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Humoral and Cellular Immune Responses of Seronegative Children Vaccinated with a Cold-Adapted Influenza A/HK/123/77 (H1N1) Recombinant Virus

ARYE LAZAR, NOBUHIKO OKABE, AND PETER F. WRIGHT*

Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Humoral and cell-mediated immune responses of young, seronegative children were assessed after intranasal vaccination with a cold-adapted influenza, A/HK/77 (H1N1) CR 35 recombinant virus. Vaccinees shedding influenza virus experienced a rise in hemagglutinin-inhibition antibody 15 to 30 days after vaccination. Vaccinees showed low but significant lymphocyte transformation to A/USSR (H1N1) by day 8 after vaccination, which decreased to prevaccination levels at 30 to 34 days. The lymphocyte transformation response occurred before serum antibody rises were detected by hemagglutinin-inhibition assay. No change in lymphocyte responsiveness was observed after vaccination as measured by phytohemagglutinin stimulation. Lymphocytes responded to in vitro incubation with inactivated influenza (H1N1) virus by producing interferon. The interferon produced was of type I and was observed in vaccinees and nonvaccinees both before and after vaccination.

The use of cold-adapted variants of influenza viruses as donors of genes to confer attenuation to wild types has provided a new tool in attempts to control influenza epidemics. The ability of those recombinants to grow at low temperatures (25°C) serves as an in vitro marker for attenuation which can be assessed in tissue culture systems before evaluation in humans. Recombinants of wild-type strains with the cold-adapted A/Ann Arbor/6/60 strain developed by Maassab (14, 15) have been shown to be attenuated and immunogenic in humans when tested in open populations (1, 3, 13). Young seronegative children are prime candidates for protection from such vaccines and represent the most sensitive population for testing attenuation, antigenicity, and genetic stability of such live respiratory viral vaccines (12, 24).

The cell-mediated immune response of seronegative children after vaccination with live influenza virus is incompletely investigated. Vaccinated infants might be expected to differ from natural infection in adults due to a relative immaturity of the immune system, primary exposure to influenza virus, and relative attenuation of the vaccine strain.

The present study examines these variables by describing the humoral and cell-mediated immune responses of young, seronegative children after vaccination with a cold-adapted recombinant of influenza A/HK/123/77 (H1N1) virus.

MATERIALS AND METHODS

Patient population. Vaccinees and controls were children aged 13 to 44 months selected for vaccine trials on the basis of seronegativity by hemagglutination inhibition (HAI) to influenza A/USSR (H1N1) virus. Children were enrolled in the Vanderbilt Pediatric Vaccine Clinic, Nashville, Tenn. A total of 15 children participated in four studies. The children were vaccinated intranasally as previously described (24). A total of 0.5 ml of a 10-fold dilution of stock influenza A/HK/123/77 (H1N1) clone 22, CR 35 (Flow Laboratories, Rockville, Md.) was administered. This was calculated to give a total of $10^{6.5}$ 50% tissue culture infective doses per vaccine dose. One child in each group was not vaccinated and served as a placebo control. Nasal washes were obtained daily for viral isolations and interferon determination starting 3 days before vaccination and continuing until day 15 after vaccine administration. Blood samples were obtained 1 day before vaccination and 1, 8, 15, and 30 to 34 days afterward. The blood samples were used for HAI antibody determination and lymphocyte studies.

Virus isolation. Daily nasal washes collected in 10 ml of phosphate-buffered saline were used for influenza isolation. Cultures were inoculated daily without freezing onto primary rhesus monkey kidney cells (RMK) and a continuous canine kidney cell line (MDCK). Cultures were inoculated at two temperatures: 32°C, permissive for growth of the cold-adapted virus, and 39°C, a temperature restrictive to growth of the vaccine strain. Tubes were hemadsorbed 5 and 10 days after inoculation with 0.1% guinea pig erythrocytes. All positive tubes were passaged at 32°C and identified as influenza A/USSR (H1N1) by HAI assay (3) with an influenza A/USSR/77-specific chicken antiserum. All original isolates containing influenza virus were subsequently titered on MDCK cells to determine the titer of virus being shed.

Other procedures. All procedures for lymphocyte collection and culture, preparation of mitogens, measurement of transformation, and interferon assay are as described in the companion paper.

RESULTS

Virus shedding. Eight of eleven vaccinated children shed influenza virus for an average of 8.4 days. Virus shedding began on day 1 or 2 after vaccination and continued in some vaccinees until day 13 (Fig. 1). The maximal level of viral replication occurred on day 6 after administration of the virus, followed by a steady decrease in titer. In one trial two vaccinees shed naturally occurring A/Brazil/78, beginning before vaccination in one child and in a contact on day 8. All A/USSR/77 isolated retained their temperature sensitivity as shown by lack of growth at restrictive temperature (39°C).

Antibody response. At the beginning of the trials, both vaccinees and placebos had a serum HAI antibody titer of 1:8 or lower. This titer was not changed 8 days after vaccine administration (Fig. 2). By 2 weeks, HAI antibody titer rose in eight virus shedders by a mean of 6.9-fold and remained at this level when measured 30 to 34 days postvaccination. Four placebo and the three nonshedding vaccinees remained seronegative.

Lymphocyte transformation (LTF) response of vaccinees to influenza A/USSR virus. The transformation responses of children's lymphocytes as response to in vitro stimulation by influenza A/USSR-inactivated virus were measured 1 day before vaccine administration (day −1) and on days 1, 8, 15, and 30 to 34 thereafter. For statistical analysis, the three children who neither shed influenza virus nor had an HAI response after vaccination were added

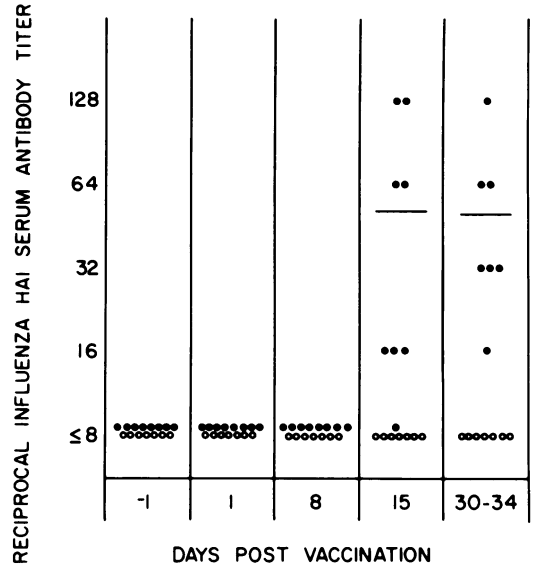

FIG. 2. *Serum HAI antibody response in children after intranasal administration of influenza A/HK/123/77 (H1N1) cold-adapted virus. Horizontal bars indicate arithmetic means. Symbols: ●, children shedding influenza H1N1 virus; ○, children not shedding influenza H1N1 virus and placebos.*

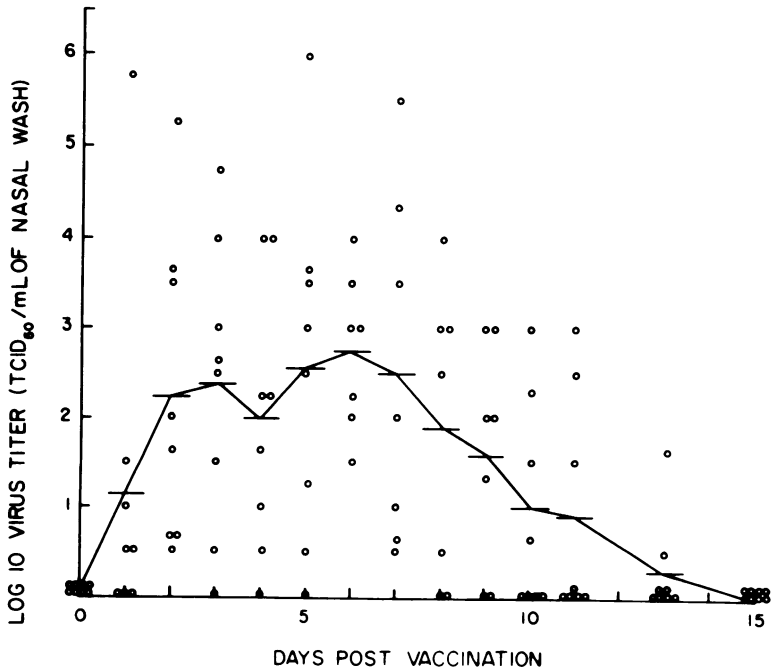

FIG. 1. *Influenza H1N1 cold-adapted virus shedding after intranasal administration of vaccine. Horizontal bars indicate arithmetic means.*

to the four placebo controls, and the LTF response of this apparently uninfected group was compared with the eight children who shed influenza A/USSR (infected group). No significant transformation response was noted on days −1 or 1 (Fig. 3). On day 8, a consistent low, but significant, response was observed in children who shed influenza virus when compared with the noninfected group ($P = 0.007$, Student's t test). This response was decreased by day 15 but was still significant ($P = 0.01$). The LTF response returned to prevaccine levels when measured 30 to 34 days postvaccination. The lack of any change in the LTF response of the nonshedders supports their inclusion in the noninfected group.

LTF response of vaccinees to phytohemagglutinin. Lymphocyte cultures of vaccinated children were incubated with phytohemagglutinin after viral administration to determine whether the LTF response was altered during vaccination. No significant changes in the LTF response of either infected or noninfected children were noted after vaccination, suggesting that the vaccine virus used in this study was not immunosuppressive as determined by this assay.

Interferon production by vaccinees' lymphocytes. Culture media of in vitro-stimulated lymphocytes from vaccinated children were used for interferon determination. Some children's lymphocytes showed a consistently high level of interferon production, whereas some showed low levels. No statistical differences in interferon levels were found between infected and uninfected children during the study period with mean levels of interferon being between 150 and 250 U. The interferon produced by lymphocytes of both groups was resistant at pH 2 and stable after 56°C incubation for 60 min and showed species specificity by lack of inhibition of vesicular stomatitis virus plaque formation on mouse cells. These tests indicate that the interferon produced was type I and reflected in vitro lymphocyte response to an interferon inducer.

Interferon in nasal washes. In vaccinees no measurable interferon could be detected in nasal washes after vaccination when daily samples were examined. In contrast, two children that experienced natural infection with A/Brazil/78 (H1N1) during the trials had peak interferon titers of 83 and 79 U at the time of maximum virus shedding.

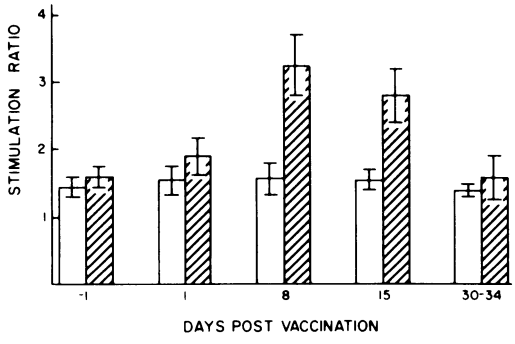

FIG. 3. *Lymphocyte transformation response to influenza A/USSR (H1N1) virus in children after intranasal administration of influenza A/HK/123/77 (H1N1) cold-adapted virus. Transformation expressed as a stimulation ratio derived by dividing the mean counts per minute incorporated in the presence of viral antigens by the mean counts per minute incorporated in the absence of virus. Data are given as means (bars) ± standard error (brackets). Striped bars represent infected children (n = 8); open bars represent uninfected children (n = 7).*

DISCUSSION

Cold-adapted influenza A/HK (H1N1) is a clinically attenuated vaccine that is genetically stable in spite of considerable replication in the upper respiratory tract. Equally important to evaluate are the effects of a live viral vaccine on the host immune system.

In the current study the in vitro transformation response of stimulated lymphocytes was noted in vaccinated children who shed influenza virus and exhibited an HAI response. This suggests that the cell-mediated immune system is involved in the immune reaction to primary infection with a live attenuated influenza virus. The early detection of LTF after vaccination is consistent with the observations of Ruben et al. (22), Dolin et al. (5), and Chow et al. (2), who found elevated blastogenic responses 10 to 14 days after vaccination with inactivated influenza virus. Since blood samples in the present study could not be obtained daily, the point of maximal transformation response after vaccination was not fully defined. But clearly the blastogenic response occurred earlier than serum antibody rises were detected by HAI assay. The return of the LTF to base line within 30 days after vaccination and the low LTF even at 1 to 2 weeks suggest a diminished lymphocyte response when compared with naturally infected children and adults with H1N1 virus.

Suppression of the immune system due to viral infection is a well-recognized phenomenon which must be considered when evaluating new live viral vaccines, (19, 23). The degree of immunosuppression resultant from influenza virus infection is controversial. Some investigators have described reduced lymphocyte transformation during influenza infection (6, 9, 10), whereas others have failed to demonstrate such

immunosuppression (11, 21). These conflicting observations could be explained by variables such as severity of infection (11) or differences in the immune response to different influenza types. Reduced immune responsiveness has not been demonstrated during vaccination with inactivated influenza virus (5, 21). One live attenuated vaccine (A/H3N2/England/8/68), on the other hand, has been shown to be immunosuppressive (9). The lack of immunosuppression as measured by phytohemagglutinin stimulation with the cold-adapted vaccine is reassuring.

Interferon production by stimulated lymphocytes during influenza infection has been investigated to only a limited extent. In the present study, the interferon produced was of the nonimmune type (type I) and was detected in vaccinees and nonvaccinees as well. These results are consistent with a nonspecific response of lymphocytes to an interferon inducer rather than a specific cell-mediated immune response. However, since type I interferon has been detected in sera and nasopharyngeal washings of patients after influenza infection (7, 8, 16, 18), it may play an important role in recovery from the disease. Vaccine infection did not induce measurable interferon in nasal washes in the present study. The macrophage-lymphocyte technique, useful for detection of immune interferon, requires two specimens of 150 and 50 ml of blood (20) and is, therefore, unsuitable for studies in young children.

Live, attenuated influenza vaccine induces lymphocyte stimulation that is of shorter duration than that seen with natural infection perhaps related to more limited virus replication. However, lymphocyte blastogenesis is detected as early as 8 days after vaccination. The possible importance of cell-mediated immunity as the earliest documented event in host's response to influenza viral infections requires further study.

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