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High Human IgG Levels in Severe Combined Immunodeficient Mouse Reconstituted with Human Splenic Tissues from Patients with Gastric Cancer

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We implanted normal peripheral blood lymphocytes (PBL) from healthy donors and splenic tissues from patients with gastric cancers into the severe combined immunodeficient (SCID) mouse, demonstrating that SCID mouse with splenic tissue can produce a high level of human immunoglobulin G (IgG). The normal PBLs at 10^7 and 10^8/mouse were implanted intraperitoneally, and three splenic tissues with a size of $3\times3\times3$ mm from gastric cancer patients were inoculated subcutaneously into the bilateral backs of the mice. At 2, 4, 6 and 8 weeks after inoculation, mice were killed, and the human IgG was assessed by an ELISA method. SCID mice with splenic tissue revealed high human IgG levels from 2 weeks after inoculation and approximately 2 mg of IgG per ml was observed at 8 weeks post-implantation, while the IgG levels in mice treated with PBLs were limited. Since the half life of the extrinsic human IgG was 10.2 days, the high level of human IgG in the SCID mice was supposed to be produced by human plasma cells in the splenic tissue from gastric cancer patients. This model was thought to be adequate for evaluating human immunological functions *in vivo*.

Key words: SCID mouse — Human immunoglobulin — Splenic tissue

Although nude mice have made it possible to grow human malignant tumor cells *in vivo*,[1] human normal cells were difficult to maintain *in vivo* until the introduction of severe combined immunodeficient (SCID) mice which accept human lymphoid and myeloid cells with retention of their functions.[2–5] Since this model was thought to be suitable to evaluate the human immune system *in vivo*, we have attempted the reconstitution of human immunological functions using human peripheral lymphocytes (PBL) and splenic tissues from patients with gastric cancer.

MATERIALS AND METHODS

Mice Male SCID mice with a CB-17 genetic background and immunocompetent CB-17 mice were kindly supplied by Dr. T. Nomura, Central Institute for Experimental Animals, Kawasaki. They were maintained under specific pathogen-free conditions using an Isorack, and fed on sterile food and water *ad libitum* in our experimental animal center. Six- to eight-week-old mice weighing 20–22 g were used for the experiments.

Preparation of human splenic tissue and peripheral blood cells After total gastrectomy combined with splenectomy carried out on patients with Stage III or IV gastric cancer, the resected spleen was stored in Hanks' balanced salt solution containing 100 U of penicillin and 100 mg of gentamycin per ml (Hanks' solution) and brought to the laboratory as soon as possible. Then, part of the red pulp of the spleen was cut into $3\times3\times3$ mm pieces with scissors in Hanks' solution. After preparation of red pulp, three splenic tissues were inoculated bilaterally into the subcutaneous tissue of SCID mice under ether anesthesia with a trocar needle. This amounted to approximately 2×10^7 spleen cells per mouse. As a control, human PBL from healthy donors were administered to SCID mice at 10^7–10^8/mouse.

Evaluation of human immunoglobulin G and surface marker of human lymphocytes At 2, 4, 6 and 8 weeks after inoculation of splenic tissue and PBL, the blood was collected from the postocular vessels, serum was separated and human immunoglobulin G (IgG) was assessed by an ELISA method. Plates were coated with 5 μg/ml of normal human IgG (Cappel, Inc., PA, USA) and unbound areas of the plate were saturated with bovine serum albumin. The inhibition of binding of peroxidase-conjugated, affinity-purified goat anti-human IgG antibody (Cappel, Inc.) to the plate by known concentrations of human IgG was determined and a standard curve was generated and used to quantify the human IgG in SCID mouse serum. At 4 and 8 weeks after the inoculation of splenic tissue, the resected splenic tissues were resected, stained routinely with hematoxylin-eosin, and examined histologically.

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To determine the elimination speed of human IgG in SCID mice, extrinsic human IgG (Beriglobin™) containing 99% purified human IgG with Fc component was bolus-administered i.v. to SCID and immunocompetent CB-17 mice at a dose of 0.05 ml (equivalent to 7.5 mg IgG) per mouse. The change of human IgG in the mice was followed by the same method mentioned above and the half life of the extrinsic IgG was calculated according to the regression equation method using an NEC 9801 personal computer with the Automated Pharmacokinetic Analysis System for PC-9801 (Nankodo, Tokyo).[6]

To examine the surface markers of lymphocytes in the sera of the reconstituted SCID mice, lymphocytes were recovered through Percoll density centrifugation and stained with murine monoclonal antibody to human OKT3, OKT4 and OKT8 (Ortho Diagnostic Systems, Tokyo). Staining with GAM-FITC (Coulter Immunology, FL, USA) was analyzed on a Coulter EPICS™ cell sorter.

RESULTS

In the Central Institute for Experimental Animals, SCID mice were randomly checked for endogenous immunoglobulin, and no "leaky mice" were found in the colony. This was also confirmed in our institute in randomly sampled SCID mice.

In our ELISA system, no human IgG was detected in SCID and immunocompetent CB-17 mice because of the species-specific antigenicity of immunoglobulin and the coefficients of variation (CV = standard deviation/mean × 100%) were less than 10% in the standard curve generation. Table I shows the human IgG in SCID mice treated with human splenic tissue or human PBL. All SCID mice treated with human PBL and human splenic tissue developed human IgG in their peripheral serum. The SCID mice with the splenic tissue revealed a high level of human IgG from 2 weeks after inoculation, and approximately 2 mg of IgG per ml was observed at 8 weeks post-implantation, while the levels of IgG in mice treated with PBLs were lower than those of mice treated with splenic tissue with statistically significant differences. The IgG levels of SCID mice treated with PBL from the healthy donor were dependent on the numbers of injected PBL, but the IgG concentration of SCID mice treated with PBL at 10^8/mouse was lower than that in mice treated with splenic tissue at 6 weeks after treatment. In addition, there were large standard deviations in IgG of PBL-reconstituted SCID mice with CV values which ranged from 112 (4 wk) to 172% (8 wk), while the CV values of IgG in the splenic tissue-reconstituted SCID mice were less than 45% (on 8 wk), showing the stable reconstruction of human IgG in the splenic tissue-implanted SCID mice.

Table II. Serum Human Immunoglobulin Levels in SCID and CB-17 Mice after Bolus Injection of Extrinsic Human Immunoglobulin (Beriglobin)

Days after injection[a]	SCID mice n=10	CB-17 mice n=5
1	925.3±303.6[b]	1253.0±961.5
3	536.3±303.6	1099.2±459.1
5	566.5±326.2	716.0±109.4
7	530.3±209.7	828.4±274.7
14	363.4±242.6	386.6±227.5
21	215.2±68.5	256.5±83.9
Half-life time[c]	10.2 days	8.7 days

a) Purified human IgG was bolus-injected i.v. at a dose of 0.05 ml (equivalent to 7.5 mg IgG) per mouse.
b) Data are shown as mean±standard deviation in μg/ml.
c) Half life was calculated according to the regression equation method.

Table I. Production of Human IgG in SCID Mice Treated with Human PBL from Healthy Donor and Splenic Tissues from Cancer-bearing Patients

W[a]	PBL 10^7 i.p.	n[b]	PBL 10^8 i.p.	n	Splenic tissue[c]	n
2	31.2±41.1[d]	4			157.3±51.4**	15
4	163.4±183.4	4			768.1±165.8**	7
6	47.6±75.8	4	462.6±51.4**	4	1616.2±435.0**	6
8	156.5±269.0	4			1914.0±857.1*	6

a) Weeks after the treatment.
b) Number of mice.
c) Three blocks of splenic tissue 3×3×3 mm in size from patients with Stage III or IV gastric cancer were inoculated s.c.
d) Data are shown as mean±SD in μg/ml of human IgG detected by ELISA.
* $P<0.01$ to SCID treated i.p. with 10^7 PBL.
** $P<0.001$ to SCID treated i.p. with 10^7 PBL.

Table III. Surface Markers of Peripheral Blood Lymphocytes from SCID Mice Treated with Human PBL or Human Splenic Tissues

	n[a]	OKT3	OKT4	OKT8	OKT4/8
Control	5	2.9±2.2	2.0±1.4	6.8±3.3	0.60
6 wk[b]	1	13.9	1.9	13.8	0.14
4 wk[c]	3	0.7±0.4[d]	0.6±0.2	5.1±3.2	0.09
8 wk[c]	4	4.7±1.5	3.7±1.4	10.3±5.6	0.80

a) Number of mice.
b) Data for SCID mice treated i.p. with 10^7 PBL at 6 weeks before the assay.
c) Data for SCID mice transplanted with splenic tissues at 2, 4 and 8 weeks before the assay.
d) Data are shown as mean±SD percentage values with respect to total PBL.

Although traces of the splenic tissue including splenic trabeculae, hematopoiesis and lymphocytes were observed at the site of splenic tissue inoculation at 4 weeks post-inoculation, only scars and chronic inflammations were found at 8 weeks after these inoculations.

The elimination of the extrinsic human IgG from SCID and CB-17 mice is shown in Table II. The pharmacokinetics fitted a one-compartment model and human IgG was eliminated monophasically in both SCID and CB-17 mice with similar peak serum concentrations. Their half lives were 10.2 and 8.7 days, respectively, and the serum IgG level declined to approximately 200 μg/ml at 21 days after i.v. injection.

The surface markers of PBL in SCID mice treated with human PBL and splenic tissue are shown in Table III. Some background was detected even in the untreated SCID mice, and no statistically significant increase of the human lymphocytes with human surface markers was observed in SCID mice treated either with splenic tissue or PBL from the healthy donor. Concerning this incomplete reconstitution of T cell surface markers, no graft-versus-host disease was encountered in any of the mice in this study.

DISCUSSION

The present results demonstrated that the reconstitution of SCID mice with human splenic tissue is a promising method to evaluate human humoral immunological functions *in vivo*. Since the half life of the extrinsic human IgG in SCID mice was 10.2 days and the transplanted splenic tissue showed no cellular components at 8 weeks post-inoculation, when a high IgG level is maintained, the circulating human IgG in the reconstituted SCID mice was thought to be produced by the adopted human plasma cells from the splenic tissue of the gastric cancer patients. Since this reconstitution of human IgG in SCID mice was also observed by the implantation of the splenic tissues from patients with hypersplenism, idiopathic thrombocytopenic purpura and huge splenic cyst in other experiments (data not shown because of the limited number of experiments), the elevation of human IgG in SCID mice was thought not to be specific for splenic cells from patients with advanced gastric cancer. Although the present paper does not provide direct evidence that the transplanted human B cells reside in the mouse B-related tissues, the results are consistent with a report by Mosier *et al.*,[7] who observed OKT19-positive human cells in the spleen of SCID mice and suggested early engraftment of long-lived memory B cells which have already switched to IgG.

On the other hand, the reconstitution of T cell surface marker was incomplete. McCune *et al.*[8] reported that SCID mice transplanted with both fetal thymus and fetal liver placed under the renal capsule produced considerable numbers of OKT4- and OKT8-positive human T cells for a period of time. This discrepancy would be caused by the different maturation of T cells in fetal thymus and liver, and splenic tissues in adult patients with gastric cancer. This suggested that the mature T cells in the splenic tissue of patients with gastric cancer cannot proliferate and circulate in SCID mice, unlike the immature T cells from the fetal thymus and liver.

Although the reconstitution of the cellular immune system *in vivo* was incomplete in this system, this model can reconstitute human IgG with the splenic tissue of patients with advanced gastric carcinomas. This system was thought to be appropriate to evaluate the humoral immunological functions of cancer-bearing patients. If one could transplant cancer cells from the same patient with a donor of splenic tissues, this method would make it possible to investigate a human monoclonal antibody to utilize the living human B cells *in vivo* which recognize the antigens of autogenic cancer cells. In addition, this model might be able to offer an appropriate *in vivo* experimental system to clarify the immunological functions of spleen cells from patients with advanced gastric cancer to elucidate the clinical significance of splenectomy in the operation for the advanced gastric cancer.

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