Since April 2009, a new influenza A/H1N1 virus has emerged and spread throughout the world. Although pandemic H1N1 vaccines have been approved in several countries, there is not enough for everyone in the world considering the manufacturing capabilities, costs, and delivery systems (10). A recent case control study suggested that some level of cross-protection against the pandemic H1N1 virus was provided by the 2008-2009 seasonal influenza virus vaccination (3). Actually, prior vaccination with seasonal influenza virus vaccines has been experimentally shown to be beneficial for pandemic preparedness (5). Two doses of seasonal influenza virus vaccines have been recommended in children to achieve higher immune responses (1). The pandemic H1N1 virus primarily affects young adults; here we ask whether additional vaccination with the trivalent inactivated seasonal influenza virus vaccines could boost the cross-reactive immunity to the pandemic H1N1 virus in young adults.

Seven young adults between the ages of 23 and 30 years, who received the first dose of the 2008-2009 trivalent inactivated seasonal influenza virus vaccine (Sanofi Pasteur) in November 2008, were immunized with the second dose of the vaccine in June 2009. Nine participants (aged 23 to 30) who received one dose of the 2008-2009 seasonal influenza virus vaccine and 11 participants (aged 21 to 28) who received no vaccination were included in this study. None of the participants had been suspected or confirmed to have the H1N1 influenza virus. Serum samples were collected at 28 days postvaccination, and serial 2-fold dilutions were subjected to hemagglutination inhibition (HI) and microneutralization assays according to the standard procedures (7). For HI assays, antibody titers of ≥40 are considered positive since they are associated with a 50% reduction in the risk of infection in human populations (2); for microneutralization assays, antibody titers of ≥160 are considered positive in adults (4).

As expected, microneutralization assays showed that no detectable neutralization antibodies against the pandemic H1N1 virus were present in the unvaccinated and once-vaccinated subject samples. Interestingly, 2 of 7 twice-vaccinated subject samples have neutralizing antibodies (titers of ≥160) to the pandemic H1N1 virus (Table 1). HI assays showed that 4 (57.1%) of 7 twice-vaccinated subject samples were positive for the pandemic H1N1 virus. This is significantly higher than the 1/11 (9.1%) and 1/9 (11.1%) positive responses in the unvaccinated and once-vaccinated groups, respectively. Most importantly, a significant increase (geometric mean titer [GMT], 40 versus 16) of antibody responses to pandemic H1N1 virus was observed in twice-vaccinated groups compared with that in the once-vaccinated group. These findings suggested that vaccination with the second dose of seasonal influenza virus vaccine significantly increased the cross-reactive antibody responses to pandemic H1N1 virus in young adults.

Currently, several countries have recommended vaccination with both seasonal and pandemic influenza virus vaccines (8, 9), while the global demands for vaccines greatly exceed the supply. Our preliminary data demonstrated that young adults can receive two doses of seasonal influenza virus vaccines to get partial cross-immunity in case of a shortage of the pandemic H1N1 vaccines. The benefits of additional vaccination with seasonal influenza virus vaccines, especially in developing countries where the pandemic H1N1 vaccines may not be available, should be carefully considered, and further laboratory and clinical investigation is warranted to clarify this issue.

We appreciate the volunteers involved in this study, and we offer many thanks to Xue-Dong Yu, Yun-Ling Wei, and Shun-Ya Zhu for technical support and Mao-Ti Wei for statistical analysis. This work was supported in part by the National 973 Plan of China (2010CB534002) and the Major Special Program of National Science and Technology of China (2009ZX10004-204).

### TABLE 1. Serum antibodies against the pandemic H1N1 virus

| No. of times vaccinated | No. of subjects | Total | HI positive | MN positive |
|-------------------------|----------------|-------|-------------|-------------|
| None                    | 11             | 1     | 0           | 0           |
| Once                    | 9              | 1     | 0           | 0           |
| Twice                   | 7              | 4     | 2           |             |

| a HI and microneutralization (MN) assays were performed using the newly isolated A/Beijing/501/2009 (H1N1) strain that was deposited in GenBank under accession no. GQ223408 to GQ223415 (6). |
| b Titer of ≥40. |
| c Titer of ≥160. |

### REFERENCES

1. Centers for Disease Control and Prevention. 2004. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. 53:1–40.

2. de Jong, J. C., A. M. Palache, W. E. Beyer, G. F. Rimmelzwaan, A. C. Boon, and A. D. Osterhaus. 2003. Haemagglutination-inhibiting antibodies to influenza virus. Dev. Biol. (Basel) 115:83–73.

3. Garcia-Garcia, L., J. L. Valdespino-Gómez, E. Laczano-Ponce, A. Jimenez-Corona, A. Higuera-Iglesias, P. Cruz-Hervert, B. Cano-Arellano, A. Garcia-Anaya, E. Ferreira-Guerrero, R. Baez-Saldana, L. Ferreyra-Reyes, S. Ponce-de-Leon-Rosales, C. Alpuche-Aranda, M. H. Rodriguez-Lopez, R. Perez-Padilla, and M. Hernandez-Avila. 2009. Partial protection of seasonal trivalent inactivated vaccine against novel pandemic influenza A/H1N1 2009: case-control study in Mexico City. BMJ 339:b3928.

4. Hancock, K., V. Veguilla, X. Lu, W. Zhong, E. N. Butler, H. Sun, F. Liu, L. Dong, J. R. DeVos, O. M. Gargiullo, T. L. Bnammer, N. J. Cox, T. M. Tumper, and J. M. Katz. 2009. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus in the elderly. Clin. Infect. Dis. 53:1392–1399.

5. Ichinohe, T., S. Tamura, A. Kawaguchi, A. Ninomiya, M. Imai, S. Itamura, T. Odagiri, M. Tashiro, H. Takahashi, H. Sawa, W. M. Mitchell, D. R. Strayer, W. A. Carter, J. Chiba, T. Kurata, T. Sata, and H. Hasegawa. 2007. Cross-protection against H5N1 influenza virus infection is afforded by intranasal inoculation with seasonal trivalent inactivated influenza vaccine. J. Infect. Dis. 196:1313–1320.

6. Jiang, T., X. Li, W. Liu, M. Yu, J. Liu, X. Yu, E. Qin, W. Cao, Q. Leng, and C. Qin. 2010. Serum antibody response to the novel influenza A (H1N1) virus in the elderly. Clin. Infect. Dis. 50:285–286.

7. Kendal, A. P., M. S. Pereira, and J. J. Skehel. 1982. Concepts and procedures from laboratory-based influenza surveillance. Centers for Disease Control, Atlanta, GA.

8. National Center for Immunization and Respiratory Diseases. 2009. Use of...
influenza A (H1N1) 2009 monovalent vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recomm. Rep. 58:1–8.

9. Woo, J. C., and C. S. Ambrose. 2009. Concomitant administration of seasonal trivalent and pandemic monovalent H1N1 live attenuated influenza vaccines. Influenza Other Respir. Viruses 3:257–259.

10. Yamada, T. 2009. Poverty, wealth, and access to pandemic influenza vaccines. N. Engl. J. Med. 361:1129–1131.

Cheng-Feng Qin*
Tao Jiang
Jian-Feng Han
Xiao-Feng Li
E-De Qin
State Key Laboratory of Pathogen and Biosecurity
Beijing Institute of Microbiology and Epidemiology
Beijing 100071, China

Qi-Bin Leng*
Institute Pasteur of Shanghai
Chinese Academy of Science
Shanghai, China

*E-mail for C.-F.Q.: cfqin@hotmail.com
*E-mail for Q.-B.L.: qbleng@sibs.ac.cn

*Published ahead of print on 10 March 2010.