Repopulation Defect of Stem Hemopoietic Cells After Retroviral Transduction of a Foreign Gene

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It is demonstrated that the proliferative potential of mouse hemopoietic stem cells does not decrease after insertion of foreign DNA and does not depend on the number of inserted DNA copies. However, transduced cells develop a repopulation defect manifesting itself as a reduced production of splenic colony-forming units in restored mice. The mice restored with transduced cells more rapidly reverse to the recipient hemopoiesis. This defect is associated with the induction of proliferation of stem hemopoietic cells by high concentration of cytokines but not with integration of the foreign gene. The repopulation defect is less pronounced in mice restored with cells stimulated for gene transduction by culturing on irradiated sublayer of long-term bone marrow cells without exogenous cytokines.

Key Words: *stem hemopoietic cell; retroviral transduction; cytokines; proliferative potential*

The stem hemopoietic cell (SHC) is a prospective target for gene transduction in the treatment of genetic abnormalities. These cells can be readily isolated in considerable amounts, and the techniques for creating long-term SHC chimeras with subsequent replacement of host hemopoietic and lymphoid cells with them have been developed [3,4]. Although the protocols of clinical trials of gene therapy of familial immunodeficiency, Gaucher's disease, etc. based on transduced SHC have been approved, the repopulation potential of transduced cells is poorly investigated. However, the repopulation potential is a very important parameter, since gene transduction requires subsequent repopulation of the target cells [1]. In order to render SHC, which are generally quiescent, accessible for a foreign gene stimulation with high concentration of cytokines is necessary [8]. This may reduce the repopulation potential of SHC. In the present study we explored this possibility on hemopoietic murine cells transduced with the human adenosine deaminase (ADA) gene. Original method of repeated isolation of bone marrow from mice enabled us to estimate for the first time the proliferative potential of individual polypotent precursors by the number of splenic colony-forming units (CFUs) with the foreign gene.

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MATERIALS AND METHODS

Twelve-sixteen-week-old (C57Bl/6×DBA$_2$) BDF$_1$ mice were used as donors (males) and recipients (females) of the bone marrow. The recipients were irradiated in an IPK installation (^{137}Cs, total dose 12 Gy, 2 sessions at a 3-h interval). Long-term bone marrow cultures were initiated and maintained by the method [2]. Donor mice were injected with 150 mg/kg 5-fluorouracil (Sigma) 2 days before experiment. Transduction of the human ADA gene was performed as described [6]. Hemopoietic cells were stimulated using two protocols. Protocol 1: 48-h culturing in the presence of high concentrations of exogenous cyto-

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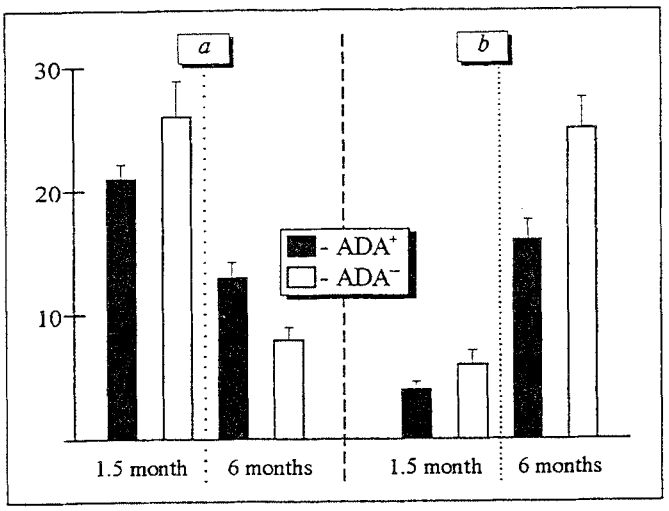

Fig. 1. Proliferative potential of ADA-positive and ADA-negative splenic colony-forming units (CFUs) isolated from the mouse bone marrow 1.5 and 6 months after transplantation of donor cells. *a*) protocol 1; *b*) protocol 2; *c*) individual sites (*1-10*) of the ADA gene integration in CFUs with the same proliferative potential (Southern blot). Ordinate: number of daughter CFUs per 11-day-old colony.

kines (100 units/ml human recombinant interleukin-6 and 50 ng/ml rat recombinant stem cell growth factor, Amgen) and then transferred into a culture of retrovirus-producing fibroblasts (hADA GP+E86) preincubated with mitomycin C in the presence of the above-mentioned growth factors. Protocol 2: donor cells were cultured for 2 days on a layer of irradiated (15 Gy) 3-4-week-old bone marrow cells, after which the layer and the cells were transferred in a culture of retrovirus-producing cells. After a 48-h incubation (both protocols), bone marrow cells were washed and injected into lethally irradiated recipients. After 1.5 months—1 year, bone marrow was collected from the recipients by femoral puncture under a light ether anesthesia. The bone marrow cells were injected into recipients irradiated for a second time. The recipients were killed on day 11, and individual colonies were isolated from the spleen. Some cells from a colony were injected into secondary recipients, and splenic colonies were counted after 8 days. This allowed us to evaluate the proliferative potential of CFUs by the number of daughter CFUs. DNA was isolated from other cells of the colony. Polymerase chain reaction was employed to confirm the donor origin of a colony (the primers specific for the Y region of male mouse DNA — 5'CTCCTGATGGACAAACTTTACG3' and 5'TGAGTGCTGATGGGTGACGG3') and the insertion of the human ADA gene (the primers specific for human ADA — 5'GACAAGCCCAAAGTAGAACTGC3' and 5'TGACCCCGAAGTCTCGCTCC3') the DNA of ADA-positive colonies was analyzed by Southern blot for the number of inserted ADA gene copies [7]. A total of 621 individual colonies were analyzed.

RESULTS

Irrespective of the mode of cell stimulation (high concentrations of exogenous cytokines or culturing with long-term cultures of stromal cells), the proliferative potential of CFUs with the foreign gene remained similar to that of CFUs without the gene both 1.5 and 6 month after restoration (Fig. 1). The proliferative potential of individual CFUs did not depend on the number of inserted ADA copies: the proliferative potentials of CFUs with 1 and 15 copies of the gene were practically the same (Fig. 1, *c*). Thus, it is demonstrated for the first time that insertion of several copies of a foreign gene does not affect the proliferative potential of the stem hemopoietic cell.

Colony-forming unit is a cell population with a short life-span: 1 month in the organism or in culture [5]. Cells investigated in this study are not SHC carrying a marker, but the "descendants" of transformed SHC. Consequently, insertion of a foreign gene does not reduce the proliferative potential of the SHC. This is important for the evaluation of the possibilities of gene therapy, irrespective of the chosen cell model. However, it was important to characterize the entire population of SHC. These cells were mobilized from the resting state, which may change their proliferative potential even without transduction. In order to check up this hypothesis, we determined the content of CFUs in the bone marrow of restored mice. It remained decreased almost 10-fold throughout the entire experimental period (Table 1). Based on the observation (1 year) that the total

TABLE 1. Concentration of CFUs (per 10^6 cells) in the Bone Marrow of Restored Mice

Protocol No.	Time after restoration, months				
	1.5	3	6	9	12
1	11.6±1.3	28.2±3.1	13.7±1.6	17.5±2.5	13.8±2.3
2	12.0±2.0	25.9±7.1	12.4±1.2	24.8±3.5	13.6±2.4

Note. Concentration of CFUs in the bone marrow of intact and irradiated BDF_1 mice restored with normal bone marrow is 150-200/10^5 cells.

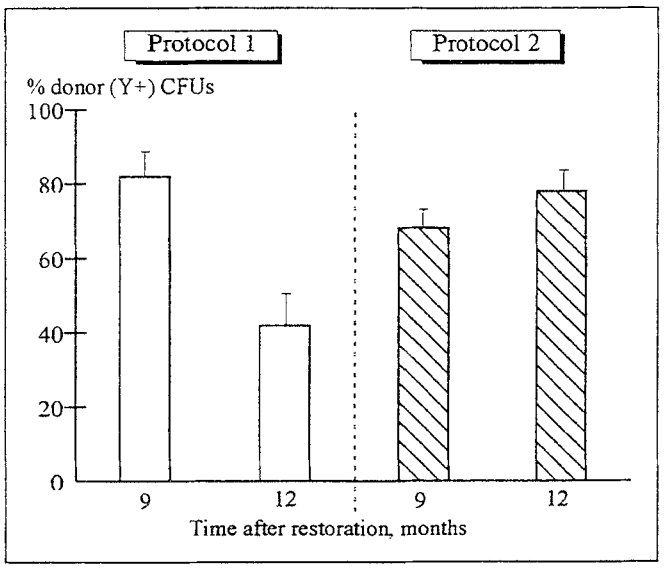

Fig. 2. Proportion of donor CFUs in the bone marrow of mice restored according to protocols 1 and 2.

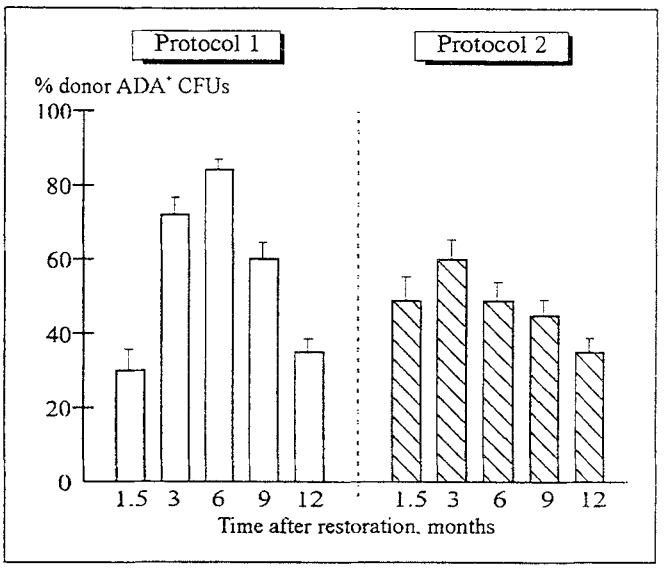

Fig. 3. Proportion of donor CFUs carrying the ADA gene in the bone marrow of mice restored according to protocols 1 and 2.

number of CFUs per SHC decreased considerably although SHC are capable of producing CFUs with normal proliferative potential, it can be hypothesized that transduction reduces the proliferative potential of SHC. If so, transduced cells are depleted at a higher rate, the depletion being faster when transduction had been performed using pharmacological concentrations of exogenous cytokines. This hypothesis was confirmed by the data presented in Fig. 2: the proportion of donor cells rapidly decreased in mice which were grafted bone marrow stimulated with exogenous cytokines. Moreover, depletion of the SHC with the foreign gene is the fastest (Fig. 3). Thus, in irradiated mice, cells subjected to strong stimulation during transduction (which increases the possibility of insertion of a foreign gene in their genome) are utilized in the first turn, which results in their rapid depletion. However, cells stimulated under physiological conditions (protocol 2) are depleted at a lower rate than cells stimulated by exogenous cytokines (protocol 1, Fig. 3). A decrease in the repopulation potential of cytokine-stimulated cells was observed in intact animals after grafting of transduced cells [9].

Thus, our results indicate that the repopulation potential of SHC subjected to retroviral transduction decreases. However, this decrease can be diminished by the use of stromal microenvironment during transduction, which provides better survival of SHC and a "more physiological" stimulation of these cells. As a result, the donor hemopoiesis remains more stable, and the probability of reversion toward the recipient hemopoiesis is lower. This can be helpful for the development of protocols for gene transduction in clinical practice.

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