COPDにおけるインフルエンザワクチンと肺炎球菌ワクチン

永井英明

気道感染症はCOPDの急性増悪をもたらすが，気道感染症を引き起こす病原体のうち，ワクチンで予防しうるものはインフルエンザウイルスと肺炎球菌である．

1. COPDに対するインフルエンザワクチン（influenza vaccine；IV）の推奨

米国の予防接種諮問委員会（Advisory Committee on Immunization Practices；ACIP）によるIV接種のガイドライン[1]では，COPDを含む慢性呼吸器疾患はIVの接種が勧められている．COPDに対するグローバルイニシアチブ（GOLD）のガイドライン[2]でも，COPDに対してIVの接種が推奨されている．COPDに対するIVの効果をみた無作為対照臨床試験では，インフルエンザの発病を76%抑え，しかもその効果はCOPDの重症度に影響を受けなかった[3]．

2. COPDに対する肺炎球菌ワクチン（pneumococcal polysaccharide vaccine；PPV）の推奨

ACIPによるPPVの接種についてのガイドライン[4]では，COPD患者はPPVの接種対象者に含まれ，接種が強く勧められている．GOLDのガイドライン[2]では2006年版からPPV接種の推奨が追記された．65歳未満かつ1秒量が40%未満のCOPD重症例では市中肺炎の合併が91%減じた[5]．

3. COPDに対するIVとPPVの併用

インフルエンザに罹患した場合，気道での細菌の排除機能が低下し，二次的に細菌性肺炎を併発して重篤化しやすい．したがって，IVとPPVの併用が有効という報告が多い．Nicholの報告[6]では，65歳以上のCOPD患者では，IVの接種により，肺炎による入院は52%減少し，死亡は70%減少したが，PPV接種を併用することにより，肺炎の入院は63%減少し，死亡は81%減少し，両者の併用効果が認められた．

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Influenza and Pneumococcal Vaccinations in Patients with COPD

Hideaki Nagai：Department of Pulmonary Diseases, National Hospital Organization, Tokyo National Hospital

独立行政法人国立病院機構東京病院呼吸器科，外来診療部長

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●原 著

肺炎球菌ワクチン再接種承認の必要性に関するアンケート調査研究

大石 和徳[1] 川上 和義[2] 永井 英明[3] 砂川 慶介[4] 渡辺 彰[5]

要旨：本邦における肺炎球菌ワクチンの再接種の実施状況と副反応の実態を明らかにすることを目的として，日本呼吸器学会，日本感染症学会の役員989名を対象に，アンケート調査を実施した．有効回答を得た385名のうち，本ワクチンの接種経験のある290名（75.3%）を調査対象者とした．この290名中，46名（15.9%）に再接種実施経験があり，252名（86.9%）は再接種の必要性を認識していた．また，その144名（49.7%）は再接種が禁忌であるが故に，患者が初回接種を控える経験をしていた．再接種の実施経験者46名のうち4名が再接種による副反応を経験していたが，いずれも軽症であった．今回の調査結果から，調査対象者の大半は再接種の必要性を認識しており，その一部は患者側の要望に応じて再接種を実施している実態が明らかになり，再接種が禁忌であることが本ワクチン接種率向上の障壁となっていることも示唆された．

キーワード：肺炎球菌ワクチン，再接種，アンケート調査
Pneumococcal polysaccharide vaccine, Revaccination, Questionnaire

緒 言

肺炎球菌は最も重要な呼吸器病原性菌であり，その菌表層は莢膜ポリサッカライドに覆われている．この莢膜ポリサッカライド（CPS）には少なくとも91種類の莢膜血清型が存在する．このCPSはT細胞非依存性抗原であり，生体内では主に血清型特異IgG2産生を誘導し，この特異抗体による補体依存性のオプソニン活性は本菌に対する感染防御の中心的役割を担っている[1]．

肺炎球菌ワクチンは23価のCPSを含有する多価ワクチンであり，その成人における敗血症や髄膜炎などの侵襲性肺炎球菌感染症（Invasive pneumococcal diseases：IPD）に対する予防効果から[1][2]，我が国では1988年に薬事承認された．この際に，米国予防接種諮問委員会（Advisory Committee on Immunization Practice；ACIP）が1982年に成人に対して14価肺炎球菌ワクチンを一回のみの接種を推奨したこともあり[3]，再接種・追加接種をしてはならない旨が添付文書に記載された．しかしながら，その後の海外における23価肺炎球菌ワクチンの再接種に関する調査においては再接種に伴う重篤な副反応は認められず，再接種のリスクは禁忌に該当しないとされている[4]~[6]．また，1997年には米国ACIPは65歳未満で肺炎球菌ワクチンを接種し，その後5年が経過した場合には再接種を推奨している[7]．我が国では，2006年10月以降，肺炎球菌ワクチンは従来の製造方法が変更されたニューモバックスNP®として臨床の現場で使用されているが，その再接種は依然禁忌のままである．近年，国内の高齢者において肺炎球菌ワクチン接種が普及するにつれて，臨床現場では肺炎球菌ワクチンの再接種承認を求める声が高まっている．

本研究では，我が国における肺炎球菌ワクチン再接種の実施状況と副反応の実態を明らかにすることを目的として，日本呼吸器学会理事，代議員および日本感染症学会の理事，評議員を対象にアンケート調査を実施したので報告する．

対象と方法

1）実態調査の内容と方法

我々は，日本呼吸器学会と日本感染症学会の協力のもとに，日本呼吸器学会の理事，代議員，および日本感染症学会の理事，評議員の総数989名を対象に，平成20年12月から平成21年1月にかけて，匿名回答による郵送アンケート調査を実施した．調査内容としては，1)肺炎球菌ワクチン再接種実施の状況，2）再接種対象の基礎疾患，3）再接種実施の動機，4）再接種による副反応の有無，5）再接種の必要性，6）再接種禁忌の与える初回接種への影響などであった．再接種実施の動機につい

〒565-0871 大阪府吹田市山田丘3—1
[1]大阪大学微生物病研究所感染症国際研究センター
[2]東北大学医学系研究科保健学専攻基礎検査医科学講座
[3]国立病院機構東京病院呼吸器科
[4]北里大学北里生命科学研究所・感染症学
[5]東北大学加齢医学研究所抗感染症薬開発研究部門
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Table 1 Questionnaires for pneumococcal vaccination and revaccination

Questions		no. of subjects (%)
Pneumococcal vaccination at the affiliated institution	no. of total subjects; 385 no. of subjects answered yes (%) no. of subjects answered no (%) or with no answer	290 (75.3) 95 (24.7)
Negative influence of the contraindication on revaccination for the first vaccination	no. of total subjects; 290 no. of subjects answered yes (%) no. of subjects answered no or with no answer (%)	144 (49.7) 146 (50.3)
Pneumococcal revaccination	no. of total subjects; 290 no. of subjects answered yes (%) no. of subjects answered no or with no answer (%)	46 (15.9) 244 (84.1)
Necessity for approval of revaccination	no. of total subjects; 290 no. of subjects answered yes (%) no. of subjects answered no or with no answer (%)	252 (86.9) 38 (13.1)

ては，医師の推奨か，患者もしくは家族の希望かのいずれかを質問した．平成21年1月末までに回収されたアンケート調査票を解析した．

成　績

1．肺炎球菌ワクチン再接種の実態調査

1）アンケート対象者

送付された989通のアンケート調査用紙のうち401通(40.5%)が回収され，そのうち解析可能な調査用紙は385通（有効回答率は96.0%）であった．回答者の性別は男性が95.3%，平均年齢は53.1歳（SD：8.5）であった．回答者の所属医療機関は，大学附属病院38.2%，国公立病院28.3%，私立病院14.5%，診療所・クリニック10.9%の順で，診療科別では呼吸器内科54.3%，一般内科13.8%，感染症内科8.8%の順であった．回答者の76.7%が内科医であった．

2）肺炎球菌ワクチン接種状況

有効回答を得た385名のうち290名（75.3%）は医療施設で本ワクチンの接種を実施しており，この290名を調査対象者とした（Table 1）．接種理由は医師の推奨が52%であり，患者本人の希望は48%であった．肺炎球菌ワクチン接種を実施した患者の基礎疾患は，慢性閉塞性肺疾患（COPD），その他の慢性肺疾患，脾摘出・脾機能不全患者，慢性心不全，糖尿病の順であった．接種を推奨している年齢については75歳以上39.3%，65歳以上38.7%，80歳以上22.1%の順であった．また，調査対象者290名のうち，144名（49.7%）から再接種が禁忌となっていることが，初回接種を控える原因となったとする回答を得た（Table 1）．さらに，これらの回答者が上記の理由から初回接種を控えたとする患者数は平均19名（SD 26.7例）であり，報告総数は2,344例であった．

3）肺炎球菌ワクチン再接種の状況と副反応

調査対象者290名のうち，46名（15.9%）の医師が再接種を実施した経験ありと回答した（Table 1）．この46名のうち11名の医師から，再接種を受けた患者49症例の臨床像が報告され，他の35名の医師からの報告は得られなかった．11名の医師の再接種患者数の内訳は，1名の医師がそれぞれ15例，8例，7例，5例，4例，3例の患者に，2名の医師がそれぞれ2例の患者に，3名の医師がそれぞれ1例の患者に再接種を実施していた．再接種を受けた患者の平均年齢は74.4歳（SD：10.3）であり，その基礎疾患の内訳は，COPDを含む慢性肺疾患が19例（38.8%），慢性心不全7例（14.3%）などであった（Table 2）．初回接種から再接種までの期間は，平均63カ月（SD：11.8）であった．再接種実施の動機は，43例（87.8%）は本人もしくは家族の希望によるものであり，医師の推奨によるものは5例（10.2%）であった．また，290名の調査対象者のうち，252名（86.9%）が再接種は必要との認識を示したのに対し，回答なし，もしくは必要なしと回答したのは38名（13.1%）に過ぎなかった．

再接種を実施した調査対象者46名のうち，4名から再接種に伴う副反応の報告があった．その内訳は注射部位の局所的腫脹が2例，発疹，筋肉痛，倦怠感は各1例であった．1例では10cm以上の腫脹も経験されていたが，アナフィラキシーなど重篤な副反応の報告はなかった．

Table 2 49 reported cases with pneumococcal revaccination during last two years by the questionnaire

Demographic features	
Male sex (%)	27 (55.1)
Age, mean years (SD)	74.4 (10.3)
Duration between primary and revaccination, mean months (SD)	63 (11.8)
Underlying diseases; no. of cases (%)	
Chronic lung diseases	19 (38.8)
COPD	13 (26.5)
Other chronic lung diseases	6 (12.2)
Chronic heart disease	7 (14.3)
Diabetes mellitus	1 (2.0)
Others	19 (38.3)
Reason for revaccination; no. of cases (%)	
Request by the patient	43 (87.8)
Request by the family	1 (2.0)
Recommendation by the doctor	5 (10.2)

SD; standard deviation, COPD; chronic obstructive pulmonary disease

考　察

我が国の成人におけるIPDの実態はこれまで明らかでなかったが，最近になってChibaらはその病院ネットワークからの報告において，小児IPD症例より成人IPD症例が多く，その致命率は22%と高い実態を明らかにした[8]．IPDの発生頻度は高齢化につれて顕著に増加することから[9]，高齢化時代を迎えた我が国においてIPDは高齢者の生命を脅かす感染症の一つと言える．一方，米国の疫学調査からその頻度がIPDの約10倍多いと推定される菌血症を伴わない肺炎に対する肺炎球菌ワクチン接種の予防効果には異論があるところである[2][10][11]．しかしながら，最近では本ワクチン接種後の成人肺炎の重症度や死亡リスクが低下するとした報告もみられる[12][13]．このような背景から，我が国の高齢者のIPD予防および肺炎重症化予防対策の一環として本ワクチン接種が薦められる．また，高齢者においては肺炎球菌ワクチン接種による血中特異抗体濃度および感染防御効果が5年以上は維持されないことから[2][14][15]，初回接種後の高齢者ではその後の追加接種が必要と考えられる．

今回のアンケート調査では，肺炎球菌ワクチン接種を実施している290名の調査対象者のうち，再接種実施経験者は15.9%である一方で，その86.9%は再接種の必要性を認識していた．また，初回接種の理由については患者本人の希望が約半数であるのに対し，再接種の理由はその89.8%が本人や家族の希望によっていた．これらの調査結果は，本ワクチンの再接種が禁忌であることを知りつつも，再接種の安全性と必要性を認識し，患者やその家族による再接種の希望に応じる医師の実態を浮き彫りにしている．また，肺炎球菌ワクチン接種経験者の約半数が再接種禁忌であるが故に，その初回接種を控える経験をしており，結果的に我が国における本ワクチン普及の障壁となっている可能性が示唆された．

一方，再接種経験のある46名の調査対象者のうち4名は再接種に伴う副反応の経験があったが，いずれも軽症であり，重篤な副反応は発生していなかった．最近，高山らも12例の高齢者を対象とした再接種例のうち，1例のみに接種部位の発赤，腫脹，疼痛などの軽症の副反応を認めたとしている[16]．一方，海外ではJacksonらが過去に肺炎球菌ワクチン接種歴のない901名，少なくとも5年前に肺炎球菌ワクチン接種歴のある513名の50歳から74歳までの成人を対象として，接種前の血清中特異IgG濃度とワクチン接種後の副反応について検討している[3]．彼らは，接種2日以内の接種部位の大きな局所反応（10.2cm以上）の頻度は，初回接種群（3%）より再接種群（11%）において有意に多く，また接種前の血中特異抗体濃度と接種部位の局所反応の頻度が相関することを報告している．この接種部位の局所反応については，局所の免疫複合体形成に基づくアルサス型反応が原因と考えられている[3][17]．また，これらの再接種に伴う局所反応は平均3日で軽快したとされる．

第二世代のELISA法を用いた高齢者に対する再接種による免疫原性の検討では，いずれも接種前の血清中特異IgG濃度（6〜7種類の血清型）は再接種後に有意に増加することが報告されている[5][6]．しかしながら，Torlingらは再接種4週後の特異抗体IgG濃度のピーク（7.47μg/ml）は初回接種4週後のピーク（19.06μg/ml）と比較して有意に低値であったとしている[5]．このよう

に，T 細胞非依存性抗原の特性から，肺炎球菌ワクチンの再接種により，初回接種時と同等程度の特異抗体産生誘導は認められるが，そのブースター効果は期待できない．

本論文はアンケートによる再接種の実態調査結果であるため，その科学的証拠としては限界がある．しかしながら，本アンケート調査研究から，1）調査対象者の大半は再接種の必要性を認識し，その一部は患者側の再接種の要望に応じていること，2）調査対象者の約半数は再接種が禁忌であるが故に，その初回接種を控える経験をしていること，3）再接種の実施経験者 46 名からの報告では，再接種に伴う重篤な副反応は認められていないことが明らかになった．今回の調査結果と国外における本ワクチンの再接種の安全性と免疫原性の成績から，我が国における高齢者に対しても再接種が早期に承認されるべきである．

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Abstract

A questionnaire study on the necessity of approval for revaccination of the pneumococcal polysaccharide vaccine

Kazunori Oishi[1], Kazuyoshi Kawakami[2], Hideaki Nagai[3], Keisuke Sunakawa[4] and Akira Watanabe[5]

[1]International Research Center for Infectious Diseases, Research Institute for Microbial Diseases, Osaka University

[2]Department of Medical Microbiology, Mycology and Immunology, Tohoku University Graduate School of Medicine

[3]Department of Respirology, National Hospital Organization Tokyo Hospital

[4]Graduate School of Infection Control Sciences, Kitasato University, Laboratory of Infectious Diseases

[5]Research Division for Development of Anti-Infective Agents, Institute of Development, Aging and Cancer, Tohoku University

To clarify the current situation of revaccination with pneumococcal polysaccharide vaccine (PPV) and the adverse effects caused by revaccination with PPV in the elderly in Japan, a questionnaire study was carried out among the 989 members of the directors and councillors of the Japanese Respirology Society and the Japanese Association for Infectious Diseases. Of 385 evaluable respondents, 290 who had had experience giving PPV immunization were regarded as the study subjects. Of whom 46 subjects (15.9%) had had experience of PPV revaccination. However, 252 subjects (86.9%) recognized that PPV revaccination is necessary. In addition, of the 290 subjects, 114 subjects (49.7%) had experienced a patient refusing the first vaccination with PPV because of contraindications for PPV revaccination. Of 46 subjects with experience of PPV vaccination, 4 subjects found adverse effects in the recipients of PPV revaccination. The adverse effects found were not serious. The present study demonstrated that most of the study subjects recognized the necessity of PPV revaccination, and in part, those subjects implementing PPV revaccination were responding to requests by patients or their family. It was also suggested that the contraindication for PPV revaccination could prevent the increase of the coverage rate of PPV.

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Immunogenicity and safety of a novel AS03$_A$-adjuvanted H1N1 2009 pandemic influenza vaccine in adults in Japan

Hideyuki Ikematsu,[1,*] Hideaki Nagai,[2] Masahiro Kawashima,[2] Yasunobu Kawakami,[1] Kazuyoshi Tenjinbaru,[3] Atsushi Maeda,[3] Ping Li,[4] Paul Gillard[5] and François Roman[5]

[1]Hara-doi Hospital; Higashi-ku, Fukuoka Japan; [2]National Hospital Organization Tokyo National Hospital; Kiyose-city, Tokyo Japan; [3]Clinical Development Vaccines; GlaxoSmithKline (GSK) Japan; [4]Global Clinical Research & Development; GSK Biologicals; Philadelphia, PA USA; [5]Global Clinical Research and Development; GSK Biologicals; Wavre, Belgium

Key words: H1N1, pandemic, influenza, Japan, adjuvant, AS03, immunogenicity, adults

Abbreviations: ATP, according to protocol; CBER, Center for Biologics Evaluation and Research, US Food and Drug Administration; CHMP, Committee for Medicinal Products for Human Use, European Medicines Agency; CI, confidence interval; HA, hemagglutinin; HI, hemagglutination inhibition; IRB, Institutional Review Board; MHWL, Ministry of Health, Labor and Welfare; SAGE, Strategic Advisory Group of Experts; SD, standard deviation; TVC, total vaccinated cohort; WHO, World Health Organization

Background and objectives: This study evaluated the immunogenicity and safety of a novel H1N1 2009 pandemic vaccine (A/California/7/2009) in Japanese adults.

Results: Following Dose 1, seroprotection rate (HI titre ≥1:40) was 95%, seroconversion rate was 94% and the geometric mean titre (GMT) was 230.3 (geometric mean fold rise [GMFR]: 26.3). Following Dose 2, seroprotection rate as well as seroconversion rate were 100%; HI antibody GMT rose to 485 (GMFR: 55.4). European and United States regulatory acceptance criteria for immunogenicity were met and exceeded following each dose of the vaccine. Solicited symptoms recorded during the 7-day post-vaccination follow-up period were of mild to moderate intensity (Grade 3: ≤4% of subjects). The most frequently reported solicited symptoms after both doses were pain at the injection site, fatigue and muscle ache. Unsolicited adverse events causally related to vaccination were reported in 18 subjects; none were of Grade 3 intensity. There were no serious adverse events.

Methods: This open-label, single-group, multi-center Phase II study enrolled 100 healthy subjects aged 20–64 years (stratification [1:1] by age: 20–40 years and 41–64 years) to receive 21 days apart, two doses of a monovalent, split-virion AS03$_A$-adjuvanted H1N1 2009 pandemic vaccine (3.75 μg hemagglutinin [HA]). Blood samples collected before vaccination and 21 days after each dose were analyzed using hemagglutination inhibition (HI) assay.

Conclusion: A single dose of AS03$_A$-adjuvanted, 3.75 μg HA H1N1 2009 pandemic influenza vaccine was highly immunogenic in Japanese adults with a clinically acceptable safety profile, thereby making it a potential candidate for mitigating A/H1N1-associated morbidity and mortality.

Introduction

A newly emergent influenza H1N1 2009 pandemic virus was first isolated in Mexico and the United States in March-April 2009.[1,2] Since then the virus has spread throughout the world and continues to cause human infections.[3] Consequently, the World Health Organization (WHO) officially raised the Pandemic Alert to Phase 6 on June 11, 2009.[4] As of May 30, 2010, more than 214 countries had reported more than 18,138 deaths.[5] Almost the entire population less than 60 years has been found susceptible to the H1N1 2009 virus.[6]

Mass vaccination can play a decisive role in limiting the spread of an influenza virus and minimize associated morbidity and mortality. The H1N1 2009 virus causing the current influenza pandemic is a triple-reassortant recombinant that originates from swine, avian influenza A and human A/H3N2 viruses. Reports indicate that it is highly unlikely that the existing seasonal influenza vaccines will confer protection against this strain.[7,8] Hence, the development of a new immunogenic vaccine with an acceptable safety profile has been a priority for vaccine manufacturers and public health authorities worldwide.

*Correspondence to: Hideyuki Ikematsu; Email: ikematsu@gray.plala.or.jp
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Table 1. Immune response in all subjects: Haemagglutination-inhibition antibodies against the A/California/7/2009 (H1N1) strain, stratified by age (ATP cohort for immunogenicity)

		Seropositivity rates			Geometric mean titres	Seroconversion rates			Geometric mean fold rise		Seroprotection rates		
Age group	Time point	N	n	% (95% CI)	Value (95% CI)	N	n	% (95% CI)	N	Value (95% CI)	N	n	% (95% CI)
All	Day 0	100	43	43 (31.9–54.7)	8.8 (7.3–10.5)	-	-	-	-	-	100	6	6 (1.9–13.6)
	Day 21	100	100	100 (95.7–100)	230.3 (177.7–298.4)	100	94	94 (86.4–98.1)	100	26.3 (20.6–33.5)	100	95	95 (87.7–98.6)
	Day 42	100	100	100 (95.7–100)	485 (420.3–559.7)	100	100	100 (95.7–100)	100	55.4 (45.6–67.2)	100	100	100 (95.7–100)
20–40 years	Day 0	50	22	44 (30–58.7)	8.9 (7.1–11.1)	-	-	-	-	-	50	3	6 (1.3–16.5)
	Day 21	50	50	100 (92.9–100)	258.3 (191.3–348.7)	50	50	100 (92.9–100)	50	29.1 (21.8–38.9)	50	50	100 (92.9–100)
	Day 42	50	50	100 (92.9–100)	505.6 (427.9–597.6)	50	50	100 (92.9–100)	50	57 (44.3–73.2)	50	50	100 (92.9–100)
41–64 years	Day 0	50	21	42 (28.2–56.8)	8.6 (6.8–10.9)	-	-	-	-	-	50	3	6 (1.3–16.5)
	Day 21	50	50	100 (92.9–100)	205.3 (145.4–290)	50	44	88 (75.7–95.5)	50	23.8 (17.4–32.6)	50	45	90 (78.2–96.7)
	Day 42	50	50	100 (92.9–100)	465.3 (384.5–563)	50	50	100 (92.9–100)	50	53.9 (42.6–68.1)	50	50	100 (92.9–100)

Previous experience with traditional seasonal influenza vaccines has demonstrated that a single dose is sufficient to induce strong immune responses in the majority of adult vaccine recipients. In contrast, two doses of non-adjuvanted H5N1 pandemic influenza vaccines with hemagglutinin (HA) contents as high as 30 μg and 90 μg conferred seroprotection rates of 67% and 58%, respectively, in an adult population that was largely naïve to the new strain.[9,10] The addition of oil-in-water emulsion-based adjuvants to prepandemic influenza vaccines has facilitated the lowering of the required antigen dose without changing the immunogenicity and safety profile of these vaccines, in addition to inducing immunogenicity against antigenically divergent strains.[10,11]

GSK Biologicals has developed a comprehensive clinical development plan to evaluate the immunogenicity and safety of its pandemic H1N1 2009 vaccine adjuvanted with $AS03_A$ (a tocopherol [11.86 mg] oil-in-water emulsion based Adjuvant System) building on preceding development of an AS03-adjuvanted H5N1 prepandemic vaccine.[12,13]

This study was conducted in a healthy adult population in Japan to determine if the immune response induced by a single dose or two doses of an $AS03_A$-adjuvanted H1N1 2009 pandemic influenza vaccine (A/California/7/2009) administered 21 days apart, could meet or exceed the immunogenicity guidance criteria in adults set by the European and the United States regulatory authorities. The study also evaluated the safety and reactogenicity profile of the vaccine in this population.

Results

Demography. The study was initiated in October 2009. A total of 100 subjects were enrolled and vaccinated. The mean age of subjects was 39.3 (standard deviation [SD]: 11.65) years and 64% were females; all subjects were of Japanese heritage. The subjects were equally distributed (50/50) into the two age strata. Both cohorts for evaluation, i.e., the According To Protocol (ATP) cohort for immunogenicity and the Total Vaccinated (intention-to-treat) Cohort (TVC) for safety included 100 subjects.

Immune response. Immunogenicity data for the ATP cohort for all subjects and stratified by age (20–40 years and 41–64 years) are presented in Table 1.

Before vaccination, 43% of subjects [20–40 years: 44%; 41–64 years: 42%] were seropositive for the A/California/7/2009 H1N1 pandemic strain; however, seroprotection rate was only 6% (HI titers ≥1:40). The HI antibody geometric mean titers (GMTs) before vaccination were low (<9) in both age strata.

The first dose of the H1N1 2009 vaccine induced an immune response against the vaccine strain that exceeded the Center for Biologics Evaluation and Research (CBER) and the Committee for Medicinal Products for Human Use (CHMP) immunogenicity guidance criteria in adults for assessment of pandemic influenza vaccines. Overall, seroprotection rate was 95%, while seroconversion rate was 94% [20–40 years: 100%; 41–64 years: 88%] against the A/California/7/2009 H1N1 strain. The HI antibody GMTs increased substantially to 230.3 [20–40 years: 258.3; 41–64 years: 205.3] with a corresponding geometric mean fold rise (GMFR) of 26.3 [20–40 years: 29.1; 41–64 years: 23.8].

Following the second dose, the immune response against the vaccine strain further increased; seroprotection rate was 100%; seroconversion rate after the second dose was 100% (in both age strata). The HI antibody GMT rose to 485 [20–40 years: 505.6; 41–64 years: 465.3], with a corresponding GMFR of 55.4 [20–40 years: 57; 41–64 years: 53.9]. The two immunogenicity guidance criteria set by the CBER and the three immunogenicity criteria set by CHMP (in adults) were met and exceeded following administration of the first and second dose of the H1N1 2009 pandemic vaccine.

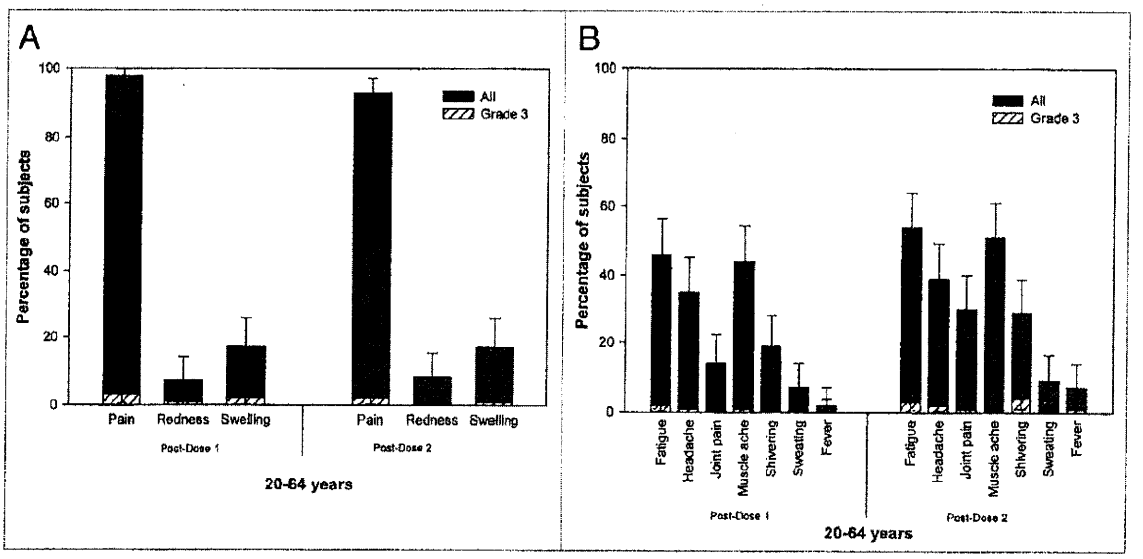

Figure 1. (A) Solicited local symptoms reported during the 7-day post-vaccination follow-up period (Total vaccinated cohort). (B) Solicited general symptoms reported during the 7-day post-vaccination follow-up period (Total vaccinated cohort).

It was observed that subjects who were seropositive before vaccination mounted a stronger immune response (higher GMT in these subjects post-Dose 1: 414.3) compared to the subjects who were seronegative before vaccination (post-Dose 1 GMT: 147.9). Following the second dose, the immune responses mounted by the initially seropositive and seronegative subjects were comparable (post-Dose 2 GMTs: 619.7 and 403.2, respectively), with a steeper increase observed in the seronegative subjects (post-Dose 2 GMFR: 80.6) than in seropositive subjects (post-Dose 2 GMFR: 33.7).

Safety and reactogenicity. The safety data for the H1N1 2009 pandemic vaccine are presented in **Figure 1A and B**. Overall, 85% of subjects reported at least one general adverse event, while 99% of subjects reported at least one local adverse event. Of these, ≤9% were of Grade 3 intensity.

Following the first vaccine dose, pain at the injection site was the most frequently reported solicited local symptom in both age strata (98%) with a median duration of four days; other solicited local symptoms persisted for a median duration of three days. Solicited local symptoms of Grade 3 intensity were reported in ≤3% of subjects. Reporting of solicited local symptoms was similar in the two age strata. The most frequently reported solicited general symptoms after the first vaccine dose were fatigue (46% with a median duration of two days; i.e., the day of vaccination and the following day) and muscle aches (44% with a median duration of three days); other solicited general symptoms persisted for a median duration of one to two days. The majority of these general symptoms were considered by the investigators to be causally related to vaccination. Solicited general symptoms of Grade 3 intensity were reported in ≤2% of subjects, all of which were considered to be related to vaccination by the investigator. A comparatively higher trend in the reporting of solicited general symptoms following Dose 1 was observed in subjects aged 20–40 years compared to subjects aged 41–64 years, especially for fatigue, headache and shivering (observed differences in incidences of symptoms were 24%, 18%, 18%, respectively).

The reporting of solicited local symptoms following the second dose of vaccination was comparable to the reporting following the first dose; pain at the injection site (93%; median duration of three days) remained the most frequently reported solicited local symptom (other solicited local symptoms persisted for a median duration of three days); Grade 3 local symptoms were recorded in ≤2% of subjects. Fatigue (54%; median duration of two days) and muscle aches (51%; median duration of three days) remained the most frequently reported solicited general symptoms; other solicited general symptoms persisted for a median duration of one to two days. Overall, Grade 3 solicited general symptoms were reported by ≤4% of subjects. There was a trend for a slight increase in solicited general symptoms after Dose 2 compared to Dose 1, in particular for joint pain, shivering and fever (however, 95% confidence intervals [CIs] overlapped). In addition, no difference was observed in the duration of solicited general symptoms following the first and second vaccine dose.

Thirty-five subjects reported at least one unsolicited adverse event. Of these, 18 were considered by the investigator to be causally related to vaccination. However, no specific clinical patterns for the occurrence of these symptoms could be identified. There were no Grade 3 unsolicited adverse events up to Day 42. None of the adverse events were of Grade 3 intensity. No marked alterations of the bio-safety laboratory parameters were observed following either vaccination.

No serious adverse events were reported up to 21 days following the second vaccine dose (Day 42). Overall, the vaccine was found to be generally well-tolerated with a clinically acceptable safety profile.

Discussion

By the end of January 2010, the estimated cumulative number of H1N1 cases in Japan was 20 million (estimated based on clinical symptoms).[14] However, the corresponding mortality rate (deaths per million) was low;[15] as of December 2009, the Ministry of Health, Labor and Welfare (MHWL) of Japan confirmed 85 deaths due to the pandemic influenza (H1N1 2009) virus in Japan.[16,17] The number of deaths is possibly to be higher as only a proportion of the suspected H1N1 deaths had laboratory confirmation. In contrast to trends observed in the United States (US), where the majority of H1N1 2009 infections and hospitalizations were reported in those aged between 18 and 64 years,[18] in Japan, most of the infections and hospitalizations were reported in children, adolescents and young adults (5–19 years);[16] H1N1 2009-related deaths in Japan were reported mostly in patients aged >20 years, a trend which was also observed in the US and Canada, where the median age for H1N1 2009-related deaths was 37 years.[19] It was also observed that the mortality rates in Japan increased with advancing age in adults, with the highest mortality rates reported in the elderly.[16]

A number of studies have been conducted worldwide using adjuvanted or non-adjuvanted vaccine formulations against H1N1 2009. Two previous studies, one conducted in adults and the elderly in the United Kingdom using a MF59-adjuvanted 7.5 μg HA H1N1 2009 vaccine[20] and another conducted in a similar age group in Australia using a non-adjuvanted 15 μg HA H1N1 2009 vaccine[21] demonstrated that a single dose of these vaccines could induce immune responses that could meet the immunogenicity guidance criteria in adults set by the US and European regulatory authorities (Day 21 seroprotection rate: 80% and 96.7%, respectively; seroconversion rate: 76% and 70.8%, respectively; GMT: 172.5 and 217.1, respectively). Two studies with a non-adjuvanted H1N1 2009 pandemic vaccine (7.5 μg–30 μg HA formulations), one in American children, adults and the elderly (Day 21 seroprotection rate: 95%/94%; seroconversion rate: 92%/83%; GMT: 747/297)[22] and another with a non-adjuvanted formulation in Chinese children, adults and the elderly (Day 21 seroprotection rate: 76.7%/89.5%/80.3%; GMT: 78.6/316.6/105.7)[23] demonstrated that the 7.5 μg HA formulation met the immunogenicity guidance criteria in adults and the elderly set by the CHMP. A study in Hungarian subjects aged 18–60 years and above using a 6 μg HA H1N1 2009 pandemic vaccine adjuvanted with aluminium phosphate adjuvant,[24] also demonstrated that a single dose can induce strong immune responses sufficient to meet the regulatory guidance criteria in adults for pandemic influenza vaccines. Other experiences with AS03-adjuvanted formulations of GSK Biologicals' H1N1 2009 pandemic vaccine has also demonstrated that a single dose of the vaccine induced immune responses in children and adults that met the CHMP and CBER immunogenicity guidance criteria for adults.[25-27]

This is the first study to evaluate the immunogenicity, safety and reactogenicity of GSK Biologicals' $AS03_A$-adjuvanted H1N1 2009 pandemic influenza vaccine in an Asian population. As observed, the immune response induced by the first dose of the $AS03_A$-adjuvanted H1N1 2009 pandemic influenza vaccine with a low antigen content (3.75 μg HA) has met and exceeded US and European regulatory acceptance criteria. The subjects in both age strata, i.e., 20–40 years and 41–64 years mounted strong immune responses against the H1N1 2009 pandemic vaccine. Despite the absence of a non-adjuvanted comparator group, the immune response induced by the H1N1 2009 vaccine is substantiated as HI antibody titres ≥1:40 are generally considered as surrogates of protection. A single dose containing 3.75 μg HA of the $AS03_A$-adjuvanted H1N1 2009 vaccine was highly immunogenic and of comparable magnitude as the immune response induced by other adjuvanted or non-adjuvanted candidate pandemic influenza vaccines with higher antigen doses.[20-23]

The data obtained from this Japanese study population are in agreement with those obtained from studies conducted using a similar H1N1 2009 vaccine in other populations.[28] The immune response mounted against the study vaccine and the reactogenicity and safety profiles in the Japanese population in this study were comparable to those observed in European adults.[28] The baseline seropositivity rate (HI GMT ≥1:10) observed in the European study was found to be high in the study participants (44%). However, the corresponding baseline seroprotection rate was low (6%) and in line with those observed in previous studies in the UK and China (baseline seroprotection rates of 4–12% and 4%, respectively),[23,24] but was lower than those observed in the US and Australian population (20–31% and up to 32%, respectively).[20,21] These results may be indicative of possible pre-existing immunity in the study population against the A/California/7/2009 strain. Exposure to H1N1 strains with similar epitopes which were in circulation before the H1N1 2009 pandemic or asymptomatic infections (considering that subjects with a history of exposure to the H1N1 2009 strain were excluded from this study) with the H1N1 2009 pandemic influenza strain in circulation at the time of this study might have contributed to this pre-existing immunity, illustrated by these relatively high baseline seropositivity rates.

Data from H5N1 studies suggest that vaccines adjuvanted with AS03 induce a persistent immune response against the vaccine strain as well as against antigenically drifted strains.[29] However, the persistence of the immune response elicited by the H1N1 vaccine adjuvanted with AS03 requires further investigation.

In conclusion, a single dose of the $AS03_A$-adjuvanted H1N1 2009 pandemic influenza vaccine with a low antigen requirement (3.75 μg HA) was highly immunogenic and generally well-tolerated in the adult population in Japan. The vaccine provides the opportunity for immunization against the H1N1 2009 pandemic influenza strain and may guide the development of vaccines to mitigate subsequent influenza pandemics.

Materials and Methods

Study vaccine. The monovalent, inactivated, split-virion H1N1 2009 vaccine (Arepanrix™ a trade mark of the GlaxoSmithKline group of companies) was manufactured by GlaxoSmithKline Biologicals, Quebec, Canada. The vaccine seed H1N1 virus was prepared from the re-assortant virus NYMC X-179A (New York Medical College, New York) generated from the

A/California/7/2009 strain based on the recommendations of the World Health Organization[30] and propagated in embryonated eggs. The $AS03_A$ used in this vaccine is an oil-in-water emulsion based Adjuvant System containing 11.86 mg of α-tocopherol.

The antigen suspension and adjuvant emulsion were presented in separate multi-dose vials; the vaccine had to be reconstituted by mixing the two components prior to administration. Each 0.5 ml vaccine dose contained 3.75 μg HA and was administered as an intramuscular injection in the deltoid of the arm.

Study design and participants. This was an open-label, single-group, multi-center (NCT00989612) Phase II study conducted at two centers in Japan. Healthy subjects aged 20–64 years at the time of the first vaccine dose were enrolled to receive 21-days apart, two doses of a monovalent pandemic influenza vaccine containing 3.75 μg HA of A/California/7/2009 H1N1-like NYMC X-179A strain, adjuvanted with $AS03_A$. Subjects were stratified into two age strata: 20–40 years and 41–64 years (allocation ratio: 1:1). Volunteers were excluded if they had received any investigational product within 30 days preceeding study start, any seasonal influenza vaccination within 14 days preceeding study start or any novel H1N1 2009 vaccine. Volunteers were also excluded if they had a history of confirmed or suspected immunosuppressive or immunodeficient condition, were under chronic administration of immunosuppressants or immune modifying drugs within six months of enrolment into the study or received any immunoglobulins and/or any blood products within three months of enrolment into the study or were suspected to be allergic to any constituent of influenza vaccines or component used in the manufacturing process of the study vaccine.

Written informed consent was obtained from all subjects prior to conducting any study-related procedures. The study was conducted in accordance with Good Clinical Practice guidelines, the Declaration of Helsinki and relevant local regulatory laws. All study related documents and procedures were approved by the appropriate Institutional review boards (IRBs).

Serological assessments. Blood samples collected before vaccination (Day 0), post-Dose 1 (Day 21) and post-Dose 2 (Day 42) were analyzed at GSK Biologicals Central laboratory, Dresden, Germany, using a validated in-house microtitre Hemagglutination Inhibition (HI) assay [cut-off: ≥1:10], with chicken erythrocytes as previously described.[31] The A/California/7/2009 vaccine strain was used as the antigen strain.

Safety and reactogenicity assessments. The subjects used diary cards to record the occurrence and intensity of solicited local symptoms (pain, redness and swelling) and solicited general symptoms (fatigue, headache, joint pain, muscle ache, shivering, sweating and fever) during the 7-day post-vaccination follow-up period after each dose. The intensity of all solicited symptoms (mild, moderate or severe) was graded on a standard three-point scale [0–3] except fever, which was graded on a four-point scale [0–4]. Grade 3 redness, swelling and induration were defined as those with a diameter of >100 mm; Grade 3 fever was defined as axillary temperatures ≥39°C, while Grade 4 fever was defined as axillary temperatures ≥40°C. Grade 3 fatigue, headache, joint pain, muscle ache, shivering and sweating were defined as symptoms that hindered normal daily activities. The occurrence of unsolicited adverse events occurring during the 21 day post-vaccination follow-up period was also recorded. Serious adverse events were recorded for the entire study period. An assessment of causality was done by the investigators for all reported adverse events. Data from the safety laboratory and urine sampling safety laboratory were tabulated over time.

Statistical analyses. The analyses for the primary immunogenicity objective was based on the ATP cohort and the analysis of safety was planned on the TVC. The immunogenicity assessments were based on the surrogate HI endpoints as required by regulatory authorities for evaluation of pandemic influenza vaccines,[32,33] and evaluated by seroconversion rate (percentage of subjects with a pre-vaccination titre <1:10 and a post-vaccination titre ≥1:40 or a pre-vaccination titre >1:10 and a four-fold increase in post-vaccination titre), seroprotection rate (a post-vaccination titre ≥1:40) and GMFR (post-vaccination fold increase in GMTs) and the associated 97.5% CIs. The occurrence of solicited and unsolicited adverse events was evaluated by the percentage of subjects with at least one solicited or unsolicited adverse event along with the 95% CIs.

The sample size was calculated taking into consideration the objective to meet and exceed the CBER and CHMP immunogenicity guidance criteria in adults for HI seroprotection rates, seroconversion rates and geometric mean fold rise, following the first or second dose of vaccination.[32,33] The results of the most recent H1N1 2009 vaccine studies conducted by GSK Biologicals, were used as references for power calculation. The study planned to enrol 100 subjects (age stratification 1:1 for 20–40 and 41–64 years) to give an estimated power of >99.68% after the first dose and >99.99%, after the second dose to meet the primary objective, assuming 90% and 95% to be the reference values for the seroconversion and seroprotection rates, respectively.

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GlaxoSmithKline Biologicals was the funding source and was involved in all stages of the study conduct and analysis (ClinicalTrials.gov Identifier: NCT00989612). GlaxoSmithKline Biologicals also took in charge all costs associated with the development and the publishing of the present manuscript. All authors had full access to the data and had final responsibility to submit for publication.

Conflict of Interest

Hideyuki Ikematsu discloses having received honoraria/paid expert testimony and travel grants from the commercial entity that sponsored the study. Hideaki Nagai discloses having received honoraria/paid expert testimony from the commercial entity that sponsored the study. Masahiro Kawashima discloses having no conflict of interest. Yasunobu Kawakami discloses having no conflict of interest. Kazuyoshi Tenjinbaru, Atsushi Maeda, Ping Li, Paul Gillard and François Roman are employees of GlaxoSmithKline Biologicals. Paul Gillard and François Roman report ownership of GSK stock options.

Trademark Statement

Arepanrix is a trademark of the GlaxoSmithKline group of companies.

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Nagai et al. BMC Infectious Diseases 2010, 10:338
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A phase II, open-label, multicentre study to evaluate the immunogenicity and safety of an adjuvanted prepandemic (H5N1) influenza vaccine in healthy Japanese adults

Hideaki Nagai[1*], Hideyuki Ikematsu[2], Kazuyoshi Tenjinbaru[3], Atsushi Maeda[3], Mamadou Dramé[4], François P Roman[4]

Abstract

Background: Promising clinical data and significant antigen-sparing have been demonstrated for a pandemic H5N1 influenza split-virion vaccine adjuvanted with $AS03_A$, an α-tocopherol-containing oil-in-water emulsion-based Adjuvant System. Although studies using this formulation have been reported, there have been no data for Japanese populations. This study therefore aimed to assess the immunogenicity and tolerability of a prepandemic (H5N1) influenza vaccine adjuvanted with $AS03_A$ in Japanese adults.

Methods: This open-label, single-group study was conducted at two centres in Japan in healthy Japanese males and females aged 20-64 years (n = 100). Subjects received two doses of vaccine, containing 3.75 μg haemagglutinin of the A/Indonesia/5/2005-like IBCDC-RG2 Clade 2.1 (H5N1) strain adjuvanted with $AS03_A$, 21 days apart. The primary endpoint evaluated the humoral immune response in terms of H5N1 haemagglutination inhibition (HI) antibody titres against the vaccine strain (Clade 2.1) 21 days after the second dose. Ninety five percent confidence intervals for geometric mean titres, seroprotection, seroconversion and seropositivity rates were calculated. Secondary and exploratory endpoints included the assessment of the humoral response in terms of neutralising antibody titres, the response against additional H5N1 strains (Clade 1 and Clade 2.2), as well as the evaluation of safety and reactogenicity.

Results: Robust immune responses were elicited after two doses of the prepandemic influenza vaccine adjuvanted with $AS03_A$. Overall, vaccine HI seroconversion rates and seroprotection rates were 91% 21 days after the second vaccination. This fulfilled all regulatory acceptance criteria for the vaccine-homologous HI antibody level. A substantial cross-reactive humoral immune response was also observed against the virus strains A/turkey/Turkey/1/2005 (Clade 2.2) and A/Vietnam/1194/2004 (Clade 1) after the second vaccine administration. A marked post-vaccination response in terms of neutralising antibody titres was demonstrated and persistence of the immune response was observed 6 months after the first dose. The vaccine was generally well tolerated and there were no serious adverse events reported.

Conclusions: The H5N1 candidate vaccine adjuvanted with $AS03_A$ elicited a strong and persistent immune response against the vaccine strain A/Indonesia/5/2005 in Japanese adults. Vaccination with this formulation demonstrated a clinically acceptable reactogenicity profile and did not raise any safety concerns in this population.

Trial registration: Clinicaltrials.gov NCT00742885

* Correspondence: hnagai-in@tokyo-hosp.jp
[1]National Hospital Organization Tokyo National Hospital, 3-1-1, Takeoka, Kiyose-city, Tokyo 204-8585, Japan
Full list of author information is available at the end of the article

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Background

The highly pathogenic influenza A H5N1 virus first emerged as a cause of death in poultry in 1996 and was identified in humans in 1997; 18 individuals in Hong Kong became severely ill, with six deaths reported, following contact with infected birds [1]. The H5N1 virus reappeared in 2003 and has since caused 295 deaths from 499 confirmed cases worldwide (World Health Organization [WHO] as of 08 June 2010) [2].

The WHO declared a pandemic alert stage 6 due to an outbreak of an influenza A virus (A/H1N1) on 11 June 2009. As of 13 June 2010, more than 214 countries have reported a total of at least 18,172 deaths [3]. However, the highly pathogenic H5N1 strain is also a potential pandemic virus and, therefore, it remains of great concern. The H5N1 virus currently meets two of the three criteria for a global pandemic strain: H5 is a haemagglutinin (HA) subtype against which most of the human population is virtually naïve, and the virus is able to replicate in humans causing severe disease and death [4]. To date, the virus has not acquired the ability for large-scale human-to-human transmission - although isolated cases have occurred [5,6].

Vaccination is a vital part of the strategy to mitigate morbidity and mortality caused by influenza pandemics [7] and is integral to the WHO global influenza preparedness plan [8]. Pandemic vaccines are produced as soon as a pandemic is declared using the specific pandemic viral strain. However, these vaccines will only be available several months after the onset of the pandemic due to the length of time required for their manufacture [8].

The efficacy of prepandemic vaccines, which are produced in advance of a pandemic, relies on the vaccine's ability to provide a breadth of protection against different, related strains, as it is not possible to predict exactly the strain that will cause such an outbreak in advance due to the progressive accumulation of antigenic changes.

Promising clinical data have been generated for a prepandemic split-virion influenza vaccine formulated with an α-tocopherol containing, oil-in-water (O/W) emulsion-based Adjuvant System, AS03. This vaccine has demonstrated a good safety profile in randomised clinical trials in a range of human populations [9-11]. AS03 adjuvantation of the H5N1 vaccine allows for a reduction in the amount of antigen required per dose in order to induce potentially protective immune responses in humans, and it can also induce strong cross-strain and cross-clade immunity as is required for an effective prepandemic vaccine [9,10,12,13]. The A/Vietnam/1194/2004 H5N1 strain was identified as having the potential to cause a human pandemic and was thus used in several AS03 candidate vaccine studies, leading to the initial approval of a prepandemic H5N1 vaccine (*Prepandrix*™ GSK Biologicals, Rixensart, Belgium) [9,10,12-14]. This vaccine has also been shown to protect against lethal heterologous challenge in an animal model [15]. A new emerging H5N1 strain was identified by the WHO sentinel laboratory (A/Indonesia/5/2005) in 2005 [16], which was subsequently recommended by the WHO for use in vaccines, and has also been employed as part of a potential prepandemic vaccine.

In January 2004, there were confirmed outbreaks of H5N1 infection in Japanese poultry, which led to increasing concern regarding the ability of the virus to infect humans. Following the development of a whole-virus, aluminium-adjuvanted H5N1 vaccine in 2007, the Japanese Ministry of Health, Labour and Welfare began domestic manufacture of this vaccine for stockpiling [17]. However, the immune response elicited by an aluminium-adjuvanted H5N1 vaccine indicated insufficient immunogenicity [18]. Clinical data on the use of alternative vaccines, such as the AS03-adjuvanted prepandemic H5N1 vaccine, in the Japanese population would therefore be of interest.

This open-label, single-arm study set out to evaluate the humoral immune response and safety of two doses of $AS03_A$-adjuvanted A/Indonesia/5/2005 H5N1 vaccine and determine its putative clinical value as a prepandemic vaccine in healthy Japanese adults.

Methods

Vaccine

The A/H5N1 monovalent split-virion recombinant influenza prepandemic candidate vaccine was manufactured by GlaxoSmithKline (GSK) Biologicals in Quebec, Canada. The vaccine contained 3.75 μg HA of the A/Indonesia/5/2005-like IBCDC-RG2; Clade 2.1 (H5N1) strain (Centers for Disease Control and Prevention [CDC], Atlanta, USA) adjuvanted with $AS03_A$ (an O/W emulsion-based Adjuvant System containing 11.86 mg of α-tocopherol).

Study design

This open-label, single-group study (NCT00742885) was conducted at two centres in Japan. The study set out to evaluate the humoral immune response generated by two doses of the adjuvanted prepandemic A/Indonesia/5/2005 H5N1 vaccine in terms of H5N1 haemagglutination inhibition (HI) antibody titres against the vaccine strain (Clade 2.1) 21 days after the second dose. Secondary and exploratory endpoints included the assessment of the humoral response in terms of neutralising antibody titres, the response against additional H5N1 strains (Clade 1 and Clade 2.2), as well as the evaluation of safety and reactogenicity. The latter were assessed in

terms of the occurrence of solicited local and general adverse events (AEs), unsolicited AEs, serious AEs (SAEs) and by the evaluation of medically attended visits and selected laboratory parameters.

Healthy Japanese men and women aged between 20 and 64 years at the time of first vaccination were eligible for inclusion if they were in good general health and provided written informed consent before enrolment. Subjects were stratified by age (20-40 years and 41-64 years) in a 1:1 ratio. Subjects were excluded from the study if they had an axillary temperature ≥37.5°C, or acute symptoms of more than mild severity on the scheduled date of first vaccination; any confirmed or suspected immunosuppressive or immunodeficient condition including history of human immunodeficiency virus (HIV) infection; administration of any registered vaccine within 30 days before study vaccination or planned administration within the first vaccination period up to blood sampling at Day 42; use of any investigational or non-registered product (drug or vaccine) within 30 days prior to study enrolment or planned use during the study period, and history of previous H5N1 vaccination; or history of H5N1 influenza infection.

Subjects received two doses of the prepandemic (H5N1) influenza candidate vaccine. The vaccine was administered intramuscularly in the deltoid region of the non-dominant arm on Day 0 and the second dose was given 21 days later in the non-dominant arm. Blood samples were collected for serological testing on Day 0, 7, 21, 42 and 182, and telephone contact was made on Day 84 to record any unsolicited AEs (Figure 1). Solicited AEs were assessed up to 7 days after each vaccination and, additionally, unsolicited AEs, including SAEs, were recorded throughout the duration of the study.

The protocol and study documents were approved by the Institutional Review Boards of the respective study centres - National Hospital Organization Tokyo National Hospital and Haradoi Hospital. The study was conducted in accordance with Good Clinical Practice (GCP) and the Declaration of Helsinki. GSK Biologicals (Wavre, Belgium) sponsored the study and was involved in all stages of the study conduct, including analysis of data. All authors had full access to the data and were involved in the analysis of data and preparation of the manuscript.

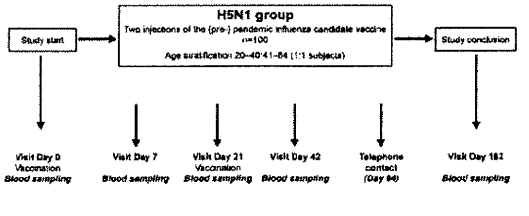

Figure 1 Trial profile.

Assessment of immunogenicity

The humoral immune response against the vaccine strain (A/Indonesia/5/2005; Clade 2.1), as well as against the heterologous strains (A/turkey/Turkey/1/2005; Clade 2.2 and A/Vietnam/1194/2004; Clade 1) was measured in terms of the standard HI antibody response according to the guidelines of the Committee for Medicinal Products for Human Use (CHMP) [19]. In addition, neutralising antibodies against the vaccine (A/Indonesia/5/2005) and one heterologous strain (A/Vietnam/1194/2004) were measured by the microneutralisation (MN) assay and are further referred to as H5N1 neutralising antibodies. Studies have shown that neutralisation assays may be more sensitive than the HI test in detecting both greater increases in antibody levels and in detecting infected individuals who are seronegative according to the HI assay [20].

Specific HI antibody titres were determined at GSK Biologicals' laboratories using methods described elsewhere [21]. Antibody titre measurements were conducted on thawed frozen serum samples with a standardised and validated micromethod using four haemagglutination-inhibiting units (HIU) of the appropriate antigens and a 0.5% horse erythrocyte suspension. Non-specific serum inhibitors were removed by subjecting the sera to heat treatment (56°C) and receptor-destroying enzyme. The sera obtained were evaluated for HI antibody levels. Starting with an initial dilution of 1:10, a dilution series (by a factor of 2) was prepared up to an end dilution of 1:20,480. The titration endpoint was taken as the highest dilution step that showed complete inhibition (100%) of haemagglutination. All assays were performed in duplicate.

The titre of H5N1 virus neutralising antibody contained in the serum was determined by an MN assay on thawed frozen serum samples. Each sample was tested in triplicate. Non-specific serum inhibitors were removed by subjecting the sera to heat treatment (56°C). A standardised amount of virus (100 infectious Unit [TCID50] in 0.05 mL) was mixed with serial dilutions of sera and incubated to allow binding of the antibodies to the virus. A cell suspension, containing a defined number of Madin-Darby canine kidney (MDCK) cells was then added to the mixture of virus and antiserum and incubated at 33°C for 7 days. After the incubation period, virus replication was visualised by haemagglutination of chicken red blood cells. The 50% neutralisation titre of a serum was calculated by the method of Reed and Muench [22].

Assessment of safety

Adverse events were classified according to the Medical Dictionary for Regulatory Activities (MedDRA). The

occurrence of AEs was recorded by the subjects themselves using diary cards. In addition, investigators solicited information on specific local AEs (swelling/induration, redness and pain at injection site) and general AEs (fever, headache, fatigue, muscle aches, sweating, joint pain and shivering) occurring within 7 days of each vaccination. Symptom intensity was assessed on a 3-point scale where grade 3 represents the most intense. For both unsolicited AEs and solicited general AEs, the investigators determined the likely relationship of vaccination to symptoms. Intensity and relationship to vaccination of unsolicited local and general AEs were recorded during a 21-day follow-up period from each vaccine administration, as well as overall (Day 0 through to Day 84). All solicited local (injection site) reactions were considered causally related to vaccination. The occurrence of SAEs was recorded during the entire study (up to Day 182).

Haematological and biochemical parameter testing was performed by SRL Medisearch Inc, Japan. The number and percentage of subjects with normal or abnormal haematological and biochemical values, and with normal or abnormal urine values at Day 0, 7 and 42, were calculated. An assessment of these haematological, biochemical and urine parameters was performed at Day 7 and 21 - all parameters were reviewed at Day 7 by the investigators before administering the second vaccine dose at Day 21. Blood parameters assessed were complete blood count, blood urea nitrogen (BUN), creatinine, alanine amino transferase (ALT) and aspartate aminotransferase (AST). Urine parameters measured were protein, glucose, blood and urobilinogen.

Statistical analysis

The statistical methods for all immunogenicity analyses were performed using the per protocol group. The primary objective of this study was to evaluate the humoral immune response induced by two doses of the H5N1 influenza candidate vaccine in terms of H5N1 HI antibody titres against the vaccine strain. The immunogenicity assessments were based on the surrogate HI endpoints as required by regulatory authorities (CBER and CHMP). In order to meet or exceed these immunogenicity guidance criteria, a target sample size of 100 subjects was required in order to ensure 90 evaluable subjects. Taking into account a 10% drop-out rate and considering a true seroconversion rate (SCR for HI antibodies was defined as the percentage of subjects with either a pre-vaccination titre <1:10 and a post-vaccination titre ≥1:40 or a pre-vaccination titre ≥1:10 and at least a 4-fold increase in post-vaccination titre) of 83.7% and a true seroprotection rate (SPR; defined as the percentage of subjects with a serum H5N1 HI antibody titre ≥1:40) of 84.3%, the proposed sample size allowed for an overall probability of above 85% of meeting the lower limits of 95% confidence intervals (CIs) for SCRs and SPRs of 40% and 70%, respectively. Ninety five percent CIs were calculated for geometric mean titres (GMTs) by exponential transformation of the 95% CI for the mean of log-transformed titres, assuming normal distribution of log-transformed titres.

The immunogenicity analysis was performed for each age stratum and overall. The humoral immune response endpoints in terms of H5N1 HI antibodies were measured using the GMTs of H5N1 HI antibody titres at Day 0, 21, 42 and 182, SCR at Day 21, 42 and Day 182 and seroconversion factors (SCF; defined as the fold increase in serum H5N1 HI antibody GMTs post-vaccination compared with Day 0) at Day 21, 42 and Day 182. The CHMP cut off for SCF is defined as a ratio of >2.5. SPRs were also calculated at Day 0, 21, 42 and 182. Seropositivity was defined as an HI titre >1:10.

For neutralizing antibodies, the endpoints (with 95% CIs) were seropositivity, GMTs and SCRs (SCR for MN antibodies was defined as the percentage of subjects with at least four-fold increase in post-vaccination neutralising antibody titres). The GMTs of neutralising antibody titres were calculated at Day 0, 42 and 182, and the SCRs in terms of neutralising antibody titres were calculated at Day 42 and 182.

The safety analysis was performed on all subjects receiving at least one vaccination (the total vaccinated cohort [TVC]). The percentage of subjects with at least one local AE (solicited and unsolicited), at least one general AE (solicited and unsolicited) and any AE during the solicited follow-up period was tabulated, with exact 95% CI after each vaccination and overall per subject considering both post-immunisation periods.

Solicited symptoms and any pain relief and/or antipyretics taken by the subject to correct the symptoms of local and/or general solicited symptoms were recorded during the 7-day follow-up period after each H5N1 vaccination.

Results

A total of 100 subjects (n = 50 for 20-40 years of age; n = 50 for 41-64 years of age) were enrolled in September 2008, all of whom received two doses of study vaccine by October 2008. The per protocol cohort was, therefore, identical to the TVC. The mean age of the vaccinated subjects (total cohort) was 40.3 years, 31.1 years for the 20-40 years' stratum and 49.6 years for the 41-64 years' stratum. The overall male-female distribution was 43% versus 57%. The demographic characteristics of the subjects involved are shown in Table 1.

Immunogenicity and cross-clade antibody titres

Only five out of 100 subjects were seropositive against the vaccine-homologous strain (A/Indonesia/5/2005;

Table 1 Demographic data of trial subjects

		20-40 Y N = 50		41-64 Y N = 50		Total N = 100	
Characteristics	**Parameters or Categories**	**Value or n**	**%**	**Value or n**	**%**	**Value or n**	**%**
Age (years)	Mean	31.1	-	49.6	-	40.3	-
	SD	5.69	-	6.04	-	10.89	-
	Median	31	-	49	-	40.5	-
	Minimum	20	-	41	-	20	-
	Maximum	40	-	63	-	63	-
Gender	Female	25	50	32	64	57	57
	Male	25	50	18	36	43	43
Race	Asian-Japanese heritage	50	100	50	100	100	100
Height (cm)	Mean	165.9	-	162.5	-	164.2	-
	SD	8.41	-	7.46	-	8.1	-
	Median	164	-	161.5	-	163	-
Weight (kg)	Mean	60.7	-	60.6	-	60.6	-
	SD	12.13	-	11.28	-	11.65	-
	Median	61	-	57.6	-	59	-

20-40 Y = subjects aged 20-40 years; 41-64 Y = subjects aged 41-64 years; N = total number of subjects;

n/% = number/percentage of subjects in a given category; value = value of the considered parameter;

SD = standard deviation

Clade 2.1) at Day 0 (before vaccination), of whom none had a seroprotective HI titre of 1:40 or more. Pre-vaccination HI GMTs against the other two vaccine-heterologous strains (A/Vietnam/1194/2004; Clade 1 and A/turkey/Turkey/1/2005; Clade 2.2) were also very low and were almost the same as for the vaccine-homologous strain.

Immune responses against the vaccine-homologous strain (A/Indonesia/5/2005; Clade 2.1) at Day 42 fulfilled and exceeded all CHMP and CBER criteria for HI antibody response (Table 2). Overall, the homologous HI SCR and SPR were found to be 91% and were comparable for the predefined age strata (90% for 20-40 years, 92% for 41-64 years). A high cross-reactive (heterologous HI) humoral immune response was observed against the A/turkey/Turkey/1/2005 strain (H5N1 Clade 2.2) and to a lower extent against the A/Vietnam/1194/2004 strain (H5N1 Clade 1) after the second vaccine administration. The HI antibody response against each of the A/turkey/Turkey/1/2005 and A/Vietnam/1194/2004 strains after two doses was similar across the predefined age strata.

Persistence of the immune response was observed on Day 182 (i.e., 6 months after the first dose); a substantial proportion of subjects (in both age strata) were still seropositive for H5N1 HI antibodies against the A/Indonesia/5/2005 strain (Clade 2.1) and the A/turkey/Turkey/1/2005 strain (Clade 2.2) and, to a lower extent, against the A/Vietnam/1194/2004 strain (Clade 1). The GMTs for H5N1 HI antibodies decreased against all three H5N1 strains compared with Day 42. On Day 182, all three CHMP criteria were still met for H5N1 HI antibody responses against the A/Indonesia/5/2005 strain (except the SPR threshold for subjects aged 20-40 years). Two of the three CHMP criteria (SCR and SCF) for H5N1 antibody response against the A/turkey/Turkey/1/2005 strain were still met in the 41-64 years age stratum.

Trends for higher HI antibody responses were observed against both vaccine homologous and heterologous strains 21 days after the second vaccine dose, in subjects who were seropositive before vaccination. This must be interpreted with caution given the low number of seropositive subjects before vaccination (N = 4 to 6).

Neutralising antibody response

Neutralising antibody response to the vaccine homologous and heterologous strains at different time points have been presented in Table 3. A marked post-vaccination response in terms of neutralising antibody titres (GMTs) was observed against both Clade 1 and Clade 2 viruses. The observed data suggested that pre-vaccination seropositivity rates against the A/Indonesia/5/2005 strain were low (11.1%) and were higher against the vaccine heterologous strain A/Vietnam/1194/2004 (50%). Following vaccination, the seropositivity rates for neutralising antibody titres against the A/Indonesia/5/2005 strain and the A/Vietnam/1194/2004 strain were increased at Day 42, reaching 100% and 95%, respectively.

The SCR for neutralising antibodies increased after the second vaccination and reached 97% and 47% at Day 42 against the A/Indonesia/5/2005 strain and the A/Vietnam/1194/2004 strain, respectively. Six months after the first vaccination, high seropositivity rates were observed in terms of H5N1 neutralising antibodies against both A/Indonesia/5/2005 (100%) and A/Vietnam/1194/2004 (92.9%). The SCR against the A/Indonesia/5/2005 and the A/Vietnam/1194/2004 strains at 6 months were 93.9% and 58.6%, respectively.

Safety

Compliance in returning safety diary cards was excellent (100%) for both general and local symptoms. There were no SAEs and no withdrawals due to AEs.

Solicited local and general AEs are shown in Figures 2 and 3, respectively, and the proportion of subjects reporting fever is shown in Figure 3. Pain at the injection site (of any grade) was the most frequently reported local symptom in the overall cohort after dose 1 (98%) and dose 2 (93%), and there was no observable difference between age strata. The next

Table 2 Haemagglutination inhibition (HI) antibody immune responses to homologous and heterologous H5N1 influenza strains following one or two doses of the AS03A-adjuvanted A/Indonesia/5/2005 (H5N1) influenza vaccine were assessed in terms of seropositivity rates, GMTs, SCRs, SCFs and SPRs

			Seropositivity rates			GMT	SCR *(Negative pre-vaccination HI titre and P/V HI titre ≥1:40, or proportion with ≥4-fold increase from pre- to post vaccination)*			SCF *(Mean GMT increase in titre >2.5 [adults]; >2.0 [aged over 60 y])*		SPR *(% subjects with P/V HI titre ≥1:40)*		
Antibody	**Group**	**D**	**N**	**n**	**% (95% CI)**	**Value (95% CI)**	**N**	**n**	**% (95% CI)**	**N**	**Value (95% CI)**	**N**	**n**	**% (95% CI)**
A/Indonesia	20-40 Y	0	50	0	0.0 (0.0-7.1)	5.0 (5.0-5.0)	-	-	-	-	-	50	0	0.0 (0.0-7.1)
		21	50	24	48.0 (33.7-62.6)	15.8 (11.0-22.8)	50	19	38.0 (24.7-52.8)	50	3.2 (2.2-4.6)	50	19	38.0 (24.7-52.8)
		42	50	45	90.0 (78.2-96.7)	156.8 (105.8-232.3)	50	45	90.0 (78.2-96.7)	50	31.4 (21.2-46.5)	50	45	90.0 (78.2-96.7)
		182	49	30	61.2 (46.2-74.8)	25.6 (17.3-38.1)	49	29	59.2 (44.2-73.0)	49	5.1 (3.5-7.6)	49	29	59.2 (44.2-73.0)
	41-64 Y	0	50	5	10.0 (3.3-21.8)	5.4 (5.0-5.8)	-	-	-	-	-	50	0	0.0 (0.0-7.1)
		21	50	27	54.0 (39.3-68.2)	15.4 (10.7-22.0)	50	16	32.0 (19.5-46.7)	50	2.8 (2.0-4.0)	50	16	32.0 (19.5-46.7)
		42	50	48	96.0 (86.3-99.5)	142.1 (104.0-194.3)	50	46	92.0 (80.8-97.8)	50	26.2 (19.2-35.8)	50	46	92.0 (80.8-97.8)
		182	50	40	80.0 (66.3-90.0)	37.4 (27.5-50.8)	50	38	76.0 (61.8-86.9)	50	6.9 (5.1-9.2)	50	38	76.0 (61.8-86.9)
A/turkey/Turkey	20-40 Y	0	50	2	4.0 (0.5-13.7)	5.7 (4.8-6.8)	-	-	-	-	-	50	2	4.0 (0.5-13.7)
		21	50	13	26.0 (14.6-40.3)	8.0 (6.1-10.4)	50	3	6.0 (1.3-16.5)	50	1.4 (1.1-1.8)	50	5	10.0 (3.3-21.8)
		42	50	30	60.0 (45.2-73.6)	24.8 (16.6-37.1)	50	27	54.0 (39.3-68.2)	50	4.4 (3.0-6.5)	50	29	58.0 (43.2-71.8)
		182	49	30	61.2 (46.2-74.8)	19.2 (13.4-27.3)	49	18	36.7 (23.4-51.7)	49	3.4 (2.4-4.7)	49	20	40.8 (27.0-55.8)
	41-64 Y	0	50	2	4.0 (0.5-13.7)	5.2 (4.9-5.5)	-	-	-	-	-	50	0	0.0 (0.0-7.1)
		21	50	18	36.0 (22.9-50.8)	9.6 (7.3-12.5)	50	7	14.0 (5.8-26.7)	50	1.8 (1.4-2.4)	50	8	16.0 (7.2-29.1)
		42	50	31	62.0 (47.2-75.3)	24.0 (16.1-35.6)	50	27	54.0 (39.3-68.2)	50	4.6 (3.1-6.8)	50	27	54.0 (39.3-68.2)
		182	50	38	76.0 (61.8-86.9)	30.7 (22.2-42.5)	50	30	60.0 (45.2-73.6)	50	5.9 (4.2-8.2)	50	30	60.0 (45.2-73.6)
A/Vietnam	20-40 Y	0	50	1	2.0 (0.1-10.6)	5.2 (4.8-5.7)	-	-	-	-	-	50	1	2.0 (0.1-10.6)
		21	50	5	10.0 (3.3-21.8)	5.7 (5.0-6.5)	50	0	0 (0-7.1)	50	1.1 (1.0-1.2)	50	1	2.0 (0.1-10.6)
		42	50	26	52.0 (37.4-66.3)	12.7 (9.5-17.1)	50	14	28.0 (16.2-42.5)	50	2.4 (1.8-3.2)	50	15	30.0 (17.9-44.6)
		182	49	17	34.7 (21.7-49.6)	8.6 (6.8-10.8)	49	2	4.1 (0.5-14.0)	49	1.6 (1.3-2.1)	49	3	6.1 (1.3-16.9)
	41-64 Y	0	50	5	10.0 (3.3-21.8)	5.9 (5.0-6.8)	-	-	-	-	-	50	2	4.0 (0.5-13.7)
		21	50	13	26.0 (14.6-40.3)	7.0 (5.8-8.5)	50	1	2.0 (0.1-10.6)	50	1.2 (1.1-1.4)	50	4	8.0 (2.2-19.2)
		42	50	24	48.0 (33.7-62.6)	13.3 (9.7-18.2)	50	12	24.0 (13.1-38.2)	50	2.3 (1.7-3.0)	50	15	30.0 (17.9-44.6)
		182	50	23	46.0 (31.8-60.7)	10.7 (8.2-13.9)	50	10	20.0 (10.0-33.7)	50	1.8 (1.4-2.4)	50	12	24.0 (13.1-38.2)

Subjects received one dose of vaccine on Day 0 and one dose on Day 21 (ATP cohort for immunogenicity and persistence).

20-40 Y = subjects aged 20-40 years; 41-64 Y = subjects aged 41-64 years; GMT = geometric mean antibody titre; N = number of subjects with available results; n/% = number/percentage of subjects; P/V = post-vaccination; 95% CI = 95% confidence interval; D = Day, 0 = pre-vaccination, 21 = 21 days post vaccination one, 42 = 42 days post vaccination one (i.e. 21 days post vaccination two), 182 = 182 post vaccination one.

Committee for Medicinal Products for Human Use (CHMP) criteria for adults 18-60 years: SCR >40%, SPR >70% and a SCF of >2.5

US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) criteria for adults <65 years of age: Lower bound of the two-sided 95% CI for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 40% and the lower bound of the two-sided 95% CI for the percentage of subjects achieving a seroprotective level of HI antibody titre ≥1:40 should meet or exceed 70%.

most frequently observed local symptom was swelling/induration, followed by redness. Grade 3 pain was reported in one subject in the 20-40 years' stratum. General solicited symptoms included fatigue (most common, reported by 71% of subjects overall) followed by muscle aches (70% overall) and headache (51% overall).

Overall, 69% of the subjects reported at least one unsolicited AE, with nasopharyngitis (18%) and injection site pruritus (18%) as the most frequently reported unsolicited AEs in the 20-40 year and 41-64 year cohorts, respectively. There were no major or clinically relevant differences between age strata for any AE and no specific clinical pattern of unsolicited AEs could be

Table 3 Neutralising antibody immune responses to homologous and heterologous H5N1 influenza strains following one or two doses of the AS03A-adjuvanted A/Indonesia/5/2005 (H5N1) influenza vaccine in terms of seropositivity rates and seroconversion rates

Antibody	Time point	N	Seropositivity % (95% CI)	N	Seroconversion % (95% CI)
A/Indonesia	PRE	99	11.1 (5.7-19.0)	-	-
	Day 42	100	100 (96.4-100)	99	97.0 (91.4-99.4)
	Day 182	99	100 (96.3-100)	98	93.9 (87.1-97.7)
A/Vietnam	PRE	100	50.0 (39.8-60.2)	-	-
	Day 42	100	95.0 (88.7-98.4)	100	47.0 (36.9-57.2)
	Day 182	99	92.9 (86.0-97.1)	99	58.6 (48.2-68.4)

Subjects received one dose of vaccine on Day 0 and one dose on Day 21 (ATP cohort for immunogenicity and persistence).
Seroconversion rate for MN antibodies: Percentage of subjects with at least four-fold increase post-vaccination neutralising antibody titres.

identified in either group. Grade 3 unsolicited AEs and grade 3 unsolicited AEs considered related to any vaccination were infrequent and similar in both age strata (20-40 years, three subjects with at least one symptom and one of these symptoms considered by an investigator to be related to vaccination; 41-64 years, three subjects reporting at least one symptom, no related symptoms). No pattern was observed regarding biochemical abnormalities.

Discussion

The results of this study demonstrate that the $AS03_A$-adjuvanted A/Indonesia/5/2005 (H5N1) vaccine was well tolerated and induced strong humoral immune responses in healthy Japanese adults.

The primary endpoints for this study were reached at 21 days after the second vaccination, with immune responses fulfilling all CHMP and CBER criteria for the vaccine-homologous HI antibody response (A/

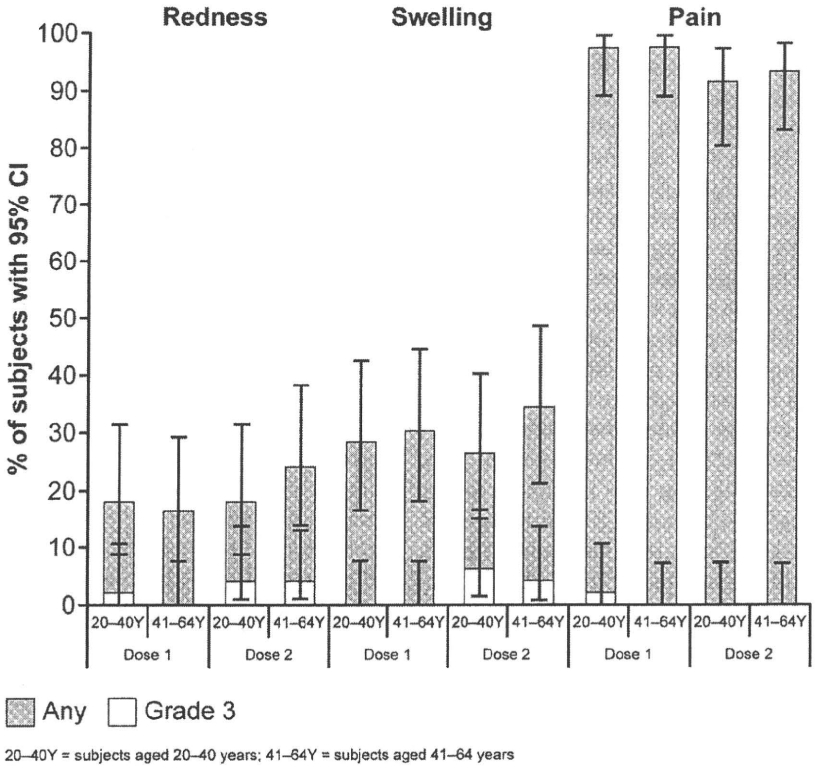

Figure 2 Solicited local symptoms of any grade and grade 3 severities, following two doses of the $AS03_A$-adjuvanted A/Indonesia/5/2005 (H5N1) vaccine, given 21 days apart.

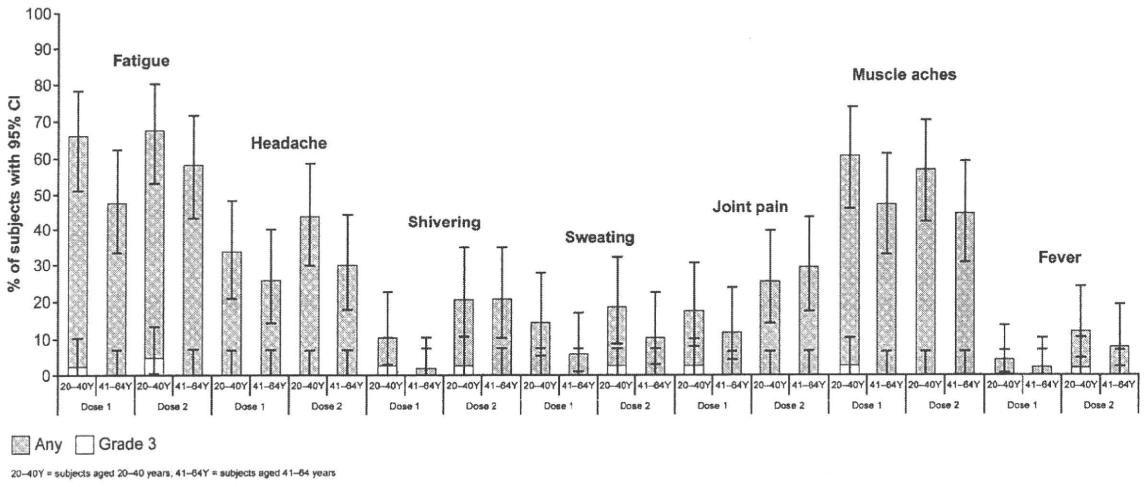

Figure 3 Solicited general symptoms of any grade and grade 3 severities, following two doses of the AS03$_A$-adjuvanted A/Indonesia/5/2005 (H5N1) vaccine, given 21 days apart.

Indonesia/5/2005, Clade 2.1). Overall, homologous HI SCR and SPR were 91% and no differences between the predefined age strata were observed (90% for 20-40 years, 92% for 41-64 years).

These responses were achieved with low doses of antigen (3.75 μg HA). The 'antigen-sparing' effect of the adjuvant AS03 was not demonstrated in this study, as no comparison was made with an unadjuvanted formulation containing 3.75 μg HA; however, the current study is in line with previous influenza vaccine studies with this adjuvant formulation which have shown that the AS03-adjuvanted H5N1 vaccines induce a substantially higher immune response than non-adjuvanted formulations [9], with antigen-sparing properties [10-12]. Therefore, the AS03 adjuvant plays a key role in providing high levels of immunity at relatively low antigen doses, which is one of the requirements for a viable pandemic vaccine in the context of mass distribution.

The H5N1 A/Indonesia/5/2005 vaccine described here was tested in a two-dose regimen with responses to the vaccine strain that met CHMP and CBER criteria only at 21 days after the second injection. This is in contrast to the GSK Biologicals' A/California/7/2009 H1N1 pandemic vaccine adjuvanted with AS03$_A$ (*Pandemrix*™) which was licensed for use in the 2009/2010 H1N1 pandemic [23]. While posology guidelines published by the European Medicines Agency indicate that a second dose of the vaccine may be desirable to achieve maximum seroprotection, data suggest that a single dose of the H1N1 vaccine may be sufficient to achieve this in healthy adults aged 18-60 years [23]. The reason for this difference may be that naturally acquired partial immunity against A/California/7/2009 (H1N1) is more common, due to prior exposure to circulating H1N1 strains with epitopes similar to those found in A/California/7/2009 (H1N1).

In the current study, SCR and SPR of more than 54% for H5N1 HI antibodies against the Clade 2.2 A/turkey/Turkey/1/2005 were observed after two doses in each age stratum, indicating that the vaccine was markedly cross-immunogenic. This was also observed, albeit to a lesser extent, against the A/Vietnam/1194/2004 (Clade 1) strain, where SCR and SPR of more than 24% were observed. This finding is similar to those of previous studies with the AS03-adjuvanted H5N1 vaccine formulated with the A/Vietnam/1194/2004 (Clade 1) strain where significant levels of cross-clade immunogenicity were observed against antigenically distinct strains of H5N1, including the strains from the other Clades [9,10,12,13]. In a study in Asian adults, following two doses of the AS03-adjuvanted H5N1 vaccine, seroprotection rates in terms of HI antibodies against the vaccine homologous (A/Vietnam/1194/2004) and heterologous (A/Indonesia/5/2005) strains were 94.3% and 50.2%, respectively. For neutralising antibodies against the A/Vietnam/1194/2004 and A/Indonesia/5/2005 (Clade 2.1) strains, seroconversion rates were 96% and 91.4%, respectively [9]. In another study in Europe, two doses of the H5N1 vaccine elicited strong immune responses against vaccine heterologous A/turkey/Turkey/1/2005 (Clade 2.2) and A/Anhui/1/2005 (Clade 2.3) strains (neutralising seroconversion rates: 75- 85%). The study also reported persistence of neutralising seroconversion rates in 60-70% of subjects, up to six months