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Highly efficient gene transfer into mobilized CD34$^+$ hematopoietic cells using serotype-5 adenoviral vectors and BoosterExpress Reagent

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Objective. **To optimize transduction efficiency of mobilized CD34$^+$ cells with serotype-5 adenoviruses (Ad5s), we investigated the activity of the chemical cocktail BoosterExpress Reagent in enhancing Ad5-mediated gene transfer into CD34$^+$ cells.**

Methods. **Enriched CD34$^+$ cells were transduced with three different Ad5s at increasing multiplicity of infections (MOIs) in the presence and absence of BoosterExpress Reagent. Percentages of transduced cells and levels of transgene expression were quantified by flow cytometry. Propidium iodide staining and colony growth were used to assess the toxicity of the transduction protocol. Expression of adenovirus receptors was investigated by flow cytometry.**

Results. **Irrespective of the Ad5 used, transduction with BoosterExpress Reagent using an MOI of 500 resulted in an average six- to seven-fold increase of transduction efficiencies and 1.5- to 2-fold increase of the levels of transgene expression, which could be detected up to 7 days post-transduction. Although BoosterExpress Reagent alone did not affect the plating efficiency of CD34$^+$ cells, adenovector transduction plus BoosterExpress Reagent significantly reduced the plating efficiency due to the marked increase of transduced cells. However, adenoviral transduction in the presence of BoosterExpress Reagent failed to significantly reduce the recovery of CD34$^+$ cells as compared with transduction in the absence of the compound. Coxsackievirus and adenovirus receptor as well as $\alpha_v\beta_3$, $\alpha_v\beta_5$, α_5, and β_1 integrins were upregulated by BoosterExpress Reagent.**

Conclusions. **BoosterExpress Reagent allows high-levels of durable transgene expression, thus making CD34$^+$ cells a suitable target for Ad5-mediated gene transfer.** © **2007 International Society for Experimental Hematology. Published by Elsevier Inc.**

Serotype-5 adenoviruses (Ad5s) require a two-step process for a productive delivery. After the fiber knob region attaches to the coxsackievirus and adenovirus receptor (CAR) on the target cell surface [1–3], clathrin-dependent viral internalization occurs through the interaction between the capsid penton base (Arg-Gly-Asp [RGD] motif) and the vitronectin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ [4]. Due to low expression of both CAR and α_v integrins, CD34$^+$ cells do not represent optimal targets for adenovirus transduction [5,6]. Despite using high multiplicities of infection (MOI), cytokine stimulation, and long incubation times, only a limited fraction of CD34$^+$ cells is susceptible to adenovectors [7–13].

However, adenovectors remain attractive vehicles for gene delivery into CD34$^+$ cells [14–18]. In fact, they infect cycling and noncycling cells, driving a very efficient gene expression and transducing up to ~8 kb of recombinant DNA. Additionally, adenovectors can be prepared at high titers and do not integrate in the genome, thus avoiding the risk for insertional mutagenesis. Several approaches have been proposed to increase Ad5-driven transduction of CD34$^+$ cells, including the addition of polycations or cationic lipids [19–23] and the improvement of the classic attachment/internalization pathways [4,24–26]. More recently, CAR-independent transduction strategies have been developed, including targeted adenovectors [27–29] and alternative serotypes, such as Ad11 and Ad35, which facilitate expression in hematopoietic cells [28,30]. Overall, adenovirus-mediated gene delivery into CD34$^+$ cells remains unsatisfactory, and approaches proposed so far to

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enhance adenoviral transduction of $CD34^+$ cells resulted in limited improvements outweighed by a substantially increased toxicity [13,31–35].

In the present study, we demonstrate that a commercially available chemical cocktail, the BoosterExpress Reagent (Gene Therapy Systems Inc., San Diego, CA, USA), remarkably enhances the efficacy of Ad5-mediated gene transfer into $CD34^+$ cells.

Materials and methods

Chemicals and cytokines

BoosterExpress Reagent (BoosterReagent #1) was purchased from Gene Therapy Systems Inc. Granulocyte-colony stimulating factor (G-CSF, Neupogen) was from Roche (Milan, Italy, EU), and stem cell factor (SCF) from StemCell Technologies Inc. (Vancouver, Canada).

$CD34^+$ cells

G-CSF-mobilized $CD34^+$ cells were enriched using the AutoMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany, EU) from the blood of cancer patients undergoing stem cell mobilization. The study was approved by the institutional Ethical Committee, and written informed consent was obtained from each patient. The purity of enriched $CD34^+$ cells was ≥95%.

Adenoviral vectors

The conventional Ad5 and the chimeric fiber-modified Ad5/F35 replication-incompetent vectors expressing green fluorescent protein (GFP) [36] were purchased from the Center for Cell and Gene Therapy (Baylor College of Medicine, Houston, TX, USA). The replication-deficient serotype-5 adenovirus encoding the human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) cDNA under the control of the cytomegalovirus (CMV) promoter was purchased from the Gene Transfer Vector Core (University of Iowa, Iowa City, IA, USA) [37]. The replication-incompetent adenovector Ad5-AP (Ad-MFG-AP) has been previously described [10]. The vector consisted of an adenovirus type 5 backbone containing the human placental alkaline phosphatase (AP) gene engineered to retain the membrane anchoring domain. Both TRAIL and AP gene products were expressed on the cell surface of transduced cells and could be detected by cytometry. Viral titers were expressed as plaque forming unit (pfu)/mL, as determined by plaque assay and tissue culture infectious dose 50 on 293A cells (Invitrogen, Milan, Italy). The MOI was calculated as pfu/cell.

Adenoviral transduction of $CD34^+$ cells

Freshly purified or frozen $CD34^+$ cells were washed twice, resuspended at 2×10^6 cells/mL in serum-free Iscove's modified Dulbecco's medium (IMDM; Cambrex Bio Science, Verviers, Belgium) and then plated in 35-mm Petri dishes. According to the standard protocol, transduction was performed by adding appropriate dilutions of adenovector stocks directly into the culture medium, allowing final MOIs ranging from 10 to 500 pfu/cell. After 2 hours of incubation (37°C, 5% CO_2), cultures were supplemented with 2× complete medium (IMDM 20% fetal bovine serum [FBS], 4 mM L-glutamine, 20 ng/mL SCF, 20 ng/mL G-CSF), to achieve a final concentration of 1×10^6 cells/mL. Four hours later, BoosterExpress Reagent was added at final dilution of 1:200, and $CD34^+$ cells were incubated overnight. The cells were then harvested by pipetting and tapping the flask, repeatedly washed (3×) in complete medium, and the transduction efficiency was finally quantified by flow cytometry. Cell viability was evaluated with the trypan blue dye exclusion test. Propidium iodide (PI; Bender MedSystems, Vienna, Austria, EU) staining was performed by adding 10 μL of PI immediately before cytometric analysis. During time course experiments, transduced cells were incubated in 1× complete medium (IMDM 10% FBS, 2 mM L-glutamine, 10 ng/mL SCF, 10 ng/mL G-CSF), and aliquots were harvested on a daily basis for 7 days and analyzed by flow cytometry. To define the role of BoosterExpress Reagent in adenovirus-mediated transduction of $CD34^+$ cells, in selected experiments the compound was added either prior to or after exposure to the vector. For preinfection treatment, $CD34^+$ cells (2×10^6 cells/mL) were incubated for 5 hours in serum-free IMDM containing BoosterExpress Reagent (1:200). The cells were then extensively washed to remove the compound and exposed to Ad5-GFP for an additional 2 hours, before being supplemented with serum-containing medium and incubated overnight. For postinfection treatment, $CD34^+$ cells (2×10^6 cells/mL) were exposed to Ad5-GFP in serum-free IMDM for 2 hours. The cells were then extensively washed to remove the unbound adenovirus and incubated in complete medium containing BoosterExpress Reagent (1:200). After overnight incubation, the cells were analyzed by flow cytometry and compared with cells transduced at the same MOI using the standard transduction protocol.

Flow cytometry

Transduction efficiency of $CD34^+$ cells was analyzed by either collecting the GFP fluorescence in the fluorescence light-1 channel or labeling the cells with the phycoerythrin (PE)-conjugated anti-TRAIL monoclonal antibody (clone Rik-2; Becton-Dickinson, San Jose, CA, USA), or the purified anti-AP monoclonal antibody (clone 3F6; Serotec, Oxford, UK) revealed by the fluorescein isothiocyanate (FITC)-conjugated F(ab′)2 fragment goat anti-mouse immunoglobulin G (Jackson ImmunoResearch, Cambridgeshire, UK, EU). Adenovirus receptor expression was analyzed using the FITC- or PE-conjugated antibodies against major histocompatibility complex (MHC) class I (clone W6/32; Sigma, Milan, Italy), $\alpha_v\beta_3$ (clone 23C6), α_4 (clone 9F10), α_5 (clone IIA1), and α_M (clone D12) (all from Becton-Dickinson). The purified anti-$\alpha_v\beta_5$ (clone P1F6) and β_1 (clone P4C10) antibodies were purchased from Chemicon (Temecula, CA, USA) and revealed by the FITC-conjugated goat anti-mouse antibody (Jackson ImmunoResearch). The anti-CAR antibody was purified from the supernatant of RmcB hybridoma cell line (American Type Culture Collection, Rockville, MD, USA) and detected using streptavidin-PE (Becton-Dickinson) [26,38]. $CD34^+$ cells were analyzed for the appropriate marker expression by two-color flow cytometry using an allophycocyanin-conjugated mouse anti-human CD34 monoclonal antibody (clone 8G12; Becton-Dickinson). Briefly, cells were washed twice in cold phosphate-buffered saline containing 2% FBS and incubated with the appropriate fluorochrome-conjugated or purified antibodies (30 minutes, 4°C). After washing, the cells were immediately analyzed, or, where required, additionally incubated with secondary reagent (30 minutes at 4°C) before being analyzed on a FACSCalibur flow cytometry system (Becton-Dickinson) using Cell Quest software (Becton-Dickinson), run on a Macintosh PowerMac G4 personal computer (Apple Computer Inc., Cupertino, CA, USA).