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Influenza haemagglutination-inhibition antibodies in Sydney

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Virus strains have been isolated from each major outbreak of influenza since 1933, and it has been found that group A strains have been responsible for almost all widespread epidemics. A sequence of variants has been obtained, each strain causing clinical influenza in many countries simultaneously, then disappearing with the emergence of the next strain. The strains fall into three subgroups; classical A strains persisted from the initial isolation of the strain PR 8 until the first A′ strains emerged in 1946, A′ strains were then obtained until the A_2 (Asian) pandemic in 1957, and A_2 strains are still being isolated. A few representative strains are shown in Table 1.

Table 1. *Representative strains of influenza A*

Group	Type	Year isolated	Country of origin
A	PR 8	1934	Puerto Rico
A′	CAM	1946	Australia
	FM 1	1947	U.S.A.
	Ned/36	1956	Netherlands
A_2	Jap 305	1957	Japan
	PAR	1957	Singapore

Influenza B, on the other hand, gives rise to localized outbreaks of infection, exhibits less antigenic variation and, on the whole, behaves as an endemic pathogen. A and B groups are distinguished serologically on the basis of a soluble complement-fixing antigen. The type-specific antigens, however, are closely bound to the virus particle and are most easily detected by the haemagglutination-inhibition technique (von Magnus, 1954).

Measurement of the haemagglutination-inhibition (HI) titres in normal subjects in the United States between 1943 and 1951 (Hilleman, Werner & Gauld, 1954) showed that, at least since 1943, almost every individual had antibodies to influenza A, and that high titres persisted even in 1951, although significant isolations of this strain had not been obtained since about 1946. Influenza A′ antibodies were present in an increasing proportion of the population between 1946 and 1951, and the average titre was also rising.

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If the antibody levels of different age groups be determined, it is found that young children have high levels only to the current strain, while adults maintain high levels to the strain prevalent in their youth, irrespective of their reaction to the current strain (Davenport & Hennessy, 1956; Hilleman *et al.* 1958). The response to infection or to vaccination with monovalent vaccines shows that, while all age groups give the greatest response to the infecting or vaccinating strain, infants and young children show little or no heterologous antibody response. Adults show a response to several strains, the heterologous rise being greatest for the strains they first encountered (Hennessy & Davenport, 1958; Culver, Lennette, Navarre & Donahue, 1958; Lief & Henle, 1960). These considerations have led Davenport, Hennessy & Francis (1953) to advance the theory that, in his heterologous antibody response to a new stimulus with influenza A, an individual recapitulates his past experience with A antigens.

After vaccination the increases induced in the levels of both homologous and heterologous antibody may be detected for about 2 or 3 years (Davis *et al.* 1961). After natural infection, antibodies probably persist for a similar period of time and this is thought to explain the 2-year cycles of influenza epidemics (Jensen, Dunn & Robinson, 1958). The maintenance of persistently high titres of A antibody long after the disappearance of the strain may be attributed to recurrent stimulation with the antigenic components common to both A and A′ strains. It should be noted, however, that experiments with guinea-pigs have shown that, in these animals at least, continued exposure to monovalent influenza vaccine gives rise to a broadening of the serological response so that heterologous antibodies may be detected (Robinson, 1961).

The A_2 (Asian) strains are distinct from the A′ group. This is shown by the lack of protection afforded by A′ vaccination against infection with A_2, by the failure of A or A′ antibody levels to rise in response to infection or vaccination with A_2 strains (Hobson & Pearson, 1961), by the great and irregular sensitivity of the virus to non-specific inhibitor, and by the irregular antibody response elicited by the virus strains (PQR variation) (Levy & Wagner, 1958).

It was therefore thought relevant to follow the changes in antibody pattern elicited in a general community following the introduction of the new A_2 strain in 1957.

Three different groups of healthy adult subjects were studied: the first in February 1958, the second in April 1961, and the third in October 1961. Influenza of the A_2 type first appeared in Sydney during July 1957, when a large number of clinical cases was seen, and several A_2 strains were isolated in this laboratory. A second wave of influenza was seen in 1959, and A_2 strains were again isolated. No widespread outbreak has since occurred in Sydney, although an A_2 strain was obtained from a fatal case which occurred in a country boarding school during April 1961.

MATERIALS AND METHODS

Sera

Sera were obtained through the courtesy of the New South Wales Red Cross Blood Transfusion Service, from consecutive blood donors in February 1958, April 1961, and October 1961. They were stored at −20° C. for up to 2 months before testing. Immediately before use they were inactivated at 56° C. for 30 min. and then periodated (Burnet & Lind, 1954).

Antigens

The antigens were manufactured from pooled allantoic fluids according to the directions of the W.H.O. Expert Committee on Influenza (W.H.O. Technical Report Series, 1953). The strains used were PR 8, FM 1, and Lee (W.H.O. Reference Strains) and PAR, an A_2 strain which was isolated in Singapore in 1957, and kindly made available by Dr E. L. French, Walter and Eliza Hall Institute, Melbourne. The same pools were used throughout, and positive control sera included with each batch of tests gave constant readings over the entire period.

Haemagglutination-inhibition tests

HI tests were performed in plastic plates, using a dropping technique. Each antigen was used at a strength of four minimal haemagglutinating doses in a volume of 0·2 ml. Each serum was used in doubling dilutions from 1/5 to 1/2560, in volumes of 0·2 ml.

A 1 % suspension of triple-washed erythrocytes drawn from a young capon was employed, and 0·85 % sodium chloride was used as the diluent.

The tests were performed thus: Doubling dilutions of the sera were made directly in the plates, in 0·2 ml. volumes, 0·2 ml. antigen was added, and mixed by tapping the sides of the plates, which were then covered and incubated at 37° C. for 1 hr. Then 0·4 ml. of the fowl erythrocyte suspension was added to each cup, and the contents again mixed. The red cells were allowed to settle at room temperature, and the results read at 30 min. The end-point was taken as 50 % inhibition of haemagglutination. Saline, antigen and positive serum controls were included with each batch.

RESULTS

Table 2 gives the actual values obtained. Some difficulty was experienced in the statistical analysis of these results since some of the individual series were badly skewed. The 'Maximum Likelihood' method of Tallis & Young (1962) was followed for the 1958 and April 1961 series. The means and variances so obtained are given in Table 3.

DISCUSSION

A_2 *antibody*

In 1958 8 % of persons showed some haemagglutination-inhibiting antibody to the A_2 strain. Compared with the results of other surveys, this value is low for the post-epidemic period. For example, Versteeg (1961) found 17 % of healthy persons

had antibodies to A_2 3 weeks after the height of the epidemic in Holland, while 85% of persons with clinical respiratory disease during the outbreak yielded A_2 antibodies after the 3 weeks interval. Similarly Jensen *et al.* (1958) found that, 3 months after the epidemic, 50% of a group of American high school students who had not been ill showed HI titres to A_2 of 1/10 or more.

Table 2. *Results of haemagglutination-inhibition tests*

Titre	1/5 or less	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280 or higher	Total
					Influenza A (PR8)					
1958	5	0	3	11	21	25	17	15	3	100
April 1961	2	5	14	22	27	15	4	4	1	93
October 1961	1	8	16	29	18	17	1	6	4	100
					Influenza A′ (FM1)					
1958	4	1	7	24	35	15	6	6	2	100
April 1961	6	7	18	22	18	16	3	3	0	93
October 1961	1	14	29	36	11	8	1	0	0	100
					Influenza A_2 (PAR)					
1958	92	1	6	1	0	0	0	0	0	100
April 1961	38	13	23	10	3	1	2	2	1	93
October 1961	16	13	19	23	4	11	8	1	5	100
					Influenza B (Lee)					
1958	0	0	3	26	25	32	13	1	0	100
April 1961	52	24	12	2	1	1	1	0	0	93
October 1961	91	8	1	0	0	0	0	0	0	100

Table 3. *Statistical analysis of results*

Strain	Mean	Variance	Square of standard deviation	Mean ± 2 × standard error
PR8 (1958)	215·7	1183·826674	180873·51	216 ± 68·8
PR8 (1961A)	77·59	116·950666	12729·7919	78 ± 21·6
FM1 (1958)	100·6	208·872453	23819·64	101 ± 28·8
FM1 (1961A)	65·21	115·113583	12557·6559	65 ± 21·4
PAR (1958)	1·840	0·24456112	27·5544	1·8 ± 1·0
PAR (1961A)	25·72	15·186286	6283·4816	26 ± 7·8
Lee (1958)	91·33	65·833391	6238·8311	91 ± 16·4
Lee (1961A)	8·067	1·825152079	192·723511	8·1 ± 2·6

However, Hilleman *et al.* (1958) found only 2·7% of persons with A_2 HI antibody titres greater than 1/5 6–12 months after the initial outbreak. The low values obtained in the present study might be attributed to a low attack rate, but there is no evidence to support this suggestion. The local strains may, however, have been poor antibody producers since A_2 strains show very marked PQR variation, and some strains isolated in Sydney at this time were in the Q phase.

Another factor which may influence the antibody titres obtained is the avidity of the testing strain for antibody. Different A_2 strains have been shown (Fukumi, 1959) to give widely different HI titres with individual sera. In the present study

the same antigen pools were used for all the tests, so this difficulty has been avoided for the purpose of comparing the 2 years.

In 1961, a little over 3 years after the pandemic, both the percentage of people with antibodies and the mean titre have increased, and the difference is highly significant. This means that the A_2 strain has persisted in the community to supersede A′ as the current strain, and may be contrasted with the observations of Versteeg (1961) who found that in Holland in 1960 only 16 % of hospital admissions showed A_2 antibodies.

Heterologous response

During the period of study both the A and A′ HI levels have fallen by a small but statistically significant amount. This is different in principle from the heterologous stimulation of A antibody reported during A′ outbreaks. This may be interpreted to mean that, while the group-specific soluble antigen is shared by A, A′ and A_2 strains, A and A′ share common type-specific antigen(s) not possessed by A_2. However, antigenic analysis of strains by the use of adsorption techniques on doubly immunized ferrets (Harboe, 1960) demonstrates some relationship between A′ and A_2 strains.

The results of surveying the healthy adult population may be compared with those obtained by Hobson & Pearson (1961) who studied the heterologous A and A′ response produced by infection and by monovalent vaccination with A_2. They also found little evidence of the recall effect previously described in response to stimulus with influenza antigens. It may be that immunological memory of the A and A′ epidemic is now fading and may disappear as completely as traces of the 1918 pandemics have vanished.

B *antibody*

Both the average titre and the percentage of persons with influenza B (Lee) HI antibodies in 1958 correspond to the levels recorded in other communities (Brown, Oligschlaeger, Legier & Schmidt, 1960; Hilleman *et al.* 1958). However, the decline observed in Sydney between 1958 and 1961 is striking and contrasts with the findings of these other observers.

The 3-year period studied is roughly similar to the time suggested for the disappearance of A antibody after infection and vaccination. The absence of any evidence of stimulation of B antibodies during this time is in striking contrast to the A_2 findings and it can be inferred that influenza B strains have been absent from the community for at least 3 years.

Further, this disappearance of B antibodies may indicate susceptibility of the community to an epidemic of influenza B during the coming winter. It is interesting that *The Lancet* of 20 January 1962 reported a widespread outbreak of influenza B in England and Wales during the preceding week.

SUMMARY

Haemagglutination-inhibiting antibodies against A, A′ and A_2 strains of influenza virus and against one strain of influenza B were estimated in sera from blood donors in Sydney in February 1958 and in April and October 1961.

The A_2 antibody increased substantially while both A and A′ antibodies declined slightly. There was a great decline in influenza B antibody during the same period.

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