6 Summary

Despite intensive efforts worldwide, all attempts to develop an effective AIDS vaccine have so far failed. Infection with HIV and the resulting disease AIDS remain a deadly threat in many areas of the world, and even in countries with an effective medical infrastructure those affected are subjected to many social and medical disadvantages. Conventional strategies for vaccine development have been shown, in the case of this retroviral agent, to be either ineffective or too dangerous: even the vaccine candidates themselves can sometimes cause disease in long-term animal studies. From the start, HIV research has tried to find the ideal vaccine, one that stimulates the immune system to a state of 'sterilising immunity' and prevents infection from occurring. As trials with such vaccines continued to fail or were not reproducible, many research groups turned their attention to developing a 'protective' vaccine. Such a vaccine, while not preventing infection, should at least stimulate the immune system enough to suppress the virus load in the body and negate the need for the daily intake of antiviral drugs. If this could be achieved, not only would the quality and length of the patient's life be considerably enhanced, but also the spread of the virus to new hosts would be prevented. Modern experimental approaches focus on the use of genetic methods to induce, by various means, a massive humoral and cellular immune response to particular HIV-proteins.

Both projects of this thesis are thematically aimed at the development of genetic HIV vaccines. First, to help elucidate the mechanism of protection induced in primates by attenuated forms of SIV, two different deletions were introduced in the *nef* gene of an RTSHIV molecular clone. Second, as a basis for a putative DNA vaccine, two codon-optimised genes corresponding to a primary isolate of a Clade A/G recombinant HIV-1 were built and inserted into an expression vector.

The first project, carried out at the Paul-Ehrlich-Institute, was planned as the basis of an animal experiment designed to determine the mechanism of protection induced in rhesus macaques by Δ*nef* mutants of SIV. This was to be made possible by the use of an SIV/HIV hybrid virus: SIVmac239 carrying an HIV reverse transcriptase sensitive to certain inhibitors i.e. an RTSHIV. By subcloning the *nef* gene it was possible using PCR techniques to produce mutants with different sized deletions that should lead to different replication efficiencies *in vivo*. The Δ*nef*-RTSHIV mutant carries a 181 base pair deletion corresponding to the entire region of *nef* gene that does not overlap with the *env* gene or the 3'-LTR. The resulting frame-shift codes for a 62 aa truncated protein comprising the 58 N-terminal amino acids of wild-type Nef and just four subsequent amino acids not present in the original Nef. The so-called

C8-Δ*nef*-RTSHIV mutant contains a 12 base-pair deletion in the *nef* gene that does not affect the reading-frame. The resulting Nef protein is deleted in amino acids 143 to 146 to give a protein 259 amino acids in length. Both mutant molecular clones were shown to produce infectious virus upon transfection. The presence of both the deletions and of the HIV reverse transcriptase was confirmed by sequencing proviruses from infected C8166 cells and PBMC. Furthermore, the sensitivity of RTSHIV deletion mutants to the inhibitory effect of the non-nucleoside-analogue reverse transcriptase inhibitor (NNRTI) Nevirapine was demonstrated *in vitro*. The use of these Δ*nef*-RTSHIV constructs in an *in vivo* rhesus study is planned.

The aim of the second major project of this thesis was to construct a virus-strain specific DNA vaccine. A collaborating research group at the RKI had characterised primary isolates of A/G recombinant HIV-1 from Nigeria that, as a result of their mosaic genomes, differ significantly from the clade B isolates of HIV-1 used as the basis for most vaccine research in Western Europe. Using molecular clones of two isolates, the *tat* and *gag* genes were sequenced and analysed for the presence of premature stop-codons and CTL epitopes (based on the available knowledge of HLA-allele distribution in Nigeria). These analyses were only used to help with the choice of isolates because the immunisation vector was to comprise entire genes containing putative CTL epitopes for all HLA-haplotypes present. Using a molecular clone of the isolate 00_200 as a basis, wild-type genes were cloned into provided expression or immunisation vectors, verified by sequencing and tested for protein expression in cell lines. Codon-optimisation, a technique needed in the group for future vaccine development, was then used to optimise the expression of these genes. The nucleotide sequence of the genes were rewritten with respect to human codon-usage, inhibitory sequences in *gag* and *tat* were removed and immunostimulatory CpG motifs were inserted into *gag*, all without changing the amino acid sequence of the resulting proteins. Short oligonucleotides of 40 or 80 bases were combined using a variety of PCR techniques to produce 303 bp *tat* constructs or 1491 bp *gag* constructs and these codon-optimised genes of isolate 00_200 were inserted into an expression vector. The expression of the wild-type and codon-optimised *gag* and *tat* genes was compared in western blot and, by cotransfection with GFP and a p24 ELISA, it was possible to show an 800-fold enhancement of expression of the codon-optimised *gag* construct. These four vectors could be used to compare the immunogenicity of the constructs in mice before use in a protection experiment in primates. The vectors provide a possible basis for a DNA vaccine specific for the region of virus origin.