TaKaRa

Nonhuman primate model for HIV-1 gene therapy using endoribonuclease MazF transduced CD4$^+$ T cells in the presence of SHIV 89.6P infection

Naoki Saito[1], Hideto Chono[1], Hiroaki Shibata[2], Naohide Ageyama[2], Yasuhiro Yasutomi[2], Junichi Mineno[1]

[1] Center for Cell and Gene Therapy, Takara Bio Inc.
[2] Tsukuba Primate Research Center, National Institute of Biomedical Innovation.

SUMMARY

MazF, an endoribonuclease derived from *Escherichia coli*, was explored for the possibility of its use to HIV-1 gene therapy. Rhesus macaques were infected with pathogenic simian/human immunodeficiency virus (SHIV 89.6P) and then autologous MazF-transduced CD4$^+$ T (MazF-T) cells were infused. MazF-T cells were detected clearly in peripheral blood throughout the experimental period. The CD4 count values increased in all rhesus macaques treated with MazF-T cells and the plasma viral load values gradually decreased. The antibodies against MazF were not detected and MazF specific immune responses were not observed. These data suggest that the transplantation of autologous MazF-T cells in the presence of SHIV is safe and low immunogenic. Long-term persistence, safety and efficacy of the MazF-T cells in the non-human primate model suggest that autologous transplantation of MazF-T cells is an attractive strategy for HIV-1 gene therapy.

BACKGROUND

MazF is an endoribonuclease which specifically cleaves ACA sequence of single-strand RNAs. The conditional expression of MazF under the control of HIV-1 LTR promoter rendered CD4$^+$ T cells resistant to HIV-1 or SHIV replication without affecting cell growth (Chono *et al*., 2011a, Okamoto *et al*., 2013). A key regulator for MazF expression in this system is HIV-1 Tat protein, which is known to activate transcription from the HIV-1 LTR. In this system, Tat induces both HIV-1 replication and MazF expression (Fig. 1). Additionally, *in vivo* safety of MazF-T cells was shown in cynomolgus macaque model in the absence of viral infection (Chono *et al*., 2011b).

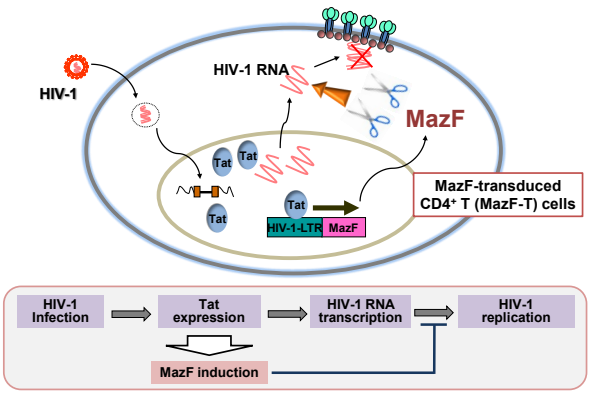

Fig. 1. Strategy of endoribonuclease MazF based anti-HIV-1 gene therapy.

RESULTS

In order to address safety, persistence, and efficacy of MazF-T cells in non-human primate model *in vivo* in the presence of viral infection, six rhesus macaques were infected with SHIV 89.6P; four were treated with MazF-T cell infusions and two were treated with control ZsGreen1-transduced CD4$^+$ T (ZsG-T) cell infusions (Fig. 2).

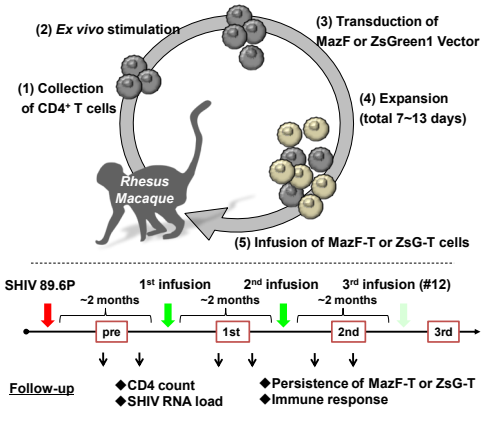

Fig. 2. Experimental design of non-human primate model for anti-HIV-1 gene therapy.

After the treatment of gene therapy, MazF-T cells were detected clearly in peripheral blood over a half year (data not shown). The CD4 count values increased with statistical significance in all four rhesus macaques treated with MazF-T cells (Fig. 3A). On the other hand, ZsG-T cells disappeared within two months after the transplantation and did not contributed to the CD4 count values. The plasma viral load values of MazF-T-transplanted rhesus macaques gradually decreased while that of ZsG-T-transplanted rhesus macaques increased rather than decreased (Fig. 3B). The antibodies against MazF were not detected and MazF specific immune responses were not observed by the interferon-γ enzyme-linked immunospot assay (data not shown). These data suggest that the transplantation of autologous MazF-T cells in the presence of SHIV is safe and low immunogenic.

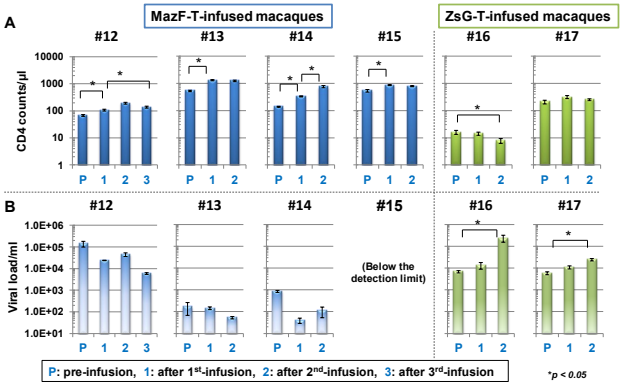

Fig. 3. Change of (A) CD4 Counts and (B)SHIV Viral load after the infusion of gene modified CD4$^+$T cells.

DISCUSSION/CONCLUSIONS

Gene therapy for HIV-1 should be aimed at reconstitution of HIV-resistant immune system as HIV-1 infection causes immunodeficiency due to reduction of CD4 cell counts in patients. It is quite important for gene modified cells not only to inhibit viral replication, but also to maintain their proper distribution with high persistence *in vivo*.

We showed that MazF-T cells, but not ZsG-T cells, survived even in the presence of SHIV with reduction of viral load, suggesting that MazF-T cells are protected from SHIV infection and showed little or no immunogenicity in rhesus macaques. In conclusion, long-term persistence, safety and efficacy in the non-human primate model suggest that autologous transplantation of MazF-T cells is an attractive strategy for HIV-1 gene therapy.

REFERENCES

Chono *et al*., 2011a, Hum Gene Ther. 22: 35-43.
Chono *et al*., 2011b, PLoS One. 6: e23585.
Okamoto *et al*., 2013, Hum Gene Ther Methods. 24: 94-103.

Summary of presentation at 19th annual meeting of Japan Society of Gene Therapy, July 4, 2013.