Local and systemic influenza haemagglutinin-specific antibody responses following aerosol and subcutaneous administration of inactivated split influenza vaccine

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An easily administered and safe vaccine is required to produce the herd immunity necessary to control influenza epidemics worldwide. A commercial quadrivalent inactivated split influenza vaccine was administered intranasally in aerosol form to a group of 46 volunteers; other groups were given the same vaccine subcutaneously and saline intranasally. The results show that mucosal stimulation via intranasal vaccination resulted in a marked increase in local HA-specific IgA antibodies, and that this stimulation was necessary for serum HA-specific IgA responses. Serum HA-specific IgA antibody levels can be used as indicators of local antigenic stimulation, providing a method for evaluating potency and antigenicity in humans of intranasal influenza vaccine. This vaccination route shows much promise for the future.

Keywords: Inactivated influenza vaccine; local IgA antibody; aerosol administration; serum HI antibody; influenza haemagglutinin

INTRODUCTION

Inactivated influenza vaccines administered subcutaneously or intramuscularly, either in the form of a whole virion or in the split or subunit form, have been the only licensed influenza vaccines in the United States, Europe and Japan[1]. Since influenza virus proliferates over the mucosal surface and causes disease instantaneously, vaccines which will stimulate both systemic and mucosal immunity are more promising for the prevention of influenza epidemics[2].

It has been reported that the parenteral administration of inactivated influenza vaccine did not induce local specific IgA responses[3]. Direct antigenic stimulation of the upper respiratory tract with a vaccine may induce local immunity. Therefore, an inactivated influenza vaccine, administered directly in the upper respiratory tract, is a good candidate vaccine which may be used safely in humans.

Systemic and local immune responses to topically administered inactivated influenza vaccine have not been fully investigated. There are a few clinical trials on local antibody responses of humans to topically administered influenza vaccines[4,5]. However, the method described for determination of local antibody responses required nasal wash solution and the results obtained were expressed only in terms of end points and not in terms of units. Lack of a sensitive method of microassay of specific antibodies present on the surface of the upper respiratory tract has long been one of the major weak points in the study of local immune responses to topically administered vaccines. We have developed a very sensitive, reliable and reproducible method of quantification of local specific antibodies to influenza haemagglutinins (HA) and investigated local specific IgA antibody responses to aerosol administration of an inactivated influenza vaccine; we compared serum haemagglutinin inhibition (HI) titres and serum specific IgA, IgM and IgG antibody levels between two groups of young adults, one of which received subcutaneous inactivated influenza vaccine and the other received aerosol administration of the same vaccine.

Methods of evaluation of the potency and antigenicity in humans, both local or systemic, are also required for an influenza vaccine administered intranasally. This study may furnish basic information for estimation of the degree of local and systemic antigenic stimulation of topically administered inactivated influenza vaccine.

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VOLUNTEERS AND METHODS

Volunteers

Ninety-two young adult volunteers were divided into two groups, the aerosol administration group and the subcutaneous administration group. Written informed consent was obtained from all volunteers. A further 46 young adult volunteers served as controls and received aerosol administration of saline.

Vaccine

Commercially available quadrivalent inactivated split influenza vaccine (0.5 ml), which was distributed during the winter of 1988–89 [A/Yamagata/120/86 (H1N1) 200 chicken cell agglutination units (CCA) ml^{-1} equivalent; A/Fukuoka/C29/85 (H3N2) 200 CCA ml^{-1} equivalent; A/Sichuan/2/87 (H3N2) 200 CCA ml^{-1} equivalent; and B/Nagasaki/1/87 100 CCA ml^{-1} equivalent] was administered by either the subcutaneous or intranasal route.

Method of administration

Aerosol administration. Forty-six volunteers received the vaccine intranasally in the form of aerosol. The vaccine was sprayed on each side of the nose at a volume of 0.25 ml each by the Jacksonian type tracheal spray. The vaccine was given four times at 1 week intervals between each administration.

Subcutaneous administration. Forty-six volunteers received subcutaneous administration of the vaccine 0.5 ml each, twice at a 4-week interval.

Collection of the samples

Preparation of the nasal swab solution. The samples were collected from the vaccinee group before administration and 7 weeks after the fourth aerosol administration of the inactivated split vaccine. Other samples were collected from the control group before administration and 6 weeks after the second administration of the saline.

A wet cotton swab, which had been soaked in 0.01 M phosphate-buffered saline (PBS), was gently rotated in the nose for 30 s. In this way at least 20 μl of mucus can be collected. The swab was mixed with 3 ml 0.01 M PBS. The solution was centrifuged for 30 min at 3000 rev min^{-1} to remove any cell debris, and NaN_3 was added at a concentration of 0.05%. The sample was stored at −20°C until assayed.

Collection of sera. Serum samples were collected before vaccination and 7 weeks after the fourth dose of aerosol vaccine or 6 weeks after the second dose of the subcutaneous vaccine.

ELISA method

ELISA of specific IgA antibodies of nasal swab solution. Details of the ELISA method have already been published[6]. In brief, a Linbro-Titertek EIA microtitration plate (Flow Laboratories Inc., McLean, VA 22102, USA) was coated with purified influenza haemagglutinin (HA) solution and was allowed to stand at 4°C for 2 days. After washing with PBS containing 0.05% Tween 20 (PBS–Tween), 50 μl of nasal swab solution or standard solution, which was the nasal swab solution collected from an adult who had received aerosol influenza vaccine every day for 7 days, was added to the wells. The plate was then incubated at 4°C overnight. After two washings with PBS-Tween, alkaline phosphatase labelled anti-human IgA goat serum conjugate diluted with PBS containing 1% bovine serum albumin was added. After incubation for 2 h the plate was washed twice with PBS and three times with demineralized water. Then 200 μl of the substrate solution (100 mg of *p*-nitrophenyl phosphate disodium dissolved in 100 ml of demineralized water with 0.53 g of Na_2CO_3, 0.02 g of $MgCl_2 . 6H_2O$ and 1 ml of 1 M HCl) was applied. After allowing to stand at 37°C for 1 h, absorbancy at 405 nm (A_{405}) was measured using a microplate photometer. The results were expressed in terms of units using the standard solution.

Total IgA concentrations of nasal swab solutions were measured by laser nephelometry (Behringwerke, Marburg, Germany) and the final results of the specific IgA values were expressed in terms of ELISA units mg^{-1} of IgA.

Determination of serum class-specific anti-influenza HA antibodies. The methods were essentially the same as that described above. Alkaline phosphatase labelled anti-human IgG, IgA and IgM goat sera were used. The values were expressed in terms of ELISA units ml^{-1} of serum.

Serum HI titres

Serum HI titres were quantified by the standard method. Haemagglutinins of the subtypes of influenza virus contained in the vaccine were used as antigens of HI tests. It should be noted that expression of the dilution of sera in Japan is different from that in the United States; a dilution of 1:8 in the United States corresponds to a dilution of 1:32 in Japan.

Statistical analysis

The χ^2 test with Yates' correction was used to compare the rises in serum HI titres or in influenza HA-specific antibodies between subcutaneous and aerosol administration groups. The Spearman–Kendall test was used to evaluate a correlation between serum-specific IgA antibody levels and HA-specific IgA antibody levels of the nasal swab solution.

RESULTS

Figure 1 shows serum HI titres (A/Sichuan H3N2) before and after administration of the subcutaneous (*Figure 1a*) or aerosol (*Figure 1b*) influenza vaccine. Greater than fourfold rises in HI titres were observed in 93% (43/46) of the subcutaneous administration group. Geometrical mean prevaccination and postvaccination titres were $2^{3.9}$ and $2^{6.9}$, respectively. Greater than fourfold elevations of serum HI titres were observed in 87% (40/46) of the aerosol vaccine administration group. Geometrical mean prevaccination and postvaccination titres were $2^{4.5}$ and $2^{7.1}$, respectively. Percentages of more than fourfold rises in serum HI titres were not significantly different between the two groups ($\chi^2 = 0.4926$, $p = 0.4827$, χ-Yates test). It should be noted that marked elevations of HI titres were observed among those with a prevaccination HI titre of less than 1:16 of the aerosol administration group.

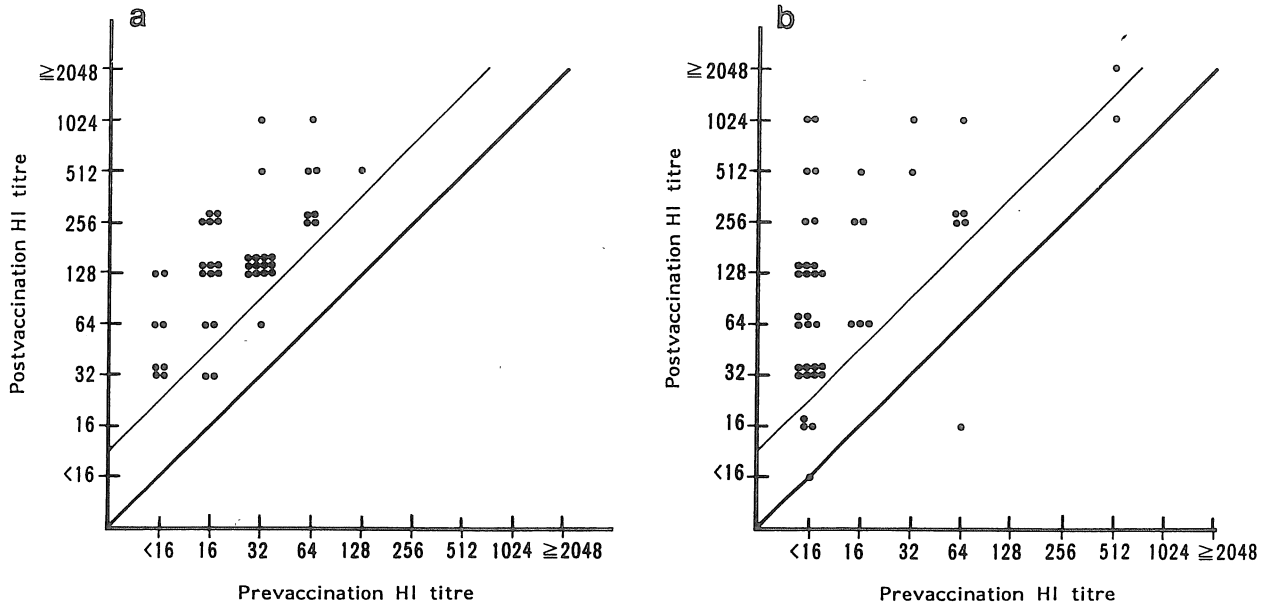

Figure 1 Serum HI titres (A/Sichuan H3N2) before and after administration of the subcutaneous (a) or aerosol (b) influenza vaccine

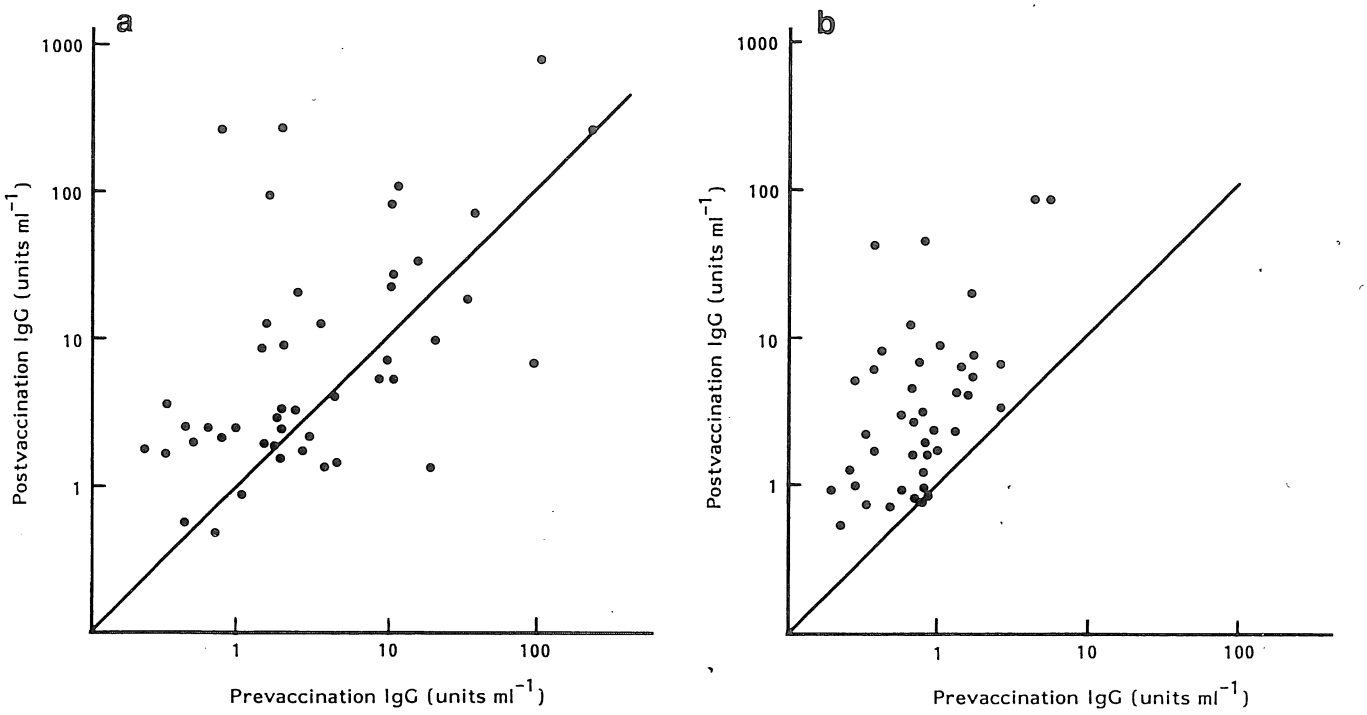

Figure 2 Serum HA (A/Sichuan H3N2) specific IgG antibody levels before and after administration of subcutaneous (a) or aerosol (b) influenza vaccine

Figure 2 shows serum HA (A/Sichuan H3N2) specific IgG antibody levels before and after administration of subcutaneous or aerosol vaccine. Greater than fourfold rises in serum HA-specific IgG antibody levels were observed in 37% (17/46) of the subcutaneous vaccine group and 48% (22/46) of the aerosol vaccine group. Percentages of greater than fourfold rises in serum HA-specific IgG antibody levels were not significantly different between the two groups ($\chi^2 = 0.7121, p = 0.3987$, χ-Yates test).

Figure 3 shows serum HA (A/Sichuan H3N2) specific IgM antibody levels before and after subcutaneous or aerosol administration of the vaccine. Greater than fourfold elevations in serum HA (A/Sichuan H3N2) specific IgM antibody levels were observed in 20% (9/45) of the subcutaneous vaccine group and in 40% (17/43) of the aerosol vaccine group. The percentages of more than fourfold rises were not significantly different between the two groups ($\chi^2 = 3.1472$, $p = 0.0760$, χ-Yates test).

Figure 4 shows prevaccination and postvaccination serum HA (A/Sichuan H3N2) specific IgA antibody levels. Greater than fourfold rises in serum HA-specific IgA antibody were noted in 4% (2/46) of the subcutaneous administration group, and in 58% (23/40) of the aerosol administration group. The aerosol administration group showed a markedly higher rate of greater than fourfold elevation than the subcutaneous administration group ($\chi^2 = 26.7937$, $p < 0.0001$, χ-Yates

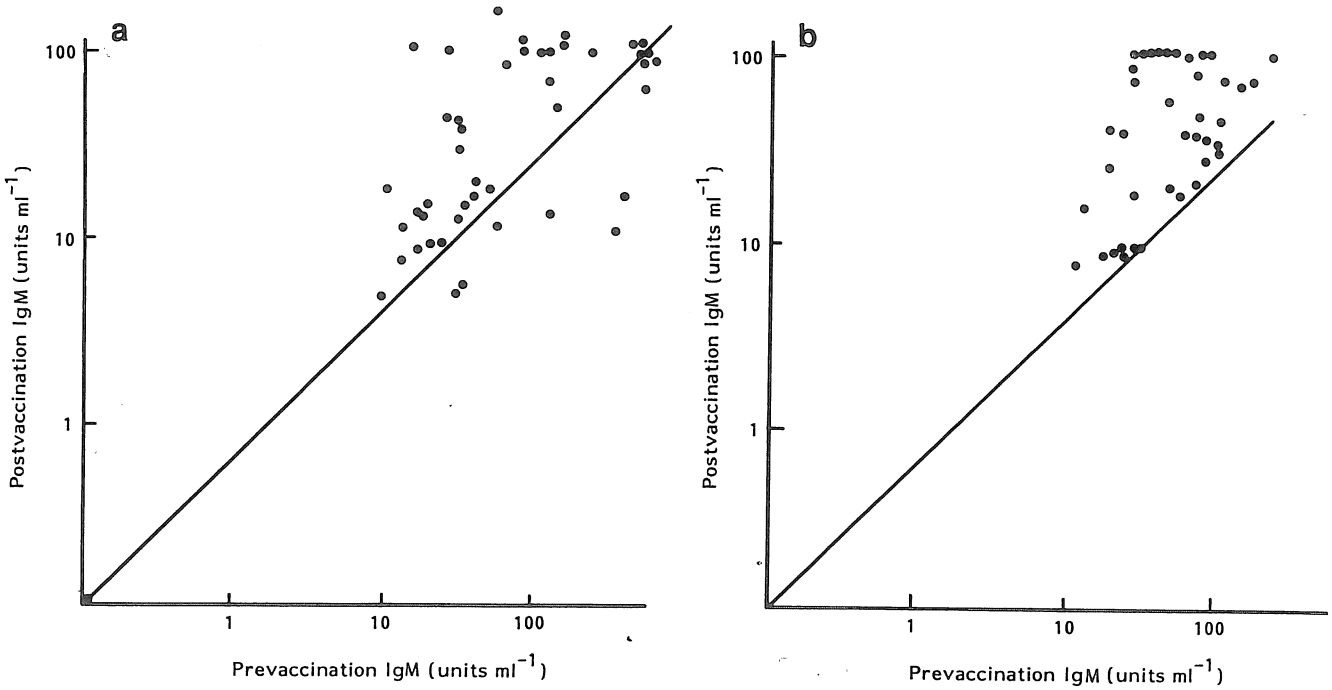

Figure 3 Serum HA (A/Sichuan H3N2) specific IgM antibody levels before and after administration of subcutaneous (a) or aerosol (b) influenza vaccine

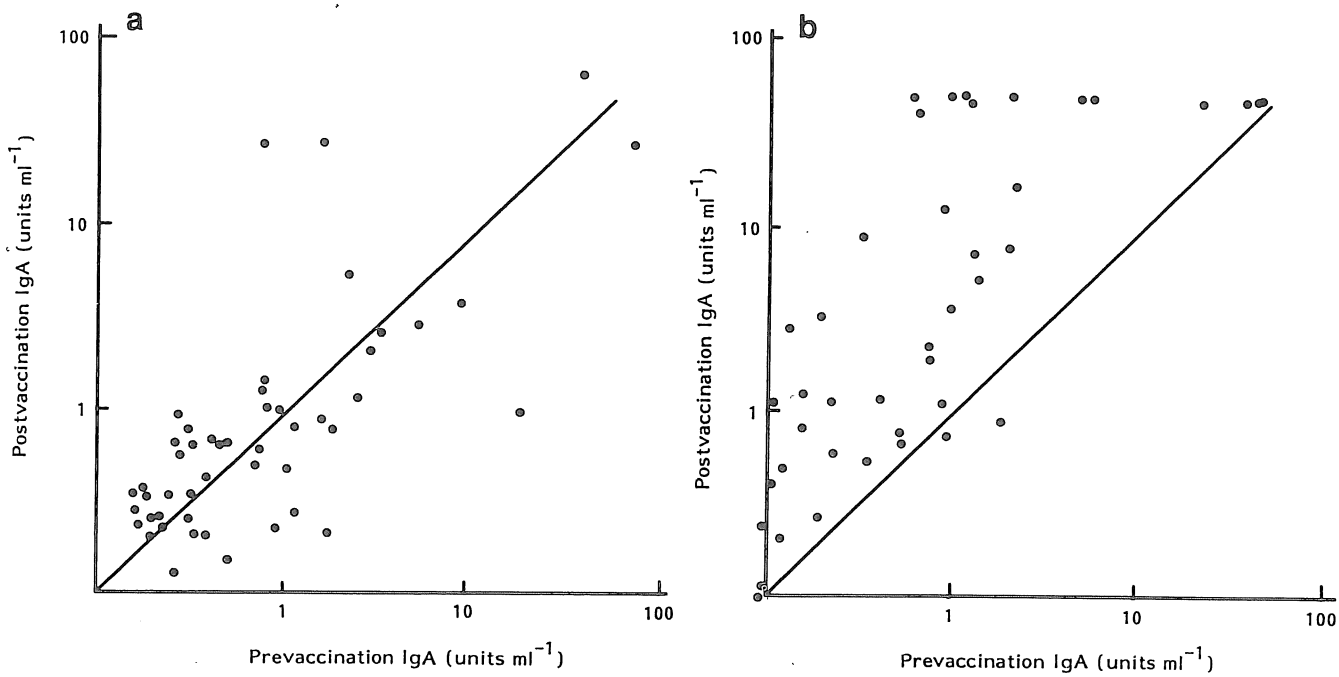

Figure 4 Serum HA (A/Sichuan H3N2) specific IgA antibody levels before and after administration of subcutaneous (a) or aerosol (b) influenza vaccine

test). Fold rises of pre- and postvaccination serum HA-specific IgA antibody levels and those of the HA-specific IgA antibody levels of the nasal swab solution showed a positive correlation, giving a correlation coefficient of 0.3538 and $p = 0.0006$ by the Spearman–Kendall test.

Figure 5 shows specific IgA antibody levels in nasal swab solutions against the haemagglutinin of A/Sichuan/2/87 (H3N2) before and after aerosol administration of the split vaccine. A greater than fourfold rise in the level was observed in 28% (12/43) of the vaccinee group, while none of the 24 controls, who received aerosol saline in place of the vaccine, showed greater than fourfold rises in the local HA-specific IgA antibody levels. The difference was statistically significant ($\chi^2 = 6.3712$, $p = 0.00115$, χ-Yates test).

DISCUSSION

Influenza virus mutates very frequently. There are two types of mutations. When a whole RNA genome is replaced by another RNA genome, the mutation is called