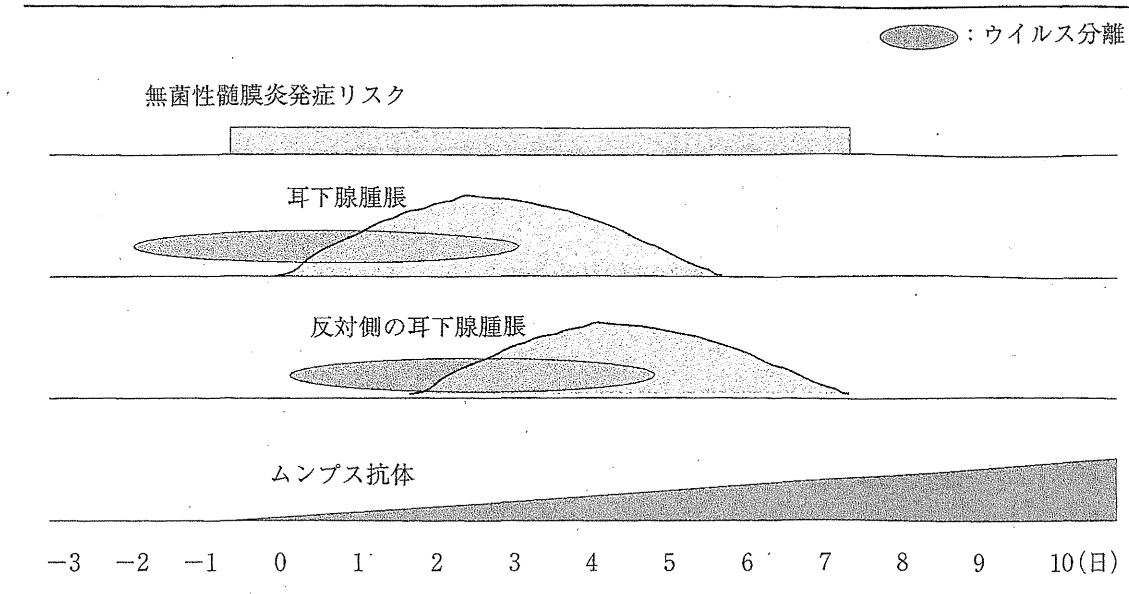

図1　ムンプスの臨床像と抗体価の上昇
発症前から唾液にムンプスウイルスを排泄している．反対側の耳下腺腫脹が7−10日遅れたときも唾液からムンプスウイルスは分離される．

から血流に入り，ウイルス血症により全身の親和性臓器に運ばれ，そこで増殖して症状や副反応が出現する．

ムンプス髄膜炎は，耳下腺腫脹前，耳下腺腫脹時，耳下腺腫脹後のいずれの時期でも発症する(**図1**)．ムンプス流行時，耳下腺腫脹を伴わずにムンプスウイルスによる髄膜炎を時に認めることがある．ムンプス発症者の50％に髄液細胞数増多を認めるが，髄膜炎を発症するのは3−10％である．年齢が高くなるほど髄膜炎の合併頻度が増加する[2]．

ムンプスの予後が悪い合併症として脳炎と難聴がある．脳炎の頻度は0.02−0.3％である．我が国の難聴の頻度は400−1,000人に1人である[10,11]．年長児では難聴出現時にめまいを合併する．ムンプス急性期に難聴に気づいた場合回復する例もあるが，回復期に難聴に気づいたときは，回復は困難である．蝸牛へのムンプスウイルス感染が関与している．

思春期以降の男子では睾丸炎を25％に発症する[5]．両側の睾丸炎は10％である．睾丸炎を発症するとその後の精子数は減少するが，不妊になるのは極めてまれである．ムンプス流行時には耳下腺腫脹を伴わずに睾丸炎を発症することがある．思春期以降の女性では乳腺炎を15−30％，卵巣炎を5％に発症する．いずれも予後の良い合併症である．妊婦がムンプスに罹患すると，第1三半期では27％が自然流産するが，第2三半期以降では特別な異常は認められない．ムンプスウイルスが関与する胎児奇形も報告されていない．その他のムンプスウイルスの合併症として，膵炎，甲状腺炎，腎腫大，心筋炎などがある．

唾液腺腫脹は唾液腺でのウイルス増殖と，リンパ球の唾液腺への浸潤により発症する．ムンプス不顕性感染者でも唾液からムンプスウイルスは分離される．ムンプスウイルスは，耳下腺腫脹が始まる3日前から耳下腺腫脹が退縮し始める腫脹5日後頃まで唾液から排泄される(**図1**)[12]．多くのムンプス例では，片側の耳下腺腫脹開始後24−48時間以内に反対側の耳下腺が腫脹するが，時に片側の耳下腺腫脹の消退後(片側の耳下腺腫脹7−10日後)に反対側の耳下腺が腫脹することがある．反対側の耳下腺腫脹時にも唾液からウイルスは分離される[12]．遅れて反対側の耳下腺が腫脹した場合は，反対側の腫脹が退縮し始めるまで登園登校の停止が必要である．なお，唾液腺は血中IgG抗体が作用し

表1 急性耳下腺腫脹例におけるムンプス抗体の特徴と診断(文献[1]より改変)

既往歴	ワクチン歴	IgM抗体	IgG抗体	診　断
なし	なし	+〜++	+	ムンプス初感染*
なし	なし	−〜+	+++†	ムンプス再感染‡
なし	なし	−	−〜+†	ムンプス以外が原因
なし	あり	+〜++	+	PVF*
なし	あり	−〜+	+++	SVF‡
なし	あり	−	+	ムンプス以外が原因
あり	なし	−	+	ムンプス以外が原因
あり	なし	−〜+	+++	ムンプス再感染‡

PVF: 一次性ワクチン不全(primary vaccine failure), SVF: 二次性ワクチン不全(secondary vaccine failure).
*急性期IgM抗体≧1.2抗体指数, 急性期IgG抗体<20.0 EIA価.
†ムンプスの顕性感染率は70%, 不顕性感染例がある.
‡急性期IgM抗体<1.2抗体指数, 急性期IgG抗体≧20.0 EIA価.

にくい臓器であり, 血中IgG抗体が存在していても唾液腺でのムンプスウイルス増殖は抑制されない. 唾液中のIgA抗体が唾液腺のムンプスウイルス増殖抑制に関与している.

5. 診断と鑑別診断

急性耳下腺腫脹をきたす疾患として, ムンプス以外にも, 化膿性耳下腺炎, 反復性耳下腺炎, 唾石などがある. 臨床診断の基準は, ムンプス流行時の48時間以上持続する急性耳下腺腫脹である. ムンプスをウイルス学的に確定診断する方法として, ①唾液からのウイルス分離, ②唾液からのウイルス遺伝子の検出, ③IgM抗体の検出, ④血清抗体の有意上昇, がある[1]. ムンプスIgM抗体および血清抗体は, 酵素免疫法(enzyme immunoassay: EIA)で測定する. 方法①および②はウイルス感染症診断の王道であるが, 保険適用されていないため臨床現場では血清抗体検査が用いられている. ワクチン後のムンプス罹患例でも唾液からウイルスは分離され, ウイルス遺伝子が検出される.

ムンプス既往歴, ムンプスワクチン歴による血清抗体のパターンと診断を表1に示した[1]. ムンプス既往者の再感染は時に経験する. 二次性ワクチン不全(secondary vaccine failure: SVF)やムンプス再感染の場合は, 早期からIgG抗体の二次免疫応答が始まっているので, IgM抗体検出の有無にかかわらずIgG抗体が20 EIA価以上の高値を示している. SVF例やムンプス再感染例では抗体価の有意上昇(EIA-IgG抗体では2倍以上)を認めないことがある. なお, ワクチン歴がないムンプス発症者の発症日のIgM抗体陽性率は70%程度であり, 臨床経過からムンプスが疑われる場合は, 第2病日以降に再検査する必要がある[13].

ムンプス髄膜炎や脳炎の診断は, 髄液からのウイルス分離またはウイルス遺伝子の検出である. 髄膜炎発症早期ほどウイルス分離率は高率であり, 髄液細胞数が増加するとウイルスは分離されにくくなる[2].

ムンプスに対する免疫を確認するためには, 感度が高いEIA法で測定する. 成人の抗体陽性率(≧4.0 EIA価)は85-90%である. ムンプスの発症予防抗体価は未確定であるが, 我が国ではEIA-IgG抗体価が4 EIA価未満のときワクチン接種を勧めている.

6. 治　療

ムンプス, ムンプス髄膜炎, ムンプス難聴, 睾丸炎に対しては対症的に治療する.

7. 予　防

ムンプス予防にはムンプスワクチンを接種する. ムンプスワクチン後の耳下腺腫脹出現頻度は1歳児が一番低く, 年齢が高くなるにつれて増加する[2]. ワクチンを安全に接種するために

は1歳での接種が勧められる.

多くの先進国ではムンプスウイルスを含むワクチンの2回接種が行われている. ムンプスワクチン初回接種の時期は1歳であり, 2回目の接種時期は, ドイツでは初回接種4-12カ月後, 米国では4-6歳である. 日本小児科学会は1歳と小学校就学1年前の2回の接種を推奨している.

ムンプスワクチンの副反応として無菌性髄膜炎がある. Jeryl Lynn株の合併率は1/1,000,000と極めて低率である. 一方, 我が国のワクチン株を含む世界の株の頻度は1/2,000-20,000であり, 自然感染時の1/100-1,000である[14]. ムンプスワクチン後の難聴, 脳炎, 睾丸炎は極めてまれである.

おわりに

ムンプスはワクチン予防可能疾患であり, 先進国や中興国では定期接種である. 我が国で2回の定期接種をしたとしても医療経済効果が認められており[15], 早期に定期接種になることが期待される.

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A New Method for the Detection of Neutralizing Antibodies against Mumps Virus

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Abstract

Neutralization test is the most reliable method of evaluating immunity against viral diseases but there is no standard procedure for mumps virus, with tests differing in the infectivity of the challenge virus, 50% plaque reduction or complete inhibition of cytopathic effects (CPE), and usage of complement. A reliable, easy, and simple neutralization test for mumps virus was developed in this study. A recombinant mumps virus expressing GFP was generated as a challenge virus. Complement was added to the neutralizing mixture at 1:200 when stocked serum samples were used. Neutralizing antibody titers were expressed as the reciprocal of the highest dilution that did not exceed two-fold of FU values (GFP expression) of the cell control wells. A total of 1,452 serum samples were assayed by inhibition of GFP expression in comparison with those examined by conventional 100% inhibition of CPE. 1,367 (94.1%) showed similar neutralizing antibody titers when examined by both methods. The GFP expression inhibition assay, using a recombinant mumps virus expressing GFP, is a simple and time- saving method.

Citation: Matsubara K, Fujino M, Takeuchi K, Iwata S, Nakayama T (2013) A New Method for the Detection of Neutralizing Antibodies against Mumps Virus. PLoS ONE 8(7): e65281. doi:10.1371/journal.pone.0065281

Editor: Yury E. Khudyakov, Centers for Disease Control and Prevention, United States of America

Received January 8, 2013; **Accepted** April 23, 2013; **Published** July 5, 2013

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Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Mumps virus is a single-stranded negative sense RNA virus, belonging to the genus *Rubulavirus* of the family *Paramyxoviridae*. The mumps virus genome encodes seven major proteins in the following gene order: nucleocapsid (N), phospho (P), matrix (M), fusion (F), small hydrophobic (SH), hemagglutinin-neuraminidase (HN), and large (L) protein genes [1]. V and I proteins are also produced from the P gene. There are two envelope glycoproteins, F and HN. The HN protein is involved in the virus attachment to sialic acid receptors on the surface of host cells. This leads to a conformational change of HN which induces further conformational change of the F protein in the cascade reaction of cell fusion [1,2]. Thus, mumps virus infection is initiated by the F and HN proteins, and neutralizing epitopes are located on these proteins [3,4].

An acute infection of mumps virus is characterized by self-limiting demonstrable swelling of the parotid glands with tenderness and several complications have been reported following parotitis, including aseptic meningitis, deafness, orchitis, and pancreatitis [1,5]. Mumps virus circulates throughout the world, and genotype classification of the wild type is useful for identifying the pathway of transmission [6]. Recently, circulating mumps virus strains have been divided into 12 genotypes from A to N (excluding E and M) based upon the sequence diversity of the SH gene [7,8]. Currently circulating strains in Japan were divided into four genotypes, B, G, J, and L [9].

The isolation of mumps virus is essential for the diagnosis of patients and for monitoring the antigenicity of wild circulating strains. The efficiency of virus isolation depends mainly upon the infectious viral load in clinical samples and the sensitivity of the cells used for isolation. Vero cells have been used, but isolation is not always successful because of the low viral load, timing of sample taking, and transportation. Several serological tests have been employed for the diagnosis of mumps virus infections and, among them, the enzyme-linked immuno-assay (EIA) was used to detect IgM antibodies for diagnosis and IgG EIA to investigate immune status [10,11]. EIA antibodies did not reflect protective immunity and a neutralization test is the most sensitive way to predict protective immunity [12,13]. Neutralization tests take a long time to obtain results and involve several complicated procedures [14,15]. The sensitivity of neutralization test was enhanced when complement was added [15]. Recently, the addition of complement was found to lead to deposition on the surface of viral particles bound with antibodies and destroyed the structure of mumps virus during the neutralization reaction [16]. Thus, the presence of complement seems to be essential for neutralization testing against mumps virus. In this study, a recombinant mumps virus expressing green fluorescent protein (GFP) was generated and the requirement for complement was examined using fresh and stocked sera.

Materials and Methods

Mumps Virus Strain

The Hoshino vaccine seed strain KO3 was developed by attenuation through 22 passages in chick embryonic cells from a wild-type mumps virus isolated in 1972 [17]. Full-length cDNA was constructed from KO3 Hoshino. The GFP sequence was inserted between the P/V and M genes (Fig. 1). GFP Hoshino was recovered from 293 T cells transfected with N, P, and L expression plasmids, and full-length cDNA under the control of T7 RNA polymerase [18]. Monolayer of Vero cells was infected with GFP Hoshino at m.o.i = 0.01 and culture fluid was stocked for challenge virus.

Virus Infectivity

Vero cells were propagated in minimum essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). Infectivity was determined based on the $TCID_{50}$ in Vero cells. The virus culture fluid was serially diluted by 10-fold and a confluent monolayer of Vero cells was infected with 100 µl of each dilution in 96-well plates. The plates were incubated for 2 h at 37°C in 5% CO_2 and MEM supplemented with 2% FBS was added. Infectivity was determined after incubation for 7 days.

Serum Samples

Eight serum samples obtained from healthy adults aged 23 to 58 years during a routine health check were used for the experiments after obtaining verbal informed consent. The remaining portion of the sera was used for preliminary experiments or as in-house control serum. Stocked serum samples (n = 185) were obtained to assess immunity against measles, mumps, rubella, and chickenpox among new students of the nursing school of Ashikaga Red Cross Hospital, Tochigi prefecture. The serological study was approved by the ethics committee of the hospital and verbal informed consent was obtained. Fresh serum samples (n = 1,452) obtained to evaluate immunity against measles, mumps, rubella, and chickenpox among new students in primary, junior high, and high schools, were used for routine yearly immunological assessments of infection control and to advise regarding vaccination for antibody negative pupils. The serological study was approved by the Health Care Center of Keio University. The purpose of the study was explained and written informed consent was obtained from their guardians. Serum samples were anonymously transferred to our laboratory, labeled with simplified numbers.

Virus Neutralization Test

The fresh serum samples were divided into several aliquots and stocked at −20°C. The samples were kept at 56°C for 30 min to inactivate the complement, serially diluted by 2-fold starting from 1:4, and mixed with the same volume of GFP Hoshino containing 100 $TCID_{50}$ of infectious virus at 37°C for 90 min for neutralization. The mixture was placed in 96-well plates in duplicate for each dilution and 25,000 Vero cells were seeded in 0.1 ml. The plates were incubated for 7 days. In order to calculate the titers automatically, the plates were processed to detect fluorescence intensity (Fluoro-Units: FU) at an emission wavelength of 528 nm and excitation wavelength of 485 nm using a fluorescence reader, FLx800 (Bio-Tek Instruments, Vermont, USA), similar to a method used to detect measles neutralizing antibodies [19]. To evaluate the requirement of complement, various concentrations of guinea pig complement (Denka Seiken, Tokyo, Japan) were added to the neutralization mixture of serially

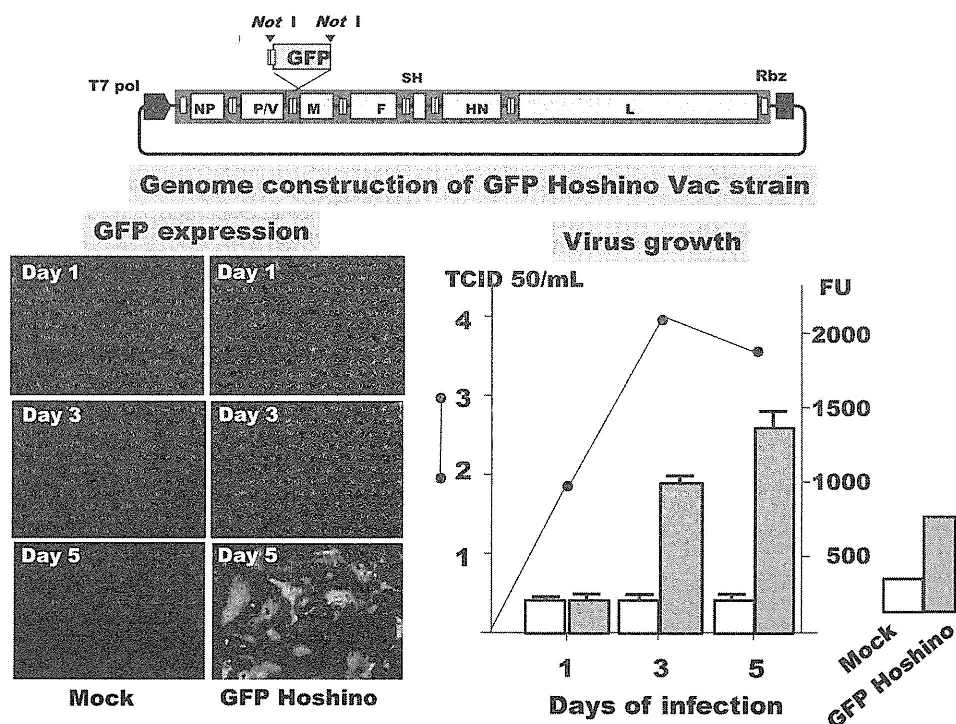

Figure 1. Genome construction of the recombinant mumps Hoshino strain expressing GFP and expression of GFP. Vero cells were infected with GFP Hoshino mumps strain at m.o.i. = 0.02 and subjected to experiments for GFP expression with fluoro EIA and microscopic examination on day 1, 3 and 5 of infection in comparison with mock-infection. Infectivity was assayed in culture supernatants on day 1, 3, and 5 of infection.

doi:10.1371/journal.pone.0065281.g001

diluted serum with challenge virus. Neutralizing antibody titers were determined as the reciprocal of the highest dilutions that did not exceed two-fold of FU values (GFP expression) of the cell control wells. Conventional neutralizing antibody titers were expressed as the reciprocal of the serum dilutions that showed 100% inhibition of CPE. Infectivity titer of the challenge virus was back-titrated in each assay, showing 50–120 $TCID_{50}$.

Statistical Analysis

Statistical significance in the neutralizing antibody titers was examined between two groups by the Mann-Whitney test. A coefficient was used for the analysis of correlation between the NT and EIA titers.

Results

GFP Expression

GFP expression and the viral growth are shown in Fig. 1. Vero cells were infected with the GFP Hoshino strain in a 24-well plate, and culture fluids were obtained 1, 3, and 5 days later. A peak infective titer of 10^4 TCID 50/ml was obtained 3 days after infection. Mean GFP expression (FU) is shown with 1.0 standard deviation (SD) in four wells in comparison with mock-infected wells. Mock-infected wells showed approximately 300 FU during the experiment, and GFP expression in infected wells increased to peak (1,300 FU) on day 5 of infection. Together with FU in fluoro-ELISA, fluoro-microscopic findings of CPE expansion with GFP expression are also shown in Fig. 1. Few CPE were observed on day 3 of infection and extensive cell fusion was noted on day 5. The development of CPE was closely related to GFP expression.

Neutralizing Antibody Titers

The results of the neutralization tests are shown in Fig. 2. Serum samples were serially diluted 2-fold from 1:4 to 1:256, and mixed with the challenge virus. The NT assay was done in duplicate. The results for one serum sample are shown in Fig. 2. CPE were observed in one well at 1:32 and none at 1:16. The conventional neutralizing antibody titer was considered to be 1:16 for 100% inhibition of CPE. The mean FU of cell control wells (mock-infected wells) was 202 FU. The mean FU of serial dilutions from 1:4 to 1:256 was 252 FU, 239 FU, 234 FU, 450 FU, 543 FU, 581 FU, and 591 FU, respectively. GFP expression increased to 450 FU at 1:32 and thus the neutralizing antibody titer for the GFP expression assay was 1:16 for inhibition of the growth of GFP Hoshino. The infective titers of the challenge virus were back-titrated, showing 50–120 TCID 50. When CPE appeared in >20% of the wells, GFP expression was >500 FU.

To evaluate the consistency of neutralizing antibody titers assayed by 100% inhibition of the appearance of CPE or GFP expression, neutralization tests for both conventional and GFP expression methods were done in 1,452 fresh serum samples. Three cut-off levels for positive GFP expression were set: 1.5-, 2.0-, and 2.5-fold increase in FU compared to cell culture controls. Among the 1,452 samples, 1,287 (88.6%) showed the similar neutralizing antibody titers when assayed by both methods using the 1.5-fold cut-off, 1,367 (94.1%) with the 2.0-fold cut-off, and 1,058 (72.9%) with the 2.5-fold cut-off. Strong similarity was noted when the cut-off was defined as a 2.0-fold of FU value in FU of the control wells.

Effect of Heat Inactivation and Addition of Complement

Eight fresh serum samples (A–H) were obtained and stocked at −80°C. Neutralizing antibody titers were examined before freeze-thawing, and after three and five rounds of freeze-thawing. The results are shown in Fig. 3. For serum A, the neutralizing antibody titer was 1:256, 1:64, and 1:128, showing no significant difference within five rounds of freeze-thawing. It decreased to 1:8 or 1:16 after inactivation at 56°C for 30 min. The other serum showed similar results. Neutralizing antibody titers did not decrease but decreased after inactivation of the complement. Complement activity would be required for neutralization tests for mumps virus.

Five fresh serum samples (A–E) were inactivated at 56°C for 30 min. When inactivated sera were used, guinea pig complement was added to the neutralizing mixture at 1:200, 1:400, 1:800, and 1:1,600. Neutralizing antibody titers were examined and mean values for three independent assays are shown in Fig. 4. Guinea pig complement did not affect the assay system without any changes in Vero cell cultures and the addition of guinea pig complement in non-inactivated sera did not influence the neutralizing antibody titers. The titer was 1:32–1:128 and dropped to around 1:8 after inactivation. The reduced neutralizing antibody titers increased to levels similar to those before inactivation when the complement was added at 1:200 or 1:400. Therefore, complement was added at 1:200 to the neutralizing mixture in the subsequent experiments.

Effect of Complement

Twenty-one fresh serum samples were obtained and neutralizing antibody titers were examined for non-inactivated and inactivated sera supplemented with complement at 1:200 in the neutralizing mixture. The results are shown in Fig. 5. The peak distribution of neutralizing antibody titers for non-inactivated samples was 1:32 and shifted to 1:64, showing no significant change in those with addition of complement.

As for the 227 stocked sera, neutralization tests were performed before and after inactivation with the addition of complement. 74 serum samples showed negative and 70 became positive, when assayed after inactivation with the addition of complement. The peak distribution of neutralizing antibody titers markedly shifted from 1:4 for non-inactivated sera (98 sera) to 1:16 after inactivation supplemented with complement (75 sera). Stocked sera were considered to lose complement activity over long periods. Therefore, the addition of complement was required when the neutralizing antibody titer was examined for the stocked sera, probably because of decreased complement activity.

Discussion

There are several serological methods of detecting mumps antibodies. Complement fixation (CF) and hemagglutination inhibition (HI) tests are not sensitive and, in addition, HI antibodies are cross-reactive to parainfluenza virus [1,10]. EIA has high sensitivity and specificity and is a simple procedure, but is not related to protective activity [11]. Neutralizing antibodies are associated with protective activity but the neutralizing test involves several complicated steps. Neutralization of an infectious virus and the preparation of cell cultures are bothersome and most time-consuming is the very last step to determine the appearance of CPE in 96-well plates. For micro-neutralization assays, there are two methods; 50% plaque reduction and complete inhibition of CPE. There have been several reports on neutralizing tests, concerning the evaluation of plaque reduction or inhibition of the appearance of CPE, infectivity of a challenge virus, and requirement of complement for neutralizing tests [12,13,14,15].

Fujino et al. [19] reported the neutralization test for measles virus using a GFP-expressing recombinant measles virus to evaluate the neutralizing antibody titer by Fluorescent EIA reader. Here, a recombinant mumps Hoshino vaccine strain expressing

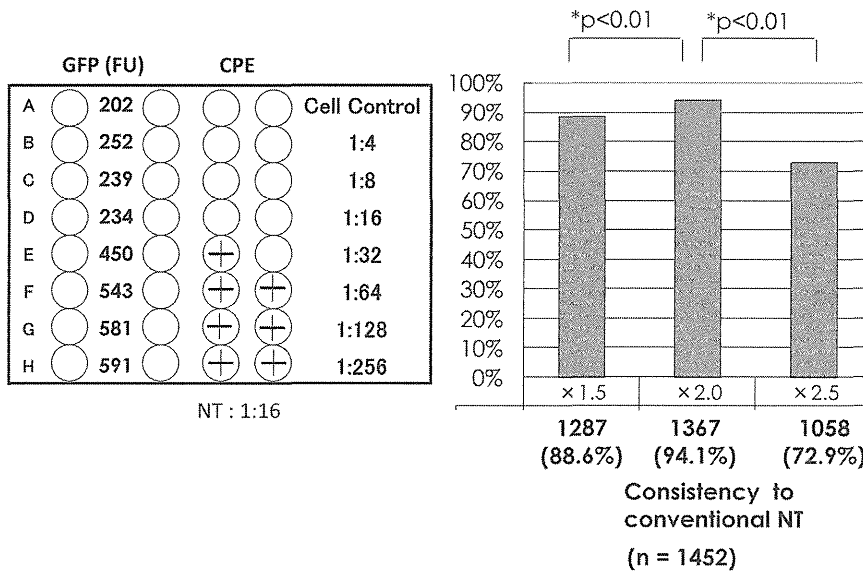

Figure 2. Relationship between the appearance of CPE and GFP expression. Serial two-fold dilutions from 1:4 to 1:256 were mixed with an equal volume of challenge virus. In the left panel, the schematic results of two neutralization methods are shown. CPE was observed in one of the two wells at 1:32, and the conventional neutralizing antibody titer was 1:16 by 100% inhibition of CPE. The mean FU value of the two cell control wells was 202 and that of the 1:32 dilution was 450, showing 1:16 of neutralizing antibody titer. Using 1,452 serum samples, the consistency of neutralizing antibody titers was compared based on different cut-off values for GFP expression: 1.5-fold, 2.0-fold, and 2.5- fold of FU values of the cell control wells.
doi:10.1371/journal.pone.0065281.g002

GFP was developed to check the expression of GFP instead of observing the appearance of CPE or plaque counting. GFP expression was examined by a fluorescent EIA reader as fluoro-units (FU). GFP expression increased as the virus genome was

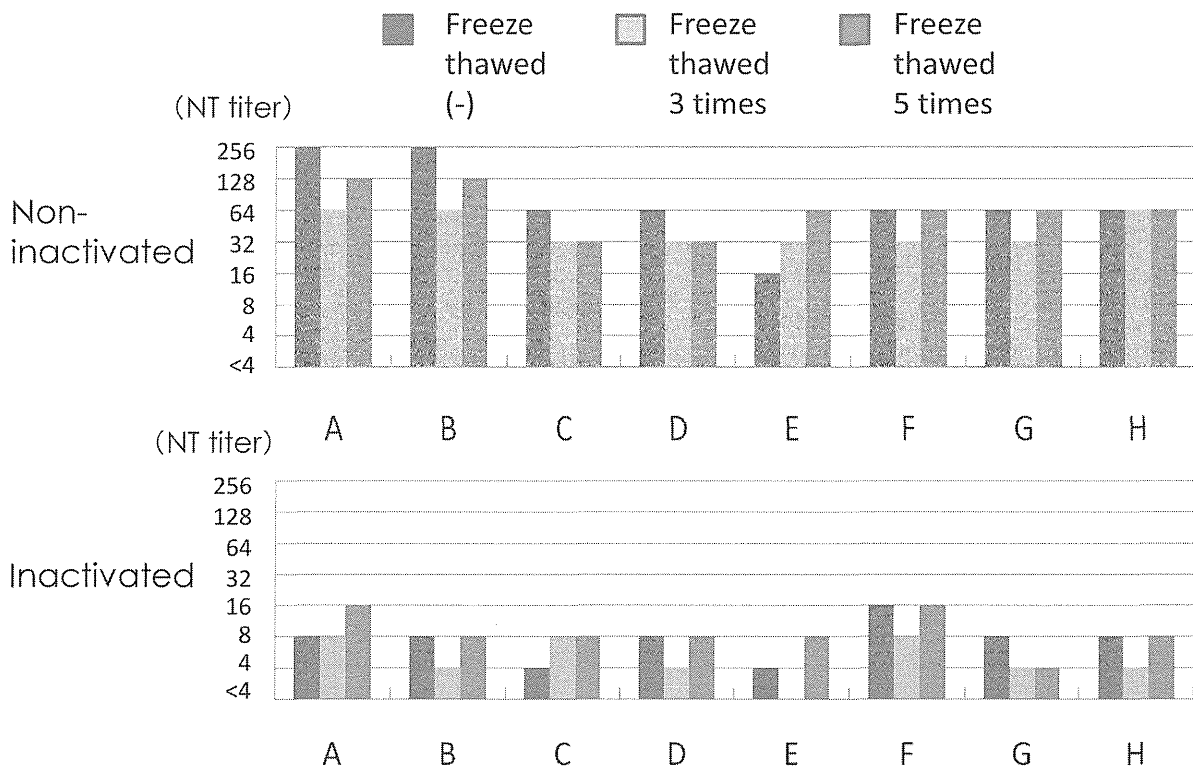

Figure 3. Effects of freeze-thawing and inactivation at 56°C for 30 min on neutralizing antibody titers. Upper panel shows the neutralizing antibody titers of eight fresh sera (A–H), without inactivation and after three or five rounds of freeze-thawing. Lower panel shows the results of neutralizing antibody titers after inactivation.
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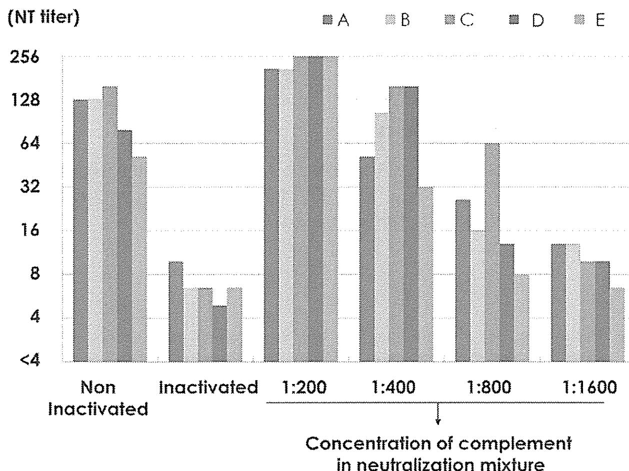

Figure 4. Neutralizing antibody titers of non-inactivated and inactivated sera with the addition of complement. Neutralizing antibody titers were examined in five sera (A–E) before and after inactivation. Complement was added at 1:200, 1:400, 1:800, and 1:1600 to the neutralizing mixture when inactivated sera were used. Each experiment was done in triplicate and mean titers were shown.
doi:10.1371/journal.pone.0065281.g004

transcribed after infection and was closely related to viral growth, as shown in Fig. 1. GFP expression in the cell control wells in a 96-well plate was approximately 200 FU. More than a two-fold increase in FU was considered positive for GFP expression (presence of CPE). Neutralizing antibody titers examined by GFP expression were similar to those by the conventional method for 100% inhibition of CPE (Fig. 2).

In several reports, the neutralizing step was performed without the addition of complement. Hishiyama et al. [15] reported that fresh guinea pig serum was required for neutralization tests for mumps virus. They used complement at 1:400 dilutions in the neutralizing mixture and the addition of complement increased the neutralizing antibodies titers. Complement has several important roles in immune responses and there are three main pathways, the classical, lectin, and alternative pathways. Complement is one of the first lines of host defense and is an important part of humoral immune responses. The complement system is immediately ready to target and eliminate viral particles and to interact with specific antibodies on the surface of a virus or infected cells [20]. Complement-dependent neutralizing antibody is reported to recognize the viral glycoproteins on the virus envelope, directly related to neutralization of Vesicular stomatitis virus [21,22], herpes simplex viruses [23,24], and West Nile virus [25]. Cooper et al. [26] reported that the deposition of antibody and complement on the surfaces of viral particles might physically interfere with infectivity in susceptible cells due to aggregation of the viral particles. However, Friedman et al. [23] suggested that complement inhibited the infection process of HSV, indicating that it affects viral replication: virus entry, uncoating, DNA transport to the nucleus, or immediate early gene expression, not requiring particle aggregation, viral lysis, or blocking of virus attachment. Johnson et al. [16] investigated the requirement of a complement system to neutralize three closely related paramyxoviruses, Simian virus 5 (SV5), mumps virus, and human parainfluenza virus type 2 (HPIV2). HPIV2 was neutralized in a complement-independent manner but neutralization of SV5 and mumps virus proceeded through alternative pathways. However, they were neutralized by different mechanisms; C3 deposition was observed on the surface of SV5 particles, resulting in aggregates. C3 deposition was also noted on the surface of mumps virus particles but they induced virion lysis through electron microscopic findings. In this sense, the presence of complement seemed to be essential for the neutralization tests for mumps virus. When fresh sera were examined for the detection of neutralizing antibodies against mumps virus, the addition of complement did not enhance the neutralizing antibody titers and the titers were stable for 5 rounds of freeze-thawing. But the complement activity was reduced after inactivation and during long-term preservation, and the addition of complement at 1:200 was required for neutralization tests against mumps virus.

EIA is simple and a large number of serum samples are handled without serial dilutions, which is suitable for surveillance but does not reflect protective immunity. A purified mumps virus antigen is used for the EIA antigen, and contains component proteins as well as viral particles. In our previous report, neutralizing antibodies assayed by the conventional method without complement showed a poor relationship to EIA titers. In the present study, there was again no significant relationship, with a low co-efficiency,

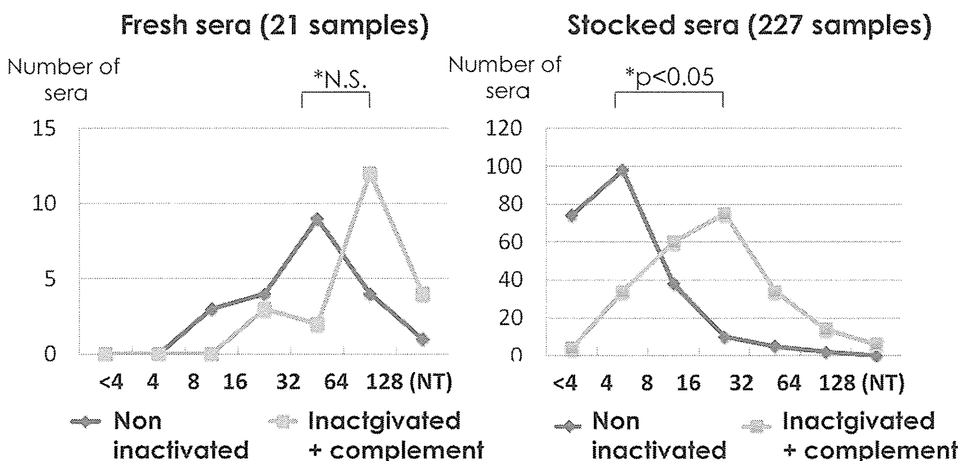

Figure 5. Effect of the addition of complement in 21 fresh and 227 stocked serum samples. Distribution of serum samples is shown for neutralizing antibody titers assayed without inactivation and for those assayed after inactivation with the addition of complement, using 21fresh serum samples (left panel). 227 stocked serum samples were assayed in a similar manner (right panel).
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examined by adding complement (data not shown). EIA-positive sera showed positive immune-fluorescent antibodies against the most abundant N protein [27]. Approximately 40–50% of the serum samples positive for neutralization test showed positive for immune-fluorescent antibodies against F or HN antigens, which are closely related to the infection process, attachment and cell fusion [27].

Using a recombinant mumps virus expressing GFP, the neutralization test was simplified via a reduction in GFP expression, counting automatically by fluorescent EIA reader. When stocked samples were used, complement was added at a concentration of 1:200.

Author Contributions

Conceived and designed the experiments: TN. Performed the experiments: KM MF. Analyzed the data: KM TN. Contributed reagents/materials/ analysis tools: MF KT SI. Wrote the paper: KM TN.

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J Infect Chemother
DOI 10.1007/s10156-013-0641-6

REVIEW ARTICLE

Vaccine chronicle in Japan

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Received: 2 May 2013
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Abstract The concept of immunization was started in Japan in 1849 when Jenner's cowpox vaccine seed was introduced, and the current immunization law was stipulated in 1948. There have been two turning points for amendments to the immunization law: the compensation remedy for vaccine-associated adverse events in 1976, and the concept of private vaccination in 1994. In 1992, the regional Court of Tokyo, not the Supreme Court, decided the governmental responsibility on vaccine-associated adverse events, which caused the stagnation of vaccine development. In 2010, many universal vaccines became available as the recommended vaccines, but several vaccines, including mumps, zoster, hepatitis B, and rota vaccines, are still voluntary vaccines, not universal routine applications. In this report, immunization strategies and vaccine development are reviewed for each vaccine item and future vaccine concerns are discussed.

Keywords Vaccine · Surveillance · MMR · DPT · Voluntary vaccines · Recommended vaccines

Abbreviations

ACIP	Advisory Committee on Immunization Practices
BCG	Bacillus Calmette–Guérin
DTaP	Acellular pertussis vaccine combined with diphtheria and tetanus toxoids
DTwP	Whole cell pertussis vaccine combined with diphtheria and tetanus toxoids
FHA	Filamentous hemagglutinin
HA	Hemagglutinin
HBV	Hepatitis B virus vaccine
Hib	*Haemophilus influenzae* type b conjugated with tetanus toxoid
HPV	Human papilloma virus vaccine
JEV	Japanese encephalitis vaccine
IPV	Inactivated polio vaccine
LAMP	Loop-mediated isothermal amplification
MMR	Measles, mumps, and rubella-combined vaccine
MR	Measles and rubella-combined vaccine
NA	Neuraminidase
NT	Neutralization test
OPV	Live oral polio vaccine
PCV7	7-valent *Streptococcus pneumoniae* conjugated vaccine with recombinant diphtheria toxoid
PT	Pertussis toxin
Tdap	Tetanus toxoid combined with a reduced concentration of diphtheria toxoid and acellular pertussis
VAP	Vaccine-associated paralytic polio
VZV	Varicella zoster virus vaccine

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Dawn of vaccines in Japan

The dawn of vaccinology was the first scientific systematic investigation of the cowpox vaccination by Edward Jenner in 1796, although several variations in approach were performed using dried pus from smallpox skin lesions in Central Asia, China, and Turkey [1]. Jenner's cowpox vaccination procedure was introduced into Japan in the Edo era by Philipp F.B. von Siebold. Sporadic nationwide outbreaks occurred at that time, which caused social, economic, and political stagnation, and doctors of herbal traditional medicine, studying Western modern medicine, wanted to use Jenner's cowpox vaccine as a preventive

Published online: 09 July 2013

procedure for smallpox. Many attempts were made to import the cowpox seed, but these did not succeed because Jenner's cowpox vaccine is a live vaccine: it was inactivated during long-term transportation or if the inoculation chain in children was interrupted. It was finally introduced to Nagasaki in 1849, bringing the vaccination scar through the idea proposed by Dr. Souken Narabayashi, who was the chief doctor of Nabeshima-Han (Saga Prefecture). The vaccination procedure became available at the Shutousyo (Vaccination Institute) in Osaka and Edo cities, which was the origin of the School of Medicine of Osaka and Tokyo Universities [2]. Jenner's cowpox vaccine gained in popularity because of its distinct effectiveness against smallpox. However, some opinions were against vaccination because of misunderstanding involving unreasonable and nonscientific rumors, as has recently been observed.

The Japanese government in the Meiji era decided that all Japanese people should be immunized with the vaccine for smallpox, which was stipulated in 1876, and a vaccination law against smallpox started in 1910. The present immunization law was implemented in 1948 under occupation by the United States (US). Issues related to vaccine development and immunization policies are summarized in Table 1. There have been two turning points for amendments to the immunization law: the compensation remedy for vaccine-associated adverse events in 1976, and the concept of private vaccination in 1994. These two turning points were attributed to vaccine-associated adverse events or accidents and lawsuits against serious neurological adverse events after immunization with vaccinia and the measles, mumps, and rubella-combined vaccine (MMR) [3]. In 1992, the regional Court of Tokyo, not the Supreme Court, set the governmental responsibility for vaccine-associated adverse events because the government did not make an effort to enlighten the public and doctors by explaining the possible adverse events associated with vaccinations, even though immunization was recommended to be compulsory [3]. This lack of information was a major reason why the government was reluctant to take active immunization strategic action, leading to the so-called long-term vaccine gap after the discontinuation, in 1993, of MMR, which had been introduced in 1989, because of the unexpectedly high incidence of aseptic meningitis caused by mumps vaccine components [4, 5]. The mechanisms behind the higher incidence of aseptic meningitis with the combined live MMR vaccine than with monovalent mumps vaccines were not clearly identified. Thereafter, new vaccines were not introduced, but many pediatric vaccines have been approved in developed countries, with the implementation of recommended vaccines, which shows that vaccine-preventable diseases should be controlled with available vaccines

Table 1 History of immunization and vaccine development in Japan

Event	Adverse events
1948: Immunization Law [Smallpox, Diphtheria, Typhoid fever, Salmonella Paratyphi, Pertussis, Tuberculosis, Typhus, Plague, Cholera, Scarlet fever, Influenza, Leptospirosis]	
1951: Preventive law against tuberculosis.	
1961: The polio vaccine was recommended.	
1962: School immunization with the influenza vaccine	Adverse events after the smallpox vaccination 1968–1970
1968: DPwT was recommended vaccination 1968–1970	
1976: Amendment of the immunization law for a compensation remedy for adverse events: Recommended obligatory [Smallpox, Diphtheria, Tetanus, Pertussis, Polio]; Temporarily [influenza, JEV]	DPT accidents 1974–1975
1977: The rubella vaccine was recommended.	
1978: The measles vaccine was recommended.	
1980: Eradication of smallpox and stopped being used.	
1981: The mumps vaccine was licensed.	MMR scandal 1989–1993
1985: The hepatitis B vaccine was licensed for the prevention of vertical transmission in1986.	
1994: Ammendment for private vaccination: Recommended [DPT, Polio, Measles, Rubella, JEV] Voluntary [influenza, VZV, Mumps]	
1995: The hepatitis A vaccine was licensed,	
2001: The influenza vaccine was recommended for the elderly >65 years.	
2005: BCG was recommended for infants 0–6 months of age.	JEV ADEM 2005
2005: The JEV vaccination was interrupted until 2009 and a booster at 14 years was stopped.	
2006: The two-dose schedule was started, using the MR combined vaccine.	
2009: Pandemic 2009 vaccines were imported from GSK and Novartis.	
2010: Hib, PCV7, and HPV were temporarily recommended.	

DPwP Whole cell pertussis vaccine combined with diphtheria and tetanus toxoids, *JEV* Japanese encephalitis virus vaccine, *MMR* Measles, mumps and rubella-combined vaccine, *VZV* Varicella zoster virus vaccine, *ADEM* Acute disseminated encephalomyelitis, *Hib* Haemophilus influenzae type b vaccine, *PCV7* 7-valent Str. pneumoniae vaccine, *HPV* Human Papilloma virus vaccine

[6–9]. *Haemophilus influenzae* type b conjugated with tetanus toxoid (Hib) was introduced in 2008, and 7-valent *Streptococcus pneumoniae* conjugated vaccine with recombinant diphtheria toxoid (PCV7) and human papilloma virus vaccines (HPV) became available in 2010. Rotavirus vaccines were introduced in 2012. Several issues concerning vaccines in Japan are discussed in this article.

Immunization law and schedule

The Japanese immunization law is complicated with double-standard categories: routine recommended and voluntary vaccination. Routine recommended vaccines consist of BCG, acellular pertussis vaccine (DTaP), measles and rubella combined vaccine (MR), inactivated polio (IPV), Hib, PCV7, HPV, and Japanese encephalitis vaccine (JEV). Voluntary vaccines are hepatitis B (HBV), mumps, varicella, and rotavirus vaccines. The difference between the two is the cost of immunization; routine recommended vaccines are principally covered by the regional government [10, 11]. Until 1994, immunization was performed by mass vaccination in regional Public Health Centers. It was replaced by private vaccination, derived from the concept that it is better that vaccinations are performed by children's family doctors who are familiar with their health conditions. Although this concept was easily accepted by general physicians, mass vaccination of BCG still continued in some regions.

In 2010, Hib, PCV7, and HPV began to be used as temporarily recommended vaccines, and the cost was partially supported by the regional governments [12]. Vaccination coverage of routine recommended vaccines is more than 90–95 % for BCG, DTaP, OPV, and MR and 80 % for JEV, whereas that of voluntary vaccines is less than 30–40 %. During 1990–2000 polyvalent combined vaccines were developed in the EU and widely used. There is no licensed polyvalent vaccine in Japan, and the vaccination schedule became much tighter than that in the 1990s, especially in very young infants less than 6 months of age (Fig. 1). Simultaneous administration of several vaccines was recommended by the Japanese Pediatric Association, as has been conducted in the US and EU [3, 4]. In March 2011, seven infants died within a week of receiving DTaP, Hib, PCV7, or BCG. The newly introduced Hib and PCV7 were temporally discontinued, but were restarted 1 month later because the risk of serious adverse events was not higher than that reported in developed countries. Simultaneous administration has been safely and effectively performed in the US and EU; however, the incidence of serious adverse events has been reported as 0.02–1 in 100,000 [13]. Therefore, simultaneous immunization is now performed without a high level of confidence. Careful surveillance monitoring and

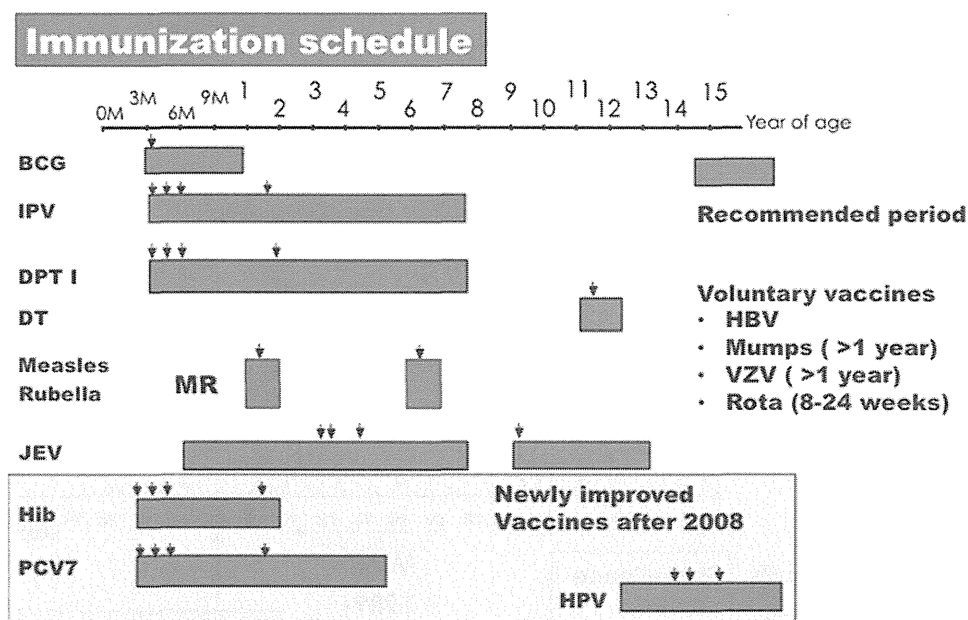


Fig. 1 Immunization schedule. BCG, IPV, DPT, DT, MR, JEV, Hib, PCV7, and HPV were recommended vaccines and HBV, Mumps, VZV, and Rota vaccines were voluntary vaccines. *Arrows* show the recommended timing for vaccinations. *BCG* Bacillus Calmette Guérin, *IPV* Inactivated polio vaccine, *DPT* Diphtheria and tetanus toxoids combined with pertussis vaccine, *DT* Diphtheria and tetanus toxoids, *MR* Measles and rubella-combined vaccine, *JEV* Japanese encephalitis vaccine, *Hib* Haemophilus influenzae type b vaccine, *PCV7* 7-valent Str. pneumoniae vaccine, *HPV* Human papilloma virus vaccine, *HBV* Hepatitis B virus vaccine, *VZV* Varicella zoster virus vaccine

scientific investigations are required to define the safety of simultaneous immunization.

Measles and rubella elimination

In Japan, live attenuated measles vaccines were developed in the 1970s, and four strains were licensed (three strains are used at present) with the implementation of recommended immunization in 1978 [14]. Five strains of live attenuated rubella vaccines (three strains are used at present) were developed and recommended for female students aged 14 years in 1977 [15]. Surveillance data and changes in the vaccination policy against measles and rubella are shown in Fig. 2. The MMR vaccine was used between 1989 and 1993 but was discontinued in 1993. Measles and rubella monovalent vaccines have been used for children aged 12–90 months since 1994 to control measles and rubella because the number of patients with congenital rubella syndrome did not decrease as a result of the vaccination of only young females since 1977.

Regarding the reporting system for measles in Japan, through 3,000 sentinel clinics or hospitals for pediatric infectious diseases and 450 clinics for adult measles surveillance, patients with clinically suspected measles were reported to Regional Health Care Centers. In the late 1990s to early 2000s, 20,000–30,000 cases of measles, including several dozen deaths, were reported yearly. A total of 2,034 cases of

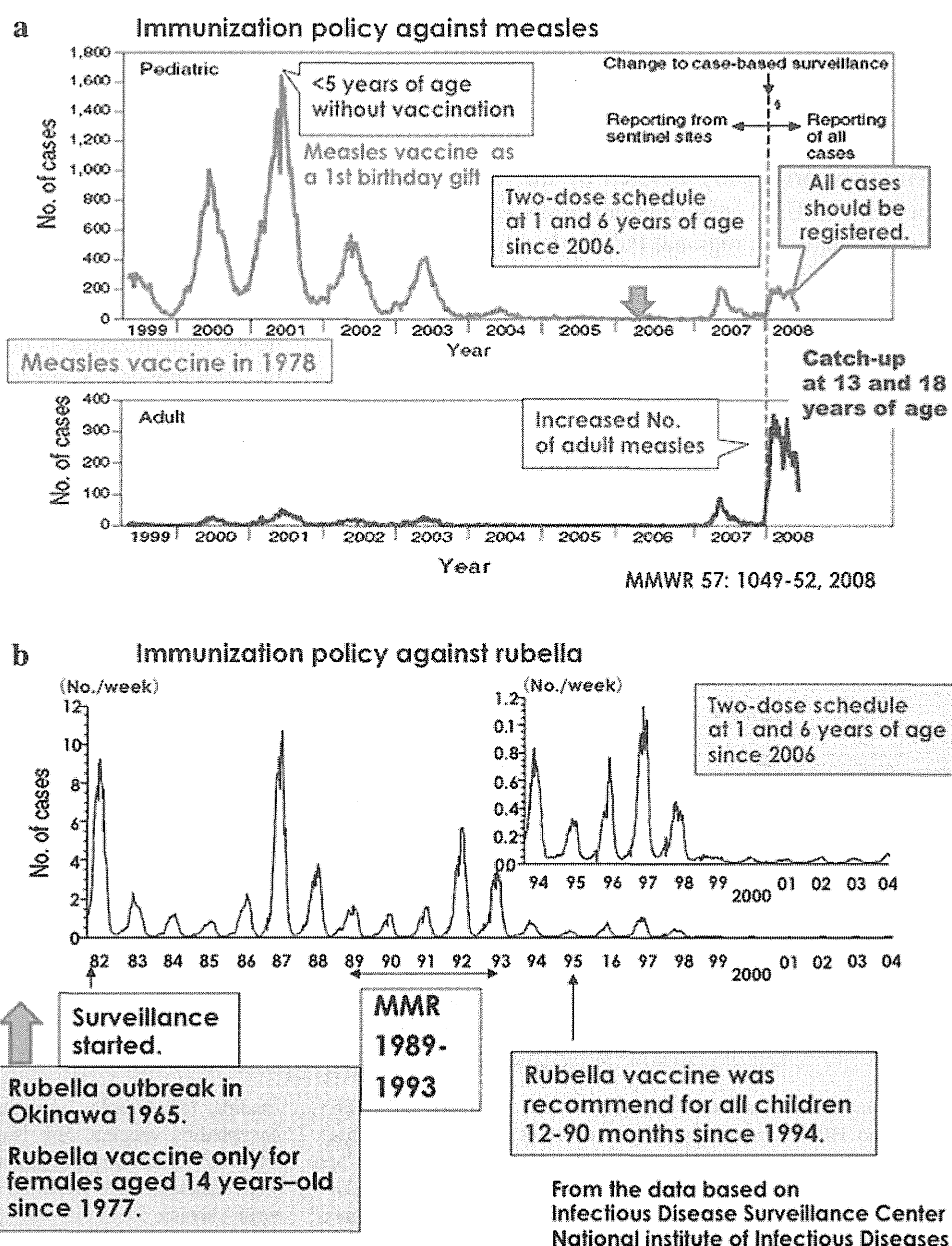


Fig. 2 Surveillance results of measles (**a**) and rubella (**b**), and the changes in immunization policies. Measles and rubella vaccines were recommended in 1978 and 1977, respectively. The MMR vaccine was used between 1989 and 1993, and the target generation of the rubella vaccine shifted from 14-year-old female schoolchildren to all infants 12–90 months of age. The two-dose schedule of the MR combined vaccine started in 2006. A catch-up campaign started in 2008 for an additional 5-year schedule for children 13 and 18 years of age. *MMR* measles, mumps, and rubella-combined vaccine

measles, including 8 deaths, were reported in a severe measles outbreak in Okinawa in 1998–1999 [16]. Many of the deaths occurred in infants under 1 year of age. A large measles outbreak was observed in 2001 in Japan. Among 33,812 reported cases, most patients were under 5 years of age and had not been vaccinated. Through a vaccination campaign to increase immunization coverage at 1 year of age, the number of reported cases decreased to 545 in 2005. The Japanese Government implemented a two-dose strategy in 2006, a combined measles and rubella vaccine (MR) for children at 1 and 6 years of age [17]. Therefore, the elimination of measles was expected. However, patients with measles were increasingly reported in March 2007, and this outbreak subsequently expanded throughout the Japanese districts, peaking in the middle of May. Furthermore, several reports indicated measles transmission by Japanese travelers or participants in an international sporting event [18–20]. This outbreak showed different characteristics, demonstrating that most patients were young adults or adolescents attending high school and university students, with a much lower proportion of young infants, at the early stage of the outbreak [21]. Cases of measles were reported in all age populations, with a total of 3,105 pediatric cases and 959 adult patients being reported in 2007. The number of patients with measles was the highest between 1 and 4 years of age, accounting for 40–50 % in 2001, which decreased to 22 % in the outbreak of 2007. A significant shift in the age distribution of cases of measles in 2007 was observed to be 10–14 years or older, accounting for 44 % in 2007 [22].

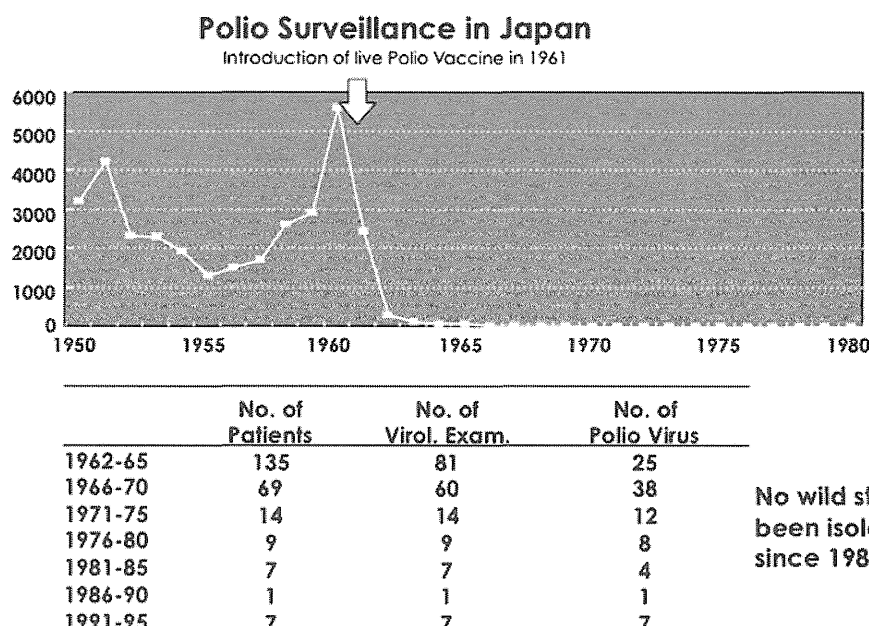
To reduce the number of patients with measles, an additional MR catch-up campaign was started for teenagers at the age of 13 and 18 years (MR III and IV) from 2008 for a 5-year schedule. After 2008, all cases with measles had to be registered, and the number of patients with measles was reduced to 457 cases in 2010 (3.58 cases per million), with some imported genotypes [23]. In 2011, measles was introduced from the EU by a journalist who was collecting the news of the earthquake, tsunami, and nuclear power disaster, and a total of 442 patients with measles were finally reported [24]. In 2012, 293 patients were reported, just on the edge of measles elimination of 1 case in 1,000,000, and most cases were identified as importations from Southeast Asia and the EU [25].

Global measles vaccination coverage increased from 72 % in 2000 to 82 % for the first dose in 2007, and the two-dose immunization strategy was recommended for countries with high coverage of the first-dose measles vaccine, at more than 95 %. Most countries (88 %) now implement the two-dose strategy [26]. However, measles transmission has increased, and outbreaks have become widespread since late 2009 in the EU region because of the failure to immunize susceptible populations [24]. The World Health Assembly updated the goal of measles elimination to a 95 % reduction in measles mortality by 2015, compared to 2000 [27].

The rubella vaccine strategy was markedly changed in 1994. Before 1989, the rubella vaccine was administered to 14-year-old girls, but the vaccine target has changed to all children aged 12–90 months. Boys more than 90 months of age and girls from 90 months to 14 years of age were not enrolled as immunization targets in the transition period. Even though a temporal catch-up campaign was conducted to cover the immunization gap, vaccine coverage was extremely low [15, 28]. According to the immunization gap in younger generations around 30 years of age, an outbreak of rubella was observed in 2011–2013, with some imported cases from Southeast Asia, resulting in congenital rubella syndrome [29]. Rubella is now prevalent (in 2013) among men around 30 years of age who have not been immunized because of the immunization gap. Through the enhanced network activity of measles and rubella elimination, the elimination of rubella has been targeted in accordance with measles elimination, using the measles and rubella combined vaccine [30, 31].

Replacement of oral polio vaccine (OPV) with inactivated polio vaccine (IPV)

Surveillance data of reported cases of polio paralysis are shown in Fig. 3. In 1960, a nationwide outbreak was observed, and approximately 5,800 patients with paralytic polio were reported. A similar level of outbreak seemed to be observed in 1961, and the Japanese government decided to import sufficient doses of OPV for all Japanese children. Within a month, 15 million doses were given to all Japanese children less than 5 years old. Around 1960, although IPV was under investigation and a clinical trial of imported OPV was also underway in Japan, the importation of OPV was politically decided. After the introduction of OPV in 1961 and afterward, the number of polio cases decreased [32]. After 1980, no wild strain was isolated from patients suspected of flaccid paralytic polio. All cases of paralytic polio were identified as vaccine-associated paralytic polio (VAP). The incidence of VAP was recently shown to be one in 1.4 million recipients in Japan. Clinical trials of domestic IPV produced from Sabin's live oral polio vaccine strains were performed beginning in 1998, but the application was withdrawn. Considering the practical way of immunization, the development of IPV combined with DTaP was more desirable than IPV alone. OPV was replaced with IPV in most developed countries, but it was delayed by the standstill of the IPV trial in Japan. Some guardians and pediatricians could not wait for the licensure of domestic DTaP/IPV and imported the IPV vaccine privately at their own responsibility. In 2012, IPV was allowed for use as a recommended vaccine imported from Sanofi and domestic



	No. of Patients	No. of Virol. Exam.	No. of Polio Virus
1962-65	135	81	25
1966-70	69	60	38
1971-75	14	14	12
1976-80	9	9	8
1981-85	7	7	4
1986-90	1	1	1
1991-95	7	7	7

Fig. 3 Polio surveillance in Japan since 1950. A peak number of patients with polio was observed in 1960, and the live polio vaccine was introduced in 1961 (*upper panel*). After 1962, the number of patients with polio decreased, and no wild strain has been isolated since 1980

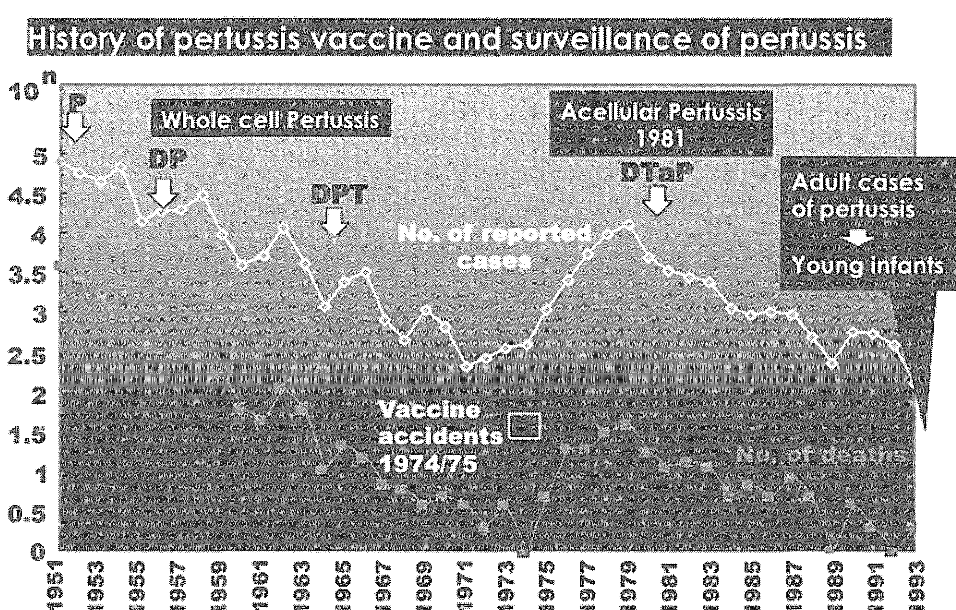


Fig. 4 History of the pertussis vaccine and surveillance of the number of reported cases of pertussis and pertussis deaths. The DPT vaccine was recommend in 1968. *P* Pertussis vaccine, *DP* Diphtheria toxoid combined with pertussis vaccine, *DPT* Diphtheria and tetanus toxoids combined with pertussis vaccine, *DTaP* acellular pertussis vaccine combined with diphtheria and tetanus toxoids

DTaP/IPV vaccines [33]. The wild poliovirus was imported in several situations from countries where wild polio has been circulating, and the high levels of vaccine coverage have been maintained. In addition to disease surveillance, environment surveillance of the vaccine for polio virus should focus on sewage monitoring [34].

Is the DTaP vaccine effective in controlling pertussis?

Pertussis is still a serious illness in young infants, and causes whooping cough, apnea, cyanosis, choking, and encephalopathy [35]. In Japan, the whole-cell pertussis vaccine was developed in 1949 and was combined with diphtheria and tetanus toxoids (DTwP). The results of pertussis surveillance and changes in vaccine strategy are shown in Fig. 4. Although febrile adverse illness was observed in 10 % of the recipients of DTwP, with local reactions of redness at 50–60 % and induration at 20 %, this vaccine was accepted. A routine immunization schedule was implemented with DTwP in 1968, resulting in a reduction in the reported cases of pertussis and pertussis deaths. In 1974–1975, two accidental deaths were reported after the administration of DTwP and, thereafter, DTwP

was temporarily discontinued. It was reintroduced for children aged 2 years old and older, or the DT vaccine was used instead of DTwP. The number of pertussis patients and pertussis deaths increased because of the low vaccine coverage [36, 37].

In 1981, a new type of acellular pertussis was developed, and a combined vaccine (DTaP) was introduced into recommended immunization practice. Principally, two types of DTaP vaccine (Biken-type, B-type; Takeda-type, T-type) were developed: the B-type consisted of two major antigens, pertussis toxin (PT) and filamentous hemagglutinin (FHA), and the T-type contained pertactin and fimbriae in addition to PT and FHA [38, 39]. Nationwide monitoring of clinical adverse events demonstrated low reactogenicity and sufficient antibody responses similar to natural infection. Since 1981, the number of pertussis patients has decreased after the acceptance of DTaP. However, the incidence of pertussis has recently been increasing in adults since 2002 in Japan, and several outbreaks on university campuses and in high schools and offices have been reported [40, 41]. Adult patients of pertussis are difficult to diagnose because of nontypical clinical features, including a prolonged cough. Also, the isolation of *Bordetella* or detection of the pertussis genome is not always successful because of the short duration of excretion of *Bordetella* influenced by the empirical administration of antibiotics or vaccination history [41, 42]. A surveillance system is currently under construction in Japan, based on a genetic diagnosis by loop-mediated isothermal amplification (LAMP) for detection of the pertussis genome [43].

DTaP was adopted by global vaccine makers because of the lower incidence of adverse events than that with DTwP, and multivalent combined vaccines, such as DTaP/Hib/IPV/HBV hexavalent vaccines, were developed based on DTaP. Pertussis is principally an infectious children's illness causing whooping and prolonged cough, and the Advisory Committee on Immunization Practices (ACIP) recommended a five-dose DTaP schedule, at ages 2, 4, 6, 15–18 months, and 4–6 years, instead of the previous DTwP in the US in 1997 [44]. In the 1990s, the incidence of pertussis at an older age increased in many countries because of waning immunity after primary childhood immunization and antigenic changes in pertussis, and adolescent pertussis was identified as the source of the transmission of pertussis to young infants through enhanced surveillance studies [45]. In Japan, the number of newborn pertussis cases increased in household contact [46]. In 2005, the tetanus toxoid, combined with a reduced concentration of diphtheria toxoid and acellular pertussis components (Tdap) vaccine, was licensed in the US, and the ACIP recommended that adolescents aged 11–18 years old should receive a single dose of Tdap for a booster immunization [47]. It is now recommended for all generations from 19 to more than 64 years of age who have not been vaccinated in the past 10 years [48]. In Japan, DT was recommended at the age of 11–12 years, and the lack of pertussis booster immunization is one of the reasons why the number of patients with pertussis has increased in adults. The booster effect of a reduced volume of DTaP was investigated instead of the DT vaccine at 11–12 years of age, and 0.2 ml DTaP induced sufficient antibodies against PT and FHA without serious adverse events [49]. Even with high vaccine coverage, the number of pertussis patients increased globally because of the short duration of vaccine immunity. Several DTaP candidates containing additional protective antigen(s) are now under investigation [50].

Does the influenza split vaccine prevent infection?

Two types of influenza virus vaccines are now globally available, inactivated and cold-adapted live attenuated vaccines. There are three types of inactivated vaccines: whole virion, split, and subunit inactivated vaccines. The whole virion inactivated vaccine induced febrile reactions after the vaccination, and thereafter the split vaccine was licensed in 1972 in Japan, which has been used for more than 40 years with a lower incidence of febrile reactions. The split vaccine is made by destroying the structure of virus using detergents and ether to remove their lipid components from the formalin-inactivated whole virion. The HA subunit vaccine is purified from the HA fragments zone [51]. Changes in immunization policies, vaccine production, and the population aged less than 15 and more than 65 years are shown in Fig. 5. The transmission of influenza was believed to be associated with contact with schoolchildren, and, thereafter, the influenza vaccine has been recommended every year as school immunization in primary schools since 1962 [52]. In the 1960s, the pediatric population (<15 years of age) was more than 20 million, and more than 25 million doses of influenza vaccine were produced. The effects of school immunization on decreasing the social impact of influenza were questionable, and a comparative study was performed. There was no difference in the number of reported cases, number of hospital visits, and cost of healthcare insurance among several cities with or without school immunization in Gunma Prefecture in the early 1980s. This study provided evidence that school immunization had no effect on reducing the impact of influenza in the community, but had a limited effect on an individual basis [53, 54]. The influenza vaccine strategy was shifted from an obligatory routine vaccine to a voluntary vaccine in 1994. School immunization was interrupted in 1995, and the total

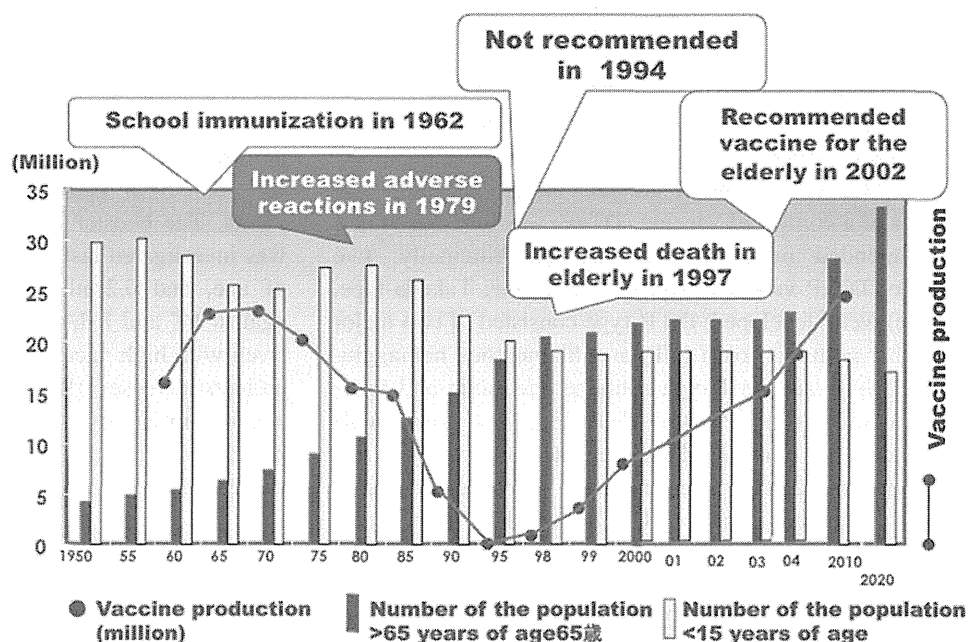


Fig. 5 Changes in the immunization strategy of the influenza vaccine, population more than 65 years and less than 15 years of age, and vaccine production in million doses

amount of vaccine produced was at its lowest, 0.35 million doses. A large outbreak of H3N2 was observed in 1997, and several deaths were reported in many nursing homes for the elderly as social topics. It has been recommended as a routine recommended vaccine for the elderly more than 65 years of age since 2002 for the benefits of vaccine recipients [55].

Three pandemics of influenza occurred in the 20th century. The most devastating pandemic dated back to 1918 and was known as Spanish flu. It was caused by a highly pathogenic H1N1 influenza virus transmitted through some animals from an avian pathogenic virus and is estimated to have killed 40–50 million people [56]. In 1957, Asian influenza A/H2N2 caused the second pandemic, and Hong Kong influenza A/H3N2 appeared as the third pandemic in 1968. Seasonal influenza outbreaks or epidemics are caused by an antigenic drift of A/H1N1 or A/H3N2, whereas these pandemics appeared as an antigenic shift, leading to a new strain, which is thought to be a re-assortment with the non-preexisting features of hemagglutinin (HA) or neuraminidase (NA) in human influenza viruses. After the 1968 pandemic of A/H3N2, several cases and small local outbreaks were reported, caused by new strains, H5, H7, or H9, and were considered to be from poultry, with H5 being very close to humans as a target for vaccine development [57]. A regional outbreak of H5 was reported in Hong Kong in 1997, and 6 of 18 patients died, causing an H5 pandemic threat [58]. Sporadic H5 transmission on poultry farms and in migratory birds has spread across Asia to the EU and Africa, and approximately 610 cases of human H5 infection have been reported at present in 2013 since 2003, showing a high mortality rate of approximately 60 %. Most cases have involved close and direct contact with poultry, with no definite evidence of human-to-human transmission. There are several barriers to human-to-human transmission: receptor usage of the HA protein, cleavage efficiency by cellular protease, and host factors. H5N1 is considered to be a target for the pandemic vaccine, and the WHO addressed sharing viruses and sequence information for a future pandemic vaccine development [57, 59]. The development of an effective and safe vaccine is expected to mitigate the threat of a pandemic.

Several types of H5 vaccines have been developed, basically based upon the HA split, subunit vaccine, or whole virion inactivated with adjuvant. In Japan, alum-adjuvanted H5N1 whole virion inactivated vaccine (WIV) (alum concentration, 300 μg/ml) was developed using a genetically engineered reassortant, the NIBRG-14 strain, originating from H5N1/A/Vietnam/1194/2004. In a clinical phase II/III trial in healthy adults, alum-adjuvanted WIV (HA protein, 15 μg) led to favorable immunogenicity, >70 % sero-conversion rate in neutralization tests (NT) antibodies, without causing any serious systemic illnesses [60]. However, when it was administered to young infants and children at a reduced dose, 7.5 or 3 μg, a high body temperature (≥38.0 °C) was observed in approximately 60 % of recipients less than 7 years of age, and, unexpectedly, NT antibody titers were higher in children than in the clinical trial in adults. These phenomena were associated with the enhanced production of inflammatory cytokines [61].

Introduction of Hib, PCV7, and HPV

Hib and PCV are the major pathogens of bacterial meningitis and invasive systemic bacteremia, and they cause serious pneumonia. In the past, bacterial infection was believed to be treatable with antibiotics through early diagnosis and was not a target for vaccine development before 2000 in Japan. However, a shift led to the development of vaccines in the late 1980s in the US. The appearance of resistant strains provided an impetus for the introduction of vaccines. In Japan, the surveillance study of the incidence of Hib meningitis was conducted, which showed the incidence was 8.3 per 100,000 children less than 5 years of age [62, 63]. These surveillance results estimated 600 cases of serious invasive Hib infection, and then, the Hib vaccine was introduced. In the postmarketing study, the practical usage of Hib simultaneously administered with DPT was confirmed to be safe and effective, similar to separate administration [64]. It was allowed in 2008, and Hib was the first vaccine imported from a foreign country. Thereafter, PCV7, HPV, and Rota vaccines were licensed. Hib, PCV7, and HPV vaccines were temporarily adopted as routine recommended vaccines in 2010 with tentative financial support and were engaged to be covered as routine recommended vaccines in 2013 [10]. After the introduction of Hib and PCV7, the incidence of serious invasive infection decreased whereas the *Streptococcus pneumoniae* 6B and 19A serotypes uncovered by PCV7 are increasing, with a higher number of penicillin-resistant strains [65, 66]. Hib infection became controlled but *S. pneumoniae* has approximately 100 serotypes, using serotype replacement after the introduction of PCV7 and PCV13 to be licensed.

Action for the routine immunization of mumps, zoster, and hepatitis B vaccines

Five live mumps vaccine strains were developed in the 1970s from domestic wild strains isolated in the 1960s and 1970s [67, 68]. MMR vaccines containing four domestic vaccine strains were used, but were discontinued because of the unexpected high incidence of aseptic meningitis. Thereafter, monovalent mumps vaccines were used and the incidence of aseptic meningitis was evaluated. We reported that the incidence of aseptic meningitis was 13/1,051 (1.24 %) in patients with symptomatic natural mumps infection and was estimated to be 0.7–1.1 % of overall infections considering asymptomatic infections, and 10/21,465 (0.05 %) in vaccine recipients [69]. Although aseptic meningitis is considered to be an apparent adverse event of the mumps vaccine, its incidence is considerably lower than among those with symptomatic natural infections. It provides informative findings for consideration of resuming the mumps vaccine as a part of a routine immunization schedule for Japanese children. Regarding mumps deafness, the incidence of deafness was previously believed to be 1 in 15,000 [70], but irreversible mumps deafness occurred at a higher incidence, in 1 case per 1,000 [71]. Mumps deafness is one of the targets for vaccine implementation. Mumps outbreaks were observed every 3–5 years because of low vaccine coverage, less than 40 %, because the vaccine was voluntary.

The varicella zoster virus vaccine OKA strain was developed in Japan in 1974, and is the only strain available in the world [72]. Initially, it was developed for immunocompromised hosts who develop serious complications with chickenpox [73]. It causes no serious adverse reaction and protects against the onset of illness by immediate inoculation within 3 days of contact with patients in pediatric wards [74]. It was allowed for use in healthy infants, but the yearly epidemiological pattern did not change because of the low vaccine coverage, less than 40 % [75].

Mumps and zoster vaccines were universal vaccines in the US and EU but were voluntary in Japan [10, 12, 75]. The hepatitis B vaccine (HBV) is still a voluntary vaccine, as HBV was developed to interrupt the carrier through vertical transmission from carrier mothers positive for the HBe antigen [76]. HBV was given at 2, 3, and 5 months of age, and the number of carriers became markedly reduced. Recently, cases of nosocomial infections or horizontal transmission cannot be neglected, and HBV should be adopted as a universal vaccination [77]. Mumps, zoster, and HBV are still voluntary vaccines in Japan although they are globally recommended as universal vaccines. These vaccines are anticipated to be routine recommended vaccines.

Requirement for future immunization

The disease surveillance system in Japan is based on 3,000 sentinel clinics or hospitals for pediatric infectious diseases and reflects the tendency of infectious diseases, not population-based incidences. The immunization strategy is decided based upon disease surveillance, and monitoring of vaccine-associated adverse events is important to assess the safety. It is now based on postmarketing surveillance in Japan and should be developed in a systematic administrative form, together with laboratory investigations. It is difficult to identify the relationship of vaccination to serious adverse events occurring within a few weeks after immunization, and, in most cases, a direct relationship could not be identified. In 2005, a serious case of acute disseminated encephalomyelitis (ADEM) was reported

after vaccination with the Japanese encephalitis vaccine (JEV). At that time, JEV was produced from purified virus particles from mouse brains infected with Japanese encephalitis virus. Therefore, JEV has the potential to cause allergic encephalomyelitis. JEV was suddenly discontinued in a shortsighted political decision, without considering the effects of blank periods without JEV. At that time, tissue-culture JEV was ready to be marketed. Comprehensive decisions are required and should be made after scientific discussion.

No organization for decision making on immunization is currently systematized in Japan, such as the Advisory Committee on Immunization Practices (ACIP) of the US [12]. An investigational Committee on Immunization was organized to propose immunization strategies to decision makers and to discuss problematic issues based on the scientific evidence. However, this committee has been organized in the administrative agency, the Ministry of Health, Labor, and Welfare. Although issues on immunization should be discussed based on scientific evidence as a third party, it belongs to the political side at present. It may be hard to listen to the clinical needs of general physicians for the improvement of immunization practice. It should be organized for the purpose of promoting public health with a longitudinal vision for immunization policies and prompt responses to the critical issues, without the influence by political changes.

Conflict of interest I have no conflict of interest regarding this manuscript.

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