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Targeting dendritic cells in humanized mice receiving adoptive T cells via monoclonal antibodies fused to Flu epitopes ☆

John P. Graham [a,b], Pierre Authie [a,b], Chun I. Yu [a,b], Sandra M. Zurawski [a], Xiao-Hua Li [a], Florentina Marches [a,c], Anne-Laure Flamar [a], Aditi Acharya [a], Jacques Banchereau [a,b,c,d], A. Karolina Palucka [a,b,c,*]

[a] The Ralph Steinman Center for Cancer Vaccines, Baylor Institute for Immunology Research, Dallas, TX 75204, United States
[b] The Jackson Laboratory, Bar Harbor, ME, United States[1]
[c] The Jackson Laboratory for Genomic Medicine, Farmington, CT, United States[1]
[d] Vaccine Research Institute, INSERM UMR955, Equipe 16, Creteil, France

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ABSTRACT

The targeting of vaccine antigens to antigen presenting cells (APC), such as dendritic cells (DCs), is a promising strategy for boosting vaccine immunogenicity and, in turn, protective and/or therapeutic efficacy. However, *in vivo* systems are needed to evaluate the potential of this approach for testing human vaccines. To this end, we examined human $CD8^+$ T-cell expansion to novel DC-targeting vaccines *in vitro* and *in vivo* in humanized mice. Vaccines incorporating the influenza matrix protein-1 (FluM1) antigen fused to human specific antibodies targeting different DC receptors, including DEC-205, DCIR, Dectin-1, and CD40, elicited human $CD8^+$ T-cell responses, as defined by the magnitude of specific $CD8^+$ T-cells to the targeted antigen. *In vitro* we observed differences in response to the different vaccines, particularly between the weakly immunogenic DEC-205-targeted and more strongly immunogenic CD40-targeted vaccines, consistent with previous studies. However, in humanized mice adoptively transferred (AT) with mature human T cells (HM-T), vaccines that performed weakly *in vitro* (i.e., DEC-205, DCIR, and Dectin-1) gave stronger responses *in vivo*, some resembling those of the strongly immunogenic CD40-targeted vaccine. These results demonstrate the utility of the humanized mouse model as a platform for studies of human vaccines.

1. Introduction

There is much interest in targeting vaccine antigens to DCs to boost vaccine immunogenicity. Seminal studies from the Nussenzweig and Steinman laboratories demonstrated the potential of monoclonal antibody (mAb)-based vaccines to cell-surface DC receptors (DC targeting vaccines) [1,2]. In these studies, a mAb-ovalbumin fusion protein specific for DEC-205, a C-type lectin receptor abundant on DCs, was 400 times more effective at eliciting ovalbumin-specific mouse $CD8^+$ T-cell responses *in vivo* than non-targeted ovalbumin. Also, targeting antigens to dectin-1 or DCIR, both C-type lectins expressed by DCs, results in antigen specific $CD8^+$ T cell responses *in vitro* [3,4]. Recent *in vitro* comparisons of DC targeting vaccines revealed differences in cross presentation [5] and identified CD40, a co-stimulatory molecule, as an optimal DC receptor target for cross presentation [6,7]. The superiority of CD40 was attributed to antigen accrual in minimally degradative compartments that, presumably, preserves antigen structure long enough for access to cross presentation machinery [6]. With the realization that early endosomal antigen targeting extends cross presentation to multiple human DC subsets (i.e., $CD1c^+$ and $CD141^+$ conventional (c)DCs), it was proposed that targeting multiple cross presenting subsets would maximize vaccine

Abbreviations: DCs, dendritic cells; c, conventional; p, plasmacytoid; HPCs, $CD34^+$ hematopoietic stem cells; FluM1, influenza M1 protein; FACS, fluorescent activated cell sorting; PBMCs, peripheral blood mononuclear cells; mAb, monoclonal antibody; APCs, antigen presenting cells; AT, adoptive transfer.

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* Corresponding author at: The Jackson Laboratory for Genomic Medicine, 10 Discovery Drive, Farmington, CT 06032, United States.
E-mail address: karolina.palucka@jax.org (A.K. Palucka).

[1] Current address.

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immunogenicity [7]. However, which DC receptors are best for antigen cross presentation *in vivo* remains unknown.

Humanized mice can be used to study the human immune system [8,9]. Human T cells develop endogenously in models like the NOD/SCID/IL-2$\gamma^{-/-}$ (NSG) mouse [9]. However, T cells in NSG are restricted to mouse MHC molecules, precluding the use of human specific vaccines [9]. Using HLA-A201 transgenic NSG mice in vaccine studies is complicated by a lack of human MHC Class II expression and subsequent lack of human MHC Class II restricted CD4$^+$ T helper cells [9]. To circumvent these issues, we chose to use a model where mature T cells from human donors are AT into humanized NOD/SCID-β2microglobulin-deficient mice transplanted with syngeneic human CD34$^+$ hematopoietic progenitor cells (HPCs) (T cell adoptive transfer humanized mouse model or "HM-T"). With HM-T, mice are reconstituted with all human DC subsets and B cells a few weeks following HPC transplant [10–12]. Subsequent AT of T cells permits analysis of memory responses to immunological stimuli. Whereas influenza challenge studies are not possible in HM-T because of the lack of human MHC molecule expression in mouse lung tissues, HM-T vaccinated with live attenuated influenza vaccine (LAIV) or with subunit vaccine (TIV) expand CD8$^+$ T cells specific to FluM1 and nonstructural protein-1 in blood, spleen, and lungs [11].

Here, we tested whether HM-T can be used for assessing DC targeting vaccines. Herein, we find that FluM1-specific human CD8$^+$ T-cell expansion using low doses of vaccine required DC antigen targeting. Importantly, results from vaccine comparisons *in vivo* differed from results generated *in vitro*. Thus, HM-T can be used to compare human-specific DC targeting vaccines.

2. Materials and methods

2.1. DC targeting vaccines

Human specific targeting IgG4 monoclonal antibody or corresponding IgG4 "isotype" control antibody fusion protein, which is not specific toward DC receptors, with heavy chain-fused FluM1 peptide (GILGFVFTL), here known as "FluM1" (i.e., "anti-CD40-FluM1 [13], anti-DCIR-FluM1 [4], anti-Dectin-1-FluM1 [3], anti-DEC-205-FluM1 [13], control "isotypeFluM1" vaccine [13]), were produced by the Baylor Institute for Immunology Research Biotechnology Core. The core antibody IgG4 sequences have been described (GenBank accession numbers KM660791 with at Kabat Q3K substitution sequence change, KM660792, JX002666, JX002667, KM246789, KM246790) or are referenced in [13]. Flamar et al. [13] describes the process of transferring the variable regions of the human specific anti-DEC-205 MG38 clone (from Ralph Steinman) onto a human IgG4 framework. The control "isotype" IgG4 sequence is described in [13] along with procedures for appending antigen sequence to the H chain C-terminus and methods of vaccine production and quality assurance such as LPS determination (<0.01 ng LPS/mg protein). The FluM1 peptide sequence appended to the H chain C-termini has two copies of the CD8$^+$ T-cell peptide (in bold) in the context of natural FluM1 sequences (underlined) [14]. ASQTPTNTISVTPTNNSTPTNNSNPKPNPASDTTEPATPT TPVTTPTTTLLPILSPLTK**GILGFVFTL**TVPSERK**GILGFVFTL**TRKNGSGETSPTSTPADSSTITPTATPTATPTI KGAS.

2.2. DC purification from humanized mice and human blood

2.2.1. Humanized mice

Sublethally irradiated NOD-SCID *B2m*$^{-/-}$ mice were transplanted with 3×10^6 CD34$^+$ HPCs isolated from G-CSF-mobilized blood of HLA-A*0201$^+$ healthy donors. All protocols were approved by the Institutional Review Board (IRB, 097-053 and 099-076) and the Institutional Animal Care and Use Committee (A01-005) at Baylor Research Institute. At 4–6 weeks post-transplant, humanized mice (without AT) were either mock inoculated with PBS or given 10 μg of CD40 fusion protein (Fig. 4 specifically) and harvested. Harvested spleens were digested for 10 min with DNase and collagenase. Following purification with Ficoll-Paque Plus density-gradient centrifugation (Stemcell Technologies, Vancouver, BC, Canada), cells were stained on ice with fluorochrome-conjugated specific antibodies. DCs were then sorted with FACSAria (BD) using Diva software (BD) (Supplementary Fig. 2).

2.2.2. Human blood

PBMCs were isolated from leukapheresis products using Ficoll-Paque Plus density-gradient centrifugation. DCs were enriched using human Pan-DC Pre-Enrichment Kit (StemCell Technologies) before FACS-sorting or use in co-culture.

2.3. Human PBMC assay for CD8$^+$ T-cell responses

Human PBMC (1×10^6/ml) were cultured with IL-2 (100 U/ml) for eight days with DC targeting vaccines. Following culture, cells were analyzed for FluM1-specific CD8$^+$ T cell responses as in [11].

2.4. Autologous DC-CD8$^+$ T-cell co-culture

CD8$^+$ T cells were isolated from PBMCs using human T-cell enrichment kits (StemCell Technologies). CD8$^+$ T cells were co-cultured with FACS sorted DC subsets or purified pan DCs (StemCell Technologies), IL-2 (10 U/ml, added at day 0 and fresh every second day), IL-7 (10 U/ml, day 0 only) for eight days in completed RPMI with 10% AB serum.

2.5. CD8$^+$ T-cell responses in vivo

Sublethally irradiated NOD/SCID-β2microglobulin-deficient mice were transplanted with human CD34$^+$ HPCs isolated from the PBMCs of granulocyte colony-stimulating factor mobilized donors. FLT3L or IFNα were not used in HM-T. ~4 weeks following injection of CD34$^+$ HPC, total T cells (autologous to CD34$^+$ HPCs) were isolated from PBMCs using human T-cell enrichment kits (StemCell Technologies). The day preceding vaccination, mice were AT i.v. with 2×10^7 total T cells in PBS. The day after adoptive transfer, mice were vaccinated i.v. with DC targeting vaccine. 75 μl of blood was obtained via retro-orbital bleeding at indicated days. CD8$^+$ T cells were stained in blood with specific antibodies and FluM1-specific MHC Class I tetramers. Following staining, blood was lysed with FACSlyse (BD) according to instructions and analyzed by FACS. Spleens and lungs were digested as described above, except that lungs were digested for 40 min. A total of ten different donors were used to create cohorts of HM-T for experiments.

3. Results

3.1. CD8$^+$ T-cell responses to CD40 targeted antigen in vitro

We first sought to determine whether a DC-targeted fusion protein vaccine encompassing canonical FluM1 epitope (GILGFVFTL) would elicit FluM1-specific CD8$^+$ T-cell responses *in vitro*. To this end, human PBMCs were cultured with titrated amounts of vaccine composed of an antibody to CD40 fused to FluM1 peptide (Fig. 1A), hereafter referred to as CD40-targeted vaccine, or a non-targeted control "isotypeFluM1" vaccine (containing an identical FluM1 sequence). After eight days of culture, flow cytometry was used to determine the expansion of antigen-specific CD8$^+$ T cells as a