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28. A Novel SCID Biotechnology for Biomedical Studies in Human

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Introduction

In the development of human culture, especially after the Industrial Revolution, enormous numbers of human-made physical and chemical agents have been produced. These toxic agents come to humans directly or after the biological concentration, whereas humans have never been exposed to those agents. Humans have been exposed to solar ultraviolet light B (UVB) by the depletion of stratospheric ozone. These environmental hazards to humans have been evaluated by the epidemiological survey, animal experiments and/or in vitro studies. For the exact estimation of the risk to human health, however, establishment of the direct methods with human organs and tissues is extremely important. For this purpose, a novel biotechnology has been established with severe combined immunodeficient (SCID) mice which cannot reject human organs and tissues immunologically [1–7].

This biotechnology also enables us to study: (1) morphology and function of human organs and tissues by the reconstruction of the human organ system in SCID mice, (2) experimental therapy of human diseases, including medicinal, immuno- and gene-therapy, (3) mutagenesis and carcinogenesis in human, including toxicity of new drugs, and (4) development of human embryos.

Improvement of SCID Mice

Scid mutation was found by Bosma in 1983 [1]. SCID mice were defective of VDJ recombination in the development of lymphocytes, showing deficient T cell and B cell function, while athymic nude mice lack T cell function, but possess normal B cell function. However, normal T and B cells appear spontaneously in 13.6–23.0 percent of the original C.B17-*scid* mice ('leaky' SCID mice) and 31.7–58.8 percent of them die of leukemia early in life (2). These characteristics give serious disadvantage to maintain human organs and tissues for long period. In fact, a majority of mouse skin allografts (> 70 percent) were rejected in leaky SCID mice.

In 1986, we started selective brother-sister inbreeding of homozygous C.B17-*scid/scid* mice which show undetectable IgG and IgM (< 1 μg/ml), and also introduced other immunodeficient genes into SCID mice. As shown in Fig. 1, 8.4 to 20.0 percent of C.B17-*scid* mice showed unusually high serum level of IgG (≥ 25 μg/ml) until F_5 generation. The incidence of such leaky SCID mice decreased after F_6 by selective inbreeding of IgG and IgM undetectable C.B17-*scid* mice. Incidence of lymphocytic leukemia was also extremely high (7.7–16.5 percent) until F_7 generation, but decreased significantly after then by selective brother-sister inbreeding of IgG and IgM undetectable C.B17-*scid* mice (Fig. 2) (40/302, 13.2 percent at F_{3-7} *vs* 46/1569, 2.9 percent at F_{8-23}, $p < 0.01$). We designated this new inbred strain as C.B17/N-*scid* after F_{20} generation. Among 443 C.B17/N-*scid* mice at F_{39-41} (early 1999), none of them (zero percent) are leaky and 13 C.B17/N-*scid* mice (2.9 percent) died of leukemia.

Leaky SCID was observed very often, when *scid* gene was introduced into a different background. Since SCID mice possess normal natural killer (NK) cell and macrophage function, *scid* gene was introduced into other inbred strains of mice, such as C57BL/6J, C57BL/6J-bg^J , C3H/HeJ, and B6C3Fe-*a/a*-Csfm^{op} mice by cross-back cross mating. It resulted in extremely high incidence of leaking of IgG (but not IgM) in *scid* homozygous mice. However, leaky SCID disappeared by the brother-sister inbreeding of *scid*-homozygous male and female (Fig. 1).

In the improved SCID mice, not only benign and malignant tumors, we succeeded to maintain normal human organs and tissues for a long period, reserving morphology and function [11, 3], and human embryos also develop well and grow in the improved SCID mice.

Transplantation Procedure

Normal human organs and tissues were treated with high concentration of antibiotics (50,000 units/ml penicillin G, 25 mg/ml panipenem, 25 mg/ml betamipron, and 50 mg/ml streptomycin sulfate) for 15 minutes and washed repeatedly with the medium containing antibiotics. Then, human tissues were transplanted subcutaneously into the SCID mice. When mice become moribund, human tissues were removed and transplanted to another set of SCID mice repeatedly to maintain human tissues beyond several mouse generations. Though SCID mice will die, human organs and tissues can survive for a long period. At the time of transfer, biopsy of human tissues were carried out to detect morphological and molecular changes. It is possible to treat human tissues with radiation and/or chemicals during these processes.

All experiments were carried out in the germ-free or SPF room of the barrier section in the Institute of Experimental Animal Sciences, Osaka University following the Osaka University Guideline for Animal Experimentation. Human tissues resected from the patients for surgical treatment of cancer were used with their permission for heterotransplantation in the SCID mice. Only the human tissues free of mycoplasma, human hepatic B and C antigens/antibodies, adult T-cell leukemia, HIV, and Wasserman reaction were accepted into the SPF room of the barrier [1–7].

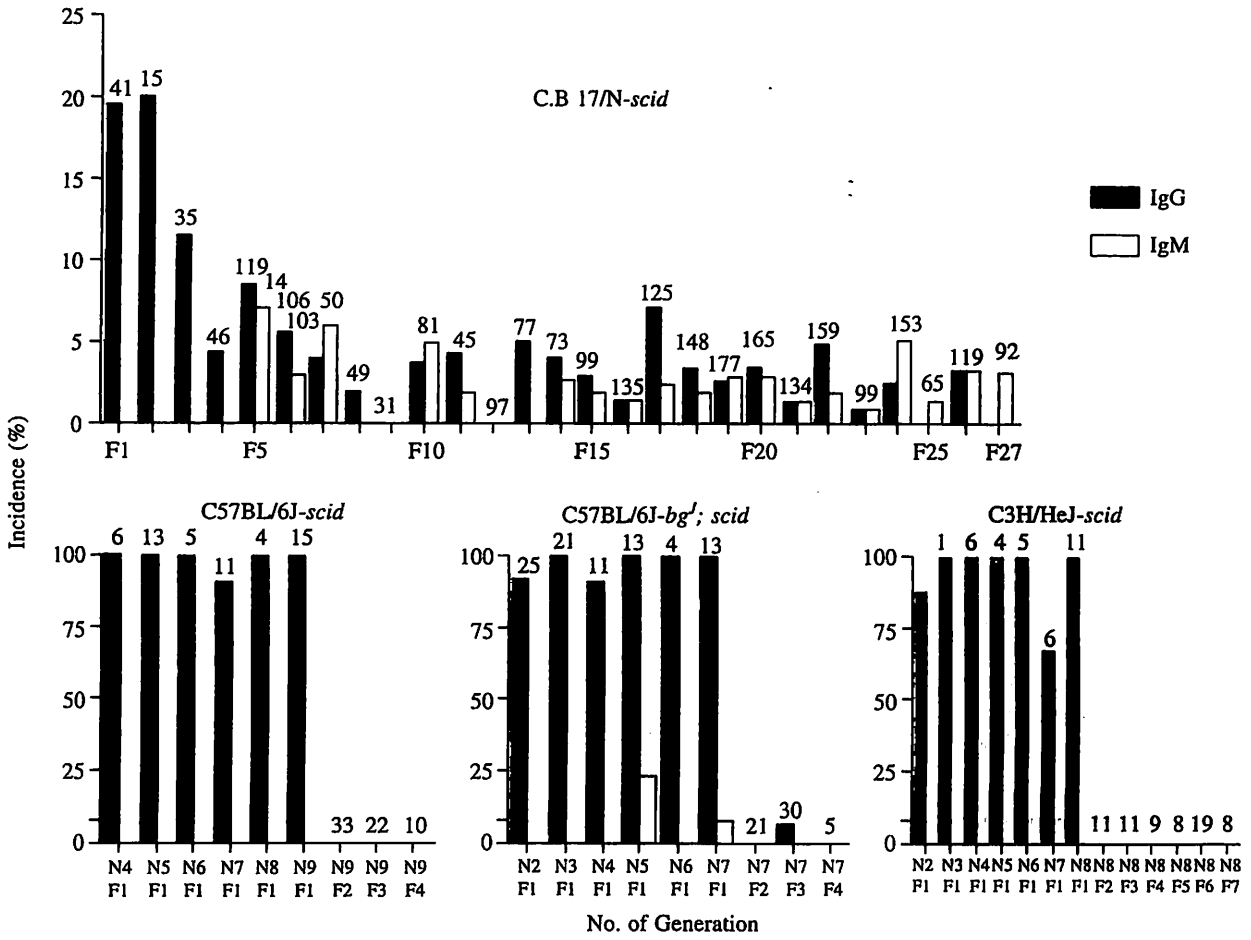

Figure 1. **Incidence of leaky SCID mice in the process of inbreeding of C.B17-*scid* mice and genetic introduction of *scid* gene into C57BL/6J, C57BL/6J-*bg*^{J} and C3H/HeJ mice. SCID mice showing serum IgG and IgM higher than 25 μg/ml were designated as leaky SCID mice. Details for measurements of IgG and IgM by ELISA were given in ref. 2 and 4. Figures on the histogram indicate numbers of SCID mice.**

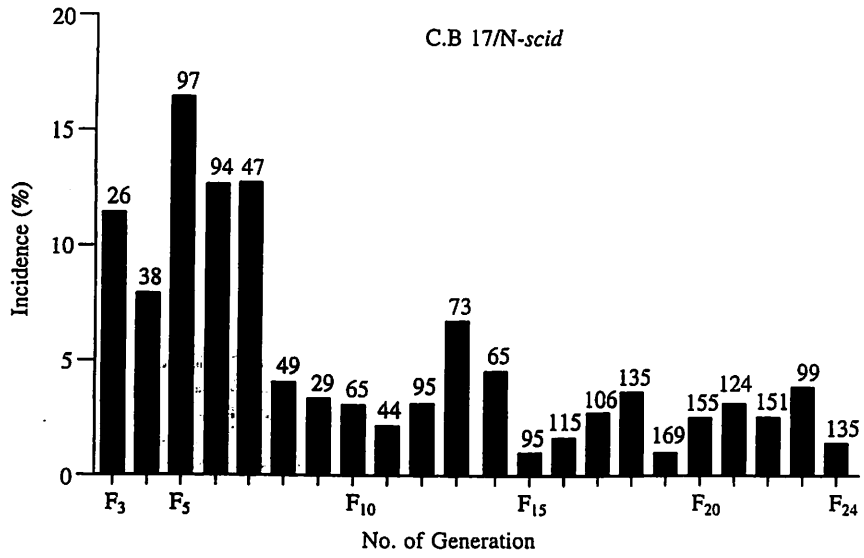

Figure 2. Incidence of thymic lymphocytic leukemia in the process of selective inbreeding of C.B17-*scid* mice. Figures on the histogram indicate numbers of SCID mice.

Human Benign and Malignant Tumors in SCID Mice

All human malignant tumors so far tested can grow more rapidly in the improved SCID mice than athymic nude mice [1–4, 10], while about half of human malignant tumors could not grow in nude mice. Furthermore, these transplanted human malignant tumors metastasized to distant organs, while metastases have never been observed in nude mice [8, 9]. Human benign tumors also could grow in the improved SCID mice for long periods (~ 3 years) over several mouse generations (Table 1). Microscopic structures of transplanted human benign and malignant tumors were well maintained in the SCID mice [1–4], and transplanted human tissues were also stable in SCID mice, for example, human chorionic gonadotropin and parathyroid hormone were secreted in the urine and blood of C.B17-*scid* mice after transplantation of hydatidiform mole (unpublished data) and parathyroid adenoma [7]. Consequently, experiments were extended to the transplantation of normal human organs and tissues to the improved SCID mice, C.B17/N-*scid*.

Maintenance of Normal Human Organs and Tissues

Normal human organs and tissues such as skin, thyroid gland, lung, liver, gastrointestinal tract, endometrium and bone marrow cells and also precancerous lesions are well maintained in C.B17/N-*scid* mice for long periods over several mouse generations (Table 1). Histological patterns of these transplanted human organs and tissues are also well maintained in the C.B17/N-*scid* mice [10]. However, human bone marrow cells could not survive longer than 6 months in our previous study with C.B17-*scid* mice from F_2 to F_7 generation [10], when survival was confirmed by the serum level of human IgG. However, higher level of human IgG was detected when bone marrow cells were injected into the improved C.B17/N-*scid*; W^v doubly mutant mice than when injected into usual

Table 1. Survival and maintenance periods of normal and precancerous human tissues in SCID mice

	No.	Survival (%)	Max. period[a] (months)	No. of transfer
1. Normal human tissues				
Skin	42	100	28	7
Thyroid gland	49	100	34	10
Lung	13	100	22	8
Liver	7	100	14	4
Gastric mucosa	18	100	21	6
Colorectal mucosa	10	100	13	5
Endometrium	7	71.4	22	6
Bone marrow cell	19	84.2	< 6[b]	1
2. Benign human tumors				
Thyroid follicular adenoma	10	100	36	13
Parathyroid adenoma	15	83.3	13	3
Parotid pleomorphic adenoma	15	100	36	11
Papilloma (tongue, larynx)	5	100	34	7
Leukoplakia (tongue)	5	100	8	2
Hydatidiform mole	18	88.9	12	4
Endometrial Hyperplasia	18	83.3	14	5
Colorectal polyp	46	63.0	37	11
Pancreas adenoma	10	50.0	13	3

(a) Some continue.
(b) Identified by human IgG in the serum of SCID mice. Usual C.B17-*scid* mice were used.

C.B17-scid mice [10]. Major differences between transplanted bone marrow cells and solid human organs are the existence of supporting tissues in solid organs. The second transfer is very difficult in human bone marrow cells.

When radio-iodine, $Na^{125}I$, was injected intravenously into SCID mice with normal human thyroid xenograft, 20 to 27 percent of radioactivities were incorporated into transplanted human thyroid gland, and 6 to 11 percent in the mouse thyroid gland for 24 hours after ^{125}I injection. Thus, transplanted human normal thyroid gland is well incorporating iodine [3]. Human thyroid hormone (T3) from human thyroid tissues were detected in the blood of SCID mice with human thyroid gland, while it was not detectable in SCID mice without human thyroid gland transplantation. Level of T3 increased about three fold by the thyroid stimulating hormone (TSH) treatment to SCID mice. These results indicate that transplanted human thyroid gland shows normal function, that is, incorporation of iodine, continuous secretion of T3 and release of T3 by the TSH treatment.

Not only the adult human tissue, various organs of human embryos 10–11 weeks old were also transplanted into SCID mice with permission. Embryonic organs differentiate and grow rapidly in the improved SCID mice. For example, tiny finger (about 1 mm diameter) forms 5mm width finger with nail four mouths after transplantation. Internal organs also develop and grow rapidly, keeping and forming their organ specific morphology.

Mutagenesis and Carcinogenesis Studies in Human Tissues

Table 2 summarizes the effects of some organ-specific mutagens/carcinogens on the normal human tissue; N-amyl-N-methyl-nitrosamine (AMN) on esophagus, 1-methyl-3-nitroso-1-nitrosoguanidine (MNNG) on gastric and colon mucosa, urethane on the lung, X- and γ-rays on thyroid gland, or UVB on the skin. When human organs and tissues were treated with specific carcinogens/mutagens repeatedly for one to two years, *p53*, K-*ras*, *c-kit* mutations were induced in human tissues, and later cancers developed in the human skin and esophagus. Mutations were detected by the Cold-SSCP and direct sequencing [4].

Table 2. Mutation and cancer induced in normal human tissues by organ-specific mutagenic and carcinogenic agents

Organs	Mutagen and carcinogen	Mutation	Cancer
Esophagus	AMN	*p53*	+
Stomach	MNNG	K-*ras*, *p53*	–
Colon	MNNG	K-*ras*, *p53*	–
Lung	Urethane	K-*ras*, *p53*	–
Lung	Dioxin (TCDD)	K-*ras*	–
Thyroid gland	X-rays, TCDD	*p53*, *c-kit*	–
Skin	UVB + A	*p53*, *c-kit*, K-*ras*	+

Ultraviolet light exposure

Solar light has continuously influenced the development, metabolism, and so on of all organisms on the earth. For human beings, it is beneficial in general, but a part of solar light, UV radiation, can be harmful. Recently, the depletion of stratospheric ozone and the consequent increase in levels of genotoxic UVB (290–320 nm) in sunlight have led to fear of a rise in the frequency of skin cancer in human populations. Consequently, we maintained human skins in the improved SCID mice, and then irradiated daily with UVB for a long period (~2 years) to confirm the direct link between UVB and the development of human skin cancer, solar (actinic) keratosis, and molecular changes in *p53*, *ras* and *c-kit* genes. We used normal human skin at breast site of breast cancer patient and human foreskin of the penis of the phimosis patients with their permission, because these skins are rarely exposed to sunlight. Ultraviolet light shorter than 290 nm was cut-off by Kodacel cellulose film, and UVB and a spectrum which is similar to solar UV on the surface of the earth was given daily to human skin on the SCID mice; 0.8 J/cm^2 on Monday, Wednesday and Saturday, and 0.2 J/cm^2 on Tuesday, Thursday, Friday and Sunday. Minimum erythema dose for Japanese is 0.03–0.15 J/cm^2. After the daily exposure to UVB, human skin became irregularly thick and brownish, whereas no changes were observed in the mouse skin, simply because the mouse skin was covered by the hair. Microscopic feature showed typical actinic keratosis [11].

After the long continued UVB irradiation with about 100 J/cm^2 in total doses, ulcerated skin tumor developed sequentially in the brownish human skin and grew rapidly. Three squamous cell carcinomas and one undifferentiated skin cancer developed. UVB induced skin tumors were transplantable to C.B17-*scid/scid* mice, but not to their wild type C.B17—+/+ mice. Furthermore, DNA extracted from UVB induced skin tumor was amplified by the primers of human *p53* and K-*ras* genes, while DNA of the mice were not. Thus, it is confirmed immunologically and genetically that induced skin tumor is malignant and has originated in human skin. This is the first experimental success to induce malignant tumor in human tissues [6].

UVB-irradiated and unirradiated human skin pieces were examined for *p53*, K-*ras* and *c-kit* mutations. *p53* mutations were often detected in the UVB-irradiated human skin, especially in about 80 percent of UVB induced actinic keratosis and all of skin cancer, although K-*ras* and *c-kit* mutations were not so often in UVB-induced actinic keratosis skin. About 85 percent of *p*53 mutations occurred at dipyrimidine sites. T-C transition at codon 242 (Cys-Arg) was specifically observed in both skin cancer and actinic keratosis, and double or triple *p53* mutations were observed in UVB-induced skin cancers. In two of three UVB induced human skin cancer, K-*ras* and *c-kit* mutations were also induced in addition to duplicated and triplicated *p53* mutations. Consequently, three to five mutations were accumulated in UVB induced skin cancer [11].

Radiation and radionuclide exposure

Thyroid gland is known to be very sensitive to radiation, and radio-iodine. Radio-therapy of thymoma patients caused thyroid cancer, and radioiodine exhausted by the Chernobyl catastrophe caused high incidence of thyroid cancer in exposed children. Thyroid gland is also the target of endocrine disrupters such as dioxins.

Normal human thyroid glands from the head and neck cancer patients were transplanted to the SCID mice. SCID mice with human thyroid glands were exposed weekly to X-rays (2 Gy) or ^{137}Cs γ-rays (1 Gy), or 0.4 MBq of Na^{131}I was injected subcutaneously in to SCID mice. With increasing doses of X- or γ-rays and repeated injection of ^{131}I, brownish thyroid glands became pale and very small, and necrotic parts increased histologically. Without radiation exposure, level of human thyroid hormone in the peripheral blood of SCID mice with human thyroid gland is same at 60 to 300 days after transplantation, indicating constant secretion of human thyroid hormone from transplanted human thyroid gland in unexposed controls. However, level of thyroid hormone decreased significantly after X- or ^{137}Cs γ-ray exposure. Results are compatible with macroscopic and histological findings.

High doses of X-rays (24 to 60 Gy) to human thyroid tissues produced *p53* mutation at codon 209 and *c-kit* mutation at codon 825, although lower doses did not. However, thyroid cancer has not yet developed, because thyroid gland of an old person is not sensitive to radiation.

Perspectives

Improvement in SCID mice enabled us to maintain normal and precancerous human organs and tissues for long periods. Although there remain some unsolved problems such as replacement of human bone marrow system, most human organs and tissues survive in mice with their normal morphology and function.

Not only to investigate the influence of radiation and environmental mutagens/carcinogens on the human health, establishment of SCID biotechnology with human tissues provides an invaluable experimental system for studying physiology, pathology, pharmacology and biochemistry of human organs and tissues, interaction between organ and organ, diagnosis and therapy/prevention of human diseases, and toxicity test of new drugs, without illegal and/or troublesome clinical trials.

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