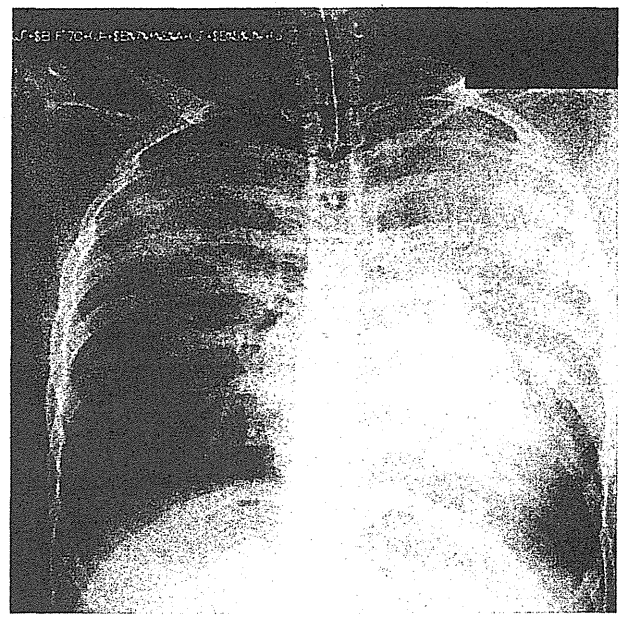
症例4 図4は，10歳台の女児で，突然の発熱，頭痛で発症．迅速検査でA型インフルエンザ陽性[後にパンデミック(H1N1)2009と判明]．翌日には胸痛も出現．第5病日，ぐったりしたため救急車で救急外来搬送された．胸部X線写真で心拡大と両肺上肺野優位に浸潤影を認め，心エコー所見とも合わせ心筋炎による心不全と肺水腫と診断された．



【図4】心筋炎とうっ血を併合した例

本例のように心筋炎から肺うっ血を合併すると，I型呼吸不全をきたす．

文献

1) File Jr TM：Community-acquired pneumonia. Lancet 362：1991-2001, 2003
2) Writing committee of the WHO consultation on clinical aspects of pandemic(H1N1)2009 influenza：Clinical aspects of pandemic 2009 influenza A(H1N1)virus infection. N Engl J Med 362：1708-1719, 2010
3) 吉田裕輔，他：人工呼吸管理例を含むパンデミックインフルエンザA(H1N1)2009肺炎3例の治療経験．日小児会誌 114：85-87, 2010
4) Hasegawa M, et al：Bronchial casts and pandemic (H1N1)2009 virus infection. Emerg Infect Dis 16：344-346, 2010

シンポジウム3

3 重症インフルエンザの臨床

○川名　明彦
防衛医科大学校 内科学（感染症・呼吸器）

成人のインフルエンザウイルス感染症の多くは自然治癒する予後良好な疾患であるが、一部で重症化する例も存在する。季節性インフルエンザでは、特に高齢者において肺炎や基礎疾患の増悪が見られ、その結果死亡する例もある。これは毎年の超過死亡という形で示されてきた。インフルエンザウイルス感染に関連する肺炎は、教科書的には
(1) 純ウイルス性肺炎、
(2) ウイルス細菌混合性肺炎、
(3) 二次性細菌性肺炎
の3つがあるとされ、成人の季節性インフルエンザでは大部分が(2)、(3)と考えられる。(1)は非常に稀であるこれは、インフルエンザウイルスが主に上気道に限局した感染症であるためと説明されてきた。2009年にパンデミックをもたらしたインフルエンザウイルス（パンデミック H1N1 2009）も、総じてみるとその病原性は季節性インフルエンザを超えるものではないと言えそうだが、純ウイルス性肺炎と考えられる重症例が比較的多く報告されている点は注目される。また、このような重症肺炎は必ずしも高齢者ばかりではなく、若年層や青壮年、基礎疾患の無いそれまで健常であった宿主においても報告されている。本シンポジウムでは、重症インフルエンザ肺炎の臨床像について考察を加えたい。インフルエンザに関連した重症肺炎は、1918年のいわゆるスペインかぜにおいても多く報告されているし、またインフルエンザA（H5N1）ウイルスのヒト感染例でも多く見られている。これらの臨床的な類似性についても言及したい。パンデミック H1N1 2009感染症では、一部で急速な増悪を示す例が見られた。したがって早い段階で重症化の所見を検知することが必要である。この観点から重症化のハイリスク要因が指摘されている。すなわち、幼児と若年小児（特に2歳未満）、妊婦、慢性呼吸器疾患（気管支喘息、慢性閉塞性肺疾患）、慢性心疾患（うっ血性心不全）、代謝性疾患（糖尿病）、慢性腎疾患、慢性肝疾患、ある種の神経疾患（神経筋疾患、痙攣性疾患）、免疫抑制状態（HIV 感染症、免疫抑制剤使用、悪性腫瘍）、長期的にアスピリン治療を受けている小児、65歳以上の高齢者、肥満（特に病的肥満）等である。入院を要した患者の約1/2は、上記の基礎疾患を一つ以上有していたとされるが、一方ICUへの入室を必要とした重症患者の1/3は基礎疾患がなかった点にも留意が必要である。酸素化障害や心肺不全の症状（呼吸困難、チアノーゼ、血痰・血性痰、胸痛、低血圧）、中枢神経系の合併症を示唆する症状・徴候（精神状態の変容、意識障害、傾眠、再発性・持続性の痙攣、錯乱、脱力、麻痺）、持続的なウイルスの増殖や、二次性細菌感染を示す検査・臨床所見（3日以上続く高熱など）ならびに重症脱水の症状（活動性の低下、めまい、尿量の減少、こん睡状態など）が見られた場合は、重症と判断して直ちに集中的な治療を開始する必要がある。治療に関しては、抗インフルエンザウイルス薬の早期治療が重要であるが、重症例については投与量の増量、長期使用も提案されている。また、新規抗インフルエンザウイルス薬の適切な使用も考慮すべきである。インフルエンザに関連したARDSの治療は、エビデンスに基づいたガイドラインに準じることが奨められる。人工呼吸は標準的な肺保護的な設定から開始する。治療抵抗性の低酸素血症に対しては、高頻度振動人工呼吸（HFO）や膜型人工肺体外循環治療（ECMO）などの先進的換気方法やの有効性が示されている。高用量コルチコステロイドの有用性については現時点では否定的である。また、二次性細菌性肺炎の予防・治療は重要である。以上の他、発表の時点における重症インフルエンザの診療に関するコンセンサスについても言及したい。

医療機関における新型インフルエンザ対策

＊防衛医科大学校
内科学2(感染症・呼吸器)
川名明彦

1．新型インフルエンザの概要

2009年春，突如として出現した豚由来の新型インフルエンザウイルス(A/H1N1)は，短期間のうちに世界に拡散し，WHO(世界保健機関)は世界的大流行(パンデミック)を宣言した．我が国でも同年5月上旬に最初の感染例が報告されて以来感染者数は増加し，夏にも終息することなく流行が続いている．

今回の新型インフルエンザは，感染者の大部分が軽症のまま回復し，抗インフルエンザウイルス薬が有効であるなど，季節性インフルエンザと類似する点も多い．しかし十代での罹患率が高く，特に基礎疾患を持つ場合は重症化のリスクが高いなど懸念材料も多い．実際我が国においても基礎疾患を持つ死亡例や小児の脳症・肺炎による重症例が報告されている．その致死率は0.5％未満と考えられるが，季節性インフルエンザよりは高い．今後，患者数が増大するとともに重症例も増加することが予想され，医療機関にはその対応が求められる．

2．感染対策

インフルエンザの感染対策の基本的事項は次の通り[1]．①標準予防策の実施(必要に応じてガウン，手袋，マスク，眼の防護)，②飛沫予防策(サージカルマスク)，③患者は可能ならば個室に収容，もしくはコホート隔離，④手指衛生の徹底，⑤咳エチケットの実施，⑥感染者の早期発見と分離，⑦流行期間中の訪問者の制限，インフルエンザ様症状のある職員の診療への関与を制限．

今回の新型インフルエンザ出現後に強調されているのが，エアロゾル産生手技を行う時のN95マスクなどの高性能マスクの着用である[2]．エアロゾル産生手技には，気管支鏡，開放性喀痰吸引，緊急挿管，心肺蘇生などが含まれる．CDCは新型インフルエンザ患者の病室に入る時もN95マスク着用を奨めている[3]．

近年インフルエンザの診断に迅速診断キットが頻用されているが，キットには次のような限界がある．①発病初期の陽性率が低い(特に新型インフルエンザでは，発病初日の陽性率は60％未満とされている)，②季節性インフルエンザと新型インフルエンザを区別できない．現在，特段の理由が無ければ行政はPCR検査によるA/H1N1の確定検査を実施していないため，臨床の現場では季節性と新型とを区別しない(できない)状況で診療を行っている．したがって新型確定例に限定せず適切なインフルエンザ感染対策を遍く実施することが重要である．

インフルエンザは流行期には市中でも感染のリスクがあるため，病院内での対策のみでは片手落ちである．職員は社会生活の中においても感染に注意する必要がある．

3．外来診療

病院外来玄関に「咳嗽，発熱などの症状のある方はマスクを着用してください」といった内容の掲示を行う．また，咳エチケットが実施できるよう手指衛生施設(擦式アルコール消毒薬の設置など)やゴミ箱の設置を行う．院内で咳をしている患者にはマスクの着用を促す．また，インフルエンザ様症状のある外来者の訪問制限を行う．

厚生労働省の運用指針によると，平素季節性インフルエンザの診療を行っている医療機関であれば，新型インフルエンザ(A/H1N1)も同様に診療を行うこととされている．その際，院内での感染予防のため，発熱患者とその他の患者については医療機関内で受診待ちの区域を分けたり，診療時間を分けるなどの方法でできるだけ導線を分けることが望ましいとされる．導線の完全な分離は困難な場合が多

＊〒359-8513 埼玉県所沢市並木3－2
E-mail kawana59@ndmc.ac.jp

いが，例えば我々の施設では問診でインフルエンザが疑わしい外来患者については待合場所を一般患者と分離し，診察室も分離している．

4．入院診療

新型に限らずインフルエンザ患者は個室への収容が望ましいとされる．個室が無い場合はコホート隔離をすることになるが，新型と季節性が同時流行した場合は迅速キットでこの両者を区別することはできず，同室に収容してよいか否かという問題が生じる．これは実は新型出現以前にも香港型(A/H3N2)とソ連型(A/H1N1)の間で同様に生じていた問題である．やむを得ずA型患者を同室にする場合は，できるだけベッド間隔を空ける，カーテンで仕切る，可能なら患者にサージカルマスクを着用させるなどの方法が現実的と思われる．ただし，エアロゾル産生手技(先述)を行う場合や，人工呼吸管理を行う場合はやはり個室収容が望ましい．

5．抗インフルエンザウイルス薬による治療

治療に関するWHOの勧告[4]の概要は次の通り．①基礎疾患が無く合併症のないインフルエンザ患者には抗インフルエンザウイルス薬による治療は必要ない．②重篤な症状を呈している場合は可及的速やかに(できれば48時間以内に)オセルタミビルによる治療を開始する(オセルタミビルが使用できない場合はザナミビルでも良い)．③この推奨は，全ての年齢層ならびに妊婦を含む．④重症化するリスクを持つ患者に対してもノイラミニダーゼ阻害薬による治療を行う．特に妊婦に対しては発症後直ちに治療を開始する．⑤必要な治療は確定診断を待たずに直ちに開始する．⑥重症例，悪化例には，投与量の増量や投与期間の延長も検討する．

全く基礎疾患の無い健常者でも重症化する例が報告されてきているので，医師が必要と判断した場合抗インフルエンザウイルス薬は躊躇せず使用するべきだろう．WHOは抗ウイルス薬を投与すべき危険な徴候として，呼吸困難，チアノーゼ，血性/膿性痰，胸痛，精神症状，3日を越える高熱の持続，低血圧，を挙げている．

6．職員への情報提供

インフルエンザの診療において，病院職員への情報提供と教育はきわめて重要である．インフルエンザはありふれた疾患だけに，全ての職員に対応策を周知しておく必要がある．ポスター，紙面での通知の他，院内電子端末を使った広報，レクチャーなどを実施することは有用である．また，抗インフルエンザウイルス薬の予防内服や，ワクチン接種の取り決めなどを事前に決定しておく必要がある．職員へのサーベイランス結果の供覧も意味がある．

7．地域における連携

新型インフルエンザ(A/H1N1)によるパンデミック時には多くの患者が発生することが予想されるが，その大部分は軽症である．一方，一部で重症患者が発生する．重症患者や合併症を持つ患者(基礎疾患，妊婦など)に適切な医療を提供するためにも，地域の医療機関における役割分担を決めておく必要があろう．軽症者は診療所やプライマリーケア医療機関へ，重症者は基幹病院や高次医療機関へ，といったシステムを構築しておくことは，限られた医療のリソースを有効に使うためにも重要と考えられる．

8．ワクチン接種

本稿執筆時点で，すでに新型インフルエンザ(A/H1N1)に対するワクチンの接種が開始されている．

一定のロットごとに市場に出てくるため，厚生労働省の提示した優先順位に従って接種を実施することとなる．医療従事者は第一の優先接種対象とされていることから，本号発行時にはすでに接種を済ませているスタッフも多いと思われる．スタッフは特段の禁忌事項がなければ，季節性・新型ともにワクチン接種を受けておくことが奨められる．また，医療機関では基礎疾患のある患者など優先接種対象者に対しては遅滞なく接種できるよう配慮する必要がある．ワクチン接種については厚労省のホームページなどに詳細が示されている．

参　考　文　献

1) CDC. Infection control guidance for the prevention and control of influenza in acute care facilities. http://www.cdc.gov/flu/professionals/infectioncontrol/healthcarefacilities.htm

2) IDSA, SHEA, APIC, ACOEM. SHEA, APIC, ACOEM, and IDSA joint position statement: healthcare personnel at high-risk for severe influenza illness: care of patients with suspected or confirmed novel H1N1 influenza A. http://www.shea-online.org/Assets/files/policy/FINAL_Joint_SHEA_APIC_IDSA_ACOEM_Position_Statement_High_Risk_HCW.pdf

3) CDC. Interim guidance for infection control for care of patients with confirmed or suspected swine influenza A (H1N1) virus infection in a healthcare setting. May 3, 2009. http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm

4) WHO. Recommended use of antivirals. 21 August 2009. http://www.who.int/csr/disease/swineflu/notes/h1n1_use_antivirals_20090820/en/index.html

THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/j.1447-0756.2011.01786.x J. Obstet. Gynaecol. Res. Vol. 38, No. 5: 793–796, May 2012

Discrepancy between human T-cell lymphotropic virus type I screening test and confirmatory tests in non-endemic areas

Masachi Hanaoka[1], Takahiko Kubo[1] and Akihiko Saitoh[2]

[1]*Division of Obstetrics, Department of Maternal, Fetal and Neonatal Medicine, and* [2]*Division of Infectious Diseases, Department of Medical Specialties, National Center for Child Health and Development, Tokyo, Japan*

Abstract

Aim: The purpose of this study was to examine the seroprevalence of human T-lymphotropic virus (HTLV)-I among pregnant women at our institution in Tokyo, Japan, which is a non-endemic area, and to investigate the results of Western blotting among pregnant women who had obtained positive results from a screening test.

Material and Methods: The seroprevalence of HTLV-I was retrospectively reviewed in 11 352 pregnant women who gave birth at the National Center for Child Health and Development in Tokyo, Japan, between 2002 and 2009. For the screening test, a chemiluminescent enzyme immunoassay was performed followed by a confirmatory Western blot test.

Results: The overall positive rate for the screening test was 0.33% (37/11 352). Western blot testing was performed in 36 of these 37 cases. Only nine patients (25%) were positive for HTLV-I by Western blot testing, seven patients (19%) were indeterminate, and 20 patients (56%) were negative.

Conclusions: In this study (carried out in a non-endemic area), the percentage of patients with a positive result from the screening test who were confirmed to be true carriers was significantly low, differing from endemic areas.

Key words: human T-lymphotropic virus, Japan, non-endemic area, pregnant women, screening test, Western blot testing.

Introduction

Human T-lymphotropic virus (HTLV)-I is the causative agent for at least two diseases: adult T-cell leukemia/lymphoma, and HTLV-I-associated myelopathy (HAM/TSP). The predominance of vertical transmission results in clustering of cases in familial or geographically discrete groups. The purpose of this study was to examine the seroprevalence of HTLV-1 among pregnant women at our institution in Tokyo, Japan, which is a non-endemic area, and to investigate the results of Western blotting among pregnant women who had obtained positive results from a screening test.

Materials and Methods

The seroprevalence of HTLV-I was retrospectively reviewed in 11 352 pregnant women who gave birth at the National Center for Child Health and Development in Tokyo, Japan, between 2002 and 2009. For the screening test, a chemiluminescent enzyme immunoassay (CLEIA) was performed for a serum sample from the patient. In total, 37 cases showed positive results for HTLV-I on the screening test. Western blotting was performed as a confirmatory testing in accordance with the 1990 World Health Organization criteria.[1] This study was approved by the ethics committee of our institution.

Received: April 4 2011.
Accepted: September 17 2011.
Reprint request to: Dr Masachi Hanaoka, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan. Email: hanaoka-m@ncchd.go.jp

Table 1 Comparison between positive screening and confirmatory tests for human T-cell lymphotropic virus in endemic and non-endemic countries

Authors/year	Country	Subjects	Number of subjects	Screening positive (%) ELISA or CLEIA	Confirmatory test by WB or immunofluorescence (%)	Confirmatory test/screening ratio	References
Arif and Ramia 1998	Saudi Arabia	Blood donors	34 541	9 (0.026%)	3 (0.009%) by WB 6 indeterminate	0.33	2
Fawaz *et al.* 2005	Saudi Arabia	Blood donors	13 443	8 (0.06%)	8 (0.06%) by WB	1	3
Al-Mufti *et al.* 1997	Kuwait	Blood donors	46 039	35 (0.076%)	10 (0.022%) by WB	0.29	4
Tamim *et al.* 2004	Lebanon	Blood donors	3 529	12 (0.3%)	2 (0.06%) by WB 1 (0.028%) by PCR	0.25	5
Knox-Macaulay *et al.* 1997	Sultanate of Oman	Blood donors	1 586	9 (0.6%)	6 (0.4%) by WB	0.67	6
Abbaszadegan *et al.* 2003	Iran	Blood donors	28 926	228 (0.77%)	219 (0.76%) by WB 8 indeterminate	0.96	7
Meytes *et al.* 1990	Jewish immigrants from Iran	Blood donors	331	24 (7.2%)	24 (7.2%) by WB	1	8
H21-tokubetsu-shitei-018 Kumamoto Prefecture 2008–2009	Japan	Pregnant women	538	3 (0.56%)	3 (0.56%)	1	9-(1)
H21-tokubetsu-shitei-018 Oita Prefecture 1988–2002	Japan	Pregnant women	105 346	1369 (1.30%)	1086 (1.03%) 283 indeterminate	0.79	9-(2)
H21-tokubetsu-shitei-018 Kagoshima Prefecture (1) 1999–2000	Japan	Pregnant women	10 480	237 (2.26%)	183 (1.75+α) 3 indeterminate	0.77	9-(3)
H21-tokubetsu-shitei-018 Kagoshima Prefecture (2) 2008–2009	Japan	Pregnant women	4 147	125 (3.01%)	106 (2.56+α) 2 indeterminate	0.85	9-(4)
H21-tokubetsu-shitei-018 Nagasaki Prefecture 1987–2006	Japan	Pregnant women	208 463	8504 (4.08%)	7265 (3.49%) 0 indeterminate	0.85	9-(5)
Present study Tokyo Prefecture 2002–2010	Japan	Pregnant women	11 352	37 (0.33%)	9 (0.079%) 7 indeterminate	0.24	—

CLEIA, chemiluminescent enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; WB, Western blotting.

Results

The overall positive rate for the screening test was 0.33% (37/11 352). Western blot testing was performed in 36 of these 37 cases (97%). Of these, only nine patients (25%) were positive for HTLV-I by Western blot testing, seven patients (19%) were indeterminate, and 20 patients (56%) were negative. The true positive rate was thus 0.079% (9/11 352). Among the seven indeterminate cases, subsequent polymerase chain reaction (PCR) testing yielded negative results in another two (2/7, 28.6%) cases and Western blot testing was repeated in two (2/7, 28.6%) cases on the decision of the physician, but the results were indeterminate (Table 1).

Discussion

In this study, the percentage of patients with a positive result from the screening test who were confirmed to be true carriers was significantly low. Kit-unique false positive rates in HTLV-I screening tests (using either particle agglutination [PA] or enzyme-linked immunosorbent assay [ELISA] methods) are the same among any population, but seroprevalence rates differ greatly among regions (Table 1). Therefore, if the seroprevalence rate in a certain region is high (endemic area), the false positive rate of the screening test for that region will be relatively low. In contrast, if the seroprevalence rate in a certain region is low (non-endemic area), the false positive rate for the screening test in that region will be relatively high. The present study showed that in a non-endemic area, the probability of screening test false positives for non-carriers was high. Western blot testing is thus very important as a confirmatory test in non-endemic areas before making a diagnosis of HTLV-I infection. Some recent studies in the Middle East[10] and Japan[9] have supported this finding. In endemic areas, seroprevalence rates of HTLV-I using ELISA and Western blotting are similar, so the confirmatory test (Western blot test)/screening test (ELISA) ratio (C/S ratio) is close to 1.0. For example, among Jewish immigrants from Iran,[8] the C/S ratio has been reported as 1.0 (Western blot, 7.2%; ELISA, 7.2%). In Nagasaki Prefecture in Japan,[9] where HTLV-I is endemic, the C/S ratio is 0.85 (Western blot, 3.5%; CLEIA, 4.1%). In contrast, in non-endemic areas, rates of HTLV-I seroprevalence according to ELISA and Western blot testing differ significantly, with a lower C/S ratio in non-endemic areas than in endemic areas. In Saudi Arabia[2] and Kuwait,[4] C/S ratios are 0.33 (Western blot, 0.009%; ELISA, 0.026%) and 0.29 (Western blot, 0.022%; ELISA, 0.076%), respectively (Fig. 1). When the kit-unique false positive rate in HTLV-I screening tests is around 0.3%, this curve fits the approximated curve in the listed studies. This curve shows that in areas where screening test positive rates are low, the probability of a screening test positive result for non-carriers is not low.

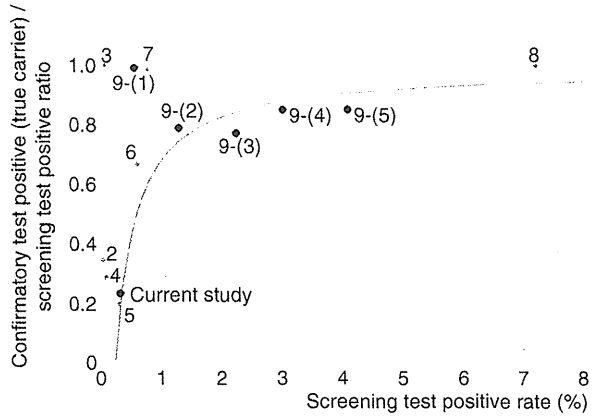


Figure 1 Correlation between screening test positive rate (%) and confirmatory test positive (true carrier)/ screening test positive ratio. Points on the graph indicate studies listed in Table 1. (◆) Study in Middle East; (●) study in Japan.

As vertical transmission is the major mode of HTLV-I infection, examining the seroprevalence of HTLV-I in pregnant women is important. As such studies should be performed, there is a relatively high probability of false positive results from HTLV-I screening tests in non-endemic areas, such as Tokyo. Diagnosis of HTLV-I infection should thus be made based on serological testing with ELISA or PA, followed by a confirmatory Western blot test. However, some individuals with positive screening results using ELISA may have indeterminate results from Western blot testing. In such cases, PCR offers an alternative diagnostic test, and is advantageous in terms of the ability to provide quantification of proviral load in the blood, which is associated with a higher incidence of HTLV-I-associated myelopathy.[11,12]

Acknowledgments

This work was supported by a grant from the Ministry of Health, Labor and Welfare of Japan (H21-tokubetsu-shitei-018).

Disclosure

None disclosed.

References

1. Acquired immunodeficiency syndrome (AIDS). Proposed WHO criteria for interpreting results from western blot assays for HIV-1, HIV-2, and HTLV-I/HTLV-II. *Wkly Epidemiol Rec* 1990; **65**: 281–283.
2. Arif M, Ramia S. Seroprevalence of human T-lymphotropic virus type I (HTLV-I) in Saudi Arabia. *Ann Trop Med Parasitol* 1998; **92**: 305–309.
3. Fawaz NA, Tamim H, Almawi WY. Low prevalence of antibodies to human T-lymphotropic virus-I/II among blood donors in eastern Saudi Arabia. *Am J Infect Control* 2005; **33**: 189–191.
4. Al-Mufti S, Voevodin A, Ahmed S, Al-Hamdan S, Al-Bisher AA. Prevalence of human T-cell lymphotropic virus type I infection among volunteer blood donors in Kuwait. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **15**: 88–90.
5. Tamim H, Musharrafieh U, Ramia S *et al.* Is seroprevalence of HTLV-I/II among blood donors in Lebanon relevant? *Am J Infect Control* 2004; **32**: 220–223.
6. Knox-Macaulay H, McDonald R, al-Busaidy S, Ali N. HTLV-I seroprevalence in the Sultanate of Oman. *Scand J Infect Dis* 1997; **29**: 217–218.
7. Abbaszadegan MR, Gholamin M, Tabatabaee A, Farid R, Houshmand M, Abbaszadegan M. Prevalence of human T-lymphotropic virus type 1 among blood donors from Mashhad, Iran. *J Clin Microbiol* 2003; **41**: 2593–2595.
8. Meytes D, Schochat B, Lee H *et al.* Serological and molecular survey for HTLV-I infection in a high-risk Middle Eastern group. *Lancet* 1990; **336**: 1533–1535.
9. H21-tokubetsu-shitei-018 supported by a grant from The Ministry of Health Labor and Welfare of Japan. The study report about mother-to-child transmission of HTLV-I. 2009. [Cited 6 November 2011.] Available from URL: http://www.mhlw.go.jp/bunya/kodomo/boshi-hoken16/dl/02_1.pdf
10. Bitar N, Hajj HE, Houmani Z *et al.* Adult T-cell leukemia/lymphoma in the Middle East: First report of two cases from Lebanon. *Transfusion* 2009; **49**: 1859–1864.
11. Miley WJ, Suryanarayana K, Manns A *et al.* Real-time polymerase chain reaction assay for cell-associated HTLV type I DNA viral load. *AIDS Res Hum Retroviruses* 2000; **16**: 665–675.
12. Nagai M, Usuku K, Matsumoto W *et al.* Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: High proviral load strongly predisposes to HAM/TSP. *J Neurovirol* 1998; **4**: 586–593.

RESEARCH PAPER

Human Vaccines & Immunotherapeutics 8:6, 1-11; June 2012; © 2012 Landes Bioscience

Safety and persistence of immunological response 6 months after intramuscular vaccination with an AS03-adjuvanted H1N1 2009 influenza vaccine

An open-label, randomized trial in Japanese children aged 6 months to 17 years

Akihiko Saitoh,[1,2,*] Akira Nagai,[3] Kazuyoshi Tenjinbaru,[4] Ping Li,[5] David W. Vaughn,[5] François Roman[6] and Tatsuo Kato[7]

[1]Department of Pediatrics; Niigata University Graduate School of Medical and Dental Sciences; Niigata, Japan

[2]Department of Pediatrics; University of California; San Diego, California USA

[3]Department of Interdisciplinary Medicine; National Center for Child Health and Development; Tokyo, Japan

[4]GlaxoSmithKline Japan; Tokyo, Japan

[5]GlaxoSmithKline Biologicals; King of Prussia; Philadelphia, PA USA

[6]GlaxoSmithKline Biologicals; Wavre, Belgium

[7]National Center for Child Health and Development; Tokyo, Japan

Key words: adjuvant, H1N1, influenza, japanese children, pandemic

Abbreviations: ATP, according-to-protocol; CBER, center for biologics evaluation & research; CHMP, committee for medicinal products for human use; CI, confidence interval; GMFR, geometric mean fold rise; GMT, geometric mean titre; HA, hemagglutinin; HI, hemagglutination inhibition; MAE, medically-attended event; pIMD, potential immune-mediated disease; SAE, serious adverse event; SCR, seroconversion rate; SPR, seroprotection rate; TVC, total vaccinated cohort; VRR, vaccine response rate; WHO, world health organization

This study evaluated the long-term persistence of immune response and safety of two doses of an A/California/7/2009 H1N1 pandemic influenza vaccine adjuvanted with AS03 (an α-tocopherol oil-in-water emulsion-based Adjuvant System) in Japanese children (NCT01001169). Sixty healthy subjects aged 6 mo–17 y were enrolled (1:1) into two study groups to receive 21 d apart, two doses of 1.9 μg haemagglutinin [HA] + $AS03_B$ (5.93 mg α-tocopherol) vaccine (6 mo–9 y) and 3.75 μg HA + $AS03_A$ (11.86 mg α-tocopherol) vaccine (10–17 y), respectively. Immunogenicity data (by haemagglutination inhibition [HI] and microneutralisation assays) to six months after the first vaccine dose are reported here. It was observed that following Dose 2, the HI immune response against the vaccine homologous strain induced by the two different dosages of the AS03-adjuvanted vaccine met and exceeded the US and European regulatory guidance criteria for pandemic influenza vaccines (seroprotection rate [SPR]/seroconversion rate [SCR]: 100%/100%; geometric mean fold rise GMFR: 146.8/57.1). Further, the immune response persisted for at least six months after the first vaccine dose wherein these regulatory criteria were still met (SPR: 100%/100%; SCR: 96.4%/89.7%; GMFR: 25.3/23.5). The neutralising antibody response was comparable to the HI immune response at Day 42 (vaccine response rate [VRR]: 100%/100%) and at Day 182 (VRR: 96.4%/82.8%). Overall, both vaccine dosages had a clinically acceptable safety profile. Thus, two doses of a 1.9 μg or 3.75 μg HA AS03-adjuvanted H1N1 2009 pandemic influenza vaccine in children aged 6 mo–17 y induced strong immune responses against the vaccine homologous strain that persisted for at least six months after the first vaccine dose.

*Correspondence to: Akihiko Saitoh; Email: asaitoh@med.niigata-u.ac.jp
Submitted: 11/07/11; Revised: 02/01/12; Accepted: 02/11/12
http://dx.doi.org/10.4161/hv.

Introduction

The emergence of a novel, swine-origin influenza A virus (H1N1 2009) that caused the first influenza pandemic of the 21st century re-affirmed the unpredictability of influenza viruses.[1] The H1N1 2009 pandemic spread rapidly across the globe leading to over 18,449 deaths in more than 214 countries.[2] The highest attack and hospitalisation rates for the H1N1 2009 pandemic virus were reported in children aged <5 y, particularly those in the first year of life, presumably due to the degree of immunological naivety of this population toward this novel strain.[1,3-5]

The first case of H1N1 2009 pandemic influenza in Japan was confirmed on May 09, 2009 and by February 05, 2010, the cumulative number of confirmed H1N1 2009 cases was estimated to have reached 20 million.[6] Significantly, a small number of deaths due to the pandemic were reported (202 deaths as of August 10, 2010).[7] As observed in other regions,[8,9] in Japan most of the H1N1 2009 pandemic influenza infections and associated hospitalisations were reported in children and adolescents.[10]

Immunisation is considered to be the most efficient method of mitigating influenza pandemic related morbidity and mortality.[11,12] In this context, the immunological naivety/lack of priming of young children to the novel H1N1 2009 strain coupled with their role in indigenous transmission of the virus made them a priority group for pandemic influenza vaccination.[13]

Based on previous experience of developing a prepandemic dose-sparing H5N1 influenza vaccine (3.75 μg haemagglutinin [HA] with AS03 [an α-tocopherol oil-in-water emulsion-based Adjuvant System]),[14-17] an AS03-adjuvanted H1N1 2009 pandemic vaccine with 3.75 μg HA content was developed for the 2009 influenza pandemic. This H1N1 2009 vaccine has been proven to be highly immunogenic (fulfilling the US and European regulatory guidance criteria for pandemic influenza vaccines) with a clinically acceptable safety profile in different populations[18,19] including adults in Japan.[20]

In October 27, 2009, a phase II, open-label study (ClinicalTrials.gov Identifier: NCT01001169) in Japanese children was initiated at the National Center for Child Health and Development, Tokyo, Japan. Healthy children aged 6 mo to 17 y received two doses of either 1.9 μg HA with $AS03_B$ (6 mo–9 y) or 3.75 μg HA with $AS03_A$ (10–17 y) H1N1 2009 vaccine intramuscularly, 21 d apart. The co-primary objectives of this study were to assess whether vaccination with two doses of the AS03-adjuvanted 1.9 μg HA or 3.75 μg HA H1N1 2009 vaccines induced an immune response against the vaccine homologous strain 21 d after the second vaccine dose (Day 42) that met and exceeded the US and European regulatory guidance criteria for pandemic influenza vaccines. The preliminary immunogenicity and reactogenicity results following the first vaccine dose (Day 21) have been published earlier in reference 21. This manuscript presents the immunogenicity and safety results from the six month follow-up phase of this study. The objectives for the follow-up phase were as follows: (a) to assess whether two doses of the study vaccine induced persistence of immunological response at Day 182 that met the US and European regulatory guidance criteria for pandemic influenza vaccines, (b) to describe homologous HI and neutralising antibody response 21 d after the second vaccine dose and at Day 182 and (c) to evaluate the safety profile of the vaccine that was administered in this pediatric Japanese population through the intramuscular route.

Results

Study population. The six month follow-up phase of this study (through Day 182) was completed on May 17, 2010.

A total of 60 subjects were enrolled to be vaccinated (Group 1.9 μg HA: 30 subjects [6–35 mo: 10; 3–9 y: 20]; Group 3.75 μg HA: 30 subjects [10–17 y]), of which 57 subjects completed the study at Day 182. The ATP cohort for immunogenicity at Day 42 and Day 182 included 58 and 57 subjects, respectively (**Fig. 1**).

The mean age of subjects in the TVC at the time of the first vaccine dose was 4.1 y (range: 7 mo–104 mo) in Group 1.9 μg HA and 13.6 y (range: 10–17.9 y) in Group 3.75 μg HA. The overall male to female ratio was 43.3%:56.7% and all subjects were of Japanese heritage.

Immunogenicity. *HI immune response.* Prior to receiving vaccination, 17.2% of subjects aged 6 mo–9 y and 60% of subjects aged 10–17 y had detectable levels of HI antibodies against the H1N1 2009 strain. The second dose of the AS03-adjuvanted vaccine elicited a strong HI immune response in both age groups that met and exceeded the CHMP guidance criteria and more stringent CBER guidance criteria for pandemic influenza vaccines at Day 42. In the 6–35 mo age stratum the sample size was small (n = 10), as a consequence the lower limit of the 95% CI for SPR was not above 70%, despite a point estimate of 100% and a high GMT value (1279.9) (**Table 1**).

Six months after the first vaccine dose (Day 182), the HI immune response against the H1N1 2009 strain still met the CHMP and CBER criteria in subjects aged 6 mo–9 y and 10–17 y; similar to the Day 42 immune response in the 6–35 mo age stratum, the lower limit of the 95% CI for SPR at Day 182 was not above 70%, despite a point estimate of 100% (with a GMT value of 154) (**Table 1**).

It is to be noted that the HI assays for the sequential time points Day 0, Day 21 and Day 42 were tested together. The Day 182 samples were tested separately without an assessment of variability from earlier time points. Due to potential assay variability, a comparative interpretation of the HI response at Day 182 with earlier time points should be done with caution.

Microneutralisation assay. Prior to receiving vaccination, 10.3% of subjects aged 6 mo–9 y and 46.7% of subjects aged 10–17 y had detectable levels of neutralising antibodies against the A/Netherlands/602/2009 strain which is antigenically similar to A/California/7/2009 strain. Twenty one days after the second vaccine dose (Day 42), 100% of subjects in both age groups were seropositive for antibodies against the A/Netherlands/602/2009 strain; corresponding GMTs were 551.1 and 702.4, respectively. The VRR was 100% in both age groups (**Table 2**). Six months after the first vaccine dose, all subjects were still seropositive for antibodies against the A/Netherlands/602/2009 strain;

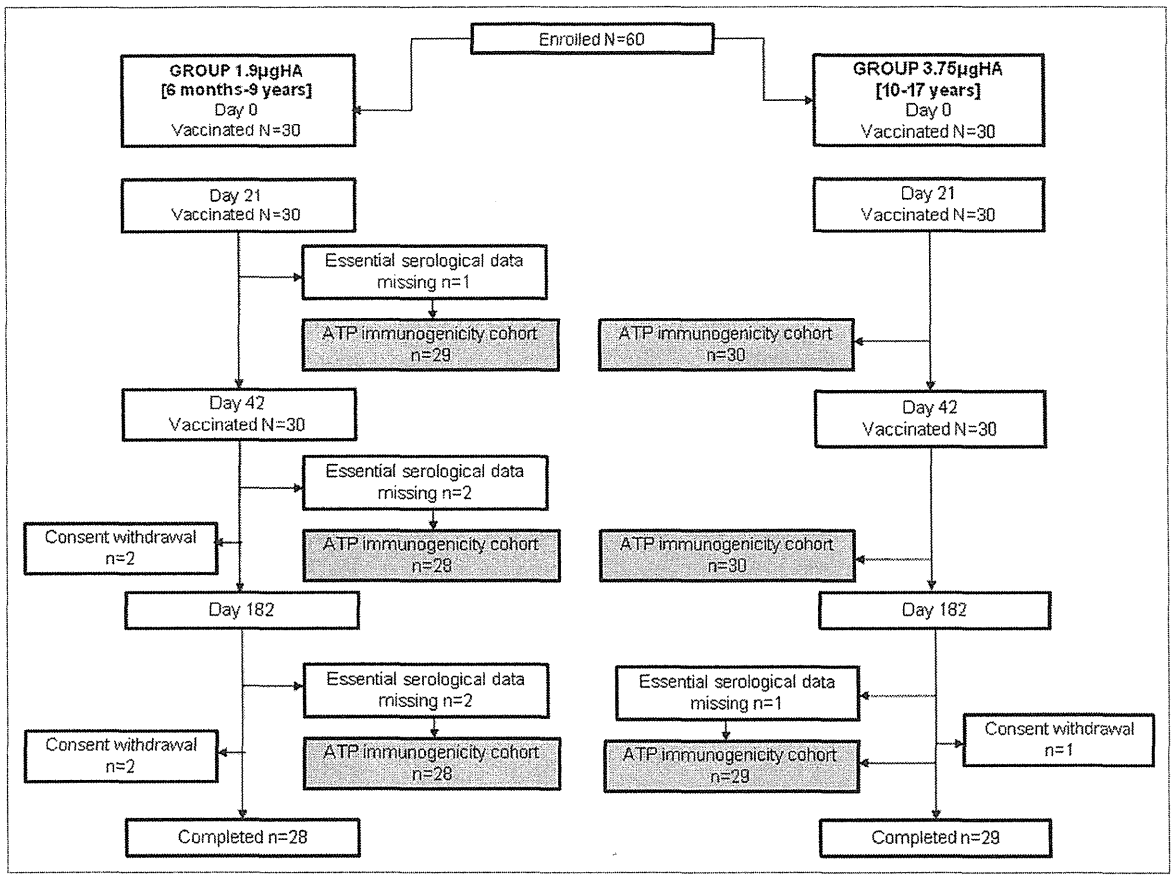


Figure 1. Study design diagram. Total vaccinated cohort (TVC): all subjects with at least one documented vaccine dose with available immunogenicity results. According-to-protocol (ATP) cohort for immunogenicity: all evaluable subjects (i.e., those meeting all eligibility criteria, with no elimination criteria during the relevant analysis interval), who received two vaccine doses and for whom assay results were available at Day 42 and Day 182. Group 1.9 µg HA: Subjects aged 6 mo-9 y received two doses of 1.9 µg HA + $AS03_B$ (5.93 mg α-tocopherol) vaccine, 21 d apart. Group 3.75 µg HA: Subjects aged 10–17 y received two doses of 3.75 µg HA + $AS03_A$ (11.86 mg α-tocopherol) vaccine, 21 d apart.

corresponding GMTs were 149.6 and 213.3. The VRR was 96.4% and 82.4% in the two age groups, respectively (**Table 2**).

Safety and reactogenicity. Overall, at least one solicited or unsolicited local symptom was reported for 70–100% of subjects in Group 1.9 µg HA (6–35 mo, 3–5 y, 6–9 y) and 100% of subjects in Group 3.75 µg HA (10–17 y); at least one solicited or unsolicited general symptom was reported for 42.9–83.3% of subjects in Group 1.9 µg HA (6–35 mo, 3–5 y, 6–9 y) and 80% of subjects in Group 3.75 µg HA (10–17 y). At least one MAE was reported in 58.3% of subjects aged 6 mo–5 y and 33.3% of subjects aged 6–9 y and 10–17 y.

Tables 3 and 4 present the percentage of subjects reporting solicited local and general symptoms overall and by age strata. Pain at injection site was the most frequently reported solicited local symptom across all age groups (overall 60%, 92.9%, 100% and 100% for subjects aged 6–35 mo, 3–5 y, 6–9 y and 10–17 y, respectively). The occurrence of pain was transient in most cases with the mean number of days being 1.8 d, 2.6 d, 2.7 d and 3.9 d for subjects aged 6–35 mo, 3–5 y, 6–9 y and 10–17 y, respectively. Overall, the occurrence of Grade 3 solicited local symptoms were infrequent; Grade 3 injection site pain was reported for two subjects aged 3–5 y and five subjects aged 10–17 y, and Grade 3 injection site swelling for two subjects aged 10–17 y. The reporting of solicited local symptoms after each of the two doses was comparable.

The most frequently reported solicited general symptoms varied across the different age groups; irritability (50% of subjects aged 6–35 mo), drowsiness (35.7% of subjects aged 3–5 y) and headache (50% of subjects aged 6–9 y and 66.7% of subjects aged 10–17 y). No fever was reported for subjects aged 6–35 mo following the first vaccine dose. However, following the second vaccine dose, four subjects (out of 10) developed fever, of which two cases of fever were of Grade 3 intensity (≥39°C). Overall, among subjects aged 6–35 mo, severe loss of appetite and irritability were reported for one subject each and Grade 3 fever for two subjects and among subjects aged 10–17 y, severe sweating was reported for one subject, Grade 3 fatigue and Grade 3 headache for two subjects each and Grade 3 fever for one subject, while no Grade 3 symptoms were reported among subjects aged 3–9 y.

Table 1. Immune response in terms of haemagglutination inhibition antibodies against vaccine homologous A/California/7/2009 strain [CBER[c]/CHMP[d] criteria] (ATP cohort for immunogenicity)

Measure	Time point	Group 1.9µg HA 6 mo-9 y						Group 3.75µg HA 10–17 y	
				Age sub-strata					
		N[a]	Overall	N	6–35 mo	N	3–9 y	N	Overall
				Value or Point estimate (95% CI[b])					
Seroprotection rates	PRE[#,^]	29	3.4% (0.1–17.8)	10	0.0% (0.0–30.8)	19	5.3% (0.1–26.0)	30	26.7% (12.3–45.9)
	Day 21^	29	100% (88.1–100)	10	100% (69.2–100)	19	100% (82.4–100)	30	96.7% (82.8–99.9)
	Day 42	28	100% (85.5–100)*	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (86.4–100)*
	Day 182	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	29	100% (88.1–100)
Seroconversion rates	Day 21^	29	100% (88.1–100)	10	100% (69.2–100)	19	100% (82.4–100)	30	90% (73.5–97.9)
	Day 42^	28	100% (85.5–100)*	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (86.4–100)*
	Day 182	28	96.4% (81.7–99.9)	9	100% (66.4–100)	19	94.7% (74.0–99.9)	29	89.7% (72.6–97.8)
Geometric Mean Fold Rise	Day 21^	29	27.1 (20.4–36.1)	10	23.5 (14.4–38.2)	19	29.3 (19.9–42.9)	30	22.1 (13.6–35.9)
	Day 42	28	146.8 (99.6–216.4)*	9	256.0 (161.4–406.1)	19	112.8 (74.6–170.5)	30	57.1 (33.5–97.3)*
	Day 182	28	25.3 (18.4–34.7)	9	30.8 (20.1–47.3)	19	23.1 (14.9–35.8)	29	23.5 (14.9–37.1)
Geometric Mean Titers	PRE[#,^]	29	6.3 (5.0–8.1)	10	5.0 (5.0–5.0)	19	7.2 (5.0–10.3)	30	15.3 (9.5–24.6)
	Day 21^	29	172 (130.1–227.6)	10	117.3 (72.2–190.8)	19	210.4 (150.4–294.5)	30	339 (238.8–481.2)
	Day 42	28	939.3 (722.9–1220.6)*	9	1279.9 (806.9–2030.4)	19	811.3 (628.4–1047.4)	30	874.3 (717.4–1065.4)*
	Day 182	28	161.9 (133.7–196.1)	9	154.0 (100.3–236.5)	19	165.8 (131.9–208.5)	29	347.9 (254.0–476.5)
Seropositivity rates	PRE[#,^]	29	17.2% (5.8–35.8)	10	0.0 (0.0–30.8)	19	26.3% (9.1–51.2)	30	60.0% (40.6–77.3)
	Day 21^	29	100% (88.1–100)	10	100% (69.2–100)	19	100% (82.4–100)	30	100% (88.4–100)
	Day 42	28	100% (85.5–100)*	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (86.4–100)*
	Day 182	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	29	100% (88.1–100)

Group 1.9µg HA: Subjects aged 6 mo-9 y received two doses of 1.9 µg HA+AS03$_B$ (5.93mg α-tocopherol) vaccine, 21 d apart. Group 3.75 µg HA: Subjects aged 10–17 y received two doses of 3.75 µg HA + AS03$_A$ (11.86mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence Interval. [c]CBER: Center for Biologics Evaluation and Research [Lower limit of 95% CI: SPR: ≥70%; SCR: ≥ 40%]. [d]CHMP: Committee for Medicinal Products for Human Use [Point estimate: SPR: >70%; SCR: >40%; GMFR: >2.5].[#]PRE: Pre-vaccination antibody titers for ATP cohort for immunogenicity at Day 21.* As per pre-defined primary co-objectives of this study, Day 42 immune response was calculated with 97.5% CI as per the CBER criteria for Group A (age 6 mo to 9 y), Group B (age 10–17 y), while at the other time points 95% CI was used. BOLD: Values of SPR and Geometric mean fold rise that did not meet the pre-specified criteria. ^PRE and Day 21 data have been presented previously in the primary manuscript *[ref. 21: Saitoh A, et al. J Japan Pediatr Soc 2011].*

Table 2. Immune response in terms of neutralising antibodies against the A/Netherlands/602/2009 strain (ATP immunogenicity cohort)

Measure	Time point	Group 1.9μg HA 6 mo–9 y						Group 3.75μg HA 10–17 y	
		N[a]	Overall	Age sub-strata				N	Overall
				N	6–35 mo	N	3–9 y		
				Value or Point estimate (95% CI[b])					
Seropositivity rates	PRE[#]	29	10.3% (2.2–27.4)	10	10.0% (0.3–44.5)	19	10.5% (1.3–33.1)	30	46.7% (28.3–65.7)
	Day 21	29	96.6% (82.2–99.9)	10	100% (69.2–100)	19	94.7% (74–99.9)	29	96.6 (82.2–99.9)
	Day 42	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (88.4–100)
	Day 182	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	29	100% (88.1–100)
Geometric Mean Titers	PRE[#]	29	4.9 (3.7–6.5)	10	4.7 (3.3–6.6)	19	5.0 (3.3–7.5)	30	11.3 (6.8–18.9)
	Day 21	29	53.8 (31.2–92.7)	10	42.5 (24.4–73.9)	19	60.8 (27–137.2)	29	121.2 (68.4–214.9)
	Day 42	28	551.1 (417–728.5)	9	623.4 (369.4–1052.1)	19	519.9 (362.3–745.5)	30	702.4 (433.5–1138)
	Day 182	28	149.6 (104.7–213.6)	9	161 (85.5–303.2)	19	144.4 (90–231.7)	29	213.3 (141.7–321)
Vaccine Response Rate	Day 21	29	51.7% (32.5–70.6)	10	60% (26.2–87.8)	19	47.4% (24.4–71.1)	29	58.6% (38.9–76.5)
	Day 42	28	100% (87.7–100)	9	100% (66.4–100)	19	100% (82.4–100)	30	100% (88.4–100)
	Day 182	28	96.4% (81.7–99.9)	9	100% (66.4–100)	19	94.7% (74–99.9)	29	82.8% (64.2–94.2)

Group 1.9 μg HA: Subjects aged 6 mo-9 y received two doses of 1.9 μg HA + $AS03_B$ (5.93mg α-tocopherol) vaccine, 21 d apart. Group 3.75 μg HA: Subjects aged 10–17 y received two doses of 3.75 μg HA + $AS03_A$ (11.86 mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence interval. [#]PRE: Pre-vaccination antibody titers for ATP cohort for immunogenicity at Day 21. Vaccine response rate defined as percentage of subjects with either a pre-vaccination titer <1:8 and a post-vaccination titer ≥1:32, or a pre-vaccination titer ≥1:8 and at least a 4-fold increase in post-vaccination titer.

Overall, 40 subjects reported at least one unsolicited adverse event upto Day 84: 17 (70.8%) in subjects aged 6 mo–5 y, 2 (33.3%) in subjects aged 6–9 y, and 21 (70%) in subjects aged 10–17 y. For subjects aged 6 mo–5 y, rhinorrhoea (7 subjects), upper respiratory tract infection and cough (6 subjects each) were the most commonly reported symptoms, while for subjects aged 6–9 y, pyrexia (2 subjects) was most frequently reported. Among subjects aged 10–17 y, there were no clear predominance of any unsolicited symptoms; axillary pain, pharyngitis, headache, cough, influenza, rhinitic allergy and acne were reported for two subjects each.

Two SAEs were reported during the entire study period. One subject in the 6–35 mo age strata presented severe febrile convulsion approximately five months after receiving the second vaccine dose which resolved in one day; the other subject in the 10–17 y age strata had a fracture in the foot approximately three months after the second vaccine dose which resolved in 72 d; none of the two SAEs were considered by the investigators to be vaccination-related. No pIMDs or fatalities were reported during the study period.

Discussion

This is the first study to report the persistence of the immunological response against the A/California/07/2009 strain in children, six months after vaccination with an AS03-adjuvanted H1N1 2009 pandemic influenza vaccine.

In this study, the 1.9 μg HA $AS03_B$-adjuvanted H1N1 2009 pandemic influenza vaccine induced a strong HI immune response in subjects aged 6 mo–9 y as evident from the high SPR/SCR (100%) following the second vaccine dose. In previous studies, similar formulations of the AS03-adjuvanted H1N1 2009 vaccine have been shown to be optimally immunogenic in subjects aged 6–35 mo and 6 mo–12 y.[19,22] Thus, the data in Japanese children conforms to the strong immunogenicity profile of the vaccine observed in other pediatric populations.

Table 3. Solicited local symptoms reported during the 7 d post-vaccination follow-up period after each vaccine dose (Total vaccinated cohort)

		Group 1.9 μg HA						Group 3.75 μg HA	
		6–35 mo		3–5 y		6–9 y		10–17 y	
		Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
		N[a] = 10	n = 10	n = 14	n = 13	n = 6	n = 6	n = 30	n = 30
		Point estimate (95% CI[b])							
Pain	Any	60.0 (26.2–87.8)	50.0 (18.7–81.3)	92.9 (66.1–99.8)	84.6 (54.6–98.1)	83.3 (35.9–99.6)	83.3 (35.9–99.6)	100 (88.4–100)	100 (88.4–100)
	Grade 3	0 (0–30.8)	0 (0–30.8)	7.1 (0.2–33.9)	7.7 (0.2–36.0)	0 (0–45.9)	0 (0–45.9)	10.0 (2.1–26.5)	6.7 (0.8–22.1)
Redness	Any	0 (0–30.8)	10.0 (0.3–44.5)	0 (0–23.2)	7.7 (0.2–36.0)	16.7 (0.4–64.1)	0 (0–45.9)	23.3 (9.9–42.3)	16.7 (5.6–34.7)
	Grade 3	0 (0–30.8)	0 (0–30.8)	0 (0–23.2)	0 (0–24.7)	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)
Swelling	Any	30.0 (6.7–65.2)	10.0 (0.3–44.5)	14.3 (1.8–42.8)	23.1 (5.0–53.8)	33.3 (4.3–77.7)	33.3 (4.3–77.7)	46.7 (28.3–65.7)	50.0 (31.3–68.7)
	Grade 3	0 (0–30.8)	0 (0–30.8)	0 (0–23.2)	0 (0–24.7)	0 (0–45.9)	0 (0–45.9)	3.3 (0.1–17.2)	3.3 (0.1–17.2)

Group 1.9 μg HA: Subjects aged 6 mo-9 y received two doses of 1.9 μg HA + $AS03_B$ (5.93mg α-tocopherol) vaccine, 21 d apart. Group 3.75 μg HA: Subjects aged 10–17 y received two doses of 3.75 μg HA + $AS03_A$ (11.86mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence interval.

A single dose of AS03-adjuvanted 1.9 μg HA H1N1 2009 vaccine in Canadian children aged 36 mo to 9 y has been found to have a protective effectiveness of 100%, 14 d following a single vaccine dose (statistically significant difference with the control group).[23] Although this value was 96% when effectiveness was assessed 10 d after vaccination, it remained at 100% in subjects aged <36 mo.[23] These observations are in agreement with the preliminary results obtained from this study in Japanese children, which reported a strong HI antibody immune response against the vaccine homologous strain (SPR and SCR 100%) after just one dose of the AS03-adjuvanted H1N1 2009 vaccine.[21]

The 3.75 μg HA AS03-adjuvanted H1N1 2009 vaccine also induced a strong HI immune response—SPR/SCR of 96.7%/90% following the first dose and 100%, following the second vaccine dose in subjects aged 10–17 y. Considering the above mentioned protective effectiveness reported in younger Canadian children (36 mo to 9 y old),[23] these immunogenicity results obtained in subjects aged 10–17 y in this study suggested that a single dose of 3.75 μg HA of AS03-adjuvanted H1N1 2009 vaccine induced a substantial protection against H1N1 2009 pandemic influenza virus.

Six months after the first vaccine dose, the HI immune response against the vaccine homologous strain were well maintained (high SPRs of 100% and SCRs of 96.4% and 89.7%, respectively), in subjects aged 6 mo–9 y and 10–17 y. Data on the long-term persistence of the immune response following pandemic influenza vaccination in children is limited. However, the observations from this study is in agreement with available data from studies in adults that the immune response induced by two doses of the 3.75 μg HA AS03-adjuvanted H1N1 2009 vaccine persists for at least six months after vaccination.[24,25]

The HI immune response against the A/California/7/2009 strain induced by the 1.9 μg and 3.75 μg HA dosages of the AS03-adjuvanted study vaccine was further corroborated when the CHMP guidance criteria[26] and the more stringent CBER guidance criteria[27] for pandemic influenza vaccines were met and exceeded, following the second vaccine dose and also six months after the first vaccine dose. The neutralising antibody titers parallel the HI immune response following each of the two vaccine doses and six months after the first vaccine dose.

Overall, the two vaccine dosages had clinically acceptable safety profiles in the respective study groups. Most solicited local and general symptoms were transient, and mild or moderate in intensity. Four cases of fever of which two were of grade 3 intensity were reported in subjects aged 6–35 mo following the second vaccine dose. Post-hoc assessments indicated that these cases may be associated with the strong increase of the humoral immune response (data not shown). Three out of these four subjects had the highest HI antibody titers (1,810 and 2,560) among children aged 6–35 mo; the remaining subject did not return for visit at Day 42. A similar observation was also made in a previous study and a possible association with increase in humoral immune response was made.[19] However, considering that the number of subjects in both studies is limited, further evaluation on a larger number of subjects would be required to ascertain the plausible reason for this observation.

When compared with the safety profile of a non-adjuvanted, trivalent seasonal influenza vaccine in a pediatric population aged between 6 mo and <18 y, the AS03-adjuvanted vaccine similar to the one used in the present study demonstrated increased frequency of solicited local symptoms, though more similar for general symptoms as well as MAEs and SAEs. However, the trend of

Table 4. Solicited general symptoms reported during the 7-d post-vaccination follow-up period after each vaccine dose (total vaccinated cohort)

		Group 1.9 µg HA						Group 3.75 µg HA	
		6–35 mo		3–5 y		6–9 y		10–17 y	
		Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
		N[a] = 10	n = 10	n = 14	n = 13	n = 6	n = 6	n = 30	n = 30
		Point estimate (95% CI[b])							
Drowsiness	Any	10.0 (0.3–44.5)	30.0 (6.7–65.2)	28.6 (8.4–58.1)	30.8 (9.1–61.4)	–	–	–	–
	Grade 3	0 (0–30.8)	0 (0–30.8)	0 (0–23.2)	0 (0–24.7)	–	–	–	–
Irritability	Any	30.0 (6.7–65.2)	40.0 (12.2–73.8)	21.4 (4.7–50.8)	15.4 (1.9–45.4)	–	–	–	–
	Grade 3	10.0 (0.3–44.5)	0 (0–30.8)	0 (0–23.2)	0 (0–24.7)	–	–	–	–
Loss of appetite	Any	10.0 (0.3–44.5)	30.0 (6.7–65.2)	28.6 (8.4–58.1)	15.4 (1.9–45.4)	–	–	–	–
	Grade 3	0 (0–30.8)	10.0 (0.3–44.5)	0 (0–23.2)	0 (0–24.7)	–	–	–	–
Fever	Any	0 (0–30.8)	40.0 (12.2–73.8)	21.4 (4.7–50.8)	15.4 (1.9–45.4)	33.3 (4.3–77.7)	0 (0–45.9)	13.3 (3.8–30.7)	23.3 (9.9–42.3)
	Grade 3	0 (0–30.8)	20.0 (2.5–55.6)	0 (0–23.2)	0 (0–24.7)	0 (0–45.9)	0 (0–45.9)	6.7 (0.8–22.1)	0 (0–11.6)
Fatigue	Any	–	–	–	–	16.7 (0.4–64.1)	16.7 (0.4–64.1)	36.7 (19.9–56.1)	36.7 (19.9–56.1)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	3.3 (0.1–17.2)	3.3 (0.1–17.2)
Gastrointestinal	Any	–	–	–	–	16.7 (0.4–64.1)	16.7 (0.4–64.1)	10.0 (2.1–26.5)	10.0 (2.1–26.5)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)
Headache	Any	–	–	–	–	33.3 (4.3–77.7)	16.7 (0.4–64.1)	40.0 (22.7–59.4)	33.3 (17.3–52.8)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	6.7 (0.8–22.1)
Joint pain at other location	Any	–	–	–	–	0 (0–45.9)	0 (0–45.9)	16.7 (5.6–34.7)	23.3 (9.9–42.3)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)
Muscle aches	Any	–	–	–	–	0 (0–45.9)	0 (0–45.9)	23.3 (9.9–42.3)	30.0 (14.7–49.4)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)
Shivering	Any	–	–	–	–	16.7 (0.4–64.1)	0 (0–45.9)	23.3 (9.9–42.3)	26.7 (12.3–45.9)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	0 (0–11.6)	0 (0–11.6)

Group 1.9 µg HA: Subjects aged 6 mo–9 y received two doses of 1.9 µg HA + $AS03_B$ (5.93 mg α-tocopherol) vaccine, 21 d apart. Group 3.75 µg HA: Subjects aged 10–17 y received two doses of 3.75 µg HA + $AS03_A$ (11.86mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence interval.

Table 4. Solicited general symptoms reported during the 7-d post-vaccination follow-up period after each vaccine dose (total vaccinated cohort)

Sweating	Any	–	–	–	–	33.3 (4.3–77.7)	0 (0–45.9)	6.7 (0.8–22.1)	10.0 (2.1–26.5)
	Grade 3	–	–	–	–	0 (0–45.9)	0 (0–45.9)	3.3 (0.1–17.2)	0 (0–11.6)

Group 1.9 μg HA: Subjects aged 6 mo–9 y received two doses of 1.9 μg HA + $AS03_B$ (5.93 mg α-tocopherol) vaccine, 21 d apart. Group 3.75 μg HA: Subjects aged 10–17 y received two doses of 3.75 μg HA + $AS03_A$ (11.86mg α-tocopherol) vaccine, 21 d apart. [a]N: Number of subjects with available results. [b]CI: Confidence interval.

slightly lower frequencies of solicited symptoms reported following the second dose as compared that after the first dose in the referenced study was reversed in the present study.[28] In another pediatric study with a non-adjuvanted, trivalent seasonal influenza vaccine in subjects aged 6–9 y and 10–13 y, a similar trend of comparatively higher reactogenicity after the second vaccine dose was observed.[29] A recent report from six studies in children showed that the frequency of solicited local and general symptoms following vaccination with a MF59-adjuvanted seasonal influenza vaccine was comparatively (although not significantly) higher than that following vaccination with a non-adjuvanted seasonal influenza vaccine.[30]

In Japan, the AS03-adjuvanted H1N1 2009 pandemic influena vaccine was approved as a 1.9 μg HA dose in children aged 6 mo–9 y and as a 3.75 μg HA dose in children aged 10 y and older, making the age-specific immunogenicity data obtained from this study particularly relevant. Also, the fact that a microneutralisation assay was used in parallel with the conventional HI assay for immunological assessments makes the findings pertinent as while HI assays are largely restricted to measuring the receptor-binding blocking activity of antibodies, theoretically, neutralisation assays can capture a broad range of anti-influenza antibody activities able to interrupt several steps of the infectious life cycle of the virus.[31,32] Further, the AS03-adjuvanted H1N1 2009 vaccines used in this study allowed dose-sparing, a property that could be beneficial in meeting the requirement for a large number of vaccine doses at the time of an influenza pandemic.

This study was restricted in drawing comparative conclusions on the persistence of the immune response following vaccination with other adjuvanted or non-adjuvanted H1N1 2009 vaccines, as it is difficult to reliably compare HI results across studies.

In conclusion, the data from this study conducted with an AS03-adjuvanted H1N1 2009 pandemic influenza vaccine establishes that, following two doses of a 1.9 μg or 3.75 μg HA in children aged 6 mo–17 y, the immune response against the vaccine homologous A/California/7/2009 strain persists for at least six months after the first vaccine dose and the US and European guidance criteria for pandemic influenza vaccines were still met. The safety data from this study added to the existing repertoire of safety data in published literature on the safety of this H1N1 2009 vaccine. In addition, it may contribute to a better understanding of the safety of intramuscular vaccination in Japan. Intramuscular injection has not been allowed in Japan since 1970s after more than three thousands cases of muscular contracture being reported after intramuscular injection of antibiotics and antipyretics, but not vaccines.[33] This issue needs to be clarified urgently given that new combination vaccines and adjuvanted vaccines are expected to be introduced in Japan in the near future.

Materials and Methods

Study design and subjects. The primary phase of the study in Japan (NCT01001169) enrolled healthy children aged between 6 mo and 17 y before study start, without history of clinically-confirmed influenza infection or previous vaccination with a novel H1N1 vaccine or with any seasonal influenza vaccine within two weeks before study start. The subjects aged 6 mo to 9 y were further stratified by age (stratification ratio: 1:2) into 6–35 mo and 3–9 y age strata by the study personnel using GlaxoSmithKline (GSK) Biologicals' internet-based central randomization system (SBIR). Subjects aged 6 mo–9 y received 21 d apart, two 0.25 ml doses of the 1.9 μg $HA/AS03_B$ vaccine (Group 1.9 μg HA) and subjects aged 10–17 y received two 0.5 ml doses of the 3.75 μg $HA/AS03_A$ vaccine (Group 3.75 μg HA). All subjects received the first vaccine dose between Oct 27, 2009 and Nov 06, 2009, and the subjects aged 6 mo–9 y received the second vaccine dose by Nov 30, 2009. The treatment and vial lists were generated at GSK Biologicals using SAS® (Cary, NC USA) to assign treatments to subjects.

Written informed consent was obtained from the parents/guardians of all subjects prior to conducting any study-related procedures. Wherever deemed necessary, informed assent was collected from the subjects. The study was conducted in accordance with the Good Clinical Practice guidelines, the Declaration of Helsinki and local regulations. All study-related documents were approved by an Institutional Review Board.

Study vaccine. The study vaccine was developed and manufactured by GSK Biologicals. The H1N1 2009 pandemic influenza vaccine was a monovalent, inactivated, split-virion antigen with an oil-in-water emulsion-based Adjuvant System AS03 (*Arepanrix*™, a trademark of GlaxoSmithKline group of companies, Belgium). The H1N1 viral seed for the vaccine was prepared from the reassortant virus NYMC X-179A (New York Medical College, New York) generated from the A/California/07/2009 strain, as recommended by the World Health Organization (WHO).[34]

The AS03-adjuvanted H1N1 2009 pandemic influenza vaccine was prepared prior to administration by mixing the antigen suspension and adjuvant emulsion (1:1), both of which were available in separate multi-dose vials. Group 1.9 μg HA received $AS03_B$—an Adjuvant System containing 5.93 mg α-tocopherol

with 1.9 μg HA (0.25 ml injection dose) and Group 3.75 μg HA received $AS03_A$—an Adjuvant System containing 11.86 mg α-tocopherol with 3.75 μg of HA (0.5 ml injection dose).[35]

The first dose of the study vaccine was intramuscularly administered on Day 0 either into the anterolateral region of the thigh in children aged <12 mo or into the deltoid of the non-dominant arm in subjects aged 12 mo or more. On Day 21, the second dose of study vaccine was administered on the opposite side.

Immunogenicity assessments. Serum samples were collected before vaccination (Day 0), 21 d after each of the two vaccine doses (Day 21 and Day 42) and six months after the first vaccine dose (Day 182).

Haemagglutination inhibition (HI) assay [cut-off: ≥1:10] using chicken erythrocytes as previously described in reference 36, was performed at GSK Biologicals' central laboratory. The samples from Day 0, Day 21 and Day 42 were tested at the same time point, while the Day 182 samples were tested at a later time point.

The viral microneutralisation assay was performed at Viroclinics Biosciences BV. The sera were used after heat treatment at 56°C for 30 min. Each serum was tested in triplicate. The assay used a constant amount of A/Netherlands/602/2009 pandemic H1N1 Influenza virus (a A/California/07/2009-like virus) mixed with serial 2-fold dilutions of serum samples. The mixture of virus and antiserum was added to Madin-Darby Canine Kidney (MDCK) cell cultures and incubated for one hour at 33–35°C. Then virus-antibody mixture was removed from the wells, cells were fed with fresh culture medium and further incubated for 6 d at 33–35°C. After the incubation period, virus replication was visualized by haemagglutination of red blood cells. The 50% neutralisation titer of a serum was calculated by the method of Reed and Muench.[37] The cut-off value of the assay was 1:8.

The assessment of the immune response was based on the seroconversion rate (SCR: percentage of subjects with pre-vaccination titer <1:10 and post-vaccination titer ≥1:40, or pre-vaccination titer >1:10 and at least 4-fold increase in post-vaccination titer), seroprotection rate (SPR: percentage of subjects with a post-vaccination titer ≥1:40) and geometric mean fold rise (GMFR: post-vaccination fold increase in geometric mean titers [GMTs]) in terms of HI antibodies against the vaccine homologous strain and on the the Vaccine Response Rates (VRRs: percentage of subjects with either a pre-vaccination titer <1:8 and a post-vaccination titer ≥1:32, or a pre-vaccination titer ≥1:8 and at least a 4-fold increase in post-vaccination titer) in terms of neutralising antibodies against a strain antigenically similar to the vaccine strain.

The outcome measures of the immune response included evaluation based on the immunogenicity criteria for pandemic influenza vaccines in adults as required by the Committee for Medicinal Products for Human Use (CHMP; point estimates for HI antibody SCR: >40%, SPR: >70% and GMFR: >2.5) and Center for Biologics Evaluation and Research (CBER; lower bound of 95% confidence interval [CI] for HI antibody for SCR: ≥40% and SPR: ≥70%).[26,27] In consideration of multiplicity of statistical analysis caused by co-primary endpoints of the study, 97.5% confidence intervals (CIs) were applied instead of 95% CIs (requirement of CBER guidance) for evaluation of the primary endpoints at Day 42.

Safety and reactogenicity assessments. Diary cards were used by parents/guardians to record solicited local and general adverse events up to seven days following each vaccine dose; unsolicited adverse events were recorded up to 84 d following the first vaccine dose; medically-attended events (MAEs), potential immune-mediated diseases (pIMD) and serious adverse events (SAEs) occurring during the entire study period were recorded.

Intensity of solicited symptoms was graded on a standard scale of (0–3), where Grade 1 symptoms were defined as those that were noticeable but did not interfere with normal activities and Grade 3 symptoms were defined as those that prevented normal activities (Grade 3 redness and swelling: diameter >100 mm; Grade 3 fever: temperature ≥39°C [≥102.2°F]). SAEs and pIMDs (subset of adverse events that include both autoimmune diseases and other inflammatory and/or neurologic disorders which may or may not have an autoimmune etiology) occurring throughout the study period were also recorded. Clinical laboratory parameters were assessed at all seven visits up to Day 182.

Statistical analyses. The sample size was calculated based on the co-primary objectives of the study using the results from the most recent studies with the H1N1 2009 vaccine as a reference. A population of 60 subjects (30 subjects in each study group) accounting for ≤10% dropout was estimated to provide a power of 84.9% to achieve the co-primary objectives, assuming log (standard deviation) for GMT to be 0.6.

The analyses of immunogenicity were performed on the According-To-Protocol (ATP) cohort that included subjects who received both vaccine doses as per protocol, complied with all protocol-defined procedures and for whom the assay results were available at the given time points (at Day 42 and Day 182). Seropositivity was defined as antibody titers greater than or equal to the cut-off value of each assay. For the purpose of GMT calculations, antibody titers below the cut-off value of each assay were substituted by half of the cut-off value.

The analyses of safety were performed on the Total Vaccinated Cohort (TVC) which included all subjects who received at least one documented vaccine dose.

Disclosure of Potential Conflicts of Interest

Dr. A. Nagai was the principal investigator, Dr. A. Saitoh and Dr. T. Kato contributed as a supervisor in this study funded by GlaxoSmithKline. All participating institutions received compensation for study involvement. Drs. K. Tenjinbaru, D. Vaughn, F. Roman and P. Li are employees of GlaxoSmithKline Biologicals. D. Vaughn and F. Roman report ownership of stock options.

Financial Disclosure

GlaxoSmithKline Biologicals was the funding source and was involved in all stages of the study conduct and analysis (ClinicalTrials.gov Identifier: NCT01001169). 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 the corresponding author had final responsibility to submit for publication.

Trademark Statement

Arepanrix is a trade mark of the GlaxoSmithKline group of companies, Belgium.

Acknowledgements

All authors participated in the implementation of the study including substantial contributions to conception and design, the gathering of the data, or analysis and interpretation of the data. All authors were involved in the drafting of the article or revising it critically for important intellectual content, and final approval of the manuscript.

We are grateful to the New York Medical College, New York for providing the vaccine virus reassortant and to the National Institute for Biological Standards and Control (NIBSC, UK) and Therapeutic Goods Administration (TGA) from the Australian Government for providing the reference standards. The authors are indebted to the participating study volunteers and their parents, clinicians, nurses and laboratory technicians at the study sites as well as to the sponsor's project staff for their support and contributions throughout the study. We are grateful to all teams of GSK Biologicals for their contribution to this study, especially Shinobu Tamura, Hiroshi Tamura and Kazunori Yagi for clinical study management and site monitoring, and Roger Bernhard and Urban Lundberg from the clinical and serological laboratory teams, Dorothy Slavin (Clinical Safety Representative) and Edith Lepine for project management. Finally the authors thank Dr. Karl Walravens for critical review of the manuscript, Avishek Pal (GSK Biologicals) for providing medical writing services and Dr. Wendy Van Doorslaer (XPE Pharma and Science, on behalf of GSK Biologicals) for editorial assistance and manuscript coordination.

References

1. Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al.; Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; 360:2605-15; PMID:19423869; http://dx.doi.org/10.1056/NEJMoa0903810.
2. Pandemic (H1N1) 2009—update 112. Geneva: World Health Organization, August 06, 2010. Available at: http://www.who.int/csr/don/2010_08_06/en/index.html. Accessed October 26, 2011.
3. Domínguez-Cherit G, Lapinsky SE, Macias AE, Pinto R, Espinosa-Perez L, de la Torre A, et al. Critically Ill patients with 2009 influenza A(H1N1) in Mexico. JAMA 2009; 302:1880-7; PMID:19822626; http://dx.doi.org/10.1001/jama.2009.1536.
4. Rojo JC, Ruiz-Contreras J, Fernández MB, Marín MA, Folgueira L. Influenza-related hospitalizations in children younger than three years of age. Pediatr Infect Dis J 2006; 25:596-601; PMID:16804428; http://dx.doi.org/10.1097/01.inf.0000220208.59965.95.
5. Centers for Disease Control and Prevention (CDC). 2009 pandemic influenza A (H1N1) virus infections—Chicago, Illinois, April–July 2009. MMWR Morb Mortal Wkly Rep 2009; 58:913-8; PMID:19713879.
6. Ministry of Health. Labour and Welfare (MHLW); Press Release (February 05, 2010). Available at: http://www.mhlw.go.jp/kinkyu/kenkou/influenza/houdou/2010/02/dl/infuh0205-05.pdf. Accessed October 26, 2011. Japanese.
7. Ministry of Health. Labour and Welfare (MHLW); Press Release (August 11, 2010). Available at: http://www.mhlw.go.jp/kinkyu/kenkou/influenza/houdou/2010/08/dl/infuh0811-01.pdf. Accessed October 26, 2011. Japanese.
8. Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. Lancet 2010; 375:1100-8; PMID:20096450; http://dx.doi.org/10.1016/S0140-6736(09)62126-7.
9. Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al.; Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. [Erratum in: N Engl J Med 2009; 361:102]. N Engl J Med 2009; 360:2605-15; PMID:19423869; http://dx.doi.org/10.1056/NEJMoa0903810.
10. Kamigaki T, Oshitani H. Epidemiological characteristics and low case fatality rate of pandemic (H1N1) 2009 in Japan. PLoS Curr Influenza 2009; December 20: RRN1139. Available at: http://knol.google.com/k/taro-kamigaki/epidemiological-characteristics-and-low/38epug6fmizmk/1#. Accessed October 26, 2011.
11. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. N Engl J Med 2009; 361:2424-35; PMID:19745215; http://dx.doi.org/10.1056/NEJMoa0907650.
12. Liang XF, Wang HQ, Wang JZ, Fang HH, Wu J, Zhu FC, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2010; 375:56-66; PMID:20018364; http://dx.doi.org/10.1016/S0140-6736(09)62003-1.
13. Strategic Advisory Group of Experts on Immunization. Report of the extraordinary meeting on the influenza A (H1N1) 2009 pandemic, 7 July 2009. Available at: www.who.int/wer/2009/wer8430.pdf. Accessed October 26, 2011.
14. Chu DWS, Hwang SJ, Lim FS, Oh HM, Thongcharoen P, Yang PC, et al.; H5N1 Flu Study Group for Hong Kong, Singapore, Taiwan and Thailand. Immunogenicity and tolerability of an AS03(A)-adjuvanted prepandemic influenza vaccine: a phase III study in a large population of Asian adults. Vaccine 2009; 27:7428-35; PMID:19683087; http://dx.doi.org/10.1016/j.vaccine.2009.07.102.
15. Nagai H, Ikematsu H, Tenjinbaru K, Maeda A, Dramé M, Roman FP. A phase II, open-label, multicentre study to evaluate the immunogenicity and safety of an adjuvanted prepandemic (H5N1) influenza vaccine in healthy Japanese adults. BMC Infect Dis 2010; 10:338; PMID:21108818; http://dx.doi.org/10.1186/1471-2334-10-338.
16. Leroux-Roels I, Borkowski A, Vanwolleghem T, Dramé M, Clement F, Hons E, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. Lancet 2007; 370:580-9; PMID:17707753; http://dx.doi.org/10.1016/S0140-6736(07)61297-5.
17. Leroux-Roels I, Bernhard R, Gérard P, Dramé M, Hanon E, Leroux-Roels G. Broad Clade 2 cross-reactive immunity induced by an adjuvanted clade 1 rH5N1 pandemic influenza vaccine. PLoS One 2008; 3:1665; PMID:18301743; http://dx.doi.org/10.1371/journal.pone.0001665.
18. Roman F, Vaman T, Gerlach B, Markendorf A, Gillard P, Devaster JM. Immunogenicity and safety in adults of one dose of influenza A H1N1v 2009 vaccine formulated with and without $AS03_A$-adjuvant: preliminary report of an observer-blind, randomised trial. Vaccine 2010; 28:1740-5; PMID:20034605; http://dx.doi.org/10.1016/j.vaccine.2009.12.014.
19. Carmona A, Omeñaca F, Tejedor JC, Merino JM, Vaman T, Dieussaert I, et al. Immunogenicity and safety of AS03-adjuvanted 2009 influenza A H1N1 vaccine in children 6–35 months. Vaccine 2010; 28:5837-44; PMID:20600478; http://dx.doi.org/10.1016/j.vaccine.2010.06.065.
20. Ikematsu H, Nagai H, Kawashima M, Kawakami Y, Tenjinbaru K, Maeda A, et al. Immunogenicity and safety of a novel AS03(A)-adjuvanted H1N1 2009 pandemic influenza vaccine in adults in Japan. Hum Vaccin 2010; 6:888-93; PMID:20980795; http://dx.doi.org/10.4161/hv.6.11.12851.
21. Saitoh A, Tamura S, Nagai A, Tsuchida N, Sako M, Maekawa T, et al. Clinical evaluation of an AS03-adjuvanted pandemic influenza H1N1 2009 vaccine in children (Preliminary report). [Japanese]. J Jap Pediatr Soc 2011; 115:578-84.
22. Waddington CS, Walker WT, Oeser C, Reiner A, John T, Wilkins S, et al. Safety and immunogenicity of $AS03_B$ adjuvanted split virion versus non-adjuvanted whole virion H1N1 influenza vaccine in UK children aged 6 months-12 years: open label, randomised, parallel group, multicentre study. BMJ 2010; 340:2649; PMID:20508026; http://dx.doi.org/10.1136/bmj.c2649.
23. Van Buynder PG, Dhaliwal JK, Van Buynder JL, Couturier C, Minville-Leblanc M, Garceau R, et al. Protective effect of single-dose adjuvanted pandemic influenza vaccine in children. Influenza Other Respi Viruses 2010; 4:171-8; PMID:20629771; http://dx.doi.org/10.1111/j.1750-2659.2010.00146.x.
24. Nicholson KG, Abrams KR, Batham S, Clark TW, Hoschler K, Lim WS, et al. Immunogenicity and safety of a two-dose schedule of whole-virion and $AS03_A$-adjuvanted 2009 influenza A (H1N1) vaccines: a randomised, multicentre, age-stratified, head-to-head trial. Lancet Infect Dis 2011; 11:91-101; PMID:21168369; http://dx.doi.org/10.1016/S1473-3099(10)70296-6.
25. Madhun AS, Akselsen PE, Sjursen H, Pedersen G, Svindland S, Nøstbakken JK, et al. An adjuvanted pandemic influenza H1N1 vaccine provides early and long term protection in health care workers. Vaccine 2010; 29:266-73; PMID:21034828; http://dx.doi.org/10.1016/j.vaccine.2010.10.038.

26. European Committee for Proprietary Medicinal Products (CHMP). Guideline on influenza vaccine prepared from viruses with the potential to cause a pandemic and intended for use outside of the core dossier context (EMEA/CHMP/VWP/263499/2006). European Agency for the Evaluation of Medicinal Products, January 24, 2007.
27. US Food and Drug Administration. (FDA) Guidance for Industry. Clinical data needed to support the licensure of pandemic influenza vaccines. US Food and Drug Administration May 2007. Available at: http://www.fda.gov/cber/gdlns/panfluvac.htm. Accessed October 26, 2011.
28. Baxter R, Jeanfreau R, Block SL, Blatter M, Pichichero M, Jain VK, et al. A Phase III evaluation of immunogenicity and safety of two trivalent inactivated seasonal influenza vaccines in US children. Pediatr Infect Dis J 2010; 29:924-30; PMID:20431425; http://dx.doi.org/10.1097/INF.0b013e3181e075bc.
29. Schmidt-Ott R, Schwarz T, Haase R, Sander H, Walther U, Fourneau M, et al. Immunogenicity and reactogenicity of a trivalent influenza split vaccine in previously unvaccinated children aged 6–9 and 10–13 years. Vaccine 2007; 26:32-40; PMID:18022736; http://dx.doi.org/10.1016/j.vaccine.2007.10.049.
30. sdsd.
31. Han T, Marasco WA. Structural basis of influenza virus neutralization. Ann NY Acad Sci 2011; 1217:178-90; http://dx.doi.org/10.1111/j.1749-6632.2010.05829.x; PMID:21251008.
32. Bachmann MF, Ecabert B, Kopf M. Influenza virus: a novel method to assess viral and neutralizing antibody titers in vitro. J Immunol Methods 1999; 225:105-11; PMID:10365787; http://dx.doi.org/10.1016/S0022-1759(99)00034-4.
33. Japan Pediatric Society Committee on Muscular Contracture. A report of muscular contracture. J Jpn Pediatr Soc 1983; 87:1067-105.
34. World Health Organization (WHO). Global Alert and Response (GAR). Pandemic influenza A (H1N1) 2009 virus vaccine—conclusions and recommendations from the October 2009 meeting of the immunization Strategic Advisory Group of Experts, December 04, 2009. Available at: http://www.who.int/csr/disease/swineflu/meetings/sage_oct_2009/en/. Accessed October 26, 2011.
35. Morel S, Didierlaurent A, Bourguignon P, Delhaye S, Baras B, Jacob V, et al. Adjuvant System AS03 containing α-tocopherol modulates innate immune response and leads to improved adaptive immunity. Vaccine 2011; 29:2461-73; PMID:21256188; http://dx.doi.org/10.1016/j.vaccine.2011.01.011.
36. Hehme NW, Künzel W, Petschke F, Türk G, Raderecht C, van Hoecke C, et al. Ten years of experience with the trivalent split-influenza vaccine, *Fluarix*™. Clin Drug Investig 2002; 22:751-69; http://dx.doi.org/10.2165/00044011-200222110-00004.
37. Reed LT, Muench H. A simple method of calculating fifty percent end point. Am J Hyg 1938; 27:493-8.