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SPECIFIC IgM ANTIBODIES IN THE SALIVA OF MUMPS PATIENTS

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Summary. — Antibodies to mumps virus were detected in 63.5% of saliva samples from mumps patients. The secretory antibody response was of primary type. Specific IgM antibodies were found in some samples collected early after the onset of disease. Specific IgA were detected in later obtained samples. Persons over 15 years of age reacted more often and more promptly than the children. The authors discuss the possible significance of prior antigenic stimulation by related paramyxoviruses (namely parainfluenzaviruses) for the intensity of local antibody response to mumps virus infection.

Key words: mumps virus; secretory antibody response; saliva

Introduction

Reports on secretory response to primary virus infections are rather scarce. Our original idea was to determine whether the examination of saliva could contribute to rapid laboratory proof of mumps. It was based on the observation of Friedman (1982), who detected IgA to mumps virus in saliva from diseased persons using RIA.

Materials and Methods

Samples of saliva (115) were collected in different intervals after the beginning of the illness. Children (40) and adults (24) with typical clinical signs of serologically confirmed mumps infection were examined. Additional samples (14) were obtained from healthy subjects with low anamnestic IgG serum titres to mumps virus. Samples of saliva were collected without prior stimulation. The saliva were heated at 56 °C for 30 min and stored at −70 °C. Before examination, each sample was diluted 1 : 4 in PBS pH 7.4 and centrifuged for 10 min at 2000 × g. The supernatants were examined for the presence of specific antibodies.

Sera of persons under examination were obtained simultaneously with the saliva samples. They were inactivated and diluted 1 : 10 in buffered saline (PBS) before investigation. Rheumatoid factor was absorbed from sera by polyacrylamide-bound human Ig (for details see Fraňková *et al.*, 1985).

Specific IgM, IgG and IgA in saliva and sera were detected by indirect immunofluorescence (IF) and by the ELISA technique. For indirect IF commercial (Sevac, Praha) FITC labelled conjugates containing swine antibodies to heavy chains of human IgM, IgG and IgA were used to stain the cell-substrates containing mumps-virus antigens.

Preparation of cells. Chorionallantoic membranes of chick embryos infected with mumps virus strain Enders (10 ID_{50}) were trypsinized on the 3rd day post-infection (p.i.). The washed cell-suspension, containing approximately 2×10^5 cells/ml was dropped on microscope slides, dried and subsequently fixed in acetone. Control IF staining with hyperimmune guinea-pig serum was used in order to verify that at least 30% of cells in each drop contained mumps virus antigens. The IF staining procedure was similar to that described previously (Fraňková *et al.*, 1986*a*). The intensity of IF was evaluated in Fluoval-microscope (Zeiss, Jena) ranging from + (weak but distinct) to + + + + (very intensive bright stain).

All examinations of sera and saliva from healthy and/or ill persons were evaluated under code. The findings evaluated as "traces" or + — (recorded in comparison of IF and ELISA results) were not considered for reliably positive.

For ELISA tests a partially purified antigen was bound to Microelisa flat bottom well plates (Dynatech). Commercial (Sevac, Praha) horse-raddish peroxidase conjugates containing swine antibodies to human Ig of different classes were used; 0.05% H_2O_2 and orthophenylendiamine as indicator were added for reading.

Antigen preparation. The mumps virus from the infected allantoic fluid was adsorbed to guinea-pig erythrocytes at 4 °C overnight and eluted in 10× smaller volume of PBS during 4 hr incubation at 37 °C. The eluate was sonicated (Sonnic Dismembrator, Dynatech) and centrifuged at 10 000× g for 30 min. The supernatant was further centrifuged at 200 000× g for 2 hr on 20% sucrorose cushion. All centrifugations were done at 4 °C. The optimal dilution of viral antigen from the bottom fraction was 1 : 40 according to box-titration with the use of reference positive serum. In this dilution the antigen was bound to Microelisa plate wells overnight at 4 °C. In ELISA tests the saliva samples were diluted serially from 1 : 20 to 1 : 160, serial dilutions of sera ranged from 1 : 100 to 1 : 5 000. The ELISA procedure was described in a previous communication (Fraňková *et al.*, 1986*b*). Optical density (OD) values were determined on ELISA-reader (Dynatech) at 488 μm. Two-fold or higher values of the examined sample related to OD value of the corresponding dilution of pooled negative serum or saliva were considered positive.

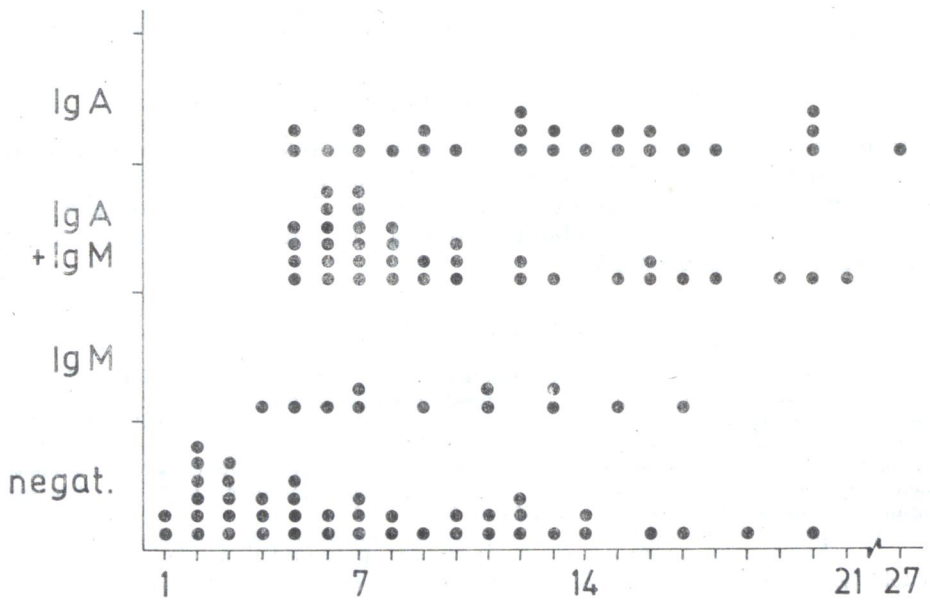

Fig. 1.
Specific antibodies in saliva of mumps-patients, days after onset of the disease
Abscissa: observation days

Table 1. The IF determination of specific antibodies in saliva

IF titre (dilution reciprocal)	Intensity of IF* (samples diluted 1 : 4)	No. of positive findings IgM	IgA	IgG
128 — >256	+++ — ++++	31	16	1
32 — 64	++	9	11	0
8 — 16	+	8	34	0

* IF intensity was evaluated independently by two workers; IF titres varied in one dilution step limit

Results

By means of indirect IF specific antibodies to mumps virus were detected in 73 out of 115 saliva samples from sick persons (63.5%). In 12 samples (16.3% of all positives) only specific IgM were found, in 36 samples (49.3% of all positives) specific IgM as well as specific IgA could be demonstrated. In 25 samples (34.2% of all positives) only specific IgA were detected. In one patient (a 65-year-old male) specific IgG were also found in saliva. None of the saliva samples from healthy persons contained specific antibodies to mumps-virus. The intensity of specific IF and the titres of specific antibodies detected in saliva of mumps-patients are summarized in Table 1.

Specific IgM antibodies were present in sera of all diseased persons. In 44 individuals the IF intensity of IgM staining in dilution 1 : 10 was evaluated + + + or + + + + and the IF titre exceeded 160. In the rest of sera the IF intensity reflecting the presence of IgM was + or + + in dilutions 1 : 10 showing IF titres in the range from 10 to 80.

Specific IgG in the sera of all patients revealed bright IF evaluated + + + or + + + + in dilution 1 : 10. The IF titres exceeded 320 in all patients. No serum IgM to mumps virus was demonstrated in healthy controls, in which serum IF titres for IgG were 80 or less.

Table 2. Clear-cut discrepancies observed between specific antibody levels in serum and saliva of certain mumps patients

Sample no.	Titres of specific antibodies			
	IgM		IgG	
	serum	saliva	serum	saliva
2, 3, 4, 23 53, 55, 57 65, 66, 98 102	>1 : 160	<1 : 4	>1 : 320	<1 : 4
8, 12, 15 21, 38, 39 47, 50	1 : 10—1 : 40	>1 : 256	>1 : 320	<1 : 4

Table 3. Specific antibodies in saliva of children in subsequent weeks after onset of clinical signs of mumps

Week	No. of samples	No. (%) of findings			
		Negatives	IgM only	IgM + IgA	IgA only
I	27	17 (62%)	3 (11%)	6 (22%)	1 (4%)
II	22	8 (36%)	4 (18%)	5 (23%)	5 (23%)
III	13	3 (23%)	2 (15%)	3 (23%)	5 (38%)
Total	62	28 (45.2%)	9 (14.5%)	14 (22.6%)	11 (17.7%)

It is evident from Table 2 that the IgM in saliva and sera from mumps patients showed no correlation. Figure 1 illustrates the relation of secretory antibody response to the time of saliva collection after onset of illness. Table 3 shows the results of examination of childrens' saliva in the weeks following the onset of overt disease. Similar data for adults are summarized in Table 4.

ELISA technique was used for examination of 61 serum and saliva samples from mumps patients along with 12 serum and saliva samples from 12 healthy persons. Sera of 4 healthy persons were IgM-positive at dilution 1 : 100, the remaining control sera were completely negative for specific IgM. Specific IgG antibodies were detected in sera from healthy persons in titres not exceeding 1000 by ELISA. In all patients sera the IgM titres were 500 or higher, specific IgG exceeded regularly the titre of 5 000. No positive finding of specific antibodies in saliva of healthy individuals was found by means of ELISA. In mumps patients all specific IF antibody findings were confirmed by ELISA. The titres of both IgM or IgA varied from 20 to 160 in individual samples. Additional 5 IgM- and 4 IgA-positive samples were found by ELISA in comparison with indirect IF examination of patients' saliva (Table 5).

Table 4. Specific antibodies in saliva of adults in subsequent weeks after onset of mumps clinical signs

Week	No. of samples	No. (%) of findings			
		Negatives	IgM only	IgM + IgA	IgA only
I	24	8 (33.3%)	2 (8.3%)	10 (41.6%)	4 (16.6%)
II	18	5 (27.8%)	1 (5.6%)	7 (38.9%)	5 (27.8%)
III	11	1 (9.0%)	0 —	5 (45.5%)	5 (45.5%)
Total	53	14 (26.4%)	3 (5.7%)	22 (41.5%)	14 (26.4%)

Table 5. Controversial results in saliva as detected by indirect IF and ELISA

Patient no.	Day after onset of the disease	Specific antibody			
		Indirect IF*		ELISA titre**	
		IgM	IgA	IgM	IgA
5	4	—	—	1 : 20	—
23	11	+	—	1 : 80	1 : 20
27	7	+	traces	1 : 20	1 : 40
41	8	—	—	1 : 20	—
41	15	+	—	1 : 40	—
46	4	—	—	1 : 20	—
46	7	—	—	1 : 20	1 : 80
46	10	—	+	1 : 40	1 : 80
56	7	traces	—	1 : 40	1 : 80
56	17	+	—	1 : 160	1 : 160

* in dilution 1 : 4
** the titres $<$ 1 : 20 are referred to as negative

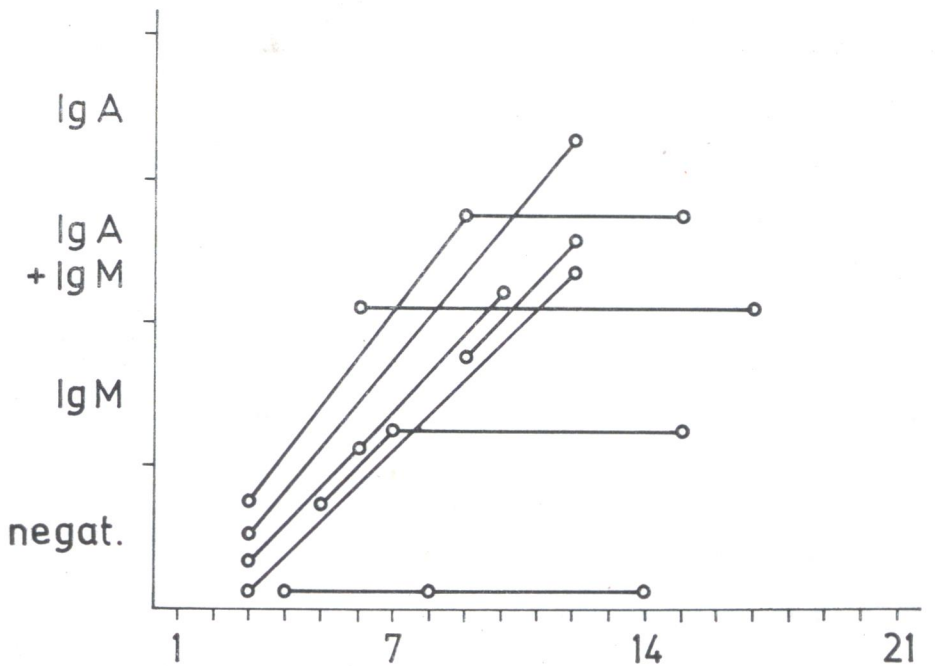

Fig. 2.
The dynamics of specific antibody response in the saliva of some patients.
Abscissa: observation days

Discussion

The secretory IgA (sIgA) are the most usually encountered immunoglobulins in saliva and other discharges. Their rapid penetration to the surface of mucous membranes is mediated by a special transport mechanism. The secretory component expressed on the membranes of salivary gland acinar cells and mucous membrane and epithelium cells operates as receptor for polymerized IgA or IgM J-chain (Mostov and Blobel, 1982; Nakamura *et al.*, 1982). Specific IgM (except for IgA- deficient persons) is not usually present on the mucous membranes in detectable amounts.

The secretory immune response alike to the whole immune system evolves during ontogenesis under the influence of antigenic stimuli from the alimentary and respiratory tracts. Repeated stimulation with food antigens as well as with those of common cell wall constituents of bacteria colonizing the mucous membranes (lipopolysacharides, peptidoglycans and other polyclonal activators) seem represent the main contributing factor (Kotani *et al.*, 1985). Repeated virus infections may play a similar role (Ganguly and Waldman, 1981).

The newborn's response to primary antigenic stimulation is weak, with the prevalence of IgM production. Repeated stimulation leads to more pronounced IgG and IgA responses. The number of IgA producing B-cells in mucous membranes increases substantially in postnatal life (Cebra *et al.*, 1985). Most of them are memory cells in an early stage of clonal proliferation (Strober and Jacob, 1985). After repeated contact with related microorganisms, the host responds by overwhelming sIgA production which may cross--react in different ways. The aim of our investigation was to assess the significance of specific sIgA detection in rapid diagnosis of mumps. Anticipating the possible transudation of serum globulins in salivary gland inflammation, we investigated the presence of specific IgA, IgG and IgM antibodies in the saliva. Although the content of specific IgG was high in sera of all patients, this antibody class was not detected in the saliva (the one 65-year-old male with probable transudation of serum proteins through gingival mucous membranes was the single exception). In the rest of saliva samples only specific IgM and/or specific IgA, i.e. the antibodies actively transferred to mucous membranes by binding the secretory component, could be demonstrated.

The antibody response to mumps virus infection starts during the incubation period; specific IgM and IgG antibodies are detectable in sera of a patients already in the early stage of illness. Specific antibodies in saliva appeared later and less regularly. Most of the patients reacted in the way of primary response, producing specific IgM on the 4th or 5th day with a switch to IgA production during the second or third week of illness (see Fig. 2). As evident from our investigations in persons over 15 years of age the secretory response to mumps virus was clearly more frequent and prompt, than in children. This may confirm that there is an anamnestic response to the common antigenic determinants of paramyxoviruses as a result of previous stimulation of the secretory response to parainfluenzavirus infection. Although we consider the finding of local specific IgM production during mumps

virus infection for interesting, we assume that the above-mentioned development mechanism of the secretory immune response might be involved as well.

The frequency of specific antibody findings in saliva corresponds roughly with results of Chiba and Nakao (1972) who observed the neutralizing capacity of saliva diluted more than 1 : 2 in 50% of mumps children. In agreement with our results, the neutralizing capacity has not been observed before the second week of disease. In contrast, Friedman (1982) using RIA regularly detected specific IgA in saliva from mumps patients from the first to the 9th week after the beginning of disease. The higher sensitivity of RIA might be the cause of higher positive rate as compared with our results. Our findings using ELISA were also more frequent than those obtained with the aid of indirect IF.

It cannot be ruled out that a part of positive antibodies in saliva detected by "solid phase" techniques may be nonspecific due to the presence of secretory antibodies reacting with parainfluenzaviruses. Cross-reactivity occurs in ELISA as shown by Van Logt *et al.* (1982), Fraňková *et al.* (1987) and others. This may also explain the low IgM titres to mumps virus found by ELISA in the sera of 4 healthy controls.

Considering the results of our investigations we conclude that for reliable proof of mumps virus infection the examination of serum was inevitable. Investigation of the secretory response to mumps virus contributes to better understanding of local antibody production in primary virus infections.

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