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Original Article

Development of the immune system in severe combined immunodeficiency mice reconstituted with transfered fetal liver cells

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Severe combined immunodeficiency (SCID) mice were immunologically reconstituted by the transfer of fetal liver cells (FLC) from BALB/c (SCID-isoFLC mice) or C57BL/6 mice (SCID-alloFLC mice). The developmental process of the immune system in the peripheral blood (PB) was almost comparable between SCID-isoFLC and SCID-isoFLC mice. Analysis of the lymphoid organs and the PB from SCID-alloFLC mice indicated that sIg^+ B cells appeared and were distributed to the periphery within 2 weeks after the transfer of FLC. It was also suggested that precursor T cells entered the thymus before 4 weeks after the transfer, and were differentiated into mature $CD4^+$ or $CD8^+$ T cells which then migrated to the periphery by 6 weeks. All of mature lymphocytes in the periphery of the SCID-alloFLC mice were shown to express donor-type H-2 antigens. Additionally, in the SCID-isoFLC mice, cell-mediated immunity such as rejection against alloantigens was functioning from 6 weeks, and humoral immune function was suggested by the detection of cells generating antigen-specific antibodies.

We discuss that development of the immune system in SCID mice receiving transferred FLC was comparable to that normally seen in the fetal and neonatal stages.

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Introduction

Studies[1,2,3] of the mechanism for the development of immunological self-tolerance have been conducted using experimental animals during the development of the immune system in fetal life; it is extremely from a technical standpoint to manipulate the immune system during fetal development even with experimental animals. If development of the immune system seen in the fetal and neonatal stages of normal mice could be made to reappear in adult mice, it would be possible to study the mechanism of self-tolerance with this model.

SCID mice discovered by Bosma et al.[4] in 1983 are the best animal model of human congenital immunodeficiency diseases in both T- and B-cell lineages. In SCID mice, bone marrow (BM)-derived cells, except for T and B cells, are supposed to be normal.[5,6] The mutation in SCID mice dose not seem to impair the hematopoietic microenvironment necessary for lymphoid differentiation. In practice, however, when normal BM or cells from long-term BM cultures (LTBMC) were transplanted to SCID mice, full reconstitution of the immune system required sublethal irradiation of the adult SCID mice.[7,8,9] In contrast, when FLC from isogenic or allogenic mice were transplanted into adult SCID mice without prior irradiation, the immune system was fully reconstituted.[10,11]

Thus, in the present study, FLC were used as the source of hematopoietic stem cells (HSCs). Then, we

studied the developmental process of the immune system and the acquisition of functions in cell-mediated immunity and humoral immunity in adult SCID mice after the transfer of normal FLC.

Materials and Methods

Mice

FOX CHASE SCID C.B-17/Icr-scid Jcl (SCID) mice, BALB/cAJcl-nu (Nude) mice, BALB/cAJcl (BALB/c) mice, C57BL/6NJcl (C57BL/6) and C3H/HeNJcl-MTV^+ (C3H/He) mice were used in the present stydy. All were supplied by Clea Japan (Tokyo, Japan) and maintained at Institute for Laboratory Animals, Tokyo Medical and Dental University, Tokyo.

Transfer of FLC to SCID mice

Individual cells from fetal livers removed from fetuses of BALB/c or C57BL/6 mice at gestation day (GD) 14 were suspended by pumping the detached cells with 23G-needle syringe in minimum essential medium (MEM). Then, free cells were washed three times with MEM. SCID mice were anesthetized by intraperitoneal injection with 0.5ml of 5% Nembutal (Dabbott Co., Osaka, Japan) in saline, and a $1x10^7$ FLC suspension was injected intravenously into the tails of SCID mice.

Immunofluorescence staining of lymphocytes and monoclonal antibodies (mAbs) for flow cytometry (FCM)

PB, 100 μl in amount, was collected from the mice, and then the sleens, thymus, and BM were removed. Free cell suspensions from the organs were prepared by pumping the detached cells with 23G-needle syringes, and washing them three times with MEM. The free cells were double-stained with variable combinations of mAbs. phycoerythin (PE)-labeled anti-L3T4 (CD4), fluorescein isothiocyanate (FITC)-labeled anti-Lyt-2 (CD8), FITC-labeled anti-Thy1. 2, FITC-labeled anti-Ig and PE-conjugated streptavidin were purchased from Becton Dickinson (California, USA). PE-labeled anti-Thy1. 2 was purchased from Caltag Laboratories Inc. (California, USA). PE-labeled anti-B220 and FITC-labeled anti-CD3 were purchased from pharmingen (San Diego, USA). FITC-labeled anti-H-$2K^b$ and biotin-labeled anti-H-$2D^d$ were purchased from Meiji Institute of Health Science (Tokyo, Japan). Flow cytometry analysis was performed with FACS-CAN (Becton Dickinson, CA, USA). Fluorescence were expressed on a log scale. Data were presented as a percentage of total viable lymphocytes gated.

Quantification of serum immunoglobulins (Igs)

Serum Igs were assessed by enzyme-linked immunosorbent assay (ELISA), by using affinity-isolated rabbit anti-mouse Igs (Dakkopatts, SA, Denmark), biotin-conjugated rabbit anti-mouse Igs (Dakkopatts), and horseradish peroxidase-conjugated streptavidin (SAPx) (Dakkopatts). The enzymatic reaction was stopped by the addition of 25 μl of 2N HCl, and absorbance of each well was measured at 490 nm with a microplate-reader (MTP-22 microphotometer Corona Electric, Japan).

The quantity of Igs in strum was represented using laboratory units (LU) in a standard with diluted serum from 12-week-old male BALB/c mice. Absorbance for the serum diluted at $1:2^{16}\times10$ was defined as 1LU. Serum samples from the mice were diluted to be in the range between 0.1 and 1.8 of absorbance on standard line.

Immunization procedure

Ovalbumin (OVA) 1mg-ml in saline was emulsified with an equal volume of incomplete Freund's adjuvant (IFA). Mice anesthetized with Nembutal were subcutaneously injected with 0.3ml of the emulsion into their four footpads. A plaque-forming cell (PFC) assay was performed at 7 days after the immunization.

PFC assay

OVA or protein-A (PA) 1mg-ml in saline, the same volume of packed sheep red blood cells (SRBC), and a ten-fold volume of 0.35 mM $CrCl_3$ in saline were mixed and incubated at 37°C for 1h. The protein-conjugated SRBC were washed three times with MEM and adjusted to 20% volume in MEM. A suspension of single cells was prepared from the spleens of immunized mice. In direct PFC, 300 μl of 0.5% agarose-MEM kept warm at 50°C, 25μl of 20% SRBC or protein-conjugated SRBC, 100 μl of 1×10^7/ml or 1×10^8/ml spleen cell suspension, and 25 μl of guinea pig serum as complement were mixed in a pre-warmed 5ml tube. In indirect PFC, 25 μl of anti-mouse Ig rabbit serum was added to the mixture. Then, 50 μl of the mixture was dropped into a petri dish (100×20mm), covered with a glass (24×24mm) and incubated overnight at 37°C.

Statistical analysis

Student's *t*-test was utilized; P values of less than 0.05 were considered to be statistically significant.

Method of skin grafting

Skin grafting was performed as described earlier by Billingham et al.[12] with slight modifications. C57BL/6 (H-2^b) and C3H/He (H-2^k) female mice were used as donors of allogenic grafts, and BALB/c (H-2^d) female mice for donors of isogenic grafts.

Experimental design

The SCID mice, ranging in age between 5 and 9 weeks, were injected with FLC from BALB/c mice or C57BL/6 mice. At the least, an interval of 2 weeks was necessary for blood collection. Thus, SCID mice were divided into 2 groups. Every odd week after the transfer of FLC, 0.1ml of the PB was collected from one group of the SCID-isoFLC or SCID-alloFLC mice; on every even week, the PB was collected from another group. For controls, the PB samples from untreated SCID mice and BALB/c mice were collected and analyzed at the same time. SCID-isoFLC mice at 14 weeks after the transfer were immunized with OVA and IFA, and used for PFC assay at 7 days after the immunization. For controls, untreated SCID mice and BALB/c mice were immunized and analyzed and analyzed, in the same way, at the same time.

To analyze the development of lymphocytes in immune organs, the BM, the spleen and the thymus were removed from SCID-alloFLC mice at 2, 4, 6, 8 or 12 weeks after the transfer. For controls, the BM, the spleen, and the thymus from untreated SCID mice and BALB/c mice were removed and analyzed at the same time. To analyze the double expression of H-2 antigens and lymphocyte markers, the PB, the spleen and the thymus were removed from the SCID-alloFLC mice at 11 weeks after the transfer.

On the other hand, skin grafts from female C3H/He, C57BL/6 and BALB/c mice were transplanted onto 4-5 week-old female SCID mice on their back side by side. Three weeks after the skin-grafting to SCID mice, FLC from isogenic BALB/c mice were transferred into those SCID mice on which the skin grafts were confirmed to be surviving without any problems. After that, skin grafts on SCID-isoFLC mice were observed.

Results

Development of immune system in PB of SCID mice after the transfer of isogenic or allogenic FLC (Figure 1, Table 1)

In the PB of SCID mice, $CD3^+$, $CD4^+$, and $CD8^+$ T cells as well as sIg^+ B cells were very small in number, less than 1%, The level of serum Igs was below the detection-limit. However, a significant percentage of $Thy1.2^+$ cells was present in the PB of SCID mice (Table 1). Thus, $CD4^+$ and $CD8^+$ T cells, sIg^+ B cells, and serum Igs, were used to monitor the development of the immune system in the PB of SCID mice after the transfer o FLC.

Table 1. Lymphocyte populations and serum Igs in the PB from BALB/c mice, nude mice, or SCID mice. The results represent means of levels in 3, 6-10 week-old, BALB/c mice, nude mice, or SCID mice. Lymphocytes in the PB from BALB/c, nude, or SCID mice were stained with a combination of PE-anti-CD4 and FITC-anti-CD8, PE-anti-CD4 and FITC-anti-CD3, or PE-anti-Thy1.2 and FITC-anti-sIg.

mice	$CD4^+$	$CD8^+$	$CD3^+$	Thy 1.2^+	sIg^+	serum Ig
BALB/c	38%	12%	50%	40%	40%	5000 LU/ml
Nude	<1%	<1%	<1%	<1%	80%	4800 LU/ml
SCID	<1%	<1%	<1%	<15%	<1%	<1LU/ml

In the PB of SCID-isoFLC mice, B cells (sIg^+) appeared within 2 weeks after the transfer of FLC, increased gradually in number, and reached the level of normal BALB/c mice in 5 weeks. T cells ($CD4^+$, and $CD8^+$ cells) appeared within 5 weeks after the transfer of FLC, increased gradually in number, and reached the level of normal BALB/c mice in 7 weeks. Serum Igs become detectable 2 weeks after transfer, and reached the level found in normal BALB/c mice in 7 weeks (Figure 1). Additionally, in the PB of SCID-alloFLC mice, BA cells (sIg^+) appeared within 2 weeks after the transfer of FLC, increased in number, and reached the plateau level found in normal BALB/c mice in 6 weeks, $CD4^+$, and $CD8^+$ T cells were detected within 6 weeks after the transfer of FLC. Although $CD8^+$ cells increased gradually in number and reached the level found in normal BALB/c mice in 7 weeks, the $CD4^+$ cells did not reach the level found in normal BALB/c mice in 10 weeks (Figure 1). The developmental process of the immune system was almost comparable between SCID-isoFLC and SCID-alloFLC mice, except that the appearance of $CD4^+$, and $CD8^+$ T cells in the PB was retarded 1 week in the latter as compared with the former.

In the PB of untreated SCID mice, H-$2D^{d+}$ cells were detected in 95.6% of lymphocytes. In the PB of SCID-alloFLC mice, H-$2K^{b+}$ cells appeared within 2 weeks after the transfer of FLC, increased gradually in number, and reached 90.8% by 10 weeks. In nearly direct contrast, H-$2D^{d+}$ cells decreased to 7.3% at 10 weeks after the transfer.

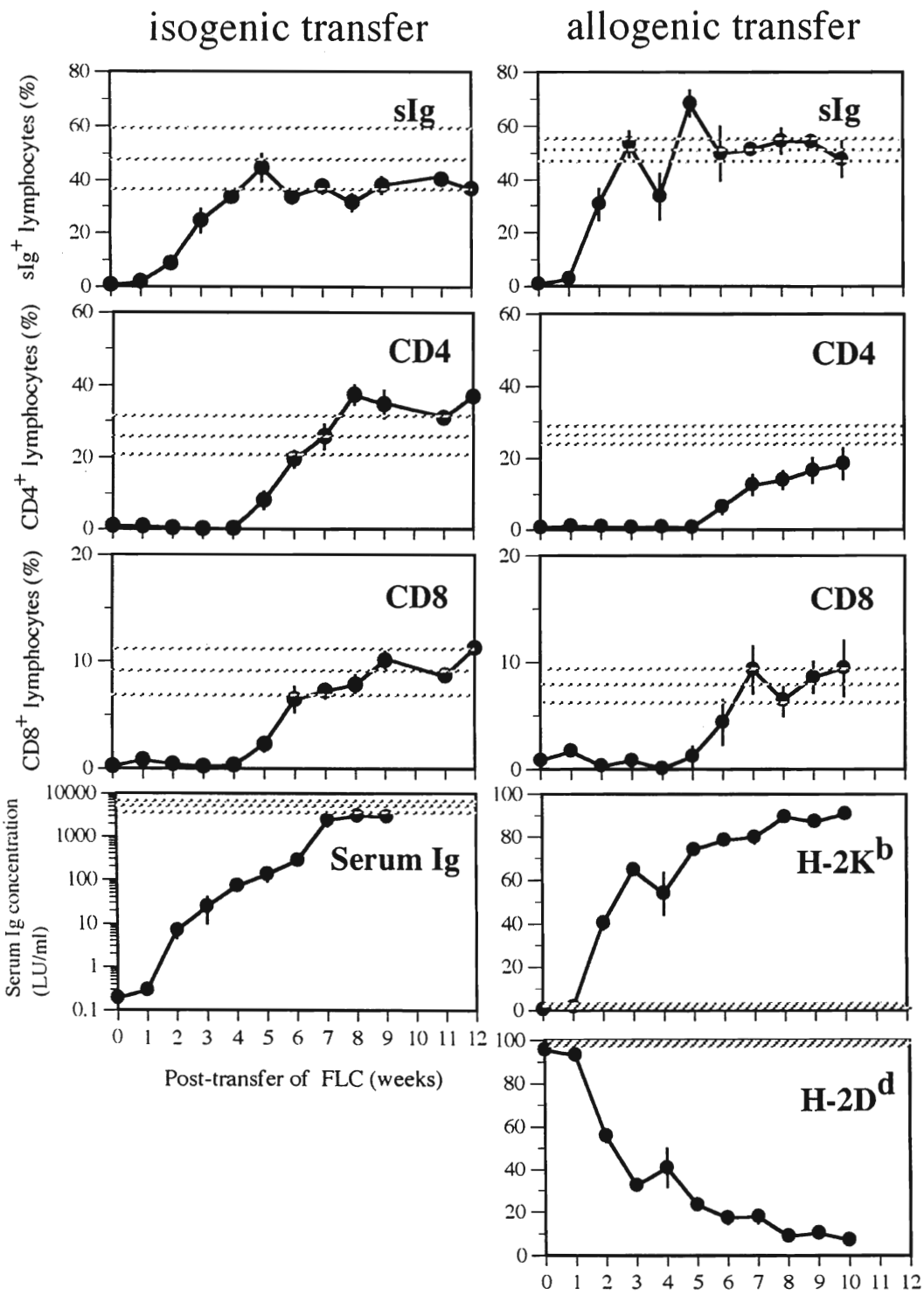

Fig 1. Development of immune system in the PB of SCID-isoFLC and SCID-alloFLC mice after the transfer of FLC. Weeks after the transfer of FLC are shown in the abscissa. The ordinate shows percentages of lymphocytes positive for each surface marker, or serum Ig concentration. In the left column, each value (solid circle) in odd weeks shows the mean of levels in 10 SCID-isoFLC mice, and each value in even weeks shows the mean of levels in 12 SCID-isoFLC mice. Vertical lines show mean ± standard error of the mean (SEM). The horizontal dotted lines indicate mean ± SEM of levels in 11, 10–12 week-old, BALB/c mice. In the right column, each value (solid circle) in odd weeks shows the mean of levels in 7 SCID-alloFLC mice, and each value in even weeks shows the mean of levels in 5 SCID-alloFLC mice. Horizontal dotted lines indicate mean ± SEM of levels in 5, 10-12 week-old, BALB/c mice.

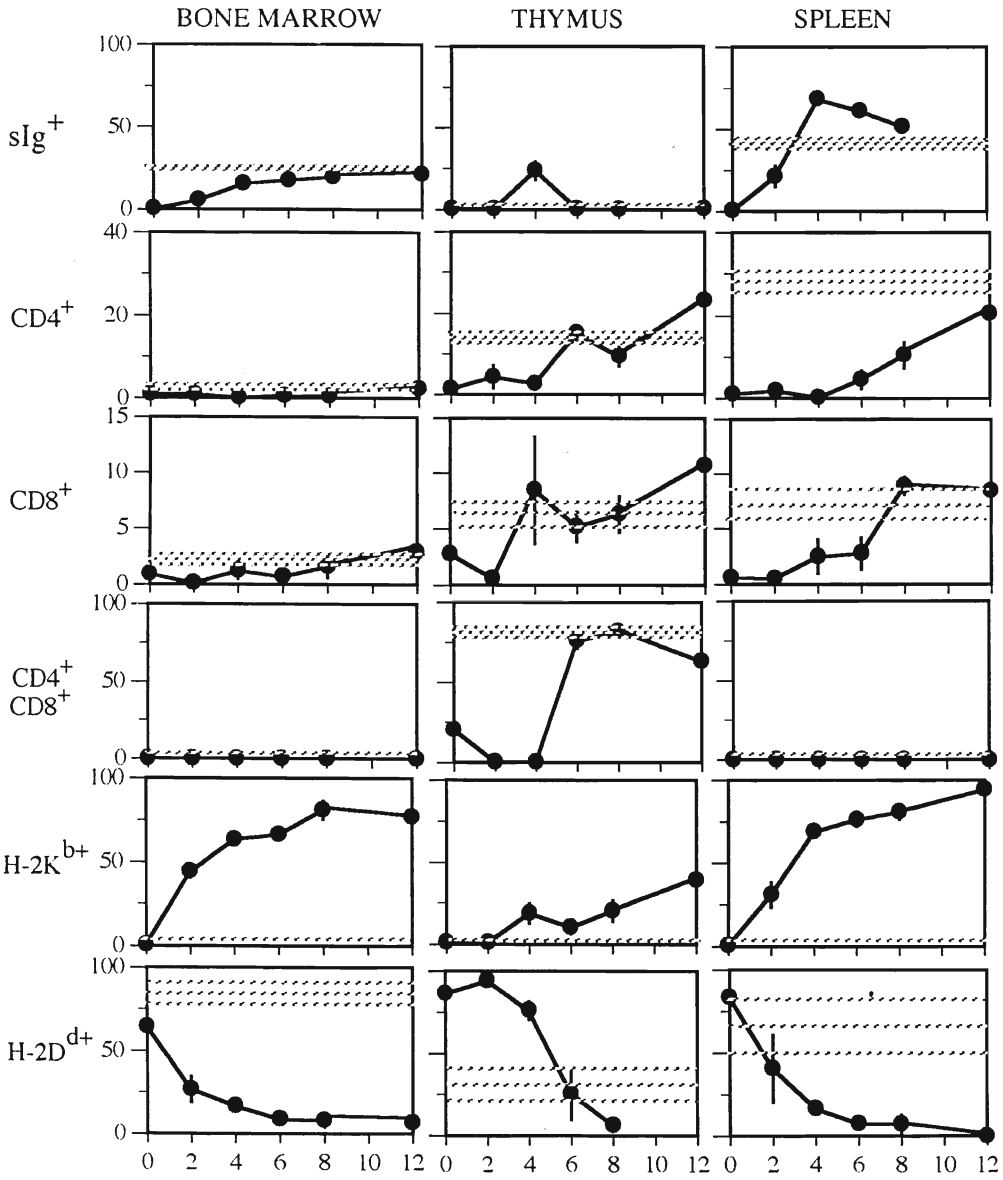

Fig 2. Development of lymphocyte populations in the BM, thymus, and spleen of SCID-alloFLC mice after the transfer of FLC. Weeks after the transfer of the allogenic FIC are shown in the adsicssa. The ordinate shows percentages of each surface marker-positive cell. Each value (solid circle) shows the mean of levels in 4 SCID-alloFLC mice. Vertical lines show mean ± SEM. Horizontal dotted lines indicate mean ± SEM of levels in 5, 10–12 week-old, normal BALB/c mice.

Development of immune system in organs of SCID mice after transfer of allogenic FLC (Figure 2, Table 2)

Figure 2 shows the time course of the development of lymphocytes in the BM, thymus, and spleen of SCID-alloFLC mice. SIg^+ appeared in the BM and spleen 2 weeks after the transfer, and reached the normal level in the spleen at 4 weeks. In the thymus, as high as 23.7% sIg^+ cells were detected transiently at 4 weeks, but they became undetectable thereafter. In the thymus, $CD4^+$ or $CD8^+$ single positive (SP) cells were detected in 4 weeks, and $CD4^+$ $CD8^+$ double positive (DP) cells appeared and reached the normal level at 6 weeks. In the spleen, $CD4^+$ or $CD8^+$ SP cells appeared in 6 weeks, and reached the normal level at 8 weeks.

In the untreated SCID mice, $H\text{-}2D^{d+}$ cells were detected in 64% of lymphocytes in the BM, 86% in the thymus, and 83% in the spleen. In SCID-alloFLC mice, $H\text{-}2K^{b+}$ cells appeared in the BM and spleen 2 weeks after the transfer of FLC, increased gradually in

Table 2. Origin of lymphocytes in the PB, spleen, and thymus from SCID-alloFLC mice. The results represent means of levels in 3 SCID-alloFLC mice at 11 weeks after the transfer of FLC. Lymphocytes in the PB, spleen, and thymus from SCID-alloFLC mice were stained with a combination of PE-anti-H-2K^b and FITC-anti-H-2D^d, CD4, THY1.2 or B220, and PE-anti-H-2D^d and FITC-anti-CD8, CD3, Thy1.2, or sIg.

a) Peripheral blood

	H-2D^{d+}	H-2D^{d-}	H-2K^{b+}	H-2K^{b+}	CD4$^+$	B220$^+$	Thy1.2$^+$	CD8$^+$	CD3$^+$	sIg$^+$
H-2K^{b+}	<1%	90%			37%	26%	53%			
H-2K^{b-}	9%	<1%			<1%	<1%	<1%			
H-2D^{d+}			<1%	9%			<1%	<1%	<1%	<1%
H-2D^{d-}			90%	<1%			43%	14%	48%	23%

b) Spleen

	H-2D^{d+}	H-2D^{d-}	H-2K^{b+}	H-2K^{b+}	CD4$^+$	B220$^+$	Thy1.2$^+$	CD8$^+$	CD3$^+$	sIg$^+$
H-2K^{b+}	<1%	96%			25%	44%	39%			
H-2K^{b-}	4%	<1%			<1%	<1%	<1%			
H-2D^{d+}			<1%	4%			<1%	<1%	<1%	<1%
H-2D^{d-}			96%	<1%			37%	17%	25%	28%

c) Thymus

	H-2D^{d+}	H-2D^{d-}	H-2K^{b+}	H-2K^{b+}	CD4$^+$	B220$^+$	Thy1.2$^+$	CD8$^+$	CD3$^+$	sIg$^+$
H-2K^{b+}	<1%	21%			16%	<1%	15%			
H-2K^{b-}	7%	<71%			77%	<1%	78%			
H-2D^{d+}			<1%	7%			<1%	<5%	<1%	<1%
H-2D^{d-}			21%	71%			98%	77%	27%	<1%

percentage, and reached 79% in the BM and 94% in the spleen by 12 weeks. In the thymus of SCID-alloFLC mice, H-2K^{b+} cells appeared in 4 weeks,and increased to no more than 39% at 12 weeks. In contrast to H-2K^{b+} cells were decreased to less than 10% in the BM, thymus, and spleen by 8 weeks after the transfer.

To determine the percentage of host- or donor-derived lymphocytes in SCID-alloFLC mice, cell populations from the PB, spleen, and thymus were studied with a two-color analysis of the expressed H-2 antigens (Table 2). The results indicated that all of CD3$^+$, CD4$^+$,CD8$^+$, Thy1.2$^+$, sIg$^+$ and B220$^+$ cells in the PB and spleen from SCID-alloFLC mice were positive for H-2K^b. In the thymus, although almost all cells were positive for Thy1.2, only 15% were positive for both Thy1.2 and H-2K^b. In contrast, 71% of the lymphocytes were negative for both H-2D^b and H-2K^b in the thymus from SCID-alloFLC mice.

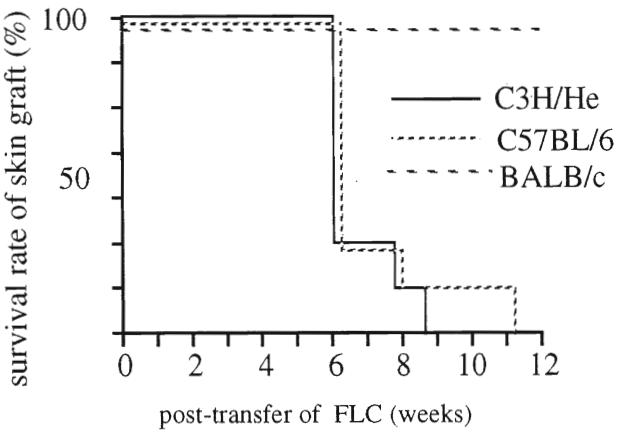

Fig 3. Survival of skin grafts from C57BL/6, C3H/He, or BALB/c mice on SCID-isoFLC mice after the transfer of FLC. Seven, 4-5 week-old, female SCID mice were given transplants of skin gtafts from female C3H/He, C57BL/6, and BALB/c mice, and then injected with isogenic FLC. Weeks after the transfer of FLC are shown in the abscissa. The ordinate shows survival rate of the skin grafts in 7 recipient SCID mice for each group.

Development of immunological functions in SCID mice after the transfer of isogenic FLC (Figure 3, Table 3)

The skin grafts from C3H/He and C57BL/6 donors survived permanently in untreated SCID mice. In SCID-isoFLC mice, however, the skin grafts from C3H/He and C57BL/6 donors were rejected between 6 and 12 weeks after the transfer of isogenic FLC. No significant difference in the survival times was

Table 3. Antibody-producing cells in SCID, BALB/c, and SCID-isoFLC mice. Values are presented as mean ± standard error of the mean (SEM) per 10^6 spleen cells. SCID, BALB/c, and SCID-isoFLC mice at 14 weeks after the transfer of FLC were immunized with OVA and IFA. Statistical significance is shown as $p<0.05$, and n. s. means not significant (Student t-test).

	Number of mice	PFC to OVA-SRBC/10^6		PFC to PA-SRBC/10^6
		direct	indirect	indirect
SCID	6	1.7±1.3	0±0	0±0
BALB/c	7	3.9±1.0	41.3±10.1	55.0±27.7
		$p<0.05$	$p<0.05$	n.s.
Reconstituted SCID	21	18.7±4.9	13.6±4.6	113.2±35.5

observed between allogenic skin grafts from C57BL/6 and C3H/He donors (Figure 3).

With regard to the antibody forming response after immunization with OVA, the counts of direct PFC for OVA in the reconstituted SCID mice were significantly higher than those of normal BALB/c mice, whereas the counts of indirect PFC were significantly lower when examined 7 days after immunization. On the other hand, the number of indirect PFC for PA in the reconstituted mice was larger than that in the BALB/c mice, although the difference was not statistically significant (Table 3).

Discussion

The developmental process of the immune system in the PB was almost comparable between SCID-isoFLC and SCID-alloFLC mice, except that the appearance of $CD4^+$ and $CD8^+$ cells was retarded 1 week in the latter as compared with the former. Analysis of the lymphoid organs and PB of the SCID-alloFLC mice also indicated that sIg^+ cells appeared in the BM, spleen, and PB at 2 weeks after the transfer. Thus, it seems that within 2 weeks after the FLC transfer, the HSCs or B cell progenitors from the fetal liver had colonized in the BM, where they differentiated into sIg^+ B cells, which were then distributed to the periphery.

With regard to the T cell ontogeny in mice, it is known that immature lymphocytes are first detected in the thymus on the 11th gestation day (GD). The lymphoid cells that first appear in the fetal thymus express no T cell receptor (TCR), CD3, CD4 or CD8 molecules on their cell surfaces, but are followed by the appearance of $CD4^+CD8^-$- and $CD4^-CD8^+$-TCR $\alpha\beta$ expressing thymocytes on the 18th and 19th GD, respectively.[13] In the adult mouse thymus, $CD4^+$, and $CD8^+$ DP, $CD4^+$ and $CD8^+$ SP cells constituted up to 80, 12 and 3%, respectively.[13] In the thymus of SCID-alloFLC mice, the $CD4^+$ and $CD8^+$ SP cells appeared in 4 weeks after the transfer and reached normal level in 6 weeks. Furthermore, the percentages of DP, $CD4^+$ and $CD8^+$ SP cells were almost the same as those observed in the normal adult mouse thymus when assessed after 6 weeks. These results suggest that HSCs or T cell progenitors of donor entered the thymus before 4 weeks after transfer of FLC, and were differentiated into mature T cells which then migrated to the periphery by 6 weeks. Thus, it appears that the development of DP, $CD4^+$, and $CD8^+$ SP cells in the reconstituted SCID mice was similar to that normally occurring in the feral life.

It was noted that as high as 24% of sIg^+ cells was recovered from the thymus around 4 weeks after FLC transfer (Figure 2). This rather unexpected finding is probably due to the fact that total number of thymocytes within the thymus at this time was still very low, resulting in an apparent increase in the proportion of sIg^+ cells. It has been reported earlier by others that a small number of B cells were present within the thymus of normal fetal, newborn, and adult mice.[14,15] These thymic B cells were reported to be involved in inducing tolerance to self-superantigens by presenting them to the developing T cells.[16]

Our results also showed that cellular immune response of SCID-isoFLC mice was apparently normal in that they rejected allogenic skin grafts 6 to 9 weeks after the transfer of FLC, i. e., during the time when the counts of $CD4^+$ and $CD8^+$ T cells in the PB approached the normal level. They also showed that humoral immune response of SCID-isoFLC mice was restored to a level where they fully responded OVA- or PA-conjugated SRBC antigens by generating PFC to these antigens. The direct PFC response of SCID-isoFLC mice was significantly higher than that of BALB/c mice, whereas the indirect PFC response of

the former was significantly lower than of the latter. The difference in the ability to generate direct and indirect PFCs may account for an immunological immaturity or slower immunological response of SCID-isoFLC mice.

In SCID-alloFLC mice, donor-type lymphocytes were detected within 2 weeks after the transfer, increased gradually in number and occupied as much as 77% of the total lymphocytes of the BM, 94% of the spleen, and 83% of the PB by 12 weeks after the transfer. When lymphocytes from SCID-alloFLC mice were analyzed by using H-2K^b antibody and antibodies to lymphocyte surface markers (CD3, CD4, CD8, Thy1.2, sIg and B220), all of T and B lymphocytes in the periphery of SCID-alloFLC mice were shown to express donor-type H-2 antigens. Thus, it was suggested that peripheral T and B cells generated de novo after the transfer of FLC were differentiated from donor-type HSCs that were present in the transferred FLC. Furthermore, it is most likely that mature lymphocytes present in the PB of SCID mice that were reconstituted with FLC from BALB/c donors also originated from the transferred FLC.

We may also note that the time course of the development of T and B cells in the FLC reconstituted SCID mice was comparable with that normally seen in the developing fetuses, although there seems to be a small delay possibly due to a lower number of stem cells available per unit body mass in this experimental model as compared with that present in normally developing fetuses.

Finally, the results presented in the paper show that the SCID-isoFLC and SCID-alloFLC mice could be a good model to analyze the interaction between self antigens and cells of the developing immune system, which is otherwise observable only in the fetal or perinatal stage of experimental animals.

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Abbreviations

FLC: fetal liver cells
SCID-isoFLC mice: SCID mice reconstituted by the transfer of isogenic FLC
SCID-alloFLC mice: SCID mice reconstituted by the transfer of allogenic FLC
PB: peripheral blood
SCID: severe combined-immunodeficiency
BM: bone marrow
LTBMC: long-term bone marrow cultures
HSC: hematopoietic stem cell
GD: gestation day
MEM: minimum essential medium
mAb: monoclonal antibody
FCM: flow cytometry
PE: phycoerythin
FITC: fluorescein isothiocyanate
Ig: immunoglobulin
ELISA: Enzyme-linked immunosorbent assay
SAPx: horseradish peroxidase conjugated streptavidin
LU: laboratory unit
OVA: ovalbumin
IFA: incomplete Freund's adjuvant
PEC: plaque-forming cell
PA: protein-A
SRBC: sheep red blood cells
TCR: T cell receptor
DP: double positive SP: single positive
SEM: standard error of the mean

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