Isolation of Rubella Virus from Patients with Progressive Rubella Pan-Encephalitis

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Summary

During a period of 7 years (1997-2003) we were received pairs of serum and CSF of 27 suspicious clinically cases from neurology department of several hospitals in Tehran. Among them 7 were serologically positive for progressive rubella panencephalitis (PRPE) and 2 were positive for sub acute sclerosing panencephalitis (SSPE). Rubella virus was isolated from 4 of 7 serologically positive cases of PRPE using three different cell culture systems.

***Key words*:** rublla, progressive, panencephalitis, interference

Introduction

Rubella is an acute febrile illness which is caused by a RNA virus and characterized by a rash, suboccipital and posterior adenopathy; and affects children and young adults. Infection in children is usually mild and self-limited; while in pregnant women may result serious apparent or in apparent abnormalities of the fetus, including neurologic and neurosensory involvement in some of the surviving infants, which is called congenital rubella syndrome (CRS). An unusual delayed-onset of rubella encephalitis has been described in several publications (Jan 1979, Townson 1975, Weil *et al* 1975) which has been named to progressive rubella panencephalitis (PRPE) or non congenital rubella encephalitis (Lebon & Lyon 1974). Similar to other slow virus diseases of central nervous system, PRPE has characteristics of a

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prolonged asymptomatic period; followed by the onset of symptoms of neural deterioration during the second decade of life. These symptoms are behavioral changes, intellectual decline, ataxia, spasticity and sometimes seizures (Wolinskys 1989). Neurological disorder leads to death within 8 years. PRPE has been mostly associated with congenital rubella; but it may be a very rare, late complication of natural childhood rubella (Abet *et al* 1983, Dayras *et al* 1980, Lebon & Lyon 1979, Wolinskey *et al* 1976). There are several reasons of evidence link rubella virus to the etiology of PRPE. Rubella antibodies in serum and CSF of affected children react with each of the structural proteins of rubella virus (Vandich *et al* 1978, Wolinskey *et al* 1982) in which rubella immune complex has been demonstrated in sera of patients (Coyle *et al* 1981). Rubella virus has been isolated from mononuclear cells in blood (Wolinsky *et al* 1979) and CSF (Squadrini *et al* 1977, Shafyi; unpublished data).

Rubella virus can be replicated without or doubtful cytopathic changes in some cell culture systems (Jawetz *et al* 1987) and the induced interference protects the cells against the cytopathic effect of other viruses. In this study, Vero, MRC-5 and RK-13 cells were used for isolation of rubella virus from suspected PRPE patients. Also, the presence or absence of rubella virus replication was proved by interference phenomena.

Materials and Methods

Patient samples. During seven years saliva, urine, serum and CSF samples of 27 patients who suspected to PRPE, transferred to our laboratory under conventional conditions. Saliva, urine and CSF were treated with antibiotic before inoculation into tissue culture.

Antibody detection. Antibodies to rubella virus and other suspected viruses such as cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), measles and mumps viruses were assayed using standard micro-complement fixation (Lennette 1969) and hemagglutination inhibition (Center for Disease Control 1970) tests.

Virus isolation. Three tissue culture systems were used for isolation of rubella virus in tissue culture: a) *Mycoplasma* free African green monkey cell line (Vero) prepared in tissue culture tube, b) human diploid cells (MRC-5) prepared in tissue culture tubes in a growth

media (DMEM+8% fetal calf serum), c) *Mycoplasma* free rabbit kidney cell line (RK-13) grown in tissue culture tube in DMEM media containing 8% irradiated fetal calf serum. At the time of inoculation, the cell sheet was washed once with PBS. Each specimen was inoculated into four tubes of each cell strain. The maintenance media consisted of 1.5ml of DMEM with 1-2% of irradiated fetal calf serum were added after 1h adsorption at room temperature. The cultures were incubated at 35ºC in stationary rocks. Media changed every 3-4 days. At days 11 postinoculation the cultures were examined microscopically to exclude the tubes with cytopathic agents. If none was positive from the points of CPE, the media of two tubes were removed and maintenance media containing challenge virus were added while, the remaining two cultures were received sterile maintenance media.

Interference assay. Quite a number of different viruses such as Echovirus 11, coxsasackie A, vesicular stomatis virus (VSV) (Indiana strain) and poliovirus can be used for the demonstration of interference phenomena. In this study, the VSV for the challenge of RK-13 cell culture and, Echo-11 for the challenge of MRC-5 and Vero cell cultures were used. Challenge dose for each tube adjusted to $100TCID_{50/0.1ml}$. Whether or not interference was positive; the remaining of unchallenged culture was harvested and freeze–thawed once then used for further passages in the same cell strain, which had been used for previous passage. Three blind passages were done before one sample to be considered as negative. The isolated viruses were confirmed by neutralization test with specific rubella goat antiserum.

Rubella antiserum. To prime the animal (goat) two inoculations with live attenuated rubella virus, Takahashi strain (Shishido *et al* 1973) with one-week interval followed by two injections with rubella hemagglutinin prepared by using the RA27/3 strain (Plotkin *et al* 1967) and treated according to Norrby instruction (Norrby 1962) were used. The neutralizing titer of this antiserum was 1:1024 and had no cross reaction with other viruses.

Results

7 out of 27 patients were serologically positive for rubella antibodies but rubella virus was isolated from only CSF of 4 of them. The results of HI antibody to rubella virus in serum and

CSF of the 4 patients indicating local synthesis of rubella antibodies intrathecally are shown in table 1.

Table 1. *Serological detection of rubella antibodies of the PRPE patients*

Patient No	Sample	HI antibody*		Complement fixation antibody*			
		Rubella	Measles	CMV	HSV	VZV	Mumps
1	Serum	2048	16	<20	20	10	8
	CSF	128	-	-	-	-	-
2	Serum	512	8	40	20	20	16
	CSF	8	-	-	-	-	-
3	Serum	1024	<8	20	40	<10	<8
	CSF	16	-	-	-	-	-
4	Serum	256	16	<20	20	10	<8
	CSF	8	-	-	-	-	-
Control	Serum	16	8	20	20	<10	8
	CSF	-	-	-	-	-	-

* Expressed as reciprocal of sample dilution

In table 2 the results of virus isolation from the CSF of the 4 patients in three different cell strains are shown. Neutralization of isolates with goat anti-rubella serum in paralel with normal serum confirmed the rubella virus. The sensitivity of the RK-13 cell strain was more than the two others. CPE in these systems is not always clear enough on primary inoculation, and tissue culture fluids may have to be passaged 3-4 times for full detection of virus.

Discussion

In sub acute sclerosing panencephalitis we succeeded to isolate the viral agent from the brain of affected patients by co-cultivation of brain biopsy and Vero cells (Mirchamsy *et al* 1977) the isolated virus remained cell-associated. In the case of PRPE, similar technique has been used for isolation of rubella virus from the brain (Cremer *et al* 1975, Weil *et al* 1975). On the

other hand Squdrini *et al* (1977) had been able to isolate the rubella virus from the CSF of patients with rubella encephalitis. In this experiment we were succeeded to isolate the Rubella virus from the CSF of 4 out of 7 PRPE patients (57%), which none of the isolates was cell associated; and free virus could be re cultured in subsequent passages. All seven patients had progressive neurological disorders which had began in the second decade of life (11~17 years old). Mean age of affected patients was 13.7 years while in SSPE patients it was around 8 years (Shafyi *et al* 1984).

Table 2. *Detection of Rubella virus interference with appropriate challenge virus in CSF*

Patient No	**Cell strain**	**CPE after challenge**			**Interpretation**
		1st passage	**2nd passage**	**3rd passage**	
1	MRC-5	± *	±	-	Rubella virus presumed present
	Vero	±	-	-	
	RK-13	- *	-	-	
2	MRC-5	+	±	-	Rubella virus presumed present
	Vero	+	±	-	
	RK-13	+	-	-	
3	MRC-5	+	+	±	Rubella virus presumed present
	Vero	+	±	-	
	RK-13	±	-	-	
4	MRC-5	Ommited	Ommited	Ommited	Ommited because of shortage of CSF sample
	Vero	±	-	-	Rubella virus presumed present
	RK-13	-	-	-	Rubella virus presumed present
Control (negative reference)	MRC-5	+	+	+	No rubella virus present in normal CSF
	Vero	+	+	+	No rubella virus present in normal CSF
	RK-13	+	+	+	No rubella virus present in normal CSF
Control (challenge virus only)	MRC-5	+	+	+	Tissue culture sensitive to related challenge virus
	Vero	+	+	+	
	RK-13	+	+	+	

*(-) Complete interference, (±) Partial interference, (+) No interference

Lebon and Lyon (1974) reported a case of PRPE in 14 years old boy with elevated level of rubella antibody both in serum and CSF with no history of rubella infection in his life. However some cases of rubella infection might be happened inapparently from the point of

clinical symptoms; and also some children exposed to rubella virus in utero may have elevated antibody titer without clinical symptoms (Butler *et al* 1965) and rubella virus persist for several years in urine, feces, throat secretion, CSF fluid and lung tissue after in utero infection (Bellanti *et al* 1965, Peckham 1972, Menser *et al* 1967) as well as serum antibody titer may remain elevated for several decades. Briefly, they concluded that some cases of PRPE might be related to intera ulterin infection and some to after birth infection. Their conclusion confirms our findings. In all 7 patients we found elevated rubella antibody titer both in serum and CSF but we could isolate rubella virus in 4 of them. Since we do not have any history of the affected patients it can assume that some of them might be infected intra uterus and some after birth. PRPE was reported in patient 12 years after childhood rubella (Wolinsky *et al* 1976).

Vandvick (1978) reported the occurrence of oligoclonal IgG and homogeneous free lambda chain in the CSF of a patient with PRPE. Evidence showed that oligoclonal IgG of serum and CSF represent rubella virus-specific antibodies specified to different antigenic components of the rubella virus. Intrathecal synthesis of rubella specific antibodies has been reported in a patient of PRPE (Schuller *et al* 1977) and in a patient of 57 years old by A.Shafyi (unpublished data) whose rubella HI antibody titer in his CSF was 1:2048 while in his serum was undetectable at the dilution of 1:8. All these indications support the hypothesis of intrathecal synthesis of rubella antibody in PRPE patients.

According to what mentioned above, investigation and titration of rubella antibody in both serum and CSF can be a fast and simple tool for diagnosis and differentiation of PRPE from other progressive neurological disorders.

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