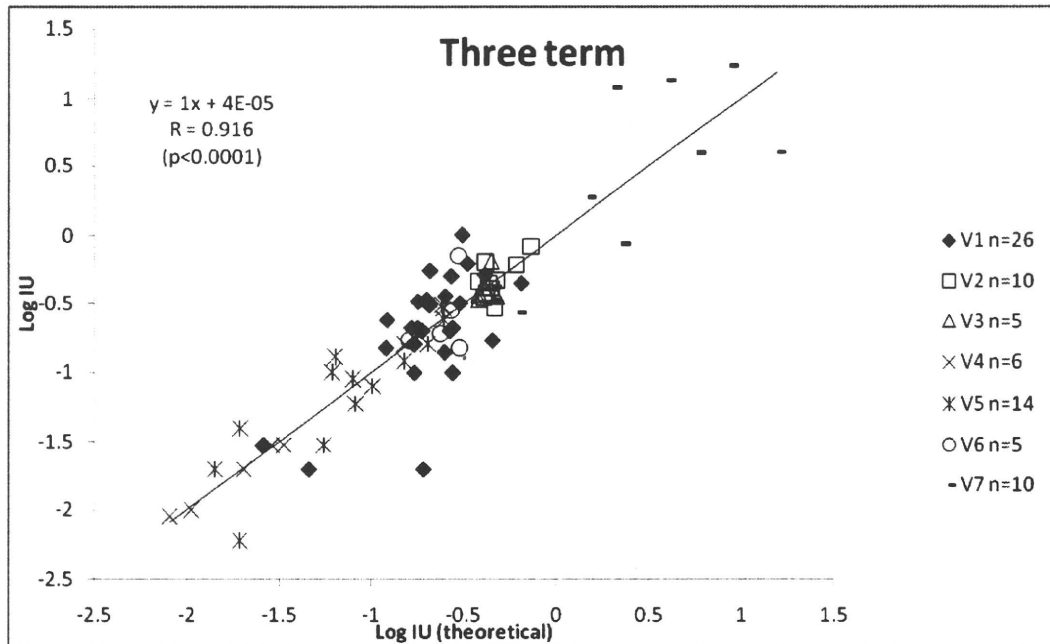


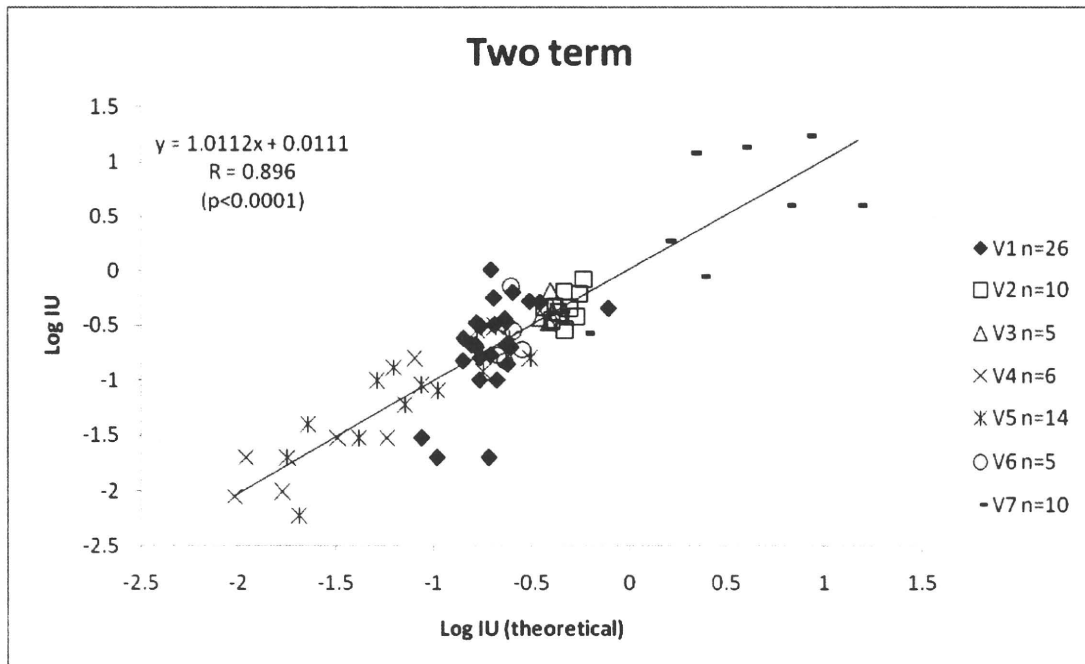
図5. マウスヒスタミン増感活性と *in vitro* 活性 (A & B) の関係



製品タイプ別の係数 (b1, b2, b3) と定数 (C) を使用した3因子重回帰分析モデル
 $\log IU(\text{推定値}) = [b1 \times \log Fet] + [b2 \times \log Fet \times \log Enz] + [b3 \times \log Enz] + C.$

製品タイプ別の係数 (3因子) 及び定数を使用することで、ADPリボシル化活性及び Binding活性からマウスヒスタミン増感活性を推定する近似式を導くことができた

図6. マウスヒスタミン増感活性と *in vitro* 活性 (A & B) の関係



製品タイプ別の係数 (b1, b3) と定数 (C) を使用した2因子重回帰分析モデル
 $\log IU(\text{推定値}) = [b1 \times \log Fet] + [b3 \times \log Enz] + C.$

製品タイプ別の係数 (2因子) 及び定数を使用することで、ADPリボシル化活性及び Binding活性からマウスヒスタミン増感活性を推定する近似式を導くことができた

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
岡部信彦、 多屋馨子	予防接種に関する Q&A	社団法人細菌製剤協会	予防接種に関する Q&A 集 2010	社団法人細菌製剤協会	東京	2010. 8.	1-155

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Baba K, Okuno Y, Tanaka -Taya K, Okabe N.	Immunization coverage and natural infection rates of vaccine-preventable diseases among children by questionnaire survey in 2005 in Japan	Vaccine			in press
多屋馨子	新時代のワクチン戦略について考える～ワクチン行政とサーベイランス～	臨床検査	54 巻 11 号.	1263-127 1	2010



An *in vitro* assay system as a potential replacement for the histamine sensitisation test for acellular pertussis based combination vaccines

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ABSTRACT

The histamine sensitisation test (HIST) for pertussis toxin is currently an official batch release test for acellular pertussis containing combination vaccines in Europe and North America. However, HIST, being a lethal endpoint assay, often leads to repeated tests due to large variations in test performance. Although a more precise HIST test based on measurement of temperature reduction after the histamine challenge is used in Asian countries, this test still uses animals. An *in vitro* test system based on a combination of enzyme coupled-HPLC and carbohydrate-binding assays with results analysed by a mathematical formula showed a good agreement with the *in vivo* HIST results based on measurement of temperature reduction after histamine challenge. The new *in vitro* test system was shown to be a potential alternative to the current *in vivo* HIST.

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1. Introduction

Pertussis (whooping cough) is still a major cause of morbidity and mortality, especially in infants, and vaccination against *Bordetella pertussis* is a key component of immunisation campaigns worldwide for the prevention of the disease. Two types of pertussis vaccines, namely whole-cell (WCV) and acellular pertussis vaccines (ACV), are recommended by WHO [1]. Both types of vaccine play an essential role in immunisation programmes worldwide. In general, ACVs are less reactogenic and there is an increasing trend towards replacing WCVs with ACVs.

Pertussis toxin in its toxoided form (detoxified) is a major protective antigen and is present in both ACVs and WCVs. Native pertussis toxin (PT) is a protein toxin which has a wide range of biological activities *in vivo* including induction of leukocytosis, histamine-sensitisation, increased insulin production with consequent hypoglycaemia, potentiation of anaphylaxis and lethality in mice [2]. PT also has been reported to increase blood–brain barrier permeability and there has been controversy regarding its possible role in neuropathology [3]. More recently, there is evidence to indicate that PT is able to induce IL-1 β production in the mouse brain. IL-1 β can affect neuroendocrine functions and modulate release of neurotransmitters, and has been claimed to play a

role in neurological reactions observed in children immunised with pertussis vaccines of high PT content [4]. Furthermore, excess residual PT activity may cause intensified sensitisation to components of diphtheria-tetanus-ACV (DTaP) combinations with subsequent severe local reaction to a booster dose in an animal model [5]. Although the biological effects of PT have been under extensive research, its mechanism(s) of toxicity is still unclear. As unmodified PT is considered too toxic for vaccine preparation, it has to be used in a stable non-toxic form. The production of toxoided PT has to be carefully controlled to prevent excessive loss of protective and T_H-epitopes on the one hand, and reversion to toxicity on the other [6,7]. Monitoring chemically toxoided PT for residual toxicity and reversion to toxicity is an essential part of the safety evaluation of ACVs and is required by regulatory authorities [8–11]. The *in vivo* histamine sensitisation test (HIST) is the official pharmacopoeial test used for detecting active PT in such preparations.

Various assay methods have been explored as alternatives to the *in vivo* HIST and most of them are based on either non-lethal animal models or cell culture systems but so far without success [12,13]. The first approach, i.e. the leukocytosis promoting assay (LPA), was shown to have poor reproducibility and large inter-laboratory variation [14]. More recently, attempts have been made to use primary vascular smooth muscle cells from rats and to measure PT induced increase in isoprenaline-induced intracellular cAMP levels or to identify gene expression after administration of PT or WCV [13]. These approaches still need the use of animals and the studies are at a very early stage. Cell culture based assay systems may be useful for

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evaluating antigens in the vaccine bulk stage but their suitability for adjuvant adsorbed final formulation that contains chemically toxoided PT remains questionable because the adjuvant usually affects the cell function/or viability and the cells may not be able to process toxin inside protein aggregates. For example, the Chinese hamster ovary (CHO)–cell assay is used for the determination of residual pertussis toxin activity in the bulk antigen but is not suitable for the assay of pertussis toxin in the final dosage form. Recently, a study has also indicated that this assay does not correlate with HIST [15,16]. Therefore, at the moment HIST is the only test requested by regulatory authorities to determine pertussis toxin activity in final formulation of pertussis containing vaccines [8–11]. However, the precise mechanism of HIST is unclear and it is difficult to standardise, especially for the lethal endpoint method, and large variations in test performance have been observed between laboratories due to mouse strain, age, sex and environmental factors [17]. Therefore, there is an urgent need for a replacement of the HIST.

Two HIST methods are currently used for the determination of residual PT in licensed acellular pertussis vaccines. One is based on lethal end point and the other measures temperature reduction after the histamine challenge [8,18,19]. The lethal endpoint method based on the induction of increased sensitivity to histamine-provoked death in mice has been widely used in North American and European countries [10,11] and its disadvantages have already been reviewed [12]. There is no internationally agreed limit on the permissible level of PT activity in ACVs. The assay remains difficult to standardise and has a history of problems associated with inconsistent results, and inter- and intra-laboratory variability. This often leads to apparently contradictory results between laboratories and to repeat tests, particularly when different mouse strains are used. A more sensitive variant of HIST based on either rectal or dermal measurement of the reduction in body temperature produced by histamine shock (temperature method) has been successfully used in Japan and other countries [8,20]. This test method provides a more precise quantitative estimate of the activity of a test vaccine relative to the activity of a reference standard and this quantitative approach forms the basis of our present study in establishing the relationship between the *in vivo* and *in vitro* assay systems.

PT has the A–B type structure typical of many other bacterial toxins, having an enzyme active A-protomer (monomer), the S-1 subunit, and a binding B-oligomer of subunits S-2 through to S-5 [21–23]. Intact B-oligomer is required for binding of the holotoxin to receptor sites on the cell surface and enables entry of the A-protomer into the cells [24]. The toxin A-protomer catalyses the ADP-ribosylation of eukaryote GTP-binding regulatory proteins, preventing hormonal inhibition of adenylate cyclase and resulting in an increase in intracellular levels of cAMP [25]. Therefore, PT is capable of initiating two types of cellular response. One results from the lectin like action of the B-oligomer moiety, while the other reflects the ADP ribosylation, and hence the abolition of the signal transduction by guanine-nucleotide-binding proteins (G-proteins) [22]. The ADP-ribosylation effects of PT have been attributed to its catalytic transfer of an adenosine diphosphate-ribose (ADP-ribose) moiety from NAD⁺ to the α -subunits of signal-transducing G-proteins [25–27]. This activity can be assayed by measuring ADP-ribosylation of a fluorescent synthetic peptide substrate using an enzymatic-HPLC coupled assay (E-HPLC) [28–32].

Although this E-HPLC assay correlates well with the toxicity observed by HIST using native PT preparations [32], it does not take account of the binding activities of the B-subunits. Chemical treatment has been widely used by manufacturers to detoxify PT and different detoxification procedures using various reagents have been shown to result in different amino acid side chain modifica-

tions and changes in conformational and epitope binding patterns of the resulting toxoided PT [7,33]. Previous results indicated that different ACV products show differences in ADP-ribosylation activity although a level which would be significant in relation to the reactivity seen in the HIST could not be defined [34]. This was to be expected as toxicity is dependent on the capacity of the enzymic moiety to enter the cell and this will be affected by chemical modification of the B-oligomer. A new functional test for PT based on the carbohydrate-binding and hence cell attachment property of the B-oligomer was developed [35].

In the present study, a refined detection strategy for an *in vitro* test system has been developed for examining both functional domains of PT based on a combination of E-HPLC and carbohydrate-binding assays. This strategy uses the E-HPLC method to measure the toxic activity of the A-subunits of the toxin and uses the carbohydrate-binding assay to measure the B-oligomer carbohydrate-binding function. Investigation was carried out on the relationship between the biochemical activities of PT found in the *in vitro* system with the *in vivo* activity shown in the HIST by the temperature measurement method using a number of ACVs of different types and formulations. A positive correlation of the two assay systems could lead to the establishment of an alternative method to the *in vivo* HIST.

2. Materials and methods

2.1. Materials

The PT used was a freeze-dried reference preparation (National Institute for Biological Standards and Control, UK (NIBSC) code 90/518). Each ampoule contained a nominal 20 μ g of PT corresponding to 2100 IU of PT activity in HIST (lethal endpoint method) when calibrated against the WHO First International Standard for Pertussis Toxin (NIBSC Code JNH-5) and was stored at -20°C [36]. For use, each ampoule of 90/518 was reconstituted with 0.2% (w/v) ovalbumin (Sigma) in PBS. Aliquots of 100 μ l (2 μ g/ml; 200 ng) were lyophilised and stored at -20°C as stock standards.

A two-component ACV, containing toxoided PT and filamentous haemagglutinin (FHA) in combination with diphtheria (D) and tetanus (T) toxoids (NIBSC code 00/486) was kindly donated by the Research Foundation for Microbial Diseases of Osaka University (Biken, Japan) and freeze-dried at NIBSC (nominal potency 11.25 units/vial). This product was used as the positive control in the E-HPLC assay as it was in stable freeze-dried form and known to have ADP-ribosylation activity.

A total of 76 batches (7 different types) of commercially available ACVs (V1–V7) were used in this study (Table 1). They comprised toxoided PT in combination with D and T toxoids (DTaP) \pm other antigens such as *Haemophilus influenzae* type b capsular polysaccharide conjugated to T (Hib), inactivated polio vaccine (IPV) and other *B. pertussis* antigens (filamentous haemagglutinin (FHA), pertactin (69K) and fimbriae 2 and 3 (Fims2/3)). Aluminium phosphate was used as the adjuvant in vaccines V1, V2, V3 while aluminium hydroxide was used in vaccines V4, V5 and V6. Samples of V7 type were adsorbed either to aluminium hydroxide or aluminium phosphate. Positive control vaccines for carbohydrate-binding assay were prepared by pooling several batches of each of the corresponding vaccine type.

The PT enzyme substrate, fluorescein-tagged G _{α 13}C20 peptide, F-VFDAVTDVIKNNLKECGLY-COOH (F-G _{α 13}C20) was custom-synthesised by AnaSpec Inc., San Jose, CA, USA and was reported to have >95% purity. Anti-PT sheep serum (Code: 97/572) was prepared at NIBSC. Peroxidase labelled anti-sheep IgG (A-3415) was obtained from Sigma. All other chemicals, unless specified otherwise, are of analytical grade and purchased from either Sigma–Aldrich or VWR-BDH (Poole, UK).

Table 1
Enzymatic and fetuin binding activities in acellular pertussis vaccines.

Vaccines							
Product code	V1	V2	V3	V4	V5	V6	V7
Number of batches	26	10	5	6	14	5	10
PTx ADP-ribosyltransferase activity (E-units/ml)							
G-Mean	3.90	4.43	1.70	2.43	4.33	3.99	2.54
Range	(2.46–6.10)	(3.93–5.64)	(1.29–2.50)	(1.89–3.25)	(2.50–5.80)	(2.93–7.88)	(0.60–10.00)
Fetuin binding activity (B-units/ml)							
G-Mean	4.53	3.85	2.85	3.41	4.60	1.36	4.83
Range	(3.16–8.37)	(3.22–5.18)	(2.17–3.61)	(2.86–3.91)	(1.72–10.16)	(0.37–10.15)	(1.56–23.14)

Detailed description of types of acellular pertussis containing combination vaccines (ACVs) and the determination of their enzymatic and fetuin binding activities are as described in the text. G-Mean: Geometric mean.

2.2. E-HPLC coupled assay

The determination of PT ADP-ribosylation activity in vaccines was performed using a synthetic fluorescein-tagged peptide substrate, F-G_{α13}C20 coupled with an HPLC method for the separation and quantitation of the ADP-ribosylated product, as described in detail previously [32,34]. A standard curve (0–400 ng/ml), constructed from the results obtained with PT reference preparation (90/518) with either Al(OH)₃ or AlPO₄ adjuvant, according to the type of adjuvant in the test sample, was used to calculate the enzymatic activity in a test sample. Vaccine sample was appropriately pre-diluted in ovalbumin (OVA, 0.2%, w/v) solution so that the ADP-ribosylation activity would be within the range of the PT (90/518) standard curve. The enzymatic activity in the neat test sample (per ml) was calculated after taking account of the sample dilution factor. The enzymatic activity was presented as E-units where 1 unit is equal to fluorescence produced by 1 μg of PT (90/518) under identical assay and analysis conditions. Unless otherwise stated, all assays met the in house assay validity criteria [34] and were performed in duplicate and the results presented as means ± standard deviation (SD) or coefficient of variation (CV%).

2.3. Carbohydrate-binding assay

The PT carbohydrate-binding activity in vaccines using either bovine fetuin (Fet; containing sialylated N-glycans) or asialofetuin (AsFet; containing mainly neutral N-glycans) as carbohydrate coat was performed as described by Gomez et al. [35]. In brief, 96-well plates (NUNC Maxisorp Microtitre plates) were coated with Fet or AsFet (1 μg) and left overnight at room temperature. At the same time, vaccines and PT were treated with freshly prepared desorption reagent (0.15 M EDTA disodium salt dissolved in 0.52 M di-sodium hydrogen orthophosphate; 1:1 (v/v)) overnight at 37 °C to release the antigen from the aluminium adjuvant. After the desorption process, vaccines were centrifuged at 2000 RPM for 10 min (Centrifuge 5415 D, Eppendorf, Germany) and supernatant, usually as neat solution and up to 3 serial dilutions in diluents (3% BSA-PBS–0.05% Tween 20) were used in the assay. To construct standard binding curves, one ampoule of PT 90/518 (20 μg) was reconstituted and diluted with PBS. The starting concentration of PT for the AsFet binding assay was 1 μg/ml and for the Fet binding assay was 0.2 μg/ml; up to 7 serial dilutions were used. Anti-PT sheep serum (NIBSC Code: 97/572; 1/80,000 dilution) and horse radish peroxidase labelled anti-sheep IgG (Sigma–Aldrich Cat. No. A-3415; 1 in 2000 dilution) were used for detection. All samples were assayed in duplicate. The relative potency of PT binding activity in vaccine to PT (90/518) was calculated using a parallel line assay via the CombiStats software (version 4.0, EDQM) and was expressed as binding (B)-units where 1 B-unit equals to binding activity produced by 1 μg of PT (90/518) under identical assay and analysis conditions.

2.4. Dermal temperature histamine sensitisation test (HIST)

The HIST was carried out according to the method described in previous publications [18,19]. In brief, NIH female mice weighing 9–11 g were randomly distributed in groups of 10 (caged in 5s and individually marked). Mice were inoculated intra-peritoneally (i.p.) with either 0.5 ml phosphate buffered saline containing 0.2% (w/v) gelatin (PBSG) as negative control, PT reference (90/518) in PBSG at doses 5.25, 1.75 and 0.58 IU/dose or with test vaccine at 1 single human dose (SHD, 0.5 ml)/mouse. Five days after the inoculation, mice were challenged i.p. with 0.5 ml of histamine solution (prepared in PBS, equivalent to 2 mg histamine base per dose). The dermal temperature of each mouse was measured 30 min after challenge using an infrared thermometer (IT-550, Horiba Ltd., Japan) according to the method described by Ochiai et al. [18]. The HIST activity in the test vaccine was calculated in comparison with that of the reference toxin groups and expressed as IU/dose.

2.5. Statistical analysis

Data analysis was performed using the statistical program in Microsoft Excel Spreadsheet (2007 version).

Predicted activity (IU) was calculated from the corresponding logarithmic values of the fetuin-binding (Fet) and the enzymatic activities (Enz) using multiple regression analysis. The establishment of consistency of the regression factors *b*₁ and *b*₂ and constant factor *C* values for the multiple regression analysis was achieved by estimating population values of *b* and *C* for each product type, e.g. batches of a particular product were randomly ordered and *b*₁, *b*₂ and *C* were calculated for the first five lots according to the method described in Fig. 5. If the estimated values were found to have sufficiently converged (consistent), that is no significant change in the value observed when additional batches are added, then these estimates of *b*₁, *b*₂ and *C* would be considered as the approximating population values.

3. Results

3.1. ADP-ribosylation and carbohydrate-binding activities in different ACVs

ADP-ribosylation and carbohydrate-binding activities were measured in all 76 batches of 7 different vaccine types and the results are presented in Table 1. Both activities were detected in all vaccine types included in this study. There was no consensus value either for enzymatic activity or the binding activity amongst these different vaccine types. Variation of the geometric means of the activities was wide between the products, ranging from 1.70 (V3) to 4.43 (V2) E-units/ml for the enzymatic activity and 1.36 (V6) to 4.83 (V7) B-Units/SHD. The level of residual PT enzymatic activity and the binding activity in these vaccine types appeared to

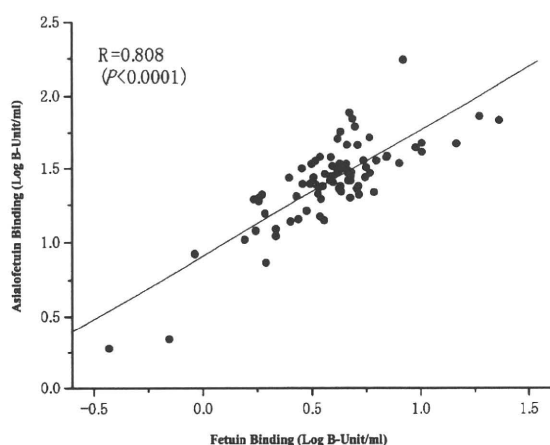


Fig. 1. Evaluation of the relationship between the binding activities to fetuin and asialofetuin in acellular pertussis containing combination vaccines (ACVs).

be product specific and batch-to-batch variability (CV%) within a particular product ranged from 18.4% to 50.4% where the numbers of batches tested for a particular type of product were ≥ 5 which is in line with previous findings [34,35].

3.2. Evaluation of relationship of Fet and AsFet binding activity in ACVs

In the present study, the carbohydrate-binding activity of PT in different types of ACVs to both Fet and AsFet were determined and the relationship between their binding activities was assessed (Fig. 1). Although the values of binding activities differed between Fet and AsFet, there was a highly significant correlation between log values of the two binding activities ($P < 0.0001$) for all products included in this study. However, each different vaccine type had its own specific range of distributions of the unit values for the two binding activities. In addition, variation of the binding activity was wide for each product (results not shown). Since PT binds best toward the fully sialylated multi-antennary structures [35], that is Fet in this study, therefore in subsequent correlation analysis to evaluate the relationship between ADP-ribosyltransferase activity and HIST, only the Fet binding data were used in order to simplify the task.

3.3. Evaluation of the relationship between ADP-ribosylation and carbohydrate-binding activities in ACVs

When the relationship between enzymatic and binding activities was examined, an overall correlation between these activities was significant ($P < 0.0001$) in the range over certain levels of the both activities (approximately 0.1 log unit, Fig. 2) with a few exceptions for individual products. Furthermore, although there was a tendency for overall positive correlation between binding and enzymatic activities ($R = 0.518$), wide batch-to-batch variation was seen for both activities even in the same type of products. This indicates that the detoxification methods used in different product types might have simultaneously affected both the binding and the enzymatic activities, but not to a consistent extent for both activities.

3.4. Establishment of a mathematical model to predict activity observed in the HIST using data from the *in vitro* biochemical assay system via multiple regression analysis

Direct comparison of *in vivo* HIST activity with the *in vitro* biochemical assays either with the enzymatic or with the bind-

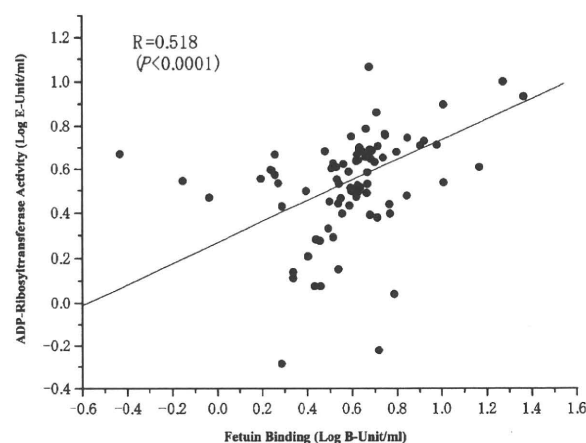


Fig. 2. Evaluation of the relationship between the fetuin binding and ADP-ribosyltransferase activities in acellular pertussis containing combination vaccines (ACVs).

ing activity did not show correlation (data not shown). Efforts were then made to develop a mathematical model by multiple regression analysis using the data from the two biochemical assays to predict the activity in HIST. In the mathematical equation, log IU is used to define the activity determined by HIST and log IU (predicted) is the predicted activity calculated from data of the two *in vitro* assays. In the first stage, a three-factor model equation was constructed: $\log \text{IU (predicted)} = b_1 \times \log \text{Fet} + b_2 \times \log \text{Fet} \times \log \text{Enz} + b_3 \times \log \text{Enz} + C$; where b_1 = regression coefficient for log Fet (binding); b_2 = regression coefficient for interaction between log Fet and log Enz (enzymatic activity); b_3 = regression coefficient for log Enz and C = the constant factor. When data for all products were analysed by this model using common regressions and constant factors (estimated from all the combined data of these products), no correlation was observed (Fig. 3a). However, when product specific regressions and constant factors were applied to the equation, good correlation was obtained between activity observed in HIST and the predicted activity calculated from the data of the *in vitro* assays (Fig. 3b and c). Since different products were usually manufactured using different detoxification processes, which would result in varying degrees of de-naturation of the PT, differences in enzymatic and binding activities between products would be expected. Hence, in a scatter plot for log IU and log IU (predicted), it was not surprising that data for a particular product would distribute in a specific data range of the graph. For example, in the case of products V1 (PT antigen is inactivated by treatment with glutaraldehyde) and V5 (PT antigen is inactivated by treatment with both formaldehyde and glutaraldehyde) (Fig. 3b and c), the log IU (predicted) values of product V1 distribute evenly in the range between -0.6 and -2.0 (Fig. 3b) while the log IU (predicted) values of product V5 distribute in a cluster between -0.2 and -1.0 (Fig. 3c). These results demonstrated that product specific regression coefficients (b_1 , b_2 and b_3) and the constant factor (C) have to be applied for the predictive calculation.

In order to assure the adequacy of the model equation, product specific regression coefficient factors were used for assessing the correlation between the predicted activity and the observed *in vivo* activity by HIST for each product separately (data not shown) as well as for the overall correlation for all products using the three-factor equation, $\log \text{IU (predicted)} = b_1 \times \log \text{Fet} + b_2 \times \log \text{Fet} \times \log \text{Enz} + b_3 \times \log \text{Enz} + C$ (Fig. 4a). A good correlation ($R = 0.916$) between the IU (predicted) and the *in vivo* IU by HIST was demonstrated and the predicted IU estimates were in agreement with measured values by HIST-

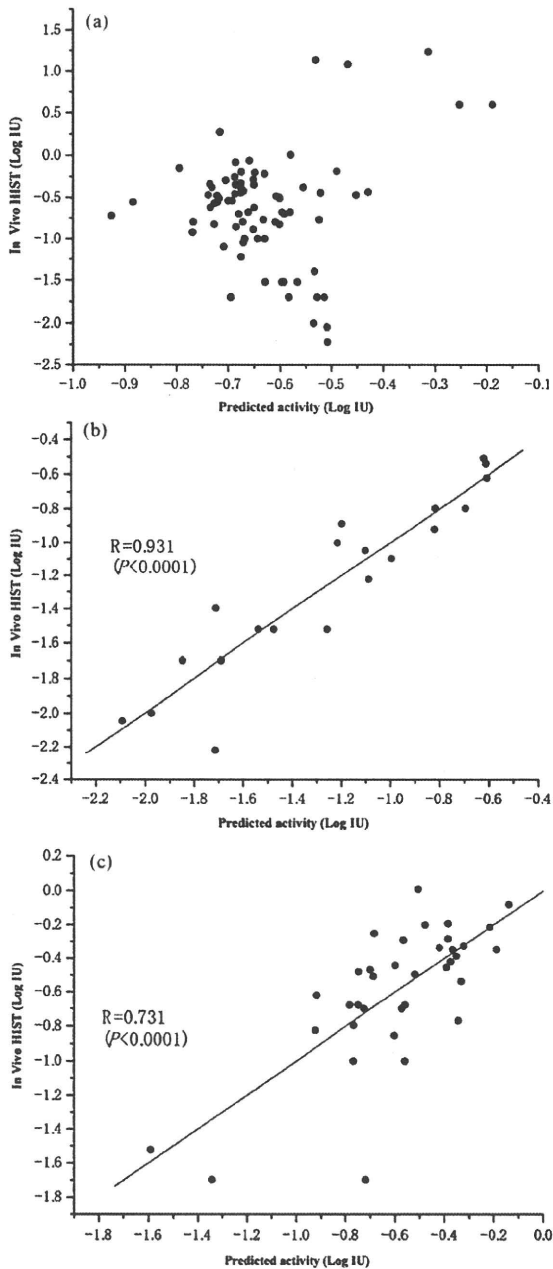


Fig. 3. Evaluation of the relationship between the predicted PT activities (calculated from the *in vitro* assays data) and the activities observed *in vivo* H1ST by the temperature method. The predicted PT activities were calculated from the *in vitro* assays data using a three-factors mathematical model equation: $\log IU (\text{predicted}) = b_1 \times \log Fet + b_2 \times \log Fet \times \log Enz + b_3 \times \log Enz + C$. $\log Fet = \log B\text{-unit/ml}$ (binding activity of PT to fetuin); $\log Enz = \log E\text{-unit/ml}$ (ADP-ribosyltransferase activity). Panel (a), data for all products were analysed using common regression (b_1 and b_2) and constant (C) factors (estimated from all the combined data of all products); Panels (b and c) data for two types of vaccines (V1 and V5) were analysed using the product specific regression and constant factors respectively.

temperature assay. In order to simplify the equation further, calculation was carried out using these data to fit into a two-factor model equation: $\log IU (\text{predicted}) = b_1 \times \log Fet + b_2 \times \log Enz + C$ (Fig. 4b). Again, good correlation between the predicted and the measured H1ST activities was found ($R = 0.896$). In view of this, the simpler two-factor equation is judged to be adequate in future data analyses. A detailed protocol showing how to calculate those

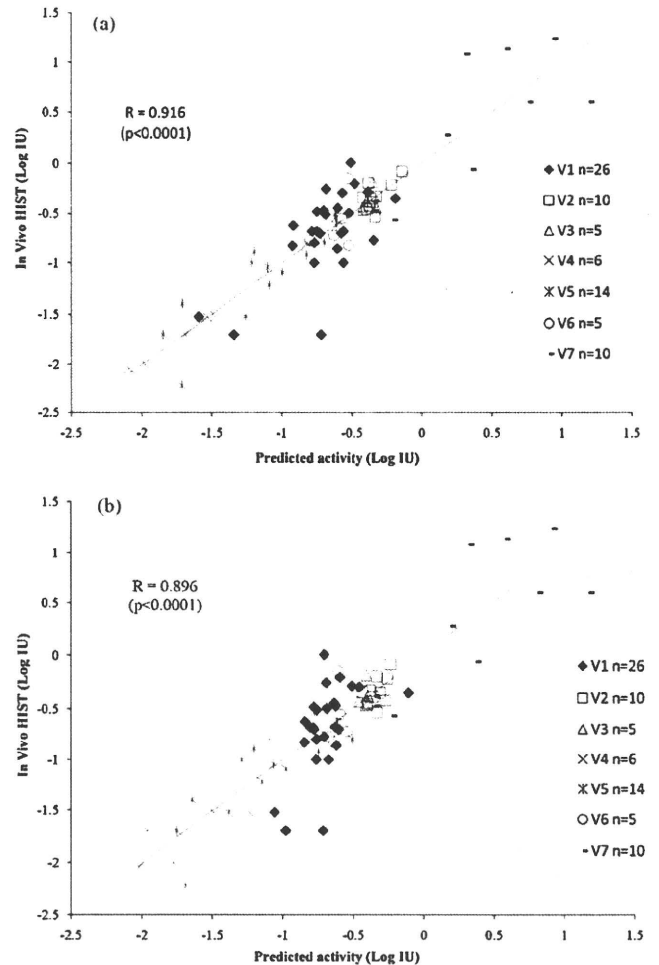


Fig. 4. Comparison of the three-factors mathematical model equation with the two-factors mathematical model equation based on product specific regression and constant factors in the determination of correlations between the *in vitro* assay system and the *in vivo* H1ST. (a) data calculated using a three-factors mathematical model equation: $\log IU (\text{predicted}) = b_1 \times \log Fet + b_2 \times \log Fet \times \log Enz + b_3 \times \log Enz + C$; (b) data calculated using a two-factors mathematical model equation: $\log IU (\text{predicted}) = b_1 \times \log Fet + b_2 \times \log Enz + C$. Abbreviations used are as described in Fig. 3 and an example how to calculate the predicted PT activity is as described in Fig. 5.

specific regression and constant factors for the two-factor model equation is presented in Fig. 5.

4. Discussion

PT is an A-B type bacterial toxin possessing two functionally distinct domains: the enzymatic A-protomer and the B-oligomer that facilitates host-cell binding and entry of the A-protomer into the cell [16]. PT in its detoxified form is an important antigen present in all current ACVs. It is usually inactivated by chemical treatments either with formaldehyde or glutaraldehyde or both, or with hydrogen peroxide, or tetra-nitromethane [37,38]. These different detoxification procedures could result in different amino acid side-chain modifications and changes in conformational and epitope binding patterns for the resulting pertussis toxoid(s) [7,33]. Glutaraldehyde or formaldehyde treatment has been reported to have differential effects on toxoided PT biological activities [39]. The precise nature and location of the effects of different toxoiding reactions on the PT molecule have not been defined and the toxoided PT present in pertussis vaccines could be modified on either the A-protomer, B-oligomer or both components of the PT

Assay	LogFet	LogEnz	HIST (LogIU)
1	X ₁₁	X ₂₁	Y ₁
2	X ₁₂	X ₂₂	Y ₂
i	X _{1i}	X _{2i}	Y _i
n	X _{1n}	X _{2n}	Y _n

Measurements on binding, enzymatic and HIST activities of *n* batches of aP based vaccine were designated as X₁₁, X₁₂, ..., X_{1i}, ..., X_{1n}, X₂₁, X₂₂, ..., X_{2i}, ..., X_{2n} and Y₁, Y₂, ..., Y_i, ..., Y_n, respectively.

$$\text{Mean } X_1 = \bar{X}_1 = \frac{\sum X_{1i}}{n}, \quad \text{Mean } X_2 = \bar{X}_2 = \frac{\sum X_{2i}}{n}, \quad \text{Mean } Y = \bar{Y} = \frac{\sum Y_i}{n}$$

$$S_{x_1x_1} = \sum X_{1i}^2 - \frac{(\sum X_{1i})^2}{n}$$

$$S_{x_2x_2} = \sum X_{2i}^2 - \frac{(\sum X_{2i})^2}{n}$$

$$S_{yy} = \sum Y_i^2 - \frac{(\sum Y_i)^2}{n}$$

$$S_{x_1y} = \sum X_{1i}Y_i - \frac{(\sum X_{1i})(\sum Y_i)}{n}$$

$$S_{x_2y} = \sum X_{2i}Y_i - \frac{(\sum X_{2i})(\sum Y_i)}{n}$$

$$b_1 = \frac{S_{x_1x_1}S_{x_2y} - S_{x_1y}S_{x_2x_2}}{S_{x_1x_1}S_{x_2x_2} - (S_{x_1x_2})^2}$$

$$b_2 = \frac{S_{x_2x_2}S_{x_1y} - S_{x_2y}S_{x_1x_1}}{S_{x_1x_1}S_{x_2x_2} - (S_{x_1x_2})^2}$$

$$C = \bar{Y} - b_1\bar{X}_1 - b_2\bar{X}_2$$

$$Y = b_1X_1 + b_2X_2 + C = b_1\text{LogFet} + b_2\text{LogEnz} + C$$

Fig. 5. Calculation procedure for the regression factors b1 and b2 and constant factor C values in the two-factors mathematical model equation. The establishment of consistency of these values was by accumulating population values of b and C for each product type, e.g. batches of a particular product were randomly ordered and b1, b2 and C were calculated for the first five batches. If no significant change in the value observed when additional batches are added, then these estimates of b1, b2 and C would be considered as the approximating population values. X₁ = log binding activity; X₂ = log Enz activity; Y₁ = log IU from HIST; i = batch number; n = number of total batches for a particular product; s = Standard deviation; ∑ = summation; \bar{X}_1 = mean.

molecule. The dual biological function of the PT molecule is likely to be fully reflected in the *in vivo* HIST. To understand better the effect of chemical detoxification on PT, an assay for measuring the B-oligomer binding activity in combination with the E-HPLC coupled assay, which measures the A-protomer activity, should provide a potentially good correlation with the *in vivo* reactivity observed by HIST [34,35].

The assay sensitivity for the HIST lethal endpoint assay varies not only between mouse strain but also between batch of mice used in different experiments. The lethal endpoint assay can only detect the PT activity level above the assay sensitivity baseline as the dose causing fatal sensitisation of mice. In the present study, the HIST based on measuring temperature reduction after histamine challenge was chosen [18,19] because this approach allows more precise estimation of residual PT activity in vaccines with better reproducibility and allows also a better quantitative correlation with the *in vitro* assay system.

All toxoided PTs in the ACVs included in this study were inactivated by treatment with either formaldehyde or glutaraldehyde or both which accounts for most ACV types in the current markets. Aldehyde groups react primarily with the free ε-amino group of lysine residues and form crosslinks with other amino acid-side chain residues, which results in inter- and intra-molecular bridging and structural conformational changes to the B-oligomer [7,40–44]. Since there are no lysine residues present in the S1 subunit, the extent of modification of A-protomer is unclear [43,45]. However, both glutaraldehyde and formaldehyde can still react with other amino acids although to a lesser extent than lysine [46,47]. Several studies showed that both the enzymatic activity and monoclonal antibody binding affinity of S1 subunit were reduced after

chemical treatment [7,33,41]. Furthermore, the intensity of the S1 subunit band decreased on SDS-PAGE gels after detoxification with formaldehyde, indicating some modification of the S1 subunit [44,48]. In the present study, a positive relationship between the binding activities and enzymatic activities was found for ACVs (Fig. 2) with the exception of a few batches that showed either very low enzymatic activity (<1.2 E-units) or binding activity (<1.25 B-units). Taking this together with previously reported results [33,41,44,48], it is suggested that the detoxification procedures used for PT in these vaccines could modify both the B- and the A-subunits, although direct evidence of changes in the A-subunit structure remains elusive.

In a previous study [35], a change of carbohydrate-binding characteristic of PT and toxoided PT was observed when using Fet (sialic acid containing glycoprotein) and AsFet (desialylated Fet). It is postulated that by measuring the binding profiles of toxoided PT to both carbohydrate types, it might be possible to differentiate different types of detoxification process in ACVs. In the present study, although we could show that there was an overall positive correlation between the two binding activities, differences in slopes of the relationship between different products were observed. This may be due to differences in proportion or types of the detoxification process amongst different products and product types (data not shown). An extended study into this aspect using well controlled and characterised detoxified samples will be needed to clarify this possibility.

In HIST (both lethal and temperature reduction), batch-to-batch variations from different manufacturers were seldom found. However, significant variation could be observed in the two *in vitro* assays even from the same manufacturer using the same detoxification procedure. This may indicate that the *in vitro* assay system has higher sensitivity and selectivity in measuring the biochemical functions of the PT or that the assay mechanisms are different between the *in vivo* and *in vitro* assay systems. In the *in vitro* assay system, the assays could distinguish HIST negative vaccine samples having very different biochemical assay profiles. For example, HIST negative samples could have a high enzymatic activity but low carbohydrate-binding activity. Conversely, they could have a high carbohydrate-binding activity but low enzymatic activity. These two are complementary functions of PT and both are required for toxicity. Other factors such as toxin translocation into host cells might also be a critical factor for toxicity.

There was no clear direct correlation between *in vivo* HIST and ADP-ribosylation activity nor between HIST and Fet binding in vaccine samples. Similar to previous findings [34], the results indicated that a value of enzymatic activity/or a value of binding activity common to all vaccines could not be defined. Furthermore, the enzymatic and binding activities did not correspond to the total PT protein content as specified in the vaccine formulations. These results are not surprising as different detoxification methods would affect the two PT subunits differently. Since direct comparison of *in vivo* HIST activity with the *in vitro* biochemical assays, either the enzymatic or binding activity, did not show correlation, effort was made to establish a mathematical model to evaluate the relationship between the *in vivo* and *in vitro* activities using multiple regression analysis. Comparison of equations consisting a two-factors (log IU (predicted) = b₁ × log Fet + b₂ × log Enz + C) and a three-factors (log IU (predicted) = b₁ × log Fet + b₂ × log Fet × log Enz + b₃ × log Enz + C) which includes a factor of interaction of log Fet and log Enz showed no statistical difference in the predicted values between these two equations (P = 0.782, by paired t-test). Hence the two-factors equation was chosen for subsequent data analysis. When data for all products were analysed by this mathematical model using common regressions and constant factors (estimated from all the combined data of these products), no correlation was observed

(Fig. 3a). When product specific regression and constant factors were applied to the equation good correlation between the activity from HIST and the predicted activity by the *in vitro* assays was obtained (Fig. 3b and c). The results indicate that HIST activity could be reflected by a combination of functions of enzymatic and binding activities. According to the mathematical model for prediction of HIST activity, the combinations of somewhat varied levels of binding and enzymatic activities may result in similar levels of HIST activity, so that a similar level of HIST activity could result from the combinations of a range of binding and enzymatic activities. Furthermore it is interesting to note that product types which were produced by different detoxification processes resulted in a different population distribution in the correlation curves between the *in vivo* HIST activity and the predicted activity by *in vitro* assays. Since different types of products were usually manufactured by a variety of chemicals and conditions which resulted in varying degrees of detoxification and differences in enzyme and binding activities between products, our results supported the conclusion that the levels of residual PT enzymatic activity, the binding activity and the *in vivo* HIST activities of these vaccine types are product specific.

In the present study, a limited number of batches for each product was examined because of restricted availability. Although results of the model equation for predicting *in vivo* HIST activity by using *in vitro* binding and enzymatic activities of PT proved that this approach worked in principle, for utilising the equation reliably for predicting residual PT toxicity in an ACV, it is essential to establish consistency of the regression factors b_1 and b_2 and constant factor C values for the equation: $\log IU(\text{predicted}) = b_1 \times \log Fet + b_2 \times \log Enz + C$. To establish such consistency, it would be necessary to obtain population values of b and C for each product type by accumulating data until they become stable. Since large batch-to-batch variations in both the *in vivo* and *in vitro* activities were observed in this study, further investigation will be necessary for identifying the values of b and C values for monitoring of such consistency. Since ACVs included in this study were inactivated by treatment with either formaldehyde or glutaraldehyde or both, the information on ACVs detoxified by other method, e.g. hydrogen peroxide is not available in the present study. Furthermore, other factors associated with the detoxification procedure such as pH, reactant concentrations, matrix and the location of the reactive amino acid in the protein could also affect the extent of chemical modification. Therefore, these factors need to be established by each manufacturer and may have to be re-evaluated when the production process changes.

The molecular action of PT involves binding of the toxin to its receptors via the B-oligomer, membrane translocation of the S1-subunit and intracellular expression of the ADP-ribosyltransferase activity catalysed by the S1-subunit [6]. The present *in vitro* assay system only addressed the binding activity and the ADP-ribosyltransferase activity but does not take into account the membrane translocation (the internal processing of the toxoided PT *in vivo*). Furthermore, since the detoxification procedure could result in molecule aggregation, the effect of such aggregates on the carbohydrate-binding assay is unclear at the moment. Further investigation is under way to address these questions.

Attempts have been made to develop alternatives to the *in vivo* HIST. Most of these were based on approaches using refinement of animal or cell-based assays. These approaches have had limited success so far and have suffered from problems in assay reproducibility, or are unsuitable for final formulations due to presence of adjuvant or preservative in the final formulations [12,13]. In the present study, the approach using the *in vitro* binding and enzymatic activities of PT in a model equation for predicting *in vivo* HIST activity showed good correlation with the measured activity in HIST which provides good prospects for an alternative to the *in vivo*

HIST. However, the successful implementation of this approach as an alternative to current *in vivo* HIST in regulatory requirement for ACVs would depend on further validation of this test system within and between products. An important step would be to organise an international collaborative study between regulatory agencies and manufacturers for method transfer as well as for further validation.

In conclusion, a mathematic model equation is established in principle for predicting *in vivo* HIST activity by using *in vitro* binding and enzymatic activities for ACVs in which PT was inactivated by treatment with either formaldehyde or glutaraldehyde or both. This indicates for the first time that a workable alternative/replacement of *in vivo* HIST is feasible. The reliability of the proposed equation is dependent on establishing a product specific consistency for the regression factors and constant factor C values. In addition, our results showed that chemical detoxification by aldehyde reagents could affect both the enzymatic and binding activities of PT molecules and that both of these activities may play roles in the HIST.

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新時代のワクチン戦略について考える

総論

7. ワクチン行政とサーベイランス

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臨 床 検 査

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ワクチン行政とサーベイランス

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SUMMARY

本邦のワクチン行政を考えるに当たり、現在国内で接種可能なワクチンの種類、現在本邦で実施されている血清疫学調査(感染症流行予測調査)、患者サーベイランス(感染症発生動向調査)、予防接種後副反応報告と接種後健康状況調査を紹介した。予防接種は感染症予防にとって最も基本的かつ重要な手段である。正しく理解して、予防可能な感染症から個人そして社会全体が守られるよう期待したい。

(臨床検査 54:1263-1271, 2010)

KEYWORDS

予防接種法, 感染症発生動向調査, 感染症流行予測調査, 副反応, VPD

れており、ワクチン行政の一元化と、米国の予防接種の実施に関する諮問委員会(ACIP)のような予防接種の実施に関する方針を議論する公的な組織の必要性が求められてきた。新型インフルエンザの発生をきっかけとして、2009年12月に厚生科学審議会感染症分科会の中に予防接種部会(座長: 独立行政法人国立成育医療研究センター, 加藤達夫総長・理事長)が新設された。また、予防接種に関連する専門学会が複数集まって、その代表者から構成される予防接種推進専門協議会(会長: 独立行政法人国立病院機構三重病院, 神谷齊名誉院長)が設立された。インフルエンザのみならず、既存の予防接種についても戦略が議論されるようになり、ワクチン先進国に向けた取り組みとして期待される。



はじめに

ワクチン行政を担当する部署は厚生労働省内でも複数にまたがっている。スケジュールや政策、ワクチンの流通や安全性などにかかわる内容は厚生労働省が担当し、新しい製剤の承認審査などは独立行政法人医薬品医療機器総合機構が担当し、承認後の製剤の国家検定などは国立感染症研究所が担当している。筆者が所属する国立感染症研究所感染症情報センターは、検定業務は行っていないが、予防接種で予防可能疾患(vaccine preventable disease; VPD)や予防接種に関する情報を収集・解析し、予防接種の有効性・安全性に関する研究を実施するとともに、国民に正しい情報を提供する役割を担っている。しかし、近年本邦はワクチン後進国あるいはワクチンギャップと言わ



予防接種の種類

—国内承認・未承認ワクチン

2010年6月現在、本邦で接種可能なワクチンを表1に記載する。2008年12月にインフルエンザ菌b型(Hib)ワクチン、2009年6月に乾燥細胞培養日本脳炎ワクチン、同年10月にA型インフルエンザHAワクチン(H1N1株)、同年12月にヒトパピローマウイルスワクチン(子宮頸癌予防ワクチン)、2010年2月に肺炎球菌(7価結合型)ワクチンの接種が可能となり、接種可能なワクチンの数は増加した。

一方、海外の一部の国では、これら以外に表2に挙げるワクチンの接種が行われている。麻疹風疹おたふくかぜ混合(MMR)、麻疹風疹おたふく

1) 国立感染症研究所感染症情報センター・室長

表1 日本で接種可能なワクチンの種類(2010年6月現在)

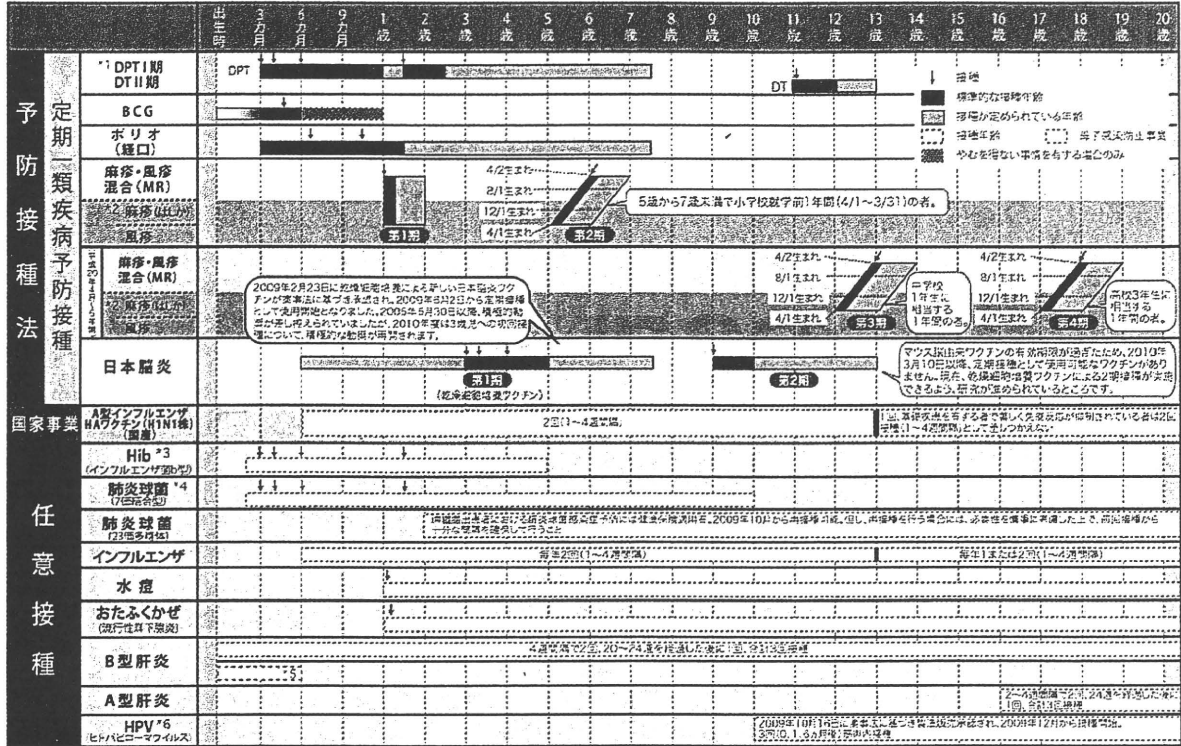
予防接種法に基づく 定期接種	生ワクチン BCG ポリオ 麻疹風疹混合(MR) 麻疹(はしか) 風疹 不活化ワクチン DPT DT 日本脳炎(乾燥細胞培養)：2009年6月接種開始 インフルエンザ
定期外接種 (任意接種)	生ワクチン 流行性耳下腺炎(おたふくかぜ) 水痘 黄熱 不活化ワクチン B型肝炎 破傷風トキソイド 成人用ジフテリアトキソイド A型肝炎 狂犬病 肺炎球菌(23価多糖体) 肺炎球菌(7価結合型)：2010年2月接種開始 ワイル病秋やみ b型インフルエンザ菌(Hib)：2008年12月接種開始 HPV(ヒトパピローマウイルス)：2009年12月接種開始 新型インフルエンザ(H5N1株)：国家備蓄 定期接種対象ワクチンを、対象以外の年齢で受ける場合
国家事業	不活化ワクチン A型インフルエンザHAワクチン(H1N1株)：2009年10月接種開始

表2 日本で承認されていないワクチンの種類(2010年6月現在)

国内未承認ワクチン	生ワクチン 麻疹風疹おたふくかぜ混合(MMR) 麻疹風疹おたふくかぜ水痘混合(MMRV) 経鼻インフルエンザウイルス 経口ロタウイルス 経口コレラ 経口腸チフス 不活化ワクチン 不活化ポリオ 不活化腸チフス 髄膜炎菌 ダニ媒介脳炎 そのほかの多価ワクチン(A型・B型肝炎混合他, 数種類)
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かぜ水痘混合(MMRV)ワクチンは国内にないが、麻疹風疹混合(MR)、麻疹、風疹、おたふくかぜ、水痘のそれぞれの単抗原ワクチンが存在する。そのため医師が特に必要と認めた場合は、これらのワクチンを異なる部位に同時接種することが可能である¹⁾。以上のことから1つずつ27日以上の間隔を空けて別の日に接種するか、あるいは

は複数のワクチンを同時に接種するかのいずれかの方法で接種が行われているのが現状である。経口ロタウイルスワクチンに関しては、国内での臨床試験が終了し、承認申請がなされているところである²⁾。また、不活化ポリオワクチンに関しては、ジフテリア百日咳破傷風混合(DPT)ワクチンとの混合(DPT+IPV)ワクチンとして、国内

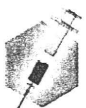


*1: D:ジフテリア, P:百日咳, T:破傷風, 赤矢印
 *2: 赤矢印は標準的な接種年齢を示す。黒矢印は任意接種を示す。黒矢印は標準的な接種年齢を示す。黒矢印は標準的な接種年齢を示す。
 *3: 2009年12月19日から国内での接種開始。生後2か月以上5歳未満の間に接種を受けることが必要と認められた場合は、接種方法を、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *4: 1回接種。接種後1週間以上経過した後に接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *5: 2009年10月16日に実施された予防接種スケジュール。生後2か月以上5歳未満の間に接種を受けることが必要と認められた場合は、接種方法を、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *6: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *7: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *8: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *9: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *10: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *11: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *12: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *13: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *14: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *15: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *16: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *17: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *18: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *19: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
 *20: 生後2〜4歳までに接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。接種方法は、生後4〜6歳の時点で接種を受ける。
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図1 日本の定期/任意予防接種スケジュール(20歳未満)
 (国立感染症研究所感染症情報センター HP(<http://idsc.nih.go.jp/vaccine/freport.html>))

で臨床治験が実施されている。

しかし、それ以外のワクチンについては、医師が個人輸入しなければ国内で受ける方法はなく、希望する場合は、国外で接種を受けることになる。医師による個人輸入は、“医師が患者の治療に際し、国内で販売されている医薬品などでは治療効果が得られず、患者救命のために外国で販売されている医薬品などを個人で輸入する場合”に行われているが、輸入に際しては煩雑な手続きが必要とされている³⁾。



予防接種スケジュール

図1に2010年4月現在の20歳未満の予防接種スケジュールを示す。なお、表1に記載した国内で接種可能なワクチンのなかで、黄熱ワクチン、狂犬病ワクチン、破傷風トキソイド、成人用ジフテリアトキソイド、ワイル病秋やみワクチン、新

型インフルエンザ(H5N1株)ワクチンに関しては、主に渡航時や特別な状況下で接種が行われるワクチンであるため、図1には含めていない。また、季節性のインフルエンザワクチンに関しては、65歳以上(一部、60~64歳)に予防接種法に基づく二類疾病として定期の予防接種(定期接種)が行われているが、図1は20歳未満を対象としているため、任意接種の枠組みに含めている。

図1の赤矢印は、標準的な接種年齢を一例として挙げたものである。しかし特に乳幼児においては、接種予定日に発熱、感冒などで体調不良となる場合も多く、予定通りに接種が進められないこともよく経験する。その場合は、必ずかかりつけ医と相談して、次の接種日を予定しておくことが接種漏れを防ぐ大切な方法である。

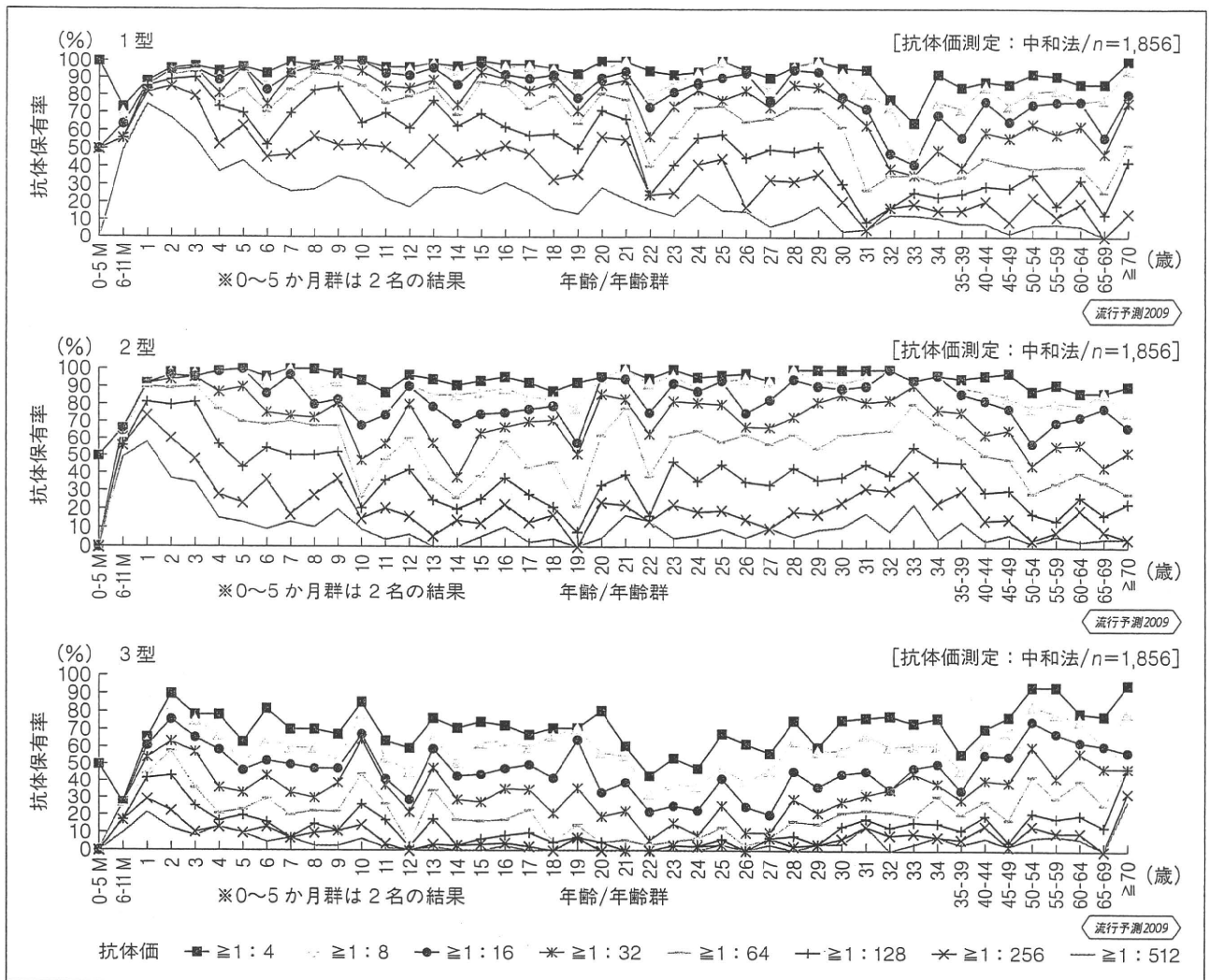
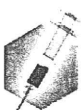


図2 年齢/年齢群別のポリオ中和抗体保有状況，2009年*

*：原則として2009年7～9月に採取された血清の測定結果(2010年2月現在暫定値)。

(2009年度感染症流行予測調査より)



予防接種で予防可能疾患 (VPD)のサーベイランス

1. 血清疫学調査(感染症流行予測調査)

定期接種対象疾患(麻疹，風疹，インフルエンザ，ポリオ，日本脳炎，百日咳，ジフテリア，破傷風)については，感染症流行予測調査事業として，本邦の国民の抗体保有率が調査されている。

この事業は，厚生労働省健康局結核感染症課が実施主体となり，都道府県，都道府県衛生研究所，国立感染症研究所が協力して行っているもので，麻疹，風疹，インフルエンザについては毎年，ポリオ，日本脳炎については2年に1回，百日咳，ジフテリア，破傷風については5年に1回の頻度で実施されている。結果は年度報告書とし

て報告されるとともに，速報として国立感染症研究所感染症情報センターのホームページに公開している⁴⁾。地域，年齢，性別，予防接種歴別に検討することによって，予防接種の有効性，感受性者数の推計が可能となり，国の予防接種スケジュールを決定するためにも用いられている。

図2に2009年度の事業結果から，ポリオを例として記載する。ポリオは現在，世界中で根絶に向けた取り組みが行われているが，本邦を含めたWHOアジア西太平洋地域では根絶された感染症であり，本邦では1980年の症例を最後に野生株ポリオウイルスによるポリオ患者はいない。そのため，抗体保有はすなわち，ポリオワクチンの接種により獲得されている。乳幼児期の高い接種率により抗体保有状況は高く維持されているが，1型ポリオウイルスについては，1970～1972年生

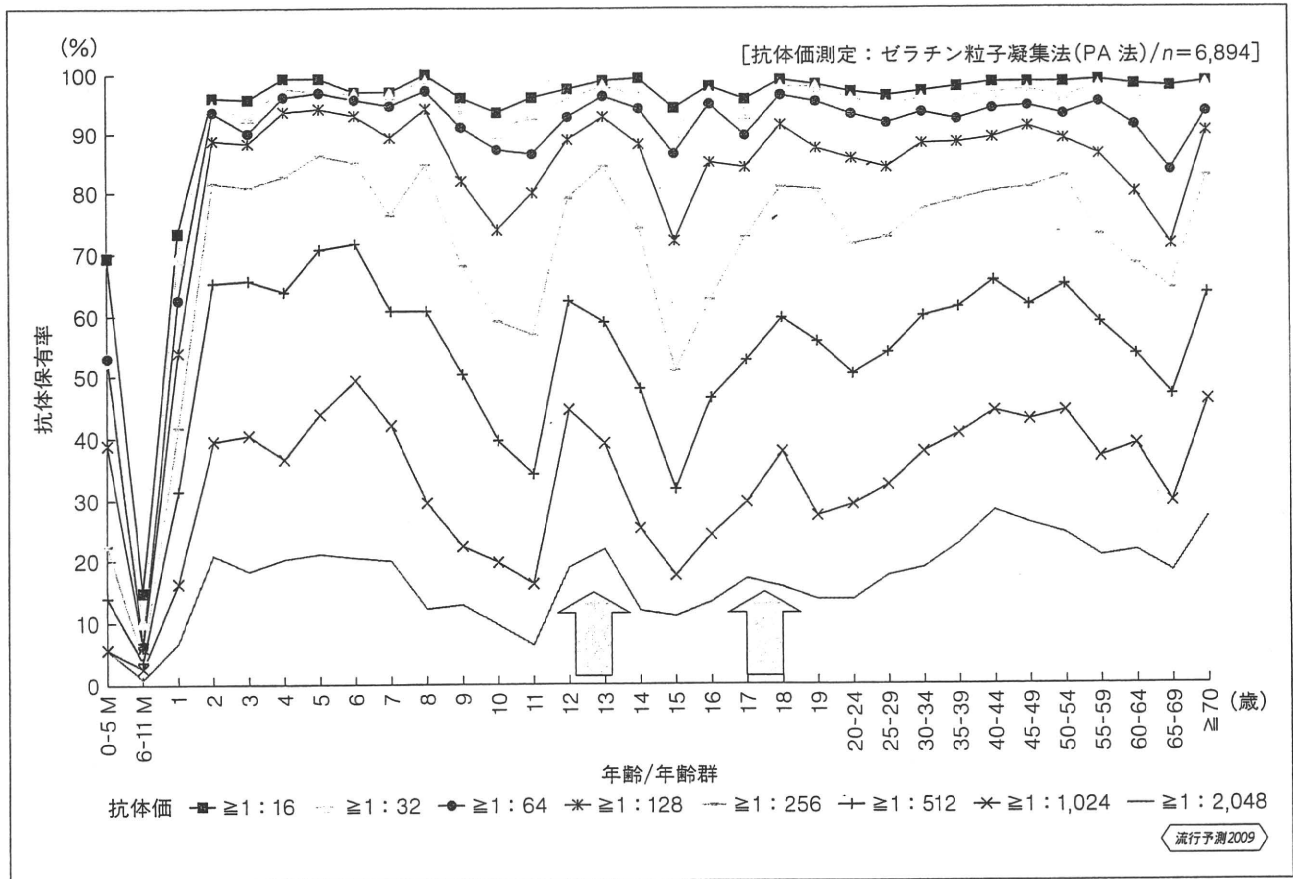


図3 年齢/年齢群別の麻疹PA抗体保有状況，2009年*

*：原則として2009年7～9月に採取された血清の測定結果(2010年2月現在暫定値)。

(2009年度感染症流行予測調査より一部改変)

まれの抗体保有率が前後の年齢群より低く、この年齢層に対してワクチンの接種が奨められている。

図3には2009年度の麻疹のゼラチン粒子凝集(PA)抗体保有状況を示したが、2008年度から始まった中学1年生、高校3年生相当年齢に対する2回目の接種の効果が結果に反映されている(図3矢印)。麻疹発症予防には少なくとも1:128以上の抗体を保有していることが必要であり、できれば1:256以上の抗体をもっていて欲しい。

2. 感染症発生動向調査

表3,4に国内で発生しているVPDの患者報告数を示した。表3は感染症法に基づいて全数報告が義務づけられている疾患の一覧であり、表4は全数把握は実施されていないが、感染症法に基づく感染症発生動向調査により疾患ごとに定められた定点医療機関を受診した患者数が報告され、サーベイランスが行われている疾患である。

急性脳炎については2003年11月5日以降、定点報告から全数把握に変更となり、痘瘡について

は、2003年11月5日以降新たに全数把握疾患に加わった。また、麻疹(定点報告のときは麻疹と成人麻疹がそれぞれ別の定点から報告されていた)と風疹は2008年1月1日以降、定点報告から全数報告疾患となったため、表3と表4に分けて記載しているが、表3は全数、表4は定点把握であるため、数字のみを単純に比較することはできない。

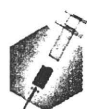
また、麻疹に代表されるように、定期接種として受けるよう努める義務(努力義務)が課せられているにもかかわらず接種率が95%以上に達しておらず、まだ多くの患者が発生している感染症もあり、日本は輸出国と非難されることも多い。一方、定期接種として実施されていない定期外(任意)接種のワクチンに関しては、水痘や流行性耳下腺炎に代表されるように、水痘は定点報告のみで毎年約25万人、流行性耳下腺炎は年によって違いがあるものの、多い年で年間約25万人、少ない年でも約6万人の患者報告があり、実際には、この数倍の患者が発生しているのが現状である。

表3 発生累積報告数(全数把握対象疾患)：感染症発生動向調査より

	1999 (14週～)	2000	2001	2002	2003	2004	2005	2006	2007	2008
痘そう*	0 (11/5～)	0	0	0	0	0
ポリオ	0	0	0	0	0	0	0	0	1	2
結核**	21,946 (4/1～)	27,737
ジフテリア	1	0	0	0	0	0	0	0	0	0
コレラ	39	58	50	51	24	86	56	45	13	45
A型肝炎	763	381	491	502	303	139	170	320	157	170
黄熱	0	0	0	0	0	0	0	0	0	0
狂犬病	0	0	0	0	0	0	0	2	0	0
日本脳炎	5	7	5	8	1	5	7	7	10	3
B型肝炎	510	425	330	332	245	241	209	228	199	174
急性脳炎***	12 (11/5～)	166	188	167	228	182
髄膜炎菌性 髄膜炎	10	15	8	9	18	21	10	14	17	10
先天性風疹 症候群	0	1	1	1	1	10	2	0	0	0
破傷風	66	91	80	106	73	101	115	117	89	120
風疹****	303
麻疹****	11,015

2000～2007年は、2009年8月4日現在報告数、2008年は、2009年1月6日現在、麻疹のみ2010年1月4日現在報告数。
*：2003年11月5日から対象疾患，**：2007年4月から対象疾患(結核統計による報告数とは異なる)，***：2003年11月4日以前は、定点把握対象疾患，****：2007年以前は、定点把握対象疾患。

(国立感染症研究所感染症情報センター HP(<http://idsc.nih.go.jp/vaccine/freport.html>))



予防接種後副反応 サーベイランス

定期接種後に副反応が発生した場合は、①予防接種実施要領¹⁾に基づいて、接種者、主治医、本人または保護者らが、予防接種後副反応報告書を市町村(特別区)長に提出し、保健所、都道府県を経由して厚生労働省に報告する、②一部の被接種者を抽出し、接種後の健康状況を調査し、保護者からハガキで返送してもらう予防接種後健康状況調査、の2つのシステムがある。

①は、ワクチンごとに報告基準が定められており、中等度あるいは健康被害救済申請前の迅速報告として比較的重症の副反応が報告される場合が多い。この報告からは異常な副反応の集積の有無や重篤な副反応の把握、ワクチンごとの副反応の

頻度を検討することが可能であり、迅速な解析が必要となる場合もある。②は、軽微な比較的頻度の高い副反応が報告されることが多く、稀で重症な副反応の把握には適していないが、接種後にみられる健康状況の変化を保護者に説明するために貢献している。しかし、すべて紙媒体の報告であるために、迅速な集計や解析が困難であり、報告書は図4に示したように、厚生労働省のホームページ²⁾に、2年程度経過してから発表されているのが現状である。

そこで、迅速かつ適切に情報を公開し、国民に正しく理解して予防接種を受けてもらうことを目的として、研究班(厚生労働科学研究 新型インフルエンザ等新興再興感染症研究事業 予防接種後健康被害審査の効率化に関する研究(研究代表者：筆者))を構築し、電子媒体での報告様式を用いた迅速な集計・解析システムの構築を現在進め

表4 発生累積報告数(定点把握対象疾患)：感染症発生動向調査より

		1999 (14週～)	2000	2001	2002	2003	2004	2005	2006	2007	2008
インフルエンザ	イ	65,471	769,964	305,441	747,010	1,162,290	770,063	1,563,662	900,181	1,212,042	621,151
急性脳炎*	基	129	149	134	108	99 (～11/4)	…	…	…	…	…
細菌性髄膜炎	基	235	256	278	300	298	379	309	350	383	412
水痘	小	162,424	275,036	271,409	263,308	250,561	245,941	242,296	265,453	245,880	224,800
成人麻疹**	基	83	426	931	440	462	59	7	39	975	…
百日咳	小	2,653	3,804	1,760	1,458	1,544	2,189	1,358	1,504	2,932	6,749
風疹**	小	2,972	3,123	2,561	2,971	2,795	4,239	895	509	463	…
ペニシリン耐性肺炎球菌感染症	基	2,129	4,321	5,254	6,132	6,447	6,692	6,233	5,294	4,840	5,217
麻疹 (成人麻疹を除く)**	小	5,875	22,552	33,812	12,473	8,285	1,547	537	516	3,132	…
無菌性髄膜炎	基	1,126	1,873	1,254	2,985	1,625	1,028	773	1,140	797	743
流行性耳下腺炎 (ムンプス)	小	69,070	132,877	254,711	180,827	84,734	127,592	187,837	200,639	67,830	65.355

イ：インフルエンザ定点(全国約5,000(内科 約2,000 および小児科 約3,000)), 小：小児科定点(小児科 全国約3,000), 基：基幹定点(内科および小児科医療を提供する300人以上収容する病院 全国約470)。

*：2003年11月5日以降、全数把握対象疾患、**：2008年以降、全数把握対象疾患。2000～2007年は2009年8月4日現在報告数。2008年は、2009年1月6日現在報告数(ペニシリン耐性肺炎球菌感染症のみ1月13日現在)。急性脳炎には麻疹脳炎、風疹脳炎、インフルエンザ脳症などが含まれます。無菌性髄膜炎にはムンプス髄膜炎など、細菌性髄膜炎にはインフルエンザ菌b型、肺炎球菌による髄膜炎などが含まれます。

(国立感染症研究所感染症情報センター HP(<http://idsc.nih.go.jp/vaccine/freport.html>))

予防接種後副反応報告書集計報告書平成19年度分
 予防接種後健康状況調査集計報告書平成19年度後期分
 予防接種後健康状況調査集計報告書平成19年度前期分
 予防接種後副反応報告書集計報告書平成18年度分
 予防接種後健康状況調査集計報告書平成18年度後期分
 予防接種後健康状況調査集計報告書平成18年度前期分
 予防接種後健康状況調査集計報告書平成18年度分(速報：MRワクチン限定)
 予防接種後副反応報告書集計報告書平成17年度分
 予防接種後健康状況調査集計報告書平成17年度後期分
 予防接種後健康状況調査集計報告書平成17年度前期分
 予防接種後健康状況調査集計報告書平成16年度後期分
 予防接種後健康状況調査集計報告書平成16年度前期分
 予防接種後副反応報告書集計報告書平成16年度分
 予防接種後副反応報告書集計報告書平成15年度分
 予防接種後健康状況調査集計報告書平成15年度前期分

図4 予防接種後副反応・健康状況調査検討会報告書

(厚生労働省 HP(<http://www.mhlw.go.jp/shingi/other.html#kenkou>))

ているところである^{6,7)}。

一方、任意接種のワクチンに関しては、ワクチンも1つの“薬”として、接種後に副反応が認められた場合においては報告されているのが実情であり、副反応モニタリング体制の検討が必要であると報告されている⁸⁾。



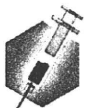
国民の誤解と 今後のワクチン行政への期待

任意接種のワクチンは、受ける必要がないワクチンと保護者に誤解されている場合が多く、国が必要と判断しているのであれば定期接種として受けるように勧奨されるはずであり、定期接種に含

まれていないワクチンは受けなくてもよいワクチンであるという誤解が国民の間に広がっているのは問題である。任意接種のワクチンの中にも、子どもたちにとって大切なワクチンが多く含まれていることを忘れてはならない。

国内では、7～8年前から定期接種に含まれていない水痘やおたふくかぜ、B型肝炎などのワクチンについても定期接種に導入する必要があるとの考えから、様々な研究が行われてきた^{9)~18)}。ほかにも数多くの研究成果が報告されているが、それらがワクチン行政に結びつかず、海外では小児の定期接種に導入することにより接種率を上昇させ、国内患者発生をゼロにしている感染症が出てきているなか、本邦では多くの患者発生が継続しているのも現状である。また、全数把握が行われていない感染症や、国としてサーベイランス自体が行われていない感染症もあり、実態を把握し予防接種の有効性と安全性を明確にするには、国としてVPDのサーベイランスの強化と予防接種後副反応報告の集計・解析を迅速かつ効率的に行い、国民に正しい情報を迅速に提供していく必要がある。

しかし、予防接種部会と予防接種推進専門協議会の発足に加えて、予防接種とVPDに関する基礎・臨床・疫学・医療経済学に関する研究がいつそう活発になることが必要である。研究成果が国の予防接種行政につなげられるような仕組み、例えば日本版ACIP(Advisory Committee Immunization Practices)が構築され、さらにそこでの議論が十分に行えるようなテーマごとのワーキンググループの構築と充実が期待される。



おわりに

予防接種は人類が感染症と戦ってきた歴史のなかで得られた大きな成果である。ワクチン未開発の疾患がまだ多数残されているなかで、VPDについては病気をよく理解するとともに、予防接種の有効性と安全性を理解して、接種対象年齢になったら可能な限り速やかに予防接種をすすめることが大切である。常に社会と個人にとっての予防接種の双方の観点から、ワクチンを考えたい。

今、本邦で必要なことは何かと考えたとき、健

康教育の中に感染症とその予防に関する項目を取り入れ、感染症の予防や対策に関する知識は予防接種を含めて子どもの頃から伝えていく必要性、予防接種後副反応の情報だけを伝えるのではなく罹患したときの重症化のリスクについても同時に正確に伝える必要性、定期接種(一類)対象ワクチンは努力義務が課せられているワクチンであって受けても受けなくてもよいワクチンではないことを伝える必要性、定期接種に含まれていないワクチンは必要性が低いワクチンという意味ではないことを伝える必要性、決められているから受けるというのではなく感染症予防のために必要な対策は何か、以上を考えた予防接種になって欲しい。

そして、受けやすい環境作りの構築とともに、ワクチンの接種費用を支払うことができる人だけが当該感染症から守られるという現在の状況は、何としても改善されて欲しい。

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の基盤構築(研究代表者:水落利明),平成21年度報告書

Summary

Surveillance of vaccine preventable diseases and vaccine policy

Keiko Tanaka-Taya¹⁾

The types of vaccine can be inoculated in Japan, the National Epidemiological Surveillance of Vaccine Preventable Diseases (NESVPD), the National Epidemiological Surveillance of Infectious Diseases (NESID), and the reports of adverse events after vaccination were summarized in this review.

Vaccination is the most fundamental and important means for the prevention of infectious diseases. I expect that people understand vaccines correctly and an individual and a society are protected from the VPD which can be prevented.

[Rinsho Kensa 54:1263-1271, 2010]

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