Rearrangements of Immunoglobulin and T-Cell Receptor Genes in Canine Lymphoma/Leukemia Cells

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ABSTRACT. Tumor cells from 15 canine lymphoma/leukemia cases were examined for genetic rearrangements of immunoglobulin and T-cell receptor (TCR) genes in parallel with cell surface antigens. Ten of these 15 cases showed rearrangements of the immunoglobulin heavy chain (IgH) gene, while 4 cases displayed TCRβ-chain gene rearrangements on Southern blot analysis. All the cases with IgH gene rearrangements had multicentric form lymphoma, and 6 of the 10 cases were cell surface immunoglobulin-positive. On the other hand, the cases with TCR gene rearrangements included atypical lymphoma / leukemia cases, and 3 of the 4 cases were Thy-1 antigen-positive. Although the tumor cell lineage of a considerable number of lymphoma/leukemia cases could not be determined by phenotypic analysis, examination of the IgH and TCR gene rearrangements disclosed the lineages of 14 of 15 cases. Genetic analysis demonstrated that the tumor cells in most canine multicentric lymphomas were formed by clonal expansion of B-lymphocyte. These findings show that studies on the rearrangements of immunoglobulin and TCR genes are very useful for understanding the cellular origin, clonality and hierarchy of canine lymphoma/leukemia cells.—KEY WORDS: canine, immunoglobulin gene, lymphoma, rearrangement, T-cell receptor gene.

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Lymphoma is one of the most common tumors in dogs, its age-adjusted annual incidence being estimated to be 24 cases per 100,000 dogs [6]. Retroviruses have been identified as causative agents of lymphoma/leukemia in many animal species, but the etiology of the disease in dogs is still unknown. As lymphomas in dogs are usually confined to specific areas of the lymphoid system in the early stage of the disease, thymic, alimentary, and multicentric forms can often be recognized [1]. Of these forms, the multicentric form is the most frequent [1, 30]. Lymphoma is sometimes associated with overspill leukemia, but acute lymphocytic leukemia is encountered as a distinct nosologic entity.

In humans and several animal species, lymphoma/leukemia cells have been categorized into B- and T-cell lineages. These B- and T-cells have been distinguished by functional criteria or by specific membrane features such as cell-surface immunoglobulin, rosette formation with erythrocytes of some species and reactivity with monoclonal antibodies against cell-surface antigens. However, in dogs as well as in humans, these particular markers are often absent or inapplicable to some tumor cells [8, 25] and so many lymphoma/leukemia cases have not yet been classified.

Recent molecular cloning of immunoglobulin and TCR genes have provided an insight into the process by which various diverse antibodies and antigen-specific TCRs are generated [10, 12, 13, 23, 32, 34]. Rearrangements of immunoglobulin and TCR genes can be used as DNA-level markers of human lymphoid neoplasms [3, 4, 7, 16, 17, 23, 24, 36]. In the present study, tumor cells from canine lymphoma/leukemia cases were examined for rearrangements of IgH and TCR genes to determine the clonality, lineage and hierarchy of the tumor cells.

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MATERIALS AND METHODS

Specimens: Fourteen cases of malignant lymphoma/lymphoid leukemia admitted to the Veterinary Medical Center, The University of Tokyo, for diagnosis and treatment were examined. The lymphoma/leukemia cells were obtained from their enlarged lymph nodes at biopsy or necropsy. Subsequent confirmation of the disease was performed by histological examination of portions of these lymph nodes. Clinical data are shown in Table 1. Single cell suspensions for surface marker analysis were prepared by gently pressing the tissues in RPMI-1640 medium. A portion of the tissue was rapidly frozen in liquid nitrogen and stored at −80°C for subsequent DNA extraction. The cell line CL-1 (Goitsuka *et al.*, submitted for publication) derived from a dog with thymic-form lymphoma was established in our laboratory, and also used.

Analysis of surface markers: Direct and indirect immunofluorescence techniques were used for the analyses of cell surface antigens. Briefly, the cells were washed twice with sorting buffer (0.8% NaCl, 0.02% KCl, 0.115% Na_2HPO_4, 0.02% KH_2PO_4 and 1% fetal calf serum). Cells were incubated with antibody to the cell surface antigen at 4°C for 30 min and washed three times with the sorting buffer. For indirect immunofluorescence, these cells were washed and incubated with FITC-labeled goat anti-mouse IgG at 4°C for 30 min. Cells were then washed and cell surface fluorescence was examined with a cytofluorometer, Cyto-Ace 150 (Japan Spectroscopic, Tokyo,

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Table 1. Clinical data on dogs

Patient	Breed	Sex	Age	Cancer	Peripheral blood blasts (/μl)
1	Welsh Corgi	Male	6 years	Multicentric	4.5×10^2
2	Shetland Sheep Dog	Female	10 years	Multicentric	5.3×10^3
3	Beagle	Female	4 years	Multicentric	Few
4	Pomeranian	Female	9 years	Multicentric	ND
5	Maltese	Female	9 years	Multicentric	ND
6	Shih Tzu	Female	7 years	Multicentric	ND
7	Shih Tzu	Male	9 years	Multicentric	4.6×10^5
8	Shih Tzu	Female	11 years	Multicentric	ND
9	Maltese	Female	4 years	Multicentric	1.3×10^4
10	Shih Tzu	Neutered male	9 years	Multicentric	ND
11	Shih Tzu	Male	9 years	Multicentric	ND
12	Shiba Dog	Female	4 years	Leukemia	1.0×10^3
13	Mixed	Female	4 months	Pulmonary mass	ND
14	Shiba Dog	Male	9 years	Alimentary	ND
15	(Cell line)	—	—	Thymic	—

ND: Not detected.

Japan). Several antibodies directed to canine lymphocytes were used. F3–20–7 [21] is a monoclonal antibody (McAb) directed to the canine Thy-1 antigen, which is a marker of T-lymphocytes. DT-2 [37] and E11 [28] were produced as McAbs reactive with surface antigens in subpopulations of canine lymphocytes. Anti-dog IgM (μ chain) and anti-dog IgG (heavy chain and light chain) antibodies (Cappel Research Products, Durham, NC) were also used to detect cell surface immunoglobulins.

DNA extraction and Southern blot analysis: Lymph node samples stored at −80°C were homogenized in liquid nitrogen, treated with lysis buffer containing 20 μg/ml proteinase K, 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 0.5% sodium dodecyl sulfate (SDS) and 10 μg/ml RNase A, extracted with phenol and chloroform, and then precipitated with ethanol. Samples of 15 μg of each DNA sample were digested with the restriction endonuclease *Bam*HI, *Hin*dIII or *Eco*RI. The DNA samples were separated by electrophoresis in 1% agarose gel and transferred to a hybridization membrane, GeneScreen *Plus* (NEN Research Products, Boston, MA). The DNA samples on the membrane were hybridized with ^{32}P-labeled probe in hybridization solution (1% SDS, 10% dextran sulfate, 1 M NaCl, 100 μg/ml salmon testis DNA and 50 mM Tris-HCl pH 7.5) at 65°C. After hybridization, the filters were washed with 2×SSC (1×SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with 1% SDS at 65°C for 30 min and autoradiographied.

DNA probes: IgH probe was prepared from a human DNA clone, Ch. H.Ig.μ-24. *Eco.Hin*d13 [29], representing the joining region (JH) of the human IgH gene containing the 3.5-kb *Eco*RI-*Hin*dIII fragment of this region. Canine TCRβ chain probe was prepared from a DNA clone, pDTB1, containing a 359-bp DNA fragment of the canine T-cell receptor β chain constant region (Cβ2), which was cloned in our laboratory (Goitsuka *et al.*, submitted for publication).

RESULTS

Phenotypic characterization of canine lymphoma/leukemia cells: Tumor cells from six cases (cases 1, 2, 3, 8, 9 and 10) with multicentric form lymphoma had cell-surface IgM or IgG. On the other hand, 3 cases (cases 11, 12 and 14) bore Thy-1 antigen detected with the monoclonal antibody F3–20–7. Six of 15 cases (cases 4, 5, 6, 7, 13 and 15), however, displayed neither cell-surface immunoglobulin nor Thy-1 antigen. DT-2 and E11 McAbs reacted with 6 and 4 cases, respectively (Table 2).

Germ-line patterns of canine IgH and TCR genes: We examined the germ-line patterns of canine IgH and TCRβ genes prior to examination of tumor DNA samples. Representative results of Southern blot using normal dog liver DNA are shown in Fig. 1. In their germ-line form, the gene segments encoding the J region of the IgH gene were present on the 5.2-kb *Bam*HI, 8.6-kb *Hin*dIII and 23-kb *Eco*RI fragments. The gene segments encoding the Cβ1 and Cβ2 genes of TCR were present on *Bam*HI fragments of 5.7 and 2.6 kb, on *Hin*dIII fragments of 6.2 and 4.1 kb and on a single 20-kb *Eco*RI fragment.

Rearrangements of the IgH gene in canine lymphoma/leukemia cells: Southern blot analysis using the *Bam*HI digest revealed that 10 (cases 1 to 10) of 15 cases of canine lymphoma/leukemia displayed IgH gene rearrangements, whereas the other 5 cases (cases 11 to 15) retained the germ-line configuration (Fig. 2a). In the DNA samples digested with *Hin*dIII, in addition to the cases 1 to 10, cases 11 and 15 also had a rearranged band (Fig. 2b). In the *Eco*RI-digested DNAs, rearrangement of IgH gene could be detected only in 8 (cases 1 to 8) of 15 cases examined (Fig. 2c). The tumor tissue DNAs of 9 cases (cases 2 to 10) showed two rearranged bands hybridizing with JH probe, suggesting rearrangements of both alleles in the IgH gene. However, case 1 showed only a single rearranged band, suggesting rearrangement and deletion of each allele. In some cases with IgH rearrangement, a faint germ-line band was detected in addition to the

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Table 2. Analyses of surface antigens and rearrangements of immunoglobulin and T-cell receptor genes

Case No.	Anatomic form	Surface markers					Genetic markers: JH probe			Genetic markers: C β probe		
		IgM	IgG	Thy-1	DT-2	E11	BamHI	HindIII	EcoRI	BamHI	HindIII	EcoRI
1	Multicentric	+	NT	−	+	+	R	R	R	G	G	G
2	Multicentric	+	−	−	+	−	R	R	R	G	G	G
3	Multicentric	−	+	−	−	−	R	R	R	G	G	G
4	Multicentric	−	NT	−	+	−	R	R	R	R*	G	G
5	Multicentric	−	−	−	+	+	R	R	R	G	G	G
6	Multicentric	−	−	−	−	−	R	R	R	G	G	G
7	Multicentric	−	NT	−	−	−	R	R	R	G	G	G
8	Multicentric	+	+	−	−	−	R	R	R	G	G	G
9	Multicentric	+	+	−	−	+	R	R	G	G	G	G
10	Multicentric	−	+	−	−	−	R	R	G	G	G	G
11	Multicentric	−	−	+	−	−	G	R*	G	G	G	R*
12	Leukemia	−	−	+	+	−	G	NT	G	R	NT	R
13	Pulmonary mass	−	−	−	−	−	G	G	G	R	R	G
14	Alimentary	−	NT	+	NT	−	G	G	G	R	R	R
15	Thymic	−	−	−	+	+	G	R*	G	R	R	R

+: Positive −: Negative, NT: Not tested, R: Rearranged, G: Germ line type, *: Possibly due to RFLP.

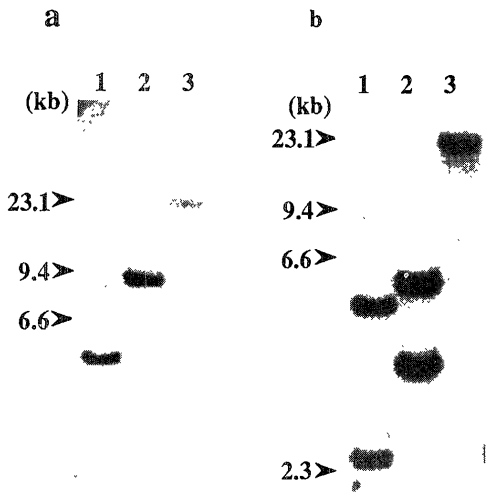


Fig. 1. Southern blot analysis of germ-line type fragments of IgH (a) and TCRβ (b) genes. Normal dog liver DNA was digested with *Bam*HI (lane 1), *Hin*dIII (lane 2), or *Eco*RI (lane 3) and subjected to Southern blot analysis using IgH J region probe (a) or TCR Cβ probe (b). Fragment sizes were estimated with *Hin*dIII-digest λ markers.

rearranged bands, indicating contamination of samples with a small amount of normal tissue. The abnormal fragments detected in cases 11 and 15 in the *Hin*dIII digests may possibly due to restriction fragment length polymorphism (RFLP) because these cases did not show any rearrangement in their *Bam*HI and *Eco*RI digests and retained a germ-line fragment in one allele which is usually deleted during the process of IgH gene rearrangement. These findings indicated that the tumor cells of 10 cases (cases 1 to 10) were committed to B-cell development at the immunoglobulin gene level. All the 10 DNA samples showing rearrangements of the IgH gene were derived from tumors of cases with multicentric form lymphoma. Furthermore, the demonstration of distinct bands by Southern blot analysis proved that these tumor cell populations represented clonal (monoclonal or at least oligoclonal) expansions of B lymphocytes.

Rearrangement of the TCR gene in canine lymphoma/leukemia cells: On Southern blot analysis of *Bam*HI digests, 5 cases (cases 4, 12, 13, 14 and 15) showed abnormal fragments hybridizing with the TCR β-chain gene probe, whereas the other 10 cases retained the germ-line configuration (Fig. 3a). In the *Hin*dIII-digested DNAs, abnormal fragments were detected in 3 cases (cases 13, 14 and 15) (Fig. 3b). In the *Eco*RI-digested DNAs, 5 cases (cases 11, 12, 13, 14 and 15) had rearrangements of TCR β-chain gene (Fig. 3c). The rearranged band in case 4 DNA digested with *Bam*HI and that in case 11 DNA digested with *Eco*RI were possibly due to RFLP because rearrangement was not detected in DNAs digested with other restriction enzymes and one allele retained germ-line configuration in these cases. These results indicated that four cases (cases 12, 13, 14 and 15) possessed rearrangements of TCRβ gene. Of these cases, cases 12 and 14 gave one rearranged band in their *Eco*RI digests, indicating TCRβ gene rearrangement on one allele and deletion on another in these cases. Case 13 also showed similar result but did not show a rearranged band in the *Eco*RI digests, probably indicating similar rearrangement and deletion of TCRβ gene on two alleles. Case 15 gave one germ-line band in *Bam*HI and *Hin*dIII digests and two rearranged fragments in an *Eco*RI digest, implying rearrangement lacking one Cβ gene on both alleles. These results indicated that the tumor cells of cases 12, 13, 14 and 15 were committed to T-cell development at the TCRβ gene level and represented

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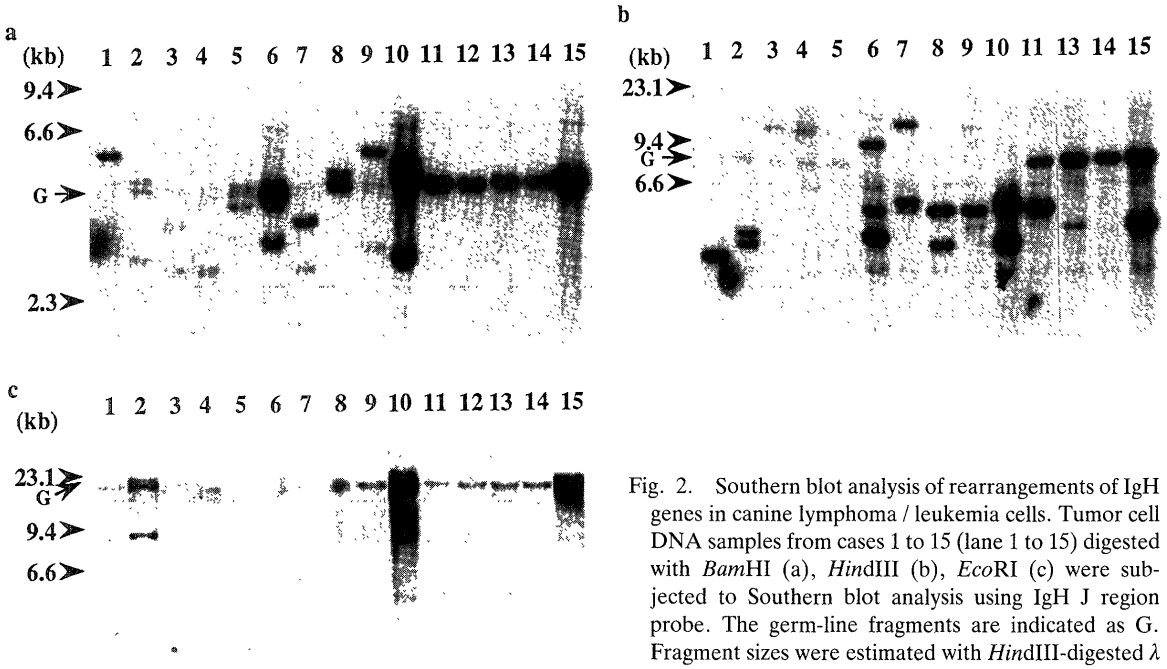


Fig. 2. Southern blot analysis of rearrangements of IgH genes in canine lymphoma / leukemia cells. Tumor cell DNA samples from cases 1 to 15 (lane 1 to 15) digested with *Bam*HI (a), *Hin*dIII (b), *Eco*RI (c) were subjected to Southern blot analysis using IgH J region probe. The germ-line fragments are indicated as G. Fragment sizes were estimated with *Hin*dIII-digested λ DNA markers.

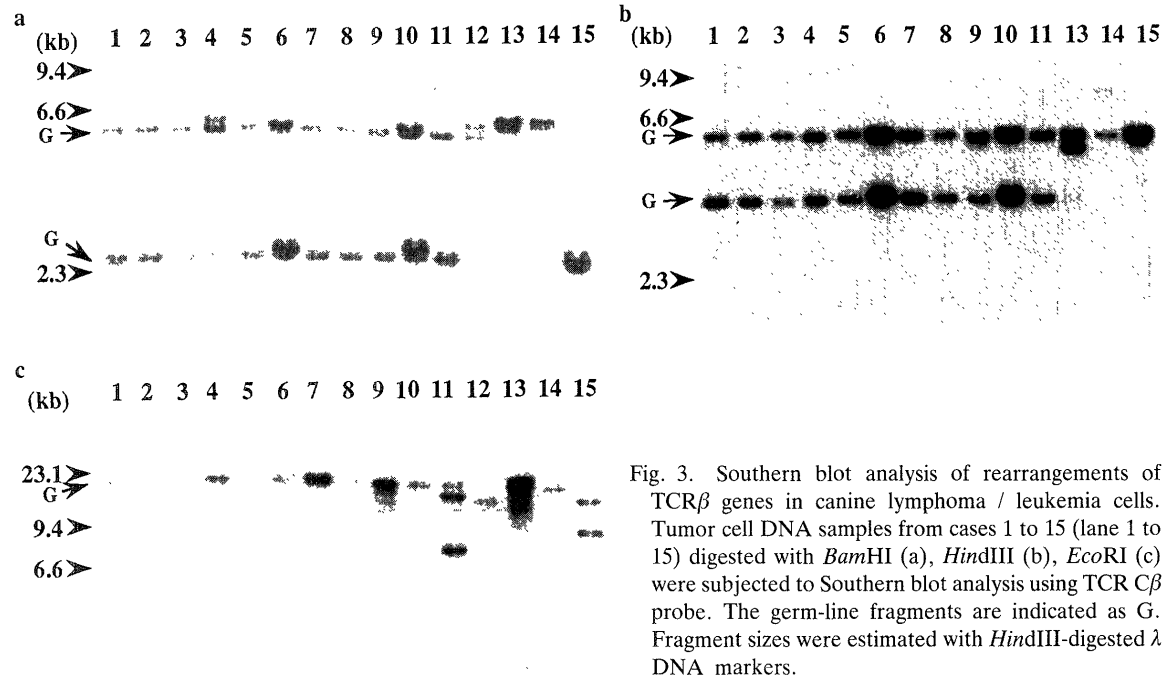


Fig. 3. Southern blot analysis of rearrangements of TCRβ genes in canine lymphoma / leukemia cells. Tumor cell DNA samples from cases 1 to 15 (lane 1 to 15) digested with *Bam*HI (a), *Hin*dIII (b), *Eco*RI (c) were subjected to Southern blot analysis using TCR Cβ probe. The germ-line fragments are indicated as G. Fragment sizes were estimated with *Hin*dIII-digested λ DNA markers.

clonal expansions of T-lymphocytes.

DISCUSSION

In the present study we classified canine lymphoma/ leukemia cells on the basis of rearrangements of the IgH and TCRβ genes. Fourteen of the 15 cases examined in this study were classified as B- or T-cell lineage by genetic analysis, although 6 cases were negative for both immunoglobulin and Thy-1 antigen. Most of the cases with surface immunoglobulin (cases 1, 2, 3, 8, 9 and 10) and Thy-1 antigen (cases 12 and 14) were found to have rearrangements of IgH and TCR genes, respectively. However case 11 which expressed Thy-1 antigen did not show any rearrangement of TCR gene. It can be considered that the tumor cells in this case might be derived from another T-cell lineage, $\gamma\delta$T-cell.

DT-2 [37] and E11 [28] were produced as McAbs

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reactive with surface antigens in subpopulation of canine T-lymphocytes [28, 37]. However, in the present study, these McAbs reacted with some tumor cells committed to B-cell defferentiation. It was reported that T-cell antigen identified by DT-2 was expressed on lymphoma cells with B-cell phenotype [2, 18]. It is conceivable that the T-cell antigens identified by DT-2 and E11 are expressed on some immature or undifferentiated B-cells.

The 6 cases showing rearrangement of IgH (cases 4, 5, 6 and 7) and TCR (cases 13 and 15) genes but lacking sIg and Thy-1 antigen were considered to be undifferentiated tumor cells (Table 2). Analyses of the rearrangements of immunoglobulin and TCR genes have several advantages over the characterization of cell surface phenotypes for determining lineages, differentiation and clonality of the lymphoid tumor cells, because these analyses can detect the initial genetic events occurring in the early stage of B- and T-cell differentiations [17]. Further investigations on the differentiation of B-cell lineage may be possibly by detections of the rearrangements of immunoglobulin κ and λ chain genes [16]. Another T-cell lineage, $\gamma\delta$T-cells may also be identified by analysis of rearrangement of TCR γ and δ chain genes [14, 27, 31]. Analyses of these gene structures may be required in some cases of lymphoma/leukemia.

The causative factor of canine lymphoma is still unknown. The viral etiology in some cases of the specific type of naturally occurring lymphoma/leukemia in many other species has been proved. For example, bovine leukemia virus is associated with B-cell lymphoma/leukemia in cattle [22] and human T-cell leukemia virus is involved in the leukemogenesis of adult T-cell leukemia in humans [11, 26, 35]. Detailed characterizations of lymphoma/leukemia cells with genetic analysis in addition to immunological and cytological examinations are considered to be very useful for identification of specific types of lymphoma/leukemia, which are necessary in further studies on the etiology of canine lymphoma/leukemia. In several human lymphomas/leukemias, such as Burkitt's lymphoma and B-cell follicular lymphoma, specific chromosomal translocation have been detected [15, 19, 33]. In these cases, oncogenes and regulatory elements of the immunoglobulin genes around the chromosomal break points were shown to be associated with tumorigenesis. Chromosomal translocation in canine lymphomas has not been studied, but analysis of rearrangements of immunoglobulin and TCR genes should be helpful for understanding their pathogenesis, because many translocation events in tumor development have been identified at the loci of immunoglobulin and TCR genes.

Detailed typing of lymphoma/leukemia cells may also be useful in predicting the prognosis of patients. Greenlee *et al.* [8] reported the association of the immunological subtype with the prognosis after chemotherapy of dogs with lymphoma. More detailed typing of the cells by genetic analysis as used in this study may provide more information on their clinical features and prognosis of canine lymphoma/leukemia. Moreover, genetic rearrangements detected in canine lymphoma/leukemia cells should also be useful to monitor the efficiency of therapy. Survival of tumor cells after therapy can be detected by demonstrating the presence of rearranged bands of immunoglobulin and TCR genes [5, 9, 20].

Canine lymphoma is a common, naturally occurring tumor and is important not only in the veterinary field but also for investigation of tumorigenesis in mammals. The present study on the rearrangements of immunoglobulin and TCR genes provide information on the origin and differentiation of tumor cells and on the mechanisms of tumorigenesis in canine lymphoma/leukemia.

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REFERENCES

1. Anderson, J., Jarrett, W. F., and Crighton, G. W. 1969. A classification of lymphoid neoplasms of domestic mammals. *J. Natl. Cancer Inst.* 32: 343–345.
2. Appelbaum, F. R., Sale, G. E., Storb, R., Charrier, K., Deeg, H. J., Graham, T., and Wulff J. C. 1984. Phenotyping of canine lymphoma with monoclonal antibodies directed at cell surface antigens: classification, morphology, clinical presentation and response to chemotherapy. *Hematol. Oncol.* 2: 151–168.
3. Arnord, A., Cossman, J., Bakhohi, N. B., Jaffe, E. S., Waldmann, T. A., and Korsmeyer, S. J. 1983. Immunoglobulin gene rearrangement as a unique clonal marker in human lymphoid neoplasms. *New Engl. J. Med.* 309: 1593–1595.
4. Cleary, M. L., Chao, J., Wranke, R., and Sklar, J. 1984. Immunoglobulin gene rearrangement as a diagnostic criterion of B-cell lymphoma. *Proc. Natl. Acad. Sci. U.S.A.* 81: 593–597.
5. d'Auriol, L., Macintyre, E., Galibert F., and Sigaux, F. 1989. *In vitro* amplification of T-cell gene rearrangements: a new tool for the assessment of minimal residual disease in acute lymphoblastic leukemias. *Leukemia* 3: 155–158.
6. Dorn, C. R., Taylor, D. O., and Hibbard, H. H. 1967. Epizootiological characteristics of canine and feline leukemia and lymphoma. *Am. J. Vet. Res.* 28: 993–1001.
7. Flug, F., Pelicci, P. G., Bonetti, F., Knowles, D. M., and Dalla-Favera, R. 1985. T-cell receptor gene rearrangements as markers of lineage and clonality in T-cell neoplasms. *Proc. Natl. Acad. Sci. U.S.A.* 82: 3460–3464.
8. Greenlee, P. G., Filippa, D. A., Quimby, F. W., Patnaik, A. K., Calvano, S. E., Matus, R. E., Kimmel, M., Hurvits, A. I., and Lieberman, P. H. 1990. Lymphomas in dogs. A morphologic, Immunologic, and clinical study. *Cancer* 66: 480–490.
9. Hansen-Hagge, T. E., Yokota, S., and Bartram, C. R.

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1989. Detection of minimal residual disease in acute lymphoblastic leukemia by *in vitro* amplification of rearranged T-cell receptor chain sequence. *Blood* 74: 1762–1767.

10. Herdrick, S. M., Cohen, D. I., Nielsen, E. A., and Davis, M. M. 1984. Isolation of cDNA clones encoding T cell-specific membrane-associated proteins. *Nature (Lond.)* 308: 149–153.
11. Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinoshita, K., Shirakawa, S., and Miyoshi, I. 1981. Adult T-cell leukemia antigen in an ATL cell line and detection of antibodies to antigen in human sera. *Proc. Natl. Acad. Sci. U.S.A.* 78: 6476–6480.
12. Honjo, T. 1983. Immunoglobulin genes. *Ann. Rev. Immunol.* 1· 499–528.
13. Honjo, T. and Habu, S. 1985. Origin of immune diversity: Genetic variation and seletion. *Ann. Rev. Biochem.* 54: 803–830.
14. Kimura, N., Takihara, Y., Akiyoshi, T., Yoshida, T., Ohshima, K., Kawara, T., Hisano, S., Kozuru, M., Okumura, M., and Kikuchi, M. 1989. Rearrangement of T-cell receptor δ chain gene as marker of lineage and clonality in T-cell lymphoproliferative disorders. *Cancer Res.* 49: 4488–4492.
15. Klein, G. 1983. Specific chromosomal translocations and the genes of B-cell-derived tumors in mice and men. *Cell* 32: 311–315.
16. Korsmeyer, S. J., Arnord, A., Bakhshi, A., Ravetch, J. V., Siebenrist, U., Hieter, P. A., Sharrow, S. O., Lebien, T. W., Kersey, J. H., Poplack, D. G., Leder, P., and Waldmann, T. A. 1983. Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemias of T-cell and B-cell precursor origins. *J. Clin. Invest.* 71: 301–313.
17. Korsmeyer, S. J., Hieter, P. A., Ravetch, J. V., Poplack, D. G., Waldmann, T. A., and Leder, P. 1981. Developmental hierarchy of immunoglobulin gene rearrangement in human leukemic pre-B-cells. *Proc. Natl. Acad. Sci. U.S.A.* 78: 7096–7100.
18. Ladiges, W. C., Keast, M., Appelbaum F., and Storb, R. 1988. Phenotypic characterization of canine lymphoma, using monoclonal antibodies and a microlymphocytotoxicity assay. *Am. J. Vet. Res.* 49: 870–872.
19. Leder, P., Battey, J., Lenoir, G., Moulding, C., Murohy, W., Potter, H., Stewart, T., and Taub, R. 1983. Translocations among antibody genes in human cancer. *Science* 222: 765–771.
20. Lee, M. S., Chang, K. S., Cabanillas, F., Freireich, E. J., Trujillo, J. M., and Stass, S. A. 1987. Detection of minimal residual cells carrying the t(14,18) by DNA sequence amplification. *Science* 237: 175–178.
21. Mckenzie, J. L. and Fabre, J. W. 1981. Studies with a monoclonal antibody on the distribution of Thy-1 in the lymphoid and extracellular connective tissues of the dog. *Transplantation* 31: 275–282.
22. Miller, J. M., Miller, L. D., Closon, C., and Gillette, K. M. 1969. Virus-like particles in phytohemagglutinin-stimulated lymphocyte cultures with reference to bovine lymphosarcoma. *J. Natl. Cancer Inst.* 43: 1297–1305.
23. Minden, M. D. and Mark, T. W. 1986. The Structure of the T cell antigen receptor genes in normal and malignant T cells. *Blood* 68: 327–336.
24. Minden, M. D., Toyonaga, B., Ha, K. M., Yanagi, Y., Chin, B., Gelfano, E., and Mak, T. W. 1985. Somatic rearrangement of T-cell antigen receptor gene in human T-cell malignancies. *Proc. Natl. Acad. Sci. U.S.A.* 82: 1221–1227.
25. Onions, D. E. 1979. B- and T-cells markers in canine lymphoma cells. *J. Natl. Cancer Inst.* 59: 1001–1006.
26. Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., and Gallo, R. C. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. U.S.A.* 77: 7415–7419.
27. Quertermous, T., Murre, C., Dialynas, D., Duby, A., Strominger, J. L., Waldman, T. A., and Seidman, J. G. 1986. Human T-cell γ chain genes: Organization, diversity, and rearrangement. *Science* 231: 252–255.
28. Szer, J., Deeg, H-J., Severns, E., and Strob, R. 1984. Characterization of DLA-D restricted suppressor cells in the dog. *Clin. Res.* 32: 360A.
29. Takahashi, N., Nakai, S., and Honjo, T. 1980. Cloning of human immunoglobulin μ gene and comparison with mouse μ gene. *Nucleic Acid Res.* 8: 1983–1991.
30. Theilen, G. H. and Maidwell, B. R. 1979. pp. 241–252. Veterinary Cancer Medicine. Lea & Feiber, Philadelphia.
31. Tkachuk, D. C., Griesser, H., Takihara, Y., Champagne, E., Minden, M., Feller, A. C., Lennert, K., and Mak, T. W. 1988. Rearrangement of T-cell δ locus in lymphoproliferative disorders. *Blood* 72: 353–357.
32. Tonegawa, S. 1983. Somatic generation of antibody diversity. *Nature (Lond.)* 302: 575–581.
33. Tsujimoto, Y., Cossman, J., Jaffe, E., and Croce, C. M. 1985. Involvement of the bcl-2 gene in human follicular lymphoma. *Science* 228: 1440–1443.
34. Yanagi, Y., Yoshikai, Y., Leggett, K., Clark, S. P., Aleksander, I., and Mak, T. W. 1984. A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin gene. *Nature (Lond.)* 308: 145–149.
35. Yoshida, M., Miyoshi, I., and Hinuma, Y. 1982. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc. Natl. Acad. Sci. U.S.A.* 79: 2031–2035.
36. Waldmann, T. A., Davis, M. M., Bongiovanni, K. F., and Korsmeyer, S. J. 1985. Rearrangements of gene for the antigen receptor on T-cell as markers of lineage and clonality in human lymphoid neoplasms. *New Engl. J. Med.* 313: 776–783.
37. Wulff, J. C., Deeg, H-J., and Storb, R. 1982. A monoclonal antibody (DT-2) recognizing canine T lymphocytes. *Transplantation* 33: 616–620.

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