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Rapid Growth and Spontaneous Metastasis of Human Germinal Tumors Ectopically Transplanted into Scid (Severe Combined Immunodeficiency) and Scid-nude^{streaker} Mice

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Xenograft acceptance, growth and spontaneous metastasis of ectopically transplanted human germinal tumors were compared among scid mice, athymic nude mice and F_2 hybrids constructed from scid and nude mice, in relation to the impairments of T and B cell functions in these mice. In scid mice which are deficient in T and B cell functions, human yolk sac tumor (YST-2) that originated from the ovary grew to enormous sizes in 100% of the animals after both subcutaneous and intraperitoneal transplantation, while only half (59.1% and 51.9%) of the subcutaneous and none of the intraperitoneal transplants were accepted in usual athymic nude mice (BALB/c-*nu/nu* and CD1-*nu/nu*). The YST-2 grew rapidly in scid mice, developing 3 to 10 times larger tumors compared to nude-streaker (AKR/J-*nu*^{str}/*nu*^{str}) and usual nude mice, respectively. Furthermore, ectopically transplanted tumors spontaneously metastasized to distant organs (mostly to the lung) in scid mice (but less frequently in leaky scid mice), while metastases have never been found in nude mice. Although a xenograft of human classic (typical or pure) seminoma of the testis has never been established in nude mice, it grows slowly in one-third (36.4%) of scid mice and very rapidly in all of scid-nu^{str} (*scid/scid; nu*^{str}/*nu*^{str}) double mutant mice. Spontaneous metastases of xenografted seminomas were also observed in distant organs (lymph node, lung, liver, spleen, and kidney). The metastastic distribution of the two human germinal tumors in scid and scid-nu^{str} mice mimics that found in human. These results (xenograft acceptance, growth of transplanted tumors and degree of metastatic spread) were compatible with the level of T and B cell impairments indicated by FACS analysis, as well as mitogen responses, serum IgG and morphological features of the thymus.

Key words: Scid mouse — Scid-nu^{str} double mutant mouse — Human germinal tumor — Rapid growth — Spontaneous metastasis

An autosomal recessive mutation that severely impairs lymphopoiesis was found in mice and used as an animal model for human severe combined immunodeficiency (SCID)[1-3] and for the transfer of a functional human immune system.[4,5] Since the scid mouse is deficient in immune functions mediated by both T and B cells,[1-3] it is expected that human malignant tumors would easily grow in scid mice,[6,7] although macrophage and NK activities seem to be within normal ranges. Recently, we found that ectopically transplanted human solid tumors metastasized spontaneously to distant organs in scid mice,[7] while in athymic nude mice spontaneous metastases seldom occurred and intravenous injection and orthotopic implantation of tumor cell lines have been adopted for studying metastasis of human tumors.[8-11]

In this study, growth and spontaneous metastasis of the subcutaneously transplanted human germinal tumors were examined in scid mice, athymic nude mice, and also in F_2 hybrids constructed from scid and nude mice, in the expectation that combination of these two immunodeficiency genes may accelerate growth and metastastic spread of transplanted human tumors. T and B cell functions were also compared among these immunodeficient mice in relation to xenograft acceptance, growth and spontaneous metastasis of these human germinal tumors. First, we used yolk sac tumor (YST-2) of the ovary, which grew slowly in the subcutaneous tissue but not in the peritoneal cavity of athymic nude mice,[12] and then tested whether xenograft acceptance occurs for human tumors such as classic seminoma, which has never been grown in athymic nude mice.[13,14]

MATERIALS AND METHODS

Mice Breeding pairs of C.B17-*scid*/+ mice were kindly provided by Dr. M. J. Bosma, Fox Chase Cancer Center, Philadelphia. From a cross of C.B17-*scid*/+ male and

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The abbreviations and gene symbols used in this paper are: scid, severe combined immunodeficiency; nu^{str}, nude-streaker. Scid, nu^{str}, and scid-nu^{str} used in the text indicate *scid/scid*, *nu*^{str}/*nu*^{str} homozygotes, and *scid/scid-nu*^{str}/*nu*^{str} double homozygotes (double mutants), respectively, unless otherwise stated.

Table I. Serum IgG Level in Rederived Homozygous C.B17-*scid/scid* Mice

Serum IgG[a] (μg/ml)	No. of mice (%)	
<1	204	(73.4)
1.1–5.0	30	(10.8)
5.1–10.0	15	(5.4)
10.1–50.0	19	(6.8)
50.1–200	10	(3.6)
Total	278	(100)

a) Wild-type C.B17 showed serum IgG levels from 500 to 2000 μg/ml (see "Materials and Methods" and Table II).

female heterozygote, C.B17-*scid/scid* homozygotes were selected based on the level of serum IgG (<1 μg/ml). Wild-type C.B17 (provided by Dr. Bosma), C.B17-*scid*/+ heterozygotes, and other inbred strains (AKR/J, AKR/J-nu^{str}, C57BL/6J, etc.) showed serum IgG levels from 500 to 2,000 μg/ml. Thereafter, C.B17-*scid/scid* homozygotes have been maintained by brother×sister mating under germ-free conditions (Department of Radiation Biology, Osaka University). In each generation, C.B17-*scid/scid* homozygous mice showing less than 1 μg/ml of serum IgG were selected and used as breeding pairs. Table I shows the serum IgG level of 278 rederived homozygous C.B17-*scid/scid* mice measured during the experiments.

A C.B17-*scid/scid* homozygous female was mated with an AKR/J-*nu*^{str}/*nu*^{str} male, and the F_1 were intercrossed to make (C.B17-*scid/scid*×AKR/J-*nu*^{str}/*nu*^{str})F_2, representing genotypes *scid/scid*; *nu*^{str}/*nu*^{str}, *scid/scid*; *nu*^{str}/+ or +/+, *scid*/+ or +/+; *nu*^{str}/*nu*^{str}, and *scid*/+ or +/+; *nu*^{str}/+ or +/+. Serum IgG was measured at about 6 weeks after birth for prescreening *scid/scid* homozygotes.[15] T and B cell functions were analyzed at the end of experiments by using a fluorescence-activated cell sorter (FACS) and by measuring the mitogen response of splenic lymphocytes.[16] *Scid/scid* homozygotes showing low to medium levels of serum IgG (>10 μg/ml) with deficient T and B cell functions by FACS and mitogen stimulation[17] were classified as *scid/scid* (leaky) mice.

Breeding pairs of AKR/J-*nu*^{str}/+ were provided from the Jackson Laboratory (Bar Harbor, ME). BALB/cAJcl-*nu/nu* and CD1-*nu/nu* were purchased from CLEA Japan (Kanagawa) and Charles River Japan (Kanagawa), respectively. All mice used for the experiments were maintained with mouse diet CRF-1 (Charles River Japan) and chlorinated water in a complete barrier condition at 23±1°C.

Heterotransplantation of human germinal tumors The human germinal tumors used were yolk sac tumor (YST-2) of the ovary and classic (i.e., typical or pure) seminoma in the testis. A xenograft of human yolk sac tumor (YST-2), which was established in athymic nude mice,[12] was provided by Dr. M. Sawada, Kure National Hospital, Hiroshima, and maintained by T. Nomura for 22 generations (Department of Radiation Biology, Osaka University). YST-2 grows slowly in the subcutaneous tissue, but has never been grown in the intraperitoneal cavity of athymic nude mice.[12] The tumor was cut to about 2 mm^3 in 0.9% NaCl solution and transplanted subcutaneously by means of a trocar needle to the back of adult mice. Tumor size was measured twice a week by using a slide caliper. About 50 days after transplantation, mice were killed by cervical dislocation and the isolated tumors were measured and weighed. Gross pathological lesions were examined macroscopically, especially for metastases to distant organs, and submitted to microscopic examinations. In some mice, tumor masses were injected into the peritoneal cavity after repeated pumping of the minced tumor masses in an 18G syringe.

Classic seminoma removed from a male patient (31 years old) by surgical operation at the Urologic Clinic of the Sumitomo Hospital (Osaka) was used for the study. About 2 to 3 mm^3 mass of tumor was transplanted subcutaneously to the back of C.B17-*scid/scid*, (C.B17-*scid/scid* × AKR/J-*nu*^{str}/*nu*^{str}) F_2-*scid/scid* ; *nu*^{str}/*nu*^{str} (scid-nu^{str} double mutant), and F_2-*scid*/+ or +/+; *nu*^{str}/*nu*^{str} mice under anesthesia with 0.77% tribromoethanol (Aldrich Chem., Co. Ltd., Milwaukee, WI). Mice were killed about 75 days after transplantation, and submitted to macroscopic and microscopic examinations. Other experimental procedures are the same as above.

IgG quantitation An enzyme-linked immunosorbent assay (ELISA) was used to quantitate serum IgG concentrations[15] for prescreening *scid/scid* homozygous mice. Microtiter wells (ELISA Plate, Sumitomo-Bakelite Ind., Tokyo) were first coated with rabbit anti-mouse IgG (Zymed, San Francisco, CA), and then serially diluted mouse test sera or standard sera (mouse immunoglobulin, ICN Immuno-Biologicals, Lisle) were added. After incubation and washing, peroxidase-conjugated rabbit anti-mouse IgG (Zymed) was added and the relative amounts of bound enzyme conjugate in the wells were ascertained by addition of *o*-phenylenediamine (Wako Pure Chemical Ind. Ltd., Osaka). The extent of hydrolysis was measured at 490 nm in an ImmunoReader NJ-2000 (Nippon InterMed, Tokyo). Serum IgG concentrations were calculated from the standard curve.

FACS analysis and mitogen response Spleen cells were analyzed for the expression of T and B cell surface antigens at the end of experiments, using two-color fluo-

Table II. T and B Cell Functions Measured in Terms of Mitogen Stimulation in Comparison with Thymus Weight and Serum Levels of IgG in Scid, Athymic Nude, and Their F_2 Hybrid Mice

Strains and genotypes[a]	No. of mice[b]	Stimulation indices[c]		Thymus weight[c] (mg)	IgG[c] (μg/ml)
		Con A	LPS		
C.B17-*scid/scid*	43	1.3±0.1	0.9±0.2	3.4±0.6	≤1
C.B17-*scid/scid* (leaky)	8	1.3±0.1	1.0±0.2	8.1±2.3	47.9±8.6
C.B17-+/+; *scid*/+	27	81.0±10.1	18.1±1.8	50.8±3.8	887.6±76.7
AKR/J-nu^{str}/nu^{str}	6	1.9±0.3	43.9±17.7	0	586.2±132.0
(C.B17-*scid/scid* ×AKR/J-nu^{str}/nu^{str})F_2					
scid/scid; nu^{str}/nu^{str} (leaky)	11	1.1±0.1	1.1±0.2	0	17.1±7.2
scid/scid; +/− (leaky)	5	1.1±0.3	1.0±0.2	7.8±1.7	23.8±6.4
+/−; nu^{str}/nu^{str}	30	2.6±0.5	29.7±8.3	0	324.2±108.9
+/−; +/−	61	45.6±4.0	18.2±1.5	43.5±2.2	310.1±42.2

a) +/− means that at least one allele is wild type.
b) Mitogen response was assayed in mice that survived at the end of the experiment.
c) Mean±SE.

rescence cytofluorometry. Spleen cells (1×10^6) were stained with FITC-conjugated mouse monoclonal anti-mouse Thy-1.1 (clone TN-26) or Thy-1.2 (clone TS) antibody (Bio-Yeda, Rehovot, Israel) versus biotinylated rat monoclonal anti-mouse Ly-1 antibody (Becton Dickinson, San Jose, CA) followed by phycoerythrin (PE)-conjugated streptavidin (Becton Dickinson) to detect T cells. Spleen cells were also stained with FITC-conjugated goat anti-mouse IgD (Nordic Immunology, Tiburg, The Netherlands) and biotinylated goat anti-mouse IgM antibodies (Capel, Organon Teknika Co., West Chester, PA) followed by PE-streptavidin to detect B cells. Stained cells (1×10^4) were analyzed on a FACStar cytofluorometer (Becton Dickinson).

T and B cell functions were also analyzed in terms of mitogen response at the end of experiments. Spleen cells (1×10^5) were stimulated for 3 days with T and B cell mitogens, 2.5 μg/ml of concanavalin A (Con A, Sigma Chemical Co., St. Louis) and 5.0 μg/ml of bacterial lipopolysaccharide (LPS, *E. coli*, 0111 B4, Difco Laboratories Inc., Detroit), respectively, and proliferative responses to the mitogens were measured in terms of incorporated amounts of ^{3}H-thymidine.[16] The stimulation index was given by the ratio of ^{3}H-thymidine incorporation obtained with Con A or LPS to that obtained in medium alone. The Con A and LPS stimulation indices in scid, nu^{str} and their F_2 hybrids are given in Table II. Mice showing stimulation indices less than 3.0 for both Con A and LPS were classified as *scid/scid* homozygotes.

RESULTS

FACS analysis and mitogen response of scid, nude, and scid-nu^{str} mice Representative FACS profiles of nu^{str}, scid, and newly constructed scid-nu^{str} mice are given in Fig. 1. In *scid/scid* homozygotes used for the experiments (Tables II and III), none or only a few (<2%) of the splenic cells stained positive for IgM and IgD, and 0 to 15% for Thy-1.2 (or Thy-1.1) and Ly-1, while 40 to 60% and 25 to 45%, respectively, stained positive in wild types and *scid*/+ heterozygotes. In AKR/J-nu^{str}/nu^{str} and (C.B17- *scid/scid* × AKR/J-nu^{str}/nu^{str}) F_2-*scid*/+ or +/+; nu^{str}/nu^{str} mice used for the experiments, 39 to 59% of splenic cells stained positive for IgM and IgD, and none or only a few (3% in one case) for Thy-1.1 (or Thy-1.2) and Ly-1. Splenic cells of scid-nu^{str} (*scid/scid*; nu^{str}/nu^{str}) double mutant mice stained negative for these T and B cell surface antigens.

T and B cell functions of these mice were analyzed by measuring the proliferative response to mitogens, as shown in Table II. No responses to mitogen stimulation by Con A and LPS were observed in any of the scid and scid-nu^{str} mice, while nu^{str} mice showed no response to Con A, but a normal response to LPS. Some scid and scid-nu^{str} mice (leaky) showed a low to medium level of serum IgG (>10 μg/ml). However, stimulation indices by Con A and LPS showed the same values as those of non-leaky scid mice (serum IgG<1 μg/ml), indicating severe impairments of both T and B cell functions irrespective of serum IgG level, although leaky scid mice had a significantly larger thymus ($P<0.01$) than non-leaky scid mice (Table II) and more premature Thy-1$^+$ and IgM$^+$ cells[17] were observed by FACS analysis of splenic lymphocytes (data not shown, but see Fig. 1).

Rapid growth and spontaneous metastasis of human yolk sac tumor in scid mice As shown in Table III, subcutaneous transplantation of YST-2 produced large local tumors in all scid mice, i.e., C.B17-*scid/scid*, C.B17-

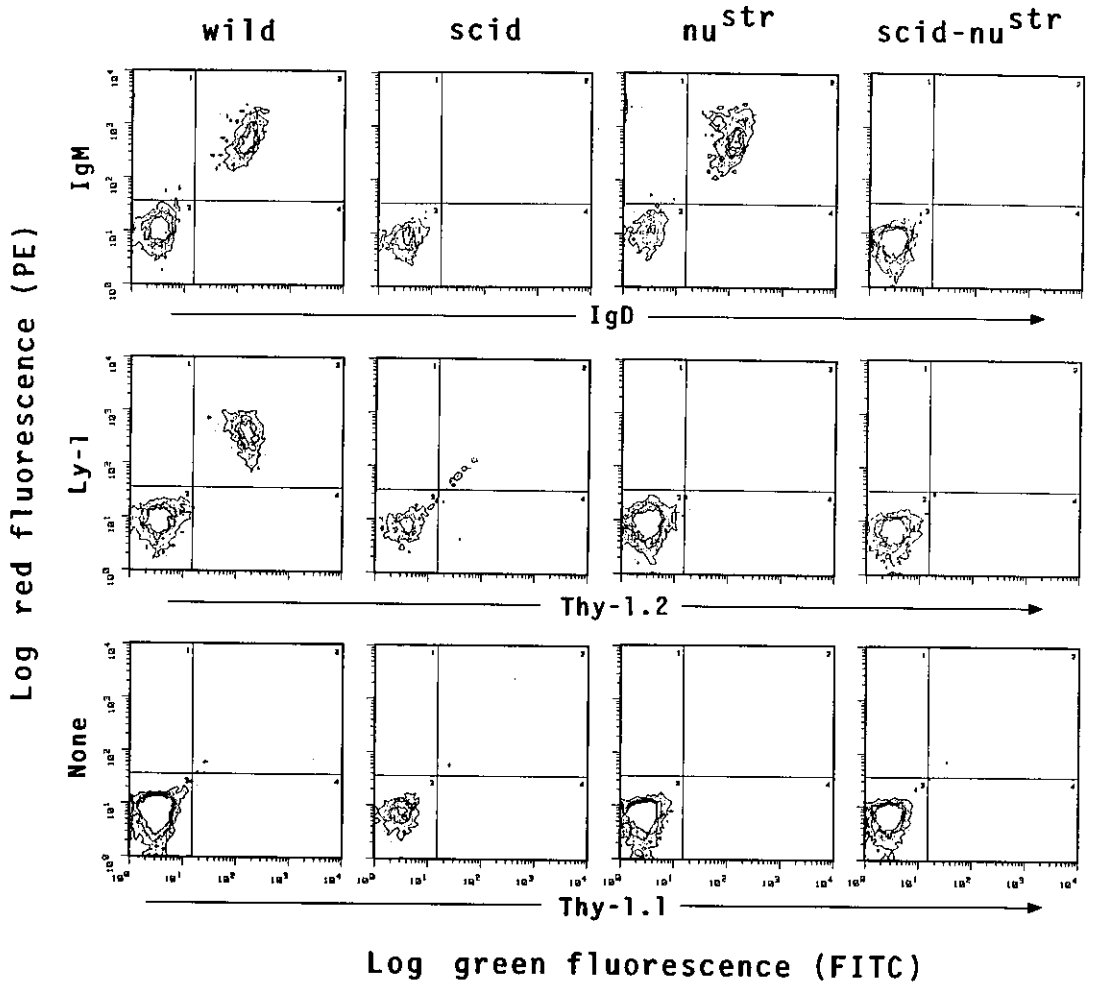

Fig. 1. Representative FACS profiles of T and B cells in the spleen of scid, nu^{str}, and scid-nu^{str} mice. Spleen cells were analyzed for the expression of cell surface antigens by two-color cytofluorometry. Details of the measurements are given in "Materials and Methods." The mice used in this figure are: wild, C.B17-+/+; scid, C.B17-*scid/scid*; nu^{str} and scid-nu^{str}, (C.B17-*scid/scid* ×AKR/J-nu^{str}/nu^{str}) F_2-nu^{str}/nu^{str} and F_2-*scid/scid*; nu^{str}/nu^{str}, respectively.

scid/scid (leaky), (C.B17-*scid/scid*×AKR/J-nu^{str}/nu^{str}) F_2-*scid/scid*; nu^{str}/nu^{str}, and F_2-*scid/scid*; +/− mice, while it did not grow in C.B17-*scid*/+, C.B17 (wild type) or F_2- +/−; +/− mice, and only half (59.1% and 51.9%) of usual athymic nude mice (BALB/cAJcl-*nu/nu* and CD1-*nu/nu*) could accept YST-2. Furthermore, subcutaneously transplanted tumors metastasized spontaneously to distant organs (mostly to the lung) in scid mice, but not in athymic nude mice (Table III). AKR-J-nu^{str}/nu^{str} and (C.B17-*scid/scid*×AKR/J-nu^{str}/nu^{str}) F_2-*scid*/+ or +/+; nu^{str}/nu^{str} mice could accept YST-2 in higher incidence than the usual athymic nude mice. Even in these nu^{str} mice, however, YST-2 has never metastasized to distant organs. Such differences were apparent when YST-2 was minced and injected into the intraperitoneal cavity. YST-2 neither grew nor metastasized in athymic nude mice, while there were diffuse and huge tumors in the intraperitoneal cavity of C.B17-*scid/scid* mice and metastases were observed in the lung and liver (Table III).

Growth of xenografts were also different between scid and nude mice. As shown in Fig. 2, subcutaneously transplanted YST-2 grew rapidly to enormous sizes in C.B17-*scid/scid* mice, which developed 3 to 10 times larger tumors than AKR/J-nu^{str}/nu^{str} and usual athymic nude mice, respectively.

Although there were no differences in mitogen responses (Table II), xenograft acceptance (Table III) and

Table III. Local Growth and Distant Metastasis of Human Yolk Sac Tumor (YST-2) Transplanted s.c. or i.p. into Scid, Athymic Nude, and Their F_2 Hybrid Mice

Strains and genotypes[a]	Local growth	Distant metastasis	
	Incidence (%)	Incidence (%)[b]	Organs[c]
A. Subcutaneous transplantation			
C.B17-*scid/scid*[d]	41/41 (100)	21/25 (84.0)	Lung (18), Kidney (1), Spleen (1), Lymph node (1), Pancreas (1)
C.B17-*scid/scid* (leaky)[e]	9/9 (100)	3/6 (50.0)	Lung (3)
C.B17-*scid*/+	0/20 (0.0)	0/20 (0.0)	
C.B17	0/8 (0.0)	0/8 (0.0)	
AKR/J-nu^{str}/nu^{str}	6/6 (100)	0/6 (0.0)	
BALB/cAJcl-*nu/nu*[d]	13/22 (59.1)	0/18 (0.0)	
CD1-*nu/nu*[d]	14/27 (51.9)	0/27 (0.0)	
(C.B17-*scid/scid* ×AKR/J-nu^{str}/*scid*)F_2			
scid/scid; nu^{str}/nu^{str} (leaky)	8/8 (100)	4/4 (100)	Lung (4)
scid/scid; +/− (leaky)[f]	10/10 (100)	3/6 (50.0)	Lung (3)
+/−; nu^{str}/nu^{str}	19/22 (86.4)	0/22 (0.0)	
+/−; +/−	0/66 (0.0)	0/66 (0.0)	
B. Intraperitoneal transplantation			
C.B17-*scid/scid*[d]	10/10 (100)	3/4 (75.0)	Lung (2), Liver (1)
BALB/cAJcl-*nu/nu*	0/6 (0.0)	0/6 (0.0)	
CD1-*nu/nu*	0/10 (0.0)	0/6 (0.0)	

a) +/− means that at least one allele is wild type.
b) Numbers of mice with distant metastases among mice killed more than 40 days after transplantation.
c) Figures in parentheses show numbers of mice with metastases in that organ.
d) Some of the data were reported previously.[7]
e, f) Leaky scid showing serum IgG levels from 13 to 170 μg/ml. One (*e*) and all (*f*) died of lymphocytic leukemia.

Table IV. Local Growth and Distant Metastasis of Human Classic (Typical) Seminoma Transplanted s.c. into Scid, Athymic Nude, and Their F_2 Hybrid Mice

Strains and genotypes	Local growth	Distant metastasis	
	Incidence (%)	Incidence (%)	Organs
C.B17-*scid/scid*	8/22 (36.4)	8/22 (36.4)	Lymph node (5), Lung (3), Liver (1), Spleen (1)
(C.B17-*scid/scid* ×AKR/J-nu^{str}/nu^{str})F_2			
scid/scid; nu^{str}/nu^{str}	4/4 (100)	4/4 (100)	Lymph node (4), Lung (2), Kidney (1)
+/−; nu^{str}/nu^{str}	0/11 (0.0)	0/11 (0.0)	

Experimental procedures and explanation of genetic terminology are given in "Materials and Methods" and in the legends to Table III.

tumor growth (Fig. 2) between leaky and non-leaky scid mice, the degree of metastatic spread of transplanted YST-2 was lower in leaky scid mice (Table III), indicating that leaked immune cells disturbed the metastatic spread of YST-2. Another disadvantage of the use of leaky scid mice was that most of them died of lymphocytic leukemia early in their lives (legend to Table III).

Growth and spontaneous metastasis of human classic seminoma in scid mice Such remarkable growth and metastasis of human tumors were confirmed with human

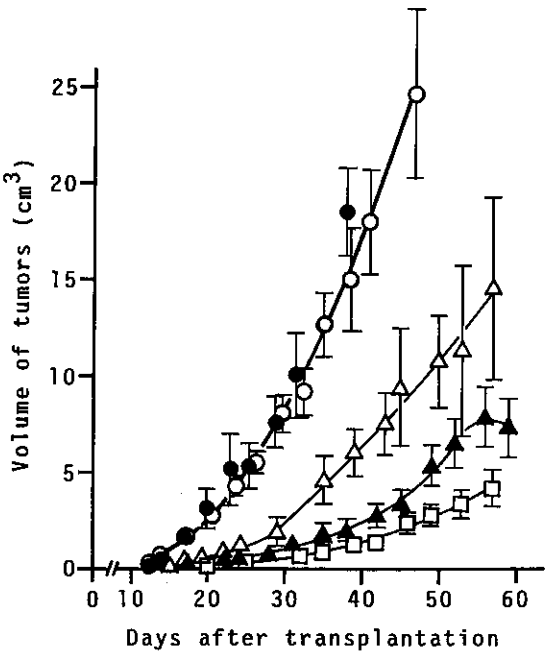

Fig. 2. Growth curve of human yolk sac tumor (YST-2) in scid and athymic nude mice. Maximum and minimum diameters of xenografts (YST-2) were measured twice a week over the fur by using a slide caliper, and tumor volume was estimated by means of Houchens' formula.[28] At necropsy, xenografts were isolated and tumor volume was measured as above. There was no difference in the ratio of tumor volume estimated by the direct measurement to that by the measurement over the fur between C.B17-*scid/scid* and nude mice. The values are 1.05 ± 0.07 and 1.03 ± 0.06 (mean $\pm$ SE) for scid and nu^{str} mice, respectively, indicating that estimated volume of tumor over the fur well reflects naked tumor volume, irrespective of the hair. Vertical bars show mean $\pm$ SE computed from the *t* distribution of the mean. Numbers of mice measured are given in Table III. The *t* test was performed between each experimental group after testing the variance ratio. There was no difference in the size of xenografted tumors between leaky and non-leaky scid mice. However, tumor sizes became significantly larger in C.B17-*scid/scid* vs. BALB/cAJcl-*nu/nu* ($P<0.01$), CD1-*nu/nu* ($P<0.01$) amd AKR/J-*nu*^{str}/*nu*^{str} ($P<0.05$) at 14, 17 and 24 days after transplantation, respectively. Differences were also significant between AKR/J-*nu*^{str}/*nu*^{str} and BALB/cAJcl-*nu/nu* ($P<0.01$) and between AKR/J-*nu*^{str}/*nu*^{str} and CD1-*nu/nu* ($P<0.01$) 21 days and 35 days after heterotransplantation of YST-2, respectively. Xenografted tumors seem larger in CD1-*nu/nu* than BALB/cAJcl-*nu/nu*, but the difference was not significant at the *P* value of 0.05. ○, C.B17-*scid/scid*; ●, C.B17-*scid/scid* (leaky); △, AKR/J-*nu*^{str}/*nu*^{str}; ▲, CD1-*nu/nu*; □, BALB/cAJcl-*nu/nu*.

classic seminoma. As shown in Table IV, seminoma grew very slowly in about one-third of C.B17-*scid/scid* mice. Nonetheless, xenografts metastasized spontaneously to distant organs such as lymph node, lung, liver, and spleen, while neither local growth nor metastasis was observed in usual athymic nude mice.[13, 14] It should be noted that seminoma grew rapidly and metastasized to distant organs (lymph node, lung, and kidney) in all of scid-nu^{str} double mutant (*sicd/scid*; *nu*^{str}/*nu*^{str}) F_2 hybrids but none of the scid$^+$-nu^{str} ($+/-$; nu^{str}/nu^{str}) F_2 hybrids which were constructed from C.B17-*scid/scid* and AKR/J-*nu*^{str}/*nu*^{str} mice (Table IV and Fig. 3).

Growth of xenografted classic seminoma showed a remarkable difference between scid and scid-nu^{str} mice (Fig. 4). Subcutaneously transplanted classic seminoma grew very rapidly in scid-nu^{str} mice, which developed about 7 to 10 times larger tumors than those in scid mice. Consequently, rapid growth and 100% metastasis of seminoma in scid-nu^{str} mice as compared with scid and nu^{str} mice are compatible with the greater impairments of T and B cells indicated by FACS profiles (Fig. 1), the morphological feature of the thymus and/or the mitogen responses (Table II). This tumor was transplantable in scid and scid-nu^{str} mice for further generations, and the xenografts and metastatic lesions showed an isozyme electrophoretically identical to human sperm-specific lactate dehydrogenase X (C_4)[18] (data not shown) and the same chromosomal abnormality as was observed in the peripheral blood lymphocytes of the patient.[7]

DISCUSSION

In the present study, it was demonstrated that ectopically transplanted human germinal tumors grew rapidly and spontaneously metastasized to distant organs in scid mice by virtue of the deficiency of both T and B cell functions (Fig. 1, Table II). Most functional mature T cells fail to develop in both scid and athymic nude mice, and natural killer (NK) and macrophage activities seem to be within normal ranges in both scid and athymic nude mice.[1-3, 19-22] However, nude mice exhibit normal B cell development for the most part, whereas scid mice do not (Fig. 1, Table II). It is, therefore, not unreasonable to suppose that both successful growth and metastasis of human tumors in scid (but not in nude) mice result from the absence of some B cell functions. B cells may play an important role in the rejection of engrafted xenogeneic tumors and their metastases, through either some unknown direct activity by B cells or the indirect action of antibodies, e.g., with antibody-dependent cytotoxic cell activity. Alternatively, some other unknown host immunity which is deficient in scid mice might be involved in the xenograft acceptance and distant metastasis. Studies on these possibilities are in progress.

There were no differences in xenograft acceptance and growth of xenografted tumors (YST-2) between C.B17-*scid/scid* and C.B17-*scid/scid* (leaky) mice (Table III

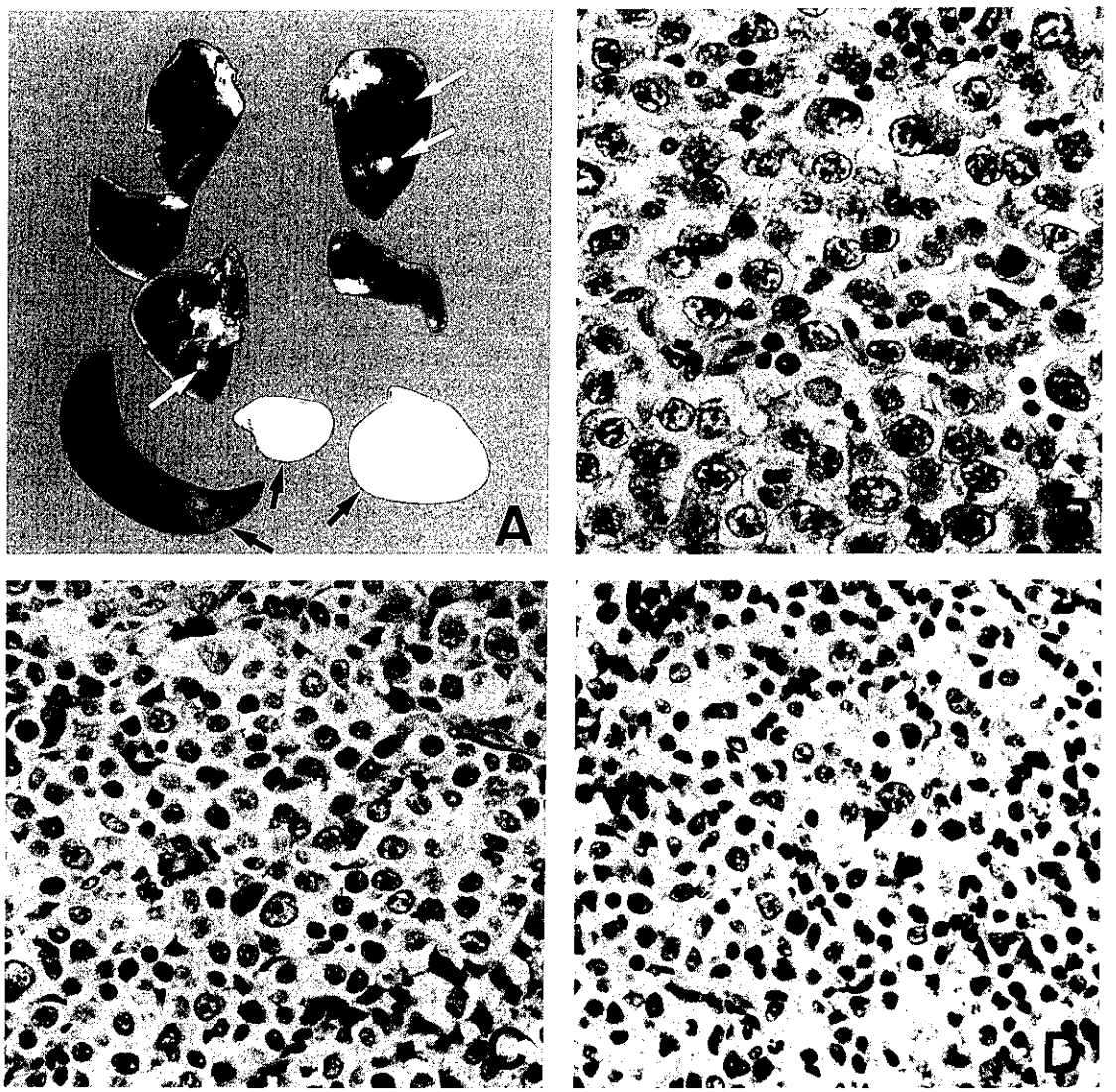

Fig. 3. Macroscopic and microscopic view of metastatic foci from the xenografts of classic seminoma in scid-nu^{str} double mutant mice. A. Metastases to the lung (multiple white foci; white arrows) (ventral view), spleen (opalescent; black arrow), and lymph nodes (white masses; black arrows) in scid-nu^{str} double mutant mice from the xenograft of classic seminoma. B. Histology of original classic seminoma from the 31-year-old patient. The seminoma cells are present in nests confined by fibrous septae containing numerous lymphocytes. The cell borders of the seminoma cells are well defined, and there are large amounts of clear cytoplasm. The nuclei show minimal variation of size and shape and contain coarse chromatin and one or two prominent nucleoli, showing the typical histological pattern of classic seminoma. H-E, ×360. C. Histology of the xenograft of classic seminoma on the back of scid-nu^{str} double mutant mice. Morphologically the same as above, but slightly smaller tumor cells are observed. H-E, ×360. D. Metastasis to the lymph node. Many seminoma cells with pale nuclei and large nucleoli are seen in the darkly stained small mouse lymphocytes. H-E, ×360.

and Fig. 2), although these leaky scid mice have been shown to develop a few functional clones of T and B cells and they rejected about half of the mouse skin grafts.[17] They must still be immunoincompetent to human tumors by virtue of mostly non-functional T and B cells. However, the degree of metastasis was lower in leaky scid mice (Table III), indicating that leaked functional clones of T and B cells[17] disturbed the metastatic spread of ectopically transplanted YST-2. Lower transplantability and lower growth rate of YST-2 in commercially obtained outbred nude mice (CD1-*nu/nu* and BALB/cAJcl-*nu/nu*) than inbred AKR/J-*nu*^{str}/*nu*^{str} mice (Table

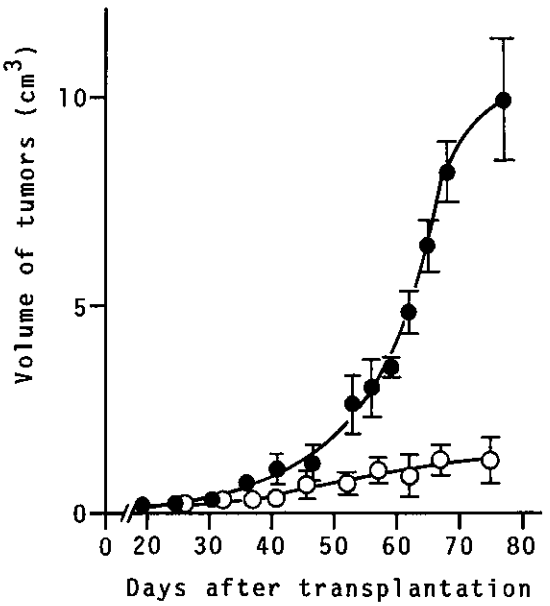

Fig. 4. Growth curve of human classic seminoma in scid mice and scid-nu^{str} double mutant mice. Details of the measurements of tumors are given in the legend to Fig. 2, and the numbers of mice measured are listed in Table IV. The *t* test yielded significant differences ($P<0.05$) in tumor sizes between scid-nu^{str} and scid mice at 52 days and later after heterotransplantation of seminoma. ●, (C.B17-*scid/scid*×AKR/J-*nu*^{str}/*nu*^{str}) F_2-*scid/scid*; *nu*^{str}/*nu*^{str}; ○, C.B17-*scid/scid*.

III and Fig. 2) also suggest that heterogeneity of some unknown genetic factors may cause the resistance to xenografts.

While it is remarkable that seminoma grew rapidly and metastasized spontaneously to distant organs in all the scid-nu^{str} double mutant mice (Table IV), the reasons are not understood, since there are no substantial differences in T and B cell functions between scid and scid-nu^{str} mice (Table II), or in NK and macrophage activities.[1, 19–22] This might reflect a greater impairment of T cell function in scid-nu^{str} mice, because there is a small thymus in scid mice in contrast to no visible thymus in scid-nu^{str} mice (Table II), and a majority of thymocytes (>90%)[1] and 0–15% of splenic cells in scid mice but not in scid-nu^{str} mice (Fig. 1) are Thy-1$^+$ premature T cells. Further elucidation will require additional studies.

In humans, the most frequent method of spreading in seminoma is by the lymphatic route. Autopsy has revealed that lymph nodes are involved in 70% of the cases; liver, 50%; lung, 40%; and kidney, 40%,[23] while distant metastasis of the yolk sac tumor is by way of both lymphatic and hematogenous routes.[23, 24] The pattern of the metastatic distribution of the two human germinal tumors in scid and scid-nu^{str} mice (Tables III and IV) mimics that in humans, although these tumors were engrafted subcutaneously into the back of mice. There may be an organ-specific affinity for the metastatic spread of these two tumors.

Metastasis of human tumors has been studied by the intravenous injection or orthotopic implantation of established tumor cell lines into relevant organs of nude mice,[8–11] and recent investigations have revealed that both tumor cell properties and host factors, e.g., oncogene activation,[10, 11] metastasis-suppressive genes,[25] basement membranes,[26, 27] etc. play an important role in modulating the metastatic activity. In addition, the present work clearly demonstrates that even ectopically transplanted human solid tumors can easily grow and metastasize to distant organs in scid mice and scid-nu^{str} mice. We believe this is the first report showing that the host's immune status controls whether or not the human tumor grows in a localized form or spontaneously metastasizes to distant organs. These human tumor-engrafted scid mice and scid-nu^{str} double mutant mice provide an invaluable experimental system to investigate the metastasis and progression of human tumors, which remain important and life-threatening problems for cancer patients. Furthermore, scid and scid-nu^{str} mice will be useful for the establishment of tumor cell lines, because we have succeeded in establishing monoclonal cell lines of human classic seminoma after the successful transplantation into these mice (unpublished data).

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REFERENCES

1) Bosma, G. C., Custer, R. P. and Bosma, M. J. A severe combined immunodeficiency mutation in the mouse. *Nature*, **301**, 527–530 (1983).

2) Custer, R. P., Bosma, G. C. and Bosma, M. J. Severe combined immunodeficiency (SCID) in the mouse; pathology, reconstitution, neoplasms. *Am. J. Pathol.*, **120**, 464–

477 (1985).
3) Dorshkind, K., Keller, G. M., Phillips, R. A., Miller, R. G., Bosma, G. C., O' Toole, M. and Bosma, M. J. Functional status of cells from lymphomyeloid tissues in mice with severe combined immunodeficiency disease. *J. Immunol.*, **132**, 1804–1808 (1984).
4) Mosier, D. E., Gulizia, R. J., Baird, S. M. and Wilson, D. B. Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature*, **335**, 256–259 (1988).
5) McCune, J. M., Namikawa, R., Kaneshima, H., Shults, L. D., Lieberman, M. and Weissman, I. L. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science*, **241**, 1632–1639 (1988).
6) Phillips, R. A., Jewett, H. A. S. and Gallie, B. L. Growth of human tumors in immuno-deficient scid mice and nude mice. *Curr. Topics Microbiol. Immunol.*, **152**, 259–263 (1989).
7) Nomura, T., Takahama, Y., Hongyo, T., Inohara, H., Takatera, H., Fukushima, H., Ishii, Y. and Hamaoka, T. SCID (Severe Combined Immunodeficiency) mice as a new system to investigate metastasis of human tumors. *J. Radiat. Res.*, **31**, 288–292 (1990).
8) Graham, S. D., Jr., Mickey, D. D. and Paulson, D. F. Detection of metastatic tumors in nude mice: brief communication. *J. Natl. Cancer Inst.*, **60**, 715–716 (1978).
9) Fidler, I. J., and Poste, G. The cellular heterogeneity of malignant neoplasms: implications for adjuvant chemotherapy. *Semin. Oncol.*, **12**, 207–221 (1987).
10) Nicolson, G. L. Tumor cell instability, diversification and progression to the metastatic phenotype: from oncogene to oncofetal expression. *Cancer Res.*, **47**, 1473–1487 (1987).
11) Thorgeirsson, U. P., Turpeenniemi-Hujanen, T., Williams, J. E., Westin, E. H., Heilman, C. H., Talmadge, J. E. and Liotta, L. A. NIH3T3 cells transfected with tumor DNA containing activated *ras* oncogenes express the metastatic phenotype in nude mice. *Mol. Cell. Biol.*, **5**, 259–262 (1985).
12) Sawada, M., Matsui, Y. and Okudaira, Y. Chemotherapy of human yolk sac tumor heterotransplanted in nude mice. *J. Natl. Cancer Inst.*, **71**, 1221–1225 (1983).
13) Okada, K. and Yoshida, O. Study of heterotransplantation of malignant urogenital tumors in nude mice: results of transplantation and the characteristics of the explants. *Hinyokika-Kiyo*, **30**, 1749–1767 (1984) (in Japanese).
14) Monagham, P., Raghavan, D. and Neville, A. M. Ultrastructural studies of xenografted human germ cell tumors. *Cancer*, **49**, 683–697 (1982).
15) Engvall, E. and Perlmann, P. Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. *J. Immunol.*, **109**, 129–135 (1972).
16) Ono, S., Takahama, Y. and Hamaoka, T. Ia-restricted B-B cell interaction. I. The MHC haplotype of bone marrow cells present during B cell ontogeny dictates the self-recognition specificity of B cells in the polyclonal B cell activation by a B cell differentiation factor, B151-TRF2. *J. Immunol.*, **139**, 3213–3223 (1987).
17) Bosma, G. C., Fried, M., Custer, R. P., Carroll, A., Gibson, D. M. and Bosma, M. J., Evidence of functional lymphocytes in some (leaky) scid mice. *J. Exp. Med.*, **167**, 1016–1033 (1988).
18) Wheat, T. E. and Goldberg, E. Sperm-specific lactate dehydrogenase C_4. *Isozymes*, **7**, 113–130 (1983).
19) Dorshkind, K., Pollack, S. B., Bosma, M. J. and Phillips, R. A. Natural killer (NK) cells are present in mice with severe combined immunodeficiency (scid). *J. Immunol.*, **134**, 3798–3801 (1985).
20) Murphy, W. J., Kumar, V. and Bennett, M. Rejection of bone marrow allografts by mice with severe combined immune deficiency (scid): evidence that natural killer cells can mediate the specificity of marrow graft rejection. *J. Exp. Med.*, **165**, 1212–1217 (1987).
21) Bancroft, G. J., Bosma, M. J., Bosma, G. C. and Unanue, E. R. Regulation of macrophage Ia expression in mice with severe combined immunodeficiency: induction of Ia expression by a T cell-independent mechanism. *J. Immunol.*, **137**, 4–9 (1986).
22) Kindred, B. Nude mice in immunology. *Prog. Allergy*, **26**, 137–238 (1979).
23) Mostofi, F. K. and Price, E. B. "Tumors of the Male Genital System," pp. 21 and 121 (1973). Armed Forces Institute of Pathology, Washington, DC.
24) Scully, R. E. "Tumors of the Ovary and Maldeveloped Gonads," pp. 233–234 (1979). Armed Forces Institute of Pathology, Washington, DC.
25) Bevilacqua, G., Sobel, M. E., Liotta, L. A. and Steeg, P. S. Association of low nm 23 RNA levels in human primary infiltrating ductal breast carcinomas with lymph node involvement and other histopathological indicators of high metastatic potential. *Cancer Res.*, **44**, 5185–5190 (1989).
26) Tupeenniem-Hujanen, T., Thorgeirsson, U. P., Hart, I. R. and Liotta, L. A. Expression of collagenase IV (basement membrane collagenase) activity in murine tumor cell hybrids that differ in metastatic potential. *J. Natl. Cancer Inst.*, **75**, 99–103 (1985).
27) Yamada, Y. Basement membranes and tumor cell metastasis. *Proc. Jpn. Cancer Assoc., 49th Annu. Meet.*, 11 (1990).
28) Houchens, D. P., Ovejera, A. A. and Barker, A. D. The therapy of human tumors in athymic (nude) mice. *In* "Proceedings of the Symposium on the Use of Athymic (nude) Mice in Cancer Research," ed. D. P. Houchens, pp. 267–280 (1978). Gustav Fisher, New York, N.Y.