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USE OF THE BLAST TRANSFORMATION TEST TO STUDY IMMUNOLOGICAL MEMORY FOR INFLUENZA A VIRUS

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In 1977, for the first time in the "modern history of influenza," virus A (H1N1) returned into epidemic circulation after an interval of 20 years. Persons over 25 years of age, i.e., those who met this virus in 1949-1956, developed influenza eight to 10 times less frequently in 1977 than younger individuals [1]. It is not clear how universal is the rule that subtype-specific anti-influenzal immunity to influenza A virus, in the mechanism of which an important role is played by cellular immunity [3], persists for a long time.

Accordingly it was decided to study immunological memory in a system of cellular immunity to influenza viruses A (H0N1) and A (H3N2) in people of different ages.

EXPERIMENTAL METHOD

Observations were made on two groups of volunteers: 1) 31 subjects aged 21-30 years, and 2) 33 persons aged 45-60 years. Blood was taken in a volume of 10 ml from the cubital vein: 2 ml was used to obtain serum and 8 ml to isolate the monocytic fraction on a Ficoll-Agipak gradient [5]. The blast transformation test (BTT) was determined from incorporation of [^{3}H]thymidine, added to the culture for 24 h in a concentration of 1 μCi/ml. Purified A (H0N1) and A (H3N2) viruses, inactivated by UV light, were used in a concentration of 25 hemagglutinating units per test sample. The reaction was read after incubation of the cultures at 37°C for 72 h. The results of the BTT were expressed as the stimulation index (SI): the ratio between the number of counts per minute of the stimulated lymphocytes and the number of counts per minute of the unstimulated lymphocytes. To detect antibodies in the blood serum the hemagglutination inhibition test (HIT) was used, and was conducted by the method recommended by WHO [7]. The level of antineuraminidase antibodies was determined by the elution inhibition test (EIT) [2, 4].

As antigens in the HIT inhibitor resistant variants of viruses were used: A/swine/Iowa/15/30 (HSw1N1), A/PR/8/34 (H0N1), A/Khabarovsk/74/77 (H1N1), A/Singapore/1/57 (H2N2), A/Hong Kong/1/68 (H3N2), A/Victoria/3/75 (H3N2), A/Texas/1/77 (H3N2), A/Bangkok/1/79 (H3N2), A/Khabarovsk/74/77 (H1N1).

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TABLE 1. Level of Antihemagglutinins and Antineuraminidase Antibodies in Blood Sera of Volunteers Tested

Test	Antigens	Antigenic formula of surface antigen taking part in reaction	Reciprocals of geometric mean antibody titers in subjects	
			group 1 (n = 28)	group 2 (n = 27)
HIT	A/swine/Iowa/15/30	H1	0	3,1
	A/PR/8/34	H0	0	9,8
	A/Khabarovsk/74/77	H1	13,9	13,9
	A/Singapore/1/57	H2	26,0	28,0
	A/Hong Kong/1/68		30,0	14,9
	A/Victoria/3/75		17,1	17,1
	A/Texas/1/77	H3	8,6	10,6
	A/Bangkok/1/79		14,9	21,1
EIT	A/Khabarovsk/74/77	N1	21,1	9,8
	A/Hong Kong/1/68		7,5	11,3
	A/Port Chalmers/1/73		8,0	13,0
	A/Texas/1/77	N2	9,2	9,2
	A/Bangkok/1/79		0	2,3

TABLE 2. Intensity of BTT in Subjects of Different Ages under the Influence of Influenza A (H0N1) and A (H3N2) Viruses

Group of subjects	Age of subjects, years	Number of subjects tested	Number of subjects with positive BTT to influenza virus				Mean stimulation index of BTT to influenza virus	
			A (H0N1)		A (H3N2)		A (H0N1)	A (H3N2)
			abs.	%	abs.	%		
1	20—30	33	18	54	26	78	1,52	1,88
2	45—60	31	20	64	17	54	1,82	1,65
							$P>0,05$	$P>0,05$

The following recombinant strains were used for the EIT: P7 (N1 of virus A/Khabarovsk/74/77, HEq1 of virus A/equine/Prague/1/56), X15 (N2 of virus A/Hong Kong/1/68, HEq1 of virus A/equine/Prague/1/56), X42 (N2 of virus A/Port Chalmers/1/73, HEq1 of virus A/equine/Prague/1/56), P9 (N2 of virus A/Texas/1/77, HEq1 of virus A/equine/Prague/1/56), P17 (N2 of virus A/Bangkok/1/79, HEq1 of virus A/equine/Prague/1/56).

The significance of differences was estimated by statistical analysis using Student's t test.

EXPERIMENTAL RESULTS

Antibodies against hemagglutinins H1, H2, and H3 and antibodies against neuraminidase N1 and N2 were found in the blood sera of the subjects of group 1, and in those of group 2 in addition antibodies were found to hemagglutinins HSw1 and H0 (Table 1). Meanwhile no statistically significant differences were found in the intensity of blast transformation of lymphocytes in subjects of the two age groups under the influence of viruses A (H0N1) and A (H3N2) (Table 2). This fact can evidently be explained by the ability of influenza virus to exhibit nonspecific mitogenic activity as well as antigen-specific activity [6].

Accordingly to study the possibility of using the BTT to determine immunological memory in a system of cellular immunity an individual analysis was undertaken of the values obtained in this test on volunteers from groups 1 and 2 (Table 3). Differences of 30% or more between SI obtained against viruses A (H0N1) and A (H3N2) were taken to be significant. In 14 subjects of group 1 and in 16 of group 2, no such differences were found. Meanwhile, in the remaining subjects of group 1 stronger reactions differed significantly more often ($P < 0.05$) against virus A (H3N2), whereas in group 2 they did so against virus A (H0N1).

TABLE 3. Differences in Intensity of Blast Transformation in Subjects of Different Ages under the Influence of Influenza A (H0N1) and A (H3N2) Viruses

Group of subjects	Age of subjects, years	Number of subjects tested	Number of subjects with no difference in intensity of BTT under the influence of A (H0N1) and A (H3N2) viruses	Number of subjects with stronger BTT under the influence of virus			
				A (H0N1)		A (H3N2)	
				abs.	%	abs.	%
1	20—30	33	14	4	21	15	79
2	45—60	31	16	14	93	1	6,7

The results indicate that the BTT can be used to study immunological memory in a system of cellular immunity to influenza A virus. The presence of antibodies against influenza A (H0N1) virus in subjects aged 45 years or more and the more active response of their lymphocytes to antigen of the same virus can be regarded as evidence of preservation of immunological memory in a system of cellular and humoral immunity to influenza A (H0N1) virus in the subjects of this age group. It can therefore be expected that should this particular virus return into epidemic circulation, persons born in 1940 or earlier will develop influenza less frequently than younger individuals.

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