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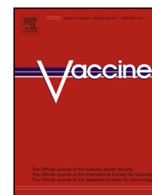

Self-adjuvanting influenza candidate vaccine presenting epitopes for cell-mediated immunity on a proteinaceous multivalent nanoplatform

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ABSTRACT

We exploit the features of a virus-like particle, adenoviral dodecahedron (Ad Dd), for engineering a multivalent vaccination platform carrying influenza epitopes for cell-mediated immunity. The delivery platform, Ad Dd, is a proteinaceous, polyvalent, and biodegradable nanoparticle endowed with remarkable endocytosis activity that can be engineered to carry 60 copies of a peptide. Influenza M1 is the most abundant influenza internal protein with the conserved primary structure. Two different M1 immunodominant epitopes were separately inserted in Dd external positions without destroying the particles' dodecahedric structure. Both kinds of DdFluM1 obtained through expression in baculovirus system were properly presented by human dendritic cells triggering efficient activation of antigen-specific T cells responses. Importantly, the candidate vaccine was able to induce cellular immunity *in vivo* in chickens. These results warrant further investigation of Dd as a platform for candidate vaccine, able to stimulate cellular immune responses.

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1. Introduction

Virus-like particles (VLP) are naturally occurring biodegradable nanomaterials that incorporate such viral features as repetitive surfaces, particulate structures and induction of innate immunity through activation of pathogen-associated molecular-pattern recognition receptors. They carry no genetic information and can be often easily produced on a large scale. VLPs are being developed as safe and effective vaccine platforms for inducing potent B- and T-cell responses. The prophylactic human vaccines based on VLPs that have been registered include vaccines against human papillomavirus (HPV) and against hepatitis B. These examples show the VLP vaccines constructed to protect against their virus of origin; however, VLPs can also be used to present foreign epitopes to the immune system. In the design of our vaccine the VLP platform built from a protein derived from the adenovirus (Ad) is used for carrying the epitopes of another virus, influenza, with the goal of establishing immunity against influenza and not against Ad infection.

The adenoviral dodecahedron (Ad Dd) is composed solely of 12 copies of a pentameric viral protein, penton base (Pb), one of two Ad proteins responsible for virus cell entry [1]. Dd attaches to receptors recognized by Pb within an Ad particle, but, in addition, it recognizes heparan sulphates, which do not serve as receptors for the Ad of origin [2]. Dd has an extraordinary propensity for intracellular entry – up to 300,000 of Dd can be seen in one cell in culture [3]. Due to its polyvalency, the vector is able to deliver several millions of foreign molecules to one cell [3]. Importantly, it is efficiently taken up by human DC and induces their maturation [4]. Dd can be stored frozen and/or lyophilized and shows remarkable stability at

Abbreviations: aa, amino acid; Ad, adenovirus; Ad3, adenovirus serotype 3; APC, antigen-presenting cells; CTL, cytotoxic T lymphocyte; Dd, dodecahedron; FCS, fetal calf serum; HA, hemagglutinin; HLA, human leukocyte antigen (a group of the most important antigens responsible for tissue compatibility); MoDC, monocyte-derived dendritic cells; NA, neuraminidase; Pb, penton base protein; PBMC, peripheral blood mononuclear cells; pDC, plasmacytoid dendritic cells; PE, phycoerythrin; PMA/Iono, phorbol 12-myristate 13-acetate/ionomycin; rwtDd, recombinant wild-type Dd; SPF, specific pathogen-free; VLP, virus-like particle.

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temperatures up to 45 °C [5]. The crystallography and site-directed mutagenesis combined with biochemical analysis showed that the N-termini of the Pbs, Dd building blocks, interlock within the particle through strand exchange, thus creating the major stabilization element of the dodecahedral particle [6].

The humoral response to currently used influenza vaccines is based on the induction of neutralizing antibodies mainly against the influenza surface glycoprotein, hemagglutinin (HA). Such neutralizing Abs block entry of the influenza virus but they are protective only against virus with closely matching HA. Antibody response builds up rather fast and declines slowly afterwards [7], while the cell-mediated immunity that does not involve Abs seems to be lasting longer [8]. The current influenza vaccines containing killed influenza virions do not induce CTL responses, because they are inefficiently processed for MHC class I presentation [9]. In addition, due to the high mutability of influenza surface proteins (antigenic drift), the current vaccines have to be produced new every year following the WHO recommendations. It is thought that the cross-reactive CTL responses may have protective potential against variants of influenza viruses that are not neutralized by antibodies [10].

The matrix protein M1 (252 aa residues) is the most abundant influenza internal virion protein with the most conserved primary structure among influenza virus proteins [11], suggestive of its utility in induction of cellular immunity. Mice immunized with matrix protein were not protected after challenge but they showed enhanced clearance of the virus from the lungs after infection, signifying involvement of cell-mediated immunity [12]. Indeed, M1 is able to elicit CTL activity in mice and generates specific $CD8^+$ T cells response in humans [13–15].

Recently, an *ex vivo* analysis of cross-reactive $CD4^+$ and $CD8^+$ memory T cell response to overlapping peptides spanning the full proteome of two influenza strains has been performed in healthy individuals [16]. M1 and nuclear protein turned out to be the immunodominant targets of cross-recognition. Those identified M1 epitopes that were recognized by human T cell repertoires are used here for the construction of a vaccine that should induce heterosubtypic T cell-mediated immunity conferring broad protection against avian and human influenza A viruses.

We exploit the features of a VLP, the adenoviral dodecahedron, for engineering a vaccination platform carrying epitopes derived from the influenza M1 protein for induction of cell-mediated immunity. A vaccine in form of Dd bearing M1 epitopes was constructed, expressed in the baculovirus system and purified. It was efficiently internalized, processed and presented by the HLA class II and cross-presented by the HLA class I molecules triggering $CD4^+$ and $CD8^+$ T cell responses. Importantly, upon chicken vaccination with Dd carrying the M1 epitopes both cellular and humoral immune responses were elicited in the absence of an adjuvant. Our data show that the proposed vaccine is able to induce strong and possibly long-lasting cell-mediated immunity.

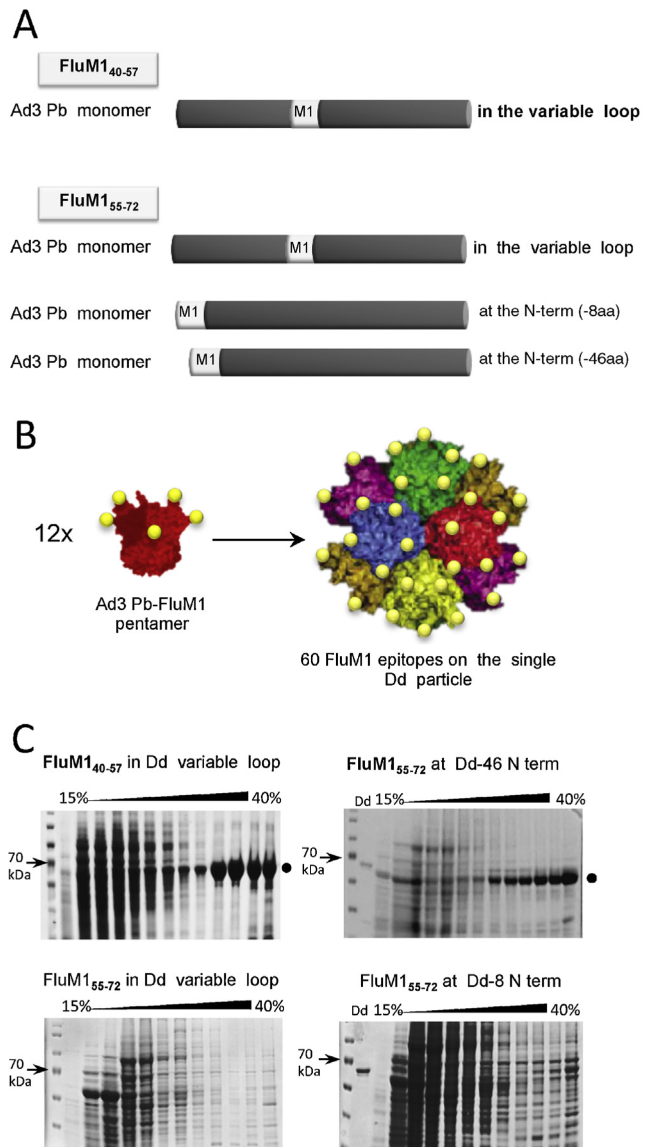

Fig. 1. Construction of dodecahedric platform bearing epitopes derived from influenza M1 protein. (A) Schematic view of prepared constructs with M1 epitopes inserted into monomers of Ad3 Pb protein that is a building block of Dd. Successful constructs are marked in bold. (B) Schematic representation of Dd bearing M1 epitopes. Upon expression in baculovirus system 12 pentameric penton bases self-assemble into dodecahedra containing 60 copies of M1 epitopes on the single particle. (C) Expression of Dd carrying M1 epitopes in the baculovirus expression system. Cells were lysed and fractionated on the sucrose density gradients as described in Materials and Methods. Portions of fractions (15 μl out of 1 ml) were run on SDS–PAGE and stained with CBB. Arrows indicate sucrose concentration from low to high. Dd – control unmodified particle.

2. Materials and methods

2.1. Construction of Dd bearing M1 epitopes

For the cloning of $FluM1_{40-57}$ epitope (EALMEWLKTRPILSPLTK) into the variable loop of penton base (Pb), the 54-long sequences of forward and reverse primers encoding the $FluM1_{40-57}$ epitope along with the fragment of Ad3 Pb were obtained from Oligo.pl (Poland). The primers (Suppl. Table 1) were amplified by PCR, yielding the fragment of 90 nucleotides. In the two subsequent steps the first PCR product was amplified using as a matrix pFastBacDual containing the Ad3 Pb sequence, with primers permitting insertion of restriction enzymes' recognition sites. In the last step the final PCR product was obtained with deleted nucleotide fragment specifying the ^{157}VTVND Pb sequence. The 1414 bp-long insert was then cloned into pFastBacDual using BamHI and PstI cloning sites. The genes encoding $FluM1_{55-72}$ epitope (LTKGILGFVFTLTVPSER) either in the variable Pb loop or at the N-terminus of the Pb were purchased from GeneArt GmbH (Germany). Three different constructs were designed. In the first one the ^{157}VTVND sequence from the variable loop was replaced by the $FluM1_{55-72}$ epitope. Two others contained the same epitope at the N-terminus of Pb devoid of 8 or 46 amino acids (Fig. 1A). The provided sequences were cloned into pFastBacDual under the control of polyhedrin promotor. The correct cloning was confirmed by sequencing.

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