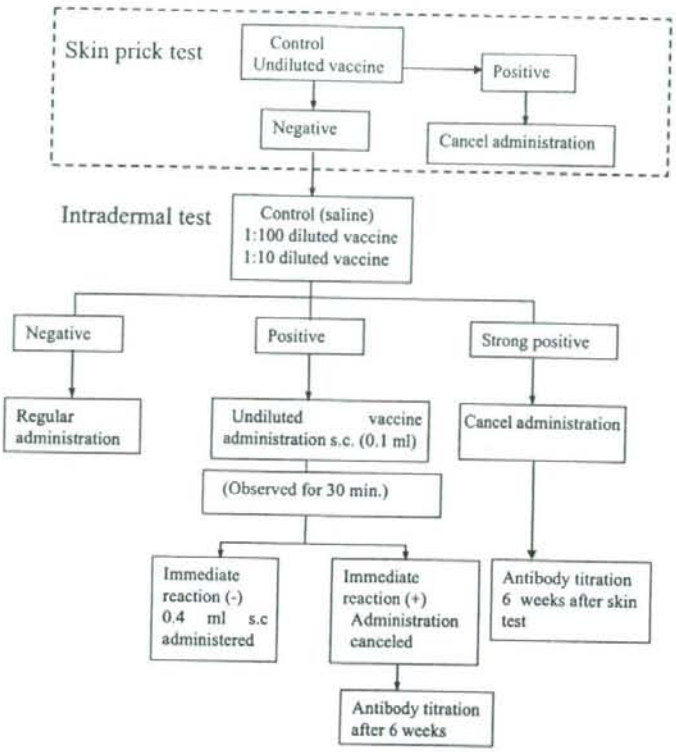

Fig. 4. Protocol of vaccine administration to high-risk allergic children (Protocol II). Intradermal test yielding the following results and assessments: wheals of 15 mm or more, or redness of 40 mm or more in diameter indicating a strong positive response; wheals of 9 mm to less than 15 mm, or redness of 20 mm to under 40 mm indicating a positive response; wheals of 5 mm to under 9 mm, or redness of 11 mm to under 20 mm indicating a doubtful positive response; a negative response being indicated by whealing/redness identical to that of the control. When the vaccines were administered, subjects with doubtful positive responses were added to the negative response group. Skin prick test results were considered positive if the resulting wheal measured 3 mm or more in diameter, or was at least twice the size of the reaction to the control.

score of class 2.2 ± 1.1. Comparing the two groups, it can be seen that there was a significant difference in both the total IgE levels and the egg white-specific IgE RAST scores. Upon assessment of the test results of subjects in whom, administration of vaccine was continued and those in whom it was discontinued, the former group had an average total IgE level of 1407.1 ± 1595.2 IU/ml and an average egg white-specific IgE RAST score of class 3.3 ± 0.8, and the latter group had an average IgE level of 2783.7 ± 3612.4 IU/ml and an average egg white-specific IgE RAST score of class 4.0 ± 0.9. There was not a significant difference in either total IgE levels or egg white-specific IgE RAST scores.

4.1.2. *Influenza vaccine*

Fig. 5b compares the responses to the simultaneously conducted intradermal 1:10 and 1:100 diluted vaccine tests in the 30 subjects. One subject showed a strong positive response (positive to the 1:100 dilution and strongly positive to the

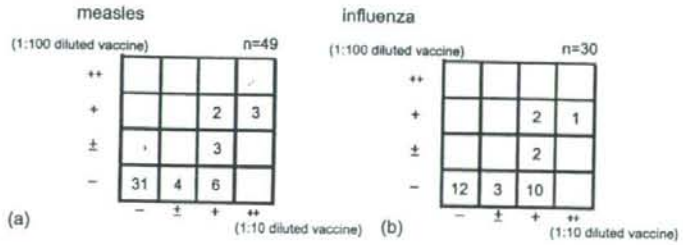

Fig. 5. Relations between vaccine concentrations (1:10 vs. 1:100 diluted vaccine) and intradermal reactions. Numbers in the cells show numbers of patients. Symbols signify the following: (−) negative, (±) false positive, (+) positive and (++) strong positive.

Table 1
Clinical characteristics of individuals whose vaccine administration in Protocol I (measles) was not completed

Patient number	Age (Y/Mo)	Sex	Diagnosis	History of immediate allergic reaction	Total IgE (U/ml)	IgE RAST score of egg white	Skin test result of diluted measles		Administration	Measles Ab titer
							1:10	1:100		
1	1 Y 3 Mo	M	AD, FA (egg)	–	863	4	++	+	Canceled	32× (HI)
2	1 Y 4 Mo	M	AD, FA (egg)	+	731	5	++	+	Canceled	128× (HI)
3	1 Y 5 Mo	M	BA, AD, FA (multiple)	–	330	3	++	+	Canceled	64× (HI)
4	2 Y 3 Mo	M	BA, AD, FA (multiple)	+	589	4	+	–	0.1 ml	32× (HI)
5	2 Y 3 Mo	M	BA, AD, FA (multiple)	–	809	3	+	±	0.1 ml	32× (HI)
6	4 Y 11 Mo	M	BA, AD, FA (egg)	+	6953	5	+	+	0.1 ml	IgG (EIA) 22.2

Y, year; Mo, month; M, male; AD, atopic dermatitis; FA, food allergy; BA, bronchial asthma.

1:10 dilution), 3 subjects (10%) tested positive to the 1:100 dilution, and 15 subjects (50%) tested positive to the 1:10 dilution.

As specified in the protocol, vaccine administration was discontinued in the one subject who had a strong positive reaction in the intradermal tests. The 14 subjects testing positive to the 1:10 diluted vaccine proceeded to be administered vaccine in instalments, and 3 of them showed local reactions at the 0.1 ml stage (first instalment), leading to cancellation of further administration, at their families' requests. The other 11 subjects showed no adverse reactions and were given the remaining vaccine doses.

Table 2 displays the profiles of a subject who had a strong positive reaction, and of the three subjects in whom administration was discontinued. One of the latter three tested positive in the intradermal tests with 1:10 diluted vaccine but negative to the 1:100 dilution.

None of the 15 subjects who proceeded to have the standard amount of vaccine administered, showed any adverse reactions to the intradermal tests.

On evaluation of the total IgE levels and egg white-specific IgE RAST scores of subjects with a positive reaction and those with a negative reaction to the intradermal test for influenza vaccine, the former (positive) subjects had an average total IgE level of 555.1 ± 797.9 IU/ml and an average egg white-specific IgE RAST score of class 3.0 ± 2.0, while the latter group had an average total IgE level of 683.4 ± 832.4 IU/ml and an average egg white-specific IgE RAST score of class 2.5 ± 1.4. Neither the total IgE levels nor the egg white-specific IgE RAST scores of the subjects having positive reactions showed a significant difference from those of subjects who reacted negatively.

Of the positive cases other than strong positives, those cases in which vaccination was possible and those in which vaccination was terminated showed respective mean values for total IgE level of 449.3 ± 583.9 and 1316.5 ± 1859.0 IU, and respective egg white-specific IgE RAST scores of classes 2.7 ± 0.6 and 3.3 ± 3.1 on average, but tyhere was no significant difference in either variable.

4.1.3. Other vaccines

Those children that gave a positive intradermal reaction to vaccine diluted 1:100 acccounted for 0/7 with varicella, 0/8 with mumps, 1/5 with rubella, 0/3 with Japanese encephalitis, and 0/1 with DPT; and those with positive intradermal reactions to vaccine diluted 1:10 numbered 2/9 with varicella, 3/8 with mumps, 4/8 with rubella, 0/4 with Japanese encephalitis, and 1/3 with DPT. All of those reacting positively with vaccine diluted 1:100 were also positive with a 1:10 dilution.

All of the positive intradermal reaction cases were also vaccinated with the standard amount of vaccine in split doses, but no adverse reactions were seen.

Table 2
Clinical characteristics of individuals whose vaccine administration in Protocol I (influenza) was not completed

Patient number	Age (Y/Mo)	Sex	Diagnosis	History of immediate allergic reaction	Total IgE (U/ml)	IgE RAST score of egg white	Skin test result of diluted measles		Administration
							1:10	1:100	
1	0 Y 10 Mo	M	AD, FA (egg)	+	2	0	+	+	Canceled
2	1 Y 9 Mo	M	AD, FA (egg)	+	ND	4	+	–	0.1 ml
3	3 Y 2 Mo	M	BA, AD, FA (multiple)	+	2631	6	+	+	0.1 ml
4	8 Y 11 Mo	M	BA, DA (multiple)	+	197	ND	++	+	Canceled

Y, year; Mo, month; M, male; AD, atopic dermatitis; FA, food allergy; BA, bronchial asthma.

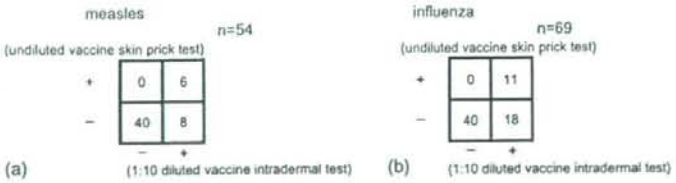

Fig. 6. Relations between skin test (1:10 diluted vaccine intradermal test vs. undiluted skin prick test) and skin test reactions. Numbers in each cell show numbers of patients. Symbols signify the following: (−) negative and (+) positive.

4.2. Comparison of undiluted vaccine prick tests and intradermal tests with 1:10 diluted vaccine

4.2.1. Measles vaccine

Fig. 6a presents the results of 54 cases of skin tests with measles vaccine, which were carried out at the same time as intradermal tests with the vaccine diluted 1:10 and prick tests with the undiluted vaccine. Undiluted vaccine prick tests yielded positive results in six subjects (11.1%), and positive intradermal reactions to 1:10 vaccine were seen in 14 (25.9%). The former (6) subjects were all included among the latter 14. Split vaccination of the 14 subjects who had positive intradermal skin tests with 1:10 vaccine resulted in local adverse reactions in 7 of the subjects. Five of these seven had negative prick test results, and in one of the five, local wheals appeared during the split vaccination, and so this procedure was terminated. The final number of terminated split vaccinations was two. The 40 subjects with negative results in the intradermal tests with vaccine diluted 1:10 showed no adverse reactions and could then be vaccinated with the vaccine.

4.2.2. Influenza vaccine

The results of the skin tests in the 69 subjects simultaneously administered prick tests with influenza vaccine and intradermal tests with influenza vaccine diluted 1:10 are presented in Fig. 6b. Eleven subjects (15.9%) had positive results in the undiluted vaccine prick test, as did 29 (42.0%) in the intradermal test with vaccine diluted 1:10. In both tests, the number of subjects with negative results was 40. Those who had positive results in the prick test were all positive in the intradermal test. Adverse reactions were seen after vaccination in four subjects, all of whom had positive intradermal reactions with 1:10 diluted vaccine, but negative results in the undiluted vaccine prick test. The number of subjects in whom vaccination was finally terminated was four (5.3%).

4.2.3. Other vaccines

In all subjects, negative results were obtained in prick tests of undiluted rubella (two subjects) and mumps (five subjects) vaccines.

In intradermal tests with vaccines diluted 1:10, the two subjects tested with rubella vaccine gave negative results, while one of the five subjects tested with mumps vaccine gave a positive result.

In the vaccinations in all cases, the standard dose was administered, and no adverse reactions were afterwards observed.

5. Discussion

With the aim of ensuring safe vaccination of children with serious allergies, we endeavored to establish a method of observing skin reactions with vaccines in such a way as to provide some warning of possible allergic reactions such as anaphylaxis. When we tested measles vaccine, as a typical live vaccine and influenza vaccine, as a typical inactivated vaccine, by trying to elicit intradermal reactions with each vaccine diluted 1:100 and 1:10 and by carrying out skin prick tests with undiluted vaccine, it be came clear that, in all cases, the method using the intradermal reaction to administration of a vaccine diluted 1:10 was superior to the others for predicting an allergic reaction.

Already at the time of the revision of the regulations governing vaccination, in the "Guidelines for Vaccination" published in 1994 [6], the method of Herman et al. [1] was recommended as a method of predicting adverse reactions when allergic children are vaccinated, but it was far too complicated and time-consuming for use in everyday clinical practice, and so a simpler and more accurate method was sought. Also, no studies have appeared that use various methods together for evaluating the relative merits of intradermal reactions in a large group of children with allergies. The present study, conducted in multiple institutions in different regions, examined three types of skin reactions to measles vaccine, influenza vaccine, as well as varicella vaccine, mumps vaccine and rubella vaccine in a total of 369 children with allergies after both their informed consent and that of their families had been obtained.

In the study of the measles vaccine that was used as a typical live vaccine, cases which gave positive reactions both in the prick test and in the intradermal reaction test that used vaccine diluted 1:100 were among those that were positive in the intradermal reaction test with vaccine diluted 1:10. Moreover, in regard to the influenza vaccine used as a typical example of an inactivated vaccine, the intradermal reaction-positive prick test subjects and the reaction-positive 1:100 dilution vaccine subjects were included among the subjects with positive intradermal reactions to 1:10 dilution vaccine. These facts either suggest that, as a method of predicting an

allergic reaction, the use of intradermal reactions to vaccine diluted 1:10 is more inclusive and superior to other methods, or they indicate that, the reactions being non-specific, the number of positive subjects increased.

First, in all subjects negative for intradermal reactions to vaccines diluted 1:10, whether these were measles or influenza vaccines, adverse reactions were not observed, and the intended vaccinations were administered. However, in the 11 children who showed positive intradermal reactions to measles vaccine diluted 1:10, the vaccine was administered in split doses, in accordance with the protocol, but 3 of these subjects manifested strong local reactions during the split vaccination, so it was not advisable to continue, and not all of the standard dose could be administered. Only one of these three subjects gave a positive intradermal reaction to the vaccine diluted 1:100. The protocol was followed, and vaccinations were not given to any of the three strongly positive subjects. Furthermore, when the 14 children who were positive for intradermal reactions at an influenza vaccine dilution of 1:10 were given split doses, 3 showed local reactions and so administration was curtailed. Two of these three had positive intradermal reactions to vaccine diluted 1:100, and one, a negative reaction. No adverse reaction was seen in the remaining 11, and the split vaccination was continued until the end. In the one child that had a strong positive reaction, vaccination was terminated.

Of the subjects mentioned above who were negative for intradermal reactions to 1:10 dilutions of measles and influenza vaccines, there were none who showed adverse reactions when the vaccines were administered, and since adverse reactions were seen in some, but not all, of those who were positive for reactions during the split administration, we considered that the intradermal reaction to 1:10 vaccine offers a reliable guide for predicting an adverse reaction, and therefore for whether to recommend the vaccination of an allergic child.

Incidentally, in relation to whether a positive intradermal reaction is useful as a predictor of an allergic reaction due to vaccination. Ogura et al. [7] reported a case in which anaphylactic shock was induced by vaccination of subjects positive for intradermal reactions to measles vaccine diluted 1:10, and considered that intradermal reactions were effectively predictive of adverse reactions. Moreover, one study reported that, in a child in whom anaphylaxis was induced by pneumococcal vaccine, skin tests gave positive results, and that skin tests are useful for diagnosing suspected IgE-dependent hypersensitivity reactions in children [8].

The backgrounds of the children who acted as subjects for vaccination in the present study indicated that 37% had total IgE levels of at least 500 IU/ml for measles vaccine and that 75% had egg white-specific IgE scores of 3 or above. In subjects with positive intradermal reactions to measles vaccine diluted 1:10, the IgE level and the egg white-specific IgE RAST score were significantly higher than those with negative reactions, but in many subjects, including those with positive reactions, it was also possible to carry out vaccination quite safely using standard doses, but since, among the positive reaction cases, there were no differences in IgE and egg white-specific IgE RAST scores in those in which vaccination was possible and those in which it was cancelled, the fact that IgE and RAST are high is no reason for not administering a vaccination. In regard to this, it has been reported in a study in children with serious allergies that no statistical difference was found between adverse reactions due to vaccination in cases of egg allergy and in those without egg allergy [2]. However, this is not a problem of ovalbumin alone, but also, undeniably, of denaturation of antibodies during the manufacturing process and of the presence of contaminants.

Immediate-type adverse reactions are almost always confined to localized rubor and wheals, with local irritation, and it is rare for them to give rise to anything of the systemic nature of anaphylactic shock. The gelatin added to a vaccine as a preservative became a social problem as the cause of adverse reactions at the time of vaccination [9–11], but more recently, as a result of removal of the gelatin component from the vaccine, the frequency of adverse reactions has decreased. In reports of anaphylaxis after vaccination with a live vaccine, the frequency of anaphylaxis due to measles vaccine in 1996 was 8.13 per 100,000 doses, but reports from the year 2000 indicate a fall to the level of 0.3 per 100,000. Currently, although not all the antigens causing the adverse reactions have been identified, it is now possible that the remaining causal antigens are those that differ from individual to individual.

In six cases in which vaccination with vaccine for measles was terminated, the viral antibody titer was determined after intradermal testing, and a rise was seen in the measles antibody titer. In a follow-up performed 2 years later, there were cases in which the antibody titer was maintained. The dendritic cells, which present antigens on their surface, are present in large numbers, and it has been reported in relation to infections and immune reactions on the skin that the skin is an important site of sensitisation [12,13]. Consequently, it is considered that antibody production is also induced by the antigens introduced into the skin by vaccination.

There have already been reports on determining the antibody titer [14] in intradermal testing of vaccines and on vaccination of small amounts of material, and others in which antibody titers are obtained after intradermal testing with a 1/10 amount of vaccine [15]. However, many unanswered questions remain as to whether or not lifelong immunity is maintained, and further investigation is needed.

Especially in very young children who have never, because of their atopic condition, eaten egg, there are cases without any history of anaphylaxis. So it is not known whether these children are at risk of anaphylaxis or not. According to a recent report in the United States, there were five cases of vaccine-associated anaphylaxis after administration of 7,644,049 vaccine doses between 1991 and 1997, and 3 of these 5 cases occurred in children with no history of anaphylaxis [16]. Whichever is true, since there are at present no other methods for predicting adverse reactions attendant

upon vaccinations, it is necessary to be aware of intradermal reactions as a means of predicting adverse reactions to vaccines. It is especially important to avoid vaccination after a strong positive reaction is obtained in an intradermal test using vaccine diluted 1:10. If the result of such a test is positive, a split vaccination should be used; if negative, vaccination must be recommended. In this way, we shall not cease to hope that every child, even an allergic child, will be able to obtain vaccinations safely.

6. Conclusions

A multi-center trial to study skin tests using undiluted and diluted vaccines was conducted on children with allergies. Intradermal tests conducted using vaccine diluted 1:100 and prick tests with undiluted vaccine were unable to pick up all of the patients at high risk for vaccination, and were thought to be inadequate for clinical purposes. In skin tests using only vaccine diluted 1:10, screening was judged to be entirely feasible.

The skin test reactions and the reactions obtained with actual vaccination did not necessarily correspond. However, after full informed consent is obtained, it is important for the physicians to reassure the parents or guardians of the child about the safety of the procedure, and to encourage them to have the vaccination done. General practitioners and non-specialist clinics tend to be less suitable for giving positive advice and encouragement, and in order to carry out the vaccinations more safely and smoothly, the method of selecting high-risk patients is preferable.

Therefore, it is to be hoped that general distribution will be made of 1:10 dilutions of vaccines. In the present study, we performed skin tests of vaccines in 369 allergic children including severe cases, but no severe adverse reactions due to the skin tests were seen, and in cases in which split vaccinations were administered, the protocol was followed and no problems of safety were encountered. With reference to the results obtained in this study, at the level of the primary care physician, simple and safe screenings can be done, and the development of a vaccination protocol that includes indications for skin tests and that enables as many allergic children as possible to be vaccinated safely, is now necessary.

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