greatly in size (311 to 366 base pairs) and number (1 to 5 bands). Further investigation of a greater number of strains from each species is required in order to determine whether the pattern obtained for a given species is truly characteristic of that species.

The results of our study suggest that fragment analysis of the intergenic spacer region is a powerful tool for the rapid identification of highly related strains. The potential of this method in species differentiation will be further investigated.

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Detection of human cytomegalovirus (HCMV) DNA from cell-free plasma of immunosuppressed patients by nested PCR: influence of nucleic acid extraction method

K. Hamprecht, G. Sorg, A. Grebenstein, M. Boniek and G. Jahn

Abt. Medizinische Virologie und Epidemiologie der Viruskrankheiten, Hygiene-Institut, Universität Tübingen, Silcherstr. 7, D-72076 Tübingen (Germany)

Human cytomegalovirus (HCMV) is an important causative organism for life and sight-threatening opportunistic infections in immunocompromised hosts, especially recipients of bone marrow or solid organ transplants and patients with AIDS. Diagnostic methods currently used for HCMV-monitoring include rapid viral isolation (detection of the 72 kD immediate early antigen in cell culture), the antigenemia assay (detection of pp65 in peripheral leukocytes) and HCMV DNA PCR from peripheral blood leukocytes. Detection of HCMV in blood cell samples is an early diagnostic marker of imminent viremia, which is associated with HCMV disease. However, there is growing evidence of the low predictive value of HCMV DNAemia as a marker of symptomatic infection[1,2]. Although detection of HCMV mRNA by RT-PCR for late HCMV proteins holds promise, none of these qualitative methods is able to discriminate HCMV disease from asymptomatic HCMV infection. Next to PCR quantification or RT-PCR, the use of plasma instead of leukocytes as material for HCMV DNA amplification seems to provide better data for the interpretation of positive PCR results.

Methods and results. We compared the results from qualitative HCMV DNA PCR from peripheral mononuclear leukocytes (PBMC) and granulocytes isolated from immunosuppressed patients with results from rapid viral isolation, conventional tube cell culture of these cells or other materials, serology, and HCMV DNA detection from plasma. DNA from blood cell fractions was isolated using a standardized protocol[2] with primer oligonucleotides from the fourth exon of the HCMV-IE1 gene. For the isolation of DNA from cell-free plasma three protocols were chosen. First, HCMV DNA was prepared by a modification of the method of Ishigaki et al.[3]. Secondly, DNA was extracted from thermally inactivated supernatants of proteinase K digestions with phenol/chloroform and precipitation with EtOH. In a third protocol, cell-free DNA was isolated from plasma by precipitation of inactivated proteinase K supernatants with polyethylene glycol (8% w/v PEG 6000) and NaCl (0.7 M) followed by phenol/chloroform extraction. With oligonucleotide primers derived from the DRB1 gene of the major histocompatibility complex (MHC) class II locus we could demonstrate that the DNA isolated from plasma was cell-free. Sensitivity and specificity of DNA amplification was estimated using an DIG-dUTP-dATP-tailed probe for hybridization of amplified products.

Conclusions. Blood cells and plasma preparations from HCMV-seropositive healthy blood donors were all nPCR negative. Detection of HCMV DNA from PBMC and granulocytes (DNAemia) of immunosuppressed patients by nPCR did not correlate with the isolation of infectious virus from these cell populations in cell culture (viremia). However HCMV could be isolated in 60% of cases from other materials of the same patient. HCMV DNA detected in blood cells persisted for up to one year in an asymptomatically infected individual after NTX. The sensitivity of HCMV DNA detection in cell-free plasma (up to 5 fg) depended on the method used for DNA isolation. The rate of HCMV DNA detection in plasma was lower than in leukocytes. In all cases of positive plasma PCR infectious virus could be isolated from any other material of the symptomatically infected patients. Therefore HCMV DNA PCR from plasma of immunosuppressed patients seems to be a suitable and easy alternative to HCMV RT/PCR for routine diagnosis of HCMV disease.

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