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# Protection by a polyvalent influenza vaccine and persistence of homologous and heterologous HI antibodies during a period of two epidemic seasons

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SUMMARY

A split-product influenza A vaccine which contained an influenza B strain (B/Hong Kong/8/73) and two influenza A strains, antigenically identical with A/Fort Dix/741/76 (Hsw1N1) and A/Victoria/3/75 (H3N2), was offered to personnel of the CPHL. Changes in the antibody status were followed with serum samples collected from 153 participants on the day of vaccination and 1, 13 and 18 months thereafter. During the two epidemic seasons in the trial period there were only four serological influenza A infections (2·6%) among the vaccinees. This is one eighth of the corresponding infection rate (22%) in the general population estimated on the basis of other indices.

The vaccinees' antibody response was strongly influenced by the age of the individual subjects. During the trial period the decrease in the antibody titres slowed down. The geometric mean titres of homologous HI antibodies were still substantially higher at the end of the period than at the beginning. This also applied to heterologous antibodies against H1N1 viruses in persons born between 1926 and 1952. In participants born after 1952, the vaccine was not able to evoke these antibodies, and in participants born in or before 1925 the boosting effect was poor.

## INTRODUCTION

Recent trials of inactivated influenza A vaccines (Hoskins, Davies & Smith, 1979; Sparks, 1979) suggested that annual revaccination, at least in certain sections of the population, confers no long-term advantage. The protective effect was limited to those vaccinees, not already immune, who were vaccinated for the first time with the most up-to-date strain. These studies were carried out during a period of a strong antigenic drift of the H3N2 viruses. Because of continuing changes in the antigenic composition of epidemic virus in those years, protection by vaccines against homologous viruses often had no practical significance. A vaccination can also evoke antibodies against previously circulating related variants and this process may impair the development of immunity against the relevant epidemic strains (Feery, Evered & Hayes, 1978; Feery, Evered & Morrison, 1979).

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Since then the antigenic drift of the H3N2 viruses seems to have slowed down, suggesting that there is a limit to the potential of this subtype to produce new antigenic variants. Besides, a previously prevalent subtype of influenza A virus, H1N1, was re-introduced into the human population. Under these circumstances the question of the duration of immunity against homologous viruses is again of current interest, and we are justified in recalling the trials (Foy, Cooney & McMahan, 1973; see also comments in Influenza Workshop V (Kilbourne *et al.* 1974)) performed at the beginning of the H3N2 subtype era, when no changes had yet taken place in the surface antigens of the virus. The observations suggested that partial but reasonably good protection by vaccination probably lasts over two years.

Our knowledge of the immunogenicity of different doses of whole-virus and split-product influenza vaccine – both monovalent and polyvalent – has increased greatly, especially during the immunization programmes, which were started in many countries after the local outbreak of an A/Swine-like influenza virus at Fort Dix, New Jersey, in early 1976 (Conference Report, 1977). In a great majority of the recent studies on the efficacy of influenza vaccines, the post-vaccination antibody status was analysed only once, shortly after administration of the vaccine.

In the present study four serial serum specimens were collected from a large proportion of adult subjects in different age groups, who received one dose of a trivalent influenza vaccine. The changes in antibody status were followed for a period which comprised two epidemic seasons. Attention was paid to the serological infection rates and to the persistence of antibodies evoked by the vaccination. In addition to the homologous HI antibodies, capable of inhibiting the haemagglutination by A/Victoria/3/75 (H3N2)-like and A/Swine-like strains, heterologous antibodies were determined against the recent epidemic viruses: A/Finland/30 (H1N1) and an A/Texas/1/77 (H3N2)-like strain, A/Finland/61/78 (H3N2).

## MATERIAL AND METHODS

### *Vaccine*

The trivalent split-product vaccine (Flupar-vaccin, Orion, Finland) contained 4000 haemagglutinating (HA) units of A/X-47 (a recombinant antigenically identical with A/Victoria/3/75 (H3N2)), 3000 units of A/X-53 (identical with A/Fort Dix/741/76 (Hsw1N1), and 3000 units of B/Hong Kong/8/73, with 0·05 mg of merthiolate and 0·75 mg of $AlPO_4$ per dose.

### *Participants and serum collections*

Altogether 197 volunteers, members of the staff of the Central Public Health Laboratory (CPHL) in Helsinki, Finland, received an intramuscular injection of 0·5 ml of the vaccine in October 1976. Blood samples were taken on the day of vaccination (I) and 4 weeks later (II). Additional samples (III and IV) were collected in November 1977 and April 1978 from vaccinees who still belonged to the staff and were willing to participate. At the end of the study period four

serial serum specimens were available from a total of 153 participants. A great majority of them, and of the whole staff, were female (88 %). The participants were classified by age into four groups on the basis of differences in pre-existing antibodies to the Hsw1N1 and H1N1 subtype viruses.

The sera were stored at − 20 °C until they were tested in autumn 1978. The pre-vaccination specimens, unlike the specimens in the other samples, were thawed once and frozen immediately in autumn 1976.

*Antibody measurements*

The principles presented by Robinson & Dowdle (1969) were followed in the HI tests. The four specimens from each participant were always tested simultaneously. Titrations were performed after removal of non-specific inhibitors by treatment with cholera filtrate (Philips-Duphar B V, Holland). Infected allantoic fluids from embryonated eggs, diluted to contain 4 HA units of virus, were used as antigens; the strains were A/Finland/23/75 (H3N2) (antigenically similar to A/Victoria/3/75), A/Finland/61/78 (H3N2) (antigenically similar to A/Texas/1/77), A/X-53 (Hsw1N1) (a recombinant; antigenically identical with A/Fort Dix/741/76), and A/Finland/30/77 (H1N1).

For geometric mean titre calculations, sera without antibody detectable at the lowest tested dilution (1/12) were considered positive at 1/6.

*The epidemics*

*Winter* 1976/77. According to the diagnostic findings of the CPHL, Finland, the outbreaks started towards the end of February 1977 and tailed off late in the spring, not ceasing until May. Both influenza A and B viruses were isolated. The influenza A strains were antigenically similar to A/Victoria/3/75 (H3N2). In a seroepidemiological survey the influenza A infection rate among pregnant women from whom serial blood specimens had been collected before and after the epidemic was 14 % (Aho, Pyhälä & Elo, 1979).

*Winter* 1977/78. The appearance of the H1N1 subtype virus in China in spring 1977 gave rise to an outbreak which reached Finland in December 1977 and terminated towards the end of March. In the same epidemic season there were also H3N2 subtype viruses circulating in the community. Both A/Victoria/3/75-like and A/Texas/1/77-like strains were isolated. The onset of the H3N2 outbreak came at the beginning of January and it ended at the beginning of April. In seroepidemiological surveys the attack rate of the H3N2 viruses varied greatly, depending on the sector of the population under examination (Pyhälä, Aho & Visakorpi, 1979). The highest rates (65–75 %) were observed among young servicemen at military training centres.

## RESULTS

*Infections*

A comparison of pre-epidemic and post-epidemic titres showed influenza A infection in only four of the 153 participants (2·6 %; Table 1). All four subjects

Table 1. *HI antibody titres of the four vaccinees considered to be infected by influenza A during the trial period*

| Strain | Sample | Subject no. 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| A/Fin/23/75 (H3N2) | I | < 12 | < 12 | < 12 | 12 |
| | II | < 12 | < 12 | < 12 | 24 |
| | III | 48 | 24 | 192 | 12 |
| | IV | 24 | 24 | 96 | 12 |
| A/X-53 (Hsw1N1) | I | < 12 | < 12 | < 12 | < 12 |
| | II | 12 | < 12 | < 12 | 96 |
| | III | < 12 | < 12 | < 12 | 24 |
| | IV | < 12 | < 12 | < 12 | 24 |
| A/Fin/61/78 (H3N2) | I | < 12 | < 12 | < 12 | < 12 |
| | II | < 12 | < 12 | < 12 | < 12 |
| | III | 12 | 12 | 48 | < 12 |
| | IV | 12 | 12 | 24 | 48 |
| A/Fin/30/77 (H1N1) | I | 24 | < 12 | 12 | < 12 |
| | II | 48 | < 12 | 24 | < 12 |
| | III | 48 | < 12 | 24 | < 12 |
| | IV | 24 | < 12 | 24 | < 12 |

Table 2. *Seropositive (HI antibodies in a titre of* $\geqslant$ 12) *vaccinees not infected by influenza A during the trial period*

| Strain | Sample | Age groups (year of birth) –1925 | 1926–42 | 1943–52 | 1953– |
|---|---|---|---|---|---|
| A/Fin/23/75 (H3N2) | I | 7/40 18% | 7/69 10% | 6/33 18% | 3/7 43% |
| | II | 27/40 68% | 52/69 75% | 26/33 79% | 5/7 71% |
| | III | 20/40 50% | 38/69 55% | 19/33 58% | 5/7 71% |
| | IV | 19/40 48% | 33/69 48% | 19/33 58% | 5/7 71% |
| A/X-53 (Hsw1N1) | I | 20/40 50% | 4/69 6% | 1/33 3% | 0/7 0% |
| | II | 35/40 88% | 57/69 83% | 29/33 88% | 2/7 29% |
| | III | 30/40 75% | 49/69 71% | 23/33 70% | 1/7 14% |
| | IV | 29/40 73% | 48/69 70% | 23/33 70% | 1/7 14% |
| A/Fin/61/78 (H3N2) | I | 6/40 15% | 4/69 6% | 3/33 9% | 1/7 14% |
| | II | 25/40 63% | 39/69 57% | 19/33 58% | 3/7 43% |
| | III | 18/40 45% | 21/69 30% | 13/33 39% | 1/7 14% |
| | IV | 16/40 40% | 17/69 25% | 11/33 33% | 1/7 14% |
| A/Fin/30/77 (H1N1) | I | 8/40 20% | 31/69 45% | 20/33 61% | 0/7 0% |
| | II | 16/40 40% | 56/69 81% | 31/33 94% | 0/7 0% |
| | III | 12/40 30% | 44/69 64% | 29/33 88% | 0/7 0% |
| | IV | 11/40 28% | 43/69 62% | 29/33 88% | 0/7 0% |

were from the age group of persons born in 1926–42. Three of them were infected in winter 1976/77 and one in 1977/78. It seems as if the infections during the first epidemic season were caused by A/Victoria/3/75 (H3N2)-like virus and that during the second season by an A/Texas/1/77 (H3N2)-like variant. In all four

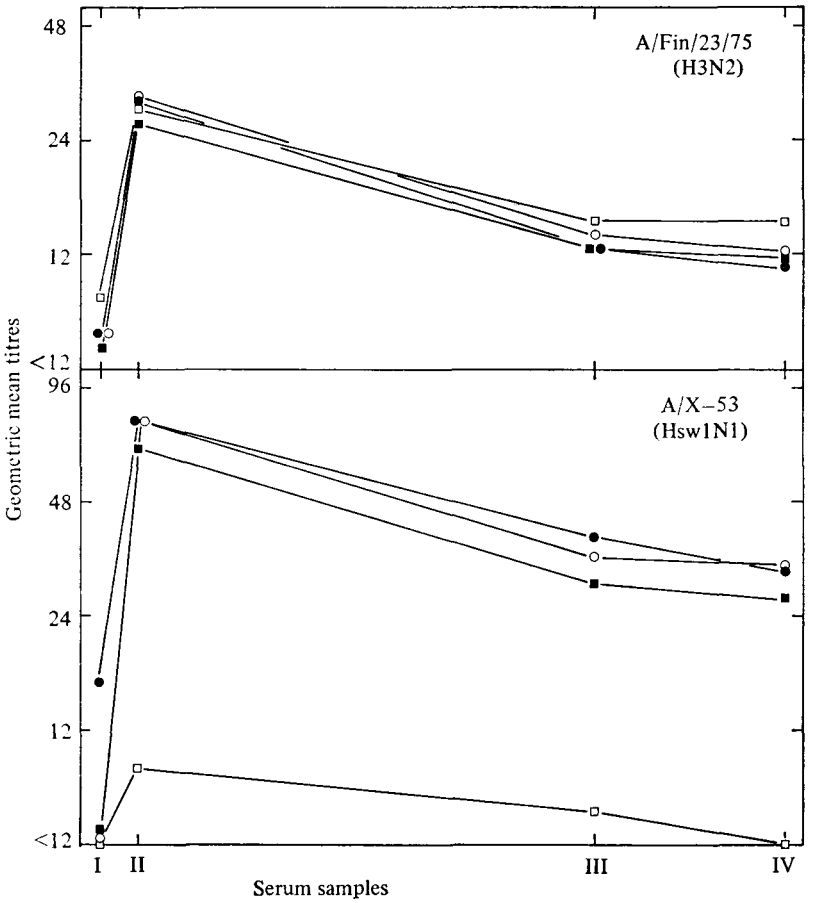


Fig. 1. Geometric mean titres of homologous HI antibodies in serial serum samples from vaccinees not infected by influenza A during the trial period. Subjects born in or before 1925 (●), in 1926–42 (■), in 1943–52 (○) and in or later than 1953 (□).

subjects the vaccination had been unable to evoke HI antibodies to the viruses responsible for the infections.

Sera from the four subjects with an influenza A infection are excluded when data concerning changes in the antibody status are given in the next section.

*Response and persistence of HI antibodies*

*Anti-A/Finland/23/75 (H3N2).* The vaccination raised the rate of seropositive persons (a titre of $\geqslant$ 12) in the different age groups from 10–43 % to 68–79 % (Table 2). The proportions then decreased but were still much higher than before the vaccination at the end of the study period, ranging from 48 to 58 %. A decreasing trend with only a slight decline, if any, during the last five months can be seen in geometric mean titres (Fig. 1) in all four age groups.

*Anti-A/X-53 (Hsw1N1).* Pre-existing antibodies were common only in persons born in 1925 or earlier. Nevertheless, in all three age groups of persons born in 1952 or earlier the vaccination raised the rate of seropositive subjects (Table 2)



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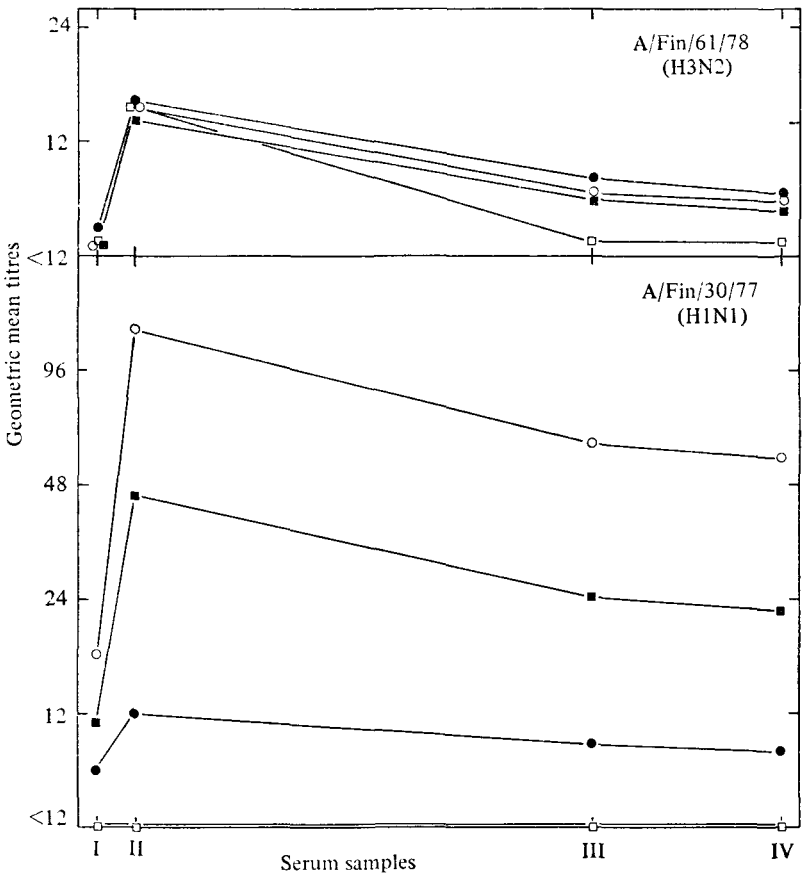


Fig. 2. Geometric mean titres of heterologous HI antibodies in serial serum samples from vaccinees not infected by influenza A during the trial period. Symbols as in Fig. 1.

as well as the geometric mean titres (Fig. 1) to about the same level. In the fourth age group, in younger subjects, the antibody response was poor. The trends showing a decrease in antibodies did not differ substantially from the trends characteristic of anti-A/Finland/23/75. At the end of the study period the rate of seropositive subjects ranged from 70 to 73% and the geometric mean titres were still from 2·1- to 5·2-fold higher than before the vaccination.

*Anti-A/Finland*/61/78 (*H3N2*). About the same proportion of individuals in the different age groups showed substantial boosting in the antibody titre to the heterologous A/Texas/1/77-like variant, A/Finland/61/78 (Table 2). The geometric mean titres (Fig. 2) were comparatively low throughout the study. At the end of the study period they were at most slightly higher than in the prevaccination samples.

*Anti-A/Finland*/30/77 (*H1N1*). There were great differences between the age groups in pre-existing antibody status (Table 2, Fig. 1). The antibodies were wholly absent in the age group of persons born after 1952, and the vaccination

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with heterologous viruses was not able to evoke them. In persons born in or before 1925, the pre-existing antibodies were at the next lowest level and the response after vaccination was the next poorest; there was only a 1·3-fold rise in the geometric mean titre. The best response (a 7·2-fold rise in the geometric mean titre) was observed in the age group which had the highest level of pre-existing antibodies, in persons born in 1943–52.

The rates of seropositive subjects and geometric mean titres were still much higher at the end of the trial period than at the beginning, in the two age groups of persons born in 1926–42 and 1943–52. A 2·0-fold rise in the geometric mean titre was still observed in the former age group and a 3·3-fold rise in the latter. The mean titres of the two age groups decreased in parallel and there was only a slight decline during the last five months.

## DISCUSSION

The vaccination was carried out as part of an immunization programme which, after the outbreak at Fort Dix in 1976, was aimed primarily against the A/Swine-like viruses. Because of the threat of pandemic influenza, the vaccine was offered without cost to those in certain high-risk groups and to medical personnel. Under these circumstances it was not possible to carry out the studies as a double-blind trial or even to form adequate control groups.

The protective effect of the trivalent vaccine during the H3N2 epidemics in the following two winters could only be evaluated by comparing serological infection rates among the vaccinees with the rates among the unvaccinated population groups examined in the surveys (Aho *et al.* 1979; Pyhälä *et al.* 1979) already referred to in Material and Methods. The total infection rate among the vaccinees (2·6 %) differed substantially from the corresponding rate among the unvaccinated pregnant women (22 %), being only about one-eighth of the figure.

However, the differences between the vaccinated and unvaccinated sectors of the population should be emphasized. Besides, there are at least two points on which the vaccine's protection can be claimed to be overestimated. (1) The post-epidemic specimens in 1977 were not collected until the autumn. Thus, in some patients, the antibodies evoked by an H3N2 infection might have decreased to a level which was not sufficient for a fourfold rise over the pre-epidemic titre. (2) It cannot be ruled out that participants affected by clinical influenza may leave the trial before the end more often than others.

On the other hand, it should be pointed out that the vaccinees were obviously not exposed as much as, for example, servicemen at military training centres, pupils at boarding-schools and patients at geriatric homes. Thus the protective effect of an influenza vaccine might be better under the circumstances prevailing in the present trial, than in some other situations considered recently (Feery *et al.* 1979; Hoskins *et al.* 1979; Sparks, 1979).

The H1N1 viruses which circulated in the community in winter 1977/78 rarely affected persons born before 1952 (Pyhälä *et al.* 1979; Pyhälä, 1979). Because of the small number of initially susceptible persons in the present study, it was not

possible to evaluate the protective effect of the vaccination against H1N1 viruses.

The antibody response of the vaccinees was influenced by the age of the individual subjects. This was unquestionably due to differences in their prior experience with influenza A viruses, especially to the priming effect of exposure to Hsw1N1, H0N1 and H1N1 subtype viruses. The observations on the ability of an Hsw1N1 vaccine to induce antibodies against H1N1 viruses in vaccinees born during or before the first era of this subtype and on the inability to evoke these antibodies in younger subjects confirm earlier findings (Noble *et al.* 1977; Monto & Ross, 1979). The comparatively low boosting of the H1N1 antibodies in the oldest age group, in participants born in 1925 or earlier, is worth further attention. It may be possible to interpret this by means of the ratio prevailing between populations of H1N1 and Hsw1N1 memory cells, and probably not by the small population of H1N1 cells alone.

During the five last months the decrease in the HI antibodies, regardless of their specificity, was found to be slower than earlier in the trial period, even in samples from individuals not infected by influenza during the study period. An inverse relation has frequently been demonstrated between the infection rate and HI antibodies against the infecting virus, induced either by a natural infection or by vaccination. At the end of the trial period the antibodies were still, as a rule, substantially higher than before vaccination. This, together with the slow decrease in antibody titres, suggests that at least some degree of protection against homologous viruses could extend beyond two epidemic seasons in most age groups.

The vaccinees showed a substantial boosting of antibodies to the A/Texas/1/77-like variant. At the end of the trial period, however, the titre of these antibodies has greatly decreased, suggesting that the vaccination no longer has a meaningful effect on protection against this variant. On the other hand, good protection after a comparatively short interval against the A/Texas/1/77-like variant by a vaccine containing only heterologous viruses, among them A/Victoria/3/75, was suggested recently (Meiklejohn *et al.* 1978).

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