comparative study of pathomorphological changes in lymphoid tissues induced by ALVs of different subgroup specificity, has not been performed.Using the chicken model we aimed at clarification of additional features, especially pathomorphological and immunological characteristics of the wasting disease induced by different strains of ALV-C. In order to achieve a proper comparison, also additional ALV subgroup (A,B,D) were included.

On the other hand, several retroviruses have been found to induce kidney tumors . MAV2 (N) is an avian nonacute oncogenic retrovirus of subgroup B ALV. In chickens infected in ovo or early after hatching it induces, with high efficiency, multiple clonal embryonic-type tumors of kidney - nephroblastomas (Watts and Smith, 1980). The model of MAV2 (N)-induced chicken nephroblastoma is based on the assumed ability of MAV2(N) retrovirus to transform cells by insertional mutagenesis, that is, by a deregulation of expression of genes hit by the proviral integration. This avian model of nephroblastoma represents a valuable tool for identifying genes involved in the malignant transformation of cells. It is assumed that macroscopic nephroblastomas arise by clonal expansion of blastema cells in which MAV2 (N) provirus has deregulated specific genes controling differenciation and proliferation (Pajer et al., 2003).

**Summary**

ALVs have a large potential to induce a broad spectrum of neoplastic and non-neoplastic disorders, particulary when congenitaly transmitted or intraembryonally inoculated, since this mode of infection provides optimal conditions for induction of persistent viraemia after hatching. Actively replicating virus reintegrates into the genome of target cells, which is a prerequisite for its accidental integration into the vicinity of a cellular protooncogene, leading finally to oncogene activation and tumour conversion of the cell. ALV-induced tumours could therefore be detected after a long latent period. In the meantime, however, the infected organism is continually supplied by a large quantity of viral antigens, which may disturb either directly or indirectly by so far unknown mechanisms different physiological functions.

The outcome of this disturbance are acute non-neoplastic diseases such as *wasting disease* detected soon after hatching. First of all, the role of age and the administered virus dose on ALV-C-induced wasting disease in chickens was examined. For efficient wasting disease induction, the embryonic stage of the host plays a critical role.The lowest virus dose

administered intraembryonally into chicken embryos and exerting the pathological activity approaches the value of 1 – 10 infectious units. As a quantitative parameter of the wasting disease we chose the body mass and lymfoid organ mass of chickens two weeks after hatching, when the disease was fully developed.An efficient induction of wasting disease in chickens by ALV, particulary by subgroup C, requires $>10^2$ infectious units inoculated in mid embryogenesis.

The subsequent effort of present study was to define the potential of several ALV-C virus strains to induce wasting disease within the first two weeks after hatching, and to compare it to other ALVs of subgroups A, B, and D.

In our case, a high virus dose was used for inoculation and all tested ALVs replicated well *in vivo.* Hence, they should readily produce wasting disease. Surprisingly, the opposite is true since ALV-A (RAV-1) and ALV-B (RAV-2), reaching the highest titres *in vivo,* exhibit no or moderate pathogenicity, respectively, and viruses of subgroups C and D, which replicate less efficiently, are highly pathogenic. Apparently, viral gene products participate in the acute pathogenicicity of ALVs and, of them, the Env protein appears to be the main candidate. An abundant knowledge has been accumulated so far on the role of the Env protein in retrovirus pathogenicity (reviewed in Svoboda et al. 2003).

When comparing the acute pathogenicity of ALV viruses of A, B, C and D subgroup specificity we have encountered notable differences among individual viral strains. The most distinctive changes were produced by viruses of subgroup C, among them only RAV-49 appeared less eficient in wasting disease induction. A moderate pathogenicity not accompanied by wasting was scored in ALV-B (RAV-2)- infected chickens, in which involution only of the thymus and bursa were found. The moderate pathogenicity of RAV-2 was surprising, as other viral strains employing the same receptor associated with cytotoxicity, MAV-2(O) of subgroup B (Hirota et al., 1980; Smith and Ivanyi, 1980) and RAV-50 of subgroup D (present study), were proven to be highly pathogenic *in vivo*. The data on RAV-2 and RAV-49 document that within both subgroups, B and C, significant differences in pathogenicity exist between individual viral strains and indicate that both, wasting and the lymphoid tissue alteration may be induced independently.

The most conspicuous histopathological changes were found in chickens infected with subgroup C and D, and less distinctively with subgroup B viruses. Alterations revealed in the thymus tissue of these animals, accompanied in some cases even with collapsed lobular architecture and slight fibrosis, resembled precocious puberty (Seemayer et al., 1984) or thymic

epithelial injury found in both children and adult patients suffering acquired immunodeficiency syndrome (Ye et al., 2004; Hazra and Mackal, 2005).

Detection of $p27_{gag}$ protein indicated replication of the virus in thymic epithelial cells. Expressed retroviral proteins may injure functions of this cellular compartment, which plays an important role in promotion and maturation of thymocytes through the action of secreted humoral substances or through the direct contact with thymocytes (Lobach and Haynes, 1987).

An observed increase of $TCR2^+$ T cells in the thymus might represent a consequence of such functional thymic disturbance, the detailed mechanism of which remains, however, unclear. An essential feature was depletion of lymphocytes in the thymus. bursa and spleen. While the number of dendritic cells in the bursa was increased, their representation in the thymus and spleen was reduced. In the spleen, however, the reduction of dendritic cells concerned only an elipsoid compartment, which in itself was also markedly reduced. The most remarkable changes in the spleen were encountered again in chickens infected with subgroup C and D viruses. Namely ALV-C viruses produced an impairment of PELS(peri-ellipsoid lymphocyte sheaths),considered for a functional equivalent of the mammalian marginal zone (Jeurissen et al., 1992) and PALS regions (peri-arteriolat lymphocyte sheaths), which is characterized by a marked reduction of B- and T cells with increased $CD8^+$ and depleted $CD4^+$ lymphocytes. These changes resembled both damage of the marginal zone and shifts in $CD8^+$ and $CD4^+$T-lymphocyte representation in the spleen of HIV-infected patients (Wilkins et al., 2003). An increased numer of macrophages in the thymus and spleen corresponded with the observed general activation of the monocyte-macrophage system. However, intact ellipsoids in RAV-1- and RAV-2- infected chickens would indicate some selectivity of this cellular compartment in responding to different viral antigens.

Morphological alteration of lymphoid organs of diseased chickens indicated a heavily impaired immune system. Ratio $CD4^+/CD8^+$ T lymfocytes in peripheral blood decreased under value 1 in chickens infected with ALV-C and ALV-D, while viruses of subgroup A and B do not induced significant decrease of this ratio. We have no data about cytotoxic activity increased $CD8^+$ lymfocytes, but it is possible that these cells don´t lack such activity. Zinkernagel and Hengartner (1994) thought, that cytotoxic $CD8^+$ T cells could be responsible for destruction of infected cells of immune system, in late stages of HIV, and by this way they could be able to induced immunodeficiency.

In accord with altered bursal morphology was a finding of severe impairment of humoral immunity in ALV-C-infected chickens that failed in mounting an antibody response to

*Brucella abortus* antigen. The severe alteration of lymphoid tissues agreed well with the inefficiency of splenic lymphocytes isolated from ALV-C-infected chickens in responding properly to concanavalin A. On the other hand, two weeks after hatching ALV-A-infected chickens have apparently preserved intact humoral immunity, and splenic lymphocytes of ALV-A-infected chickens responded to stimulation by concanavalinA, which is in accordance with absence of morphological changes of lymphoid organs in these chickens.

The affinity of ALVs for lymphoid tissues resulting in severe morphological alteration of the thymus, bursa and spleen followed by subversion of immune regulatory functions appears to be an unifying aspect of ALV acute pathogenicity. However, in tissue sections from infected animals irrespective of the viral strain, only some B lymphocytes stained positively for the viral Gag protein, indicating active viral replication. Instead, other lymphoid cellular compartments such as macrophages, thymic epithelium, follicular dendritic cells and splenic ellipsoid cells were largely infected. In the pathogenesis of ALV-induced immune damage the direct toxic effect of viruses, namely those of subgroup B and D, has been invoked. However, the direct toxicity may not be a principal cause of lymphoid tissue depletion; more likely lymphocytes are attacked in an indirect way, as it has been shown in another retroviral infection (Alimonti et al., 2003).

The striking differences in acute pathogenicity of individual strains of ALVs offer a unique experimental system for analysis of viral genome regions playing a significant role in establishing wasting disease. Based on analysis of pathogenic activity chimeric viruses between ALV-A and ALV C, gene *env*, respectively its surface unit, is the primary determinant of pathogenic response resulting from ALV infection. The other regions of viral genome have only slight module effect, especially C (constant region). All observed parameters of wasting disease (reduction of body mass, involution of thymus, bursa and spleen, also ratio $CD4^+/CD8^+$ T lymphocytes in peripheral blood) was exhibited in chickens infected with chimeric viruses of subgroup C specifity.

Despite a large body of our knowledge about adverse effects of retroviral infections, the mechanism responsible for wasting disease in infected hosts has not yet been fully clarified. In this respect, comparative studies of the wasting disease and immunosuppression employing different experimental models appear valuable, because they could bring new insights into the pathogenesis of acute retrovirus-induced diseases.

Contrary to pleiotropic effects of wasting disease, *nephroblastomas* represents a retrovirus pathogenic manifestation within one cell transformed in consequence of retroviral

insertional mutagenesis and give rise to macroscopic tumor of kidney.

Morphological picture of nephroblastomas confirms, that this group of clonal tumors is very heterogenous. The most prominent alterations that was observed were imperfectly differentiated tubules-with or with-out glomeruli, smaller or larger aggregations of unorganized and apparently undifferentiated cells and unusual spherical formations not known from normal nephrogenesis but reminiscent of origins of the normal tubule formation. We call them nests of pseudonephrogenesis. In more differentiated nephrons supplemented with glomeruli, the cystic dilations of nephron tubular segments appeared frequently. Based on qualitative and quantitative representations of these structures, samples were divided into four major classes, 0, I, II, and III. Class 0 samples represent infected tissue with a prevalence of normal renal structures and sporadic cystic dilations of tubules. The nests of abnormal nephrogenesis, which were considered the most evident symptoms of malignant transformation, were missing in class 0, but were constantly present in all other classes. Classes I and II included tumors with more or less differentiated nephrons, respectively, various numbers of cystic dilations of tubules, and a growing proportion of unorganized cells. Samples belonging to class III contained only the nests of pseudonephrogenesis and unorganized cells.

There was a correlation between the tumor morphology class and the tumor size. Forty nephroblastomas were distributed into classes I to III, as described above, and ordered according to their mass. In general, class III members, the least differentiated nephroblastomas, clearly displayed greater size, although a rather high size variation within each class was registered. We suggest that the size variation is mainly caused by different growth rates of each individual tumor clone and not by a different time of a target cell infection because the pool of target cells for transformation (nephrogenic blastema cells) fades away rapidly within the first few days after hatching.

It has been assumed that the malignant renal cell arises as late as several specific regulatory pathways in it have been distorted by MAV2 integration. Since these pathways are constituted by cascades of functionally connected genes, the provirus does not have to hit one particular gene in order to deregulate the pathway. That is probably why the efforts to identify the crucial nephroblastoma-specific integrations had only a limited success (Westaway et al., 1986; Joliot et al., 1992). That is also probably why nephroblastomas display histopathological variations, since distinct deregulated genes have a different impact on the phenotype of transformed cells. Nevertheless, deregulation of some specific genes might contribute to malignant transformation more efficiently than activation/inhibition of others, and such genes should constitute a set of 'common integration sites' of the MAV2 provirus in nephroblasto-

ma. Since retroviral integration is in principle site-unspecific (Brown, 1997), the existence of a common site of integration found in a limited number of independent tumor clones must be a result of the selection process: only cells in which the MAV2 provirus has hit the proper gene or combination of genes give rise to a tumor.

Analysis of integration sites have revealed the transcriptional factor *foxP1* to be one of the common sites of MAV2 proviral integration in chicken nephroblastoma.The integration events are clustered around the second coding exon. Therefore N-truncated protein could arise. On the one hand, full-length FoxP1 is consider to be a tumor suppressor gene in epithelial malignancies (Banham et al., 2001), on the other hand N-truncated small isoforms seems to contribute to oncogenic transformation (Brown et al., 2008).

Chicken *foxP1* included several alternative promotors and alternative splicing sites, for that reason various foxP1 isoform could arise. A level of foxP1 mRNA in nephroblastomas was equivalent, no increased gene expression influnced by retroviral integration was observed. Differences were noticed in the protein expression, nuclear or cytoplasmic location of FoxP1 was detected. It is possible that the expression of alternatively spliced Foxp1 proteins may explain the presence of cytoplasmic staining that was observed in some tumors (Fox et al., 2004). The virally altered *foxP1* might interfere (in a dominant-negative fashion) with a normal function of the gene and support malignant transformation.