表3 麻疹肺炎と脳炎の病型

| 病型 | 特徴 | 治療・予後 |
|---|---|---|
| 1. 肺炎 | | |
| ・ウイルス性肺炎 | 免疫反応による間質性肺炎<br>AC ブロック，KL-6 の上昇 | 成人では重症<br>パルス療法 |
| ・巨細胞性肺炎 | 主として免疫不全者に認める<br>麻疹ウイルスの直接浸潤 | 予後不良 |
| ・細菌性肺炎 | 細菌の二次感染による肺炎 | 抗生剤投与 |
| 2. 脳炎 | | |
| ・麻疹後脳脊髄炎 | 自己免疫的機序による組織障害 | ステロイド投与 |
| ・麻疹封入体脳炎 | 主として免疫不全者に認める<br>麻疹ウイルスの直接浸潤 | 予後不良 |
| ・SSPE | SSPE ウイルスによる直接浸潤<br>持続感染 | 予後不良 |

SSPE：亜急性硬化性全脳炎　　（庵原[3] 2006 より引用一部改変）

① 発疹出現 7～14 日後に免疫学的機序により発症する麻疹後脳脊髄炎（急性散在性脳脊髄炎（ADEM）に類似），② 発疹出現 1 か月後頃に麻疹ウイルスの直接浸潤により発症する麻疹封入対脳炎，③ 発疹出現 8～10 年後に麻疹ウイルス M 蛋白が変異した SSPE ウイルスの持続感染により発症する SSPE の 3 種類がある[8]。

発症病態から麻疹後脳脊髄炎の治療にステロイドパルス療法が試みられているが，治療成績は不明である。麻疹封入対脳炎は T 細胞系の免疫不全宿主に認める合併症で，予後不良である。SSPE の治療としてイノシプラノベクスの経口投与，インターフェロン α の脳室内投与が行われるようになり，SSPE の進行は抑制されるようになったが，生命予後の改善は認められていない。なお，麻疹ワクチンの接種率向上により，SSPE 発症者数は激減している[9]。

角膜軟化症・角膜潰瘍は，栄養状態が悪かった時代にはわが国でも認められた合併症であり，現在はビタミン A が欠乏している途上国で多く認められている。細菌の二次感染を合併すると失明するリスクが高くなる。

細胞性免疫の一時的低下（約 4 週間）は，麻疹ウイルスが感染した樹状細胞やリンパ球がアポトーシスをきたすために発症する。結核が蔓延していた時代には，麻疹後に結核を合併する児を認めていたが，麻疹と結核の流行が激減した現在では，結核の合併は認められていなくなっている。

麻疹による合併症の発症を予防する最高の手段は，麻疹ウイルスを含む生ワクチンを 95%以上の接種率で 2 回接種し，流行を排除することである[10]。中途半端な接種率を続けていては，流行時に成人の麻疹発症リスクを高めるだけである[2]。

表4 麻疹抗体レベルと感染制御

| 免疫レベル | NT 抗体価* | 臨床症状 | 抗体反応 |
|---|---|---|---|
| 高い | ≧32 倍 | なし | なし |
| 中等度 | 4～16 倍 | なし | 上昇† |
| 低い | 2 倍 | 軽症発症‡ | 上昇 |
| 陰性 | <2 倍 | 典型発症‡ | 上昇 |

*大部分の人で認められる抗体レベル。
†不顕性感染により抗体のブースターを認める。
‡顕性感染（麻疹では感染予防よりも発症予防が大切）。
（注）EIA-IgG 抗体価 2.0EIA 単位，PA 抗体価 128 倍が NT 抗体価 2 倍に相当する。

## ⑦ 症状経過，検査所見からみた予後判定

T 細胞系の免疫不全宿主では，麻疹ウイルスの増殖が持続し，巨細胞性肺炎や封入対脳炎を合併するため予後不良である。また，成人が麻疹に罹患すると免疫応答が強いため，ウイルス性肺炎を合併するリスクが高く，注意深い観察が必要である。

麻疹ウイルスに曝露されたとき，ホストが持っている免疫レベルにより，臨床反応および免疫反応が異なっている（表 4）。特異免疫が陰性の場合は，典型的な臨床像を呈するとともに抗体は陽転化する。免疫レベルが低い場合は軽症に経過すると同時に免疫は賦活され（clinical infection，顕性感染），免疫レベルが中等度の場合は臨床症状を呈

しないが免疫は賦活され(subclinical infection, 不顕性感染), 免疫レベルが高い場合は感染が阻止されるため免疫も賦活されない。臨床症状の重さと周囲への感染力は関係しており, 修飾麻疹例の周囲への感染力は典型例と比べ軽減している。

麻疹に対する感染防御には, 抗体だけではなく細胞性免疫や粘膜免疫も働いていること, ヒトはヘテロな集団であることなどから, 抗体の顕性感染予防レベル, 不顕性感染予防レベルをクリアに線引きすることは困難であり, 報告者によってもその値は異なっている。現在のところ, 大部分のヒトの顕性感染を予防できる抗体レベルは, 120～200mIU/ml, 不顕性感染を予防できる抗体レベルは, 500～1,000mIU/ml とされている[11~13]。この国際単位を麻疹 NT 抗体に当てはめると, 顕性感染を予防する抗体レベルは $2^2$(4 倍)に相当し, 不顕性感染を予防するレベルは $2^5$(32 倍)に相当する(表 4)[14]。EIA-IgG 抗体は, EIA 価を 22.2 で除した値が国際単位(IU/ml)に近似し[15], 120mIU/ml は 2.66EIA 価, 200mIU/ml は 4.44EIA 価に相当する。

現在, わが国で麻疹の免疫状態を調べるために用いる麻疹抗体測定方法として優れているのは, NT 法と EIA 法であり, 2007 年の 6 月から一部のコマーシャルラボで測定が可能となった PA 法も免疫状態を調べるのに優れた方法である。EIA 法と PA 法は麻疹抗原に結合する抗体の蛋白量を測定しているため, 感染防御にかかわる抗体を直接測定していない問題点はあるが, NT 法 4 倍にほぼ相当する抗体価は, EIA 法では 4.0EIA 価, PA 法では 256 倍である[16]。

2007 年における成人での麻疹流行後, 医療関係者・教育保育関係者や医療・教育・保育の実習にかかわる学生の麻疹抗体を測定し, 感染のリスクがある人に麻疹ワクチン接種が勧められるようになった。麻疹ワクチン接種を勧める抗体価の基準に関しては, 安全を期して高い抗体価(8.0EIA 価: 360mIU/ml)を設定し, 発症リスクの高いヒトすべてにワクチン接種を勧める考え方と[15], 抗体測定とワクチン接種のコストを考え, 大部分のヒトの発症を予防できるレベル(120～200mIU/ml 未満)でワクチン接種を勧める考え方がある[11,12,14]。麻疹 NT 抗体 4 倍未満, EIA-IgG 抗体 4.0EIA 価未満, PA 抗体 256 倍未満が後者の麻疹ワクチン接種の適応となる抗体レベルである。


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**EL16—2　小児急性発疹症の臨床**

# ウイルス感染症診断に必要な検査とその読み方

庵原　俊昭

## 1．はじめに

ウイルスがホストに感染すると，ウイルスの直接侵襲とそれに対するホストの免疫応答により臨床症状が出現する[1]．ウイルス感染により臨床症状が出現したのがウイルス感染症であり，臨床症状の出現や症状の軽重に，生来免疫から獲得免疫までの総合的な免疫力が関与している．本稿では，ウイルス感染症診断時にウイルス学的診断が必要な場合や診断方法について解説する．

## 2．ウイルス検査が必要なとき（表1）

ウイルス検査が必要なときを表1に示した[2]．麻疹や風疹のように，ワクチンの接種率向上により流行が小さくなった疾患を診断するときや，新型インフルエンザ出現時の早期診断をするときなど，疫学的視点からウイルス学的確定診断が必要な機会が増加している．昨年度の麻疹流行時，県別の定点当たりの麻疹報告数と風疹報告数は有意の正の相関を示しており[3]，麻疹ワクチン接種後の軽症麻疹を風疹と誤って診断した可能性が示唆されている．なお，2008年1月から麻疹と風疹は全数報告となり，診断にあたっての実験室診断の必要性が示されている．

## 3．ウイルス学的診断方法

ウイルス学的診断方法には，病原体検査により診断する方法と血清抗体検査により診断する方法がある．目的に応じて両者の併用や使い分けが大切である．

### 1）病原体検査

病原体検査のゴールドスタンダードは，病巣からのウイルス分離である[4]．ウイルス分離にあたっては，適切な部位から適切な方法でサンプルを採取し，適切な方法で輸送することが大切である．病巣からウイルスが分離されず，病巣以外の部位からウイルスが分離されたときは，他のウイルス学的検査結果や臨床像などから総合的に診断する必要がある．なお，目的とするウイルスによりウイルス分離に用いる細胞が異なっており，コマーシャルラボにウイルス分離を依頼するときは，目的ウイルスを連絡することが大切である．

ベッドサイドで簡単にできるウイルス検査法として，抗原迅速検査がある．ウイルスの種類にかかわらず，多くの迅速検査では，$10^4$/ml以上のウイルス量があると陽性と判定される．

近代的ウイルス病原体検査法として，PCR法やLAMP法などウイルス遺伝子を検出する方法が開発され，更に感染したウイルス量を定量するreal time PCR法やreal time LAMP法が開発された．逆転写酵素（RT）でウイルスRNAをDNAに転換させてからウイルス核酸の増幅を図るRNAウイルスよりも，サンプルから抽出したウイルス核酸をそのまま増幅するDNAウイルスの方が，検出感度が良好である．また，増幅されるウイルス量とウイルス感染症の病像とは関連しており，ウイルス量が多いほど一般に重症である[5]．

### 2）血清抗体検査

血清抗体の測定方法には種々の方法がある[2]．ウイルス感染の感染防御に働く抗体を直接測定しているのが中和（NT）法であり，赤血球凝集抑制（HI）法も感染防御に関わる抗体を測定している．一方，酵素免疫（EIA）法や粒子凝集（PA）法は抗体の総蛋白量を測定しており，必ずしも感染防御に働く抗体を直接測定していない欠点がある．しかし，風疹HI抗体とEIA抗体はよく相関し（図1），麻疹NT抗体（100％細胞変性効果抑制にて判定）とEIA抗体の間においても，NT抗体64倍までは0.91の傾きでよく相関し，128倍を越えるとNT抗体と比較するとEIA抗体は低く表示されるが，両者は有意に相関している（図2）．以上の結果は，風疹や麻疹のEIA法は，NT法やHI法と同様に定量性があることを示している．

血清IgM抗体の測定は，全身性ウイルス感染症の初感染の診断に有用である．しかし，麻疹では発疹出現0～2日では，IgM抗体の陽性率は70～80％程度であり[6]，臨床経過から麻疹が強く疑われるときは，5病日以降に再検することが大切である．

全身性ウイルス感染症再感染時の急性期では，IgM

国立病院機構三重病院院長（小児科）
著者連絡先：（〒514-0125）津市大里窪田町357　国立病院機構三重病院小児科　庵原　俊昭

表 1　ウイルス検査を必要とするとき

①疫学的視点から検査を必要とするとき
- ワクチンなどにより流行規模が小さくなった感染症を確定診断するとき
- 地域流行しているウイルス感染症を診断するとき
- 患者を早期に発見し，防疫上の措置を行う必要があるとき
- 特定のウイルスに対する変異や薬剤感受性を調べるとき（例：インフルエンザ）
- 院内感染防止のためワクチン予防可能疾患に対する免疫状態を調べるとき

②患者の予後を推定し，治療方針を決定する必要があるとき
- 類似した病像を呈する感染症の鑑別診断を行うとき（例：肝炎）
- 治療が可能なウイルス感染症を診断するとき（特に診断が困難なとき）
- 免疫不全者（児）におけるウイルス感染症を診断するとき
- ワクチン後のウイルス再感染を診断するとき

③献血や手術時などにおいて他者への感染防止を図るとき（HBV，HCV，HIV）
④予防接種後に生じた臨床反応の原因を明らかにするとき

（文献 2．一部改変）

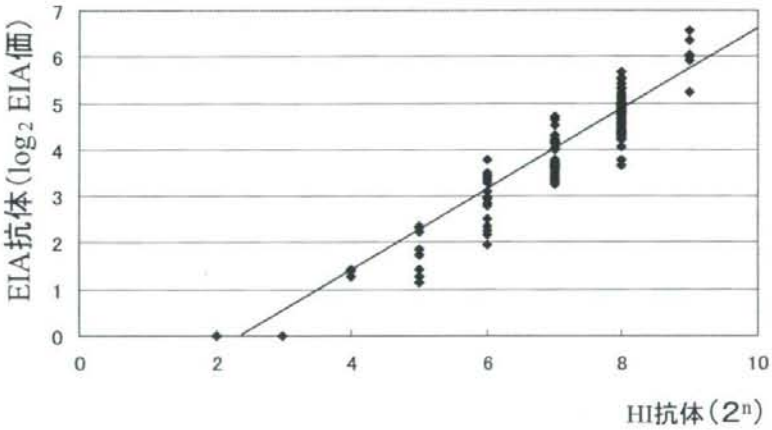
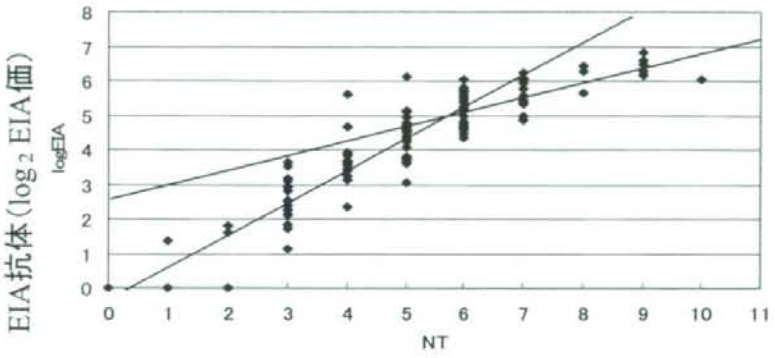



図 1　風疹 HI 抗体価（$2^n$）と EIA 抗体価（$\log_2$EIA 価）との関係

抗体陰性，IgG 抗体高値，IgG 抗体の avidity は強い，が一般的である．しかし，再感染時でも IgM 抗体は検出される時があり，このような症例は IgM 抗体が検出されない症例よりも，臨床症状がより典型的である[7]．再感染時のホストの免疫力に応じてウイルスが増殖し，増殖したウイルス量が多いと，IgM 抗体産生細胞が反応して IgM 抗体が産生されると考えられている．なお，ウイルス感染症初感染時には，抗原との結合力が弱い IgG3 分画に属する抗体が先ず出現し，その後 IgG1 分画に属する抗体が出現するが，再感染時には，抗原との結合力が強い（avidity が強い），中和活性が高い IgG1 分画に属する抗体が発症早期から上昇する[1]．

麻疹，風疹，ムンプス，水痘では，ワクチン後の免疫力の評価や感染既往の評価に血清抗体が測定される．免疫力の評価に適した抗体測定方法を表 2 に示した[2]．麻疹では PA 法，風疹では LA 法も免疫力の評価に適した方法である．これらの感染症では，抗体陽性レベル，発症予防レベル，感染予防レベルの抗体価がそれぞれ異なっている[8]．多くの人の発症予防レベル，感染予防レベルの抗体価を表 2 に示した．発症予防レ




$NT \leqq 2^6$: R=0.9263(P<0.0001)、Y=0.91X-0.24
$NT \geqq 2^6$: R=0.7059(P<0.0001)、Y=0.40x+2.72

図2　麻疹NT抗体価（$2^n$）とEIA抗体価（$log_2$EIA価）との関係

表2　MMRV抗体の発症予防レベルと感染予防レベル*

| 感染症 | 測定方法 | 陽性閾値 | 発症予防L* | 感染予防L* |
|---|---|---|---|---|
| 麻疹 | mNT | 2倍 | ≧4倍 | ≧32倍 |
| | EIA | 2.0 | ≧4.0 | ≧16.0 |
| 風疹 | HI | 8倍 | ≧16倍 | ≧64倍 |
| | EIA | 2.0 | ≧4.0 | ≧16.0 |
| 水痘 | IAHA | 2倍 | ≧4倍† | ≧32倍† |
| | EIA | 2.0 | ≧4.0† | ≧16.0† |
| ムンプス | EIA | 2.0 | ≧4.0† | ≧16.0† |

*多くの人(vast majority:98%)の発症予防レベルおよび感染予防レベル
†：麻疹，風疹の結果から推定されるレベル
(注) 麻疹mNT陽性者の20%程度はHI法では陰性になるが，麻疹HI陽性者全員はmNT法，EIA法で陽性

ベル以下の抗体価の人に対しては，ワクチンの追加接種が必要である．なお，発症予防レベル以上であっても，ワクチンの追加接種を希望する場合はワクチンを接種すべきである．多くの例でワクチン接種により免疫の賦活が期待される．

## 4. まとめ

ウイルス検査法とその読み方について解説した．対象とするウイルス感染症によりウイルス検査法は異なっている．目的に応じた適切な検査方法やサンプル採取方法を選択すべきである．

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# Dermal testing of vaccines for children at high risk of allergies

Kazuko Sugai [a,*], Ayako Shiga [a], Kenji Okada [b], Tsutomu Iwata [c], Hideo Ogura [d], Kihei Maekawa [e], Shumpei Yokota [a]

[a] *Department of Pediatrics, Yokohama City University, Fukuura 3-9, Kanazawa-ku, Yokohama, Kanagawa 239-0004, Japan*
[b] *Department of Pediatrics, National Hospital Organization Fukuoka National Hospital, Yakatabaru 4-39-1, Minami-ku, Fukuoka 811-1394, Japan*
[c] *Department of Child Health and Development, Tokyo Kasei University, Kaga 1-18-1, Itabashi-ku, Tokyo 173-8602, Japan*
[d] *National Hospital Organization Kochi National Hospital, Asakura-nishi-cho 1-2-25, Kochi 780-8077, Japan*
[e] *Kanagawa University of Human Services Faculty of Health and Social Work, Heisei-cho 1-10-1, Yokosuka, Kanagawa 238-8522, Japan*

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**Abstract**

Vaccinations for children with allergic diseases often need to be postponed or terminated because of the presumed risk of an immediate-type allergic reaction such as anaphylaxis. A new skin test protocol for predicting allergic reactions using the vaccine itself and the following stepwise vaccination method were developed and tested. Intradermal tests using 1:10 and 1:100 diluted measles vaccine indicated that the former was superior to the latter because a positive reaction against 1:10 diluted vaccine was found in 28.6% of 49 patients with severe allergic diseases including bronchial asthma, atopic dermatitis, food allergies and allergies to two or more allergens with high levels of IgE, as compared with the reaction against 1:100 diluted vaccine in 10.2% of the patients. Patients negative for 1:10 skin tests were safe from the following full-dose vaccine shots. Three patients showed very strong local reactions against measles vaccine, and avoided receiving the following full-dose shot. Positive reactions to skin tests of 1:10 diluted vaccine were found in 11 patients, who were given stepwise vaccinations. Three patients had adverse reactions, and two of them had been negative for 1:100 skin tests. In the case of influenza vaccine, skin tests were again more sensitive to 1:10 than to 1:100 diluted vaccine, because 3 out of 14 patients with positive reactions showed immediate-type adverse reactions against the following stepwise vaccinations, and 1 of them was negative for the 1:100 skin test. Moreover, the results of the skin prick test (undiluted vaccine) and the intradermal skin test (1:10 diluted vaccine) indicated that the latter was more useful in both cases of measles (54 patients) and influenza vaccine (69 patients). Overall, the skin test using 1:10 diluted vaccine was the more suitable for predicting an immediate-type reaction to measles and influenza vaccinations. Patients having negative 1:10 skin tests can be expected to show no adverse reactions to the remaining injections and even the positive subjects will complete the course of vaccine doses by the stepwise method.


*Keywords:* Dermal test; Allergic children; Vaccination

## 1. Introduction

Vaccinations should be readily available for children in order to safeguard their health. However, since vaccinations are artificial injections of antigens into the body and can elicit strong allergic reactions in some children, they are often postponed or completely avoided in children with allergic diseases, for fear of liability falling later upon the doctor.

However, skin tests to predict allergic reactions to vaccinations, such as the Herman et al. technique [1], the Ogura technique [2], and various methods devised by individual institutions specializing in allergology are used, but have not yet become widely adopted for reasons such as that the methods are too complicated. Moreover, they have not been subjected to strict controlled trials. Primary care physicians tend to be hesitant to prescribe vaccinations for allergic children,

* Corresponding author. Tel.: +81 45 787 2670; fax: +81 45 787 0461.
*E-mail address:* kazgoto@bc.mbn.or.jp (K. Sugai).

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probably because no simple evidence-based method exists. We have therefore devised a protocol that will allow primary care physicians to make proper judgments of safety, and where the risk of an allergic reaction is predicted, to ensure vaccination safety with the cooperation of major local hospitals and specialized hospitals. The effectiveness of, and possible adverse reactions to, this protocol were studied.

## 2. Subjects and methods

### 2.1. Subjects

The subjects were children suffering from allergic diseases who were drawn from outpatients of the pediatrics departments of following four institutes: Yokohama Minami Kyosai Hospital, Fukuoka National Hospital, Tokyo University, and Kochi National Hospital. They were chosen according to the presence of the following seven characteristics, and were subjected to skin testing for vaccination: (1) severe bronchial asthma (according to the classification [3] of the Japanese Society of Pediatric Allergy and Clinical Immunology), (2) severe atopic dermatitis (according to the Japan Dermatological Association classification [4]), (3) food allergy undergoing dietetic treatment by eliminating certain foods, (4) history of anaphylaxis, (5) history of drug allergy, (6) the presence of IgE antibodies specific for two or more types of food antigens, and (7) a history of immediate-type reactions after vaccination.

The criterion for diagnosis of food allergies is at least one of the following: (1) a history of immediate-type or a distinct delayed reaction after consumption of allergenic food(s) and (2) a positive reaction in either a food elimination test or a food tolerance test. Subjects with positive reactions in skin prick tests for specific foods were included in the study.

A skin test involving a vaccine and its diluted form was performed on the above children.

### 2.2. Comparative study of intradermal tests using 1:10 and 1:100 dilutions of vaccine

#### 2.2.1. Measles vaccine

Intradermal tests using 10× and 100× dilutions of measles vaccines were conducted on a total of 133 subjects (85 boys, 48 girls). Of the vaccines used, 66 were manufactured by Chiba Kessei Laboratories (Chiba, Japan), 41 by Takeda Pharmaceutical Company Limited (Tokyo, Japan), and 26 by the The Kitasato Institute (Saitama, Japan). This last group all contained 0.2% (w/v) gelatin (Plionex). Of the 133 subjects, 36 (27.1%) had a history of immediate-type allergy reactions including anaphylaxis. Of these, 24 had a history of sensitivity to chicken eggs.

There were 98 infants (73.7%) under 2 years of age; 107 (80.5%) with atopic dermatitis; 80 (60.2%) with food allergies; 36 (27.1%) with bronchial asthma, including those with other allergic diseases.

Of the 116 cases in which the total serum IgE level was measured, 80 cases (69.0%) showed levels less than 500 IU/ml, but in 19 subjects (16.4%), the level exceeded 1000 IU/ml. In addition, of the 113 subjects tested, 76 (67.2%) had specific IgE radio allergosolvent test (RAST) scores of class 3 or above to egg white (Fig. 1a).

#### 2.2.2. Influenza vaccine

Intradermal tests using 10× dilution and 100× dilution of influenza vaccine were conducted on 46 subjects (20 boys, 26 girls). Of the vaccines used, 23 were manufactured by the Research Foundation for Microbial Diseases of Osaka University (Osaka, Japan) and 23 by the Chemo-Sero-Therapeutic Research Institute (Kumamoto, Japan). The influenza vaccines produced by these two manufacturers were already gelatin-free at the time of this study. Of the 46 subjects, 21 (45.7%) had histories of immediate-type allergic reactions including anaphylaxis, and of these, 19 had a history of hypersensitivity to eggs.

In terms of age group, 15 subjects (32.6%) were under 2 years of age, 21 (45.7%) were between 2 and 6 years old, and 10 (21.7%) were 6 years of age and above. As for disease background, 36 subjects (78.3%) had had atopic dermatitis, 30 (65.2%), food allergies, and 29 (63.0%), bronchial asthma.

In 26 cases, or 61.9% of the 42 cases where the total serum IgE level was measured, this level was less than 500 IU/ml, but 13 subjects (31.0%) had a total serum IgE level above 1000 IU/ml. In addition, of the 41 subjects tested, 20 (48.8%) had an egg white-specific IgE RAST score of class 3 or above (Fig. 1b).

#### 2.2.3. Other vaccines

Intradermal tests on other vaccines were conducted on a small scale, using 10× and 100× dilutions. The tests were performed on the varicella vaccine in nine subjects (six boys, three girls), the mumps vaccine in eight subjects (five boys, three girls), the rubella vaccine in eight (seven boys, one girl), the Japanese encephalitis vaccine in four (two boys, two girls), and the diphtheria pertussis tetanus (DPT) vaccine in three boys.

Of the vaccines used, the varicella vaccine was manufactured in the Research Foundation for Microbial Diseases of Osaka University, the mumps vaccine (containing gelatin) by the Kitasato Laboratory, four samples of rubella vaccine by the Takeda Pharmaceutical Company Limited and four by the Research Foundation for Microbial Diseases of Osaka University (all of which were gelatin-free), the Japanese encephalitis vaccine by the Takeda Pharmaceutical Company Limited, and the (gelatin-free) DPT vaccine by the Research Foundation for Microbial Diseases of Osaka University.

The intradermal test for varicella vaccine was conducted on subjects aged 1 year and 3 months to 5 years and 8 months, of which three showed immediate-type allergic reactions including anaphylaxis and scoring between 24.1 and 6953 IU/ml in total IgE levels.

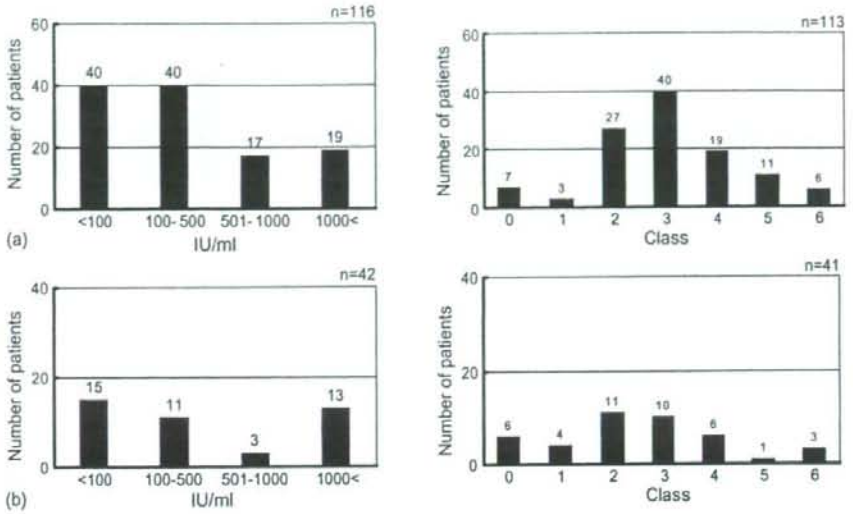


Fig. 1. Total serum IgE and IgE RAST scores of ovalbumin in patients tested in Protocol I. (a) Measles and (b) influenza.

The intradermal test for mumps vaccine involved subjects aged 1 year and 1 month to 9 years and 5 months, of which two showed immediate allergic reactions including anaphylaxis, with total IgE levels of 44.4 and 6953 IU/ml.

The age range of the subjects given the intradermal test for rubella vaccine was between 1 year and 4 months and 3 years and 6 months. Five of the children showed immediate-type allergic reactions including anaphylaxis, with total IgE levels ranging between 68 and 5654 IU/ml.

The intradermal test for Japanese encephalitis vaccine was performed using subjects aged 2 years and 2 months to 7 years and 1 month. Three of them showed immediate allergic reactions including anaphylaxis, with total IgE levels between 105 and 2478 IU/ml.

Subjects of the intradermal test for DPT vaccine aged between 9 months and 1 year and 2 months, and two showed immediate allergic reactions including anaphylaxis. The total IgE level was measured in only one subject, aged 1 year and 2 months, who showed a total IgE level of 1800 IU/ml.

### *2.3. Comparative study of undiluted vaccine prick tests and 1:10 dilution intradermal tests*

#### *2.3.1. Measles vaccine*

Undiluted vaccine prick tests and intradermal tests with 1:10 diluted vaccine were conducted on 76 subjects (47 boys, 29 girls).

Of the vaccines used, 58 were manufactured by Chiba Kessei Laboratories and 18 by the Takeda Pharmaceuticals, and all were gelatin-free. Seventeen subjects (22.4%) had a history of immediate allergic reactions including anaphylaxis, 15 of which were egg hypersensitivities. There were 65 subjects (85.5%) who were 2 years old or less. The disease histories included atopic dermatitis in 63 subjects (82.9%), food allergies in 68 (89.5%), and bronchial asthma in 18 (23.7%). Total blood serum IgE levels were less than 500 IU/ml in 35 of the 67 subjects (52.2%), and exceeded 1000 IU/ml in 22 (32.8%). Sixty-two of the 72 subjects (86.1%) tested for egg white-specific IgE RAST scores proved to be class 3 or above (Fig. 2a).

#### *2.3.2. Influenza vaccine*

Undiluted vaccine prick tests and intradermal tests using 1:10 diluted influenza vaccine were performed on 75 subjects (44 boys, 31 girls).

Of the vaccines used, 48 were manufactured by the Chemo-Sero-Therapeutic Research Institute, 25 by the Research Foundation for Microbial Diseases of Osaka University, and 2 by Chiba Kessei Laboratories, and all were gelatin-free. Twenty-three subjects (30.7%) had a history of immediate allergic reactions including anaphylaxis, 13 of which were egg hypersensitivities. There were 27 subjects (36%) who were 2 years of age or less, 39 (52%) between 2 and 6 years of age, and 9 (12%) over 6. The disease histories included atopic dermatitis in 53 subjects (70.7%), food allergies in 69 (92%), and bronchial asthma in 37 (49.3%). Total blood serum IgE levels were less than 500 IU/ml in 36 of the 71 subjects (50.7%), and exceeded 1000 IU/ml in 27 (38%). Forty-seven subjects (65.3%) of the 72 tested were found to have an egg white-specific IgE RAST score of class 3 or above (Fig. 2b).

#### *2.3.3. Other vaccines (mumps vaccine, rubella vaccine)*

Undiluted vaccine prick tests and 10× dilution intradermal tests were also conducted using mumps and rubella vaccines. The mumps vaccine test was conducted on five subjects (three boys, two girls), and the rubella vaccine test on two subjects (a boy aged 1 year 8 months, and a girl aged 1 year 7 months).

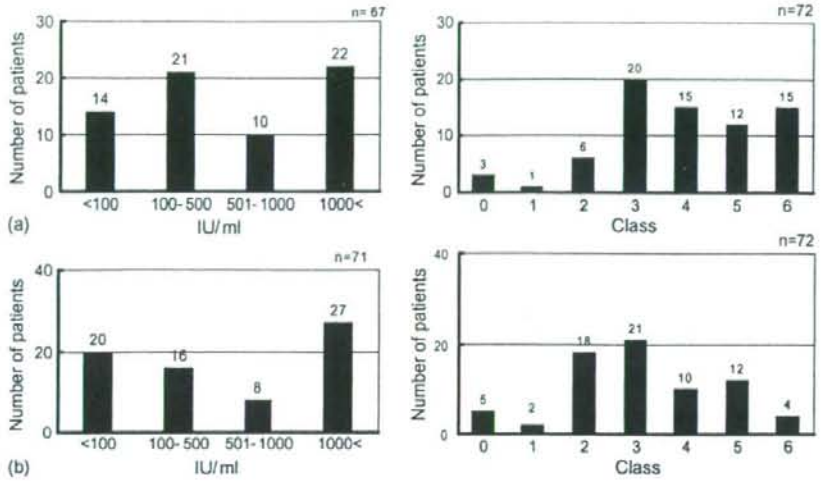


Fig. 2. Total serum IgE and IgE RAST scores of ovalbumin in patients tested in Protocol II. (a) Measles and (b) influenza.

The mumps vaccines used (containing gelatin) were manufactured by the Kitasato Institute and the rubella vaccines (gelatin-free), by the Research Foundation for Microbial Diseases of Osaka University.

The mumps vaccine test subjects were aged between 1 year 7 months and 6 years 4 months. Two subjects had had a history of immediate allergic reactions including anaphylaxis, and the total blood serum IgE levels ranged between 24.7 and 5654 IU/ml.

## 3. Methods

### *3.1. Intradermal test methods using 1:10 and 1:100 diluted vaccine*

Intradermal tests using vaccine diluted 1:10 and 1:100 were performed according to the following methods (Protocol I; Fig. 3). Intradermal injections into the forearm of 0.02 ml each of 1:10 dilution, 1:100 dilution, and a control liquid (physiological saline used as vehicle) were simultaneously performed and assessed after 15 min. Assessment followed Torii's grading system [5]: wheals of 15 mm or more/redness of 40 mm or more in diameter indicating a strong positive response, wheals of 9 mm to less than 15 mm/redness of 20 mm to under 40 mm indicating a positive response, wheals of 5 mm to under 9 mm/redness of 11 mm to under 20 mm indicating a doubtful positive response, and 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.

To subjects showing a negative response to the intradermal tests, the standard amount of vaccine was administered, and immediate-type allergic reactions were observed after 30 min. To those showing positive responses to the intradermal test, 0.1 ml of undiluted vaccine was administered, and if no immediate-type allergic reactions could be detected, either locally or generally after 30 min, the remaining undiluted vaccine was administered and reactions were observed after a further 30 min. If an allergic reaction was observed after the initial administration, the remaining administration was cancelled and tests for the respective virus antibody levels were conducted 6 weeks later. In subjects showing a strong positive response to the intradermal test, vaccine administration was aborted and virus antibody levels were measured 6 weeks later using the HI method or the EIA method. Virus antibody tests were conducted using the measles virus HI reagent or the measles virus IgG (EIA), both manufactured by Denka Seiken Company Limited (Tokyo, Japan). For the measurement of specific IgE antibody levels, CAP-RAST (the FEIA method) manufactured by Pharmacia Co. (NJ, USA) was used. The administration of antihistamines after the skin tests and before vaccination was left to the discretion of the vaccinating physician.

As statistical procedures, the Mann–Whitney *U*-test was used, with $P < 0.05$ indicating significant difference, in comparing total IgE levels and egg white-specific IgE RAST scores between subjects whose intradermal tests were positive and those testing negative, and among the former, between those to whom further administration could be conducted, and those for whom administration was terminated.

### *3.2. Methods for undiluted vaccine prick tests and intradermal tests with 1:10 diluted vaccine*

The undiluted vaccine prick test and intradermal tests using 1:10 diluted vaccine were conducted according to the method shown in Protocol II (Fig. 4). The needles used for the prick tests were the standard needles regularly used in

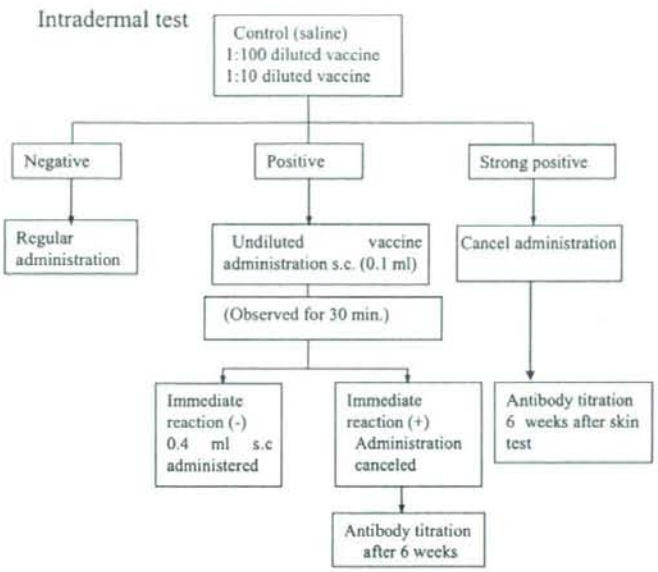


Fig. 3. Protocol of vaccine administration to high-risk allergic children (Protocol I). Intradermal test yieding the following results and assessments: wheals of 15 mm or more, or redness of 40 mm or more in diameter indicating a strong positive response; wheals of 9 mm to less than 15 mm, or redness 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, or redness identical to that of the control. When the vaccines were administered, subjects with doubtful positive responses were added to the negative response group.

the respective institutions, mainly bifurcated needles made by ALO Laboratories Inc. (OH, USA). 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.

## 4. Results

### *4.1. Comparative study of intradermal tests using vaccines diluted 1:10 and 1:100*

#### *4.1.1. Measles vaccine*

Fig. 5a compares the test results obtained from the 49 subjects on whom 1:10 and 1:100 diluted vaccine intradermal tests were simultaneously performed. The intradermal reactions were positive in 5 subjects (10.2%) in the 1:100 dilution test, and 14 subjects (28.6%) in the 1:10 dilution test. All of the subjects testing positive to the 1:100 dilution also tested positive to the 1:10 dilution.

Following the protocol, for all subjects with a strong positive reaction to the intradermal test using a 1:10 dilution (all of whom had also tested positive to the 1:100 dilution), further vaccine administration was aborted. In 11 subjects testing positive to the 1:10 dilution (including 1 subject with a negative response and 1 subject with a questionable positive response to the 1:100 dilution test), further administration was conducted in instalments. However, three subjects showed strong local reactions at the 0.1 ml instalment stage (including one subject who had tested positive to the 1:100 dilution), and upon the requests of subjects' family members, administration at the standard dosage was not conducted. None of the subjects showed strong general secondary reactions. Table 1 presents the profiles of three subjects who had a strong positive response and three subjects to whom dose administration had to be discontinued. Of the latter, one subject tested positive to the 1:10 dilution but negative to the 1:100 dilution intradermal test. In the subjects to whom administration was discontinued, tests for measles virus antibody levels were conducted after the intradermal tests, but in all cases the antibody levels had risen (Table 1). Of these, one subject (number 3) in whom follow-up tests were conducted, scored a measles virus antibody HI level of 32 times the original level, 2 years after the intradermal tests had been conducted.

No secondary reactions were seen in the subjects who proceeded to be given the standard vaccine dosage after testing negative to intradermal tests.

In assessment of the total IgE levels and egg white-specific IgE RAST scores in those subjects testing positive to the measles vaccine intradermal test and those testing negative, the positive response group had an average total IgE level of $1538.0 \pm 1985.9$ (mean $\pm$ S.D.) IU/ml, with an average egg white-specific IgE RAST score of class $3.7 \pm 0.9$; the negative response group had an average total IgE level of $364.4 \pm 532.1$ IU/ml and an egg white-specific IgE RAST

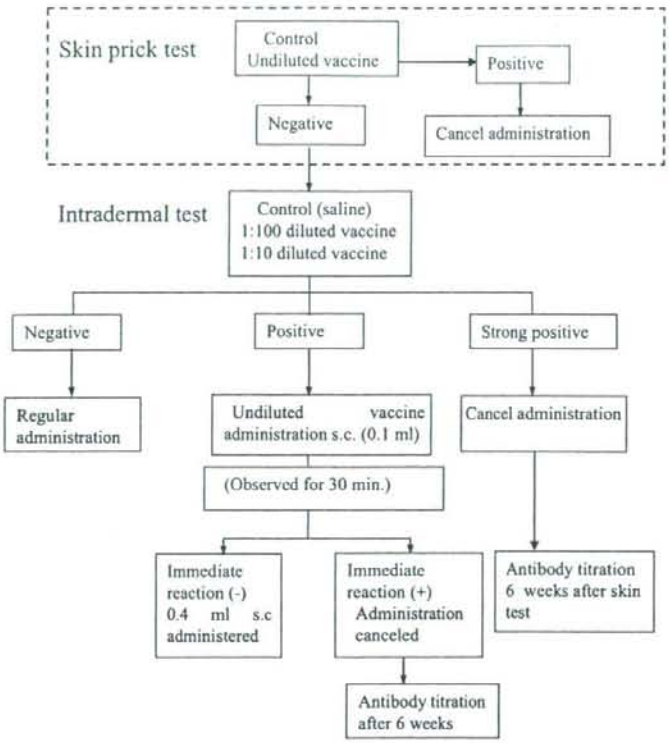


Fig. 4. Protocol of vaccine administration to high-risk allergic children (Protocol II). Intradermal test yielding the following results and assessments: wheals of 15 mm or more, or redness of 40 mm or more in diameter indicating a strong positive response; wheals of 9 mm to less than 15 mm, or redness 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 $2.2 \pm 1.1$. 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 $1407.1 \pm 1595.2$ IU/ml and an average egg white-specific IgE RAST score of class $3.3 \pm 0.8$, and the latter group had an average IgE level of $2783.7 \pm 3612.4$ IU/ml and an average egg white-specific IgE RAST score of class $4.0 \pm 0.9$. 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

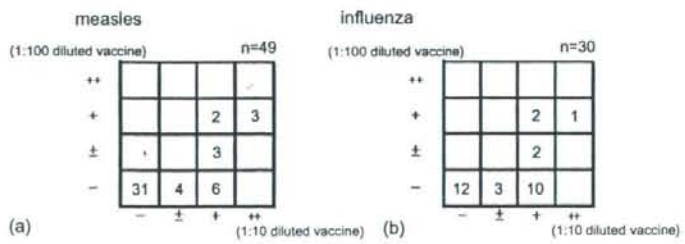


Fig. 5. Relations between vaccine concentrations (1:10 vs. 1:100 diluted vaccine) and intradermal reactions. Numbers in the cells show numbers of patients. Symbols signify the following: (−) negative, (±) false positive, (+) positive and (++) strong positive.

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 $555.1 \pm 797.9$ IU/ml and an average egg white-specific IgE RAST score of class $3.0 \pm 2.0$, while the latter group had an average total IgE level of $683.4 \pm 832.4$ IU/ml and an average egg white-specific IgE RAST score of class $2.5 \pm 1.4$. 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 $449.3 \pm 583.9$ and $1316.5 \pm 1859.0$ IU, and respective egg white-specific IgE RAST scores of classes $2.7 \pm 0.6$ and $3.3 \pm 3.1$ 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.

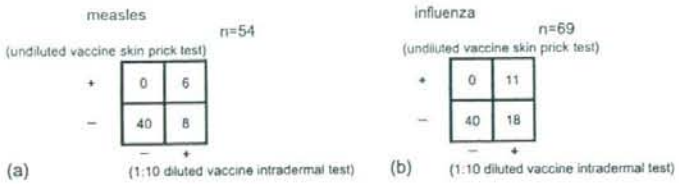


Fig. 6. Relations between skin test (1:10 diluted vaccine intradermal test vs. undiluted skin prick test) and skin test reactions. Numbers in each cell show numbers of patients. Symbols signify the following: (−) negative and (+) positive.

### *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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