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## 要 旨

全国規模で収集した侵襲性感染症由来の肺炎球菌における薬剤耐性化の現況とワクチネーションの基盤となる莢膜型について述べた。

肺炎球菌のβ-ラクタム系薬剤耐性は、その作用標的である細胞壁合成酵素のPBP1A, PBP2X, およびPBP2Bをコードする遺伝子変異から解析すると、PBP変異のないgPSSPは19.9%、1~2遺伝子変異したgPISPは48.3%、3遺伝子変異したgPRSPは31.8%の割合であった。またそれらの株の80%はマクロライド系薬剤耐性遺伝子も保持していた。

莢膜型は多岐にわたっていたが、7価コンジュゲートワクチン(7PCV)型に含まれる4, 6B, 9V, 14, 18C, 19F, 23F型の菌は全体の73.8%を占めていた。それらの型にはgPRSPが圧倒的に多く、また2歳以下の症例が80%以上を占めており、乳幼児への7PCVの導入は肺炎球菌による重症感染症例の減少に寄与するものと考えられた。

## はじめに

肺炎球菌は、小児あるいは成人が市中において罹患する呼吸器感染症の原因菌としてもっとも検出頻度の高い細菌である。特に、抗菌薬が発達した現代においても、本菌によって敗血症や化膿性髄膜炎を惹起した場合には、しばしば重篤な後遺症の残存や致命的となりうる<sup>1)</sup>。このような臨床的に重要な肺炎球菌において、本菌による疾患に対する基本的な治療薬であるβ-ラクタム系薬剤耐性菌(PRSP)が注目されて久しい<sup>2)3)</sup>。

そもそも、PRSPに関する最初の報告は1970年代に遡るが、それ以降、この耐性菌が臨床的に注目されたのは治療薬の主体がペニシリン系

薬剤を処方している欧米からであり、セフェム系薬剤が主体のわが国においては長い間ほとんど問題にならなかった耐性菌である。

本邦においてPRSPが臨床上の関心事となったのは、1988年の本菌による小児化膿性髄膜炎例の報告に始まる<sup>4)</sup>。筆者らは1993年から全国規模で肺炎球菌を収集し、薬剤感受性や耐性遺伝子レベルでの疫学調査、病原性にかかわる莢膜の血清型別を含めた疫学研究を開始し、その成績を報告してきている<sup>5)</sup>。

ここでは、肺炎球菌による感染症の予防策となるワクチネーションの疫学的基礎となる薬剤耐性化の現況と莢膜型の成績について、小児の侵襲性感染症由来株の成績を中心に述べる。

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# **Simultaneous and Rapid Detection of Causative Pathogens in Community-acquired Pneumonia by Real-time PCR (1167)**

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## **Pneumococcal Global Serotype Project**

### **Summary report of Stage 1/Version 1 analysis**

#### **Background**

Pneumococcal vaccine has been chosen as the pilot vaccine for the Advanced Market Commitment (AMC), a novel funding mechanism to encourage the development of vaccine products and capacity for developing world markets. As part of the AMC process, a Target Product Profile (TPP) for pneumococcal vaccines has to be established. The TPP will describe minimum characteristics required for a pneumococcal vaccine to be eligible for AMC funding. The WHO has been charged with developing the TPP. The WHO requested GAVI's PneumoADIP to undertake a global pneumococcal serotype project (GSP) and analysis, a summary of which is provided here.

Vaccines based on capsular polysaccharides, pneumococcal protein antigens or both, may be developed during the AMC period. Both types of vaccines are potentially eligible for AMC funding. While the TPP should be structured to be equally relevant to both vaccines based on the capsular polysaccharides and protein antigens, the literature on the distribution of protein antigens is not yet well developed and therefore, the capsular serotype distribution is a better parameter on which to set health impact and effectiveness benchmarks for the TPP in the meantime.

The current "state of the art review" of the global and regional serotype distribution of disease causing strains was published in 2000 by Hausdorff et al. However, the data in that review are now mostly over 10 years old and there are key limitations to the existing published analyses. In the interim substantial new data have become available that make this the right time to conduct a new, comprehensive review of data, and to use that data for determining the AMC TPP.

The specific objectives of the GSP included:

- To identify and collect all existing published and unpublished data with information on serotypes of pneumococci causing invasive disease in children less than five years of age, globally.
- To analyze the data collected to understand what variables impact the serotype distribution
- To provide the TPP Expert Committee with an analysis and summary of the serotype burden among young children globally and by region (by sub-region if possible).

## Importance of the Anti-Interferon Capacity of Sendai Virus C Protein for Pathogenicity in Mice<sup>∇</sup>

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The Sendai virus (SeV) C protein blocks signal transduction of interferon (IFN), thereby counteracting the antiviral actions of IFN. Using HeLa cell lines expressing truncated or mutated SeV C proteins, we found that the C-terminal half has anti-IFN capacity, and that K<sup>151</sup>A, E<sup>153</sup>A, and R<sup>154</sup>A substitutions in the C protein eliminated this capacity. Here, we further created the mutant virus SeV Cm\*, in which K<sup>151</sup>A, E<sup>153</sup>K, and R<sup>157</sup>L substitutions in the C protein were introduced without changing the amino acid sequence of overlapped P, V, and W proteins. SeV Cm\* was found to lack anti-IFN capacity, as expected. While the growth rate and final yield of SeV Cm\* were inferior to those of the wild-type SeV in IFN-responsive, STAT1-positive 2fTGH cells, SeV Cm\* grew equivalently to the wild-type SeV in IFN-nonresponsive, STAT1-deficient U3A cells. SeV Cm\* was thus shown to maintain multiplication capacity, except that it lacked anti-IFN capacity. Intranasally inoculated SeV Cm\* could propagate in the lungs of STAT1<sup>-/-</sup> mice but was cleared from those of STAT1<sup>+/+</sup> mice without propagation. It was found that the anti-IFN capacity of the SeV C protein was indispensable for pathogenicity in mice. Conversely, the results show that the innate immunity contributed to elimination of SeV in early stages of infection in the absence of anti-IFN capacity.

Sendai virus (SeV) belongs to the genus *Respirovirus* of the subfamily *Paramyxovirinae*; it exclusively infects respiratory epithelial cells of rodents and causes fatal bronchopneumonia. Experimental infection of mice with SeV is frequently used as a model of viral pathogenesis of respiratory disease (25, 31, 37). SeV is an enveloped virus with a linear, nonsegmented, negative-sense RNA genome of 15,384 nucleotides and contains six genes in the order 3'-(leader)-N-P-M-F-HN-L-(trailer)-5' on the genome. Monocistronic mRNAs are transcribed by the viral RNA polymerase, which is composed of the L and P proteins (17).

However, the P gene gives rise to multiple protein species by a process known as RNA editing and by the use of an overlapping open reading frame. The P protein is translated from the unedited mRNA, which is the exact copy of the P gene. The V protein is translated from the edited mRNA, in which one nontemplated G residue is cotranscriptionally inserted to the editing position. Among transcripts from the P gene, one G is inserted into approximately 20% of transcripts. Less frequently (<5%), two G residues are inserted and the W protein is synthesized (25). Therefore, the N-terminal 316 amino acids of the P, V, and W proteins produced from the coding region before the editing site are in common, and their C termini are unique (38). The V protein is expressed by members of viruses in the subfamily *Paramyxovirinae*, except for some viruses belonging to the genus *Respirovirus*. The unique C-terminal region of V proteins contains seven cysteine residues that are

highly conserved among paramyxoviruses, forms zinc finger-like motifs, and binds Zn<sup>2+</sup> (22, 39, 49, 56) and also binds one of the intracellular RNA sensor molecules, MDA5 (1, 59). The W protein is potentially synthesized by viruses which produce the V protein, according to the sequence database. The presence of the W protein has been identified only in SeV and Nipah virus (38, 48).

The C protein is translated from the -1 reading frame relative to the P, V, and W common frame of the respective mRNAs. The C protein is expressed by viruses belonging to three genera (*Respirovirus*, *Morbillivirus*, and *Henipavirus*), but is not expressed by viruses belonging to two genera (*Rubulavirus* and *Avulavirus*) of the subfamily *Paramyxovirinae* (38). The SeV P, V, and W proteins are initiated at the AUG codon at position 104, whereas four initiation sites at positions 81, 114, 183, and 201 are used for translation of the SeV C proteins, C', C, Y1, and Y2, respectively (2). Among the initiation sites of the C protein, the first is a non-AUG codon (ACG) (2, 16, 50). Translation of the C', C, Y1, and Y2 proteins is terminated at the same position, 725.

Interferon (IFN) antagonism of some paramyxoviruses is known because preinfection or persistent infection of cells with paramyxoviruses such as SeV or human parainfluenza virus type 3 (hPIV3) enhances the growth of heterologous postinfecting IFN-sensitive viruses, such as Newcastle disease virus (NDV) and vesicular stomatitis virus (VSV) (19, 40). Anti-IFN capacity was recently shown to be associated with viral proteins encoded by the P gene of the paramyxoviruses. The V proteins of simian virus 5 (SV5) (4, 51), SV41 (45), mumps virus (35), hPIV2 (45, 47), NDV (21, 48), and measles virus (46, 57) counteract IFNs, whereas the C proteins of SeV and hPIV3 counteract IFNs (7, 12, 41). For Nipah virus, the V, W, C, and

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## SARS-CoV spike protein-expressing recombinant vaccinia virus efficiently induces neutralizing antibodies in rabbits pre-immunized with vaccinia virus

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### Abstract

A vaccine for severe acute respiratory syndrome (SARS) is being intensively pursued against its re-emergence. We generated a SARS coronavirus (SARS-CoV) spike protein-expressing recombinant vaccinia virus (RVV-S) using highly attenuated strain LC16m8. Intradermal administration of RVV-S into rabbits induced neutralizing (NT) antibodies against SARS-CoV 1 week after administration and the NT titer reached 1:1000 after boost immunization with RVV-S. Significantly, NT antibodies against SARS-CoV were induced by administration of RVV-S to rabbits that had been pre-immunized with LC16m8. RVV-S can induce NT antibodies against SARS-CoV despite the presence of NT antibodies against VV. These results suggest that RVV-S may be a powerful SARS vaccine, including in patients previously immunized with the smallpox vaccine.

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**Keywords:** SARS coronavirus; Recombinant vaccinia virus; LC16m8

### 1. Introduction

In November 2002, an influenza-like acute pneumonia designated as severe acute respiratory syndrome (SARS) by the World Health Organization, first emerged in China and spread to 29 countries within a few months. By July 2003, 8098 probable cases with 774 deaths were

reported ([www.cdc.gov/mmwr/mguide\\_sars.html](http://www.cdc.gov/mmwr/mguide_sars.html)). The etiologic agent of SARS was identified as a novel type of coronavirus (CoV) that was genetically distinct from previously characterized members of the Coronaviridae family [1–3]. Like other coronaviruses, SARS-CoV is a positive stranded RNA virus with an approximately 30 kb genome encoding non-structural proteins as well as structural proteins, including spike, envelope, membrane and nucleocapsid. Spike protein is a type I transmembrane glycoprotein that mediates binding to the host cell receptor using an amino-terminal S1

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# 予防接種の現状と対策 2

## 細菌に対するワクチン

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### ● はじめに

わが国における細菌に対するワクチンは、肺炎球菌荚膜ポリサッカライドワクチンとインフルエンザ b 菌 (Hib) ワクチン、ジフテリア・百日咳・破傷風 3 種混合ワクチン (DPT ワクチン) が実施可能である。これらのワクチンの特徴と現状について解説し、問題点についても述べる。

### ● 肺炎球菌荚膜ポリサッカライドワクチン

肺炎球菌荚膜ポリサッカライドワクチン (肺炎球菌ワクチン) とは肺炎球菌の荚膜多糖体成分でできた成分ワクチンである。肺炎球菌の荚膜はその抗原性から 90 種類以上の血清型に分類されているが、このワクチンには 23 種類の血清型の荚膜成分が含まれている。なお、米国などで小児に使用されている 7 価の結合型ワクチンはわが国では今のところ使用できない。米国予防接種諮問委員会 (ACIP) は 1997 年に肺炎球菌感染症の予防が推奨される対象について示した<sup>1)</sup>。このなかで、65 歳以上の高齢者に対してはワクチンの有効性が証明され、臨床的な利益がある A ランクの推奨をしている。わが国での接種対象を、表 1 に示す。

肺炎球菌ワクチンは基本的に重篤な副反応が少なく、安全なワクチンである。妊婦への接種は原則禁止であるが、ほかに禁忌となる対象はない。ワクチン接種に伴う副反応の多くは注射

表 1 23 価肺炎球菌荚膜ポリサッカライドワクチンの接種対象者

- 2 歳以上で肺炎球菌による重篤疾患に罹患する危険が高い次のような個人および患者
- (1) 脾摘患者における肺炎球菌による感染症の発症予防  
(脾臓摘出後の 2 歳以上の場合のみ保険適応)
  - (2) 肺炎球菌による感染症の予防
    - 1) 鎌状赤血球疾患、あるいはその他の原因で脾機能不全である患者
    - 2) 心・呼吸器の慢性疾患、腎不全、肝機能障害、糖尿病、慢性髄液漏等基礎疾患のある患者
    - 3) 高齢者
    - 4) 免疫抑制作用を有する治療が予定されている者で治療開始まで少なくとも 14 日以上の余裕のある患者

局所の疼痛、熱感、腫脹、発赤といったもので 5% 以上に認められるが、治療を要するものは少ない。わが国における本ワクチンの接種状況は 1988 年の接種開始時には年間 5000 人程度の接種実績であったが、近年は増加傾向にあり 2002 年以降は年間 15 万人以上となっている。しかし、現在でも 65 歳以上の人口に対する肺炎球菌ワクチンの接種率は 3% 前後であり、米国における接種率の 64.26%<sup>2)</sup> とはいまだ大きな隔りがある。

問題点として、本ワクチンを 5 年以内に再接種した場合、強いアナフィラキシーや局所の強い副反応のおそれがあることがあげられる。米

## Comparative Immune Responses of Patients with Chronic Pulmonary Diseases during the 2-Year Period after Pneumococcal Vaccination<sup>∇</sup>

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**Antibody responses to a 23-valent pneumococcal vaccine for *Streptococcus pneumoniae* serotypes 6B, 14, 19F, and 23F in 84 patients with chronic pulmonary diseases over a 2-year period after vaccination were examined by using a third-generation enzyme-linked immunosorbent assay. Of these patients, 28 (31%) were low responders who had developed increases of at least twofold in the levels of serotype-specific immunoglobulin G (IgG) in sera for none of the four serotypes at 1 month after vaccination. Although no specific clinical features of low responders were evident, their prevaccination levels of IgG for all serotypes were higher than those of responders. In responders, the levels of IgG specific for serotypes 14 and 23F in sera were greatly increased 1 month after vaccination and those specific for serotypes 6B and 19F were moderately increased. In contrast, no significant increases in the levels of IgG specific for serotypes 6B, 19F, and 23F in the low responders during the same period were found, but the levels of IgG specific for serotype 14 did increase. Although a rapid decline in the levels of IgG for all serotypes in responders between 1 month and 6 months after vaccination was found, the levels of IgG specific for serotypes 14 and 23F in sera remained higher than the prevaccination levels for at least 2 years after vaccination. These data suggest the need for the revaccination of responders but not low responders among patients with chronic pulmonary diseases. Revaccination as early as 3 years postvaccination is recommended for responders to increase the reduced levels of IgG in sera, especially those specific for the weak vaccine antigens.**

*Streptococcus pneumoniae* is an important cause of pneumonia and serious invasive diseases in children and adults (4, 13, 14). The increased rate of drug-resistant pneumococci in recent years emphasizes the need for preventing pneumococcal infections by vaccination with the 23-valent pneumococcal polysaccharide vaccine (PPV) (3, 16, 19, 28).

Patients with chronic pulmonary diseases, such as chronic obstructive pulmonary diseases (COPD), are highly susceptible to pneumonia or acute exacerbation caused by *S. pneumoniae* (25). Since previous investigators reported the efficacy of PPV for preventing invasive pneumococcal diseases in patients, including those with chronic pulmonary diseases and other chronic illnesses, PPV is recommended for these patients (8, 9, 26). The nature of the effects of PPV in preventing pneumonia or acute exacerbation among patients with chronic pulmonary diseases, however, remains controversial (1, 11, 27, 30).

Antibodies to pneumococcal capsular polysaccharide (PPS) and complement provide protection against *S. pneumoniae* strains with homologous or cross-reactive capsular serotypes

(18). Using a variety of methodologies, previous investigators have reported the concentrations of PPS-specific immunoglobulin G (IgG) in sera from patients with chronic pulmonary diseases, including COPD (7, 11, 22, 29). No studies, however, have examined the levels of serotype-specific IgG in sera from patients with chronic pulmonary diseases by using the third-generation enzyme-linked immunosorbent assay (ELISA) that has recently been recommended by the World Health Organization (31).

Two previous studies reported a substantial proportion of poor responders to PPV among elderly adults or patients with COPD who were receiving steroid therapy (12, 21). However, these studies failed to demonstrate the kinetics of the immune responses of this group. In addition, antibody avidity is an indicator of the strength with which an antibody binds to a complex antigen, and high-avidity antibodies are superior to low-avidity antibodies in terms of opsonophagocytic killing of *S. pneumoniae* (2, 20). No previous studies have examined the avidities of antibodies in sera from patients with chronic pulmonary diseases before and after pneumococcal vaccination.

The objective of this study, therefore, was to examine the concentrations of serotype-specific IgG and the avidity of IgG in sera from patients with chronic pulmonary diseases by using the third-generation ELISA before and after pneumococcal vaccination. We also attempt to characterize a subset of low responders among these patients and demonstrate the differ-

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## Increased Rates of Intense Nasopharyngeal Bacterial Colonization of Vietnamese Children with Radiological Pneumonia

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ANH, D.D., HUONG, P.L.T., WATANABE, K., NGUYET, N.T., ANH, N.T.H., THI, N.T., DUNG, N.T., PHUONG, D.M., TANIMURA, S., OHKUSA, Y., NAGATAKE, T., WATANABE, H. and OISHI, K. *Increased Rates of Intense Nasopharyngeal Bacterial Colonization of Vietnamese Children with Radiological Pneumonia*. Tohoku J. Exp. Med., 2007, 213 (2), 167-172 — Acute lower respiratory infection (ALRI), primarily pneumonia, is the leading cause of death in children under the age of five. Bacterial ALRI is preceded by asymptomatic bacterial colonization. Bacterial colonization, therefore, may have an important role in the development of pneumonia in children. This case-control study was conducted in order to determine if intense bacterial colonization was increased in the nasopharynx of pediatric patients with ALRI. One hundred-sixty four pediatric patients with ALRI and 70 healthy children < 5 years of age were enrolled in Hanoi, Vietnam between 2001 and 2002. Bacterial pathogens were isolated from nasopharyngeal secretions and quantitatively cultured. Of 164 patients, 91 were diagnosed as having radiological pneumonia (PN group) and 73 as having acute bronchitis (AB group). Intense growth of any bacterial pathogen ( $\geq 10^6$  colony-forming units/ml) was highest in the PN group (49.4%), followed by the AB group (28.8%), with healthy children having the lowest (17.1%). Patients with intense bacterial growth were more likely to develop pneumonia, but not acute bronchitis, than were patients with light or no bacterial growth. The results of this case-control study suggest that the vertical spread of intense bacterial pathogens colonized in the nasopharynx to the lower airway leads to bacterial pneumonia in children under the age of five. ——— radiological pneumonia; children; bacterial colonization; Vietnam; *Streptococcus pneumoniae*

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## Strain-Specific Pulmonary Defense Achieved after Repeated Airway Immunizations with Non-Typeable *Haemophilus Influenzae* in a Mouse Model

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KOYAMA, J., AHMED, K., ZHAO, J., SAITO, M., ONIZUKA, S., OMA, K., WATANABE, K., WATANABE, H. and OISHI, K. *Strain-Specific Pulmonary Defense Achieved after Repeated Airway Immunizations with Non-Typeable Haemophilus Influenzae* in a Mouse Model. *Tohoku J. Exp. Med.*, 2007, **211** (1), 63-74 — Strain-specific immune responses may play a critical role in the acute exacerbation of chronic obstructive pulmonary disease (COPD) caused by *Haemophilus influenzae* (NTHi), and the outer membrane protein P2 is one of surface antigens of NTHi, which may contribute to the strain-specific protective immunity. We examined whether repeated airway immunizations with killed-NTHi strains bearing different P2 molecules were capable of inducing protective immunity against homologous or heterologous strains in the lungs of a mouse model. Three different strains of NTHi were used in this study. Three serial intratracheal (IT) immunizations of a single strain or three different strains of NTHi led to the production of cross-reactive immunoglobulins G and A in bronchoalveolar lavage fluids. Three serial IT immunizations with a single strain enhanced the bacterial clearance of the homologous strain in the lungs, but no enhancement of bacterial clearance was found with three serial IT immunizations of heterologous strains. The enhancement in bacterial clearance, therefore, appears to be primarily strain-specific. Enhanced bacterial clearance of a heterologous strain was also found after three serial IT immunizations of a single strain among two of the three strains employed for bacterial challenge. These findings suggest that P2 molecules and surface antigens other than P2 are involved in the development of pulmonary defense against NTHi in mice. Our data may explain, in part, why patients with COPD experience recurrent NTHi infections. ——— non-typeable *Haemophilus influenzae*; outer membrane protein P2; pulmonary defense; chronic obstructive pulmonary disease; acute exacerbation

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## Lack of Efficacy of High-Dose Intravenous Immunoglobulin Treatment of Severe Thrombocytopenia in Patients with Secondary Dengue Virus Infection

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**Abstract.** Because most cases of secondary dengue virus infection are associated with an increased level of platelet-associated IgG, a high dose of intravenous immunoglobulin (IVIG) may have an effect on the development of severe thrombocytopenia in this disease. A randomized, controlled study was conducted with two treatment groups consisting of a treatment (IVIG) group ( $n = 15$ ) and a non-treatment (non-IVIG) group ( $n = 16$ ) to determine whether a high dose of IVIG is effective in hastening the recovery from thrombocytopenia in patients with secondary dengue virus infection. No significant difference was found in the baseline demographic data between the two groups. No adverse effect of IVIG was observed, but no effect in hastening the recovery of platelet counts was found in patients with secondary dengue infections. The lack of efficacy of IVIG suggests that platelet clearance by macrophages through Fc  $\gamma$  receptors is not a primary mechanism in this disease.

### INTRODUCTION

Dengue virus types 1–4 induce a wide spectrum of clinical manifestations, including hemorrhagic manifestations associated with thrombocytopenia and increased vascular permeability. Secondary infections, which are commonly observed in dengue-endemic areas, are more likely to constitute a risk factor for dengue hemorrhagic fever (DHF).<sup>1</sup> Although dengue virus-induced bone marrow suppression decreases platelet synthesis, an immune mechanism of thrombocytopenia resulting in increased platelet destruction appears to be operative in patients with DHF.<sup>2,3</sup> This disease is now highly endemic in more than 100 tropical countries, and the number of cases has increased dramatically during the past three decades.<sup>3,4</sup> More than 1,000 deaths occur annually due to DHF, and no specific treatment is currently available.

The high frequency of elevated platelet-associated IgG (PAIgG) in idiopathic thrombocytopenic purpura (ITP) suggests that PAIgG is involved in the mechanisms of thrombocytopenia.<sup>5,6</sup> Platelets coated with IgG autoantibodies, which form PAIgG, undergo accelerated clearance through Fc $\gamma$  receptors that are expressed on tissue macrophages. Intravenous immunoglobulin (IVIG) is currently a widely accepted treatment option for ITP. Therapeutic activity of IVIG in the amelioration of ITP appears to involve the mechanism of competitive inhibition of activating Fc  $\gamma$  receptors on phagocytic macrophages in the mononuclear phagocytic system by IVIG-sensitized erythrocytes.<sup>7</sup> Recent studies demonstrated that the levels of PAIgG levels were inversely correlated with platelet count in patients in the acute phase of secondary dengue virus infections.<sup>8,9</sup> An increased level of PAIgG was observed in 73.8–80.8% of patients with secondary dengue virus infection. These data indicate that the formation of PAIgG in patients with secondary dengue virus infection may result in thrombocytopenia due either to plate-

let clearance by macrophages or to platelet lysis. This disease can be classified as a dengue virus-induced ITP. It was hypothesized, therefore, that Fc  $\gamma$  receptor blockade by a high dose of IVIG might inhibit the development of severe thrombocytopenia caused by secondary dengue virus infection. A previous case report had suggested this effect in a patient with dengue fever (DF).<sup>10</sup>

### MATERIALS AND METHODS

**Patients and study design.** The present randomized, controlled study was conducted to determine the efficacy of a high dose of IVIG in hastening the recovery of platelets or inhibiting the development of severe thrombocytopenia in patients with secondary dengue virus infection. Thirty-six patients clinically suspected of being infected with dengue virus who fulfilled the inclusion criteria were admitted and enrolled in the study on the first day of admission (day 1) to San Lazaro Hospital (Manila, The Philippines) between October and November 2005. Dengue hemorrhagic fever was diagnosed according to World Health Organization (WHO) criteria.<sup>11</sup> There were two inclusion criteria for these patients: 1) an acute phase of dengue illness (within 5 days after the onset of illness) verified by the particle agglutination test for dengue IgM,<sup>12</sup> and 2) severe thrombocytopenia (platelet count between 20,000  $\mu$ L and 80,000/ $\mu$ L) without prominent manifestation of bleeding or shock. The exclusion criterion was a present history of platelet transfusion either before or after admission to the hospital. The study was reviewed and approved by the Bioethics Committees of San Lazaro Hospital and St. Luke's Medical Center. Parents or guardians of all patients provided written informed consent.

Of the patients enrolled, 34 were confirmed to be infected with the dengue virus infected on the basis of a positive result by IgM-capture enzyme-linked immunosorbent assay or reverse transcription-polymerase chain reaction.<sup>13,14</sup> Three patients were diagnosed as having primary infections, and 31 patients were diagnosed as having secondary infections by a hemagglutination inhibition test.<sup>15</sup> Laboratory tests were conducted at St. Luke's Medical Center (Quezon City, The Phil-

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# Possible Prevalence and Transmission of Acute Respiratory Tract Infections Caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* among the Internally Displaced Persons in Tsunami Disaster Evacuation Camps of Sri Lanka

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## Abstract

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**Objective** The objective of this prospective study was to investigate the status of acute respiratory tract infections caused by *Haemophilus influenzae* and *Streptococcus pneumoniae* in tsunami disaster evacuation camps.

**Methods** Nasopharyngeal swabs (NP) of 324 internally displaced persons (IDP) in 3 different tsunami disaster evacuation camps of Sri Lanka were collected between March 18th and 20th, 2005, and analyzed for MIC,  $\beta$ -lactamase production, serotypes, PCR and pulsed-field gel electrophoresis (PFGE).

**Results** Many IDP had respiratory symptoms and the prevalence of cough and/or sputum was 84%, 70.5% and 64.7% in the three camps. Twenty-one *H. influenzae* from 20 IDP and 25 *S. pneumoniae* from 22 IDP were isolated from the NP. All *H. influenzae* isolates were nontypeable, and 5 were  $\beta$ -lactamase producing. Seventeen pneumococci were susceptible, 5 showed intermediate resistance and 3 were fully resistant to penicillin G. Molecular analysis showed the 21 *H. influenzae* strains had 13 PFGE patterns and 25 pneumococci had 16 PFGE patterns. All 4 different PFGE patterns of *H. influenzae* strains were detected in a few IDP in camps 1 and 3, and 5 different PFGE patterns of serotype 3, 22A, 9A, 10A and 11A pneumococci were detected in a few IDP in camps 1 and 3.

**Conclusion** Our data indicate acute respiratory tract infections caused by various types of *H. influenzae* and *S. pneumoniae* appear to have been prevalent, some of which were potentially transmitted from person to person in tsunami disaster evacuation camps.

**Key words:** *Haemophilus influenzae*, *Streptococcus pneumoniae*, tsunami, internally displaced persons, acute respiratory tract infection, evacuation camp

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## Introduction

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The tsunami that occurred on December 26th, 2004 struck

southeast Asia and affected 12 countries. At least 310,000 people died, and many millions were left destitute (1). After the tsunami, many people continued to live in evacuation camps in the affected countries for various periods of times.

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# Mechanisms underlying glycosylation-mediated loss of ecotropic receptor function in murine MDTF cells and implications for receptor evolution

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A *Mus dunni* tail fibroblast (MDTF) cell line is highly resistant to infection by ecotropic Moloney murine leukemia virus (Mo-MLV). The cationic amino acid transporter type 1 (CAT1) paralogues of murine NIH 3T3 and MDTF cells (mCAT1 and dCAT1, respectively) contain two conserved *N*-linked glycosylation sites in the third extracellular loop (ECL3, the putative Mo-MLV binding site). Glycosylation of dCAT1 inhibits Mo-MLV infection, but that of mCAT1 does not. Compared with mCAT1, dCAT1 possesses an Ile-to-Val substitution at position 214 and a Gly insertion at position 236 in the ECL3. To determine the residues responsible for the loss of dCAT1 receptor function, mutants of mCAT1 were constructed. The mCAT1/insG receptor (with a Gly residue inserted at mCAT1 position 236) had greatly reduced Mo-MLV receptor function compared with mCAT1. Treatment of mCAT1/insG-expressing cells with tunicamycin, an *N*-linked glycosylation inhibitor, increased the transduction titre. In addition, the reduced susceptibility to Mo-MLV observed with mCAT1/insG-expressing cells correlated with impaired binding of Mo-MLV. These results show that a single amino acid insertion confers mCAT1 receptor properties on dCAT1 and provide an important insight into the co-evolution of virus–host interactions.

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## INTRODUCTION

The entry of ecotropic murine leukemia virus (Eco-MLV) into host cells is initiated by the interaction between the viral envelope glycoprotein (Env) and its receptor, cationic amino acid transporter type 1 (CAT1) (Albritton *et al.*, 1989; Kim *et al.*, 1991; Wang *et al.*, 1991). The CAT1 protein is a 14-transmembrane multi-spanning molecule comprised of seven extracellular and six cytoplasmic loops with two conserved *N*-linked glycosylation sites (Asn 223 and Asn 229). The third extracellular loop (ECL3) appears to be critical for ecotropic retrovirus receptor function (Albritton *et al.*, 1989; Overbaugh *et al.*, 2001; Sommerfelt, 1999; Taylor *et al.*, 2003). Previous studies have shown that the integrity of the <sup>235</sup>YGE<sup>237</sup> motif in ECL3 is essential for MLV receptor function (Albritton *et al.*, 1993; Yoshimoto *et al.*, 1993).

Susceptibility to Eco-MLV is restricted to murine and certain rat cells. Glycosylated murine CAT1 paralogue

(mCAT1) receptors retain full Mo-MLV receptor function (Kubo *et al.*, 2002; Wang *et al.*, 1996). Hamster CAT1 (hCAT1) orthologues fail to function as the Eco-MLV receptor but can be rendered functional after treatment with tunicamycin, an inhibitor of *N*-linked glycosylation (Miller & Miller, 1992; Wilson & Eiden, 1991). In addition, the rat CAT1 orthologue (rCAT1) and the *Mus dunni* CAT1 paralogue (dCAT1) function poorly as Mo-MLV receptors due to glycosylation-dependent inhibition (Eiden *et al.*, 1993, 1994; Kubo *et al.*, 2002; Tavoloni & Rudenholz, 1997). The ECL3 of rCAT1 is 2 aa longer than the ECL3 of mCAT1 (due to deletion of the Lys residue at position 222 and insertions of Ser, Pro and Leu at positions 226–228 compared with mCAT1). We previously reported that the extra amino acids in rCAT1 (compared with mCAT1) are associated with the inhibition of Eco-MLV infection by rCAT1 glycosylation (Kubo *et al.*, 2004).

## 2 風 疹

rubella

風疹は幼児と学童を中心に初冬から夏にかけて5年おきの流行を繰り返していたが、男女幼児への定期予防接種により大流行は制圧された。2006年4月より麻疹・風疹混合(MR)ワクチンが定期接種として導入され、わが国でも疾患排除をめざす段階に入った。一方、未接種・未罹患の小児、成人が残存しているため、その層への任意接種が重要で、局所流行と先天性風疹症候群の再燃にはなお注意が必要である。

### ■疫 学

1981～82、87～88、92～93年の大流行後、全国的な流行は制圧されたが、なお地域流行が残り、2004年には年間10例の先天性風疹症候群児が報告された。流行は冬から夏(特に初夏)に多くみられるが、今後の散発例の疫学は不明である。1994年の予防接種法改正後、中学生の接種率(移行措置)は低迷し、若年成人や成人男性を中心に未接種・未罹患の風疹感受性者が数百万人残存している。感染者の平均年齢が上昇し、成人の割合が増加している。感染症法では5類疾患。風疹は定点報、先天性風疹症候群(報告基準あり)は全数報告である。

### ■臨 床 像

- 潜伏期 約14～21日、平均16～18日。
- 発疹が3日間程度持続する。発疹は5mm程度のピンク色の孤在性の発疹で、癒合傾向が少ない。顔面、頸部、頭部、軀幹、四肢へと広がり、色素沈着や落屑なく消失する。成人では発疹が出血性になることがある。
- 発熱は発疹の出現と相前後して出現する。40～60%は無熱または微熱。
- リンパ節腫脹は発熱や発疹に先行し、耳介後部、後頭部、頸部に多くみられ、数週間持続する。圧痛がある。
- 眼球結膜の軽度充血、軟口蓋の赤い点状粘膜疹(Forschheimer斑)。
- 思春期、成人では関節腫脹がよくみられる。
- その他、咽頭痛、頭痛、倦怠感を呈することもある。
- 不顕性感染は約30%。

### ■検査所見

特異的なものはない。末梢血液像では白血球減少と比較的リンパ球増多、異型リンパ球増多がみられる。CRPなどの炎症所見は陰性または弱い。ときに血小板減少がみられる。肝機能異常を示すことあり。



## A comparative study of the incidence of aseptic meningitis in symptomatic natural mumps patients and monovalent mumps vaccine recipients in Japan

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### Abstract

To compare the incidence of aseptic meningitis associated with symptomatic natural mumps infection and in mumps vaccine recipients, we conducted a prospective comparative study. Consecutive samples of 1051 children with mumps were enrolled by 10 pediatricians and 21,465 vaccine recipients by 143 pediatric primary care practitioners, from January 1, 2000 to January 1, 2003. Parents used a daily diary to record symptoms during the period of illness (15 days) or 30-day period following immunization. Mumps infection was confirmed by virus isolation and/or detection of mumps virus genome in salivary and CSF samples. The incidence of aseptic meningitis was 13/1051 (1.24%) in patients with symptomatic natural mumps infection and was estimated to be 0.7–1.1% of overall infection in considering asymptomatic infection, and 10/21,465 (0.05%) in vaccine recipients. Although aseptic meningitis is a clear side effect of the mumps vaccine, the incidence is considerably lower than among those with symptomatic natural infection. Our results provide an informative data for consideration to resume mumps vaccine as a part of routine immunization schedule for Japanese children.

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**Keywords:** Aseptic meningitis; Mumps vaccine; Natural mumps

### 1. Introduction

Epidemic parotitis is a common infantile infectious disease caused by the mumps virus belonging to the paramyxoviridae RNA virus; mumps virus is also a well known

cause of aseptic meningitis in children [1,2]. Live attenuated Measles-Mumps-Rubella (MMR) vaccine is approved in most countries, with effective immunization reducing annual outbreaks of mumps, and vaccine-associated aseptic meningitis is not a matter of concern [3,4]. In Japan, MMR vaccine was licensed in December 1988 and recommended for infants as a part of their basic immunization. Because of unexpectedly high incidence of aseptic meningitis caused by the mumps vaccine component, MMR vaccine was discontinued in 1993 [5,6]. Since then, monovalent measles and rubella vaccines have been recommended for children over 1 year of age, but mumps monovalent vaccine has been optional. Consequently, we have experienced annual outbreaks of mumps

**Abbreviations:** MMR, Measles-Mumps-Rubella; RT-PCR, reverse transcription-polymerase chain reaction; CSF, cerebrospinal fluid; SAGPJ, Society of Ambulatory and General Pediatrics of Japan; CPE, cytopathic effect; P, phosphoprotein; HN, hemagglutinin-neuraminidase protein; CI, confidence interval

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## ポリオワクチン関連麻痺 (vaccine-associated paralytic poliomyelitis)

宮崎千明

### ◎ はじめに

経口生ポリオワクチン (oral polio vaccine : OPV) に関連する麻痺 (vaccine-associated paralytic poliomyelitis : VAPP) は、治療の落とし穴というよりワクチンの落とし穴である。本稿では、OPV に不可避の VAPP の臨床疫学的特徴と、最近話題の cVDPV (circulating vaccine-derived poliovirus : 生ワクチン由来株の伝搬)、不活化ポリオワクチン (inactivated polio vaccine : IPV) の開発状況を述べる。

### ◎ わが国のポリオ

ポリオウイルスには 1 型、2 型、3 型があり、ワクチンには OPV (経口) と IPV (注射) がある。わが国では 1960 年の大流行を期に、1961 年からの OPV の一斉投与により劇的に疾患が減少し、1981 年の 1 型ポリオ症例を最後に野生株ポリオウイルス症例はみられていない。急性弛緩性麻痺症例調査や環境水調査から、わが国には野生株ウイルスは常在しない (排除された) と考えられ、2000 年に国内根絶宣言が出された。

#### 1 ポリオワクチン関連麻痺とは

野生株流行がなくなる一方で、1970~2000 年までの 31 年間に生ワクチン株が分離されたポリオ患者が 20 人発生し (頻度は 300~400 万回に 1 人)、接触者からの発症が 17 人出ている (投与者 250 万人あたり 1 人)。生ワクチン株の毒力復帰によって服用者または周囲の接触者が麻痺を起こすことを VAPP という。

臨床的には服用後 4~30 日の間に、脊髄前角炎 (MRI が診断に有用) が起こり、四肢の非対称性の弛緩性麻痺を起こす。下肢に多いが嚥下や呼吸筋が障害される場合もある。また、髄液細胞増多と軽度蛋白増加がみられる。発生率の男女比は 8 対 1 で圧

倒的に男子に多い。髄液からのウイルス分離は診断価値が高いが頻度は低く、通常、糞便中から分離を試み OPV 株かどうかの判定を行う。増殖の良い 2 型や強毒復帰しやすい 3 型が多い。他のエンテロウイルスによる弛緩性麻痺、ギランバレー症候群 (対称性麻痺)、横断性脊髄炎 (腱反射亢進) などが鑑別診断としてあげられる。

服用者の VAPP は 1 回目の接種時に多く、反復性肛門周囲膿瘍男児に多い。接触者は服用乳幼児の父親でワクチン未接種者が多いようである。定期接種の服用者の VAPP は予防接種法で救済されるが、接触者の VAPP に対しても 2004 年 4 月 1 日より救済事業 (補償) が始まった。

伝染病流行予測事業によれば、1975~1977 年生まれの年齢層で 1 型に対する抗体価が低い。3 型は広い年齢層で抗体陽性率がやや低い傾向にあり、2 型には高い抗体陽性率を示している。VAPP は 2 型または 3 型が多く未服用者に出やすいので、1 型抗体のみを心配するのは科学的にはバランスを欠くが、VAPP 予防には服用児と同日に未接種の保護者も服用するのがよいだろう。

2 circulating vaccine-derived poliovirus とは予防接種率の低い数カ国 (ハイチ、ドミニカ、フィリピン、エジプトなど) で、他のエンテロウイルスとの間で遺伝子組換えを起こして毒力復帰したワクチン由来株がヒト-ヒト間で地域流行し、ワクチン未接種者に麻痺を起こすことが知られてきた。これを cVDPV とよぶ。

#### 3 不活化ポリオワクチンの開発

北欧など一部地域では当初から IPV のみでポリオをコントロールしてきたが、OPV を使用してきた先進国では VAPP を避けるため、麻痺の起こらない IPV に最近切り替えてきた。

## 生ワクチンと不活化ワクチン

宮崎千明\*

Chiaki Miyazaki

### はじめに

現行ワクチンはその性状により、生ワクチン、不活化ワクチン、トキソイドに分類される。便宜的にトキソイドも含めて不活化ワクチンと呼称する場合もある。歴史的には、ポリオや麻疹、インフルエンザなど、同じ疾患に対して生ワクチンと不活化ワクチンの両方が開発されたものもある。表1に現在国内外で市販されているワクチンを分類して示した。

### 1. 生ワクチンと不活化ワクチンの違い

生ワクチンは弱毒化した病原体（細菌またはウイルス）の比較的少量を生体内に接種する。接種された弱毒株は体内で増殖し、細胞性免疫と液性免疫の両方を惹起するので、自然感染に近い免疫になる。1回の接種で比較的強い免疫を惹起できる利点がある一方、体内で増殖するため、発熱や発疹などの全身的な臨床症状を伴うことがある。また、細胞性免疫不全児への安全性が問題になる、妊婦に接種できない、母からの移行抗体が残存する時期やγ-グロブリン投与後には体内増殖が不十分になり、免疫ができないことがあるなどの問題もある。かつて生ワクチンの免疫は終生免疫と考えられていたが、麻疹ワクチン接種後の軽症麻疹などの経験から、ワクチン免疫は自然感染免疫よりやはり弱く、流行が縮小し自然感染による追加免疫がなければ獲得した免疫が低下して感染し発症しうることが明らかになってきた。米国では麻疹、風疹、ムンプス、水痘などは2回接種が原

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表1 生ワクチンと不活化ワクチン

	生ワクチン	不活化ワクチン
国内認可	BCG ポリオ（経口） 麻疹（M） 風疹（R） MR ムンプス（M） 水痘（V） 黄熱* 種痘（備蓄） 国産 MMR（発売中止）	百日咳（P） ジフテリア（D、トキソイド） 破傷風（T、トキソイド） DPT、DT、T 日本脳炎（マウス脳） インフルエンザ B型肝炎 A型肝炎 狂犬病 肺炎球菌（23価莢膜多糖体） インフルエンザ b 菌（Hib） ウイルス・秋やみ コレラ（注射）
国内未認可	MMR（輸入） MMRV ロタウイルス インフルエンザ（経鼻） 日本脳炎 腸チフス（経口） コレラ（経口）	日本脳炎（組織培養） ポリオ（注射） 肺炎球菌（7価結合型） ヒトパピローマウイルス 髄膜炎菌 腸チフス（注射） インフルエンザ H5N1（備蓄）*

注：ほかに不活化多価混合ワクチンなどがある。

\* プレバンデミックワクチン（全粒子アジュバント添加）は2007年中に製造承認予定

\* 黄熱ワクチンは国内未承認ながら検疫所で接種可能

則になった。

麻疹生ワクチンによる接種後1週間前後の発熱と発疹はまさに体内でワクチン株ウイルスが増殖し、それに対して生体（宿主）が反応した結果である。逆に、生ワクチン接種後1~2日に発熱や発疹がみられた場合には、その臨床症状（有害事象）は生ワクチンの増殖によるものではない可能性が高い。

一方、不活化ワクチンは実験的に大量に培養した細菌やウイルスをホルマリンなどによって無

## ワクチンの新しい投与方法

宮崎千明\*

Chiaki Miyazaki

### はじめに

ワクチンの新しい投与方法には、ワクチンの性状と投与経路の2つの側面がある。従来の不活化ワクチンは、病原体そのもの、または感染防御（または発症予防）に有効な抗原、すなわち蛋白質に注目し、それを選択して投与してきた。しかし、蛋白質だけでなく、ペプチド、多糖体、DNAなどもワクチンになりうるようになってきた。

一方、生ワクチンは体内増殖を期待して主に注射で抗原を宿主に与えてきた。例外はポリオの生ワクチン（経口接種）、種痘とBCG（経皮接種）であったが、経鼻接種がインフルエンザで実用化され、粘膜免疫が再認識されてきた。

### I. DNA ワクチン

DNA ワクチンはまったく新しい発想で登場してきた。ウイルスの感染防御抗原をコードする遺伝情報を含むが、ウイルス粒子の複製に必要な遺伝情報を欠いた遺伝情報をプラスミドやレトロウイルスベクターに組み込み、それを宿主細胞内に入れることにより、宿主細胞はウイルス抗原を産生し自身の細胞表面に表現したり、細胞外に出すが、感染性粒子はできないので感染はそこで頓挫し、体内でウイルスが増殖することはない。細胞性免疫、とくにCTL活性を誘導しやすい、温度に安定、強毒復帰がない、多価化が容易などの利点があるといわれている。通常、生理食塩水に溶解したDNAワクチンを注射などで筋肉内に投与する。遺伝子の発現効率や安全性の検討がなされている

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が、まだ実用化していない。

### II. ベクターウイルスに載せて

すでに弱毒化されているウイルスワクチン株や、人で増えるが病原性のないウイルス株に別の病原ウイルスの中和（感染防御）に関連する抗原決定基をコードする遺伝子のみを組み込んで、新しい組み替え生ワクチンを作成して接種する方法である。この方法をとれば、宿主細胞表面には複数のウイルス抗原が表出され、培養や弱毒化が困難なウイルスに対しても、細胞性免疫でも惹起することができる。

### III. 新しいアジュバントの開発

不活化ワクチンのアジュバント（免疫増強剤）として長くアルミニウム化合物（水酸化アルミニウムやリン酸化アルミニウム）が使用されてきた。そのほか、結核菌の細胞壁骨格（CWS）、合成ムラミルジペプチド（MDP）、サポニン、リポソーム、スクアレン、コレラトキシンBサブユニット、肺サーファクタント、ほか多数のアジュバントが精力的に研究されている。スクアレンを含むMF59の使用が欧州のメーカーで始まっている。

*Haemophilus influenzae* type b や肺炎球菌のワクチンは莢膜多糖体が感染防御抗原になるが、これはT細胞非依存性抗原なので乳幼児に対して免疫惹起力が弱い。したがって、破傷風トキソイドや髄膜炎菌の外膜などの他の蛋白質（キャリア蛋白）と結合させて乳幼児にも免疫惹起力を高めて実用化された。これらは結合型（conjugate）ワクチンとよばれる。

## 日本ではまだ使われていない開発済みのワクチン

—日本で認可されていない理由と認可の見通し—

宮崎千明\*

Chiaki Miyazaki

海外大手ワクチンメーカーはこの10年間、競って新ワクチンの開発を進めてきた。一方、わが国では国産MMRワクチンの導入と頓挫以来、開発速度が減少し海外との格差が目立ってきた。表に海外ではすでに開発済みであるが、まだ日本で認可されていないワクチンをあげた。

ロタウイルス、髄膜炎菌、ヒトパピローマを対象疾患とする各ワクチンは日本になく、他のワクチンは既存ワクチンの混合多価化されたものか、もしくは改良されたものである。

日本が海外からワクチンを輸入して認可をとろうとする場合、海外の製造所（もしくは、提携した日本の製造所）が独立行政法人医薬品医療機器総合機構（PMDA：以下、総合機構）にワクチンの臨床試験実施を申し出、総合機構のアドバイスをを受けて1相～3相試験を実施し、その後データをまとめて承認申請を出す。海外で数億の接種実績のあるワクチンでも、日本に導入する場合は臨床試験が求められる。海外データの利用方法についての基準は明確でない。

近年、ワクチンを含む新薬の臨床試験の実施については厳しい基準（good clinical practice：GCP）が設けられており、手間と費用がかさむ。また、日本では予防接種法の定期接種として実施されれば接種率は高いが、法に基づかないワクチンは有料・任意接種となり接種率が上がらないという事情もあり、定期接種化の見通しが立たないと日本への導入意欲が上がらない。また、国も戦略的見

表 国内未承認ワクチン

生ワクチン	不活化ワクチン
MMR（輸入）	日本脳炎（組織培養）
MMRV	ポリオ（注射）
ロタウイルス	肺炎球菌（7価結合型）
インフルエンザ（経鼻）	コレラ
日本脳炎	ヒトパピローマウイルス
腸チフス（経口）	髄膜炎菌（4価、4価結合型）
	腸チフス（注射）

地から国内製造所を保護し国内での安定供給を図ってきた歴史的経緯もあり、最近になるまで外資系の手メーカーの日本進出の動きは鈍かった。

申請から承認までの期間が長いこともマイナス要因になっている。臨床治験や申請書類の不備、審査する総合機構生物製剤審査部の人員の不足、副反応に対する国や国民の厳しい目なども審査期間を長くしている一因であろう。各ワクチンの現状について略解するが、詳細は他稿を参照されたい。

### 各ワクチンの現状

#### 1. MMR ワクチン

1989年4月から1993年4月までの4年間に、国産4種（統一株と3種の自社株）のMMRワクチンが上市され、ムンプスワクチン（とくに統一株に含まれた占部株）による無菌性髄膜炎の多発で頓挫した。その後、化血研が米国メルク社製のMMR II®（髄膜炎を非常に起こしにくいといわれているムンプスワクチン Jeryl Lynn 株を含有するワクチン）を日本に導入すべく、麻疹単抗原ワクチン

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## ワクチン開発の過程

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### I. ワクチン開発の基礎

#### 1. ワクチン開発の前提

まず、対象となる感染性疾患の臨床、疫学、病原体の特徴、発症病理、感染免疫機構などを考え、ワクチン開発の意味があるかどうかを吟味する。一疾患一病原体である（あるいは少数の血清型しかない）、疾患が重篤になる、患者発生数が多い、終生免疫ができる（二度罹りなし）場合には開発のターゲットは明確である。天然痘、麻疹、風疹、ポリオなどがこれにあたる。しかし、かぜ症候群を起こす多くのウイルス群などのように、同じ疾患名であっても病原体の数が多く、かつ中和抗体がクロスしない場合には、開発をあきらめるか、特定のタイプに絞り込んで開発が行われる。

実際の開発にあたっては感染・発症・メカニズムとその防御メカニズムを研究し、生ワクチン、不活化ワクチン、トキソイドなどのワクチンの種別の選択、ターゲットにする抗原の選択、投与経路の選択などが吟味される。

#### 2. ワクチンとしての評価

ワクチンの最終評価は広く使用された場合の感染（または発症）防御効果であるが、開発段階ではそれを検証することが困難な場合も少なくないので、動物モデルを使った感染防御実験や中和抗体の上昇を確認し、ヒトへの臨床応用を考えることになる。第3相臨床試験でも感染防御効果をみることは容易でないので、抗体の上昇を目安に評

価がなされる場合がほとんどである。抗体測定以外にも、ツベルクリン反応のように細胞性免疫の上昇を目安にすることもまれにある。最近の海外の新ワクチンの臨床試験では数千人～数万人を対象として2重盲検試験で有効性と安全性の評価が行われることが増えてきた。

### II. ワクチンの開発

#### 1. 生ワクチンの開発

生ワクチン株の開発には、麻疹、風疹、水痘、ムンプス、黄熱などの野外強毒株を種々の培養細胞や発育鶏卵などで継代培養して、ヒトに対して弱毒株をつくる方法が一般的である。低温継代なども併用して低温馴化株（低温でよく増殖する）や温度感受性株（高温で増えにくい）を得ることもある。ワクシニアウイルスはヒトの病原体（天然痘ウイルス）に似て非なる他（宿主）の病原体（起源は不明確である）であり、BCGはウシ型結核菌を長期継代培養して弱毒化したものである。

また最近では、インフルエンザ生ワクチンやロタウイルスワクチンのように、弱毒親株に、ターゲットにしたい抗原決定基を規定する遺伝子を組み合わせて reassortant ウイルスを作成する場合もある（図1）。

弱毒化と免疫原性とは相反することが多く、最終的にはヒトへの接種により十分な免疫原性があり、かつ副反応が許容範囲（tolerable）の株が選択される。そして、温度感受性や種々の動物に対する免疫原性、ブラックサイズなどの生物学的弱毒マーカーが選定され、株の管理に用いられる。最近では強毒株とワクチン株の全塩基配列を比較して、弱毒化の機序を突きとめる研究が進んでい

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