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Safe and Efficient Methods of Autologous Hematopoietic Stem Cell Transplantation for Biomedical Research in Cynomolgus Monkeys

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We have established safe and efficient methods for autologous hematopoietic stem cell (HSC) transplantation in cynomolgus monkeys (*Macaca fascicularis*) that include regimens of supportive care to ensure survival during hematopoietic reconstitution following otherwise lethal total body irradiation. Eleven young adult cynomolgus monkeys were studied. Bone marrow was aspirated from the ilium and/or tuber ischiae after administration of recombinant human stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF). Using the immunomagnetic selection method, $CD34^+$ cells were then isolated (90 to 95% pure) as a fraction containing HSCs. Just prior to transplantation, the animals received myeloablative total body irradiation—500 to 550 cGy daily for two days. The monkeys re-infused with $CD34^+$ cells developed moderate to severe myelosuppression, with some animals requiring intravenous hyperalimentation, antibiotic administration, and blood transfusion. Hematopoiesis was restored in all animals after transplantation. It took 12 days, on average, until the peripheral white blood cell count reached more than 1,000 cells/μl. Up to two years after transplantation, signs of radiation-induced pneumonitis or other radiation-related disorders were not evident at the aforementioned dose of irradiation. This transplantation model will be useful for testing new approaches using HSCs for therapy of many diseases and will offer unique insights into the biology of these cells.

After many years of study of the identity of potential blood-forming stem cells called hematopoietic stem cells (HSCs), researchers have begun exploring their therapeutic use. Currently, no other type (adult, fetal, or embryonic) of stem cell has attained such status. Transplantation of HSCs is now routinely used to treat patients with cancer and other disorders of the blood and immune systems (1-3). Despite vast clinical experience with HSCs, we do not yet have an accurate in vitro method to distinguish HSCs from other cells harvested from bone marrow or peripheral or cord blood. Animal transplantation has proved to be the only reliable method for assay of HSCs (4-6). Cells capable of restoring multi-lineage hematopoiesis in recipient animals through self-renewal and differentiation can be called HSCs.

Therefore, transplantation of presumable HSCs into humans would be the most reliable method to assess human HSCs, but it is impossible to design such experiments. Instead, xenograft models of human hematopoiesis have been used for the study of in vivo engraftment and proliferation potentials of human HSCs. Until now, only two xenotropic transplantation models have been available. One recipient is the non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mouse and the other is the fetal sheep (7, 8). These models take advantage of the animal's immunologically naive state. Since NOD/SCID mice are severely immunodeficient and fetal sheep are immunologically immature, human HSCs can engraft and generate their progeny in these animals. The behavior of human HSCs, however, might be different in xenotropic recipients. The relevance of these xenograft models to natural human in vivo hematopoiesis remains unclear.

On the other hand, autologous transplantation models are ideal for evaluating engraftment, proliferation, and differentiation of HSCs, since natural hematopoiesis can be studied in these models. Although mouse transplantation models have been widely used for assay of murine HSCs, these models may not reliably predict the biology of HSCs in larger animals such as humans. Large animal species may provide far more appropriate preclinical models that will more closely reflect human HSC characteristics and behavior (9). Among large animals, nonhuman primates may provide the best models because of their close phylogenetic relationship to humans (10, 11). In the study reported here, we used safe and efficient methods for autologous transplantation of immunoselected $CD34^+$ cells in cynomolgus monkeys (*Macaca fascicularis*).

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Materials and Methods

Breeding. A large-scale cynomolgus monkey breeding colony derived from animals of the Philipines, Indonesia, and Malaysia was established in 1978 at Tsukuba Primate Center, National Institute of Infectious Diseases (Ibaraki, Japan) (12). More than 700 animals have been bred over the past 22 years, with 201 normal births being obtained, on average, from 229 pregnancies (live birth rate; 87.8%) in a year. Approximately 180 cynomolgus monkeys, including juvenile monkeys and retired breeders, were supplied for research every year. Individual housing system and timed mating system are effective for prevention of horizontal infection, resulting in establishment of a specific-pathogen-free (SPF) colony that is free of blood parasites, intestinal parasites, herpes virus B, simian varicella virus, and simian immunodeficiency virus. Our SPF definition does not include more ubiquitous or poorly defined viral flora of nonhuman primates (foamy viruses, adenoviruses, other herpes viruses, or reoviruses) (13). These animals are periodically screened to maintain health status. These highly screened monkeys with detailed health records have been used in a variety of biomedical research experiments, including the study reported here.

Animal housing. Eight monkeys born in Tsukuba Primate Center were enrolled in our study. In addition, animals 099053, 099061, and 099056 were imported from China (Animal Care, Tokyo, Japan), were quarantined for five weeks, and were kept in Tsukuba Primate Center. All monkeys were two to four years old and weighed 2.1 to 3.3 kg. They were healthy, without signs of disease at annual health examinations. In addition, animals of this study were further certified free of simian type-D retrovirus as well as herpes virus B, simian varicella virus, and simian immunodeficiency virus. All animals were individually housed in stainless steel cages under temperature conditions between 23 and 27°C, humidity between 50 and 70%, 12 air changes/h, and a 12/12-h light/dark cycle, and were fed 70 g of commercial monkey chow (Type AS, Oriental Yeast, Chiba, Japan) and 200 g of fruits daily unless otherwise indicated. This study was strictly subjected to the Rules for Animal Care and Management of Tsukuba Primate Center (14) and the Guiding Principles for Animal Experiments Using Nonhuman Primates formulated by the Primate Society of Japan (15). The protocol of the experimental procedures was approved by the Animal Welfare and Animal Care Committee of the National Institute of Infectious Diseases (Tokyo, Japan).

Bone marrow harvest. Three weeks before bone marrow harvest, a central venous catheter, used with a tether and a jacket, was placed in each animal to allow administration of fluids, antibiotics, and transfusions. The catheter was tunneled subcutaneously and brought out through the skin of the back. For autologous blood donation, animals received recombinant human erythropoietin (Chugai, Tokyo, Japan; 150 IU/kg of body weight) subcutaneously three times a week during the three weeks prior to bone marrow harvest. Autologous blood (20 to 30 ml) was drawn once a week and saline was then infused for volume replacement. A total of 60 to 90 ml of peripheral blood was obtained from each animal and stored at 4°C in a bag containing the anticoagulant acid-citrate dextrose prior to use as an autologous blood transfusion at the time of bone marrow harvest (16).

All animals (except No. 396042, No. 396051 and No. 396053) received recombinant human stem cell factor (SCF, 50 to 200 μg/kg; Amgen, Thousand Oaks, Calif.) and recombinant human granulocyte colony-stimulating factor (G-CSF, 10 to 100 μg/kg; Chugai) subcutaneously daily for five days (17, 18). After cytokine administration, 50 ml of bone marrow was aspirated from the iliac crest and/or tuber ischiae of monkeys under isoflurane (A.D.S.1000; Shin-ei, Tokyo, Japan)-induced general anesthesia into a syringe that had been rinsed with preservative-free heparin. Concurrently, the stored autologous blood was re-infused. After bone marrow harvest, animals received butorphanol tartrate (0.5 mg/kg, intramuscularly) daily for 3 days to alleviate bone pain associated with the bone marrow harvest.

Preparation of $CD34^+$ cells. From the harvested bone marrow, the nucleated cell fraction was obtained by red blood cell lysis by addition of ACK buffer (155 m*M* NH_4Cl, 10 m*M* $KHCO_3$, and 0.1 m*M* EDTA; Wako, Osaka, Japan). Enrichment of $CD34^+$ cells was performed using magnet beads conjugated with a monoclonal anti-CD34 antibody (clone 561) (Dynal, Lake Success, N.Y.). The purity of $CD34^+$ cells was assessed by use of flow cytometry with another monoclonal anti-CD34 antibody (clone 563; PharMingen, San Diego, Calif.). The $CD34^+$ cells were cultured for four days until re-infusion in Dulbecco's modified Eagle's medium (DMEM; Gibco, Gaithersburg, Md.) supplemented with 10% fetal bovine serum (FBS; Gibco), recombinant human interleukin 6 (IL-6, 50 ng/ml; Ajinomoto, Osaka, Japan), recombinant human thrombopoietin (TPO, 100 ng/ml; Kirin, Tokyo, Japan), recombinant human SCF (100 ng/ml; Amgen), recombinant human Flt-3 ligand (FL, 100 ng/ml; Research Diagnostics, Flanders, N.J.) and antibiotics (100 U of penicillin [Banyu, Tokyo, Japan] and 0.1 μg of streptomycin [Meiji, Tokyo, Japan]/ml) (Table 1).

Total body irradiation. Prior to irradiation, a non-absorbent antibiotic (polymyxin B sulfate, 5×10^4 U/kg; Pfizer, Brooklyn, N.Y. or kanamycin sulfate, 50 mg/kg; Meiji) was orally administered to animals for three days to sterilize the gastrointestinal tract. Microbial contamination on animals' body surface was decreased by immersion in an iodine bath before irradiation. Animals under general anesthesia by administration of ketamine hydrochloride (Ketalar, 10 mg/kg; Sankyo, Tokyo, Japan) and xylazine hydrochloride (Seraktar, 0.5 mg/kg; Bayer, Leverkusen, Germany) received myeloablative total body x-ray irradiation (PANTAK HF-420, Shimazu, Tokyo, Japan). Irradiation was conducted at a dose of 500 cGy (all monkeys except No. 296113 and No. 296116) or 550 cGy (No. 296113 and No. 296116) daily for 2 days (total 1,000 or 1,100 cGy, dose rate: 10 to 15 cGy/min) just prior to transplantation (19, 20). Since the x-ray device was originally intended for industrial use, the energy spectrum was altered to be similar to that of γ-radiation for medical use by addition of a specific filter (Al 0.5 mm + Pb 0.1 mm + Cu 0.3 mm + Al 1.0 mm; Shimazu).

Transplantation and supportive care. After total body irradiation, the first animal (No. 396042) was re-infused intravenously with whole autologous bone marrow nucleated cells. The subsequent 10 animals were re-infused intravenously with autologous $CD34^+$ cells. After re-infusion of the cells, animals were kept in an intensive care unit with high efficiency particulate air (HEPA)-filtered airflow and were fed sterilized commercial monkey chow from the day of irradiation until the peripheral white blood cell (WBC) count reached 5,000 cells/μl. Recombinant human G-CSF (Chugai) was administered intravenously to animals at a dosage of 5 to 10 μg/kg once a day from the day when the

Table 1. Bone marrow harvest from cynomolgus monkeys

Animal no.	Sex	Age (yr)	BW (kg)	Priming G-CSF (μ/kg)	Priming SCF (g/kg)	Total nucleated cells ($\times 10^9$)	$CD34^+$ Cell numbers ($\times 10^9$)	$CD34^+$ Proportion in the total nucleated cells (%)	Culture conditions
396042	F	2	2.3	0	0	NA	NA	NA	-
396051	F	3	2.4	0	0	0.70	3.3	0.5	-
306053	F	3	2.3	0	0	066	9.7	1.5	IL3/IL6/SCF/FL
396058	F	3	2.3	100	200	2.69	37.5	1.4	IL3/IL6/SCF/FL
396060	F	3	2.1	10	50	0.72	17.0	2.4	IL6/SCF/FL
296102	F	3	2.3	50	50	1.63	16.7	1.0	IL6/SCF/FL/TPO
099053*	M	3	2.5	50	50	1.47	10.4	0.7	IL6/SCF/FL/TPO
099061*	M	4	3.3	50	50	1.56	45.0	2.9	IL6/SCF/FL/TPO
099056*	M	2	2.7	50	50	2.08	38.4	1.8	IL6/SCF/FL/TPO
296113	F	4	3.2	50	50	0.74	11.3	1.5	IL6/SCF/FL/TPO
296116	F	4	3.3	50	50	1.22	11.6	1.0	IL6/SCF/FL/TPO
Average	-	3	2.6	-	-	1.35	20.1	1.5	

*Imported from China.
F = female; M = male; BW = body weight; G-CSF = granulocyte colony-stimulating factor; SCF = stem cell factor; IL = interleukin; FL = Flt-3 ligand; TPO = thrombopoietin; and NA = not applicable.

WBC count was < 1,000 cells/μl until the WBC count reached 5,000 cells/μl. Every day after irradiation, animals also received a histamine H_2-receptor-blocking agent (famotidine, 4 mg, intravenously; Yamanouchi, Tokyo, Japan) to prevent gastric ulcer development. Three times a week during the period of myelosuppression, blood was drawn from animals under ketamine hydrochloride (Ketalar, 10 mg/kg; Sankyo, Tokyo, Japan) general anesthesia to obtain a serum biochemical profile and a complete blood count. The serum biochemical profile included sodium, potassium, chloride, total protein, albumin, globulin, blood urea nitrogen, creatinine, alanine transaminase, aspartate transaminase, and C-reactive protein (CRP) values. Transfusion of irradiated (2,000 cGy) freshly obtained whole blood (approx. 30 ml/transfusion) was administered to treat anemia (hemoglobin concentration < 7 g/dl). Transfusion of platelet-rich plasma was done to treat thrombocytopenia (platelet count < 40,000 cells/μl). Blood-donor monkeys (body weight > 5 kg) were grouped by blood type and were cross-matched before transfusion. Antibiotics were administered when the animals had high fever (> 38°C) or increased CRP value. Antibiotics were chosen from results of bacteriologic culture and antimicrobial susceptibility testing when possible. Diarrhea was a common adverse effect of total body irradiation. *Lactobacillus* and *Bifidobacterium* spp. were administered when radiation-related diarrhea was prolonged.

Results

Bone marrow harvest. Autologous blood from each animal was stored before bone marrow harvest. To avoid development of anemia, animals received recombinant human erythropoietin subcutaneously prior to blood donation (16). Removal of 20 ml of blood was performed once a week (total, 60 to 90 ml of blood) safely without adverse effect.

We administered SCF and G-CSF to eight monkeys for five days just prior to bone marrow harvest to expand hematopoietic stem/progenitor cells in the bone marrow (17, 18). Fig. 1A shows the mean and standard deviation of peripheral WBC counts from monkeys (n = 3) not administered SCF and G-CSF. On the other hand, administration of these cytokines resulted in a mean increases in the WBC count to 46,000 (range, 16,000 to 67,000) cells/μl (Fig. 1B).

Adverse effects associated with the cytokine administration, such as fever or anorexia, were not observed. After a five-day

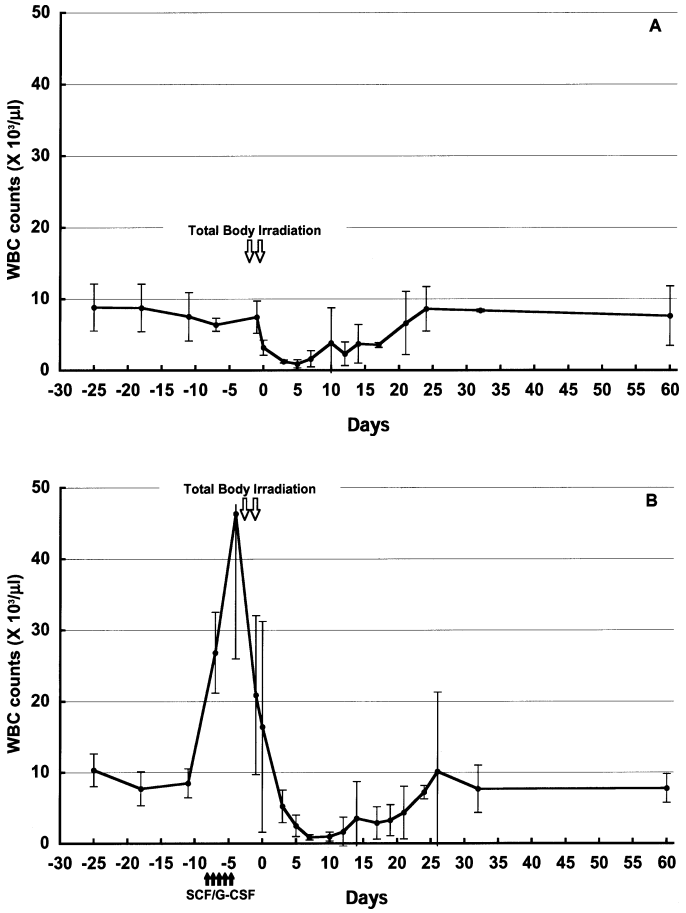

Figure 1. Peripheral white blood cell (WBC) counts before and after transplantation, which was conducted on day 0. Data are expressed as mean and standard deviation of WBC counts from animals administered (B, n = 8) or not administered (A, n = 3) stem cell factor (SCF) and granulocyte colony-stimulating factor G-CSF. Administration of these cytokines (shaded arrows in B) resulted in an increase in the WBC count between 16,000 and 67,000 (mean, 46,000) cells/μl. After total body irradiation (500 to 550 cGy times two, open arrows in A and B), the WBC count decreased to < 1,000 cells/μl. To facilitate granulocyte recovery, the G-CSF was administered intravenously to animals at a dosage of 10 μg/kg once a day from the day when the WBC count became < 1,000 cells/μl until the WBC count reached 5,000 cells/μl.

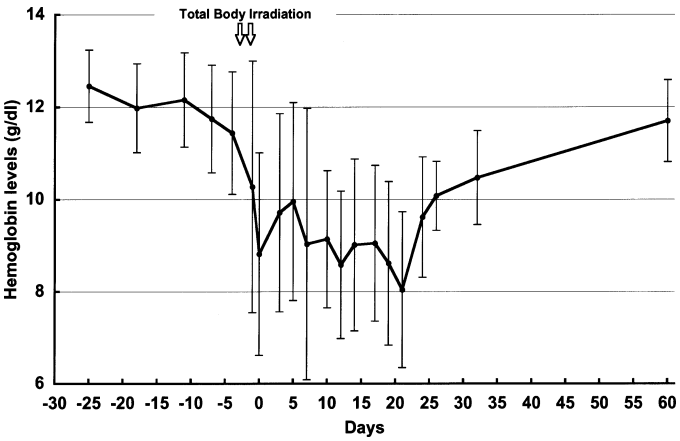

Figure 2. Blood hemoglobin concentrations (g/dl) before and after transplantation, which was conducted on day 0. Mean values for all monkeys (n = 11), with or without cytokine administration, are shown, since administration of SCF and G-CSF did not affect hemoglobin concentration. Bars indicate the standard deviation. Freshly obtained, irradiated (2,000 cGy), whole blood and/or stored autologous blood were administered to anemic animals when the blood hemoglobin value was < 7 g/dl.

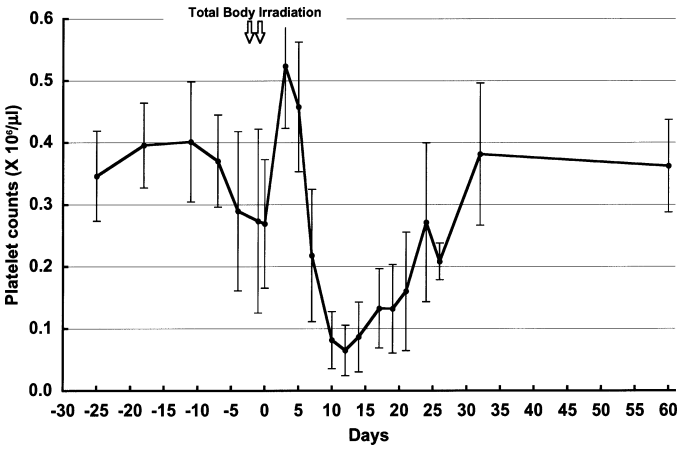

Figure 3. Blood platelet counts before and after transplantation, which was conducted on day 0. Mean values for all monkeys (n = 11), with or without cytokine administration, are shown, since administration of SCF and G-CSF did not affect platelet count. Bars indicate the standard deviation. Freshly obtained, irradiated (2,000 cGy), whole blood or platelet-rich plasma was administered to thrombocytopenic animals when the platelet count was < 70,000 cells/µl.

Table 2. Clinical course of autologous hematopoietic stem cell transplantation in cynomolgus monkeys

Animal no.	White blood cells Nadir (cells/µl)	White blood cells > 1,000/µl	Platelets Nadir (cells/µl)	Platelets > 50,000/µl	Blood transfusion	Body temperature > 38°C	Peak CRP value (mg/dl)
396042	1,300	NA	83,000	NA	-	0 Days	4.69
396051	400	Day 13	23,000	Day 15	3 Times (total 80 ml)	4 Days	10.26
396053	500	Day 10	67,000	NA	2 Times (total 50 ml)	2 Days	8.56
396058	700	Day 12	26,000	Day 17	4 Times (total 75 ml)	2 Days	0.6
396060	300	Day 14	40,000	Day 14	3 Times (total 45 ml)	0 Days	1.02
296102	700	Day 10	145,000	NA	-	2 Days	0.18
099053	700	Day 14	80,000	NA	1 Time (25 ml)	0 Days	1.77
099061	700	Day 10	69,000	NA	1 Time (30 ml)	0 Days	3.81
099056	1,200	NA	109,000	NA	-	2 Days	1.02
296113*	200	-	16,000	-	3 Times (total 60 ml)	2 Days	10.01
296116	500	Day 14	45,000	Day 17	1 Time (60 ml)	0 Days	10.39
Average	700	Day 12	64,000	Day 15	2 Times (total 50 ml)	1.1 Days	4.76

*Died due to accidental catheter fracture at 3 weeks after transplantation.
CRP = C-reactive protein.

administration of SCF and G-CSF, 50 ml of bone marrow was harvested while the stored autologous blood was re-infused. The decrease in hemoglobin values observed prior to transplantation (Fig. 2) was a complication of the bone marrow harvest operation. The transient increase in platelet count observed after transplantation (Fig. 3) may have been secondary to the decrease in hemoglobin values.

Preparation of CD34$^+$ cells. The CD34 is a cell-surface marker of undifferentiated HSCs. Although recent reports suggest that all HSCs may not express CD34 (21), clinical CD34$^+$ cell transplantation has been successfully conducted, using immunoselected CD34$^+$ cells in a variety of HSC transplantation and gene therapy studies (22, 23). The CD34$^+$ cells were isolated from bone marrow cells by use of commercially available magnet beads conjugated to a monoclonal antibody (clone 561) that recognizes human and cynomolgus monkey CD34$^+$ cells (24). On average, sorted CD34$^+$ cells accounted for 1.5% of total nucleated bone marrow cells. The purity of CD34$^+$ cells ranged from 90 to 95%. The numbers of CD34$^+$ cells were generally higher in bone marrow harvested from monkeys administered SCF and G-CSF, compared with monkeys not administered the cytokines (Table 1). However, correlation between numbers of harvested CD34$^+$ cells and dose of SCF and G-CSF administration was not apparent, presumably because even the lowest administered dose of SCF and G-CSF seemed to reach biologically optimal serum values.

The CD34$^+$ cells were cultured ex vivo for four days prior to reinfusion, since ex vivo culture of CD34$^+$ cells is required for many applications, such as ex vivo expansion of HSCs and genetic manipulation of the cells. We used the standard culture conditions for primate CD34$^+$ cells that included several cytokines (SCF, FL, and TPO) (20, 25).

Transplantation and myelosuppression. Just prior to transplantation, the animals received myeloablative total body irradiation (500 to 550 cGy) daily for two days (19, 20). Animal 396042 initially received all bone marrow nucleated cells, and subsequent animals received immunoselected autologous CD34$^+$ cells. From the day of irradiation until hematopoiesis was restored, all animals were kept in the intensive care unit with HEPA-filtered airflow. Although animal 396042 that received all bone marrow nucleated cells developed only slight myelosuppression, the other 10 animals receiving CD34$^+$ cells had moderate to severe myelosuppression (Table 2).

After transplantation, all but two animals experienced neu-

tropenia (WBC < 1,000 cells/μl) (Fig. 1). On average, the WBC count nadir was 700 cells/μl, and 12 days was required for the WBC count to reach a value > 1,000 cells/μl. Some animals experienced anemia (hemoglobin concentration < 7g/dl) (Fig. 2), and five animals required blood transfusion (20 to 40 ml) one to three times. Three animals experienced thrombocytopenia (platelet count < 40,000 cells/μl) and required platelet-rich plasma transfusion (Fig. 3). Fifteen days was required for the platelet count to reach > 50,000 cells/μl. Overall, eight of 11 animals required whole blood or platelet-rich plasma transfusion for treatment of anemia and/or thrombocytopenia (Table 2). Numbers of harvested $CD34^+$ cells and days required for hematopoietic recovery were not correlated. When the harvested $CD34^+$ cell numbers reached 1 to 2×10^6 cells/kg, further increase did not appear to accelerate recovery.

Allelic difference has been reported among regional populations of rhesus macaques, possibly leading to variable outcome of transplantation studies (26). Although three animals of this study were imported from China, they did not experience a different outcome after transplantation, compared with that of the animals from the in-house breeding colony.

Complications. Six animals had high fever (body temperature > 38°C) and increase in C-reactive protein (CRP) values during myelosuppression. Fever subsided after administration of antibiotics (Table 2). Enrofloxacin, ceftazidime, aminoglycoside, or other antibiotics were chosen according to antimicrobial susceptibility test results. *Enterococcus faecalis*, *Enterobacter cloacae* or *Staphylococcus aureus* was isolated from culture of the blood from three animals. Some animals developed nausea and anorexia during myelosuppression. Intravenous hyperalimentation, including amino acids, fat emulsion, and vitamins, was conducted to supplement intake of food and water in those animals. Radiation-associated diarrhea ceased within three weeks after irradiation in all animals.

Animal 396051 had high serum alanine transaminase and aspartate transaminase activities, which may have been drug associated since the animal was receiving ceftazidime. The values completely normalized after change of antibiotics. Animals 396053 and 396060 had high blood urea nitrogen and creatinine values, presumably due to dehydration. Values normalized after administration of fluids. Other abnormal serum biochemical values were not observed.

Three weeks after transplantation, animal 296113 died due to accidental catheter fracture. Otherwise all procedures were safely conducted. Up to two years after transplantation, complications, such as radiation pneumonitis or other radiation-related disorders, were not observed.

Discussion

We have established protocols of autologous $CD34^+$ cell transplantation in cynomolgus monkeys. Hematopoietic reconstitution was observed around two weeks after transplantation in all animals. Although some animals required antibiotic administration, blood transfusion, and intravenous hyperalimentation, procedures were performed safely with few adverse effects. The focus of this study was on the use of bone marrow as a source of $CD34^+$ cells. Our preliminary data, however, indicate that CD34-selected peripheral blood cells can also be used for hematopoietic restoration (our unpublished data).

Results of this study documented a fourth large animal model for CD34-selected hematopoietic cell transplantation in addition to that of baboons (27), rhesus macaques (28), and dogs (29). Compared with the dog, the other animals mentioned are closer phylogenetically to humans, and more reagents, including cytokines and antibodies, are available that were originally developed for humans (24). Although baboons may provide the best animal model among them because of its phylogenetic relatedness to humans, there is no clear evidence that the hematopoietic system of baboons would resemble that of humans more than does that of macaques. In addition, the macaque model has the advantages of animal availability, cost, and small size, compared with the baboon model. On the other hand, among macaque monkeys, the advantages of using cynomolgus monkeys over rhesus monkeys are full seasonal breeder and smaller body size. Full seasonal breeding capacity is important in maintaining a self-sustaining breeding colony without need for introduction of new breeders. The self-sustaining breeding system has made it possible to establish our SPF colony and to accumulate background data and set normal limits (30-32).

There are a few disadvantages to using monkeys, compared with other smaller animals such as mice, for biomedical research. Although it is not a true disadvantage, monkeys, like humans, are outbred, allowing the potential of substantial individual differences between animals. Other disadvantages are fewer monkey models for human diseases, compared with mice (33, 34), and that primate research costs are substantially higher (35).

The study reported here included administration of a five-day course of SCF and G-CSF prior to bone marrow harvest to expand immature hematopoietic cells in bone marrow (17, 18), and ex vivo culture of $CD34^+$ cells for four days (25). The $CD34^+$ cells were stimulated overall for nine days (in vivo for five and ex vivo for four days) prior to re-infusion. One may claim that these stimulated cells lost their engraftment ability (25, 36) and that hematopoietic restoration was attributable to endogenous recovery. To address the issue, we genetically modified $CD34^+$ cells with retroviral vectors under the same cytokine-stimulated conditions, followed by re-infusion of the cells into each monkey. The genetically modified hematopoietic cells were still detected in monkeys one year after transplantation (37). This result clearly indicates that the re-infused cells engrafted and generated their progeny, resulting in the hematopoietic restoration in vivo.

Animals were irradiated with 1,000 to 1,100 cGy for myeloablation. At issue is whether this dose of irradiation can really cause “full” myeloablation. If not, endogenous recovery could occur. To answer the question, it would be necessary to test whether animals, after total body irradiation with 1,000 to 1,100 cGy, could survive without infusion of cells. We, however, could not design such experiment because of considerations of animal welfare. It has been reported that 1,300 cGy may be required for “full” ablation (100% lethal) but that this dose would cause radiation-induced pneumonitis at much higher frequency, compared with the dose of 1,000 cGy (19). Since myeloablative irradiation is associated with high systemic toxicity or potential damage to bone marrow stroma, projects are needed to assess how much the dose of radiation can be reduced without losing engraftment efficiency of reinfused HSCs, or whether alternative methods for myeloablation, such as cyclophosphamide administration, can be used instead of total body irradiation.

Recently, researchers have observed, principally in murine models, that hematopoietic cells appear to be able to form other kinds of cells, such as liver, muscle, and blood vessels (38), although cell fusions may in part account for such change of phenotype (39, 40). If plasticity of hematopoietic cells can be applied to human cells, it may eventually be possible to use hematopoietic cells to replace a wider array of cells and tissues than was initially thought. Our nonhuman primate model will provide an important framework for such future clinical studies.

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