Enzyme-linked immunosorbent assay (ELISA) for detection of specific IgA antibodies to mumps virus

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SUMMARY A sensitive enzyme-linked immunosorbent assay (ELISA) is described for detection of IgA antibodies to mumps virus. Specific mumps IgA antibodies could be demonstrated in 10 patients with mumps virus infections. No specific mumps IgA antibodies (titres <1/40) were detected by ELISA in 46 control sera (healthy adults; hospitalised patients with various other diseases). The potential application of the ELISA mumps IgA technique in serodiagnosis of mumps infections is discussed.

Mumps is best known as a mild childhood disease, but it can have complications such as orchitis and meningoencephalitis. It is one of the most common causes of viral meningitis.¹ There are several techniques for the detection of mumps antibodies. These include a neutralisation test (NT),² complement fixation (CF),³ haemagglutination inhibition (HI),⁴ single radial immunodiffusion, mixed haemadsorption and haemolysis inhibition.⁵ A highly sensitive radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA) for detection of specific mumps IgG antibodies have also been developed recently in a number of laboratories.⁶⁻⁷ Ukkonen *et al* developed an ELISA to detect mumps-specific antibodies.⁸ Friedman recently showed induction of mumps-specific secretory IgA antibodies in saliva by RIA.⁹

In the present study we describe an ELISA for determination of IgA antibodies to mumps virus in serum. The results obtained for the sera examined were compared with those obtained by CF for the same sera.

Material and methods

ANTIGEN PREPARATION

Vero cells were grown in RPMI-1640 culture medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 200 µg/ml streptomycin, at pH 7.2. The Vero cell cultures were split. One of the resulting sister cultures was infected, and the other was the source of the control antigen. The Jeryl Lynn strain of mumps was

grown in Vero cells in Dulbecco's modified Eagle's medium (DMEM, glucose, 4.5 g/l, without sodium pyruvate), supplemented with 2% FCS. The cultures were incubated at 37°C. Cytopathic effects became apparent two to three days after inoculation with a 1/20 dilution of crude viral lysate.

Cells were washed twice with phosphate-buffered saline (PBS) without Ca²⁺ and Mg²⁺, and stored at -70°C. They were removed from the glass by repeated freezing and thawing. The infected and control cell suspensions were homogenised by sonication for 1 min in a Bransonic 12 sonifier. The sonicate was centrifuged at 1400 g for 10 min and the supernatant fluid was diluted with PBS to a final protein concentration of 0.2 mg/ml, as determined by the method of Lowry *et al*,¹⁰ to be used as antigen or control antigen. The same mumps antigen preparation was used for both serologic methods (CF and ELISA).

HUMAN SERA

Seventy-four sera were examined including sera from 30 medical students, eight laboratory workers, eight hospitalised patients with various diseases, and 28 sera of 10 patients with clinically and serologically diagnosed mumps infections, and were stored at -20°C until use. Twentyfold or twofold serial dilutions of sera were prepared in PBS containing 0.05% Tween 20 (PBST) and tested on both mumps antigen and control antigen.

ELISA

The procedure is a previously described modification¹¹ of the ELISA technique described by Bidwell *et al*.¹² The assay was carried out on polystyrene,

U-shaped microtitre plates of Nunc (Microtest 96 U-1182). Each well received 6 μ g mumps antigen or control antigen in a volume of 0.03 ml. The plates were dried overnight at room temperature and were stored at -70°C . Before use, the antigen-coated plates were thoroughly washed with PBST, and this buffer was used for sera and conjugate dilutions as well as for rinsing the plates after each stage of the procedure. Volumes of 0.025 ml human serum dilutions to be tested were dropped into appropriate wells, and the plates were incubated at 37°C for 1 h. The plates were rinsed and incubated for 1 h at 37°C with 0.025 ml/well diluted peroxidase-linked rabbit antihuman IgA (specific for α chain) obtained from Dakopatts, Copenhagen. The conjugate was diluted to 1/60 in PBST. After 1 h, the plates were rinsed again, and 0.1 ml of a distilled water solution of 0.08% 5-amino-salicylic acid with 0.005% H_2O_2 (pH 6.1) was added to each well. The enzymatic reaction was stopped by the addition of 0.1 ml NaOH (1 N), and the whole reaction mixture was transferred to tubes and diluted with 1 ml H_2O . Absorbance at 450 nm was measured with a 300 n Gilford Microsample spectrophotometer. Dilutions of sera beginning with 1/20 were tested on both mumps antigen and control antigen.

In each experiment known positive and negative sera were included as well as 10 wells containing no serum dilutions (diluent alone). An average background absorption level was determined from these ten wells.

COMPLEMENT FIXATION (CF)

The CF assay was carried out as described by Sever¹³ with 2 units of lyophilised guinea-pig complement (Behringwerke, FRG) and 3 units of hemolysin (Difco, USA).

Results

The effect of different components of the ELISA system was examined in order to find the optimal conditions for the detection of IgA antibodies to mumps virus.

DETERMINATION OF SERUM TITRE FOR MUMPS IgA BY ELISA

Fig. 1 demonstrates the titration curves of a serum positive for IgA directed against mumps virus with an ELISA titre of 1/640, and a mumps IgA-negative serum. The serum titre was defined as the intersection between the titration curve using viral antigen and the corresponding curve obtained with the control antigen, as previously described.¹⁴ The negative sera had control antigen curves either identical to,

Fig. 1 Positive and negative mumps specific IgA sera as determined by ELISA. Titration curves of a serum positive for mumps-specific IgA (○) and a negative serum (Δ). Solid lines, solid symbols: serum tested on mumps antigen. Dashed lines, open symbols: serum tested on control Vero antigen. ELISA mumps IgA titre determined for the positive sera was 640. The absorbance at 450 nm given in the figure represents the optical density of the undiluted samples.

insignificantly lower than, or slightly higher than viral antigen curves.

CONJUGATE AND ANTIGEN CONCENTRATIONS

A known positive and a known negative serum were tested on different concentrations of mumps antigen and of control antigen. No significant change in the titre of the positive serum was observed when the concentration of the mumps antigen used was in the range of 200–300 μ g/ml, whereas with 50–100 μ g/ml, serum titres were lower. When the negative serum was checked, it was negative with all concentrations of mumps antigen/control antigen examined. A concentration of 200 μ g/ml mumps and control antigen was chosen for use in further experiments.

A dilution of 1/60 of the peroxidase-conjugated rabbit antihuman IgA was chosen for standard use. Under these conditions, 20 min were necessary to obtain the maximal colour reaction with 5-aminosalicylic acid.

Mumps antibody titres measured in sera by specific IgA-ELISA and CF at acute and convalescent stages

Patient No	Age (yr) and sex	Days after onset	Clinical diagnosis	Titre	
				CF	ELISA
1	F 39	8	mumps meningitis	16	2560
		12		16	2560
		25		8	640
2	F 30	4	mumps	<4	640
		15		8	1280
		26		16	1280
		61		16	160
		101		8	160
3	M 27	—*	mumps parotitis	<4	<40
		5		4	160
		36		16	1280
		156		NT	160
4	M 3½	10	mumps meningitis	16	320
		24		64	160
5	F 4½	8	mumps meningitis	64	160
		10		64	160
		19		128	2560
6	M 5	8	mumps meningitis	4	1280
		24		32	160
7	M 7	6	mumps meningitis	8	640
		21		128	160
8	F 22	5	mumps	4	40
		10		64	160
		15		32	640
9	M 10	5	mumps	4	160
		9		8	640
10	M 7	3	mumps	16	80
		24		128	80

* = two years before onset
NT = not tested.

REPRODUCIBILITY

We examined the reproducibility of the technique by testing one positive serum in 25 different experiments. The titre was reproducible within a twofold range.

MUMPS PATIENTS

Twenty eight sera from 10 mumps patients were tested for mumps virus antibodies by CF and by the ELISA techniques (Table). For all the patients mumps-specific IgA was detected by ELISA in all serum samples available. In one of the patients (case 3), a single serum sample taken two years before onset of mumps parotitis showed no mumps specific IgA antibodies (titre <1/40). A marked decrease of mumps IgA titre was already seen after about 20–25 days in four of the mumps patients. In serum samples of cases 2 and 3 mumps IgA antibodies were still observed (titre 1/160), 101 and 150 days respectively after onset of illness.

CONTROL ADULTS

Forty-six sera of blood donors (see "Human sera" under "Material and methods") were evaluated for mumps-specific IgA antibodies by ELISA and by CF. In all sera no mumps IgA antibodies (titre <1/40) were observed by ELISA. Five of these sera were negative for mumps antibodies by the CF test (titre

<1/4) while the others were positive (titres 1/4 to 1/16). The possibility that mumps IgA antibodies might be detected at titres lower than 1/40 in control sera needs to be examined.

Discussion

In the present study we have developed an enzyme-linked immunosorbent assay (ELISA) for detection of IgA antibodies to mumps virus. In our examinations we found high titres of IgA antibodies specific for mumps in the sera of 10 mumps patients studied. Detection of high IgA titres in mumps patients by the ELISA technique described indicates that the method has potential for serodiagnosis of mumps infections since no mumps IgA antibodies (titre <1/40 by ELISA) were found in 46 control sera of healthy adults and patients hospitalised with various other diseases. In one serum sample available three days after onset of illness, IgA antibodies were already observed (titre 1/80) by ELISA. However, before one accepts this mumps IgA ELISA technique as a standard technique for serodiagnosis of mumps infections, (i) the possible cross reactivity with other parainfluenza viruses needs to be examined since there is a report of one patient out of 12 with parainfluenza virus 3 infection (as determined by a rise in CF titre) having a rise in IgG titre

to mumps when tested by ELISA;⁸ (ii) the persistence of the specific IgA antibodies needs to be carefully examined in larger population samples. We found that in two patients IgA titres seemed to decline after about one month, but were still positive about four to six months after onset of illness. Studies which have been carried out with respect to persistence of IgA antibodies after other viral infections have not yielded uniform results. In patients with Epstein-Barr virus (EBV) mononucleosis and influenza, IgA antibodies appeared in the early stages of disease and were not detected 10 weeks after onset.^{15,16} Conversely, Halonen *et al.*¹⁷ by a highly sensitive radioimmunoassay (RIA) have shown that IgA antibodies to rubella viruses persist in most rubella patients for at least several months. The longest persistence time of IgA recorded is over four years.¹⁸ Recently, it became evident that also IgM antibodies induced during various viral infections persist longer than was previously thought when sera were examined by less sensitive techniques.¹⁹⁻²² It seems that the persistence of IgA antibodies depends upon the virus involved, individual variations among the patients, and most importantly, on the sensitivity of the method used to detect specific IgA.

Recent investigations indicate that serum IgA antibody is involved in the transport of a foreign antigen from the circulation into the bile.²³⁻²⁷ Very little is known about the role of serum IgA antibodies in viral infections. Of particular interest is the finding of Henle and Henle that nearly all nasopharyngeal carcinoma patients tested prior to specific therapy had IgA antibodies to EBV viral capsid antigen (VCA).²⁸ HSV-2 IgA antibodies to membrane antigen were found to be detected significantly more frequently and at higher titres in patients with cervical dysplasia and neoplasia than among matched controls.²⁹

Specific IgA antibodies can be detected in both primary and recurrent varicella zoster virus (VZV) and cytomegalovirus (CMV) infections by the ELISA technique.^{11,30,31} There have been suggestions that mumps virus may be associated with neurological, autoimmune and malignant diseases.³²⁻³⁷ Examination of sera of persons with such disorders for the presence of IgA antibodies to mumps virus is warranted. The technique described herein may be useful for such studies as well as for routine diagnostic serology.

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