</th>
<th>% Positive for mumps infection (no. positive/no. tested) among:</th>
<th>Healthy controls</th>
<th>Patients with parainfluenza virus infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mumps patients (days after onset of disease)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-3</td>
<td>4-12</td>
<td>13-48</td>
</tr>
<tr>
<td>Positive IgM test</td>
<td>83 (19/23)</td>
<td>94 (32/34)</td>
<td>96 (43/45)</td>
</tr>
<tr>
<td>NT, &lt;2; NP</td>
<td>76 (16/21)</td>
<td>77 (24/31)</td>
<td>5 (2/41)</td>
</tr>
<tr>
<td>ELISA, &gt;1,000</td>
<td>14 (1/70)</td>
<td></td>
<td>0 (0/12)</td>
</tr>
<tr>
<td>Titer in NP ELISA. 4 ×</td>
<td>78 (18/23)</td>
<td>62 (21/34)</td>
<td>64 (44/66)</td>
</tr>
<tr>
<td>titer in GP ELISA</td>
<td>0 (0/50)</td>
<td></td>
<td>0 (0/12)</td>
</tr>
<tr>
<td>OD490 in ELISA for NP IgG3,</td>
<td>62 (13/21)</td>
<td>88 (29/33)</td>
<td>95 (41/43)</td>
</tr>
<tr>
<td>&gt;0.5</td>
<td>0 (0/50)</td>
<td></td>
<td>0 (0/12)</td>
</tr>
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</table>

reactivity, and higher total IgG to GP than to NP in their acute-phase samples. In contrast, one patient expressed IgM and IgG3 to NP in her acute- and convalescent-phase samples, and NP IgG titers rose significantly. The NT and GP IgG titers were low and stationary. The patient had an afebrile illness with swollen parotid glands, and the mumps diagnosis seemed less certain in this patient. For the three last patients described, the GP IgG and NT titer rises seemed to be diagnostic.

DISCUSSION

This study addresses two major issues in the choice of methods for measurement of mumps antibodies, i.e., optimal methods for determination of mumps immunity and current mumps infection. The reference method for mumps immunity was the NT, and for current infection it was the μ capture ELISA.

The specificity and sensitivity of the NT were not established in the study, but neutralizing antibodies are considered to correlate well with immunity in most viral infections. Our own findings of absence of NT titers in acute samples from all of the patients with typical mumps infection and appearance of such antibodies after at least 3 weeks of disease support the assumption of the relevance of NT antibodies as indicators of mumps immunity. The NT is, however, a cumbersome assay to be used for large seroepidemiological studies. Therefore, evaluation of assays simpler to perform, such as ELISAs and the HIG assay, were made.

The GP ELISA correlated best with the NT. Protection against viral disease by antibodies to GPs has also been shown (26). High sensitivity (100%) and high specificity (90%) was obtained with the chosen titer limit of 500. Although these values seem satisfactory, the 90% specificity means that the immune status in 24% (17 of 70) of NT-positive and NT-negative samples with GP IgG titers between 500 and 1,000 could not be firmly established. Though the antigens used were highly purified, cross-reactive antibodies were probably measured. The results indicate that one type of cross-reactive antibody is directed to other paramyxoviruses. Cross-reactive autoantibodies could probably be another reason for positive ELISA results with NT-negative samples. Monoclonal antibodies reactive with paramyxoviruses and cellular antigens have been previously described (21), which supports this assumption. A third explanation for the discrepant results of complement fixation tests on virus-bound and soluble antigens (7). The mumps GP-NT titer difference was not found in patients with parainfluenza infections. The NT could also be used to diagnose mumps infections but, since neutralizing antibodies appear late in the course of the disease, the NT has little practical value in the diagnosis of current infections.

As with other viral antigens (12), IgG1 was found to be the dominant IgG subclass to the mumps antigens, both in healthy seropositive individuals and in patients with mumps infections. The reactivity was similar to that found for total IgG, and thus the IgG1 assay had no additional value compared with the assays for total mumps IgG. The NP IgG3 assay may be used to diagnose a mumps infection in a single sample. In sera from patients with probable mumps infection there was a striking parallel in the appearance of IgG3 to the mumps NP antigen and IgM in the μ capture ELISA, though IgG3 normally appeared later than IgM. Since the patient with a probable false-positive IgM reaction also had a positive NP IgG3 titer and the patients with borderline IgM reactions had low NP IgG3 values, the NP IgG3 assay did not give a higher sensitivity or specificity than did the IgM assay.

Specific IgG4 reactive with the mumps GP antigen was found in 5% of mumps-seropositive individuals but had no diagnostic value. IgG4, probably directed to egg proteins, was found in 40% of all samples. Egg-reactive antibodies of subclass IgG4 have been described previously (10). Interestingly, IgG4 reactivity could not be measured by conventional IgG ELISA. NT-negative samples with high IgG4 levels to GP were frequently negative in the GP assay for total IgG. Lower sensitivity of the total-IgG ELISA than of the IgG subclass ELISA is a possible explanation.

An effort was made to determine whether patients with previous mumps experience or with infections complicated by meningitis or orchids differed from patients with ordinary...
mumps in immunoglobulin class or subclass reactivity to the two mumps antigens. Though some atypical antibody patterns were noted in such patients, no statistically significant differences between the groups could be found.

In conclusion, the best method(s) for determination of immunity to mumps seems to be a combination of screening by ELISA followed by an NT for sera with uncertain results. For diagnosis of current infection, the requirements of high sensitivity and specificity and rapid diagnosis were best fulfilled by the IgM capture ELISA. The NP IgG3 assay could also be used for diagnosis of mumps infection in single samples, but IgG3 antibodies often appeared a few days after IgM antibodies.

LITERATURE CITED