Acta virol. **26**: 481—487, 1982

RADIOIMMUNOASSAY OF MEASLES VIRUS ANTIBODIES IN SSPE

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Received October 28, 1981; revised April 12, 1982

Summary. — A sensitive radioimmunological assay (RIA) was introduced for detection of measles virus IgG and IgM antibodies. The hyperimmune response to measles virus could be demonstrated more precisely by RIA than by haemagglutination inhibition (HI). The ratio between RIA and HI antibody titres was decidely higher in sera and cerebrospinal fluids (CSF) of patients with subacute sclerosing panencephalitis (SSPE) than in those of other groups tested.

Key words: *measles virus antibody*; *SSPE*; *radioimmunoassay*

Introduction

Elevated levels of measles virus antibodies in CSF are considered diagnostically significant for SSPE. In SSPE patients, measles virus antibody represents about 75% of total CSF IgG and nearly 10 to 20% of total serum IgG (Mehta *et al.*, 1977). Moreover, reduced serum to CSF ratio of measles virus antibodies (Link *et al.*, 1973; Sever *et al.*, 1974) as well as the presence of oligoclonal predominantly measles virus specific IgG in CSF (Vandvik and Norrby, 1973) indicate the local production of measles virus antibodies within the central nervous system. The relatively low sensitivity of conventional serological techniques such as complement fixation and haemagglutination inhibition tests and also the restricted specificity of antibodies detected by these methods, allows to obtain limited information about measles virus antibodies in sera and CSF of patients with SSPE.

This paper presents data comparing the results of detection of measles antibodies in sera of healthy persons, of children with acute measles, in sera and CSF of patients with various neurological diesase as detected by RIA.

Materials and Methods

Samples. Serological examinations were carried out in sera and CSF coming from 21 boys and 18 girls of 3 to 14 years age. The clinical diagnosis of SSPE was based on the following criteria: progression of clinical signs of cerebral lesions to the loss of cerebral cortex function, paretic collodial gold curve or elevated IgG in the serum or in CSF and characteristical EEG. Sera and CSF were also sampled from 51 patients with multiple sclerosis (16), epilepsy (17), extrapyramidal syndrome (9), meningoencephalitis of unknown etiology (5) and other neurological diseases (4).

The tests were performed also with 4 sera of 2 to 6 years old children with clinical manifestations of measles. The samples were taken on the first and the second weeks after the onset of disease. Single serum samples taken from 5 to 9 years old healthy children were included as well.

Measles virus antigens. The SSPE strain LEC and Edmonston strain of the measles virus were kindly supplied by Prof. E. Norrby, Karolinska Institutet, Stockholm. L-16 strain was isolated from live measles virus vaccine produced by the Institute of Viral preparations in Moscow, U.S.S.R., and the Boston strain of measles virus was received from the Institute of Sera and Vaccines, Prague, Č.S.S.R. The viruses were multiplied and semipurified according to Tyrrell and Norrby (1978).

Briefly, the confluent monolayer of Vero cells was infected at multiplicity of infection (m.o.i.) of 0.05 $TCID_{50}$/cell in MEM with 2% inactivated calf seruml. By 72 hr, the medium was changed and the extracellular virus was harvested on the days 4-6 post infection. The medium was clarified at 10,000 × g for 10 min and filtered through No O Munktell's filter paper. The virus was pelleted from the clarified medium at 40,000 × g for 1.5 hr in Beckmann J-21-C centrifuge. The pellet was suspended in TE buffer (0.005 mol/l Tris-HCl, 0.001 mol/l EDTA, pH 7.4) to 1% of the original volume and sonicated for 15 min at 20 kHz and 12—16 μm in 150 Wyatt MSE apparatus. After removing of aggregated material by centrifugation at 5000 × g, the virus suspension was used in RIA.

Radioimmunoassay procedure. The solid-phase RIA used in this study was originally developed to detect cytomegalovirus antibodies (Jankowski *et al.*, 1980). Briefly, serial dilutions of serum (starting from dilution 1 : 100) or CSF (starting from dilution 1 : 10) specimens were incubated with measles virus antigen coated wells in polystyrene microtrays. After incubation for 3 hr at room temperature the serum and CSF were removed and washed with phosphate buffer, pH 7.2, containing 0.05% Tween 20 and 0.5% calf albumin. An aliquot of ^{125}I labelled rabbit Ig rised against Fc-fragment of IgG or μ chain of IgM (Dako Lab., Copenhagen, Denmark) containing 150 000 counts per min (cpm) was added to each well. Specific activity of ^{125}I labelled Ig (according to Greenwood *et al.* (1963) ranged from 133—188 MBq/mg protein. After incubation for 1 hr at 36 °C, gamma radiation was measured in individual wells (Packard counter). In each experiment le five serum of CSF samples were included as negative controls. From ten wells filled with these five serum (diluted 1 : 100) or CSF samples (diluted 1 : 10) a "negative" mean count per minute (c.p.m.) value was calculated. Subsequently, for each serum (CSF) dilution, the cpm value was divided by the negative mean to positive/negative yield ratio (P/N ratio). P/N ratios equal to or greater than 2 were considered positive. The titer of measles virus antibodies was defined as a highest sample dilution yielding a P/N value of 2 or greater.

In measles virus IgM antibody tests the samples were previously absorbed with protein A bound to Sepharose (Pharmacia) in order to eliminate non-specific reactions if rheumatoid factor was present in the sample. After absorption, about 94—97% of IgG and 34—40% of IgM were removed from sera as measured by radial immunodiffusion test (Mancini *et al.*, 1966). Control experiments were also performed with sera taken from patients with rheumatoid arthritis disease containing IgM rheumatoid factor and IgG measles virus antibodies. With all of these samples positive results were obtained for RIA IgM antibody, when untreated sera were added on wells with viral antigen. After Sepharose-protein A treatment all samples were negative for IgM class measles antibody in the RIA test.

Haemagglutination inhibition test (*HIT*). HIT was carried out by a micromethod using twofold dilutions of serum or CSF, 4 units of measles virus antigen and 0.05 ml of 1% *Cercopithecus aethiops* erythrocytes. Edmonston B strain viral antigen was prepared by the tween-ether method according to Norrby (1968). Serum samples were heated at 56° C for 30 min and absorbed with 10% suspension of monkey red blood cells.

Results

Some measles virus antibody titres by RIA were 50—51, 200 times higher than those detected by HIT (Fig. 1). Measles antibody titres in all sera from SSPE cases determined by RIA were at least 1,600-fold higher than those obtained by HIT. In control groups the titre was decidely lower; in the majority of samples tested it was ranging between 100—200. Two samples ori-

ginating from patients with multiple sclerosis, revealing titre 800 being the only exception. In all CSF samples from patients with SSPE the titres of measles antibodies in RIA exceeded 3 200. In some CSF samples taken from neurological cases other than SSPE, low titres of measles antibody were detected (1:20—1:400). This could have been due to a high sensitivity of radioimmunoassay, because in the majority of these samples the measles virus antibodies were not found by HIT.

The simultaneous examination of antibody occurrence in serum and CSF of individual patients allowed to calculate the serum to CSF antibody ratio, an important value in the diagnosis of SSPE. From 45 samples tested, a low antibody ratio (≤ 80) was measured in all but four cases by RIA and in all but two cases by HI test.

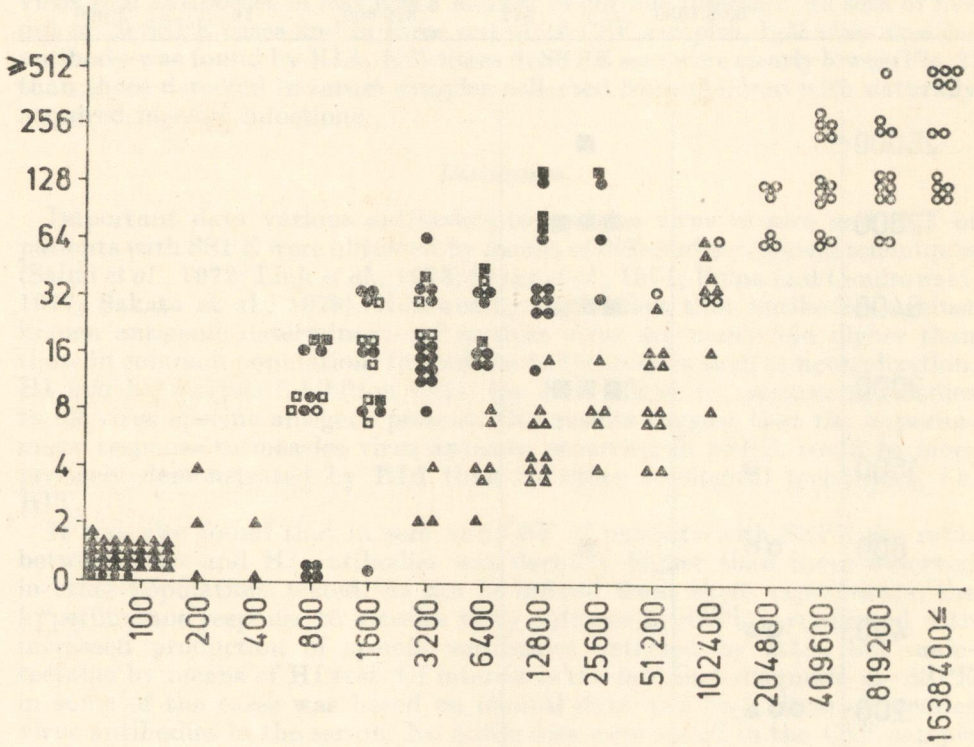

Fig. 1.

Measles IgG antibody titre in sera and CSF from SSPE cases and other groups as tested by HI and RIA

Abscissa: RIA titres; ordinate: HI titres.

Symbols: SSPE — sera (○), CSF (△); other neurological diseases — sera (●), CSF (▲); measles — — sera (■); control group — sera (□)

Table 1. Fluctuation of measles virus IgG antibody titres in serum and CSF of patients with SSPE as detected by HIT and RIA

Patients	Sampling date	Antibody titre in serum		Antibody titre in cerebrospinal fluid	
		HIT	RIA	HIT	RIA
K. R.	29.12.1978	512	819 200	2	800
	3.02.1979	128	409 600	2	3 200
	6.06.1979	128	409 600	4	12 800
	3.09.1979	256	409 600	8	51 200
W. A.	3.01.1980	256	819 600	32	12 800
	6.04.1980	128	204 800	32	3 200
	7.05.1980	128	409 600	16	6 900
R. A.	2.03.1980	256	102 400	16	3 200
	6.08.1980	256	409 600	16	25 600
T. K.	3.04.1980	156	204 800	32	12 800
	5.06.1980	512	819 600	16	1 600

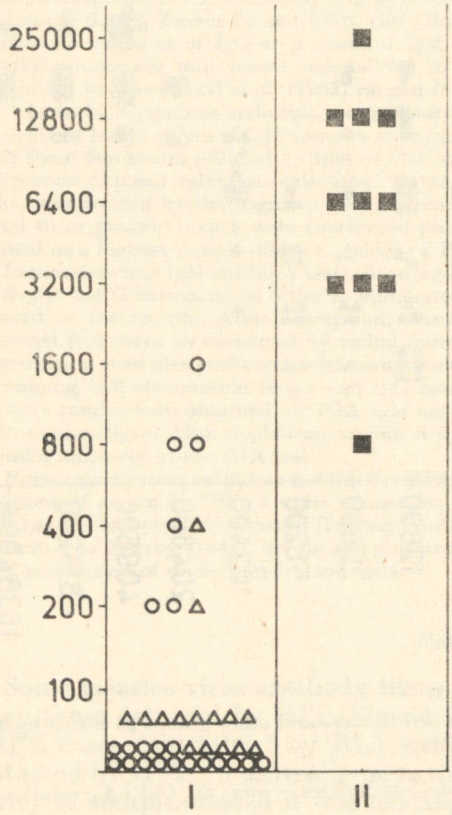

Fig. 2.
RIA of measles virus IgM antibodies Sera (○) and CSF (△) from SSPE cases; sera of measles cases (■); I — — SSPE, II — measles; ordinate: RIA titres.

Table 1 shows the longitudinal determination of measles virus antibody in serum and CSF of 4 patients with SSPE as detected by HIT and RIA. These results suggest that fluctuation of measles virus antibody level in sera and CSF of patients with SSPE could be visualized independently by RIA and HI tests. It is probable due to the differences in the antibody populations measured by each method. On the other hand, such results could be associated with the strain used for preparation of antigen for RIA. The radioimmunonological test was primarily performed with antigens of LEC virus strain. But no differences in the results of specific RIA antibody were obtained, if the samples from SSPE, measles and neurological patients were tested simultaneously with Edmonston B, L-16, Boston and LEC strain measles virus antigens.

Present evidence is far conclusive as to whether the persistence of measles virus IgM antibodies in SSPE is a marker of chronic infection. In sera of five out of 20 SSPE cases and in three out of 16 CSF samples, IgM class measles antibody was found by RIA. IgM titres in SSPE sera were clearly lower (Fig. 2) than those detected in serum samples collected from children with naturally acquired measles infections.

Discussion

Important data various antibodies to measles virus in sera and CSF of patients with SSPE were obtained by means of different serological techniques (Salmi *et al.*, 1972; Link *et al.*, 1973; Sever *et al.*, 1974; Polna and Cendrowski, 1977; Sakata et al., 1979). Noteworthy is the fact that antibodies against known antigenic determinants of measles virus are many-fold higher than those in common population. In contrast to techniques such as neutralization, HI and haemolysis inhibition tests, the RIA allows to measure antibodies to all virus specific antigens present. Our results suggest that the hyperimmune response to measles virus antigens occurring in SSPE, could be more precisely demonstrated by RIA than by other serological techniques, i.e. HIT.

It was also found that in sera and CSF of patients with SSPE the ratio between RIA and HI antibodies was decidely higher than those observed in other populations tested. As can be infered from these experiments, the hyperimmune response to measles virus antigens in SSPE is connected with increased production of specific antibodies detected by RIA, but undetectable by means of HI test. Of interest is the fact that diagnosis of SSPE in some of the cases was based on clinical data and high titres of measles virus antibodies in the serum. No antibodies were found in the CSF sample of these patients by HI and neutralization tests (Polna *et al.*, 1970).

On the other hand, the presence of measles virus antibodies in CSF of SSPE patients may result from their local production or from the increased permeability of the blood-brain barrier (Salmi *et al.*, 1972). The last phenomenon occures in many neurological disorders and some of them clinically resemble SSPE (Kalimo *et al.*, 1977). RIA technique may be very useful for verification of above-mentioned diagnostically difficult cases of SSPE.

Serum and CSF levels of measles virus antibodies increased and decreased at different stages of SSPE. Changes were found particularly in HI antibody response during longer period of clinical observation. The titres of neutralizing and fluorescence antibodies were less fluctuating (Polna and Cendrowski, 1977; Polna et al., 1980). In our longitudinal examinations of sera and CSF from patients with SSPE an interdependent fluctuation of antibody levels to measles virus was detected by HIT and RIA. Other authors using the ELISA and RIA techniques also observed relatively high titres of measles virus antibodies as compared to those detected by HIT in a few samples from SSPE patients (Kahane *et al.*, 1979; Sakata *et al.*, 1979). However, in contrast to our findings, Sakata *et al.* (1979) obtained a high RIA to HI measles antibody titre ratio in sera of patients with multiple sclerosis and other neurological disorders. This discrepancy could be connected with differences in the RIA procedure, because the authors in question used cells persistently infected with measles virus as antigens. Such an interpretation is supported by following data: 1) virion antibodies and antibodies to antigens on virus infected cells reacted at different dynamics; 2) binding and saturation kinetics of antibodies from SSPE patients to virus infected cells differed from that observed with antibodies from adult convalescence sera (Joseph *et al.*, 1976).

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