

# Serum IgM and IgA responses in influenza A infections

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**SYNOPSIS** Immunofluorescent serum IgM and/or a significant level of IgA antibody was detected in 87% of 39 cases of current or recent influenza A infection from two to 84 days after the onset of illness. Secondary IgM staining occurred in 5% of sera and a significant correlation was found between complement-fixing and class-specific antibodies. It was estimated that the immunofluorescent test could be diagnostic in 64% of single sera with levels of CF antibody between 32 and 256, and because anti-s antibody was detected in the IgM test this test did not differentiate primary and secondary influenza A infection.

Patients with influenza are frequently admitted to hospital too late in the illness for positive virus isolation. Because the detection of specific serum IgM and IgA antibodies is a valuable aid in recent rubella infection (Banatvala, Best, Kennedy, Smith, and Spence, 1967) it was decided to evaluate the application of this method in 45 cases of confirmed influenza infection and 19 control patients.

## Materials and Methods

### PATIENTS

Over the two winters of 1971-73, 100 sera from 64 selected patients in the north Gloucestershire clinical area were examined by immunofluorescence for influenza A IgM and IgA antibodies. Twenty-five patients diagnosed as current influenza A infection had four-fold or greater antibody rises by the complement-fixation test (CFT), 14 cases of recent infection had CFT antibody levels equal to or greater than 512, six cases had influenza A virus isolated 10-49 months before serum sampling (follow-up cases), and 19 cases which did not yield influenza virus on culture and had no CFT antibody rise or levels of antibody greater than 128, were classified as non-influenzal respiratory infection.

### IMMUNOFLUORESCENCE

Four ounce bottles of secondary monkey kidney cell monolayers were inoculated with 250 TCD<sub>50</sub> of a field strain (A/England/640/72) of influenza A2 virus similar to the Hong Kong/68 variant (H3N2).

After incubation, rolling at 33°C in serum-free 199 medium for 70 to 72 hours the cells were treated with versene and centrifuged at 500 rpm (MSE minor) for two minutes. The supernatant was removed and the cells were resuspended in 30 drops of phosphate-buffered saline pH 7.4 (PBS). Three drops of cells were put onto each of 10 slides, air dried, acetone fixed at room temperature for two minutes and redried before the addition of patient's serum diluted 1 in 5. Incubation at 37°C for one hour in a moist chamber was followed by washing twice in PBS for 10 minutes. Appropriate antihuman globulin conjugate diluted to contain 4-6 units was applied and incubated for one hour at 37°C.

Washing was repeated and cell spots were mounted in glycerol-PBS 9/1 before examination under a  $\times 40$  dry objective using a Tyoda microscope with Polaron HB0 100 mercury vapour light source and Balzer FITC no. 3 interference primary filter. All test sera positive at a 1 in 5 dilution were titrated and the endpoint was read as that dilution giving 2 plus fluorescence. Confirmation of the presence of virus antigen in cells was made using influenza A specific rabbit antiserum and antirabbit globulin conjugate (Nordic Pharmaceuticals). All sera were tested on uninfected monkey kidney cells, 20 sera were also tested on influenza B infected cells, and eight sera also on cells infected with a field strain (A/England/267/73) similar to the A/England/42/72 variant (H3N2) of influenza A2. IgM and IgA antihuman globulin conjugates were obtained from Nordic Pharmaceuticals, and IgG antihuman globulin conjugate from Wellcome Reagents Limited.

## OTHER VIRAL PROCEDURES

Complement-fixation microtechnique tests were carried out based on the method of Bradstreet and Taylor (1962), and the isolation of influenza viruses by the method of Higgins, Ellis, and Boston (1963). Influenza viruses were typed using the complement-fixation test and subtyped by haemagglutination inhibition.

## LATEX TESTS

Latex tests for rheumatoid factor were carried out using Latex reagent (Difco Labs) with sera diluted 1 in 20.

## ABSORPTION OF ANTIGLOBULINS FROM SERA

Sera positive in the IgM test were absorbed with aggregated  $\gamma$ -globulin prepared by adding an equal volume of saturated ammonium sulphate to pooled human sera and allowing precipitation to occur at 4°C for 30 minutes. The aggregate was washed three times in PBS and resuspended in a four times volume of phosphate-buffered saline. Three volumes of test serum was added to four volumes of aggregated  $\gamma$ -globulin suspension and after overnight incubation at 4°C the mixture was centrifuged at 3000 rpm for 10 minutes. Nine volumes of PBS was added to the supernatant to make a 1 in 5 dilution, which was tested for influenza A IgM antibody by immunofluorescence.

## Results

IgM antibody was present in 28/39 (72%) (table I) of patients with either current or recent influenza A infection but not in follow-up or non-influenzal cases. Similarly IgA antibody was present in 38/39 (97%), 3/6 (50%), and 3/19 (16%) cases respectively. However, in the three follow-up and three non-influenzal cases with IgA antibody, this was present in low titre (less than 1 in 20). Thus in currently and recently infected patients IgM and/or a significant level (1 in 20 or greater) of IgA antibody was present in 34/39 (87%) cases and not at all in the remaining 25 follow-up or non-influenzal cases. IgM and/or significant IgA antibodies were present in 20/25 (80%) of current and 14/14 (100%) of recent influenza A infections. It is likely that the low titres of IgA of 1/5 and 1/10 in the follow-up and non-influenzal cases represented residual antibody following previous infection.

The phenomenon of secondary IgM staining due to the presence in sera of antiglobulin along with specific IgG antibody (Fraser, Shirodaria, and Stanford, 1971) occurred in five of the 100 sera tested (table II), and these were excluded from the IgM antibody analysis (tables I and III). Only two of these five 'false-positive' IgM sera were predictable on the basis of positive latex and IgG antibody tests. Fifteen other sera in the series were positive both in

Diagnostic Category	No. Patients Tested	Mean Age (yr)	Time of Sera after Onset of Illness		No. with True IgM Antibody	No. with IgA Antibody		No. with IgM and/or IgA ( $\geq 1/10$ ) Antibodies
			Range	Mean		$\geq 1/5$	$\geq 1/20$	
Current influenza A	25	47	1-45 days	14 days	16	24	16	20 (80%)
Recent influenza A	14	58	2-84 days	18 days	12	14	12	14 (100%)
Follow-up influenza A	6	52	10-49 mths	31 mths	0	3	0	0 (0%)
Non-influenzal respiratory infection	19	65	1-42 days	8 days	0	3	0	0 (0%)
Total	64	—	—	—	28	44	28	34

Table I Immunofluorescent serum IgM and IgA influenza A antibodies

Diagnostic Category	Total Sera Tested	Sera Showing Secondary Staining				
		No.	No. Latex Test Positive	No. IgG Antibody Positive	Absorption with Aggregated $\gamma$ -Globulin	
					IgM Titre <sup>1</sup>	
Before	After					
Current influenza A	50	2	2	1	5, 20	<5, <5
Recent influenza A	19	1	1	1	20	<5
Follow-up influenza A	11	1	0	1	5	<5
Non-influenzal respiratory infection	20	1	0	0	5	<5
Total	100	5	3	3	—	—

Table II Sera showing secondary IgM antibody staining

<sup>1</sup>Reciprocal of endpoint dilution.

Complement-fixation Antibody Titre	No. with True IgM Antibody	No. with IgA Antibody $\geq 1/20$	No. with IgM and/or IgA ( $\geq 1/20$ ) Antibody	Total Sera Tested
< 8	0	0	0	7
8	0	0	0	6
16	0	0	0	7
32	1 (20%)	1 (20%)	1 (20%)	5
64	1 (17%)	3 (50%)	3 (50%)	6
128	4 (57%)	5 (71%)	6 (86%)	7
256	6 (86%)	4 (57%)	6 (86%)	7
512	19 (79%)	21 (88%)	24 (100%)	24
Total	31 (44%)	34 (49%)	40 (56%)	69

Table III Relation between complement-fixation and class-specific antibodies in sera from 39 current and recent influenza A infections

latex and IgG tests and were expected to give false positive IgM results. These did not show any reduction in apparent IgM antibody titre after absorption with aggregated  $\gamma$ -globulin and were thought to contain specific IgM antibody.

The mean true IgM titre detected was 1 in 10 with a range from 5 to 40 and occurred two to 43 days after the onset of respiratory infection. The mean significant IgA antibody titre was 1 in 80 with a range from 20 to over 1000 and these levels were detected from two to 84 days after the onset of illness. (None of 21 sera from currently infected cases was IgM antibody positive or contained significant IgA antibody in the first seven days after the onset of illness.)

No IgM or IgA antibody to influenza B was detected in 20 sera with IgM or IgA antibody to influenza A and eight sera with these latter antibodies gave similar titres when tested with cells infected with a field strain of influenza A similar to A/England/42/72.

With sera containing IgM and/or IgA influenza A antibodies, fluorescence was seen both in the nucleus and cytoplasm indicating that at least anti-s antibody was being measured in this test. This observation was confirmed when the presence of class-specific antibody was related to the CF titres of sera (table III). All sera with CF titres equal to

or greater than 1 in 512 were positive for IgM and/or contained significant IgA antibodies, while none of the sera with CF titres less than 1 in 32 was positive. In the CF titre range 32-256, 16/25 (64%) sera gave significant results in IgM and IgA immunofluorescence antibody tests. Thus a diagnosis of recent influenza A infection would have been possible in 64% of single sera containing these titres of CF antibody. The mean CF titre in the 31 sera from follow-up and non-influenza cases was 1 in 16 with a range from less than 1 in 8 to 1 in 256 and, as previously stated, none of these contained IgM or significant IgA antibody.

#### Discussion

This report and that of others (table IV) shows that serum IgM and IgA antibodies are detectable by immunofluorescence following natural infection with influenza A virus. Compared with rubella infection titres are significantly lower but are present over a similar time period after the onset of illness. IgM antibody declined after 43 days to undetectable levels. IgA antibody was detectable at a significant level (1 in 20 or greater) for 84 days after the onset of illness and persisted at lower levels for two years at least. The application of immunofluorescent IgM and IgA antibody to the diagnosis of recent

Source	Virus	No. Cases	Percentage with IgM Antibodies	Percentage with Significant <sup>1</sup> IgA Antibodies	Percentage with IgM and/or Significant IgA Antibodies <sup>1</sup>	Titres of IgM Antibody Present <sup>2</sup>		Titres of IgA Antibody Present		Days after Illness Detected
						Range	Mean	Range	Mean	
Brown & O'Leary (1971)	Influenza A	10	100	100	100	8-64	32	8-32	16	1-26
De Silva <i>et al</i> (1973)	Influenza A	13	31	23	54	12-80	45	6-324	40	No data
Urquhart (1974)	Influenza A	39	72	72	87	5-40	10	5-1000	40	2-84
Craddock-Watson <i>et al</i> (1972)	Rubella	11	82	100	100	8-2048	128	4-2048	256	1-78

Table IV Comparison of immunofluorescent IgM and IgA serum antibodies in confirmed influenza A and rubella infections

<sup>1</sup>As determined by individual authors.

<sup>2</sup>Reciprocal of endpoint dilution.

influenza A infection is limited by (1) the need for absorption of sera with aggregated  $\gamma$ -globulin to detect the 5% false positive IgM sera; (2) the need for titration of IgA antibody-positive sera to determine whether a significant level is present; and (3) the relationship between CF antibody titre and class-specific antibody content of sera, the latter being useful diagnostically only when the CF titre is between 32 and 256. However, in these latter cases, where the attempted isolation of virus is negative and a second serum is not available to show a conventional antibody rise, the immunofluorescent test for IgM and IgA antibodies may be diagnostic in 64% of cases when single sera are examined.

A point of interest from this series is that, taking into account the age of patients with current or recent influenza A infection (table I), it is likely that many had previous influenza A infection. Because influenza anti-s IgM antibody was detected in these cases, one may say that the presence of IgM antibody does not distinguish between primary and secondary infection with influenza A virus and this finding is similar to that of Ross and McDaid (1972) in varicella-zoster infection.

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