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Efficient induction of cross-presentating human B cell by transduction with human adenovirus type 7 vector

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ABSTRACT

Although human autologous B cells represent a promising alternative to dendritic cells (DCs) for easy large-scale preparation, the naive human B cells are always poor at antigen presentation. The safe and effective usage record of human adenovirus type 7 (HAdV7) live vaccines makes it attractive as a promising vaccine vector candidate. To investigate whether HAdV7 vector could be used to induce the human B cells cross-presentation, in the present study, we constructed the E3-defective recombinant HAdV7 vector encoding green fluorescent protein (GFP) and carcinoembryonic antigen (CEA). We demonstrated that naive human B cells can efficiently be transduced, and that the MAPKs/NF-κB pathway can be activated by recombinant HAdV7. We proved that cytokine TNF-α, IL-6 and IL-10, surface molecule MHC class I and the CD86, antigen-processing machinery (APM) compounds ERp57, TAP-1, and TAP-2. were upregulated in HAdV7 transduced human B cells. We also found that CEA-specific IFNγ expression, degranulation, and *in vitro* and *ex vivo* cytotoxicities are induced in autologous $CD8^+$ T cells presensitized by HAd7CEA modified human B cells. Meanwhile, our evidences clearly show that Toll-like receptors 9 (TLR9) antagonist IRS 869 significantly eliminated most of the HAdV7 initiated B cell activation and $CD8^+$ T cells response, supporting the role and contribution of TLR9 signaling in HAdV7 induced human B cell cross-presentation. Besides a better understanding of the interactions between recombinant HAdV7 and human naive B cells, to our knowledge, the present study provides the first evidence to support the use of HAdV7-modified B cells as a vehicle for vaccines and immunotherapy.

1. Introduction

Although there are three main types of professional antigen-presenting cells (APCs), macrophages, dendritic cells (DCs), and B cells, it is proposed that DCs have a more central and competent role in antigen presentation and T cell activation [1]. Therefore, the majority of studies to date have focused on DCs as the main source of professional APCs, even though human DC vaccination studies are often hampered by complex long-term culture protocols, high experimental costs, and a lack of adequate DC cell numbers [2–4]. In contrast, large numbers of autologous B cells can be easily prepared from the blood of patients. In addition, unlike DCs, intravenously administrated B cells readily migrate to immune inductive sites in secondary lymphoid tissues. Hence, B cells, another professional APC, represent a promising alternative to DCs for cell vaccine development [5–8].

However, naive B cells are poor at antigen presentation, which may in part be due to the relatively low expression of costimula-

Abbreviations: APC, antigen-presenting cell; ARD, acute respiratory disease; APM, antigen-processing machinery; CEA, carcinoembryonic antigen; CPE, cytopathic effect; Ct, threshold cycle; CTL, cytotoxic T lymphocytes; DC, dendritic cell; DSG-2, desmoglein 2; ELISA, enzyme-linked immunosorbent assay; GFP, green fluorescent protein; HRP, horseradish peroxidase; HAdV7, human adenovirus type 7; hCAR, human coxsackievirus-adenovirus receptor; ODN, oligonucleotide; PBMC, peripheral blood mononuclear cell; p.i, post infection; RT-PCR, reverse transcriptase-polymerase chain reaction; RT, room temperature; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TLR9, Toll-like receptors 9.

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tory molecules [9–11]. Previous studies have demonstrated that the TLR9 agonist, CpG ODN, can activate naive B cells and increase the surface expression of costimulatory and MHC molecules, resulting in an enhanced antigen presentation function in these cells [12–17]. Therefore, novel strategies combining TLR9 activation and antigen gene delivery are promising for the induction of B cell cross-presentation and cytotoxic T lymphocytes (CTL) response.

Previous studies have confirmed that adenovirus can induce APCs maturation via TLR9 recognized viral DNA and it was found that the TLR9 pathway was preferentially activated by CD46-utilizing adenoviruses [18–22]. Therefore, as a CD46-utilizing adenovirus, human adenovirus type 7 (HAdV7) was proposed to be effective for B cell activation. More importantly, the Food and Drug Administration (FDA, USA)-approved non-attenuated live HAdV7 vaccine has been used for over 25 years to protect more than 10 million U.S. military recruits from acute respiratory disease (ARD), and to reduce the overall morbidity rate by about 50% [18,23–27]. The safe and effective usage record of live vaccines makes HAdV7 attractive as a promising vaccine vector candidate. Mucosal or systematic recombinant HAdV7 vaccines against HIV, HBV, RSV, and H1N5 infection have been developed and reported [28–30], suggesting that recombinant HAdV7 could deliver vector-encoded antigens to APCs. In these regards, we propose that if HAdV7 could efficiently deliver antigen-encoding genes to human naive B cells, the TLR9-activated B cells could, in turn, cross-present the vector-encoded antigen and induce an antigen-specific CTL response.

To explore this possibility, we constructed an E3-defective recombinant HAdV7 vector encoding green fluorescent protein (GFP) and carcinoembryonic antigen (CEA). We demonstrate that naive human B cells are efficiently transduced and activated *in vitro* by recombinant HAdV7 vectors. We investigated the ability of recombinant HAd7CEA-transduced human naive B cells to process and to present CEA to autologous $CD8^+$ T cells. We also examined the *in vitro* and *ex vivo* cytotoxicities of CEA-specific $CD8^+$ T cells induced by HAd7CEA-modified B cells. At the same time, we provided evidence for the involvement of TLR9 in the activation of human naive B cells APC function by recombinant HAdV7.

2. Material and methods

2.1. Enzymes, kits, virus strains, cell lines, and animals

Unless otherwise indicated, enzymes and kits were purchased from Takara Co. (Dalian, China) or New England BioLabs, Inc. (Ipswich, MA, USA). HAdV7 strain WZ1 was originally isolated from a child with ARD [31]. The A549, SW480 (HLA-A2+/CEA+), MCF-7 (HLA-A2+/CEA-), and LoVo (HLA-A2-/CEA+) cell lines were obtained from the China Center for Type Culture Collection (Wuhan, China). These cells were maintained in culture medium according to the supplier's specifications. Female CB.17 SCID mice (6–8 weeks) were provided by the Animal Center at Wenzhou Medical University (Wenzhou, China) and housed in pathogen-free conditions.

2.2. Construction of an infectious clone of HAdV7

The infectious DNA clone containing the full-length HAdV7 genome was constructed using the highly efficient homologous recombination system in *Escherichia coli* BJ5183 (Strategene, CA, USA) as described before [32–34]. The shuttle plasmid pMD18Ad7TLR was constructed to contain the left and right end of HAdV7 genome (Fig. 1A). The left terminal homologous fragment, Ad7L, was PCR-amplified from the extracted viral genomic DNA using the primer pair, Ad7LF_PmeI and Ad7LR_NotI (Table 1), while the right terminal homologous fragment, Ad7R, was PCR-amplified using the primer pair, Ad7RF_NotI and Ad7RR_PmeI (Table 1). After digestion with *Not* I, the Ad7L and Ad7R PCR products were cloned into pMD18T (Takara Co. using three-fragment ligation). The resulting shuttle plasmid, pMD18TAd7LR, was linearized using *Not* I, and dephosphorylated with calf alkaline phosphatase (Fig. 1A). The purified HAdV7 genomic DNA and the linearized shuttle plasmid were cotransformed into competent *E. coli* BJ5183 cells by electroporation (Bio-Rad Laboratories, Inc, USA).

Table 1
Primers for PCR.

Primers	Sequences (5′ → 3′)
Ad7LF_PmeI	GGGTTTAAACCTCTCTATTTAATATACCTT
Ad7LR_NotI	CCGCGGCCGCGGAACTCGTCAGGTTTAA
Ad7RF_NotI	CCGCGGCCGCAAGCCACCCCTCGCGGATA
Ad7RR_PmeI	GGGTTTAAACCTCTCTAATATAATATACCTT
CEAF_NheI	CGAAGCTAGCATGGAGTCTCCCTCGGCCCC
CEAR_BspEI	GCGCTCCGGACTATATCAGAGCAACCCCAACC
β2mF	GTGCTCGCGCTACTCTCTCT
β2mR	TCAATGTCGGATGGATGAAA
TAP-1F	ACGTCCACCCTGAGTGATTC
TAP-1R	AGCTTTTCCCTAAACTTCTGGG
TAP-2F	ATCCCTCACTATTCTGGTCGT
TAP-2R	TGTAGGTGAAGCAGCCTCC
ERp57F	GCTAGAACTCACGGACGACA
ERp57R	TCAGGGTTGGATATCCACTG
LMP-2F	CATCTACTGTGCACTCTCTG
LMP-2R	CAGCTGTAATAGTGACCAGG
LMP-7F	CAGACACAGACATGACAACC
LMP-7R	GCCACATGAGTGTCTTACTG
GAPDHF	ACCACAGTCCATGCCATCAC
GAPDHR	TCCACCACCCTGTTGCTGTA

After overnight growth on Luria–Bertani (LB) agar plates supplemented with ampicillin, smaller colonies were picked and incubated overnight. The minipreped plasmid DNA from these colonies was digested by *Pme* I, and recombinants yielding an approximately 30 kb large fragment were retransformed into *E. coli* EZ10 cells (Clontech Laboratories, Mountain View, CA, USA) for further restriction analysis to confirm their plasmid structure. Finally, to determine the infectivity of the recombinant clone, pMD18TAd7, *Pme* I-linearized recombinant plasmid DNA was transfected into A549 cells using lipofetamine 2000 (Invitrogen, Carlsbad, CA, USA), and the cells were examined for evidence of cytopathic effect (CPE).

2.3. Construction of an E3-defective recombinant HAdV7GFP and HAd7CEA

HAdV7 genomic DNA was double digested with *Avr* II/*Spe* I, and the resulting 8.3 kb fragments were gel purified, blunted using a DNA blunting kit, and linked with blunted and dephosphorylated pMD18T to produce the shuttle vector, pAd7E3. The *Ase* I/*Mlu* I restriction fragment of pEGFP C1 (Clontech Laboratories), containing a CMV-eGFP-SV40 expression cassette, was blunted and linked to *Eco* RI-digested, blunted, and dephosphorylated pAd7E3 to construct the rescue and reporter plasmid, pAd7E3GFP. Dephosphorylated 5.7 kb *Eco* RV-digested fragments of pAd7E3GFP and the *Eco* RI-linearized infectious clone plasmid, pMD18TAd7, were cotransformed into *E .coli* BJ5183 cells. The resultant recombinant plasmids were screened by restriction analysis, linearized by *Pme* I, and transfected into A549 cells as described in Section 2.2 to produce recombinant HAd7GFP (Fig. 1B).

To construct the CEA-expressing recombinant HAd7CEA, human CEA cDNA was amplified from the total RNA of LoVo cells by reverse transcriptase-polymerase chain reaction (RT-PCR) using the primer pair, CEAF_NheI and CEAR_BspEI (Table 1). The PCR products were then cloned into the *Nhe* I/*Bsp* EI sites of pAd7E3GFP to produce the shuttle vector, pAd7E3CEA. Subsequently, the dephosphorylated 7.1 kb *Eco* RV-digested fragments of pAd7E3CEA and the *Eco* RI-linearized infectious clone plasmid pMD18TAd7 were cotrans-