Journal of Biotechnology 156 (2011) 218–226

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Transient proteasome inhibition as a strategy to enhance lentiviral transduction of hematopoietic CD34+ cells and T lymphocytes: Implications for the use of low viral doses and large-size vectors

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ARTICLE INFO

Article history:
Received 2 May 2011
Received in revised form 8 August 2011
Accepted 1 September 2011
Available online 10 September 2011

Keywords:
Lentiviral transduction
Transduction efficiency
Hematopoietic stem cells
T lymphocytes

ABSTRACT

The proteasome system restricts lentiviral transduction of stem cells. We exploited proteasome inhibition as a strategy to enhance transduction of both hematopoietic stem cells (HSC) and T lymphocytes with low dose or large-size lentiviral vectors (LV). HSC showed higher transduction efficiency if transiently exposed to proteasome inhibitor MG132 (41.8% *vs* 10.7%, $p < 0.0001$). Treatment with MG132 (0.5 μM) retained its beneficial effect with 3 different LV of increasing size up to 10.9 Kb ($p < 0.01$). We extended, for the first time, the application of proteasome inhibition to the transduction of T lymphocytes. A transient exposure to MG132 significantly improved lentiviral T-cell transduction. The mean percentage of transduced T cells progressively increased from 13.5% of untreated cells, to 21% ($p = 0.3$), 30% ($p = 0.03$) and 37% ($p = 0.01$) of T lymphocytes that were pre-treated with MG132 at 0.1, 0.5 and 1 μM, respectively. MG132 did not affect viability or functionality of HSC or T cells, nor significantly increased the number of integrated vector copies. Transient proteasome inhibition appears as a new procedure to safely enhance lentiviral transduction of HSC and T lymphocytes with low viral doses. This approach could be useful in settings where the use of large size vectors may impair optimal viral production.

1. Introduction

Gene-transfer strategies targeting hematopoietic stem cells (HSC) and T lymphocytes hold great potentials for important clinical applications (Bonini et al., 1997; Horn et al., 2007; Kohn, 2010; Neschadim et al., 2007), correcting various defects of the haematopoietic system (Aiuti, 2002; Aiuti et al., 2009; Aiuti and Roncarolo, 2009; Aiuti et al., 2002; Kohn, 2008) and improving adoptive immunotherapy strategies (Johnson et al., 2009; Varela-Rohena et al., 2008; Yang et al., 2008). Their effective and safe scale-up into clinical settings would require high gene-transfer efficiency with a low number of vector copies transferred per cell. Lentiviral vectors (LV), with their ability to transduce cells with low proliferation rates are ideal vehicles to target both HSC and T lymphocytes (Ailles et al., 2002; Cavalieri et al., 2003; Manganini et al., 2002; Naldini et al., 1996; Sirven et al., 2001; Verhoeyen et al., 2003).

Preclinical models have shown that LV can efficiently transduce HSC which retain the ability to self-renew and subsequently differentiate into all of the hematopoietic lineages (Ailles et al., 2002; Piacibello et al., 2002). LV may transfer tumor-specific T-cell receptors (Cavalieri et al., 2003; Circosta et al., 2009; Verhoeyen et al.,

Abbreviations: CB, cord blood; eGFP, enhanced green fluorescent protein; FL, flt3/flk2 ligand; HSC, hematopoietic stem cells; HLA, human leukocyte antigen; IL-6, interleukin-6; IL-2, interleukin-2; LV, lentiviral vectors; MLR, mixed lymphocyte reaction; MOI, multiplicity of infections; PBMC, peripheral blood mononuclear cell; PGK, phosphoglycerate kinase; PHA, phytohemagglutinin; P/S, penicillin/streptomycin; SCF, stem cell factor; TPO, thrombopoietin; VCM, virus-conditioned medium; ΔLNGFR, low-affinity nerve growth factor receptor.

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doi:10.1016/j.jbiotec.2011.09.001

2003), transgenes encoding for specific cytokines or conferring drug-resistance and even mediating T cell inactivation if required (Bonini et al., 1997; Lupo-Stanghellini et al., 2010; Sangiolo et al., 2007). LV have already been used in phase I clinical trials with safe and relatively efficient gene delivery to T cells that have good *in vivo* persistence (Kohn, 2007).

The obtainment of high and stable transduction efficiency is crucial for the success and possible clinical translation of gene-transfer strategies with both HSC and T-lymphocytes. The transfer and integration of high numbers of vector copies into target cells might address this issue; however, it could increase the risk of oncogenic transformation (Baum, 2007; Baum et al., 2003; Hacein-Bey-Abina et al., 2003) and the preparations of high-titer virus-conditioned medium (VCM) are not always possible. In large-scale transduction, like those required for clinical trials, technical limitations and safety concerns make it necessary or desirable to conduct transductions with low multiplicity of infection (MOI) (Aiuti and Roncarolo, 2009; Matrai et al., 2010; Persons, 2009; Smith et al., 2004). Moreover, in recent years, large-size LV, carrying additional reporter or selectable genes have become more commonly used (Amendola et al., 2005). The increased size of the LV results in lower titer VCM preparations; VCM titer decreases in a semilogarithmic manner as insert size and proviral length increase (Kumar et al., 2001), consistent with reduced packaging efficiency (Terwilliger et al., 1989). Titers of bidirectional vectors were reported to be approximately 10-fold reduced in comparison to unidirectional vectors (Maetzig et al., 2009). New strategies to maximize the LV transduction efficiency with low MOI and large-size vectors are needed and would help the clinical application of gene transfer approaches. Recent studies showed that the proteasome activity restricts LV entry and integration into various types of stem cells (Santoni de Sio et al., 2008). Transient pharmacological inhibition of the proteasome significantly enhanced LV transduction of HSC (Santoni de Sio et al., 2006, 2008). The exact mechanism of this effect is not completely known. Santoni De Sio et al. showed that the proteasome action occurs mainly at post entry steps and it does not seem to be mediated by the activation of nuclear factor-kB, a major signaling pathway modulated by the proteasome, or to correlate with high proteasome activity; interestingly the interaction of the virus core with cyclophilin A, a molecule with an important role in the uncoating process, is required to maximize the effect of the proteasome inhibitor on the infection pathway (Santoni de Sio et al., 2008).

In the present work we extended these findings, exploiting the proteasome inhibition as a strategy to improve the LV transduction of HSC with low MOI or large size LV, then applied for the first time this approach to improve the transduction efficiency of differentiated human T-lymphocytes.

2. Materials and methods

2.1. Lentiviral vectors

VSV-G pseudotyped third-generation LV were produced by transient four-plasmid co-transfection into 293T cells as described (Amendola et al., 2005). The transfer vector pRRL.sin.PPT.hPGK.eGFP.Wpre of 3.8 Kb (LV-PGK.eGFP) was described elsewhere (Follenzi et al., 2000). The 5.4 Kb bidirectional LV pCCL.sin.cPPT.SV40polyA.CTE.eGFP.mCMV.hPGK.ΔLNGFR.Wpre (LV-eGFP.mCMV.hPGK.ΔLNGFR) was kindly provided by Prof. Naldini (Amendola et al., 2005). The 7.4 Kb bidirectional LV pCCL.sin.cPPT.SV40polyA.CTE.eGFP.mCMV.hPGK.c-KIT.Wpre (LV-eGFP.mCMV.hPGK.c-KIT) was obtained by replacing the 842 bp ΔLNGFR fragment on LV-eGFP.mCMV.hPGK.ΔLNGFR with the 2931-bp c-KIT coding fragment cleaved from the pENTR™221 plasmid (Invitrogen Ltd, http://www.invitrogen.com). The 10.9 Kb bidirectional LV pCCL.sin.cPPT.SV40polyA.CTE.ΔLNGFR.mCMV.hPGK.Bcr/Abl.Wpre (LV-ΔLNGFR.mCMV.hPGK.Bcr/Abl) was obtained by cloning the 5.4 Kb p210 Bcr/Abl coding fragment (cleaved from pSLX CMV vector, kindly provided by Griffin J.D., Dana Farber Cancer Institute, Boston) into the bidirectional LV pCCL.sin.cPPT.SV40polyA.CTE.ΔLNGFR.mCMV.hPGK.Wpre kindly provided by Prof. Naldini. Physical titers for lentiviral vector stocks were determined based on p24 antigen content (HIV-1 p24 ELISA kit; PerkinElmer, Milano, Italy). Functional titers were determined upon the expression of eGFP or ΔLNGFR by FACS analysis after limiting dilution on Hela cells.

2.2. Proteasome inhibition

Proteasome inhibitor MG132 was purchased from Calbiochem. For $CD34^+$ cell transduction, MG132 was added to the culture medium, at a concentration of 0.5 μM, 6 h before the addition of VCM and maintained until cells were rescued 16–20 h after transduction. For T cell transduction, MG132 was added to the culture medium at concentrations ranging from 0.1 to 1 μM. Six hours later cells were washed and VCM added for transduction.

2.3. Collection and transduction of $CD34^+$ cells

Umbilical cord blood (CB) was obtained, after written informed consent, at the end of full-term pregnancies, after clamping and cutting of the cord, by drainage of blood into sterile collection tubes containing the anticoagulant citrate-phosphate dextrose. We conducted our studies in compliance with the principles of the Declaration of Helsinki. $CD34^+$ cells were obtained with MiniMACS (Miltenyi Biotech, Gladbach, Germany) separations as previously described (Piacibello et al., 1999).

Virus conditioned media (VCM) were prepared and titered according to standard procedures (Dull et al., 1998; Follenzi et al., 2000). Before transduction $CD34^+$ cells were prestimulated for 24 h with flt3/flk2 ligand (FL) and stem cell factor (SCF), (both at 100 ng/ml and kindly provided by Amgen, Thousand Oaks, CA, http://www.amgen.com), thrombopoietin (TPO), (a gift from Kirin Brewery Co. Tokyo, http://www.kirin.co.jp/english) and interleukin-6 (IL-6) (Peprotech, Rocky Hill, NJ, http://www.peprotech.com) both at 20 ng/ml, in serum-free medium (StemSpam; StemCell Technologies, Vancouver, BC, Canada). For each LV transduction, 1×10^5 prestimulated $CD34^+$ cells were resuspended in StemSpam medium with the same cytokines described above. VCM was added at an MOI of 5 for 16–20 h, cells were harvested, washed twice and used to initiate stroma-free expansion cultures.

2.4. Collection and transduction of human T lymphocytes

Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood by Ficoll-Hypaque gradient separation. Before transduction, cells were cultured for 72 h at a density of 1×10^6/ml in RPMI (Sigma–Aldrich Co., St. Louis, MO, USA) supplemented with heat-inactivated 10% fetal bovine serum (FBS; Sigma–Aldrich Co., St. Louis, MO, USA) and 1% penicillin/streptomycin (P/S) (Sigma–Aldrich Co., St. Louis, MO, USA) in the presence of interleukin-2 (IL-2) (Proleukin, Novartis, Holzkirchen, Germany, http://www.proleukin.com/) and monoclonal antibody (mAb) anti CD3 (Pharmingen, San Diego, CA) at concentrations of 100 IU/ml and 100 ng/ml respectively.

Transduction was carried out in flat-bottomed 48-well plates (Corning, Costar). For each LV transduction, 5×10^5 cells were resuspended in 0.5 ml of RPMI, supplemented with heat-inactivated 10% FBS, 1% P/S and IL2 (100 IU/ml). VCM was