5 Summary

The genus *Mycobacterium* comprises highly pathogenic as well as opportunistic or apathogenic species exhibiting a great variability with respect to their growth rates but also their ability to persist or multiply on the cellular level. Intracellular persistence is a key feature of virulence of *M. tuberculosis*, the causative agent of human tuberculosis. The intention of this work was to find out whether or to which degree the permeability of the mycobacterial outer membrane affects the intracellular persistence. For this purpose the major porin of *M. smegmatis* (*mspA*), which is lacking in slow-growing mycobacteria, was expressed in *M. bovis* BCG. Quantification of bacterial growth on agar plates demonstrated clearly increased growth of the *M. bovis* BCG derivative expressing MspA. Transposon mutagenesis proved the *mspA* gene to be responsible for the growth enhancement. Intracellular multiplication of the *M. bovis* BCG derivative in the mouse macrophage cell line J774A.1 and the human pneumocyte cell line A549 was also clearly enhanced.

In contrast to this finding, deletion of *mspA* in *M. smegmatis* increased its intracellular persistence in *A. castellanii* and murine bone marrow macrophages. Deletion of *mspA* together with another homologous porin *mspC* in another mutant strain of *M. smegmatis* resulted in decreased growth in broth culture while it significantly enhanced intracellular persistence in murine bone marrow macrophages, the mouse macrophage cell line J774A.1 and *A. castellanii*, respectively. Complementation of deletions by expression of *mspA* in the porin mutant strains resulted in restoration of the wild type phenotype with respect to intracellular persistence.

These data show that the permeability of the mycobacterial cell wall affects the intracellular persistence. These findings also suggest that intracellular persistence of mycobacteria depends, inter alia, on the balance between “walling-off” towards the hostile environment and the uptake of required compounds in the nutrient-depleted phagosomal environment.

Furthermore, the gene *porM1* encoding a porin homologous to MspA was characterized in *M. fortuitum*. PorM1 was present in different strains of *M. fortuitum* and in the closely related non-pathogenic *M. peregrinum*. Analysis of expression patterns of *porM1* showed divergent expression profiles among the members of the *M. fortuitum*-group. Due to the ability of *M. fortuitum* to persist and due to the existence of porins like PorM1, this

species represents an appropriate model to study the impact of mycobacterial porins on persistence.