blood-collections, while the other was a plateletpheresis from an O-donor. Donor and donation characteristics were similar among the TMA-positive and -negative donations ($p > 0.05$ for all variables, Fisher's exact test). In a multivariable model adjusted for age, donors with residence in the San Juan metropolitan area were approximately three times more likely than donors residing outside of the metropolitan area to be TMA-positive (adjusted odds ratio, 3.0; 95% confidence interval, 0.9-10.1).

The five blood donations that had the highest S/CO ratios on initial TMA testing were the only specimens to be TMA-reactive at a dilution of 1:16 (Table 2). Four of these five specimens were positive by RT-PCR and had quantifiable viral loads ranging from 2×10^3 to 8×10^7 viral RNA copies per mL. Three were identified as DENV-2 and the other as DENV-3. DENV was cultured from three of the four specimens, two by mosquito inoculation and one in cell culture. DENV-2 and DENV-3 were the predominant serotypes in circulation in Puerto Rico in 2005.

Serologic testing of the 12 TMA-positive blood donations revealed that only 1 was IgM-positive and 9 were IgG-positive by ELISA (Table 2). The lack of IgG antibody titers in Specimens 1, 4, and 8 indicates no previous dengue infections in these patients. The presence of IgG antibodies in the absence of IgM antibodies could reflect evidence of previous infections in Specimens 2, 3, 5, 7, 9, 10, 11, and 12, and IgG titers equal or greater than 1:163,840 in Specimens 3, 7, 9, and 10 indicate a recent or current secondary infection in those patients.[32] The presence of IgG in the sole donor with IgM antibodies (Specimen 6) could similarly be reflective of recent or current infection.

Other than the 12 TMA-positive specimens, there were an additional three IR specimens with S/CO ratios on initial testing of 1.00, 1.03, and 11.58 and on repeat testing of 0.92, 0.40, and 0.07, respectively. All were negative on PCR, IgM MAC-ELISA, and virus recovery. They were, however, positive on IgG ELISA. In the WNV TMA assay, an S/CO ratio of greater than or equal to 17 has a positive predictive value for confirmation of 95 percent (ARC data, unpublished); it is likely that this relationship is the same for DENV TMA.

TABLE 1. Characteristics of all and TMA-positive blood donors in Puerto Rico, September 20 to December 5, 2005*

Characteristic	All donors (n = 16,521)	TMA-positive donors (n = 12)
Age (years)	37.0 (13-85)	36.5 (16-65)
Male	10,654 (64.5)	8 (67)
Donation status		
First-time donor	5,056 (30.6)	5 (42)
Repeat donor	11,465 (69.4)	7 (58)
Region of residence		
San Juan Metropolitan Area	6,631 (40.1)	8 (67)
East	5,182 (31.4)	3 (25)
West	4,706 (28.5)	1 (8)
Phlebotomy procedure		
Whole blood	15,838 (95.9)	11 (92)
Plateletpheresis	627 (3.8)	1 (8)
Plateletpheresis/RBC pheresis	48 (0.3)	0 (0)
Double RBC pheresis	7 (0.0)	0 (0)
Leukapheresis	1 (0.0)	0 (0)
Donation type		
Allogeneic	16,400 (99.3)	12 (100)
Directed	67 (0.4)	0 (0)
Autologous	54 (0.3)	0 (0)
Region of donation site		
San Juan Metropolitan Area	8,984 (54.4)	8 (67)
East	3,870 (23.4)	4 (33)
West	3,667 (22.2)	0 (0)

* Data are reported as median (range) or number (%).

TABLE 2. Results of supplementary testing of TMA IR specimens (n = 12)

	TMA test Gen-Probe (S/CO ratio)*			Supplementary testing CDC dengue branch					
Specimen	Initial test	Second test	1:16	PCR†	Number viral RNA/mL	IgM‡	IgG	Cell culture	Mosquito inoculation
1	31.96	26.99	27.73	D2	7.14×10^3	0.229	Negative	Negative	D2
2	30.31	31.28	28.78	D3	8.12×10^7	0.337	1:10,240	Negative	D3
3	29.22	27.86	27.12	D2	7.74×10^5	0.409	1:163,840	D2	Negative
4	29.17	24.84	22.92	D2	2.0×10^3	0.229	Negative	Negative	Negative
5	23.89	20.59	8.54	Negative	Undetected	0.469	1:2,560	Negative	Negative
6	21.22	5.28	0.21	Negative	Undetected	8.870	1:160	Negative	Negative
7	17.78	23.10	0.15	Negative	Undetected	0.409	1:655,360	Negative	Negative
8	17.41	18.44	0.31	Negative	Undetected	0.198	Negative	Negative	Negative
9	17.24	21.05	0.33	Negative	Undetected	1.540	1:163,840	Negative	Negative
10	5.97	7.73	0.15	Negative	Undetected	0.440	1:655,360	Negative	Negative
11	4.08	4.15	0.13	Negative	Undetected	0.368	1:10,240	Negative	Negative
12	1.53	5.56	0.60	Negative	Undetected	0.270	1:2,560	Negative	Negative

* S/CO ≥ 1 considered to be reactive.
† D2 = DENV-2, D3 = DENV-3.
‡ >2.000 considered positive.

Nine of the 12 repeat-reactive samples had S/CO values in one or both tests of 17 or greater.

DISCUSSION

This study, and a similar one recently conducted using donations in Honduras, Brazil, and Australia,[29] are the first to document the presence of dengue viral nucleic acid in blood donations. In Puerto Rico, nearly 1 in 1000 donations was positive for the presence of dengue viral nucleic acid by TMA. Furthermore, live virus was recovered from three of the 12 TMA-positive donations, indicating that at least these 3 were capable of transmitting infection to recipients. The prevalence of dengue viral nucleic acid in blood donations in this study was similar to that estimated for WNV in the areas experiencing outbreaks in the continental United States in 2002[35] before universal screening using minipool NAT was implemented in July 2003.[22] Assuming an annual prevalence rate of 0.73 per 1000 (as found in this study) and that each donation is made into a mean of 1.45 transfusable components,[36] there may be as many as 56 potentially viremic donations and 81 components generated from the approximately 77,000 blood donations collected annually by the ARC in Puerto Rico. Dengue incidence is highly seasonal and varies considerably from year to year,[37,38] however, so the prevalence of potentially viremic donors could be considerably higher or lower than this figure at any given time. Furthermore, the three IR specimens lacking reproducible results in repeat TMA testing may have been true-positive specimens but with lower viral loads. If the case, this would underestimate the true prevalence of TMA positivity.

The unlinked study design did not permit contact with the recipients of the TMA-positive donations to assess whether transmission occurred. Nevertheless, virus was cultured from three donations and the viral loads of the four RT-PCR–positive donations indicate that their transfusion would have resulted in inocula orders of magnitude greater than the amount of virus secreted in the saliva of *Aedes* mosquitoes, documented to be as low as 10^2 viral particles per secretion.[39] The RT-PCR assay used in this study had lower sensitivity than the TMA assay, and it was not possible to assess the viral load of the RT-PCR–negative specimens.

Our results indicate the feasibility of NAT as a screening strategy for DENV, as has been successfully used for WNV. Of concern, we found that simulated minipool NAT (dilution 1:16) would not have detected the majority (7 of 12, or 58%) of the TMA-positive specimens; however, the experience with WNV suggests that not all of these donations may be infectious. Approximately 30 percent of WNV NAT–positive donations have viral loads below the limits of detection by minipool NAT and can only be detected by screening of individual donations.[23,40] Although WNV has been transmitted from transfusions detectable only by individual unit screening and with an estimated level of viremia as low as 0.06 plaque-forming units (PFUs) per mL (1 PFU is approximately 400 viral copies),[41] most donations only detectable by individual unit screening had IgM and IgG antibodies and were likely not infectious given the fact that nearly all WNV transfusion transmissions have occurred from antibody-negative donations.[22,25,42] Unfortunately, this same marker of infectivity is not applicable to dengue because of the high prevalence of preexisting, cross-reactive dengue antibodies in the population and the complex and variable serologic response after secondary dengue infection.[32,43]

The global incidence of dengue has risen more than 30-fold in the past 50 years. In areas where dengue is endemic, however, transfusion transmission of the agent is rarely investigated for many reasons, including the fact that this mode of transmission is difficult to prove against a background of endemic dengue. In such cases, the distinction between a recipient infection via mosquito-borne transmission as opposed to transfusion transmission may be too complex to distinguish. Furthermore, many dengue-endemic countries lack hemovigilance systems with sufficient resources to investigate cases of recipient infection that are potentially related to transfusion of blood components. Finally, sophisticated laboratory testing may not be readily available in many dengue-endemic countries and such testing is required to distinguish dengue from other arboviral infections as well as distinguishing current dengue infection from prior infections.

In contrast, when WNV entered the United States, it was against a background of a naïve population. This permitted the laboratory linkage of multiple transfusion recipients with WNV infection to a single infected donor within several clusters of WNV cases. Infectious virus and/or viral RNA could also be recovered from retrieved cocomponent plasma units; in these cases, WNV was readily identified in the absence of competing arboviral infections. The transmissibility of WNV via blood transfusion has been established, and our findings documenting the presence of DENV RNA in the Puerto Rican blood supply, at a level comparable to that which triggered screening of the US blood supply for WNV in 2003, highlight the risks to transfusion safety posed by emerging diseases such as the vector-borne flaviviruses. Further evaluation is required to assess the risk of dengue transmission by TMA-positive donations and the cost and benefit of routine dengue screening in endemic regions.

ACKNOWLEDGMENTS

The authors acknowledge the following individuals for their contribution to this research: Mrs Dilia Magaly Borges and Mr Antonio de Vera (ARC-Puerto Rico) for providing data specific to the PR-region of ARC; Dr Mark Beatty (Pediatric Dengue Vaccine

Initiative) for assisting with protocol development; Mr Gilberto Santiago and Mr Mark Verduin (Dengue Branch, CDC) for laboratory support; Mr Michael Johansson (Dengue Branch, CDC) for statistical support; and Dr Ed Notari (ARC), Dr Mathew Kuehnert (Office of Blood, Organ, and other Tissue Safety (proposed), CDC), Mr Joshua Smith, Dr Kate McElroy, Dr D. Fermín Argüello, and Dr Wellington Sun (Dengue Branch, CDC) for their scientific input in preparing the manuscript.

REFERENCES

1. Endy TP, Chunsuttiwat S, Nisalak A, Libraty DH, Green S, Rothman AL, Vaughn AL, Ennis FA. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng phet, Thailand. Am J Epidemiol 2002;156:40-51.
2. Rodriguez-Figueroa L, Rigau-Perez JG, Suarez EL, Reiter P. Risk factors for dengue infection during an outbreak in Yanes, Puerto Rico in 1991. Am J Trop Med Hyg 1995;52: 496-502.
3. Burke DS, Nisalak A, Johnson DE, Scott RM. A prospective study of dengue infections in Bangkok. Am J Trop Med Hyg 1988;38:172-80.
4. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis 2000;181:2-9.
5. Nishiura H, Halstead SB. Natural history of dengue virus (DENV)-1 and DENV-4 infections: reanalysis of classic studies. J Infect Dis 2007;195:1007-13.
6. Halstead S. Dengue hemorrhagic fever—a public health problem and a field for research. Bull World Health Organ 1980;58:1-21.
7. Haemorrhagic D. Fever: diagnosis, treatment, prevention and control, 2nd ed. Geneva, Switzerland: World Health Organization; 1997.
8. Thang Hung N, Trong Lan N. Improvement of case management—a key factor to reduce case-fatality rate of dengue hemorrhagic fever in Southern Viet Nam. Dengue Bull 2003;27:144-8.
9. Chareonsook O, Foy H, Teeratkul A, Silarug N. Changing epidemiology of dengue hemorrhagic fever in Thailand. Epidemiol Infect 1999;122:161-6.
10. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG, Gubler DJ; Hawaii Dengue Outbreak Investigation Team. Dengue fever, Hawaii, 2001-2002. Emerg Infect Dis 2005;11:742-9.
11. Moore C. Aedes albopictus in the United States: current status and prospects for further spread. J Am Mosq Control Assoc 1999;15:221-7.
12. Ramos M, Mohammed H, Zielinski-Gutierrez E, Hayden MH, Lopez JL, Fournier M, Trujillo AR, Burton R, Brunkard JM, Anaya-Lopez L, Banicki AA, Morales PK, Smith B, Munoz JL, Waterman SH, The Dengue Serosurvey Working Group. Epidemic dengue and dengue hemorrhagic fever at the Texas-Mexico Border: results of a household-based seroepidemiological survey, December 2005. Am J Trop Med Hyg 2008 Mar;78(3):364-9.
13. Reiter P, Lathrop S, Bunning M, Biggerstaff B, Singer D, Tiwari T, Baber L, Amador M, Thirion J, Hayes J, Seca C, Mendez J, Ramirez B, Robinson J, Rawlings J, Vorndam V, Waterman S, Gubler D, Clark G, Hayes E. Texas lifestyle limits transmission of dengue virus. Emerg Infect Dis 2003; 9:86-9.
14. Tsang C. Local dengue fever cases in 2002. Hong Kong, China: Public Health and Epidemiology Bulletin—Department of Health; 2002.
15. Rigau-Perez JG, Vorndam AV, Clark GG. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994-1995. Am J Trop Med Hyg 2001;64:67-74.
16. Tan FL-S, Loh DL, Prabhakaran K. Dengue haemorrhagic fever after living donor renal transplantation. Nephrol Dial Transplant 2005;20:447-8.
17. Chen LN, Wilson ME. Nosocomial mucutaneous transmission and other routes of transmission. Emerg Infect Dis 2005;11:775.
18. De Wazieres B, Gil H, Vuitton D, Dupond J. Nosocomial transmission of dengue from a needle stick injury. Lancet 1998;351:498.
19. Nemes Z, Kiss G, Madarassi EP, Peterfi Z, Ferenczi E, Bakonyi T, Ternak G. Nosocomial transmission of dengue [letter]. Emerg Infect Dis 2004;10:1880-1.
20. Wagner D, de With K, Huzly D, Hufert F, Weidmann M, Breisinger S, Eppinger S, Kern WV, Bauer TM. Nosocomial acquisition of dengue. Emerg Infect Dis 2004;10:1872-3.
21. Hirsch J, Deschampes C, Lhuillier M. Metropolitan transmission of dengue by accidental inoculation at a hospital. Ann Intern Med 1990;141:629.
22. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, Stobierski MG, Signs K, Newman B, Kapoor H, Goodman JL, Chamberland ME; West Nile Virus Transmission Investigation Team. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
23. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 2005;353:451-9.
24. Chien LJ, Liao TL, Shu PY, Huang JH, Gubler DJ, Chang GJ. Development of real-time reverse transcriptase PCR assays to detect and serotype dengue viruses. J Clin Microbiol 2006;44:1295-304.
25. Busch MP, Tobler LH, Saldanha J, Caglioti S, Shyamala V, Linnen JM, Gallarda J, Phelps B, Smith RI, Drebot M, Kleinman SH. Analytical and clinical sensitivity of West Nile virus RNA screening and supplemental assays available in 2003. Transfusion 2005;45:492-9.
26. Giachetti C, Linnen JM, Kolk DP, Dockter J, Gillotte-Taylor K, Park M, Ho-Sing-Loy M, McCormick MK, Mimms LT,

McDonough SH. Highly sensitive multiplex assay for detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J Clin Microbiol 2002;40:2408-19.
27. McCormick MK, Dockter J, Linnen JM, Kolk D, Wu Y, Giachetti C. Evaluation of a new molecular assay for detection of human immunodeficiency virus type 1 RNA, hepatitis C virus RNA, and hepatitis B virus DNA. J Clin Virol 2006;36:166-76.
28. Linnen JM, Deras ML, Cline J, Wu W, Broulik AS, Cory RE, Knight JL, Cass MM, Collins CS, Giachetti C. Performance evaluation of the PROCLEIX West Nile virus assay on semi-automated and automated systems. J Med Virol 2007;79: 1422-30.
29. Linnen JM, Vinelli E, Sabino EC, Tobler LH, Hyland C, Lee TH, Kolk DP, Broulik AS, Collins CS, Lanciotti RS, Busch MP. Dengue viremia in blood donors from Honduras, Brazil and Australia. Transfusion DOI: 10.1111/j.1537-2995.2008.01772.x
30. Linnen JM, Deras ML, Cline J, Wu W, Broulik AS, Cory RE, Knight JL, Cass MM, Collins CS, Giachetti C. Performance evaluation of the PROCLEIX West Nile virus assay on semi-automated and automated systems. J Med Virol 2007;79: 1422-30.
31. Burke DS, Nisalak A, Ussery MA. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J Clin Microbiol 1982;16:1034-42.
32. Miagostovich MP, Nogueira RM, dos Santos FB, Schatzmayr HG, Araujo ES, Vorndam V. Evaluation of an IgG enzyme-linked immunosorbent assay for dengue diagnosis. J Clin Microbiol 1999;14:183-9.
33. Rosen L, Gubler D. The use of mosquitoes to detect and propagate dengue viruses. Am J Trop Med Hyg 1974;23: 1153-60.
34. Gubler DJ, Kuno G, Sather GE, Velez M, Oliver A. Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. Am J Trop Med Hyg 1984;33:158-65.
35. Biggerstaff BJ, Petersen LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 2003;43:1007-17.
36. Marshall DA, Kleinman SH, Wong JB, AuBuchon JP, Grima DT, Kulin NA, Weinstein MC. Cost-effectiveness of nucleic acid test screening of volunteer blood donations for hepatitis B, hepatitis C and human immunodeficiency virus in the United States. Vox Sang 2004;86:28-40.
37. Rigau-Perez JG. Dengue activity in Puerto Rico during an interepidemic period (1995-1997). Am J Trop Med Hyg 2001;64:75-83.
38. Rigau-Perez JG. The reappearance of dengue-3 and a subsequent dengue-4 and dengue-1 epidemic in Puerto Rico in 1998. Am J Trop Med Hyg 2002;67:355-62.
39. Kraiselburd E, Gubler DJ, Kessler MJ. Quantity of dengue virus required to infect rhesus monkeys. Trans R Soc Trop Med Hyg 1985;79:248-51.
40. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.
41. Montgomery SP, Brown JA, Kuehnert M, Smith TL, Crall N, Lanciotti RS, Macedo de Oliveira A, Boo T, Marfin AA; 2003 West Nile Virus Transfusion-Associated Transmission Investigation Team. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. Transfusion 2006;46:2038-46.
42. Petersen LR, Epstein JS. Problem solved? West Nile virus and transfusion safety. N Engl J Med 2005;353:516-7.
43. Gubler DJ, Sather GE. Laboratory diagnosis of dengue and dengue hemorrhagic fever. In: Homma A, Cunha JF, editors. Proceedings of the international symposium on yellow fever and dengue. Rio de Janeiro, Brazil: Rio de Janeiro; 1988. p. 291-322.

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008 年 6 月 13 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. Ehrlich, H.J. et al, New Engl. J. Med., 358, 2573-2584 (2008)	公表国 オーストリア	
販売名（企業名）						

研究報告の概要

本稿では，抗 H5N1 型鳥インフルエンザワクチンの安全性及び有効性を検討する無作為化用量漸増第 1・2 相試験の試験成績について記載した。Baxter Bioscience 社が同ワクチンを開発し，試験を実施した。当該ワクチンの主な特徴は，サルの腎臓細胞の培養株（ベロ細胞）で作成された自然発生するウイルス株 A/Vietnam/1203/2004 を利用していることである。ミョウバンアジュバントによる作用も検討し，ウイルス全体をワクチンとして使用した。有効性エンドポイントとして，ワクチンの（i）ヘマグルチニン阻害を生じさせる能力，（ii）中和抗体を誘発する能力，（iii）注射 21 日後にセロコンバージョンを生じさせる能力を検討した。各被験者に対し，それぞれ 3.75，7.5，15 又は 30 ug のヘマグルチニン抗原を含有するワクチンをアジュバントとともに，もしくは 7.5 又は 15 ug の抗原を含有するワクチンをアジュバントなしで 21 日の間隔をおいて 2 回投与した。免疫寛容は非常に良好に成立した。いずれのワクチン処方でも，注射部位の軽度疼痛（被験者の 9～27%）及び頭痛（被験者の 6～31%）が最も高頻度に報告された有害事象であった。有効性に関する限り，免疫応答はアジュバントなしの処方を投与した被験者において最も高い割合で認められたが，いずれの処方でも第 21 日目から第 42 日目で中和抗体の抗体価は同程度に増加した。さらに，ウイルス株 A/Indonesia/05/2005 及び A/Hong Kong/156/1997 に対する交差中和が認められた。再度，アジュバントなしの処方が最も高い免疫原性を示した。本試験では重要な用量反応性の関連が示されなかったことから，今後の開発にあたってアジュバントなしの 7.5 ug の処方が選択された。

使用上の注意記載状況・その他参考事項等

BYL-2008-0338

報告企業の意見	今後の対応
抗 H5N1 型鳥インフルエンザワクチンが利用可能となれば、パンデミックの発生及び拡大を防ぐために有効であろう。血漿由来製剤工程におけるウイルス除去は、インフルエンザウイルス除去に対しても有効と考えられる。弊社製品の製造工程に使用されている血漿分画成分は，製造工程中のウイルスバリデーションにおいて，インフルエンザウイルスと同様のエンベロープ RNA ウイルスである HIV（レトロウイルス）の不活化・除去能が確認されている。各成分の製造工程における不活化・除去能は以下のとおり。 • アルブミン・カッター及びコージネイト FS の製造工程培地に使用されているヒト血清アルブミン：17.8 log 以上 • プラスマネート・カッター，コージネイト FS 及びコージネイト FS バイオセットの製造工程培地に使用されている加熱ヒト血漿タンパク：15 log 以上 • コージネイト FS の製造工程に使用されているトランスフェリン：9.1 log 以上 • ベタフェロン皮下注，ゼヴァリン イットリウム (^{90}Y) 静注用セット及びゼヴァリン インジウム (^{111}I) 静注用セットの製造工程に使用されているヒト血清アルブミン：9.98 log 以上	現時点で新たな安全対策上の措置を講じる必要はないと考える。

ORIGINAL ARTICLE

A Clinical Trial of a Whole-Virus H5N1 Vaccine Derived from Cell Culture

Hartmut J. Ehrlich, M.D., Markus Müller, M.D., Helen M.L. Oh, M.D., Paul A. Tambyah, M.B., B.S., Christian Joukhadar, M.D., Emanuele Montomoli, Ph.D., Dale Fisher, F.R.A.C.P., Greg Berezuk, M.S., Sandor Fritsch, Ph.D., Alexandra Löw-Baselli, Ph.D., Nina Vartian, Ph.D., Roman Bobrovsky, Ph.D., Borislava G. Pavlova, Ph.D., Eva Maria Pöllabauer, M.D., Otfried Kistner, Ph.D., and P. Noel Barrett, Ph.D., for the Baxter H5N1 Pandemic Influenza Vaccine Clinical Study Team

ABSTRACT

BACKGROUND

Widespread infections of avian species with avian influenza H5N1 virus and its limited spread to humans suggest that the virus has the potential to cause a human influenza pandemic. An urgent need exists for an H5N1 vaccine that is effective against divergent strains of H5N1 virus.

METHODS

In a randomized, dose-escalation, phase 1 and 2 study involving six subgroups, we investigated the safety of an H5N1 whole-virus vaccine produced on Vero cell cultures and determined its ability to induce antibodies capable of neutralizing various H5N1 strains. In two visits 21 days apart, 275 volunteers between the ages of 18 and 45 years received two doses of vaccine that each contained 3.75 μg, 7.5 μg, 15 μg, or 30 μg of hemagglutinin antigen with alum adjuvant or 7.5 μg or 15 μg of hemagglutinin antigen without adjuvant. Serologic analysis was performed at baseline and on days 21 and 42.

RESULTS

The vaccine induced a neutralizing immune response not only against the clade 1 (A/Vietnam/1203/2004) virus strain but also against the clade 2 and 3 strains. The use of adjuvants did not improve the antibody response. Maximum responses to the vaccine strain were obtained with formulations containing 7.5 μg and 15 μg of hemagglutinin antigen without adjuvant. Mild pain at the injection site (in 9 to 27% of subjects) and headache (in 6 to 31% of subjects) were the most common adverse events identified for all vaccine formulations.

CONCLUSIONS

A two-dose vaccine regimen of either 7.5 μg or 15 μg of hemagglutinin antigen without adjuvant induced neutralizing antibodies against diverse H5N1 virus strains in a high percentage of subjects, suggesting that this may be a useful H5N1 vaccine. (ClinicalTrials.gov number, NCT00349141.)

From the Department of Global Research and Development, Baxter BioScience (H.J.E., G.B., S.F., A.L.-B., N.V., R.B., B.G.P., E.M.P., O.K., P.N.B.), and the Department of Clinical Pharmacology, Medical University of Vienna, Vienna General Hospital (M.M., C.J.) — both in Vienna; Changi General Hospital (H.M.L.O.) and the National University of Singapore and National University Hospital (P.A.T., D.F.) — all in Singapore; and the University of Siena, Siena, Italy (E.M.). Address reprint requests to Dr. Müller at the Department of Clinical Pharmacology, Medical University of Vienna, Vienna General Hospital (AKH), Währinger Gürtel 18-20, 1090 Vienna, Austria, or at markus.mueller@meduniwien.ac.at.

Drs. Ehrlich and Müller contributed equally to this article.

N Engl J Med 2008;358:2573-84.

THE EMERGENCE OF A NEW HUMAN INfluenza pandemic caused by an avian virus strain is possible. Vaccination against pandemic influenza is considered to be the most effective option to limit its spread. However, the conventional approaches to the manufacture of influenza vaccines have a number of disadvantages and raise concern about whether sufficient quantities of an effective vaccine can be made available early enough at the onset of a pandemic to have a major effect on public health.[1] In addition, clinical studies of conventional split-vaccine formulations without adjuvant have shown poor immunogenicity.[2,3] It has been suggested that whole-virus vaccines have the potential to be more immunogenic than split-virus or subunit vaccines in previously unvaccinated populations.[4,5] The first clinical study of a whole-virus vaccine against avian influenza H5N1 virus showed that a substantially reduced antigen dosage (10 μg) with an alum formulation induced seroconversion in nearly 100% of subjects.[6]

All these studies were carried out with vaccines manufactured by conventional methods (i.e., with the use of embryonated chicken eggs and modified, attenuated reassortant viruses produced by reverse genetics).[7] We have devised a strategy for the development of an H5N1 vaccine that involves the use of a wild-type virus (i.e., the strain circulating in nature) grown in a Vero cell culture. This strategy has the advantage that the lead time for pandemic vaccine production can be reduced, since the generation of attenuated reassortants is not required, although the requirement for the use of enhanced biosafety level 3 (BSL-3) facilities for such a strategy is a relative drawback. In addition, cell culture provides a robust manufacturing platform that eliminates dependence on embryonated chicken eggs, which would be an advantage in the event of limited availability of such eggs during a pandemic caused by a highly pathogenic avian virus. This technique was used to develop a whole-virus vaccine that was highly immunogenic in animal models.[8] We report on the safety and immunogenicity of this vaccine, using formulations with and without alum adjuvant.

METHODS

STUDY DESIGN AND OBJECTIVE

From June 2006 through September 2006, we enrolled a total of 284 men and women between the ages of 18 and 45 years in a randomized, partially blinded (between groups) clinical trial at three sites: one in Austria and two in Singapore. The study was designed by its sponsor, Baxter. Data were collected by the investigators and were held and analyzed by Baxter. The manuscript was written by a subgroup of industry and academic authors; all authors contributed to the content, had full access to the data, and vouch for the completeness and accuracy of the data and data analysis.

The appropriate local review boards and ethics committees approved the protocol for the study, which was conducted in compliance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki. The study investigators were unaware of assignments to study groups. (For details of the study design, see the Supplementary Appendix, available with the full text of this article at www.nejm.org.)

The objective was to identify the immunogenicity and safety of various doses of inactivated H5N1 whole-virus vaccine in formulations with and without adjuvant. The primary immunogenicity outcome was the number of subjects with hemagglutination-inhibition and neutralizing antibodies to the vaccine strain (A/Vietnam/1203/2004) 21 days after the first and second doses of vaccine. The primary safety outcome was any systemic reaction after the first and second doses.

VACCINE

The monovalent avian influenza H5N1 whole-virus vaccine (Baxter) was produced with the wild-type strain A/Vietnam/1203/2004, which was obtained from the Centers for Disease Control and Prevention and was inactivated with formalin and ultraviolet light. The vaccine was manufactured in Vero cell culture in an enhanced BSL-3 facility (as required for wild-type H5N1 virus), as described previously.[9]

RANDOMIZATION AND FOLLOW-UP

Subjects were eligible to participate if they were clinically healthy, understood the study procedures, provided written informed consent, and agreed to keep a daily record of symptoms. Women were required to have a negative pregnancy test at screening and before each vaccination.

Subjects were recruited in three study cohorts in a dose-escalating manner and were randomly assigned to receive two 0.5-ml injections into the deltoid muscle at an interval of 21 days (range,