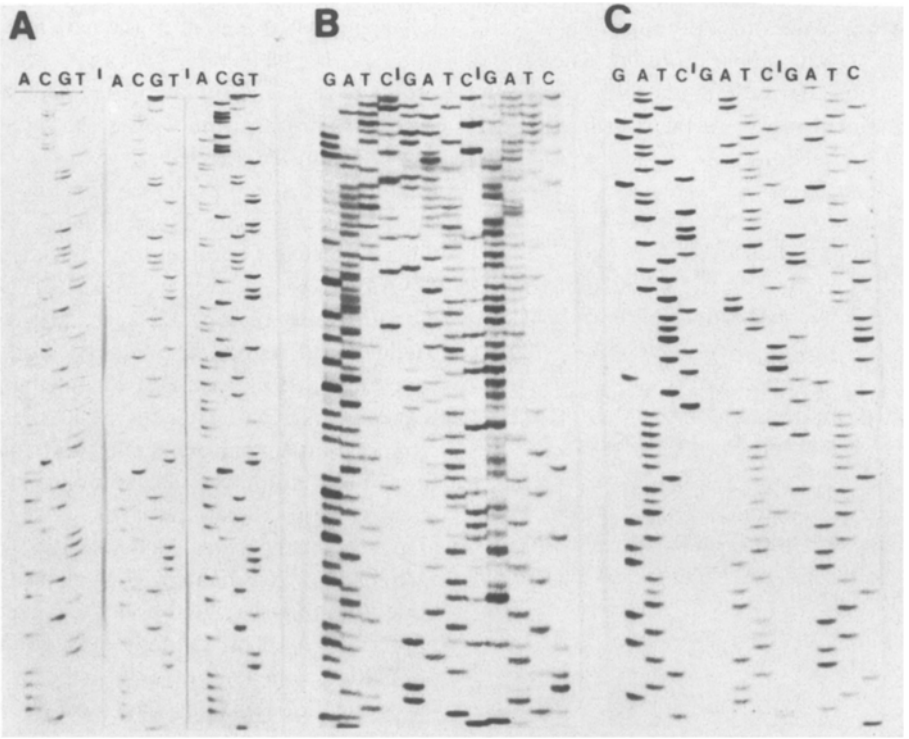

Figure 1. Amplicons from the *pol* gene (*A*) and a hypervariable region of the *env* gene (*B*) were sequenced directly. (*C*) shows the same hypervariable region of the *env* gene, but the amplicons were cloned prior sequencing.

e.g. during therapy with reverse transcriptase inhibitors.

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Diagnostic value of human cytomegalovirus DNA PCR regarding different clinical specimens

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Human cytomegalovirus (HCMV) is a major pathogen in immunocompromised individuals, especially organ transplant recipients or HIV-infected patients, and a common infectious cause of congenital abnormalities. Furthermore, HCMV is associated with neurological disorders, e.g. encephalitis or polyradiculopathy in AIDS patients.

After primary infection HCMV, like other herpesviruses, develops a life-long persistence, possibly in monocytic blood cells[4]. In immunosuppressed patients reactivation of the virus or superinfection leads to active infections with often fatal prognosis. A rapid, sensitive, and specific diagnosis is a prerequisite for the successful treatment of HCMV infections[2].

Efforts in HCMV diagnosis have been made by replacement of the time-consuming tissue culture methods by the more rapid 'centrifugation culture' coupled with indirect immunofluorescence assay. Furthermore, PCR techniques have been established to detect viral DNA (or RNA) in several different clinical specimens. Comparing the results of 'centrifugation culture' and PCR for the monitoring of transplant recipients, especially bone marrow recipients, DNA detection has been shown to be a sensitive and early marker of active HCMV infections[3].

In our laboratory, PCR techniques have been established to detect HCMV DNA in different clinical specimens like PBLs, urine, bronchial lavage and cerebrospinal fluid (CSF). For HCMV PCR it has been

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*,