Europ. J. Cancer Vol. 17, pp. 61–69

0014-2964/81/0101-0061 $02.00/0

Heterotransplantation of Natural Bovine Lymphosarcoma to Nude Mice*

M. ONUMA,† K. MATSUMOTO,‡ H. KODAMA,† Y. FUJIMOTO,§ R. MORIGUCHI,§ T. MIKAMI† and H. IZAWA†

†*Department of Epizootiology, Faculty of Veterinary Medicine,* ‡*Institute for Animal Experiment, School of Medicine, and* §*Department of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo* 060, *Japan*

Abstract—*Nude mice of BALB/c background were utilized for the heterotransplantation of tissues derived from 4 forms of natural bovine lymphosarcoma. The growing tumors have been established from adult case tumor (6 out of 22 attempts), from calf case tumor (2 out of 5 attempts), from skin case tumor (18 out of 30 attempts) and from thymic case tumor (3 out of 11 attempts). In all, successful tumor growth was observed in 29 out of 68 transplanted natural bovine lymphosarcoma cells. Transplanted tumors from skin case grew fast, metastasized and then became lethal to the host. However, transplanted adult case tumors regressed and no spread of tumor cells was observed.*

INTRODUCTION

ENZOOTIC bovine leukosis (EBL) or the adult form of bovine lymphosarcoma (ALS) is the most common neoplastic disease in cattle induced by bovine leukemia virus (BLV) and it is contagious [1]. Sporadic bovine leukosis (SBL) is rare and its etiology is unknown. Three forms of SBL are recognized; calf form (CLS), thymic form (TLS) and skin form (SLS).

Recent studies on cell surface marker or the antigen of bovine lymphosarcoma cells revealed the following: (1) the specific tumor-associated antigen was found on neoplastic lymphoid cells from cattle with EBL, but not on the cells from cattle with SBL [2], and (2) the origin of neoplastic cells of EBL is the B cell but that of SBL is not [3–5].

Since SBL is very rare, it is very difficult to examine the properties of SBL tumor cells. Therefore it is highly desirable to maintain these tumor cells in laboratory animals.

The use of nude mice for the heterotransplantation of tumors has been widely adapted for studies on human neoplasms [6–8]. However, only few studies have been done with neoplasms from domestic animals [9, 10]. Recently, Irvin *et al.* [11] have reported the heterotransplantation of the transformed cell line derived from bovine lymphosarcoma in irradiated nude mice.

This communication describes the heterotransplantation of natural bovine lymphosarcoma to nude mice.

MATERIALS AND METHODS

Nude mice

The nude mice utilized were of BALB/c strain, and were bred under specific pathogen-free conditions. New-born mice less than 1 week old and adult mice 4–6 weeks old were used. Irradiation was carried out using X-rays with 150 rads.

Tumor specimens

The tumor specimens used for transplantation were obtained from four different forms of natural bovine lymphosarcoma as listed in Table 1. Diagnosis of the lymphosarcoma was confirmed by clinical and histological examinations. Histologically, these tumors consisted of massive infiltrations of neoplastic cells. Fresh bovine tumors were collected in Eagle's minimal essential medium containing 10% fetal calf serum and antibiotics. Tumor cells were teased out with scissors and forceps. The cells were washed twice with PBS and then pelleted down by low speed (1000 rev/min for 5 min). Viability of the cells was determined by trypan blue dye exclusion. About 3×10^7 living cells were injected into the subcu-

Accepted 10 July 1980.
*This work was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

Table 1. BLV antibody, BLV and percentage of B cells in cattle with lymphosarcoma used

Cattle with lymphosarcoma			Lymphocyte		BLV	B cell†
Case No.	Form	Age (yr)	(cmm)	BLV*	antibody	(%)
131	ALS	10	1848	+	+	45
125	CLS	0.5	47,600	−	−	2.4
124	SLS	4.5	2430	−	−	2.0
135	TLS	1.8	2891	−	−	3.9
150	TLS	2.0	74,688	−	−	7.5

*BLV was detected by SA.
†B cells were detected by the surface immunoglobulin immunofluorescence test [3].

taneous layer of the back of nude mice using 18 gauge of needle. Tumors were passed from mouse to mouse by surgically removing tumor tissue after anesthesia by ether.

Histological analysis

Materials for light microscopic examination was fixed with 10% formalin. Paraffin sections were routinely stained with hematoxylin and eosin.

Karyotypic analysis

Chromosome analyses were made on cells cultured for short-term periods (2–3 days) without the addition of a mitogen. Samples of growing cells were exposed to Colcemid (0.0025 mg/ml of medium) for 5 hr. The cells were then centrifuged, treated with a hypotonic solution (0.075 M KCl) for 10 min and fixed in Carnoy fixative. Slides were stained with Giemsa.

Serological examinations

To detect antibodies to BLV, agar gel immunodiffusion and complement fixation tests were performed [12, 13]. A direct fluorescent antibody (FA) test was performed to detect BLV antigen as previously described [12, 13] using fluorescein isothiocyanate (FITC)-conjugated V34 γ-globulin [13].

Syncytia assay

Syncytia assay (SA) was performed to detect BLV using F81 cells as indicator cells [14].

RESULTS

The presence of BLV and antibody to BLV and the percentages of B cells in cattle with lymphosarcoma used for heterotransplantation are shown in Table 1. BLV and antibody to BLV were detected in case No. 131 but not in the other cases. The percentage of B cells in lymph node tumor of No. 131 was higher than that in normal lymph nodes of cattle. However, the percentages of B cell from the other cases (125, 124, 135 and 150) tested were lower than that in normal bovine cells.

The results of heterotransplantation of 5 bovine lymphosarcoma cases into nude mice are listed in Table 2. The growing tumors have been established from adult case tumor (6 out of 22 attempts), from calf case tumor (2 out of 5 attempts), from skin case tumor (18 out of 30 attempts) and from thymic case tumor (3 out of 11 attempts). In all, successful tumor growth was observed in 29 out of 68 transplanted natural bovine lymphosarcoma cells.

Representative growth curves of 4 natural bovine lymphosarcoma in nude mice are shown in Fig. 1. Compared to tumors from CLS and TLS, the intervals of appearance after inoculation of tumors from SLS and ALS were relatively short. In SLS tumors, tumor growth was rapid and the mice eventually died because of the tumor. However, tumor regression was observed in nude mice transplanted with ALS tumors. In CLS or TLS tumors in nude mice, tumor growth was relatively slow, took 2–3 weeks to reach maximum size and then remained with the same appearance for 1–2 weeks until the mice were killed for further experiments.

Monolayer cultures were established from all transplanted tumors in the nude mice. BLV and BLV antigen was detected in cultured cells from nude mice tumors injected with No. 131 ALS tumor by SA and FA, respectively. However, no BLV antigen or BLV was detected in cultured cell from nude mice tumors injected with 125 CLS, 124 SLS

Table 2 Results of heterotransplantation of bovine lymphosarcoma in nude mice

Source of bovine lymphosarcoma		Nude mice injected	1st passage* Local s.c. growth	1st passage* Spread	2nd passage* Local s.c. growth	2nd passage* Spread	3rd passage* Local s.c. growth	3rd passage* Spread
ALS (131)	Lymph node tumor	New born	2/5	–	2/3	–		
		Adult	0/6					
		Adult+X†	2/8	–				
CLS (125)	Lymph node tumor	Adult	1/1	–	1/4	–		
SLS (124)	Lymph node tumor	New born	1/2	+	1/2	+	1/2	–
		Adult	2/7	+	2/4	+	3/3	–
		Adult+X	3/4	+	5/6	+		
TLS (135)	Thymus tumor	Adult	0/6					
TLS (150)	Lymph node tumor	Adult	3/5	–				

*Passage number in nude mice.
†Adult nude mice were irradiated with 150 rads of X-rays.
‡Number of mice with tumors/number of mice injected.

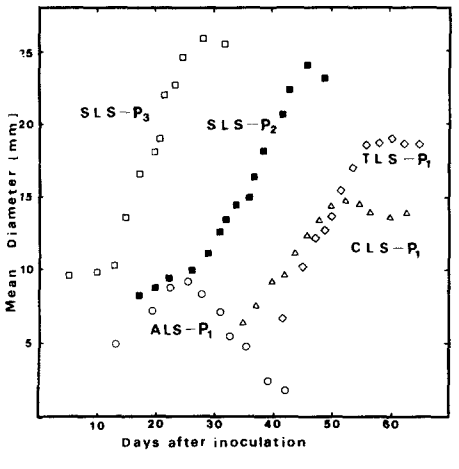

Fig. 1. Growth curves of four forms of natural bovine lymphosarcoma implanted in nude mice. $P_{1,2,3}$: *passage number in nude mice.*

and 150 TLS tumors. Karyological analysis showed that the tumors induced in the nude mice tested (13 cases) were of bovine origin but not of mouse origin (Fig. 2).

Nude mice tumors as well as original bovine lymphosarcoma used for transplantation were histologically examined. The original tumors of skin case and adult case revealed lymphoblastic lymphosarcoma (Figs. 3a and 5a), whereas the tumor of calf case and thymic case showed lymphocytic lymphosarcoma (Figs. 4a and 6a). The neoplastic lymphoid cells of transplanted tumors derived from 4 bovine lymphosarcoma cells in nude mice were morphologically similar to those of the respective original tumors (Figs. 3b, 3c, 4b, 5b, 5c and 6b). The transplanted tumors were composed of a uniform population of neoplastic lymphoid cells. Mitosis was frequently observed in the nude mice tumors derived from skin case tumor. Metastasis was found in the liver, spleen, lungs and axillary lymph nodes in the nude mice derived from skin case tumor but not in the transplanted nude mice derived from adult case, calf case and thymic case tumors.

DISCUSSION

In spite of the wide use of congenitally athymic nude mice for the heterotransplantation of human neoplasms, there has been relatively little documentation of their use in studies of neoplasms from domestic animals. Oughton and Owen [10] successfully transplanted a number of canine neoplasms to nude mice. Three specimens of dog gastric cancer with local invasion were also transplanted to nude mice [15]. The successful transplantation of bovine squamous cell carcinoma tissue into nude mice has been reported [9]. Irvin *et al.* [11] reported that

cultured bovine lymphosarcoma cells grew only in irradiated nude mice and indicated that a high degree of immunosuppression is required. In the present experiment, however, the tumor growth was observed in both the irradiated and non-irradiated nude mice when primary tumor fragments were transplanted to nude mice. This is in contrast to the findings of Morgan *et al.* [16] transplanting canine lymphosarcoma into nude mice. Lymphosarcoma cells from 8 dogs failed to grow in nude mice. Following whole body X-irradiation, however, a further lymphosarcoma grew successfully and was subsequently transplanted to both irradiated and non-irradiated nude mice.

The role of virus etiology of EBL has been reviewed and it has been found that BLV can cause persistent lymphocytosis or lymphosarcoma on inoculation into cattle [17] and sheep [18, 19] but the efficacy of BLV to induce tumors in goats seem rather weak [20, 21]. In the present work, successful tumor growth in nude mice was observed in 6 out of 22 transplanted ALS tumors. BLV antigen and BLV was detected in cultured cells from these nude mice tumors. Karyological analysis of reisolated tumor cells showed bovine karyotype but not mouse karyotype. This suggests that the tumor growth was simply dependent on the continued division of bovine lymphosarcoma cells transferred from bovine to nude mice, and not on viral transformation or invasion of host cells.

The etiology of SBL is unknown. Tumor cells from EBL originate from a B cell, while those from SBL do not [3–5]. The specific tumor-associated antigen was found on neoplastic lymphoid cells from EBL but not from SBL [2, 4]. Inoculation of SBL tumor materials into experimental calves and sheep resulted in induction of anti-BLV antibody and lymphosarcoma [22]. In this reported case, however, accidental contamination by BLV remains a plausible possibility because no BLV sequences were detected in sporadic tumors by molecular hybridization tests [23]. Transmission of SBL to homologous and heterologous animals has never been successful. The present work appears to represent the first successful heterotransplantation of natural bovine lymphosarcoma from both EBL and SBL to nude mice.

It is interesting that one of the transplanted SBL tumors (SLS) grew very fast and the tumor became lethal to the host. Spread of tumor cells at the vascular site of liver, spleen and lung was also demonstrated. However, transplanted EBL tumor regressed and no spread of tumor cells was observed. These differences may be due to the different origins of tumor cells of EBL and SBL; however, the exact reasons are not yet clear. Experiments on the pathological and biological differences in nude mice when tumor cells from EBL and SBL were transplanted to them are now in progress.

The present experiments indicate that nude mice can be used as a potential laboratory animal model system for differentiation of EBL and SBL.

Acknowledgements—We are grateful to Drs. K. Matsukawa, Department of Pathology, College of Dairy Agriculture, Ebetsu, and S. Ichijo, Department of Veterinary Internal Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, for obtaining lymphosarcoma materials and to Dr. Y. Miyake, Department of Veterinary Obstetrics, Faculty of Veterinary Medicine, Hokkaido University, for assistance with karyotypic analysis. We also thank Miss Y. Munakata for preparation of the manuscript.

REFERENCES

1. G. H. Theilen, D. L. Dungworth, J. Lengyel and L. S. Rosenblatt, Bovine lymphosarcoma in California. I. Epizootiologic and hematologic aspects. *Hlth Lab. Sci.* **1,** 96 (1964).
2. M. Onuma and C. Olson, Tumor-associated antigen in bovine and ovine lymphosarcoma. *Cancer Res.* **37,** 3249 (1977).
3. M. Onuma, T. Honma, T. Mikami, S. Ichijo and T. Konishi, Studies on the sporadic and enzootic forms of bovine leukosis. *J. comp. Path.* **89,** 159 (1979).
4. M. Onuma, I. Takashima and C. Olson, Tumor-associated antigen and cell surface marker in cells of bovine lymphosarcoma. *Ann. Rech. vet.* **9,** 825 (1978).
5. I. Takashima, C. Olson, D. M. Driscoll and L. E. Baumgartener, B-lymphocytes and T-lymphocytes in three types of bovine lymphosarcoma. *J. nat. Cancer Inst.* **59,** 1205 (1977).
6. B. C. Giovanella, S. O. Yim, A. C. Morgan, J. S. Stehlin and L. J. Williams, Metastases of human melanomas transplanted in nude mice. *J. nat. Cancer Inst.* **50,** 1051 (1973).

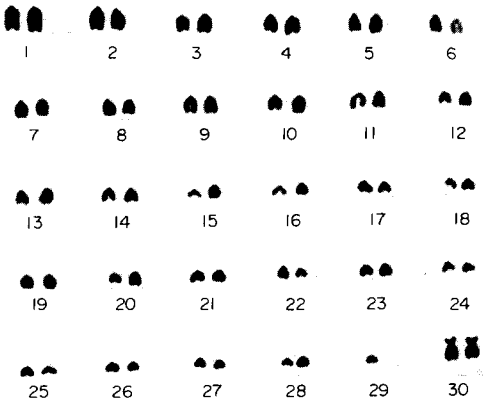

Fig. 2. Karotype of culture cells after skin case tumor was passed through a nude mouse. A karotype of 29 *acrocentric pairs and a pair of sex chromosomes is characteristic of bovine origin.*

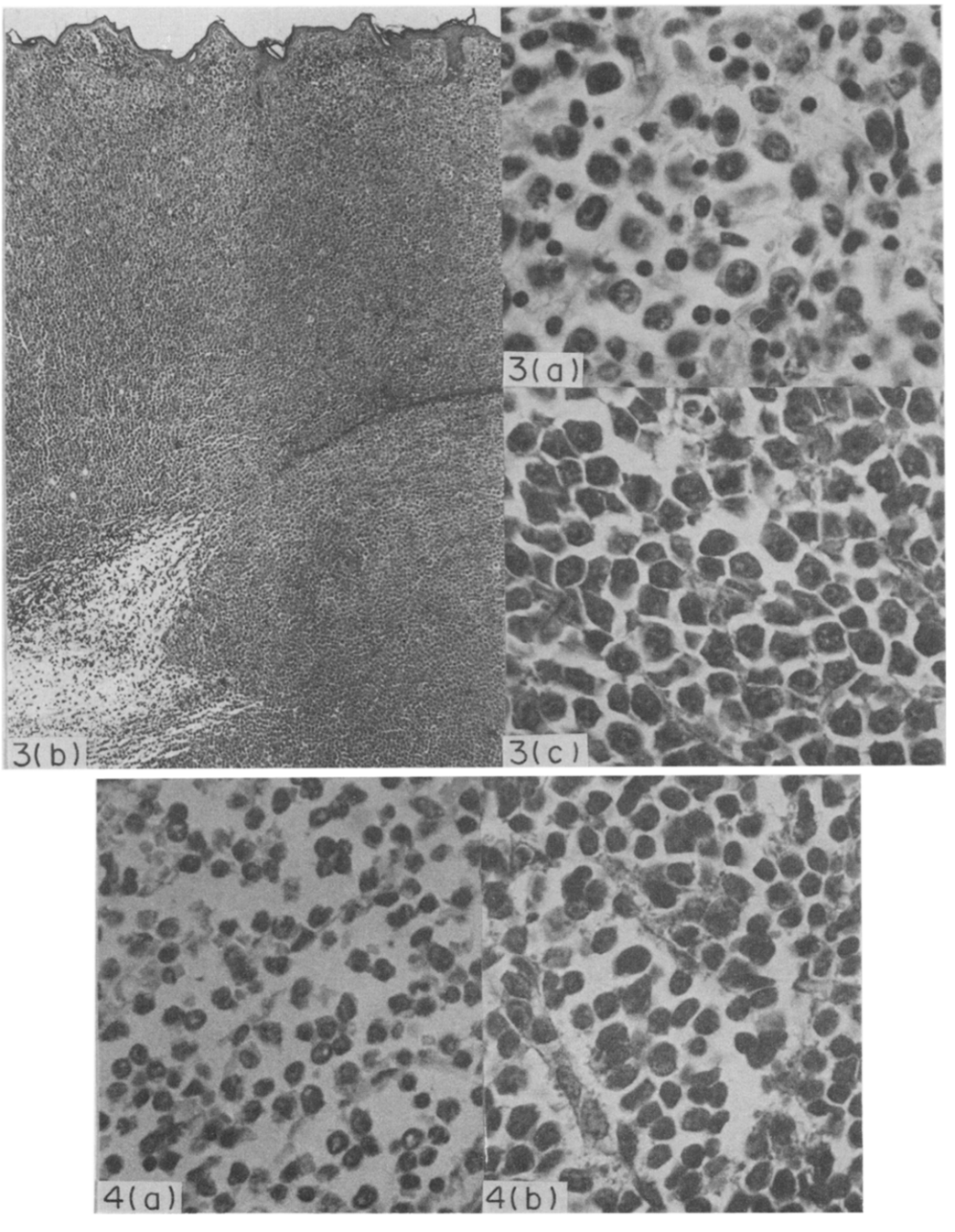

Fig. 3. Histology of the original skin case tumor and its transplanted tumor in nude mice. (a) Lymphoblastic lymphosarcoma in skin case (No. 124): urinary bladder. The tumor shows varying degrees of cellular maturity. Lymphoblastic cells are predominant and various sized lymphocytic cells are seen together. Hematoxylin–eosin (H–E), × 700. (b) Lymphoblastic lymphosarcoma in the transplanted tumor in nude mouse: skin. The tumor develops in a nodular manner in the subcutaneous tissue and diffusely in the corium of the inoculated site. H–E, × 102. (c) Higher magnification of (b). The tumor cells have large round nuclei with prominent nucleoli and irregular cell outlines. H–E, × 700.

Fig. 4. Histology of the original calf case tumor and its transplanted tumor in nude mice. (a) Lymphocytic lymphosarcoma in calf case (No. 125): Lymph node. The tumor cells have some irregularity in cellular maturity. H–E, × 700. (b) Lymphocytic lymphosarcoma in the transplanted tumor in nude mouse: skin. The tumor consists of a uniform population of mature lymphocytic cells. They look somewhat larger than those of the original tumor cells. H–E, × 700.

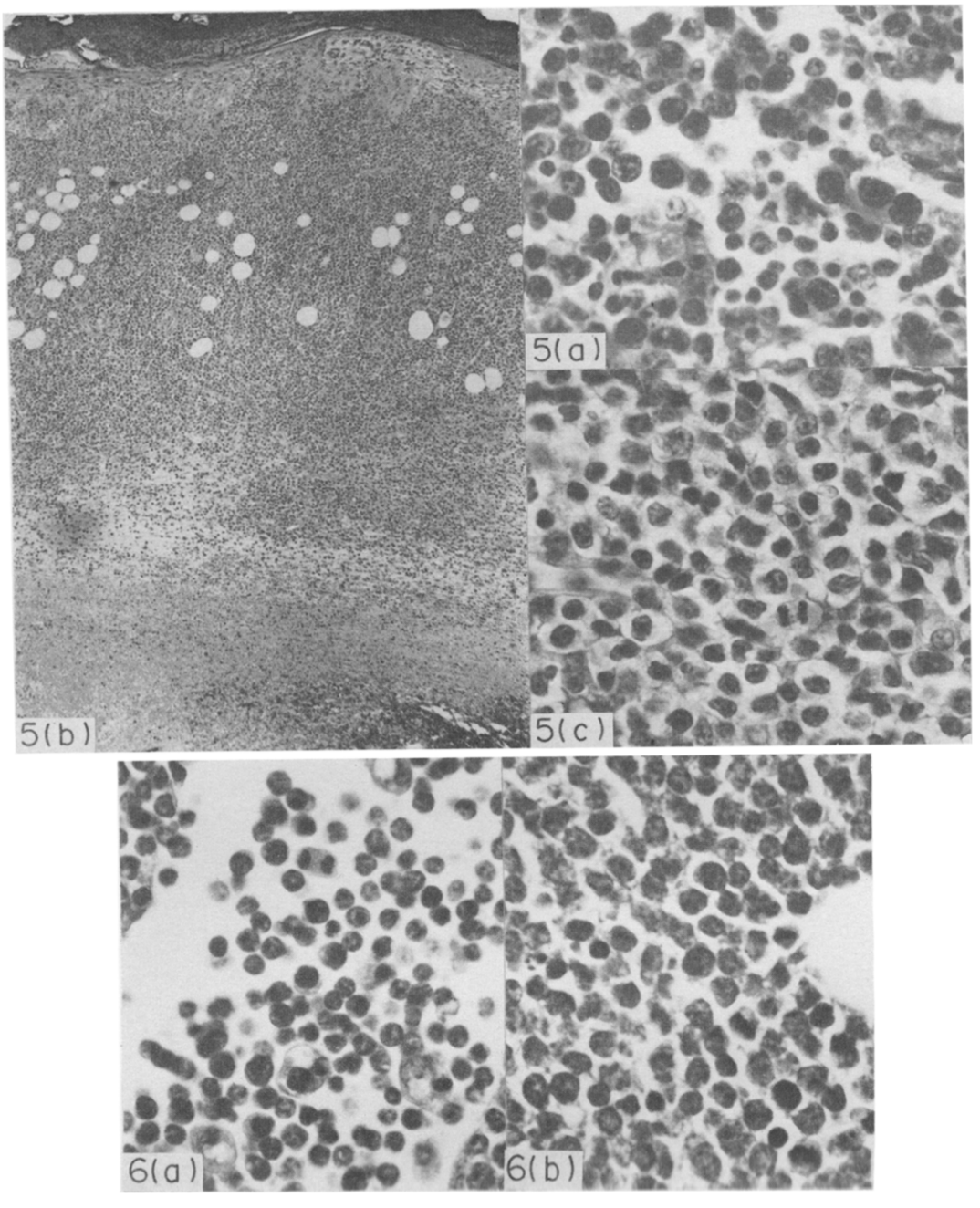

Fig. 5. Histology of the original adult case tumor and its transplanted tumor in nude mice. (a) Lymphoblastic lymphosarcoma in adult case (No. 131): submaxillary lymph node. Tumor consists of various sized cells of lymphocytic series, but lymphoblastic cells are predominant. H–E, × 700. (b) The transplanted tumor derived from adult case develops in a nodular manner in the subcutaneous tissue and diffusely in the corium of the inoculated site, but the tumor cells in the subcutaneous tissue shows extensive necrosis. H–E, × 102. (c) Higher magnification of (b). Lymphoblastic lymphosarcoma. The tumor cells show a polygonal or somewhat stellate shape and an irregular cytoplasmic contour, suggesting cytoplasmic processes. H–E, × 700.

Fig. 6. Histology of the original thymic case tumor and its transplanted tumor in nude mice. (a) Lymphocytic lymphosarcoma in thymic case (No. 150): lymph node. The tumor cells have some irregularity in cellular maturity and mitosis can be seen. H–E, × 700. (b) Lymphocytic lymphosarcoma in the transplanted tumor in nude mice: skin. The tumor consists of a uniform population of rather large mature lymphocytic cells. H–E, × 700.

7. C. O. Povlsen and J. Ryggard, Heterotransplantation of human adenocarcinomas of the colon and rectum to the mutant nude mice. A study of nine consecutive transplantations. *Acta pathol. microbiol. scand.* **A79,** 159 (1971).
8. J. Ryggard and C. O. Povlsen, Heterotransplantation of a human malignant tumor to nude mice. *Acta. pathol. microbiol. scand.* **A77,** 758 (1969).
9. D. Hoffman, P. A. Jennings and P. B. Spradbrow, Transplantation of bovine squamous cell carcinoma into congenitally athymic nude mice. *Vet. Record* **5,** 348 (1977).
10. S. M. J. Oughton and L. N. Owen, Transplantation of dog neoplasms into the mouse mutant nude. *Res. vet. Sci.* **17,** 413 (1974).
11. A. D. Irvin, G. G. D. Brown, G. K. Kanhai and D. A. Stagg, Transplantation of bovine lymphosarcoma cells to athymic (nude) mice. *Res. vet. Sci.* **22,** 53. (1977).
12. M. Onuma, L. E. Baumgartener, C. Olson and L. D. Pearson, Fetal infection with bovine leukemia virus in sheep. *Cancer Res.* **37,** 4075 (1977).
13. M. Onuma, C. Olsen, L. E. Baumgartener and L. D. Pearson, An ether-sensitive antigen associated with bovine leukemia virus infection. *J. nat. Cancer Inst.* **55,** 1155 (1975).
14. M. Onuma, S. Watarai, T. Mikami and H. Izawa, Cell fusion activity of bovine leukaemia virus. *J. gen. Virol.*, **48,** 421 (1980).
15. T. Taguchi, M. Fujita and M. Usugane, Heterotransplantation of various human and canine tumors into nude mice. In *Proceedings of the Second International Workshop on Nude Mice.* (Edited by T. Nomura, N. Ohsawa, N. Tamaoki and K. Fujiwara) p. 305. University of Tokyo Press, Tokyo (1977).
16. D. R. Morgan, L. N. Owen and D. E. Onions, Growth of canine lymphosarcoma in X-irradiated and non-irradiated athymic (nude) mice. *Europ. J. Cancer* **14,** 1353 (1978).
17. L. D. Miller, J. M. Miller and C. Olson, Inoculation of calves with particles resembling C-type virus from cultures of bovine lymphosarcoma. *J. nat. Cancer Inst.* **48,** 423 (1972).
18. C. Olson, L. D. Miller, J. M. Miller and H. E. Hoss, Transmission of lymphosarcoma from cattle to sheep. *J. nat. Cancer Inst.* **49,** 1463 (1972).
19. M. J. Van der Maaten and J. M. Miller, Induction of lymphoid tumors in sheep with cell-free preparation of bovine leukemia virus. *Bibl. Haemat.* **43,** 377 (1976).
20. H. E. Hoss and C. Olson, Infectivity of bovine C-type virus for sheep and goat. *Amer. J. vet. Res.* **35,** 633 (1974).
21. A. A. Ressang, J. C. Boars, J. Calafat, N. Mastenbroek and J. Quak, Studies on bovine leukemia. III. The haematological and serological response of sheep and goats to infection with whole blood from leukemic cattle. *Zbl. vet. Med.* **B. 23,** 662 (1976).
22. C. Olson, L. D. Miller, J. M. Miller and K. G. Gillette, Progress on transmission of bovine lymphosarcoma. *Bibl. Haemat.* **36,** 476 (1970).
23. A. Burny, F. Bex, H. Chantrenne, Y. Cleuter, D. Deketel, J. Ghysdael, R. Kettman, M. Leclercq, J. Leunen, M. Mammerickx and D. Portetelle, Bovine leukemia virus involvement in enzootic bovine leukosis. *Advanc. Cancer Res.* **28,** 251 (1978).