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Strain specificity of serum antibody to the haemagglutinin of influenza A (H3N2) viruses in children following immunization or natural infection

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SUMMARY

The specificity of serum anti-HA antibody from children immunized or infected with A/Victoria/75 (H3N2) or A/Texas/77 (H3N2) virus was examined using the single radial haemolysis test together with adsorption of antibody with three antigenic variants A/Hong Kong/68 (H3N2), A/Port Chalmers/73 (H3N2) and A/Victoria/75 (H3N2). The majority of young children reacted to vaccination or infection by producing strain-specific (SS) antibody to the homologous virus. A small proportion of children's sera contained cross-reacting (CR) antibodies capable of reacting with the haemagglutinins of all antigenic variants of the subtype including A/HK/1/68. In contrast, most adults reacted immunologically to either vaccination or infection by producing CR antibody, reacting with all variants of the antigenic subtype including the prototype virus A/HK/1/68 (H3N2).

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INTRODUCTION

The haemagglutinin (HA) of influenza virus induces virus neutralizing and haemagglutination-inhibiting (HI) antibody in the immunized or infected host (reviewed by Potter & Oxford, 1979). A number of studies have indicated a correlation between the level of such antibodies and resistance to infection (Morris *et al.* 1966; Potter *et al.* 1977). At the molecular level, the antigenic structure of the HA is complex and the molecule possesses at least two (Laver, Downie & Webster, 1974; Virelizier, Allison & Schild, 1974) and perhaps as many as five (Gerhard, 1976; Rovnova *et al.* 1979) different antigenic determinants. The relative importance in protection against influenza infection of antibody to the different antigenic determinants and the immunogenic nature of the HA is not known at present. However, it has been clearly established both in animals (Virelizier, 1975) and in man (Schild *et al.* 1977; Couch *et al.* 1979; Kasel *et al.* 1979; Oxford *et al.* 1979) that, following immunization or infection, at least two classes of antibodies are induced, one of which is narrowly specific, reacting with the HA antigens of the homologous and closely related virus strains (strain-specific, SS) whilst the other is more broadly specific (cross-reactive, CR) reacting with a wide range of antigenic variants within the antigenic subtype. Recent work has established that SS antibody has a higher intrinsic virus neutralizing capacity (per μg IgG) than CR antibody (Haaheim & Schild, 1980), and thus induction of SS antibody by vaccination, might be expected to give better protection against infection than do CR antibodies.

Most studies in man on the specificity of anti-HA antibodies induced by inactivated influenza vaccines have been carried out in 'immunologically primed' adults, i.e. individuals who had previous immunological experience, through natural infection with one or more viruses of the H3N2 subtype before immunization. Following immunization with inactivated influenza vaccines most such individuals produced mainly antibodies reacting with the CR determinants of the HA antigens of H3N2 strains (Kasel *et al.* 1976; Schild *et al.* 1977; Couch *et al.* 1979; Kasel *et al.* 1979; Oxford *et al.* 1979). In contrast, SS antibody to the HA of the vaccine virus was produced in only a small proportion of vaccinees. It is possible that this apparently paradoxical antibody response is partly responsible for the rather low efficacy of inactivated influenza virus vaccines in adults. (Schild *et al.* 1977).

In the present study we have analysed the specificities of the anti-HA antibody response in children who received inactivated A/Victoria/3/75 (H3N2) vaccine and demonstrate that, in contrast to adults immunized with the same vaccines, children produced largely SS antibody to the homologous virus in the vaccine.

MATERIALS AND METHODS

Viruses

Influenza A viruses were grown in embryonated hens' eggs by standard procedures. Concentrated and purified virus preparations for serological work were prepared as described previously (Skehel & Schild, 1971).

Inactivated influenza A/Victoria/3/75 (H3N2) vaccine

This was a purified surface antigen vaccine (SANDOVAC) containing essentially only the haemagglutinin and neuraminidase antigen of the A/Vic/75 (H3N2) virus in aqueous suspension. Its potency was approximately 15 μg per 0·5 ml dose.

Vaccination schedule and serum samples

Children aged 3–6 years received a single dose of vaccine i.m. and serum samples were collected at the time of vaccination (day 0) and at day 28. The sera were provided by Dr H. Bachmayer and Dr E. Liehl, Sandoz Research Institute, Vienna. In a separate study adults at the Clinical Research Centre, Harrow, were immunized with one dose of the same vaccine and the sera were collected on the day of vaccination and 14 days later.

Post-epidemic serum collection

Sera from persons of various age groups with HI titres $\geq$ 40 versus A/Tex/77 (H3N2) virus were selected from a post-epidemic collection of sera taken in October 1978. An outbreak of the A/Tex/77-like variant had occurred 6–7 months earlier (Haaheim, 1979). Nineteen sera from children aged 2–9 years with a mean age of 4·5 years and 24 sera from individuals from the age of 10–76 years with a mean age of 30·6 years were tested.

Single radial haemolysis test

Ten per cent (v/v) suspensions of freshly washed sheep erythrocytes (Oxoid) were made up in physiological saline buffered with 0·05 M HEPES buffer, pH 6·5. Chromium chloride was freshly diluted 1/400 in physiological saline from a 2·25 M solution (Oxford *et al.* 1979). Purified virus was added (10 μg virus protein per ml of 10% erythrocyte suspension) and within 10 min at 4 °C visible haemagglutination occurred and a half volume of the freshly diluted $CrCl_3$ solution was then added. The mixture was allowed to stand at 4 °C for 5 min with occasional mixing. The cell suspension was sedimented by gentle centrifuging (1000 rev/min for 5 min), washed once in 0·05 M HEPES pH 6·5 buffer containing 0·2% (w/v) bovine serum albumin (BSA) and once in phosphate buffered saline (PBS) pH 7·2 containing 0·2% BSA. Immunoplates containing the virus sensitized red blood cells and fresh guinea-pig complement in agarose gel were prepared as described previously (Schild, Oxford & Virelizier, 1976). The prepared plates or sensitized red blood cells could be stored at 4 °C for several weeks before use.

Analysis of anti-HA antibody in human sera

Paired human sera taken before and after immunization or single post-epidemic sera were analysed for anti-HA antibody specificity in SRH immunoplates containing erythrocytes sensitized with A/HK/68, A/Vic/75 or A/Tex/77 antigens. Analysis of the CR and SS antibody content of human sera was performed essentially as described previously (Oxford *et al.* 1979). Briefly, undiluted serum (40 μl) was heated (56 °C for 30 min) to destroy complement and incubated for 30 min at room temperature after mixing with 10 μl volumes of A/HK/68, A/Vic/75 or A/Tex/77 antigens (purified virus containing 10–15 mg/ml virus protein) or with 10 μl PBS as control. Volumes of 20 μl of the virus–serum mixture were then added to the wells of the SRH immunoplates. Preliminary tests established that the dose of virus antigen used for adsorption in these experiments was in excess of that required to adsorb the SS or CR antibody. In certain experiments sera were adsorbed with other viruses of the H3N2 antigenic subtype including A/England/42/72 (H3N2), A/Port Chalmers/73 (MRC-11 recombinant) and A/Scotland/74. Control viruses included B/HK/73 and A/PR/8/34 (HON1). SRH plates were incubated overnight at 37 °C and the diameter of the lysis zones measured using a calibrating viewer (Transdyne General Corporation).

RESULTS

Occurrence of SS and CR antibodies in post-immunization sera

Analysis of the specificity of the antibody response in both children and young adults receiving inactivated influenza vaccine is presented in Table 1. The antibody response in adults was characterized by the production of CR antibodies in 82% of vaccinees. Only 36% of adults produced SS antibody to the A/Vic/75 virus in the vaccine. In contrast the majority, (78%) of post-vaccination childrens' sera contained antibody reacting with the SS antigenic determinant of the vaccine virus A/Vic/75 virus and fewer sera showed antibody rises to the common CR determinant(s) shared between A/HK/68 and A/Vic/75 viruses and the SS_{HK} determinant(s).

The antibody response in any individual serum was often complex, and some sera had detectable antibody rises to more than one of the different antigenic determinants studied, namely SS_{HK}, SS_{Vic} and CR, although certain children reacted to only a single group of antigenic determinants. Six children had detectable rises in antibody to both SS_{HK} and SS_{Vic} antigenic determinants and ten children had antibody rises to SS_{Vic} and CR antigenic determinants.

Distribution of SS and CR antibodies in a post-epidemic serum collection

The data presented in Table 2 indicate that a high proportion of children had specific (SS) antibodies to the HA antigen of A/Tex/77 virus, whereas only 7 out of 24 sera from adults (29%) had detectable antibody of this kind. The pattern of antibody prevalence therefore differed markedly between the children and adults and was very similar to that following vaccination described above. In

Table 1. *Specificity of anti-influenza HA antibody in pre- and post-immunization sera from children and young adults*

		No. of sera with following antibody								
		SS_{HK}			SS_{Vic}			CR		
Sera from	No. tested	Pre	Post	% Rises	Pre	Post	% Rises	Pre	Post	% Rises
Children[1]	23	3	6	26	8	18	78	11	16	39
Adults[2]	22	6	10	46	1	8	36	10	19	82

Pre, Post: sera taken pre- and post-immunization.

SS_{HK}: strain-specific antibody to HA of A/HK/1/68 (H3N2) virus; CR cross-reactive antibody; SS_{Vic} strain-specific antibody to HA of A/Victoria/75 (H3N2) virus.

[1] Age range 3–6 years.

[2] Age range 19–30 years.

Table 2. *Specificity of anti-influenza HA antibody in a post-epidemic serum collection from children and adults*

		No. and (%) of sera with following antibody		
Sera from	No. tested	SS_{HK}	SS_{Tex}	CR
Children[1]	19	0	18 (95%)	10 (53%)
Adolescents and adults[2]	24	21 (88%)	7 (29%)	24 (100%)

[1] Age range from 2–9 years.

[2] Age range from 10–76 years.

SS_{HK}: strain-specific antibody to HA of A/HK/1/68 (H3N2) virus; SS_{Tex}: strain-specific antibody to HA of A/Texas/1/77 (H3N2) virus; CR: cross-reactive antibody.

particular, the frequency of presence of SS_{HK} antibody was very different between the two age groups. None of the children's sera had detectable antibody to the SS_{HK} antigenic determinant, in contrast to sera in the older age group in which 88% had specific anti A/HK/68 HA antibodies.

In order to investigate in more detail the spectrum of antibodies present, a number of post-epidemic sera from both children and adults were analysed in more detail using adsorption techniques with successively prevalent antigenic variants of the H3N2 subtype, i.e. A/HK/68, A/PC/73 and A/Tex/77 (Figs 1 and 2). Figure 1 shows the reactions in SRH immunoplates of two representative sera from individuals aged 3 and 52 years respectively. The serum from a three-year-old child gave zones only in the A/Tex/77 plate, and SRH reaction was not reduced by absorption with A/HK/68 virus indicating the strain-specific nature of the antibody. The area of the zone was reduced by 50% after adsorption with A/PC/73 virus, thus indicating the presence of a population of antibodies specific for A/Tex/77 but not reacting with A/PC/73 HA. Absorption with A/Tex/77 virus resulted in complete removal of the haemolysis zone. The serum from a 52 year old person, on the other hand, reacted in both immunoplates showing the presence of antibody cross reacting with A/HK/68 and A/Tex/77 (CR antibody). Reactivity

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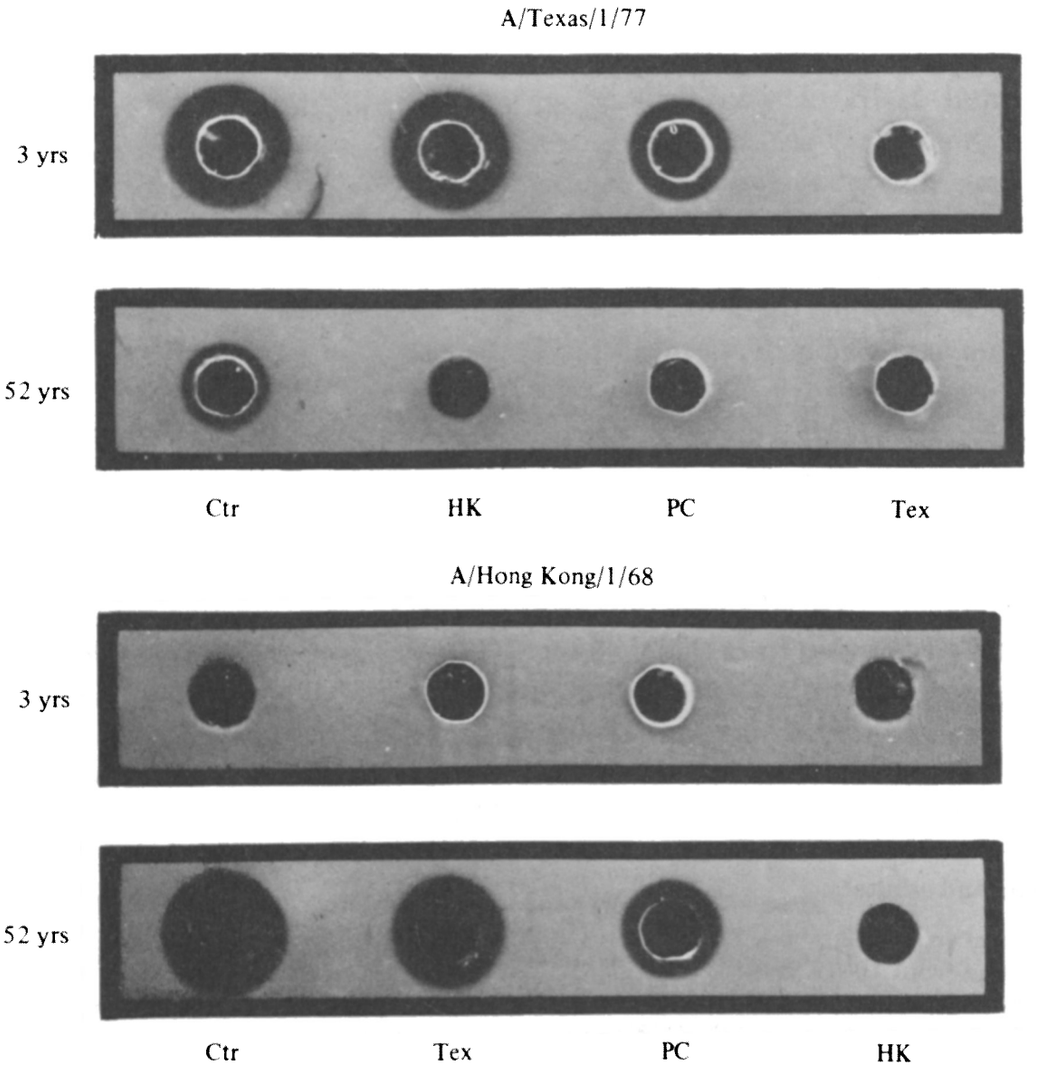

Fig. 1. Specificity and potency of anti-HA antibody of two representative post epidemic sera. The sera from a three-year-old child and an adult of 52 years were tested in SRH immunoplates containing the H3N2 viruses A/Texas/1/77 and A/HK/1/68. The absorbing viruses were, as a control, A/PR/8/34 (HON1) (Ctr), and the successive H3N2 variants A/HK/1/68 (HK), A/Port Chalmers/1/73 (PC) and A/Texas/1/77 (Tex). The serum from the child gave zones of haemolysis only in the A/Texas/ plate, whereas the serum from the adult gave zones in both immunoplates. The adsorption patterns indicated that the serum from the child had SS_{Tex} antibody as well as some 'narrow-range' CR antibody since the size of the haemolysis zone on the A/Texas/77 immunoplate was reduced after adsorption with A/PC/73 virus, whereas the serum from the older person had no SS_{Tex} antibody but did possess SS_{HK} and CR antibodies.

on the A/Tex/77 plate was not removed after absorption of the serum with A/PC/73 and A/HK/68 viruses, suggesting that SS_{Tex} antibody was not present.

Figure 2 presents quantitative aspects of the SRH adsorption data for some representative individual sera. The upper row (Fig. 2*a–c*) shows results obtained from three typical children's sera which contained antibodies reacting with SS_{Tex} antigenic determinants. Thus, no haemolysis zones were detected in the A/HK/68 SRH plates and the zone sizes in the A/Tex/77 SRH plates were not reduced to any large extent after adsorption with A/HK/68 virus. The serum of a seven-year-old

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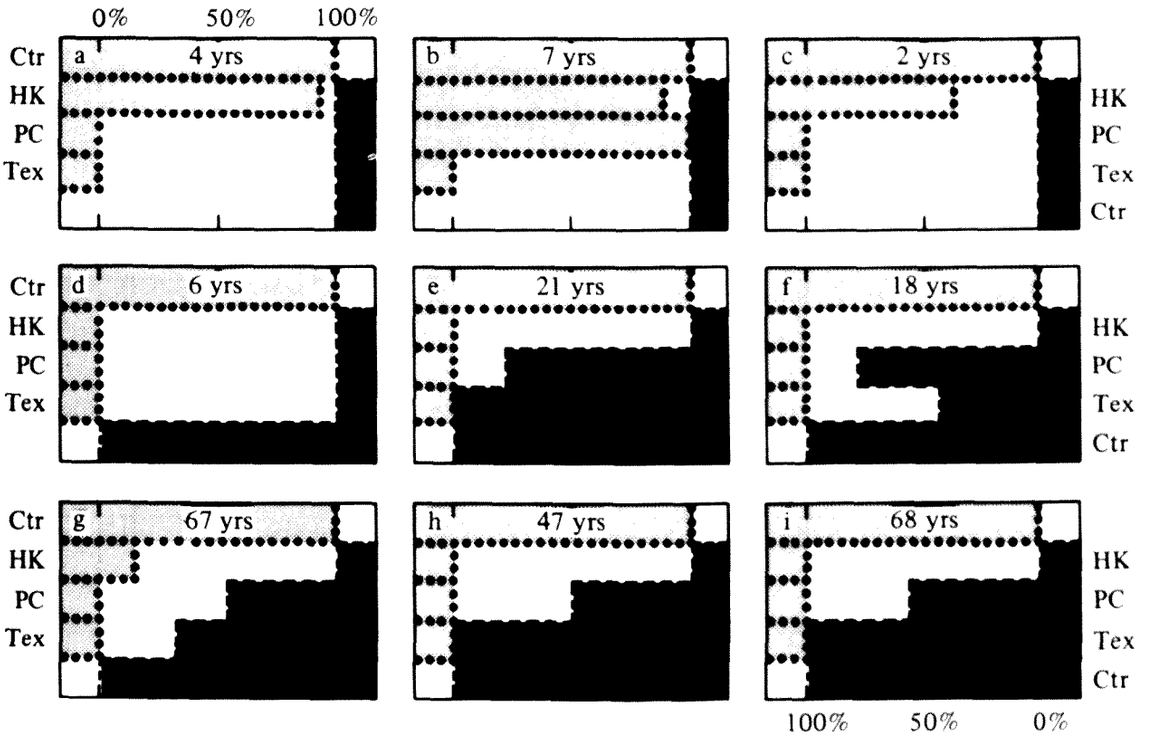

Fig. 2. Quantitative SRH absorption data for some representative individual post-epidemic sera. The horizontal histograms represent per cent SRH zone areas of haemolysis after absorption with A/PR/8/34 (H0N1) virus as a control (Ctr) or with the H3N2 variants A/HK/1/68 (HK), A/Port Chalmers/1/73 (PC), and A/Texas/1/77 (Tex). The lighter-shaded histograms illustrate SRH haemolysis reactions on the A/Texas immunoplate, the scale running from left to right, whereas the darker-shaded histograms represent SRH reactions obtained on the A/HK immunoplate, the scale running from right to left.

The upper panel (Fig. 1*a–c*) shows antibody patterns of childrens' sera, none of which gave positive reactions in the HK/68 plate, indicating that no SS_{HK} antibodies were present. All these three sera had antibody to A/Tex/77 virus, of which very little could be absorbed by HK/68 virus, suggesting that these sera had SS_{Tex} antibody. Since some antibody could be removed by adsorption with A/PC/73 virus (Fig. 1*a*, *c*) these two sera had in addition 'narrow range' CR antibody. The serum from a six-year-old child, shown in Fig. 1(*d*), had only CR antibodies reacting in both SRH plates and completely absorbed by cross-adsorption with the heterologous strains. The remaining sera (Fig. 1(*e*), (*f*), middle panel, and Fig. 1(*g*)–(*i*), lower panel) show adsorption patterns obtained with some representative adult sera, all of which had CR antibody as well as some SS_{HK} antibody. None of them, except for the serum shown in Fig. 1(*g*) had any evidence of having SS_{Tex} antibody.

child shown in Fig. 2*b* reacted on the A/Tex/77 immunoplate and the haemolysis zone was only reduced by prior adsorption of the serum with the homologous virus A/Tex/77: the antibodies were of a highly specific SS_{Tex} specificity. The antibodies in sera 2a and 2c were rather less specific and could be removed by adsorption with the A/PC/73 virus variant. In contrast, and rather exceptionally, the serum from a six-year-old child, shown in Fig. 2*d*, reacted exclusively with the CR determinant(s) shared between the H3N2 test strains and haemolysis zones in A/Tex/77 and A/HK/68 SRH plates were reduced to non-detectable levels by prior adsorption of the serum with heterologous virus.

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Individual adult sera illustrated in Fig. 2*e–i* exhibited broader patterns of serological reactivity. Thus, the sera reacted in the A/HK/68 plate and there were varying degrees of reduction in haemolytic zone sizes following adsorption with the different influenza A viruses. Nevertheless, each antiserum revealed a unique spectrum of reacting antibodies. Unlike the childrens' sera, none of these latter sera possessed antibodies which reacted exclusively with the specific SS_{Tex} antigenic determinants.

DISCUSSION

Experimental studies have established that SS antibody is more efficient in conferring passive immunity in influenza infected animals than is CR antibody (Virelizier, 1975) whilst SS antibody has a higher neutralizing capacity per quantity of IgG than CR antibodies *in vitro* (Haaheim & Schild, 1979). The present study establishes that SS antibody was induced by vaccination in a very high proportion of young children, whereas it was induced in only a low proportion of adults. It might be anticipated, therefore, that the protective efficacy of inactivated influenza vaccine currently estimated to be around 70% in adults (Smith, 1976) may be higher in children following first vaccination with a new antigenic subtype. In recent reports (Hoskins *et al.* 1979; Sparks, 1979) describing annual vaccination of healthy young boys at boarding schools a good protection rate against clinical influenza was induced by the vaccine containing the first variant within the H3N2 subtype (A/HK/1/68) but subsequent annual revaccination did not result in a reduction in influenzal morbidity. We would predict that the first immunization induced SS antibodies to the homologous virus whereas re-immunization stimulated less protective CR antibodies.

It is clear from the present study that production of SS and CR antibodies are not mutually exclusive and that many individuals, particularly adults, produce high titres of antibody reacting with both these groups of antigenic determinants. High levels of CR antibodies, shown to have some protective effect both in animal models (Virelizier, 1975; Haaheim & Schild, 1980) and in man (Couch *et al.* 1979; Kasel *et al.* 1979), might be the preferable type of antibody response after vaccination. Another unknown and perhaps important variable at present is the respective longevity of antibody to SS and CR antigenic determinants and this should now be determined experimentally.

The individual immunological response to the influenza HA molecule was more often complex in adults than in children and it is not unexpected that individuals should vary in their immunological response to such a large glycoprotein molecule with multiple antigenic determinants. Post-immunization adult sera may contain a number of antibody populations, one reacting specifically with the homologous virus in the vaccine or causing the infection such as A/Tex/77 (H3N2) virus, and another antibody population reacting more broadly to include A/HK/68 (H3N2) virus. Individual sera also differed in their reaction with closely related viruses. Thus, a particular individual, as illustrated in Fig. 1, had antibodies reacting only with A/Tex/77 virus, whilst another had SS antibodies reacting

with both A/Tex/77 and A/HK/68 virus. Many individuals possessing immunological memory to a particular SS or CR antigenic determinant of the virus causing a first infection may be 'sidetracked' in their response to SS determinants of the next encountered variant within the subtype.

Antigenic determinants of the HA which are important in immunity to influenza infection may now be identified using virus mutants selected in the presence of monoclonal antibodies to influenza HA (Webster & Laver, 1980; Yewdell *et al.* 1979). Such studies are in progress and preliminary results show that antibodies to these mutants are less commonly found in children's sera (Haaheim, L. R., personal communication; Natali, A., personal communication), thus confirming the immunologically narrow reacting properties of anti-HA antibodies induced in children's sera compared to adult sera.

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