Varicella zoster virus DNA in throat swabs of vaccinees

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Abstract

Ninety throat swabs from 30 healthy children who received varicella vaccine were tested for varicella zoster virus DNA by the polymerase chain reaction. Seroconversion was observed in 27 of 30 (90%) vaccinees. The positive rates were 7.4% (2/27) at one week and 0% (0/27) at four weeks after vaccination. These rates were considerably lower than those in normal children with varicella.

Varicella, caused by primary infection with varicella zoster virus, is a common and highly contagious disease of childhood. The study of the pathogenesis of varicella zoster virus infection had been limited by technical problems with the laboratory detection of it. We established a polymerase chain reaction method for detecting varicella zoster virus recently,¹ and we have used it to examine throat swabs of normal children with varicella. We could detect the viral DNA in 26.2% (11/42) of the children during the incubation period and in 89.7% (35/39) after clinical onset of the disease.²

Live attenuated varicella vaccine, developed in Japan in the early 1970s, has been widely used in normal children in Japan since being licensed in 1987. We describe here the results of our attempts to detect viral DNA in the pharynx of normal varicella vaccine recipients by the polymerase chain reaction technique.

Methods

This study was conducted at the paediatric

outpatient clinic of Showa Hospital over a three month period in 1990. Thirty healthy children of both sexes who had no history of varicella, 1 to 9 years of age, received live attenuated varicella vaccine (lot 016 and 017, Oka strain, Biken). Two sera (before and four weeks after vaccination) and three throat swabs (before one week and four weeks after vaccination) were obtained from each of them. One throat swab was also obtained from each of 15 healthy children who were used as negative controls; their ages ranged from 2 to 14 years. All swabs were placed in 3 ml of phosphate buffered saline and stored at -80°C before analysis. After the project was explained parental consent was obtained.

The serum samples were examined for humoral immunity to varicella zoster virus by the two assays previously described; the immune adherence haemagglutination assay³ and the indirect fluorescent antimembrane antibody assay.⁴

The throat swabs were tested for varicella zoster virus DNA by the polymerase chain reaction; this has been described previously.² Briefly, DNA samples extracted from the throat swabs were first heated at 94°C for 10 minutes to denature the DNA and then subjected to 30 amplification cycles. Annealing was performed at 60°C for 2 minutes, extension at 72°C for 5 minutes, and denaturation at 90°C for 1 minute. The pair of primers used in this study were a region of glycoprotein I, and the length of amplified product was 651 base pairs. The preliminary testing made sure that the primers amplified Oka varicella zoster virus from lots 016 and 017. The amplification reaction was carried out in a DNA thermal cycler (Perkin-Elmer/Cetus). Direct gel electrophoresis with ethidium bromide staining and Southern blot hybridisation with a homologous ^{32}P -labelled cloned DNA probe (figure) were applied for detection of the amplified product. In this system varicella zoster virus DNA could be detected at a level of 5×10^3 copies by ethidium bromide staining, and 50 copies by Southern blot hybridisation. Varicella zoster virus DNA extracted from cells infected with the Oka strain was detected by direct gel electrophoresis and by Southern blot hybridisation and was used as a positive control in every experiment.

Results

The table shows the age and sex of the 30 vaccinees, vaccine lot number, and the results of serological and polymerase chain reaction studies. Clinical observations were made daily

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Autoradiograph after Southern blot hybridisation with ^{32}P -labelled probe for 16 pharyngeal swabs and a positive control. Samples No 14 (case 12, one week after vaccination) and No 16 (case 5, one week after vaccination) contain varicella zoster virus DNA; bp = base pairs.

The results of serological and polymerase chain reaction studies in the vaccinees. Day 0 is the day of vaccination

Case No	Age/sex	Vaccine lot	Antibodies (IAHA/FAMA)		PCR analysis		
			Day 0	Day 28	Day 0	Day 7	Day 28
1	1/F	016	<2/<4	8/16	-	-	-
2	3/F	016	<2/<4	8/32	-	-	-
3	2/M	016	<2/<4	16/16	-	-	-
4	2/M	016	<2/<4	8/16	-	-	-
5	4/F	016	<2/<4	8/8	-	+	-
6	1/F	016	<2/<4	8/4	-	-	-
7	1/F	016	<2/<4	16/16	-	-	-
8	2/M	016	<2/<4	8/8	-	-	-
9	4/F	016	<2/<4	64/64	-	-	-
10	2/M	016	<2/<4	16/4	-	-	-
11	1/F	016	<2/<4	8/8	+	-	-
12	2/F	016	<2/<4	4/4	-	+	-
13	5/M	016	<2/<4	<2/<4	-	-	-
14	2/F	016	<2/<4	16/16	-	-	-
15	1/M	016	<2/<4	16/8	-	-	-
16	2/M	016	<2/<4	8/8	-	-	-
17	9/M	016	<2/<4	64/32	-	-	-
18	1/M	016	<2/<4	8/8	-	-	-
19	3/M	016	<2/<4	16/8	-	-	-
20	8/F	016	<2/<4	8/8	-	-	-
21	6/F	016	<2/<4	4/4	-	-	-
22	4/M	016	8/4	128/64	-	-	-
23	1/F	017	<2/<4	16/8	-	-	-
24	5/M	017	<2/<4	32/32	-	-	-
25	1/M	017	<2/<4	4/4	-	-	-
26	5/F	017	<2/<4	32/32	-	-	-
27	4/M	017	<2/<4	32/32	-	-	-
28	7/F	017	<2/<4	8/8	-	-	-
29	1/F	017	<2/<4	16/8	-	-	-
30	3/M	017	<2/<4	8/4	-	-	-

Not detected (-) and detected (+) by Southern blot hybridisation. IAHA=immune adherence haemagglutination, FAMA=fluorescent antimembrane antibody; PCR=polymerase chain reaction.

by their mothers for four weeks after vaccination, and no systemic reactions were recorded. In 27 of 30 (90%) vaccinees, seroconversion was observed by both the serological assays. The viral DNA could be detected in three of 90 (3.3%) throat swabs by the polymerase chain reaction with Southern blot hybridisation. No viral DNA could be detected by the method with direct gel electrophoresis. One positive swab was found before vaccination and two swabs were positive one week after vaccination. The three vaccine recipients whose swabs contained varicella zoster virus DNA showed seroconversion by the both serological assays. In the swabs of negative controls no viral DNA was amplified specifically.

Discussion

Isolation of varicella zoster virus from the pharyngeal area is usually difficult in normal children with varicella. One explanation for this is that cell culture technique is not sensitive enough to detect the virus. Recently we established a polymerase chain reaction method for detecting varicella zoster virus DNA and this has been proved to be much more sensitive than viral culture with the previous studies.^{1 2} The viral DNA was amplified in 100% (20/20) of vesicle samples and in 83.3% (5/6) of crusts from normal children with varicella.¹ Identification of the viral DNA from throat swabs in 26.2% (11/42) during the incubation period and in 89.7% (35/39) after clinical onset² indicates

that the pharynx is a site for replication of the virus in normal children with varicella.

A live varicella vaccine has been extensively used in Japan, and its safety and effectiveness have been recognised for immunocompromised and immunocompetent children. The magnitude of viral replication of the vaccine virus is less than that of the wild type virus, and it has been considered to be unlikely that the vaccine virus spreads widely to susceptible children. In two institutions in Japan no infection was observed to be transmitted clinically or serologically from normal and handicapped vaccine recipients without a rash to susceptible children.^{5 6} Our investigation of varicella zoster virus DNA in throat swabs by the polymerase chain reaction demonstrated that positivity rate and quantity of detected viral DNA in normal vaccine recipients were considerably lower than those in normal children with varicella. These results may suggest that the Oka strain and the wild type strain of varicella zoster virus have different sites of replication in healthy children. If transmission of the virus occurred mainly by airborne route from the pharynx, our data might explain the low transmissibility of the virus from vaccine recipients.

Although the varicella zoster virus DNA was detected in the swab of a vaccinee before vaccination (case 11), we believe it was not contamination and non-specificity. The viral DNA was not amplified in negative controls, and the primers used were certified as having no homology with five other human herpesviruses (herpes simplex virus types 1 and 2, human herpesvirus-6, human cytomegalovirus, and Epstein-Barr virus).¹ We presume that the swab was taken just after unknown exposure to a wild strain and varicella was prevented by immediate inoculation of vaccine.

The polymerase chain reaction is very sensitive and is useful to investigate the pathology of varicella zoster virus infection. To justify our speculations, further studies using the method should be carried out with more samples taken. Studies should also be attempted on blood specimens obtained from immunocompromised and immunocompetent vaccinees.

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