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

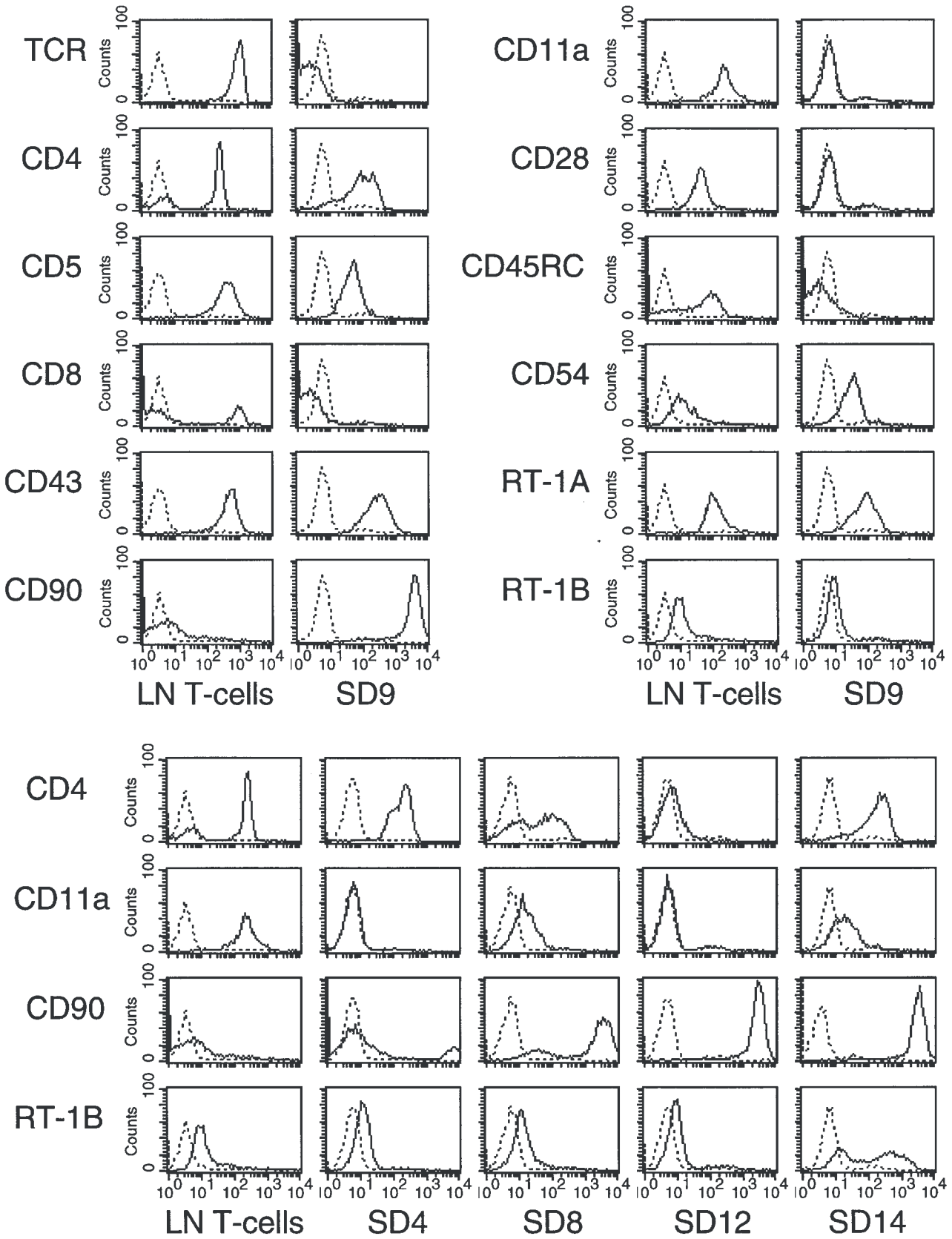

Fig. 7. Immunophenotype of haematological neoplasms in SD/Cub rats
Histograms represent the expression of the respective markers of a representative lymphoma (SD9/96) next to the phenotype of normal lymph node T cells derived from SD/Cub rats [A]. Heterogeneity of the lymphomas is shown in [B] for the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

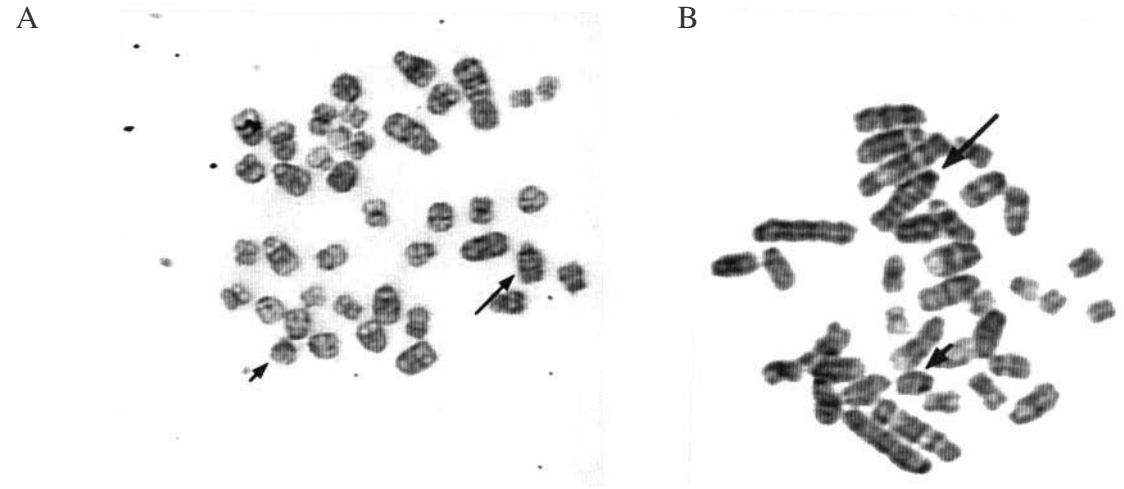

Fig. 8. G-banded metaphases obtained from SD lymphomas: [A] SD10/96 lymphoma, [B] SD9/96 lymphoma
Variation in the most frequent rearrangement of chromosome 11 termed as der(11q); critical region 11q (q11-q12). The arrows mark the homologous pair of chromosomes 11; short arrow – normal shape, long arrow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

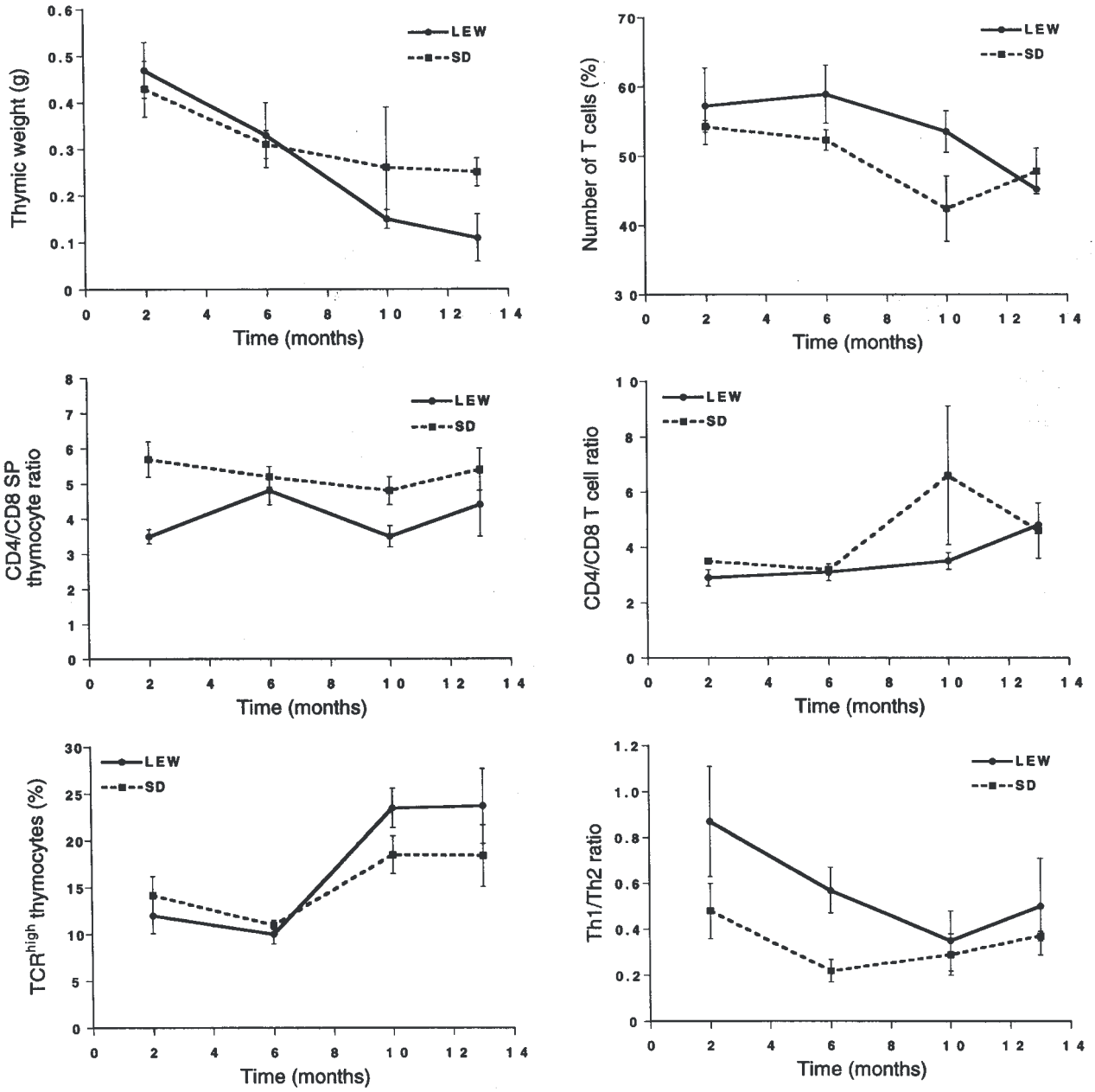

Fig. 9. Effect of ageing on the T-cell compartment of SD/Cub and Lewis rats
Age-dependent changes in the thymus [A] and the peripheral lymph nodes [B] are determined by measuring the thymic weight ([A], upper diagram), CD4/CD8 single positive, mature thymocyte ratio ([A], middle diagram), percentage 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).

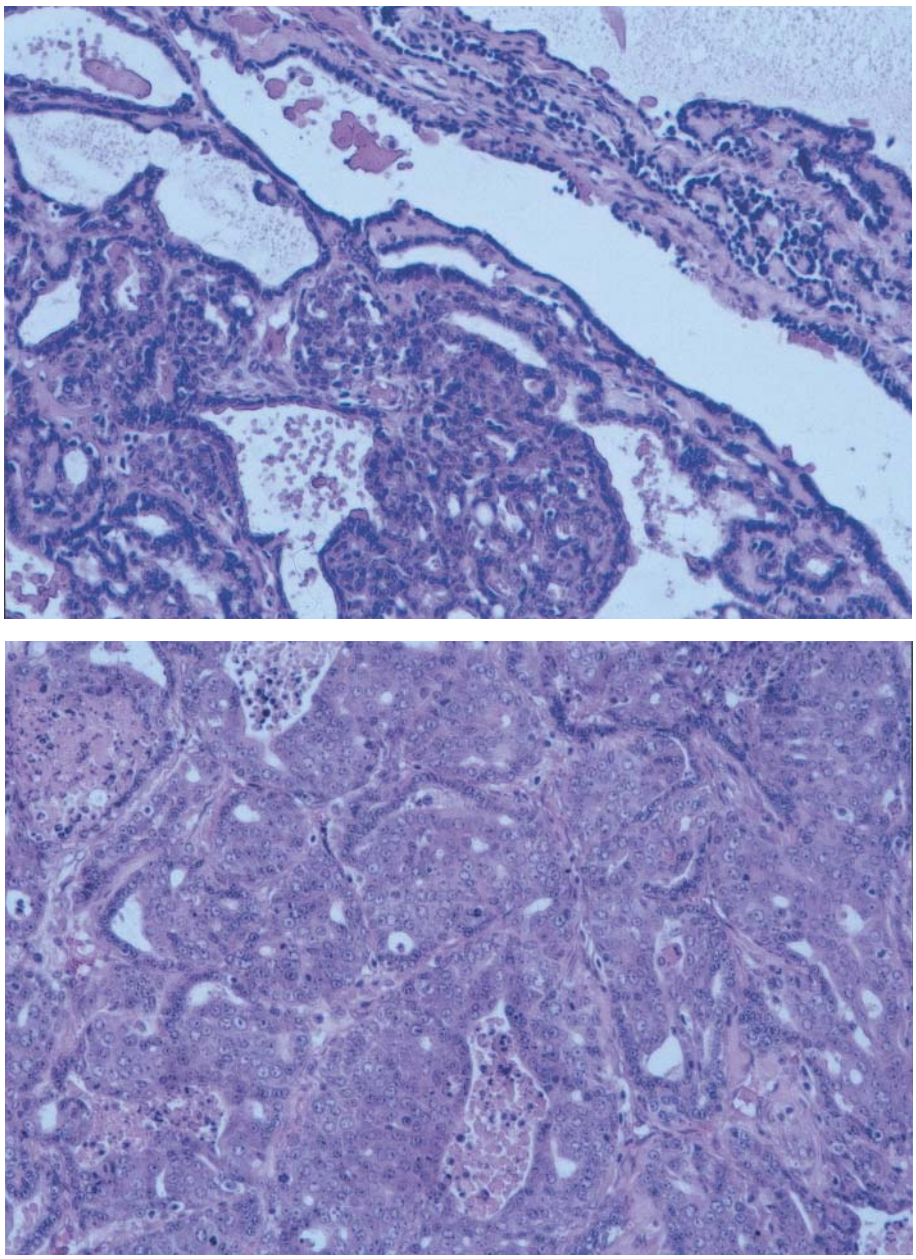

Fig. 10. Structures of eccrine spiradenoma predominating within a primary tumour [A], solid nests of epithelial cells of the carcinomatous component of the tumour [B], SD/Cub female rat (H&E, magnification 100x)

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

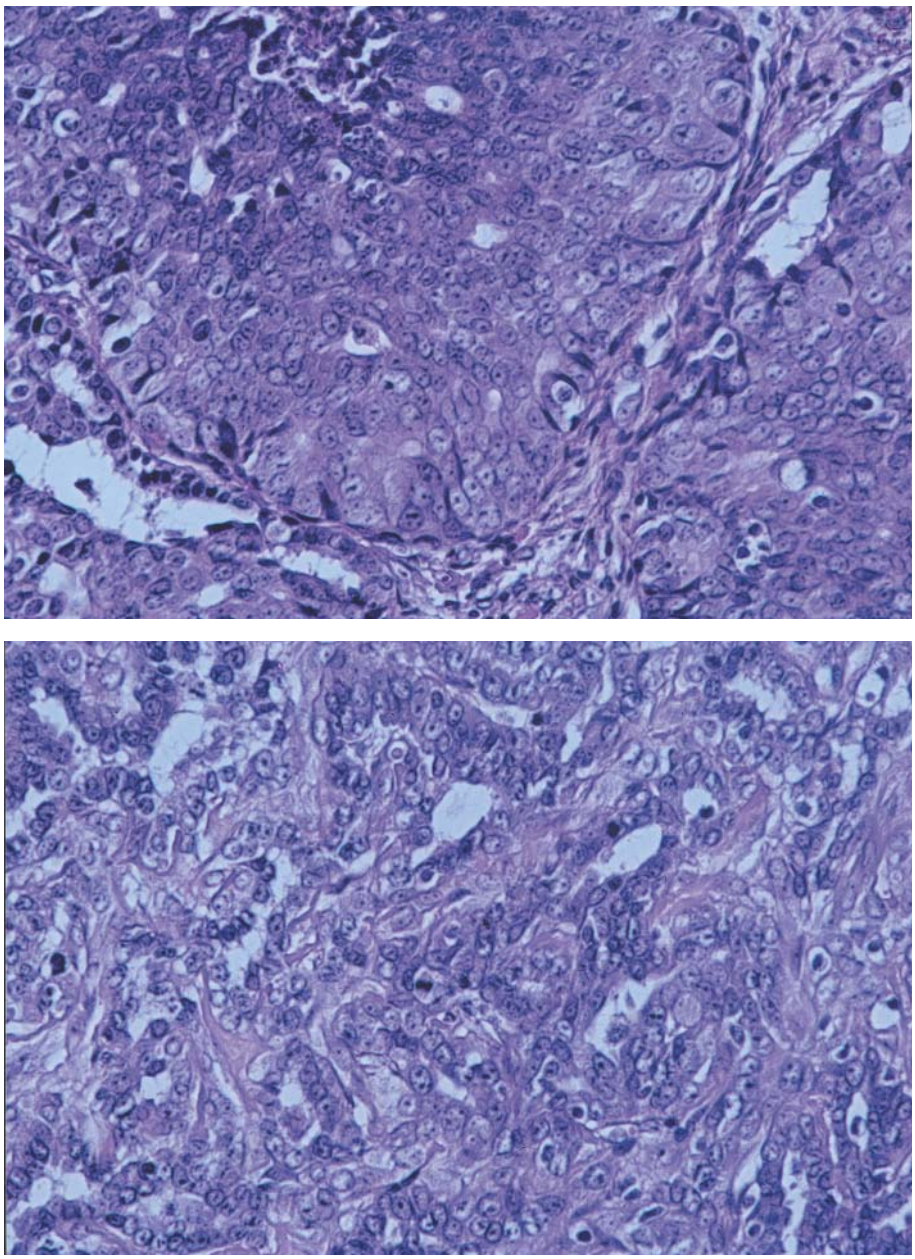

Fig. 11. Third passage of the tumour presented in Figure 10 Solid nests of carcinoma [A], irregular tubular structures within the carcinoma [B], SD/Cub female rat (H&E, magnification 200x)

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.

Acknowledgement

We gratefully acknowledge the excellent technical assistance of Ms. Anna Kolářová and Dr. Jitka Hanzalová.

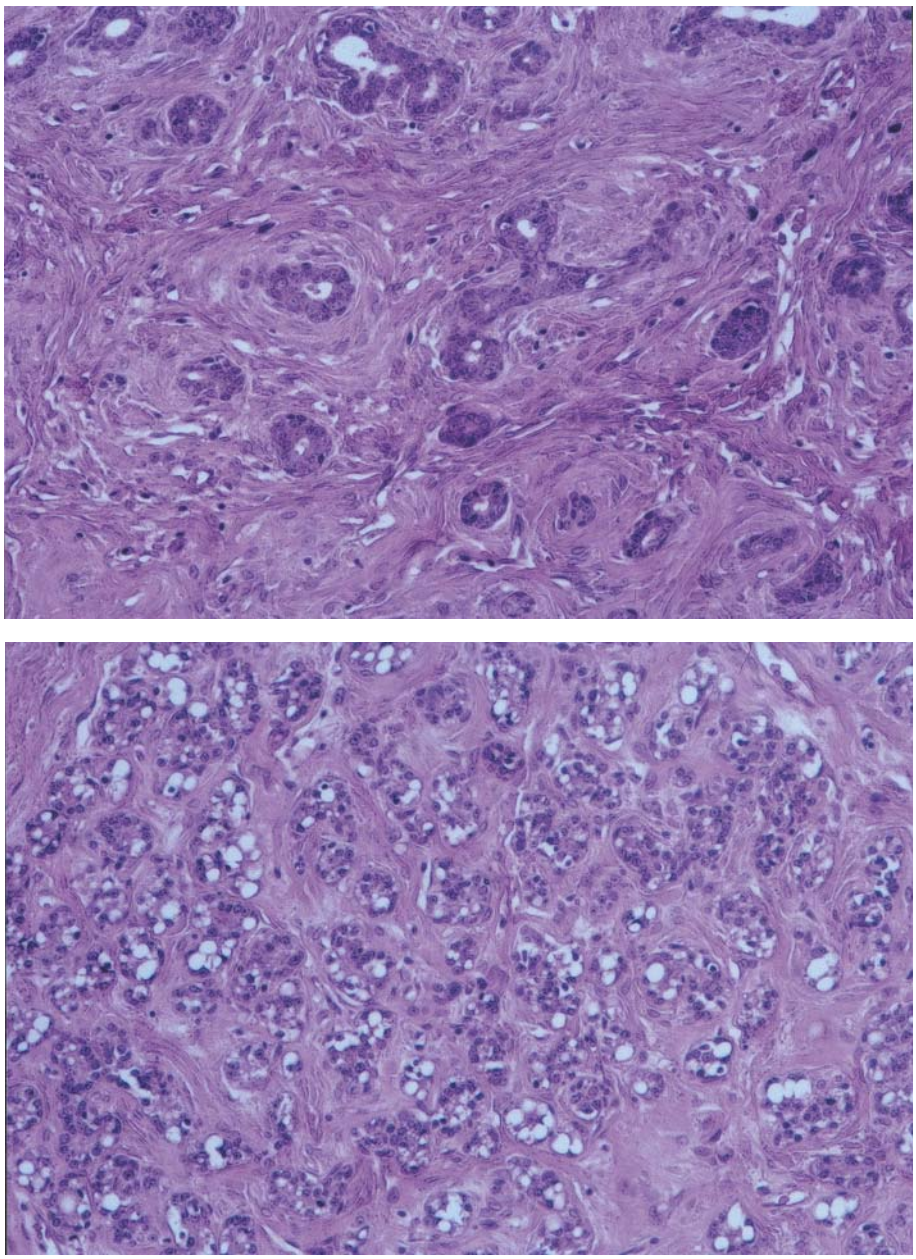

Fig. 12. SD/Cub rat fibroadenoma
Neoplastic tubular structures within the dense stroma [A], and adenomyoepithelial component of the tumour [B], SD/Cub male rat (H&E, magnification 100x)

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.