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Study of rubella candidate vaccine based on a structurally modified plant virus

Ekaterina A. Trifonova [a, *], Vladimir A. Zenin [b], Nikolai A. Nikitin [a], Maria S. Yurkova [b], Ekaterina M. Ryabchevskaya [a], Egor V. Putlyaev [a], Ekaterina K. Donchenko [a], Olga A. Kondakova [a], Alexey N. Fedorov [b], Joseph G. Atabekov [a], Olga V. Karpova [a]

[a] Department of Virology, Faculty of Biology, Lomonosov Moscow State University, 1-12 Leninskie Gory, Moscow, 119234, Russian Federation
[b] Group of Molecular Biotechnology, Federal State Institution «Federal Research Centre «Fundamentals of Biotechnology» of the Russian Academy of Sciences», Leninsky pr., 33, Moscow, 119071, Russian Federation

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ABSTRACT

A novel rubella candidate vaccine based on a structurally modified plant virus – spherical particles (SPs) – was developed. SPs generated by the thermal remodelling of the tobacco mosaic virus are promising platforms for the development of vaccines. SPs combine unique properties: biosafety, stability, high immunogenicity and the effective adsorption of antigens. We assembled *in vitro* and characterised complexes (candidate vaccine) based on SPs and the rubella virus recombinant antigen. The candidate vaccine induced a strong humoral immune response against rubella. The IgG isotypes ratio indicated the predominance of IgG1 which plays a key role in immunity to natural rubella infection. The immune response was generally directed against the rubella antigen within the complexes. We suggest that SPs can act as a platform (depot) for the rubella antigen, enhancing specific immune response. Our results demonstrate that SPs-antigen complexes can be an effective and safe candidate vaccine against rubella.

1. Introduction

Rubella is a highly contagious disease caused by a virus agent (rubella virus, genus *Rubivirus*, family *Togaviridae*). The major complication of rubella is its teratogenic effects on the fetus, especially in the early weeks of pregnancy. Infection can lead to spontaneous abortion, fetal death or children born with congenital rubella syndrome (CRS). CRS is associated with multiple disabilities that can require lifelong care, including hearing impairment, cataracts and congenital heart disease (Hinman et al., 2002; Plotkin et al., 2013; Wesolowski et al., 2015). The death of the fetus or congenital malformations can occur in 80–85% of infections during the first trimester of gestation (Petrova et al., 2016). All rubella vaccines used throughout the world are based on attenuated virus strains, and most of them have been produced in human cell lines that have come from aborted fetuses. Despite the effectiveness and relative safety of attenuated rubella vaccine, it is important to note that live vaccines containing replicating viruses carry the risk of reversion to the virulent form (Hongxuan, 2013), and that propagation of viruses in human cell lines is a rather expensive process. Additionally, pregnant women, children with AIDS and children with other immunodeficiencies cannot be vaccinated with current attenuated rubella virus vaccines (Petrova et al., 2016). Therefore, the development of new, safe and stable non-replicating vaccines against the rubella virus is desirable.

Plant viruses and their virus-like particles (VLP) are promising tools for the production of novel candidate vaccine compositions (Denis et al., 2008; Karpova et al., 2012; Lebel et al., 2014; Lico et al., 2008; Mathieu et al., 2013; Trifonova et al., 2014). One of the main advantages of plant viruses usage is biosafety, since they are not

Abbreviations: A_4, tetraepitope A, a recombinant antigen of rubella virus glycoprotein E1; CP, coat protein; CRS, congenital rubella syndrome; IM, intramuscular immunisation; LPS, Lipopolysaccharide; PapMV, papaya mosaic virus; PVX, potato virus X; SPs, spherical particles of structurally modified plant virus; TMV, tobacco mosaic virus; VLP, virus-like particles.

* Corresponding author.
E-mail address: trifonova@mail.bio.msu.ru (E.A. Trifonova).

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infectious for animal cells (Nikitin et al., 2016). Generally, plant viruses and VLP serve as carriers (platforms) for antigenic determinants of infectious agents. Antigenic determinants of the pathogen can be exposed on the surface of plant virus capsids by means of genetic engineering or chemical conjugation (Cañizares et al., 2005; Choundry et al., 2009; McCormick and Palmer, 2008; Rioux et al., 2012). It should be emphasised that evidence is being accumulated showing that plant viruses can serve not only as platforms for foreign antigen presentation, but also as effective adjuvants (Acosta-Ramírez et al., 2008; Karpova et al., 2012; Manuel-Cabrera et al., 2012; Rioux et al., 2014; Trifonova et al., 2014).

Our group has demonstrated that spherical particles (SPs) generated by thermal remodelling of the tobacco mosaic virus (TMV) also have immunopotentiating activity (Karpova et al., 2012; Trifonova et al., 2014). TMV (genus *Tobamovirus*, family *Virgaviridae*) is a rod-shaped virus with helical symmetry (diameter 18 nm, length 300 nm). The virion consists of 2130 identical 17.5 kDa protein subunits and a single-stranded RNA. At 94 °C, helical TMV transforms into RNA-free SPs (Atabekov et al., 2011). TMV remodelling to SPs is associated with changes in secondary structure (Dobrov et al., 2014). TMV coat protein (CP) has a high proportion of secondary structure, with 50% of the residues in α or 3_{10} helices, and 10% in β-structure, in addition to numerous reverse turns. The CP core consists of a right-handed, four anti-parallel α-helix bundle (Namba et al., 1989). The protein in SPs contains 14% of α-helices, 32% of β-structures and 54% of unordered sequences (Dobrov et al., 2014). The size of SPs depends on the initial concentration and aggregation state of TMV, and varies from 50 to more than 1000 nm (Atabekov et al., 2011; Trifonova et al., 2015). Increasing the initial virus concentration leads to the formation of spherical particles of a larger size (Atabekov et al., 2011). Prolonged storage of TMV preparation can result in changes in virus aggregation state. This, apparently, raises the average diameter of SPs. Information about the aggregation state of the viral sample plays a crucial role in obtaining SPs, since this allows more precise control of particle size through changes in the concentration of TMV (Trifonova et al., 2015). SPs are very stable to external factors, highly immunogenic and biodegradable (Atabekov et al., 2011; Nikitin et al., 2011). The unique feature of SPs is their ability to non-specifically adsorb foreign proteins on the surface. We have demonstrated that the mixing of SPs with various antigens of human, animal and plant pathogens leads to the formation of SPs-protein complexes (Karpova et al., 2012; Nikitin et al., 2013). It is noteworthy that SPs act as an effective adjuvant and stimulate an immune response to antigens adsorbed on their surface (Karpova et al., 2012; Trifonova et al., 2014).

Previously, we designed complexes of SPs with an antigen containing four repeats of epitope A (tetraepitope A, (A_4)) of E1 rubella virus glycoprotein (Karpova et al., 2012). E1 is a structural glycoprotein of the virions' envelope, and it is the main target for neutralising antibodies against rubella (Chaye et al., 1993; Petrova et al., 2016). Epitope A, also known as peptide SP15, represents highly conservative amino acid sequences from 208 to 239 of glycoprotein E1 (Petrova et al., 2016). This sequence has been described by many researchers as a region which contains major rubella virus epitopes (Chaye et al., 1992; Corboba et al., 2000; DuBois et al., 2013; Li et al., 2014; Meitsch et al., 1997; Wolinsky et al., 1993). There are at least two neutralising, and one hemagglutinating epitopes within this region (Chaye et al., 1992). SP15 effectively induces the production of polyclonal antibodies, which are able to neutralise the rubella virus and block a virion's excretion *in vitro* (Corboba et al., 2000; Wolinsky et al., 1993). Here, we present the results of a study of candidate rubella vaccine based on SPs-tetraepitope A complexes.

2. Methods

2.1. Bacterial expression of tetraepitope A

Bacterially expressed recombinant tetraepitope A of rubella virus E1 glycoprotein (E1 gene GenBank Accession Number AB072387.1) was used for composition formation. All recombinant DNA procedures were carried out by standard methods (Sambrook et al., 1989). The recombinant protein was expressed from the pQE-30 plasmid vector and was composed of 6 His residues at the N-terminus and four repeats of the epitope A (32 amino acid residues (MNYTGNQQSRWGLGSPNCHGPDWASPVCQRHS) x 4). A schematic diagram of recombinant antigen is shown in Fig. S1. A restriction fragment was ligated into the corresponding sites of the expression vector, as described previously (Ivanov et al., 1994). All methods used were in accordance with Sambrook et al. (1989). The purification of (His)6-tagged protein from the bacterial culture was followed by the general procedure described by the manufacturer (QIAGEN) for denaturing Ni-NTA chromatography. Determination of the endotoxin level in recombinant protein was carried out using a LAL reagent in a semi-quantitative gel clot assay, in accordance with the manufacturer's protocol (Charles River Endosafe, USA).

2.2. Obtaining SPs and SPs-tetraepitope A complexes

SPs were obtained as described earlier, with incubation at a temperature of 94 °C (Atabekov et al., 2011; Trifonova et al., 2015). For *in vitro* assembly of SPs-A_4 complexes, 10 μg of A_4 was incubated with 100 μg of SPs for 20 min in water at 25 °C. For stability studies, complexes were incubated in 0.15 M NaCl or in water at 25 °C for 10, 20, 30 and 60 min.

2.3. Immunisation of mice

Female BALB/c mice, who were six to eight weeks old and weighing 20–24 g, were used, in groups of ten. They were primed, and received a further two boosts, at 14-day intervals, of different doses of SPs-A_4 complexes in 100 μL volume (SP to A_4 ratio 10:1), by intramuscular injection, as specified in the "Results" section. (Intramuscular injection was to both quadriceps, with 50 μL volume per site, without anesthesia). Other groups of mice were immunised with either 10 μg of A_4 with alum-based adjuvant (50% v/v of Imject Alum, Thermo Scientific, USA, lot #PD201123A), or A_4 alone. The final volume of each preparation for immunisation was 100 μl. The volume ratio of Imject Alum to A_4 (1:1) was chosen according to the manufacturer's protocol. The dose of aluminium hydroxide per immunisation was 2 mg. Control groups were immunised with PBS (100 μl) or with rubella live attenuated vaccine (Microgen, Russia), in a dosage of 200 TCID50. Sera were collected through a small incision of mouse tail veins, at defined time points; these were prior to the first and second boosts, and two weeks after the second boost. Total IgG, as well as IgG1, IgG2a and IgG2b, levels in sera were measured by indirect ELISA, as described below. Animal weight was monitored twice a week, throughout the study.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The wells were coated with antigen (either A_4 or SP) at 1 μg/well at 4 °C overnight, and blocked with 2% skimmed milk and 0.05% Tween-20 in PBS for 2 h. Murine sera were diluted in PBST 1:50 in the first well and titrated with 1:3 dilutions. Binding of sera was conducted for 1 h at 37 °C. Anti-mouse HRP conjugate against IgG was from Promega (USA). Goat anti-mouse HRP conjugates against IgG1, IgG2a and IgG2b, and rat anti-mouse HRP conjugate against IgE, were from Abcam (Cambridge, MA, USA). Staining was