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Review

Current issues with the immunization program in Japan: Can we fill the "vaccine gap"?

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ABSTRACT

The "vaccine gap" is a term which has been used in Japan to indicate that the current immunization program is behind compared to the programs in other developed countries. The current national immunization program (NIP) which was established under the Japanese Immunization Law includes only six vaccines (eight targeted diseases), and the rest of available vaccines have been categorized as voluntary vaccines, which require out-of-pocket expense in order for the patients to receive them. This has led the vaccination rates for the voluntary vaccines remaining low, and the incidence of the target diseases remaining high. In addition, there are a few domestic rules that exist for immunizations including (1) subcutaneous injection is the standard method of vaccination, (2) the thigh is not considered to be the common site of vaccination in infants, and (3) the intervals of administration of inactivated and live vaccines are strictly determined by law. Along with the "vaccine gap" and the domestic rules, some movements to improve our current NIP are underway; including increased calls to change the NIP from civilians and professionals, the establishment of a group by the representatives from 13 medical professional societies asking the government to consider the immunization policy a "national policy" and seeking the establishment of a new and reorganized national immunization technical advisory group (NITAG). In addition, the Vaccination Subcommittee of Health Sciences Council was formed in the government to reform the current Immunization Law and NIP, which established a new national program for three voluntary vaccines funded by a temporary budget. We hope these new movements will fill the "vaccine gap" and that the NITAG will help ensure that vaccine policy becomes a national policy, and will provide necessary vaccinations without out-of-pocket expense to protect children in Japan from vaccine preventable diseases.

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Abbreviations: Hib, *Haemophilus influenzae* type b; VZV, varicella zoster virus; NIP, national immunization program; HPV, human papillomavirus vaccine; PCV7, seven-valent conjugate pneumococcal vaccine; HBV, hepatitis B virus; VPD, vaccine-preventable diseases; NITAG, National immunization technical advisory group; DTaP, diphtheria, tetanus-toxoid, and acellular pertussis; DTwP, diphtheria, tetanus-toxoid, and whole cell pertussis; MMR, mumps, measles, rubella; JPS, Japan Pediatric Society; IPV, inactivated polio vaccine; BCG, Bacille de Calmette et Guérin; OPV, oral polio vaccine; ACIP, Advisory Committee on Immunization Practices; VAPP, vaccine-associated poliomyelitis paralysis.

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1. Introduction

The "vaccine gap" is a term which has been used for the last decade to indicate that the immunization program in Japan has been behind compared to the programs in other developed countries [1]. The best example is that the *Haemophilus influenzae* type b (Hib) vaccine, which has been known to be safe and the most effective vaccine for preventing invasive Hib infections [2], was introduced in Japan in 2008, which was more than 20 years later than other countries. In addition, some important vaccines, including the mumps vaccine, and varicella zoster virus (VZV) vaccine, have been available in Japan for the last two decades; however, they have not been in the national immunization program (NIP). Furthermore, the Hib vaccine, human papillomavirus vaccine (HPV), seven-valent conjugate pneumococcal vaccine (PCV7), and rotavirus vaccine have only recently been introduced in Japan since 2008; however, none of them is part of the NIP. Lastly, the hepatitis B virus (HBV) vaccine has so far only been used as a selective vaccination; and the universal HBV vaccination has not yet been included in the NIP. All these vaccines not in the NIP have been categorized as voluntary vaccines, as opposed to vaccines under the Japanese Immunization Law. To receive the voluntary vaccines, individuals must pay out-of-pocket expense, which has been a major obstacle to increasing the vaccination rates for each voluntary vaccine and decreasing the incidence of vaccine-preventable diseases (VPD).

The reasons why the "vaccine gap" exists are multi-factorial; a long history of fear about vaccinations, the existence of the Immunization Law imposing strict rules for immunization practice, the ineffectiveness of a systematic national surveillance system for VPD, insufficient resources of vaccine education for both medical personnel and civilians, and the lack of an effective national immunization technical advisory group (NITAG). In this review, we summarize these factors contributing to the "vaccine gap" and discuss a few current issues related to immunization in Japan.

2. The history of vaccine fears in Japan

The initial Immunization Law was launched in 1948, with the goal of decreasing the incidence of endemic diseases, such as smallpox, diphtheria, polio, tetanus, pertussis, tuberculosis, etc. After the significant improvement of the sanitary status and the distribution of available vaccines under the revised immunization law, the incidence of the endemic diseases in Japan decreased significantly. During the subsequent period, the vaccination rates for the targeted diseases were high, because receiving vaccines was considered a duty, and there was a penalty if citizens did not receive the required vaccines. Furthermore, Japanese scientists contributed to develop some novel vaccines to the world, including the VZV vaccine in 1974 [3] and the DTaP (diphtheria, tetanus-toxoid, and acellular pertussis) vaccine in 1981 [4].

There were two major events that impacted the immunization program in Japan. First, two fatalities after DTwP (diphtheria, tetanus-toxoid, and whole cell pertussis) vaccination were reported in 1975, and the vaccine was withheld for six years until a new acellular pertussis combination vaccine was available [4]. After that event, civilians started to have doubts about receiving the immunization because the risks of vaccination were emphasized by the mass media. As expected, the temporary discontinuation of the DTwP vaccine led to the resurgence of 13,000 pertussis cases and 20 deaths reported in 1979 [4]. The second event was in 1989 when the mumps, measles, rubella (MMR) vaccine caused vaccine-related aseptic meningitis due to the mumps component of the vaccine. The incidence of meningitis was estimated to be one case in every 500–900 vaccinations [5]. The vaccine was withdrawn from the market in 1993 based on the Japanese government's decision, and monovalent measles and rubella vaccines were recommended for children >1 year of age and a mumps monovalent vaccine has become an optional vaccine. Twelve years were required for the marketing of a new combination vaccine of measles and rubella, without the mumps component, and the lack of a combination vaccine with the mumps component is the reason why the disease is still endemic in Japan and many children have been suffering from its complications [6]. The delay of introducing the mumps strain causing less aseptic meningitis, which was carried out in order to protect domestic Japanese vaccine manufacturers, was criticized by the vaccine authorities [7]. The Japanese government was also sued several times for being responsible for vaccine adverse effects in the 1980s and 90s, including severe adverse events after small pox immunization. In addition, there were negative campaigns against the influenza vaccine by both citizens and medical professionals doubting its effectiveness and believing it to cause serious adverse effects. After these multiple events, the government has had difficulty in defending their vaccination policy, because they hesitate to be responsible for their decisions related to immunization. In 1994, the Immunization Law was revised, and the immunization was changed from a civic "duty" to an "effort duty", and mass immunization in schools was thus discontinued and changed to immunization on an individual basis. These movements decreased the vaccination rates in general and led to a failure to introduce new vaccines between 1991 and 2007, which led to the creation of the "vaccine gap". Only two new vaccines were licensed in Japan between 1990 and 2007 (hepatitis A virus, and measles and rubella combination vaccine) compared to 17 vaccines (including combination vaccines) introduced in the US during the same period.

3. Factors contributing to the "vaccine gap"

3.1. Vaccines under the law and voluntary vaccines

There is a unique classification of vaccines in Japan; vaccines defined by the immunization law, and voluntary vaccine not regulated by Japanese law (Table 1). Several important vaccines, including the mumps vaccine, VZV vaccine, and HBV vaccine have remained categorized as voluntary vaccines which require individuals to pay out-of-pocket, and considers them to be less important vaccines compared to the vaccines under the law. This led to low vaccination rates for these voluntary vaccines, and the incidence of the target diseases has remained high [8]. In contrast, the use of the HBV vaccine has been limited to children whose mothers are positive for the HBV surface antigen and individuals at high risk for HBV. Although the HBV carrier rate used to be high in Japan and the rate has decreased significantly due to selective immunization, we nevertheless hope to reduce the rate even further in order to reduce the drop out of selective immunization and to prevent

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Table 1
A comparison of vaccines under the immunization law and voluntary vaccines for children in Japan.

	Vaccines under the immunization law	Voluntary vaccines
Regulated by the immunization law	Yes	No
Vaccination fee	Almost free of charge (50% provided by the government 50% by the local sector)	Out-of-pocket expense
Compensation for adverse effects	By the immunization law	By the PMDA law
Vaccines	Diphtheria, pertussis, tetanus vaccine BCG Oral polio vaccine Measles rubella vaccine Japanese encephalitis vaccine Diphtheria tetanus vaccine	*Haemophilus influenzae* type b vaccine[a] 7 valent pneumococcal conjugate vaccine[a] Hepatitis B virus vaccine[b] Mumps vaccine Varicella zoster virus vaccine Human papillomavirus vaccine[a] Influenza vaccine Hepatitis A virus vaccine Rotavirus vaccine

PMDA: Pharmaceutical and medical devices agency.
[a] Supported by the temporary budget for fiscal years 2010–2011 and 2011–2012.
[b] Selective immunization is paid by the national health insurance system.

horizontal transmission through intra-familial, intra-institutional, or sexual routes. Japan is surrounded by countries with a high incidence of HBV infection [9]. In addition, genotype A, which tends to shift to chronic hepatitis, is the predominant genotype accounting for up to 60% of acute HBV infections in Japan [10]. Furthermore, it is estimated that one third of pediatric HBV carriers were transmitted the disease by non-vertical transmission [11]. These facts strongly emphasize the importance of universal HBV vaccination. The new vaccines introduced after 2008, including the Hib vaccine, HPV vaccine, PCV7, and rotavirus vaccine, have also been categorized as voluntary vaccines as of March, 2012. The fee for receiving these vaccines for parents has been high, and the economic burden associated with these VPD has been increasing.

3.2. Subcutaneous vs. intramuscular vaccination

Currently, subcutaneous vaccination is the standard method of vaccination in Japan. Intramuscular injection is limited to specific vaccines, including the HPV vaccine, the adjuvanted 2009 A/H1N1 vaccine, and the HBV vaccine for subjects older than 10 years of age. The reason why intramuscular injection has been restricted is that there was a report with an accumulation of approximately 3700 cases with contracture of the gluteal quadriceps muscle in the 1970s due to the frequent intramuscular injection of antibiotics or antipyretics for the treatment of common respiratory infections [12]. Although no case of muscle contracture has been reported due to the injection of vaccines, the Japanese Pediatric Society (JPS) made a statement that there is no safe place for intramuscular injection in children in 1972 to reduce unnecessary injections for common respiratory infections [12]. Since then, intramuscular injections to children have remarkably decreased, but the majority of vaccines have been administered subcutaneously.

However, intramuscular injection is known to be superior to subcutaneous injection [13]; it causes fewer local reactions such as pain, redness, and swelling, and results in equal or greater immunogenicity in children immunized with the diphtheria, tetanus-toxoid vaccine [14]. In infants that received a tetravalent combination vaccine (diphtheria, pertussis, Hib and IPV), intramuscular injection also showed fewer local reactions and equal immunogenicity compared to subcutaneous injection [15]. Because intramuscular injection is the standard method of vaccination for the majority of vaccines (except for some live vaccines) in other countries, and has benefits compared to subcutaneous injection, intramuscular injection should be reconsidered as a method of vaccination for Japanese children.

3.3. Anatomical site of vaccinations

The most common location used for the vaccination of children in Japan is the lateral side of the upper arms. The anterior frontal aspects of the thighs have not been used as the site of injection due to the fear of muscle contracture. When simultaneous vaccination is required to provide appropriate vaccines for children, especially in early infancy, Japanese physicians have started to have questions about where to inject multiple vaccines in the small area of the upper arms in infants. In addition, it has been reported that it is best to separate the injection sites by at least one inch if the same anatomical site is used for intramuscular injection [16], but there have been no such studies regarding subcutaneous injection at the same anatomical site. To provide a sufficient location for vaccination, the anterior frontal aspects of the thighs should be included as a location of vaccination for Japanese children.

3.4. Obstacles impacting simultaneous vaccination

Simultaneous vaccination is a common and safe practice used to vaccinate children [17], and it is known to be efficacious to provide vaccines in a timely manner in order to appropriately protect children from VPD, to save time for caregivers and medical care personnel, and also to decrease the medical costs [18]. However, this practice has not been well distributed and understood in Japan, because there had been no need to perform simultaneous vaccination due to the lack of necessary vaccines, especially during early infancy. Following the introduction of the Hib vaccine and PCV7, there has been a need for simultaneous vaccination. Currently, there are doubts about the safety and efficacy of simultaneous vaccination voiced by both medical professionals and civilians. In addition, there have been issues about where to inject multiple vaccines when using only the bilateral upper arms in young infants.

To reduce the number of shots for young infants and children, it is necessary to develop and introduce combination vaccines. To date, due to the limited use of combination vaccines in Japan, simultaneous vaccination is a necessary practice to protect children from VPD. This message was clearly noted by the JPS in 2011. However, there has been a gap between the statement and actual practice. As stated above, the usefulness of combinations vaccines has been confirmed by several studies to decrease the numbers of vaccinations and increase the vaccination rates [19–21]; however, there are currently only three combination vaccines available in Japan; the DTaP, DT, and MR vaccines produced by the domestic vaccine companies. Moreover, there is currently no combination vaccine containing components of HBV, Hib, or an inactivated polio

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vaccine (IPV). At this moment, the numbers of shots that should be completed with the current JPS recommended immunization program during early infancy is high. To widely distribute simultaneous vaccination to protect children from VPD beginning from early infancy in Japan, both medical professionals and civilians need to understand the importance and safety of simultaneous vaccination. Furthermore, the introduction of combination vaccines to reduce the number of shots and increase the vaccination rates is urgently needed.

3.5. Rules about the vaccination intervals

Under the immunization law, the intervals at which different inactivated vaccines and live vaccines are given are strictly set to be greater than six days and greater than 27 days, respectively. These numbers were established by the Immunization Law to ensure that the responsible vaccine could be identified if an adverse event occurred after vaccination. These intervals prevent the general public from getting their vaccinations in a timely manner, especially after receiving lives vaccines [Bacille de Calmette et Guérin (BCG) and oral polio vaccine (OPV)] during early infancy. In the United States, the intervals of different vaccines are only set when parenteral live vaccines are given (28 days) [22]; therefore, these rules should be reconsidered to increase the vaccination rates and increase the opportunities for the vaccination of Japanese children.

3.6. The lack of an effective national immunization technical advisory group (NITAG)

The NITAG is important because it makes decisions that determine the national policy of vaccination; however, such a group does not exist in the current Japanese system. There have been a few committees organized by separate departments of the Ministry of Labor, Welfare, and Health to discuss issues related to immunization; however, there was little discussion regarding the long-term vision of national vaccination strategies and such committees have not been held either regularly or continuously. Because current infectious disease epidemiology clearly indicates that several VPD are still endemic in Japan and affect Japanese children, it is necessary to consider developing a vaccine policy setting system in Japan [23].

4. Current issues

4.1. Refusal of the oral polio vaccine due to fear of vaccine-associated poliomyelitis paralysis (VAPP)

Although the development of IPV using Sabin-derived vaccine was initiated in the 1990s in Japan, there has been delay of in the process for its production and authorization, which has therefore led to the current problematic situation, namely that Japan is the only developed country still routinely using the OPV as of March, 2012. There have been many programs on television and articles in newspapers describing the fears of vaccine-associated poliomyelitis paralysis (VAPP), which estimated the incidence of VAPP as 1.4 cases per one million vaccinations in Japan for the last 15 years based on the number of cases that have been recognized and reported as VAPP, but have not been confirmed virologically [24]. This led to a decrease in the OPV vaccination. Although a combination vaccine including Sabin-derived, inactivated polio and DTaP is expected to be licensed in Japan by the end of fiscal year 2012 at the latest (March, 2013), and the Director of the Ministry of Health, Labor, and Welfare has been trying to facilitate the process, there are caregivers who have had their children receive an imported and unlicensed IPV in Japan by paying for the vaccine out-of-pocket, and more than 20,000 children have been vaccinated this way. If adverse effects occur due to the vaccine, the government cannot be held responsible; the patients will have to be compensated by the insurance system of the importing company, with strict limitations for compensation. Some parents have been waiting for the IPV + DTaP combination vaccine, and have not had their children receive the OPV, which leads to the risk of developing a wild polio infection if the disease moves into Japan. The JPS warned the public about this situation and that everyone should avoid an unvaccinated status, because there are still some outbreak cases of wild polio that have occurred in various countries, including cases in China in August 2011 [25]. This issue will continue until the new Sabin-derived IPV + DTaP vaccine is licensed.

4.2. Temporary withholding of the Hib and PCV7 vaccines after a report of seven fatalities

On March 8, 2011, the Hib vaccine and PCV7 were temporarily withheld due to a report of accumulation of seven fatalities that occurred one to seven days after simultaneous vaccinations with the Hib vaccine and/or PCV7 and/or the DTaP vaccine or BCG. Detailed case analyses demonstrated that there was no causative relationship between these deaths and the vaccines according to an expert committee organized by the Ministry of Health, Labor, and Welfare. Two of the cases had severe congenital heart diseases; three patients had risk factors for sudden infant death syndrome; and one case was reported to have a human metapneumovirus infection. The accumulated fatality rates (including adverse events) were 0.13 and 0.15 per one million vaccinations for Hib and PCV7 in Japan between 2005 and 2010, respectively, which have been reported to range between 0.04–1.0 and 0.1–0.6, respectively, in other countries. No specific lots were identified to be responsible for causing the events. Additionally, no deviation in the process of vaccine certification was found. Therefore, the Scientific Committee assembled by the government concluded that there was no relationship between the vaccines and the fatal events, and vaccinations with both vaccines were resumed on April 1, 2011, 22 days after the interruption. Although no causal relationship between the vaccines and fatalities were identified, the following sentences were added to the package inserts for Hib and PCV7; Physicians need to notify their patients or the patients' guardians that there is an option for single vaccination, and single vaccination should thus be considered, especially for children with underlying diseases. These specific notes in the package inserts, which were added without the authorization of vaccine specialists, led to physician confusion regarding whether simultaneous vaccination is safe for Japanese children.

5. New movements to improve the immunization system in Japan

Although these critical issues have been discussed for decades [26], some important movements to improve our current NIP are underway. First, both the general public and medical professionals have voiced a desire to change the NIP, and these voices have become stronger every year. Approximately 2.7 million signatures from civilians and medical professionals led by the Japanese Medical Association were collected and presented to the government asking them to improve the NIP [27]. Second, representatives from 13 medical professional societies gathered to ask the government to consider immunization policy as a "national policy" and seeking the establishment of a new NITAG system to provide expert opinions for the government [23]. Finally, along with these movements, the government launched the Vaccination Subcommittee of Health Sciences Council to discuss the reform of the current Immunization Law and NIP in Japan [28]. All of these new movements led to the

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government's decision to establish a new national program for the Hib vaccine, PCV7, and HPV vaccines funded by a temporary budget. The government is further considering continuing this budget and including these vaccines into the NIP, along with other important vaccines currently categorized as voluntary vaccines, such as the universal HBV vaccine, VZV vaccine, and mumps vaccine. Furthermore, the JPS launched a new immunization schedule which put the vaccines in an order of requirement and does not distinguish between the vaccines under the law and voluntary vaccines [29]. It is hoped that these new movements will reform the immunization law and improve the NIP in Japan, and that this will lead to the government providing necessary vaccines for all children without out-of-pocket expenses for the guardians in order to make sure that all children are protected from VPD.

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We dedicated this manuscript to Dr. Hitoshi Kamiya (1943–2011) who devoted his life to improving the immunization systems in Japan.

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RESEARCH PAPER

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Safety and persistence of immunological response 6 months after intramuscular vaccination with an AS03-adjuvanted H1N1 2009 influenza vaccine

An open-label, randomized trial in Japanese children aged 6 months to 17 years

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Key words: adjuvant, H1N1, influenza, japanese children, pandemic

Abbreviations: ATP, according-to-protocol; CBER, center for biologics evaluation & research; CHMP, committee for medicinal products for human use; CI, confidence interval; GMFR, geometric mean fold rise; GMT, geometric mean titre; HA, hemagglutinin; HI, hemagglutination inhibition; MAE, medically-attended event; pIMD, potential immune-mediated disease; SAE, serious adverse event; SCR, seroconversion rate; SPR, seroprotection rate; TVC, total vaccinated cohort; VRR, vaccine response rate; WHO, world health organization

This study evaluated the long-term persistence of immune response and safety of two doses of an A/California/7/2009 H1N1 pandemic influenza vaccine adjuvanted with AS03 (an α-tocopherol oil-in-water emulsion-based Adjuvant System) in Japanese children (NCT01001169). Sixty healthy subjects aged 6 mo–17 y were enrolled (1:1) into two study groups to receive 21 d apart, two doses of 1.9 μg haemagglutinin [HA] + $AS03_B$ (5.93 mg α-tocopherol) vaccine (6 mo–9 y) and 3.75 μg HA + $AS03_A$ (11.86 mg α-tocopherol) vaccine (10–17 y), respectively. Immunogenicity data (by haemagglutination inhibition [HI] and microneutralisation assays) to six months after the first vaccine dose are reported here. It was observed that following Dose 2, the HI immune response against the vaccine homologous strain induced by the two different dosages of the AS03-adjuvanted vaccine met and exceeded the US and European regulatory guidance criteria for pandemic influenza vaccines (seroprotection rate [SPR]/seroconversion rate [SCR]: 100%/100%; geometric mean fold rise GMFR: 146.8/57.1). Further, the immune response persisted for at least six months after the first vaccine dose wherein these regulatory criteria were still met (SPR: 100%/100%; SCR: 96.4%/89.7%; GMFR: 25.3/23.5). The neutralising antibody response was comparable to the HI immune response at Day 42 (vaccine response rate [VRR]: 100%/100%) and at Day 182 (VRR: 96.4%/82.8%). Overall, both vaccine dosages had a clinically acceptable safety profile. Thus, two doses of a 1.9 μg or 3.75 μg HA AS03-adjuvanted H1N1 2009 pandemic influenza vaccine in children aged 6 mo–17 y induced strong immune responses against the vaccine homologous strain that persisted for at least six months after the first vaccine dose.

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Introduction

The emergence of a novel, swine-origin influenza A virus (H1N1 2009) that caused the first influenza pandemic of the 21st century re-affirmed the unpredictability of influenza viruses.[1] The H1N1 2009 pandemic spread rapidly across the globe leading to over 18,449 deaths in more than 214 countries.[2] The highest attack and hospitalisation rates for the H1N1 2009 pandemic virus were reported in children aged <5 y, particularly those in the first year of life, presumably due to the degree of immunological naivety of this population toward this novel strain.[1,3-5]

The first case of H1N1 2009 pandemic influenza in Japan was confirmed on May 09, 2009 and by February 05, 2010, the cumulative number of confirmed H1N1 2009 cases was estimated to have reached 20 million.[6] Significantly, a small number of deaths due to the pandemic were reported (202 deaths as of August 10, 2010).[7] As observed in other regions,[8,9] in Japan most of the H1N1 2009 pandemic influenza infections and associated hospitalisations were reported in children and adolescents.[10]

Immunisation is considered to be the most efficient method of mitigating influenza pandemic related morbidity and mortality.[11,12] In this context, the immunological naivety/lack of priming of young children to the novel H1N1 2009 strain coupled with their role in indigenous transmission of the virus made them a priority group for pandemic influenza vaccination.[13]

Based on previous experience of developing a prepandemic dose-sparing H5N1 influenza vaccine (3.75 μg haemagglutinin [HA] with AS03 [an α-tocopherol oil-in-water emulsion-based Adjuvant System]),[14-17] an AS03-adjuvanted H1N1 2009 pandemic vaccine with 3.75 μg HA content was developed for the 2009 influenza pandemic. This H1N1 2009 vaccine has been proven to be highly immunogenic (fulfilling the US and European regulatory guidance criteria for pandemic influenza vaccines) with a clinically acceptable safety profile in different populations[18,19] including adults in Japan.[20]

In October 27, 2009, a phase II, open-label study (ClinicalTrials.gov Identifier: NCT01001169) in Japanese children was initiated at the National Center for Child Health and Development, Tokyo, Japan. Healthy children aged 6 mo to 17 y received two doses of either 1.9 μg HA with $AS03_B$ (6 mo–9 y) or 3.75 μg HA with $AS03_A$ (10–17 y) H1N1 2009 vaccine intramuscularly, 21 d apart. The co-primary objectives of this study were to assess whether vaccination with two doses of the AS03-adjuvanted 1.9 μg HA or 3.75 μg HA H1N1 2009 vaccines induced an immune response against the vaccine homologous strain 21 d after the second vaccine dose (Day 42) that met and exceeded the US and European regulatory guidance criteria for pandemic influenza vaccines. The preliminary immunogenicity and reactogenicity results following the first vaccine dose (Day 21) have been published earlier in reference 21. This manuscript presents the immunogenicity and safety results from the six month follow-up phase of this study. The objectives for the follow-up phase were as follows: (a) to assess whether two doses of the study vaccine induced persistence of immunological response at Day 182 that met the US and European regulatory guidance criteria for pandemic influenza vaccines, (b) to describe homologous HI and neutralising antibody response 21 d after the second vaccine dose and at Day 182 and (c) to evaluate the safety profile of the vaccine that was administered in this pediatric Japanese population through the intramuscular route.

Results

Study population. The six month follow-up phase of this study (through Day 182) was completed on May 17, 2010.

A total of 60 subjects were enrolled to be vaccinated (Group 1.9 μg HA: 30 subjects [6–35 mo: 10; 3–9 y: 20]; Group 3.75 μg HA: 30 subjects [10–17 y]), of which 57 subjects completed the study at Day 182. The ATP cohort for immunogenicity at Day 42 and Day 182 included 58 and 57 subjects, respectively (**Fig. 1**).

The mean age of subjects in the TVC at the time of the first vaccine dose was 4.1 y (range: 7 mo–104 mo) in Group 1.9 μg HA and 13.6 y (range: 10–17.9 y) in Group 3.75 μg HA. The overall male to female ratio was 43.3%:56.7% and all subjects were of Japanese heritage.

Immunogenicity. *HI immune response.* Prior to receiving vaccination, 17.2% of subjects aged 6 mo–9 y and 60% of subjects aged 10–17 y had detectable levels of HI antibodies against the H1N1 2009 strain. The second dose of the AS03-adjuvanted vaccine elicited a strong HI immune response in both age groups that met and exceeded the CHMP guidance criteria and more stringent CBER guidance criteria for pandemic influenza vaccines at Day 42. In the 6–35 mo age stratum the sample size was small (n = 10), as a consequence the lower limit of the 95% CI for SPR was not above 70%, despite a point estimate of 100% and a high GMT value (1279.9) (**Table 1**).

Six months after the first vaccine dose (Day 182), the HI immune response against the H1N1 2009 strain still met the CHMP and CBER criteria in subjects aged 6 mo–9 y and 10–17 y; similar to the Day 42 immune response in the 6–35 mo age stratum, the lower limit of the 95% CI for SPR at Day 182 was not above 70%, despite a point estimate of 100% (with a GMT value of 154) (**Table 1**).

It is to be noted that the HI assays for the sequential time points Day 0, Day 21 and Day 42 were tested together. The Day 182 samples were tested separately without an assessment of variability from earlier time points. Due to potential assay variability, a comparative interpretation of the HI response at Day 182 with earlier time points should be done with caution.

Microneutralisation assay. Prior to receiving vaccination, 10.3% of subjects aged 6 mo–9 y and 46.7% of subjects aged 10–17 y had detectable levels of neutralising antibodies against the A/Netherlands/602/2009 strain which is antigenically similar to A/California/7/2009 strain. Twenty one days after the second vaccine dose (Day 42), 100% of subjects in both age groups were seropositive for antibodies against the A/Netherlands/602/2009 strain; corresponding GMTs were 551.1 and 702.4, respectively. The VRR was 100% in both age groups (**Table 2**). Six months after the first vaccine dose, all subjects were still seropositive for antibodies against the A/Netherlands/602/2009 strain;

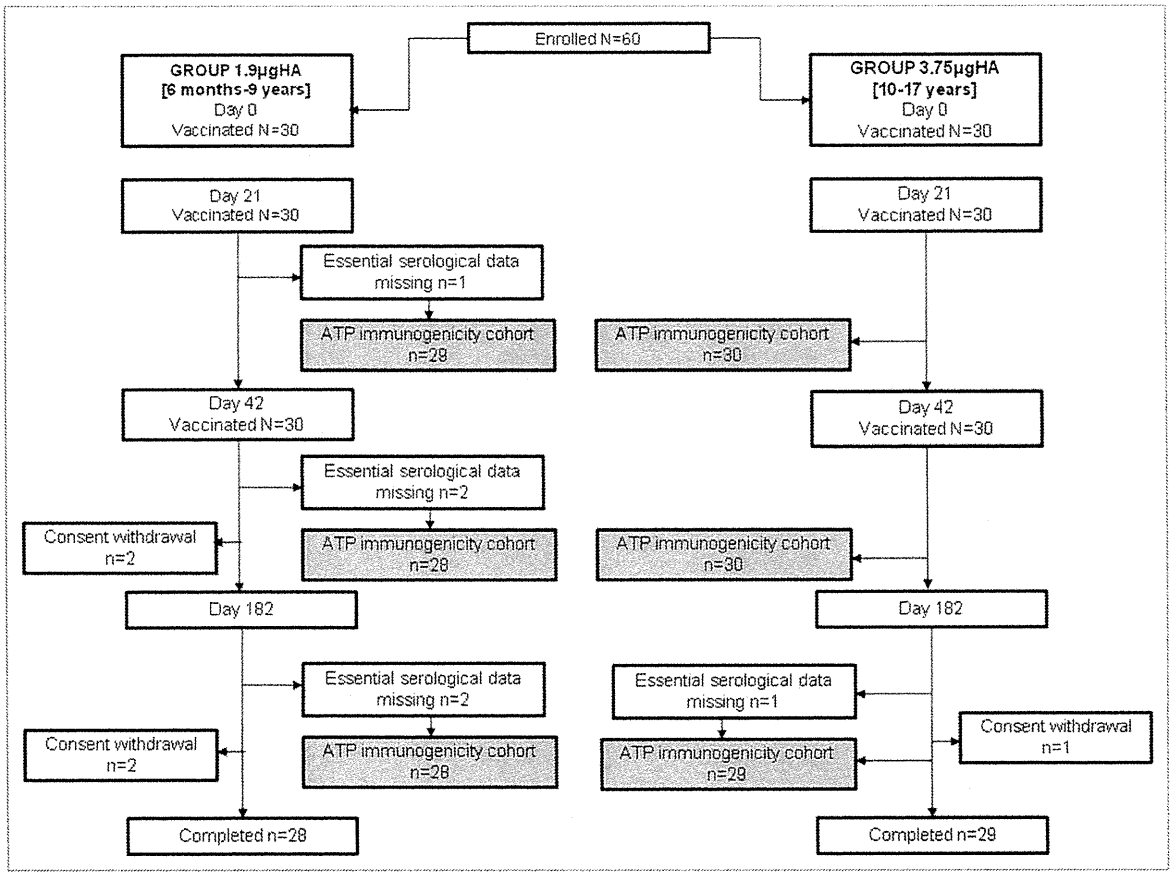

Figure 1. Study design diagram. Total vaccinated cohort (TVC): all subjects with at least one documented vaccine dose with available immunogenicity results. According-to-protocol (ATP) cohort for immunogenicity: all evaluable subjects (i.e., those meeting all eligibility criteria, with no elimination criteria during the relevant analysis interval), who received two vaccine doses and for whom assay results were available at Day 42 and Day 182. Group 1.9 µg HA: Subjects aged 6 mo-9 y received two doses of 1.9 µg HA + $AS03_B$ (5.93 mg α-tocopherol) vaccine, 21 d apart. Group 3.75 µg HA: Subjects aged 10–17 y received two doses of 3.75 µg HA + $AS03_A$ (11.86 mg α-tocopherol) vaccine, 21 d apart.

corresponding GMTs were 149.6 and 213.3. The VRR was 96.4% and 82.4% in the two age groups, respectively (**Table 2**).

Safety and reactogenicity. Overall, at least one solicited or unsolicited local symptom was reported for 70–100% of subjects in Group 1.9 µg HA (6–35 mo, 3–5 y, 6–9 y) and 100% of subjects in Group 3.75 µg HA (10–17 y); at least one solicited or unsolicited general symptom was reported for 42.9–83.3% of subjects in Group 1.9 µg HA (6–35 mo, 3–5 y, 6–9 y) and 80% of subjects in Group 3.75 µg HA (10–17 y). At least one MAE was reported in 58.3% of subjects aged 6 mo–5 y and 33.3% of subjects aged 6–9 y and 10–17 y.

Tables 3 and 4 present the percentage of subjects reporting solicited local and general symptoms overall and by age strata. Pain at injection site was the most frequently reported solicited local symptom across all age groups (overall 60%, 92.9%, 100% and 100% for subjects aged 6–35 mo, 3–5 y, 6–9 y and 10–17 y, respectively). The occurrence of pain was transient in most cases with the mean number of days being 1.8 d, 2.6 d, 2.7 d and 3.9 d for subjects aged 6–35 mo, 3–5 y, 6–9 y and 10–17 y, respectively. Overall, the occurrence of Grade 3 solicited local symptoms were infrequent; Grade 3 injection site pain was reported for two subjects aged 3–5 y and five subjects aged 10–17 y, and Grade 3 injection site swelling for two subjects aged 10–17 y. The reporting of solicited local symptoms after each of the two doses was comparable.

The most frequently reported solicited general symptoms varied across the different age groups; irritability (50% of subjects aged 6–35 mo), drowsiness (35.7% of subjects aged 3–5 y) and headache (50% of subjects aged 6–9 y and 66.7% of subjects aged 10–17 y). No fever was reported for subjects aged 6–35 mo following the first vaccine dose. However, following the second vaccine dose, four subjects (out of 10) developed fever, of which two cases of fever were of Grade 3 intensity (≥39°C). Overall, among subjects aged 6–35 mo, severe loss of appetite and irritability were reported for one subject each and Grade 3 fever for two subjects and among subjects aged 10–17 y, severe sweating was reported for one subject, Grade 3 fatigue and Grade 3 headache for two subjects each and Grade 3 fever for one subject, while no Grade 3 symptoms were reported among subjects aged 3–9 y.

Table 1. Immune response in terms of haemagglutination inhibition antibodies against vaccine homologous A/California/7/2009 strain [CBER[c]/CHMP[d] criteria] (ATP cohort for immunogenicity)

Measure	Time point	Group 1.9µg HA 6 mo-9 y						Group 3.75µg HA 10–17 y	
				Age sub-strata					
		N[a]	Overall	N	6–35 mo	N	3–9 y	N	Overall
				Value or Point estimate (95% CI[b])					
Seroprotection rates	PRE#,^	29	3.4% (0.1–17.8)	10	0.0% (0.0–30.8)	19	5.3% (0.1–26.0)	30	26.7% (12.3–45.9)
	Day 21^	29	100% (88.1–100)	10	100% (69.2–100)	19	100% (82.4–100)	30	96.7% (82.8–99.9)
	Day 42	28	100% (85.5–100)*	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (86.4–100)*
	Day 182	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	29	100% (88.1–100)
Seroconversion rates	Day 21^	29	100% (88.1–100)	10	100% (69.2–100)	19	100% (82.4–100)	30	90% (73.5–97.9)
	Day 42^	28	100% (85.5–100)*	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (86.4–100)*
	Day 182	28	96.4% (81.7–99.9)	9	100% (66.4–100)	19	94.7% (74.0–99.9)	29	89.7% (72.6–97.8)
Geometric Mean Fold Rise	Day 21^	29	27.1 (20.4–36.1)	10	23.5 (14.4–38.2)	19	29.3 (19.9–42.9)	30	22.1 (13.6–35.9)
	Day 42	28	146.8 (99.6–216.4)*	9	256.0 (161.4–406.1)	19	112.8 (74.6–170.5)	30	57.1 (33.5–97.3)*
	Day 182	28	25.3 (18.4–34.7)	9	30.8 (20.1–47.3)	19	23.1 (14.9–35.8)	29	23.5 (14.9–37.1)
Geometric Mean Titers	PRE#,^	29	6.3 (5.0–8.1)	10	5.0 (5.0–5.0)	19	7.2 (5.0–10.3)	30	15.3 (9.5–24.6)
	Day 21^	29	172 (130.1–227.6)	10	117.3 (72.2–190.8)	19	210.4 (150.4–294.5)	30	339 (238.8–481.2)
	Day 42	28	939.3 (722.9–1220.6)*	9	1279.9 (806.9–2030.4)	19	811.3 (628.4–1047.4)	30	874.3 (717.4–1065.4)*
	Day 182	28	161.9 (133.7–196.1)	9	154.0 (100.3–236.5)	19	165.8 (131.9–208.5)	29	347.9 (254.0–476.5)
Seropositivity rates	PRE#,^	29	17.2% (5.8–35.8)	10	0.0 (0.0–30.8)	19	26.3% (9.1–51.2)	30	60.0% (40.6–77.3)
	Day 21^	29	100% (88.1–100)	10	100% (69.2–100)	19	100% (82.4–100)	30	100% (88.4–100)
	Day 42	28	100% (85.5–100)*	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (86.4–100)*
	Day 182	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	29	100% (88.1–100)

Group 1.9µg HA: Subjects aged 6 mo-9 y received two doses of 1.9 µg HA+AS03$_B$ (5.93mg α-tocopherol) vaccine, 21 d apart. Group 3.75 µg HA: Subjects aged 10–17 y received two doses of 3.75 µg HA + AS03$_A$ (11.86mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence Interval. [c]CBER: Center for Biologics Evaluation and Research [Lower limit of 95% CI: SPR: ≥70%; SCR: ≥ 40%]. [d]CHMP: Committee for Medicinal Products for Human Use [Point estimate: SPR: >70%; SCR: >40%; GMFR: >2.5].#PRE: Pre-vaccination antibody titers for ATP cohort for immunogenicity at Day 21.* As per pre-defined primary co-objectives of this study, Day 42 immune response was calculated with 97.5% CI as per the CBER criteria for Group A (age 6 mo to 9 y), Group B (age 10–17 y), while at the other time points 95% CI was used. BOLD: Values of SPR and Geometric mean fold rise that did not meet the pre-specified criteria. ^PRE and Day 21 data have been presented previously in the primary manuscript *[ref. 21: Saitoh A,* et al. *J Japan Pediatr Soc 2011].*

Table 2. Immune response in terms of neutralising antibodies against the A/Netherlands/602/2009 strain (ATP immunogenicity cohort)

Measure	Time point	Group 1.9μg HA 6 mo–9 y						Group 3.75μg HA 10–17 y	
				Age sub-strata					
		N[a]	Overall	N	6–35 mo	N	3–9 y	N	Overall
				Value or Point estimate (95% CI[b])					
Seropositivity rates	PRE[#]	29	10.3% (2.2–27.4)	10	10.0% (0.3–44.5)	19	10.5% (1.3–33.1)	30	46.7% (28.3–65.7)
	Day 21	29	96.6% (82.2–99.9)	10	100% (69.2–100)	19	94.7% (74–99.9)	29	96.6 (82.2–99.9)
	Day 42	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (88.4–100)
	Day 182	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	29	100% (88.1–100)
Geometric Mean Titers	PRE[#]	29	4.9 (3.7–6.5)	10	4.7 (3.3–6.6)	19	5.0 (3.3–7.5)	30	11.3 (6.8–18.9)
	Day 21	29	53.8 (31.2–92.7)	10	42.5 (24.4–73.9)	19	60.8 (27–137.2)	29	121.2 (68.4–214.9)
	Day 42	28	551.1 (417–728.5)	9	623.4 (369.4–1052.1)	19	519.9 (362.3–745.5)	30	702.4 (433.5–1138)
	Day 182	28	149.6 (104.7–213.6)	9	161 (85.5–303.2)	19	144.4 (90–231.7)	29	213.3 (141.7–321)
Vaccine Response Rate	Day 21	29	51.7% (32.5–70.6)	10	60% (26.2–87.8)	19	47.4% (24.4–71.1)	29	58.6% (38.9–76.5)
	Day 42	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (88.4–100)
	Day 182	28	96.4% (81.7–99.9)	9	100% (66.4–100)	19	94.7% (74–99.9)	29	82.8% (64.2–94.2)

Group 1.9 μg HA: Subjects aged 6 mo-9 y received two doses of 1.9 μg HA + $AS03_B$ (5.93mg α-tocopherol) vaccine, 21 d apart. Group 3.75 μg HA: Subjects aged 10–17 y received two doses of 3.75 μg HA + $AS03_A$ (11.86 mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence interval. [#]PRE: Pre-vaccination antibody titers for ATP cohort for immunogenicity at Day 21. Vaccine response rate defined as percentage of subjects with either a pre-vaccination titer <1:8 and a post-vaccination titer ≥1:32, or a pre-vaccination titer ≥1:8 and at least a 4-fold increase in post-vaccination titer.

Overall, 40 subjects reported at least one unsolicited adverse event upto Day 84: 17 (70.8%) in subjects aged 6 mo–5 y, 2 (33.3%) in subjects aged 6–9 y, and 21 (70%) in subjects aged 10–17 y. For subjects aged 6 mo–5 y, rhinorrhoea (7 subjects), upper respiratory tract infection and cough (6 subjects each) were the most commonly reported symptoms, while for subjects aged 6–9 y, pyrexia (2 subjects) was most frequently reported. Among subjects aged 10–17 y, there were no clear predominance of any unsolicited symptoms; axillary pain, pharyngitis, headache, cough, influenza, rhinitic allergy and acne were reported for two subjects each.

Two SAEs were reported during the entire study period. One subject in the 6–35 mo age strata presented severe febrile convulsion approximately five months after receiving the second vaccine dose which resolved in one day; the other subject in the 10–17 y age strata had a fracture in the foot approximately three months after the second vaccine dose which resolved in 72 d; none of the two SAEs were considered by the investigators to be vaccination-related. No pIMDs or fatalities were reported during the study period.

Discussion

This is the first study to report the persistence of the immunological response against the A/California/07/2009 strain in children, six months after vaccination with an AS03-adjuvanted H1N1 2009 pandemic influenza vaccine.

In this study, the 1.9 μg HA $AS03_B$-adjuvanted H1N1 2009 pandemic influenza vaccine induced a strong HI immune response in subjects aged 6 mo–9 y as evident from the high SPR/SCR (100%) following the second vaccine dose. In previous studies, similar formulations of the AS03-adjuvanted H1N1 2009 vaccine have been shown to be optimally immunogenic in subjects aged 6–35 mo and 6 mo–12 y.[19,22] Thus, the data in Japanese children conforms to the strong immunogenicity profile of the vaccine observed in other pediatric populations.

Table 3. Solicited local symptoms reported during the 7 d post-vaccination follow-up period after each vaccine dose (Total vaccinated cohort)

		Group 1.9 μg HA						Group 3.75 μg HA	
		6–35 mo		3–5 y		6–9 y		10–17 y	
		Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
		N[a] = 10	n = 10	n = 14	n = 13	n = 6	n = 6	n = 30	n = 30
		Point estimate (95% CI[b])							
Pain	Any	60.0 (26.2–87.8)	50.0 (18.7–81.3)	92.9 (66.1–99.8)	84.6 (54.6–98.1)	83.3 (35.9–99.6)	83.3 (35.9–99.6)	100 (88.4–100)	100 (88.4–100)
	Grade 3	0 (0–30.8)	0 (0–30.8)	7.1 (0.2–33.9)	7.7 (0.2–36.0)	0 (0–45.9)	0 (0–45.9)	10.0 (2.1–26.5)	6.7 (0.8–22.1)
Redness	Any	0 (0–30.8)	10.0 (0.3–44.5)	0 (0–23.2)	7.7 (0.2–36.0)	16.7 (0.4–64.1)	0 (0–45.9)	23.3 (9.9–42.3)	16.7 (5.6–34.7)
	Grade 3	0 (0–30.8)	0 (0–30.8)	0 (0–23.2)	0 (0–24.7)	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)
Swelling	Any	30.0 (6.7–65.2)	10.0 (0.3–44.5)	14.3 (1.8–42.8)	23.1 (5.0–53.8)	33.3 (4.3–77.7)	33.3 (4.3–77.7)	46.7 (28.3–65.7)	50.0 (31.3–68.7)
	Grade 3	0 (0–30.8)	0 (0–30.8)	0 (0–23.2)	0 (0–24.7)	0 (0–45.9)	0 (0–45.9)	3.3 (0.1–17.2)	3.3 (0.1–17.2)

Group 1.9 μg HA: Subjects aged 6 mo-9 y received two doses of 1.9 μg HA + $AS03_B$ (5.93mg α-tocopherol) vaccine, 21 d apart. Group 3.75 μg HA: Subjects aged 10–17 y received two doses of 3.75 μg HA + $AS03_A$ (11.86mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence interval.

A single dose of AS03-adjuvanted 1.9 μg HA H1N1 2009 vaccine in Canadian children aged 36 mo to 9 y has been found to have a protective effectiveness of 100%, 14 d following a single vaccine dose (statistically significant difference with the control group).[23] Although this value was 96% when effectiveness was assessed 10 d after vaccination, it remained at 100% in subjects aged <36 mo.[23] These observations are in agreement with the preliminary results obtained from this study in Japanese children, which reported a strong HI antibody immune response against the vaccine homologous strain (SPR and SCR 100%) after just one dose of the AS03-adjuvanted H1N1 2009 vaccine.[21]

The 3.75 μg HA AS03-adjuvanted H1N1 2009 vaccine also induced a strong HI immune response—SPR/SCR of 96.7%/90% following the first dose and 100%, following the second vaccine dose in subjects aged 10–17 y. Considering the above mentioned protective effectiveness reported in younger Canadian children (36 mo to 9 y old),[23] these immunogenicity results obtained in subjects aged 10–17 y in this study suggested that a single dose of 3.75 μg HA of AS03-adjuvanted H1N1 2009 vaccine induced a substantial protection against H1N1 2009 pandemic influenza virus.

Six months after the first vaccine dose, the HI immune response against the vaccine homologous strain were well maintained (high SPRs of 100% and SCRs of 96.4% and 89.7%, respectively), in subjects aged 6 mo–9 y and 10–17 y. Data on the long-term persistence of the immune response following pandemic influenza vaccination in children is limited. However, the observations from this study is in agreement with available data from studies in adults that the immune response induced by two doses of the 3.75 μg HA AS03-adjuvanted H1N1 2009 vaccine persists for at least six months after vaccination.[24,25]

The HI immune response against the A/California/7/2009 strain induced by the 1.9 μg and 3.75 μg HA dosages of the AS03-adjuvanted study vaccine was further corroborated when the CHMP guidance criteria[26] and the more stringent CBER guidance criteria[27] for pandemic influenza vaccines were met and exceeded, following the second vaccine dose and also six months after the first vaccine dose. The neutralising antibody titers parallel the HI immune response following each of the two vaccine doses and six months after the first vaccine dose.

Overall, the two vaccine dosages had clinically acceptable safety profiles in the respective study groups. Most solicited local and general symptoms were transient, and mild or moderate in intensity. Four cases of fever of which two were of grade 3 intensity were reported in subjects aged 6–35 mo following the second vaccine dose. Post-hoc assessments indicated that these cases may be associated with the strong increase of the humoral immune response (data not shown). Three out of these four subjects had the highest HI antibody titers (1,810 and 2,560) among children aged 6–35 mo; the remaining subject did not return for visit at Day 42. A similar observation was also made in a previous study and a possible association with increase in humoral immune response was made.[19] However, considering that the number of subjects in both studies is limited, further evaluation on a larger number of subjects would be required to ascertain the plausible reason for this observation.

When compared with the safety profile of a non-adjuvanted, trivalent seasonal influenza vaccine in a pediatric population aged between 6 mo and <18 y, the AS03-adjuvanted vaccine similar to the one used in the present study demonstrated increased frequency of solicited local symptoms, though more similar for general symptoms as well as MAEs and SAEs. However, the trend of

Table 4. Solicited general symptoms reported during the 7-d post-vaccination follow-up period after each vaccine dose (total vaccinated cohort)

		Group 1.9 µg HA						Group 3.75 µg HA	
		6–35 mo		3–5 y		6–9 y		10–17 y	
		Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
		N[a] = 10	n = 10	n = 14	n = 13	n = 6	n = 6	n = 30	n = 30
		Point estimate (95% CI[b])							
Drowsiness	Any	10.0 (0.3–44.5)	30.0 (6.7–65.2)	28.6 (8.4–58.1)	30.8 (9.1–61.4)	–	–	–	–
	Grade 3	0 (0–30.8)	0 (0–30.8)	0 (0–23.2)	0 (0–24.7)	–	–	–	–
Irritability	Any	30.0 (6.7–65.2)	40.0 (12.2–73.8)	21.4 (4.7–50.8)	15.4 (1.9–45.4)	–	–	–	–
	Grade 3	10.0 (0.3–44.5)	0 (0–30.8)	0 (0–23.2)	0 (0–24.7)	–	–	–	–
Loss of appetite	Any	10.0 (0.3–44.5)	30.0 (6.7–65.2)	28.6 (8.4–58.1)	15.4 (1.9–45.4)	–	–	–	–
	Grade 3	0 (0–30.8)	10.0 (0.3–44.5)	0 (0–23.2)	0 (0–24.7)	–	–	–	–
Fever	Any	0 (0–30.8)	40.0 (12.2–73.8)	21.4 (4.7–50.8)	15.4 (1.9–45.4)	33.3 (4.3–77.7)	0 (0–45.9)	13.3 (3.8–30.7)	23.3 (9.9–42.3)
	Grade 3	0 (0–30.8)	20.0 (2.5–55.6)	0 (0–23.2)	0 (0–24.7)	0 (0–45.9)	0 (0–45.9)	6.7 (0.8–22.1)	0 (0–11.6)
Fatigue	Any	–	–	–	–	16.7 (0.4–64.1)	16.7 (0.4–64.1)	36.7 (19.9–56.1)	36.7 (19.9–56.1)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	3.3 (0.1–17.2)	3.3 (0.1–17.2)
Gastrointestinal	Any	–	–	–	–	16.7 (0.4–64.1)	16.7 (0.4–64.1)	10.0 (2.1–26.5)	10.0 (2.1–26.5)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)
Headache	Any	–	–	–	–	33.3 (4.3–77.7)	16.7 (0.4–64.1)	40.0 (22.7–59.4)	33.3 (17.3–52.8)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	6.7 (0.8–22.1)
Joint pain at other location	Any	–	–	–	–	0 (0–45.9)	0 (0–45.9)	16.7 (5.6–34.7)	23.3 (9.9–42.3)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)
Muscle aches	Any	–	–	–	–	0 (0–45.9)	0 (0–45.9)	23.3 (9.9–42.3)	30.0 (14.7–49.4)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)
Shivering	Any	–	–	–	–	16.7 (0.4–64.1)	0 (0–45.9)	23.3 (9.9–42.3)	26.7 (12.3–45.9)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)

Group 1.9 µg HA: Subjects aged 6 mo–9 y received two doses of 1.9 µg HA + $AS03_B$ (5.93 mg α-tocopherol) vaccine, 21 d apart. Group 3.75 µg HA: Subjects aged 10–17 y received two doses of 3.75 µg HA + $AS03_A$ (11.86mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence interval.

Table 4. Solicited general symptoms reported during the 7-d post-vaccination follow-up period after each vaccine dose (total vaccinated cohort)

Sweating	Any	–	–	–	–	33.3 (4.3–77.7)	0 (0–45.9)	6.7 (0.8–22.1)	10.0 (2.1–26.5)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	3.3 (0.1–17.2)	0 (0–11.6)

Group 1.9 μg HA: Subjects aged 6 mo–9 y received two doses of 1.9 μg HA + $AS03_B$ (5.93 mg α-tocopherol) vaccine, 21 d apart. Group 3.75 μg HA: Subjects aged 10–17 y received two doses of 3.75 μg HA + $AS03_A$ (11.86mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence interval.

slightly lower frequencies of solicited symptoms reported following the second dose as compared that after the first dose in the referenced study was reversed in the present study.[28] In another pediatric study with a non-adjuvanted, trivalent seasonal influenza vaccine in subjects aged 6–9 y and 10–13 y, a similar trend of comparatively higher reactogenicity after the second vaccine dose was observed.[29] A recent report from six studies in children showed that the frequency of solicited local and general symptoms following vaccination with a MF59-adjuvanted seasonal influenza vaccine was comparatively (although not significantly) higher than that following vaccination with a non-adjuvanted seasonal influenza vaccine.[30]

In Japan, the AS03-adjuvanted H1N1 2009 pandemic influena vaccine was approved as a 1.9 μg HA dose in children aged 6 mo–9 y and as a 3.75 μg HA dose in children aged 10 y and older, making the age-specific immunogenicity data obtained from this study particularly relevant. Also, the fact that a microneutralisation assay was used in parallel with the conventional HI assay for immunological assessments makes the findings pertinent as while HI assays are largely restricted to measuring the receptor-binding blocking activity of antibodies, theoretically, neutralisation assays can capture a broad range of anti-influenza antibody activities able to interrupt several steps of the infectious life cycle of the virus.[31,32] Further, the AS03-adjuvanted H1N1 2009 vaccines used in this study allowed dose-sparing, a property that could be beneficial in meeting the requirement for a large number of vaccine doses at the time of an influenza pandemic.

This study was restricted in drawing comparative conclusions on the persistence of the immune response following vaccination with other adjuvanted or non-adjuvanted H1N1 2009 vaccines, as it is difficult to reliably compare HI results across studies.

In conclusion, the data from this study conducted with an AS03-adjuvanted H1N1 2009 pandemic influenza vaccine establishes that, following two doses of a 1.9 μg or 3.75 μg HA in children aged 6 mo–17 y, the immune response against the vaccine homologous A/California/7/2009 strain persists for at least six months after the first vaccine dose and the US and European guidance criteria for pandemic influenza vaccines were still met. The safety data from this study added to the existing repertoire of safety data in published literature on the safety of this H1N1 2009 vaccine. In addition, it may contribute to a better understanding of the safety of intramuscular vaccination in Japan. Intramuscular injection has not been allowed in Japan since 1970s after more than three thousands cases of muscular contracture being reported after intramuscular injection of antibiotics and antipyretics, but not vaccines.[33] This issue needs to be clarified urgently given that new combination vaccines and adjuvanted vaccines are expected to be introduced in Japan in the near future.

Materials and Methods

Study design and subjects. The primary phase of the study in Japan (NCT01001169) enrolled healthy children aged between 6 mo and 17 y before study start, without history of clinically-confirmed influenza infection or previous vaccination with a novel H1N1 vaccine or with any seasonal influenza vaccine within two weeks before study start. The subjects aged 6 mo to 9 y were further stratified by age (stratification ratio: 1:2) into 6–35 mo and 3–9 y age strata by the study personnel using GlaxoSmithKline (GSK) Biologicals' internet-based central randomization system (SBIR). Subjects aged 6 mo–9 y received 21 d apart, two 0.25 ml doses of the 1.9 μg $HA/AS03_B$ vaccine (Group 1.9 μg HA) and subjects aged 10–17 y received two 0.5 ml doses of the 3.75 μg $HA/AS03_A$ vaccine (Group 3.75 μg HA). All subjects received the first vaccine dose between Oct 27, 2009 and Nov 06, 2009, and the subjects aged 6 mo–9 y received the second vaccine dose by Nov 30, 2009. The treatment and vial lists were generated at GSK Biologicals using SAS® (Cary, NC USA) to assign treatments to subjects.

Written informed consent was obtained from the parents/guardians of all subjects prior to conducting any study-related procedures. Wherever deemed necessary, informed assent was collected from the subjects. The study was conducted in accordance with the Good Clinical Practice guidelines, the Declaration of Helsinki and local regulations. All study-related documents were approved by an Institutional Review Board.

Study vaccine. The study vaccine was developed and manufactured by GSK Biologicals. The H1N1 2009 pandemic influenza vaccine was a monovalent, inactivated, split-virion antigen with an oil-in-water emulsion-based Adjuvant System AS03 (*Arepanrix*™, a trademark of GlaxoSmithKline group of companies, Belgium). The H1N1 viral seed for the vaccine was prepared from the reassortant virus NYMC X-179A (New York Medical College, New York) generated from the A/California/07/2009 strain, as recommended by the World Health Organization (WHO).[34]

The AS03-adjuvanted H1N1 2009 pandemic influenza vaccine was prepared prior to administration by mixing the antigen suspension and adjuvant emulsion (1:1), both of which were available in separate multi-dose vials. Group 1.9 μg HA received $AS03_B$—an Adjuvant System containing 5.93 mg α-tocopherol

with 1.9 μg HA (0.25 ml injection dose) and Group 3.75 μg HA received $AS03_A$—an Adjuvant System containing 11.86 mg α-tocopherol with 3.75 μg of HA (0.5 ml injection dose).[35]

The first dose of the study vaccine was intramuscularly administered on Day 0 either into the anterolateral region of the thigh in children aged <12 mo or into the deltoid of the non-dominant arm in subjects aged 12 mo or more. On Day 21, the second dose of study vaccine was administered on the opposite side.

Immunogenicity assessments. Serum samples were collected before vaccination (Day 0), 21 d after each of the two vaccine doses (Day 21 and Day 42) and six months after the first vaccine dose (Day 182).

Haemagglutination inhibition (HI) assay [cut-off: ≥1:10] using chicken erythrocytes as previously described in reference 36, was performed at GSK Biologicals' central laboratory. The samples from Day 0, Day 21 and Day 42 were tested at the same time point, while the Day 182 samples were tested at a later time point.

The viral microneutralisation assay was performed at Viroclinics Biosciences BV. The sera were used after heat treatment at 56°C for 30 min. Each serum was tested in triplicate. The assay used a constant amount of A/Netherlands/602/2009 pandemic H1N1 Influenza virus (a A/California/07/2009-like virus) mixed with serial 2-fold dilutions of serum samples. The mixture of virus and antiserum was added to Madin-Darby Canine Kidney (MDCK) cell cultures and incubated for one hour at 33–35°C. Then virus-antibody mixture was removed from the wells, cells were fed with fresh culture medium and further incubated for 6 d at 33–35°C. After the incubation period, virus replication was visualized by haemagglutination of red blood cells. The 50% neutralisation titer of a serum was calculated by the method of Reed and Muench.[37] The cut-off value of the assay was 1:8.

The assessment of the immune response was based on the seroconversion rate (SCR: percentage of subjects with pre-vaccination titer <1:10 and post-vaccination titer ≥1:40, or pre-vaccination titer >1:10 and at least 4-fold increase in post-vaccination titer), seroprotection rate (SPR: percentage of subjects with a post-vaccination titer ≥1:40) and geometric mean fold rise (GMFR: post-vaccination fold increase in geometric mean titers [GMTs]) in terms of HI antibodies against the vaccine homologous strain and on the the Vaccine Response Rates (VRRs: percentage of subjects with either a pre-vaccination titer <1:8 and a post-vaccination titer ≥1:32, or a pre-vaccination titer ≥1:8 and at least a 4-fold increase in post-vaccination titer) in terms of neutralising antibodies against a strain antigenically similar to the vaccine strain.

The outcome measures of the immune response included evaluation based on the immunogenicity criteria for pandemic influenza vaccines in adults as required by the Committee for Medicinal Products for Human Use (CHMP; point estimates for HI antibody SCR: >40%, SPR: >70% and GMFR: >2.5) and Center for Biologics Evaluation and Research (CBER; lower bound of 95% confidence interval [CI] for HI antibody for SCR: ≥40% and SPR: ≥70%).[26,27] In consideration of multiplicity of statistical analysis caused by co-primary endpoints of the study, 97.5% confidence intervals (CIs) were applied instead of 95% CIs (requirement of CBER guidance) for evaluation of the primary endpoints at Day 42.

Safety and reactogenicity assessments. Diary cards were used by parents/guardians to record solicited local and general adverse events up to seven days following each vaccine dose; unsolicited adverse events were recorded up to 84 d following the first vaccine dose; medically-attended events (MAEs), potential immune-mediated diseases (pIMD) and serious adverse events (SAEs) occurring during the entire study period were recorded.

Intensity of solicited symptoms was graded on a standard scale of (0–3), where Grade 1 symptoms were defined as those that were noticeable but did not interfere with normal activities and Grade 3 symptoms were defined as those that prevented normal activities (Grade 3 redness and swelling: diameter >100 mm; Grade 3 fever: temperature ≥39°C [≥102.2°F]). SAEs and pIMDs (subset of adverse events that include both autoimmune diseases and other inflammatory and/or neurologic disorders which may or may not have an autoimmune etiology) occurring throughout the study period were also recorded. Clinical laboratory parameters were assessed at all seven visits up to Day 182.

Statistical analyses. The sample size was calculated based on the co-primary objectives of the study using the results from the most recent studies with the H1N1 2009 vaccine as a reference. A population of 60 subjects (30 subjects in each study group) accounting for ≤10% dropout was estimated to provide a power of 84.9% to achieve the co-primary objectives, assuming log (standard deviation) for GMT to be 0.6.

The analyses of immunogenicity were performed on the According-To-Protocol (ATP) cohort that included subjects who received both vaccine doses as per protocol, complied with all protocol-defined procedures and for whom the assay results were available at the given time points (at Day 42 and Day 182). Seropositivity was defined as antibody titers greater than or equal to the cut-off value of each assay. For the purpose of GMT calculations, antibody titers below the cut-off value of each assay were substituted by half of the cut-off value.

The analyses of safety were performed on the Total Vaccinated Cohort (TVC) which included all subjects who received at least one documented vaccine dose.

Disclosure of Potential Conflicts of Interest

Dr. A. Nagai was the principal investigator, Dr. A. Saitoh and Dr. T. Kato contributed as a supervisor in this study funded by GlaxoSmithKline. All participating institutions received compensation for study involvement. Drs. K. Tenjinbaru, D. Vaughn, F. Roman and P. Li are employees of GlaxoSmithKline Biologicals. D. Vaughn and F. Roman report ownership of stock options.

Financial Disclosure

GlaxoSmithKline Biologicals was the funding source and was involved in all stages of the study conduct and analysis (ClinicalTrials.gov Identifier: NCT01001169). GlaxoSmithKline Biologicals also took in charge all costs associated with the development and the publishing of the present manuscript. All authors

had full access to the data and the corresponding author had final responsibility to submit for publication.

Trademark Statement

Arepanrix is a trade mark of the GlaxoSmithKline group of companies, Belgium.

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All authors participated in the implementation of the study including substantial contributions to conception and design, the gathering of the data, or analysis and interpretation of the data. All authors were involved in the drafting of the article or revising it critically for important intellectual content, and final approval of the manuscript.

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