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Aqueous and oil-adjuvant influenza vaccines and iso-immunization to group A substance

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INTRODUCTION

Because pregnant women tend to suffer severely from influenza they form one of the groups for whom influenza vaccine has been advised (Davenport, 1962). When Springer & Tritel (1962) reported that a concentrated extract of influenza vaccines stimulated a significant rise in anti-A iso-agglutinins in group O or B volunteers and Dr Springer was quoted (American Heart Association, cited by U.S. Public Health Service, 1962) as having warned that this might lead to ABO haemolytic disease in infants, much concern was caused.

Sussman & Pretshold (1963) were unable to demonstrate the presence of group A substance in commercial influenza vaccines but conflicting evidence as to whether anti-A antibodies were actually stimulated by similar vaccines was presented by Davenport (cited by Influenza Surveillance, 1962) and Mathieson, Banner & Harris (1963).

In view of this controversy it seemed appropriate to use the opportunity and specimens presented by two trials of influenza vaccines to test whether antibodies to group A red cells were elicited in volunteers of blood group O.

MATERIALS AND METHODS

Trials

In the first trial 339 volunteers, medical students at the Queen's University of Belfast in the first to fifth years of their course, received two inoculations and had four blood samples taken over a period November 1962 to February 1963.

All volunteers were randomly allocated to receive as first inoculation either 0·5 ml. of a saline placebo or 0·5 ml. of an aqueous polyvalent inactivated influenza virus vaccine (*Invirin*, Glaxo) subcutaneously. From this dose of vaccine each volunteer received 7500 haemagglutinating units (H.A.U.) of virus. The

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viruses in this, as in the other vaccines, had been propagated in embryonated eggs.

The second inoculation was given in January 1963, 2 months after the first inoculation. The volunteers were again distributed into two groups by an independent process. Each volunteer was given either 1·0 ml. of *Invirin*, containing 15,000 H.A.U. of virus, subcutaneously or 0·25 ml. of an oil-adjuvant inactivated vaccine intramuscularly. This adjuvant vaccine contained 3000 H.A.U. of virus of the same strains and in the same proportions as in *Invirin*. The effect of the adjuvant was to give this lesser amount of virus an antigenic effect approximately similar to that in 1·0 ml. of *Invirin*.

The blood samples were taken at the time of inoculation and 3 weeks after the first inoculation and 4 weeks after the second. The sera were separated and stored at −20° C. The blood group of each volunteer was determined at the time of the third bleeding.

In the second trial 97 volunteers, 68 first year medical students and 29 nurses from the Royal Victoria or the Royal Maternity Hospitals, Belfast, were given 0·25 ml. of an oil-adjuvant inactivated influenza virus vaccine (*Admune*, Evans Medical) intramuscularly. This dose contained 3500 H.A.U. of virus.

Specimens of blood were taken at the time of inoculation and one and three months after inoculation. The blood group of each volunteer was ascertained at the time of the first bleeding.

During the second trial a survey of the incidence of anti-A_1 haemolysins in the sera of group O donors to the Northern Ireland Blood Transfusion Service was made in an attempt to detect any seasonal variation which might affect the interpretation of the results from the trial.

Methods

Saline iso-agglutinin tests

A 5% (v/v) suspension of washed standard human A_1 red cells in saline was added to equal volumes of a range of serum dilutions from 1/1 to 1/512. The tubes were read after $1\frac{1}{2}$ hr. at room temperature and the final end point was established microscopically. All sera of an individual were examined together.

Haemolysin tests

To drops of serum were added equal volumes of 5% (v/v) saline suspensions of standard human A_1 red cells. After incubation at 37° C. for $1\frac{1}{2}$ hr. the degrees of haemolysis were recorded as:

No haemolysis, trace haemolysis (T), slight colouring of the supernatant fluid, partial haemolysis (P), or complete haemolysis (C).

Significance tests

The '*t*'-test was used and significance was read at the 5% level.

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RESULTS

In the first trial 146 of the volunteers were of blood group O. The design of this trial was such that the effect of the first inoculation of vaccine could be compared with the effect of placebo after 3 weeks and after 2 months.

The mean pre-inoculation iso-agglutinin titres of the placebo and vaccine groups were different and the antibody titres of the placebo group also rose over the period of the test (see Fig. 1). However, it was possible to show that the group receiving vaccine experienced a greater rise in antibody by 3 weeks after inoculation ($0{\cdot}05 > P > 0{\cdot}0025$) and by 2 months after inoculation ($0{\cdot}005 > P > 0{\cdot}0025$).

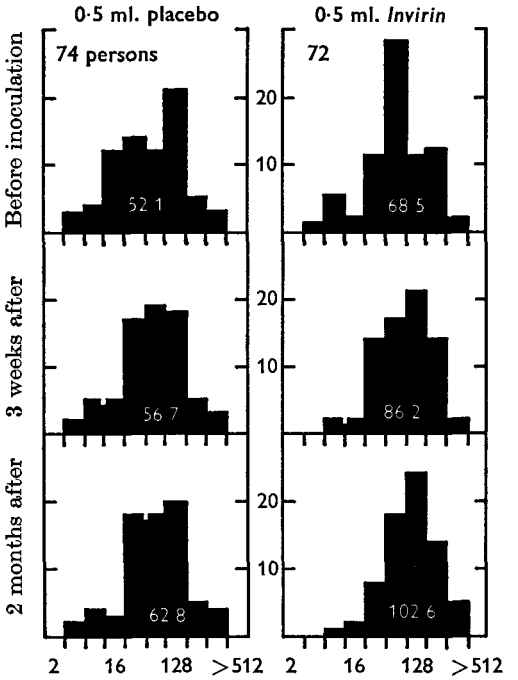

Fig. 1. Levels of anti-A iso-agglutinins in volunteers before and at intervals after the inoculation of saline placebo or 0·5 ml. *Invirin*. Geometric mean titres (G.M.T.) inserted.

When the effect of the two vaccines was compared after the second inoculation (see Fig. 2) no difference in the behaviour of antibody titres emerged ($0{\cdot}3 > P > 0{\cdot}2$). This lack of difference was not, apparently, affected by which inoculum the volunteers had received in the first part of the trial.

Of the 97 volunteers in the second trial, 44 were of group O. Levels of anti-A iso-agglutinins did not rise much after the inoculation of *Admune*. Geometric mean titres were:

Before inoculation	54·7
One month after	56·4
Three months after	68·2

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The increase in the first month was within chance limits ($0{\cdot}7 > P > 0{\cdot}6$) but over 3 months was just significant ($0{\cdot}05 > P > 0{\cdot}025$). However, in the absence of a group given placebo no adjustment could be made for the upwards drift unassociated with vaccine which was noticed in the first trial.

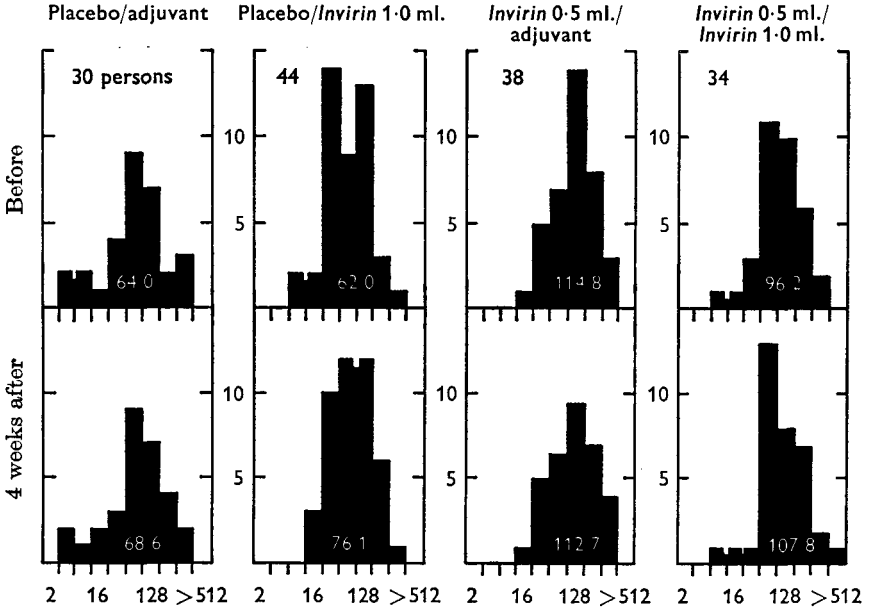

Fig. 2. Levels of anti-A iso-agglutinins in volunteers, who had been given placebo or *Invirin* 2 months previously, before and 4 weeks after either adjuvant influenza vaccine or *Invirin* (G.M.T. inserted).

Table 1. *The percentage incidence of anti-A_1 haemolysins active at 37° C. in group O blood donors and in group O volunteers receiving oil-adjuvant influenza vaccine* (Admune)

			Haemolysis			Total numbers
			Complete	Partial	Trace	
November	Before vaccine	Donors	0 %	4·7 %	11·1 %	342*
		Volunteers	0	4·4	0	44
December	1 month	Donors	0·01 %	3·4 %	11·1 %	1495
		Volunteers	0	2·2	0	44
January	2 months	Donors	0	2·0 %	7·7 %	1887
February	3 months	Donors	0 %	2·9 %	7·4 %	1506
		Volunteers	0	2·2	8·9	44

* Examination of donor sera started only in the latter part of November.

During the second trial sera were tested to find the effect of the vaccine used on the production of haemolysins active against A_1 red cells at 37° C.

Only weak haemolysins were found in the pre-inoculation sera of few of the volunteers and the sera taken after vaccination showed no clear tendency for haemolysins to be increased (see Table 1). Over the period of the trial the variations

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in haemolysin incidence of the volunteers' sera were not greater than those seen in the sera of unselected group O blood donors (see Table 1).

Over this same period, the effect of the standard dose of 1500 units of equine anti-tetanic serum (A.T.S.), administered for the usual indications, on the production of haemolysins was investigated in a number of casualty patients attending the Royal Victoria Hospital. Blood samples were taken before the prophylactic A.T.S. and again about 10 days later in some cases and a month later in others. While only a few pairs of suitable specimens from group O patients were available there did seem to be some tendency for haemolysin to be stimulated (see Table 2).

Table 2. *The effect of* 1500 *units of equine anti-tetanic serum* (A.T.S.) *on anti-*A_1 *haemolysins in group O patients*

		Before A.T.S.	After A.T.S.
(i) 9–15 days between sera	Trace haemolysis	—	1
Four patients	Partial haemolysis	—	1
	Complete haemolysis	1	2
(ii) 1 month between sera	Trace haemolysis	3	1
Six patients	Partial haemolysis	1	3
	Complete haemolysis	0	1

In all these tests for haemolysin the sera were tested fresh, within hours of being taken. This excluded the error introduced by adding extraneous serum as a source of complement, but increased the day-to-day test variations. While many of the donor sera were taken in Belfast, the mobile unit collected blood from many towns in Northern Ireland and this could be expected to increase the spread of results.

DISCUSSION

From the evidence described there does appear to be an association between the administration of influenza vaccine and a rising titre of saline reactive iso-antibodies to group A substance. However, from the slight increase in antibody in the volunteers given the placebo inoculation of saline, it is clear that more than one factor was operating.

If the vaccines had stimulated the formation of haemolysins, as an index of immune-type antibodies, it would have appeared more significant in relation to haemolytic disease of the new-born than the rise of saline-reactive agglutinins actually observed, as these latter types of antibodies are less prone to cross the placenta (Tovey, 1945). However, the relation of any pre-existing antibody to ABO type haemolytic disease is obscure, as shown by the frequency with which such cases occur in first-born children (Levine, Vogel & Rosenfield, 1953) and may not occur in succeeding susceptible infants (Crawford, Cutbush & Mollison, 1953).

The adjuvant vaccine did not appear to stimulate the formation of haemolysins and the fact that the administration of A.T.S. did elicit haemolysins weakens the force of the suggestion that the Northern Ireland population, with its low incidence of haemolysins, might be inherently insensitive to the relevant antigenic stimuli.

However, it is possible that the aqueous vaccine, *Invirin*, could have been more active in this respect than the adjuvant vaccine as might be suggested by the iso-agglutinin studies.

SUMMARY

Influenza vaccines used in trials appeared to stimulate a small increase of saline-reactive anti-A_1 antibodies in group O volunteers. There was no evidence that haemolysins were elicited by adjuvant vaccine. It is unlikely that influenza vaccines would cause ABO haemolytic disease in infants.

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