Original Articles

Intraembryonic Avian Leukosis Virus Subgroup C (ALV-C) Inoculation Producing Wasting Disease in Ducks Soon after Hatching

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Abstract. We have studied the pathogenic changes in Khaki Campbell ducks injected in mid embryogenesis with ALV subgroup C virus *td* daPR-C derived from a molecular clone. The employed duck flock was shown to be highly genetically homogeneous and was controlled for the absence of current infections. Clear symptoms of wasting disease, which appeared since one week post hatching, represented the early consequence of the virus infection. They were manifested by decreased body weight, including clear involution of thymic tissue and pronounced anaemia. Microscopically, thymuses of infected animals displayed lymphatic depletion, clearly visible in the lobular cortex. Similarly, in the bursa Fabricii follicles, a marked reduction of the cortical layer and a decrease in folicullar centres was revealed. A decrease in the antibody response correlated with bursa Fabricii atrophy. The clear signs of anaemia were confirmed by haematological measurements, red blood cell count, haematocrit value and haemoglobin included. On the basis of these and additional observations we propose that inoculation of duck embryos provides a suitable model for analysis of the wasting disease produced by ALV-C.

Avian retroviruses and later retroviruses isolated from mammals and other animal classes have been studied preferentially from the point of view of their oncogenicity. Later, it became apparent that in addition to either acute or delayed tumorigenic activity, retroviruses are responsible for a broader spectrum of pathogenic activities (Coffin et al., 1997).

Avian leukosis viruses (ALV) represent the first thoroughly investigated retroviral group, their pathogenicity included. They were classified according to their envelope glycoprotein properties to several subgroups (A through J) (Payne et al., 1992). Viruses belonging to each subgroup interact with a respective receptor on the cell surface, which allows virus entry into the cell. As revealed recently, receptors of subgroup B viruses can be engaged in producing cytopathic consequences after binding to its cognate receptor, which is homologous to the tumour necrosis factor receptor (Brojatsch et al., 1996). It has been already revealed that subgroup B produces suppression of blastomogenic response to phytohaemagglutinin (Smith and van Eldik, 1978; Rup et al., 1982). Especially MAV–2(O), subgroup B helper virus derived from avian myeloblastosis virus (AMV), was shown to produce prominent symptoms of wasting disease in chickens that had been infected intraembryonally (Hirota et al., 1980; Smith and Ivanyi, 1980). This wasting disease was accompanied by a decrease of body weight and by bursal and thymic tissue hypoplasia.

Reticuloendotheliosis viruses (REV) represent another retrovirus group encountered in avian species. These retroviruses are most probably of mammalian origin and have been heterotransmitted to avian hosts relatively recently (Martin et al., 1999). They were shown to produce wasting disease characterized by thymus reduction, anaemia and immunosuppression when injected to neonatal chickens (Mussman and Twiehaus, 1971, Witter 1984). The involvement of viral genome regions in producing REV pathogenicity was approached by Filardo et al. (1994), who on the basis of recombinant study concluded that co-operative *gag* and *env* gene action is of primary importance.

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Abbreviations: ALV – avian leukosis virus, AMV – avian myeloblastosis virus, DEF – duck embryo fibroblasts, DHBV – duck hepatitis B virus, HE – haematoxylin-eosin, PBS – phosphate-buffered saline, REV – reticuloendotheliosis virus.

In this study we investigated the outcome of experimental retrovirus heterotransmission using chicken ALV-C for infection of duck embryos.

Despite the ALV-C absence in ducks under natural conditions, this virus infects and replicates efficiently, in contrast to other subgroups, in duck cells which harbour a so far unidentified subgroup C receptor (Payne et al., 1992). Previously, we have also demonstrated that ALV-C can establish long-term persistent infection in the duck host (Trejbalová et al., 1999).

Here we report that ALV-C inoculated in mid embryogenesis causes clear symptoms of wasting disease soon after hatching and is also responsible for additional pathogenic consequences, including haematological disorders.

Material and Methods

Experimental animals and breeding conditions

Khaki Campbell ducks employed in this study were originally obtained from the Institute for Poultry Breeding and Improving at Ivanka pri Dunaji, Slovakia, in 1958. This flock underwent serial selection steps of brother x sister mating and was found to be genetically homogenous using SAA polymorphic markers (Stepanets et al., 2001).

The animals are regularly examined by the state veterinarian. The presence of pathogens such as Salmonella, Pasteurella, and Mycobacterium were tested recently in our flock of ducks with a negative outcome. The presence of an aetiological agent inducing duck hepatitis type I (picornavirus) can also be excluded, since no changes were observed in 9-day-old chicken embryos inoculated into the allantoic sac with duck liver homogenates (Woolcook and Fabricant, 1997). The absence of duck hepatitis B virus (DHBV) sequences in the liver from DNA of our ducks was also documented (Stepanets et al., 2001). Moreover, no typical inflammatory or degenerative changes in the liver tissue were found by histologic examination, further confirming the absence of a specific hepatitis-inducing agent in our flock of ducks (data not shown). No tumour formation has ever been observed, either.

Inoculation of duck embryos with ALV-C

Khaki Campbell duck embryos were inoculated essentially as described previously (Nehyba et al., 1990). Briefly, duck embryos in mid embryogenesis (14^{th} day of incubation) were infected by injection of 100 µl of the virus (10^5–10^7 i.u.) into the chorioallantoic vein. The virus used represented the progeny of a molecular clone of a transformation-defective mutant of the Prague strain Rous sarcoma virus (referred to as *td* daPR-C), obtained by transfection of duck embryo fibroblasts (DEF) (Geryk et al., 1996). For inoculation, the virus passaged once on DEF was used. The origin of the molecular clone of *td* daPR-C and its nucleotide sequence was presented by Kashuba et al. (1993).

In control sham-inoculated animals, instead of virus suspension, 100 µl of culture medium were injected into the chorioallantoic vein of duck embryos in mid embryogenesis.

Immunization of ducks with heat-inactivated Brucella abortus

Ducks were immunized with *Brucella abortus* following an essentially established protocol (Hašková and Svoboda, 1959). Briefly, at the age of 28 days, preimmune sera were collected from all experimental animals. Starting the age of 33 days, ducks were inoculated with heat-inactivated *Brucella abortus*, strain Weibridge S99 (kindly provided by Dr. P. Plačkov, Bioveta Ivanovice na Hané, Czech Republic), twice a week for a period of one month. Together, nine injections of the bacterial antigen were applied to ducks. A single immunization dose represented 6 x 10^7 bacteria per 10 g of the body weight and was administered in 0.5 ml of phosphate-buffered saline (PBS) intravenously into the jugular vein. The dose, which was calculated separately for control and infected ducks on the basis of the average body weight of animals determined at the age of one month, was used for the whole immunization period. Three days after the last immunization, duck sera were collected for determination of antibody titres, which was performed as follows: 0.5 ml of sera serially diluted with PBS were mixed with 0.5 ml of Brucella Abortus Antigen for Slow Agglutination (Bioveta, Ivanovice na Hané, Czech Republic) 10x diluted with PBS, thoroughly mixed, and incubated 20 h at 37ºC, then 1 h at room temperature**.** Pronounced agglutinates with either pellucid or slightly opalescent supernatant were scored as positive reactions (+++ and ++, respectively). The titres were expressed as reciprocals of the terminal serum dilution producing the positive reaction.

Haematology and histology

The birds were bled from the jugular vein into EDTA K tubes (Sarstedt, Nürnbrecht, Germany) just before sacrifice and blood samples were subjected to analysis in a Coulter ONYX machine (Beckman Coulter, Inc., Fullerton, CA). Since this machine has not been equipped with a software allowing it to count the real number of animal erythrocytes, we had to first define a coefficient for correlation of the obtained data. The correlation coefficient, the value of which was set down to 0.65, represents the ratio of a mean erythrocyte count determined by other techniques to the mean count determined by the Coulter machine, in blood samples from 10 control and infected ducks of the same age as experimental animals. Accordingly, the final values represent erythrocyte counts obtained by the Coulter machine multiplied by 0.65.

All sacrificed and dead animals were subjected to necropsy. Organ samples were fixed in 4% buffered formalin, pH 7.2, for at least 24 h, embedded in paraffin, stained with haematoxylin and eosin (HE).

Apoptosis assay

Tissue portions of thymus were excised from ducklings at the age of one day and 13 days, preserved in 10% buffered formalin and embedded in paraffin. Tissue sections of 5 μm were mounted onto slides and stained for apoptotic cells by the TUNEL assay, using Apop Tag-Peroxidase kits (Oncor, Gaithersburg, MD), following the manufacturer's recommendations. Slides were counterstained with methyl green.

Thymic apoptosis was determined by microscopy, enumerating apoptotic cells in five power-fields per slide (magnification 600x), and is expressed as means ± SD in % of total cells per field.

Statistical data analysis

The time profiles of changes were statistically investigated within control (uninoculated and sham-inoculated) animals and ALV-C-infected animals. Data fractionated in this way required simultaneous application of different statistical procedures suitable for comparative evaluation from both quantitative and qualitative viewpoints.

In the case of data with asymmetric distribution (e.g. growth kinetics data), standard logarithmic transformation was used prior to application of any parametric methods (ANOVA, t-tests) (Parkin and Robinson, 1992). If necessary, the final estimates of the geometric mean were corrected to reduce bias due to small n (Kleijnen, 1987; Parkin et al., 1990).

Evaluation of time profiles of the followed parameters predominantly involved one-way ANOVA models testing the differences among experimental variants under verified hypotheses of normality of distribution and homogeneity of variance. Tukey multiple-range test or standard two-sample t-test were applied subsequently for detailed inspection of differences among variants.

Results

Signs of wasting disease in ducks intraembryonally infected with ALV-C

When following the ALV-C-infected ducklings soon after hatching, we noticed their growth retardation accompanied by high mortality. To get better insight into the ALV-C pathogenicity in ducks, we first measured the kinetics of the body growth during the first month after hatching in animals infected intraembryonally or post hatching and compared it with controls. As documented in Table 1, no significant differences were found between three categories of animals – uninoculated controls, sham-inoculated controls, and post-hatching infected ducks. In comparison to these animals, about 50% mortality and a significantly lower body mass were observed in ducks inoculated with ALV-C intraembryonally. In all animals that died within three weeks after hatching (24 ducks), we noted an almost complete stagnation of the body mass, whereas in surviving animals (20 ducks), significant growth retardation appeared starting the second week of their life. This retardation, however, was usually surmounted several months later (almost around the 6–8th month of age), when infected ducks reached the same body mass as controls.

In most cases, the surviving animals were retarded in growth and cachectic, with only a very thin subcutaneous fat layer. Their muscle system was hypoplastic, the liver slightly enlarged and pale. The red bone marrow had not been replaced by fatty marrow inside the bone cavities, except for the pneumatic bones.

These data clearly indicate that the vital functions of the duck organism were severely altered by the replicating virus administered intraembryonally, but not post hatching. The role of the virus is further underlined by the observation that unspecific intervention during the embryonic period, represented by inoculation of medium instead of the virus, did not have a negative effect upon the growth of ducklings (Table 1). What factors contribute to the survival of some infected animals is not known. We compared the degree of viraemia in a small group of one-week-old ducks, which included both wasting and almost normally developed animals, and found no marked difference.

Pathologic changes of lymphoid tissues, haematologic disorders and lowered immunologic reactivity of ducks intraembryonally infected with ALV-C

All dead ducklings were dissected to identify pathologic changes associated with ALV-C infection. The most prominent findings were thymus atrophy and an increased size of the spleen. To characterize these findings quantitatively, a new experimental group of ducks was established. Animals were sacrificed at 1st, 4th and 8th week after hatching and the relative masses of their organs were determined.

As shown in Fig. 1, the relative mass of the thymus in ALV-C-infected ducks was significantly lower when compared to control animals during the whole observation period. On the other hand, however, the relative masses of the bursa and spleen of infected animals were significantly increased during 8 weeks of observation.

To better understand the gross pathologic changes found in infected ducks, a detailed histologic examination of affected tissues was performed with samples collected from groups given in Fig. 1 and from dead animals.

The most conspicuous changes were encountered in the thymus. In contrast to the controls (Fig. 2A), the thy-

Table 1. Kinetics of body growth of control and ALV-C-infected ducks

Ducks[a]	Sample size (n)	Body mass (g)[b] Week 0	Week 1	Week 2	Week 3	Week 4
Controls uninoculated	41	**41.1** (39.7; 42.4)^{A}	**97.5** (98.2; 102.2)^{A}	**227.1** (211.4; 242.8)^{A}	**452.6** (426.8; 478.4)^{A}	**629.2** (591.6; 666.8)^{A}
Controls sham-inoculated	21	**39.1** (36.1; 42.1)^{A}	**98.3** (87.7; 108.9)^{A}	**211.9** (193.7; 230.1)^{A}	**435.7** (402.3; 468.9)^{A}	**603.1** (542.5; 663.7)^{A}
ALV-C infected post-hatching	18	ND[c]	**96.3** (90.5; 102.1)^{A}	**243.7** (229.2; 258.3)^{A}	**452.3** (429.5; 475.1)^{A}	**667.1** (639.7; 695.5)^{A}
ALV-C infected intraembryonally - surviving	20	**40.0** (39.3; 40.7)^{A}	**81.1** (70.1; 92.1)^{A}	**167.2** (144.6; 190.1)^{B}	**275.5** (222.7; 328.3)^{B}	**409.6** (331.1; 488.1)^{B}
ALV-C infected intraembryonally - died	24[d]	**40.3** (39.8; 40.8)^{A}	**48.0** (40.5; 55.5)^{B}	**69.8** (58.5; 81.1)^{C}		

[a]In addition to uninoculated control ducks, also ducks inoculated intravenously in mid embryogenesis with 0.1 ml of culture medium were included in the experiment (sham-inoculated). Similarly, two types of ALV-C-infected animals were analysed: infected post-hatching (10^7 IU/0.1 ml of the virus inoculated into the leg vein on the day of hatching) and infected intraembryonally (10^7 IU/0.1 ml of the virus inoculated intravenously in mid embryogenesis). [b]Mean values are supplied with 99% confidence limits (in parentheses). Within a single column, estimates followed by the same superscript letter (A, B or C) are not significantly different (one-way ANOVA followed by Tukey multiple range test; $P < 0.01$). [c]Not determined. [d]The animals died mostly after the first week. In the second week, 9 animals were still alive; however, none of them survived until the third week of age.

muses of infected animals showed pronounced lymphatic depletion, clearly visible in the lobular cortex (Fig. 2B). On the other hand, the medulla was slightly enlarged, similarly as Hassal's corpuscles, in which regressive changes were visible, accompanied by pronounced vacuolization and, in some cases, by cell degeneration. Particularly, in the first week after hatching, a boundary between the cortex and the medulla mingled.

The spleens of the infected animals displayed mild swelling and hyperaemia. A moderate hypertrophy of white pulp has also been observed. In bursa Fabricii follicles, a marked reduction of the cortical layer and a moderate decrease in folicullar centre lymphocytes were observed (Fig. 2C, 2D). These changes were not reflected by a decrease of bursa Fabricii mass (Fig. 1). This might have resulted from fibrotization of perifollicular connective tissue that we have observed. Other organs were pale and did not reveal any pathological changes.

Using the TUNEL assay, we measured the degree of apoptosis in young animals. In thymic samples that were obtained from one-day-old ducklings inoculated intraembryonally with retrovirus, i.e. before any signs of thymus involution appeared, we found $1.7 \pm 0.4\%$ of cells ApopTag positive, as compared to $0.2 \pm 0.06\%$ positive cells in control animals at the same time. Thus, a marked increase of apoptotic stain (8.5-fold) was found in the thymus of ducklings that were inoculated with retrovirus (Fig. 3A, 3B).

Comparison of samples from thymic tissues, at an age when growth retardation in differently treated ducklings became visible (around day 13), revealed similar staining for apoptosis, showing about 0.5% of cells being apoptotic in both retrovirus-inoculated and control animals (data not shown).

The general paleness of organs observed in ALV-C-infected ducklings indicated disorders of haematopoiesis. Therefore, in all animals of this experimental group the blood was collected just before sacrifice and some haematological parameters (red blood cells count, haematocrit value, and haemoglobin) were determined. As shown in Fig. 4, values given for infected animals were significantly lower when compared to controls during the whole observation period, i.e. during the first two months after hatching. These findings document that the anaemia, which was fully developed already in the first week after hatching and which persisted over the 8th week of age, can represent one of the important factors contributing significantly to the growth retardation of the infected ducks.

Based on the striking alteration of lymphoid tissues in ALV-C-infected ducks, we also anticipated functional insufficiency of duck immunity. To verify this hypothesis and to analyse at least the humoral component of the duck immune system, we immunized by repeated administration of *Brucella abortus* both, control and ALV-C-inoculated ducks. Data given in Table 2 clearly point out the lower capacity of two-month-old infected ducks to mount potent humoral response against the bacterial antigen. It should be noted that none of both, control and infected duck preimunne sera contained natural agglutination antibodies directed aginst *Brucella abortus* (not shown).

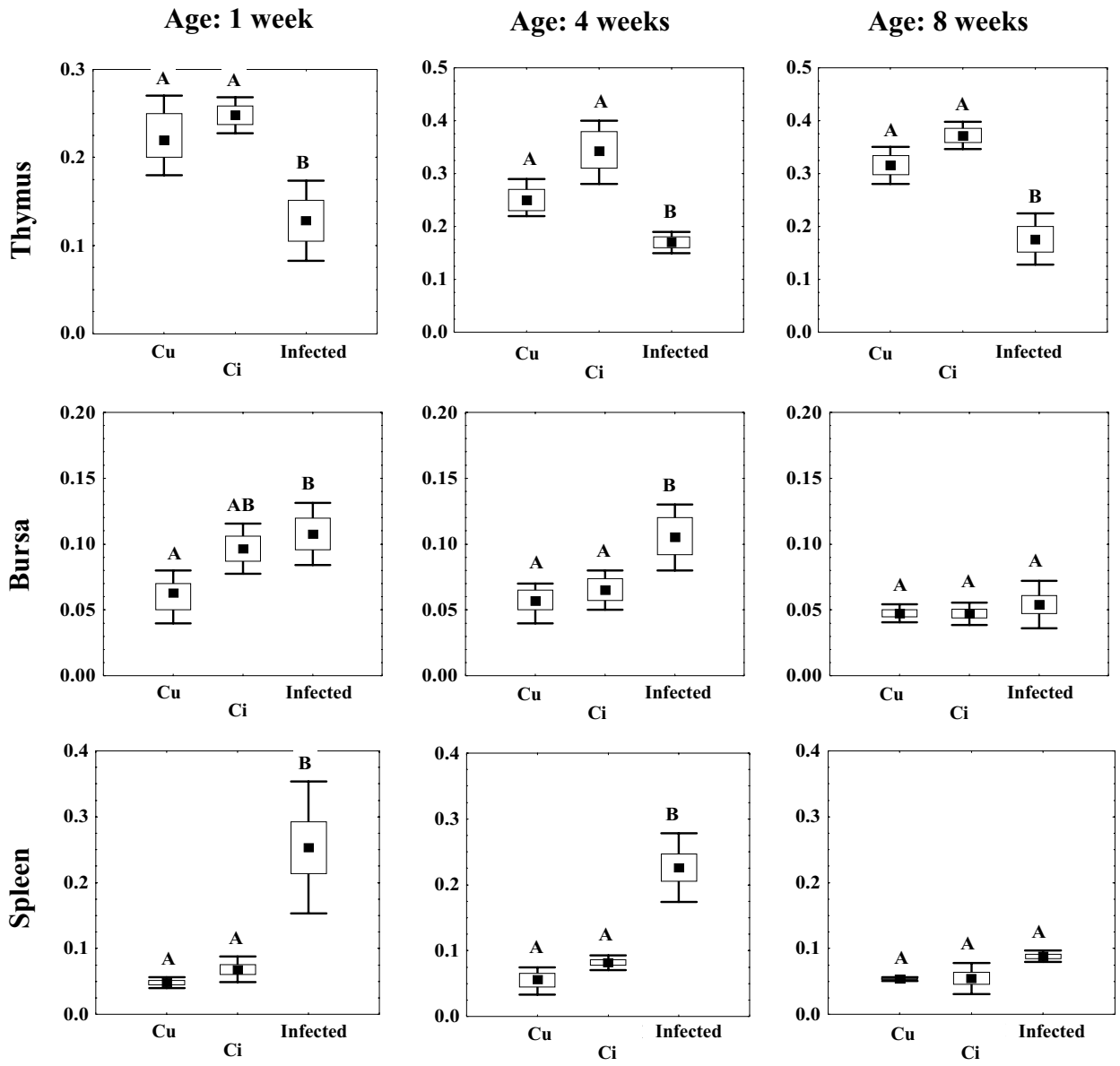

Fig. 1. Comparative evaluation of weight (in g) of selected organs in control and ALV-C-infected ducks. Ordinate: Cu (control uninoculated ducks), Ci (control sham-inoculated ducks - inoculated with 0.1 ml of culture medium intravenously in mid embryogenesis), infected (inoculated with 10^7 IU/0.1 ml of ALV-C intravenously in mid embryogenesis). Abscissa: organ weights expressed as the ratio of organ to body weight x 100. Points: mean value; boxes: standard error of mean estimate; whiskers: 99% confidence limits of mean estimate. Within each sampling time and organ separately, the compared experimental variants (Cu; Ci; infected) marked by the same capital letter are not significantly different ($P < 0.01$). In each experimental group and in each sampling time 5–7 ducks were examined. The only exception were groups of sham-inoculated controls aged 1 and 8 weeks, in which only three animals were included.

Discussion

In this report we have focused on the specific early pathogenic consequences of ALV-C infection of ducks. We have selected this foreign host because it is lacking endogenous retroviral loci (ev) (Frisby et al., 1979), and mainly because even after prolonged observation, no signs of avian leukosis have been observed. Therefore, the pathogenic activity of ALV-C can be evaluated, thus excluding a bias of secondary effects of tumorigenesis (Payne, 1970). In addition, ALV-C heterotransmission should serve as a suitable model for the study of consequences resulting from infection of a foreign host, which became an important issue of retrovirus research (Weiss and Vrangham, 1999).

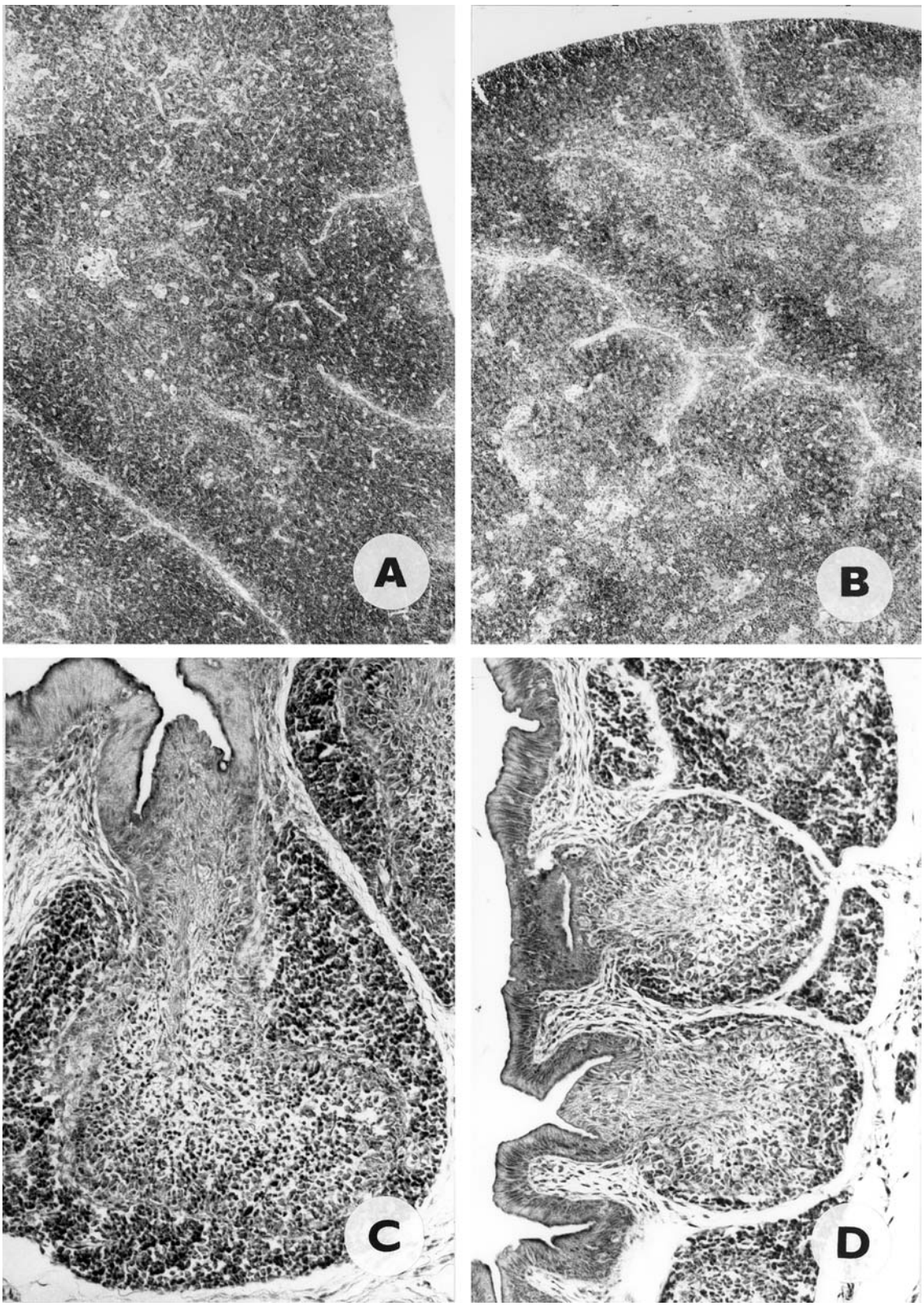

Fig. 2A. Normal structure of thymus in a control 13-day-old duck. Cortex and medulla contain many lymphocytes. HE, magnification 100x.
Fig. 2B. Severe lymphatic depletion of thymus in an ALV-inoculated 13-day-old duck. Marked lymphocytic reduction of cortex. HE, magnification 100x.
Fig. 2C. Normal structure of bursa in the control 13-day-old duck. Cortex and medulla of follicles are rich in lymphocytes. HE, magnification 210x.
Fig. 2D. Marked atrophy of bursa in the ALV-C-inoculated 13-day-old duck. Lymphocytic depletion in cortical parts of follicles is clearly visible. HE, magnification 210x.

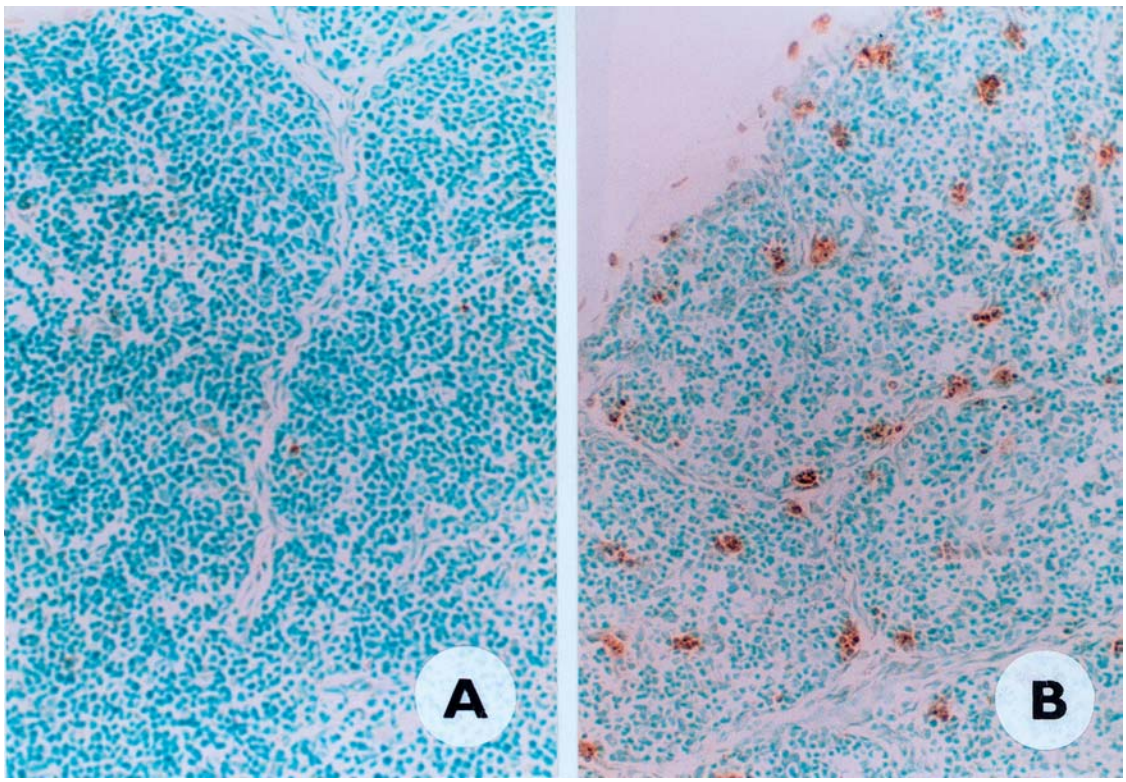

Fig. 3A. Thymus of a control newly hatched duckling stained for apoptosis. Magnification 200x.
Fig. 3B. Thymus of an infected newly hatched duckling stained for apoptosis. Magnification 200x.

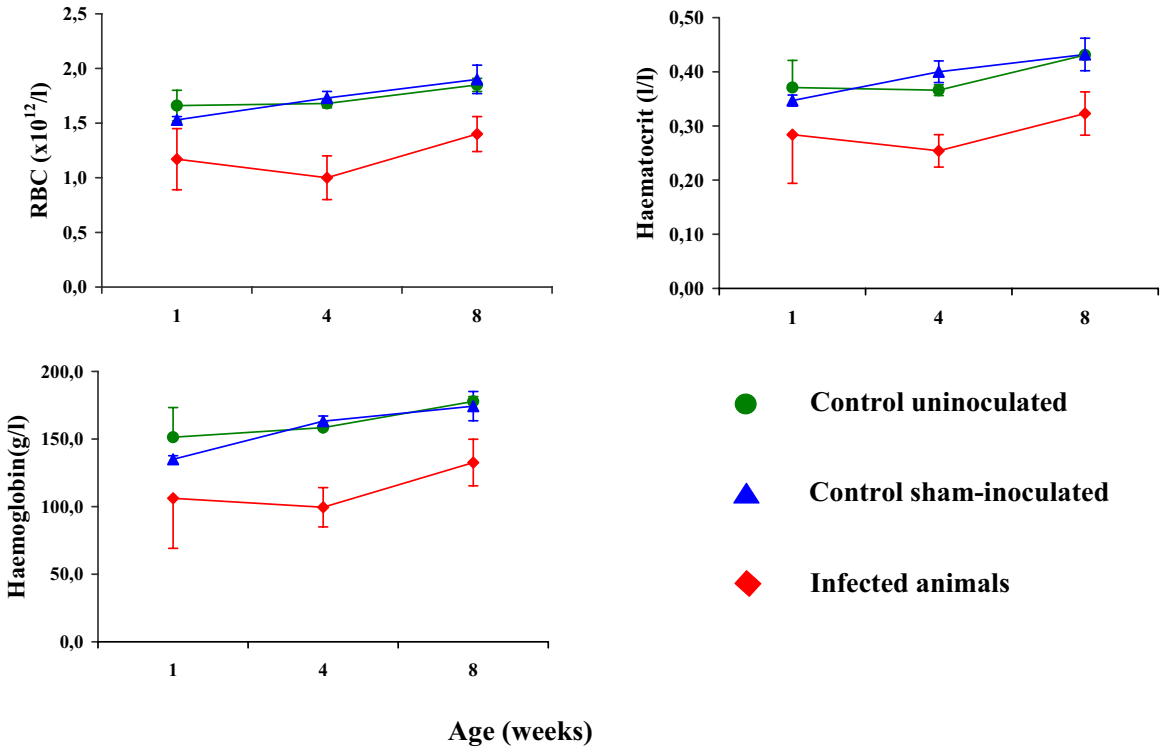

Fig. 4. Comparative evaluation of selected parameters of anaemia in control and ALV-C-infected ducks.
Ducks were bled at indicated times from the jugular vein and anaemia parameters were determined using a Coulter ONYX machine. RBC – red blood cells. For determination of RBC counts, the values obtained by the Coulter machine were multiplied by a correlation coefficient 0.65 (for details see Material and Methods). For the number of analysed animals see text to Fig. 1. Ducks were bled just before sacrifice. In infected ducks all the measured values within each sampling time were significantly different from the values identified in control animals ($P < 0.01$), whereas mutually, values in control uninoculated and sham-inoculated ducks were not significantly different.

Table 2. Immunologic response of ducks to Brucella abortus antigen[a]

Ducks[b]	Sample size	Titres of antibodies[c]
Controls uninoculated	5	**1 408.0** (514.5; 2301.5)[A]
Controls sham-inoculated	5	**1024.0** (588.7; 1459.3)[A]
ALV-C-infected intraembryonally - surviving	9	**355.6** (221.1; 489.9)[B]

[a]Ducks were inoculated twice a week with heat-inactivated *Brucella abortus* (6 x 10^7 cells/0.5 ml PBS/10 g of body weight). The first immunization dose was administered into the jugular vein of 33-day-old ducks. Altogether nine injections were performed, the last being applied at the age of 60 days. [b]For explanation see text to Table 1. [c]Sera were collected on the third day after the last injection of *Brucella abortus*. The titre of antibodies was determined in serially diluted sera using inactivated *Brucella abortus*. 100–50% efficiencies of *Brucella abortus* agglutination with pellucide or slightly opalescent supernatant were scored as positive reactions (+++ and ++). The titres are expressed as reciprocals of the terminal serum dilution producing the positive reaction. Mean values are supplied with 99% confidence limits (in parentheses). Estimates in the column followed by the same superscript letter (A or B) are not significantly different ($P < 0.01$).

In agreement with our previous observation (Karakoz et al., 1980a, b; Bozhkov et al., 1987) made on chickens, we found profound anaemia during the first weeks post hatching in the intraembryonally infected animals. The anaemia encountered in ducks does not appear to be haemolytic, because of splenomegaly absence. In further experiments we have to decide whether this anaemia corresponds to the aplastic or hypochromic type.

Serious anaemia was described by Smith and Schmidt (1982) after inoculation of newly hatched chickens with ALV subgroups B and D. On the contrary, RAV-7 as a representative of subgroup C produced very mild signs of this disease, significant in only two points of the observation period.

Why the ALV-C in our hands produced severe anaemia and pathologic changes in other lymphoid organs? There are two factors that should influence the outcome of ALV-C infection. First, we inoculated the virus in mid embryogenesis (14^{th} day of incubation), which made possible an efficient spread of infection before immunological maturity. Immunologically immature animals are in general more sensitive to pathogenic agents. Second, for our experiments we employed molecularly cloned *td* daPR-C. It represents the PR-RSV-C transformation-defective mutant (*td*) lacking the v-*src* coding region. In addition, this mutant was isolated from RSV repeatedly passaged in duck cells, where it acquired the ability to replicate the transforming virus (da). When compared with PR-RSV-C, *td* daPR-C differed in a set of single nucleotide substitutions (Kashuba et al., 1993), which might contribute to its pathogenicity. In additional experiments not described in this paper we also tested the consequences of intraembryonic inoculation of *td* PR-C (Vogt, 1971) that has not been passaged in ducks. Using this mutant we have also observed wasting and increased mortality of infected animals, but with some delay. At the age of five months, 63% of *td* PR-C-infected animals died as compared with 90% of *td* daPR-C-infected ones. Controls either non-infected or sham-inoculated exhibited only low mortality (14 and 18%, respectively). From both the observation of chickens (Karakoz et al., 1980a) and ducks, the pathogenic activity of *td* daPR-C was more pronounced than that of *td* PR-C, which suggests that various ALV-C strains differ in their pathogenicity *in vivo*. What differences are involved is not known, but it is likely that they are not due to the virus ability to replicate in ducks, because we have previously shown that both *td* PR-C and *td* daPR-C reach the same titres after infection of duck fibroblasts (Schimakage et al., 1979; Geryk et al., 1980). Relevant to the pathogenicity of ALV-C *in vivo* are also recently obtained results revealing that in addition to ALV subgroups B and D, subgroup C also produces cytopathic plaques on cells of the DF-1 chicken cell line (Himly et al., 1998; Schaefer-Klein et al., 1998). We also tested the immunosuppression in infected ducks by immunization of infected and control animals with *Brucella abortus*. There has been a significant decrease in antibody production in the infected animals, which agrees with the pathologic changes found in bursa Fabricii.

Thymus atrophy represents a further important consequence of ALV-C infection of ducks. This was confirmed by a significant loss of thymus weight and lymphatic depletion in the thymic lobular cortex, as well as by regressive changes in Hassal's corpuscles, visible especially 7 to 21 days post hatching. This agrees with our previous histochemical observation, which demonstrated that the virus was expressed in thymus and in Hassal's corpuscles (Trejbalová et al., 1999). Interestingly enough, similar changes in the cortex were found 14 days after inoculation of pathogenic SIV in juvenile rhesus macaques (Wykrzykowska et al., 1998). These changes were later rebounded. This fits again with our observation, because our experimental animals surviving three weeks later regained normal thymus sizes. In the SIV experiment the peak of apoptosis in thymus was revealed 14 days after inoculation, which corresponds to early occurrence of apoptosis in our infected animals.

We can exclude that any other pathogenic virus responsible for the wasting disease symptoms was present in ALV-C inoculum, because we injected the progeny of *td* daPR-C obtained from a molecular clone. The

animals employed for the experiments were tested for the absence of common pathogens, DHBV included, but they were not reared under specific pathogen-free conditions, these not yet being developed for ducks.

As a result of all the above-mentioned pathologic changes, the characteristic picture of the wasting disease emerged, accompanied by a serious loss of weight. In spite of the fact that two thirds of infected animals died during three weeks after hatching, the remaining animals, also suffering a loss of weight, recovered during the following months and represent the carriers of persistent virus infection (Trejbalová et al., 1999). Thus, ALV-C inoculation of duck embryos appears to provide a suitable model for further characterization of ALV-induced immunodeficiency.

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