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Lentivirus Vector Mediated Hematopoietic Stem Cell Gene Transfer of Common Gamma-Chain Cytokine Receptor in Rhesus Macaques

Dong Sung An. M.D., Ph.D.

UCLA AIDS Institute and Department of Hematology and Oncology, David Geffen School of Medicine, UCLA, Los Angeles, California 90095, USA
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Abstract We examined the efficiency of marking, gene expression, and transplant of bone marrow and peripheral blood CD34 +cells using a lentivirus vector in non-human primate model systems. *In vitro* cytokine stimulation was not required to achieve efficient transduction of CD34 +cells resulting in marking and gene expression of the reporter gene encoding enhanced green fluorescent protein (EGFP) following transplant of the CD34 +cells. Monkeys transplanted with mobilized peripheral blood CD34 +cells resulted in EGFP expression in 1 to 10% of multilineage peripheral blood cells, including red blood cells and platelets, stable for 5 years to date. The relative level of gene expression utilizing a lentiviral vector bearing a RhMLV promoter is 2- to 10-fold greater than that utilizing a lentivirus vector bearing the cytomegalovirus immediate-early promoter. In contrast, in animals transplanted with autologous bone marrow CD34 +cells, multilineage EGFP expression was evident initially but diminished over time. We further tested our lentivirus vector system by demonstrating gene transfer of the human common gamma-chain cytokine receptor gene (γc), deficient in X-linked SCID patients and recently successfully used to treat disease. Marking was 0.42 and 0.001 HIV-1 vector DNA copy per 100 cells in two animals. To date, all EGFP- and γc-transplanted animals are healthy. This system may prove useful for expression of therapeutic genes in human hematopoietic cells.

Key words: gene therapy, lentiviral vector, hematopoietic stem cell, rhesus macaque, common gamma chain cytokine receptor gene

Introduction

Lentivirus vectors have been proposed as potential vectors for human hematopoietic progenitor cell gene transfer.[1] These vectors have a number of advantages, in particular the ability to transduce nondividing cells.[2] Thus, lentivirus vectors should in theory provide effective transduction of hematopoietic progenitor. However, these potential advantages and their origin also emphasize the need to adequately assess the properties of lentivirus vectors prior to use in humans. The nonhuman primate model system is ideal for evaluation of the efficiency of marking and the efficiency and maintenance of gene expression and for initial safety testing regarding introduction of potential human therapeutic genes.

Here we report on the use of a lentivirus vector gives long-term expression in rhesus macaque hematopoietic cells following transplant of transduced mobilized CD34 + peripheral blood (PB) cells in the absence of *in vitro*

cytokine stimulation. The use of this vector demonstrates that marking is more efficient in mobilized PB cells than in bone marrow (BM) cells. Finally, the vector was used to express the human common gamma-chain cytokine receptor gene (γc) in lymphocytes of rhesus macaques.

Results

Transduction of immunoselected mobilized PB- and BM-derived CD34 +cells with a lentivirus vector. We compared immunoselected mobilized PB and BM CD34 +cells of the rhesus macaques that were treated with SCF and G-CSF for their efficiency of lentivirus transduction and reconstitution of rhesus macaque hematopoietic system *in vivo*. Nonhuman primate immunoselected PB and BM CD34 +cells of four animals were transduced with lentiviral vector in the absence of further cytokine stimulation *ex vivo* and were analyzed for EGFP expression by flow cytometry 12 h postinfection. 15 and 20% of the transduced PB CD34 +cells were EGFP positive for animals 96E035 and 95E132, respectively. In comparison, 52 and 53% of the transduced BM CD34 +cells were EGFP positive (animals 96E041 and 95E131, respectively). Lentivirus vector transduction is achieved in the rhesus macaque CD34 +cells of either PB or BM origin without further cytokine stimulation *ex vivo*.

Transplantation of rhesus macaque with the lentivirus vector-transduced PB CD34 +cells. Transduced CD34 +cells were infused into irradiated rhesus macaques to study gene expression, multilineage marking, and long-term reconstitution. Two animals received autologous transplants with transduced PB CD34 +cells. Marking and expression of the vector were monitored in different hematopoietic lineages by flow cytometric analysis for EGFP expression and were further confirmed by quantitative DNA PCR. EGFP expression was detected in PB cells from macaques transplanted with autologous PB CD34 +cells beginning at 1 week after transplant. In both animals, EGFP marking was observed in granulocyte, monocyte, lymphocyte, red blood cell, and platelet populations and has been stable for 5 years to date. We also examined the presence of the vector DNA in PB of animals 95E132 and 96E035 by quantitative DNA PCR analysis at 22 weeks posttransplantation. The highest EGFP-marked animal (95 E132) had highest amount of vector DNA (2. 2 copies per 100 cells).

EGPF expression from RhMLV promoter was higher than that from CMV promoter in rhesus macaque PBMC. Previously, we showed that the lentiviral vector bearing the Rh-MLV LTR promoter has 5- to 10-fold-higher EGFP expression in human T lymphocytes than another lentiviral vector, bearing the CMV immediate-early promoter in vitro.[3] We therefore examined the fluorescence intensity of EGFP expression in multiple lineages of rhesus hematopoietic cells and compared their mean fluorescence intensities (MFI) of EGFP expression to that of the previously described animal (RC505) that was transplanted with CD 34 +cells transduced by the CMV promoter-bearing lentiviral vector.[4] The MFI of EGFP expression in the transduced hematopoietic cells of animal RC505 has been stably maintained since week 13 posttransplantation and remains unchanged for 5 years to date. We found that the lentiviral vector with Rh-MLV promoter consistently gave 2- to 10-fold-greater levels of gene expression than the CMV promoter in granulocyte, monocyte, lymphocyte, RBC, and platelet populations. To date, this higher level of EGFP expression has been stably maintained for 5 years. Taken together, we confirm that the RhMLV promoter allows a higher level of EGFP expression in multiple lineages of rhesus macaque hematopoietic cells.

Long-term marking was not achieved in rhesus macaques transplanted with lentiviral vector-transduced BM CD34 +cells. Two animals (96 E041 and 95E131) received autologous transplants with lentiviral vector-transduced BM CD34 +cells. Low percentages of EGFP +cells were initially detected in PBMC of both animals and were found to diminish gradually over time. In one animal (96E041), the EGFP marking in all lineages was lost at 32 weeks posttransplantation. In the second animal (95 E131), EGFP marking was lost in granulocytes, monocytes, platelets, and RBC at 26 weeks posttransplantation. Marking was observed only in the lymphocyte population at 26 weeks posttransplantation. To date, EGFP marking

in the lymphocyte population has been stable for 61 weeks.

Gene transfer of human γc. We further tested the potential of our lentiviral vector system by modeling gene transfer of human common γc. Mutations of the common c have been identified in X-linked SCID patients and have been shown to contribute to the impaired lymphocyte development in these patients.[5] Transplantation of the CD34 +cells that were transduced with retrovirus vector carrying the γc cDNA has been shown to restore normal lymphocyte development and functions in the X-linked SCID patients and animal models.[6] We inserted human γc cDNA in the place of EGFP cDNA in the lentiviral vector. Human common c expression from the vector was confirmed in HeLa cells and rhesus macaque primary PBMC in vitro. Two animals were transplanted with autologous PB CD34 +cells transduced with the HIV-1 vector bearing human common γc. The gene transfer of γc was determined by quantitative DNA PCR analysis to be 0.42 and 0.001 copies/100 cells. Cell surface expression of γc was determined by flow cytometric analysis on the rhesus macaque lymphocyte population. Expression of the γc was stable for 27 weeks.

Discussion

The modeling of gene therapy vectors in nonhuman primate model systems is critical for evaluation of potential efficacy in the clinical setting. Here, we use a lentivirus vector bearing a RhMLV-related promoter to demonstrate marking in multiple lineages of hematopoietic cells. The CD34 +cells were not stimulated in vitro prior to transduction. The levels of gene expression are significantly higher than that of the non-self-inactivating lentivirus vector utilizing an internal CMV promoter. The level of marking is stable for approximately 5 years to date for those animals transplanted with mobilized PB CD34 +cells. All animals are healthy. Thus, this study represents the first demonstration of the use of lentivirus vectors to transduce non-ex vivo cytokine-stimulated CD34 +cells in a primate.

In contrast to mobilized PB, transduction and transplant of mobilized BM cells did not result in efficient marking. Indeed, in the two animals, marking gradually declined, with loss of marking in most lineages by 26 (95E 131) and 32 (96E041) weeks after transplant. There was approximately a 3-week time difference in the rate of reconstitution utilizing PB CD34 +cells compared to BM CD34 +cells. Thus, these results may be due to more efficient reconstitution utilizing PB CD34 +cells or alternatively mobilization of CD34 +target cells from BM to the PB. Our results are consistent with those observed in human clinical studies.[7]

Having developed conditions for transplant and marking, we tested those strategies with a potential human therapeutic gene. Cavazzana-Calvo et al. demonstrated therapeutic benefit in humans following transplant of X-linked SCID patients, using a murine retrovirus vector.[6] We therefore used that gene to model potential human therapeutic gene transfer strategies utilizing the lentivirus gene transfer approach described here. We achieved marking and expression of human common γc using the lentivirus vector in lymphocytes of the rhesus macaque, utilizing non-cytokine-stimulated CD34 +cells. Although the levels are relatively low, we anticipate that similar to the transplant into X-linked SCID patients with an oncoretrovirus vector,[6] under conditions of selective pressure, the cγ transduced cells would be expanded. Thus, these studies in nonhuman primate animals provide model strategies and the basis for the future application of lentivirus vectors to potentially treat human diseases.

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