articles

molecular pharmaceutics

A Single-Component CD40-Targeted Adenovirus Vector Displays Highly Efficient Transduction and Activation of Dendritic Cells in a Human Skin Substrate System

Nikolay Korokhov,*,† Sam C. Noureddini,† David T. Curiel,‡ Saskia J. A. M. Santegoets,§ Rik J. Scheper,§,|| and Tanja D. de Gruijl||

VectorLogics, Inc., 550 11th Street South, Birmingham, Alabama 35294, Division of Human Gene Therapy, Departments of Medicine, Pathology, and Surgery, and Gene Therapy Center, University of Alabama at Birmingham, Birmingham, Alabama 35294, Department of Pathology, Vrije Universiteit Medical Center, Amsterdam, The Netherlands, and Division of Immunotherapy, Department of Medical Oncology, Vrije Universiteit Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

Received January 18, 2005

Abstract: Dendritic cell (DC) based tumor vaccination usually involves the administration of ex vivo generated autologous DC. Transduction of DC by viral vectors in vivo has been proposed as a more standardized and easily clinically applicable approach. Previously, we have reported that an Ad5 vector targeted to CD40 via genetic capsid incorporation of CD40L achieves selective transduction of DC in vitro. In the present study, we evaluate the ability of this vector to deliver transgenes in a stringent human substrate system. We report the capacity of this CD40-targeted vector to infect, with high efficiency, cutaneous DC resident in human skin explants, while simultaneously inducing their activation and maturation. This latest generation of single-component, fully targeted vectors should make feasible the clinical testing of in vivo DC-targeted vaccines.

Keywords: Adenovirus; dendritic cells; targeting; CD40 ligand

Introduction

A variety of strategies to achieve active immunization exploiting dendritic cells (DC) have been proposed. These diverse approaches seek to capitalize on the central role played by DC in the generation of potent and long lasting immune responses. The majority of these efforts have sought to achieve direct antigen loading of DC by modification of these cells ex vivo,[1−3] by both genetic and nongenetic methods. Despite the rapid clinical translation of this approach, significant disadvantages may accrue to the ex vivo modification of DC. In this regard, harvest of autologous precursors and the subsequent generation of large numbers of DC is laborious and costly. Over and above these considerations, the optimal ex vivo DC preparation to achieve proper migration to lymph nodes and CTL activation upon in vivo transfer remains to be established. By targeting and triggering DC in vivo, their natural functions may be exploited to effect the priming of CTL.[4−7] Direct in vivo administration of DC-targeted vectors may thus present a more attractive and standardized alternative for vaccination.

* Corresponding author. Mailing address: VectorLogics, Inc., 550 11th Street South, Birmingham, AL 35294. Tel: (205) 933-8378. Fax: (205) 933-5836. E-mail: nkorokhov@vectorlogics.com.

† VectorLogics, Inc.

‡ University of Alabama at Birmingham.

§ Department of Pathology, Vrije Universiteit Medical Center.

|| Division of Immunotherapy, Department of Medical Oncology, Vrije Universiteit Medical Center.

(1) Gunzer, M.; Grabbe, S. Dendritic Cells in Cancer Immunotherapy. *Crit Rev. Immunol.* **2001**, *21*, 133−145.

(2) Kirk, C. J.; Mule, J. J. Gene-Modified Dendritic Cells for Use in Tumor Vaccines. *Hum. Gene Ther.* **2000**, *11*, 797−806.

(3) Nencioni, A.; Brossart, P. New Perspectives in Dendritic Cell-Based Cancer Immunotherapy. *BioDrugs* **2001**, *15*, 667−679.

(4) Corr, M.; von Damm, A.; Lee, D. J.; Tighe, H. In Vivo Priming by DNA Injection Occurs Predominantly by Antigen Transfer. *J. Immunol.* **1999**, *163*, 4721−4727.

10.1021/mp050002w CCC: $30.25
Published on Web 04/14/2005

To this end we have employed retargeting approaches to route adenoviral (Ad) vectors via the CD40 pathway as a means to circumvent the paucity of the virus's primary receptor, the coxsackie and adenovirus receptor (CAR), on the surface of DC and to selectively target DC in situ. CD40-targeted Ad-mediated transduction of DC has been successfully realized through the use of bispecific antibody conjugates[8] or through bispecific adapter proteins consisting either of single chain antibody (scFv)-scFv[9] or of soluble CAR-scFv fusions.[10] With these CD40-targeted Ad complexes we have shown an enhanced and more selective transduction as well as a simultaneous CD40-dependent maturation of DC.[8,11,12] However, these approaches required the production of several components followed by physical association to form targeted vector complexes. Evidently, the presence of an excess of free targeting ligands in analyzed samples leaves open questions about the causal component of DC activation and the ability of targeting ligands as integral components of viral particles to induce this process with similar efficiency. Direct incorporation of the TNF-like domain of human CD40L into the Ad capsid,[13] preserving the targeting ability of the genetically modified Ad vector, now allows us to answer these questions and offers a single-component targeted vector for direct clinical application.

Here, we show the ability of such a vector encoding both GFP and the carcinoembryonic antigen (CEA) to infect monocyte-derived dendritic cells (MoDC) with high efficiency. Importantly, we demonstrate that intradermal (i.d.) injection of the Ad vector in human skin explants results in targeted, enhanced gene transfer to migrating DC, as well as their phenotypic maturation.

Materials and Methods

Cell Lines. Human embryonal kidney cell line 293 was purchased from the American Type Culture Collection (ATCC; Manassas, Va). Generation, properties, and maintaining of cell lines 293.CD40 and 293FΔTAYT, which are derivatives of cell line 293 expressing human CD40 or a mutant form of the fiber, respectively, have been described previously.[13]

Genetic Engineering. The CEA expression cassette was first assembled into a transfer vector by subcloning of the 2.2-kb *Not*I-*Hin*dIII fragment of pBlu-CEA plasmid (kindly provided by Dr. Jeffrey Schlom, NIH) into *Not*I-*Hin*dIII-digested pAdTrack-CMV.[14] The resultant plasmid pAdTrack-CEA-GFP codes CEA and GFP genes under the control of individual identical CMV promoters surrounded by adenoviral sequences that allow homologous recombination with rescue vectors.

Recombinant Ad genomes containing the CEA- and GFP-expressing cassettes in place of the E1 region and Ad5 fiber or FF-CD40L genes was generated by two consequent homologous DNA recombinations in *Escherichia coli* as previously described.[15] The *Pac*I-*Pme*I fragment isolated from plasmid pAdTrack.CEA-GFP and *Cla*I-linearized rescue plasmid pVK500C were used for the first recombination. pVK500C, which contains an Ad5 genome with modified *Cla*I site in the E1 region and deleted fiber gene, is a derivative of pTG3602. The resultant plasmid containing Ad genome with the CEA- and GFP-expressing cassettes in place of the E1 region and deleted fiber gene was designated pVL.CEA-GFP. Next, recombinant Ad genomes incorporating FF-CD40L or fiber genes in addition to the CEA and GFP expressing cassettes were derived by homologous recombination with *Swa*I-linearized plasmid pVL.CEA-GFP essentially as described previously.[14] Details of all genetic engineering procedures are available upon request.

(5) Akbari, O.; Panjwani, N.; Garcia, S.; Tascon, R.; Lowrie, D.; Stockinger, B. DNA Vaccination: Transfection and Activation of Dendritic Cells as Key Events for Immunity. *J. Exp. Med.* **1999**, *189*, 169−178.

(6) Bouloc, A.; Walker, P.; Grivel, J. C.; Vogel, J. C.; Katz, S. I. Immunization through Dermal Delivery of Protein-Encoding DNA: A Role for Migratory Dendritic Cells. *Eur. J. Immunol.* **1999**, *29*, 446−454.

(7) Condon, C.; Watkins, S. C.; Celluzzi, C. M.; Thompson, K.; Falo, L. D., Jr. DNA-Based Immunization by in Vivo Transfection of Dendritic Cells. *Nat. Med. (N.Y.)* **1996**, *2*, 1122−1128.

(8) Tillman, B. W.; de Gruijl, T. D.; Luykx-de Bakker, S. A.; Scheper, R. J.; Pinedo, H. M.; Curiel, T. J.; Gerritsen, W. R.; Curiel, D. T. Maturation of Dendritic Cells Accompanies High-Efficiency Gene Transfer by a Cd40-Targeted Adenoviral Vector. *J. Immunol.* **1999**, *162*, 6378−6383.

(9) Brandao, J. G.; Scheper, R. J.; Lougheed, S. M.; Curiel, D. T.; Tillman, B. W.; Gerritsen, W. R.; van den Eertwegh, A. J.; Pinedo, H. M.; Haisma, H. J.; de Gruijl, T. D. Cd40-Targeted Adenoviral Gene Transfer to Dendritic Cells through the Use of a Novel Bispecific Single-Chain Fv Antibody Enhances Cytotoxic T Cell Activation. *Vaccine* **2003**, *21*, 2268−2272.

(10) Pereboev, A. V.; Asiedu, C. K.; Kawakami, Y.; Dong, S. S.; Blackwell, J. L.; Kashentseva, E. A.; Triozzi, P. L.; Aldrich, W. A.; Curiel, D. T.; Thomas, J. M.; Dmitriev, I. P. Coxsackievirus-Adenovirus Receptor Genetically Fused to Anti-Human Cd40 Scfv Enhances Adenoviral Transduction of Dendritic Cells. *Gene Ther.* **2002**, *9*, 1189−1193.

(11) Tillman, B. W.; Hayes, T. L.; DeGruijl, T. D.; Douglas, J. T.; Curiel, D. T. Adenoviral Vectors Targeted to Cd40 Enhance the Efficacy of Dendritic Cell-Based Vaccination against Human Papillomavirus 16-Induced Tumor Cells in a Murine Model. *Cancer Res.* **2000**, *60*, 5456−5463.

(12) de Gruijl, T. D.; Luykx-de Bakker, S. A.; Tillman, B. W.; van den Eertwegh, A. J.; Buter, J.; Lougheed, S. M.; van der Bij, G. J.; Safer, A. M.; Haisma, H. J.; Curiel, D. T.; Scheper, R. J.; Pinedo, H. M.; Gerritsen, W. R. Prolonged Maturation and Enhanced Transduction of Dendritic Cells Migrated from Human Skin Explants after in Situ Delivery of Cd40-Targeted Adenoviral Vectors. *J. Immunol.* **2002**, *169*, 5322−5331.

(13) Belousova, N.; Korokhov, N.; Krendelshchikova, V.; Simonenko, V.; Mikheeva, G.; Triozzi, P. L.; Aldrich, W. A.; Banerjee, P. T.; Gillies, S. D.; Curiel, D. T.; Krasnykh, V. Genetically Targeted Adenovirus Vector Directed to Cd40-Expressing Cells. *J. Virol.* **2003**, *77*, 11367−11377.

(14) He, T. C.; Zhou, S.; da Costa, L. T.; Yu, J.; Kinzler, K. W.; Vogelstein, B. A Simplified System for Generating Recombinant Adenoviruses. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2509−2514.

(15) Chartier, C.; Degryse, E.; Gantzer, M.; Dieterle, A.; Pavirani, A.; Mehtali, M. Efficient Generation of Recombinant Adenovirus Vectors by Homologous Recombination in Escherichia Coli. *J. Virol.* **1996**, *70*, 4805−4810.

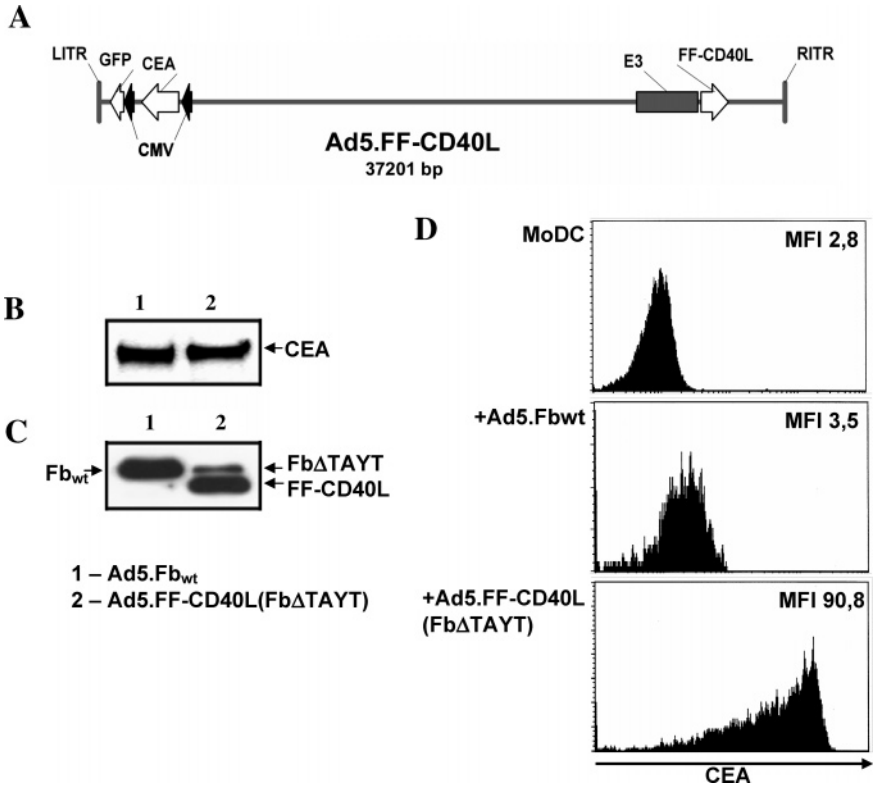

Figure 1. Construction and functional properties of CD40-targeted Ad vectors. (A) Map of Ad.FF-CD40L virus, containing the CEA and GFP genes, cloned in tandem with separate CMV promoters. (B) Western immunoblot analysis of CEA expression by 293.CD40 cells transduced by control (lane 1) or CD40-targeted (lane 2) Ad vectors. CD40-positive 293.CD40 cells were transduced with Ad5.FF-CD40L(FbΔTAYT) or Ad5.Fb$_{wt}$ at an MOI of 200 viral particles/cell. Twenty-four hours post-transduction, the cells were washed with PBS, harvested, and lysed in cell culture lysis reagent (Promega, Madison, WI). Aliquots of cell lysates (50 μg of total protein) were used for Western blot analysis with anti-CEA mAb. (C) Integration of FF-CD40L and modified Ad5 fiber proteins into mosaic Ad5.FF-CD40L(FbΔTAYT) virions. Aliquots of 10^{10} viral particles of Ad5.Fbwt (lane 1) and Ad5.FF-CD40L (lane 2) were fully denatured, and viral proteins were separated by SDS−PAGE. The wild-type (Fb$_{wt}$) and mutant (FbΔTAYT) Ad5 fibers as well as the FF-CD40L chimera were detected on the blots with MAb 4D2. (D) The expression of CEA in MoDC infected by Ad vectors in vitro. Mean fluorescence indices (MFI), related to isotype control staining, are indicated. Results shown are representative of three experiments.

Viruses. Viruses were rescued according to previously described methods.[16] To obtain Ad.FF-CD40L containing FF-CD40L chimeras in combination with the Ad5 mutant fiber, the rescued virus was amplified on 293FΔTAYT cells. Ad vectors were isolated from infected cells and purified by equilibrium centrifugation in CsCl gradients as previously described.[17]

Western Blot Analysis. Samples were incubated in Laemmli sample buffer at 96 °C for 5 min and separated on a 4−20% gradient polyacrylamide gel (Bio-Rad). The proteins were electroblotted onto a polyvinylidene difluoride (PVDF) membrane, and the blots were developed with the WesternBreeze immunodetection system (Invitrogen) according to the manufacturer's protocol using either the 4D2 or murine anti-CEA antibodies as primary probes. 4D2 anti-fiber murine mAb was obtained from Jeffery Engler (University of Alabama at Birmingham), and the anti-CEA mAb was obtained from Maine Biotechnology Services (Portland, ME).

Adenoviral Infection of MoDC. Monocyte-derived dendritic cells (MoDC) were generated from plastic-adherent monocytes in the presence of GM-CSF and IL-4, and characterized as described previously.[8] Briefly, peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood of normal human volunteer donors by density centrifugation over Lymphoprep (Nycomed AS, Oslo, Norway). PBMC were suspended at a concentration of 3−5 million cells per milliliter in Iscove's modified Dulbecco's medium (IMDM) containing 50 units/mL penicillin-streptomycin, 1.6 mM L-glutamine, 0.01 mM 2-ME (complete medium), and 10% FCS and were allowed to adhere to the bottom of plastic culture flasks (NUNC, Intermed, Denmark) at 37 °C. The adherent cells were cultured for an additional 6 days in medium supplemented with 1000 units/

(16) Krasnykh, V.; Belousova, N.; Korokhov, N.; Mikheeva, G.; Curiel, D. T. Genetic Targeting of an Adenovirus Vector Via Replacement of the Fiber Protein with the Phage T4 Fibritin. *J. Virol.* **2001**, *75*, 4176−4183.

(17) Graham, F. L.; Prevec, L. Methods for Construction of Adenovirus Vectors. *Mol. Biotechnol.* **1995**, *3*, 207−220.

mL rIL-4 (Centraal Laboratorium van de Bloedtransfusiedienst, Amsterdam, The Netherlands) and 100 ng/mL GM-CSF (Schering-Plough, Madison, NJ). Adherent MoDC released by 0.5 mM EDTA were pooled with nonadherent MoDC. These cells were characterized by the presence of CD1a and CD11c expression as well as the absence of other lineage markers, such as CD3, CD14, CD19, and CD56, by FACS. These cells also demonstrated typical DC morphology, such as dendritic processes and clustered cells.

For all infections, MoDC were incubated with the Ad vectors at a multiplicity of infection (MOI_{vp}) of 1000 viral particles (vp) per cell, in 100 μL of serum-free IMDM medium, at 37 °C for 2 h, after which the cells were washed and cultured overnight in complete IMDM medium with 10% FCS.

Ad Infection of Skin Explants and DC Migration. Human skin specimens were obtained after informed consent from patients undergoing corrective breast or abdominal plastic surgery. Six millimeter skin biopsies were cultured on rafts according to previously described methods,[12] and were subsequently injected intradermally (i.d.) with 10^9 viral particles (vp) per biopsy, or with plain medium as a negative control. Ad vectors were injected into the dermis in a total volume of 10 μL. Following injection, the explants (12−20 samples/condition) were cultured, floating freely on HPS-containing medium, with their epidermal side up, before their removal 2 days later. The explants were discarded, and the medium, containing migrated cells, was harvested and pooled per test condition. FACS analyses were subsequently performed. Details of all procedures with skin explants are described elsewhere.[12]

Flow Cytometric Analysis of DC. For detection of CEA the cells were incubated with an anti-CEA mAb (Clone II-7, DAKO, Glostrup, Denmark) and stained with a PE-labeled goat ($F[ab]_2$)-anti-mouse conjugate (DAKO) following permeabilization with Cytofix/Cytoperm (BD Biosciences, San Jose, CA) according to the manufacturer's instructions. To assess maturation, DCs were stained with PE-labeled anti-CD80, CD86 (BD Biosciences Pharmingen, San Diego, CA), or CD83 (Immunotech, Marseille, France) mAbs. Samples were analyzed using a FACSCalibur and CellQuest FACS.

Results and Discussion

Construction and Functional Characterization of a CD40-Targeted, GFP- and CEA-Encoding Ad Vector. To design an Ad vector targeted to CD40-expressing cells, we have previously modified the virus tropism by using the TNF-like domain of human CD40L, which is a ligand for CD40.[18] It was accomplished by the incorporation into virus capsid of a protein chimera comprising structural domains of three different proteins: the Ad serotype 5 fiber, phage T4 fibritin, and the human CD40 ligand (CD40L). The tumor necrosis factor-like domain of CD40L retained its functional tertiary structure upon incorporation into this chimera and allowed the virus to use CD40 as a surrogate receptor for cell entry.[13]

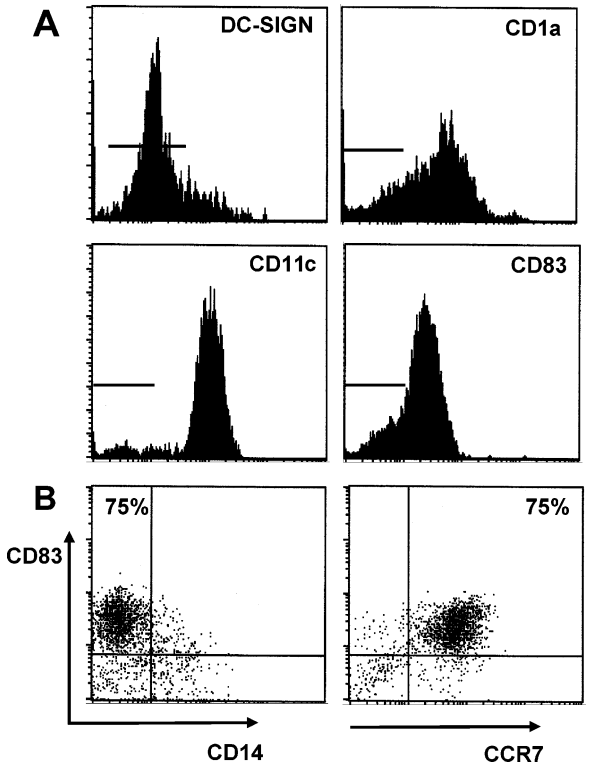

Figure 2. Phenotype of activated, skin-emigrated DC, 2 days after the start of skin explant culture. For flow cytometric analysis DC were gated by their characteristic high forward and side scatter properties.[12] Analysis revealed (A) the majority to express CD1a, CD11c, CD83, but not DC-SIGN (markers indicate the corresponding isotype controls) and (B) a small population of CD14+CD83− cells as well as coexpression of CD83 and CCR7 (75% indicating the majority of CD83+CCR7+CD14− skin-emigrated DC). Data shown are representative of at least three experiments.

To determine the potential of the CD40-targeted Ad vectors for in situ antigen loading of DC, we sought to construct a single-component recombinant vector encoding CEA, targeted to CD40. The gene encoding CEA, as well as the GFP reporter gene, were cloned in tandem behind separate CMV promoters into the E1 region of the Ad.FF-CD40L genome (Figure 1A). Two variants of the GFP/CEA encoding Ad vectors were generated and propagated: the variant with the wild-type fiber ($Ad.Fb_{wt}$) and a mosaic variant containing the FF-CD40L chimeric fiber and a full-size Ad5 fiber engineered to lack CAR-binding ability (FbΔTAYT). The presence of this mutated fiber protein, as previously reported,[13] increases the infectivity of the CD40-targeted vector. This goal was accomplished by propagating Ad5.FF-CD40L in 293FΔTAYT cells derived from 293 cells to constitutively express an Ad5 fiber whose binding to CAR was abolished by the previously described deletion.[19] This additional vector amplification step resulted in a virus preparation designated Ad5.FF-CD40L (FbΔTAYT), which contained viral particles randomly incorporating both types

(18) van Kooten, C.; Banchereau, J. Cd40-Cd40 Ligand. *J. Leukocyte Biol.* **2000**, *67*, 2−17.

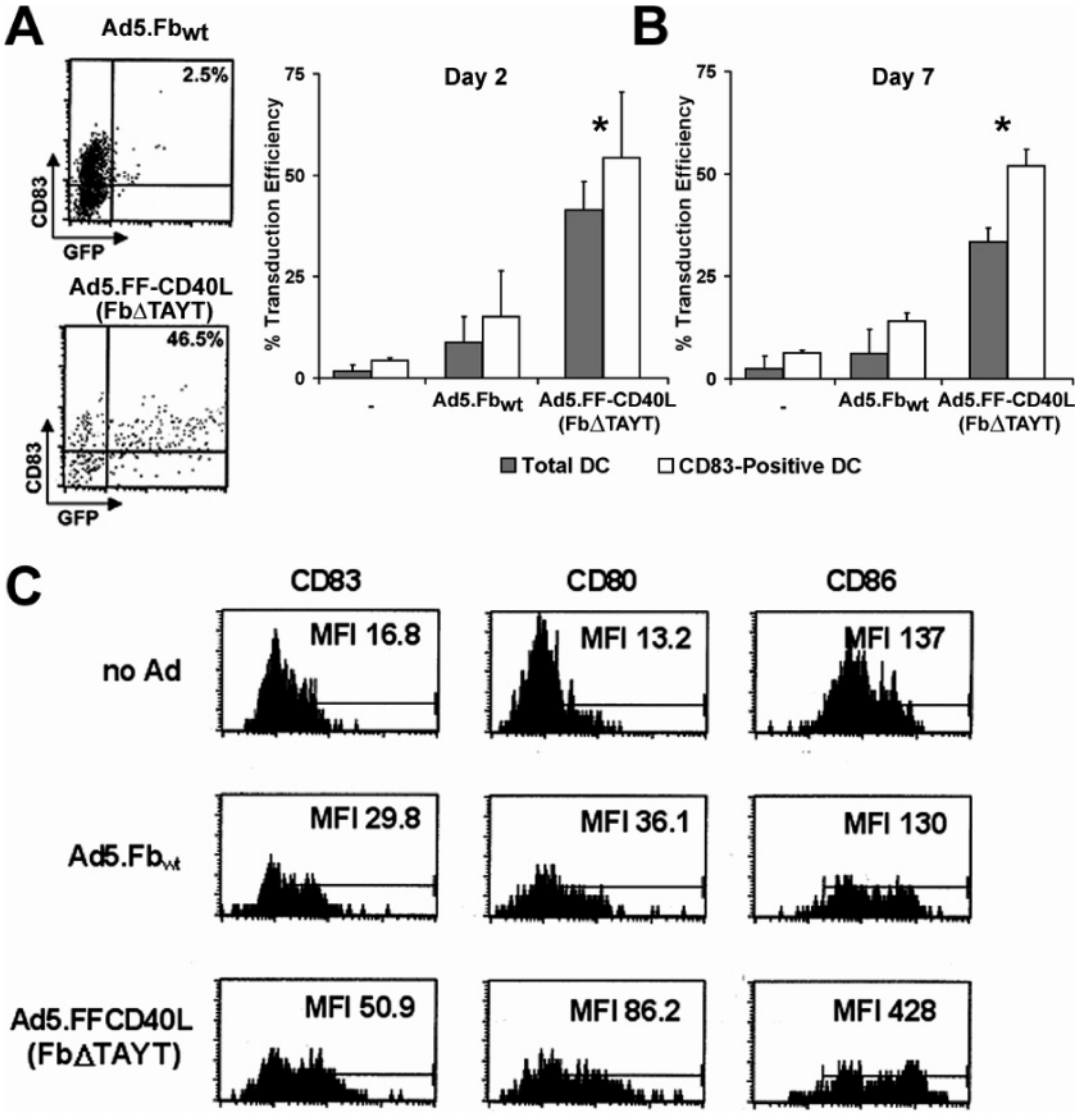

Figure 3. Transduction efficiency of skin-emigrated DC subsequent to i.d. injection of CD40-targeted Ad vectors in human skin explants. Six millimeter skin biopsies were i.d. injected at day 0 with medium, cultured on rafts, and then injected with medium (−, panel B) or with 10^9 vp of the indicated GFP-encoding Ad vectors. The biopsies were cultured (free-floating on HPS-containing medium) and removed after 2 days, at which time emigrated DC were harvested (day 2), as well as 5 days later (day 7). (A) GFP expression was determined via FACS analysis in relation to CD83 expression on day 2 migrated DC: the percentages of CD83 and GFP positive DC are indicated. (B) Means of transduction efficiencies (in percentage GFP expression; significantly higher for both total DC and CD83+ DC upon CD40-targeted Ad infection as compared to wtAd infection, (*) $P < 0.005$) for skin-emigrated DC at day 2 and day 7 ($n = 3$) are shown. Error bars indicate standard deviations. (C) Markers of maturation exhibited by migrated DC at day 7 postinjection of Ad vectors in human skin explants. (MFI: mean fluorescence intensity.) The histograms indicate the distribution of positive cells relative to the negative isotype control.

of modified fibers (Figure 1C). The functionality of the CEA expression cassette was confirmed by immunoblot analysis of cell lysates obtained from 293.CD40 cells transfected by these vectors (Figure 1B). In addition, we performed in vitro gene transfer to DC in order to determine CEA expression as well as functionality of our modified CD40-targeted vectors. To this end, Ad.Fb$_{wt}$ and Ad.FF-CD40L(FbΔTAYT) were tested in parallel on monocyte-derived dendritic cells (MoDC) with CEA (and GFP; not shown) expression levels ascertained by FACS analysis (Figure 1D). The targeted mosaic vector achieved superior transduction of the CD40 positive DC (MOI 1000 vp/cell), as evident by the increase in CEA expression levels.

Gene Transfer Analysis of CD40-Targeted Vector in Human Skin Explant Model and Maturing Effects on Skin-Emigrated DC. To demonstrate the utility of the

(19) Roelvink, P. W.; Mi Lee, G.; Einfeld, D. A.; Kovesdi, I.; Wickham, T. J. Identification of a Conserved Receptor-Binding Site on the Fiber Proteins of Car-Recognizing Adenoviridae. *Science* **1999**, *286*, 1568−1571.

CD40-targeted Ad vector for in vivo transduction of DC, we employed the previously described human skin explant model.[12] In this system, targeted DC infection in the context of complex tissue structure could be ascertained for cutaneous DC. Ad vectors were injected intradermally, and the injected skin explants were cultured for 2 days, floating in medium with their epidermal side up, after which the migrated DC were harvested. As described previously,[12] the vast majority of skin-emigrated cells at this time display a typical DC morphology and a phenotype consistent with mature, migrated Langerhans cells, expressing CD1a, CD11c, and CD83, but no DC-SIGN (see Figure 2A) and high levels of HLA-DR, -ABC, CD40, CD54, CD80, and CD86 (not shown).[12] Double staining revealed a relatively small population of CD14+ cells, which were CD83 negative (Figure 2B). As the chemokine receptor CCR7 has been identified as a key regulator that governs trafficking of skin DC,[20] mediating both DC entry into the lymphatic vessels and migration of the DC to the paracortical T cell areas in draining LN,[21] we sought to determine CCR7 expression on skin-emigrated DC. At day 2 after migration we found clear CCR7 expression, exclusively in combination with the maturation marker CD83 (Figure 2B). Migrated DC, harvested either two or seven days after viral injection, were analyzed for GFP transgene and CD83 expression levels via FACS analysis (Figure 3A). Results showed that the CD40-targeted mosaic vector exhibited superior in situ DC transduction when compared to Ad5.Fb_{wt} with significant increases in (CD83+) DC transduction efficiencies ($P < 0.005$, Figure 3B). Moreover, stable transduction of (CD83+) skin-emigrated DC was observed up to seven days after in situ infection (Figure 3B).

Because the utility of this vaccination approach is predicated upon maturation of DC with accompanied upregulation of costimulatory molecules, we then further analyzed the maturing effects on the skin-emigrated DC, postinfection and -migration. To this end, migrated DC were screened by flow cytometry for the upregulation of DC maturation and costimulatory markers, namely, CD83, CD80, and CD86. After i.d. injection of the Ad vectors, expression levels of these cellular markers were assessed at day 7 of culture (Figure 3C). We previously showed that Ad vectors targeted to CD40 on cutaneous DC by antibody conjugates could maintain DC maturation levels during this period subsequent to migration, whereas untargeted Ad could not.[12] Similarly, higher expression levels of the tested maturation markers were maintained upon in situ infection with the Ad.FF-CD40L(FbΔTAYT) CD40-targeted vector. Importantly, stable long-term CCR7 and CD83 expression, indicative of a T cell stimulatory phenotype and a LN homing capacity, was observed in activated cutaneous DC up to 7 days postmigration (data not shown).

These studies have provided validation of our CD40-targeting vector schema in a stringent, human substrate system. The ability of the FF-CD40L vectors to accomplish efficient DC transduction with direct cellular activation in an in vivo context is highly consonant with the vector's planned employment in human vaccination approaches. As such, this vector should now open the way for targeted, cell-specific gene delivery in vivo, relevant to a range of gene therapy applications.

Acknowledgment. The authors thank Sinéad Lougheed for excellent technical assistance and Dr. Joanne T. Douglas for helpful discussion and support. This study was supported by Grant R01 CA 86881. D.T.C. is an equity holder in VectorLogics, Inc.

MP050002W

(20) Ohl, L.; Mohaupt, M.; Czeloth, N.; Hintzen, G.; Kiafard, Z.; Zwirner, J.; Blankenstein, T.; Henning, G.; Forster, R. Ccr7 Governs Skin Dendritic Cell Migration under Inflammatory and Steady-State Conditions. *Immunity* **2004**, *21*, 279−288.

(21) MartIn−Fontecha, A.; Sebastiani, S.; Hopken, U. E.; Uguccioni, M.; Lipp, M.; Lanzavecchia, A.; Sallusto, F. Regulation of Dendritic Cell Migration to the Draining Lymph Node: Impact on T Lymphocyte Traffic and Priming. *J. Exp. Med.* **2003**, *198*, 615−621.