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Short communication

Persistence of varicella-zoster virus DNA in elderly patients with postherpetic neuralgia

Ravi Mahalingam[1], Mary Wellish[1], John Brucklier[1] and Donald H Gilden[1,2]

Departments of [1]Neurology and [2]Microbiology, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Box B182, Denver, Colorado 80262, USA

The most common complication of zoster in the elderly is postherpetic neuralgia, operationally defined as pain persisting longer than 1–2 months after rash. The cause of postherpetic neuralgia is unknown. Using polymerase chain reaction, we detected varicella zoster virus DNA in blood mononuclear cells from 11 of 51 postherpetic neuralgia patients, but not in any of 19 zoster patients without postherpetic neuralgia, or in any of 11 elderly individuals without a history of zoster. Blood mononuclear cells from nine of 27 serially-bled postherpetic neuralgia patients were positive for varicella zoster virus DNA; six were positive once, and three patients were positive more than once. Our results indicated that postherpetic neuralgia may be related to persistence of varicella zoster virus.

Keywords: VZV; postherpetic neuralgia; PCR

Varicella zoster virus causes chickenpox (varicella), becomes latent in dorsal root ganglia, and reactivates decades later, usually after age 60, to produce shingles (zoster). Zoster is characterized by pain and a vesicular rash on an erythematous base limited to one – three dermatomes. Pain usually lasts 1–2 months. Pain that persists longer is operationally defined as postherpetic neuralgia (PHN). Although the mechanism of PHN is unknown, VZV-specific proteins and DNA have been detected in peripheral blood mononuclear cells (MNCs) of patients with PHN 1 – 4 years after acute zoster, but not in zoster patients without PHN (Vafai *et al*, 1988). In contrast, VZV DNA is found in uncomplicated zoster patients for 3 – 7 weeks after rash, but not later, coinciding with the period of pain (Gilden *et al*, 1988). Together, these data suggest that persistence of VZV is associated with PHN. To determine whether prolonged pain might be related to VZV persistence, we prospectively analyzed blood MNCs from zoster patients with or without PHN, and from elderly individuals with no history of zoster for the presence of VZV DNA.

We studied 81 subjects (44 women and 37 men) > 60 years old; 51 had PHN for 1 to 20 years, 19 had a history of zoster without PHN, and 11 had no history of zoster (Table 1). Of the 51 PHN patients, serial blood samples were obtained from 27 patients over a period of a few weeks to 4 years and analyzed by PCR for VZV DNA. Serum was stored at –20°C. Since heparin inhibits PCR amplification (Holodniy *et al*, 1991), blood was also collected in tubes containing EDTA. Blood was resuspended in Hanks' balanced salt solution and centrifuged in a Ficoll-Hypaque gradient. Mononuclear cells (5×10^6 cells per ml) were isolated from blood as described (Gilden *et al.*, 1987) except that proteinase K was used at 100 mg per ml. The final concentration of DNA was adjusted to be 10^7 cell equivalents per 100 µl.

The propagation of VZV in African green monkey kidney (BSC-1) cells, extraction of DNA (Gilden *et al*, 1982, 1987) and determination of antibody to VZV by enzyme immunoassay (Forghani *et al*, 1984) have been described. Oligonucleotide primers (Operon Technologies, Alameda, CA) were selected from the sequence of VZV (Davison and Scott, 1986). The VZV UL40 primer 1 (5'-TCACACA-CAATCGGATGTTGC-3') is located between nucleotides 75280 and 75300, primer 2 (5'-ATCGCTTGAGCATAGTGGTGG-3') between nucleotides 75593 and 75613, and the UL40 internal oligonucleotide (5'-ATACGGTGACAGGCTATA-CAACGGAA-3') between nucleotides 75349 and 75374.

Polymerase chain reaction (PCR) and analysis of products have been described (Mahalingam *et al*, 1990). Our minor modifications included hot-start PCR as described by Chou *et al* (1992). The

Correspondence: R Mahalingam
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researcher performing the PCR was blinded to the samples. In a typical reaction, 1–5 μg (10^5 – 10^6 cell equivalents) of blood MNC DNA was used as the template in a 100μl reaction mixture. The optimum concentration of $MgCl_2$ was 0.015 M, although previously we had used 0.05 M $MgCl_2$ with VZV gene 40 primers (Gilden *et al*, 1992). The total number of cycles was 35–40. Synthetic oligonucleotides representing a region internal to the amplified fragment were end-labeled with [γ-^{32}P]adenosine triphosphate (7000 Ci per mM; ICN, Costa Mesa, CA) as described (Mahalingam *et al*, 1990; Sambrook *et al*, 1987). Five μl of a total 100 μl volume of the PCR mixture containing VZV-infected cell DNA and 12 μl from the blood MNC DNAs were used for analysis by Southern blot hybridization (Mahalingam *et al*, 1990). All 81 subjects were seropositive for VZV. DNA from MNCs was analyzed by PCR for the presence of VZV gene 40. Positive and negative controls were included in each analysis.

VZV gene 40-specific sequences were detected at least once in 11 of 51 (21%) zoster patients with PHN, but not in any of 19 elderly zoster patients without PHN, or in any of 11 elderly subjects without a history of zoster (Table 1, Figure 1). The proportion of PHN patients positive for VZV DNA differed significantly between the three groups (χ^2 test for a 2 × 3 contingency table = 7.09 with 2 degrees of freedom, $P < 0.01$). The significance was enhanced when the two control groups were combined into a 2 × 2 contingency table and compared to PHN patients ($P = 0.004$, Fisher's exact test). The levels of statistical significance leave little doubt that the difference between PHN patients and the control groups is not random.

These data are consistent with our previous detection of VZV DNA in MNCs of PHN patients months to years after skin rash (Vafai *et al*, 1988; Devlin *et al*, 1992), and strongly suggest that PHN is related to virus persistence. In one of our studies (Devlin *et al*, 1992), we also found VZV DNA in MNCs of four adults > 60 years old who did not have a history of zoster. Of the four elderly controls

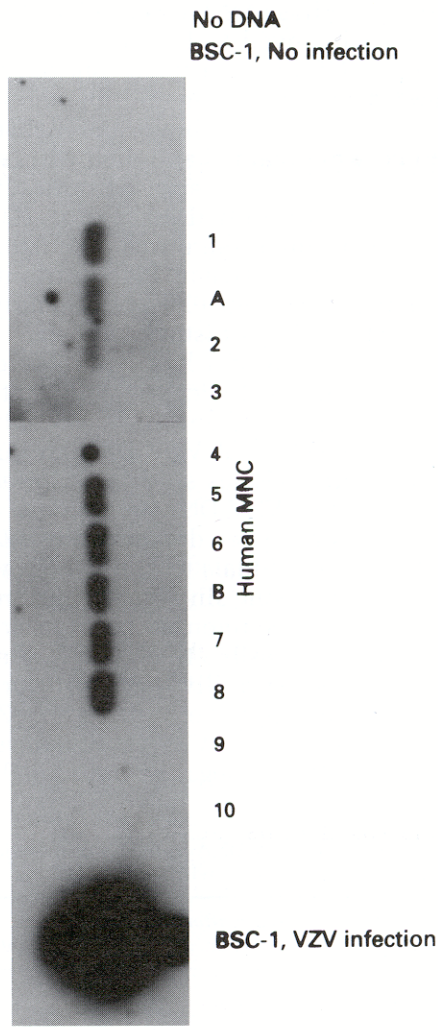

Figure 1 Detection of VZV gene 40 DNA by PCR in MNCs from elderly subjects with PHN. Total DNA was extracted from uninfected BSC-1 cells (BSC-1, no infection), MNCs from two individuals with acute zoster (A and B), 10 individuals postherpetic neuralgia (1-10), and VZV-infected BSC-1 cells (BSC-1, VZV infection) and used in PCR. As a control, DNA was omitted from one reaction tube (no DNA). VZV gene 40 sequences in the products (250 bp) were detected using a synthetic oligonucleotide probe.

Table 1 Presence of VZV DNA in MNCs from elderley zoster patients with or without PHN, and from elderly individuals with no history of zoster

Clinical features	*Age range (years)*	*Number of positives*[a]	*Number of subjects*
Zoster with PHN	60-85	11 (4 men; 7 women)	51 (22 men; 29 women)
Zoster without PHN	60-79	0	19 (9 men; 10 women)
Elderly without zoster	61-75	0	11 (6 men; 5 women)

[a] determined by PCR

Blood samples were obtained from all of the subjects in the three study groups, MNCs isolated, DNA prepared and analyzed by PCR for the presence of VZV gene 40 sequences as shown in Figure 1. PCR analysis was performed more than once on all samples. A sample was scored positive if VZV DNA was detected at least once by PCR.

in that study whose MNCs were VZV DNA-positive, one subject believed to be a control actually had acute zoster. Of the other three control subjects, two were gravely ill and taking multiple medications for cardiovascular and cerebrovascular disease at the time blood was obtained and died within 4 months; only one subject was otherwise healthy. The sample size in our earlier study (Devlin *et al*, 1992) was too small to determine if there was a statistically significant difference between PHN patients, elderly zoster patients without PHN, and elderly seropositive subjects with no history of zoster. This led to our analysis herein of a larger number of subjects, which revealed a statistically significant difference between PHN patients and the two control groups. Unlike the detection of VZV DNA in three control subjects in our previous study (Devlin *et al*, 1992), we did not detect VZV DNA in MNCs of controls in this study. Our earlier detection of VZV DNA in three elderly individuals (two of whom were gravely ill) is not surprising since subclinical reactivation of VZV is well-documented (Gilden *et al*, 1992), and it is always possible that MNCs from a random elderly seropositive individual, with or without a history of zoster, might be positive for VZV DNA by PCR. In our experience, VZV DNA is never found in MNCs from VZV-seronegative individuals (data not shown).

Table 2 Presence of VZV DNA in MNCs from serial blood samples of elderly zoster patients with PHN

Subject	*Age/sex*	*Years after zoster*	*Number of positives*	*Number of blood samples*
1	62F	4	5	17
2	65M	3	1	13
3	66F	5	1	17
4	72M	8	1	11
5	79F	7	3	13
6	66M	3	2	6
7	65F	2	1	2
8	79F	4	1	4
9	74F	1	1	3
10	73F	20	0	3
11	76M	3	0	3
12	85F	2	0	2
13	71F	2	0	3
14	77M	2	0	4
15	63F	2	0	3
16	77M	6	0	3
17	78M	4	0	4
18	79F	7	0	3
19	71M	3	0	2
20	62M	2	0	2
21	66F	10	0	2
22	75F	6	0	2
23	69F	5	0	2
24	83F	5	0	2
25	75M	4	0	6
26	67F	3	0	4
27	68M	5	0	2

Serial blood samples were obtained from individual patients over a period of a few weeks to 4 years and analyzed as described in Figure 1 and Table 1. A single blood sample was obtained at each draw.

Table 3 Presence of VZV DNA in MNCs from serial blood samples of elderly zoster patients without PHN

Subject	*Age/sex*	*Years after zoster*	*Number of positives*	*Number of blood samples*
1	79F	3	0	5
2	78F	4	0	4
3	67M	3	0	4
4	77F	4	0	4
5	67F	15	0	4
6	78M	1	0	3
7	60F	5	0	2
8	62M	7	0	2
9	72M	31	0	4
10	71M	2	0	2

Serial blood samples were obtained from individual zoster patients without PHN over a period of 2 weeks to 1 year and analyzed as described in Figure 1 and Table 1. A single blood sample was obtained at each draw.

Of the 51 PHN patients, 27 were accessible for serial analyses. VZV gene 40 DNA sequences were found in nine PHN patients 1 to 8 years after zoster (Table 2): six were positive once, and three were positive more than once. Of the three PHN patients who were positive more than once, one 62-year-old woman was positive on five of 17 occasions, one 79-year-old woman was positive on three of 13 occasions, and one 66-year-old man was positive on two of six occasions (Table 2). Analysis of serial blood samples at equivalent times after zoster from 10 elderly zoster patients without PHN, ranging in age from 60 to 79 years, who had zoster 1 – 31 years ago, did not reveal VZV gene 40 DNA (Table 3).

Because blood samples were obtained at equivalent times after zoster in patients with and without PHN, it is unlikely that virus DNA in MNCs of PHN patients is due to fragments of the VZV genome which might have been engulfed weeks or months before the onset of PHN. Although our current search was limited to VZV gene 40 DNA, we are currently looking for other regions of the VZV genome. We are also estimating the number of copies of virus DNA in VZV-positive MNC samples; our preliminary reconstitution experiments indicated that up to 10 copies of VZV DNA can be detected in 1 μg of human MNC DNA (data not shown).

While the cause of PHN is unknown, the possible presence of VZV in ganglia at a level greater than that found during latency, together with two microscopic analyses which revealed inflammatory infiltrates of mononuclear cells, often around dying neurons, in ganglia from PHN patients 1–2 years after acute zoster (Smith 1978; Watson *et al*, 1991), may account for persistent pain. It is not possible to examine ganglia during life from PHN patients for the presence of VZV or inflammation, but our detection of VZV in MNCs of PHN patients, but not in those of control zoster patients without PHN, provides indirect evidence that VZV may be present in

ganglia of PHN patients at a greater level than during latency. It is possible that MNCs trafficking through such ganglia encounter and engulf virus whose DNA is then amplified by PCR. We are arranging to analyze ganglia obtained at autopsy from individuals who suffered from PHN at the time of death. If a greater virus burden can be demonstrated in these ganglia than has been found during latency (Mahalingam *et al*, 1993), this would provide a rationale for aggressive treatment of PHN patients with antivirals. In addition, the existence of ganglionitis without rash is further supported by the presence of radicular pain up to 100 days preceding zoster (Gilden *et al*, 1991), so-called preherpetic neuralgia.

Finally, none of our PHN patients' MNCs was positive for VZV DNA every time, and even in the three PHN subjects whose MNCs were positive more than once, the same three subjects' MNCs were negative at other times. The presence of VZV DNA in some PHN patients' MNCs some, but not all, of the time may reflect the chance occurrence of MNCs encountering virus in ganglia and blood sampling at that same time. Support for such a hypothesis would come from finding VZV DNA in MNCs of PHN patients restricted to macrophages. Alternatively, VZV DNA in MNCs of PHN patients may represent a primary interaction of the virus with B or T cells, as with Epstein-Barr virus and B cells. Identification of the MNC type(s) that contains VZV DNA in PHN patients should distinguish between these two possibilities.

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