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The effect of ABO blood groups on the incidence of epidemic influenza and on the response to live attenuated and detergent split influenza virus vaccines

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SUMMARY

The effect of blood group status on the incidence of epidemic influenza A (H3N2) infections and on serological response to influenza vaccination with killed subunit and live attenuated vaccines have been investigated during comparative vaccine trials in Western Australia. A significantly higher incidence of epidemic influenza was observed in subjects of blood group B compared with those of other blood groups, regardless of whether they had serological evidence of previous exposure to H3N2 antigens or not. Volunteers of different blood groups exhibited similar seroconversion frequencies to both vaccines after the administration of two doses, but a significantly higher proportion of blood group A subjects seroconverted after receiving their first dose of live attenuated vaccine compared with those of other blood groups. Although this finding was inconsistent with the increased incidence of epidemic influenza in subjects of blood group B, it is discussed in terms of the methods employed to obtain attenuation. Higher geometric mean HI antibody titres were observed in blood group O subjects after the administration of killed subunit vaccine. The results described in this report supported the contention that genetic factors linked to ABO blood groups may play a role in susceptibility to infection with influenza A virus, but that any association must be indirect.

INTRODUCTION

The relationship of ABO blood group status and the incidence of influenza infections has been studied by a number of workers (McDonald & Zuckerman, 1962; Potter & Schild, 1967; Tyrrell, Sparrow & Beare, 1968; Potter, 1969; Cuadrado & Davenport, 1970; Evans, Shepard & Richards, 1972), but their findings have been inconsistent. In a study of 701 servicemen admitted to sick quarters with influenza A (H2N2) infections, diagnosed serologically or by virus isolation, it was found that a significantly higher proportion of servicemen of blood group O were infected than those of other blood groups (McDonald & Zuckerman, 1962). This observation was supported in later studies by Potter &

Schild (1967) and, to a lesser extent, by Tyrrell *et al.* (1968). In the former, haemagglutination-inhibiting (HI) antibody in sera from civilians of blood group O was found to be significantly more common than in sera from similarly aged subjects of blood group A. Tyrrell *et al.* (1968), using volunteers experimentally infected with virulent influenza A (H2N2) or B virus strains, also found an increased seroconversion frequency in subjects of blood group O with a higher incidence of virus isolations, but volunteers of blood group A developed clinical symptoms more commonly than those of group O. Further evidence indicative of an increased susceptibility of blood group O subjects was obtained in a study of military recruits in Argentina, Brazil and Colombia (Cuadrado & Davenport, 1970). A significantly higher proportion of antibody-positive sera to both influenza A (H2N2) and A (H1N1) were observed from recruits of blood group O compared with those of blood group A, but a similarly high proportion was also noted in recruits of blood group B in all three countries.

Results reported by Evans *et al.* (1972) and Potter (1969) were inconsistent with the above findings. In one study, a higher incidence of antibody-positive sera to influenza A (H2N2) was found in subjects of blood group A, whereas in a second study, a higher incidence was observed in subjects of blood groups AB and B (Evans *et al.* 1972). Potter (1969), however, was unable to obtain any significant difference in the incidence of HI antibodies to influenza A (H2N2) in the sera from individuals of blood group O or A collected in 1966, and suggested that repeated exposure of a population to infection might result in obscuring genetically determined variations in susceptibility.

A partially novel antigenic series of influenza A viruses began in 1968 with the isolation of A/Hong Kong/1/68 (H3N2). Evans *et al.* (1972) determined the incidence of infection with A/Hong Kong (H3N2) in a group of Yale University students who had no prior immunity, but found no differences in the ABO blood group preferences. A comparative clinical trial of live attenuated and killed subunit influenza A (H3N2) vaccines, which was undertaken in Western Australia in 1973–4 (Mackenzie, Mackenzie, Lloyd & Dent, 1975), presented an ideal opportunity for further examination of the effect of blood group status on the incidence of epidemic influenza of the H3N2 antigenic series, and also for determining the effect that blood groups might exert on the responses to live attenuated and subunit vaccines.

MATERIALS AND METHODS

The volunteers, vaccines, clinical schedules and haemagglutination-inhibiting antibody assays have been described in detail previously (Mackenzie *et al.* 1975). In brief, 715 volunteers from three localities in Western Australia (Perth, Collie & Busselton) were randomly assigned to three groups: group A received two doses of a live attenuated influenza A virus vaccine administered intranasally 2 weeks apart; group B received two doses of a saline control administered by the same route and schedule as group A; and group C received two doses of a killed subunit influenza vaccine administered by deep subcutaneous injection 4 weeks apart. Only male volunteers between the ages of 18 and 55, who were

in good health and not in influenza high-risk categories, were permitted to receive the live virus vaccine.

The live vaccine, 'Alice' strain, was developed by Recherche et Industrie Thèrapeutique, Belgium, as a stable inhibitor-resistant variant of MRC-2, a recombinant isolated by Dr G. C. Schild from a cross between A/England/42/72 (H3N2) and A/PR8/34 (H0N1). This recombinant retained the haemagglutinin and neuraminidase antigens of the A/England/42/72 parent. Each dose contained $10^{7\cdot3}$ egg infectious units in 0·6 ml. The killed subunit vaccine was a commercially available preparation manufactured by the Commonwealth Serum Laboratories, Melbourne. Each dose contained 16000 haemagglutinating units of A/England/42/72 and 8000 haemagglutinating units of B/Roma/1/67 in 1·0 ml.

Blood samples were collected from volunteers immediately before vaccination to determine pre-vaccination humoral antibody titres, and subsequently before the second dose of vaccine, and at 7, 30 and 50 weeks after vaccination. Serum samples were treated with cholera filtrate to destroy non-specific viral inhibitors and then titrated in parallel for haemagglutination-inhibiting (HI) antibody. Four haemagglutinating units of influenza strain MRC-7, which contained the antigenic determinants of A/England/42/72, were incubated with serial twofold serum dilutions for 1 h at 37 °C before the addition of a 0·5% suspension of fowl erythrocytes. Post-epidemic sera were also tested against 4 haemagglutinating units of A/Perth/2/73 which was antigenically similar to A/Port Chalmers/1/73.

Although 1048 volunteers were employed in the vaccine trial, ABO blood groups were determined in only 715 cases. The blood groups were estimated macroscopically on tiles by the method of Dunsford & Bowley (1955) using anti-A and anti-B blood group antibody preparations obtained from Commonwealth Serum Laboratories, Melbourne.

RESULTS

Incidence of ABO blood groups

The distribution of ABO blood groups in Western Australia has been determined by the Red Cross Blood Transfusion Service using Busselton as a model community (Davey, Rosman & Curnow, 1973), but a similar distribution has been observed elsewhere in the state (M. G. Davey, personal communication). No significant differences were found in blood group incidences among the volunteers in this study compared to the rest of the community (Table 1).

HI antibodies for A/England/42/72 in the sera from individuals of different blood group status before vaccination

Table 2 gives the proportion of volunteers with HI antibody against influenza virus A/England/42/72 at a serum dilution 1/6 or greater, before vaccination. No significant differences were observed between subjects with different blood groups, although the proportion of blood group B subjects with residual levels of antibody was slightly higher than those of other groups. Subjects of blood group AB were not considered further because of their lack of sufficient numbers for statistical analysis.

Table 1. *Incidence of ABO blood groups in Western Australia and in the trial volunteers*

	Busselton survey*		Vaccine trial	
Blood group	Number	Percentage	Number	Percentage
O	1795	48·8	335	46·9
A	1364	37·1	273	38·2
B	387	10·5	81	11·3
AB	131	3·6	26	3·6

* From the data of Davey *et al.* (1973).

Table 2. *Distribution of HI antibody titres of volunteers before vaccination*

		Volunteers with serum HI antibody titres of 6 or greater	
Blood group	Number of volunteers	Number	Percentage
O	335	220	65·7
A	273	195	71·4
B	81	61	75·3
AB	26	17	65·4

Table 3. *Effect of blood group status on the ability of volunteers with pre-vaccination titres of* 96 *or less to respond to live and subunit influenza vaccines*

			Subjects seroconverting		
Vaccine	Blood group	No. of volunteers	After 1st dose	After 2nd dose	Sero-conversion (%)
Live	O	132	53*	46	75
	A	105	63*	24	83
	B	30	9*	15	80
Subunit	O	97	73	9	84
	A	71	54	7	86
	B	27	20	3	85

* Seroconversion after the 1st dose of live attenuated vaccine was significantly greater for volunteers with blood group A, χ^2, O–A = 9·22, $P = < 0{\cdot}005$; B–A = 8·44; $P = < 0{\cdot}005$; O–B = 1·07 not significant.

Effect of blood group status on response to vaccination

A fourfold or greater rise in serum HI antibody titres was assumed to be indicative of seroconversion. The effect of blood group status on the ability of the vaccines to invoke seroconversion in volunteers with pre-vaccination HI titres of 96 or less is shown in Table 3. A slightly higher percentage of subjects with blood group A seroconverted after receiving both vaccines, but the differences in seroconversion between blood groups for each vaccine were not significant. However, significantly more volunteers with blood group A seroconverted after receiving the first dose of live attenuated vaccine.

Table 4. *Geometric mean HI titres and longevity of response to live and subunit influenza vaccines in volunteers who seroconverted and who had pre-vaccination HI titres of 96 or less*

Vaccine	Blood group	Geometric mean HI titres: Pre-vaccination	2 or 4 weeks*	7 weeks	50 weeks
Live	O	9	33 ± 14†	104 ± 20	60 ± 18
	A	9	57 ± 14	139 ± 22	72 ± 20
	B	10	32 ± 15	114 ± 20	79 ± 20
Subunit	O	10	381 ± 48	479 ± 32	135 ± 30
	A	9	283 ± 44	323 ± 28	125 ± 26
	B	10	236 ± 48	411 ± 40	153 ± 62

* Serum samples collected 2 weeks post-vaccination from live virus vaccinees and 4 weeks post-vaccination from subunit virus vaccinees.

† Geometric means are shown plus or minus two standard deviations.

The geometric mean HI titres in sera collected immediately before the administration of the second vaccine dose, and at 7 and 50 weeks post-vaccination, from volunteers who seroconverted and who had pre-vaccination titres of 96 or less, are shown in Table 4. No significant differences were observed between blood group status and geometric mean HI titres after immunization with the live attenuated vaccine, or in the longevity of the responses. Blood group O subjects who received the subunit vaccine, however, were found to have significantly higher geometric mean HI titres than volunteers with blood groups A or B after the first dose of vaccine, and higher titres than volunteers with blood group A after the second dose of vaccine (7 weeks), but no differences were observed in the longevity of the responses.

Effect of blood group status on the incidence of epidemic influenza

An influenza epidemic occurred in Western Australia with the majority of cases falling between mid-October and mid-December 1973, approximately $3\frac{1}{2}$–$5\frac{1}{2}$ months after vaccination. The epidemic was unusual in that it was much later in the year than normal and two antigenically distinct strains of influenza were isolated. The two strains were antigenically related to A/England/42/72 and A/Port Chalmers/1/73. A fourfold or greater rise in serum HI antibody titres between 7 and 50 weeks post-vaccination was construed as evidence of infection with epidemic influenza. All sera were titrated against both strains of virus using MRC-7 (antigenically similar to A/England/42/72) and A/Perth/2/73 (antigenically similar to A/Port Chalmers/1/73).

Previous results have suggested that serum HI antibody titres of 48 or less might indicate potential susceptibility to influenza (Mackenzie, 1977). The effect of blood group status on the incidence of epidemic influenza was therefore determined in volunteers with pre-epidemic HI titres of 48 or less (Table 5). A greater proportion of volunteers with blood group B (31·1%) were found to have serological evidence of infection than the blood group O (17·7%) or blood group A

Table 5. *Incidence of epidemic influenza in volunteers with blood groups A, O and B who had pre-epidemic HI antibody titres of 48 or less*

Pre-epidemic titre	Blood group	No. of volunteers	No. with evidence of seroconversion*	% with evidence of seroconversion
⩽ 6	O	57	18	31·6
	A	36	7	19·4
	B	12	6	50
12	O	36	7	19·4
	A	29	6	20·7
	B	12	5	41·7
24	O	40	3	7·5
	A	27	4	14·8
	B	9	2	22·2
48	O	42	3	7·1
	A	36	1	2·8
	B	12	1	8·3
Total	O	175	31	17·7
	A	128	18	14·1
	B	45	14	31·1†

* Including all cases of seroconversion to A/England/42/72 and A/Port Chalmers/1/73.
† Significant by χ^2 test, O–B = 3·95, $P = < 0{\cdot}05$; A–B = 6·42, $P = < 0{\cdot}025$.

Table 6. *Incidence of epidemic influenza in volunteers of blood groups A, O and B regardless of pre-epidemic HI titre*

Blood group	No. of volunteers	Incidence of epidemic seroconversion	
		Number	Percentage
O	328	32*	9·8
A	266	19	7·1
B	65	14	21·5

* $\chi^2_{(1)}$ O–B = 7·3, $P = < 0{\cdot}01$; A–B = 12·1, $P = < 0{\cdot}001$; O–A = 1·28, Not significant.

(14·1 %) volunteers, the difference being significant in a χ^2 test. However, if the incidence of epidemic influenza was estimated from all participants of the trial regardless of pre-epidemic titre, a calculation more in keeping with previous studies, the increased proportion of blood group B subjects with serological evidence of infection was even more significant. The results are shown in Table 6. The relative incidence of infections with A/England/42/72 and A/Port Chalmers/1/73 were approximately 2:1 respectively, and was not significantly different between the three blood groups. For logistic reasons, it was not possible to monitor volunteers for clinical influenza during the epidemic period. Instead each subject was provided with a questionnaire which was used in an attempt to assess the incidence of clinical infection (Mackenzie *et al.* 1975). No significant information

was obtained, however, to correlate clinical symptoms with serological evidence of infection between volunteers with different blood groups.

DISCUSSION

A number of reports have indicated a relation between blood group status and the incidence of influenza A virus infections, but the findings have not been uniform. They have either demonstrated an increased susceptibility to epidemic (McDonald & Zuckerman, 1962) or experimentally administered (Tyrrell *et al.* 1968) influenza infections, and a higher incidence of HI antibody-positive sera (Potter & Schild, 1967), in blood group O subjects compared with those of other blood groups, or have presented variable results ranging from increased incidences of antibody-positive sera from subjects of blood groups O and B (Cuadrado & Davenport, 1970), blood group A (Evans *et al.* 1972) and blood groups AB and B (Evans *et al.* 1972), to an absence of any difference in the proportion of antibody-positive sera from subjects of blood groups O and A (Potter, 1969).

This latter study lead Potter to suggest that repeated exposure of a population to a succession of epidemics caused by influenza strains of the same antigenic series might result in obscuring or obliterating genetically determined degrees of susceptibility. Most of the above studies were conducted in the period during which strains of the H2N2 influenza A antigenic series were prevalent (the years 1957–68), with the approximate collection dates of sera being 1958–61 (McDonald & Zuckerman, 1962), 1961–3 (Potter & Schild, 1967), 1962–6 (Evans *et al.* 1972), 1964 (Cuadrado & Davenport, 1970) and 1966 (Potter, 1969). In support of his suggestion, Potter (1969) was unable to detect differences in the incidence of antibody-positive sera between subjects of blood groups O and A after 9 years and several waves of H2N2 epidemics, nor, with the same sera, differences in antibodies to previous H1N1 (1946–57) or H0N1 (prior to 1946) antigenic series. Similarly, McDonald & Zuckerman (1962) found no evidence of blood group preferences to H1N1 influenza strains. Cuadrado & Davenport (1970), however, observed higher incidences of antibody-positive sera (sera collected in 1964) to the H1N1 strain, FM/1, in subjects of blood groups O and B.

In 1968, a partially novel antigenic series began with the isolation of A/Hong Kong/1/68 (H3N2). Evans *et al.* (1972) found no blood group preferences to A/Hong Kong among 276 students, despite a lack of prior immunity, in apparent contradiction to Potter's hypothesis. In the present study 68% of our volunteers had evidence of previous exposure to the H3N2 antigenic series, with a slightly greater incidence of positive sera in subjects of blood group B. Despite this relatively high frequency of volunteers with evidence of prior exposure to H3N2 antigens, a significantly higher proportion of blood group B volunteers had serological evidence of infection in the subsequent epidemic period to the two H3N2 epidemic strains, A/England/1/72 and A/Port Chalmers/1/73. This increased susceptibility of blood group B volunteers was particularly evident if all volunteers were included, regardless of pre-epidemic titre (Table 6), but the incidence was also significant among volunteers with pre-epidemic titres of 48 or less (Table

5), who might be considered as potentially susceptible to epidemic influenza (Mackenzie, 1977). Furthermore, blood group B volunteers exhibited an increased susceptibility whether or not they had evidence of pre-exposure to H3N2 antigens (Table 5) and an increased incidence of epidemic seroconversions was observed in volunteers of blood group O who had little or no pre-epidemic antibody.

Two other studies have also implicated a relation between blood group B and influenza A infections, although in each case with a virus strain belonging to a different antigenic series and in parallel with an increased incidence of antibody-positive sera in subjects of a second blood group, H1N1 and H2N2 virus strains and blood group O (Cuadrado & Davenport, 1970) and H2N2 virus strains and blood group AB (Evans *et al.* 1972). In terms of these results, it is unfortunate that subjects of blood group B were not included in the studies reported by Potter & Schild (1967) and Potter (1969).

The effect of blood group status on the response to live attenuated and killed subunit influenza vaccines has not been examined previously. Although volunteers of different blood groups exhibited similar seroconversion frequencies to both vaccines after the administration of two doses, a significantly higher proportion of blood group A subjects seroconverted after receiving their first dose of live vaccine compared with those of other blood groups. This finding would tend to suggest that blood group A subjects were more susceptible to infection with the live vaccine, but such an explanation is difficult to reconcile with the increased incidence of epidemic seroconversion in subjects of blood group B, particularly since both the vaccine and the epidemic strains were of similar H3N2 antigenicity. The vaccine strain, however, was an avirulent inhibitor-resistant variant of a recombinant derived from H3N2 and H0N1 parents which had retained H3N2 antigenicity (Mackenzie *et al.* 1975), whereas the epidemic strains were virulent and sensitive to serum inhibitors. However, it is difficult to ascribe an alteration in the relationship between blood groups and susceptibility to either the acquisition of inhibitor-resistance or the presence of H0N1 genomic fragments in the vaccine strain. Tyrrell *et al.* (1968) observed a higher incidence of clinical symptoms in blood group A subjects experimentally infected with an influenza A (H2N2) strain and also to influenza B strains, but no differences were found in this study between post-vaccination symptoms reported by blood group A subjects and those of other blood groups (Mackenzie, unpublished observations).

The post-vaccination geometric mean HI antibody titres after administration of killed subunit vaccine were significantly higher in blood group O volunteers than the titres from volunteers of other blood groups. However, no comparisons can be made between this finding and either those presented above or those reported by other investigators, since the subunit vaccine was administered by deep subcutaneous injection. The responses were therefore not subject to infection in the respiratory tract and the antigens were presented in a totally different environment. The virus for subunit vaccine was prepared in embryonated hens' eggs and may have incorporated substances antigenically related to human blood group A antigen, which have been shown to be present in eggs and in whole influenza virus vaccines prepared in eggs (Springer & Tritel, 1962), but

Potter & Schild (1967) have shown that anti-A antibodies did not interfere with haemagglutination-inhibition, and it would appear unlikely that they would influence antigen processing. Thus no explanation can be proposed at this time to account for the increased geometric mean HI titres in the sera of blood group O volunteers.

The longevity of antibody responses to both vaccines were similar for all blood groups.

The results presented in this report, together with the findings of other investigators, have exposed the wide variation that exists between blood groups and influenza A infections. That statistically significant relationships have been demonstrated for each blood group is evidence for an indirect association, and supports the contention of Muschel (1966) that genetic factors linked to ABO blood groups play a role in susceptibility to infection or in the ability to make antibodies to influenza (or both), but in a totally undetermined manner. Two other cases of genetic factors influencing the outcome of influenza infection have been reported. Spencer, Cherry & Terasaki (1976) found that individuals of HL-A type BW16 were resistant to the same live attenuated vaccine as used in the present study. The live virus vaccinees responded poorly in terms of geometric mean HI antibody titres, but killed virus vaccinees responded normally. It is possible that further work will expose a similar variability in HL-A type-linked resistance as has been found for blood groups. The genetic resistance observed in inbred A2G mice after intranasal and intracerebral administration of influenza (Lindenmann, Lane & Hobson, 1963; Lindenmann, 1964) was shown to be associated with a single dominant autosomal gene. Thus, unlike the inherent variability due to an indirect genetic relationship, this resistance might be expected to be uniform with full penetrance.

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REFERENCES

Cuadrado, R. R. & Davenport, F. M. (1970). Antibodies to influenza viruses in military recruits from Argentine, Brazil and Colombia. Their relation to ABO blood group distribution. *Bulletin of the World Health Organization* **42**, 873–84.

Davey, M. G., Rosman, D. L. & Curnow, D. H. (1973). Blood groups and their correlations with disease and biochemical variables in the population of Busselton, Western Australia. *Proceedings of the Australian Society for Medical Research* **3**, 146–7.

Dunsford, I. & Bowley, C. C. (1955). *Techniques in Blood Grouping*. Edinburgh: Oliver and Boyd.

Evans, A. S., Shepard, K. A. & Richards, V. A. (1972). ABO blood groups and viral disease. *Yale Journal of Biology and Medicine* **45**, 81–92.

Lindenmann, J. (1964). Inheritance of resistance to influenza virus in mice. *Proceedings of the Society for Experimental Biology and Medicine* **116**, 506–9.

Lindenmann, J., Lane, C. A. & Hobson, D. (1963). The resistance of A2G mice to myxoviruses. *Journal of Immunology* **90**, 942–51.

McDonald, J. C. & Zuckerman, A. J. (1962). ABO blood groups and acute respiratory disease. *British Medical Journal* ii, 89–90.

MACKENZIE, J. S. (1977). Influenza subunit vaccine: antibody responses to one and two doses of vaccine and length of response with particular reference to the elderly. *British Medical Journal* i, 200–2.

MACKENZIE, J. S., MACKENZIE, I., LLOYD, J. & DENT, V. (1975). Comparative trials of live attenuated and detergent split influenza virus vaccines. *Journal of Hygiene* **75**, 425–43.

MUSCHEL, L. H. (1966). Blood groups, disease and selection. *Bacteriological Reviews* **30**, 427–41.

POTTER, C. W. (1969). HI antibody to various influenza viruses and adenoviruses in individuals of blood groups A and O. *Journal of Hygiene* **67**, 67–74.

POTTER, C. W. & SCHILD, G. C. (1967). The incidence of HI antibody to influenza virus A/2/Singapore/1/57 in individuals of blood groups A and O. *Journal of Immunology* **98**, 1320–5.

SPENCER, M. J., CHERRY, J. D. & TERASAKI, P. I. (1976). HL-A antigens and antibody response after influenza A vaccination. Decreased response associated with HL-A Type W16. *New England Journal of Medicine* **294**, 13–16.

SPRINGER, G. F. & TRITEL, H. (1962). Blood group A active substances in embryonated chicken eggs and their relation to egg-grown virus. *Science, New York* **138**, 687–8.

TYRRELL, D. A. J., SPARROW, P. & BEARE, A. S. (1968). Relation between blood groups and resistance to infection with influenza and some picornaviruses. *Nature, London* **220**, 819–20.