FULL PAPER *Parasitology*

Erythrocyte-Replaced Mouse Model for Haemoparasite Studies: Comparison of NOD/shi-*scid* and C.B-17/Jcl-*scid* Mouse upon Acceptability of Human Erythrocytes

Chiaki ISHIHARA[1)], Aya ZAMOTO[1)], Masayoshi TSUJI[1)], Qiang WEI[1)], Ichiro AZUMA[2)] and Kyoji HIOKI[3)]

[1)]School of Veterinary Medicine, Rakuno gakuen University, Bunkyo-dai, Ebetsu 069–8501, [2)]Hakodate National College of Technology, 14–1 Tokura-cho, Hakodate 042–8501 and [3)]Central Institute of Experimental Animals, 1430 Nogawa, Miyamae-ku, Kawasaki 216–0001, Japan

(Received 28 August 2002/Accepted 8 April 2003)

ABSTRACT. The erythrocyte-exchanged chimera mouse model has become to be a significant tool for studying animal and human (hu) protozoan haemoparasites, though the usefulness of this model varies depending primarily on the acceptability of xenogeneic red blood cells (RBC). To find a superior recipient in comparison with C.B-17/Jcl mouse with severe combined immuno-deficiency (*scid*) mutation, we examined in this report the non-obese diabetes (NOD)/shi-*scid* mouse, a recently available strain of SCID. When 2.5×10^8 of fluorescent dye-labeled hu-RBCs were transfused, C.B-17*scid* mouse eliminated them logarithmically by a simple linear regression, while NOD-*scid* mouse eradicated hu-RBCs by a unique two-step fashion, i.e., a potent but only briefly functioning RBC eradication followed by a weak steadily functioning step. The means of regression line constance ± their standard deviations (SD) of 205 C.B-17*scid* and of 213 NOD-*scid* mice for their short- and long-lasting steps were –0.73 ± 0.63, –0.53 ± 0.25 and –0.16 ± 0.10, respectively. Hu-RBC half-lives determined from these means of C.B-17*scid* mice and of NOD-*scid* mice for the short- and long-living steps were 3.6, 4.9 and 16.3 hr, respectively. Higher hu-RBC acceptability of NOD-*scid* mouse, especially at their long-lasting step, was also demonstrated under at an activated state of mouse innate immunity. Treatment with 1.0 mg heat-killed *Candida* cells caused an acceleration of hu-RBC elimination in both mouse strains but the magnitudes for the short- and long-living steps of NOD-*scid* mice evaluated by "stimulation index" were only 1/2.6 and 1/7.6 of C.B-17*scid* mice, respectively.

KEY WORDS: *Babesia*, NOD/shi-*scid*, protozoan hemoparasite, SCID mouse, *Theileria*.

J. Vet. Med. Sci. 65(8): 831–837, 2003

Most of protozoan erythro-parasites of man and domestic animals are known to rarely be cultivable *in vitro*, and not infect into any small laboratory animals because of their strict host specificity. Using the severe combined immunodeficiency mice with T and B lymphocyte-deficiency mutation (*Prkdc*^{scid} [4] hereafter called *scid*), a unique animal model of which circulating red blood cells (RBC) were replaced almost completely with mature xenogeneic RBCs has been developed recently [2, 5, 27, 28, 40–43]. Many of the parasites in bovine *Babesia*, bovine *Theileria* and canine *Babesia* groups have been shown to proliferate successfully in the mouse model, however, some, such as human malaria parasites, show poor growth probably because of rapid eradication of hu-RBCs from their blood circulation [2, 5, 27, 28, 40–43]. Indeed, the period maintained in circulation of hu-RBC has been described as only about 1/25-fold to the bovine's [19].

Ways to extend hu-RBC life time in SCID mouse's blood circulation have been investigated, including a priming method by which hu-RBCs are transfused frequently at beginning of the replacement to saturate the reticulo-endotherial system [5], a method to eliminate mouse RBCs selectively by introducing intravenously with an anti-mouse RBC rat monoclonal antibody 2E-11 [28], or to inhibit hu-RBC eradication system competitively by treating mice with possible sugar ligands, such as mannose [21] or sulfated carboxymethyl chitin [20]. A few previous report has also implicated the influences of genetic background of SCID mouse, as likely it has extremely been demonstrated in many reports for human hematopoietic cell and tumor cell engraftments [14, 16, 18, 24, 39], and NOD-*scid* mouse is suggested to be a better host for hu-RBC replacement [27, 30]. These hu-RBC transfusion studies, however, describe the results using only a limited number of SCID mouse and no direct comparison has there been performed between NOD-*scid* mouse and C.B-17*scid* or NIH-*bg nu Xid* mouse [5, 43]. Moreover, the extent of RBC maintenance which has not been estimated particularly is very variable presumably because of the difference in the recipient mice such as the leaky phenotype and as well as the difference in the levels of their innate immunity [19, 22].

To reduce the risk of failure in the RBC replacements we assayed SCID mice in advance for about two years by a simple hu-RBC clearance tests. The results indicate clearly the excellence in the extent of hu-RBC maintenance in NOD/shi-*scid* mice than in C.B-17/Jcl-*scid* mice not only at a normal but also at an accelerated level of mouse innate immunity. NOD/shi-*scid* mice are also characterized by their smaller individual difference in the extent of hu-RBC maintenance and as well as by a unique two-step hu-RBC eradication fashion.

MATERIALS AND METHODS

Mice: NOD/shi-*scid* mice which were developed in Central Institute of Experimental Animals, Kawasaki, were maintained in the laboratory animal facility in Rakuno gakuen University. Fox CHASE C.B-17/Jcl-*scid*/Jcl mice,

5 to 8 weeks of age, less than 10 μg per m*l* of serum Ig, and free from infections with *Pseudomonas aeruginosa, Escherichia coli* O115, *Salmonella, Pasteurella, Bordetella bronchiseptica, Corynebacterium kutscheri, Clostridium piliforme, Mycoplasma pulmonis*, mouse hepatitis virus and Sendai virus were purchased from CLEA (Tokyo). They were housed in a vinyl film isolator at around 23°C, and were provided autoclaved tapped water and γ-ray-irradiated pelleted diet (Funabashi Co., Funabashi) *ad libitum*. All animals were treated according to Laboratory Animal Control Guideline in Rakuno gakuen University which is basically in conformity with the American Association of Laboratory Animal Control Guidelines from the National Institute of Health, U.S.A.

Activation of mouse innate immunity: *Candida guilliermondii* strain SW5 used in this experiment has been described as an agent that cause a significant acceleration of transfused hu-RBC eradication from blood circulation in asymptomatic C.B-17*scid* mice [22]. Heat-killed *Propionibacterium acnes* C7 cells are described elsewhere as a potent non-specific stimulant to tumorcidal and bactericidal activities of mice and guinea pigs [3]. *C. guilliermondii* SW5 and *P. acnes* C7 were grown in tryptose phosphate broth (Difco, Detroit, Mich. U.S.A.), killed at 121°C for 20 min, washed 3 times with phosphate buffered saline (PBS) and stored at –20°C before use. They were thawed and suspended in PBS at 15 mg/m*l*, and sonicated at 20 kHz, 50 watts for 1 min (VP-5s sonicator, Taitec Co., Tokyo); Protein assay was according to the manufacture's instructions with BCA reagent (Pierce, Lockford, I.L. U.S.A.). We also used thioglycolate medium (Brewer TGC; Difco, Detroit, Mich. U.S.A.), which can activate non-specific host resistance by accelerating mainly phagocytosis but not *Salmonella*-cidal [7], *Listeria*-cidal [15] nor tumorcidal activities [8]. Responsiveness of SCID mouse to these reagents was evaluated by a single or a paired hu-RBC tracking tests as mentioned below. Splenomegaly was measured and the spleen index was calculated as "spleen index"={spleen weight/body weight} × 100.

Red blood cells and their tracking in the blood circulation of mice: Human (hu) O type red blood cells (RBC) taken from normal adult volunteers were washed 3 times with anti-coagulant acid citrate dextrose (ACD) and labelled by the method described previously with PKH-26-GL cell linker dye at an optimal concentration of 1×10^{-8} M (Sigma, St. Louis, MO. U.S.A.) per 4×10^{7} RBCs [19]. To evaluate how long SCID mouse maintain hu-RBCs in their blood circulation, 2.5×10^{8} PKH labelled cells in 0.1 m*l* ACD were injected intravenously, and a 10 μ*l* volume of blood was collected periodically from 5 min to 5 hr (NOD-*scid* mice) or 6 hr (C.B-17*scid* mice). Hu-RBCs in the blood were counted under a fluorescent microscope (BH-2, Olympus, Tokyo) and a regression line and it's constance was determined by the method of least squares. Acceleration of hu-RBC eradication under at an activated state of mouse innate immunity was evaluated by an "stimulation index", which was calculated individually using formula "stimulation index"= a - a',

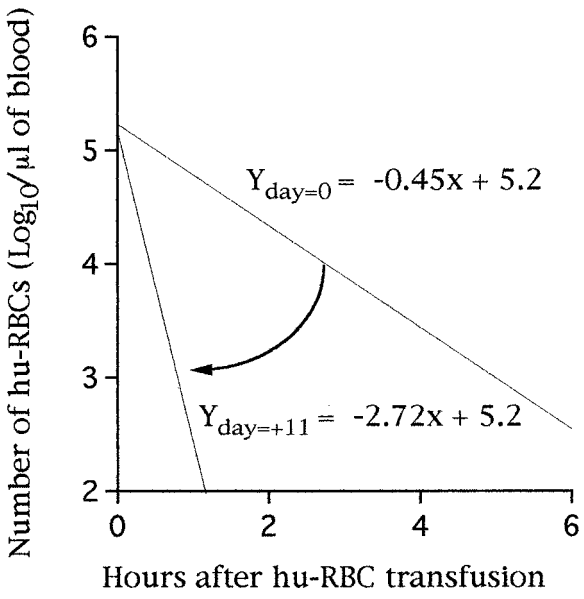

Fig. 1. Determination of stimulation index. For each SCID mouse, a paired hu-RBC tracking test was performed 5 hr before and 11 days after the treatment with heat-killed *Candida* cells, heat-killed *Propionibacterium* cells or TGC. The regression line was determined for each test and the stimulation index was calculated using formula "stimulation index" = a-a', where "a" and "a" were the constances of corresponding regression lines. In case of this figure; stimulation index = (–0.45) – (–2.72) = 2.27.

where "a" and "a" were the constances of the regression lines determined from the tests before and after the treatment with heat-killed *C. guilliermondii* cells, with *P. acnes* cells or with TGC (Fig. 1).

Statistics: Comparison was done between the two means of the clearance or stimulation indexes and statistical significance was calculated by one-tailed Student's *t* test. Goodness of fit testing of normality in the population distributions was done by chi-square method.

RESULTS

Acceptability of human erythrocytes into NOD/shi-scid *and C.B-17/Jcl* scid *mouse*: RBC tracking test was performed to determine how NOD-*scid* and C.B-17*scid* mice maintained hu-RBCs in their blood circulation. Figure 2 depicts typical results suggesting a simple linear regression for the hu-RBC eradication in C.B-17*scid* mice but in NOD-*scid* mice it seems to be composed at least of two elements, a potent short-living hu-RBC eradication followed by a weak long-lasting hu-RBC eradication step. This was assured by assessing 79 C.B-17*scid* and 213 NOD-*scid* mice as if their frequency distributions of "constance-difference", each of which was calculated individually as a difference between the two regression line constances either for the data given within 1 hr or after the RBC transfusion, was approximated to normal with mean "zero". Only the mean

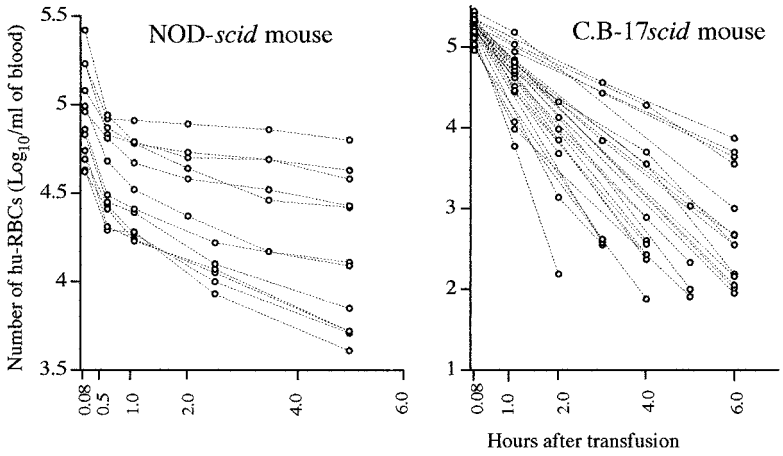

Fig. 2. Currency of transfused hu-RBCs in the blood circulation of NOD/shi-*scid* and C.B-17/Jcl *scid* mice. Number of hu-RBCs remained in circulation were counted under a microscope by the indicated time intervals after intravenous injection with 2.5 $\times 10^8$ PKH-26 dye-labelled hu-RBCs. Each line represents the results of each mouse.

Table 1. Effect of timing of the treatment of heat-killed *Candida* cells on the elimination of transfused hu-RBC in C.B-17*scid* mice[a)]

Days after treatment	Stimulation index		Comparison of the two groups
	Candida-treated	PBS control	
5	3.55 ± 3.36	–0.05 ± 0.24	P<0.05[b)]
11	2.76 ± 1.62	–0.01 ± 0.17	P<0.001
15	1.25 ± 0.63	–0.03 ± 0.16	P<0.001

a) Mean of "stimulation index" and its standard deviation (SD) was determined after testing 8 mice in each experimental group.
b) Comparison was made between the two means of "stimulation index". P values were calculated by Student's *t* test.

of C.B-17*scid* mice (0.07 ± 0.19) but not of NOD-*scid* mice (0.37 ± 0.21) was approximated to "zero" yet their distributions were both determined as normal at levels of $p<0.05$ (C.B-17*scid* mice) and $p<0.01$ (NOD-*scid* mice). The mean regression constance and it's SD for 205 C.B-17*scid* mice was –0.73 ± 0.63 and the hu-RBC half life calculated by using this mean was 3.6 hr. While the means and their SDs for the short-living and the long-lasting hu-RBC elimination steps of 213 NOD-*scid* mice were –0.53 ± 0.25 and –0.16 ± 0.10 respectively, and the hu-RBC half-lives calculated by these means were 4.9 and 16.3 hr, respectively.

Acceptability of human erythrocytes to NOD-scid and C.B-17 scid mice under at an activated state of mouse innate immunity: Attempts to replace RBCs and to infect subsequently with haemoparasites have there been achieved at various levels of mouse innate immunity, from negligible to extremely enhanced. We, therefore, examined next the extent of hu-RBC maintenance in NOD-*scid* mouse at an elevated level of mouse innate immunity. Treatment of C.B-17*scid* mice with heat-killed *Candida* cells at either timing caused a significant shortening of hu-RBC life as judged by "stimulation index" (Table 1). Thus, we next compared the two SCID strains by this index at 11 days post the treatment. The levels of hu-RBC eradication acceleration in NOD-*scid* mice in response to 1 mg *Candida* cells were as low as only 1/2.6 and 1/7.6 by their short-living and long-lasting steps respectively as that determined in C.B-17*scid* mice (Fig. 3, Table 2). Heat-killed *Propionibacterium* cells and TGC, however, did not induced significant effect in any group of mice except for the long lasting hu-RBC elimination step of the NOD-*scid* mice, which had been treated with 1.0 mg of heat killed *Propionibacterium* cells (Fig. 3, Table 2). This inferior responsiveness of NOD-*scid* mice was also demonstrated by splenomegaly measurement in the groups given with 1.0 mg *Candida* cells. *Propionibacterium* cells, which induced only a weaker splenomegaly even in C.B-17*scid* mice and as well as in NOD-*scid* mice, did not provoked a significant difference between the two strains of SCID mice (Table 3).

DISCUSSION

In the present study we performed a simple RBC tracking test and characterized NOD/shi-*scid* mice by a unique two-step hu-RBC eradication fashion. They cleared transfused hu-RBCs by two steps, a potent short-functioning step fol-

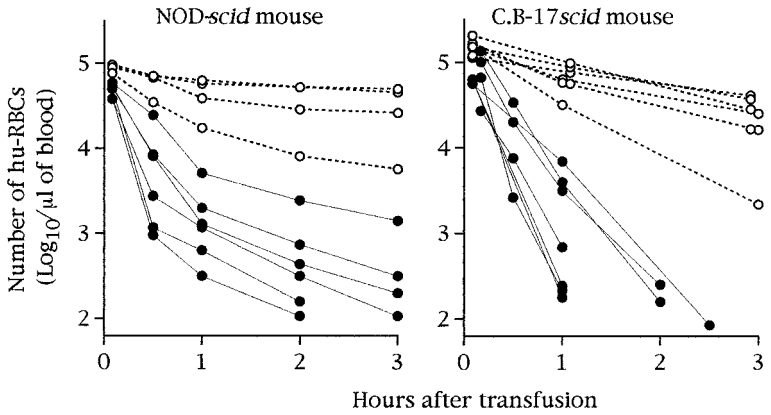

Fig. 3. Acceleration of hu-RBC clearance at an elevated level of mouse innate immunity. Currency of transfused hu-RBCs in circulation was examined twice in each mouse 5 hr before (open circle) and 11 days after (closed circle) the treatment with 1.0 mg of heat-killed *Candida* cells.

Table 2. Reduced acceleration of hu-RBC eradication of NOD-*scid* mice at an elevated level of mouse innate immunity in comparison with that of C.B-17*scid* mice by "stimulation index"[a)]

Treatments		C.B-17*scid* mice		NOD-*scid* mice			Comparison of the two strains of mice[b)]	
	dose	0–5 hr	n[c)]	< 1 hr	1 hr <	n[c)]	< 1 hr	1 hr<
Candida	1.0 mg	2.12 ± 1.45***[d,e)]	16	0.83 ± 0.68***	0.28 ± 0.11***	12	P<0.001	P<0.01
	0.5 mg	0.53 ± 0.26***	12					
Propionibacterium	1.0 mg	0.49 ± 0.83[NS]	12	0.12 ± 0.24[NS]	0.11 ± 0.14*	7	NS	NS
Thioglycolate	2 m*l*	0.32 ± 0.46[NS]	32	0.17 ± 0.16[NS]	0.06 ± 0.11[NS]	13	NS	NS
PBS	2 m*l*	0.06 ± 0.30	18	0.02 ± 0.15	0.003 ± 0.09	14		

a) Hu-RBC eradication test was done twice for each mouse 5 hr before and 11 days after the treatment with heat-killed *Candida* cells, *Propionibacteriuum* cells, TGC or PBS. A regression line for each C.B-17*scid* mouse and the two of each NOD-*scid* mouse, one for the short functioning (<1 hr) and another for the long-lasting (1 hr <) step, were determined. The "stimulation index" was calculated as described in the text.
b) Between the strains mice, the two "stimulation indexes" of the groups with identical treatment were compared by Student's *t* test. NS; not significant.
c) Number of mice examined.
d) Mean ± standard deviation of "stimulation index".
e) Within a strain of mice, comparisons were made between the two means of "stimulation index", one for the treated and another for the PBS control group, by F-test. ***; p<0.01, *; p<0.05.

Table 3. Splenomegaly of NOD-*scid* and C.B-17*scid* mice[a)]

Treatments	dose	Spleen index[b)] C.B-17*scid* mice	n[c)]	NOD-s*cid* mice	n[c)]	Comparison of the two strains of mice[d)]
Candida	1.0 mg	0.53 ± 0.32***[e)]	23	0.33 ± 0.05***	14	P<0.05
	0.5 mg	0.31 ± 0.10**	6			
Propionibacterium	1.0 mg	0.30 ± 0.08 **	7	0.24 ± 0.10*	10	NS
PBS	2 m*l*	0.22 ± 0.03	18	0.19 ± 0.03	22	

a) Spleen and body weights was measured 14 days after intraperitoneal treatment with the heat-killed *Candida* cells, *Propionibacterium* cells and PBS. Spleen index was calculated individually as described in the text.
b) Mean ± standard deviation of spleen index.
c) Number of mice examined.
d) Between the strains mice, the two the groups with identical treatment were compared by Student's *t* test. NS; not significant.
e) Within a strain of mice, comparisons were made between the group with the treatment and the PBS control group, by F-test. ***; p<0.001, **; p<0.01, *; p<0.05, NS; not significant.

lowed by a weak steady-functioning step, while in C.B-17*scid* mice hu-RBC elimination proceeded logarithmically by a simple linear regression. The mean regression constances ± SDs determined for C.B-17*scid* mice (–0.73 ± 0.63) and for the short- (–0.53 ± 0.25) and the long-living (–0.16 ± 0.10) steps of NOD-*scid* mice revealed that NOD-*scid* mice kept hu-RBCs in circulation much longer periods than C.B-17*scid* mice with smaller individual differences. This hu-RBC acceptability of NOD-*scid* mice is likely to be much significant at such a peculiar condition of RBC replacement as to transfuse with a big amount of 2×10^9 or more hu-RBCs, since hu-RBC eradication mechanism of NOD-*scid* mouse, especially the short-living mechanism, might be kept in saturation and in paralysis as long as the hu-RBC level was maintained by repeated transfusions [5]. Furthermore, a superior hu-RBC acceptability of NOD-*scid* strain was examined even under at an activated state of mouse innate immunity. Surprisingly, the accelerated levels of hu-RBC eradication in NOD-*scid* mice as judged by "stimulation index" was only 1/2.6 for their short-living step and 1/7.6 for the long-lasting step to that of C.B-17/Jcl*scid* mice, which were given 11 days before with heat-killed *Candida* cells (Table 2).

Natural killer cells, macrophages and polymorphonuclear cells in C.B-17*scid* mouse are reported to exhibit a normal or even enhanced activity [1, 34], while NOD-*scid* mice demonstrate reduced NK cell activity, have functional macrophage abnormalities, and lack hemolytic complement fixation capability [6, 23, 32, 33, 37]. The defects of NK cell activity, in particular, are described in many reports to have a major impact on graft survival for the transplantations of human hematopoietic stem cells upon the data from targeted depletion of NK cell activity with specific antibodies such as anti-asialo-GM1, anti-N.K.-1.1. and TM- s1 and on the data from flow cytometric analysis [9, 10, 35, 36, 39, 40]. Elimination of xenogeneic RBCs from blood circulation, however, is primarily described to be attributable to the macrophage functions, particularly through their recognitions by lectin like receptors [17, 19–21]. Several T cell-independent macrophage abnormalities have been identified in NOD mouse, including impaired bone marrow precursor proliferation and differentiation in response to CSF-1 [33], defect in LPS-stimulated IL-1 secretion [26, 37], aberrant down regulation of MHC class I expression in IFN-γ treated macrophage [33], and deficiencies of FcγRI [11] and FcγRII expression [25]. Although the detailed mechanism is not clear at present, a part of these macrophage defects alone or in combination with NK cell defect may contribute to the superior hu-RBC maintenance in NOD/shi-*scid* mouse and should concern to the unique two-step hu-RBC eradication fashion in this strain of mouse. Therefore, at least the defects of FcγRI and FcγRIIb molecules alone are unlikely to account for the enhanced level of hu-RBC engraftment in the NOD-*scid* mice.

In summary, we studied variables that assure to establish an hu-RBC substitute mouse model and demonstrated for the first time that NOD/shi-*scid* mice eradicated the transfused hu-RBCs by an unique two-step fashion with the smaller individual differences of the mean regression constance. This and a finding that NOD/shi-*scid* mice eradicated transfused hu-RBCs more slowly than C.B-17*scid* mice did at an elevated and as well at a normal level of mouse innate immunity meet requirements of an hu-RBC substituted mouse model. Thus, the use of NOD/shi-*scid* mice for hu RBC replacement may facilitate to propagate human haemoparasites, enable to isolate and even to give a clone of those parasites, and advance studies into the pathogenesis of human babesiosis. Indeed, in our separate study *B. microti* is successfully isolated and cloned from a human patient who was found 1999 in Japan by using hu-RBC-substituted NOD/shi-*scid* mouse model [31, 44].

ACKNOWLEDGEMENTS. This work was supported in part by Grant-in-Aid from Japanese Ministry of Education, Science and Culture of Japan and from Gakujutsu-Frontier cooperative research in Rakuno gakuen University. The authors are grateful to Mr. Hajime Miyake, Miss Yoko Saito, Miss Akiko Uebayashi, Miss Chiaki Daita, Miss Nami Oshitari, and Miss Keiko Sano for technical assistance.

REFERENCES

1. Ansell, J. D. and Bancroft, G. J. 1989. The biology of the SCID mutation. *Immunol. Today* **10**: 322–325.
2. Arai, S., Tsuji, M., Kim, S-J., Nakade, T., Kanno, Y. and Ishihara, C. 1998. *Babesia canis* infection in canine red blood cell-substituted SCID mice. *Int. J. Parasitol.* **28**: 1429–1435.
3. Azuma, I., Yamawaki, M., Yoshimoto, T., Saiki, I., Uemiya, M., Tanio, Y., Tokuzen R., Yasumoto, K. and Yamamura, Y. 1979. Antitumor activity of cell-wall skeleton of *Propionibacterium acnes* C7 in mice and guinea pigs. *Gann* **70**: 737–748.
4. Araki, R., Fujimori, A., Hamatani, K., Mita, K., Saito, T., Mori, M., Fukumura, R., Morimyo, M., Muto, M., Itoh, M., Tatsumi, K., and Abe, M. 1997. Nonsense mutation at Tyr-4046 in the DNA-dependent protein kinase catalytic subunit of severe combined immune deficiency mice. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 2438–2443.
5. Badell, E., Pasquetto, V., Van Rooijen, N. and Druihe, P. 1995. A mouse model for human malaria erythrocytic stages. *Parasitol. Today* **11**: 235–237.
6. Baxter, A. G. and Cooke, A. 1993. Complement lytic activity has no role in the pathogenesis of autoimmune diabetes in NOD mice. *Diabetes* **42**: 1574–1578.
7. Briles, D. E., Lehmeyer, J. and Forman, C. 1981. Phagocytosis and killing of *Salmonella typhimurium* by peritoneal exudate cells. *Infect. Immunity* **33**: 380–388.
8. Burger, C. J. and Elgert, K. D. 1983. Level of macrophage induction during tumor growth: primed or activated? *Immunol. Commun.* **12**: 285–290.
9. Christianson, S. W., Greiner, D. L., Schweitzer, J. B., Gott, B., Beamer, G. L., Shweitzer, P. A., Hesselton, R. M. and Shultz, L. D. 1996. Role of natural killer cells on engraftment of human lymphoid cells and on metastasis of human T-lymphoblastoid leukemia cells in C57BL/6J-*scid* mice and in C57BL/6J-*scid bg* mice. *Cell. Immunol.* **171**: 186–199.
10. Flavell, D. J., Warnes, S. L., Noss, A. L. and Flavell, S.U.

2000. Anti-CD7 antibody and immunotoxin treatment of human CD7(+) T-cell leukaemia is significantly less effective in NOD/LtSz-*scid* mice than in C.B-17*scid* mice. *Br. J. Cancer* **83**: 1755–1761.

11. Gavin, A. L., Hamilton, J. A. and Hogarth, P. M. 1996. Extracellular mutations of non-obese diabetic mouse FcγRI modify surface expression and ligand binding. *J. Biol. Chem.* **271**: 17091–17099.
12. Gavin, A. L., Tan, P. S. and Hogarth, P. M. 1998. Gain-of-function mutations in FcγRI of NOD mice: implications for the evolution of the Ig superfamily. *EMBO J.* **17**: 3850–3857.
13. Greiner, D. L., Shultz, L. D., Yates, J., Appel, M. C., Perdrizet, G., Hesselton, R. M., Schweitzer, I., Beamer, W. G., Shultz, K. L., Pelsue, S. C., Leif, J.H. and Rajian, T. V. 1995. Improved engraftment of human spleen cells in NOD/LtSz-*scid/scid* mice as compared with C.B-17*scid* mice. *Am. J. Pathol.* **146**: 888–902.
14. Greiner, D. L., Hesselton, R. A. and Shultz, L. D. 1998. SCID mouse models of human stem cell engraftment. *Stem Cells* **16**: 166–177.
15. Harrington-Fowler, L., Henson, P. M. and Wilder, M. S. 1981. Fate of *Listeria monocytogenes* in resident and activated macrophages. *Infect. Immun.* **33**: 11–16.
16. Hesselton, R.M., Greiner, D. L., Mordes, G. P., Rajian, T. V., Sullivan, J. L. and Shultz, L. D. 1995. High levels of human peripheral blood mononuclear cell engraftment and enhanced susceptibility to human immunodeficiency virus type 1 infection in NOD/LtSz-*scid/scid* mice. *J. Infect. Dis.* **172**: 974–982.
17. Horn, S., Gopas, J. and Bashan, N. 1990. A lectin-like receptor on murine macrophage is involved in the recognition and phagocytosis of human red cells oxidized by phenylhydrazine. *Biochem. Pharmacol.* **39**: 775–780.
18. Hudson, W. A., Li, Q., Le, C. and Kersey, J.H. 1998. Xenotransplantation of human lymphoid malignancies is optimized in mice with multiple immunologic defects. *Leukemia* **12**: 2029–2033.
19. Ishihara, C., Tsuji, M., Hagiwara, K., Hioki, K., Arikawa, J. and Azuma, I. 1994. Transfusion with xenogeneic erythrocytes into SCID mice and their clearance from the circulation. *J. Vet. Med. Sci.* **56**: 1149–1154.
20. Ishihara, C., Shimakawa, S., Tsuji, M., Arikawa, J. and Tokura, S. 1995. A sulfated chitin, SCM-chitin III, inhibits the clearance of human erythrocytes from the blood circulation in the erythrocyte-transfused SCID mice. *Immunopharmacology* **29**: 65–71.
21. Ishihara, C., Hiratai, R., Tsuji, M., Yagi, K., Nose, M. and Azuma, I. 1998a. Mannan decelerate the clearance of human red blood cells in SCID mouse. *Immunopharmacology* **38**: 223–228.
22. Ishihara, C., Miyamoto, A., Kim, S-J., Arai, S., Taniyama, H., Maejima, K. and Tsuji, M. 1998b. *Candida guilliermondii* infection in SCID mice in association with the acceleration of the elimination of transfused human red blood cells. *Exp. Anim.* **47**: 69–73.
23. Kataoka, S., Satoh, J., Fujiya, H., Toyota, T., Suzuki, R., Itoh, K. and Kumagai, K. 1983. Immunologic aspects of the non-obese diabetic (NOD) mouse. Abnormalities of cellular immunity. *Diabetes* **32**: 247–253.
24. Lapidot, T., Fajerman, Y. and Kollet, O. 1997. Immune-deficient SCID and NOD/SCID mice models as functional assays for studying normal and malignant human hematopoiesis. *J. Mol. Med.* **75**: 664–673.
25. Luan, J. J., Monteiro, R. C., Sautes, C., Fluteau, G., Eloy, L., Fridman, W. H., Bach, J. F. and Garchon, H. J. 1996. Defective FcγRII gene expression in macrophages of NOD mice: genetic linkage with up-regulation of IgG1 and IgG2b in serum. *J. Immunol.* **157**: 4707–4716.
26. Markees, T. G., Serreze, D. V., Phillips, N. E., Sorli, C. H., Gordon, E. J., Shultz, L. D., Noelle, R. J., Woda, B. A., Greiner, D. L., Mordes, J. P. and Rossini, A. A. 1999. NOD mice have a generalized defect in their response to transplantation tolerance induction. *Diabetes* **48**: 967–974.
27. Moore, J. M., Kumar, N., Shultz, L. D. and Rajian, T. V. 1995. Maintenance of the human malarial parasite, *Plasmodium falciparum*, in *scid* mice and transmission of gametocytes to mosquitoes. *J. Exp. Med.* **181**: 2265–2270.
28. Nakamura, Y., Tsuji, M., Arai, S. and Ishihara, C. 1995. A method for rapid and complete substitution of the circulating erythrocytes in SCID mice with bovine erythrocytes and use of the substituted mice for bovine hemoprotozoa infections. *J. Immunol. Methods* **188**: 247–254.
29. Prins, J. B., Todd, J. A., Rodrigues, N. R., Ghosh, S., Hogarth, P. M., Wicker, L. S., Gaffney, E., Podolin, P. L., Fisher, P. A., Sirotina, A. and Peterson, L. B. 1993. Linkage on chromosome 3 of autoimmune diabetes and defective Fc receptor for IgG in NOD mice. *Science* **260**: 695–697.
30. Rajian, T. V., Moore, J. M. and Shultz, L. D. 1996. Immunodeficient mice as hosts for hemoparasitic infections. *Parasitol. Today* **12**: 479–485.
31. Saito-Ito, A., Tsuji, M., Wei, Q., He, S., Matsui, T., Kohsaki, M., Arai, S., Kamiyama, T., Hioki, K. and Ishihara, C. 2000. Transfusion-acquired, autochthonous human babesiosis in Japan: Isolation of *Babesia microti*-like parasite with hu-RBC-SCID mice. *J. Clin. Microbiol.* **38**: 4511–4516.
32. Serreze, D. V., Gaedeke, J. W. and Leiter, E. H. 1993a. Hemopoietic stem-cell defects underlying abnormal macrophage development and maturation in NOD/Lt mice: defective regulation of cytokine receptors and protein kinase C. *Proc. Natl. Acad. Sci. U.S.A.* **90**: 9625–9629.
33. Serreze, D. B., Gaskins, H. R. and Leiter, E. H. 1993b. Defects in the differentiation and function of antigen presenting cells in NOD/Lt mice. *J. Immunol.* **150**: 2534–2543.
34. Shibata, S., Asano, T., Noguchi, A., Naito, M., Ogura, A. and Doi, K. 1998a. Peritoneal macrophages play an important role in eliminating human cells from severe combined immunodeficient mice transplanted with human peripheral blood lymphocytes. *Immunology* **93**: 524–532.
35. Shibata, S., Asano, T., Noguchi, A., Kimura, H., Ogura, A., Naiki, M. and Doi, K. 1998b. Enhanced engraftment of human peripheral blood lymphocytes into anti-murine interferon gamma monoclonal antibody-treated C.B-17*scid* mice. *Cell. Immunol.* **183**: 60–69.
36. Shpitz, B., Chambers, C. A., Singhal, A. B., Hozumi, N., Fernandes, B. J., Roifman, C. M., Weiner, L. M., Roder, J. C. and Gallinger, S. 1994. High level functional engraftment of severe combined immunodeficient mice with human peripheral blood lymphocytes following pretreatment with radiation and anti-asialo GM1. *J. Immunol. Methods* **169**: 1–15.
37. Shultz, L. D., Schweitzer, P. A., Christianson, S. W., Gott, B., Schweitzer, I. B., Tennent, B., Mckenna, S., Mobraaten, L., Rajian, T. V., Greiner, D. L. and Leiter, E. H. 1995. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-*scid* mice. *J. Immunol.* **154**: 180–191.
38. Tournoy, K. G., Depraetere, S., Meuleman, P., Leroux-Roels, G. and Pauwels, R. A. 1998. Murine IL-2 receptor beta chain blockade improves human leukocyte engraftment in SCID

mice. *Eur. J. Immunol.* **28**: 3221–3230.

39. Tournoy, K. G., Depraetere, S., Pauwels, R. A. and Leroux-Roels, G. G. 2000. Mouse strain and conditioning regimen determine survival and function of human leukocytes in immunodeficient mice. *Clin. Exp. Immunol.* **119**: 231–239.
40. Tsuji, M., Hagiwara, K., Takahashi, K., Ishihara, C., Azuma, I. and Siddiqui, W. A. 1992. *Theileria sergenti* proliferates in SCID mice with bovine erythrocyte transfusion. *J. Parasitol.* **78**: 750–752.
41. Tsuji, M., Terada, Y., Arai, S., Okada, H. and Ishihara, C. 1995a. Use of the Bo-RBC-SCID mouse model for isolation of a *Babesia* parasite from grazing calves in Japan. *Exp. Parasitol.* **81**: 512–518.
42. Tsuji, M., Ishihara, C., Arai, S., Hiratai, R. and Azuma, I. 1995b. Establishment of a SCID mouse model having circulating human red blood cells and a possible growth of *Plasmodium falciparum* in the mouse. *Vaccine* **13**: 1389–1392.
43. Tsuji, M., Fujioka, H., Arai, S., Taniyama, H., Ishihara, C. and Aikawa, M. 1996. A mouse model for cerebral babesiosis. *Parasitol. Today* **12**: 203–205.
44. Wei, Q., Tsuji, M., Zamoto, A., Kohsaki, M., Matsui, T., Shiota, T., Telford III, S. R. and Ishihara, C. 2001. Human babesiosis in Japan: isolation of *Babesia microti*-like parasites from an asymptomatic transfusion donor and from a rodent from an area where babesiosis is endemic. *J. Clin. Microbiol.* **39**: 2178–2183.