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A recombinant varicella vaccine harboring a respiratory syncytial virus gene induces humoral immunity

Kouki Murakami[a,b], Masaaki Matsuura[b], Megumi Ota[a], Yasuyuki Gomi[b], Koichi Yamanishi[c], Yasuko Mori[a,*]

[a] Division of Clinical Virology, Kobe University Graduate School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
[b] Kanonji Institute, Seto Center, The Research Foundation for Microbial Diseases of Osaka University, 4-1-70, Seto-cho, Kanonji 768-0065, Kagawa, Japan
[c] The Research Foundation for Microbial Diseases of Osaka University, 3-1, Yamada-oka, Suita 565-0871, Osaka, Japan

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ABSTRACT

The varicella-zoster virus (VZV) Oka vaccine strain (vOka) is highly efficient and causes few adverse events; therefore, it is used worldwide. We previously constructed recombinant vOka (rvOka) harboring the mumps virus gene. Immunizing guinea pigs with rvOka induced the production of neutralizing antibodies against the mumps virus and VZV.

Here, we constructed recombinant vOka viruses containing either the respiratory syncytial virus (RSV) subgroup A fusion glycoprotein (RSV A–F) gene or RSV subgroup B fusion glycoprotein (RSV B–F) gene (rvOka-RSV A–F or rvOka-RSV B–F). Indirect immunofluorescence and Western blot analyses confirmed the expression of each recombinant RSV protein in virus-infected cells. Immunizing guinea pigs with rvOka-RSV A–F or rvOka-RSV B–F led to the induction of antibodies against RSV proteins. These results suggest that the current varicella vaccine genome can be used to generate custom-made vaccine vectors to develop the next generation of live vaccines.

1. Introduction

Varicella-zoster virus (VZV) is a ubiquitous human herpesvirus that, as its name suggests, causes varicella (chicken pox) and zoster (shingles). Varicella is characterized by viremia and skin lesions [1]. A live attenuated varicella vaccine, called the Oka vaccine strain (vOka), was developed from the Oka parental strain [2], which is a Japanese clinical isolate that was attenuated by passaging in semi-permissive guinea pig embryo fibroblasts [3]. Because vOka is efficient and causes few adverse events, it is the only vaccine strain to be used worldwide, especially for immunizing children [4–7].

Another advantage of vOka is that foreign genes can be easily inserted into the viral genome. Thus, vOka has been used as a vector for expressing the Epstein–Barr virus membrane glycoprotein (gp350/220) [8], hepatitis B surface antigen [9], human immunodeficiency virus env [10], and herpes simplex virus type2 glycoproteins B and D [11]. We recently cloned the genomes of the VZV Oka parental strain [12] and vOka [13] into a bacterial artificial chromosome (BAC) vector and successfully reconstituted both recombinant viruses from the BAC genome. Using this system, we constructed a recombinant vOka, which encoded a mumps virus hemagglutinin–neuraminidase and/or fusion protein [14–16], and showed that recombinant vOka are potentially useful as polyvalent vaccines that provide protection against both VZV and mumps viruses.

Respiratory syncytial virus (RSV) causes serious lower respiratory tract diseases, such as viral pneumonia and bronchiolitis, in infants and young children [17]. RSV infection most often occurs between the ages of 6 months and 2 years, and may be associated with severe disease [18]. It can also affect the elderly and those with immunodeficiency, resulting in serious complications [19,20]. A formalin-inactivated RSV vaccine was developed in the 1960s, which was not effective at eliciting neutralizing antibody responses; furthermore, it failed to protect against RSV infection and, interestingly, people that received the vaccine suffered more severe symptoms upon subsequent natural infection [21–23].

Abbreviations: BAC, bacterial artificial chromosome; CMVp, cytomegalovirus promoter; IFA, immunofluorescence analysis; PFU, plaque-forming unit; RSV, respiratory syncytial virus; RSV G, RSV glycoprotein; RSV F, RSV fusion glycoprotein; vOka, oka vaccine strain; VZVvaricella-, zoster virus.

* Corresponding author. Tel.: +81 78 382 6272; fax: +81 78 382 6879.
E-mail address: ymori@med.kobe-u.ac.jp (Y. Mori).

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Thus, an effective vaccine that protects against RSV is urgently required.

RSV is a negative-sense single stranded RNA virus belonging to the family *Paramyxoviridae* [24]. The viral genome encodes 11 proteins [25]; of these, the RSV fusion glycoprotein (RSV F) and glycoprotein (RSV G) are major transmembrane glycoproteins targeted by neutralizing antibodies [26–29]. RSV is divided into two subgroups, A and B, according to the antigenicity of RSV G [30]. Previous studies used parainfluenza virus type 3 [31], sendai virus [32], Newcastle disease virus [33], or measles virus [34] as vectors to express the RSV F and G antigen. It has been shown that Simian varicella virus (SVV) expressing RSV glycoprotein G and second matrix protein M2 genes, could induce antibodies to SVV and RSV antigen in Rhesus macaques [35].

Here, we generated a novel recombinant vOka expressing the subtype A or B RSV F antigen (rvOka-RSV A–F and rvOka-RSV B–F). Guinea pigs immunized with rvOka-RSV A–F or rvOka-RSV B–F produced neutralizing antibodies against VZV and RSV.

Thus, these results may show that currently available vOka genome would be a promising candidate of vaccine vector for next generation.

2. Materials and methods

2.1. Cells and viruses

MRC-5 cells, derived from human lung fibroblasts, were cultured in modified Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. HEp-2 cells, derived from a human cervix cancer (ECACC, Catalogue No.: 85011412) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS. Vero cells and MeWo cells were cultured in DMEM supplemented with 10% FBS. The vOka (Biken) and rvOka viruses were propagated in MRC-5 cells, as described previously [12]. The RSV Long strain was kindly provided by Dr. Takahashi (Osaka Prefectural Institute of Public Health) and the RSV 9320 strain was obtained from ATCC. Both RSV strains were grown in HEp-2 cells and the supernatants were used for the neutralizing antibody test.

2.2. Antibodies

An anti-RSV F monoclonal antibody (2F7; Abcam) was used for immunofluorescence analysis (IFA) and Western blotting. The anti-VZV gB rabbit antibody for IFA has been described previously [36].

2.3. Construction of RSV antigen-encoding expression plasmids

RSV F was obtained as follows. Total RNA was extracted from a RSV subgroup A 200/2004 clinical isolate and a subgroup B 9320 strain using the QIAamp Viral RNA Mini Kit (Qiagen) and used as the template for RT-PCR. RSV F gene was generated using the One Step RNA PCR Kit (TAKARA BIO) and primer pair specific for 200/2004 strain RSV F (RSV A–F) or 9320 strain RSV F (RSV B–F). These gene fragments were then cloned into pCAGGS [37] to generate RSV A–F/pCAGGS and RSV B–F/pCAGGS. Plasmids containing the RSV gene expression cassette were generated and then used to construct the plasmid used for two-step Red-mediated recombination [38]. These RSV fragments were then inserted into the pcDNA3.1(+)-aphAI plasmid along with the I-SceI-aphAI fragment [16]. The resulting plasmids were named pcDNA3.1(+)-aphAI-RSV A–F and pcDNA3.1(+)-aphAI-RSV B–F.

2.4. Generation of the vOka-BAC genome containing the RSV gene

Escherichia coli GS178 harboring vOka-BAC DNA were generated as previously described [12,39]. DNA fragments containing the VZV ORF 13 upstream region, the cytomegalovirus promoter (CMVp) region, the aphAI region, the RSV gene, the bovine growth hormone polyadenylation signal (BGHpA), and the VZV ORF 13 downstream region were amplified along with primer pair, and pcDNA3.1(+)-aphAI-RSV A–F or pcDNA3.1(+)-aphAI-RSV B–F. The amplified products were then transformed into competent *E. coli* GS1783 cells [40] harboring the vOka-BAC genome. The product was inserted into the vOka-BAC genome by two-step Red-mediated recombination [16]. The vOka-BAC ORF13 gene was then replaced with the RSV gene expression cassette. The vOka-RSV A–F-BAC or vOka-RSV B–F-BAC genomes were then extracted from cells using a NucleoBond BAC100 kit (Macherey-Nagel). Finally, the vOka-RSV A–F-BAC or vOka-RSV B–F-BAC genomes were digested with BamHI and the products were separated in 0.5% agarose gels.

2.5. Reconstitution of infectious virus from the vOka-BAC genome

Recombinant viruses (rvOka, rvOka-RSV A–F, and rvOka-RSV B–F) were reconstituted as previously described [12,13].

2.6. Indirect immunofluorescent assay and Western blot analysis

IFA was performed as described previously [36,41]. Western blot analysis was performed as described previously [15].

2.7. Viral growth analysis

The growth kinetics of rvOka-RSV A–F and rvOka-RSV B–F were compared with those of the original rvOka. MRC-5 cells were infected with these viruses by cell-to-cell infection. The infected cells were harvested at 2 days post-infection by treating with trypsin and transferred onto newly prepared MRC-5 cells with a 20-fold dilution. After 24, 48, 72, and 96 h of incubation at 37 °C, cell-free viruses were collected from infected cells by sonication as described previously [13]. Infectious viruses titers were then determined in a plaque-formation assay, calculated as PFU/ml.

2.8. Inoculation of guinea pigs with recombinant virus

Four-week-old guinea pigs were immunized with each recombinant cell-free virus. An equal titer of each recombinant cell-free virus was administered via the intratracheal route [42]. Briefly, each animal received two 500 μl doses of virus suspension (4.0×10^5 pfu/ml), with an interval of 2 weeks between doses. Two weeks after the second administration, blood samples were collected. The animals received a final vaccination 4 weeks after the second vaccination. Four weeks after the final vaccination, blood samples were collected by cardiac puncture. Serum was obtained and inactivated by heating to 56 °C for 30 min before use in the serological tests. This study was approved by the Institutional Animal Care and Use Committee (Permission number: P130517-R1) and carried out according to the Kobe University Animal Experimentation Regulations.

2.9. Serological analyses

Antibody titers in the sera were determined in an IFA. Vero cells transfected with RSV A–F or B–F expression plasmids were cultured in chamber slides and then used to determine the presence of RSV F antibodies. Briefly, the chamber slides were fixed with ice-cold

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