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FIBRILLAR ANTI-CELLULAR ANTIBODY ASSOCIATED WITH MUMPS AND MEASLES INFECTION

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SUMMARY

Fibrillar anti-cellular IgM antibody was found in sera of 8/10 children with acute mumps infection and in 8/12 children with acute measles infection. Absorption experiments showed that the antibody was against cellular components in cells of human origin and was unrelated to either viral antibody or to RF. Similar antibody was previously found in patients with MS.

INTRODUCTION

When testing sera by immunofluorescence for virus-specific immunoglobulin M (IgM), anti-cellular antibody was found in tissue culture cells in twenty-seven out of forty-three patients with multiple sclerosis (MS) compared with one of forty-three healthy control subjects (Millar *et al.*, 1971). Some of these sera also had IgM specific for measles or mumps viruses. The type of staining appeared to be similar to the staining of the microfilamentous network described by Farrow, Holborow & Brighton (1971), when sera from patients with acute infective hepatitis containing IgM smooth muscle antibody (SMA) were tested on tissue culture cells. We had observed this pattern of staining occasionally when testing for virus-specific IgM antibodies in acute viral infections, therefore we made a detailed study to determine its incidence in sera from patients with acute mumps and measles infections.

MATERIALS AND METHODS

Patients

Serum specimens were obtained from ten children with mumps and six children with measles in the acute stage of illness and in convalescence. Acute sera were obtained from six further measles patients. Control sera were obtained from ten healthy children of a similar age group who were in hospital for minor plastic surgery; control sera were also obtained from healthy adult blood donors. Sera were stored at −20°C until tested.

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Detection of anti-cellular and virus-specific IgM and IgG antibodies in serum

(i) *Antigen.* Sera were tested on both infected and uninfected HEp_2 cells growing on coverslips. These were seeded thinly so that the cells were well spread out, and in the case of infected preparations approximately 50% of the cells were infected. They were fixed in fresh acetone for 10 min at room temperature, air-dried for 30 min and stored at −20°C in the presence of silica gel. Uninfected cells were also fixed by dipping them for 30 sec in isopentane cooled in liquid nitrogen (Farrow *et al.*, 1971). All experiments were carried out on a single batch of each type of coverslip employed.

(ii) *Fluorescein conjugates.* Sheep anti-human IgM conjugated with fluorescein isothiocyanate (FITC) (Wellcome Reagents, Ltd) was used. Sheep anti-human IgG (Wellcome Reagents, Ltd) was conjugated with FITC (Sigma Chemical Company) in our own laboratory.

(iii) *Absorption of conjugates and standardization.* Both conjugates were absorbed with mouse liver powder, HEp_2 cells and BHK21 cells. The anti-human IgM conjugate was tested and evaluated for specificity on an indirect viral system (Chantler & Haire, 1972). The optimum staining titre of both conjugates was assayed on an indirect viral system, and at these dilutions neither gave non-specific fluorescence of either infected or uninfected cells when treated with normal human sera.

(iv) *Absorption of sera.* Sera, diluted 1/5 were absorbed overnight at +4°C with HEp_2 cells. Approximately 4×10^7 cells were used to absorb 0·5 ml of original serum. Six sera were also absorbed with heat-aggregation Cohn Fraction II human γ-globulin. Four sera were also absorbed with semi-purified mumps virus; 15 ml of egg-grown virus of haem-agglutinating titre of 1/320 was concentrated by spinning at 30,000 rev/min for 2 hr, and the resulting pellets were used for the absorption, also overnight at +4°C. All sera were inactivated by heating them at 56°C for 30 min.

(v) *Staining.* Dilutions of patients' absorbed sera were applied to the appropriate acetone fixed infected and non-infected coverslip preparations for 45 min at 35°C. After thorough washing the conjugate was applied for 45 min at 35°C. After a final wash each coverslip was mounted in glycerol-saline at pH 8·3 and the preparations were examined with a Reichart Fluoropan microscope equipped with an IQ light source, FITC-3 (Balzer) interference filter, a Wratten secondary filter, and a cardioid dark ground condenser.

Anti-cellular antibody was also assessed on isopentane-fixed uninfected HEp_2 cells.

Rheumatoid factor

Sera, diluted 1/5 were tested for rheumatoid factor (RF) using IgG coated latex (Hyland RA-test).

RESULTS

The titres of IgM anti-cellular antibody, mumps-specific IgM and mumps-specific IgG antibodies and RF tests in sera from ten children with mumps are shown in Table 1. The significant rise in titre of mumps-specific IgG antibody between the acute and the convalescent specimens confirms the diagnosis; further evidence of active infection is given by the presence of mumps-specific IgM antibody (Haire & Hadden 1970). Staining by mumps IgG antibody shows mainly cytoplasmic aggregates of antigen, while staining by mumps IgM antibody shows a more superficial type of staining.

Anti-cellular IgM antibody is present in 8/10 of the mumps patients; in 3/8 it is present in the acute sera only, while in the remaining five patients it is present in both acute and convalescent sera. The staining appears fibrillar and is most distinctive when the cells are well spread out; it is seen in the uninfected cells of the virus-infected coverslip preparations (Fig. 1), and also appears the same in both acetone- and isopentane-fixed preparations of uninfected cells, the latter method of fixation tending to make the staining more intense. In addition to the typical appearance in cells which are well spread out, the staining appears as perinuclear crescents which may have a 'tail' towards the cell periphery.

TABLE 1. Titres of anticellular IgM antibody, mumps-specific IgM and IgG antibodies and presence of RF in ten patients with clinical mumps

Sex and age of patients	Days after onset of illness	Anti-cellular IgM antibody	Mumps antibody in		RF at 1/5 dilution of serum
			IgM	IgG	
M. 6	3	—	10	10	−
	27	—	20	80	−
F. 5	3	20	40	10	++
	28	—	10	80	−
M. 5	3	20	40	20	+
	22	—	20	80	+
F. 6	2	10	20	20	++
	20	—	10	160	++
*F. 6	2	10	10	5	++
	22	10	40	80	+++
M. 7	1	5	5	5	−
	19	10	20	80	+
M. 7	1	—	5	10	−
	19	—	10	80	+
†F. 8	3	20	20	5	++
	36	40	40	160	++
M. 12	6	5	5	5	++
	40	5	5	160	++
‡M. 5	4	40	80	40	−
	36	20	40	80	−

End point = +++ strong staining. − = Negative at 1/5.

The results of a screening test of sera, diluted 1/10, for anti-cellular IgM antibody and measles-specific IgM antibody on 'paired' sera from six children with measles and six acute phase sera from children are shown in Table 2. Anti-cellular IgM antibody is present in 8/12 of the patients, although in one of these it is weak (±).

Anti-cellular IgG antibody was seen in neither the mumps nor the measles series. There did not appear to be any association between the presence of anti-cellular IgM antibody

and the presence of RF; the incidence of the latter factor was much higher in mumps than in measles infection.

The antibody titres of anti-cellular IgM and mumps-specific IgM antibodies of 'paired' sera from children, tested in parallel (i) unabsorbed, (ii) absorbed with HEp_2 cells and (iii) absorbed with HEp_2 cells and heat-aggregated γ-globulin are shown in Table 3. The absorptions did not alter the titres significantly.

In a further experiment 'paired' sera from two mumps patients were absorbed by three methods and were tested in parallel for anticellular and mumps-specific IgM antibodies.

TABLE 2. Anti-cellular and measles-specific IgM antibodies in sera tested at 1/10 dilution from twelve patients with clinical measles

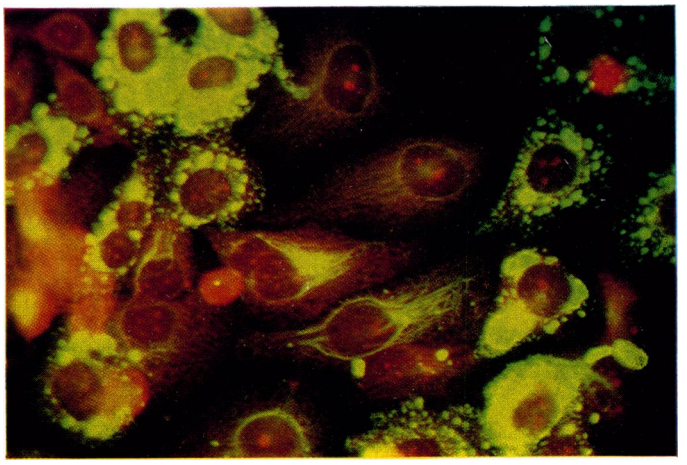
Sex and age of patient	Days after onset of illness	Anti-cellular IgM antibody at 1/10 serum dilution	Measles-specific IgM antibody at 1/10 serum dilution	RF at 1/5 serum dilution
F. 6	2	+++	+++	−
	27	−	±	−
M. 5	2	+	+++	−
	32	+	±	±
F. 5	3	−	+++	ND
	49	−	−	ND
F. 6	4	+++	+++	−
	25	+	+	−
F. 18	2	+	+++	−
	37	−	±	+
M. 6	4	+++	+++	−
	32	−	−	−
M. 6	3	+++	+++	−
M. 6	2	+++	+++	−
M. 5	1	−	+	−
M. 6	2	−	+++	ND
M. 2	2	−	+	−
M. 5	2	±	+++	−

ND = not tested.

The absorptions were (i) with HEp_2 cells and heat-aggregated γ-globulin (ii) twice with HEp_2 cells and (iii) with HEp_2 cells and semi-purified mumps virus. In the first patient the second method of absorption, a method more extensive than usual, removed the fibrillar anti-cellular staining, while it reduced its titre in the second patient. This type of absorption did not alter the titre of the viral antibody. Absorption with virus removed the viral antibody, but only reduced the anti-cellular antibody in titre (Table 4).

Five of the healthy control children (four females and six males) had mumps-specific IgG antibody and all of the same group had measles-specific IgG antibody at a serum dilution of

(a)



(b)

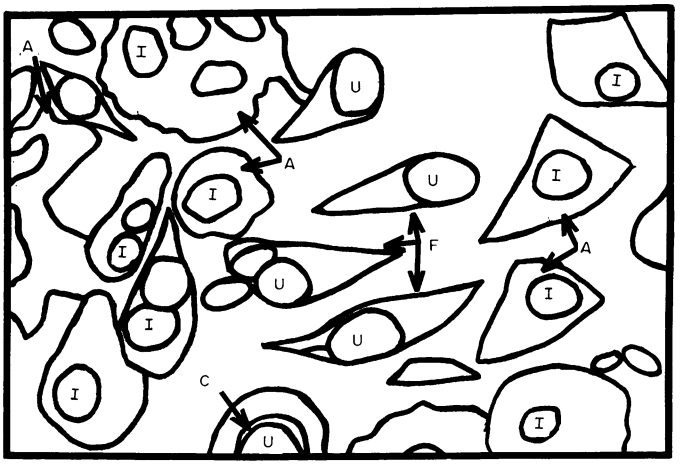


FIG. 1. (a) HEp_2 cells, uninfected and infected with mumps virus, stained with serum from patient with mumps taken 3 days after onset of symptoms and with anti-human IgM conjugated with FITC. (b) Line drawing of photomicrograph to illustrate various types of staining patterns. Abbreviations: F = fibrillar anti-cellular IgM staining; C = crescentic peri-nuclear anti-cellular staining (IgM); U = uninfected HEp_2 cell; I = HEp_2 cell infected with mumps virus; A = aggregates of viral antigen.

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1/5; viral-specific IgM, anti-cellular IgG and anti-cellular IgM antibodies were not found in the same sera at this dilution.

Of the seventeen adult blood donors (ten females and seven males) of ages ranging from

TABLE 3. Titres of anti-cellular and mumps-specific IgM antibodies in 'paired' mumps sera tested (i) unadsorbed, (ii) after adsorption with HEp_2 cells and (iii) after adsorption with HEp_2 cells and heat aggregated γ-globulin

Sex and age of patient	Days after onset of illness	Anti-cellular antibody in serum with			Mumps antibody in serum with		
		(i) unads.	(ii) ads. HEp_2	(iii) ads. HEp_2 and γ-globulin	(i) unads.	(ii) ads. HEp_2	(iii) ads. HEp_2 and γ-globulin
*F. 6	2	10	10	10	10	10	10
	22	10	10	10	40	40	20
†F. 8	3	20	20	20	20	20	20
	36	40	40	40	40	40	40
‡M. 5	4	40	40	20	80	80	160
	36	20	20	20	40	40	40

* Denotes patient in Table 1.
† Denotes patient in Table 1.
‡ Denotes patient in Table 1.

TABLE 4. Titres of anti-cellular and mumps-specific IgM antibodies in 'paired' mumps sera tested (i) after absorption with HEp_2 cells and heat aggregation γ-globulin, (ii) after double absorption with HEp_2 cells and (iii) after absorption with HEp_2 cells and concentrated mumps virus

Sex and age of patient	Days after onset of illness	Anti-cellular antibody in serum abs. with			Mumps antibody in serum abs. with		
		(i) HEp_2 and γ-globulin	(ii) Hep_2 (twice)	(iii) HEp_2 and virus	(i) HEp_2 and γ-globulin	(ii) HEp_2 (twice)	(iii) HEp_2 and virus
*F. 8	3	20	–	10	20	20	–
	36	40	–	20	40	40	–
†M. 5	4	20	10	10	80	80	–
	36	20	10	10	40	40	–

* Denotes patient in Table 1.
† Denotes patient in Table 1.
– = Negative at 1/10.

22 to 58 yr who had serological evidence of previous mumps infection (mumps-specific IgG antibody), one only had anticellular antibody in IgM. In this instance the staining was of a more generalized anti-cytoplasmic type and differed from the fine fibrillar staining described in this paper. Similarly of the fifty-six adult blood donors (twenty-nine females

and twenty-seven males) of ages ranging from 22 to 62 yr with serological evidence of previous measles infection, only one, the individual referred to above, again had anti-cellular antibody of similar type. RF was not present in this serum nor in any of the mumps positive sera, but was present in five of the measles positive group.

DISCUSSION

When HEp_2 tissue culture cells, of human origin, are treated first with sera from children with acute mumps or measles infection and then with fluorescein-labelled anti-human IgM, the fibrillar pattern of staining appears similar to the IgM anti-cellular antibody which we found in MS (Millar *et al.*, 1971). The antigen stained may be the same as Farrow *et al.* (1971) has shown in cultures of diploid human foetal lung cells using SMA positive sera and anti-human IgM conjugate (FITC). These workers stress that isopentane fixation of the tissue culture cells is essential for showing this particular antibody. We have found fixation in fresh acetone satisfactory, though the resulting staining was duller. However in looking for viral antibody acetone fixation is more useful as cell morphology is less altered and the location of viral antigen is better preserved. Probably both methods of fixation reveal whatever antigenic determinants are stained. This might well be, as Farrow *et al.* (1971) suggested, a filamentous network which is part of the cell membrane located just below the cell surface.

There does not appear to be any association between the presence of RF and IgM fibrillar anti-cellular staining. Removal of RF from sera by absorption with γ-globulin did not alter significantly the titres of either anti-cellular or anti-viral antibodies: this finding confirmed the specificity of the IgM staining (Fraser, Shirodaria & Stanford, 1971; Shirodaria, Fraser & Stanford, 1972). The results of the other absorption experiments suggest that fibrillar anti-cellular antibody differs from anti-viral antibody.

The high incidence of fibrillar anti-cellular IgM antibody in membrane virus infections—acute mumps and acute measles—is striking compared with the absence from normal healthy children and from previously infected adults. Similar IgM antibody was found in patients with MS and not in control subjects. In both instances it may be assumed that, as the antibody is IgM in type, the immunogenic stimulus is active (Svehag & Mandel, 1964). It is interesting that the IgM from MS and following virus infection produces the same staining pattern, but I do not suggest without further evidence that these viruses have a role in the pathogenesis of MS.

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