Doctoral Thesis

Heat shock protein 65 of Tropheryma whippelii molecular characterization, phylogenetic analysis and detection of antibodies in patients

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Heat shock protein 65 of *Tropheryma whippelii*: molecular characterization, phylogenetic analysis and detection of antibodies in patients

A dissertation submitted to the
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for the degree of
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presented by

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SUMMARY

Whipple’s disease is a systemic infection of humans associated with a wide variety of intestinal as well as extraintestinal manifestations. The causative agent, *Tropheryma whippelii*, has so far never been cultivated on artificial media. The molecular detection of this rare bacterium is currently based on PCR-mediated amplification of a part of its 16S ribosomal RNA gene (16S rDNA), of the 16S-23S internal transcribed spacer (ITS), or of the 23S rDNA.

Recently, *T whipplei* DNA has been found in duodenal biopsies and gastric juice of patients without Whipple’s disease. In a follow-up study, saliva and dental plaques of the same patients were screened for the presence of *T. whippelii* DNA (see Chapter 2). Six out of the 14 previously PCR-positive persons but none of the 17 controls had *T. whippelii* DNA in their saliva (the same ITS-type). Our results suggest that Whipple bacteria are ubiquitous environmental or commensal organisms causing Whipple’s disease in a particular subset of individuals, possibly those with an as yet uncharacterized immunological defect.

We wanted to confirm these results with a target completely different from the rRNA genes used in all these studies. The heat shock protein 65 (Hsp65) and the gene coding for it (*hsp65*) are ubiquitous and therefore most probably also found in *T. whippelii*. Using broad-spectrum primers, we have amplified, cloned and sequenced a 620 bp-fragment of the *T. whippelii* heat shock protein (*hsp65*) gene from the heart valve of a patient with Whipple’s endocarditis (see Chapter 3). With primers derived from this sequence a semi-nested PCR was established. Seventeen patients with or without clinical signs of Whipple’s disease shown to be positive by 16S rDNA-PCR and/or ITS-PCR were also positive by *hsp65*-PCR. Clinical samples from 33 control patients remained negative. We conclude that the *hsp65*-PCR represents a sensitive and specific alternative assay for the molecular diagnosis of Whipple’s disease.

Only recently, Raoult and coworkers were able to cultivate *T. whippelii* in a human cell line. With bacterial DNA isolated from these cell cultures and with primers derived from the previously determined 620 bp-fragment, we determined the unknown up- and downstream regions of the *hsp65* gene of *T. whippelii* by genome walking (see Chapter 4). After determination of the entire gene sequence (1623 bp), the corresponding amino acid sequence provided a basis for evolutionary comparisons. The phylogenetic analysis obtained showed higher bootstrap values than those from the 16S rDNA data set and confirmed that *T. whippelii* is indeed a member of the Gram-positive bacteria with high G+C content (actinobacteria). Only slight differences to trees based on 16S rDNA gene sequences were observed.
Recombinant plasmids carrying the partial and the entire *T. whippelii hsp65* gene were constructed, and expression yielded recombinant proteins of 32.8 and 65 kDa, respectively (see Chapters 5 and 6). Immunoblot analysis revealed only weak antibody response. The entire Hsp65 was then purified by affinity chromatography and used in a quantitative
ZUSAMMENFASSUNG

(Aktinobakterien) gehört. Es wurden nur kleine Differenzen zu den 16S rDNA-Stammbäumen beobachtet.

Rekombinante Plasmide, die das partielle und ganze T. whipplei hsp65-Gen tragen, wurden konstruiert. Die Expression ergab Proteine von 32.8 respektive 65 kDa (Kapitel 5 and 6). Im Immunoblot zeigte sich nur eine schwache Antikörper-Antwort. Das ganze Hsp65 wurde mittels Affinitätschromatographie gereinigt und in einem Enzyme-linked