TYPING OF HUMAN HERPESVIRUS-6 DNA BY RESTRICTION ENDONUCLEASE CLEAVAGE OF THE POLYMERASE CHAIN REACTION PRODUCT

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Summary. – A 591 bp portion of the *Hind*III H (pZVH14) fragment of human herpesvirus-6 (HHV-6) strains GS (type A) and R-147 (type B) DNA was amplified by the polymerase chain reaction (PCR) using specific primers. While *Alu*I cleaved the amplified DNA of both types of HHV-6, *Eco*RV cut the type A but not the type B DNA; *vice versa*, *Hae*III cleaved the type B but not the type A DNA.

Key words: Human herpesvirus–6; DNA types A and B; restriction endonuclease cleavage

Tropism of HHV-6 isolates to different lymphoid cell lines and the reactivity of monoclonal antibodies to certain HHV-6 glycoproteins (e.g. gp82/105) predicts that the isolates of this virus fall into two types represented by strains GS (type A) and Z-29 (type B) (Ablashi *et al.*, 1991). Furthermore, restriction endonuclease cleavage patterns of HHV-6 DNA and/or their PCR products resulted in a similar grouping of HHV-6 isolates (Aubin *et al.*, 1991). Comparison of restriction endonuclease profiles of HHV-6 DNA coming from various sources with that of the prototype laboratory strains confirmed that two cleavage patterns exist, namely one resembling strain GS and/or U1102 (type A), and an another resembling strain Z-29 (type B) (Ablashi and Shalahuddin, 1992). Partial sequencing of PCR products of different length varying from 163 bp to 591 bp which had been prepared from lymphadenopathy strains GS and U1102 (type A) and from exanthem subitum isolates (type B) showed that approximatelly 4 – 5% of nucleotides may differ between DNAs of these two types (Rajčáni *et al.*, 1994). This divergence, and the existence of polymorphic HHV-6 DNA regions, typical for each subtype was confirmed also by using oligonucleotide probes specific for each type which hybridize to the PCR product of one type but not to the similar product of the other type (Drobiski *et al.*, 1993). A 591 bp portion in the *Hind*III H (pZVH14) fragment (Josephs *et al.*, 1991; Razzaque *et al.*, 1993) of HHV-6 DNA (nt 884-1476) was amplified from the strains GS (type A) and R-147 (type B) by PCR and sequenced (Rajčáni *et al.*, 1994). The sequencing showed a missing A in position 1251 causing the difference in the originally postulated and real number of nucleotides. Computer analysis of the region in question showed that the fragments of both types differ at least in 28 restriction sites of 20 enzymes which cut the PCR product of one but not other type; in addition, the enzymes which cut the PCR products of both types show at least 13 different restriction sites. For practical reasons we chose *Eco*RV which cuts the type A PCR product at nt 1162 producing two fragments, 278 bp and 313 bp in size. In contrast, *Hae*III cuts the type B PCR product at nt 1160, giving two similar fragments, 276 bp and 315 bp in length (Fig. 1). The latter two enzymes do not cleave the PCR product of the opposite type, and *Alu*I cleaves the PCR products of both types.

HHV-6 strains GS, R-147 and the isolate Strasbourg (S-610) were propagated in HSB-2 cells and in human blood cord leukocytes, respectively. The cells were grown in

Abbreviations: HHS-6 = human herpesvirus-6; FITC = fluorescein isothiocyanate; PBS = phosphate buffered saline; PCR = polymerase chain reaction

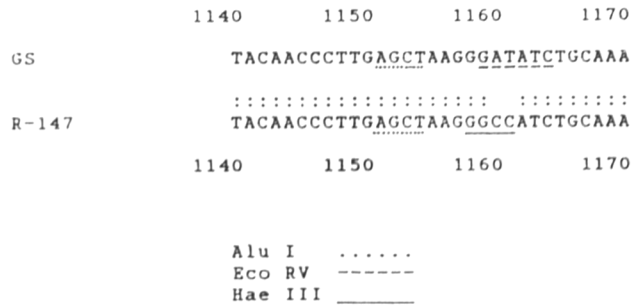

Fig. 1
The region of the *Hind*III H (pZHV14) fragment of HHV-6 DNA cut by *Alu*I, *Eco*RV and *Hae*III

RPMI-1640 medium supplemented with 10% foetal calf serum and antibiotics. The human blood cord leukocytes were stimulated with phytohaemagglutinin (5 µg/ml) and interleukin-2 (2 U/ml). The cells were checked for the presence of HHV-6 antigens by indirect immunofluorescence using human anti-HHV-6 serum and anti-human IgG FITC-labelled conjugate. Antigen-producing cells were harvested, washed in PBS by low speed centrifugation and treated with proteinase K (200 µg/ml) in a buffer containing non-ionic detergents (50 mmol/l KCl, 10 mmol/l Tris-HCl pH 8.3, 25 mmol/l $MgCl_2$, 0.1 mg/ml gelatine, 0.45% NP-40 and 0.45% Tween-20). After digestion for 2 hrs at 56 °C, the proteinase was inactivated at 96 °C for 10 mins and the mixture was used for PCR without further DNA extraction. Ten µl of cell extract corresponding to 10^5 cells were used per reaction. The primers

P3 (5'-CAT ACT AGA ACA ACA GAG AG-3', forward) and
P4 (5'-ATT TTA AAG ATT ACG GAC TT-3', reversed)

were used. The conditions of PCR were described previously (Rajčáni *et al.*, 1994). The reaction mixture from the Perkin Elmer GeneAmp PCR reagent kit was employed according to the recommendations of the manufacturer (Perkin-Elmer Cetus). The template was denatured at 92 °C for 7 mins, cycled 40 times (denaturation at 96 °C for 1 min, annealing at 50 °C for 2 mins and elongation at 72 °C for 3 mins extended by 5 secs during each cycle (Perkin Elmer DNA Thermal cycler 480). Electrophoresis of the amplified and/or digested DNA fragments was run in 1.5% agarose (NU-Sieve, Seakem GTG, Rockland, ME, USA) in TBE buffer (45 mmol/l Tris-HCl pH 8, 45 mmol/l boric acid, 1 mmol/l EDTA). After the run, gels were stained with ethidium bromide. The amplification products were digested in the presence of 2U of the corresponding enzyme in 20 µl of reaction volume for 2 hrs at 37 °C. An example of typical

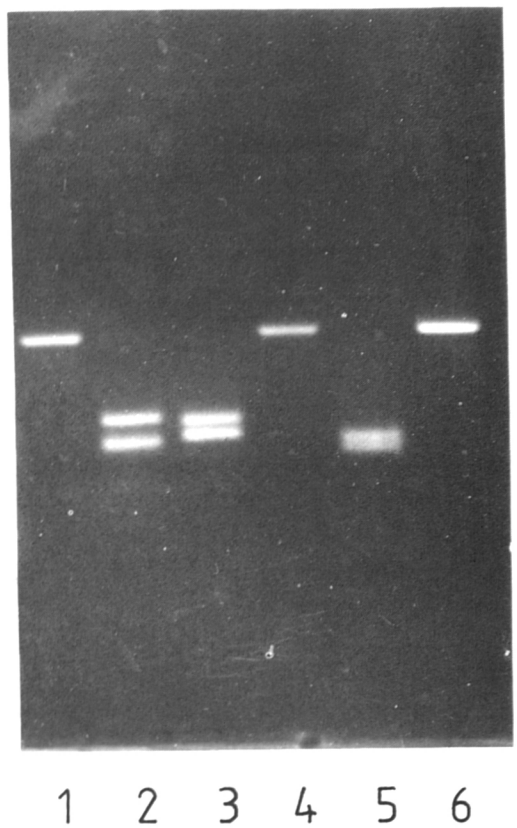

Fig. 2
Gel electrophoresis of PCR products and their *Eco*RV digests of DNA of HHV-6 strains GS and isolate S-610
Lane 1: The 591 bp PCR product from the strain GS. Lane 2: the 269 bp and 322 bp *Alu*I cleavage products from the sample in lane 1. Lane 3: the 276 bp and 315 bp *Eco*RV cleavage products from the sample in lane 1. Lane 4: the PCR product from the strain isolate S-610. Lane 5: the 269 bp and 240 bp *Alu*I cleavage products from the sample in lane 4. The 82 bp cleavage product is not visible in this gel. Lane 6: the sample from lane 4 digested with *Eco*RV. There is no cleavage.

digestion is shown in Fig. 2. While *Alu*I cleaved both PCR products, i.e. type A as well as type B, *Eco*RV cleaved the type A product but not the type B product. Similar results were obtained with the Rochester isolate of strain R-147 originating from *exanthema subitum* (data not shown). In contrast, *Hae*III digested the PCR product type B but not type A (data not shown). Further work is in progress to confirm the usefullnes of our approach for typing a variety of clinical isolates.

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