Resistance to dihydroartemisinin.

Eric Legrand, Beatrice Volney, Jean-Baptiste Meynard, Philippe Esterre, Odile Mercereau-Puijalon

by the same method, where a similar safety profile has been seen after >15 million vaccinations in humans.

This is the first study that reports that an inactivated whole virus vaccine with an aluminum phosphate adjuvant system against influenza A (H5N1) was safe and immunogenic in humans after only 1 injection. This study reports the lowest effective dose used to cause immune response. Other trials used much higher maximum doses and required 2 injections 21 or 28 days apart (*8–10*). Using the lowest possible amount of the antigen and fewer injections is essential for increasing the production capacity of vaccine manufacturers in a pandemic (*2*).

Using 1, instead of 2, injections will shorten the time needed to develop immune response by 3–4 weeks. Unlike previous studies on influenza A (H5N1) vaccines that reported only data from 21, 28, or 56 days after the final vaccination (*8–10*), we report data up to 90 days. The lower dose and fewer injections required to trigger an immune response can be at least partially explained by using a whole virus vaccine and an aluminum phosphate adjuvant system. The use of a different adjuvant system than ours may have influenced the results of other trials (*9*,*10*). Other investigators used a modified HI method with horse erythrocytes, which are known to be more sensitive for influenza A (H5N1) subtype than the conventionally used turkey or chicken erythrocytes (*8*,*9*). Thus, if horse erythrocytes had been used in our study, the vaccine would likely have been even more immunogenic.

This study found fewer, less frequent, and milder side effects than did other trials of influenza A (H5N1) vaccines published so far (*8–10*). This could possibly be explained by the smaller dose used. Also, the endotoxin content of 0.1 IU/mL in our vaccine was much smaller then the allowed amount of 100 IU/mL by standards (*5*).

We report an inactivated whole virus vaccine that is safe and immunogenic in healthy adults and that requires a low dose and only 1 injection to trigger an immune response. We are conducting trials in elderly persons and children.

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References

1. Ungchusak K, Auewarakul P, Dowell SF, Kitphati R, Auwanit W, Puthavathana P, et al. Probable person-to-person transmission of avian influenza A (H5N1). N Engl J Med. 2005;352:333–40.
2. Dennis C. Flu-vaccine makers toil to boost supply. Nature. 2006;440:1099.
3. Takatsy G. Purified precipitated virus obtained by a new simple method. Acta Med Acad Sci Hung. 1952;3:185–91.
4. License number: OGYI-T-8998/01. Budapest: National Institute of Pharmacology; 1995.
5. European Committee for Proprietary Medicinal Products. Note for guidance on harmonization of requirements for influenza vaccines, March 1997 (CPMP/BWP/214/96). Brussels: European Agency for the Evaluation of Medicinal Products; March 12, 1997.
6. European Committee for Proprietary Medicinal Products. Guideline on dossier structure and content for pandemic influenza vaccine marketing authorisation application (CPMP/VEG/4717/03). Brussels: European Agency for the Evaluation of Medicinal Products; April 5, 2004.
7. Klimov A, Cox N. Serologic diagnosis of influenza virus infections by hemagglutination inhibition. Influenza laboratory course. Atlanta: Centers for Disease Control and Prevention; 2003.
8. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immungenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med. 2006;354:1343–51.
9. Bresson JL, Perronne C, Launay O, Gerdil C, Saville M, Wood J, et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet. 2006;367:1657–64.
10. Lin J, Zhang J, Dong X, Fang H, Chen J, Su N, et al. Safety and immunogenicity of an inactivated adjuvanted whole-virion influenza A (H5N1) vaccine: a phase I randomised controlled trial. Lancet. 2006;368:991–7.

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Resistance to Dihydroartemisinin

To the Editor: The title of the letter by Cojean et al. (*1*) is misleading. The data presented essentially point to an absence of in vitro resistance to dihydroartemisinin (dhART) in the panel of African isolates studied, with 1 of 397 isolates having an elevated 50% inhibitory concentration (IC_{50}) for dhART. The S769N *PfATPase6* mutation associated with in vitro resistance to artemether (*2*) was observed in 1 isolate. This mutant isolate had a low IC_{50} for dhART, but its IC_{50} for artemether has not been tested. Since the relationship between in vitro susceptibility to artemether and dhART is still uncertain (*3*), these data do not disprove the association of a *PfATPase6* S769N polymorphism with elevated IC_{50} for artemether that was observed in isolates from French Guiana (*2*).

Worth noting is that the association of the S769N *PfATPase6* polymorphism with elevated IC_{50} for artemether was confirmed in an isolate collected in French Guiana in 2005; that isolate had an IC_{50} for artemether of 127 nmol/L. Molecular typing identified 2 clonal types, 1 with a wild-type *PfATPase6* allele and 1 with a S769N

single mutant. After 3 weeks of in vitro cultivation without drug, the mutant allele was no longer detected and the IC_{50} for artemether was 8.2 nmol/L. This finding suggests poor fitness of the mutant allele under standard culture conditions.

The observation of an additional case of in vitro resistance to artemether in French Guiana 3 years after the first cases is of concern. Reinforcement of surveillance is needed as is clarification of the relationship of in vitro susceptibility to artemether and artesunate, the derivatives currently included in artemisinin-based combination therapies (ACTs). Surveillance and clarification would be particularly timely since emerging clinical or parasitologic failures to some ACTs have been reported (*4*,*5*).

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References

1. Cojean S, Hubert V, Le Bras J, Durand R. Resistance to dihydroartemisinin. Emerg Infect Dis. 2006;12:1798–9.
2. Jambou R, Legrand E, Niang M, Ing NK, Lim P, Volney B, et al. In vitro artemether resistance of *Plasmodium falciparum* field isolates and point mutations pf the SERCA-type PfATPase6. Lancet. 2005;366:1960–3.
3. Brockman A, Price RN, van Vugt M, Heppner DG, Walsh D, Sookto P, et al. *Plasmodium falciparum* antimalarial drug susceptibility on the north-western border of Thailand during five years of extensive use of artesunate-mefloquine. Trans R Soc Trop Meg Hyg. 2000;94:537–44.
4. Grandesso F, Hagerman A, Kamara S, Lam E, Checchi F, Balkan S, et al. Low efficacy of the combination artesunate plus amodiaquine for uncomplicated falciparum malaria among children under 5 in Kailahun, Sierra Leone. Trop Med Int Health. 2006;11:1017–21.
5. Bukirwa H, Yeka A, Kamya MR, Talisuna A, Banek K, Bakyaita N, et al. Artemisinin combination therapies for treatment of uncomplicated malaria in Uganda. PLoS Clin Trials. 2006;1:e7

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Resistance to Dihydroartemisinin

In Response: The original title of our article was "Lack of *Plasmodium falciparum* in Vitro and Genomic Resistance to Dihydroartemisinin in Travelers Returning to France from Africa." EID's shortening of the title (*1*) led to the perception that the letter title was misleading, but it was not on purpose. We have recently tested the 50% inhibitory concentration for artemether of the S769N *PfATPase6* isolate that we had kept in liquid nitrogen, and it showed susceptibility.

We underline that the previously reported clinical or parasitologic failures to some artemisinin-based combination therapies (*2*,*3*) were not synonymous with the emergence of resistance to artemisinin compounds. In the study by Grandesso et al., a combination of artesunate plus amodiaquine was given to children <5 years of age who lived in an area in which amodiaquine alone was ineffective to adequately treat uncomplicated falciparum malaria in 1 of 3 cases at day 28 (*2*). Such a combination (artesunate plus amodiaquine) was nearly equivalent in 1 of 3 cases to a 3-day artesunate monotherapy, which may fail to completely cure children because of the short half-life of artesunate. In the study by Bukirwa et al., no recrudescence occurred in patients treated with artesunate plus amodiaquine and only 2 of 199 patients treated with artemether plus lumefantrine experienced recrudescence at day 28 (*3*). As Birkiwa et al. themselves acknowledged, artemether plus lumefantrine was not administered with food, and it is known that lumefantrine is absorbed better when it is taken with a small amount of fat. Thus, the clinical failures observed did not necessarily reflect *P. falciparum* resistance to artemisinin compounds.

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References

1. Cojean S, Hubert V, Le Bras J, Durand R. Resistance to dihydroartemisinin. Emerg Infect Dis. 2006;12:1798–9.
2. Grandesso F, Hagerman A, Kamara S, Lam E, Checchi F, Balkan S, et al. Low efficacy of the combination artesunate plus amodiaquine for uncomplicated falciparum malaria among children under 5 in Kailahun, Sierra Leone. Trop Med Int Health. 2006;11:1017–21.
3. Bukirwa H, Yeka A, Kamya MR, Talisuna A, Banek K, Bakyaita N, et al. Artemisinin combination therapies for treatment of uncomplicated malaria in Uganda. PLoS Clin Trials. 2006;1:e7.

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