[S4-4]

CD7-Specific Single Chain Antibody Mediated Delivery of siRNA to T Cells Inhibits HIV Replication in a Humanized Mouse Model

Hongseok Ban[1], Priti Kumar[2], Nahyun Kim[1], Changson Choi[1], Premlata Shankar[2], and Sang-Kyung Lee[1]*

[1]*Department of Bioengineering, Hanyang University,*
[2]*Immune Disease Institute and Department of Pediatrics, Harvard Medical School, Boston, MA, USA*

Abstract

A major hurdle to the development of RNA interference as therapy for HIV infection is the delivery of siRNA to T lymphocytes which are difficult cells to transfect even *in vitro*. We have employed a single chain antibody to the pan T cell surface antigen CD7 was conjugated to an oligo-9-arginine peptide (scFvCD7-9R) for T cell-specific siRNA delivery in NOD/SCIDIL2γ-/- mice reconstituted with human peripheral blood lymphocytes (Hu-PBL). Using a novel delivery, we first show that scFvCD7-9R efficiently delivered CD4 siRNA into human T cells *in vitro*. *In vivo* administration to Hu-PBL mice resulted in reduced levels of surface CD4 expression on T cells. Mice infected with HIV-1 and treated on a weekly basis with scFvCD7-9R-siRNA complexes targeting a combination of viral genes and the host coreceptor molecule CCR5 successfully maintained CD4/CD3 T cell ratios up to 4 weeks after infection in contrast to control mice that displayed a marked reduction in CD4 T cell numbers. p24 antigen levels were undetectable in 3 of the 4 protected mice. scFvCD7-9R/antiviral siRNA treatment also helped maintain CD4 T cell numbers with reduced plasma viral loads in Hu-PBL mice reconstituted with PBMC from donors seropositive for HIV, indicating that this method can contain viral replication even in established HIV infections. Our results show that scFvCD7-9R could be further developed as a potential therapeutic for HIV-1 infection.

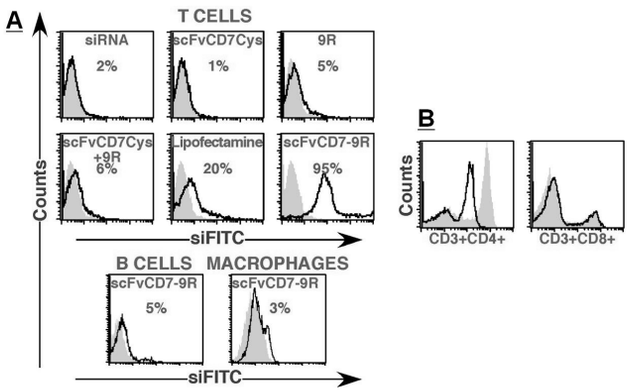
Background and significance

scFvCD7 is a mouse single chain antibody specific to human CD7 and has been successfully used for delivery of immunotoxins and soluble FasL to human T leukemia cells with no adverse effects (1). We conjugated scFvCD7 to a positively-charged oligo-9R peptide (scFvCD7-9R) to enable siRNA binding and delivery into T cells. Because there are no small animal models that can be infected with HIV-1,

considerable efforts have been invested to adapt immunodeficient mice as animal models for HIV-1 by engrafting them with human immune cells and tissues. We have used the recently validated humanized NOD/Lt-SCIDIL2rc-/- mouse (2), as a preclinical model for validating our delivery approach as a step towards RNAi based therapy for HIV-1.

Results

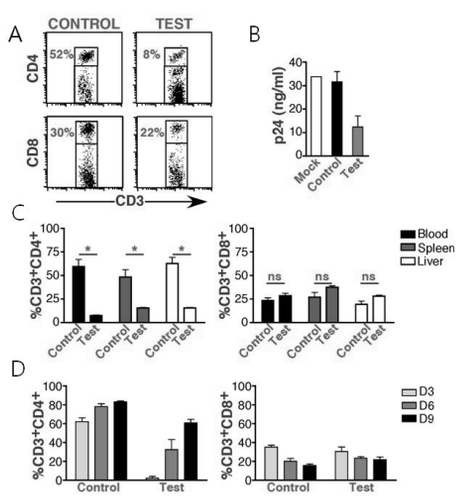
- scFvCD7-9R specifically delivers siRNA and silences target gene expression in cells that express surface CD7.



(A) Purified human $CD3^+$ T cells (upper panels), $CD19^+$ B cells and differentiated $CD14^+$ monocyte-derived macrophages (bottom panel) were treated with 200 pmol FITC-labeled siRNA alone or siRNA mixed with the indicated reagents (black histograms). Grey, filled histograms represent mock-transfected cells. scFvCD7-9R specifically delivered FITC-siRNA very effectively into purified human $CD3^+$ T cells. (B) PHA activated PBMC were treated with 400 pmole anti-huCD4 siRNA complexed to scFvCD7-9R and CD4 and CD8 expression levels (black histograms) on $CD3^+$ T cells monitored 60 h later. The mean fluorescent intensity of CD4 was reduced by almost a log on $CD3^+$ T cells. The silencing was specific since CD8 expression remained unaffected.

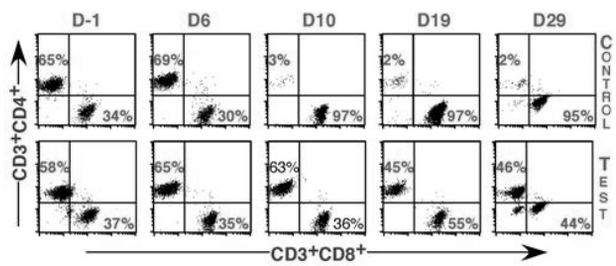
- scFvCD7-9R/siRNA mediated gene-silencing in T cells *in vivo* in Hu-PBL mice.

NOD/SCIDIL2rgc-/- mice were injected ip with healthy donor PBMC (Hu-PBL mice). After 15 days, groups of 3 mice were injected iv with 50 mg of siLuc (control) or siCD4 (test) siRNAs complexed to scFvCD7-9R twice (16 h apart) and human CD3+ T cells in peripheral blood, spleen and liver analyzed for CD4 and CD8 expression 60 h later. Representative dot plots from one mouse (A) and cumulative data from 3 mice (C) are shown. Surface expression of CD4 on T cells was significantly reduced in scFvCD7-9R/siCD4 treated, but not in control siLuc-treated mice. (B) PBMC isolated from treated Hu-PBL mice were PHA-stimulated and infected with HIVIIIB at a moi of 3. Culture supernatant collected on day 10 after infection was tested for p24 antigen levels in triplicate by ELISA. HIV-1



p24 levels were significantly reduced in culture supernatants from treated cells confirming reduced permissibility to viral infection after knockdown of CD4 expression. (D) Mice were treated with siRNA as in (A) three times and CD4 and CD8 expression in peripheral blood T cells determined on days 3, 6 and 9 after the last injection. Silencing was maximal during the first 3 days and expression was gradually regained by 9 days.

- scFvCD7-9R complexed to anti-HIV siRNAs can efficiently prevent HIV infection in HU-PBL mice challenged with HIV-1.



Hu-PBL mice were treated iv with 50 mg siCCR5 or control siLuc 14 days after reconstitution. Two days later, the mice were intraperitoneally infected with 10,000 TCID50 of HIVBaL and subsequently either mock-treated (n=2) or treated with a combination of 50 mg of siCCR5/vif/tat (test, n=4) or siLuc (control, n=4) complexed to scFvCD7-9R as indicated in (A). CD3/CD4/CD8 T cell levels were monitored by flow cytometry.

Conclusion

scFvCD7 conjugated to 9R is a promising tool for therapeutic delivery of antiviral siRNAs to T cells for the treatment of AIDS. Multiplexing antiviral siRNAs with scFvCD7-9R can serve as effective antiviral treatment analogous to combination antiretroviral therapy for HIV in a clinical setting.

References

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