shown necessary to isolate and purify the DNA. Routinely, PBLs from citrate blood, cells enriched from urine or bronchial lavage were lysed in a detergent-containing buffer and incubated with proteinase K. DNA was purified by phenol/chloroform extraction and ethanol precipitation. Recently, time consuming latter steps could be minimized by use of QIAGEN spin-columns. Detection of HCMV in CSF seems to be somewhat difficult for various reasons, e.g. low cell and virus concentrations or presence of inhibitory substances in CSF. CSF probes were used for PCR either in the 'native' form after incubation at 100 °C for 10 min or prepared as described for the other specimens. When HCMV DNA is isolated from CSF using the QIAGEN kit, the DNA was concentrated by ethanol precipitation after purification.

Generally, we used a one-step PCR for detection of HCMV DNA. Sensitivity of the PCR ranged between 50 and 100 viral copies per test. Under these conditions PBLs from the majority (about 93%) of healthy seropositive or seronegative individuals were negative for HCMV DNA, indicating that one-step PCR does not 'erroneously' indicate latent HCMV in PBLs[1,3].

In bone marrow transplant recipients, negative for HCMV DNA at the time of transplantation, active HCMV infections could be detected by PCR 1 to 2 weeks earlier than by centrifugation culture and before observation of the first clinical symptoms. But some latently infected bone marrow recipients became positive for HCMV DNA in PBLs before transplantation after whole body irradiation or chemotherapy. Only in some patients is a positive PCR result of prognostic value for the development of HCMV disease.

In a further study we investigated the significance of HCMV DNA detection in the CSF of patients with neurological disorders. When we investigated CSF probes from more than 20 individuals without any signs of inflammatory CNS disease, no HCMV DNA could be amplified despite the fact that some of the patients were positive for HCMV DNA in the peripheral blood. On the other hand, HCMV DNA could be detected in the CSF of a patient suffering from encephalitis. After the acute phase of the illness the virus was shown to be eliminated from the CSF and several months later also from the peripheral blood.

Conclusions. In transplant recipients, a positive HCMV PCR in PBLs is a sign of the possible development of HCMV disease which should be confirmed by further diagnostic approaches. However, detection of HCMV DNA in the CSF appears to be a specific marker of pathological processes, exemplified in a case of HCMV encephalitis.

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Molecular characterization of the p83/100 proteins of various *Borrelia burgdorferi* sensu lato strains

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A protein with a molecular weight of approx. 83–100 kDa (p83/100, p93) is an immunodominant protein of the late immune response in patients with Lyme borreliosis[4]. This chromosomally encoded borrelial protein[3] is usually expressed in low amounts, not present in the periplasmic space and associated with the protoplasmic cylinder[2] or the flagella[1]. These p83/100 homologues[4] share a high degree of identity (88–99%) and the glycosylated (Thilo Schlott, unpublished). The aim of this work was to get new insights into the molecular structure of the p83/100 molecules and to explore whether p83/100 sequence polymorphism correlates with previous typing methods.

Methods and results. The complete p83/100 coding region of *Borrelia burgdorferi* sensu lato strains PBi (*B. garinni*, OspA serotype 4), TN (*B. garinii*, OspA serotype 6), Gö2 (*B. garinii*, OspA serotype 6) and PBre (*B. burgdorferi* sensu stricto, OspA serotype 1) were PCR-amplified, cloned and sequenced. After alignment studies an internal fragment of the p83/100 molecule turned out to be the most heterologous region (approx. aa 390–540). Therefore, a part of this internal region of p83/100 was determined from an additional 11 *B. burgdorferi* sensu lato strains (PLe, PGau, PLj7, PWudI, PKa2, 297, T255, B. pac., TIsI, T25 and WABSou). Sequence-specific oligonucleotides were used for PCR amplification. DNA sequencing was performed on an ABI 373 DNA-sequencer (*Taq*,

dye-terminator reactions). Sequence analysis of the p83/100 internal part showed an 18 bp insertion typical for *B. burgdorferi* sensu stricto strains and a 90 bp deletion typical for all investigated *B. afzelii* strains. Due to the insertions/deletions, the size of these PCR-amplified internal p83/100 fragments varies between the three species. The only exception was the internal fragment of *B. burgdorferi* sensu stricto, strain *B. pacificus*, which did not show the 18 bp insertion. Restriction analysis of PCR fragments with *Dra*I revealed three patterns, correlating to the species grouping.

Conclusions. As in OspA-serotyping experiments[5], the *B garinii* group (OspA-sterotype 3–7) showed highest diversity within this internal fragment of p83/100, whereas the *B. afzelii* group (OspA-type 2) and the *B. burgdorferi* sensu stricto group (OspA-type 1) were nearly identical. Determination of the size of the PCR products as well as restriction fragment length polymorphism analysis (*Dra*I) can be used for classification into the three species of *B. burgdorferi* sensu lato. Since p83/100 is chromosomally encoded, this protein might be a more stable marker for classification than the plasmid-encoded OspA. In contrast to the flagellin gene a subclassification of the *B. garinii* group is possible due to the diversity of the p83/100 internal fragment.

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Highly sensitive semi-quantitative method to detect amplified viral DNA in the microtitre format

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In many clinical situations it is important to know the virus titre of people chronically infected with HBV, HCV or other viruses, for example to monitor the success of an interferon treatment[2]. Nested PCR is useful to detect nucleic acids with sufficient sensitivity, but is hard to quantitate. We have developed a simple chemiluminescent method that allows the detection of HBV and HCV nucleic acids as sensitively as nested PCR. This method is performed in the microtitre format which makes working with many samples much easier.

Extraction. We compared different published or our own methods to extract HBV DNA (proteinase K, NaOH-/KOH-lysis, microwave or heat treatment, acetone) or HCV RNA (proteinase K, GITC, heat or QiaAMP Blood Kit, Qiagen)[1]. In our hands a modified NaOH-lysis method (from the Boehringer ES300 protocol) worked best for extraction of HBV DNA from serum (10 µl serum + 20 µl 0.2 N NaOH: 1 h 37 °C, + 30 µl 0.2 M TrisCl, pH 7.5; 20 µl of this mixture were used for PCR). Standard GITC extraction worked best for HCV.

Chemiluminescent detection of amplified nucleic acids. During PCR one biotinylated primer is integrated into the amplified PCR fragment. This amplicon is immobilized on white microtitre plates that are coated with covalently linked streptavidin (Dr. J. Diment, Kodak clinical diagnostics). After denaturation this template is hybridized to a DNA probe that is labelled with peroxidase. Target-bound peroxidase is detected by chemiluminescence in an Amerlite Analyzer.

As detection probe we tested single stranded or double stranded DNA of different lengths, or a labelled oligo. Ds and ss probes were labelled with ECL direct nucleic acid labelling system, Amersham; the oligo was 5'-labelled with ECL 5'thiol oligolabelling system, Amersham. The best probe in our hands was the single stranded probe (HBV or HCV probe not overlapping with the used primers, cloned into pBluescriptII SK$^+$, Stratagene, made single stranded with the helper phage R408, Stratagene). The detection limits were: ss-probe 8 pg, ds-probe 32 pg, and oligo 500 pg.

Hybridization was performed for 4 h in urea-containing hybridization buffer (ECL system) at 42 °C on a heated microtitre plate shaker (AIP4, Pasteur Diagnostics). Prehybridization decreased the sensitivity.

After the nucleic acid detection system had been optimized we compared nested PCR, the Boehringer ES 300 system and our inhouse method using a panel of sera obtained from a trial of Eurohep to standardize PCR for the detection of HBV DNA. The following titres were obtained for one serum by endpoint dilution and calculation according to ref. 3: nested PCR 2.21×10^9/ml, Boehringer ES 300 2.3×10^9/ml, and inhouse method 3.43×10^9/ml. The titres that were determined by nested PCR and both semiquantitative methods were identical for this serum and others.