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IMMUNOFLUORESCENT IgG AND IgM ANTIBODIES IN HUMAN SERA BY ENTEROVIRUS INFECTIONS

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Summary. — IgG and IgM antibodies to selected enterovirus serotypes (P 2, CB 1—5, E 2, 4, 20), to prototype collection strains and to fresh isolates were examined by immunofluorescence in paired sera of 11 children and 4 adults in whom enterovirus had been isolated, or a rise of specific antibodies had been proved by neutralization test. One six-month- and one seven--month-old child had antibodies of both classes to the isolated virus strain and to poliovirus only. Children from eight month onwards and adults had antibodies to the majority of enterovirus serotypes tested. Among all enteroviruses tested, heterologous reactions were observed not only with IgG but also with IgM class antibodies.

Key words: enteroviruses, IgG and IgM antibodies, immunofluorescence

Introduction

The problem of rapid enterovirus detection methods remains very topical for enteroviruses which are frequent human pathogens. None of the available serological tests appears fully satisfactory as many authors report the occurence of heterologous reactions (Bussel *et al.*, 1962; Dôrries and Ter Meulen, 1983; Holonen *et al.*, 1959; Katze and Crowell, 1980; King *et al.*, 1983; Kraft and Melnick 1952; Morgan-Capner and McSorley 1983; Minor *et al.*, 1979; Pattison, 1983; Schmidt *et al.*, 1968).

The experience we gained in this respect when using the indirect immunofluorescence for diagnosing enterovirus infections in a selected group of patients is presented in this contribution.

Materials and Methods

Paired sera examined in this study were acute and convalescent sera from a group of 15 patients of both sexes 6 month to 49 years old who had been found positive by enterovirus isolation or by a rise of neutralization antibodies from 1982 to 1985 either in the hospital or in out-patient care.

The presence of IgG and IgM class antibodies in the sera was determined by an indirect immunofluorescence method using the anti-human IgG and IgM swine conjugates obtained from the Institute of Sera and Vaccines in Prague.

Table 1. Immunofluorescent antibodies in children

Patient	Age	Virus isolated	Type of antibody	Titres of antibodies to Coxsackie B		
				1	2	3
1	6 m.	CB 5	G	<8/<8*	<8/<8	<8/<8
			M	<4/<4	<4/<4	<4/<4
2	7 m.	CB 5	G	<8/<8	<8/<8	</8<8
			M	<4/<8	<4/<8	<4/<8
3	11 m.	CB 5	G	16/32	<8/16	<8/8
			M	4/8	<4/<8	<4/<8
4	10 m.	CB 5	G		16/32	16/>32
			M	<4/4	4/8	<4/4
5	8 m.	E 20	G	8/8	8/8	8/8
			M	4/<4	<4/<4	<4/<4
6	2 y.	E 20	G	32/32	32/64	
			M	8/16	8/16	8/16
7	11 m.	CB 3	G	32/32	32/32	32/64
			M	32/64	32/32	16/16
8	7 y.	CV 4	G	64/64	64/64	64/64
			M	32/16	32/32	16/8
9	8 y.	CB 4	G	64/128	64/128	64/128
			M	8/16	16/32	8/16
10	9 y.	CB 4	G	32/128	32/64	16/128
			M	4/16	8/16	<4/8
11	9 y.	neg.	G	128/256	128/256	128/128
			M	32/32	16/16	8/8

* first sample/second sample

Mouse monoclonal antibody to human IgG was supplied by Dr. L. Rozprimová (Institute of Sera and Vaccines). It was prepared from the hybridoma HB 57 ascitic fluid using the caprylic acid technique. The anti-mouse swine conjugate was obtained from Institute of Sera and Vaccines.

The antigens used were the prototype strains of the following enterovirus serotypes: Poliovirus (P) type 2, vaccination strain P 712 Ch 2 a b; coxsackie virus B (CB) type 1—5; echovirus (E) type 2, 4 and 20. Fresh enterovirus isolates used also as antigens were the serotypes CB 5 and E 20 determined by the neutralization test with W.H.O. reference sera and monotype sera of the Institute of Poliomyelitis and Virus Encephalitides, U.S.S.R.

The preparations to be examined for the presence of antibody were mostly prepared from BGM, exceptionally RD cells. Suspensions of virus-infected cells were dropped on slides, air dried, and fixed in acetone. The preparations were either immediately processed or stored at —20 °C till examined.

Table 1 (continued)

Titres of antibody to						
Coxsackie B		ECHO			Polio	Isolated
4	5	2	4	20	2	strain
<8/<8	<8/<8	<8/<8	<8/<8		8/16	32/32
<4/<4	<4/<4	<4/<4	<4/<4		4/8	16/16
<8/<8	4/4	<8/<8	<8/<8		16/32	32/128
<4/<4	8/8	<4/<8	<4/<8		8/8	32/32
8/16	16/16	16/32	16/32		16/32	32/64
4/8	<4/<4	<4/<8	4/8		8/16	8/8
32/32	16/16	8/16	8/16		64/64	32/32
<4/4	<4/4	4/8	<4/8			16/16
16/16		8/8	8/8	16/16	32/32	8/8
4/4		8/16	8/8	8/8	16/16	4/4
64/64		32/32	64/128	32/32	32/32	
8/8		4/8	32/32	8/8	8/16	
32/32		32/32	32/32		64/64	
16/32		8/16	16/32		32/32	
64/64		64/64	64/64		128/128	
16/8		16/16			32/32	
64/128		64/128	64/128		64/128	
8/16		4/8	4/8		16/32	
16/64		32/128	32/128		32/128	
<4/16		4/16	8/8		4/16	
64/128		128/128	64/128		64/128	
8/8		16/16	8/8		8/16	

Results

Immunofluorescent antibodies in children

Eleven children aged 6 months to 9 years were tested for the presence of specific IgG and IgM class antibodies. From ten children enterovirus was isolated in the acute phase of disease. Patient No 11 revealed only an increase of neutralizing antibodies to CB 3 (Table 1).

The virus coming from the stool of children 1—4 was identified as CB 5. These children belonged to a group of patients hospitalized simultaneously for respiratory infection. The specific IgG and IgM antibodies to the isolated virus strain showed titres higher then those against the prototype strain CB 5. All children had been previously immunized against polio. Children

Table 2. Immunofluorescent antibodies in adults

Patient	Age years	Virus isolated	Type of antibody	Titres of antibodies to virus						
				Coxsackie B				ECHO		Polio
				1	2	3	4	2	4	2
1	31	E 4	G	32/256*	16/128	16/128	16/64	32/128	32/128	32/128
			M	8/16	4/16	4/32	4/16	8/16	8/32	8/32
2	44	E 4	G	128/128	128/128	64/64	64/64	128/128	128/128	64/64
			M	16/16	16/16	16/16	16/16	16/16	16/16	16/16
3	37	neg.	G	32/32	64/32	16/16	16/16	32/32	16/16	64/64
			M	8/8	8/8	4/4	$<4/<4$	8/8	4/4	8/8
4	49	neg.	G	32/128	64/64	64/128	64/128	16/64	64/128	32/64
			M	8/32	4/32	8/32	4/16	4/8	4/32	8/8

* first sample/second sample

1 and 2 (6 and 7 months) had antibodies to the majority of enterovirus serotypes tested. Children 5—11 were treated for respiratory infection or meningitis. In child 8 the virus was isolated from nasal washing, in the remaining children from stools. A significant rise of IgG and IgM class antibodies was observed in child 10 only; this child had antibodies of both classes to the majority of enterovirus serotype tested and not only to virus CB 4 isolated from the child. In the remaining children of this group the antibody titres in the 1st and 2nd sample of the serum were similar or identical.

The presence of IgM class antibodies in child 10 was also tested using the mouse monoclonal antibody to human IgM. The titres in the 1st and 2nd serum sample were as follows: CB 1: not tested/16; CB 2: <4/16; CB 4: <4/16; E 2: not tested/16; E 4: 4/8; P 2: 4/16.

Immunofluorescent antibodies in adults

The antibodies found in the sera of 4 adults are shown in Table 2. E 4 was isolated from liquor of patient 1, and from nasal washing of patient 2. The remaining two patients had a simultaneous rise of neutralizing antibody titres to several serotypes. Significant rises of immunofluorescent antibodies of both classes were in patient 1 and 4 simultaneously again to the majority of enterovirus serotypes tested.

Discussion

Detectability of IgG and IgM class antibodies to a greater number of enterovirus serotypes increased with the increasing age: child 1 (6 months) had antibodies to the isolated virus strain and to poliovirus only, child 7 (11 months) had antibodies of both classes to all enterovirus serotypes tested. In the first group of 4 children the specific antibodies to the isolated CB 5 virus strain were at titres considerably higher then those seen in the prototype strain. This implies the isolation of a CB 5 variant other than that of the collection strain Faulkner.

A definite demonstration of the existence of heterologous antibodies was seen in child 10 showing a significant rise of titres to almost all enterovirus serotypes tested. The age-related cross-reactivity of IgM antibodies to CB type 1—6 viruses was also proved by King *et al.* (1983) who used ELISA.

Older children and adults had IgM antibodies to the majority of enterovirus serotypes used. This may be ascribed to the prolonged persistence of IgM class antibodies (Mertens, 1982, found the IgM antibody to E 11 virus to persist for 6 to 10 months postinfection), or to the widespread incidence of enteroviruses among the population, or to the occurrence of cross reactions. A detailed elucidation of enterovirus cross-reactivity including description of the structural proteins involved in these reactions, has been recently presented by Reigel *et al.* (1985).

We conclude that the demonstration of IgM class antibodies to enteroviruses by immunofluorescence is not a reliable proof of either recent or

past infection and, therefore, this method cannot serve as a rapid diagnostic tool in contrast to some other virus infections.

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