Developments in mucosal influenza virus vaccines

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Immunogenicity and efficacy of aerosol inactivated split influenza virus vaccines, which are threefold the strength of the vaccines for parenteral use, and cold-adapted reassortant live influenza virus vaccines were evaluated. Mucosal immune responses were evaluated by quantifying specific IgA antibody of the nasal swab solution, and systemic immune responses were evaluated by determining serum haemagglutination inhibition antibody levels. Efficacy of the aerosol inactivated vaccine was evaluated by a challenge test using live vaccine virus. It was concluded that mucosally administered inactivated influenza virus vaccine stimulated systemic and mucosal immune responses more strongly than live influenza virus vaccine and manifested a much stronger booster effect than live vaccine. Mucosal administration of inactivated influenza virus vaccine was effective in preventing infection by live vaccine virus.

Keywords: Mucosal vaccine; influenza virus vaccine; local IgA response

In the past it was reported and believed that parenterally administered inactivated influenza virus vaccines induced local IgA responses. A comparative study on systemic and local immune responses to intranasally administered cold-adapted live influenza virus vaccine and parenterally administered inactivated influenza virus subvirion vaccine in seronegative adults was performed. The report concluded that parenterally administered inactivated influenza vaccine induced higher titres of serum antibodies than live virus vaccine and stimulated a nasal wash IgG response more strongly than live vaccine virus, and that 38% of recipients of inactivated vaccine had local IgA responses compared with 83% of those who received live virus vaccine[1]. Topical or aerosol application of inactivated influenza vaccine to the upper respiratory tract was reported to be immunogenic in primed subjects but the route was supposed to be inferior to subcutaneous routes in the induction of either serum or nasal antibody[2]. However, in contrast to those previous observations and assumptions that parenteral administration of inactivated influenza vaccine induced local IgA responses, it has now been found that parenterally administered inactivated influenza vaccines stimulate antibodies mainly in the blood, whereas studies in mice suggest that oral or nasal administration of inactivated influenza vaccine virus induced a local IgA response[3]. Another overview mentioned that inactivated influenza virus vaccines given by nasal drops, sprays or small-particle aerosol induced local antibody and protection against challenge[4]. Resistance to influenza infection in humans has been correlated with influenza haemagglutinin (HA) antibody in nasal washings. It has been assumed that in humans antibody present in either the local or systemic compartment can contribute to resistance to illness caused by influenza virus, but that there is a possibility that to be protective, HA antibody must be present at the mucosal surface, having been produced either locally or derived from serum[5]. These recent observations and assumptions indicate a preference for a vaccine that can induce local immune responses of upper respiratory mucosal surfaces as well as systemic immune responses to a vaccine given parenterally which can stimulate systemic immune responses only. The current concept of common mucosal immune systems states that the mucosal immune system is completely independent of the systemic immune system[6]. It is now clear that to stimulate the mucosal immune system a vaccine should be administered directly to mucosal surfaces. Upper respiratory mucous membrane surfaces provide a reasonable route of administration of vaccines for upper respiratory viral diseases such as influenza.

Influenza is such a common disease that most people have some degree of immunity to influenza virus. Therefore, an influenza virus vaccine which can enhance immunity of partially immune people as well as endorse immunity in non-immune people may be preferable.

There are two candidate mucosal influenza virus vaccines which can be administered intranasally: cold-adapted reassortant live influenza virus vaccine[7] and aerosol concentrated inactivated split influenza virus vaccine[8–10]. The purpose of this paper is to report the results of a crossover test of these two types of mucosal

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0264-410X/94/14/1303–08

vaccines in order to compare immunogenicities of mucosally administered live vaccine and inactivated vaccine and to evaluate the efficacy of mucosally administered inactivated vaccines by a challenge test taking live vaccine virus as the challenge virus.

MATERIALS AND METHODS

Volunteers

Volunteers, who had been lving in the same school dormitory for a long period of time and had close contact with each other, were divided into two completely comparable groups. The two groups were matched with regards to sex, age, socioeconomic status and status of medical care. The first group was composed of 35 volunteers, aged 16 or 17, comprising 17 boys and 18 girls. The second group was composed of 40 volunteers, aged 16 or 17, comprising 20 boys and 20 girls. No selection was made with regards to preimmunization antibody levels. The first group initially received one 0.5 ml dose of trivalent cold-adapted live reassortant influenza vaccine intranasally and then after 6 weeks received two 0.5 ml doses each of aerosol trivalent concentrated inactivated split influenza vaccines at an interval of 1 week. The second group each received initially two 0.5 ml doses of aerosol inactivated vaccine intranasally at an interval of 1 week and then after 6 weeks, one 0.5 ml dose of live vaccine. Written informed consent was obtained from all volunteers.

Vaccines

A 1 ml plastic disposable syringe with a tip was used to administer the vaccine intranasally (*Figure 1*). The droplet diameters of the mists of liquid sprayed out of the above-mentioned syringe were measured by use of Phase Doppler Particle Analyzer (Aerometrics, USA, imported by Seika Sangyo Co. Ltd, Shinzyuku-ku, Tokyo). The droplet diameters ranged from 5 to 150 μm (*Figure 2*).

Aerosol inactivated vaccine was threefold the strength of commercially available trivalent inactivated split influenza vaccine [A/Yamagata/32/89(H1N1) 900 CCA ml^{-1} equivalent, A/Beijing/352/89(H3N2) 750 CCA ml^{-1} equivalent, B/Bangkok/163/90 750 CCA ml^{-1} equivalent]. The vaccine was supplied by the Research Foundation for Microbial Disease of Osaka University, Suita City, Osaka, Japan.

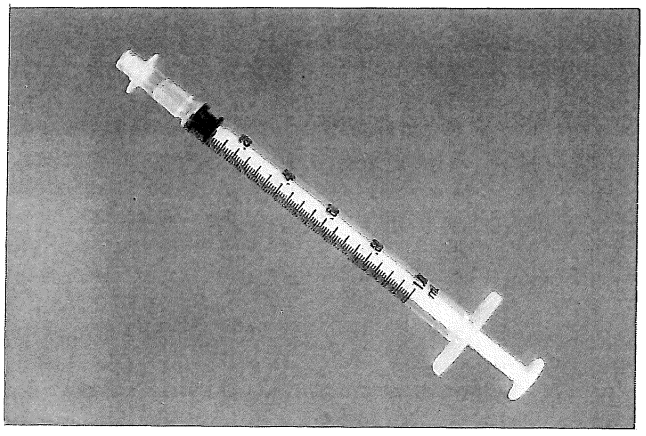

Figure 1 Disposable syringe (capacity 1 ml) with tip. A total of 0.5 ml vaccine was sprayed into two sides of the nose

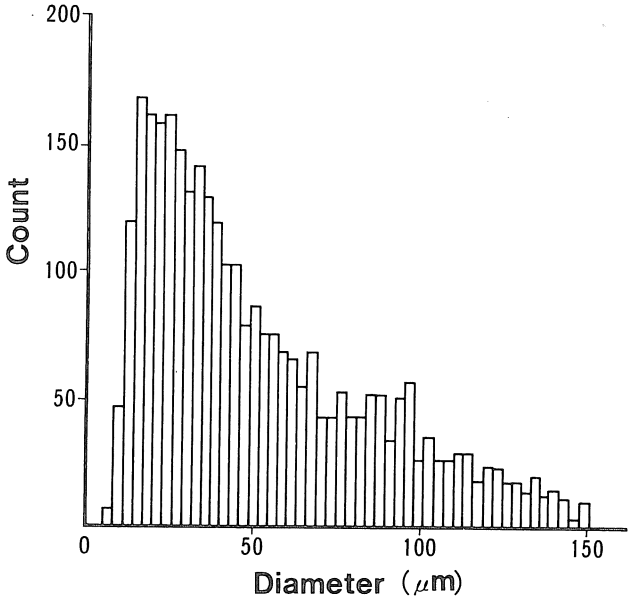

Figure 2 Distribution of droplet diameter of mists sprayed out from the apparatus in *Figure 1*

The live influenza vaccine was trivalent influenza cold-adapted reassortant live influenza vaccine [AA-CR 125; A/Kawasaki/9/86 (H1N1) × A/Ann Arbor/6/60 (H2N2) $10^{7.5}$ p.f.u. ml^{-1}, AA-CR 179; A/Beijing/352/89 (H3N2) × A/Ann Arbor/6/60 (H2N2) $10^{7.5}$ p.f.u. ml^{-1}, AA-CRB 181; B/Bangkok/163/90 × B/Ann Arbor/1/66 $10^{7.5}$ p.f.u. ml^{-1}], supplied by the Chemo-Sero Therapeutic Institute, Kumamoto City, Japan. The cold-adapted reassortant live vaccines were antigenically identical with the respective type A H1N1, A H3N2 and B inactivated vaccines.

Specimens

The serum samples and nasal swab solutions were collected from each individual before and 6 weeks after the administration of the vaccines. A cotton swab was soaked in 0.01 M phosphate-buffered saline (PBS) and then applied into the nose. The swab was gently rotated in the nose for 30 s on each side. By this procedure at least 20 μl of mucus could be collected. The swab was mixed with 3 ml 0.1 M PBS. The superanatant was stored at −20°C until assayed.

ELISA

Details of the ELISA method have been published[9,10]. In brief, 50 μl of nasal swab solution or standard solution collected from a hyperimmune adult were placed in the wells of a Limbro-Titertek EIA microtitration plate (Flow Laboratories, Inc., McLean, VA 22102, USA), which were precoated with purified influenza haemagglutinin (HA) solution. The plate was incubated and washed. Then alkaline phosphate-labelled anti-human IgA goat serum conjugate was added to the wells. After incubation and washings, a substrate solution was applied. Absorbance at 405 nm was measured using a microplate photometer. The results were expressed in terms of units using the standard. Total IgA concentrations of the nasal swab solutions were measured by a laser nephelometer (Behringwerke, Marburg, Germany). The final results of the specific IgA values were expressed in terms of ELISA units mg^{-1} IgA.