Reactivation of chickenpox contracted in infancy

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Abstract

Varicella zoster virus DNA in mononuclear cells was studied by the polymerase chain reaction to obtain virological evidence of reactivation in the children who had contracted chickenpox in infancy. The results appear to explain why chickenpox in infancy is a risk factor for herpes zoster in immunocompetent children.

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Varicella zoster virus causes chickenpox as primary infection; it can later be reactivated, causing herpes zoster. Based on lymphoproliferative assay studies of immunocompromised patients^{1,2} and elderly subjects,³ the mechanism of this reactivation is thought to be a deficiency in specific cellular immunity. However, the causes of herpes zoster in immunocompetent children remain unclear.

Chickenpox in infancy is a risk factor for herpes zoster in healthy children.^{4,5} A low response of specific immunity after chickenpox in infancy has been reported, and thereafter subclinical reactivation in the form of significant changes in specific humoral and/or cellular immunity was observed.⁶

To obtain virological evidence for the viral reactivation described above, we studied the varicella zoster virus DNA, as determined by the polymerase chain reaction (PCR), in the peripheral blood mononuclear cells (PBMCs) of children who had contracted chickenpox in infancy and children with malignancy.

Subjects and methods

The subjects were 27 normal children who were studied within 72 hours of the onset of chickenpox, 11 children aged less than 12 months and 12 over 12 months who had blood taken five or more weeks after the onset of chickenpox, and 17 children on maintenance treatment for malignancy who either had had chickenpox in the past (n=16) or had been immunised with varicella vaccine (n=1). We confirmed the diagnosis and past history of chickenpox by varicella zoster virus specific IgG and/or IgM antibody with the enzyme linked immunosorbent assay.

PBMCs were separated from heparinised blood by Ficoll-Hypaque gradient centrifugation. DNA extraction was carried out from 2×10^6 cells of PBMCs using a SepaGene kit (Sanko Junyaku, Tokyo).⁷ PCR was done as described by Koropchak *et al*⁸ and Ozaki *et al*⁹ using oligonucleotide sequences from varicella

zoster virus gene 31. The sequences of the outer⁸ and inner primers were 5'-GAGGAAGTTGAAGCCAGATC-3' and 5'-CTTCCAGTTCCAACCAACCG-3', 5'-TCCAACCAACCGTTAAATGA-3' (57877-57897) and 5'-TTGAAGCCAGATCAATATTC-3' (57663-57683), respectively. After electrophoresis on a 2.5% agarose gel, a band of 235 base pairs was seen when samples contained varicella zoster virus DNA. We confirmed that some of the band can be hybridised with the inner probe by Southern blot.⁸

Results

The varicella zoster virus DNA sequences were detected in 21 (77.8%) of 27 children in the acute phase of chickenpox. Six of these had contracted chickenpox at <1 year old and all six were positive for the PCR. When blood samples were drawn at five weeks to 62.5 months after the onset, five (45%) of 11 children who had contracted chickenpox at <1 year old were positive on at least one sample for the PCR, whereas none of the 12 children who had contracted it at ≥ 1 year old were positive ($p < 0.02$, Fisher's test; table 1). The longest period between the onset of chickenpox and the time blood was drawn among the positive samples was 3 years and 10 months. Six (35%) of the 17 patients with malignancy during maintenance treatment were positive for the PCR (table 2). The healthy subjects with a positive PCR at least five weeks after the onset were not recognised as having herpes zoster or

Table 1 Results of the PCR for the varicella zoster virus DNA sequences in PBMCs from the children who had contracted chickenpox at five or more weeks after the onset

Subject No	Age the child contracted chickenpox	Time after the onset (weeks)	PCR
<1 Year old at onset of chickenpox (age in months)			
1	5	5	Negative
		25	Negative
2	4	6	Positive
		20	Negative
3	11	7	Negative
4	10	8	Negative
5	9	9	Positive
6	9	16	Negative
7	5	20	Positive
		24	Positive
8	7	30	Positive
		38	Negative
9	1	52	Negative
10	9	144	Negative
11	2	184	Positive
		188	Negative
≥ 1 Year old at onset of chickenpox (age in years)			
1	2-3	5	Negative
2	2-8	5	Negative
3	2-3	5	Negative
4	1-8	8	Negative
5	1-8	8	Negative
6	1-9	16	Negative
7	2-9	32	Negative
8	8-8	144	Negative
9	6-9	172	Negative
10	7-2	172	Negative
11	13-8	240	Negative
12	7-5	250	Negative

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Table 2 Results of the PCR for the varicella zoster virus DNA sequences in PBMCs from the patients with malignancy who had contracted chickenpox

Patient No*	Age/sex	Diagnosis†	Past history of chickenpox	PCR
1	14/M	NHL	3 Years	Negative
2	5/M	ALL	1 Year	Negative (after 10 weeks)
3	20/F	CML	Unknown	Negative
4	4/F	Neuroblastoma	Unknown	Positive
5	16/M	NHL	5 Years	Positive
6	2/F	Wilms' tumour	4 Months	Negative (after 24 weeks)
				Positive
				Positive (after 26 weeks)
				Negative (after treatment of herpes zoster)
7	14/F	ALL	3 Months	Positive
				Negative (after 26 weeks)
8	16/M	ALL	3 Years	Positive
9	13/F	AML	4 Years	Negative
10	8/F	ALL	Vaccination	Negative
11	14/F	Sarcoma	4 Years	Negative
12	6/M	APL	3 Years	Positive
				Positive (after 10 weeks)
				Negative (after treatment of herpes zoster)
13	16/M	Sarcoma	Unknown	Negative
14	6/M	ALL	1 Year	Negative
15	17/M	NHL	5 Years	Negative
16	9/M	MDS	3 Years	Negative
17	8/M	ALL	1 Year	Negative

*Patients Nos 3, 4, and 11 already had herpes zoster and No 9 had contracted a second form of chickenpox during maintenance therapy. Nos 6 and 8 developed clinical herpes zoster at 10 and 28 weeks, respectively, after the positive PCR. No 7 was treated with oral acyclovir for three weeks because of recurrent stomatitis.

†NHL, non-Hodgkin's lymphoma; ALL, acute lymphocytic leukaemia; CML, chronic myelocytic leukaemia; APL, acute promyelocytic leukaemia; MDS, myelodysplastic syndrome.

recurrent chickenpox during this observation period. However, two of the six patients with malignancy who had been positive for the PCR developed clinical herpes zoster at 10 and 28 weeks, respectively.

Discussion

The positive rate for the PCR of PBMCs in the acute phase of chickenpox in this study was 78% (21 of 27 children) within 72 hours after the onset as determined by the nested double PCR. This compares with rates of 67% (eight of 12 patients) within 24 hours after the onset as reported by Koropchak *et al.*,⁸ 69% (24 of 35 patients) within 24 hours by Sawyer *et al.*,¹⁰ and 100% (12 of 12 patients) within 72 hours by Ozaki *et al.*⁹

Patients with malignancy are at high risk for herpes zoster because they are immunocompromised. In this study, we detected varicella zoster virus DNA in PBMCs from six of the 17 patients with malignancy during maintenance treatment without clinical signs of herpes zoster and chickenpox. Wilson *et al.*¹¹ reported the detection of varicella zoster virus DNA in PBMCs from seven (19%) of 37 varicella zoster virus seropositive bone marrow transplant recipients by the same PCR method of Koropchak *et al.*⁸ and demonstrated virological evidence of subclinical reactivation of the virus, which had been previously suggested by significant changes in the antibody titres.¹ Devlin *et al.* reported the detection of viral

amplification products by the PCR from PBMCs in four (25%) of 16 normal elderly people of 60 years old or older and in a 39 year old woman without signs of herpes zoster.¹²

Ozaki *et al.* reported that detection of varicella zoster virus DNA in PBMCs decreased with time and was undetectable after 15 days from the onset.¹³ Our preliminary experiment (data not shown) showed that the latest detection was at day 14 and day 21 after chickenpox in the children who had contracted chickenpox at ≥ 1 year and at < 1 year of age, respectively. Therefore, we think that the positive PCR in the normal children at five or more weeks after the onset suggests subclinical reactivation of the virus rather than chronic infection in PBMCs after acute infection. This virological evidence of viral reactivation in children who had contracted chickenpox in the first year of life appears to explain why chickenpox in infancy is a risk factor for herpes zoster in normal children.

Informed consent was obtained from the parents or guardians of the children according to the guidelines of the United States Department of Health and Human Services and those of Kawasaki Medical School.

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