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Immunogenic properties of polyomavirus-derived recombinant virus-like particles: activation of murine spleen cell-derived dendritic cells

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Virus-like particles (VLPs) generated by the heterologous expression of viral structural proteins possess a high immunogenicity and have been exploited as potential vaccines. As evidenced for different VLPs, their strong immunogenicity is driven by activation of dendritic cells (DCs), the most potent antigen-presenting cells. Recently, we have demonstrated that yeast-expressed hamster polyomavirus-derived VP1 assembled to VLPs induce strong B- and T-cell responses in mice. The present study was aimed at evaluating the ability of recombinant hamster polyomavirus VP1 VLPs to activate murine spleen cell-derived DCs. For this purpose, we investigated the uptake of chimeric fluorescent VLPs by murine spleen-cell derived DCs, observed their phenotypic changes during the incubation with VLPs and evaluated DC activation by analysing the cytokine IL-12 secretion level. In addition, we evaluated the functional activity of DCs treated with VLPs by analysing their capability to activate naļve T-helper cells isolated from the spleens of syngeneic mice. The activation of T-helper cells by VLP-treated DCs was evaluated by the secretion of cytokine IL-2 in DC/T cell co-cultures. The fluorescence microscopy analysis demonstrated an efficient uptake of VP1-derived fluorescent VLPs by immature DCs *in vitro*. Incubation with VP1-derived VLPs induced morphological changes of DCs indicative of their maturation. Following incubation with VLPs, significant amounts of cytokines IL-12 and IL-2 were determined in DC cultures and DC/T cell co-cultures, respectively. In conclusion, recombinant yeast-expressed VP1-derived VLPs efficiently induce an activation of murine spleen cell-derived DCs. This may serve as a basis for the strong immunogenicity of recombinant VP1-derived VLPs.

Key words: virus-like particles, hamster polyomavirus, major capsid protein, dendritic cells, immunogenicity

INTRODUCTION

Recombinant viral proteins with their intrinsic capacity to self-assemble to highly-organized virus-like particles (VLPs) represent promising vaccine candidates. This is evidenced by the fact that the first recombinant vaccine for human use is based on VLPs derived from the yeast-expressed surface antigen of hepatitis B virus. Other VLP-based vaccines are already in clinical trials [1, 2]. Because of their repetitive structure, VLPs induce a strong humoral immune response [2, 3]. In addition, VLPs have been found to stimulate T helper and cytotoxic T cell responses [4].

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Previously, we have efficiently generated VLPs by yeast expression of the major capsid protein VP1 of different polyomaviruses [5, 6]. Hamster polyomavirus major capsid protein VP1 derivatives assembled to VLPs have been shown to induce strong B- and T-cell responses in mice [3, 7]. Moreover, chimeric VP1-derived VLPs harbouring foreign epitopes at certain surface-exposed sites have been successfully used as a tool for hybridoma technology to generate monoclonal antibodies of the desired specificity [8]. These data suggested that hamster polyomavirus VP1 protein expressed in yeast and self-assembled to VLPs is a strong immunogen able to activate both B cells T-helper cells even in the absence of adjuvants.

Dendritic cells (DCs) are a family of professional antigen-presenting cells with a unique capacity to trigger

primary immune response [9]. Immature DCs located in peripheral tissues capture and process antigens and then migrate to regional lymph nodes where they activate T cells. Following the acquisition of antigens, maturation of DCs is induced, which is associated with morphological changes of DCs as well as up regulation of co-stimulatory molecules and certain cytokines necessary to initiate the cell-mediated immune response [9, 10]. A wide range of stimuli, including infectious virus and bacterial antigens, can induce maturation of DCs. The immunogenicity of protein antigens is enhanced by using adjuvants that provide DC-activating signals. Most of protein antigens applied without adjuvants are not able to activate DCs and therefore are non-immunogenic.

In this study, we have assessed the effects of hamster polyomavirus VP1-derived VLPs on murine spleen cell-derived DCs. We have investigated the uptake of recombinant VLPs by DCs, their activation, and their ability to activate naïve T cells. We found that hamster polyomavirus VP1-derived VLPs were capable of inducing DC activation and maturation sufficient to initiate primary immune reactions.

MATERIALS AND METHODS

Antigens

In this study, three different types of hamster polyomavirus-derived VLPs: major capsid protein VP1 without any insert (VP1) and two chimeric proteins with either inserted GFP (VP1/eGFP) or 120-long hantavirus nucleocapsid protein (VP1/1-VR120) were used. For studying the uptake of VLPs by murine DCs, chimeric VP1/eGFP VLPs containing a single insert of enhanced green fluorescent protein (eGFP) in hamster polyomavirus VP1 were applied [8]. For the activation of DCs, the antigens VP1 and VP1/1-VR120 were used [3]. All VP1-derived VLPs were expressed in yeast *S. cerevisiae* and purified by centrifugation in CsCl gradients according to previously published protocols [5, 3]. The formation of VLPs was confirmed by electron microscopy [3].

Experimental mice

For the isolation of DC and T-helper cells, 6–8 week-old BALB/c mice obtained from the Institute of Immunology of Vilnius University were used.

Preparation of murine dendritic cell culture

Spleen cell-derived murine DCs were prepared as described previously [11]. Briefly, spleen cells of naïve BALB/c mice were seeded into 6-well-plates at a density of 10^6 cells/ml (5 ml/well) and incubated for 2 h in a CO_2-thermostat at 37 °C. The cells were cultivated in RPMI-1640 growth medium supplemented with 10% fetal bovine serum (Biochrom, Germany). Non-adherent cells were removed and the two cytokines, granulocyte-monocyte colony stimulating factor (GM-CSF; BD PharMingen, 10 μg/vial) and interleukin 4 (IL-4; BD PharMingen, 10 μg/vial) were added to the growth medium to give final concentrations of 10 ng/ml. After incubation of the DCs for 6 days (144 h) with the cytokines GM-CSF and IL-4, the respective VLPs were added to the DC cultures at a concentration of 10 μg/ml.

Fluorescence microscopy analysis of dendritic cells

The uptake of chimeric VP1/eGFP VLPs was monitored by an inverted fluorescence microscope (Olympus IX-70, Olympus, Japan) after 24 and 48 h of incubation with VP1/eGFP VLPs (10 μg/ml). Morphological changes of DCs were observed during 6–8 days of incubation with VLPs.

Isolation and characterization of T helper cells

T helper cells were isolated from the spleen of a naïve BALB/c mouse using a kit for magnetic cell separation (CD4+ T cell isolation kit, Miltenyi Biotec, Germany). The content of magnetically separated T cells was estimated by fluorescence microscopy using a FITC-labeled mouse CD3-specific antibody (BD PharMingen). For this purpose, the cells were directly stained with FITC-labeled anti-CD3 by incubation for 30 min in the dark. The content of fluorescent CD3-positive cells in the magnetically separated cell population was estimated using an Olympus IX-70 inverted fluorescence microscope.

Stimulation of dendritic cells with virus-like particles for cytokine assays

The ability of VLPs (VP1, VP1/eGFP, and VP1/1-VR120) to stimulate murine DCs was evaluated by two parameters: (i) IL-12 secretion, (ii) the ability to activate syngeneic T cells. For the measurement of IL-12 secretion, on day 6 following the incubation with GM-CSF and IL-4 the DCs were recovered from 6-well plates and seeded into 24-well plates at a density of 5×10^5 cells/well. The VLPs VP1, VP1/eGFP and VP1/1-VR120 were added to the DC cultures at a concentration of 10 μg/ml. As a positive control, lipopolysaccharide (LPS) was used (10 μg/ml; Sigma). After incubation for 48 h, DC supernatants were collected for IL-12 assay and stored at –20 °C until required for analysis. To evaluate the ability of DCs to activate syngeneic T cells, co-cultures of DCs and T cells were prepared and after incubation with the respective antigens the level of IL-2 was measured. T cells were added to the DC cultures treated for 48 h with the respective VLPs (10 μg/ml). The DC/T-cell ratio in the co-cultures was 1:20. After the addition of T cells, a fresh growth medium was added, containing the same concentration of the respective VLPs (10 μg/ml), and the co-cultures were further incubated for 96 h. After incubation, the supernatants of DC/T-cell co-cultures were collected for IL-2 assay and stored at –20°C until analysis.

Cytokine assays

Interleukin 12 (IL-12) and interleukin 2 (IL-2) concentrations in DC cultures or DC/T cell co-cultures were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) using Mouse IL-12 Opt-EIA Set and Mouse IL-2 Opt-EIA Set according to the recommendations of the supplier (BD PharMingen, San Diego, USA).

RESULTS

Uptake of fluorescent virus-like particles by dendritic cells and induction of dendritic cell maturation

Antigen capture by the antigen-presenting cells (DCs) is the first step in the initiation of the immune response. Strong immunogens induce maturation of DCs. Only mature DCs that express co-stimulatory molecules, and cytokines are able to present the antigen to T cells and trigger the cellular immune response. Therefore, in order to evaluate the ability of hamster polyomavirus VP1-derived VLPs to activate DCs we investigated the uptake of fluorescent VLPs by murine spleen-cell derived DCs of naïve (non-immunized) BALB/c mice and the morphological changes of DCs following the incubation with VLPs. Fluorescence microscopy analysis demonstrated an efficient uptake of fluorescent VLPs (chimeric protein VP1/eGFP) by DCs: after 24 h of incubation the fluorescent VLPs were detected inside the DCs (Fig. 1a). The cells were round-shaped, which is typical of immature DCs. After 6 days (144 h) of incubation with fluorescent VLPs, morphological changes of DCs were observed: the cells became dendritic-like structures typical of mature DCs (Fig. 1b).

Activation of murine spleen cell-derived dendritic cells by virus-like particles

Activated DCs are characterized by the enhanced expression of certain co-stimulatory molecules and cytokines, such as IL-12. Therefore, we evaluated the VLP-mediated activation of DCs by analysis of IL-12 secretion by DC cultures treated with VLPs. Antigens VP1, VP1/eGFP and VP1/1-VR120 added to DC cultures induced a high IL-12 secretion after 48 h of incubation (Fig. 2). The levels of IL-12 in VLP-treated DC cultures were in the range of 50–160 pg/ml. In comparison, the mean concentration of IL-12 in control DC cultures treated with *E. coli* lipopolysaccharide (LPS), a potent DC stimulator, was 195 pg/ml. In contrast, the levels of IL-12 in DC cultures incubated for 48 h with cytokines IL-4 and GM-CSF without the addition of VLPs were in the range of 0–15 pg/ml. Compared to this basal level, the IL-12 secretion level was increased 3- to 10-fold by incubation with the VLPs, indicating the specificity of the VLP-mediated activation of DCs.

Activation of syngeneic T helper cells by dendritic cells treated with virus-like particles

The functional activity of DCs treated with VLPs was demonstrated by their capability to activate naïve T-helper cells isolated from the spleens of non-immunized BALB/c mice. To evaluate the purity of the magnetically separated spleen cell population, the cells were directly immuno-stained with a FITC-labeled anti-CD3 antibody. Fluorescence microspopy confirmed that magnetically separated cells were CD3-positive, i.e. T cells (Fig. 3). The activation of T-helper cells by VLP-treated DCs was evaluated by the secretion of cytokine IL-2 in DC/T cell co-cultures. Significant amounts of IL-2 (in the range of 45–120 pg/ml)

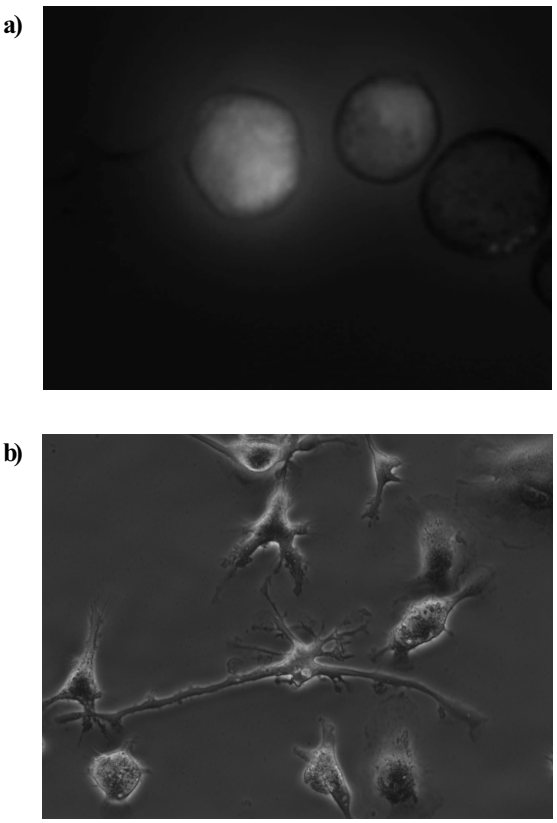

Fig. 1. Demonstration of the uptake of recombinant VLPs by murine DCs and the VLP-induced maturation of DCs: fluorescence microscopy analysis of DCs incubated with fluorescent VP1-derived VLPs VP1/eGFP for 24 h (a) and 144 h (b)

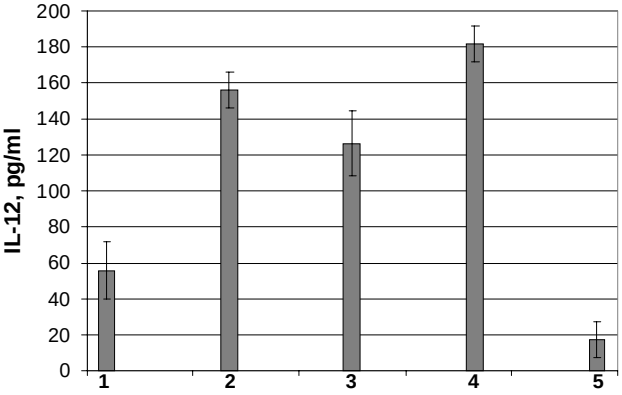

Fig. 2. Activation of murine spleen-cell derived DCs by recombinant VLPs: production of IL-12 (pg/ml) by murine spleen-cell derived DCs incubated for 48 h with (1) VP1; (2) VP1/1-VR120; (3) VP1/eGFP; (4) LPS; (5) medium alone. Mean values (±SD) from triplicates are shown

were determined in DC/T cell co-cultures after 96 h (4 days) of incubation with different VLPs: VP1, VP1/eGFP and VP1/1-VR120 (Fig. 4, columns 1–3). In contrast, the basal level of IL-2 in DC/T cell co-cultures incubated for 96 h without the addition of VLPs did not exeed 20 pg/ml (Fig. 4, column 4). Thus, compared to DC/T cell

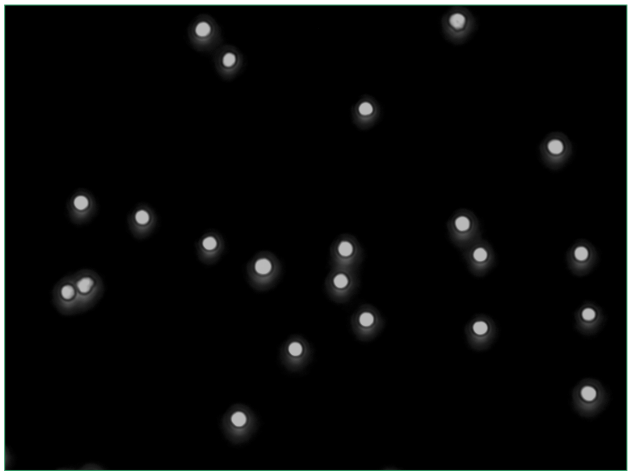

Fig. 3. Characterization of magnetically separated murine spleen cell-derived T helper cells by fluorescence microscopy. Magnetically separated cells were directly immunostained with FITC-labeled anti-CD3 antibodies

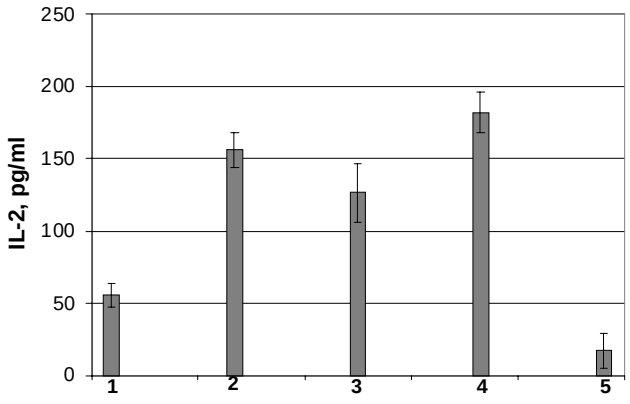

Fig. 4. Activation of naļve T helper cells by VLP-treated DCs: production of IL-2 (pg/ml) by murine DC/T cell co-cultures incubated for 96 h with (1) VP1; (2) VP1/1-VR120; (3) VP1/eGFP; (4) LPS; (5) medium alone. Mean values (±SD) from triplicates are shown

co-cultures incubated with medium alone, following incubation with VLPs a 2- to 6-fold increase of IL-2 concentration was observed. These data demonstrate the functional activity of DCs treated with VLPs, i.e. their ability to present the antigen and activate naïve T-helper cells.

DISCUSSION

In this study, we have demonstrated the ability of hamster polyomavirus-derived recombinant VLPs to activate antigen presenting cells, which is a key step in triggering cellular immune response. Dendritic cells (DCs) represent the most potent antigen-presenting cells that capture and process antigens [9, 10]. Following the uptake, antigens induce activation and maturation of DCs. Mature DCs are phenotypically characterized by a dendritic-like structure and increased expression levels of various surface molecules such as class I and class II MHC, costimulatory molecules CD80, CD86 and others. In addition, they secrete cytokines such as IL-12, which are important to induce T cell responses. Only mature DCs are capable to present the antigens to T helper cells and induce their activation [9].

As evidenced for different VLPs, their strong immunogenicity is driven by activation of DCs. Previous studies with papilloma- and parvovirus-derived VLPs demonstrated their ability to induce activation of DCs and T cell priming [4, 10, 12]. Here, we have employed hamster polyoma virus VP1-derived fluorescent VLPs to demonstrate their uptake by murine spleen cell-derived DCs. We have also demonstrated the ability of hamster polyomavirus-derived recombinant VLPs to induce the secretion of cytokine IL-12, which suggested their activation. In addition, we have observed morphological changes of immature DCs after the capture of recombinant VLPs indicative of their maturation. In the second approach, we have demonstrated the functional activity of VLPs-treated DCs, e.g., their ability to prime naïve syngeneic T helper cells. In our study, we have used three different types of hamster polyomavirus-derived VLPs: major capsid protein VP1 without any insert (VP1) and two chimeric proteins with either inserted GFP (VP1/eGFP) or 120-long hantavirus nucleocapsid protein (VP1/1-VR120, [3]). VP1-based chimeric proteins containing inserts of different size and origin were shown to be highly immunogenic, therefore, we expected them to induce a strong DCs activation [3, 7, 8]. Indeed, the chimeric VLPs induced higher cytokine expression levels than did VP1 alone. This difference might be explained by a more efficient capture of chimeric VLPs by DCs due to a slightly different solubility of chimeric VLPs as compared to VP1 VLPs [3].

Taken together, the results obtained within this study on murine spleen cell-derived DC cultures and DC/T cell co-cultures characterized hamster polyomavirus-derived recombinant VLPs as strong immunogens capable of activating DCs and efficiently priming naïve T cells. This is in agreement with our previous data demonstrating that hamster polyomavirus-derived VLPs efficiently promote the development of highly-specific IgG antibodies, indicating that VLPs activate both B cells and T-helper cells providing the necessary signals for Ig class switching and affinity maturation [3, 8]. The efficient activation of DCs demonstrated in the current study may serve as a basis for the strong immunogenicity of hamster polyomavirus VP1-derived recombinant VLPs.

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L. Šamonskytė, I. Kučinskaitė, A. Gedvilaitė, K. Sasnauskas, A. Žvirblienė

POLIOMOS VIRUSO BALTYMO PAGRINDU SUKURTŲ REKOMBINANTINIŲ, Į VIRUSUS PANAŠIŲ DALELIŲ IMUNOGENINĖS SAVYBĖS: PELĖS DENDRITINIŲ LĄSTELIŲ AKTYVACIJA

Santrauka

Į virusus panašios dalelės (VPD), gaunamos ekpresuojant virusų baltymus heterologinėse sistemose, yra labai imunogeniškos ir todėl naudojamos vakcinoms kurti. Skirtingų VPD tyrimai rodo, kad didelį jų imunogeniškumą nulemia dendritinių ląstelių (DL) – efektyviausių antigeną pateikiančiųjų ląstelių – aktyvacija. Kaip rodo mūsų ankstesni tyrimai, mielėse ekspresuotas žiurkėno poliomos viruso VP1 baltymas, formuojantis VPD, sukėlė stiprų imunizuotų pelių B ir T ląstelių atsaką. Šio darbo tikslas buvo įvertinti VPD, gautų iš žiurkėno poliomos viruso rekombinantinio baltymo VP1, sugebėjimą aktyvinti DL, išskirtas iš pelės blužnies. Tam tikslui mes tyrėme chimerinių fluorescuojančių VPD patekimą į DL, stebėjome jų fenotipinius pokyčius inkubacijos su VPD metu, taip pat įvertinome DL aktyvaciją matuodami citokino IL-12 sekrecijos lygį. Be to, mes įvertinome DL funkcinį aktyvumą tirdami jų sugebėjimą aktyvinti naiviąsias T ląsteles, išskirtas iš singeninių pelių blužnies. T ląstelių aktyvacija buvo įvertinta pagal citokino IL-2 sekrecijos lygį bendroje T ląstelių ir DL kultūroje. Fluorescencine mikroskopija nustatyta, kad fluorescuojančios VP1 baltymo VDP efektyviai patenka į nesubrendusias DL *in vitro*. Inkubacija su VP1 baltymo VPD sukėlė DL morfologinius pokyčius, kurie liudija DL brendimą. Inkubuojant su VPD, gerokai padidėjo citokino IL-12 koncentracija DL kultūroje ir citokino IL-2 koncentracija bendroje T ląstelių ir DL kultūroje. Galima padaryti išvadą, kad rekombinantinėse mielėse ekspresuotos VP1 baltymo VPD efektyviai aktyvina DL, išskirtas iš pelės blužnies. Tuo gali būti paaiškintas didelis VPD imunogeniškumas.