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Serum IgG subclass antibody responses in children vaccinated with influenza virus antigens by live attenuated or inactivated vaccines

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Summary

To ascertain whether live attenuated or inactivated vaccines can be considered equivalent, we examined the primary antibody response of children following vaccination with influenza virus antigens in three different formulations. Nine children received cold recombinant vaccine (CRV) containing A/Korea/82 (H_3N_2) and A/Dunedin/83 (H_1N_1) variants. Eight of these children responded to HA of the H_3N_2 subtype and the major portion of the elicited antibody was in the IgG1 subclass. Antibody of low titer in the IgG2 and IgG3 subclasses was detected in two and six serum specimens, respectively. Six of the nine children administered with CRV responded to the H_1 antigen and only IgG1 antibody was detected. Serum specimens from eight children less than one year of age (5 less than 6 months of age) who had developed an antibody response to trivalent inactivated vaccine (TIV) vaccination were examined. High levels of IgG1 antibody to purified H_3 were detected in all eight children. Low titers of antibody in IgG2 and IgG3 subclasses were detected in two and five children, respectively. Antibody responses to purified H_1 showed a similar subclass distribution. In order to examine secondary response, eight children primed by immunization with TIV vaccine were subsequently given a single booster dose of purified hemagglutinin (HA) conjugated to diphtheria toxoid (HA-D). In 6/8 specimens antibody rises were detected to purified H_3 and H_1 antigens. Prior to the HA-D immunization, low levels of HA specific IgG1 antibody were detected in all serum specimens and vaccine induced responses were primarily of the IgG1 subclass. These data indicate that HA responses in the IgG subclasses are similar following immunization with antigen presented in different

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formulations to primed and unprimed children. In contrast to results in the mice, children produced predominantly IgG1 serum antibody following immunization with these three vaccine formulations.

Influenza virus; Serum IgG

Introduction

Previous studies from this laboratory have shown that the physical form in which influenza virus antigens are given to mice can influence the IgG subclass of the host serum antibody response (Balkovic et al., 1987). Mice infected with either virulent or non-virulent mouse adapted influenza viruses produce predominantly IgG2a antibodies to the hemagglutinin (HA) surface glycoprotein, whereas mice immunized with purified hemagglutinin-neuraminidase (HANA-flu) produced high levels of IgG1 antibody. Low levels of antibody in the other three subclasses were observed in both groups of mice. More recently, a study has shown that IgG2a antibodies are preferentially elicited by infection with a variety of viruses and that immunization with soluble proteins induces antibodies of the IgG1 subclass (Coutelier et al., 1987). Moreover, these antibody sub-class differences are associated with the secretion of different lymphokines (either interleukin 4, 5 or gamma interferon) by either of two distinct subsets of T-helper cells (Vitetta et al., 1987). To determine whether similar antibody sub-class differences occur in humans, we exposed young children to influenza antigens by immunization with an experimental bivalent cold recombinant vaccine (CRV), a commercial trivalent inactivated vaccine (TIV), or trivalent HA conjugated to diphtheria toxoid vaccine (HA-D).

Materials and Methods

Study populations

In the autumn of 1985, 10 children (6 younger than one year and four older than one year) with no detectable antibody to HA were administered two doses of a bivalent influenza A CRV containing A/Korea 1/82 (H_3N_2) and A/Dunedin 6/83 (H_1N_1) $10^{5.7}$ virus.

Thirty children (15 three to five months of age and 15 six to 14 months of age) with underlying heart or lung disease were vaccinated with two 0.25 ml doses of TIV four weeks apart and one dose of inactivated A/Taiwan/1/86 vaccine given with the second TIV dose (study performed at the University of Colorado, Denver, CO). Antibody to HA was not detected in the prevaccination specimens by either HAI or Elisa.

In addition, 11 children previously immunized with two doses of TIV were administered with a trivalent conjugate vaccine containing 15 μg of each HAs rep-

resenting the H/Taiwan/1/86 (H_1N_1), A/Leningrad/360/86 (H_3N_2) and B/Ann Arbor/1/86 influenza strains. The purified HAs were individually covalently attached to diphtheria toxoid and then mixed to produce the trivalent vaccine. Serum specimens were collected before and four weeks after vaccine administration. Paired serum specimens were available from eight children. Serum antibody to both HAs present in the TIV vaccine were detected in all eight prevaccination specimens.

Antisera

Cappel laboratories (Westchester, PA) was the source of affinity purified goat anti-mouse alkaline phosphatase antibodies. Mouse monoclonal antibodies specific against human IgG1, IgG2, IgG3, and IgG4 were obtained as follows: HP6014 for IgG2, HP6047 for IgG3, HP6023 for IgG4 (Chemicon, Burlingame, CA), NL-16 (Unipath, U.K.) or HP 6012 (Chemicon) for IgG1. These monoclones were furnished as purified preparations of 1 mg protein per ml.

Antibody determination

Monoclones directed against the IgG1, IgG2 and IgG3 subclasses were evaluated using samples of purified human IgG1, IgG2 and IgG3 ranging from 0.1 to 100 ng per well. Antibody of the three subclasses was detected with low but comparable efficiency, 10 to 20 ng of immunoglobulin for a 0.2 optical density reading. Serum specimens collected before and four weeks after each vaccine dose from children who developed an antibody response by HIA tests to both H_3N_2 and H_1N_1 virus components were assayed. HA representative of A/Mississippi/85 (H_3N_2) and A/Chile/83 (H_1N_1) were used as antigen. Immunoglobulin G subclass antibody responses in serum were determined by an ELISA assay as described previously (Murphy et al., 1981). Microtiter plates were coated with 50 ng per well of purified HA. After incubation overnight, the plates were post-coated with 10% fetal calf serum (FCS) in phosphate-buffered saline (PBS). After washing, dilutions of serum prepared in PBS containing 1% FCS and 0.2% Tween-20 were added to the wells. Mouse monoclones were added at 1:1000 (1 μg per ml) dilution and allowed to react for 20 h. After washing, goat anti-mouse alkaline phosphatase conjugate was added. After 4 h incubation, the plates were washed and para-nitrophenylphosphate was added. Absorbancies were determined on a Dynateck plate reader at 412 nm.

Results

Monoclonal antibody specificity and sensitivity

The monoclones directed against IgG1, IgG2 and IgG3 subclasses were found to be specific (Table 1). Lack of a purified human IgG4 preparation prevented characterization of the monoclone directed to it but no crossreactivity with other subclasses was observed. A lower reactivity was initially observed with the IgG2

TABLE 1
Specificity of mouse monoclonal antibodies directed against human IgG subclasses

Coating immunoglobulin	Monoclonal antibodies with specificity for the indicated human immunoglobulin[a]			
	G1[a]	G2	G3	G4
IgG1	≥2.0[b]	0.04	0.06	0.05
IgG2	0.19	1.20	0.05	0.04
IgG3	0.13	0.07	1.70	0.04
None	0.14	0.05	0.06	0.06

[a]All monoclonal antibodies were tested at a dilution of 1:1000. Experiments in other laboratories have shown that the IgG4 specific moncolone used here is specific and capable of performing in this type of assay (Hamilton, 1987).
[b]Values represent absorbancies (412 nm) recorded 1 h after addition of enzyme substrate.

monoclone but lengthening the reaction time from 4 h to 20 h improved the sensitivity.

Serum IgG subclass antibody responses following immunization with CRV

Ten children were immunized with CRV vaccine containing antigens representing the A/Korea/82 (H_3N_2) and A/Dunedin/85 (H_1N_1) viruses. A rise in serum neutralizing antibody was detected with A/Philippines/2/82 virus in eight of nine children who completed the study; sera rises in IgG antibody to the HA of A/Mississippi/1/85 in ELISA tests (data not shown) were also observed in those eight children. When the distribution of serum antibodies to this HA in the IgG subclasses was studied, high levels of antibody in the IgG1 subclass were detected in all eight serum specimens (Table 2), while antibodies of the IgG2 and IgG3 subclasses were detected in only two and six specimens respectively, and at much lower titers. Antibodies of the IgG2 and IgG3 subclasses were detected in sera which contained the highest

TABLE 2
Serum IgG subclass antibody levels to purified HA derived from A/Mississippi/85 virus following two doses of CRV[a]

Child No.	Antibody titer in the indicated IgG subclass		
	G1	G2	G3
1	3,200	<200	429
2	22,290	493	303
3	2,790	<200	< 200
4	22,290	<200	1600
5	38,800	530	650
6	204,800	<200	1,838
7	4,850	<200	<200
8	44,570	<200	3200

[a]Vaccine contained CR viruses representing the A/Korea/82 (H_3N_2) and A/Dunedin/83 (H_1N_1) variants. One child failed to develop an ELISA antibody response and one child was not evaluated because of inadequate serum specimens. Antibody of the IgG4 subclass was not detected in any of the serum specimens.

TABLE 3
Serum IgG subclass antibody levels to purified HA derived from A/Mississippi/85 (H_3N_2) virus following two doses of TIV[a]

High risk children	Antibody titers in the indicated IgG subclass		
	G1	G2	G3
Under 6 months			
1	11,940	<200	280
2	9.050	<200	200
3	2,790	200	570
4	2,990	<200	<200
5	7,350	<200	<200
Over 6 months			
1	8,380	<200	<200
2	23,800	<200	570
3	25,140	830	540

[a]Vaccine contained antigens of A/Mississippi/85 (H_3N_2), A/Chile/83 (H_1N_1), and B/Ann Arbor/86 viruses. A monovalent split product vaccine containing antigens of A/Taiwan/1/86 (H_1N_1) virus was also given with the second dose. Antibody of the G4 subclass was not detected in these specimens.

concentrations of IgG1 antibody.

Rises in IgG antibody to the HA of A/Chile/83 (H_1N_1) were observed in six of these children and a similar analysis was performed. Only antibodies of the IgG1 subclass were detected and the titers were low (range 400–3100).

Serum IgG subclass antibody responses following immunization with TIV

Antibody responses in the IgG subclasses from children receiving their first exposure to influenza virus antigens by immunization with TIV are given in Tables 3 and 4. Antibody to the HA of A/Mississippi/85 was predominantly of the IgG1

TABLE 4
Serum IgG subclass antibody levels to purified HA derived from A/Chile/83 (H_1N_1) virus following two doses of TIV[a]

Children	Antibody titer in the indicated IgG subclass		
	G1	G2	G3
Under 6 months			
1	>12,800	320	115
2	3,400	220	<100
3	8,300	180	<100
4	3,300	<100	<100
5	>25,600	1100	380
Over 6 months			
1	3,400	280	<100
2	3,100	280	<100
3	4,600	<100	<100

[a]Vaccine contained antigens of A/Mississippi/85 (H_3N_2), A/Chile/83 (H_1N_1), and B/Ann Arbor/86 viruses. A monovalent split product vaccine containing A/Taiwan/1/86 (H_1N_1) virus antigens was also given with the second dose. Antibody of G4 subclass was not detected in these specimens.

TABLE 5
Serum IgG subclass antibody levels to purified HA derived from A/Mississippi/85 virus following immunization HA-D[a]

Child No.	Antibody titer in the indicated IgG subclass		
	G1	G2	G3
1	6400	173	305
2	9600	200	399
3	395	<100	<100
4	1168	<100	<100
5	5000	195	180
6	6400	<100	<100
7	9600	<100	220
8	200	<100	<100

[a]The vaccine contained hemagglutinins representing the A/Taiwan/1/86 (H_1N_1), A/Leningrad/360/86 (H_3N_2) and B/Ann Arbor/1/86 strains of influenza virus. The purified hemagglutinins were individually conjugated to diphtheria toxoid and were mixed to form a trivalent vaccine. Antibody of the IgG4 subclass was not detected in these specimens.

subclass (Table 3). Two children produced antibody of IgG2 subclass and five produced antibody of the IgG3 subclass but the levels were low. Production of IgG2 and IgG3 antibody usually occurred in children who produced the highest levels of IgG1 antibody. The subclass distribution of antibodies to the HA of A/Chile/83 (H_1N_1) for these same serum specimens was similar (Table 4). Antibodies of the IgG1 subclass represented the majority and when present the IgG2 and IgG3 antibodies were of a low level. The distribution of H_3 and H_1 antibodies among the IgG subclasses was similar following one and two doses of TIV (data not shown).

Serum subclass antibody responses following vaccination with HA-D vaccine

Serum specimens from children who received HA-D vaccine one or two years after immunization with TIV were examined to determine the distribution of anti-HA among the IgG subclasses. Prior to immunization with HA-D, low levels of HA specific IgG1 antibody were detected in all serum specimens. As shown in Table 5, the subclass profile of antibodies directed to the HA of A/Mississippi/85 virus was not different from that induced by infectious virus or inactivated split-product vaccine. Again, HA antibodies were predominantly of the IgG1 subclass. When IgG2 and IgG3 were produced, serum levels were low. Antibodies to the HA of A/Chile/83 had a similar distribution among the IgG subclasses (data not shown).

Discussion

Immunization of children not previously exposed to the influenza virus with either live CRV or subvirion TIV induced similar serum IgG subclass antibody responses to the HA protein. Similarly, in primed children immunization with HA-D, purified HA covalently attached to diphtheria toxoid induced predominantly

antibody of the IgG1 subclass. Although the diphtheria toxoid should have provided additional recognition sites for the supplemental T-helper lymphocyte clones, the subclass distribution of antibody to HA was not changed. These findings in humans are in contrast to expectations from murine antibody responses (Balkovic et al., 1987).

Balkovic et al. demonstrated that following influenza virus infection, mice produce serum antibodies predominantly of the IgG2a subclass with low level of the IgG2b subclass, whereas mice immunized with purified HA or HANA-flu produce serum antibodies of the IgG1 subclass. This difference in immunoglobulin heavy chains has been shown following a wide variety of virus infections and immunization with soluble proteins in murine systems (Coutelier et al., 1987). Moreover, recent studies in mice indicate that this variation in heavy chain isotype is a result of the subclass of T-helper cell being utilized by the antigen specific B cell (Sanders et al., 1988). Sanders et al. further show that the utilization of T-helper 1 subset of lymphocytes in the immune response induces the B cells to switch from the production of IgM to IgG2a antibody by production of interferon gamma, whereas T-helper 2 subset by producing interleukin 4 and interleukin 5, induces the B cells to switch from the production of IgM to the production of IgG1. In mice, antibodies of the IgG2a and IgG2b subclasses have been shown to be more active in complement fixation (CF) reactions, antibody dependent cellular cytotoxicity (ADCC) reactions and binding to the Fc receptors on macrophages (Spiegelberg, 1974).

There is, at this time, no evidence of the existence of functional different subsets of T helper lymphocytes in humans, but if they do exist, they apparently do not alter the IgG isotype of antibody response to immunization with various forms of the influenza HA. However, in humans, antibodies of the IgG1 subclass are most active in CF and ADCC reactions, binding to the Fc receptors of macrophages. While IgG3 antibodies can also participate, they are present in serum at much lower concentrations (Hamilton, 1987). In mice, the IgG subclass most active in CF and ADCC reactions is the IgG2a subclass.

Although heavy chain isotypes are a marker for T-helper lymphocyte utilization in mice, it is possible that differences in CF, ADCC, or other immunological responses such as delayed hypersensitivity may be more pronounced in children. These functional assessments as well as the specificities of antibodies to the relevant antigens need to be established before these different vaccine formulations can be considered equivalent in unprimed individuals.

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