Analysis of HCMV glycoprotein B DNA fragments with non-radioactive and radioactive SSCP

T. Binder, T. Berg and C. A. Schmidt

Abteilung für Innere Medizin (Hämatologie und Onkologie), Universitätsklinikum Rudolf Virchow, Spandauer Damm 130, D-14050 Berlin (Germany)

Human Cytomegalovirus (HCMV) is a member of the Herpesvirus family. Reactivation of latent infection has increasingly become a factor contributing to the morbidity and mortality associated with AIDS and organ transplantation. Viral transmission by the donor organ is most likely in patients HCMV seronegative prior to transplantation receiving a graft from a seropositive donor[4]. These patients are at high risk of acquiring primary infection. Latent cytomegalovirus can reactivate in seropositive patients after organ transplantation or infection with HCMV of a donor strain. The major immunogenic envelope glycoprotein B (gB) of HCMV is expressed as a precursor molecule of about 906 amino acids that is cleaved at a specific proteolytic site (Aa 460/461) to form a disulphide-linked complex. The major variable locus spans the gB proteolytic cleavage site region from codon 448 to 480 [1,2,5].

Mutations in amplified DNA fragments can be detected by single-strand conformation polymorphism analysis (SSCP). This method provides a rapid and sensitive method for identifying known and unknown mutations and polymorphisms in amplified DNA fragments. SSCP is most frequently performed using radioactive labelled PCR products[3]. Several non-radioactive detection procedures with silver staining or ethidium bromide staining are employed. We also used radioactive SSCP analysis and developed another non-radioactive SSCP detection method, which is based on the use of biotinylated primers. After electrophoresis and membrane transfer, biotinylated DNA can be detected with an enzymatic dye reaction (streptavidin alkaline phosphatase conjugate) or chemiluminescence.

For sample preparation, a simple alkaline lysis procedure of buffy coat leukocytes was performed[4]. As primers sense HCMVgB-1 (nt 1276–1295) and antisense HCMVgB-2 (nt 1505–1524), which covered all known HCMV glycoprotein B sequences, were used[1,2,5]. After PCR under standard conditions 5 µl of nonradioactive PCR product (2 µl of radioactive product) were diluted with 5 µl (8 µl) formamide dye. Double stranded DNA fragments were denatured by heating for 5 min to 95 °C and were incubated for 5 min in icewater. Five µl of the diluted solutions were transferred to a 0.5 × MDE gel (AT Biochem, USA) in a standard vertical sequencing apparatus. Gels were run with constant power (6.5 Watt) for 16 h. Separated PCR products were transferred to positively charged nylon membranes by dry blotting, crosslinked using UV light and detected as recommended by the supplier (Boehringer Mannheim, FRG), but streptavidin-AP was used instead of anti-DIG-AP antibodies. The radioactive ssDNA was detected by autoradiography[3].

The combination of biotinylated sense and non-biotinylated antisense primers allows the identification of sense and antisense strands. Because the genetic information is contained in both DNA strands, the use of only one biotinylated primer allows detection of mobility shifts (mutations). This facilitates the analysis of SSCP gels, especially when in the case of virus variability analysis of more than one virus strain in the sample is necessary.

1 Chou, S., and Dennison, K. M., J. infect. Dis. *163* (1991) 1229.
2 Lehner, R., Stamminger, T., and Mach, M., J. clin. Microbiol. *29* (1991) 2494.
3 Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K., and Sekiya, T., Proc. natl. Acad. Sci. USA *86* (1989) 2766.
4 Schmidt, C. A., Oettle, H., Neuhaus, P., Wiens, M., Timm, H., Willborn, F., and Siegert, W., Transplant *56* (1993) 872.
5 Spaete, R. R., Thayr, R. M., Probert, W. S., Masiarz, F. R., Chamberlain, S. H., Rasmusen, S. H., Merigan, T. C., and Pachl, C., Virology *167* (1988) 207.

Detection of cytotoxin gene (vacA) and cytotoxin-associated antigen (cagA) of *Helicobacter pylori* by using the polymerase chain reaction (PCR)

F. Chahoud, N. Ade, E. Pieringer-Müller and H. Hof

Institut für Medizinische Mikrobiologie und Hygiene, Fakultät für Klinische Medizin der Universität Heidelberg, Theodor-Kutzer-Ufer, D-68135 Mannheim (Germany)

In 1987 Leunk et al.[1] described a vacuolating cytotoxic effect of *H. pylori* on cell cultures. This cloned cytotoxin[2] is an immunogenic protein with a molecular weight of 87 kDa. Its expression is linked to a surface exposed immunodominant antigen with a molecular weight of 127 kDa. This cagA antigen is used in serological tests and its gene has been cloned and sequenced[3].

Telford et al.[4] demonstrated the cytopathic effect of the cytotoxin in combination with the cagA antigen in a mouse model and concluded that the cytotoxin gene is present but not expressed in noncytotoxic strains, whereas the cagA gene is completely absent in cytotoxin negative strains.

Table. Distribution of the vacA und cagA genes in 91 clinical *H. pylori* isolates.

	cagA +	*cagA* −
vacA +	51.7%	13.2%
vacA −	18.7%	16.5%

The intention of our investigation was to identify the cytotoxin and the cagA gene in well characterized *H. pylori* isolates by means of the polymerase chain reaction (PCR).

The 91 *H. pylori* isolates used in the present study were isolated from human gastric biopsies from 68 patients. We were able to detect the vacA gene in 64.8% and the cagA gene in 70.3% of our *H. pylori* isolates 51.7% harboured both genes, whereas 16.5% lacked both genes. With our selected primer pairs we could detect the cag A gene without concomitant vacA in 18.7% and the vacA gene without concomitant cagA in 13.2% of the tested strains.

In a significant portion of our tested *H. pylori* strains neither the cytotoxin gene nor the cagA gene could be detected, which is in contrast to the results of the above mentioned research groups. One possible explanation for these contradictory results is the chosen primer pairs. Additional specific primers for different parts of the genes may give us comparable results.

To evalute the PCR results further it seems necessary to show the cytopathic effect of *H. pylori* strains with different expression of the vacA or cagA genes in a cell culture system.

1 Leunk, R. D., Johnson, P. T., David, B. C., Draft, W. G., and Morgan, D. R., J. med. Microbiol. *26* (1988) 93.
2 Burroni, D., Dell'Orco, M., Commanducci, M., Bugnoli, M., Rappuoli, R., and Telford, J. L., Acta Gastroenterol. Belg. *56* (1993) 62.
3 Tummuru, M. K. R., Cover, T. L., and Blaser, M. J., Infect. Immun. *61* (1993) 1799.
4 Telford, J. L., Ghiara, P., Dell'Orco, M., Comanducci, M., Burroni, D., Bugnoli, M., Tecce, M. F., Censini, S., Covacci, A., Xiang, Z., Papini, E., Montecucco, C., Parente, L., and Rappuoli, R., J. expl Med. *179* (1994) 1653.

Identification of new oral treponemes by comparative sequence analysis of in vitro amplified 16S rRNA genes

B.-K. Choi[a], C. Wyss[b] and U. B. Göbel[a]

[a]*Institut für Mikrobiologie und Hygiene, Universitätsklinikum Charité, Dorotheenstr. 96, D-10117 Berlin (Germany)*
[b]*Zahnärztliches Institut, Universität Zürich, Plattenstr. 11, CH-8028 Zürich (Switzerland)*

Rapid and unequivocal identification of new bacterial isolates is a prerequisite to determine their aetiological role in inflammatory processes. This is particularly true for oral treponemes associated with periodontal infections. Molecular genetic evidence suggested the presence of a large number of yet uncultured treponemal phylotypes identified by comparative 16S rRNA sequence analysis[1].

To study the phenotypic traits, physiological properties and pathogenicity of these newly identified organisms we have cultured subgingival plaque material on the semi-solid, defined medium OMIZ-W1. Treponemal isolates were subjected to microscopic examination and biochemical analysis, e.g. SDS-PAGE and API-zym enzyme test. Twenty-three isolates could not be identified by phenotypic analysis and were selected for comparative 16S rRNA sequence analysis. Molecular genetic analysis identified six isolates as *T. socranskii*. The remaining 17 isolates were characterized by sequences identical to previously described 'cluster 17' treponemes[1]. They exhibited a marked phenotypic heterogeneity and could not be grown in NOS medium. All attempts failed to identify these very small 'cluster 17' treponemes by in situ hybridization.

In conclusion, we have isolated and characterized a new group of small oral treponemes, which may represent a new species. Due to their marked phenotypic heterogeneity they could only be classified by molecular genetic analysis.

1 Choi, B. K., Paster, B. J., Dewhirst, F. E., and Göbel, U. B., Infect. Immun. *62* (1994) 1889.