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Haemagglutination-inhibiting (HI) antibodies against four prototype strains of influenza A virus in different age groups

By A. L. TERZIN, S. DJURIŠIĆ, B. VUKOVIĆ and V. VUJKOV
Medical Faculty, Novi Sad, Yugoslavia

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SUMMARY

Sera of 197 apparently well persons were tested for residual haemagglutination-inhibiting antibodies against live Hong Kong/68, A/FM/47 and A/PR/34 strains. Sera of 62 well persons, regularly exposed to contacts with swine, were tested against an inactivated A/New Jersey/76 antigen.

Those born some time before and during a certain influenza era showed a significantly greater proportion of homologous residual titres against the subtype prevailing in that influenza era, than those born after the termination of the same era.

In each of the seven age groups tested both the percentage of positives and the geometric mean titres were usually highest against the Hong Kong strain (representing the most recent era); the next highest were those against the FM1 strain and the lowest were those against the PR8 strain (representing the most distant of these three influenza eras).

The serological involvement of donors exposed to regular contacts with swine was relatively stronger against the New Jersey antigen than the response of other serum donors shown against the other three, more recent, prototypes of influenza virus A. The oldest age groups showed significantly lower antibody response against the PR8, FM1 and Hong Kong strains (but not against the New Jersey antigen) than the next one or two of the younger age groups.

INTRODUCTION

Although, in any age group, individual sera show a considerable variation in HI titres against a particular antigen, a comparison of the mean titres and of the percentages of sero-positive reactors in representative groups of persons is known to be a suitable tool for sero-epidemiological studies.

It is also known that a population's past exposure to various subtypes of influenza viruses can be reconstructed by ascertaining the age distribution of HI antibodies against selected prototype strains (Davenport, Hennessy & Francis, 1953).

This study shows the age-specific serological involvement of a population group in Yugoslovia as a reflexion of past influenza eras characterized by prevalence of different subtypes of influenza virus.

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The collection of serum specimens tested was completed several months before the reappearance of the A (H1N1) strains, related to the virus A/FM/1/47 (W.H.O., 1977).

MATERIALS AND METHODS

Blood donors, serum specimens and serology

Sera were collected from persons of various age groups, with no manifest symptoms of illness. All donors were residents of Voyvodina. However, with respect to past exposures to the dominant subtypes during the respective influenza eras, the population sampled could be considered reasonably representative of Yugoslavia as a whole.

In April 1976, 62 farmers and members of their families, all of whom had in their professional capacity been exposed to regular contacts with pigs, were bled. In the period April–August 1977 sera were collected from 197 persons of various professions. The years of birth of the donors bled in 1976 (and their age in years at the date of bleeding) were as follows: 1896–1914 (62–80), 1936–54 (22–40), and 1957–72 (4–19). The same data for persons bled in 1977 were: 1914–27 (50–63), 1928–32 (45–49), 1933–37 (40–44), 1938–42 (35–39), 1943–47 (30–34), 1948–52 (25–29) and 1953–57 (20–24).

Sera drawn in 1976 were treated with RDE and titrated in twofold dilutions from 1/10 to 1/1280, against the inactivated antigen A/New Jersey/76 (Hsw1 N1) obtained from the Virus Diseases Unit, W.H.O., Geneva (W.H.O., 1976).

The sera drawn in 1977, after treatment with trypsin and periodate, were tested in twofold dilutions from 1/8 to 1/2048 for HI titres against allantoic fluids infected with the following prototype strains: A/PR/8/34, A/FM/1/47 and A/Hong Kong/1/68. One volume of serum was mixed with 1 volume of trypsin (Difco, 1/250) solution (4 mg/ml) in phosphate buffer (0·1 M, pH 8·1) and immediately heated for 30 min at 56 °C. After cooling, 3 volumes of M/90 potassium periodate were added, and after holding for 25 min at room temperature the serum dilution was mixed with 3 volumes of 1% glycerol in saline.

Determinations of HI titres were carried out by the standard pattern method (Expert Committee on Respiratory Virus Diseases, 1959).

Calculations

In calculations of the geometric mean titres and the percentages of positives, from the results obtained with New Jersey antigen titres $<1/10$ were taken as 1/4 and titres of 1/20 or greater as positive, while with results obtained with PR8, FM1 and Hong Kong antigens titres $< 1/8$ were taken as 1/4 and titres of 1/16 or greater as positive.

The statistical significance of the differences observed between frequencies compared has been evaluated by the chi-square test (Snedecor, 1962) or according to the 'Vierfelder-test' tables of Diem (Diem, 1960).

Table 1. *HI titres in* 197 *serum donors of various age groups*

Age groups (years)	No. of sera tested	HI titres against influenza: PR strain < 8	8	16	32	64	128	256	FM strain < 8	8	16	32	64	128	256
20–24	17	16	1	—	—	—	—	—	11	5	1	—	—	—	—
25–29	16	14	1	1	—	—	—	—	5	2	5	2	2	—	—
30–34	16	12	3	—	1	—	—	—	4	2	4	5	1	—	—
35–39	35	19	3	8	4	1	—	—	7	7	6	12	3	—	—
40–44	52	9	10	14	15	3	—	1	11	8	14	13	3	3	—
45–49	25	3	7	6	7	2	—	—	10	4	2	6	3	—	—
50–63	31	20	3	7	—	1	—	—	17	3	6	3	1	1	—
Not recorded	5	1	1	2	—	1	—	—	1	1	2	1	—	—	—

Table 2. *HI titres in* 197 *serum donors of various age groups*

Age groups (years)	No. of sera tested	HI titres against the Hong Kong strain: < 8	8	16	32	64	128	256	512	1024	2048
20–24	17	—	—	—	5	5	4	2	1	—	—
25–29	16	—	1	1	4	3	5	1	1	—	—
30–34	16	1	—	—	5	6	4	—	—	—	—
35–39	35	2	—	3	8	14	4	4	—	—	—
40–44	52	1	—	4	14	14	9	6	2	2	—
45–49	25	—	2	4	5	7	5	2	—	—	—
50–63	31	2	5	1	5	13	3	2	—	—	—
Not re-	5	—	—	2	1	2	—	—	—	—	—

RESULTS AND DISCUSSIONS

The 197 blood samples were drawn in April–August 1977. The serological findings in specimens drawn in any of these months showed no significant difference from the findings in sera taken in any of the other months. Results matched according to the age of the patients showed no detectable serological differences between the sexes. Consequently only the serological findings of different age groups are reported here.

Tables 1–3 show the age-specific frequencies of HI titres found against four prototype strains. From the raw data shown in these tables we can compute the percentages and geometric means shown in Table 4, as well as the significance of the differences between the antibody patterns observed in the various age groups.

The results shown in Table 4 can be summarized as follows:

(1) Persons born some time before and during a certain influenza era showed a significantly greater proportion of homologous residual titres against the subtype prevailing in that influenza era than persons born after the termination of that era. This finding proved to be significant at a level of $P = < 0{\cdot}001$ ($\chi^2 = 19{\cdot}2$) when comparing the findings with PR8 virus in age groups 1933–42 with these in age groups 1943–52.

Table 3. *HI titres in 62 serum donors of various age groups after treatment of serum with RDE*

Age groups (years)	No. of sera tested	HI titres against the New Jersey strain							
		< 10	10	20	40	80	160	320	640
4–19	17	3	11	3	—	—	—	—	—
22–40	27	2	14	6	5	—	—	—	—
62–80	18	—	—	—	—	9	6	2	1

A significance at $P = 0{\cdot}01$ level (according to the tables of Diem) was found when comparing the difference between findings with FM1 virus in persons born between 1948–52 with those in persons born between 1953–7. The finding that persons born in the second part of the A-prime era showed a lower serological response to the FM1 strain than those born before or during the first half of the same era seems to be compatible with the well known antigenic drifting characterizing the era of A-prime variants.

The difference between the serological involvement against the New Jersey antigen of the younger age groups (born between 1936 and 1972) and the oldest group (born between 1896 and 1914) was at the significance level of $P = < 0{\cdot}001$.

Findings with the Hong Kong strain did not show such a pattern, owing to the relative recentness of the Hong Kong era.

(2) Usually, in each of the seven age groups tested, both the percentage of positives and the geometric mean titres were highest against the Hong Kong strain (representing the most recent era), the next highest were those against the FM1 strain, and the lowest were against the PR8 strain (representing the most distant of these three influenza eras).

(3) The magnitude of serological involvement of the youngest age group (persons born after 1957) against the New Jersey antigen was higher than against FM1 and lower than the response against the Hong Kong strain found in the youngest age group tested. Young adults (born between 1936 and 1954) showed a response against the New Jersey antigen higher than against the PR8 and lower than against the FM1 strain. The oldest donors (born before 1914) showed a greater response against the New Jersey strain than that shown by the oldest age group tested against the Hong Kong strain. Such a relatively strong response against the New Jersey strain seems to be associated with the fact that all sera tested against the New Jersey antigen were taken from farmers regularly exposed to contacts with swine.

(4) When compared with persons born between 1928 and 1932, the oldest age group (born between 1914 and 1927) showed a significantly lower antibody response against the PR8 strain ($\chi^2 = 5{\cdot}3$; $P = 0{\cdot}02$). Against the FM1 strain the antibody response in the oldest age group was significantly lower ($\chi^2 = 5{\cdot}0$; $P = 0{\cdot}025$) than in the group of persons born between 1933 and 1937.

Persons born between 1914 and 1927 also showed a significant decline of antibody response against the Hong Kong strain ($\chi^2 = 7{\cdot}3$; $P = 0{\cdot}01$) compared with persons born between 1933 and 1937.

Table 4. *Approximate correspondence between the age groups of the 259 persons tested, the epidemiological prevalence of subtypes and the antibody patterns found*

		Donors' years of birth and results against antigens								
			PR8		FM1		Hong Kong		New Jersey		
Prototype of test strains	Influenza eras and prevalence of subtypes	Years of birth	%	g.m.t.	%	g.m.t.	%	g.m.t.	Years of birth	%	g.m.t.
A/Hong Kong/ 1/68 (H3N2)	A2-like Hong Kong 1968–	—	—	—	—	—	—	—	1957–72	17·6	9·6
A/Singapore/ 1/57 (H2N2)	A2(Asian) 1957–68	—	—	—	—	—	—	—			
A/FM/1/47 (H1N1)	A prime 1946–57	1953–57	0	4·1	5·9	5·3	100	81·7	—	—	—
	'swine-like'	1948–52	6·3	4·6	56·3	12·3	93·8	66·8			
A/PR/8/34 (H0N1)	A0 1928–46	1943–47	6·3	5·2	62·5	14·1	93·8	51·5	1936–54	40·7	14·1
	(PR8: 1934–43)	1938–42	37·1	8·0	60·0	15·1	94·3	52·5			
	'swine-like'	1933–37	63·5	15·4	63·5	15·6	98·1	72·2	—	—	—
		1928–32	60·0	15·2	44·0	11·6	92·0	48·5	—	—	—
A/New Jersey/ 8/76 (HswN1)	'Spanish flu' 1918–28	1914–27	25·8	6·4	35·5	8·4	77·4	38·3	—	—	—
	A2-like strains around 1900	—	—	—	—	—	—	—	1896–1914	100·0	132·0

% Percentage positive. g.m.t. = geometric mean titre.

This phenomenon of lower antibody response in the oldest age group was not shown by sera of farmers exposed to regular contacts with swine, when tested against the New Jersey antigen.

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