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Influenza vaccine viruses and the development of seasonal vaccines: A Japanese perspective

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ABSTRACT

In Japan, the Ministry of Health, Labour and Welfare (MHLW) designates one specific virus strain for each component of the quadrivalent seasonal influenza vaccine, and four domestic manufacturers produce egg-based influenza vaccines with the same formulation (inactivated, split-virus) using uniform vaccine strains. Thus, discussions of the development of effective seasonal influenza vaccines so far has focused solely on the antigenic match between the vaccine strains and epidemic viruses. However, in 2017, the Japanese selection system of vaccine viruses demonstrated that even a candidate vaccine virus that is antigenically similar to the predicted circulating viruses is not necessarily suitable for vaccine production, given lower productivity of the vaccine. Taking this experience into account, the MHLW reformed the scheme of vaccine strain selection in 2018, and instructed the Vaccine Epidemiology Research Group created by the MHLW to probe how the virus strains for the seasonal influenza vaccine should be selected in Japan. In this context, a symposium, entitled "Issues of the Present Seasonal Influenza Vaccines and Future Prospects", was held as part of the 22nd Annual Meeting of the Japanese Society for Vaccinology in 2018, and subjects related to the influenza vaccine viruses were discussed among relevant administrators, manufacturers, and researchers. This report summarizes the presentations given at that symposium in order to convey the present scheme of vaccine virus selection, the evaluation of the resulting vaccines, and the efforts at new vaccine formulation in Japan. Notably, from March 2022, the MHLW has launched a discussion of the merits of the seasonal influenza vaccines produced by foreign manufacturers.

1. Introduction

Skepticism about influenza vaccine effectiveness (VE) overwhelmed Japanese society during the late 1980s and the 1990s [1]. This skepticism was attributable primarily to low-quality studies that involved disease misclassification, confounding, selection bias or ecological study design, which reported results suggesting little effectiveness of the vaccine. However, this apparent lack of VE has generally been interpreted as being reflective of antigenic mismatch between the vaccine strains and circulating viruses [2]. Thus, the selection of vaccine strains has been a focus of discussion regarding the development of effective influenza vaccines, occurring in an environment in which Japanese manufacturers produce and supply egg-based seasonal influenza vaccines with the same formulation (inactivated, split-virus) using the uniform virus strains designated by the Ministry of Health, Labour and Welfare (MHLW). The peculiar process used in Japan so far seems to have functioned as a barrier of sorts to the entry of foreign vaccine manufacturers into the Japanese market for seasonal influenza vaccines [3].

Abbreviations: MHLW, The Ministry of Health, Labour and Welfare of Japan; NIID, National Institute of Infectious Diseases, Japan; WHO, World Health Organization; WHOCCs, WHO Collaborating Centres for Influenza; CVVs, candidate vaccine viruses; HA, hemagglutinin; HI, hemagglutination inhibition; NT, neutralization test; VE, vaccine effectiveness; RCT, randomized controlled trial; ILI, influenza-like illness; RT-PCR, reverse transcription-polymerase chain reaction; ccCVVs, cell culture candidate vaccine viruses; pccCVVs, potential cell culture candidate vaccine viruses; VERG, Vaccine Epidemiology Research Group created by the MHLW.

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Recently, it has been reported that human influenza A(H3N2) viruses easily undergo egg-adaptive mutations when passaged in embryonated chicken eggs, a process used to obtain high-growth vaccine viruses [4]. In Japan, one A(H3N2) virus strain was first selected for the 2017–2018 season's vaccine because of that strain's antigenic similarity to the predicted circulating viruses. However, this strain was replaced by another strain immediately following the initiation of that season's vaccine production, after the initially selected strain was shown to have unexpectedly low vaccine productivity [5,6].

This experience prompted the concerned parties to reconsider the process for the selection of vaccine strains, not only from the view of antigenic similarity to the epidemic strains, but also regarding the amount of vaccine supply, clinical VE, and vaccine formulae. Thus, the concerns surrounding the present seasonal influenza vaccine related to the vaccine viruses were discussed with regard to the issues of the administrative system (author HK), vaccine productivity (TH), VE (WF), and cell culture-derived vaccines (EN), with the assistance of a moderator (YH), during a symposium held as part of the 22nd Annual Meeting of the Japanese Society for Vaccinology in December 2018. From the view of appropriate vaccine virus selection and adequate vaccine supply, discussion of the merits of the seasonal influenza vaccines produced by foreign manufacturers, has been initiated by the MHLW as of March 2022 [7].

2. The scheme of strain selection for the seasonal influenza vaccine

In Japan, influenza vaccines and vaccination have attracted much public attention, given that approximately 26 million 1-mL vials of quadrivalent inactivated vaccine are distributed in any given year, with approximately half of the Japanese population receiving vaccination in a given season. Furthermore, influenza vaccines are unique in that the vaccine composition is revised annually. Typically, the MHLW designates one specific virus strain for each vaccine component, and four manufacturers then produce vaccines using the same strains according to the MHLW's notification. Thus, the selection process for influenza vaccine strains in Japan is quite different from the ones in other countries (Table 1).

Previously, the National Institute of Infectious Diseases (NIID) of Japan would identify and recommend a single candidate strain for each virus subtype/lineage of influenza vaccine, considering the recommendation of the World Health Organization (WHO), antigenic match to the domestic circulating viruses (the latest dominant circulating strains), proliferative properties of the virus, etc. Based on the NIID's recommendation, the MHLW subsequently would then authorize the designated strain for vaccine production after hearing the views of the Health Science Council (hereinafter "the Council") of the MHLW [3].

For the 2017–2018 season, the NIID recommended A/Saitama/103/2014 (CEXP-002) (hereinafter "the Saitama strain") for the A(H3N2) vaccine component; this recommendation was based, in part, on the observations that the Saitama strain was less prone to egg-adaptive mutation, and was antigenically more similar to the predicted circulating viruses, than were another candidate, A/Hong Kong/4801/2014 (X-263) (hereinafter "the Hong Kong strain") [8]. On the basis of this recommendation, the MHLW designated (in May 2017) the Saitama strain for use in that year's vaccine; however, the MHLW then switched this designation to the Hong Kong strain in July 2017. This change was instituted because, after starting vaccine production using the Saitama strain, protein recovery in the splitting process was noted to be too low to provide the amount of antigen required for production of the vaccine supply [5,6]. This delay in strain selection resulted in a vaccine shortage at the beginning of that influenza season, and prompted the MHLW to reconsider the process for selecting vaccine strains.

In February 2018, the Subcommittee for Review of Strains for Producing Seasonal Influenza Vaccines (hereinafter "the Subcommittee") was newly established within the Council, and the role of the NIID was changed to being that of referring their views on a few selected candidate strains per vaccine component to the Subcommittee. Thus, starting in 2018, selection of a single vaccine strain for the next season has been the responsibility of the Subcommittee, which makes a selection based on the antigenicity information provided by the NIID and the productivity assessment data provided by vaccine manufacturers [9]. The MHLW then designates one specific strain for each component based upon the deliberations of the Subcommittee/Council, with the goal of maximizing the benefit to public health based on the strain's potential VE and expected amount of vaccine supply [3].

In the 2018–2019 season, the Subcommittee/Council had extensive discussions on the reports from the NIID and manufacturers, which focused on two candidate strains (B/Colorado/06/2017 and B/Maryland/15/2016(NYMC BX-69A)) for the B/Victoria lineage [10]. The NIID endorsed B/Colorado and B/Maryland in that order, based on data from the US that demonstrated 97 % and 65 % antigenic similarity, respectively, to the recently circulating viruses. On the other hand, vaccine manufacturers estimated that vaccine production using each respective strain would provide 26.02 million and 27.79 million 1-mL vials. Considering these data together, the Subcommittee/Council ultimately selected B/Maryland as the more appropriate strain for incorporation into the 2018–2019 season's vaccine [11,12].

3. Assessment of virus properties related to the productivity of vaccines

The WHO Global Influenza Surveillance and Response System has been monitoring worldwide influenza activity using the information on regional epidemics and circulating viruses reported by the National Influenza Center of each country [13]. Additionally, the WHO Collaborating Centres for Influenza (WHOCCs) assess antigenic and genetic characteristics of recent viruses with the potential for use in vaccine production. On the basis of these data, the WHO annually recommends virus strains to be incorporated into seasonal influenza vaccines intended for use in the northern and southern hemispheres in February and September, respectively [14]. The WHO designates the strains being recommended for each vaccine component in the form of "-like viruses" and presents this information together with a list of candidate vaccine viruses (CVVs) that are antigenically equivalent to the recommended strain [8,10].

In Japan, the NIID, one of the WHOCCs, narrows the list down to a few CVVs based on the viruses' properties, including antigenicity and vaccine productivity, as well as domestic influenza activity. Subsequently, the MHLW selects a single specific strain from among the short-listed CVVs for each vaccine component, with the goal of maximizing the benefits to public health [9]. For the deliberations of the NIID and the Subcommittee/Council/MHLW, the Japanese vaccine manufacturers (the Japan Association of Vaccine Industries) play an important role in providing data on the properties of CVVs related to vaccine productivity [3].

Subsequently, the manufacturers produce seasonal influenza vaccines using the uniform virus strains designated by the MHLW and ship these vaccines after confirming that these products pass the national test [3]. The current influenza vaccine generated for use in Japan is quadrivalent, that is, includes four components (one each of the A(H1N1), A (H3N2), B/Yamagata, and B/Victoria lineages), and contains 15 μg hemagglutinin (HA) protein per

Table 1
Comparison of the Selection Process for Influenza Vaccine Strains.*

	Japan	USA	Europe
Review Process	(Up to 2017) The NIID identified and recommended a single specific strain per vaccine component based on the following information: • WHO-recommended vaccine strains • Antigenicity of viruses recently circulating in Japan • Growth properties of candidate strains The MHLW authorized one NIID-recommended strain after hearing the views of the HSC. (After 2018) The NIID and manufacturers provide their views on antigenicity, growth properties and productivity for a few selected strains per vaccine component to the Subcommittee of the HSC. The Subcommittee then selects one strain based on the above information.	VRBPAC deliberates on vaccine strain selection based on the following information: • Data showing the effectiveness of the previous season's vaccine • Information on global influenza activity • Surveillance report in the US • Antigenicity of candidate strains • Information on the vaccine production process from manufacturers After holding public hearings (where any preregistered participant can listen to the discussion and pose questions), the Committee members vote to decide the virus strains to be recommended to the FDA.	CHMP and BWP convene an Ad-hoc Influenza Working Group meeting of experts from member countries to deliberate on the selection of vaccine strains and the timetable for applications for partial changes of vaccine strains for the year based on the following information: • WHO's global surveillance (explained by WHOCC, London) • WHO-recommended vaccine strains • Antigenicity and growth properties of candidate strainsBefore the meeting, each manufacturer is requested to bring data (such as data regarding growth properties and productivity) and to participate. After the BWP puts together its recommendation for virus strains, CHMP approves the BWP's recommendation.
Decision	MHLW	FDA	EMA
Designation	One specific strain	"-like viruses"	Multiple strains
Advantages	• The near identical vaccine quality among manufacturers • Minimized burden on the individual manufacturer for potency testing and regulatory approval • Uncomplicated national testing because of uniform vaccine strains	• Early start in vaccine production since manufacturers themselves select the strains • Immediate exclusion by manufacturers of virus strains unsuitable for vaccine production • Since the working seeds of more than one strain are prepared, the prompt switch from one production strain to another when issues arise such as the first virus not growing well is possible	
Disadvantages	• Time taken to finalize strain selection • Difficulty for foreign manufacturers to enter into the Japanese market • Not applicable for formulations other than egg-based vaccines	• Manufacturers' responsibility for selection of production strains and subsequent assessment of resultant vaccine products • Tendency to prioritize availability rather than suitability in selecting production strains • Potential variance in vaccine quality including efficacy among manufacturers • Difficulty in predicting the timing and amount of vaccine supply for a whole country	

NIID, National Institute of Infectious Diseases; MHLW, The Ministry of Health, Labour and Welfare of Japan; HSC, Health Science Council of the MHLW; VRBPAC, Vaccines and Related Biological Products Advisory Committee; FDA, Food and Drug Administration; CHMP, Committee for Medicinal Products for Human Use; BWP, Biologics Working Party; WHOCC, WHO Collaborating Centre for Influenza; EMA, European Medicines Agency.
* Drawn-up based on related reference [3].

0.5- mL vaccine dose (i.e., is provided at a concentration of 30 μg HA per mL). In practice, bulk solutions of the individual components are prepared separately by propagating vaccine viruses, and the resulting solution is then blended so as to contain the required amount of HA for each virus in the final formulation. During these procedures, the manufacturers may find that a specific virus among the four exhibits a markedly low vaccine productivity, given that the viruses are not identical in terms of growth properties, etc. Such a virus requires a longer interval to yield a sufficient volume of bulk solution, thereby causing difficulties in supplying the necessary amounts of vaccine on schedule. The productivity assessment for CVVs is therefore regarded as essential in selecting the vaccine strains. Three major parameters on which the vaccine manufacturers place importance for the productivity assessment are the proliferation of the virus, the shape of the virus, and the recovery in the splitting process.

The first and most critical parameter is the proliferative properties of the virus. In vaccine production, differences in viral proliferation of 2-fold or more have been observed among virus strains. The proliferative ability is evaluated by subjecting each influenza virus suspension (obtained by culturing in embryonated chicken eggs) to 30 % sucrose density centrifugation; the protein concentration of the resulting viral concentrate is used as a measure of viral density. The findings obtained by this method show strong correlation with the yield of purified virus in actual vaccine production and therefore is considered crucial to evaluating the proliferative properties of CVVs.

Second, the shape of the virus also is an important element in assessing vaccine productivity. To reduce the bioburden associated with vaccine production, a filtration process is incorporated into the production line of the bulk solution. Under electron microscopy, influenza viruses propagated in embryonated chicken eggs normally have a spherical structure with the diameter of approximately 100 nm, but occasionally have filamentous shape larger than the pore size of the filter membrane, thereby resulting in decreased virus recovery [15,16]. For this reason, manufacturers examine the shapes of CVVs using a transmission electron microscope before filtration, with the intent of avoiding large losses in the filtration process.

Third, the recovery rate in a splitting process recently has been added to the parameters used to assess vaccine productivity. The purified viruses obtained in vaccine production next are split with ether. Historically, it has been assumed that protein recovery in this splitting process remains almost constant, regardless of the vaccine strain. However, the Saitama strain, one of the CVVs used as the A(H3N2) component in the 2017–2018 season's vaccine, exhibited a notably lower recovery rate, a feature of the virus that was observed only after vaccine production had been initiated [5,6]. Given this adverse experience, the manufacturers have since additionally implemented laboratory-scale assessments of the recovery rate in the splitting process, with the goal of improving the evaluation of the CVVs' properties related to the productivity of vaccine.

Thus, the Japan Association of Vaccine Industries is making efforts to achieve a precise assessment of vaccine productivity for CVVs, with the goal of contributing to the quality of vaccine virus selection and stable vaccine supply, under the guidance of the MHLW and NIID.

4. Epidemiologic evaluation of influenza vaccine effectiveness

Seasonal influenza vaccine strains are currently selected according to best scientific knowledge, including worldwide seasonal influenza activity, antigenic and genetic characteristics of recent influenza viruses, and the extent of proliferation of candidate strains. Although the extent of antigenic match between candidate strains and predicted circulating strains is also considered, such evaluations are usually based on laboratory experiments using post-infection ferret antisera and circulating strains from the previous influenza season. However, it is critical to assess actual VE in the human population during seasons in which the vaccines are distributed; such assessments require appropriately conducted epidemiologic studies.

Among various epidemiologic studies, interventional trials including randomized controlled trials (RCTs), represent the strongest means of assessing the preventive or therapeutic effects of factors in the human population, followed by cohort studies. With respect to influenza VE, however, even an excellent RCT only provides time-, place-, and subject-specific observations, and not conclusive findings, given that: (1) the characteristics of circulating influenza viruses differ by time and place; (2) the proportion of subjects having pre-existing immunity differs by time, place, and age group; and (3) vaccine strains differ by time (i.e., season) [17]. When the outcome measure is defined as laboratory-confirmed influenza, both RCTs and cohort studies have difficulties achieving "equal intensity" of follow-up due to a disparity in healthcare-seeking attitudes between vaccinated and unvaccinated subjects. Investigators therefore must expend great effort in performing active surveillance for outcome confirmation throughout the influenza season [17].

At present, test-negative design (a modified case-control study) is considered the most desirable strategy for estimating influenza VE against laboratory-confirmed influenza. Among eligible subjects who visit a clinic or hospital due to influenza-like illness (ILI) during the influenza season, subjects with test results for influenza infection are recruited in the study and then are classified as either cases (positive test results for influenza) or controls (negative test results for influenza). Influenza vaccination status among cases is compared with that of controls to calculate VE. Since patients with ILI are expected to visit a clinic or hospital immediately after the onset of symptoms, healthcare-seeking attitude is likely similar between cases and controls. Thus, a strength of test-negative influenza VE studies is their ability to minimize misclassification of diseases and confounding by differences in health care-seeking attitudes [17]. Detailed mechanisms for how test-negative design reduces confounding by healthcare-seeking attitudes have been described elsewhere [18,19,20]. The number of influenza VE studies using test-negative designs has increased dramatically over the last decade. Presently, test-negative designs enable the monitoring of influenza VE across seasons, and several studies (performed by the Global Influenza Vaccine Effectiveness Collaboration) have contributed (confidentially) to the WHO's recommendations on seasonal influenza vaccine composition [14].

Accumulating evidence from test-negative-design studies indicates that inactivated influenza vaccines provide moderate protection against laboratory-confirmed influenza. One meta-analysis summarized the findings from 56 influenza VE studies that were published between January 2004 and March 2015 and used test-negative designs with real-time reverse transcription-polymerase chain reaction (RT-PCR) to define case/control status in an outpatient setting. Pooled VEs according to type or subtype were 61 % (95 % CI: 57–65) for A(H1N1)pdm09, 33 % (26–39) for A(H3N2), and 54 % (46–61) for type B. The lower VE for A(H3N2) may reflect antigenic mismatch between vaccine strains and circulating strains; however, it is worth noting that the VE estimate for A (H3N2) differed among age groups, showing values of 43 % for pediatric age groups, 35 % for working-age adults, and 24 % for older adults [21]. Another meta-analysis examining VE against hospitalization with laboratory-confirmed influenza among adults, which included 30 studies using a test-negative design between the 2010–2011 and 2014–2015 seasons, also showed that the influence of antigenic mismatch on VE against A(H3N2) was substantial in the elderly. Pooled VE (95 % CI) among subjects aged 16–64 years and $\geq$65 years was 59 % (38–80) and 43 % (33–53), respectively, when vaccine strains and circulating strains were antigenically similar, whereas the VE was 46 % (30–61) and 14 % (−3 to 30), respectively, when vaccine strains and circulating strains were antigenically variant [22]. An interpretation of influenza VE beyond antigenic match, including birth cohort effects, is required, as proposed in a report by Canadian researchers [23].

In Japan, very few studies have used RT-PCR to confirm case/-control status in test-negative designs intended to evaluate influenza VE. Since rapid diagnostic testing for influenza is routine clinical practice in Japan, clinicians conduct large studies with test-negative designs easily without regard for the underlying methodology. It should be noted that if subjects are limited to those who received the clinician-ordered test in a routine clinical setting, application of the test would depend on the likelihood of having influenza (outcome) or influenza vaccination status (exposure), thus resulting in biased sampling (non-representativeness) of study subjects, thereby compromising the validity of the study [17,20].

To maximally eliminate selection bias, an influenza VE study by the Vaccine Epidemiology Research Group created by the MHLW (VERG) [2] applied "active recruitment of study subjects from eligible patients according to pre-defined disease criteria" and "active application of the test to study subjects in a systematic manner", regardless of exposure and outcome status [24]. Clinicians need to understand that the most important issue in epidemiologic studies is validity, and not the size of the study.

5. A feasibility study on the use of a cell culture-derived vaccine as a seasonal influenza vaccine

Currently, the influenza vaccine is manufactured using embryonated chicken eggs or cultured cells. The egg-based process for production of influenza vaccines has been utilized since the 1940s; this process has repeatedly demonstrated efficient vaccine production. However, the production rate for this vaccine is affected by the supply of embryonated chicken eggs. Therefore, egg-based vaccines are not suitable for the production of pandemic vaccines, which require rapid production when a pandemic occurs. On the other hand, stocks of tissue culture cells used for vaccine production, which are stored at deep-freeze temperatures, always are available for vaccine production. For this reason, Japan plans to use cell culture-derived vaccines during pandemics.

To date, circulating human influenza viruses, especially A (H3N2), have proven to be difficult to propagate in embryonated chicken eggs. To develop high-growth vaccine viruses, clinical isolates are passaged several times in eggs. Consequently, the resulting high-growth viruses have egg-adaptive mutations, which occasionally affect the antigenicity of the virus [4]. To avoid this deleterious effect on the antigenicity of vaccine viruses and to maintain the condition of the production lines for a pandemic influenza vaccine, Japan has been considering the use of cell culture-derived vaccines as seasonal influenza treatments [25].

In our feasibility study, in cooperation with four Japanese vaccine manufacturers, we developed seasonal influenza vaccine

viruses that are completely cell culture-derived without passaging through eggs. Procedures for the development of these cell culture-derived vaccine viruses were based on a strategy provided by the WHO [26]. In brief, the following steps were performed by the WHOCC: (1) virus isolation from clinical samples using qualified cells, and (2) antigenic and genetic characterization of the virus isolates and potential cell culture candidate vaccine viruses (pccCVVs). The development of pccCVVs was carried out by the manufacturers using qualified cell lines, such as Madin-Darby canine kidney (MDCK) cells, Vero cells (African green monkey kidney cells), and EB66 cells (duck embryonic stem cells). The NIID performed the above procedures as a WHOCC member. To test the antigenicity of the virus isolate, a ferret antiserum was raised against a WHO-designated cell-propagated prototype virus (prototype virus), and the reactivity of this antiserum was assessed against a virus isolate derived from a clinical sample. Specifically, the reactivity was determined by means of either a hemagglutination inhibition (HI) test or a neutralization test (NT). When the antibody titer observed in response to the viral isolate was $\leq$2-fold lower than that observed in response to the prototype virus, the antigenicity of the virus isolate was considered similar to that of the prototype virus. These viruses then were provided to the manufacturers as parent viruses for generating pccCVVs.

Over the past five influenza seasons, 25, 49, 11, and 11 viruses of A(H1N1)pdm09, A(H3N2), B/Victoria, and B/Yamagata (respectively) have been isolated (using qualified cell lines) from clinical samples. Among these isolates, the viruses showing antigenicities similar to prototype viruses from the corresponding seasons were provided to the four Japanese manufacturers.

The manufacturers propagated the parent viruses in cells qualified for vaccine production. The antigenicity of the resulting viruses, that is, the pccCVVs, was examined by either an HI test or an NT using antisera produced by infecting ferrets with the pccCVVs or with the corresponding prototype viruses. According to the WHO criteria for assessing the antigenicity of a ccCVV, the antiserum produced in response to the prototype virus should recognize the pccCVV with a titer $\leq$2-fold lower than the homologous titer for the prototype virus. In addition, the antiserum raised against the pccCVV should recognize the prototype virus at a titer $\leq$2-fold lower than the homologous titer for the pccCVV. Once the antigenicity of the pccCVV met the abovementioned criteria, the pccCVV was considered as a ccCVV for vaccine production.

Progress in the development of vaccine viruses by the manufacturers has varied depending on the manufacturer. Currently, two of the four manufacturers have succeeded in developing ccCVVs to generate quadrivalent vaccines in qualified cell lines. These viruses satisfied the criteria for assessing the antigenicity of a ccCVV. The other manufacturers have generated two or three ccCVVs of A (H3N2), B/Victoria, and B/Yamagata, but have failed to develop a ccCVV of A(H1N1)pdm09 because the antigenicity of the isolates has been unsuitable for a ccCVV.

Regarding cell-adaptive mutations in pccCVVs, specific mutations for a particular cell line generally have not been observed (to date). Genetic analysis of the HA-encoding genes demonstrated that mutations leading to similar amino acid changes tended to occur at the same position in pccCVVs derived from the same parent virus, even though each manufacturer developed the pccCVVs in different cells. Analysis of next-generation sequencing data showed that most of the mutations resulted in a mixture of original and substituted amino acids at the same position in the HA protein, while a few mutations resulted in complete amino acid substitution. The influence of the former class of mutations on the antigenicity of the virus was weaker than that of the latter class.

To determine whether the antigenicity of the resultant ccCVVs was similar to that of the circulating viruses isolated during the corresponding influenza season, we performed either an HI test or an NT (plaque reduction assay) using ferret antisera. The available ccCVVs of A(H1N1)pdm09, A(H3N2), B/Victoria, and B/Yamagata were used to infect four different groups of ferrets. The antisera collected from these animals generally reacted well with the corresponding circulating viruses, with a $\leq$4-fold reduction in titers compared to the homologous titers of the ccCVVs. Accordingly, these ccCVVs were defined as antigenically similar to the circulating viruses isolated during the corresponding influenza season, suggesting that (in the present feasibility study) the cell culture-derived vaccine viruses seem to avoid the deleterious effects of egg-adaptive mutations on the antigenicity of vaccine viruses. In this study, however, antigenicity was determined by means of ferret antisera. Before cell culture-derived seasonal influenza vaccines can be used in the clinic, the immunogenicity of the vaccine in humans will need to be investigated.

6. Discussions

In the general process of selecting influenza vaccine strains, the WHO annually recommends the virus strains for the seasonal influenza vaccine, and each country subsequently assesses the suitability of those strains for vaccine production in the light of the domestic situation and designates its own vaccine strains in the form of "-like viruses" or multiple strains for each subtype/lineage. Vaccine manufacturers usually are free to select any strain from the designated viruses in producing their vaccines [3].

On the other hand, in Japan, the MHLW designates one specific strain for each vaccine component, and four manufacturers produce the egg-derived influenza vaccines with the same formulation using the uniform strains. In this particular situation, the antigenic similarity between vaccine strain and predicted circulating viruses has been regarded as a key index for selecting a single strain from among the CVVs, a step that is believed to directly impact VE in the upcoming season. However, little attention has been paid to the fact that the extent of antigenic match is evaluated using antisera produced by infecting ferrets with the CVVs. Humans are likely to exhibit immune responses distinct from those of ferrets, given pre-existing immunity induced by previous virus exposure [2,3].

In the 2017–2018 season, the WHO recommended A/Hong Kong/4801/2014-like viruses for use as an A/H3N2 strain in egg-derived vaccines for use in the northern hemisphere, together with a list of CVVs [8]. For vaccine production in Japan, the MHLW designated the Saitama strain, the antigenicity of which was more similar to the predicted circulating viruses than was the Hong Kong strain, although both strains appeared on the WHO's list of CVVs. The Saitama strain was reported to have acquired only a limited number of egg-adaptive mutations when passaged in eggs, while the Hong Kong strain used in the prior season's vaccine had accumulated a substantial number of mutations when passaged in eggs [5,6].

However, the Saitama strain was replaced by the Hong Kong strain immediately after vaccine production was initiated, after unexpectedly low vaccine productivity was noted for the Saitama strain. Specifically, given the reduced productivity of the Saitama strain, the vaccine supply was predicted to achieve only 71 % of that in the previous season if the Saitama strain were to be retained as a vaccine strain [5,6]. The switch to the Hong Kong strain, which was antigenically less similar to the predicted circulating strains, raised concerns of poor VE. There was even a heated discussion, with opinions raised that it would be more appropriate to produce an "effective vaccine" using the Saitama strain rather than to produce an "ineffective vaccine" using the Hong Kong strain, in which case the resulting vaccine shortage would be made up for by prophylactic use of antivirals.

To investigate whether the change of vaccine virus was rational or not, monovalent influenza A(H3N2) vaccines containing the Saitama or Hong Kong strain were specially prepared and the immunogenicity of these two vaccines was compared in a randomized controlled trial involving healthy adults. The resulting virus NT assays against the Saitama and Hong Kong strains and a circulating A/Osaka/188/2017(H3N2) strain in the corresponding season revealed better or equivalent immunogenicity for the Hong Kong strain vaccine compared to the Saitama strain vaccine [27].

These experiences prompted the MHLW to restructure the selection process for the influenza vaccine strain, and induced vaccine manufactures to pay more attention to the viral properties related to the productivity of vaccine. As a result, a two-step review system consisting of the NIID and Subcommittee/Council was instituted for the selection of vaccine viruses as of 2018, wherein the second step is completely separated from the NIID [3,9]. Since then, the Subcommittee/Council has chosen a single strain from a few selected CVVs according to the available information regarding both potential VE (i.e., antigenic match to the predicted circulating viruses) and expected amount of vaccine production (i.e., virus properties related to the productivity of vaccine), data that are provided by the NIID and the vaccine manufacturers, respectively [11].

In Japan, the discussion on the development of effective influenza vaccines seems (to date) to have focused solely on achieving antigenic match between the vaccine strain and circulating viruses, despite the fact that data on antigenic match originated from laboratory experiments employing post-infection ferret antisera. Under these circumstances, the VERG has continued monitoring influenza VEs using test-negative design since the 2013–2014 season [2,17,24]. A series of VERG studies in pediatric subjects has demonstrated moderate but significant VE even when the dominant circulating A(H3N2) viruses were poorly matched to the respective season's vaccine strain [24]. Moreover, in the 2017–2018 season when the A(H3N2) vaccine component was switched from the Saitama strain to the Hong Kong strain, VE against A (H3N2) was still significant and higher than usual (VE: 67 %, 95 % CI: 29–85) [28]. Thus, the VE as examined in the human population also has become understood as an important element in evaluating vaccine viruses.

Egg-adaptive mutations of vaccine viruses may have deleterious effects on the antigenicity of vaccine viruses. To cope with these changes, cell culture-derived seasonal vaccines are being investigated in the development of effective vaccines [25]. This approach also is considered useful for maintaining a production line in Japan for vaccines against pandemic influenza. At present, two manufacturers have successfully developed ccCVVs to produce quadrivalent vaccines. Furthermore, the HA content in the vaccine thus prepared by one manufacturer was well assigned by the single radial immunodiffusion method. In developing ccCVVs according to the strategy specified by the WHO [26], antigenicity has been assessed (so far) by means of ferret antisera. For the clinical use of cell culture-derived seasonal influenza vaccine in Japan, studies in the human population will be required in order to obtain essential data, such as for immunogenicity, as illustrated by the events surrounding the Saitama and Hong Kong strains.

Furthermore, in terms of strain selection for the cell culture-derived vaccines, each manufacturer uses different culture cells that are qualified for vaccine production, and the strains that proliferate most efficiently in each manufacturer's cells can then be used for vaccine production. Thus, the development of cell culture-derived vaccines against seasonal influenza is expected to raise another complication for the system of vaccine virus selection in Japan [3]. There is no doubt that the present system, whereby the manufacturers produce influenza vaccines using uniform strains designated by the MHLW, will present a challenge for the production of cell culture-derived vaccines in Japan.

In the series of discussions at the symposium that formed the basis for this paper, issues in developing effective influenza vaccines in Japan seemed to converge on the topics of description of vaccine viruses and demonstration in human studies. Many of these points appear in the report "Consideration for the Future Selection Process of Influenza Vaccine Strains in Japan", prepared by the VERG in close cooperation with the NIID and domestic and foreign vaccine manufacturers [3]. In March 2022, the MHLW cited that report when initiating a discussion on the merits of the seasonal influenza vaccines produced by foreign manufacturers [7].

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All authors attest that they meet the ICMJE criteria for authorship.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: 'Takao Hozawa is an employee of Denka Co., Ltd. in Tokyo, Japan. All other authors declare that they are free of competing interests.'

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References

[1] Hirota Y, Fedson DS, Kaji M. Japan lagging in influenza jabs. Nature 1996;380:18.
[2] Hirota Y, Ozasa K, Nakano T. Vaccine epidemiology: Its role in promoting sound immunization programs in Japan. Vaccine 2017;35:4787–90.
[3] Fukushima W, Ohfuji S, Kase T, Ito K, Hirota Y. Consideration for "The Future Selection Process of Influenza Vaccine Strains in Japan." Research Grant for Investigation of Promotion of Policies for Emerging/Re-emerging Infectious Diseases and Immunization from the Ministry of Health, Labor and Welfare, Japan. Shared Research Report (Fiscal 2019). Available at: https://souseikaiglobal.com/cerc-publications/the-future-selection-process-of-influenza-vaccine-strains-in-Japan.pdf [accessed April 27, 2023].
[4] Parker L, Wharton SA, Martin SR, Cross K, Lin Y, Liu Y, et al. Effects of egg-adaptation on receptor-binding and antigenic properties of recent influenza A (H3N2) vaccine viruses. J Gen Virol 2016;97:1333–44.
[5] The R&D and Production and Distribution Committee, Immunization and Vaccines Section, Health Science Council, Ministry of Health, Labor and Welfare, Japan. Influenza vaccines for 2017-2018 season (Document 1 at the 16th meeting held on August 25, 2017) [in Japanese]. Available at: https://www.mhlw.go.jp/file/05-Shingikai-10601000-Daijinkanboukouseikagakuka-Kouseikagakuka/0000175510.pdf [accessed April 27, 2023].
[6] National Institute of Infectious Diseases. Selection process of influenza vaccine viruses in 2017-2018 season. IASR (Infectious Agents Surveillance Report) 2017;38: 225-6. [in Japanese]. Available at: https://www.niid.go.jp/niid/ja/allarticles/surveillance/2413-iasr/related-articles/related-articles-453/7671-453r05.html [accessed April 27, 2023].

[7] The R&D and Production and Distribution Committee, Immunization and Vaccines Section, Health Science Council, Ministry of Health, Labor and Welfare, Japan. Development of seasonal influenza vaccines in the foreign countries (Document 2 at the 27th meeting held on March 23, 2022) [in Japanese]. Available at: https://www.mhlw.go.jp/content/10906000/000917576.pdf [accessed April 27, 2023].
[8] World Health Organization. Influenza A(H3N2) egg-derived candidate vaccine viruses for development and production of vaccines for use in the 2017-2018 northern hemisphere influenza season. Available at: https://cdn.who.int/media/docs/default-source/influenza/cvvs/cvv-northern-hemisphere-2017-2018/summary_a_h3n2_cvv-egg_nh1718_update.pdf?sfvrsn=5b1999ef_15 [accessed April 27, 2023].
[9] The R&D and Production and Distribution Committee, Immunization and Vaccines Section, Health Science Council, Ministry of Health, Labor and Welfare, Japan. Setting up the Subcommittee for Review of Strains for Producing Seasonal Influenza Vaccines (Document 1 at the 17th meeting held on February 19, 2018) [in Japanese]. Available at: https://www.mhlw.go.jp/file/05-Shingikai-10601000-Daijinkanboukouseikagakuka-Kouseikagakuka/0000203083.pdf [accessed April 27, 2023].
[10] World Health Organization. Influenza B Victoria lineage egg-derived candidate vaccine viruses for development and production of vaccines for use in the 2018-2019 northern hemisphere influenza season. Available at: https://cdn.who.int/media/docs/default-source/influenza/cvvs/cvv-northern-hemisphere-2018-2018/summary-b-vic-cvv-egg-nh1819.pdf?sfvrsn=9f55371a_8 [accessed April 27, 2023].
[11] The Subcommittee for Review of Strains for Producing Seasonal Influenza Vaccines, The R&D and Production and Distribution Committee, Immunization and Vaccines Section, Health Science Council, Ministry of Health, Labor and Welfare, Japan. Influenza vaccine strains for 2018–2019 season (Document 2-4, 3-1, 3-2, and 3-3 at the 1st meeting held on April 11, 2018) [in Japanese]. Available at: https://www.mhlw.go.jp/stf/shingi2/0000203178.html [accessed April 27, 2023].
[12] National Institute of Infectious Diseases. Selection process of influenza vaccine viruses in 2018-2019 season. IASR 2018;39:195–7 [in Japanese]. Available at: https://www.niid.go.jp/niid/ja/allarticles/surveillance/2436-iasr/related-articles/related-articles-465/8429-465r06.html [accessed April 27, 2023].
[13] Hay AJ, McCauley JW. The WHO global influenza surveillance and response system (GISRS): A future perspective. Influenza Other Respir Viruses 2018;12:551–7.
[14] World Health Organization. Recommended composition of influenza virus vaccines for use in the 2018–2019 northern hemisphere influenza season. Available at: https://www.who.int/publications/m/item/recommended-composition-of-influenza-virus-vaccines-for-use-in-the-2018-2019-northern-hemisphere-influenza-season#:~:text=It%20is%20recommended%20that%20quadrivalent,2%2F87%20lineage)%3B%20and (accessed April 27, 2023).
[15] Dadonaite B, Vijayakrishnan S, Fodor E, Bhella D, Hutchinson EC. Filamentous influenza viruses. J Gen Virol 2016;97:1755–64.
[16] National Institute of Infectious Diseases. Selection process of influenza vaccine viruses in 2016–2017 season. IASR 2016;37:225–7 [in Japanese]. Available at: https://www.niid.go.jp/niid/ja/allarticles/surveillance/2350-iasr/related-articles/related-articles-441/6878-441r05.html [accessed April 27, 2023].
[17] Fukushima W, Hirota Y. Basic principles of test-negative design in evaluating influenza vaccine effectiveness. Vaccine 2017;35:4796–800.
[18] Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. Vaccine 2013;31:2165–8.
[19] Foppa IM, Haber M, Ferdinands JM, Shay DK. The case test-negative design for studies of the effectiveness of influenza vaccine. Vaccine 2013;31:3104–9.
[20] Ozasa K, Fukushima W. Commentary: Test negative design reduces confounding by healthcare-seeking attitude in case-control studies. J Epidemiol 2019;29:279–81.
[21] Belongia EA, Simpson MD, King JP, Sundaram ME, Kelley NS, Osterholm MT, et al. Variable influenza vaccine effectiveness by subtype: A systematic review and meta-analysis of test-negative design studies. Lancet Infect Dis 2016;16:942–51.
[22] Rondy M, El Omeiri N, Thompson MG, Levêque A, Moren A, Sullivan SG. Effectiveness of influenza vaccines in preventing severe influenza illness among adults: A systematic review and meta-analysis of test-negative design case-control studies. J Infect 2017;75:381–94.
[23] Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Winter AL, Dickinson JA, et al. Beyond antigenic match: Possible agent-host and immuno-epidemiological influences on influenza vaccine effectiveness during the 2015–2016 season in Canada. J Infect Dis 2017;216:1487–500.
[24] Fukushima W, Morikawa S, Fujioka M, Matsushita T, Kubota M, Yagi Y, et al. for the Vaccine Epidemiology Research Group of the Ministry of Health, Labour and Welfare Japan. Influenza vaccine effectiveness in children under 6 years: summary findings from 2013-2014 season to 2016-2017 season. IASR. 2018;39:197–9 [in Japanese]. Available at: https://www.niid.go.jp/niid/ja/allarticles/surveillance/2436-iasr/related-articles/related-articles-465/8430-465r07.html [accessed April 27, 2023].
[25] The R&D and Production and Distribution Committee, Immunization and Vaccines Section, Health Science Council, Ministry of Health, Labor and Welfare, Japan. Study on putting cell culture-derived seasonal influenza vaccine to practical use (Document 3 at the 11th meeting held on February 19, 2016) [in Japanese]. Available at: https://www.mhlw.go.jp/file/05-Shingikai-10601000-Daijinkanboukouseikagakuka-Kouseikagakuka/siryou3_1.pdf [accessed April 27, 2023].
[26] World Health Organization. Antigenic characterisation of seasonal influenza viruses isolated in vaccine-qualified cell line, December 2015, WHO/HSE/PED/GIP/GISRS/2015.01. Available at: http://apps.who.int/iris/bitstream/10665/201995/1/WHO_HSE_PED_GIP_GISRS_2015.01.pdf?ua=1 [accessed April 27, 2023].
[27] Kase T, Inoue M, Morikawa S, Kumashiro H, Hiroi S, Nakata K, et al. Comparison of immunogenicity between candidate influenza A(H3N2) virus vaccine strains in Japan: A randomized controlled trial using a monovalent vaccine of A/Saitama/103/2014 (CEXP-002) and A/Hong Kong/4801/2014 (X-263). Vaccine 2020;38:6524–32.
[28] Fukushima W, Morikawa S, Matsumoto K, Fujioka M, Matsushita T, Kubota M, et al. Influenza vaccine effectiveness in children: 2017–2018 season. Annual report of the Vaccine Epidemiology Research Group of the Ministry of Health, Labour and Welfare Japan. pp 27–39, 2019 [in Japanese]. Available at: https://mhlw-grants.niph.go.jp/system/files/2018/182111/201818022A_upload/201818022A0009.pdf [accessed April 27, 2023].