Response to influenza virus vaccination in vertical HIV infection

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Abstract

Objective—To assess the antibody response to influenza vaccine of children vertically infected with HIV.

Design—Prospective study in HIV infected children vaccinated during the winter of 1994-5.

Setting—Family HIV clinic at St Mary's Hospital, Paddington.

Subjects—25 children, aged 1-11 years, vertically infected with HIV.

Main outcome measures—Responses to influenza antigens (H1N1-A /Taiwan /1/86, H3N2-A /Shandong /9/93, B /Panama /45/90) were tested by haemagglutination inhibition. Antibody responses were assessed according to clinical symptoms and immune function, stratified according to the 1994 revised classification for HIV infection in children.

Results—23 children (92%) had either very low or no detectable antibody before vaccination. New protective antibody responses were made by 10 children (40%): in seven to a single antigen, in two to two antigens, and in one to all three antigens. For each antigen there was an overall small increase in the mean geometric titre of antibody produced, but this only reached a protective level for antigen H1N1 and for children with minimal symptoms. Less symptomatic children were significantly more likely to produce a protective antibody response to influenza vaccination. No association was found between immune function, as measured by CD4 count, and vaccine response.

Conclusions—Only vaccination of the least symptomatic HIV infected children against influenza is likely to be effective. This will not only protect them against influenza, but will also protect other more immunosuppressed and vulnerable members of their families.

(*Arch Dis Child* 1997;76:215-218)

Keywords: influenza vaccination; paediatric HIV infection.

Children with HIV are well known to have increased susceptibility to viral and bacterial respiratory infections, but although there have been case reports of persistent respiratory infection with influenza, including fatal interstitial lung disease,¹ neither the wider spectrum of influenza disease in HIV infected children nor the immune response to influenza vaccination has been widely studied.

Where influenza disease has been examined in other groups of immunosuppressed children, infection was found to be more prevalent in oncology patients than in sibling or community controls, although in that study the cohort was too small to assess for degree of severity of complications.² A recent retrospective review of influenza B infection in solid organ transplant recipients showed that over a three year period 9% of children developed culture proved influenza B infection with severe complications including encephalopathy, respiratory failure, and concurrent allograft rejection.³ The authors stated that as a consequence of this review the protective role of influenza immunisation in transplant patients was under investigation.

The relatively small numbers of immunosuppressed children make collection of community based data on influenza infection difficult. However, current guidelines within the United Kingdom and the USA recommend yearly vaccination against influenza for children with immunosuppression and those with chronic respiratory disease.^{4,5} Despite this recommendation, the level of uptake of vaccination has been low.⁶

There are two types of influenza vaccine available worldwide for children, given as single or double doses one month apart: (1) intramuscular or subcutaneous killed split or subunit virions; (2) live attenuated cold adapted virions, given intranasally. Only the killed, split/subunit vaccine is licensed for use in the United Kingdom. Killed whole virus vaccines are not recommended for children as they have frequent side effects. An antibody titre of 1:40 or a fourfold rise in titre at eight weeks after the first dose of vaccine is considered to be a protective antibody response.⁷ Sustained protection against influenza is poorly maintained because of the continual antigenic drift in prevalent strains of the virus, so annual vaccination is advised. The influenza vaccine contains two A strains (H1N1, H3N2) and one B strain of the virus, and the antibody response to antigenic determinants of each strain can be assessed.

In healthy younger children who are naive to influenza infection, vaccination often produces a low antibody response. Children do not always produce a uniform antibody response to the different strains of the influenza vaccine.⁸⁻¹⁰ In some studies, live attenuated cold adapted vaccines have been more immunogenic in younger children than killed virus vaccine, as they infect the respiratory mucosa and set up a sustained local anti-influenza response.¹¹ The

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Accepted 4 November 1996

T cell mediated immune response and other types of immune responses to natural influenza infection or vaccination have not been examined in healthy children. Only killed vaccine has been recommended for immunosuppressed children.⁵

Following the United Kingdom recommendations for immunocompromised children, in the autumn of 1994 HIV infected children attending the St Mary's Hospital family clinic were vaccinated against influenza. In this cohort all the children immunised had vertically acquired HIV infection. The aim of the study was to examine the antibody responses of the children to influenza vaccine and relate these to their immune and clinical status.

Patients and methods

Twenty five children over 6 months of age with vertically acquired HIV infection (median age 5 years, range 1 to 11 years) whose parents gave consent were immunised. All were vaccine naive. Two children only received one dose of vaccine because of parental choice. Twelve children who were not immunised were either receiving immunoglobulin infusions (1); terminally ill (5); irregular attenders (5); or refused vaccination (1).

Using the 1994 revised classification for HIV infection in children,¹² by clinical category: two children had no clinical signs or symptoms (group N), five had minimal (group A), 13 had moderate (group B), and five had severe signs and symptoms or an AIDS diagnosis (group C). According to the age related immunological scoring for CD4 count, eight had no evidence of suppression (group 1), nine had moderate suppression (group 2), and eight had severe suppression (group 3). The median CD4 percentage for the group was 21% (range 1% to 47%), within the moderate range for immunosuppression. At some time 11 of the 25 children had been p24 antigenaemic; all of these children were in symptom group B or C. Thirteen children were receiving zidovudine treatment.

The inactivated vaccine used contained highly purified influenza virus subvirions with the haemagglutinin and neuraminidase antigens of the A and B strains recommended by the World Health Organisation for the 1994-5 season (Fluzone, Servier Laboratories). The recommended dose of vaccine was given subcutaneously in two doses, one month apart (6 to 47 months of age, 0.25 ml; 4 to 12 years, 0.5 ml). Antibodies to influenza were tested by haemagglutination inhibition in a predose specimen and at the next follow up clinic visit (four to 24 weeks).

Responses to the influenza antigens, H1N1-A /Taiwan /1/86, H3N2-A /Shandong /9/93, B /Panama /45/90, were assessed in terms of geometric mean titre and protective response. As the antibody levels are measured on an ordinal scale (<10, 10, 20, 40, 80, 160, etc) to simplify analysis of changes in geometric mean titre, levels below the minimal detectable level were assigned a score of 5 (one half of the minimum detected level). A level of 40 or

more was considered to be a clinically protective response. These results were compared with the clinical and immunological status of the children.

STATISTICS

For analysis of the data, as only two children were in the no symptoms group (N) they were combined with the five children in the minimal symptoms group (A). The analysis of geometric mean titre was performed using an analysis of variance technique. Each antibody response was used as a dependent variable in a within patient analysis of variance to investigate whether the response to vaccine was associated with clinical symptoms or immunological status. Log(2) conversion of the antibody levels was used to remove the skewness in the variables. For the analysis of protective response the Fisher's exact and χ^2 tests of association were used. The program used was STATA 3.1.

Results

The recommended time to sample for a response to flu vaccine is eight weeks after the first dose. In this group of patients the median time for obtaining the postvaccination specimen was 14 weeks, with a range of four to 24 weeks. Two children who, by parental choice, only received one dose of vaccine were sampled at four weeks. However, the timing of postvaccination specimens did not appear to affect the results, since equal numbers of positive and negative results occurred before and after eight weeks (data not shown).

PROTECTIVE ANTIBODY RESPONSES

Twenty three (92%) of the children had either very low or no detectable antibody response to the three influenza antigens before vaccination. One child had a protective level of antibody to H1N1 and another had protective level of antibody to all three antigens, probably reflecting previous infection. New protective antibody responses were made by 10/25 children (40%). Seven children made a new response to a single antigen (H1N1 $\times$ 1, H3N2 $\times$ 5, B $\times$ 1), this included a boosted response to H3N2 in the child with an initial response to all three antigens and a new response to B in the child with an initial response to H1N1. Two children made new responses to two antigens (H1N1 and H3N2; H1N1 and B). Only one child made a new response to all three antigens.

Less symptomatic children in the clinical group N/A were significantly more likely to produce an antibody response to any component of the influenza vaccine than those in the more symptomatic groups B or C ($p = 0.01$). When the children were examined by immunological groups, there was no apparent statistical difference in response (table 1). Three of the 13 children receiving zidovudine treatment produced a new antibody response, as did six of the 12 not receiving treatment. Only two of the 11 children with p24 antigen produced a new antibody response, whereas seven of the 14 without p24 antigen did so. The group was too small to show any effect of age on antibody

Table 1 Production of new boosted protective vaccine responses to the three influenza antigens, H1N1, H2N3, and B, in children vertically infected with HIV. Stratified according to clinical and immunological status: Clinical groups: N/A, no/minimal symptoms; B, moderate symptoms; C, severe symptoms or AIDS. Immunological groups: 1, no immunosuppression; 2, moderate immunosuppression; 3, severe immunosuppression

	Clinical status									Immunological status								
	N/A (n=7)			B (n=13)			C (n=5)			1 (n=8)			2 (n=9)			3 (n=8)		
Protective new antibody response to 1, 2, or 3 influenza antigens	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
No of responders	5	—	1	1	2	—	1	—	—	3	—	—	3	1	1	1	1	—
Proportion responding to vaccine	0.86			0.23			0.2			0.38			0.56			0.25		

response. Children with lymphoid interstitial pneumonitis (LIP) often suffer more frequent respiratory infections. In this small group an equal number of the non-vaccine responders had or did not have LIP (7:8). Of the vaccine responders the majority did not have LIP (9:1). Most of these children were in the minimal symptom group N/A (6/10).

GEOMETRIC MEAN TITRE OF ANTIBODY

When the actual titre of antibody produced was examined and the rise in antibody titre for each child calculated it was apparent that in most cases there was a rise in titre of antibody. However, the mean levels of antibody and the increased level in each category are low, generally below the protective level (table 2). By symptom category the mean titre exceeded the level considered to give a protective response only in the H1N1 subtype and in children in group N/A. By immunological category, most groups show an increase in the antibody level; however, the mean concentrations of antibody in each group were low, with none exceeding the level required for a protective response (table 2).

Discussion

This small study is the first to examine a homogeneous group of vaccine naive children vertically infected with HIV. The majority (23, 92%) of the children were susceptible to influenza infection. The response to vaccination was poor with only 10 children (40%) producing a new or boosted protective response to one or more antigens, and only one child to all three antigens. Other studies of young healthy children, unprimed to influenza, have shown that they respond less well to vaccination than previously exposed older persons, but there is

usually a response in more than 50% of children.^{8,9} Only two of the 25 children in the group had a protective level of antibody to all three antigens; in one child these were present before vaccination, and the response to only one antigen was boosted after vaccination. A protective response to vaccination was associated with fewer clinical symptoms, but not with the immune status of the children, as measured by CD4 count. Unfortunately, in this study it was not possible to compare vaccine responses directly with those of children uninfected with HIV.

Our findings concur with those of Chadwick *et al* who recently examined the serological response to influenza A antigens in children with HIV and controls vaccinated with trivalent inactivated influenza vaccine.¹³ Comparison of the ratios of rise in geometric mean titre before and after vaccination in this study shows a better response in uninfected children. The proportion of HIV infected children achieving a fourfold rise in antibody to both influenza A antigens was 44%, compared with 70% for controls (no statistical difference). CD4 count did not correlate with serological response to influenza A antigens, but subjects with an AIDS diagnosis had a significantly lower response to vaccination.

It is of interest that in both studies immunosuppression, as demonstrated by CD4 count (by age adjusted category in our cohort), did not affect the response to vaccination. A different system of assessment of symptomatology was used within the two studies, but here again the studies agree that more severe symptoms, including an AIDS diagnosis, are associated with a poor response to vaccination. In both studies only antibody responses to vaccination were examined and although these were poor it is possible that the immunised children might still have gained some undetected, useful T cell mediated cytotoxic response.

This poor vaccine response to influenza is mirrored in the responses of HIV infected children to other vaccines, including measles, mumps, rubella and pneumococcal vaccine, particularly in those with more advanced disease. Where previous infection with influenza has occurred, vaccination may generate a more effective anamnestic response than that which occurs in the naive children. In this study group there were only two minimally symptomatic children with previous antibody responses, and they made boosted responses to

Table 2 Geometric mean titres (95% confidence limits) of antibody to the three influenza antigens, H1N1, H2N3, and B, before and after vaccination, in children vertically infected with HIV. Stratified according to clinical and immunological status

	Clinical status			Immunological status		
	N/A	B	C	1	2	3
H1N1 before	13.5 (2.7-67.1)	5.3 (4.7-5.9)	5.0 (5.0-5.0)	11.9 (3.0-47.1)	5.0 (5.0-5.0)	5.5 (4.4-6.7)
H1N1 after	44.2 (11.4-171.5)	8.1 (4.9-13.3)	5.0 (5.0-5.0)	16.8 (3.6-78.5)	11.3 (5.3-25.7)	8.4 (4.3-16.5)
H3N2 before	11.0 (5.1-24.1)	6.2 (4.8-8.0)	5.0 (5.0-5.0)	6.5 (3.5-12.0)	8.6 (5.1-14.4)	5.9 (4.5-7.8)
H3 N2 after	26.9 (6.8-107)	9.5 (4.8-18.6)	8.7 (1.9-40.6)	7.7 (2.8-21.5)	25.2 (8.1-78.0)	9.2 (4.2-18.8)
B before	6.7 (3.3-13.9)	5.3 (4.7-5.9)	5.0 (5.0-5.0)	6.5 (3.5-12.0)	5.0 (5.0-5.0)	5.5 (4.4-6.7)
B after	13.5 (6.0-30.4)	7.3 (4.4-12.0)	5.7 (3.9-8.4)	6.5 (3.5-12.0)	10.8 (6.2-18.9)	7.7 (3.4-17.4)

A mean of 5 denotes samples having a concentration of antibody below the detection limit of the assay.

vaccination. This group was too small to show any relation of vaccine response to age or lymphoid interstitial pneumonitis.

It has been suggested that foreign antigens, such as vaccines, presented to HIV infected patients might increase replication of HIV through activation of the immune system and thus promote progression of immune dysfunction. Studies of the HIV viral load in HIV infected adults after influenza vaccination have produced controversial results, with only short term rises in virus replication, and there have been none in children.^{14 15} However, the degree of immune stimulation caused by vaccination is likely to be far less than that caused by a severe or protracted natural infection.

Thus if vaccination could be detrimental it is all the more important not only to ascertain whether influenza vaccines are immunogenic in the HIV infected child but also to gain a better understanding of natural influenza infection in these children. A larger scale examination of influenza infection rates and antibody responses to vaccination in whole family units affected by HIV, including the immunocompetent uninfected members and immunosuppressed infected members, would be required to answer these questions.

From this study we conclude that only vaccination of the least symptomatic HIV infected children against influenza is likely to be effective. This winter we shall only vaccinate children in symptom categories N, A, and B. This could not only protect them against influenza, but could also protect other more immunosuppressed and vulnerable members of their families.

We thank Mr Paul Laidler for excellent technical assistance.

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