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Review

Recombinant measles AIK-C vaccine strain expressing heterologous virus antigens

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ABSTRACT

Further attenuated measles vaccines were developed more than 50 years ago and have been used throughout the world. Recombinant measles vaccine candidates have been developed and express several heterologous virus protective antigens. Immunogenicity and protective actions were confirmed using experimental animals: transgenic mice, cotton rats, and primates. The recent development of measles vaccine-based vectored vaccine candidates has been reviewed and some information on recombinant measles vaccines expressing respiratory syncytial virus proteins has been shown and discussed.

1. Introduction

The measles virus is a member of the genus *Morbillivirus*, the family *Paramyxoviridae*, order *Mononegavirales* and consists of 15,894 nucleotides that encode six major structural proteins. The nucleoprotein (N), phosphoprotein (P), large protein (L), and genomic RNA constitute the ribonucleoprotein complex (RNP) as transcription and replication units. Two envelope spike glycoproteins, hemagglutinin (H) and fusion (F), are present on the surface of infectious particles and execute the process of virus attachment and cell fusion. The M protein contributes to viral assembly and maturation [1]. Measles virus was first isolated in 1954 by Enders and Peebles from a patient and the Edmonston strain was further propagated in human kidney or alantoic fluid fibroblastic cells [2]. The AIK-C measles vaccine strain was developed from the wild-type Edmonston strain through small plaque cloning in sheep kidney cells and chick embryonic cells at 32.5 °C [3,4]. The AIK-C strain is one of the candidate vaccine strains for the Expanded Programme on Immunization (EPI) to overcome maternally conferred immunity, thereby resulting in a high seroconversion rate in young infants at 6 months of age [5–7]. Over 20 million doses have been administered mainly in Japan, and no serious adverse events have been reported in post marketing surveillance.

2. Characteristics of the AIK-C strain

The AIK-C strain shows optimal virus growth at 33 °C with small plaques, but extremely poor or no growth at 39–40 °C, demonstrating temperature sensitivity (*ts*). These biological markers have been used to validate vaccine production and some responsible genes were identified [3,4]. The AIK-C vaccine strain was developed from the Edmonston strain and its full-length sequence was reported, in which 31 amino acid substitutions were found relative to the Edmonston wild-type [8]. The recent development of molecular genomic analysis has allowed reverse genetics to be performed in order to generate the infectious measles virus [9]. The reverse genetics of the AIK-C vaccine strain was explored to investigate the specific genome regions responsible for its unique biological characteristics. F and HA protein expression experiments revealed that Leu at position 278 of the F protein was responsible for the formation of small plaques in Vero cells and Phe at position 278 of the F protein of the Edmonston strain induced large plaques in Vero cells [10]. Infectious cDNA clones having F278Leu and F278Phe were generated: a recombinant virus having F278Leu induced small plaques, whereas large plaques were generated by a virus having F278Phe. However, this position did not influence the *ts* characteristics [10].

The replication and transcription of the measles virus are regulated by interactions between the N, P, and L proteins, and a measles

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Table 1
Recombinant measles vaccine-based virus expressing heterologous antigens.

MV vector	Insertion site	Inserted targets		Experimental animals	Authors [ref. No.]
Edm. Zagreb	P/M	SARS-Corona virus	Nucleocapsid, Spike	CD46+, IFN-receptor- mice	Liniger et al. [22]
Berna Moraten	P/M	HPV	L1	CD46+, IFN-receptor- mice	Cantarella et al. [23]
	P/M, H/L	HIV	gp140, Gag-pol,	CD46+, IFN-receptor- mice	Liniger et al. [24]
Edmonston B	P/M, H/L	Mumps virus, SIV	Mumps F, HN, SIV env, etc.	CD46+, IFN-receptor- mice	Wang et al. [25]
Schwarz	P/M	SARS Corona virus	Spike	CD46+, IFN-receptor- mice	Escriou et al. [26]
	P/M	HIV	HIV Gag, RT, Nef	Cynomolgus macaques	Stebbings et al. [27]
	P/M	West Nile virus	Envelope	Squirrel monkey	Brandler, et al. [28]
	P/M	Dengue viruses I-IV	Envelope + partial M	CD46+, IFN-receptor- mice	Brandler et al. [29]
	P/M, H/L	HIV	Gag (P/M), Env (H/L)	CD46+, IFN-receptor- mice	Guerbois et al. [30]
	F and H: replaced	SIV	gp160+MVF cytoplasmic	ND	Mourez et al. [31]
	P/M	Chikungunya virus	C-E3-E2-6K-E1	CD46+, IFN-receptor- mice	Brandler et al. [32]
Edmonston B, Wild	N/P	Nipah virus	G	African green monkey	Yoneda et al. [33]
Moraten/Schwarz	P/M	Hepatits C,	E1, E2	CD46+, IFN-receptor- mice	Reyes-del Valle et al. [34]
Edm. Zagreb	P/M	RSV, EB virus	SV F, EB gp350	Cotton rats, Rhesus Macaques	Mok et al. [39]
AIK-C	P/M	RSV	F, G	Cotton rats	Sawada et al. [40]
AIK-C	P/M	RSV	NP, M2, F	Cotton rats	Yamaji et al. [41]

MV: measles virus, Edm. Zagreb: Edmonston Zagreb, HIV: human immunodeficiency virus, HPV: human papilloma virus, SIV: simian immunodeficiency virus, RSV: respiratory syncytial virus, EB: Epstein Barr virus, CD46+, IFN-receptor- mice: human CD46 transgenic and interferon receptor knockout mice.

mini-genome system, in which the luciferase reporter gene was inserted between the measles leader and trailer sequences, was developed to investigate transcription and replication activities [11]. Limited or no luciferase activity was observed when the P protein expression plasmid from the AIK-C strain was used in the mini-genome assay at 37 °C or higher temperatures, whereas the P expression plasmid from the Edmonston strain did not influence luciferase activity at 37 °C or higher temperatures. The P protein of the AIK-C strain was responsible for the *ts* characteristics and Pro at position 439 of the P protein was a critical point for the *ts* phenotype based on the findings of a mini-genome assay using the chimerical P expression plasmids constructed from the AIK-C and parental Edmonston strains. A recombinant measles virus having Pro at position 439 of the P protein was generated, and showed the *ts* phenotype. However, a recombinant measles virus having Leu at position 439 of the P protein did not [11].

3. Development of recombinant vaccines

Currently available vaccines are categorized into two types: live attenuated and inactivated vaccines. Inactivated vaccines principally induce humoral antibodies, whereas both humoral and cellular immune responses are induced by live attenuated vaccines. Therefore, a live attenuated vaccine has clinical benefits in addition to strong immunity: a basically single or two-dose immunization provides long-term immunity with a relatively lower cost. Clinical adverse events are more frequently observed with live vaccines than with inactivated vaccines because of the growth of the vaccine strain in the body. Numerous difficulties are associated with developing a new live vaccine by conventional procedures through consecutive passages in primary cell cultures. Immunogenicity and safety profiles are confirmed in experimental animal models. Final availability is established in a large-scale phase III clinical trial; however, the number of participants can be limited. Many effective live attenuated vaccines are known to be effective and safe through a long history of clinical use. Therefore, currently available live vaccine-based vectored vaccines are also expected to be theoretically safe and immunogenic.

Although a number of recombinant virus vectors derived from the vaccinia virus Ankara strain, poxviruses, adeno-associated viruses, and human parainfluenza virus type III have been developed, some virus vectors were not derived from the licensed vaccine strains, without clinical experience [12–16]. There is no predictable biomarker relevant to the vaccine safety in experimental animals. Thus, large-scale clinical trials should be conducted for these vectors in order to assure the safety of the vector. Virus vectors derived from currently available vaccines are theoretically considered to be safe and effective. Among the currently licensed vaccines, the yellow fever vaccine has been used as a live attenuated vaccine-virus vector for the development of recombinant Japanese encephalitis, dengue, and West Nile virus vaccines [17–19]. The pre M + E region of the yellow fever vaccine was replaced by that of the other flaviviruses. Of these, the yellow fever vaccine-based Japanese encephalitis virus vaccine (ChimeriVax-JE) has been licensed.

The live attenuated measles vaccine is a more popular than the yellow fever vaccine. It induces efficient humoral and cellular immunity and its long clinical use has guaranteed its safety [20]. In addition, the measles virus theoretically replicates in the cytoplasm and there is no evidence to suggest that the genome is integrated into host DNA. Recent developments in the reverse genetics of the measles virus have allowed recombinant measles virus cDNA to be artificially manipulated, thereby, contributing to a better understanding of the mechanisms underlying virus replication, transcription, and pathogenesis. The live attenuated measles vaccine has been investigated for the recombinant virus vector besides the yellow fever vaccine. Several strains are now available throughout the world and attenuation mechanisms are supposedly different for each strain [21]. Two groups are now extensively working on recombinant measles vaccine candidates using licensed measles vaccine strains from Dr. Naim, Berna Biotech [22–25], Dr. Tangy, Pasteur Institute [26–32], and others [33,34]. Their construction strategies, targets, and experimental models are summarized in Table 1. They targeted the unmet needs of the vaccines against HIV, SARS corona, West Nile, dengue, Nipah, chikungunya, and hepatitis C viruses. Through conventional strategy, effective live or inactivated vaccines were not developed against these targets. The targeted genome regions were inserted at the N/P, P/M, or H/L junctions. Most popular insertion site is at the P/M junction. When a heterologous gene was inserted at the N/P junction, amounts of mRNA of the P gene decreased, causing poor virus growth. Mourez et al. [31] reported a different strategy by which the F and H genome regions of the measles virus were replaced by the chimeric DNA fragment of the simian immunodeficiency virus (SIV) gp160 genome fused with the cytoplasmic region of measles F protein genome, which interacted with the M protein. The immunogenicity of the recombinants was examined in transgenic mice expressing human CD46 with the deletion of the IFN-α/β receptor or non-human primates. There is no proper experimental animal for evaluating immune responses for measles. Transgenic mice depleted of IFN systems are not appropriate to investigate the immunogenicity following administration of

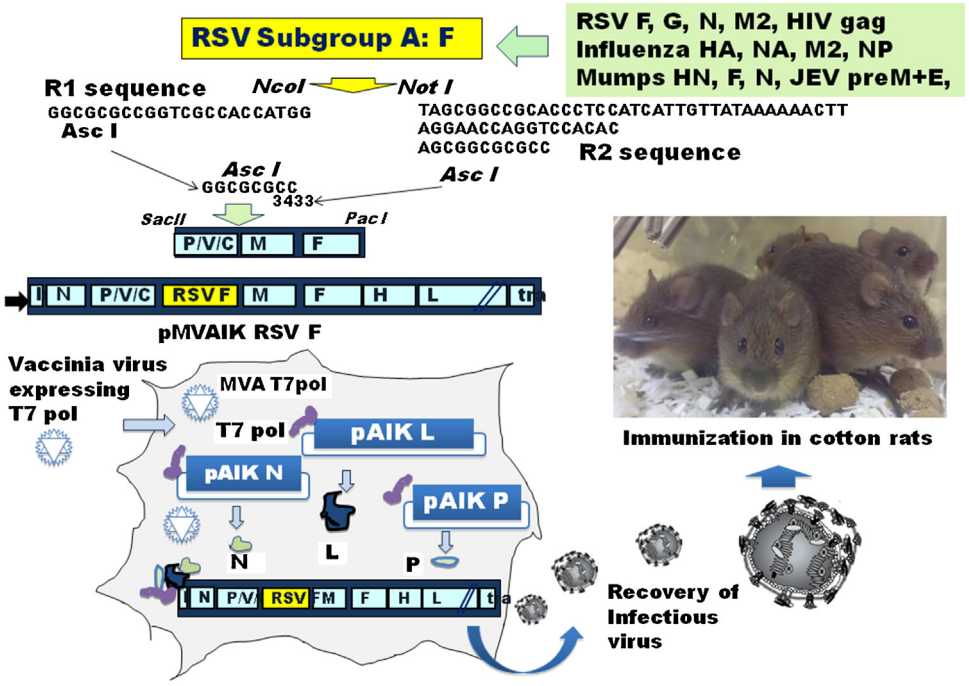

Fig. 1. Experimental strategy for the recombinant measles virus expressing heterologous antigens. Three experimental steps are involved. (1) Cloning of heterologous genome: heterologous virus genomes encoding the protective antigen were cloned using *Nco* I and *Not* I. (2) Construction of measles AIK-C vector: *Asc* I restriction enzyme site was introduced at the 3433 genome position at the P/M junction, and the R1, R2 sequences, and *Nco* I and *Not* I sites were added. A cloning vector was constructed from nucleotide position 2040 of the *Sac* II site and at 7238 of the *EcoT22I* site. (3) Infectious virus recovery: 293T cells were infected with vaccine virus expressing T7 RNA polymerase and were transfected with full length of infectious cDNA, together with helper plasmids expressing measles N, P, and L proteins. Then, cotton rats were immunized.

recombinant measles-vectored vaccine candidates. In our preliminary experiments, cotton rats (*Sigmodon hispidus*) were susceptible for measles virus infection. Measles virus genome was detected from regional draining lymph nodes and virus was isolated. Neutralizing antibodies were produced in cotton rats three weeks after immunization of AIK-C measles vaccine strain [35].

4. History of the respiratory syncytial virus (RSV) vaccine

The RSV vaccine has been anticipated for many years, among the many unmet needs of vaccines. RSV is one of the most common causes of lower respiratory tract infections in infants and young children worldwide. The peak age for serious RSV infections is less than 6 months and most children have had an RSV infection by two years of age. Serious complications have been reported especially in newborn babies born prematurely with lung diseases, or those with congenital heart diseases. RSV also causes lower respiratory tract infections in the elderly and immuno-compromised hosts, as well as in the pediatric population [36,37].

The formalin-inactivated RSV vaccine (FI-RSV) was developed after the discovery of RSV. However, it failed to induce protective immunity and resulted in a paradoxical tragedy of increasing the severity of subsequent RSV infections [36]. Since then, many challenges were performed. The classical biological selection of cold-adapted or *ts* mutants or reverse genetically engineered vaccine candidates were not appropriately attenuated, and subunit vaccines of the fusion (F) or glycoprotein (G) did not provide long-term protective immunity [38]. The reasons for the serious outcome of FI-RSV were extensively investigated, and FI-RSV was found to induce a skewed Th2 response. A balanced Th1 and Th2 response is essential for the development of a RSV vaccine [38]. Therefore, live-vectored vaccine candidates have been investigated using human parainfluenza, bovine parainfluenza, and Sendai viruses [38]. The measles vaccine-vectored RSV vaccine has also been reported. Mok et al. [39] reported recombinant measles virus cDNA with the insertion of RSV F at the P/M junction and Epstein–Barr (EB) virus gp350 protein at the H/L junction. The infectious virus was recovered and induced neutralizing antibodies against RSV but not against EBV.

5. Development of recombinant measles vaccine expressing RSV antigens

The experimental design for the construction of recombinant cDNA from the AIK-C strain and immunogenicity are shown in Fig. 1. The *Asc* I restriction enzyme site was introduced by adding the GGCGCG sequence at the 3433 genome position of the P/M junction. The R1 and R2 sequences were added, and a cloning vector was constructed from nucleotide position 2040 of the *Sac* II site and at 7238 of the *EcoT22I* site. The open reading frames of the heterologous virus genome encoding the protective antigen were cloned at the restriction enzyme sites, *Nco* I and *Not* I. They were introduced into full-length infectious cDNA using *Sac* II and *Pac* I sites. They were then transfected into 293 T cells together with helper plasmids expressing the measles N, P, and L proteins under the control of T7 RNA polymerase. Infectious virus particles were rescued through blind passages in Vero cells at 32.5 °C in 5% CO_2.

Recombinant measles viruses expressing the fusion (F) or glyco (G) proteins of RSV subgroup A (MVAIK/RSV/F or MVAIK/RSV/G) were developed. These viruses induced cross protective neutralizing antibodies (NT) against RSV subgroups A and B in cotton rats. The infectious virus was not recovered from the lung tissues of the cotton rats immunized with recombinant viruses after the challenge with RSV subgroup A, but the protective effects were not sufficient after the challenge with RSV subgroup B [40]. Although no detectable RSV was recovered from immunized cotton rats after the challenge, very mild inflammatory pneumonitis was observed.

Recombinant measles viruses expressing RSV M2-1 or NP (MVAIK/RSV/M2-1 and MVAIK/RSV/NP) were generated, together

with MVAIK/RSV/F. No detectable neutralizing antibody against RSV was induced in cotton rats immunized with recombinant measles virus expressing M2-1 or NP, whereas high titers of neutralizing antibody were induced after immunization with those expressing F or G protein. MVAIK/RSV/M2-1 and MVAIK/RSV/NP induced strong CTL responses, increasing the number of $CD8^+$ IFN-γ^+ cells in spleen cells stimulated with the NP, M2, and F peptides, or inactivated RSV antigen [41]. No infectious virus was recovered from lung homogenate following the challenge. A histological examination of the lung tissues demonstrated a significant reduction in inflammatory reactions without alveolar damage, despite negative for neutralizing antibodies. Both neutralizing antibody and CTL responses are essential for the protection from infection and mitigating the inflammatory responses.

When considering a clinical usage of recombinant measles vaccine candidates expressing RSV antigen in young infants, growth inhibition of vaccine virus was supposed because of maternally conferred immunity. When cotton rats were immunized beforehand with different doses of AIK-C vaccine strain, a significant antibody response was demonstrated after immunization with MVAIK/RSV/F in cotton rats with PA titer $\leq$320. Considering the protection for newborn or young infants in clinical setting, adult immunization of recombinant virus (MVAIK/RSV/F) would be expected before pregnancy. But, the experimental model is hardly developed because of different immunological system in rodents.

Due to its clinical use for several decades, the measles vaccine is considered to be safe and immunogenic, thereby providing long-term memory, and maybe a promising platform for developing a new vaccine. Several issues remain to be resolved: the acceptability of DNA-engineered vaccines in public, clinical guidelines for safety profiles, and regulatory guidelines for the manufacture of these vaccines.

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