Vaccine 26 (2008) 3242–3251

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Cellular and humoral immune responses to chimeric EGFP-pseudocapsids derived from the mouse polyomavirus after their intranasal administration

Jan Frič[a,b,1], Martin Marek[a,1], Veronika Hrušková[a], Vladimír Holáň[a,b], Jitka Forstová[a,*]

[a] Faculty of Science, Charles University in Prague, Viničná 5, 128 44 Prague 2, Czech Republic
[b] Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic

ARTICLE INFO

Article history:
Received 1 November 2007
Received in revised form 6 March 2008
Accepted 5 April 2008
Available online 23 April 2008

Keywords:
Mouse polyomavirus pseudocapsids
Chimeric VLPs
Antigen carrier and adjuvant

ABSTRACT

Mouse polyomavirus (MPyV) VP1-pseudocapsids carrying enhanced green fluorescent protein (EGFP-VLPs) were used for intranasal immunization of mice. EGFP-VLPs induced strong anti-VP1 but not anti-EGFP antibody production. *In vitro* restimulation with antigen-pulsed bone marrow-derived dendritic cells (BMDCs) induced remarkable T-cell proliferative response specific for both VP1 and EGFP antigen and IL-2 and IFN-γ production. Surprisingly, no specific cytotoxic activities against VP1 and EGFP proteins were detected. After intranasal administration of EGFP-VLPs, as well as after polyomavirus infection, a moderate reduction of $CD4^+CD25^+Foxp3^+$ T cells was observed in spleens but not in lymph nodes and peripheral blood, suggesting that both MPyV virions and pseudocapsids are able to induce changes in distribution of regulatory T cells. Treatment of EGFP-VLPs pulsed BMDCs with inhibitors of endosomal acidification proved that presentation of peptides on MHCgp class II is dependent on acidic endosomal environment. Substantial decrease of CD4-specific T-cell proliferation in the presence of proteasome inhibitor suggests that MHCgp class II might load VPL-derived peptides processed by proteasomes. Thus, polyomavirus derived VLPs appear to be promising delivery and adjuvant vehicles for therapeutic proteins.

1. Introduction

Efficient delivery of antigens to cells of the immune system still remains an important objective in the vaccination research. Virus-like particles (VLPs) derived from different viruses can be used as direct immunogens that stimulate cellular and humoral responses. In addition, they can be manipulated to carry heterologous epitopes for delivery into the cells *in vitro* and *in vivo*.

Murine polyomavirus (MPyV) is a small non-enveloped tumorogenic DNA virus. Its genome encodes three structural proteins VP1, VP2 and VP3. The MPyV capsid with icosahedral symmetry is composed of 72 capsomeres formed by pentamers of the major structural protein, VP1. The minor structural proteins of MPyV, VP2 and VP3, are not exposed on the surface of the capsid structure. The common C-terminus sequence of either VP2 or VP3 interacts with the central cavity of the VP1 pentamer. VLPs based on MPyV capsids can be formed by the major structural protein, VP1 only. VP1 is able to spontaneously self-assemble into VLPs either during its expression in insect or yeast cells, or *in vitro,* from purified VP1 pentamers. VP1 is responsible for interaction of the viral particle with the sialysed ganglioside receptor. VP1-VLPs enter a variety of cell types including human cells, with the efficiency comparable with that of the native virions. Several studies demonstrated the ability of MPyV-based VLPs to deliver genes for expression to many tissues of mouse [1–3]

Recently, VLPs have become broadly studied for vaccination strategies, primarily against the viral antigens forming the capsid surface [4–6] but also with the aim to induce immune response against heterologous antigens introduced into the pseudocapsid [7]. Immunization protocols have included VP1 antigen/DNA complexes [8] or chimeric VP1 VLPs with therapeutical peptides exposed on the surface of particles, inserted into VP1 surface loops [9,10] or inside pseudocapsids [2]. Intranasal immunization with VLPs has been shown to generate strong antibody production and to activate T-cell response without any other adjuvants. In our

Abbreviations: APC, antigen-presenting cells; BMDC, bone marrow-derived dendritic cell; BSA, bovine serum albumin; CFSE, carboxyfluorescein diacetate succinimidyl ester; CFA, complete Freund's adjuvant; CM, complete culture medium; cpm, counts per minute; CTL, cytotoxic T lymphocytes; EGFP, enhanced green fluorescent protein; DC, dendritic cell; EGFP-VLPs, pseudocapsids composed of VP1 major capsid protein and EGFP-t-VP3 fusion protein; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICFA, incomplete Freund's adjuvant; mAb, monoclonal antibody; MPyV, mouse polyomavirus; PBS, phosphate-buffered saline; pDC, pulsed BMDC; PI, proliferation index; t-VP3, truncated VP3 minor capsid protein (C-terminus 105–204 amino acids); Treg, regulatory T cells; VLPs, empty artificial virus-like particles; VP1-VLPs, pseudocapsids composed of VP1 major capsid protein of MPyV.

* Corresponding author. Fax: +420 221 951 729.
E-mail address: jitkaf@natur.cuni.cz (J. Forstová).
[1] These authors contributed equally to this work.

0264-410X/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2008.04.006

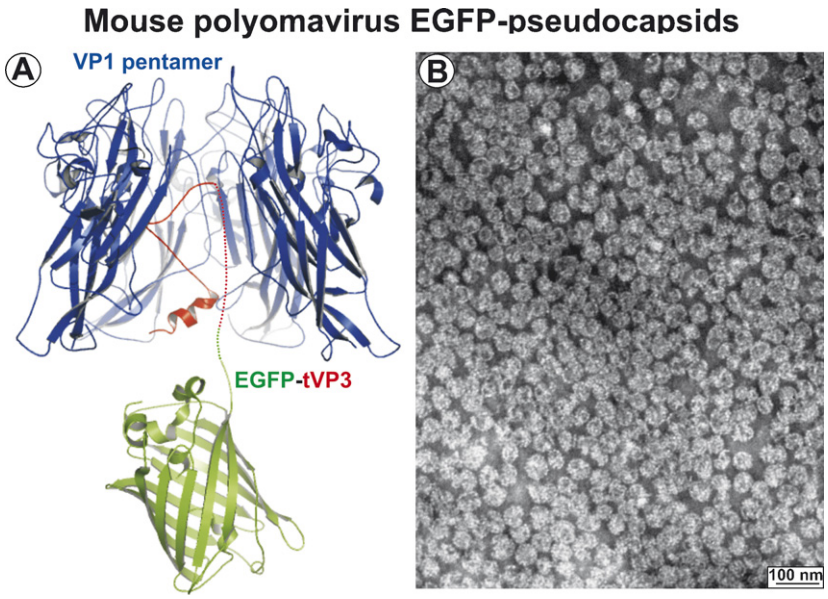

Fig. 1. Structure and architecture of the analyzed EGFP capsid-like particles derived from mouse polyomavirus. (A) View-through, showing the macromolecular interaction of VP1 pentamer (depicted in blue) with the C terminus of minor structural protein VP3 (depicted in red) fused with the EGFP protein (depicted in green). (B) Electron microscopy: EGFP-VLPs purified from insect cell lysates by density gradient were attached to carbon-coated grids, contrasted by negative staining and visualized by electron microscopy. Detailed design, production, properties, and characterization of chimeric EGFP-VLPs were described by Bouřa et al. [11].

previous paper [11], we studied, in *in vitro* experiments, model MPyV-derived chimeric pseudocapsids carrying enhanced green fluorescent protein (EGFP) fused with the C-terminal part of the VP3 minor protein inside the particles. We showed that particles entered mouse and human dendritic cells (DC) efficiently and in part colocalized with lysosome marker and with ubiquitine. However, neither VLPs composed of VP1 only nor EGFP-VLPs upregulated expression of costimulatory molecules CD80, CD83, CD86 of DC *in vitro*. Also, in concentrations used (up to 10^5 particles per cell), the particles did not change the migratory capacity of human DCs in response to MIP-3b, the competence to stimulate T-cell proliferation, or the ability of phagocytosis. On the other hand, they did induce secretion of IL-12 by DCs.

In the present work, we analyzed responses of the mouse immune system after intranasal immunization with VLPs. We have focused on the study of EGFP-VLPs as an efficient system for delivery of the EGFP antigen to the mouse immune system and to a more detailed characterization of the immune response. We have also made the first study to characterize processing of the capsid protein and the carried antigen in various intracellular compartments, which may lead to different types of antigen presentation in context with MHCgp class I or II. Our data have shown that intranasal immunization with EGFP-VLPs induces a vigorous antibody response and activates antigen-specific proliferation of $CD4^+$ T cells. In addition, an involvement of antigen processing in the acidic endosomal compartment of APC has been documented. The results are discussed with respect to the potential use of VLPs for vaccination purposes.

2. Materials and methods

2.1. Mice

Female mice of inbred strain BALB/c at the age of 7–10 weeks obtained from the breeding unit of the Institute of Molecular Genetics (Academy of Sciences, Prague, Czech Republic) were used in the experiments. The experiments were approved by the local Animal Ethics Committee.

2.2. Preparation of mouse polyoma VP1 and VP1/EGFP-t-VP3 capsid-like particles

Insect cells (*Spodoptera frugiperda* – Sf9) were infected with recombinant baculoviruses carrying the gene for VP1 only [12] or both genes for VP1 and EGFP-t-VP3 [11] in doses of 10 PFU per cell. Cells were harvested 72 h post infection, lysed, and capsid-like particles were purified by CsCl and sucrose gradients as described previously [13]. The amounts of prepared capsid-like particles were determined by the standard Bradford method and their quality was checked by electron microscopy. Purity of VLPs was further tested for protein and nucleic acid contaminations (see AppendixB-supplementary data). The structure of EGFP-VLPs is shown in Fig. 1.

2.3. Mouse polyomavirus propagation and purification

For mouse polyomavirus propagation, 3T6 Swiss albino fibroblasts, cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS; Gibco, Paisley, UK) and 4 mM glutamine were infected with mouse polyomavirus (strain A2), with multiplicity of infection of 0.1 PFU per cell. After 7 days, cells were harvested and virions were purified by the standard ultracentrifugation procedure according to Türler and Beard [14]. The amount of viral particles was estimated by hemagglutination and by Bradford protein concentration analysis.

2.4. Non-VLP recombinant EGFP production

For EGFP production of non-VLP origin, the insect cells (*Spodoptera frugiperda* – Sf9) were infected with recombinant baculoviruses carrying the gene for EGFP protein under the late p10 promotor. Cells were harvested 72 h post infection, lysed, and EGFP was precipitated at 80% saturation of ammonium sulphate. The dissolved sample enriched in EGFP was dialyzed against phosphate-buffered saline (PBS) and concentrated by polyethylene glycol (Serva, Heidelberg, Germany); all steps were carried out at 4 °C. The quality of preparation was analyzed by sodium dodecyl