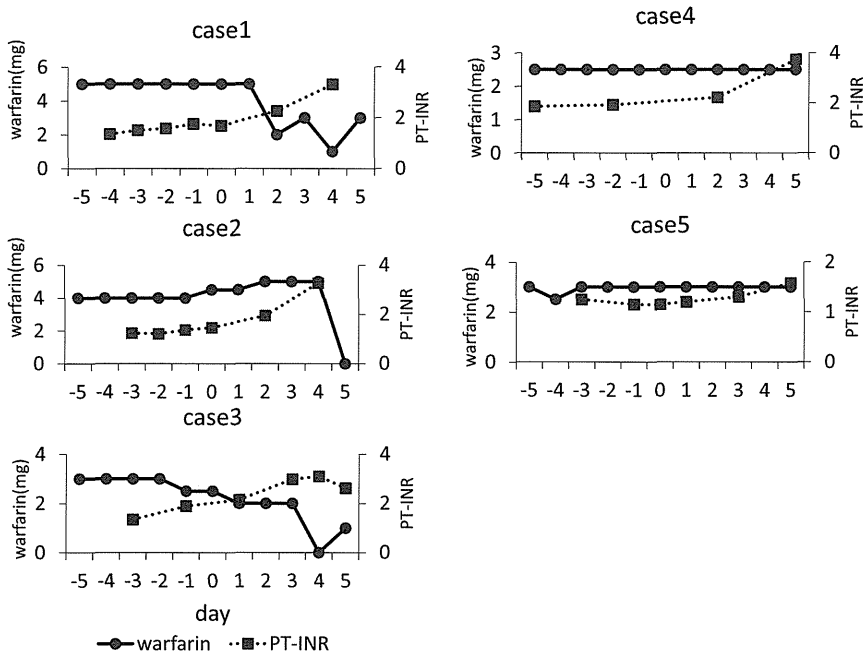

Figure 2. Relationship between the dose of warfarin and the PT-INR in the five patients. We defined the start date of linezolid treatment as day 0 and investigated changes in the dose of warfarin and the PT-INR before and after the administration of concomitant therapy for five days. Consequently, the PT-INR values increased during concomitant linezolid administration in all cases. In Cases 1, 2 and 3, the dose of warfarin was reduced or withdrawn after the concomitant administration of warfarin and linezolid. In Case 4, the dose of warfarin was not changed. In Case 5, the dose of warfarin was not changed, except for day -4.

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The administration of concomitant warfarin and linezolid was started on days 8, 13, 14 and 21 in the postoperative cases. In order to determine the changes in the PT-INR values in the cases treated without linezolid, we investigated the PT-INR and dose of warfarin from days 8 through 28 after surgery for mitral insufficiency. The control group included four patients, with a mean age of 74.3±5.7 years. Fig. 3 shows the relationship between the dose of warfarin and the PT-INR in this group. The PT-INR increased slowly in control Case 1, although this increase was not as high as that observed among the patients treated with concomitant linezolid. Meanwhile, the PT-INR values changed when the dose of warfarin was changed in control Cases 2 and 3. In control Case 4, a decrease in the dose of warfarin resulted in a simultaneous decrease in the PT-INR.

Discussion

There are some caveats to interpreting the results of the statistical analyses. Because the available sample size was small, our data analysis should be used only to generate hypotheses, not as a confirmatory analysis.

In previous studies, the PT-INR values decreased when warfarin and linezolid were simultaneously administered in healthy adults, although the decrease was not clinically problematic (7). In contrast, our data analysis showed a tendency toward an increase in the PT-INR when the dose of warfarin was decreased. We speculated that the changes in the postoperative PT-INR values in the control group were influenced by the dose of warfarin. However, the dose of warfarin was increased prior to the administration of concomitant warfarin and linezolid in only one case. Therefore, we do not believe that the increase in the PT-INR values was influenced by surgery. In addition, we speculated that the increase in the PT-INR values was perhaps due to the effects of infectious disease. However, although the degree of inflammatory reactions observed before the concomitant administration of linezolid was high, the PT-INR values only increased when the inflammatory reactions decreased. Therefore, we do not consider the rise in the PT-INR values to be the result of infection. These results suggest the possible presence of a drug interaction between warfarin and linezolid.

Patients with liver and kidney disease and hypoalbuminemia are reportedly at risk of hemorrhage when being treated with warfarin (8, 9). However, no significant signs of hemorrhage were reported after the concomitant administration

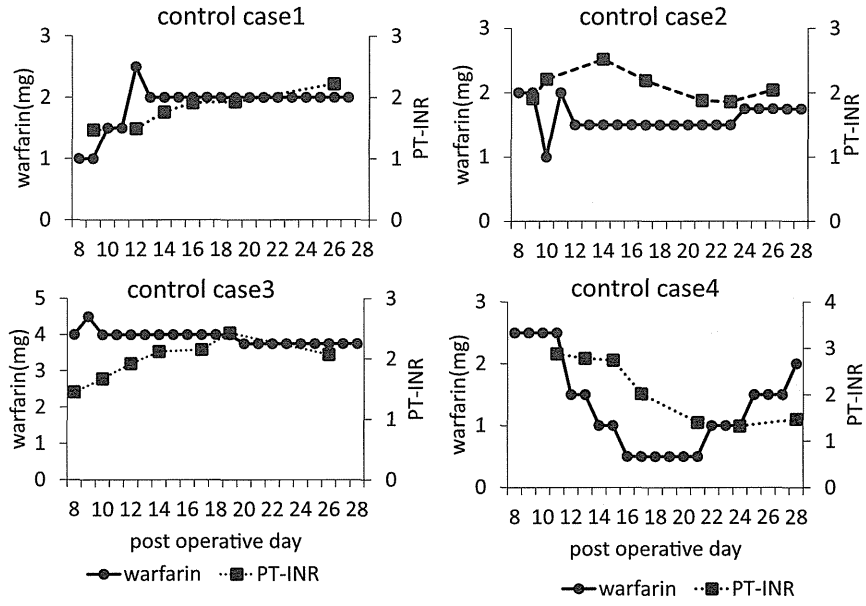

Figure 3. Relationship between the dose of warfarin and the PT-INR among the four subjects in the control group. We defined the date of surgery as day 0 and investigated changes in the dose of warfarin and the PT-INR from days 8 through 28. Consequently, the PT-INR values increased slowly in control Case 1, although the rise in the PT-INR was not as high as that observed among the patients who received concomitant linezolid administration. Meanwhile, the PT-INR values changed when the dose of warfarin was changed in control Cases 2 and 3. In control Case 4, a decrease in the dose of warfarin resulted in a simultaneous decrease in the PT-INR.

of linezolid in this study, suggesting that these diseases did not affect our subjects' laboratory values. Linezolid is reportedly mostly metabolized in the tissues of healthy adults, with the main site of metabolism being the liver (3). The metabolism of linezolid has been shown to not be affected by cytochrome P450, and this drug is believed to be primarily oxidized non-enzymatically by reactive oxygen species distributed throughout the body (10). Therefore, the interaction observed between warfarin and linezolid in this study may not have been due to metabolism by the cytochrome system.

In addition to the actions of metabolic enzymes, drug interactions with warfarin are believed to occur via substitutions for protein binding and involvement with the effects of vitamin K (2). The rate of protein binding of linezolid is low (31%) (3), which reflects its superior capacity for tissue transfer. Since linezolid is rapidly transferred to the tissue after administration, an interaction between warfarin and linezolid via protein binding in the circulation is unlikely. In the present survey, the changes in the albumin levels from before the initiation of concomitant treatment with linezolid and warfarin to the time of the highest PT-INR measurement were not significant, and the PT-INR values increased after the concomitant administration of the two drugs; therefore, the effects of protein binding were minimal. Some antibiotics reportedly strengthen the effects of warfarin by decreasing the levels of intestinal vitamin K-producing bacteria. Similarly, it has been reported that linezolid possesses an antibacterial activity for Bifidobacterium producing vitamin K (11). Hence, we considered that linezolid may potentiate the effects of warfarin by lowering the level of vitamin K, thus increasing the PT-INR.

The difficulty in managing the coagulation system under concomitant therapy with rifampicin and warfarin is well documented (12). Our survey found that the PT-INR reaches a maximum on day 4 of concomitant warfarin and linezolid administration in early cases and day 20 in late cases, suggesting that it is also difficult to manage the PT-INR during the concomitant administration of these drugs and that periodic monitoring of the coagulation system is required.

In the present study, we demonstrated the possibility for an interaction between warfarin and linezolid in patients undergoing cardiovascular surgery. In this study, warfarin was administered to prevent postoperative thromboembolism in all patients. Warfarin is also used in non-postoperative patients and is indicated for the prevention of atrial fibrillation-associated thromboembolism. In future studies, the survey period should be extended and the number of cases and targets should be increased. In addition, differences in the indications for warfarin therapy should be investigated. It is also necessary to clarify the mechanism of interaction between these two drugs. Based on a review of the literature, we speculate that linezolid increases the actions of warfarin by lowering the vitamin K level.

The coagulation system must be monitored by measuring the PT-INR in order to determine the need to change the dose of warfarin when concomitantly administering linezolid. Sufficient monitoring is also necessary after the com-

pletion of concomitant treatment.

This article concerns the content of a study that attracted the attention of the manager of the fourth Japan Society of Chemotherapy, West Japan Branch Activation Committee Special Prize.

The authors state that they have no Conflict of Interest (COI).

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J Infect Chemother 21 (2015) 296–301

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original article

The N-terminal fragment of PA subunit of the influenza A virus effectively inhibits ribonucleoprotein (RNP) activity via suppression of its RNP expression

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ARTICLE INFO

Article history:
Received 28 October 2014
Received in revised form
17 December 2014
Accepted 23 December 2014
Available online 9 January 2015

Keywords:
Influenza A virus
RNA polymerase
Ribonucleoprotein
PA subunit
Inhibitor

ABSTRACT

The influenza RNP, which is formed from PB1, PB2, PA, NP subunits, and vRNA, is autonomously replicated and transcribed in the infected cell. The simplest method to inhibit RNP activity is to impair the formation of the RNP. Thereupon we confirmed whether the peptides/fragments mimicking one of RNP components can interfere with their formation. During the process of this inhibitory study we found interesting suppression of protein expression of the RNP components by the N-terminal fragment of PA subunit. Especially, we found two residues (D108 and K134) on the fragment that were critical for the suppression. Furthermore, we determined the combination of three amino acids (P28, M86 and E100) on the fragment that are important for the strong suppression, and identified the minimum essential region (residues from 1 to 188) of the PA subunit that allowed its suppression. Our findings indicate that the N-terminal fragment of PA subunit may become one of candidates for an effective inhibitor of influenza RNP activity.

1. Introduction

The influenza A virus is a single-stranded negative-sense RNA virus belonging to the family *Orthomyxoviridae*. The influenza virus has RdRp, which is a heterotrimeric complex that includes three subunits: PB1, PB2 and PA. These subunits are assembled with nucleoproteins (NP) and viral RNA (vRNA), and form RNP [1,2].

PA, one of the RNP components, is known as a multi-functional protein, which provides RNA synthesis as well as proteolytic and endonuclease activity [3–8]. By Sanz-Ezquerro et al., it was firstly observed that the full-length and N-terminal PA induced degradation of co-expressed proteins [3,4]. PA also is known to be involved in an endonuclease activity that cleaves a capped structure from host mRNA to provide a primer for its viral transcription [6–8]. More recently, it was established that endonuclease active sites of N-terminal PA subunit were required for suppression of protein expression by two groups [9,10].

On the other hand, we previously showed that an incompatible combination of RNP components impaired own RNP activity, and indicated the importance of PA [11]. We had an idea that the simplest method to inhibit RNP activity is to impair the formation of the RNP. Accordingly, we focused on the PA, and investigated whether the fragment mimicking the PA subunit could be used as an inhibitor against influenza RNP activity. This unique study means that the influenza virus is inhibited by own proteins. During the process of this inhibitory study we found an interesting suppression of protein expression of influenza RNP components, which was actually correlated with its N-terminal region of PA, and not with its C-terminal region. We presumed that this mechanism of suppression against influenza RNP components might be correlated with proteolytic and/or endonuclease activities, which have previously been mentioned [3,4,9,10].

Abbreviations: RNP, ribonucleoprotein complex; RdRp, RNA-dependent RNA polymerase; WSN, A/WSN/33 (H1N1); NT, A/NorthernTerritory/60/68 (H3N2); HK, A/HongKong/56/97 (H5N1); VN, A/Vietnam/1194/2004 (H5N1); SW, A/Kurume/K0910/2009 (H1N1); vNA, viral-like neuraminidase gene; vLUC, viral-like luciferase gene.

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http://dx.doi.org/10.1016/j.jiac.2014.12.007
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In the present study, we further characterized suppression of RNP expression by N-terminal PA fragment to obtain a more efficient inhibitor. Namely, we identified the minimum region, two essential and three important residues on the N-terminal fragment, which were related to its effective suppression of influenza RNP activity. The aim of this study is to obtain the peptides/fragment that can effectively inhibit influenza RNP activity.

2. Materials and methods

2.1. Strains and plasmids

cDNA clones isolated from the following influenza strains were used in this report: WSN, NT, HK, VN, and SW strains [6,12–14]. The PB1, PB2, PA, and NP expression plasmids of WSN, NT, HK, VN, and SW have previously been described [12–15]. The pPOLI/WSN/vNA for expressing a viral RNA of the influenza NA gene has also been described [16].

2.2. Construction of mutants and fragments

In order to construct the deleted PA and the point-mutants, site-directed mutagenesis was performed as previously described [11,13,14]. To avoid structural damage of the protein, the NT/PA was separated at amino acid position 212, which was previously shown to be a sensitive site for trypsin [6], producing NT/PA/N212. To confirm these sequences, the open reading frames were fully sequenced by the outside order (FASMAQ co., Japan).

2.3. Luciferase reporter assay

To screen the RNP activity via the activity of luciferase, a pPOLI/vLUC vector was constructed by substituting the influenza NA sequence in a pPOLI/WSN/vNA with *firefly* luciferase sequence. A subconfluent monolayer of HEK 293 T cells (human embryonic kidney cell) [6,12,17] in E-MEM medium with 10% fetal bovine serum in a 6-well plate was transfected with 0.2 μg each of WSN/PA, WSN/PB1 WSN/PB2, WSN/NP, and vLUC expression vector via Lipofectamin 2000 (Invitrogen). For the inhibition assay, 0.2 μg vector, such as the fragment of PA expression vector, was also co-transfected. In order to determine the dose-dependency of the fragment, various concentrations of VN/PA N212 vector (0.04–1.0 μg/well) were co-transfected with a constant concentration of WSN/RNP vectors (0.2 μg/well). After transfection, cells were lysed at 30 h using Cell Culture Lysis Buffer (CCLB) (Promega). Luciferase activity was measured using a Luminometer Lumat LB 9507 (Berthold, Germany), and was calculated as a relative light unit (RLU).

2.4. RNA isolation and primer extension assay

To reconstitute the RNP of influenza A virus and analyze the RNP activity, a subconfluent monolayer of HEK 293 T cells transfecting vectors of WSN/RNP components and PA fragment were prepared as described in the luciferase reporter assay. pPOLI/WSN/vNA was used as a vector expressing viral RNA in substitution for pPOLI/vLUC. WSN/RNP activities were analyzed via primer extension assay as descried in previous reports [6,12,17]. All transcripts were visualized by 6% polyacrylamide gel containing 7 M urea in TBE buffer, and were detected by autoradiography.

2.5. Western blotting

To confirm the expression level of the protein, WSN/RNP was reconstituted with/without the PA fragments as described in the luciferase-reporter assays. The extracted lysate were confirmed by western blotting with each specific antibody against PB1, PB2, PA, NP [13,14], or beta-actin as an internal control using 12% SDS-PAGE.

3. Results

3.1. Inhibitory effect of NT/PA subunits against a WSN/RNP

A recent study indicated that incompatible combinations of RNP, e.g., NT/PA, WSN/PB1, WSN/PB2, and WSN/NP, diminished its RNP activity [11]. Therefore, we first confirmed whether the full-length NT/PA inhibits WSN/RNP activity (Fig. 1A). However, a significant difference in the inhibitory effect was not observed in the presence of full-length NT/PA compared with the empty vector (Fig. 1B). We next confirmed whether the fragment mimicking NT/PA subunit relates to the inhibitory effect against WSN/RNP activity. Consequently, WSN/RNP activity was significantly reduced by the NT/PA/N212 (Fig. 1B).

3.2. Inhibitory effect of N-terminal PA fragment from the other strains

To confirm whether the inhibitory effect is specific to this strain, the fragments derived from the other strains were additionally produced. Both SW/PA/N212 and VN/PA/N212 fragments showed the strongest inhibitory effect (Fig. 2A). The same result was obtained from a primer extension assay (Fig. 2B). For the following studies, the VN/PA/N212 fragment was chosen because it has the highest inhibitory effect and is a well-documented strain. The N-terminal fragment (VN/PA/N212) showed an exceedingly strong inhibitory effect from low concentration whereas the C-terminal fragment (VN/PA/C504) showed no inhibition (Fig. 2C).

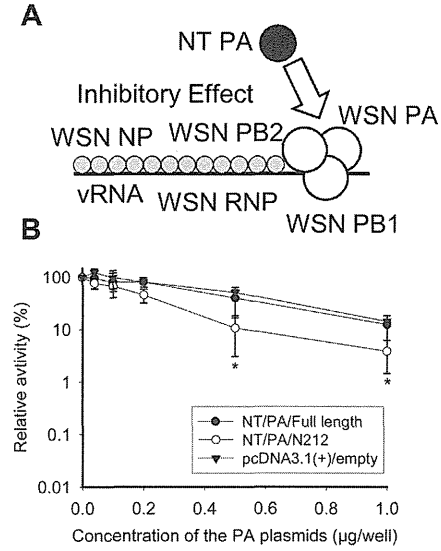

Fig. 1. Confirming the inhibitory effect of the NT/PA subunit against WSN/RNP activity. (A) The model of the study for the inhibitory effect of the NT/PA subunit against WSN/RNP activity. (B) Dose-dependency of the inhibitory effects. The WSN/RNP activities were estimated by a luciferase reporter assay. The relative WSN/RNP activities without the inhibitor are expressed as 100% activity. The standard deviations and significant differences were calculated from three independent trials. * indicates statistically significant differences at <0.05 based on a Student's t-test (n = 3).

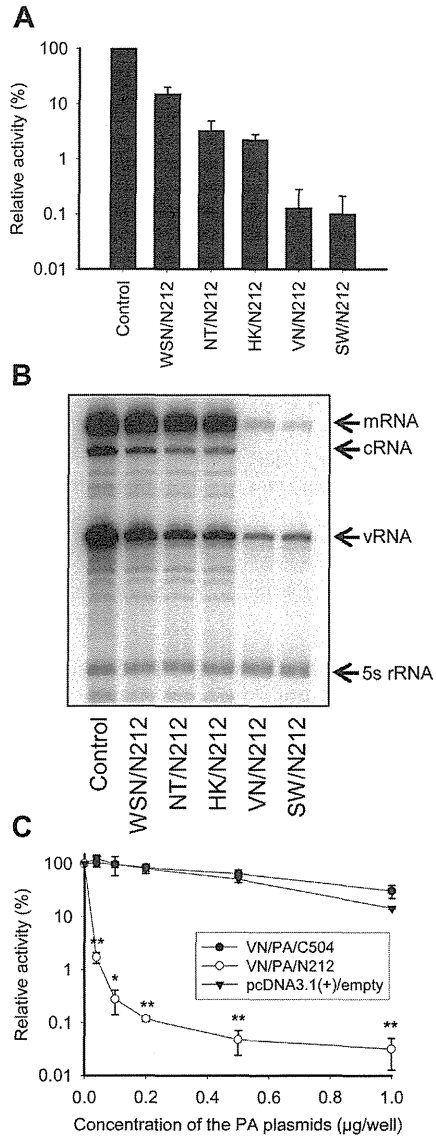

Fig. 2. Comparison of the inhibitory effects of the N-terminal fragments derived from PA subunits WSN, NT, HK, VN, and SW using a luciferase reporter assay (A) and a primer extension assay (B). (C) Comparison of the inhibitory effects by N and C-terminal fragments derived from VN/PA subunit. The WSN/RNP activities were estimated by a luciferase reporter assay. The relative WSN/RNP activities without the inhibitor are expressed as 100% activity. The standard deviations and significant differences are calculated from three independent trials. * and ** indicate statistically significant differences at <0.05 and <0.01, respectively, using a Student's *t*-test ($n = 3$).

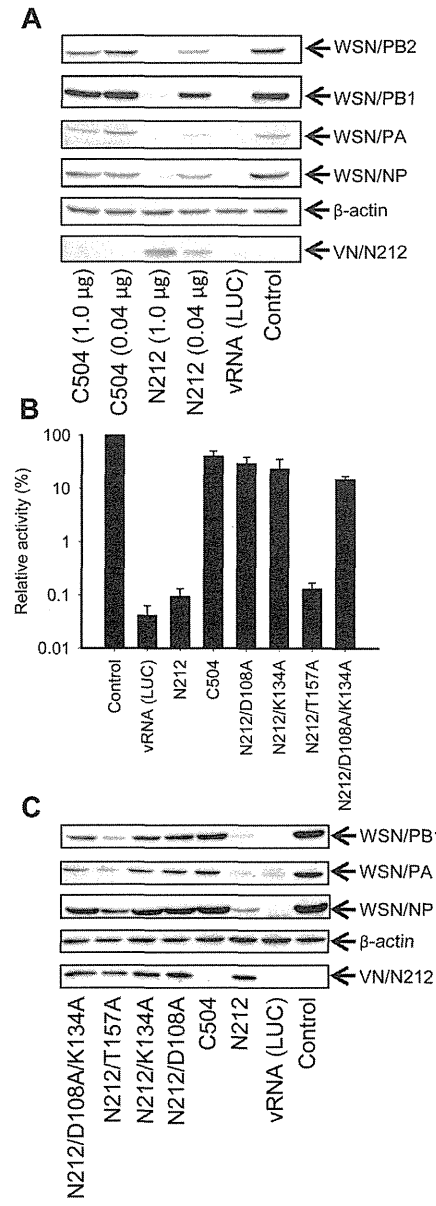

Fig. 3. Checking the protein expression levels of WSN/RNP and fragments. (A) Representative protein expression levels of the WSN/RNP components with either N- or C-terminal PA. (B) Determination of an important active site on the N-terminal fragment for the inhibitory effect. (C) Representative protein expression levels of WSN/RNP components in the presence of the fragments which either an endonuclease (D108 and/or K134) or proteolytic (T157) active site was substituted with an alanine. The vRNA (LUC) means that only luciferase vector was transfected to 293 T cell. A reproduction of these results was confirmed by at least two independent trials.

3.3. Confirming protein expression levels of RNP components and fragments

To investigate the mechanism behind the severe loss of RNP activity by the VN/PA/N212, the expression levels of the RNP and VN/PA/N212 were confirmed. The protein expressions of WSN/RNP components were severely impaired in the presence of the VN/PA/N212 (Fig. 3A).

To confirm that each active site (D108, K134, or T157A) is involved in the reduction of the WSN/RNP expression, WSN/RNP activities (Fig. 3B) and protein expression levels of WSN/RNP components (Fig. 3C) were checked in the presence of fragments with inactivation of each active site. The strong inhibitory effect in the presence of VN/N212/T157A was still remained, whereas they were disappeared in both of the mutations of VN/N212/D108A and VN/N212/K134A.

3.4. Determination of the essential region for a strong inhibitory effect

To find the region that is essential to the strong suppression, WSN/RNP activity was measured in the presence of VN/PA/N212

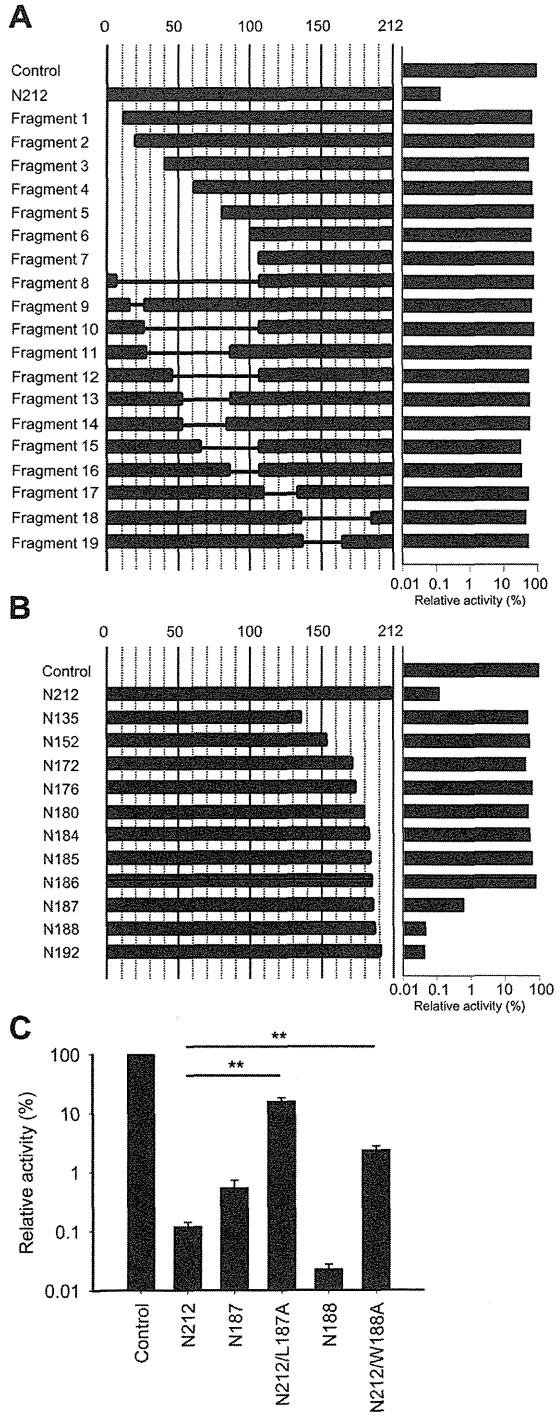

Fig. 4. Important region and amino acid of the VN/PA/N212 for an effective suppression. (A) Deleted mutants of the N or middle region of the VN/PA/N212. (B) Deleted mutants of the C-terminal region of the VN/PA/N212. Fragment numbers and deleted regions are indicated on the left side of the panel. The average was calculated from two independent trials, as indicated in the right side of the panel. (C) Fragments point-mutated at the position(s) 187 and/or 188. The relative RNP activities without the inhibitory PA fragment are expressed as 100% activity. The standard deviations and significant differences are calculated from three independent trials. ** indicates statistically significant differences at <0.01, using a Student's t-test (n = 3).

that was further truncated (Fig. 4A). However, the suppressions were all absent among fragments from nos.1 to 19. Next, the RNP activities were measured for fragments with deleted distal regions (Fig. 4B). Dramatic changes were observed with regard to suppression when either N187 or N188 vector was co-transfected as inhibitor, although no reductions were obtained with fragments from N135 to N186.

In order to identify the importance of positions 187 and 188 on the fragment, they were substituted with the alanine on VN/PA/N212, and the inhibitory effects were measured (Fig. 4C). The suppressions of these fragments were significantly attenuated, compared with that of VN/PA/N212 (wild type).

3.5. The amino acid on the PA fragment that is important for a strong suppression

Since a significant difference in the suppression was observed between VN/PA/N212 and WSN/PA/N212 (Fig. 2A), we determined which of the amino acid are required for the strong suppression. By multiple alignments, we found 10 differences in the amino acids between VN/PA/N212 and WSN/PA/N212, and then these amino acids on VN/PA/N212 were serially substituted with those on WSN/PA/N212 (Fig. 5A). The inhibitory effects against WSN/RNP activity were measured by fragments with each mutation via a luciferase-reporter (Fig. 5B) and a primer-extension assay (Fig. 5C). When the change in amino acid position 100 was accumulated in VN/PA/N212 (shown as Mut. 10), the suppression of WSN/RNP activity was almost disappeared (Fig. 5B). Significant changes were also found with changes to amino acid positions 28, 86 and 100 on the VN/PA/N212 (Fig. 5B and C). Thereupon, only these amino acids on the VN/PA/N212 were substituted with those of WSN. For a control of counterpart, three positions on the WSN/PA/N212 were also substituted with those on the VN. As shown in Fig. 5D, three amino acids (28, 86 and 100) were determined to be important for strong suppression of WSN/RNP activity, because the suppression was complementarily changed by these substitutions.

4. Discussion

We found interesting suppression of WSN/RNP activity by PA fragment, which actually correlated with the N-terminal PA subunit and not with the C-terminal region (Figs. 1B and 2C). This result was very interesting, because we originally hypothesized that the PB1 binding site, which is part of C-terminal PA subunit, is required for a competitive inhibition against WSN/RNP activity, whereas the PB1 binding site was unimportant.

A very interesting result was also observed when protein expressions of WSN/RNP components were compared with and without inhibitory fragments (Fig. 3A). Namely, all components of the WSN/RNP were severely decreased in the presence of VN/PA/N212, although beta-actin was not affected. Additionally, we confirmed that VN/PA/N212 didn't affect the *Renilla* luciferase activity as internal control (data not shown). Previously, some groups had also identified that the N-terminal functions of the PA subunit were required for the suppression of protein expression [3,4,9,10]. These results indicate that the inhibition of WSN/RNP activity by VN/PA/N212 depends on the reduction of protein expression of WSN/RNP components. Moreover, the activity and protein expressions of WSN/RNP were rescued by inactivating its endonuclease catalytic sites (D108A and K134A) on the VN/PA/N212 (Fig. 3B and C). These results suggest that the VN/PA/N212 impairs the protein expressions of RNP components via its endonuclease activity, although the detailed mechanism still remains unclear.

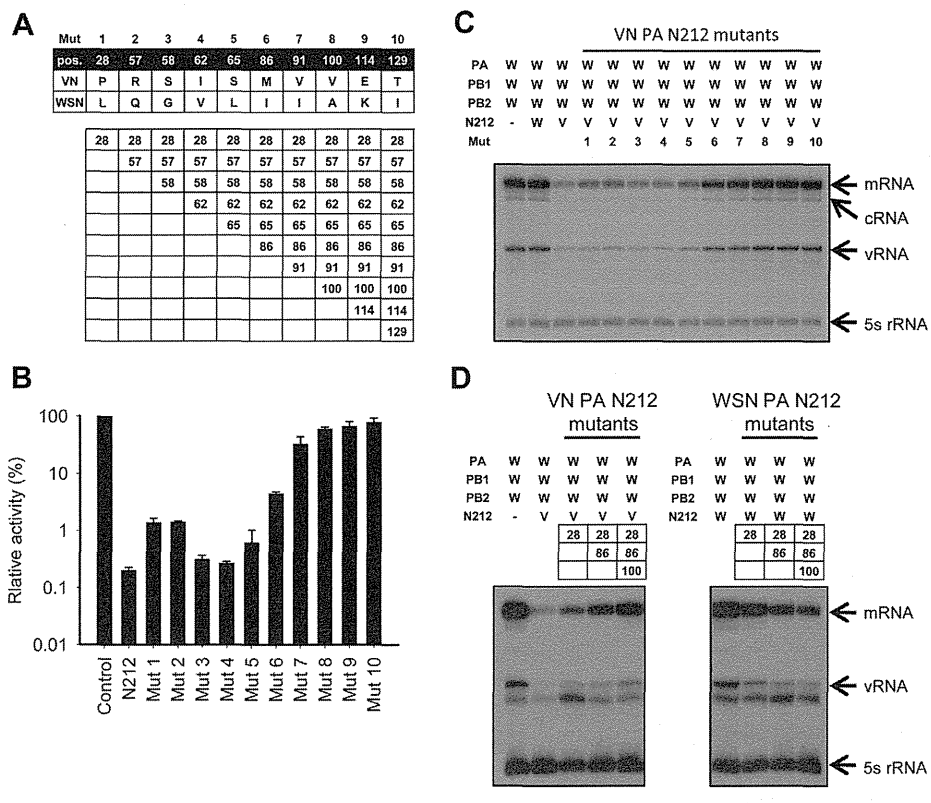

Fig. 5. Comparison of the inhibitory effect on WSN and VN/PA fragments. (A) Comparing the different amino acids on WSN and VN/PA subunits and the constructed fragments are indicated in the lower panel. Comparison of the inhibitory effect of each of the mutants derived from the VN/PA/N212 using a luciferase reporter assay (B) and a primer extension assay (C). (D) Comparison of the inhibitory effect of the VN and WSN/PA/where three points of amino acids (28, 86, and 100) were substituted. The numbers in the upper panel of (D) represent the substituted positions of an amino acid on the fragment. The standard deviations are calculated from three independent trials.

Our data indicated that the region comprised by amino acid positions 189 to 212 was not needed for suppression of WSN/RNP activity (Fig. 4B). Structural studies have shown that the position 189 lies between the 6th and 7th alpha-helix of the endonuclease domain of PA subunit [7,8,18]. These results suggest that the regions from the 1st to the 6th alpha helixes are required, but that the 7th alpha helix is unimportant for suppression of RNP activity. Previously, Sanz-Ezquerro *et. al.* suggested that 1–247 residues were found to be important whereas 1–186 residues was not enough to induce the suppression of protein in their system [4]. Our data are consistent with their data, and we further identified that the minimum essential region for the suppression was that of 1–188 residues of the N-terminal PA subunit.

The amino acids at positions 28, 86, and 100 were found to be the amino acids that are important for strong suppression of WSN/RNP activity (Fig. 5B and C). These amino acids are involved near the catalytic site of endonuclease [6–8], indicating again somehow the endonuclease activity on the fragment is involved in the suppression of RNP expression and that it is controlled by the combination of these three amino acids (positions 28, 86, and 100).

More recently, Jagger and Desmet *et. al.* showed how PA-X was important for the suppression of protein synthesis [9,10]. PA-X is a new protein that was found as a ribosomal frame-sifting protein, derived from the N-terminal PA cording region. Though the full-length of PA-X is not covered by our essential region of the VN/PA/N188 that is needed for strong suppression of WSN/RNP (Fig. 4B), the mechanism of the suppression may be involved into the PA-X functions.

During the process of the inhibitory study by PA subunit we found an interesting suppression of influenza RNP components, which was actually correlated with the endonuclease active sites (D108 and K134) of its N-terminal PA subunit. Furthermore, we newly identified the essential region (1–188 residues) and the combination of three amino acids (P28, M86 and E100) that was important for strong suppression. The natural target for the endonuclease of PA subunit is a host mRNA, which is a process that is referred to as cap-snatching. It remains unclear how the PA subunit can distinguish between host and viral mRNAs. In the present study, it is suggested that the PA subunit may lose control of its endonuclease activity and suppress the protein expression of WSN/RNP components by its fragmentation, although the direct mechanism is still unclear.

Our results indicate that the N-terminal fragment (1–188 residues) of PA subunit may become one of candidates for the inhibitor of influenza RNP activity via suppression of protein expression of the RNP components. This is very unique inhibition, because it means that influenza virus is inhibited by the fragment derived from own proteins. On the other hand, the cap-snatching mechanism is involved in the toxicity of the influenza virus, because this is what shuts off the host mRNA expression. Thus, our findings should help elucidate the molecular mechanism of the endonuclease activity on PA subunit, and should aid with developing new sights for the inhibition of the influenza A virus. Both of these could help reduce the influenza infection and toxicity in the future.

Conflict of interest

The authors declare that they have no conflict and interest.

Acknowledgments

This study was supported by grants from the Promotion of Science and the Ministry of Education, Science, Sports and Culture, Japan, Grant-in-Aid for Scientific Research (C), 24591590, 2012–2014, and the Ishibashi Foundation for the Promotion of Science.

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トピックス　Ⅰ．今日の肺炎球菌感染症

今日の肺炎球菌感染症

要　旨

小児用7価肺炎球菌結合型ワクチン（PCV7）の公費助成開始により，我が国のワクチン血清型による小児の侵襲性肺炎球菌感染症（invasive pneumococcal disease：IPD）は激減した．一方，非PCV7含有血清型による小児IPDが増加し，血清型置換が明確となった．結果的に，小児IPD罹患率は，PCV7導入前に比較して，2013年度までに57%減少した．さらに，65歳以上の高齢者におけるIPDの原因菌にも血清型置換が認められた．

〔日内会誌　104：2301～2306，2015〕

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Key words　侵襲性肺炎球菌感染症，肺炎球菌ワクチン，血清型置換

はじめに

肺炎球菌（*Streptococcus pneumoniae*）は主に乳幼児の鼻咽頭に高頻度に保菌されている[1)]．肺炎球菌による保菌は，本菌による感染症に先行して発生し，市中における菌の水平伝播に重要な役割を果たす[2)]．本菌は小児，成人に中耳炎，副鼻腔炎や菌血症を伴わない肺炎などの非侵襲性感染症や髄膜炎や菌血症を伴う肺炎などの侵襲性肺炎球菌感染症（invasive pneumococcal disease：IPD）を引き起こす．このIPDとは，通常無菌的であるべき検体から肺炎球菌が分離された疾患を指す．近年，主に先進諸国において，小児用7価肺炎球菌結合型ワクチン（PCV7）の定期接種導入後に肺炎球菌感染症の発生動向，原因菌の血清型分布が大きく変化している．本稿においては海外，国内の肺炎球菌感染症の実態について記述する．

1．海外の疫学状況

欧米諸国において，小児へのPCV7の定期接種導入後に肺炎球菌感染症の疾病負荷は有意に減少した[3)]．米国ではPCV7導入7年後において，導入前と比べて，全てのIPD罹患率とPCV7ワクチン血清型によるIPD罹患率はそれぞれ45%，94%減少し，一方ではPCV7に含まれない19Aなどの非PCV7血清型によるIPD罹患率が増加し，血清型置換（serotype replacement）が明確になった[4)]．さらに，65歳以上の高齢者においてもPCV7ワクチン血清型によるIPD罹患率も92%減少した[4)]．英国，ウェールズではPCV7導入4年後に，PCV7ワクチン血清型による2歳以下のIPD罹患率が98%減少し，65歳以上の高齢者のIPD罹患率の81%の減少が報告された[5)]．

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Knowledge and Strategy in Pneumococcal Vaccines for General Physicians. Topics：I. Pneumococcal infection：update

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表　年齢群別の侵襲性肺炎球菌感染症の症例数，致命率，罹患数
（2013年4月～2015年1月）

年齢グループ	症例数	死亡患者数	致命率（%）	罹患率（/10万人・年）
5歳未満	640	6	0.94	6.55
5～14歳	72	1	1.39	0.34
15～64歳	762	47	6.17	0.52
65歳以上	1,615	147	9.10	2.85
全年齢	3,089	201	6.51	1.31

（文献6の表1より作図）

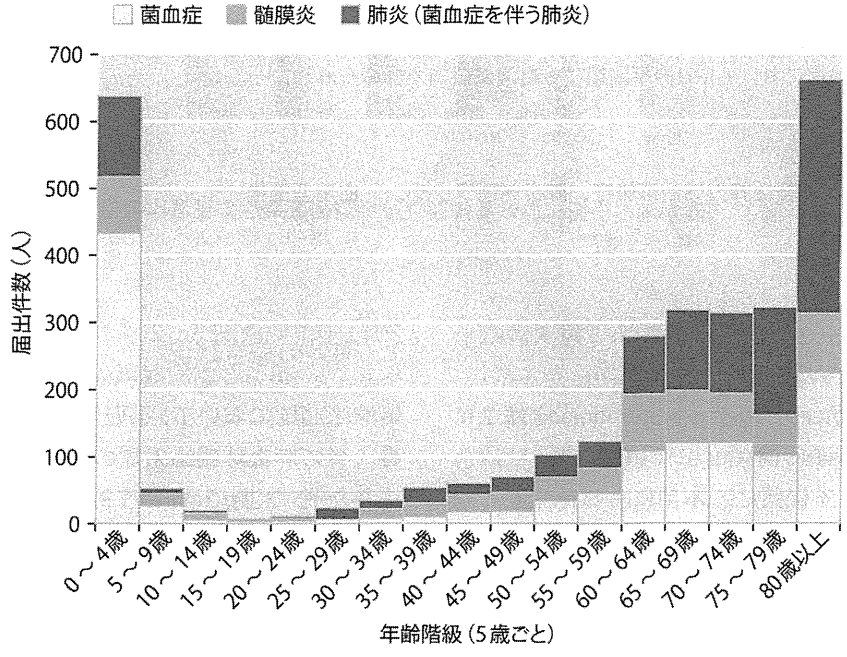

図1　侵襲性肺炎球菌感染症の発生動向と臨床病型（2013年4月～2015年1月）
（文献6より引用）

2. 我が国の小児，成人のIPDの発生動向調査

2013年4月から2015年1月までの感染症法に基づく感染症発生動向調査ではIPDの報告総数は3,089例であり，全国で約1,500例の症例が報告されている（表）[6]．また，罹患率（/10万人・年）は5歳未満で6.55，65歳以上では2.85と小児の方が高齢者より高かった．年齢別のIPD症例の届け出患者数では，5歳未満の小児と60歳以上の成人における二峰性分布を示すことがわかる（図1）．また，IPDの病型の構成では，5歳未満の小児では菌血症が大半を占めていた．成人では60歳以上で届出数が増加し，病型としては菌血症を伴う肺炎，菌血症，髄膜炎の順に多かった．予後については，3,089症例中，201例が死亡していた．とりわけ65歳以上の高齢者では，報告時点での致命率は9.10%と高かった（表）．

3. 我が国の小児IPDの疫学状況

我が国において，PCV7は2009年10月に製造

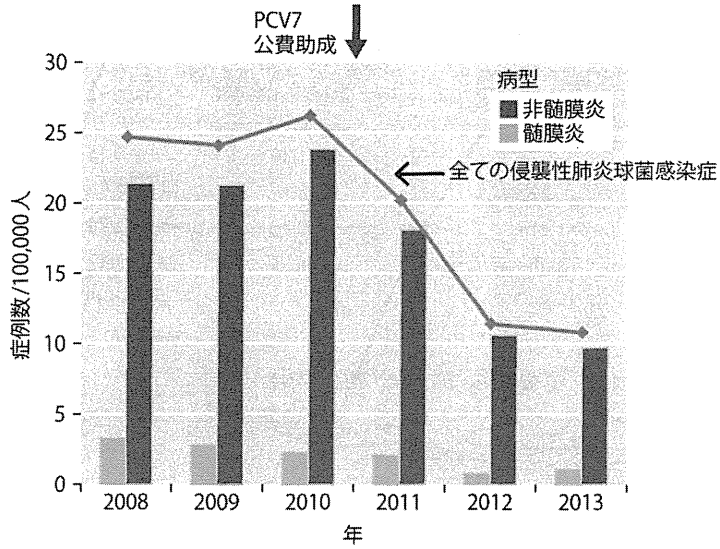

図2　5歳未満の小児における侵襲性肺炎球菌感染症の罹患率の推移
（文献7より改変）

販売承認され，2010年11月には5歳未満の小児に対するPCV7接種の公費助成が拡充された．その後，PCV7は2013年4月から定期接種ワクチンとなり，同年11月には13価肺炎球菌結合型ワクチン（PCV13）に置き換わった．成人用の23価莢膜ポリサッカライドワクチン（PPSV23）は1988年3月に輸入承認され，2006年にはニューモバックスNPとして製造販売承認された．その後，2014年10月から65歳以上の成人などを対象として定期接種ワクチン（B類疾病）となった．また，2014年6月にPCV13に対する製造販売承認の用法に65歳以上の高齢者が追加された．

我が国において，2007年から始まった「ワクチンの有用性向上のためのエビデンス及び方策に関する研究」（庵原・神谷班）において，PCV7の公費助成後の小児のIPD罹患率は，2008～2010年に比較して2013年度までに57％減少し，5歳未満の人口10万人あたり10.8まで低下した（図2）[7]．

さらに，PCV7の血清型特異的な効果に着目すると，PCV7含有血清型の小児IPDの罹患率は2011年までに32％，2012年までに85％，2013年までに98％と劇的な減少を認めた（図3）．結果的に，2010年の小児IPDの原因菌のPCV7含有血清型の割合は78.5％であったのに対し，定期接種化後の2013年には3.3％に低下した．一方，PCV13に含まれるがPCV7に含まれない血清型（PCV13-PCV7）が2010年には11.6％であったものが2013年には47.8％に増加した．このPCV13-PCV7の血清型では19Aが27.9％と最も多かった．また，PCV13にも含まれない血清型（24F，15A，15Cなど）によるIPD症例の割合は，2012年（41.5％），2013年（48.9％）に増加し，我が国の小児IPDにおいてもPCV7導入後の血清型置換が明確になっている．

4．我が国の成人のIPDの疫学状況

厚生労働省指定研究班「成人の重症肺炎サーベイランス構築に関する研究」を全国10道県において実施し，2013年4月から2014年11月までに152例を登録した[8]．152例の年齢中央値は70歳（範囲：25歳～94歳），男性が97例（64％）

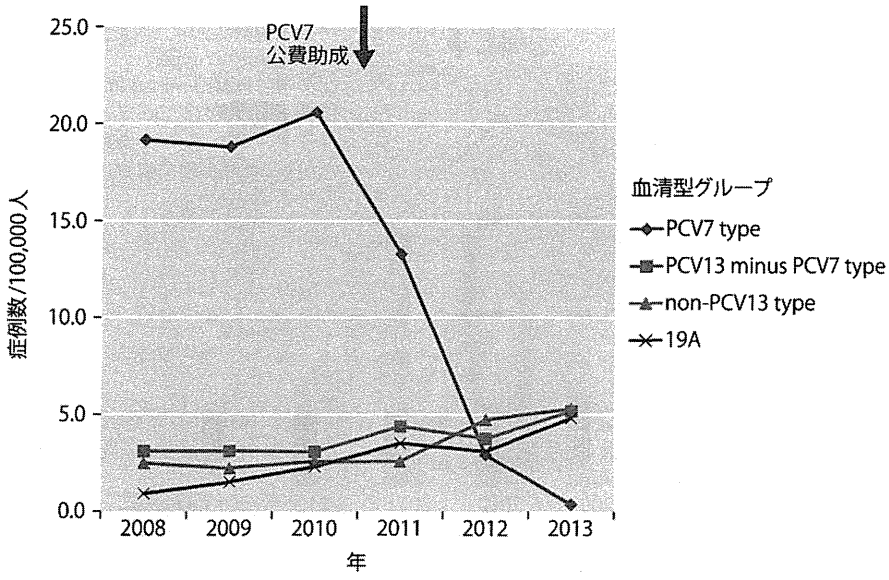

図3　5歳未満の小児における侵襲性肺炎球菌感染症：血清型別の罹患率の推移
（文献7より改変）

であった．喫煙者は67例（44%），基礎疾患のある患者は107例（70%）で，うち免疫不全を伴う患者は64例（42%）であった．PPSV23の接種歴がある患者は5例（3%）であった．人工呼吸器管理は36例（24%），集中治療室管理は35例（23%）であった．主な病型は菌血症を伴う肺炎86例（57%），髄膜炎25例（16%），菌血症のみ24例（16%）で髄膜炎，菌血症が比較的若年に偏る傾向にあった．死亡例は32例（致命率21%）であった．このように，成人のIPD患者で基礎疾患を有する症例は70%と多く，42%が免疫不患者であった．基礎疾患のない患者に比べて，免疫不全患者の原因菌としてPCV13非含有血清型の割合（63%）が高く，菌血症の病型を取る割合（25%）が高かった．

2013年4月から2015年1月までの22カ月間に収集された224株の成人IPD患者の原因菌の血清型別分離率を図4に示した[9]．分離頻度の高い血清型は3，19A，22Fの順であった．2006～2007年に実施された国内の成人IPD患者の血清型分布の調査（PCV7，PCV13，PPSV23に含まれる血清型の割合；34.0%，61.5%，85.4%）と比較して[10]，PCV7，PCV13，PPSV23に含まれる血清型の割合はそれぞれ12.5%，46.0%，66.5%と減少していた．また，非PCV13血清型である10A, 22F, 6Cなどの割合が増加していた．

5. 我が国の成人の肺炎球菌性肺炎の疫学状況

2010～2012年に国内で実施された成人市中肺炎と医療ケア関連肺炎の調査では，肺炎球菌性肺炎の割合は市中肺炎が17.1～23.2%，医療ケア関連肺炎が12.7～18.4%であった[11,12]．また，国内の肺炎球菌性肺炎例の調査では，全ての肺炎球菌性肺炎のうち，菌血症を伴う肺炎の頻度は4～5%であった[13,14]．また，2011年9月から2013年1月の期間に国内4カ所の医療機関で実施された市中発症肺炎（市中肺炎と医療ケア関連肺炎）の疫学的調査では，罹患率と死亡率の推定値（95%信頼区間）は1,000人・年あたり16.9（13.6～20.9），0.7（0.6～0.8）とされている[13]．年齢依存性の罹患率の増加は，

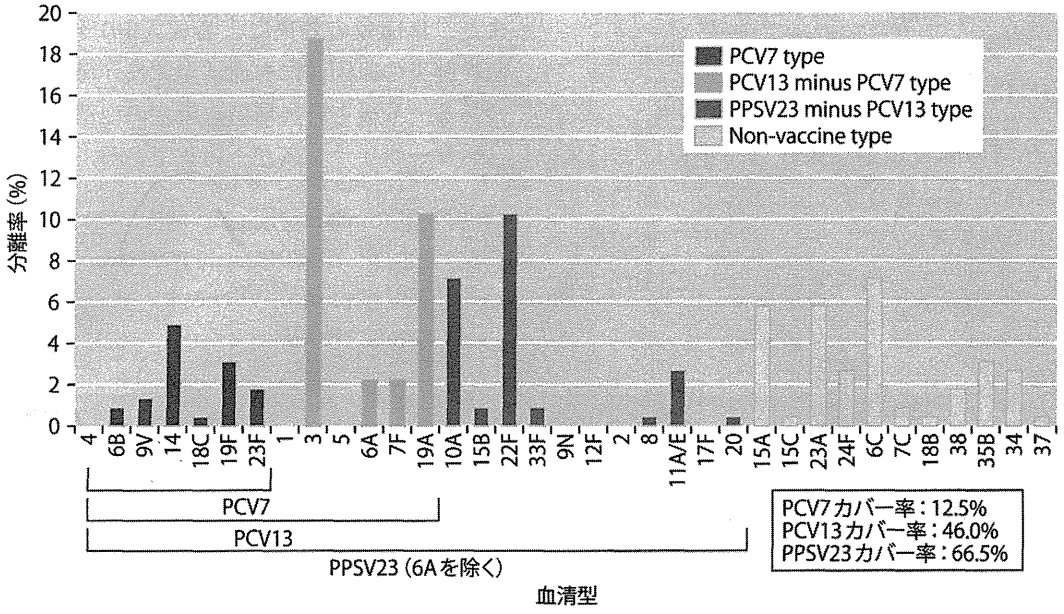

図4　成人の侵襲性肺炎球菌感染症患者の原因菌の血清型別分離率：2013年～2014年度（n＝224）
（文献9の表2より改変）

女性に比べて男性において顕著であった．罹患率は85歳以上の男性において最も高かった．また，本調査では市中肺炎の罹患率は65歳以上で高くなり，医療ケア関連肺炎の罹患率は75歳以上で増加することが示されている．本研究における成人の市中発症肺炎患者由来の100株の喀痰由来の肺炎球菌の血清型分布の検討では，分離頻度の高い血清型は3，11A/E，6Cの順であった．2003～2005年に実施された国内の成人市中肺炎の血清型分布（PCV7，PCV13，PPSV23に含まれる血清型の割合；42.3%，73.1%，80.8%）と比較して，PCV7，PCV13，PPSV23に含まれる血清型の割合はそれぞれ23%，54%，67%と減少していた[15]．また，非PCV13血清型である11A/E，6C，35Bなどの割合が増加していた．

6. 小児から成人への菌伝播の可能性

我が国の小児に対するPCV7導入後にみられた成人IPD患者および市中発症肺炎患者の原因菌の血清型分布において，PCV7に含まれる血清型の減少とPCV7に含まれない血清型の増加が認められた．これらの所見はすでに欧米で報告されている血清型置換と考えられ，小児におけるPCV7導入に伴う集団免疫効果に起因すると推察された．また，このような小児に対するPCV7の導入が間接的に成人のIPDを減少させた事実は，小児の鼻咽頭に保菌された肺炎球菌が成人に伝播する可能性を示唆している．

小児と成人間の肺炎球菌の伝播について，過去の我が国の研究では医療機関で小児とその親から採取された肺炎球菌のDNAパターンが一致することが報告されている[16]．また，最近の海外研究によれば，成人の菌血症を伴わない肺炎球菌性肺炎の発症は小児と成人の接触に有意に関連することが報告されている[17]．本論文における子供と成人の接触の定義は，1）16歳未満の子供と同じ世帯に同居，2）肺炎発症前4週間に8時間以上の接触があった，3）小児とフルタ

イムで接触する職業（教師など）とされている．

おわりに

我が国の肺炎球菌感染症は，小児におけるPCV7/PCV13接種の導入後にワクチン血清型の小児IPDが激減した．しかし，ワクチン血清型に代わり非ワクチン血清型による小児IPDが増加傾向にあり，血清型置換が明確になっている．また，集団免疫効果による成人IPD及び肺炎球菌性肺炎の原因菌にも血清型置換が確認されつつある．今後も肺炎球菌感染症の血清型分布を含むサーベイランスが不可欠である．

著者のCOI（conflicts of interest）開示：本論文発表内容に関連して特に申告なし

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特集◉呼吸器感染症の最新動向

重症肺炎と肺炎球菌ワクチンの動向

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Key words：肺炎球菌ワクチン，重症肺炎，侵襲性肺炎球菌感染症

Ⅰ 重症肺炎

肺炎は病原微生物により発症する肺実質の炎症であり，臨床診断では新たに出現した呼吸器症状（発熱，咳嗽，喀痰など）と新たに出現した胸部画像陰影（胸部X線，胸部CT画像など）を満たすものとされる。現在，国内における肺炎の分類は市中肺炎，医療・介護関連肺炎，院内肺炎（人工呼吸器関連肺炎を含む）となっている[1)2)]。肺炎と診断したら，まず発症場所により分類を行う（**図1**）。肺炎では起炎菌を考慮した抗菌薬を選択するべきだが，肺炎の母体となる宿主要因や環境などにより，病原微生物の種類，薬剤感受性などが異なる。起炎菌の同定には時間を要するため，上記肺炎分類は異なる治療戦略への対応に有用である。初期治療には幅広い菌種をカバーする広域スペクトラム抗菌薬で治療を開始し（経験的治療），起炎菌の同定，薬剤感受性試験結果が判明した後に狭域スペクトラム抗菌薬に変更する（de-escalation）。

肺炎は脳血管疾患を抜いてわが国の死因第3位となった。わが国の肺炎死亡率を年齢別にみると，65歳以上の高齢者で増加し，85歳以上では10万人あたり1,000～5,000人の死亡率を示している（**図2**）。高齢者肺炎死亡の増加が死因統計の肺炎増加の主要因であることを示しており，高齢化の進行に伴い，今後もますます増加するものと見込まれている。

市中肺炎の起因菌は**図3**に示すとおり肺炎球菌が最も多く[3)]，全体の1/4強，原因菌の判明したもののうち約40％を占める。続いてインフルエンザ菌が多く，マイコプラズマや肺炎クラミジアが続く。市中肺炎の重症例は肺炎球菌，インフルエンザウイルス，レジオネラ菌が多く[4)]，致死率も高い。医療・介護関連肺炎でも最も多い原因分離微生物は肺炎球菌であり，肺炎球菌感染症に対する対策が重要である。血液や髄液のような無菌検体から肺炎球菌が検出される重篤な感染症は侵襲性肺炎球菌感染症（IPD）として特に重症度の高い疾患と考えられるが，厚生労働省班研究（生方班）のサーベイランスの結果から，小児では髄膜炎や肺炎を伴わない菌血症が多かったのに対し，65歳以上の高齢者では肺炎に伴う菌血症の割合が高かった（**図4**）[5)]。IPDは致命率が高く，高齢者肺炎

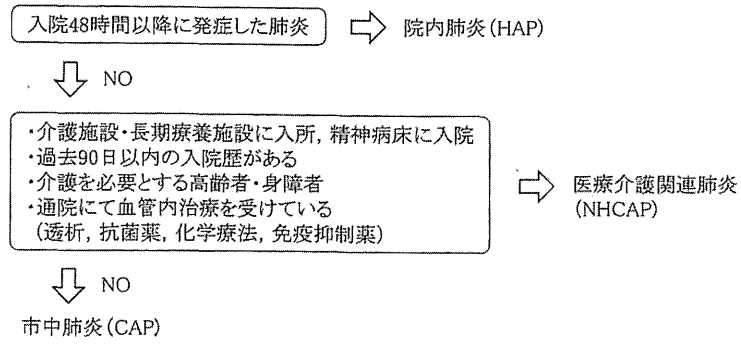
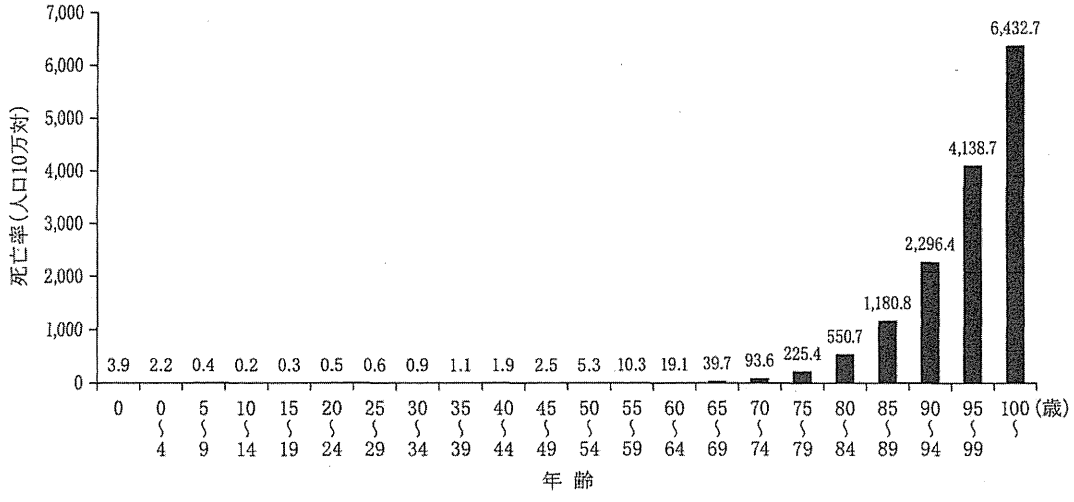


図1 肺炎の発症場所による分類



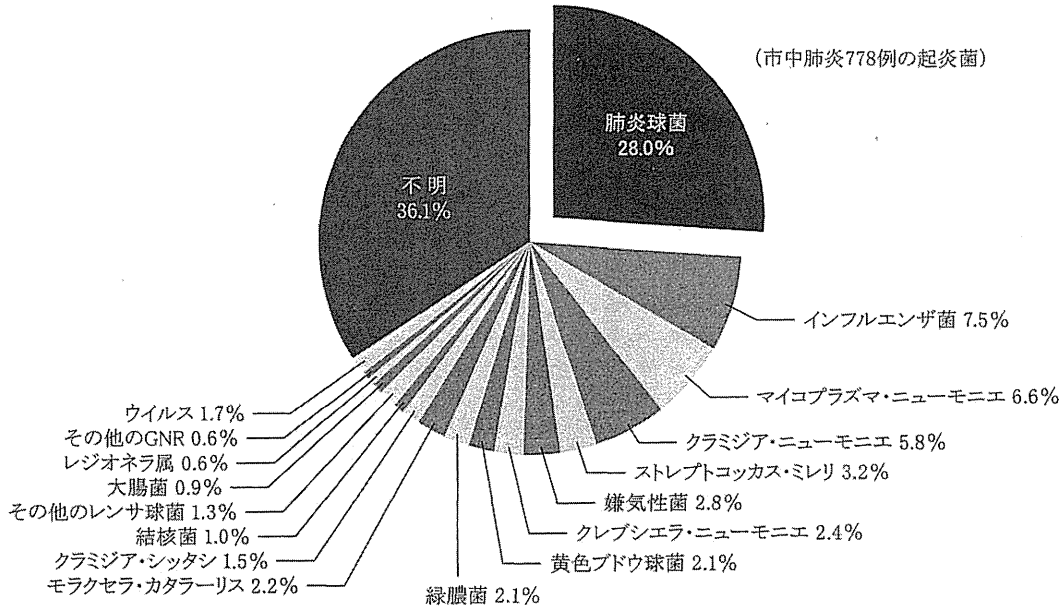
(厚生労働省：人口動態統計年報主要統計表〔2010年〕より作図)

図2 年齢別肺炎による死亡率

の中にも多くのIPD症例が含まれていることから，IPDの発症予防，早期治療が重要である。肺炎の診断時にはできるだけ早期に血液培養2セットを採取することを心がけ，高齢者，免疫低下状態などのハイリスク群には肺炎球菌ワクチン接種を勧める。

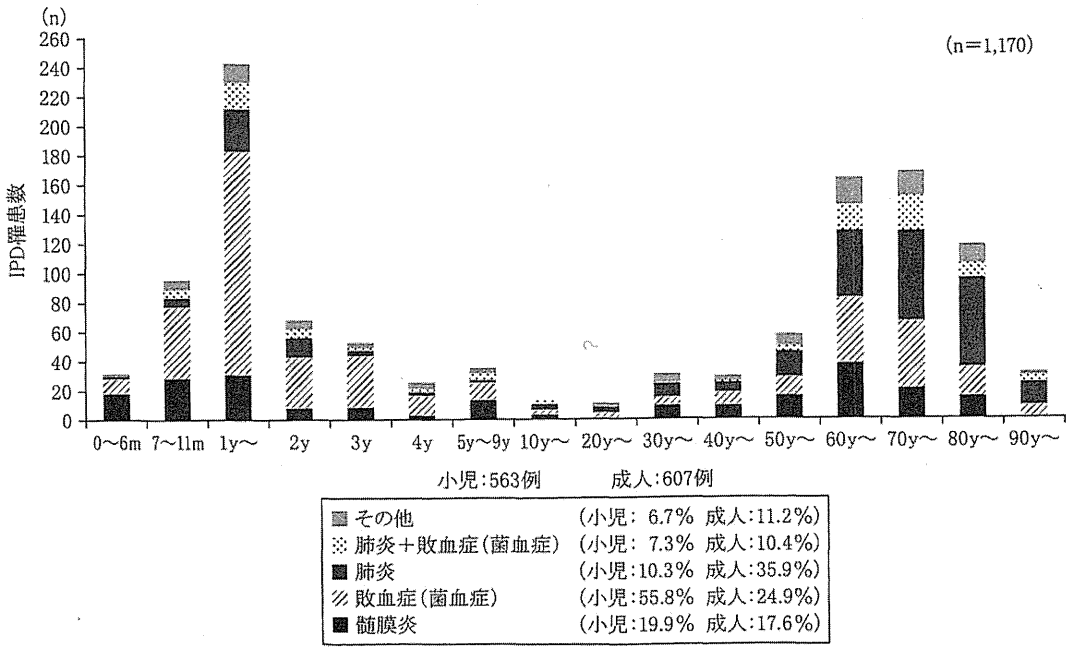
II 肺炎球菌ワクチンの動向

現在国内で使用可能な肺炎球菌ワクチンには大きく2種類あり，莢膜多糖体型肺炎球菌ワクチン（PPSV）と蛋白結合型肺炎球菌ワクチン（PCV）がある。2010年11月に5歳未満の小児に対する7価結合型ワクチン（PCV7）接種の公費助成が開始された。2013年4月にPCV7の定期接種化がなされ，同年11月にはPCV13（PCV7に6種類の血清型多糖を加えたワクチン）に置き換えられた。厚生労働省班研究（庵原・神谷班）の調査結果から，2011年10月時点でのワクチン公費助成前後の比較では，髄



対象・方法：1994年から7年間に倉敷中央病院に入院した成人市中肺炎778例に対し，原因微生物についての前向き調査を行った。

図3 市中肺炎の原因微生物の分離頻度



対象・方法：全国344の医療機関の患者検体から分離された侵襲性感染症由来株のうち，肺炎球菌につき分析した（2010年4月～2013年3月，n=1,170）。

図4 侵襲性肺炎球菌感染症の年齢分布と疾患の内訳

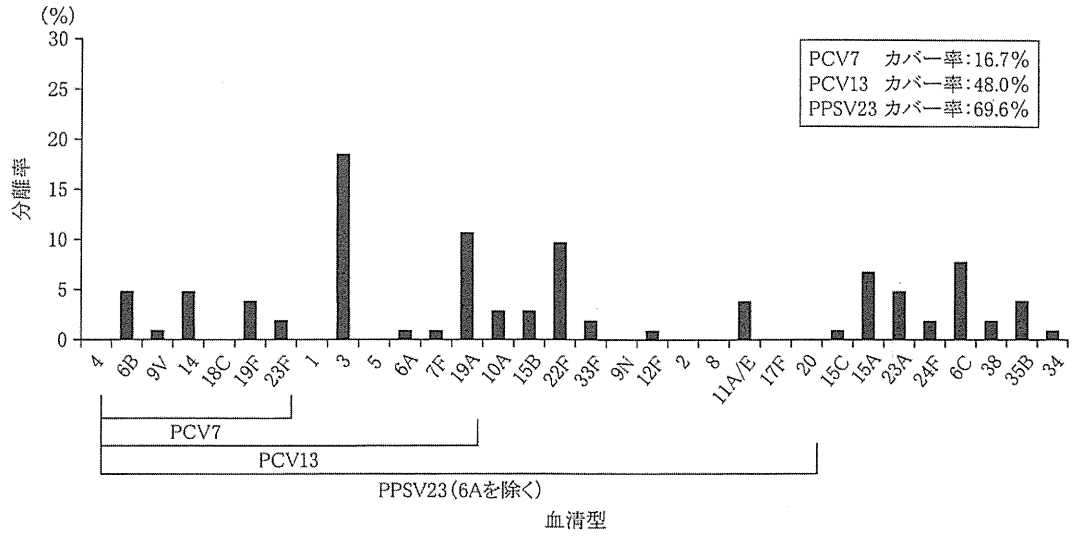


図5 2013年度に分離された侵襲性肺炎球菌感染症患者由来の原因菌の血清型分布

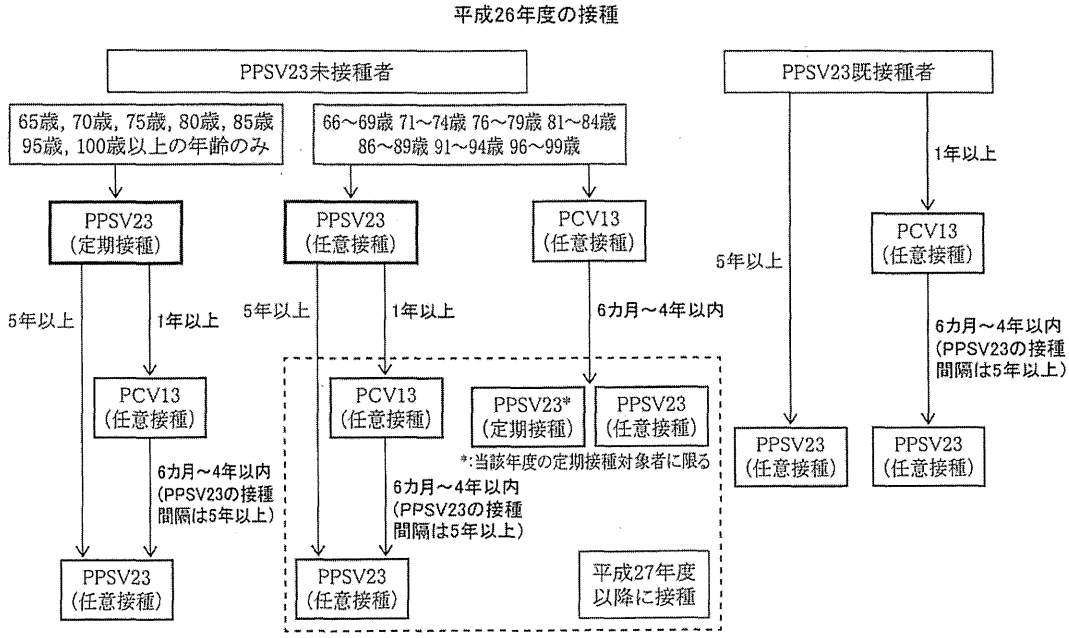
膜炎で92％の減少，菌血症を伴う非髄膜炎で82％の減少となっている[6]。

ワクチン定期接種が先行して行われた米国ではワクチンでカバー可能な莢膜型のIPDを減少させる一方，カバーされていない莢膜型のIPDの比率が増加するという血清型置換（Serotype replacement）がみられた[7]。わが国においても血清型置換は重要な課題であり，モニタリングが必要とされている。現在，厚生労働省班研究（大石班）において1道9県を対象に「成人の重症肺炎サーベイランス構築に関する研究」が行われており，2013年の中間報告（**図5**）において，2006～2007年に実施された国内の成人IPD患者野血清型分布と比較してワクチンカバー率が低下していることが明らかとなった[8]。

わが国の成人肺炎球菌ワクチン接種率は約20％と米国の70％と比較して大きな開きがあり，接種率の向上が課題となっている。2014年10月から65歳以上と60歳以上65歳未満の者で重度の障害を有する者を対象としてPPSV23が定期接種化された。5歳刻みで接種対象者を定め，5年間で全高齢者に接種可能となる見込みである。

わが国ではPCV13は定期接種に用いられていないが，米国ACIPは2014年9月にMMRW誌上でPCV13とPPSV23の連続接種やワクチン接種歴のある65歳以上成人に対する追加接種を推奨した[9]。これを受けてわが国でも日本呼吸器学会ワクチン検討WG委員会と日本感染症学会ワクチン委員会の合同委員会において2015年1月にワクチン接種の考え方を発表した（**図6**）。同委員会の見解としては，PCV13は国内でのデータが乏しいことから，定期接種とはせず任意接種（自己負担約1万円）となった。

2015年3月にPCV13の大規模無作為化試験（CAPITA）の結果がBontenらにより報告された[10]。オランダで施行された約85,000例の高齢者を対象とし，PCV13がカバーする莢膜型が原因である肺炎球菌肺炎を46％，IPDを75％削減するという結果であった（**図7**）。これまでの肺炎球菌ワクチンの効果に関するエビデンスはIPDの減少に関しては高い信頼があったものの，市中肺炎に関して減少効果は十分ではなかった。本研究結果は市中肺炎に対し



注意

#1. 今回の考え方はPPSV23の定期接種措置と米国ACIPの推奨を参考に作成された。

#2. 定期接種対象者が，定期接種によるPPSV23の接種を受けられるように接種スケジュールを決定することを推奨する。

#3. PPSV23未接種者に対して両ワクチンを接種する場合には，上記#2を勘案しつつ，PCV13→PPSV23の順番で連続接種することが考えられる。

#4. PCV13とPPSV23の連続接種については海外のデータに基づいており，日本人を対象とした有効性，安全性の検討はなされていない。

#5. 定期接種は平成26年10月～平成31年3月までの経過措置に準ずる。

#6. 今回の考え方は3年以内に見直しをする。

図6 65歳以上の成人に対する肺炎球菌ワクチン接種の考え方

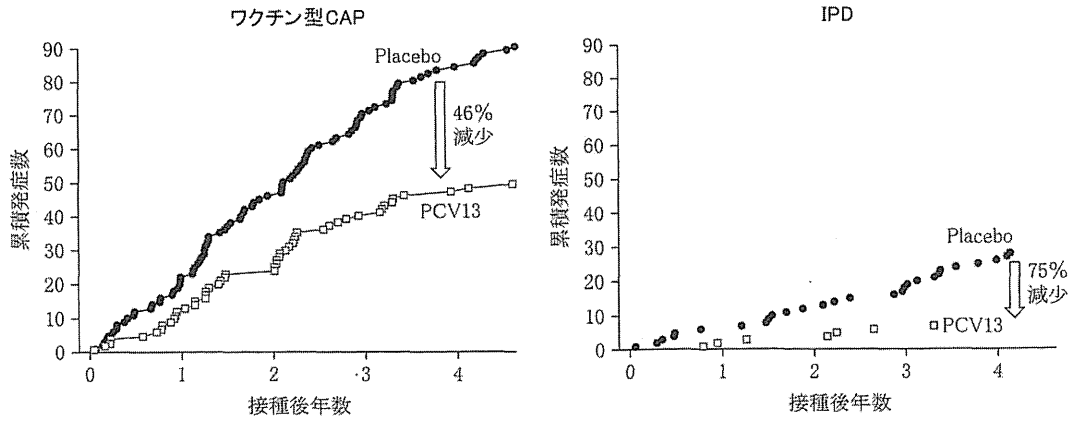


図7 PCV13接種後の市中肺炎および侵襲性肺炎球菌感染症の累積発症数