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Bispecific chimeric antigen receptors targeting the CD4 binding site and high-mannose Glycans of gp120 optimized for anti–human immunodeficiency virus potency and breadth with minimal immunogenicity

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Abstract

Background aims. Chimeric antigen receptors (CARs) offer great potential toward a functional cure of human immunodeficiency virus (HIV) infection. To achieve the necessary long-term virus suppression, we believe that CARs must be designed for optimal potency and anti-HIV specificity, and also for minimal probability of virus escape and CAR immunogenicity. CARs containing antibody-based motifs are problematic in the latter regard due to epitope mutation and anti-idiotypic immune responses against the variable regions. *Methods.* We designed bispecific CARs, each containing a segment of human CD4 linked to the carbohydrate recognition domain of a human C-type lectin. These CARs target two independent regions on HIV-1 gp120 that presumably must be conserved on clinically significant virus variants (i.e., the primary receptor binding site and the dense oligomannose patch). Functionality and specificity of these bispecific CARs were analyzed in assays of CAR-T cell activation and spreading HIV-1 suppression. *Results.* T cells expressing a CD4-dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DCSIGN) CAR displayed robust stimulation upon encounter with Env-expressing targets, but negligible activity against intercellular adhesion molecule (ICAM)-2 and ICAM-3, the natural dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin ligands. Moreover, the presence of the lectin moiety prevented the CD4 from acting as an entry receptor on CCR5-expressing cells, including $CD8^+$ T cells. However, in HIV suppression assays, the CD4-DCSIGN CAR and the related CD4-liver/lymph node-specific intercellular adhesion molecule-3-grabbing non-integrin CAR displayed only minimally increased potency compared with the CD4 CAR against some HIV-1 isolates and reduced potency against others. By contrast, the CD4-langerin and CD4-mannose binding lectin (MBL) CARs uniformly displayed enhanced potency compared with the CD4 CAR against all the genetically diverse HIV-1 isolates examined. Further experimental data, coupled with known biological features, suggest particular advantages of the CD4-MBL CAR. *Discussion.* These studies highlight features of bispecific CD4-lectin CARs that achieve potency enhancement by targeting two distinct highly conserved Env determinants while lacking immunogenicity-prone antibody-based motifs.

Key Words: *adoptive cellular therapy, C-type lectin, carbohydrate recognition domain, CD4, chimeric antigen receptor, functional cure, human immunodeficiency virus, mannose binding lectin*

Introduction

Although combination antiretroviral therapy has had a transformative impact on the global human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) pandemic [1,2], the inability of current regimens to eradicate the infection has stimulated research toward curative approaches [3–5]. Adoptive immunotherapy with natural or engineered T cells directed to kill specific target cell types is a propelling focus in the cancer field [6–8] and offers considerable promise against viral pathogens [9] including HIV [10,11]. Particularly noteworthy successes against certain hematologic malignancies have been achieved with T cells genetically modified to express a chimeric antigen receptor (CAR) [12,13]. CAR-T cell technology is under active investigation for its potential in HIV cure approaches [14]. CAR constructs have

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been designed to recognize the HIV-1 Env glycoprotein expressed on the surface of infected cells, using as targeting motif either a region of the primary HIV receptor CD4 [15–23] or a single chain variable fragment (scFv) from an anti-HIV monoclonal antibody (mAb) including the new generation of broadly neutralizing antibodies [24–26]. Indeed, first-generation CD4 CAR constructs were examined in early clinical studies, with minimal effects on HIV burden [27–30].

We have proposed that the CAR approach has great potential in efforts to achieve an HIV functional cure, whereby long-term (life-long?) suppression of virus replication can be maintained in the absence of antiretroviral therapy even though the virus has not been fully eradicated [31]. To achieve such durable suppression, we believe that the CAR must not only be highly potent and HIV-specific, but also minimally susceptible to viral escape and immunogenicity. Our approach is to design bispecific CARs containing CD4 linked to a second Env-binding moiety that provides two important advantages compared with the monospecific CD4 CARs described by others: enhanced potency against genetically diverse HIV-1 primary isolates, and absence of HIV entry receptor activity of the CD4 moiety, an important concern for CAR-expressing $CD8^+$ T cells that might otherwise be rendered susceptible to HIV infection.

Here we report bispecific CD4-based CARs in which the second moiety is the carbohydrate recognition domain (CRD) of a human C-type lectin [32,33]. The CRD binds to the dense oligomannose patch that is universally displayed on Envs of genetically diverse HIV-1 primary isolates [34]. The data indicate improved potency compared with our previously described bispecific CAR containing CD4 linked to a scFv against the conserved coreceptor binding region of gp120 [22], with minimized concerns of mutational escape and anti-idiotypic immune responses that threaten the long-term efficacy of CARs with antibody-based targeting motifs.

Materials and methods

Peripheral blood mononuclear cells and cell lines

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats from healthy anonymous donors from the National Institutes of Health (NIH) Blood Bank using Ficoll-Hypaque gradient separation and subsequently cultured in AIM-V medium (ThermoFisher) supplemented with 5% human AB serum (Valley Biomedical) and recombinant human interleukin-2 (IL-2; Chiron). Aliquots were frozen using Recovery Cell Culture Freezing Medium (ThermoFisher). To generate CAR-bearing effector cells, PBMCs were washed once and resuspended in AIM-V+5% human AB serum containing 300 IU/mL IL-2 and 50 ng/mL of the anti-CD3 monoclonal antibody OKT3 at 2 x 10^6 cells/mL. The cells were plated in the wells of a 24-well plate (2 mL/well) and cultured at 37°C in 5% CO_2 for 2 days.

HEK293T (ATCC) and 293GP (BD Biosciences) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 25 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 2 mmol/L L-glutamine and 1% sodium pyruvate. Chinese hamster ovary (CHO) cells were grown in DMEM containing 10% FBS, 2 mmol/L glutamine, 1% nonessential amino acids, and 25 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer. In addition, 250 nmol/L methotrexate (MTX) was added to the growth medium of CHO-*env* cells, which constitutively express the Env glycoprotein from HIV-1 isolate IIIB [35]. All cell culture media contained 100 U/mL penicillin and 100 µg/mL streptomycin and the cells were grown at 37°C in 5% CO_2.

CAR-encoding retroviral constructs

The CARs in this study were all cloned into the pMSGV-1 gammaretrovirus vector as described previously [36]. Within each construct the indicated target moiety is linked to a hinge region, a transmembrane region and the cytoplasmic signaling domains of CD28 and CD3 zeta. The negative control (referred to as 139 CAR), contains a scFv from human Mab 139, which is specific for a variant of the epidermal growth factor (EGF) receptor only found on glioma cells [37]. All of the CD4-containing CARs contain domains 1 and 2 of human CD4. All targeting motifs were synthesized by GenScript, codon optimized for human expression, and subcloned into the appropriate plasmid backbone. The CD4-dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DCSIGN) CAR point mutants were generated by oligonucleotide directed mutagenesis using primers from LifeTech. Schematic representations of the CAR constructs are shown in Figure 1A, and the corresponding amino acid sequences are presented in Supplementary Figure S1 (Supplemental Materials).

Gammaretrovirus vector production and transduction of PBMCs

The gammaretroviral vectors carrying the CAR transgenes were prepared as described previously [38]. Briefly, supernatants from 293GP cells (BD Biosciences) co-transfected with the retroviral vector plasmid and the plasmid encoding RD114 envelope protein were collected at 48 h after transfection (using Lipofectamine 200 reagent from Life Technologies) and stored at –80°C.