7 Summary

Investigations into the Integration of Nucleotide Sequences of the Reticuloendotheliosis Provirus into the Genome of Fowlpox Virus

The first aim of this study was to find out by molecularbiological methods to which extent REV–specific DNA was integrated into the DNA of fowlpox virus (FPV) that caused field–outbreaks in Germany during the recent years. For this purpose a multiplex–PCR, which detects the gene of the 4b–core–protein of FPV as well as gag–, pol– und env–genes of REV and the long terminal repeats of REV, was established. In most investigated DNAs the four sequences specific for REV were detected. Additionally a chimeric FPV–REV–PCR–product was found in most DNAs. This indicates the presence of an integrated, almost complete REV–provirus (fvRP). In addition three DNAs were investigated further by long–distance–PCR to confirm this. The detection of the integrated fvRP was not possible with primers specific for FPV that flank the integration site of the fvRP, but only with primers specific for the LTR of REV. By adjacent restriction enzyme analysis the result was confirmed. Furthermore the integration site of the fvRP in a field–DNA and in the vaccine strain HP B as well as the chimeric FPV–REV–PCR–product from the field–DNA were sequenced. By these means a remnant of the LTR of about 300 bp was detected also in the vaccine strain. Comparison of all three obtained sequences with published sequences showed only one single base–pair substitution. This demonstrated a high conservation of the integration site of FPV–strains from three continents.

A qPCR was established to determine the ratio between DNA specific for FPV and DNA specific for REV in some field–DNAs and in different passages *in vitro* of a field isolate. In all investigated field–DNAs the FPV subpopulation with integrated fvRP predominated. When passaged *in vitro*, the field isolate lost the fvRP at a rate of about 0.5 (50 %) until the 16th passage. After 32 passages no DNA specific for REV was detectable by PCR.

Further extensive serological investigations of field sera for antibodies against FPV and REV were carried out. As there was no commercial ELISA available for the detection of antibodies against FPV, an indirect ELISA for this purpose was established. The sensitivity was 85.3 %, the specificity 100 %. Afterwards the repeatability of the obtained results of the ELISA was tested. The intraassay–coefficients showed a good repeatability. The interassay–coefficients were not satisfying. One explanation was an insufficient stability of the coating antigen when partly used plates were stored at 4°C resp. frozen a second time. In comparison to other methods for detecting antibodies against FPV the ELISA was marginally less sensitive than indirect immunfluorescence and more sensitive than AGP.

The investigation of sera from flocks that were exposed to natural infection with FPV for antibodies against FPV and REV showed a proportion of about 50 % with antibodies against FPV and a higher proportion with antibodies against REV. There were some differences between the flocks. A similar proportion of sera from flocks that were vaccinated against FPV had antibodies against FPV, but only in two of ten investigated flocks chickens had antibodies against REV. This makes clear, that the REV–antibodies were induced by the integrated fvRP and not by an accidental coinfection with REV. This also shows that most of German FPV–strains are able to induce antibodies against REV.

Two experimental infection studies were done to investigate the humoral immune response against FPV and REV after intracutaneous infection with different *in vitro* passages of a field isolate and with the vaccine strain HP B. In both trials the low passaged field isolate with the integrated fvRP induced no antibodies against FPV. The proportion of birds with antibodies against REV varied depending on the infection dose. In contrast to that the vaccine strain induced antibodies against FPV in all birds but not against REV independently from the infecting dose. The highly passaged field isolate, in which the fvRP could not be found by PCR, induced antibodies against FPV only in a few birds and no antibodies against REV. So it was assumed, that the integrated fvRP contributes to the suppression of the antibody response against FPV after an infection with a field isolate. In the first trial the birds were reinfected after five weeks by intravenous application of the low passaged field isolate. About half the birds that previously had been infected with the field isolate developed antibodies against FPV. The proportion of birds with antibodies against REV increased marginally. The birds that had been vaccinated previously showed no antibody response against REV. The reason for this may be that the vaccination prevented the replication of the FPV field isolate and so the development of antibodies. After the intracutaneous infection with the low passaged field isolate and after the intravenous reinfection DNA specific for REV was detected in the peripheral blood mononuclear cells. FPV–DNA could only be detected in one of all examined samples. This indicates that infectious REV–virions can be formed from the FPV field isolate with integrated fvRP.