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NOVEL APPROACH FOR DESIGNING ROTAVIRUS VACCINE CANDIDATE BASED ON TWO PLANT VIRUSES

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Abstract

Rotavirus A (genus *Rotavirus*, family *Reoviridae*) is still the main cause of viral gastroenteritis in children under 5 years. Existing attenuated vaccines have serious disadvantages, including the risk of potential reversion to pathogenic form, and side effects, the most dangerous of which is intussusception. Moreover, they occurred to be less effective in developing countries, where the most rotavirus-associated deaths are recorded. The development of an effective recombinant rotavirus A vaccine is an actual assignment; herewith the selection of effective and safe adjuvant is the key point for that. Plant viruses are very promising for innovative vaccine designing; they possess high immunostimulating properties, safe for humans and mammals and can serve as a carrier for pathogens' epitopes. Here we suggest an approach for rotavirus A vaccine development that involves two plant viruses: Alternanthera mosaic virus (AltMV) and Tobacco mosaic virus (TMV) as simultaneously epitope carriers and adjuvants. Spherical particles (SPs) generating by the heating of tobacco mosaic virus were used as an adjuvant and platform for presentation of obtained in our previous study chimeric recombinant protein ER6, which is an AltMV coat protein (CP) fused with the epitope RV14 (RLSFQLMRPPNMTP) of rotavirus A antigen VP6. Epitope RV14 are able to induce protective immune response and is conservative for the majority of rotavirus A strains therefore its usage gives hope to the successful overcoming of one of the main difficulties in rotavirus A vaccine development: wide serological diversity. In present work, effective adsorption of ER6 on the SPs surface leading to the SPs-ER6 complexes formation without loss of ER6 antigenic specificity was demonstrated. Two immune antisera with specificity to RV14 epitope within ER6 were obtained. The first serum was obtained via anti-ER6 sera depletion with AltMV CP and recombinant AltMV CP (AltMV rCP), which was expressed in *Escherichia coli* but did not contain RV14 sequence. The second serum was obtained by a direct immunization with synthetic peptide RLSFQLMRPPNMTP. These sera were utilized for studying RV14 within SPs-ER6 complexes. By means of immunofluorescent microscopy, SPs-ER6 complexes were demonstrated to interact with both depleted serum and anti-RV14 serum. Therefore, rotavirus epitope was confirmed to keep its ability to interact with antibodies within obtained complexes. Considering unique adjuvant properties of spherical particles and characteristics of selected epitope obtained SPs-ER6 complexes can be thought as a promising component for recombinant rotavirus A vaccine. Moreover, it can be hoped that the suggested in present work approach, involving the usage of TMV SPs as a platform and adjuvant for chimeric AltMV CP, containing pathogen's epitope, will be useful not only for rotavirus A vaccine development but for designing of vaccines against other pathogens of humans or farm animals.

Keywords: plant viruses, Tobacco mosaic virus, spherical particles, platform-carrier, platform-adjuvant, Alternanthera mosaic virus, chimeric recombinant antigen, recombinant vaccines, rotavirus infection

Rotavirus infection is the main cause of viral gastroenteritis in infants and young children [1]. In 2016, on average, more than 40% of children under the age of five experienced rotavirus diarrhea, while the number of deaths was 128,500 (95% uncertainty interval 104500-155600) [2]. Currently licensed live attenuated vaccines, the monovalent Rotarix® (GlaxoSmithKline Biologicals S.A., Belgium) and pentavalent RotaTeq (Merck & Co., Inc., USA) have proven to be quite effective in high-income countries. However, these oral vaccines can rarely cause serious side effects, the most dangerous of which is intussusception [3-5]. In addition, the majority of deaths associated with rotavirus are recorded in developing countries where the effectiveness of these vaccines is lower than in high-income countries [3, 6-9]. Thus, the designing of a non-replicating new-generation rotavirus vaccine is highly desirable [10, 11].

Recombinant rotavirus antigens (genus *Rotavirus*, family *Reoviridae*) associated with protein carriers or virus-like particles (VLP) are promising candidates for safe and effective rotavirus vaccine [3]. The rotavirus virion is a three-layered particle with icosahedral symmetry [12]. The main rotavirus antigens are the following structural proteins: VP6 forming the intermediate capsid [11, 13, 14], VP7 composing the outer capsid [13, 15], VP4, which forms spikes [15, 16], and VP5* and VP8* derived from the VP4 by proteolysis [17]. VP6 is conserved for rotavirus strains of group A [11, 18] causing human diseases. Despite the fact that VP6 is not located on the surface of the virion it plays a key role in the formation of a protective immune response. The VP6 is able to induce almost 100% protection against rotavirus in mice after the first immunization [11]. CD4+ T cells have been shown to be the main immune cells providing protective immunity [19, 20]. In addition, some studies indicate that VP6-specific IgA, after entering the cell via transcytosis, are able to inhibit transcription. They are supposed to bind rotavirus bilayer particles intracellularly that leads to steric blocking of messenger RNA-releasing channels [21]. The 14-amino acids length epitope of the protein VP6, RV14 (RLSFQLMRPPNMTP, positions 289-302), can induce an immune response comparable to that induced by the whole VP6 molecule, also providing almost 100% protection in mice [20, 22]. Therefore, the epitope RV14 is of particular interest as an antigenic determinant to design rotavirus vaccines.

Plant viruses and their VLPs are a promising tool to design novel vaccine formulations [23-29]. Safety for humans [23] and high adjuvant properties [29-34] provide the attractiveness of virions and VLPs of plant viruses for applying in vaccines as carriers of pathogen recombinant antigens. For the virions and VLPs of the *Alternanthera mosaic virus* (AltMV) (genus *Potexvirus*, family *Alphaflexiviridae*) adjuvant properties have been previously demonstrated, and the AltMV coat protein (CP) has the ability to form RNA-free filamentous VLPs, which are stable under physiological conditions [35]. In our previous studies, we used AltMV CP as a carrier for the RV14 epitope and obtained a 25-kDa chimeric recombinant protein ER6 (Epitope of Rotavirus protein VP6) [36]. However, it is known that individual proteins even with greater molecular weight often demonstrate low immunogenicity in themselves, and the use of an adjuvant is necessary to enhance immune response. In particular, for ~ 88-kDa chimeric protein, which is a rotavirus protein VP6 fused with a maltose-binding protein, the crucial importance of the presence an adjuvant to induce protective immunity was described [18, 19, 37].

We have previously shown that heating the *Tobacco mosaic virus* (TMV) (genus *Tobamovirus*, family *Virgaviridae*) virions leads to the remodeling of this

rod-shaped virus with a helical structure into spherical particles (SPs) that do not contain RNA. The safety and high immunostimulating potency have been demonstrated for SPs [34, 38, 39].

In this paper, we have proposed for the first time an approach to design a vaccine candidate against rotavirus based on two plant viruses with different structures. We used SPs as a platform for adsorption and an adjuvant for the chimeric antigen of rotavirus – ER6. It has been demonstrated that, despite the small size (14 amino acid residues), the antigenic determinant of rotavirus remains available for antibodies in the SPs-ER6 complexes. This gives reason to believe that the presented epitope will induce the production of antibodies, which are necessary to protect against rotavirus infection. The described approach, which includes the use of the plant virus coat protein to create a chimeric antigen containing the pathogen epitope, and the simultaneous TMV SPs application as a platform-adjuvant, can also be expected to allow integrating short epitopes into the compositions of recombinant vaccine candidates against various infectious agents of humans and farm animals.

Our goal was to construct antigenically active complexes based on the chimeric antigen of the rotavirus (ER6) and structurally modified viral particles derived from TMV after thermal remodeling.

Materials and methods. Purified TMV from infected tobacco plants (*Nicotiana tabaccum* L.) and SPs were prepared according to the description [38]. AltMV from infected tobacco plants (*Nicotiana benthamiana* L.) was purified according to Donchenko et al. [35]. For AltMV coat protein isolation, salt deproteinization with 2 M LiCl was applied.

The recombinant rotavirus antigen ER6 was expressed in the *Escherichia coli* system as per the protocols [23]. To produce a recombinant AltMV coat protein (AltMV rCP), the DNA fragments encoding ER6 protein but not containing the RV14 epitope sequences was amplified from the pQE-60-ER6 plasmid carrying an insert for ER6 protein. For amplification, we used a pair of primers 5′-ATACGGATCCAGTACTCCATTTCCTCAAGTCACCCA-3′ and 3′-GTATAGCTTCTCCGGTGGTGGGAGGTATTG-5′ with BamHI and HindIII restriction endonuclease sites at the 5′- and 3′-ends respectively and Encyclo PCR kit (Evrogen, Russia); PCR program: 2 min at 96 °C; 30 s at 95 °C, 30 s at 64 °C, 45 s at 72 °C (26 cycles); 1 min at 72 °C. The BamHI and HindIII (Thermo Scientific, USA) sites were used for cloning the recombinant DNA fragment into pQE-30 vector (Qiagen N.V., Germany). All genetic engineering manipulations were performed using conventional techniques [40]. Further procedures for obtaining AltMV rCP were the same as for the ER6 protein [36].

For electron microscopy, the preparations were loaded on copper mesh grids for electron microscopy covered with carbon-coated collodion support films and kept for 15-20 s. The excess of the preparation was removed by filter paper; in the case of SPs-ER6, the meshes were negatively contrasted with a 2% uranyl acetate solution for 15-20 s. The preparations were analyzed using a JEM-1011 electron microscope (JEOL, Ltd, Japan) equipped with an Orius™ SC1000 W digital camera (Gatan, Inc., USA). The average size of the SPs was measured using the ImageJ software (National Institutes of Health, USA); the mean M and ±SD was calculated for 100 particles.

For immune sera, white outbred mice aged 6-8 weeks were immunized intraperitoneally three times with a 2-week interval. For the first immunization, 10 μg of ER6 protein or 25 μg of synthetic peptide RLSFQLMRPPNMTP (Almabion, Russia) in 0.2 ml sterile PBS (7 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4, 137 mM NaCl, 2.7 mM KCl) was mixed with an equal volume of Freund's complete adjuvant (Sigma-Aldrich Chemie GmbH, Germany) to a homogeneous

suspension. In the second and third immunization, instead of Freund's complete adjuvant, Freund's incomplete adjuvant (Sigma-Aldrich Chemie GmbH, Germany) was used. For obtaining control non-immune serum, the mouse was immunized three times with 0.2 ml PBS. Blood samples were collected 2 weeks after the third immunization. Blood serum was prepared by centrifugation at 10,000 g for 5 min. The antibody titer was determined by indirect enzyme-linked immunosorbent assay (ELISA). Medium sorption plates (Greiner Bio-One GmbH, Austria) were coated with the corresponding antigen at a concentration of 10 μg/ml. Free binding sites were blocked with 1% milk powder (PanReac AppliChem ITW Reagents, Spain) in PBS. All sera were tested in 3-fold dilutions. The secondary antibodies were antibodies against mouse IgG conjugated with horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc., USA) at a dilution of 1:20,000; 3,3′,5,5′-tetramethylbenzidine was a substrate, the reaction was stopped with sulfuric acid added to a final concentration of 1 M. The absorbance in the wells was measured at 450 nm (a Multiskan FC microplate photometer, Thermo Scientific, USA). The titer was expressed as the serum dilution at which the absorbance value corresponds to the mean value of the block +3SD.

For depletion of antiserum, 200 μg AltMV CP was added to 100 μl of anti-ER6 serum, incubated for 30 min at 37 °C, and the precipitate was removed by centrifugation at 10000 g. To 50 μl of the supernatant, 270 μg of AltMV rCP was added, incubated at 4 °C overnight, and centrifuged at 10000 g in the cold, the supernatant was used as depleted serum.

For immunoblotting, proteins were separated by electrophoresis in an 8-20% gradient polyacrylamide gel with sodium dodecyl sulfate (SDS-PAAG) and transferred from the gel to an Amersham™ Hybond® P polyvinylidene fluoride (PVDF) membrane (GE Healthcare — Life Sciences, USA) using a Pierce™ Power Blotter transfer system (Thermo Fisher Scientiific, USA). The membrane was exposed to depleted mouse anti-ER6 serum (1:2000) and secondary antibodies to mouse IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc., USA) (1:20,000). After the treatment with Amersham™ ECL™ substrate (GE Healthcare — Life Sciences, USA), a chemiluminescent signal was detected by the ChemiDoc XRS+ gel documentation system (Bio-Rad Laboratories, Inc., USA). Gels were stained with Coomassie® Brilliant Blue G 250 (SERVA Electrophoresis, Germany). PageРишпler Plus Prestained Protein Ladder (Thermo Scientific, USA) was used.

For immunofluorescence microscopy, the SPs-ER6 sample was loaded on formvar-coated coverslips. After 10 min exposure, the excess of the sample was removed, the coverslips were incubated for 1 h with blocking solution (PBS with 1% bovine serum albumin — BSA and 0.05% Tween 20), then for 1 h with 1:50 dilution of the corresponding serum in the blocking solution. The coverslips were washed thrice with a washing solution (PBS with 0.25% BSA and 0.05% Tween 20) and incubated for 45 min with secondary antibodies to mouse IgG conjugated to Alexa 546 fluorophore (Invitrogen, USA; 1:100 dilution in the blocking solution). After secondary antibodies bound, the preparations were washed thrice with the washing solution and once with PBS, then rinsed with water and dried in air. Immediately before the study, the preparations were treated with a photo-protector 1,4-diazabicyclo[2.2.2]octane and examined under an Axiovert 200M fluorescence microscope (Carl Zeiss, Germany) equipped with an ORCAII-ERG2 integrated camera (Hamamatsu Photonics, Japan).

Results. SPs-ER6 complexes obtaining. At the first stage, the previously designed rotavirus recombinant antigen ER6 [36] was adsorbed on the surface of spherical particles.

ER6 is a chimeric AltMV CP fused at the C-terminus with the RV14 epitope (RLSFQLMRPPNMTP) of the VP6 protein of rotavirus A (Fig. 1, A). Figure 1 schematically shows the structure of the ER6 (see Fig. 1, A) and the location of its structural and functional components within the rotavirus (see Fig. 1, B) and AltMV (see Fig. 1, C) virions. The antigenic determinant (RV14) is a fragment of the rotavirus intermediate capsid protein VP6 (see Fig. 1, B), and AltMV CP is the epitope carrier [36].

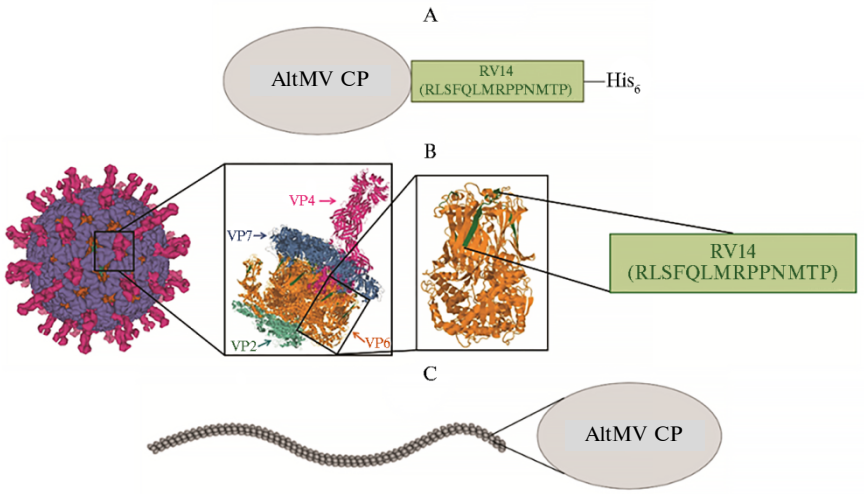

Fig. 1. The scheme of the recombinant antigen ER6 [36] containing RV14 epitope (RLSFQLMRPPNMTP) of the rotavirus A intermediate capsid protein VP6.

A. ER6: AltMV CP — *Alternanthera mosaic virus* coat protein, epitope RV14, His_6 — His-tag.

B. RV14 (RLSFQLMRPPNMTP) epitope position in rotavirus: virion (PDB: 4V7Q) [41-43]; rotavirus capsid proteins structure (PDB: 4V7Q) [41, 43, 44]; VP6 trimer (PDB: 1QHD) [43-45]; RV14 scheme (RV14 within VP6 is marked in green).

C. An AltMV virion (ViralZone) [46] and the AltMV CP schematic image.

Image is not to scale.

SPs prepared from TMV to form complexes with the protein ER6, have a regular spherical shape, are electron-dense and detectable without contrasting (Fig. 2). The average size of the obtained particles measured by electron microscopy was 986±109 nm.

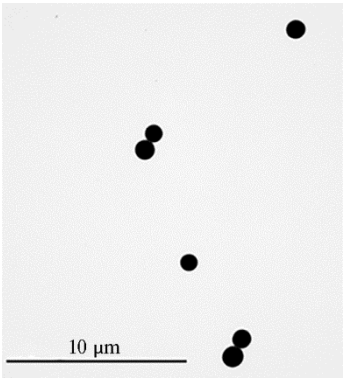

Fig. 2. Spherical particles obtained by thermal treatment of TMV virions. Transmission electron microscopy (JEM-1011, JEOL, Ltd, Japan; digital camera Orius™ SC1000 W, Gatan, Inc., USA).

To obtain SPs-ER6 complexes, the SPs were incubated with the ER6 in MilliQ water overnight at room temperature. Based on previous studies [39], the 10:1 mass ratio of SPs and ER6 was selected.

SPs-ER6 complexes were detected by immunofluorescence microscopy with serum to ER6 (the titer of antibodies to ER6 determined by ELISA was 1:11,284,633). This serum was obtained by immunization of white mice with ER6 in presence of the Freund's adjuvant.

Immunofluorescence analysis clearly revealed the SPs-ER6 complexes (Fig. 3). The image in the fluorescence mode (see Fig. 3, A) fully corresponds to the position of the SPs observed in the phase contrast mode (see Fig. 3, B). Comparison of these images allows us to conclude that all SPs in the field of view are ER6-coated. Despite the large number of SPs are detected in the field of view in

the phase contrast mode in the control sample, which were not treated with serum to ER6 (see Fig. 3, C), nothing detects in fluorescent mode (see Fig. 3, D) that confirms the specificity of the interaction. This indicates that the antigenic specificity of the ER6 protein does not change during adsorption on SPs.

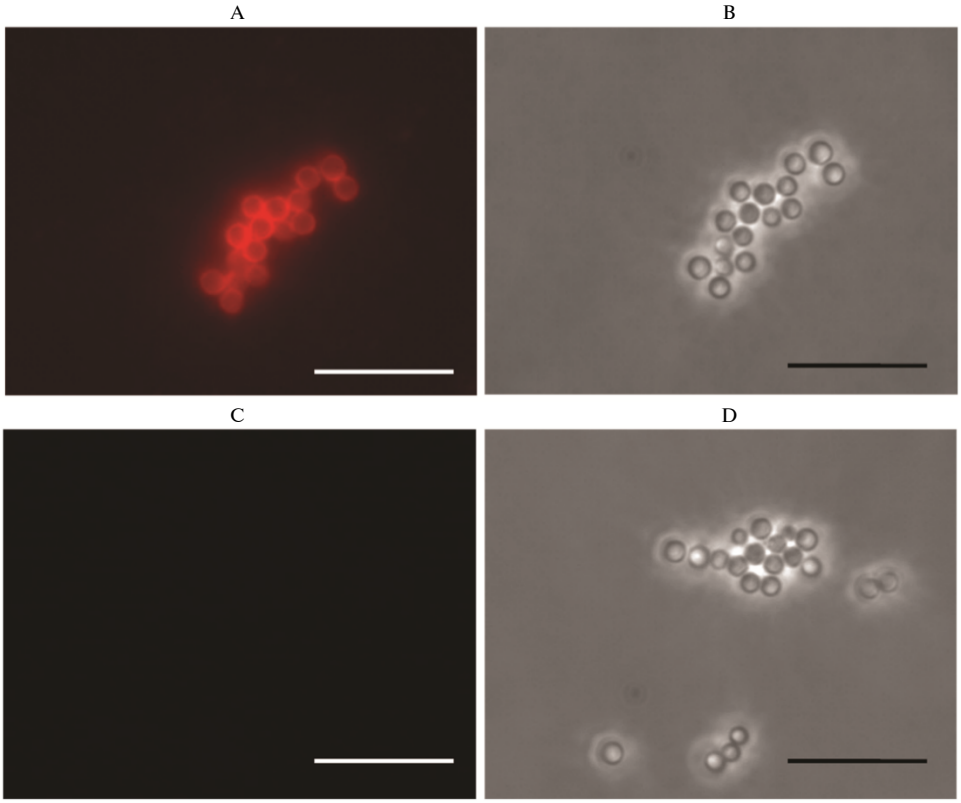

Fig. 3. Recombinant antigen ER6, which is the Alternanthera mosaic virus coat protein fused with RV14 epitope of the rotavirus A protein VP6, can be effectively adsorbed on spherical particles (SPs), derived from the Tobacco mosaic virus coat protein, forming SPs-ER6 complexes. The complexes were treated with immune serum to ER6 and secondary anti-species antibodies conjugated to Alexa 546 fluorophore (A, B) and only secondary antibodies (control) (C, D). Scale bar 10 microns. Immunofluorescence microscopy, the fluorescence (A, C) and phase contrast (B, D) modes (Axiovert 200M microscope, Carl Zeiss, Germany, ORCAII-ERG2 integrated camera, Hamamatsu Photonics, Japan).

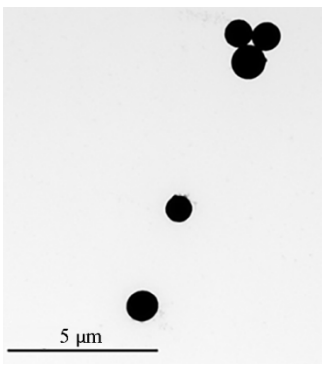

Fig. 4. SPs-ER6 complexes of spherical particles (SPs), derived from the Tobacco mosaic virus coat protein, and the recombinant antigen ER6, which is the Alternanthera mosaic virus coat protein fused with RV14 epitope of the rotavirus A protein VP6 . Transmission electron microscopy, contrasting with 2% uranyl acetate (JEM-1011, JEOL, Ltd, Japan; digital camera Orius™ SC1000 W, Gatan, Inc., USA).

Electron microscopy showed that the formation of complexes with the recombinant protein ER6 does not lead to a change in the morphology of SPs (Fig. 4). The shape and size of the SPs-ER6 complexes turned out to be identical to those of ER6-free SPs (see Fig. 2).

Rotavirus A epitope keeps its antigenic specificity within the SPs-ER6 complexes. We used two antisera specific for the RV14 region of ER6 to assess the availability of the RV14 epitope for antibodies in the SPs-ER6 complexes.

One of the sera is twice depleted serum to ER6. The first stage of depletion was performed with native AltMV CP, and the second one with recombinant AltMV CP expressed in *E. coli* and purified by the same method as for ER6. After removing the precipitate, we assessed the specificity of the interaction of the depleted serum with the antigens ER6, AltMV CP, and AltMV rCP by indirect ELISA. The titer of depleted serum to AltMV CP and to AltMV rCP was low, 1:79424 and 1:85046, respectively, while the titer to ER6 remained relatively high, the 1:1,021,968.

In addition the depleted serum ability to interact with ER6, AltMV CP and AltMV rCP was examined by immunoblot analysis. A clear interaction with the ER6 protein (Fig. 5, lane 2) was demonstrated, while the reaction with AltMV CP (see Fig. 5, lane 1 on the right) and AltMV rCP (see Fig. 5, line 3 on the right) was extremely weak. We did not observe interactions with control samples: TMV, PVX and molecular weight markers (Fig. 5, lanes 4 and M on the right, respectively).

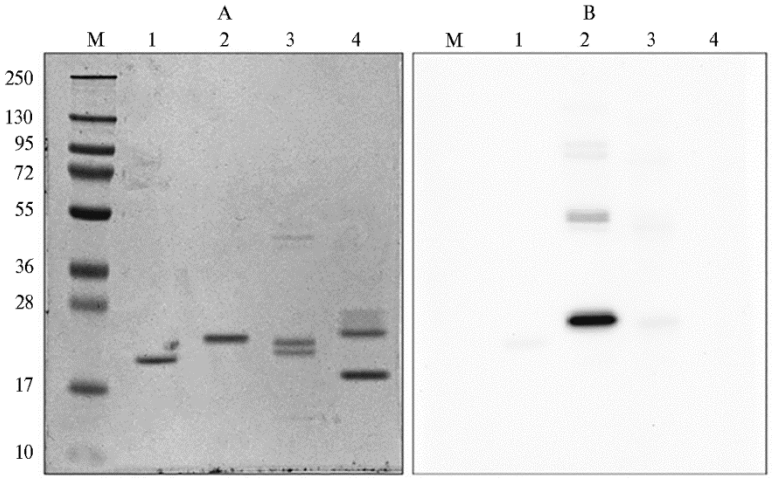

Fig. 5. Immunoblot analysis of interaction specificity between depleted antiserum and recombinant antigen ER6, which is the Alternanthera mosaic virus coat protein fused with epitope RV14 of the rotavirus A protein VP6 (left — electrophoretic separation, right — immunoblotting): 1 — Alternanthera mosaic virus coat protein (AltMV CP), 2 — ER6, 3 —AltMV rCP (the sequence of epitope RV14 is absent in the recombinant protein), 4 — TMV and PVX (controls), M — molecular weight markers (PageRuler Plus Prestained Protein Ladder, Thermo Scientific, USA); molecular weights are indicated in kDa). Serum to ER6 was depleted with AltMVP CP and AltMV rCP; SDS-PAAG electrophoresis (8-20%), staining with Coomassie® Brilliant Blue G 250.

Therefore, the depleted serum remains specific to ER6, while it almost completely lacks specificity to AltMV CP and to AltMV rCP.

The second serum we used to assess antigenic specificity of the SPs-ER6 complexes was the serum against the individual peptide RV14. Short peptides are known to be extremely low immunogenic. Nevertheless, in this work, we attempted to obtain antiserum against synthetic peptide RV14 and for this purpose four mice were immunized. Despite quite a large amount of the peptide (25 μg per mouse) added together with Freund's complete adjuvant, three animals did not develop an immune response. Only one collected serum contained antibodies to the RV14 peptide. The titer determined by indirect ELISA with RV14 as antigen was 1:226143, which is considerably higher compared to 1:3088 in control (non-immune serum).

In order to examine anti-RV14 serum specificity to ER6 antigen, we also used non-immune serum and nonspecific antigen AltMV rCP as controls. The titer of anti-RV14 serum to specific antigen ER6 was 1:183971 while the titer to

nonspecific antigen AltMV rCP was 1:7101. Non-immune serum titer to antigen ER6 was 1:8682, therefore, the efficacy of the interaction of anti-RV14 serum with ER6 was more than 20 times higher compared to controls.

Considering the immune serum against RV14 peptide was proven to react with ER6 specifically, it was also applied in analysis of the SPs-ER6 complexes.

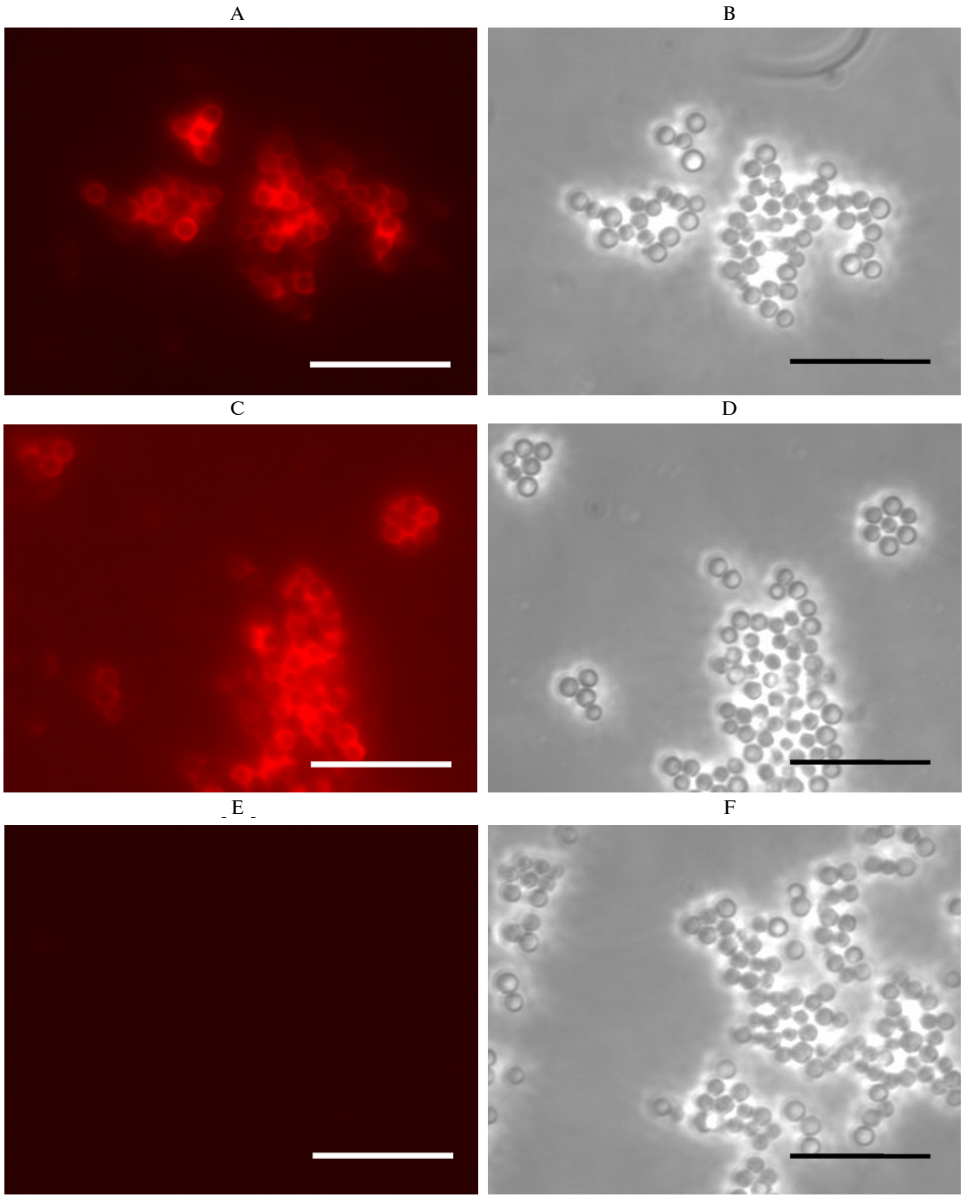

Fig. 6. RV14 epitope of rotavirus A protein VP6 is available for interaction with antibodies within the SPs-ER6 complexes. SPs are spherical particles derived from TMV coat protein. Recombinant antigen ER6 is the Alternanthera mosaic virus coat protein fused with RV14 epitope of the rotavirus A protein VP6. SPs-ER6 complexed were treated with a depleted anti-ER6 serum (A, B), with anti-RV14 serum (C, D), and with secondary antibodies only (E, F) (control). Secondary antibodies are conjugated to Alexa 546 fluorophore. Scale bar 10 μm. Immunofluorescence microscopy in fluorescence (A, C, E) and phase contrast (B, D, E) modes (an Axiovert 200M microscope, Carl Zeiss, Germany, ORCAII-ERG2 integrated camera, Hamamatsu Photonics, Japan).

By means of immunofluorescence microscopy the SPs-ER6 complexes were demonstrated to be recognized by both depleted serum against ER6 (Fig. 6, A) and anti-RV14 serum (see Fig. 6, B). The positions of the detected SPs

corresponded in fluorescence and phase contrast modes (see Fig. 6, B, D). The complete absence of fluorescence in the not-treated with primary antibodies control sample (see Fig. 6, E, F) excludes unspecific binding of secondary antibodies.

These results indicate that the rotavirus epitope RV14 is available for interaction with antibodies and retains its antigenic specificity within the SPs-ER6 complexes.

Various researchers have demonstrated that the designing of plant viruses chimeric proteins fused with pathogens epitopes can lead to the formation of VLPs, which are able to induce immune response to the corresponding epitopes [31, 32, 47, 48]. In particular, based on Papaya mosaic virus (genus *Potexvirus*, family *Alphaflexiviridae*), which is close relative to the AltMV, chimeric VLPs carrying influenza virus [31, 48] or hepatitis C virus [47] epitopes on their surface were obtained. The authors believe that multivalent structure of VLP provides the immunogenicity in this case [30, 31, 47]. In our previous studies, we for the first time used AltMV for designing chimeric recombinant antigen carrying epitope of rotavirus protein VP6. The protein ER6, which is AltMV CP fused to the epitope of rotavirus protein VP6, was obtained. ER6 was recognized by commercial polyclonal antisera to rotavirus A, thus it was proved to be a promising antigen for the development of recombinant rotavirus A vaccines. However, ER6 was unable to form VLPs in the absence of viral RNA [36]. Recombinant proteins themselves possess low immunogenicity and their use requires adding an adjuvant [49, 50]. In this work, we propose SPs derived of TMV virions by thermal remodeling as a platform-adjuvant for a ER6-based vaccine. SPs are stable under physiological conditions, safe for humans and mammals, biodegradable and can adsorb various proteins on their surface that lead to the SPs-antigen complexes formation. Moreover, SPs were previously demonstrated to be able to increase in several times the titer of antibodies to the antigen administrated within complexes with SPs, therefore, SPs have high immunostimulating properties [38, 39]. In this work, rotavirus A recombinant antigen ER6 was efficiently adsorbed on the spherical particles with the SPs-ER6 complexes formation. These complexes were detected by indirect immunofluorescence microscopy with anti-ER6 serum. All SPs were showed to be coated with the ER6. The interaction of primary antibodies with ER6 located on the SPs surface indicates that adsorption does not lead to a loss of the chimeric protein antigenic specificity.

To assess the prospects for using the SPs-ER6 complexes as a component of a rotavirus vaccine, it was also important to make sure that the rotavirus epitope RV14 within such complexes is available for antibodies. To address the problem, two immune sera specific to RV14 were prepared: the depleted anti-ER6 serum and the anti-RV14 serum obtained by immunization with the synthetic peptide. As a result of depletion with AltMV CP and AltMV rCP, the serum retained mainly those antibodies for the production of which the RV14 was necessary. The titer of depleted anti-ER6 serum to the antigen ER6 was more than 10 times higher than to the antigens AltMV rCP and AltMV CP. This serum almost completely lost the ability to recognize both native and recombinant CP of AltMV in immunoblotting but at the same time effectively bounded ER6. The relatively large amounts of antibodies specific to the RV14 region of the protein ER6 allows suggesting that AltMV CP, acting as a carrier of RV14, might also act as adjuvant, enhancing the immune response to the rotavirus epitope. Another serum to RV14 was obtained by direct immunization with the synthetic peptide RV14. Both sera were applied to examine the antigenic specificity of the rotavirus epitope in SPs-ER6 complexes by indirect immunofluorescence microscopy. The SPs-ER6 complexes were proved to react with both depleted anti-ER6 serum and anti-RV14 serum. This indicates that the ER6 keeps the rotavirus antigenic specificity within

the SPs-ER6 complexes, which confirms the correctness of AltMV CP selection as a carrier for the rotavirus epitope. Taking into account the previously shown adjuvant properties of SPs, it can be assumed that the immune response to the antigenic determinant of rotavirus will be greatly enhanced due to formation of complexes with SPs.

Therefore, based on the coat protein of one plant virus, the Alternanthera mosaic virus (AltMV), we have constructed a recombinant antigen ER6 carrying the epitope RV14 of the rotavirus A protein VP6. As well as based on the coat protein of another plant virus, the Tobacco mosaic virus (TMV), we obtained spherical particles (SPs) that form complexes with ER6. Chimeric protein was demonstrated to retain its antigenic specificity during adsorption on SPs. In turn, SPs was previously shown to have high adjuvant activity. This suggests that the obtained SPs-ER6 complexes containing an epitope of the rotavirus protein VP6 and modified coat proteins of simultaneously two plant viruses (AltMV and TMV) can serve as a component of a new safe recombinant vaccine against rotavirus infection. The proposed approach can be further applied to design recombinant vaccines against pathogens of humans and farm animals.

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