Review

Relevant Animal Model of Human Lymphoblastic Leukaemia/Lymphoma – Spontaneous T-Cell Lymphomas in an Inbred Sprague-Dawley Rat Strain (SD/Cub)

(T-cell / lymphoma / leukaemia / rat model / immunophenotype / cytogenetics / cell cycle / immunochemistry / experimental therapy)

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Abstract. More than a decade of experimental work in an inbred subline of Sprague-Dawley rats having high incidence of spontaneous T-cell lymphoma/leukaemia is reviewed. Longitudinal follow-up of biological characteristics (growth, survival, haematology) of both multiple cases of primary disease and s.c. passaged lymphomas as well as comparative immunophenotypic and karyotypic studies are concluded. In these T-cell lymphomas (mostly CD4 positive), arising on the same genetic background of the inbred SD strain, the aberrations involving chromosome 11 have been recognized as a typical non-random cytogenetic marker. This unique rat model of lymphoblastic lymphomas/leukaemias, relevant to human pathology, seems to be very suitable for testing different anticancer therapeutic strategies, as it is documented by results of a number of various protocols conducted in our laboratory.

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Abbreviations: ALL – acute lymphoblastic leukaemia, ATLL – human adult T-cell leukaemia/lymphoma, Bcl2 – protein coded by the *bcl2* oncogene, CD and arabic numerals – clusters of differentiation molecules, CD80 – rat homologue of the human CD28/CTLA-4 ligand (B7-1), der(11) – structural aberration on chromosome 11, Ets2 – avian erythroblastosis virus E26 (v-ets) oncogene homologue, FAB – French-American-British classification, G-banded – Giemsa staining, HTLV-1 – human T-cell lymphotropic virus, Ig1 – immunoglobulin light chain, lambda gene cluster, MDM2 – nuclear protein capable of binding to and inactivating p53 protein (feedback regulation of p53 function), MHC – major histocompatibility complex, Mox2 – cell surface protein (thymocyte, antigen identified by monoclonal antibody MRC – OX2), MST – mean survival time, $p21^{WAF/CIPI}$ – universal cyclin-CDK inhibitor, PBL – peripheral blood lymphocytes, RT1.A; RT1.B; RT1.D; RT6 – histocompatibility loci of rat, s.c. – subcutaneous, SD/Cub – Sprague-Dawley rats/Charles University Biology, SD1/90 to SD14/97 – lymphoma lineages (arabic numerals: order/year), sIg – surface immunoglobulin, $TCR_{\alpha\beta}$ – T-cell receptor of the αβ type, TCR^{high} – phenotype of mature thymocyte, Th1, Th2 – subpopulations of T-helper lymphocytes, Vpreb1 – immunoglobulin lambda Vpreb1 chain, wt p53 – wild-type protein coded by tumour suppressor gene *TP53*, WT31 – murine IgG1 anti-CD3/T-cell receptor antibody.

Within all human malignancies, haematological neoplasms represent the cause of mortality and morbidity in 5% and 3% of cases, respectively. During the last 30 years, marked improvement of the overall survival of patients with haematological malignancies has been observed. This is the result of the development of new anticancer drugs and therapeutic strategies, as well as of the growing knowledge about the biology and pathology of the different cell lineages. Many of these findings have been obtained by using animal models. Malignant lymphomas are neoplasms that arise from B, T or NK cells at various stages of normal lymphocyte development. The large number of different types and their wide range of morphologic features reflect the complexity of the haematopoietic system. Morphology, i.e. histology and cytology, alone is not adequate to classification of malignant lymphomas. Additional immunophenotyping performed by utilizing either flow cytometry or immunohistochemical techniques is a powerful tool for establishing the correct diagnosis.

Cytogenetic methods such as classical G-banding and fluorescence *in situ* hybridization (FISH) methods are of special interest/advantage. Molecular genetic methods are particularly useful for analysis of tumours in which the histological and immunophenotypic data are not conclusive.

In this article we review the results obtained in a unique rat model of (non-Hodgkin) T-cell lineage-derived lymphomas.

Spontaneous haematological malignancy in the Prague inbred subline of Sprague-Dawley rats – the history

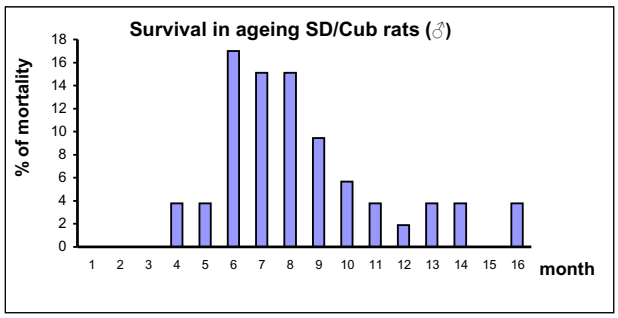
The finding of haematological malignancies in the Prague inbred line of Sprague-Dawley (SD) rats was first reported in 1984 by Klír and co-workers. The disease occurred regularly in their animal facility in both sexes, with the incidence of approximately 17 % in the total rat population of the Prague inbred line of SD rats. The disease has been investigated with respect to pathological mechanisms of its development and transplantability (Svoboda et al. 1989). Both haematological and electron-microscopic analysis of peripheral blood lymphocytes (PBL) in terminally diseased animals and the clinical course characterized the disease as acute lymphoblastic leukaemia type L2 (SD ALL) according to the FAB classification. Solid lymphomas were formed in young healthy syngeneic rats after s.c. inoculation of either PBL, spleen cell suspension or submandibular lymph node cells taken from terminally diseased rats. Identical clinical features accompanied the progressive *in situ* growth of subcutaneous secondary neoplasms as they were noticed in the primary diseased rats. The progression of disease was accompanied by paralysis of hind limbs, anaemia, and cachexia followed by infiltration of parenchymatous organs with neoplastic cells (Klír et al. 1987, Svoboda et al., 1989).

Since 1991 until now the breeding of inbred Sprague-Dawley rats has continued in the animal facility of our institute (SD/Cub) under conventional conditions. Our SD/Cub strain is highly inbred due to repeated brother-sister mating for all this time. The isohistogenicity of the strain has been proved by intrastrain skin grafting (broad double-ring system). Longitudinal study of the primary spontaneous and/or secondary disease after transplantation of neoplastic cells into the subcutis of SD/Cub rats allowed us to precisely define its clinical and biological parameters. This highly defined model of haematological malignancy in SD/Cub rats is believed to be a valuable *in vivo* model for understanding malignant processes and its treatment.

Incidence of disease

In the early 80's, the original SD rat strain was characterized by spontaneous occurrence of ALL in 17% of animals of both sexes beginning at the age of 8 months (Klír et al., 1984). Three independent longitudinal follow-up studies on the incidence of spontaneous haematological malignancy in ageing SD/Cub rats were performed in our animal facility in three independent sessions during the years 1995–1996 (Otová et al., 1997), 1996–1997 and 2000–2001. These studies revealed an increased incidence of spontaneous disease compared with the data published previously (Klír et al., 1984). Figure 1a,b summarizes the data obtained from three separate long-term studies of disease and mortality incidence of SD/Cub rats.

[A]



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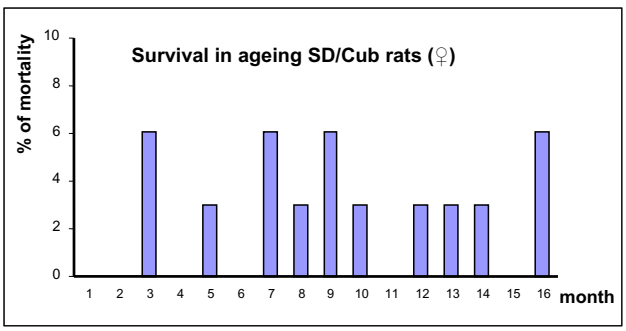

Fig. 1. Mortality of SD/Cub male [A], and female [B] rats in consequence of spontaneous lymphoma – longitudinal study

In contrast to the original inbred SD rat strain, a significant difference in the incidence of the disease was found between SD/Cub males (87%) and females (36%). Variation was not observed in the incidence of disease only, but also in terms of the age of mortality. The peak of mortality in SD/Cub males started from month 6 to month 9 of age. However, in SD/Cub females two peaks of mortality were found. The first one at month 3, the second started from month 7 and persisted to month 9. Compared with the data collected in the early 80's, in our study mortality occurred in both sexes at a younger age.

Animal models, in general, serve as appropriate experimental tools for the understanding of human diseases. In this article we have focused on haematological malignancies spontaneously arising in rats; we have compared our findings obtained in SD/Cub rats with the incidence of spontaneous leukaemias/lymphomas in other rat strains. Spontaneous haematological neoplasm incidences have been described in several rat strains. Rarely, the precise clonality has been reported. In Fischer 344 rats, the incidence of spontaneous leukaemia/lymphoma was reported to be relatively high (Haseman, 1983; Losco and Ward, 1984). Further investigation of the Fischer 344 model by Haseman et al. (1998) revealed that the most frequently occurring neoplasms were pituitary gland adenomas, testicular adenomas in males, and mammary gland fibroadenoma in females; for mononuclear cell leukaemia, different rates were found depending on the gender: 50.5% in males compared to 28.1% in females. In Wistar rats, the proportion of lymphomas did not exceed 6.9% in two separate studies (Walsh and Poteracki, 1994; Poteracki and Walsh, 1998). Sporadic spontaneous transplantable acute lymphatic leukaemia has also been described in the Lewis rat (Křemen et al., 1980), as well as one case of lymphoma in inbred WAB/Not rats (Middle et al., 1981). In other substrains of Sprague-Dawley rats (different from the inbred Prague strain of Sprague-Dawley rats) lymphoma has been observed in 0.65%, and the large granular lymphocyte lymphoma in 0.6% of the animals (Frith, 1988). In aged Sprague-Dawley rats, the incidence of malignant lymphocytic lymphoma has been reported to be 1.9% (Chandra et al., 1992), while other authors (Zwicker et al., 1992) did not report any primary lymphoma in 1435 examined Sprague-Dawley individuals. Finally, a significantly increased incidence of lymphomas of B-cell origin has been recognized in diabetes-prone BB rats (Seemayer et al., 1982; Meehan et al., 1993).

From these data, it is obvious that the spontaneous development of lymphomas of T-cell origin (*vide infra*) in the highly inbred Prague strain of Sprague-Dawley rats is rather unique.

Clinical features of the disease

Clinical symptoms of the end stage in primary disease included anaemia, cachexia, and relatively frequent spinal cord paralyses (Otová et al., 1993). At autopsy, the majority of primary diseased rats showed an enlargement of the submandibulary lymph nodes and spleen; lymph nodes in other localizations were enlarged only sporadically. The level of infiltration of parenchymatous organs with lymphoma cells corresponded well to the stage of clinical progression. Subcutaneous administration of lymphoid cell suspensions isolated from spontaneously diseased animals resulted in *in situ* growing tumours in syngeneic recipients. The clinical and histopathological course of the disease was similar in both primary and secondary diseased rats (Otová at al., 1997).

In order to further characterize these malignancies, 11 lymphomas were collected during the years 1990-1997. Individual lymphomas were designated SD1/90, SD4/91, SD5/92, and SD7/95 to SD14/97. The lymphomas were transferred by subcutaneous injection of 10^5 cells in 2-month-old SD/Cub males or females in agreement with their original gender. As shown in Fig. 2, the average survival time, as observed in the group of six recipients of each of these different SD lymphomas, revealed marked variability. The mean survival time of individual lymphoma lineages varied between 25 days (SD8/96) and 70 days (SD1/90), respectively (Fig. 2).

As shown in Fig. 3a,b, the numbers of leukocytes of some, but not all, ageing SD/Cub rats were increased at the terminal stage of disease in peripheral blood. This was due to an increased number of lymphocytes; lymphoblasts were not present in the examined samples of peripheral blood (Fig. 3a,b). Haematological examination of recipients of three different subcutaneously growing tumours also revealed increased numbers of total leukocytes, as well as total lymphocytes, in the peripheral blood (Fig. 4a,b).

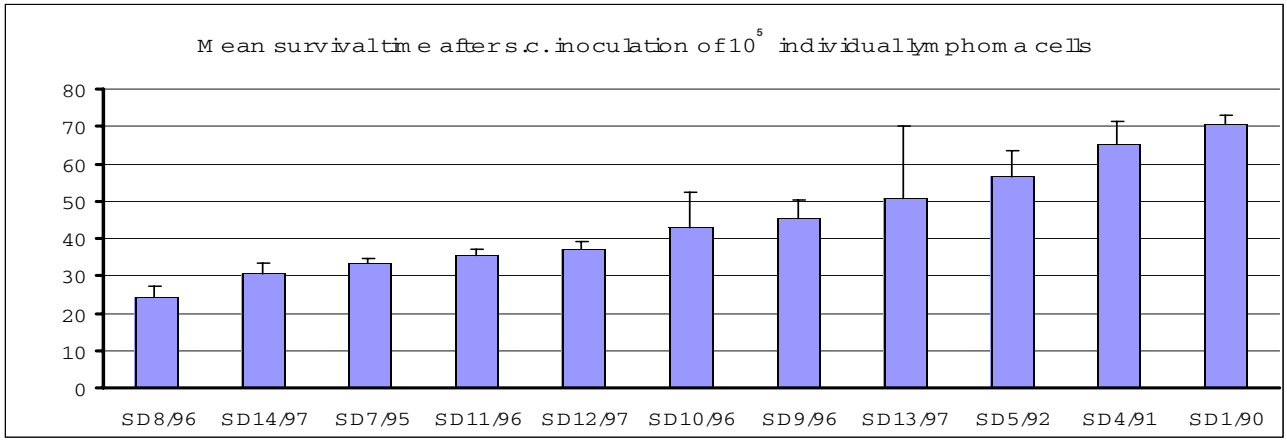

Fig. 2. Survival of the syngeneic SD/Cub recipients after injection of cell suspensions originating from different SD lymphomas

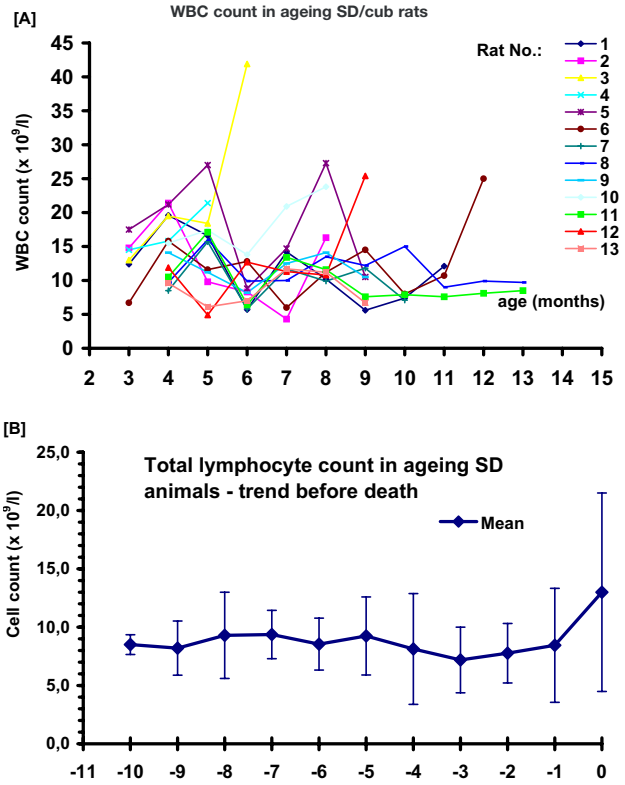

Fig. 3. Longitudinal study of the white blood cell count (WBC) in ageing SD/Cub rats
[A] WBC count in individual SD/Cub rats, [B] total lymphocyte count, trend before death

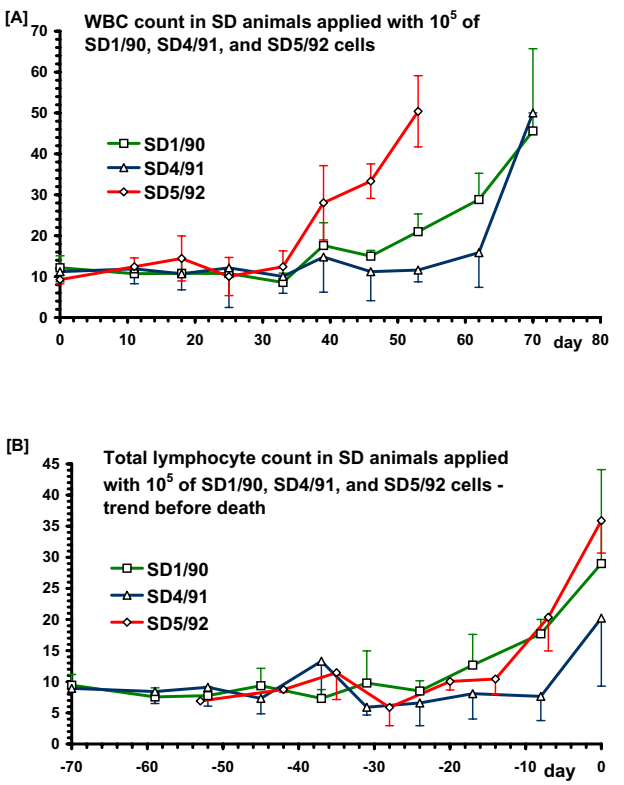

Fig. 4. White blood cell count in SD/Cub rats after inoculation of lymphoma cell suspensions into subcutis
[A] WBC count, [B] total lymphocyte count, trend before death

Morphology

For light microscopy, lymphoma samples were fixed in 10% buffered formaldehyde, routinely processed and embedded in paraffin. Tissue sections were stained with haematoxylin and eosin and Giemsa's stain. Ten out of 11 examined lymphomas consisted of medium-sized lymphoblastic cells with scanty, moderately basophilic cytoplasm. The nuclei showed inconspicuous nucleoli and had fine chromatin (Fig. 5). However, in SD1/90 lymphoma there was, apart from these medium-sized cells, an admixture of large immunoblastic cells. These immunoblasts had moderately abundant cytoplasm, oval nuclei with coarse chromatin and large nucleoli (Fig. 6) (Otová et al., 1999a; Bobková et al., 2000).

Electron microscopy revealed retrovirus-like particles in the lymphoma cells. Retrovirus-like particles were present in lymph node cells of primary diseased rats and in the lymphoma cells isolated from the first passage of malignant cells into the subcutis of syngeneic animals. Furthermore, an enzyme with reverse transcriptase activity has been isolated from the neoplastic cells. These findings support the idea of a retroviral origin of the neoplasms (Schramlová et al., 1994).

Human adult T-cell leukaemia/lymphoma (ATLL) is a postthymic lymphoproliferative neoplasm of T cells caused by human T-cell lymphotropic virus (HTLV-1). Clinically, human ATLL is characterized by lymphadenopathy (70%), splenomegaly (31%), hepatomegaly (27%), skin lesions (41%), hypercalcaemia (74%) and trombocytopenia (17%). ATLL is usually present in two major clinical forms: a) leukaemia (75%) either acute, chronic or smouldering, and b) lymphoma (25%). A retrospective review of human ATLL samples from 1990 to 2000 showed the presence of a polymorphous population of lymphocytes ranging from small bland-appearing lymphocytes to large atypical ones with bizarre, multilobulated nuclei with coarse chromatin and prominent nucleoli. The cytoplasm was deeply basophilic, with occasional vacuoles. The tumour cells in all cases tested were positive for CD2, CD3, CD4, CD5, and CD25 and were negative for CD7, CD8, CD16, CD56, CD57 (Dahmoush et al., 2002). The incidence of familiar ATLL in endemic regions such as Japan, the Carribean region, and North and South America has also been reported. The disease was accompanied with HTLV-1 positivity (Prates et al., 2000). Recently, a case of human ATLL unrelated to HTLV-1 was announced (Nakase et al., 2000). The phenotype of tumour cells revealed $CD7^+$, $CD5^+$, $CD3^+$, $WT31^-$, $CD4^-$, $CD8^-$, $CD25^-$, and the karyotype showed a 5q-, and a t(12;18). HTLV-1-unrelated ATLL is a very rare disease in the human population.

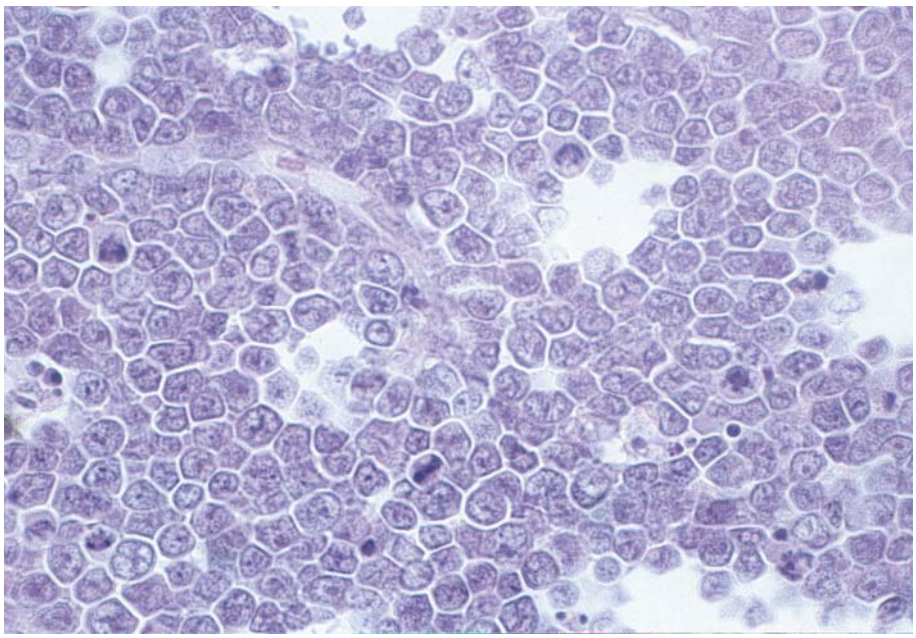

Fig. 5. Morphological appearance of SD7/95 lymphoma
Uniform medium-sized lymphoblastic cells containing large nuclei with fine chromatin structure (Giemsa, original magnification 160x)

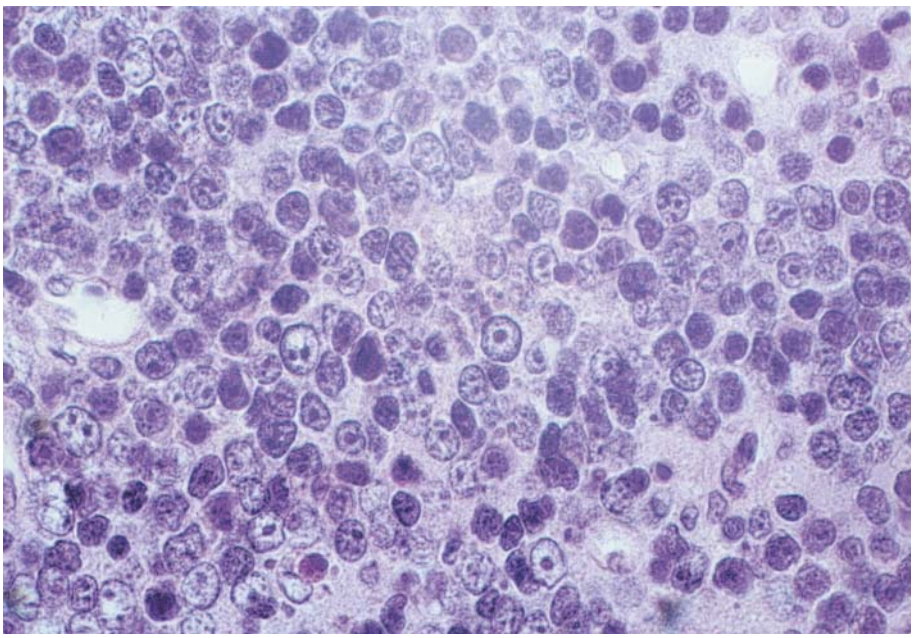

Fig. 6. Morphology of SD1/90 lymphoma
Predominating population of medium-sized lymphoblastic cells with large nuclei and fine chromatin structure with the admixture of large immunoblastic cells with pale oval nuclei containing large nucleoli (Giemsa, original magnification 160x)

Immunohistochemistry

The proteins p53, $p21^{WAF1/CIP1}$, MDM2 and Bcl2, all known to be involved in cell-cycle regulation and/or cell survival, were visualized immunohistologically using an avidin-biotin peroxidase complex technique LSAB2 kit (DAKO, Glostrup, Denmark). Sections from paraffin blocks were used for examination. The antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The evaluation of positive cells was estimated randomly in 50 high-power fields using the image analysis system LUCIA G (Laboratory Imaging Ltd., Prague, Czech Republic).

The expression of the Bcl2 protein was found in all 11 examined lymphomas. This corresponds very well with the *bcl2* oncogene survival-promoting function. Expression of p53 was detected in only one (SD1/90) of the lymphomas (Otová et al., 1999a; Bobková et al., 2000). Since it is impossible (under the common conditions in cycling cells) to visualize the wild-type (wt) form of p53, the recognized form in SD1/90 represents the mutant form of the *TP53* gene. Finally, a certain number of p21-positive cells was detected in the p53-negative SD10/96 lymphoma. The detection of the p21 protein indirectly shows the growth-suppressing activity of the wt p53 protein in lymphoma cells, since the wt p53 is the transcriptional factor of the $p21^{WAF1/CIP1}$ gene, which inhibits cell cycling. The expression of the protein MDM2, which allows cells to recover from G1 arrest as induced by p53, was not observed in any of the examined lymphoma samples (Bobková et al., 2001).

Immunophenotyping

Originally, four individual spontaneous SD/Cub haematological neoplasms, repeatedly passaged subcutaneously for a long period in our laboratory, were used to examine their antigenic profile by immunofluorescent microscopy. The absence of sIg and MHC class II determinants (OX-3; OX-6) and positive reaction with anti-T-cell monoclonal antibodies (OX-7 – Thy 1.1/CD90; W3/13 – leukosialin/CD43) determined the T-cell origin of these four lymphomas (Otová et al., 1988).

More recently, the immunophenotype of a set of 11 randomly chosen SD/Cub lymphomas was investigated by flow cytometry. The cells examined included: a) cells isolated from submandibulary lymph nodes of primary diseased rats, and b) lymphoma cells grown after passage into the subcutis of syngeneic recipients. At first, the screening was performed using mouse anti-rat CD4 (OX-8) and CD8 (OX-38) monoclonal antibodies (PharMingen, San Diego, CA) to describe the basic immunophenotype of the lymphoma cells. The immunophenotype of submandibular lymph node cells of primary diseased rats was analysed in eight of the 11 lymphomas. Submandibular lymph node cells as well as the $1^{st}/2^{nd}$ passage of three rats were not examined (SD1/90, SD4/91, and SD5/92).

In primary disease (as shown in Table 1), six out of eight primary lymphomas/disease were $CD4^+CD8^-$, in the two other cases (SD10/96 and SD12/97), a mixture of $CD4^+CD8^-$ and $CD4^-CD8^-$ double negative lymphoid cells was present. However, the presence of these $CD4^+CD8^-$ lymphoid cells was lost in lymphoma SD9/96 ($CD4^-CD8^-$ immunophenotype) upon repeated passage of neoplastic cells in young healthy SD/Cub recipients. On the other hand, repeated passage of neoplastic cells resulted in the appearance of $CD4^-CD8^-$ double negative lymphoid cells alongside the $CD4^+CD8^-$ lymphoid cells (Table 1) in three lymphomas (SD8/96, SD13/97, and SD14/97). Furthermore, one of the three lymphomas that was only analysed after the 15^{th} passage (SD1/90) consisted of only $CD4^-CD8^-$ double negative cells; $CD4^+CD8^-$ markers were present on the surface of both SD4/91 and SD5/92 lymphomas.

Next, the 11 lymphomas were examined in more detail by flow cytometry using a panel of monoclonal antibodies specific for T-cell markers including adhesion and activation molecules according to the method described by Homma et al. (1977). In agreement with previous findings, they were immunophenotyped as T cells based on the concomitant expression of CD5, CD43, and with one exception (SD4/91), CD90. However, none of the lymphomas expressed $TCR_{\alpha\beta}$. Eight lymphomas were CD4 single positive, and three, SD1/90 (15^{th} passage), SD12/97 (2^{nd} passage) and SD13/97 (2^{nd} passage), were double negative. With respect to adhesion and activation molecules, all lymphomas expressed CD54 and RT1.A (MHC class I), but none expressed CD11b, CD25, CD28, CD 134 or RT6. Heterogeneity was observed for several other markers: SD8/96 was positive for CD11a; SD11/96 and SD12/97 were slightly positive for CD45RC. SD14/97, being positive for CD11a, CD45RC, and especially RT1.B/RT1.D, was phenotypically the most interesting lymphoma (Otová et al., 1999c). The phenotype of a representative lymphoma as well as the heterogeneity of the lymphoma phenotype is shown in Fig. 7a,b.

We conclude that our longitudinal flow cytometry follow-up identified all haematological neoplasms of SD/Cub rats as T-cell lymphomas with only minor phenotypical heterogeneity.

Cytogenetics

The set of 11 individual cases of SD lymphomas has also been studied cytogenetically. Chromosome studies were performed on preparations of either submandibular lymph node cells and/or s.c. growing lymphomas using common cytogenetic methods. For detailed karyology, G-banded preparations were used. The detailed description of chromosome rearrangements in individual lymphomas has been described before (Sladká and Otová, 1994; 1998).

Metaphase cells in 10 neoplasms possessed chromosome numbers near diploidy, most of them being pseudodiploid with one or two constant chromosome markers. SD8/96 lymphoma contained a hyperdiploid number of chromosomes already in cells isolated from submandibular lymph nodes. The hyperploid number of chromosomes was still present after ten passages. The most frequent chromosomal changes involved chromosome 11, where the translocation form of trisomy 11 or der(11) (the latter one previously designated as 11q+ aberration) was observed. Chromosome 11 abnormalities were found in all 11 lymphomas studied, suggesting thus to be a non-random change (Table 1). In five cases this aberration was already found in submandibular lymph node cells of primary diseased rats. The q arm of chromosome 11 (q11q12) is supposed to be the critical region involved in the genesis or progression of SD lymphomas (Fig. 8a,b). These results are in agreement with our previous observations in nine cases of SD ALL (Sladká et al., 1988).

Detailed gene mapping of the rat chromosome 11(q11q12) region is not yet available. So far, no specific rat gene or homologue to classical mouse or human genes involved in carcinogenesis is known to map to this specific region. In proximity to this region there have been provisionally mapped genes (*Cd80*, *Ets2*, *Ig*l, *Mox*2, and *Vpreb*1) whose mouse or human homologues are involved in haematopoietic cell differentiation, possibly having the oncogenic potential (Ushijima, 2002).

Table 1. Description of the basic immunophenotype and karyotype of the set of 11 individual cases of SD lymphomas
The lymphoma cells have been examined by flow cytometry using mouse anti-rat CD4 (OX-8) and CD8 (OX-38) monoclonal antibodies, and cytogenetically by G-banding.

Lymphoma line	Autopsy at	Tissue	Immunophenotype	Karyotype
	Control cells	Submandibular LN*	$CD4^+CD8^-$(53%), $CD4^-CD8^+$(26%)	n = 42; diploidy
SD1/90***	15th passage	lymphoma	$CD4^-CD8^-$	42,X0; -3, +2 microsomes; pseudodiploidy
SD4/91***	15th passage	lymphoma	$CD4^+CD8^-$	42,XX,11q+; pseudodiploidy
SD5/92***	15th passage	lymphoma	$CD4^+CD8^-$	42,XX,der(11,13); pseudodiploidy
SD7/95	Primary disease	Submandibular LN*	$CD4^+CD8^-$	42,XY,11q+ ;pseudodiploidy
	1st passage	lymphoma	$CD4^+CD8^-$	42,XY,11q+; pseudodiploidy
	10th passage	lymphoma	$CD4^+CD8^-$	42,XY,11q+; pseudodiploidy
SD8/96	Primary disease	Submandibular LN*	$CD4^+CD8^-$	42,XY,11q+; hyperdiploidy (8%)
	1st passage	lymphoma	$CD4^+CD8^-$(18%) / $CD4^-CD8^-$(82%)	hyperdiploidy, microchromosomes
	10th passage	lymphoma	$CD4^+CD8^-$(55%)/ $CD4^-CD8^-$(43%)	45,XY,der11, 2 mar; 43,XY, mar
SD9/96	Primary disease	Submandibular LN*	$CD4^+CD8^-$	42,XX,11q+; pseudodiploidy
	1st passage	lymphoma	$CD4^+CD8^-$	42,XX,11q+; pseudodiploidy
	10th passage	lymphoma	$CD4^-CD8^-$	42,XX,der (7),der (11); pseudodiploidy
SD10/96	Primary disease	Submandibular LN*	$CD4^+CD8^-$(41%) / $CD4^-CD8^-$(47%)	Nt**, not assessable
	2nd passage	lymphoma	$CD4^+CD8^-$(49%) / $CD4^-CD8^-$(50%)	42,XY,der (11); pseudodiploidy
	5th passage	lymphoma	$CD4^-CD8^-$	42,XY,der (11); pseudodiploidy
SD11/97	Primary disease	Submandibular LN*	$CD4^+CD8^-$	42,XY;
	2nd passage	lymphoma	$CD4^+CD8^-$	42,XY;
	5th passage	lymphoma	$CD4^+CD8^-$	43,XY,+11
SD12/97	Primary disease	Submandibular LN*	$CD4^+CD8^-$(36%) / $CD4^-CD8^-$(50%)	Nt**, not assessable
	2nd passage	lymphoma	$CD4^+CD8^-$(26%) / $CD4^-CD8^-$(74%)	42,XX;
	5th passage	lymphoma	$CD4^-CD8^-$	42,XX, der (11); pseudodiploidy
SD13/97	Primary disease	Submandibular LN*	$CD4^+CD8^-$	42,XY, der (11); pseudodiploidy
	2nd passage	lymphoma	$CD4^+CD8^-$(26%) / $CD4^-CD8^-$(70%)	42,XY, der (11); pseudodiploidy
	5th passage	lymphoma	$CD4^+CD8^-$(29%) / $CD4^-CD8^-$(60%)	42,XY, der (11); pseudodiploidy
SD14/97	Primary disease	Submandibular LN*	$CD4^+CD8^-$	42,XX; 42,XX,der(11); pseudodiploidy
	2nd passage	lymphoma	$CD4^+CD8^-$	42,XX; 42,XX,der(2); 43,XX,+11; 43,XX,+11,der(2)
	5th passage	lymphoma	$CD4^+CD8^-$(51%) / $CD4^-CD8^-$(49%)	42,XX; 42,XX,der(11); 42,XX,der(2); 43,XX,+11, der(2)

* LN - lymph nodes, ** Nt - in cellular suspension no convenient metaphases were found, *** Primary disease as well as 1st or 2nd passage of lymphoma cells were not examined

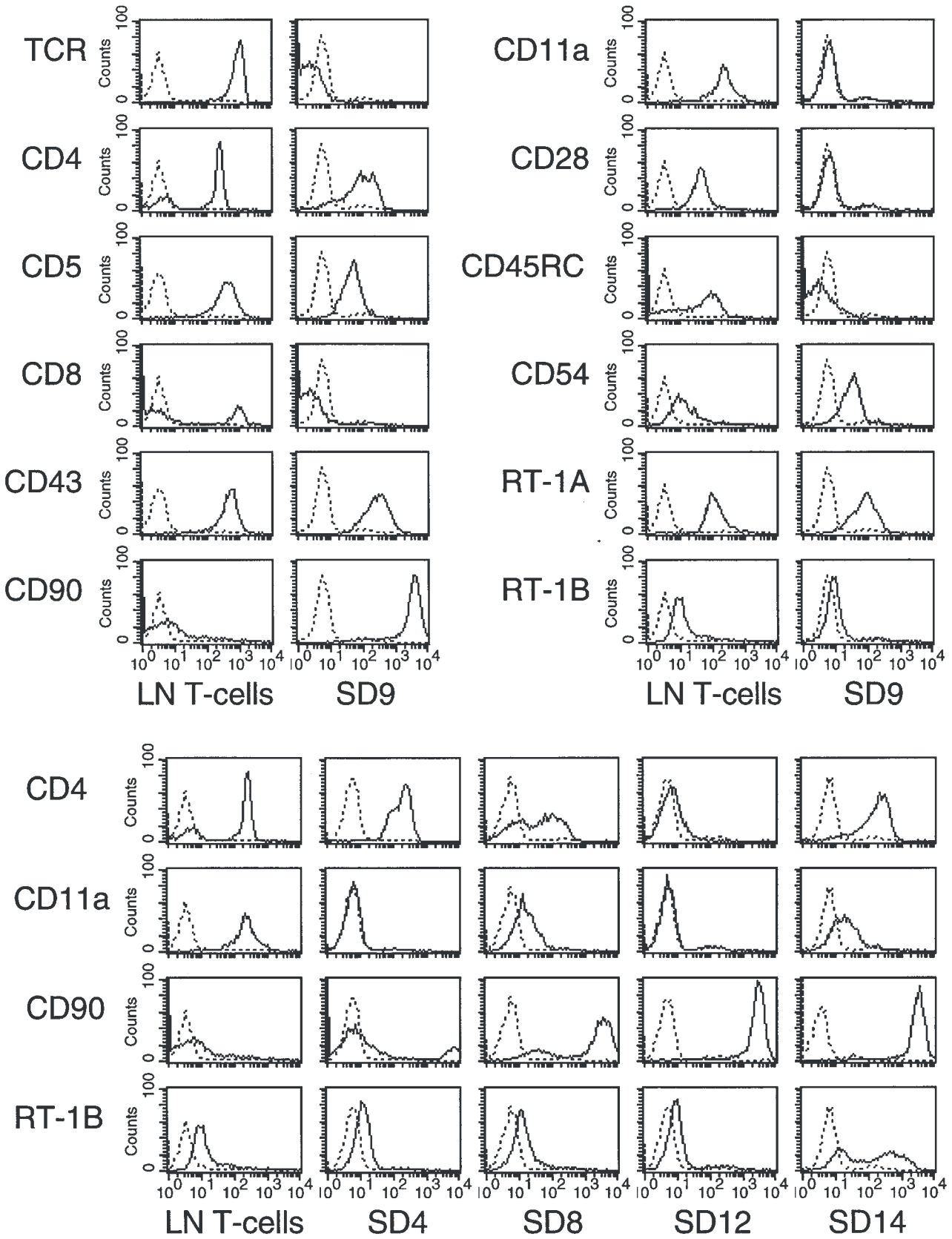

Fig. 7. Immunophenotype of haematological neoplasms in SD/Cub rats
Histograms represent the expression of the respective markers of a representative lymphoma (SD9/96) next to the phenotype of normal lymph node T cells derived from SD/Cub rats [A]. Heterogeneity of the lymphomas is shown in [B] for the relevant lymphomas and markers. Solid lines represent the expression of the respective marker, whereas the dotted lines represent the isotype controls.

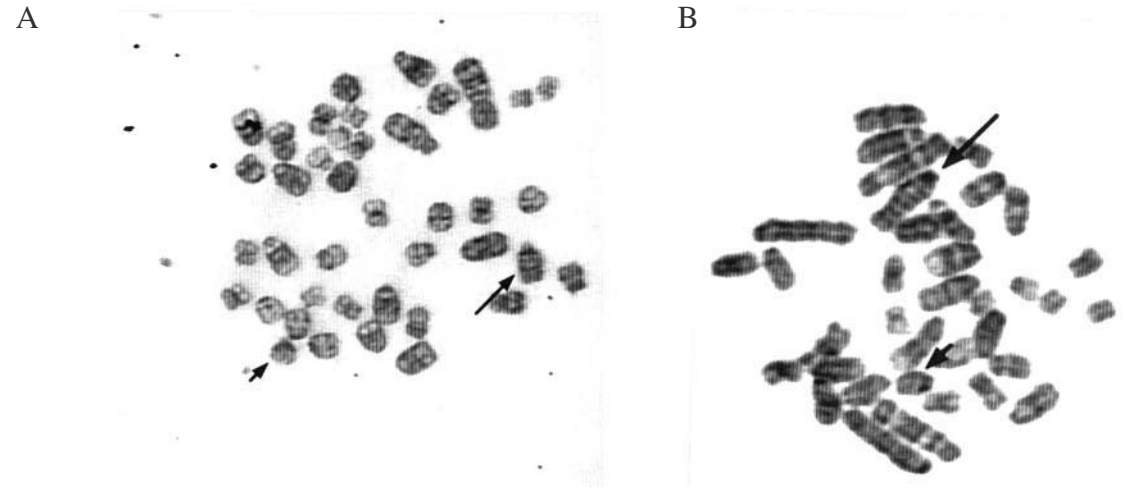

Fig. 8. G-banded metaphases obtained from SD lymphomas: [A] SD10/96 lymphoma, [B] SD9/96 lymphoma
Variation in the most frequent rearrangement of chromosome 11 termed as der(11q); critical region 11q (q11-q12). The arrows mark the homologous pair of chromosomes 11; short arrow – normal shape, long arrow – acquired aberration.

Aetiology of haematologic malignancies in the SD/Cub strain

As already mentioned above, a retroviral infection is suspected to be involved in the high incidence of lymphoma in the Prague SD/Cub inbred strain. Since the onset of disease differs in terms of age between male and female animals, as well as between the SD/Cub strain and the original SD strain, we have examined the effect of ageing on the immune system of SD/Cub rats as compared to lymphoma-resistant Lewis rats. Hereto the thymus was analysed for thymic weight, CD4/CD8 single positive mature thymocyte ratio, and the presence of TCR^{high} thymocytes as already described by Homma (Homma et al., 1997). Furthermore, peripheral T cells were analysed for the absolute number in the submandibular and mesenteric lymph nodes, the CD4/CD8 T-cell ratio, and the phenotypically determined Th1/Th2 ratio as described by Beijleveld (Beijleveld et al., 1996). The results are presented in Fig. 9a,b and reveal that the age-dependent involution of the thymus, as concluded from the thymic weight and percentage of TCR^{high} thymocytes, is somewhat retarded in the SD/Cub rats as compared to Lewis rats (Fig. 9a). Especially in the first half year of life, the SD/Cub rats have a lower Th1/Th2 ratio (Fig. 9b). Since Th1 cells are considered to be involved in the immunosurveillance for malignancies, the relatively low Th1/Th2 ratio in SD/Cub rats may be a facilitating factor for the outgrowth of the retrovirally induced haematologic neoplasms early in life of this rat strain.

Incidence of other malignancies in the SD/Cub strain

During the longitudinal search for lymphoma incidence in the Prague SD/Cub inbred strain we found two spontaneous tumours that differed from the clinical features of haematological malignancy in SD/Cub rats. Both of them grew subcutaneously; the first case in the axilla, the second in the area of the neck.

In case 1, the tumours microscopically showed mixed patterns, with a dominant component of eccrine spiradenoma, with variable proportions of a cylindromatous component and foci of less differentiated solid carcinoma (Fig. 10a,b). The suspension of primary tumour cells was transplanted into the subcutis of syngeneic rats and histologically examined again after the third passage. The structures of prevalently solid carcinoma with occasional tubular structures predominated in the third passage of this tumour (Fig. 11a,b).

The tumour in case 2 was microscopically composed of a mixture of fibroadenoma with the areas of adenomyoepithelioma (Fig. 12a,b). The passaging of this tumour to syngeneic rats was unsuccessful.

SD lymphomas – a tool for testing various anticancer strategies

SD lymphomas of T-cell origin were repeatedly used with success for various experimental testing of several antitumour therapy strategies. During the last few years, we have used SD T-cell lymphomas to investigate the promising antitumour potency of a prospective group of the acyclic nucleotide analogues: 9-[2-(phosphonomethoxy)ethyl] derivatives of adenine (PMEA), 2,6-diaminopurine (PMEDAP), and guanine (PMEG) (Otová et al., 1997), and N^6-substituted 2,6-diaminopurine (Valeriánová et al., 2001). For description of these compounds see reviews Holý (1993); Naesens et al. (1997).

The mechanism of the antiproliferative effect of PMEA, PMEDAP, and PMEG was investigated in detail. These nucleotide analogues are phosphorylated by cellular kinases to their diphosphates (analogues of

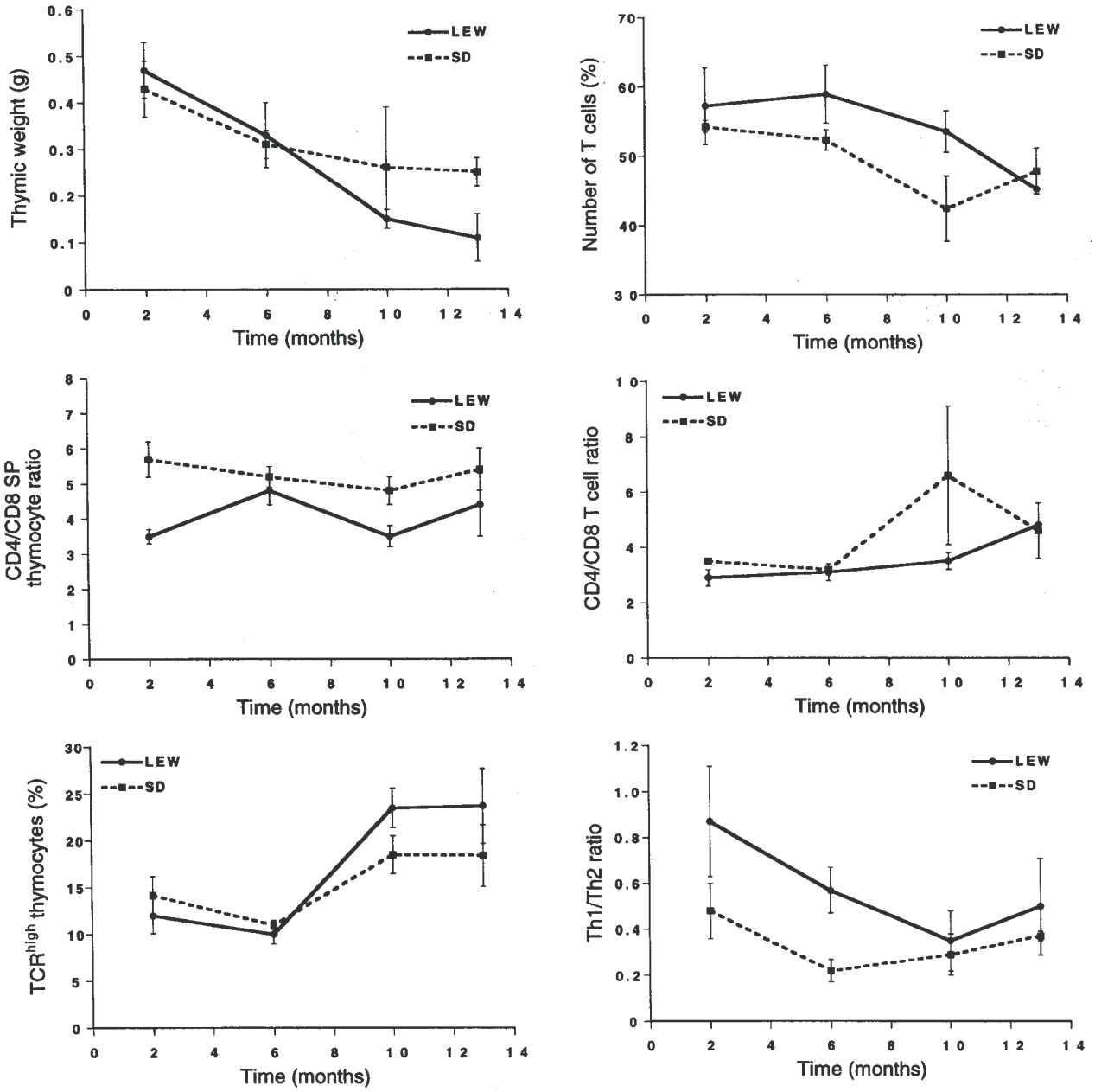

Fig. 9. Effect of ageing on the T-cell compartment of SD/Cub and Lewis rats
Age-dependent changes in the thymus [A] and the peripheral lymph nodes [B] are determined by measuring the thymic weight ([A], upper diagram), CD4/CD8 single positive, mature thymocyte ratio ([A], middle diagram), percentage of TCR^{high} thymocytes ([A], lower diagram), absolute number of lymph node T cells ([B], upper diagram), CD4/CD8 T cell ratio in peripheral lymph nodes ([B], middle diagram), and the phenotypically determined Th1/Th2 ratio in peripheral lymph nodes ([B], lower diagram). Solid lines represent the data obtained from Lewis rats; dotted lines represent the data obtained from SD/Cub rats.

nucleoside-5'-triphosphates) (Kramata et al., 1995; Birkuš et al., 1998; Birkuš et al., 1999), which inhibit replicative DNA polymerases (Kramata et al., 1996; Birkuš et al., 1998; Birkuš et al., 1999).

A dose- and time-dependent ability of PMEDAP to induce apoptosis in treated subcutaneously growing lymphomas was observed (Otová et al., 1999a; Bobková et al., 2000). The antitumour effect of PMEA and/or PMEDAP was highly significant at the beginning of treatment. Decreased progression of lymphoma growth lasted only during the administration of the compound(s), but with diminishing effect. After drug cessation, progression of neoplasia was re-established. The failure in the single therapy with PMEDAP could be overcome by combined therapy with docetaxel (Bobková et al., 2001).

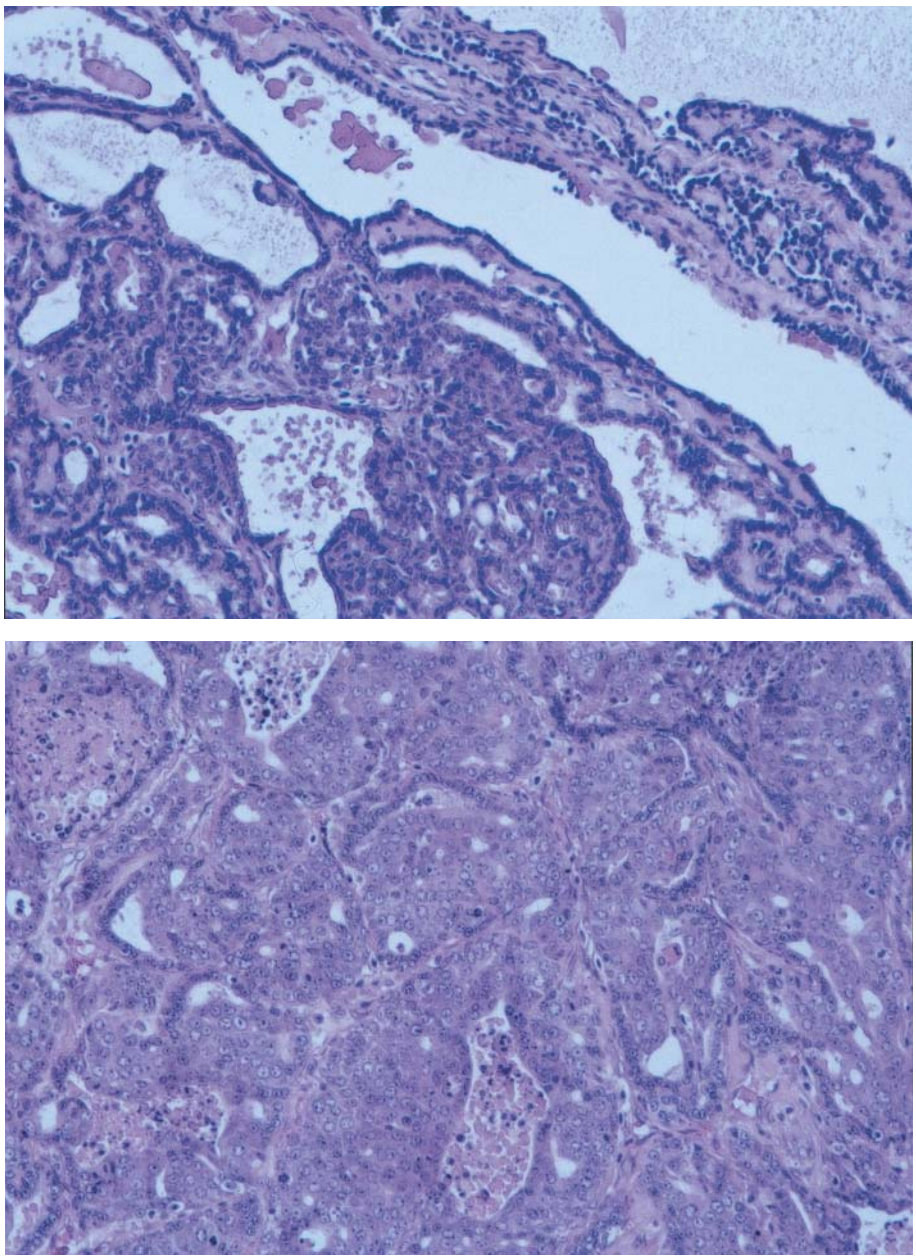

Fig. 10. Structures of eccrine spiradenoma predominating within a primary tumour [A], solid nests of epithelial cells of the carcinomatous component of the tumour [B], SD/Cub female rat (H&E, magnification 100x)

SD lymphomas were also used as a model system to investigate the therapeutic effect of local administration of recombinant murine interleukin-2 (IL-2) or heat shock (Otová et al., 1996; 1999b). The anticancer effect of heat shock, either alone or in combination with the drug PMEDAP, was studied in SD/Cub s.c. growing lymphomas. A significant anticancer effect was induced by repeated sessions of heat shock. Much stronger therapeutic effects were observed when combined with PMEDAP (Otová et al., 1999b). In IL-2-treated rats, the lymphomas exhibited weight reduction, large necrotic areas, and no dissemination of neoplastic cells into parenchymatous organs was revealed. Thymus hypoplasia was a constant picture of the histopathology. The $CD4^+/CD8^+$ ratio showed temporary reduction of $CD4^+$ and increase of $CD8^+$ cells in IL-2-treated rats (Otová et al., 1996).

Conclusion

Our longitudinal clinical, histopathological, and haematological examination combined with the flow cytometric follow-up has changed our original view on the disease in the Prague inbred subline of Sprague-Dawley rats. The disease previously described as acute lymphoblastic leukaemia (Klír et al., 1984, 1987; Svoboda et al., 1989) has been reclassified. All SD/Cub rat haematological malignancies investigated in the period 1990-2001 appeared to be T-cell derived lymphomas with a leukaemic phase in end-stage disease. In primary disease, non-random chromosomal markers have been found in lymph node cells only, and have definitely not been detected in bone marrow. In rats with s.c. inoculated lymphomas, the bone marrow infiltration has been reported only in end-stage disease, paralleling the

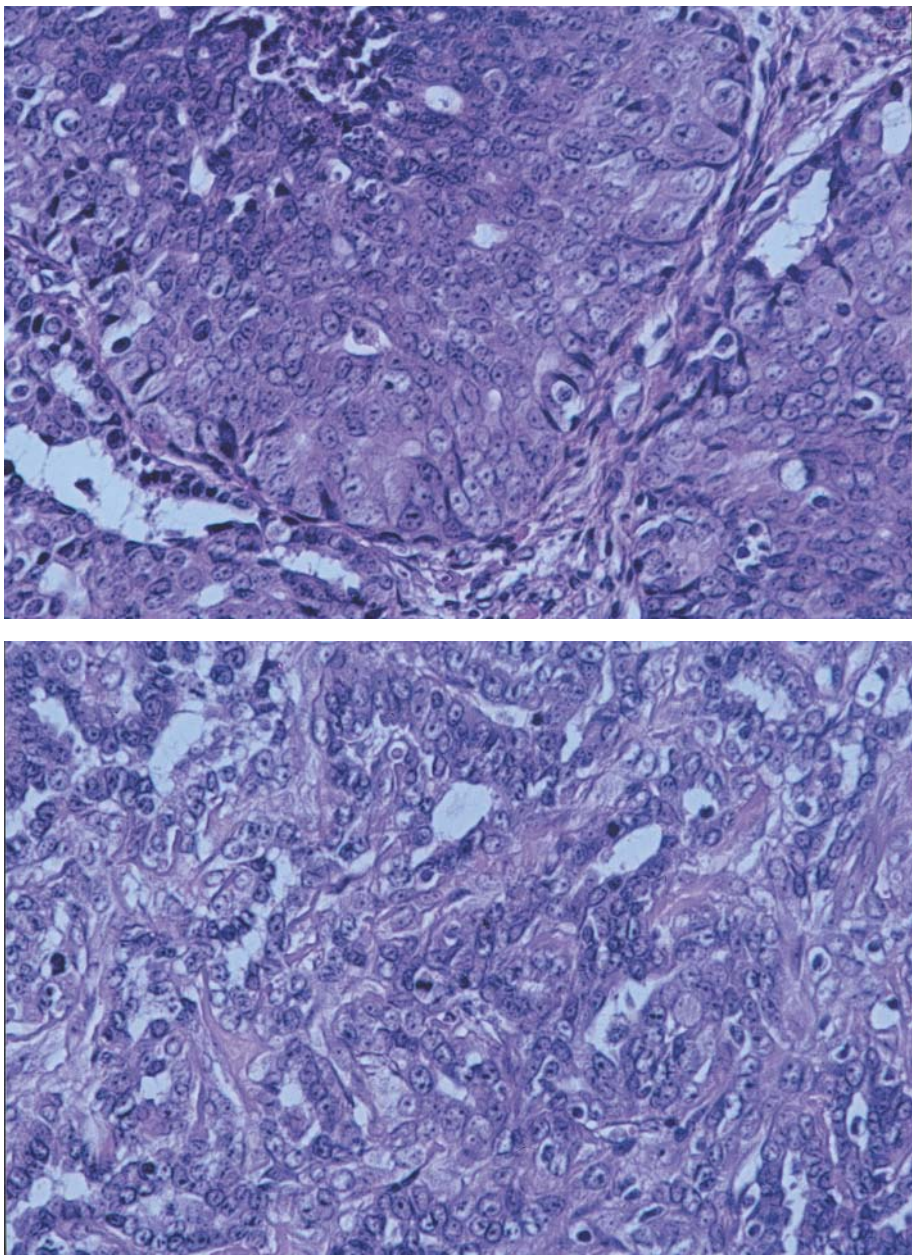

Fig. 11. Third passage of the tumour presented in Figure 10 Solid nests of carcinoma [A], irregular tubular structures within the carcinoma [B], SD/Cub female rat (H&E, magnification 200x)

infiltration of parenchymatous organs. The onset of the disease is earlier when compared to the data obtained in 1984. Also, the incidence of disease increased from 17% to 87% in the male population and 36% in the female population, respectively. These differences might be due to the further inbreeding of the SD strain in comparison to the original strain.

Our highly defined SD/Cub rat model of haematological malignancy can serve as a relevant model of human haematological neoplasia, since the T-cell lymphomas obtained in our model exhibited similar phenotypic markers as have been found in some human adult T-cell leukaemias/lymphomas.

In summary, the highly inbred Prague strain of Sprague-Dawley rats with regular incidence of spontaneous T-cell lymphomas represents an animal model of human haematological malignancies. Our experiments have revealed that the SD/Cub lymphomas exhibit several basic phenotypic characteristics similar to human T-cell lymphomas. To elucidate the basic principles of malignant transformation of T lymphocytes, the investigation of spontaneous T-cell lymphomas in SD/Cub inbred rats (Prague subline) will continue by methods of molecular genetics. This unique breed can also serve as a relevant model for investigating the development of malignant transformation of T cells as well as for testing the anticancer effect of new compounds.

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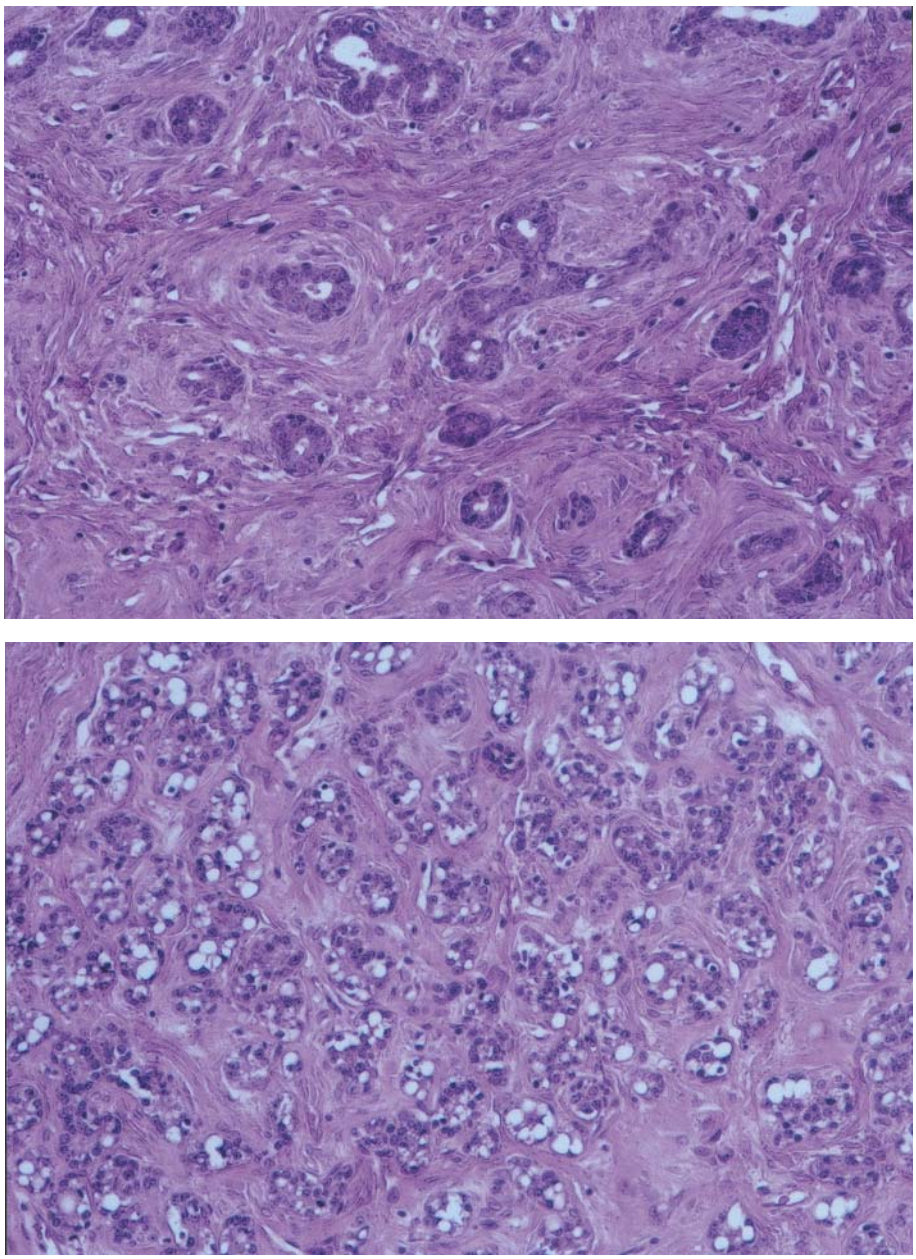

Fig. 12. SD/Cub rat fibroadenoma
Neoplastic tubular structures within the dense stroma [A], and adenomyoepithelial component of the tumour [B], SD/Cub male rat (H&E, magnification 100x)

References

Beijleveld, L. J. J., Groen, H., Broeren, C. P. M., Klatter, F. A., Kampinga, J., Damoiseaux, J. G. M. C., Van Breda Vriesman, P. J. C. (1996) Susceptibility to clinically manifest cyclosporin A (CsA)-induced autoimmune disease is associated with interferon-gamma (IFN-γ)-producing $CD45RC^{+}RT6^{-}$ T helper cells. *Clin. Exp. Immunol.* **105,** 486-496.

Birkuš, G., Kramata, P., Votruba, I., Otová, B., Otmar, M., Holý, A. (1998) Nonproteolyzed form of DNA-polymerase ε from T-cell spontaneous lymphoma of Sprague-Dawley inbred rat: isolation and characterization. *Collect. Czech. Chem. Commun*. **63,** 723-731.

Birkuš, G., Votruba, I., Holý, A., Otová, B. (1999) 9-[2-(Phosphonomethoxy)ethyl]adenine diphosphate (PMEApp) as a substrate toward replicative DNA polymerases α, δ, ε and ε*. *Biochem. Pharmacol*. **58**, 487-492.

Bobková, K., Otová, B., Marinov, I., Mandys, V., Panczak, A., Votruba, I., Holý, A. (2000) Anticancer effect of PMEDAP – monitoring of apoptosis. *Anticancer Res.* **20,** 1041-1048.

Bobková, K., Gut, I., Mandys, V., Holý, A., Votruba I., Otová, B. (2001) Antitumor activity of a combined treatment with PMEDAP and docetaxel in the Prague inbred Sprague-Dawley/Cub rat strain bearing T-cell lymphoma. *Anticancer Res.* **21,** 2725-2732.

Chandra, M., Riley, M. G., Johnson, D. E. (1992) Spontaneous neoplasms in aged Sprague-Dawley rats. *Arch. Toxicol.* **66,** 496-502.

Dahmoush, L., Hijazi, Y., Barnes, E., Stetler-Stevenson M., Abati, A. (2002) Adult T-cell leukemia/lymphoma. *Cancer* **96,** 110-116.

Frith, C. H. (1988) Morphologic classification and incidence of hematopoietic neoplasms in the Sprague-Dawley rat. *Toxicol. Pathol.* **16,** 451-457.

Haseman, J. K. (1983) Patterns of tumor incidence in two-year cancer bioassay feed studies in Fischer 344 rats. *Fundam. Appl. Toxicol*. **3,** 1-9.

Haseman, J. K., Hailey, J. R., Morris, R. W. (1998) Spontaneous neoplasms in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. *Toxicol. Pathol*. **26,** 428-441.

Holý, A. (1993) Antiviral acyclic nucleotide analogues. In: *Antibiotics and Antiviral Compounds,* eds. Krohn, K., Kirst, H., Maag, H., pp. 455-462, VCH Verlagsgesellschaft mbH, Weinheim.

Homma, M., Van Breda Vriesman, P. J. C., Damoiseaux, J. G. M. C. (1997) Defective de novo thymocyte maturation in cyclosporin A (CsA)-induced autoimmunity: expression of costimulatory and activation molecules. *Clin. Exp. Immunol.* **110,** 79-85.

Klír, P., Svoboda, T., Přibylová, M., Tachecí, O. (1984) Spontaneous mononuclear cell leukemia in Sprague-Dawley rats. Hematology and transplantation experiment II. Z. Versuchstierkd., **30,** 51-52.

Klír, P., Svoboda, T., Přibylová, M., Tachecí, O. (1987) Spontaneous mononuclear cell leukemia in Sprague-Dawley rats, a possible model. *Deutsch. TierArztl. Wschr*. **92,** 21.

Kramata, P., Černý, J., Birkuš, G., Votruba, I., Otová, B., Holý, A. (1995) DNA- polymerases α, δ, and ε from T-cell spontaneous lymphoblastic leukemia of Sprague-Dawley inbred rat: isolation and characterization. *Collect. Czech. Chem. Commun.* **60,** 1555-1572.

Kramata, P., Votruba, I., Otová, B., Holý, A. (1996) Different inhibitory potency of acyclic phosphonomethoxyalkyl nucleotide analog toward DNA-polymerases alfa, delta and epsílon. *Molecul. Pharmacol.* **49,** 1005-1011.

Křemen, J., Havlíček, F., Pohlreich, P. (1980) Spontaneous transplantable lymphatic leukemia in Lewis rats (KPH-Lw-I). *Neoplasma* **27,** 197-202.

Losco, P. E., Ward, J. M. (1984) The early stage of large granular lymphocyte leukemia in the F344 rat. *Vet. Pathol.* **21,** 286-291.

Meehan, C. J., Krajewski, A. S., Butcher, G. W., Smith, W., Baird, J. D. (1993) Lymphoma in the BB/E rat: c-myc translocation identified. *J. Pathol*. **170,** 87-93.

Middle, J. G., Robinson, G., Embleton, M. J. (1981) Naturally arising tumors of the inbred WAB/Not rat strain. Classification, age and sex distribution, and transplantation behavior. *J. Natl. Cancer Inst*. **67,** 629-636.

Naesens, L., Snoeck, R., Andrei, G., Balzarini, J., Neyets, J., De Clercq, E. (1997) HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphonate analogues: a review of their pharmacology and clinical potential in the treatment of viral infections. *Antivir. Chem. Chemother.* **8,** 1-23.

Nakase, K., Hasegawa, M., Tsuji, K., Ikeda, T., Tamaki, S., Tanigawa, M., Miyanishi, E., Shiku, H. (2000) HTLV-1 unrelated adult T-cell leukemia/lymphoma with unique phenotype and karyotype. *Am. J. Hematol*. **64,** 64-66.

Otová, B., Křen, V., Sladká, M., Klír, P. (1988) Spontaneous acute lymphoblastic leukemia in Sprague-Dawley rats. I. Immunogenetic analysis of four individual leukemias. *Neoplasma* **35,** 315-320.

Otová, B., Sladká, M., Blažek, K., Schramlová, J., Votruba, I., Holý, A. (1993) Cytostatic effect of 9-(2-phosphonomethoxyethyl)adenine (PMEA). II. Lymphoblastic leukemia in Sprague-Dawley rats. *Folia Biol. (Praha)* **39,** 142-149.

Otová, B., Panczak, A., Šímová, J., Jandlová, T., Bubeník, J., Blažek, K., Schramlová, J., Marinov, I. (1996) Treatment of transplanted spontaneous rat T-cell leukaemia with local administration of recombinant murine interleukin-2. *Folia Biol. (Praha)* **42,** 339-344.

Otová, B., Holý, A., Votruba, I., Sladká, M., Bílá, V., Mejsnarová, B., Lešková, V. (1997) Genotoxicity of purine acyclic nucleotide analogs. *Folia Biol. (Praha)* **43,** 225-229.

Otová, B., Sladká, M., Panczak, A., Marinov I. (1997) Biological characteristics of spontaneous transplantable T-cell lymphomas in inbred Sprague-Dawley/Cub rat. *Transpl. Proc.* **29,** 1754-1755.

Otová, B., Zídek, Z., Holý, A., Votruba, I., Sladká, M., Marinov, I., Lešková, V. (1997) Antitumor activity of novel purine acyclic nucleotide analogs PMEA and PMEDAP. *In Vivo* **11,** 163-168.

Otová, B., Francová, K., Franěk, F., Koutník, P., Votruba, I., Holý, A., Sladká, M., Schramlová, J. (1999a) 9-[2-(Phosphonomethoxy)ethyl]-2,6-diaminopurine (PMEDAP) – a potential drug against hematological malignancies – induces apoptosis. *Anticancer Res.* **19,** 3173-3182.

Otová, B., Mráz, M., Mandys, V., Panczak, A., Schramlová, J., Votruba, I., Holý, A. (1999b) Therapeutic effect of heat shock on T-cell lymphoma in inbred Sprague-Dawley rat. *Folia Biol. (Praha)* **45,** 121-131.

Otová, B., Sladká, M., Damoiseaux, J., Panczak, A., Mandys, V., Francová, K., Kudláčková, M. (1999c) Characterization of T-cell lymphomas in the Prague inbred Sprague-Dawley/Cub rat strain: a model of spontaneous hematologic malignancy. *Transpl. Proc.* **31,** 1618-1619.

Poteracki, J., Walsh, K. M. (1998) Spontaneous neoplasms in control Wistar rats: a comparison review. *Toxicol. Sci*. **45,** 1-8.

Prates, V., Cobos, M., Bouzas, B., Napal, J., Bordone, J., Milone, J. (2000) The first report of familial adult T-cell leukemia/lymphoma in Argentina. *Leuk. Lymphoma* **37,** 225-227.

Seemayer, T. A., Schurch, W., Kalant, N. (1982) B cell lymphoproliferation in spontaneously diabetic BB Wistar rats. *Diabetologia* **23,** 261-5.

Schramlová, J., Otová, B., Černý, J., Blažek, K. (1994) Electron-microscopic demonstration of virus particles in acute lymphoblastic leukaemia in Sprague-Dawley rats. *Folia Biol. (Praha)* **40,** 113-118.

Sladká, M., Otová, B. (1994) Chromosome 11 participation in acute lymphoblastic leukaemia in Sprague-Dawley rats. *Folia Biol. (Praha)* **40,** 173-183.

Sladká, M., Křen, V., Klír, P. (1988) Spontaneous acute lymphoblastic leukaemia in Sprague-Dawley rats, II. Cytogenetic analysis of nine individual leukemias. *Neoplasma* **35,** 379-388.

Sladká, M., Otová, B. (1998) Cytogenetic changes in Sprague-Dawley rat lymphomas in the course of in vivo passaging. *Folia Biol. (Praha)* **4,** 143-150.

Svoboda, T., Jiřička, Z., Klír, P. (1989) Spontaneous acute lymphoblastic leukemia in Sprague-Dawley rats, III. clinico-pathologic observation. *Neoplasma* **36,** 149-154.

Ushijima, T. (2002) Report on Rat Chromosome 11. http://srcc.gen.gu.se/rcc11/srcc11report.html

Valeriánová, M., Votruba, I., Holý, A., Mandys, V., Otová, B. (2001) N^6-substituted derivatives of PMEDAP: antitumor activity against T-cell lymphoma. *Anticancer Res.* **21,** 2057-2064.

Walsh, K. M., Poteracki, J. (1994) Spontaneous neoplasms in control Wistar rats. *Fundam. Appl. Toxicol.* **22,** 65-72.

Zwicker, G. M., Eyster, R. C., Sell, D. M., Gass, J. H. (1992) Spontaneous brain and spinal cord/nerve neoplasms in aged Sprague-Dawley rats. *Toxicol. Pathol.* **20,** 576-584.