Analysis of Varicella-Zoster Virus DNAs of Clinical Isolates by Endonuclease HpaI

By YASUHIKO HAYAKAWA, TOSHIKO YAMAMOTO, KOICHI YAMANISHI AND MICHIAKI TAKAHASHI*
Department of Virology, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka, Japan

(Accepted 6 June 1986)

SUMMARY
The DNAs of 20 strains of varicella-zoster virus (VZV) isolated from epidemiologically unrelated individuals, and of 15 strains isolated from vesicles of vaccinees with varicella or zoster after vaccination, were compared by restriction enzyme cleavage using HpaI. Differences were found in the sizes of the HpaI-F, -G and -K fragments of the wild strains. The gel migration patterns of the HpaI-F and -G fragments, but not of the HpaI-K fragment, were polymorphic in the different strains isolated from the vaccinees. The effects of serial passages in vitro and in humans on the genome stability of VZV were investigated by HpaI analysis. The DNA profiles of the HpaI-K fragments from six isolates recovered from room-mates infected in a single outbreak were identical, but the mobilities of their HpaI-F and -G fragments varied. The DNA profiles of the Oka vaccine virus after 10 and 85 passages in human embryo cells differed only in the HpaI-F fragment. The profiles of these fragments in DNA derived from two isolates obtained at different times from a vaccinee with varicella followed by zoster were compared with those of the Oka (parental) and Oka (vaccine) strains, and identical results were obtained for the two viruses. In addition, the same DNA profiles of HpaI fragments were obtained from three sequential isolates from one person and also from two isolates from another with varicella and zoster. Thus, it was concluded that: (i) three variable fragments (HpaI-K, -F and -G) were not changed in the DNAs of isolates derived from the same patient; (ii) HpaI-K was stable both on passage in vitro and after human transmission in the case of the same outbreak, but was different among all wild-type strains isolated in epidemiologically unrelated outbreaks; (iii) HpaI-F was very unstable both on passage in vitro and in human infections by either vaccine or wild-type strains; (iv) HpaI-G was not influenced by passage in vitro but varied among wild-type strains. Using physical maps of VZV DNA established by others, three variable regions on the viral genome were identified. One was located near the 0.16 coordinate, which is covered by HpaI-K (variable region I, VRI). Another was represented by HpaI-F (VRII), the most unstable fragment, and mapped at about the 0.35 coordinate. The third was VRIII near the right terminus, covered by HpaI-G.

INTRODUCTION
Varicella-zoster virus (VZV) is a human herpesvirus that causes varicella in children on primary infection, and zoster, which is attributed to reactivation of latent virus (Takahashi, 1983). In recent years, herpesvirus DNAs have been analysed by digestion with restriction enzymes, and differences in DNA sequences have been found between different isolates. This technique is now used in molecular epidemiological studies of herpesviruses (Roizman & Tognon, 1983; Hayward et al., 1975; Locker & Frenkel, 1979; Whalley et al., 1981; Lewis et al., 1984; Ambinder et al., 1985).

Several groups have attempted to distinguish different strains of VZV by digestion of their DNAs with restriction enzymes and examination of the fragment patterns after separation by agarose gel electrophoresis (Ecker & Hymar, 1981; Martin et al., 1982; Straus et al., 1983). A live
Table 1. *VZV* strains used in this study

<table>
<thead>
<tr>
<th>Type</th>
<th>Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild varicella or zoster</td>
<td>Tsuchiyama, Inoue, Watanabe, Kawaguchi, Ellen</td>
</tr>
<tr>
<td>Zoster</td>
<td>Morita, Kato, Takenaka</td>
</tr>
<tr>
<td>Varicella in vaccinees</td>
<td>TAK, ABE, OSA, FMO, KAK, KIT, MAT, WAD, KAJ, FUR</td>
</tr>
<tr>
<td>Zoster: OYA</td>
<td></td>
</tr>
<tr>
<td>Varicella in room-mates in an outbreak</td>
<td>MOT, MOI, MUR, ICH, HIR, SEK</td>
</tr>
<tr>
<td>Zoster: ICI(1), ICI(2), ICI(3)</td>
<td></td>
</tr>
<tr>
<td>Varicella and then zoster in the same patients</td>
<td>ICI(1), ICI(2), ICI(3)</td>
</tr>
<tr>
<td>Zoster: ICI(2), ICI(3), MOR(2)</td>
<td></td>
</tr>
<tr>
<td>Varicella and then zoster in a vaccinee</td>
<td>MEV, MEZ</td>
</tr>
<tr>
<td>Zoster: MEV, MEZ</td>
<td></td>
</tr>
<tr>
<td>Isolates from vesicle or blood of a vaccinee</td>
<td>TAJ(V), TAJ(L)</td>
</tr>
</tbody>
</table>

Varicella vaccine (Oka strain) has been developed recently, and genomic differences between it and wild-type strains have been identified by this method (Ecker & Hyman, 1981; Martin *et al*., 1982; Hayakawa *et al*., 1984). The profiles of DNA fragments obtained after *HpaI* digestion may be useful for distinguishing the vaccine virus from wild strains (Hayakawa *et al*., 1984).

In this paper, we report polymorphism of *VZV* genome DNA in isolates from epidemiologically unrelated individuals and from vaccinees, and also a comparison of the profiles obtained by *HpaI* digestion of DNAs of viruses passaged *in vitro* and in humans. The locations of variable regions in the DNA sequence are also presented.

**METHODS**

**Cells.** Human embryonic lung (HEL) cells were grown and maintained in a mixture of equal volumes of Eagle's MEM and Medium 199. This medium was supplemented with 10% and 3% calf serum for growth and maintenance, respectively. Cells at between the seventh and 12th passages were used for the experiments.

**Viruses.** All the strains used in this study are listed in Table 1. Four strains of *VZV* (Tsuchiyama, Inoue, Watanabe and Kawaguchi) isolated from vesicles of patients with varicella and three strains (Morita, Kato and Takenaka) isolated from vesicles of patients with zoster were used as wild-type strains. The Ellen strain, originally isolated in the U.S.A., was obtained from Dr H. Kamiya (Mie University, Tsu City, Japan). Viruses passaged two to 12 times in HEL cells were used in experiments. Thirteen strains (MEV, MEZ, TAK, ABE, OSA, FMO, KAK, OYA, KIT, MAT, WAD, KAJ and FUR) were isolated from vesicles that developed in children early (15 to 27 days) or late (4 to 12 months) after vaccination with the Oka strain of *VZV* and tested after two to seven passages in HEL cells. The FUR and KIT strains were isolated from patients who developed varicella after vaccination, and were determined to be wild-type strains by *HpaI* analysis. The MOT, MOI, MUR, ICH, HIR and SEK strains were isolated from patients who were in the same room of a hospital and suffered varicella sequentially within 1 month. The ICI(1), ICI(2) and ICI(3) strains were isolated from a child who had suffered from varicella and then zoster. The times between isolation of ICI(1) and ICI(2), and of ICI(2) and ICI(3) were 8 months and 3 days, respectively. The MOR(1) strain was isolated from a leukaemic patient with varicella and the MOR(2) strain was isolated 1 month later from a vesicle of the same patient who had developed zoster. The TAJ(V) and TAJ(L) strains were isolated at the same time from a vesicle and the blood respectively, of a vaccinee. Strains MEV, MEZ, KAK, OYA and KAJ were obtained from Dr K. Baba (Osaka University), strains KIT, ICI, MOR, MOT, MUR, HIR, SEK, FMO, FUR, MAT and WAD from Dr H. Kamiya, and strains TAK and ABE from Dr N. Katsushima (Yamagata City Hospital).

The Oka vaccine strain (passaged 11 times in HEL cells at 34 °C, 12 times in guinea-pig embryo fibroblast cells at 37 °C, and seven to nine times in human diploid cells at 37 °C) was also used. The Oka (parental) strain was isolated from a patient with varicella and used as the parent strain of Oka vaccine. None of the viruses was passaged in HEL cells more than 20 times except the Kawaguchi strain, which was passaged about 40 times.

**Purification of viral DNA.** *HpaI* restriction endonuclease digestion and electrophoresis in agarose gel. Viral DNA purification, *HpaI* digestion and agarose gel electrophoresis have been described previously (Hayakawa *et al*., 1984). The following DNAs were used as molecular weight markers: λ CI857 S7, 48 kilobases (kb); *EcoRI*-digested...
pSC101, 9 kb; EcoRI-digested pBR322, 4.3 kb; a triple digestion of pBR322 (PstI, PstI and BamHI), 2.3, 1.42 and 0.65 kb; HindIII-digested λ CI857 S7 DNA, 23, 9.1, 6.5, 4.3, 2.3 and 2.0 kb.

Exonuclease III digestion. Three μg VZV (Oka vaccine) DNA was suspended in 10 μl reaction mixture (50 mM-Tris–HCl pH 8.0, 0.5 mM-MgCl2) and digested by 0.5 units exonuclease III (Bethesda Research Laboratories) at 37 °C for 30 min. After phenol extraction and ethanol precipitation, DNA was resuspended in high salt reaction buffer and digested with HpaI.

Nick translation of DNA. The preparation of probe DNA was nick-translated using Amersham’s nick translation kit and labelled with [32P]dCTP (4000 Ci/mmol). After incubation for 1 h at 15 °C, labelled DNA was separated on Sephadex G-50 in TEN buffer (10 mM-Tris–HCl pH 7.6, 1 mM-EDTA, 20 mM-NaCl). The DNA was precipitated by addition of 200 μl 3.3 M-ammonium acetate, 20 mM-EDTA, 440 μl 95% ethanol and then placed in a solid CO2-ethanol bath. The mixtures were centrifuged for 4 min and the precipitates were washed twice with 70% ethanol-0.5 ra-ammonium acetate and once with 95% ethanol. The DNA probe was heated at 100 °C for 5 min and then cooled in ice before use.

Blotting and hybridization. DNAs were treated with appropriate enzymes, and the digests were fractionated on 0.5% agarose gels and transferred to nitrocellulose by the method of Southern (1975). Hybridization was carried out under stringent conditions using 6 x SSC at 65 °C for 24 h, and sheets were washed under stringent conditions at 65 °C for 3 h with 1 x SSC. Blots were examined after autoradiography.

**RESULTS**

**HpaI cleavage profiles of wild-type and vaccine-derived strains**

*HpaI* has been reported to be useful for distinguishing between viruses isolated from vaccinees and wild-type viruses (Hayakawa et al., 1984). The fragments obtained by *HpaI* digestion of the DNAs of the Oka vaccine strain, 11 wild-type strains and strains isolated from vesicles of 11 vaccinees with varicella or zoster after vaccination, were analysed to determine the degree of variation in molecular weight and stability of each fragment. The profiles of *HpaI*-F and -G digests differed between wild-type viruses and viruses derived from vaccinees. The size of the *HpaI*-K fragment differed among different wild strains, but the *HpaI*-K fragments of strains derived from different vaccinees showed the same mobility (Fig. 1). In various strains, the range of variation in molecular weight of *HpaI*-F was about 300 base pairs and that of *HpaI*-K was 150 base pairs. The range of variation of *HpaI*-G in the various strains was within 50 base pairs.

**Comparison of HpaI-F, -G and -K fragments of isolates from vesicles of room-mates in a hospital within 1 month**

There was an epidemic of varicella among leukaemic children in a hospital, and six children had chickenpox within 1 month. The virus was isolated from each patient and its DNA was analysed. Patients MOT and MOI were cases of primary infection, followed by patients MUR and ICH 2 weeks later (cases of secondary infection). Two or 3 weeks later, tertiary infection occurred in patients HIR and SEK. The cleavage patterns of these six strains are shown in Fig. 2. There was some variation in the density and distance between the *HpaI*-F and -G fragments among the primary group (MOT, MOI), the secondary group (MUR, ICH) and the tertiary group (HIR, SEK), but no differences could be detected in the *HpaI*-K fragment.

**Stability of HpaI fragments during serial passages in vitro**

Next, vaccine virus was serially passaged up to 85 times in HEL cells, the DNAs of viruses at different passage levels were digested with HpaI, and the profiles of the digests in agarose gels were compared. The profile of the *HpaI*-F fragment of the Oka (vaccine) virus was rather indistinct at the 2nd and 10th passage, and this became pronounced by the 85th passage (Fig. 3). However, the molecular size of the *HpaI*-K fragment in viruses at these three passage levels was the same. These results indicate that during passage of the virus in vitro, the molecular size of the *HpaI*-F fragment became heterogeneous, but that of the *HpaI*-K fragment was stable.

**Comparison of DNAs of viruses isolated from the individual patients with varicella and then zoster, and of viruses isolated from a vesicle and the blood of the same patient**

A leukaemic patient inoculated with the Oka vaccine strain developed a few vesicles in the weeks after vaccination. The patient suffered from zoster 1 year later. Virus was isolated from
Fig. 1. *HpaI* cleavage analysis of 11 wild-type strains, a vaccine strain and 11 isolates from vaccinees. The DNAs of the various VZV isolates were digested with *HpaI*, the fragments were separated by agarose gel electrophoresis and stained with ethidium bromide. Partial cleavage of the DNAs of the Oka (parental), Watanabe and Kawaguchi strains was detected. DNA fragments are named alphabetically in order of molecular weight. White arrows indicate the bands of fragments *HpaI*-F, -G and -K, which showed polymorphism between different strains. The left lane shows the positions of the DNA mol. wt. markers (described in Methods) separated on the same 0.5% gel. w, Wild-type; o, Oka vaccine-derived strain; v, varicella; z, zoster. Since the figure is composed of photographs from separate gels, relative distances cannot be compared, but the results clearly show the heterogeneity of fragments F, G and K.
vesicles of the patient when he had varicella (MEV) followed by zoster (MEZ). The DNAs of the Oka (parent) and the Oka (vaccine) strains and the two isolates (MEV and MEZ) were prepared and digested with HpaI. As shown in Fig. 4, there was no detectable difference in the DNA profiles of these viral isolates. Thus, the viruses isolated from the vesicles during varicella and zoster may have been derived from the same original virus, that is the vaccine strain. Next, five strains isolated from two persons on different occasions were examined by HpaI digestion. The MOR(1) and MOR(2) strains were isolated from one patient, and the ICI(1), ICI(2) and ICI(3) strains were isolated from another patient (see Methods). Both developed varicella and then zoster. Fig. 5(a) shows that the HpaI-G, -F and -K fragments of DNAs of viruses from individual patients were apparently identical, and that no detectable change occurred in such isolates. The DNAs of two isolates [TAJ(V) and TAJ(L)] obtained at the same time from the vesicles and the blood of one vaccinee were analysed electrophoretically after HpaI digestion, but there was no detectable difference in the gel profiles of the DNAs from these isolates (Fig. 5b).

**Mapping and some properties of variable regions (HpaI-F, -G and -K)**

The above results indicate that there are at least three variable regions detectable by HpaI digestion of the VZV genome. We examined the locations of these regions in VZV DNA.
Fig. 3. HpaI analysis of the Oka vaccine strain passaged in HEL cells in vitro. The arrowheads indicate the positions of the HpaI fragments F and K of viruses passaged 2, 10 and 85 times. The band of HpaI-F becomes more diffuse as passage times increase.

Fig. 4. Comparison of the DNAs of isolates from a vaccinee, who suffered from varicella and then zoster 1 year later. DNAs were digested with HpaI and subjected to agarose gel electrophoresis. Conditions for enzyme digestion are described in Methods. Lane 1, Oka parent; lane 2, Oka vaccine; lane 3, MEV; lane 4, MEZ. Digestion with HpaI was incomplete in lanes 1 and 2.

The HpaI-K fragment was isolated from an agarose gel, labelled and used as a hybridization probe with filters carrying VZV DNA fragments produced by digestion with BamHI, EcoRI, XbaI, BanIII, BglII, HpaI, KpnI or SalI. The hybridization profiles (Fig. 6a) showed that the probe hybridized strongly with BamHI-F, EcoRI-M, -P, -Q and -H, XbaI-H and -E, KpnI-A, and
Fig. 5. Comparison of *Hpa*I cleavage of DNAs of VZV isolated from lesions of two related individuals who contracted varicella in the same outbreak (a) and simultaneous isolates from a lesion and the blood of one patient (b). The history of infection is described in Methods.

*Sal*I-D and -C. It also recognized a few other bands weakly. This probe might also have contained other fragments such as *Hpa*I-J, since it was difficult to isolate one fragment completely by this method. The strong bands may therefore have hybridized with *Hpa*I-K and the weak bands with *Hpa*I-J. This speculation was proved true by another hybridization experiment. When the *Bam*HI-L fragment of VZV, which maps at about coordinate 0·6 of the VZV genome (see Fig. 8), was cloned into pBR322 and used as a DNA probe, *Hpa*I-J hybridized strongly with it (Fig. 6b). Moreover, the cloned *Eco*RI-P fragment, which is located at about 0·15, hybridized to *Bam*HI-F, *Bgl*I-B, *Eco*RI-D, *Hind*II-A, *Kpn*I-A, *Sst*I-A, *Xba*I-E, *Pst*I-C and *Hpa*I-K (Fig. 6c). From these observations, *Hpa*I-K was presumed to be located at about the 0·16 coordinate.
VZV genome variable regions

Fig. 6. Autoradiographs of blot hybridizations showing VZV DNA fragments after digestion with several restriction enzymes and their hybridization with the purified HpaI-K fragment and several other VZV fragments cloned in pBR322. DNA was digested with BamHI (Ba), BglII (Bg), BglII (Bl), EcoRI (Ec), HindIII (Hi), KpnI (Kp), SstI (Ss), XbaI (Xb), PstI (Ps), SalI (Sa), BamHI (Bn) or HpaI (Hp), and subjected to electrophoresis on 0.5% agarose gel and stained with ethidium bromide. After the photograph was taken, the gel was blotted onto a nitrocellulose filter. Hybridization conditions are described in Methods. (a) Hybridization analysis to determine the location of the HpaI-K fragment. HpaI-K DNA recovered from an agarose gel was used as the probe. This DNA was slightly contaminated with HpaI-J. (b) Hybridization profile to determine the location of the BamHI-L fragment. Cloned BamHI-L was used as the probe. (c) Hybridization with EcoRI-P as probe to determine the HpaI-K locus. (d) Hybridization with EcoRI-O as probe to determine the HpaI-F locus. (e) BamHI-J probe hybridized with HpaI-G and other fragments. The left lanes of (a), (b), (d) and (e) contain DNA mol. wt. markers; HindIII-digested λ phage DNA was used (from top to bottom, 23, 9-1, 6-5, 4-3, 2-3 and 2-0 kb). The abbreviated names of restriction enzymes at the top of the figure (left to right) correspond to the order of lanes in each of the figures of the ethidium bromide staining (left part) and hybridization (right part).

Second, HpaI-F was mapped using a cloned EcoRI-O fragment as probe. EcoRI-O hybridized with HpaI-F (Fig. 6d). This probe also hybridized with BamHI-I, BglII-K and -S, HindIII-A and -E, KpnI-A and -E, SstI-A, XbaI-K and -N, and PstI-B and -E. From these results, HpaI-F was presumed to be located near the 0-3 coordinate of the VZV map, and that part of the fragment covered EcoRI-O.

Finally, HpaI-G was mapped using a probe of cloned BamHI-J, which is located in the large inverted repeated sequence. The BamHI-J probe hybridized not only to HpaI-G but also to HpaI-A, -I and -M (Fig. 6e). Furthermore, this probe strongly hybridized with XbaI-A, -B, -D and -I, PstI-A, SalI-K, -P, -Q and -S, HindIII-C and -M, and SstI-B and -F. In order to determine which inverted repeated sequence contained the HpaI-G fragment, exonuclease III was used to destroy the terminal fragments of VZV that were digested by HpaI. Fig. 7 shows that the terminal fragments were HpaI-G and HpaI-T. From this result, HpaI-G was assumed to be located at coordinates 0-94 to 1-00, in the terminal repeated sequence (TRs) (Fig. 8).

DISCUSSION

Several groups have attempted to distinguish different strains of herpesviruses, and have shown that isolates from epidemiologically unrelated patients can be distinguished by digestion
Fig. 7. Exonuclease III digestion to determine terminal \textit{HpaI} fragments of the VZV genome. DNA of the Oka vaccine strain was treated with exonuclease III to digest DNA 3' termini, and then digested with \textit{HpaI}. DNAs treated (b) and untreated (a) with exonuclease III were electrophoretically separated through a 0.6% agarose gel, and profiles were compared under u.v. light. The arrows indicate the \textit{HpaI} fragments digested by exonuclease III.

of virus DNAs with restriction enzymes and separation of the digests on agarose gel (Roizman \& Tognon, 1983; Richards \textit{et al.}, 1979; Zweerink \textit{et al.}, 1981; Straus \textit{et al.}, 1983; Martin \textit{et al.}, 1982). We have analysed the DNAs of clinical isolates from patients with varicella or zoster using ten restriction enzymes (\textit{HpaI}, \textit{BgII}, \textit{KpnI}, \textit{SalI}, \textit{EcoRI}, \textit{BamHI}, \textit{HindIII}, \textit{PvuII}, \textit{PstI} and \textit{XhoI}). Four of these enzymes were found to reveal small but detectable differences in the migration patterns of the fragments (Hayakawa \textit{et al.}, 1984). \textit{HpaI} was found to be particularly useful, since the F, G and K fragments of DNAs from different strains showed slightly different rates of migration (Fig. 1). This enzyme was therefore used for analysis of further clinical isolates.

All of the epidemiologically unrelated VZV strains reported here (30 patients) were found to be different by \textit{HpaI} analysis except for isolates from the same patient. The size of \textit{HpaI}-F was altered by passage \textit{in vitro} 85 times but not after 10 passages.

Six sequential isolates from patients in an outbreak in one room in a hospital had a \textit{HpaI}-K fragment with the same mobility, but distinct \textit{HpaI}-G and -F fragments, as judged by the density and mobility of bands (Fig. 2). There have been few reports on the isolation of viruses and comparison of DNA profiles on agarose gel after serial VZV infections. In the present study, it was clearly shown that during consecutive infections of three humans, \textit{HpaI}-K was not changed, but \textit{HpaI}-F and -G slightly changed, which suggests that no deletion, addition or point mutations had occurred at \textit{HpaI} cleavage sites themselves, but rather that small (about 50 to 150 base pairs) deletions and additions of DNA sequences occurred in some region within the \textit{HpaI}-F and \textit{HpaI}-G fragments.

Multiple VZV isolates recovered from the same patient gave identical cleavage profiles with the restriction enzyme, even in \textit{HpaI}-F, as shown in Fig. 4 and 5. Thus, there was no detectable genomic variation during the 1-year latent infection [MEV and MEZ, ICI(1), ICI(2) and ICI(3),
Fig. 8. Physical maps of the VZV genome showing the locations of the variable regions. The physical maps of BamHI, BglII, EcoRI, HindIII and PstI sites were determined by Casey et al. (1985). Ecker et al. (1984) reported EcoRI, HindIII and KpnI sites. Mishra et al. (1984) also mapped HindIII, SalI, XbaI, BamHI, BglII, BglII, EcoRI, HpaI, PstI, SmaI and XhoI sites; Davison & Scott (1983) determined the Bgl, XbaI, PstI, SstI, KpnI, XhoI, PvuII, EcoRI and SalI maps. The positions of the three HpaI fragments was not exactly determined in our experiments but their maximum extents are indicated by dotted lines and their minimum extents from hybridization experiments are shown as solid boxes. At the top of the figure, open arrows indicate the inverted repeated sequences, L and S indicate long segment and short segment respectively, Us and UL denote unique short region and unique long region respectively. M.u., Map units.

MOR(1) and MOR(2)], and no variation was observed in the isolates obtained at the same time from different vesicles or different tissues [ICI(2) and ICI(3), TAJ(V) and TAJ(L)]. Similar results were reported by Straus et al. (1984). These results showed that all the VZV DNA fragments obtained with restriction enzymes were stable during a single infection in vivo. This stability of the DNA from isolates from one patient may be due to a lower frequency of DNA replication, as compared with multiple infections through several persons where repeated virus replication may cause increased DNA rearrangements.

Straus et al. (1983) demonstrated four typically variable regions in the VZV genome. They mapped two of these variable regions detected from the gel profiles within the unique long
Table 2. Variable regions, biological markers and variability between VZV isolates

<table>
<thead>
<tr>
<th>Variable region</th>
<th>VRI</th>
<th>VRII</th>
<th>VRIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Map position</td>
<td>0.16</td>
<td>0.28-0.36</td>
<td>0.94-1.00</td>
</tr>
<tr>
<td>Variable restriction fragments</td>
<td>HpaI-K, EcoRI-P</td>
<td>HpaI-F, BglI-F, KpnI-E, SalI-E</td>
<td>HpaI-G</td>
</tr>
<tr>
<td>Range of variation (base pairs)</td>
<td>150</td>
<td>300</td>
<td>150</td>
</tr>
<tr>
<td>Different patients*</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Different origin (wt)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Same origin (wt)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Same origin (vac)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Same patient</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>wt</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vac</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* wt, Wild-type; vac, Vaccinee.

region (U₁), and the other two within the two inverted repeated regions flanking each side of the unique short region (U₃). We have mapped the three variable regions detected by HpaI enzyme analysis of the VZV genome; the results are summarized in Table 2 and Fig. 8. Hybridization experiments showed the location of the three regions (VRs): VRI was located at 0.16 covered by HpaI-K, VRII was located between the 0.30 and 0.40 coordinates, and VRIII was located near the right terminus at 0.94 to 1.0. These regions include those in the previous report of Straus et al. (1983). A physical map of VZV DNA obtained using 11 restriction enzymes including HpaI has been reported by Mishra et al. (1984). However, the locations of the HpaI-F, -G and -K fragments obtained in this work are different from those in their map. This discrepancy is probably because their nomenclature is different from ours. What we have named HpaI-K, -F and -G (shown as k, f and g in Fig. 8) may correspond to their -N, -G and -H, respectively.

HpaI-K, which differs in molecular size among different strains, contains VRI. This polymorphism was also observed in the EcoRI-P fragment. Although VRI is not diagnostic for virus attenuation, both fragments (HpaI-K and EcoRI-P) can be used as Oka vaccine markers in DNA analysis. Judging from the results of agarose gel electrophoresis, HpaI-K of a particular isolate was very stable both during passage in vitro up to 85 times (Fig. 3) and in human infection (Fig. 2).

It is not clear at present from our results why HpaI-K is more stable than HpaI-F during limited passage in vitro and in humans. This might be resolved by a study of DNA replication or DNA recombination based on the analysis of DNA sequences. The amount of variation in HpaI-F (VRII) as reflected by variation of BglI-F, KpnI-E and SalI-E fragments reached about 300 base pairs (Hayakawa et al., 1984), the same level as in HpaI-F itself (Fig. 8). VRII differs in some properties from VRI and VRIII. All isolates from epidemiologically unrelated individuals and viruses passaged in vitro (Fig. 3) or by human transmission (Fig. 2) showed DNA polymorphism in this region. This variable region was so unstable that DNA fragments covering it could not be used for distinguishing strains. Ecker et al. (1984) found single-stranded regions in such fragments (HindIII-E and EcoRI-G) by electron microscopy, and recognized significant heterogeneity in these fragments. Since herpesviruses contain nicks and gaps in their genomes (Roizman, 1979), VRII might be a 'hot spot' in a nicked sequence, and so readily produced heterogeneous DNA fragments.

Finally, the polymorphism of HpaI-G, which showed variations in molecular size of less than 50 base pairs, was difficult to detect in different strains because this variation was small and the molecular weight of the HpaI-G fragment was high. However, some differences were observed in the size of this fragment in different strains. VRIII may not be influenced by passage in vitro (Fig. 3) and may be relatively stable in different strains. HpaI-G is located in the terminal and inverted repeat sequences. Davison & Scott (1985) reported that a G + C-rich unit reiterated five or more times is located near the origin of DNA replication. DNA sequences in the same region as above were determined for several different strains by Casey et al. (1985), and the
G + C-rich repeats were reported to vary in copy number in the different strains. Moreover, the reiteration shows sequence homology with the tandemly repeated sequences in the short repeat region of herpes simplex virus types 1 and 2 (Davison & Wilkie, 1983), which have been proposed to be used as recombination signals (Casey et al., 1985). Possibly, the polymorphism at VRIII in VZV is the result of DNA recombination.

DNA polymorphism detected by restriction enzymes may be caused by two mechanisms, point mutation affecting the restriction site, and addition or deletion of DNA sequences at specific sites as shown by Roizman & Tognon (1983). Our results indicate that the heterogeneity of the three VRs may not be caused by point mutations at the recognition sequence for restriction enzymes, but by rearrangement of a specific DNA sequence.

REFERENCES


(Received 6 January 1986)