ANALYSIS OF A PREVIOUS SMALLPOX VACCINATION STUDY: ESTIMATION OF THE TIME PERIOD REQUIRED TO ACQUIRE VACCINE-INDUCED IMMUNITY AS ASSESSED BY REVACCINATION

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Abstract. The time interval required to develop immunity after vaccination, in the event of a bioterrorist attack using variola virus, is yet to be clarified. In this article, a historical vaccination study conducted in Japan in 1929 was re-examined. Forty-four previously vaccinated and 44 unvaccinated children were involved. After successful first round primary (or re-) vaccination, all children underwent revaccination at variable intervals. Absence of a major reaction (vaccine 'take') after revaccination was taken as a sign of immunity conferred by first round primary (or re-) vaccination. Univariate analysis was employed to examine the relationship between vaccine 'take' and the exposure variables. Maximum likelihood estimates of the time period required to develop immunity were obtained using a simple logit model. The interval between vaccinations was significantly associated with vaccine 'take' in both the previously unvaccinated ($p < 0.01$) and vaccinated ($p < 0.01$) groups, and the median interval required for immunity after vaccination was estimated to be 6.4 [95% Confidence Interval (CI): 5.8, 7.1] and 4.3 days (95 % CI: 4.1, 4.7), respectively. Obtained estimates were consistent with previous observations, and the logistic fits reasonably explained the discrepancy among previous suggestions. The findings suggest that it is necessary to vaccinate exposed susceptible individuals within 3 days after exposure to ensure disease protection, and within at least 5 days (for those previously unvaccinated) to provide a certain level of protection; the probability shows a dramatic decline hereafter.

INTRODUCTION

Despite the global eradication of smallpox (Fenner *et al*, 1988), there is a serious threat of future bioterrorist attacks using its causative agent, variola virus. Even though smallpox has the longest history of vaccination, and despite the enormous numbers of studies concerned with the protective effect of vaccinations, the effectiveness of postexposure vaccination remains a burning question. As it is difficult to determine whether individuals vaccinated after possible exposure were actually infected, former evidence showing numbers of cases by the interval between postexposure vaccination and onset of disease fails to provide us with a denominator for estimating the time-period required to develop immunity (*ie,* the number of protected individuals) (Hanna, 1913; Mortimer, 2003). Even though the post-exposure dates of vaccination were ignored in the study design, a lowered, but not statistically significant, frequency of cases among those who underwent post-exposure vaccination was confirmed in

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India during the early 1960s (Rao *et al*, 1968). Further, Lyons and Dixon (1953) suggested that successful vaccination during the first 7 days after exposure would almost always prevent the disease; however, their evidence was based on individual experiences from several outbreaks. This was partly re-examined based on an outbreak in West Pakistan in 1967 by investigating the difference in the frequency of cases dichotomized at 10 days (Mack *et al*, 1972), but the lack of information on those actually exposed and consequently protected made it difficult to draw definitive conclusions. Although the results of a statistical study indicate that vaccination up to 3.2 days after exposure might protect against disease, this study again relied on the frequency distribution of a limited number of cases (Eichner and Schwehm, 2004). Thus, the question regarding the time period required to acquire vaccine-induced immunity is yet to be clarified.

Immediately after discovery of the cowpox vaccine by Edward Jenner in the late 18th century, Francis Jeffrey (1807), a colleague of Jenner, confirmed the protective effect of vaccination by challenging cowpox vaccination with smallpox inoculation (variolation). Following this early experiment, Dr Luigi Sacco (1809), an Italian physician, inoculated himself and others several times with cowpox, suggesting the usefulness of revaccination in confirming the effectiveness of prior vaccination, and thus, vaccine-induced immunity. Observation of localized infection, a characteristic skin reaction consisting of vesicle formation at the site of vaccination, now recognized as vaccine 'take', is interpreted as a sign of successful vaccination of a susceptible host (Rosenthal *et al*, 2001). Using this logic, the development of immunity after vaccination can be assessed by observing a major reaction to revaccination performed shortly after primary vaccination. In other words, the presence of a reaction to revaccination performed immediately after primary vaccination (within several days) denotes a non-immune state after primary vaccination, and the frequency of 'take' at variable intervals between vaccinations allows estimation of the time period required to develop immunity.

Although there has been considerable debate concerning the issue of reintroducing routine vaccination as preparedness for bioterrorist attacks, public mass vaccination prior to an actual event is currently not recommended (Fauci, 2002; Pennington, 2003). As an alternative, it is crucial to clarify the time period required to develop vaccine-induced immunity, ensuring the protective effect of postexposure vaccination in relation to the time lag between exposure and vaccination. In this paper, I evaluate a historical study of smallpox vaccination conducted in 1929 in Japan to investigate the development of vaccine-induced immunity in relation to time after vaccination. Consequently, the time period required to develop immunity is estimated.

MATERIALS AND METHODS

Background to the original study

Dr Itsuo Oyamada, a Japanese infectious disease physician at Osaka City Momoyama Hospital (presently unified with Osaka City General Hospital), examined the reaction to revaccination among 88 children successfully vaccinated several days previously (Oyamada, 1929). This method of immunity evaluation is convenient, and a number of similar trials were conducted during the late 19th and early 20th century (Acland, 1897; von Pirquet, 1906). Dr Oyamada used the vaccine classified as 'variolae vaccine', which conventionally denotes the vaccine first developed by Jenner for cowpox (Jenner, 1800). The viral strain in this study, however, was not cowpox but was one of the vaccine strains obtained from the 'retrovaccination' technique primarily achieved by taking the variola virus from human lesions back to a cow. According to Dr Jokai Iguchi

(1929), the leading smallpox specialist in Japan at the time of the study, the vaccine based on retrovaccination was introduced to Japan in 1917 and thereafter maintained by the National Institute of Communicable Diseases (presently The Institute of Medical Science, The University of Tokyo). As originally recommended in the Japanese Vaccination Law (Iguchi, 1929) two to four inoculations were usually made per vaccination. The inoculations were performed no less than 2 cm apart at small cross-incisions (5 mm in length) on the outer aspect of the upper arm made using a lancet (Oyamada, 1929). Revaccination immediately following primary (or re-) vaccination was performed using the opposite arm.

The experimental design

Of the 88 children involved, 44 were previously vaccinated and 44 were unvaccinated. All underwent successful first round primary (or re-) vaccination (all showed vaccine 'take') followed by revaccination at variable intervals (Fig 1). The interval between vaccinations, as allocated by Dr Oyamada based on previous experiments (Sacco, 1809; Acland, 1897; von Pirquet, 1906), ranged from 2 to 49 days. The absence of vaccine 'take' after revaccination was considered by Dr Oyamada as a sign of vaccine-induced immunity conferred by the first round primary (or re-) vaccination. Information on age, the interval between vaccinations, and the observed number of vaccine 'take' results to the first round primary (or re-) vaccination was obtained for each subject (Oyamada, 1929). Although partial names of each individual were given in the records, information concerning sex was unavailable.

The definition of successful vaccine''take' employed by Dr Oyamada was almost the same as what was later defined as a major reaction, 'Jennerian vesicles' at the vaccination site, by the World Health Organization (WHO) (World Health Organization, 1964; Fenner *et al*, 1988). Skin reactions to revaccination (second round vaccination) were examined daily and only the presence of obvious vesicular and, thereafter, pustular lesions was defined as vaccine 'take'. Otherwise, the response was defined as''non-take', or rather as a sign of immunity conferred by first round primary (or re-) vaccination. Individuals defined as 'non-take' included those who showed no reaction or an obvious immediate hypersensitivity reaction only.

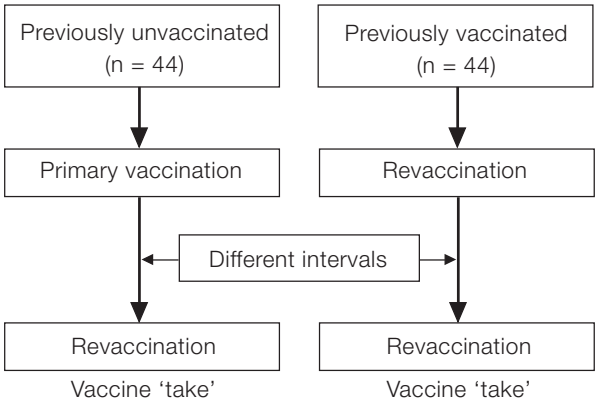

Fig 1–Flow chart of the original study. Forty-four previously unvaccinated and 44 vaccinated children were involved in the original study. All 88 children underwent two vaccinations: all were successfully vaccinated (with vaccine 'take') at first round primary (or re-) vaccination and then received revaccination at variable intervals. The presence of a vaccine 'take' reaction after revaccination was used as the outcome of the present study. Although a third revaccination was made in the original study, this information was ignored here to simplify interpretation of the results.

Outcome and exposure variables

The outcome in the present study was the presence of a major reaction, vaccine 'take', following revaccination (second round vaccination). Those showing vaccine 'take' after revaccination were interpreted as still being susceptible even several days after first round primary (or re-) vaccination, while the absence of 'take' denoted the development of vaccine-induced immunity. The outcome

was therefore measured as a dichotomous variable. Although the potential association between the outcome and interval between vaccinations was suggested in the original study, I firstly examined the univariate associations between the outcome and each exposure variable (age, number of 'take' in the first round, and the interval between vaccinations); these exposure variables were measured as continuous variables. Furthermore, the probability of vaccine 'take' in relation to the interval between vaccinations was investigated to obtain an estimate of the median duration required to develop vaccine-induced immunity.

Statistical analysis

To examine univariate associations between the binary response variable and continuous exposure variables, the Wilcoxon-Mann-Whitney test was used. The level of statistical significance was set at $\alpha = 0.05$. Maximum likelihood estimates of the median interval required to develop vaccine-induced immunity (the interval with a 50 % probability of 'take') were obtained using a logit model. In the logit model, the probability of vaccine 'take', $p(Y=1)$, is given by

$$p(Y=1) = \frac{\exp(k)}{1+\exp(k)} \qquad \text{(eqn. 1)}$$

where k is a linear regression equation given by

$$k = a + b_1 \times X_1 + b_2 \times X_2 + \ldots + b_n \times X_n. \qquad \text{(eqn. 2)}$$

In the above equation, a represents the intercept, and b_n is the coefficient of the predictor variable X_n (*ie*, b_1 is the coefficient of the interval between vaccinations, X_1). In estimating the median time required to develop immunity using univariate analysis, I ignored other explanatory (confounding) variables not associated with the outcome in univariate analyses. Since the median time yields $p(Y=1) = 0.5$, the estimate of X_1 ($= t_m$) is given by $t_m = -a / b_1$. Replacing a with t_m and b_1, eqn.1 is transformed to:

$$p(Y=1) = \frac{\exp\{b_1 \times (X_1 - t_m)\}}{1+\exp\{b_1 \times (X_1 - t_m)\}} \qquad \text{(eqn. 3)}$$

The maximum likelihood estimate of t_m is obtained by minimizing the binomial deviance of the model from the obtained data. Ninety-five percent confidence intervals (CI) were derived from the profile likelihood. All statistical data were analysed using the statistical software JMP IN ver. 5.1 (SAS Institute Inc, Cary, NC).

RESULTS

Descriptive data and univariate analysis

Characteristics of the outcomes and exposure variables in the study population are summarized in Table 1. Twenty-five (56.7%) and 32 (72.7%) of the previously unvaccinated and vaccinated individuals, respectively, showed no vaccine 'take' after revaccination (second round vaccination). The age of the previously vaccinated group was less deviated than that of the previously unvaccinated group.

Table 2 shows the results of univariate analyses. In both groups, the interval between vaccinations showed a significant negative association with vaccine 'take' ($p < 0.01$ and $p < 0.01$ for previously unvaccinated and vaccinated groups, respectively). Neither age ($p = 0.45$ and $p = 0.72$) nor the number of major reactions after the first round primary (or re-) vaccination ($p = 0.65$ and $p = 0.93$) was significantly associated with the outcome.

Time period required to develop vaccine-induced immunity

Using a logit model, the time interval required to develop vaccine-induced immunity was shown to be significantly associated with vaccine 'take' after adjusting for confounding variables; the estimated coefficient of predictor variables (and standard error, SE) was -1.85 (0.58) and -1.08 (0.42) for the previously unvaccinated ($p < 0.01$) and vaccinated ($p = 0.01$) groups, respectively. The median time

Table 1
Observed outcomes and exposure variables of a smallpox vaccination study conducted in Japan in 1929.

	Previously unvaccinated (n= 44)		Previously vaccinated (n= 44)	
Outcome (vaccine 'take')	19 (43.3 %)		12 (27.3 %)	
Exposure variable:	Mean	SD	Mean	SD
Age (months)	23.8	18.0	54.2	20.4
Interval[a] (days)	9.8	10.0	5.6	2.2
Number of successful reactions [b]	3.8	0.9	3.8	0.5

[a] Interval between the first and second vaccinations; [b]Number of vaccine 'take' in the first round primary (or re-) vaccination

Table 2
Univariate analysis using non-parametric independent two-group comparisons: exposure variables in relation to vaccine 'take'.

	Previously unvaccinated		Previously vaccinated	
Exposure variable	z-statistic[a]	p-value[b]	z-statistic[a]	p-value[b]
Age	-0.75	0.45	-0.36	0.72
Interval[c]	-5.27	<0.01	-3.35	<0.01
Number of successful reactions [d]	-0.46	0.65	-0.08	0.93

[a]Wilcoxon-Mann-Whitney, two-sample z-statistic was used to test the hypothesis that two unmatched samples were from populations with the same distribution; [b]Two-sided; [c] Interval between the vaccinations; [d]Number of vaccine 'take' at the first round primary (or re-) vaccination

interval required was estimated to be 6.4 days (95% CI: 5.8, 7.1) and 4.3 days (95% CI: 4.1, 4.7) for the previously unvaccinated and vaccinated groups, respectively. The χ^2 test revealed no significant deviation between observed and predicted frequency ($\chi^2 = 3.05$, $p = 0.08$ and $\chi^2 = 2.70$, $p = 0.10$, respectively). According to the obtained logistic fits (Fig 2), the probability of vaccine 'take' for both groups becomes extremely small when the median interval is larger than the obtained upper 95 % CI estimates.

DISCUSSION

This study investigated the historical records of a smallpox revaccination study conducted in the early 20th century in Japan. The study involved 44 previously vaccinated and 44 unvaccinated children, all of whom received two vaccinations at different intervals. The interval between vaccinations was significantly associated with vaccine 'take' after revaccination (second round vaccination). Those in the previously vaccinated group were older than those in the unvaccinated group; however, this was expected since primary vaccination is compulsory above a certain age. And age was not associated with vaccine 'take'. Based on a logit model, maximum likelihood estimates of the time required to develop immunity were 6.4 days for the previously unvaccinated and 4.3 days for the previously vaccinated groups. Since these estimates reflect the time between vaccination and dis-

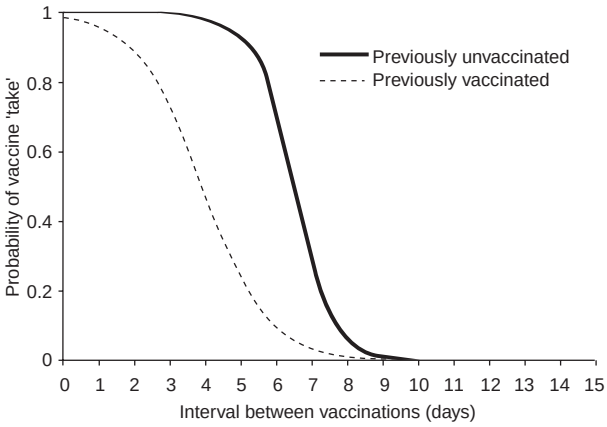

Fig 2–Logit model showing the probability of vaccine 'take' stratified by the interval between vaccinations. Probability of vaccine 'take' for those previously vaccinated (dotted line) and unvaccinated (solid line). The probability declines earlier for those previously vaccinated than unvaccinated, indicating that prior vaccination shortened the time to acquire vaccine-induced immunity.

ease onset (as measured by the presence of a major reaction in this study), it is difficult to use the results shown here to determine the maximum time period within which individuals should be vaccinated after actual exposure to smallpox. In other words, clarification requires elucidation of the time between exposure and vaccination. However, assuming the deterministic mean of the incubation period is 12 days, crude interpretation of the obtained estimates indicates that vaccination of exposed individuals is necessary within at least 5.6 days after exposure. Moreover, for those previously vaccinated, preferably within a few years, as in this study, vaccination within a maximum of 7.7 days after exposure is expected to result in 50% protection against the disease. Therefore, prior vaccination extended the maximum time interval by 2.1 days.

The obtained findings are consistent with the observations of Lyons and Dixon (1953) who proposed an approximate cut-off point of at least 7 days after exposure. It is worth noting that Ricketts implicitly provided the exact same assumption in his historical documentation in the early 20th century, published before explicit descriptions were obtained from direct observations (Ricketts and Byles, 1908). After wide distribution of this information by Dixon (1962), this assumption was partly confirmed by comparing the frequency of smallpox cases dichotomized at a rather optimistic cut-off point of 10 days after exposure (Mack *et al*, 1972). On the other hand, a statistical model implied that vaccination was only effective within 3.2 days after exposure (Eichner and Schwehm, 2004), and Delphi analysis revealed that post-exposure vaccination could be assumed to be 80-93% effective during the first 3 days after exposure and 2-25% thereafter (Massoudi *et al*, 2003). This suggestion that 'vaccination within 72 hours promises protection' was also widely accepted during the Smallpox Eradication Program (Dixon, 1962; Rao, 1972). This assumption is also supported by recent laboratory evidence demonstrating that the cell-mediated response within 4 days after exposure might have a protective effect (Kennedy *et al*, 2004). Nevertheless, the above pessimistic estimates are also consistent with the present findings, especially the logistic fit results of the previously unvaccinated children (Fig 2). Here, the probability of vaccine 'take' declined very close to zero at more than 9 days after primary vaccination, indicating that the probability of escaping disease is perhaps extremely high during the first 3 days after exposure.

Although the absence of vaccine 'take' at revaccination could imply immunity, the Expert Committee on Smallpox of the World Health Organization (WHO) documented that interpretation of a major reaction to revaccination was sometimes difficult (World Health Organization, 1964; Fenner *et al*, 1988). The major reason for this is that in field practice a negative response to revaccination after sev-

eral months or years has often been mistaken as evidence of immunity (World Health Organization, 1964). Nevertheless, Dr Oyamada successfully overcame this problem by evaluating revaccination performed several days after first round primary (or re-) vaccination, rather than estimating the duration of vaccine-induced immunity after two vaccinations conducted at longer intervals. Second, in evaluating the reaction to revaccination, it was previously considered technically difficult to give definite opinions as to the expected actual vaccine 'take' (Rao, 1972). Thus, for reaction interpretation, the WHO committee proposed an original definition consisting of 'major' and otherwise 'equivocal' reactions whereby the efficacy of the latter is doubtful and suggests the requirement of further revaccination (World Health Organization, 1964; Fenner *et al*, 1988). Even though Dr Oyamada's experiment took place earlier than this recommendation, he successfully excluded 'equivocal' reactions in his study design. As a result, I believe the examination of Dr Oyamada's study is useful for clarification of the immune response immediately after vaccination.

Since the vaccine used at the time of this study did not undergo virological evaluation, it might therefore have been of low potency (Cockburn *et al*, 1957). While this might partly explain why Dr Oyamada's experiment resulted in unsuccessful replication, recent studies have demonstrated that even diluted vaccines can provide immunogenicity (Frey *et al*, 2002; Talbot *et al*, 2004). Thus, it is speculated that even a low potency vaccine is more efficacious than previously thought. Moreover, the obvious association between vaccine 'take' and the interval between vaccinations confirms the appropriateness of Dr Oyamada's study design. Although it is perhaps imprecise to simply use vaccine 'take' to determine immune status (Rosenthal *et al*, 2001), it is evident that only those reactions correlated with clinical results are useful in estimating disease protection. In other words, it is rather difficult to use laboratory experiments alone as an interpretation of actual protection. Moreover, in the absence of smallpox, laboratory evidence relies on limited classical evaluation of pock counts to disentangle the correlation (Cockburn *et al*, 1957), and consequently, results of vaccine 'take' are perhaps more helpful.

One of the biggest advantages of this study in evaluating the time-period required to develop immunity is the presence of a denominator for estimation (*eg*; that is, the number of protected individuals). Although the sample size for the estimation was small (and thus, variance of the maximum likelihood estimates are potentially biased), the logit model obtained using the interval between vaccinations as an explanatory variable enabled interpretation of the discrepancy among previous suggestions. One important conclusion that can be drawn from the presented results is that, in the event of a bioterrorist attack, vaccination should be conducted within at least 3 days after exposure to ensure protection, and within at least 5 days after exposure (or 7 days for previously vaccinated individuals) to provide a certain level of protection; the probability shows a dramatic decline hereafter. Given the agreement with previous laboratory findings (Frey *et al*, 2002; Kennedy *et al*, 2004; Talbot *et al*, 2004), these given intervals are recommended to ensure the protective effects of post-exposure vaccination.

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Since vaccination induces a specific long-lived response to infectious agents, it becomes a basis for preventive medicine. In a human population, immunological memory of individuals shapes the so-called herd or community immunity crucial for national health.Â Among the greatest achievements of that time was complete eradication of smallpox and partial elimination of measles, polio, diphtheria, tetanus and tuberculosis (reported in a few developed regions in Europe and North America).Â - relevance: samples should be representative of the studied group or object; studies should be conducted in accordance with the principles of modern epidemiology; - continuity: continuity of surveillance, i.e. a possibility to reproduce a study over the course of a few years Vaccine-induced immunity fades over time and the loss of protection differs with each disease. Mathematical models, based on cases from outbreaks as well as antibody levels and their decay, project how long immunity lasts in people who have received a full vaccine regimen.Â The HPV estimate is based on a model of the Cervarix vaccine. Waning pertussis immunity is estimated from outbreak cases per year following a fifth dose of vaccine and before a subsequent booster. The smallpox estimate draws on data from six outbreaks a century ago and assesses protection from disease, not infection. Many vaccine immunogenicity trials are conducted in an equivalence or nonin-feriority framework. The objective of such trials is to demonstrate that the immuno-genicity of an investigational vaccine is comparable or not less than that of a control vaccine. In Chap. 6, the statistical analysis of such trials is explained, both for trials with an antibody response as endpoint and trials with seroprotection or serocon-version as endpoint. The standard analysis of lot consistency data is known to be conservative, but a simple formula is presented which can be used to decide if the lot sample sizes