J. Hyg., Camb. (1969), **67**, 67
Printed in Great Britain

HI antibody to various influenza viruses and adenoviruses in individuals of blood groups A and O

By C. W. POTTER

Department of Medical Microbiology and Virus Research Laboratories, University of Sheffield

(*Received* 22 *June* 1968)

INTRODUCTION

The relationship between blood group and the incidence of certain virus infections in man has been studied by several workers (reviewed by Allison, 1965). A study of servicemen admitted to sick-quarters with acute respiratory infections during the period 1956–61 indicated that infection by A 2 influenza virus was more common in persons of blood group O than in persons of blood group A (McDonald & Zuckerman, 1962). In addition, antibody to A 2 influenza virus was found in a significantly higher proportion of serum specimens from civilian persons of blood group O than from similar aged persons of blood group A (Potter & Schild, 1967). These independent studies suggest that susceptibility of man to infection by A 2 influenza virus is not uniform, and that the variability is genetically linked to blood-group status.

To extend these observations on the susceptibility of persons of blood groups A and O to A 2 influenza virus infection, the incidence of antibody to representative A_0, A, A_1 and A_2 influenza viruses was carried out in serum specimens collected in 1966. A study of antibody to adenovirus types 3 and 7 was also performed as McDonald & Zuckerman (1962) observed a significantly higher infection rate by adenovirus for servicemen of blood group A as compared with blood group O.

MATERIALS AND METHODS

Serum specimens

Serum specimens were obtained during the period April–October 1966 from 369 persons of known age and blood group A or O. The majority of the serum specimens were from Sheffield blood donors. The remainder, and all those from individuals under 18 years of age, were specimens submitted to the Sheffield Children's Hospital for antistreptolysin 'O' testing.

Serum from a further 382 persons of blood group A or O and of known age were examined for antibody to adenovirus. These specimens were collected in 1961–3 from children admitted to the Sheffield Children's Hospital for treatment of trauma or accidents or from children with a confirmed diagnosis of appendicitis. Specimens from older age groups were from 150 women attending antenatal clinics and from blood donors.

All sera were from persons living in the Sheffield region.

Viruses

A/Swine (Shope Sw. 15, 1930), A/PR 8 (1934), A 1/FM 1 (1947) and A 2/Singapore/1/57 were stock strains adapted to growth in the allantoic cavity. Virus pools were prepared by allantoic inoculation of 10-day fertile fowl eggs with 10^{-3} or 10^{-4} dilutions of seed virus. After incubation at 35° C. for 48 hr., allantoic fluids were harvested, pooled and stored in sealed ampoules at −70° C.

Adenovirus types 3 and 7 were cultured on HeLa cells maintained in Eagle's basal medium with 2 % inactivated calf serum, 0·88 g./l. sodium bicarbonate and antibiotics. When complete cytopathic effect was observed, cultures were frozen to −70° C. and thawed twice, centrifuged at 3000 rev./min. for 30 min. and the supernatant fluids stored in sealed ampoules at −70° C.

Haemagglutination inhibition (HI) tests

Serological tests for HI antibody to influenza viruses were carried out by standard methods in Perspex plates (W.H.O., 1953) using eight haemagglutinating units of virus (50 % end-point). Before testing, serum specimens were incubated for 18 hr. at 37° C. with 5 volumes of cholera filtrate (N. V. Philips Duphar, Amsterdam) and subsequently heated at 56° C. for 1 hr. Fowl erythrocytes (0·5 % suspension in phosphate buffered saline (PBS)) were used.

Serum specimens tested for HI antibody to adenovirus types 3 and 7 were pretreated by the methods described by Rosen (1960). HI tests were carried out by incubating 0·2 ml. of virus, containing four haemagglutinating units, for 1 hr. at room temperature with an equal volume of pretreated serum dilution. After this time, 0·2 ml. of 1 % Patas monkey cells were added and the tests were further incubated at 37° C. for 1 hr. HI antibody titres were taken as the highest serum dilution which gave 50 % inhibition of virus haemagglutination.

RESULTS

Incidence of HI antibody in serum specimens to influenza viruses

The overall incidence of HI antibody at 1/6 or greater serum dilution to four influenza viruses is given in Fig. 1. The proportions of serum specimens with antibody at various ages differed with the various strains. HI antibody to A 2/Singapore/1/57 was detected in 224 of 369 specimens (61 %) from persons aged 11–60 years; the highest incidence was found in persons aged 16–20 years (91 %) and the lowest in persons aged 51–60 years (29 %). HI antibody to A 1/FM 1, A/PR 8 and A/Swine was found in 142 (38 %), 136 (37 %) and 104 (28 %) of serum specimens, respectively. Antibody to A 1/FM 1 virus was not found in serum specimens from persons aged less than 16 years and the highest incidence (82 %) was found in serum specimens from persons aged 21–25 years. HI antibody to A/PR 8 and A/Swine was not detected in persons aged less than 21 years and 26 years, respectively. The highest incidence for HI antibody to A/PR 8 virus was found in persons aged 26–30 years (86 %) and to A/Swine virus in persons aged 51–60 years (91 %).

This age distribution of HI antibody to influenza viruses was very similar to that reported by other workers (Schild & Stuart-Harris, 1965).

HI antibody to influenza virus in serum specimens from persons of blood groups A and O

The sera tested included 209 specimens from persons of blood group O and 160 specimens from persons of blood group A. The incidence of HI antibody at

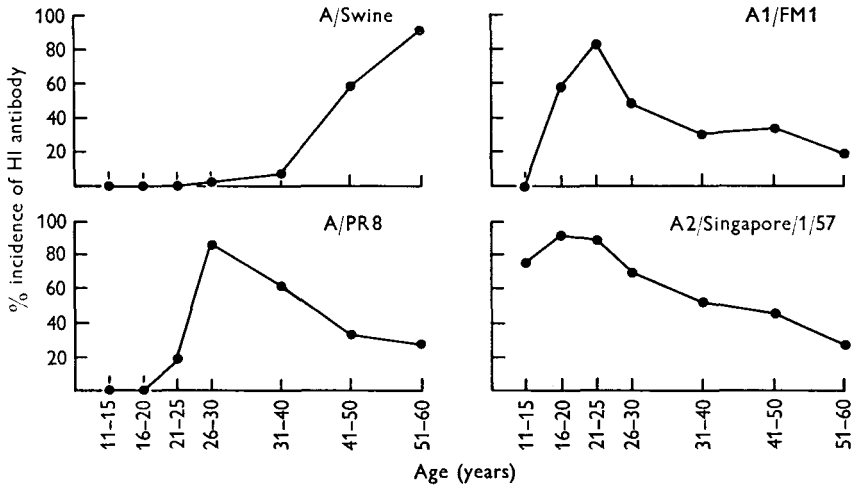

Fig. 1. Percentage incidence of HI antibody to four influenza viruses in serum specimens from individuals of various ages.

Table 1. *Incidence of HI antibody in serum specimens from persons of blood groups A and O to four influenza viruses*

Age (years)	Blood group	No. with HI antibody to A/swine	A/PR8	A1/FM$_1$	A2/Sing/1/57
11–15	O	0/18*	0/18	0/18	14/18 (78)
	A	0/11	0/11	0/11	8/11 (73)
16–20	O	0/18	0/18	10/18 (56)	16/18 (89)
	A	0/17	0/17	10/17 (59)	16/17 (94)
21–25	O	0/31	7/31 (23)	23/31 (74)	28/31 (90)
	A	0/25	3/25 (12)	23/25 (92)	22/25 (88)
26–30	O	0/30	26/30 (87)	15/30 (50)	21/30 (70)
	A	1/19 (5)	16/19 (84)	8/19 (42)	13/19 (68)
31–40	O	5/45 (11)	30/45 (67)	13/45 (29)	23/45 (51)
	A	0/25	13/25 (52)	8/25 (32)	14/25 (56)
41–50	O	25/40 (62·5)	13/40 (32·5)	14/40 (35)	16/40 (40)
	A	13/24 (54)	9/24 (37·5)	7/24 (29)	14/24 (58)
51–60	O	24/27 (89)	10/27 (37)	2/27 (7)	7/27 (26)
	A	36/39 (92)	9/39 (23)	10/39 (26)	12/39 (31)

* $\frac{\text{No. positive}}{\text{No. tested}}$. Figures in parentheses indicate percentage positive.

https://doi.org/10.1017/S0022172400041437 Published online by Cambridge University Press

1/6 or greater serum dilution to each of four influenza viruses is given for persons of blood groups O and A separately and in seven age groups in Table 1. The findings are shown in percentages in Fig. 2. HI antibody to A/Swine virus was detected in four of the seven age groups; in two groups, aged 31–40 years and 41–50 years, a higher percentage of serum specimens from persons of blood group O contained antibody compared with persons of blood group A whilst the reverse was found in the age groups 26–30 years and 51–60 years. HI antibody to A/PR8 virus was present in five of the seven age groups tested; a higher proportion of antibody positive sera from persons of blood group O, compared with persons of blood

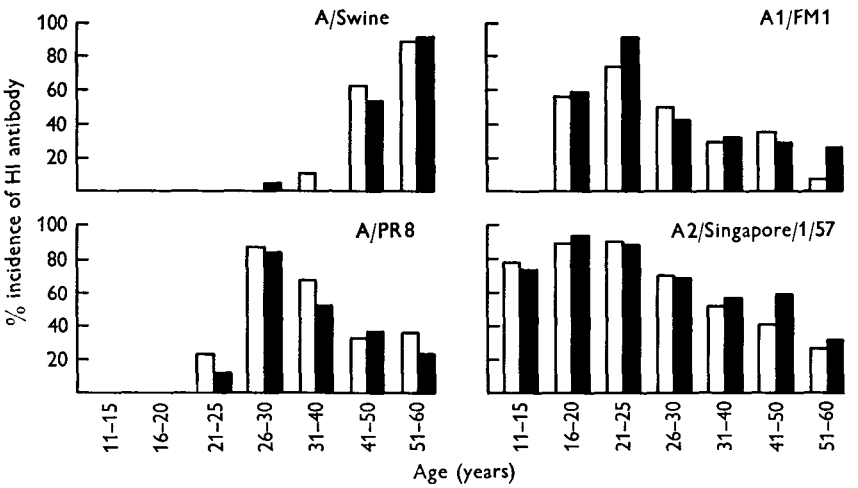

Fig. 2. Percentage incidence of HI antibody to influenza viruses in serum specimens from individuals of blood groups A and O of various ages. □, Blood group O; ■, blood group A.

group A, was found in four of the five groups. HI antibody to A/FM1 and A/Singapore/1/57 was found in a greater proportion of persons of blood group O compared with persons of blood group A, in two of six and three of seven of the age groups, respectively. None of these differences, either individually or collectively, were significant (χ^2 test (Yates correction); $P = > 0{\cdot}05$).

HI antibody in serum specimens to adenovirus types 3 *and* 7

HI antibody titres to adenovirus types 3 and 7 were determined for each of 382 serum specimens from individuals of blood groups A and O. The incidence of HI antibody to adenovirus type 3 at 1/10 dilution or greater (Table 2) rose from 30% in children aged 1–5 years to a maximum of 62% for persons aged 21–25 years. HI antibody to adenovirus type 7 was detected in 7% of serum specimens from persons aged 1–5 years and increased to a maximum of 41·5% in persons aged 26–30 years.

Figure 3 shows the incidence of HI antibody to adenovirus types 3 and 7 in persons of blood group A and O separately. The incidence of antibody to adenovirus type 3 was greater in persons of blood group O than in persons of blood group A in

the three youngest age groups; for persons aged 16 years and older, the incidence of antibody in persons of blood group O was essentially the same as in persons of blood group A. HI antibody to adenovirus type 7 was found in a higher proportion of persons of blood group O aged 1–25 years than in similarly aged persons of blood group A. For persons aged 26 years and over, the incidence of HI antibody to adenovirus type 7 was the same for persons of blood groups A and O.

Table 2. *Incidence of HI antibody to adenovirus types* 3 *and* 7 *in serum specimens from individuals of various ages*

		HI antibody to	
Age (years)	No. tested	Adenovirus-3	Adenovirus-7
1–5	30	9 (30 %)	2 (7 %)
6–10	65	24 (37 %)	9 (14 %)
11–15	54	22 (41 %)	12 (22 %)
16–20	51	25 (49 %)	13 (23·5 %)
21–25	60	37 (62 %)	21 (34 %)
26–30	53	30 (57 %)	22 (41·5 %)
31–40	52	25 (48 %)	21 (40 %)
41–50	17	7 (41 %)	5 (29 %)

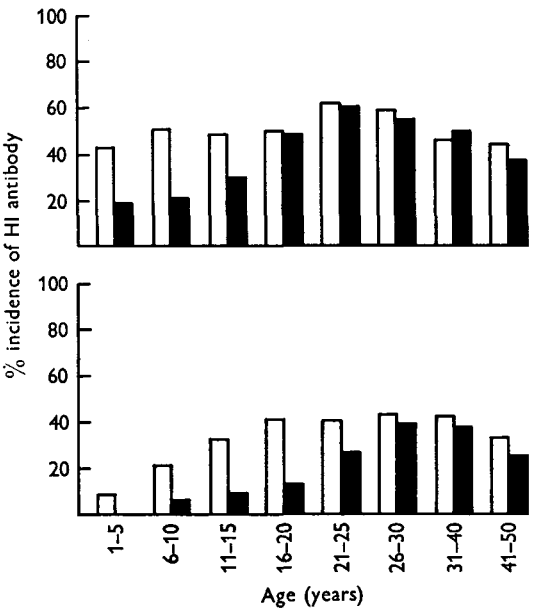

Fig. 3. Percentage incidence of HI antibody to adenovirus types 3 and 7 in serum specimens from individuals of blood group A and O of various ages. □, Blood group O; ■, blood group A.

The significance of the variable distribution of HI antibody to adenovirus types 3 and 7 for persons of blood groups A and O is shown in Tables 3 and 4. For persons aged 1–15 years HI antibody was detected in a significantly higher proportion of persons of blood group O, compared with persons of blood group A. Only in the

https://doi.org/10.1017/S0022172400041437 Published online by Cambridge University Press

age group 6–10 years was the difference significant for an individual age group. HI antibody to adenovirus type 7 was detected in a significantly greater number of persons of blood group O, compared with persons of blood group A, for individuals aged less than 21 years. However, in no individual age group was the difference significant.

Table 3. *Distribution of HI antibody to adenovirus type* 3 *in sera from individuals of blood group A and O*

		HI antibody to Adenovirus type 3			
Age (years)	Blood group	No. tested	No. positive	No. negative	χ^2 (Yates correction)
1–5	O	14	6 (4·2)	8 (9·8)	1·078
	A	16	3 (4·8)	13 (11·2)	
6–10	O	33	17 (12·2)	16 (20·8)	4·887*
	A	32	7 (11·8)	25 (20·2)	
11–15	O	31	15 (12·6)	16 (18·4)	1·1323
	A	23	7 (9·4)	16 (13·6)	

Figures in parentheses indicate expected values.
* $P = < 0{\cdot}05$.
Total χ^2 values (Yates correction). $\chi^2 = 7{\cdot}0973$; $n = 3$; $P = < 0{\cdot}05$.

Table 4. *Distribution of HI antibody to adenovirus type* 7 *in sera from individuals of blood groups A and O*

		HI antibody to Adenovirus type 7			
Age (years)	Blood group	No. tested	No. positive	No. negative	χ^2 (Yates correction)
1–5	O	14	2 (1·0)	12 (13·0)	2·1436
	A	16	0 (1·0)	16 (15·0)	
6–10	O	33	7 (4·6)	26 (28·4)	1·8631
	A	32	2 (4·4)	30 (27·6)	
11–15	O	31	10 (7·0)	21 (24·0)	2·7506
	A	23	2 (5·0)	21 (18·0)	
16–20	O	22	9 (5·6)	13 (16·4)	3·5412
	A	29	4 (7·4)	25 (21·6)	
21–25	O	37	15 (13·0)	22 (24·0)	0·6982
	A	23	6 (8·0)	17 (15·0)	

Figures in parentheses indicate expected values for age groups 1–20 years. χ^2 (Yates correction) = 10·2985; $n = 4$; $P = < 0{\cdot}05$.

DISCUSSION

Influenza A 2 virus first appeared in 1957; at this time the entire population was immunologically susceptible to infection (Mulder, 1957). A study of serum specimens collected in the period 1961–3, after the first few years of prevalence of A 2 influenza virus, indicated that infection had occurred in a greater proportion of persons of blood group O than of persons of blood group A (Potter & Schild, 1967). These findings were consistent with those of McDonald & Zuckerman (1962). The

https://doi.org/10.1017/S0022172400041437 Published online by Cambridge University Press

present study of serum specimens collected in 1966, from the same population but one exposed to further waves of infection by A 2 influenza virus, indicated that past infection had occurred with the same frequency in persons of blood group A and O. In addition, evidence of past infection by A/Swine, A/PR 8 and A 1/FM 1 was found with the same frequency for persons of the two blood groups.

The changes in antibody distribution for influenza A 2 virus with respect to blood groups may be due to changes in the immune status of the population following successive waves of infection. Infections in the first waves of A 2 influenza would occur predominantly in the more susceptible blood group O persons. Later waves of infection would occur in a heterogeneous population with certain individuals possessing, as the result of past infection, protective antibody and others being antibody-negative. The incidence of influenza in less susceptible, blood group A, persons would increase with successive waves of infection as blood-group status does not relate to absolute resistance. Thus, repeated exposure of a population to epidemics of A 2 virus influenza would result in obscuring or obliterating genetically determined degrees of susceptibility.

HI antibody to adenovirus types 3 and 7, two of the three adenovirus serotypes most prevalent in military populations (Hilleman *et al.* 1957; McDonald *et al.* 1958), was detected in a higher proportion of persons of blood group O than in persons of blood group A in the younger age groups only. In older age groups the incidence of antibody was the same for persons of blood groups A and O.

The explanation suggested to account for the changes in antibody status for influenza viruses may be extended to the studies with adenoviruses. In the younger age groups infections by adenovirus types 3 and 7, as indicated by the presence of HI antibody, occur in a significantly greater proportion of persons of blood group O than persons of blood group A. From the results of the present study, this is seen for the age groups 1–10 years for adenovirus type 3 and age 1–20 years for adenovirus type 7. After these ages adenovirus infections will occur with relatively greater frequency in antibody negative, blood group A persons. This conclusion is indicated as in older age groups serological evidence of part infection is found with equal frequency in persons of both blood groups. The ages when infections by adenovirus types 3 and 7 occur more frequently in blood group A persons, compared with persons of blood group O, include a high proportion of military personnel, and it was in such a group that McDonald & Zuckerman (1962) reported a higher infection rate by adenovirus for persons of blood group A compared with those of blood group O.

SUMMARY

Serum specimens collected in 1966 from individuals of different age groups were studied for the presence of haemagglutination inhibition (HI) antibody to four influenza viruses. All sera were from individuals of blood group A or O and in no instance was the incidence of antibody to a virus strain significantly greater for persons of blood group O compared with similar aged individuals of blood group A. This finding for HI antibody to A 2/Singapore/1/57 is different from similar studies of serum specimens collected in 1961–3. It is suggested that the change in the

immune status of a population, with reference to blood-group status, is due to repeated exposure to infection; this changing pattern of immune status is discussed.

Similar studies of HI antibody to adenovirus type 3 and 7 in human sera from persons of blood group A and O shows a changing pattern with increasing age. These results are consistent with the findings for influenza virus and are discussed. Repeated exposure of a population to infection results in obscuring genetically determined variations in susceptibility.

I would like to thank Professor M. G. McEntegart, Professor of Medical Microbiology, and Professor C. H. Stuart-Harris, Professor of Medicine, for their advice and criticism, and Miss D. Coles, F.I.M.L.T., and Mrs P. Robinson for expert technical assistance.

REFERENCES

ALLISON, A. C. (1965). Genetic factors in resistance against virus infections. *Arch. ges. Virusforsch.* **17**, 280.

HILLEMAN, M. R., GAULD, R. L., BUTLER, R. L., STALLONES, R. A., HEDBERG, C. L., WARFIELD, M. S. & ANDERSON, S. A. (1957). Appraisal of occurrence of adenovirus-caused respiratory illness in military populations. *Am. J. Hyg.* **66**, 29.

MCDONALD, J. C., WILSON, J. S., THORBURN, W. B., HOLLAND, W. W. & ANDREWS, B. E. (1958). Acute respiratory disease in the R.A.F., 1955–7. *Br. med. J.* ii, 721.

MCDONALD, J. C. & ZUCKERMAN, A. J. (1962). ABO blood groups and acute respiratory virus disease. *Br. med. J.* ii, 89.

MULDER, J. (1957). Asian influenza in the Netherlands. *Lancet.* ii, 334.

POTTER, C. W. & SCHILD, G. C. (1967). The incidence of HI antibody to influenza virus A2/Singapore/1/57 in individuals of blood groups A and O. *J. Immun.* **98**, 1320.

ROSEN, L. (1960). A haemagglutination-inhibition technique for typing adenoviruses. *Am. J. Hyg.* **71**, 120.

SCHILD, G. C. & STUART-HARRIS, C. H. (1965). Serological epidemiological studies with influenza A viruses. *J. Hyg., Camb.* **63**, 479.

WORLD HEALTH ORGANIZATION (1953). Expert Committee on Influenza, *Tech. Rep. Ser. Wld Hlth Org.* no. 64.