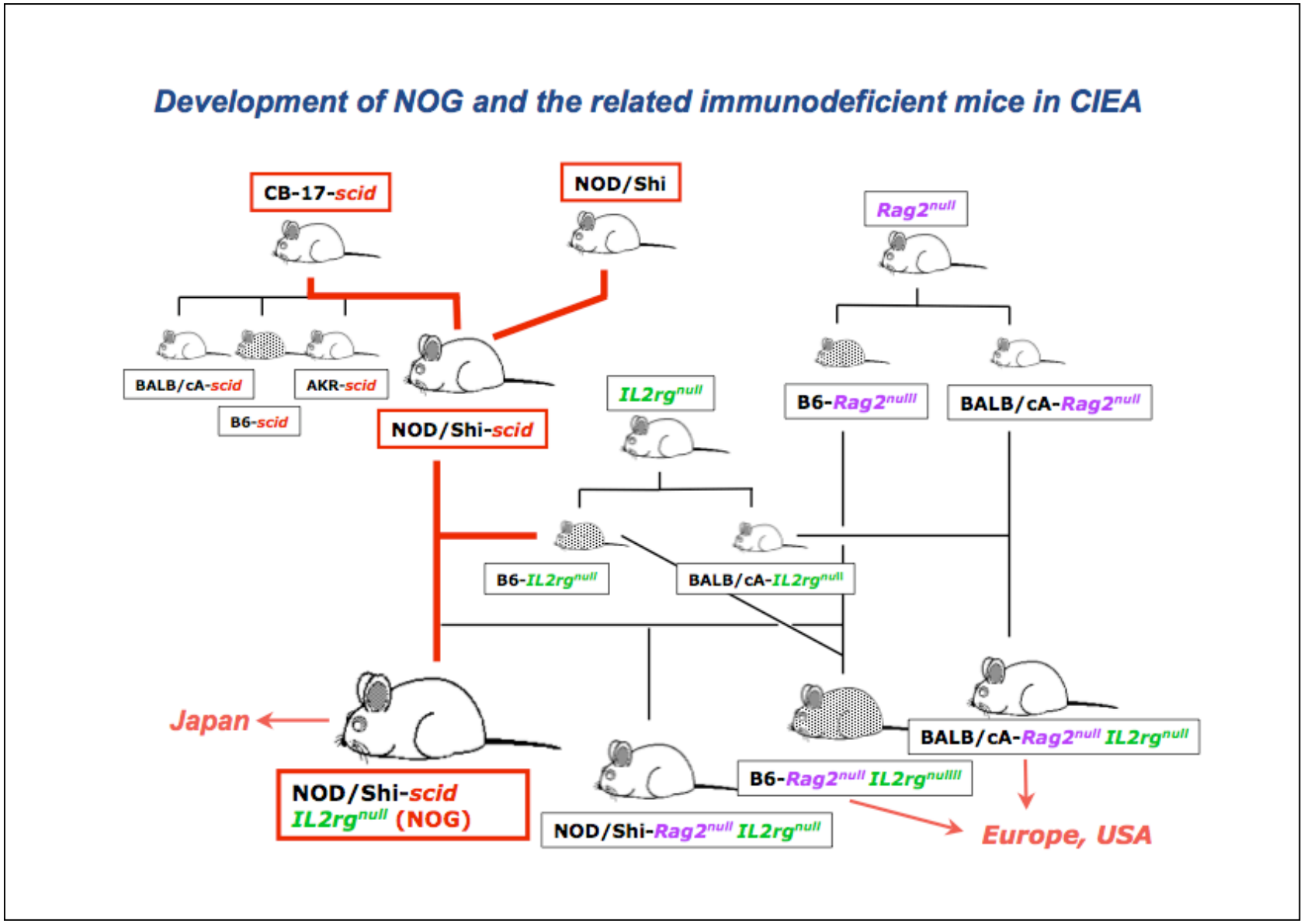
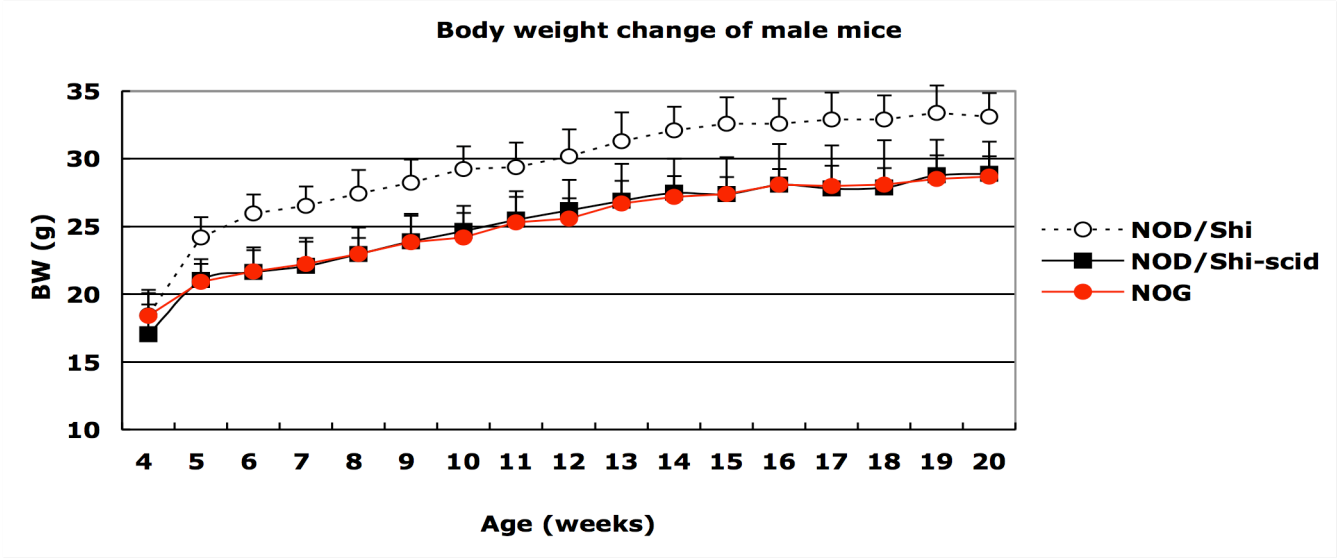
Development of NOG mice

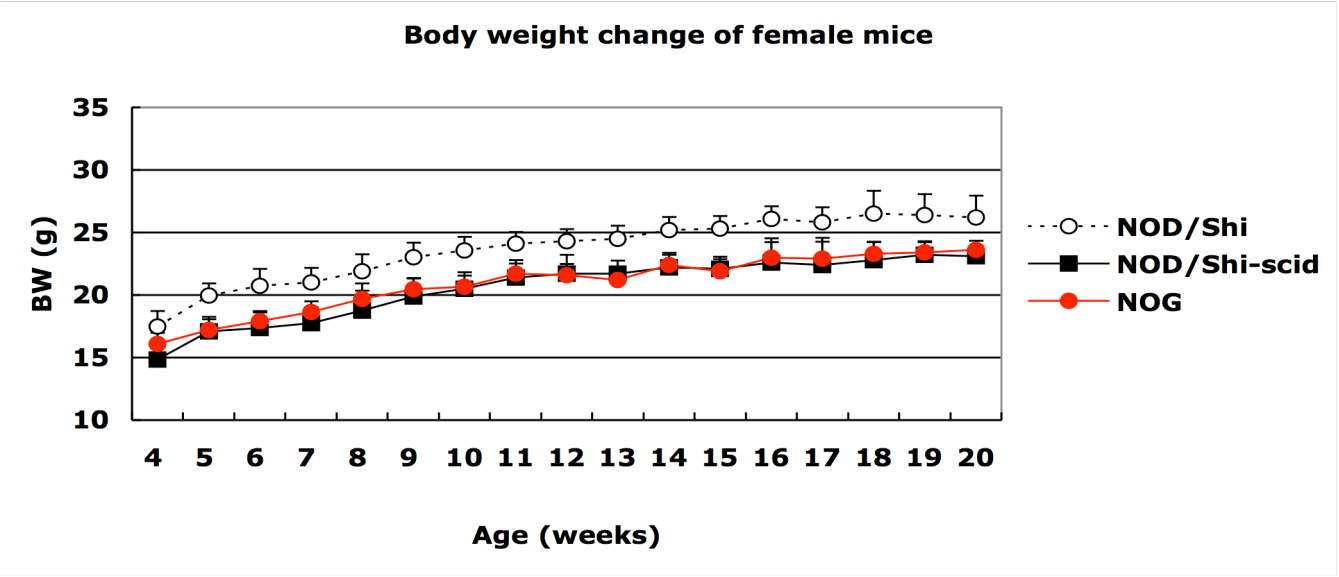


General characteristics of NOG mice

1. T and B cell deficient
2. NK cell deficient
3. Reduced macrophage and dendritic cell function
4. Complement activity deficient
5. No incidence of lymphoma
6. No T, B cell leakiness
7. Long life span
8. Sensitive for irradiation
9. Sensitive against microbiological pathogens
10. High engraftment for xeno-transplants

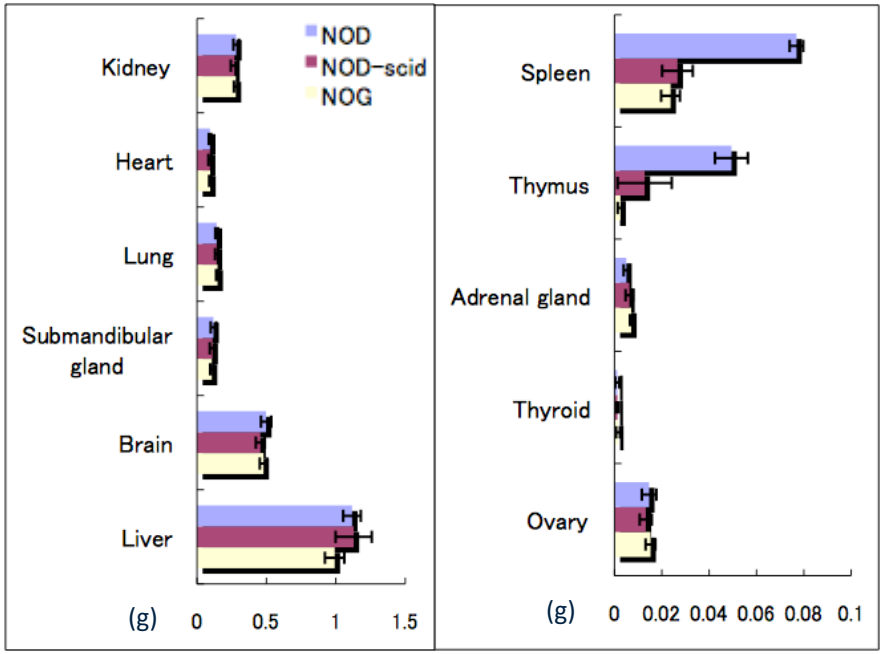
Basic characteristics ----- Body weight



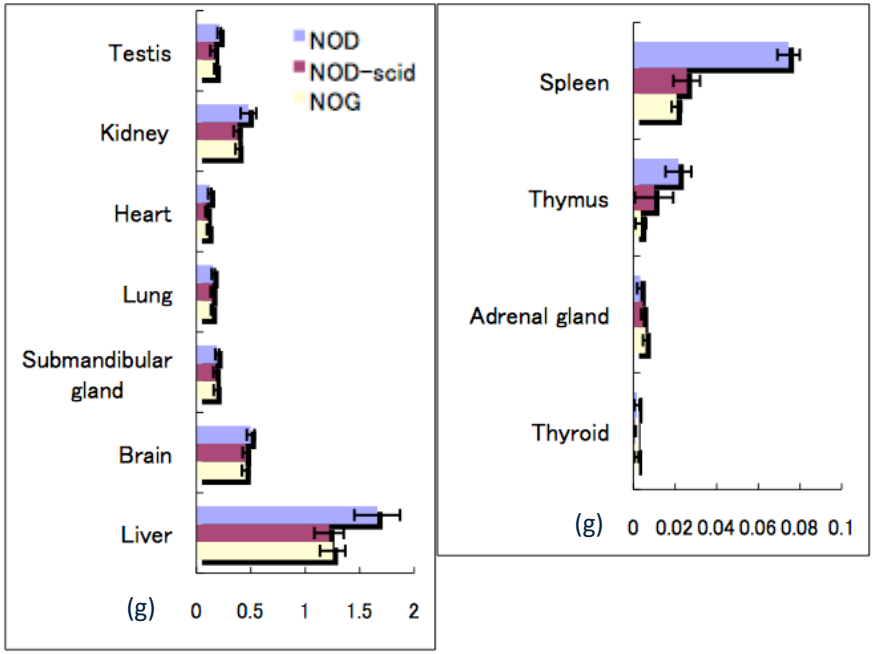


Basic characteristics ----- Organ weight

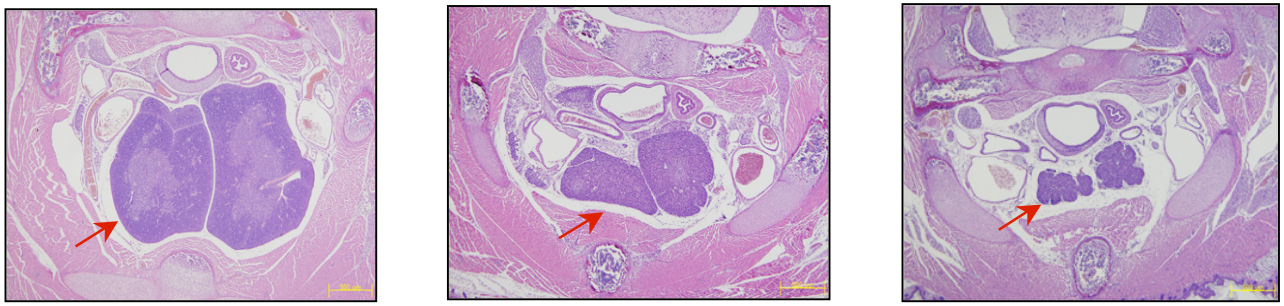
female



male



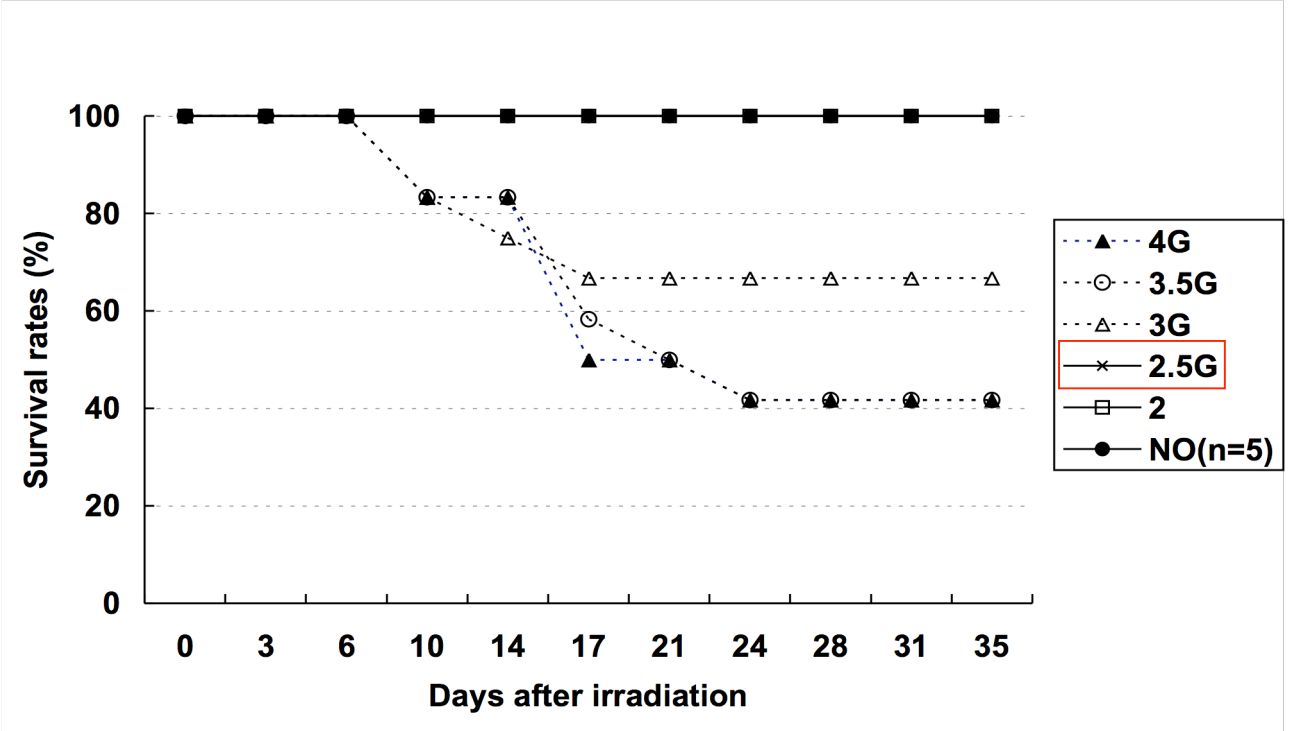
Histology of thymus from newborn mice



NOD *NOD/SCID* *NOG*

Basic characteristics ----- Irradiation sensitivity

Survival rates of NOG mice after irradiation



Five mice in each group were irradiated with 2 to 4 Gy with using an X-ray device (MBR-1505R, Hitachi Medical Co., Tokyo) at age of 8 weeks.

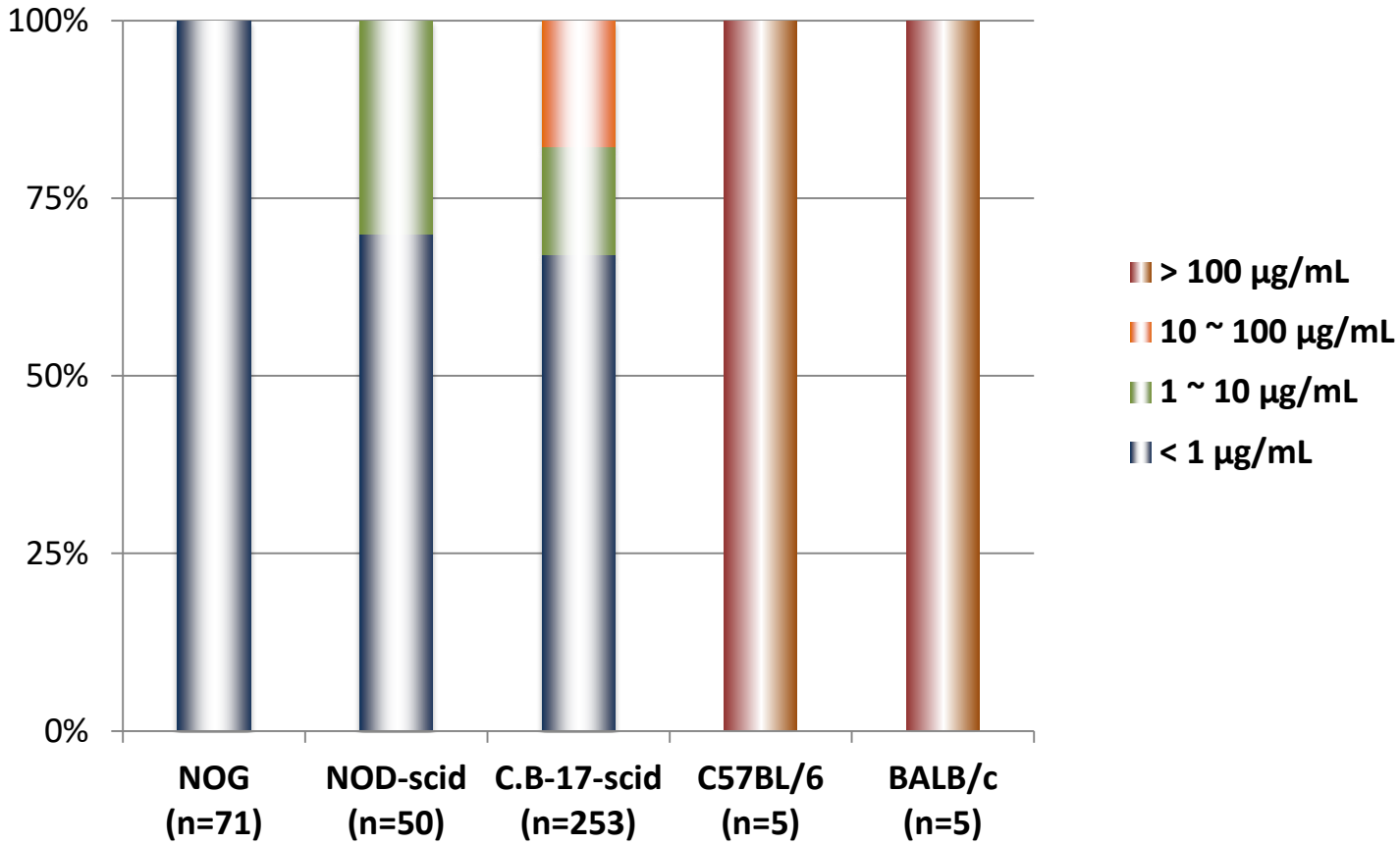
Lymphocytes Leakiness in Immunodeficient Mice

Mouse strain	Spleen test	No. of potisive mice/ No. of mice* (range, µg/ml)				
	CD19+	CD3+	CD4+	CD8+	TCRVb	TCRVgd
C.B-17-*scid*	6/10 (0.5 - 6.3)	1/10 (9.3)	1/10 (6.5)	0/10 -	1/10 (7.1)	1/10 (2.1)
NOD-*scid*	0/9 -	9/9 (2.0 - 13.8)	6/9 (1.0 - 12.5)	8/9 (0.6 - 7.8)	9/9 (0.9-12.4)	4/9 (0.5 - 1.1)
NOG	0/18 -	0/18 -	0/18 -	0/18 -	0/18 -	0/18 -
IQI	4/4 (34.7 - 59.9)	4/4 (21.5 - 34.9)	4/4 (17.9 - 25.5)	4/4 (1.5 - 3.5)	4/4 (20.0 - 33.2)	4/4 (0.7 - 1.3)

Mouse strain	Peripheral blood test	No. of potisive mice/ No. of mice* (range, µg/ml)				
	CD19+	CD3+	CD4+	CD8+	TCRVb	TCRVgd
C.B-17-*scid*	4/10 (0.5 -0. 8)	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -
NOD-*scid*	0/9	2/9 (2.4 - 3.2)	2/9 (1.53 - 2.26)	2/9 (0.8 - 2.1)	4/9 (0.5 - 4.4)	0/9
NOG	0/18 -	0/18 -	0/18 -	0/18 -	0/18 -	0/18 -
IQI	4/4 (28.1 -45.5)	4/4 (14.4 - 31.3)	4/4 (10.4 - 23.9)	4/4 (2.6 - 5.1)	4/4 (13.7 - 30.0)	3/4 (0.5 - 1.4)

* No. of positive mice means the number of mice with more than 0.5% of cells stained, since a value of less than 0.5 % was considered as nonspecific staining.

No B cell leakiness in NOG mice

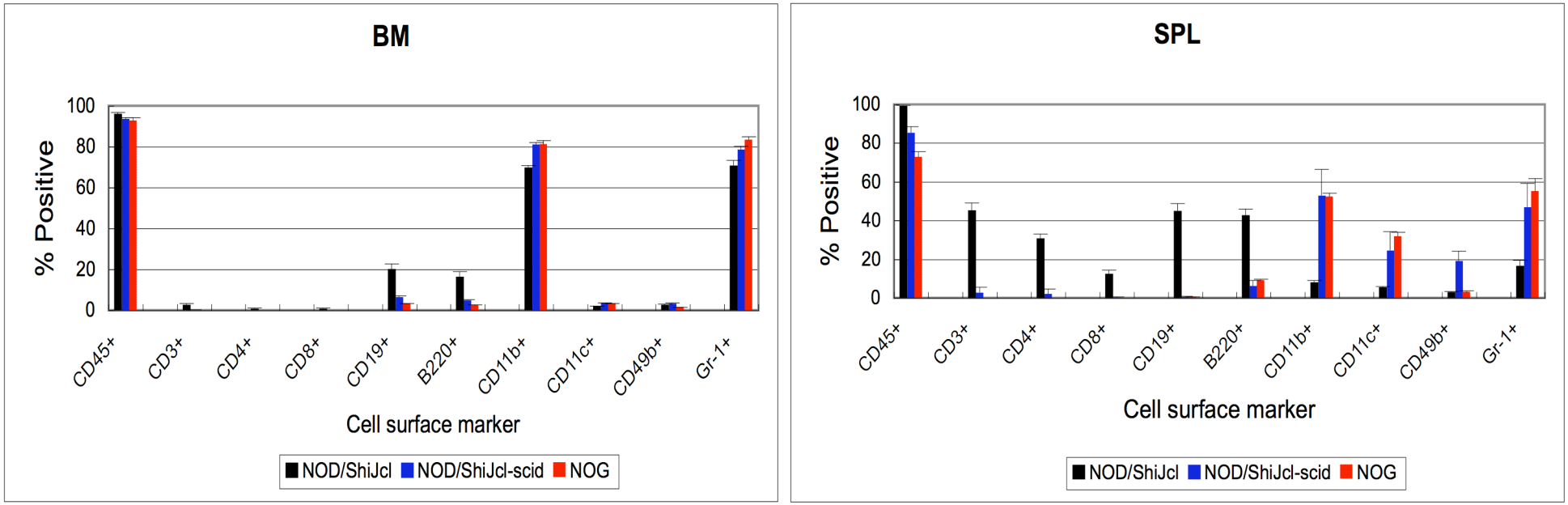


NOG: 7-10 months old
NOD-scid: 6-7 months old
C.B-17-scid: 6-9 months old*
C57BL/6 & BALB/c: 12 weeks old

***Ig M+G+A levels in sera of C.B-17- scid were measured in 1989.**

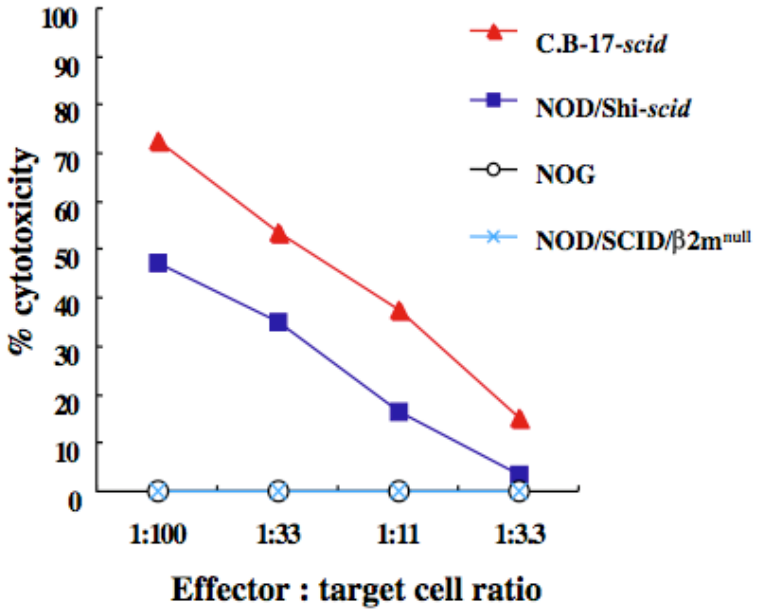
Basic characteristics ----- Hematology

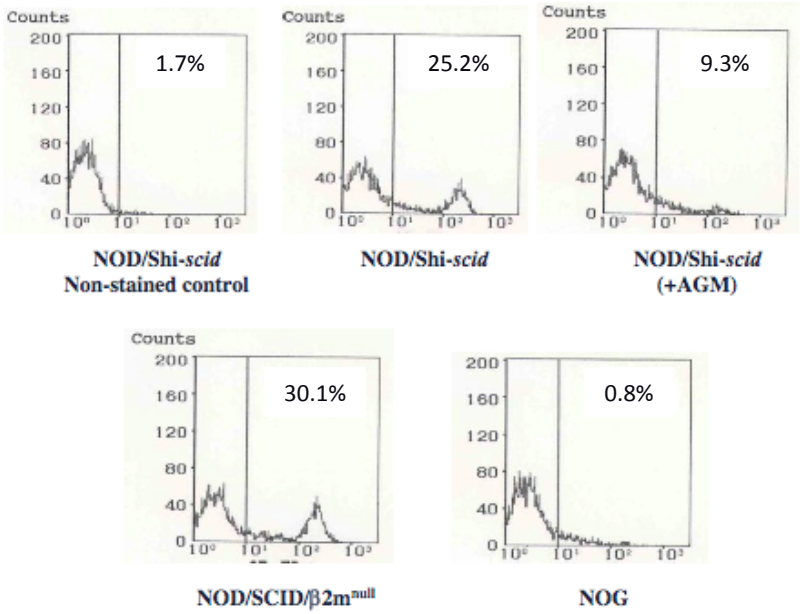
Murine cells in bone marrow and spleen



Bone marrow and spleen were obtained from 12 weeks-old NOG mice. Single cell suspensions prepared from them according to the ordinal manner were stained with FITC- or PE-labeled anti-mouse CD45+, CD3+, CD4+, CD8+, CD19+, B220+, CD11b+, CD11c+, Gr-1+ and analyzed with FACSCanto (BD sciences, CA).

Immunological characteristics ----- NK cell defect



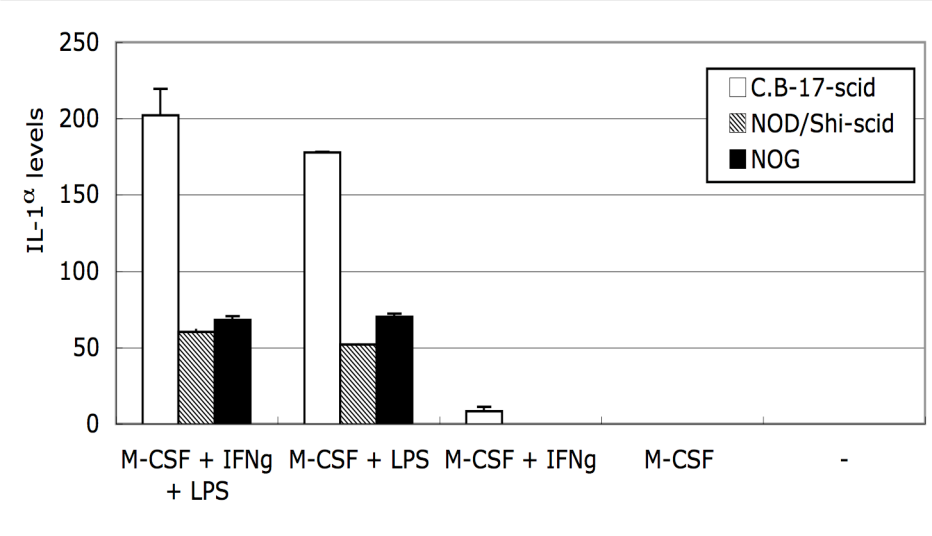


NK cell Activity of NOG mice

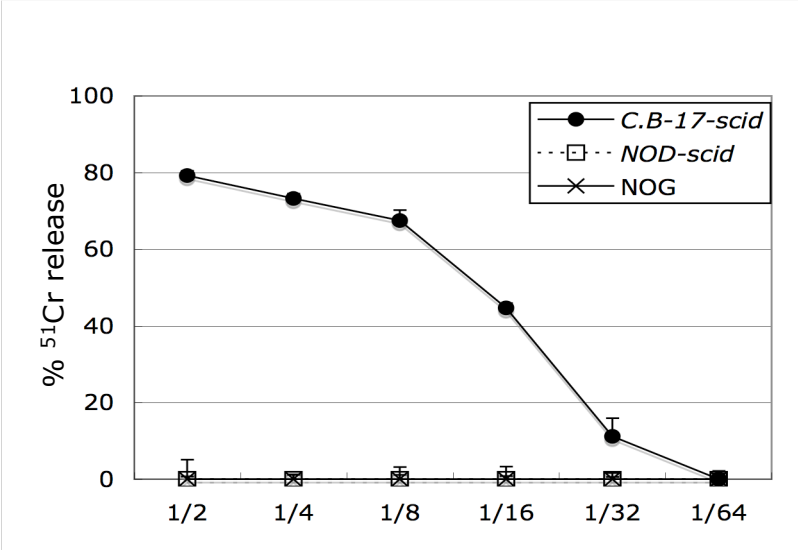
The NK cell activity was determined by a cytotoxicity test using NK sensitive YAC-1 cells as a target cell. Mice were intraperitoneally inoculated with 100 mg of polyinosinic-polycytidylic acid (poly I:C, SIGMA Chemical Co., St. Louis, MO) to stimulate NK cell activity for 48 hr before assay. Spleen cells were separated from 4 mice of each strain of mice, pooled and co-cultured with ^{51}Cr-labeld YAC-1 cells as target cells for 4 hr at 37 ^{o}C in 5% CO_2. The supernatants harvested were assayed on a gamma counter. The present specific ^{51}Cr release was calculated using the following formula, where X is the mean experimental release from triplicate wells. Total release (T) was determined from wells with ^{51}Cr labeled YAC-1 cells and 1H HCl, and spontaneous release (S) was determined from wells with ^{51}Cr –labeled YAC-1 cells and medium: % specific release = [(X-S)/(T-S)] x 100.

Immunological characteristics
----- Macrophage and Complement activity

Reduced IL-1 production from macrophages



Defect of hemolytic complement activity in serum



IL-1 production from bone marrow cells

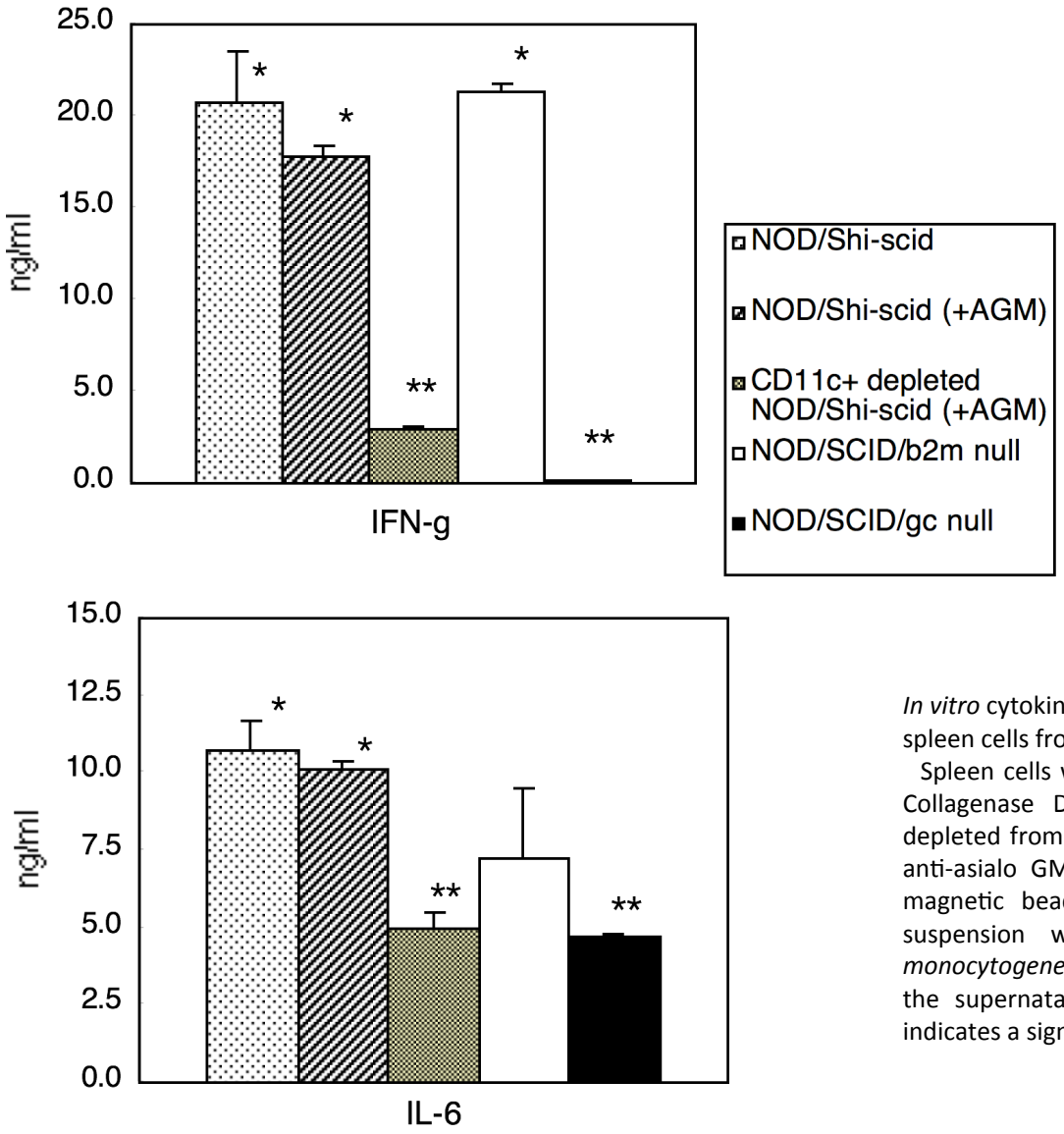
IL-1 production from bone marrow cells stimulated with IFN-g and LPS was determined. Bone marrow cells were cultured with 500 U/ml human rM-CSF, with and without 10 U/ml rat rIFN-g and cultured for 4 days at 37 ^{o}C in 5% CO_2. After 4 days, the medium was replaced with fresh medium alone or with medium containing *Escherichia coli* LPS at 10 mg/ml. After an additional 24 hr incubation period, the culture supernatants were harvested and assayed for IL-1a levels using ELISA kits. The amount of IL-1a in the supernatants was expressed as the absorbance at 405 nm.

Complement-dependent hemolytic activity

Complement-dependent hemolytic activity in sera were determined by measurement of ^{51}Cr released in the supernatants after 30 min incubation of mouse sera and ^{51}Cr labeled SRBC/anti SRBC antibody conjugates. Spontaneous release (S) was determined from wells with ^{51}Cr SRBC-Ab conjugate in media. Total release (T) was determined from wells with ^{51}Cr SRBC-Ab conjugates and 100 ml 2% SDS. Percent specific release = [(X-S)/(T-S)] x 100.

from Ito, M. *et al.* NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* 100, 3175-3182. (2002).

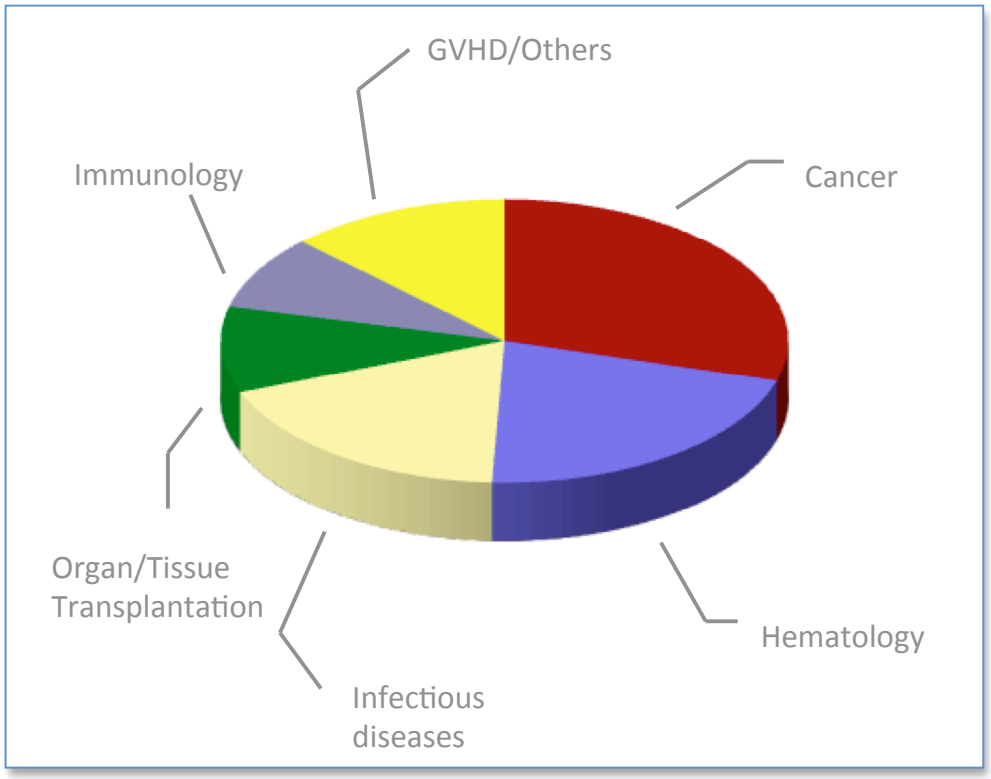
No production of IFNγ in NOG mice



In vitro cytokine production of *L. monocytogenes*-stimulated spleen cells from 3 strains of mice.

Spleen cells were separated after injection of 1 ml of 1 mg/ml Collagenase D solution into the spleen. $CD11c^+$ cells were depleted from spleen cells from NOD/Shi-*scid* mice treated with anti-asialo GM1 antiserum, using anti-CD11c antibody labeled magnetic beads, by magnetic cell sorter (MACS). The cell suspension were co-cultured with 10^7 of heat-killed *L. monocytogenes* for 8 hours at 37 ^oC. The IFN-g and IL-6 levels in the supernatants were determined using ELISA kits. Asterisk indicates a significant difference (* vs **: $P<0.01$).

Collaborative studies with Japanese Academic Society



Application

1. Infectious disease model
    - HIV-1 infection
    - ATL infection
    - EBV infection model
    - Hodgkin's disease model
2. Cancer model
    - Liver metastasis
    - Multiple myeloma
    - Acute myeloid leukemia
3. Human tissue or organ model
    - Model with human ovary
    - Model with human liver
    - Model with human endometrium
4. Other models
    - GVHD model
    - Efficacy test model for thrombopoietic drugs
    - Safety test for human cell (ES, iPS, gene-manipulated cells) transplantation