Jpn. J. Cancer Res. 83, 894–898, August 1992

Production of Human Immunoglobulin G Reactive against Human Cancer in Tumor-bearing Mice with Severe Combined Immunodeficiency Reconstituted with Human Splenic Tissues

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Splenic tissues derived from patients with gastric cancer were implanted into mice with severe combined immunodeficiency (SCID) and then the mice were challenged with COLO-205, a human colon cancer cell line. Production of human immunoglobulin G (IgG) reactive against the COLO-205 cells was detected by enzyme-linked immunosorbent assay in sera from the reconstituted and tumor-bearing SCID mice. The titers of the reactive IgG relative to total IgG in the sera of SCID mice began to increase from one week after implantation of the tumor cells, and became 10- to 100-fold higher than that in the donor's serum by 3–4 weeks. This model using implantation of human cancer cells in SCID mice reconstituted with human splenic tissues would facilitate further studies of human cancer immunology.

Key words: SCID mouse — Human immunoglobulin — Splenic tissue

Mice with severe combined immunodeficiency (SCID),[2] which congenitally lack functional T- and B-lymphocytes,[1] have been reported to accept human lymphoid and myeloid cells with all their associated functions.[2] Furthermore, it has been demonstrated that production of specific human antibody is induced after immunization with tetanus toxoid or hepatitis B core antigen in SCID mice reconstituted with human peripheral blood lymphocytes (PBL).[3,4] In our recent study, SCID mice reconstituted with splenic tissues derived from patients with gastric cancer were found to produce a higher level of human immunoglobulin G (IgG) in comparison with those reconstituted with human PBL.[5] In the present study, we examined the reactivity of human IgG produced in response to COLO-205 human colon cancer cells, which were implanted into SCID mice after reconstitution with splenic tissues from patients with gastric cancer.

MATERIALS AND METHODS

Mice Male SCID mice with a CB-17 genetic background were kindly supplied by Dr. T. Nomura, Central Institute for Experimental Animals (Kawasaki). The animals were maintained under specific pathogen-free conditions using an Isorack™, and fed on sterile food and water *ad libitum* in the Experimental Animal Center, Keio University School of Medicine. Six- to eight-week-old mice weighing 20–22 g were used for the experiments.

Cell lines COLO-205, a well differentiated human colon cancer cell line established by Semple *et al.*,[6] was provided by the Pathology Division, National Cancer Center Research Institute, Tokyo. C-1, a human colon cancer cell line, was kindly provided by Dr. K. Nagai, Tokyo Medical College. Exponentially growing monolayer cultures of both cell lines were maintained in a humidified atmosphere of 5% CO_2/95% air at 37°C in RPMI-1640 medium (Kaken, Tokyo) supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Suspensions of both cell lines were obtained enzymatically by incubation for 10 min at 37°C using a 1:4 mixture of 0.25% trypsin (Gibco, Grand Island, NY) and Versene (Gibco).

Implantation of splenic tissues and cancer cells Splenic tissues were derived from 12 patients with advanced gastric cancer who underwent splenectomy combined with total gastrectomy at Keio University Hospital between February and November, 1991. Human splenic tissues were implanted into SCID mice as reported previously.[5] Each resected spleen was rinsed immediately in Hanks' balanced salt solution containing 100 U/ml penicillin and 100 μg/ml streptomycin (Hanks' solution) and brought to the laboratory as soon as possible. After part of the splenic red pulp had been cut into $3\times3\times3$-mm pieces with scissors in Hanks' solution, three of the tissue pieces were inoculated bilaterally into the subcutaneous tissue of SCID mice under ether anesthesia using a trocar needle. Within 7 days after implantation of the splenic tissues, 1×10^7 COLO-205 cells/100 μl RPMI-

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[2] Abbreviations: SCID, severe combined immunodeficiency; IgG, immunoglobulin G; PBL, peripheral blood lymphocytes; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

1640 were injected subcutaneously. Control mice were processed in parallel without implantation of COLO-205 cells.

Quantification of human total IgG At various intervals after COLO-205 implantation, blood was collected from the postocular vesseles of each mouse and the serum was separated. Human total IgG levels expressed as both total (total human IgG) and as that reactive against COLO-205 cells (COLO-205-reactive human IgG) were assessed by enzyme-linked immunosorbent assay (ELISA).

1) Determination of total human IgG Each well of 96-well microplates was coated with 5 μg/ml normal human IgG (Cappel, West Chester, PA) dissolved in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). After blocking for 2 h with 5% BSA in PBS, serial dilutions in 2% BSA in PBS of serum obtained from each SCID mouse reconstituted with human splenic tissues (SCID-sp mouse) and serum from the corresponding donor of the splenic tissue that had been stored frozen, were added and further incubated for 1 h. Human IgG levels were determined using 2 μg/ml peroxidase-conjugated affinity-purified goat anti-human IgG (Cappel) in 1% BSA in PBS followed by addition of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (Sigma, St. Louis, MO) and 20 μl of 30% H_2O_2 dissolved in 20 ml citrate buffer (Zymed, San Francisco, CA). After the reaction had been stopped by addition of H_2SO_4, the optical density was determined at 630 nm. Several washes with PBS were carried out after each step. Known concentrations of human IgG diluted in 2% BSA in PBS were assessed to generate a standard curve.

2) Determination of COLO-205-reactive human IgG Each well of 96-well microplates was coated with COLO-205 cells prepared by centrifugation in 0.25% glutaraldehyde diluted in PBS after overnight incubation with the cell suspension at a cell concentration of 8×10^6 cells per well. Human IgG levels in serial dilutions of serum obtained from each SCID-sp mouse and stored

Table I. Human IgG Levels Expressed as Total IgG and IgG Reactive against COLO-205 and C-1 Cells in SCID-sp Mice in the 5th Week after Reconstitution

Patient number	Total IgG in patient's serum[c]	SCID-sp without COLO-205[a]			SCID-sp with COLO-205[b]		
		Total[d]	205-reactive[e]	C-1-reactive[f]	Total[d]	205-reactive[e]	C-1-reactive[f]
1	9.16	1.34[g] (0.17)	0.365 (0.105) [n=4][h]	0.260 (0.023)	1.57 (0.23)	19.4 (4.0) [n=4]	0.255 (0.050)
2	8.45	2.30 (0.73)	0.296 (0.133) [n=4]	0.201 (0.036)	2.35 (0.68)	3.87 (1.78) [n=4]	0.298 (0.128)
6	11.9	1.53 (0.27)	0.295 (0.164) [n=4]	0.220 (0.076)	1.51 (0.50)	4.78 (2.88) [n=5]	0.218 (0.082)
9	6.27	0.796 (0.300)	0.289 (0.141) [n=4]	0.209 (0.061)	1.10 (0.42)	1.67 (0.73) [n=4]	0.320 (0.146)
11	8.10	2.07 (0.40)	0.267 (0.168) [n=5]	0.204 (0.059)	2.23 (0.35)	25.3 (13.4) [n=5]	0.385 (0.129)

a) SCID mice reconstituted with splenic tissues from the patient, but not implanted with COLO-205 cells.
b) SCID mice reconstituted with splenic tissues from the patients, and implanted with COLO-205 cells.
c) Total human IgG levels in mg/ml detected by ELISA in the patient's serum.
d) Total human IgG levels in mg/ml detected by ELISA in sera of SCID-sp mice with or without COLO-205.
e) Levels of human IgG reactive against COLO-205 cells in sera of SCID-sp mice detected by ELISA. Data are shown as ratios relative to the corresponding patient's serum in terms of absolute titer.
f) Levels of human IgG reactive against C-1 cells in sera of SCID-sp mice detected by ELISA. Data are shown as ratios relative to the corresponding patient's serum in terms of absolute titer.
g) Data are shown as mean (standard deviation) evaluated 5 weeks after implantation of splenic tissues and 4 weeks after COLO-205 implantation.
h) Number of mice evaluated.

serum from the corresponding donor of the splenic tissue were determined according to the same ELISA method as used for the determination of total human IgG. Stored sera from SCID-sp mice known to be reactive and non-reactive to COLO-205 cells were used as positive and negative controls in every quantification of the COLO-205-reactive human IgG levels, and the titer corresponding to an optical density of twice that of the negative control at the lowest dilution was considered to be detectable. Parallel determinations of human IgG levels reactive against C-1 cells (C-1-reactive human IgG) were perfomed using C-1 cells for each sample. Levels in the sera of SCID-sp mice were evaluated as the ratio relative to that in the corresponding donor's serum.

In both ELISA systems used for determinations of the total, and the COLO-205-reactive human IgG, the coefficients of variation were less than 10% and human specificity was confirmed by their non-reactivity with sera from non-manipulated SCID or immunocompetent CB-17 mice.[5]

3) Determination of the relative titer Since the total human IgG levels varied among the reconstituted mice and increased steeply in each SCID-sp mouse after splenic tissue implantation,[5] the relative titers of the COLO-205-reactive and the C-1-reactive human IgG were determined according to Duchosal *et al.*[4] as follows: ratio relative to the corresponding donor's serum in terms of absolute titer × (total IgG in the donor's serum/total IgG in the serum of SCID-sp mouse). This allowed clear differentiation between the passive and active changes in the COLO-205-reactive and the C-1-reactive human IgG, and was thought to be a more reliable assessment of the putative immune response in a given mouse with time or between SCID-sp mice bearing tissues derived from a common donor.[4]

RESULTS

Production of COLO-205-reactive human IgG was observed in SCID mice given implants of splenic tissues from 5 of 12 patients. In the sera from all of several mice bearing splenic tissues from the other 7 patients, no COLO-205-reactive human IgG was detectable, although the levels of total human IgG were also elevated (data not shown). The COLO-205-reactive human IgG in SCID mice without COLO-205 challenge was significantly increased by the additional implantation of COLO-205 cells, whereas the total IgG levels were essentially equivalent to each other (Table I). In the sera of these SCID-sp mice, the relative titer of COLO-205-

Table II. Relative Titers of Human IgG Reactive against COLO-205 and C-1 Cells in the SCID-sp Mice

Patient	SCID-sp without COLO-205[a]		SCID-sp with COLO-205[b]	
	205-reactive	C-1-reactive	205-reactive	C-1 reactive
1	2.34 (0.76)[c] [n=4][d]	1.97 (0.52)	115.6 (33.7) [n=4]	1.55 (0.50)
2	1.43 (1.22) [n=4]	0.825 (0.346)	13.1 (3.56) [n=4]	1.06 (0.23)
6	2.21 (0.74) [n=4]	1.67 (0.35)	34.5 (11.0) [n=5]	1.70 (0.16)
9	2.16 (0.45) [n=4]	1.70 (0.23)	11.8 (3.06) [n=4]	1.96 (0.94)
11	1.03 (0.57) [n=5]	0.828 (0.243)	86.4 (43.3) [n=5]	1.36 (0.31)

Relative titer was calculated as follows: ratio relative to the corresponding patient's serum in terms of absolute titer × (total human IgG in the patient's serum/total human IgG in the serum of SCID-sp mouse).

a) SCID mice reconstituted with splenic tissues from the patients, but not implanted with COLO-205 cells.

b) SCID mice reconstituted with splenic tissues from the patients, and implanted with COLO-205 cells.

c) Data are shown as mean (standard deviation) evaluated 5 weeks after implantation of splenic tissues and 4 weeks after COLO-205 implantation.

d) Number of mice evaluated.

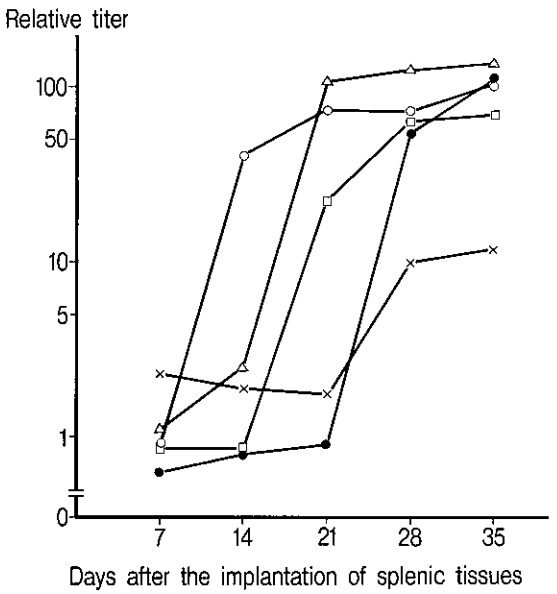

Fig. 1. Change in relative titers of human IgG reactive against COLO-205 cells as a function of time in five tumor-bearing SCID-sp mice reconstituted with splenic tissues from patient number 11. The mice were implanted with the splenic tissues on day 0 and further implanted with COLO-205 cells on day 4. The ratios relative to the patient's stored serum in terms of absolute titer of human IgG reactive against COLO-205 cells in each mouse serum were assessed by ELISA on each day indicated, and the relative titers of the reactive human IgG were calculated as follows: ratio relative to the patient's serum in absolute titer × (total human IgG in the patient's serum/total human IgG in the sera of SCID mice). The total human IgG level in the stored serum from patient 11 was 8.1 mg/ml. ○, ●, △, □ and × indicate the data obtained from each of five individual mice.

reactive human IgG was elevated as much as 100-fold in comparison with that in the donor's serum (Table II). However, the C-1-reactive human IgG was not increased by the COLO-205 challenge (Tables I and II). Data for SCID-sp mice bearing splenic tissue from patient number 11 are shown in Fig. 1, where the relative titer of COLO-205-reactive human IgG began to increase from one week after COLO-205 implantation, and reached a plateau level after 3 to 4 weeks, with a range of levels from 12- to 130-fold higher than those in the donor's serum. One of the reasons for this variation might have been the different parts of the spleen implanted into each mouse.[4]

DISCUSSION

It has been suggested that SCID mice reconstituted with human PBL could provide a promising model for analysis of human immune functions *in vivo*.[7] Recent studies using tetanus toxoid or hepatitis B core antigen have shown that PBL from individuals who have not come into contact with antigens for many years can be stimulated to produce specific antibodies in this SCID mouse model.[3,4] In the present study, we demonstrated that production of such antibodies can also be induced against human cancer cells. Therefore, the SCID-sp model would be appropriate for studying human cancer immunology. It would be possible to investigate a human monoclonal antibody by utilizing the human B-lymphocytes in this SCID-sp model.[7] At present, however, the reconstitution of human T-lymphocyte functions in SCID mice is considered to be insufficient in terms of surface markers of human T-lymphocytes.[5,8] This was also supported by the result that the COLO-205 tumors could also grow in SCID-sp mice, with an equivalent growth rate to that in untreated SCID mice (data not shown).

Recently, we have newly established a SCID-sp model using subctaneous implantation of human splenic tissues, allowing a more stable production of human IgG in SCID mice than in the case of reconstitution with PBL.[5] This was also the case with the present study, because the tumor-bearing SCID mice reconstituted with PBL failed to produce high levels of the COLO-205-reactive human IgG (data not shown). This model using implantation of human cancer cells in SCID mice reconstituted with human splenic tissues would facilitate further studies of human cancer immunology.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Tatsuji Nomura, the Central Institute for Experimental Animals, for the supply of SCID mice.

(Received March 6, 1992/Accepted May 13, 1992)

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