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SEPARATION OF ADENOVIRUSES INTO SUBGROUPS IN CONNECTION WITH THEIR HEMAGGLUTININATING ACTIVITY AND THE PROPERTIES OF THE HEMAGGLUTININS

[Following is the translation of an article by R. S. Brovzin and S. Ye. Zolotarevskaya, Institute of Virology named D. I. Ivanovskogo, ul. 30-ya, Moscow, published in the Russian-language periodical Vopr. Virusol. (Problems of Virology), No 8, 1967, pages 87-91. It was submitted on 23 Feb 1968.]

Earlier [1] we made a study of the resistance of hemagglutinating and infectious activity of various serotypes of adenoviruses to a number of physico-chemical factors. It was revealed that there were 3 subgroups of adenoviruses which differed in the degree of resistance of hemagglutinins. Of these subgroups a and b (correspondingly types 3, 7, 21 and 11 and 16) are included in the first group of Rosen's classification, and subgroup c (types 10, 15, 24) in the second group of the same classification.

The present paper presents the results of a study of the agglutinating activity of adenoviruses of types 3, 7, 8-11, 13-17, and 19-27 in respect to erythrocytes of man, monkeys, rats, mice, and guinea pigs, the hemagglutinating activity to which may be used for the differentiation of the group of adenoviruses from other groups of viruses. The study was made with a calculation of the individual sensitivity of erythrocytes of different animals of the same species. These data in respect to a number of serotypes supplement the data of Rosen [2, 3] and Sauer and Wignau [4] and agree with the data from authors [5, 11, 12] who studied single serotypes of adenoviruses. In addition to this we made a detailed study of agglutination with guinea pig erythrocytes, agglutination of adenoviruses of types 19, 22, 23, 24, 25, and 27 with erythrocytes of white mice, and of the dependence of the titers of hemagglutinins on the individual peculiarities of the erythrocytes of white rats; a different hemagglutinating activity was revealed in strains of type 21.

On the basis of all these data - ours and from the literature - we deemed it possible to propose an additional division of the first group according to Rosen's classification into 2 subgroups, and the second group - into 4 subgroups.

Materials and Methods

The investigations were conducted both with reference strains of adenoviruses of the above indicated types and with strains of types 3, 7, 8, 11, and 21 which we isolated. The adenoviruses were cultivated in cell cultures of transplanted lines of HeLa, Mv-2

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and F1 in the presence of medium No 123 and with the addition of it of 2.5-5% of inactivated horse or chicken serum.

The agglutinating activity of adenoviruses was studied in respect to erythrocytes of rhesus and grivet monkeys, white laboratory rats, white mice, guinea pigs, and human blood type O.

The hemagglutination reaction (HI) was set up mainly by the method of Rosen (1950). The reaction was conducted at 4, 20, and 37°.

In the work the results of the HI are cited for optimum temperature conditions. For adenoviruses which agglutinated monkey erythrocytes the optimum temperature for adsorption and precipitation of erythrocytes is 37°, and for those which agglutinated rat erythrocytes - 4°.

Results

The results of the study of the hemagglutinating activity of various serotypes of adenoviruses and the dependence of this activity on the individual peculiarities of erythrocytes from individual specimens of the same species of animals are presented in tables 1-4.

There is interest in the results of the study of the hemagglutinating activity of the type 21 adenovirus reference strain, isolated by Doll and associates [5] from the conjunctiva of the eyes of trachoma patients in Saudi Arabia, and a strain isolated in the Soviet Union by V. M. Zhdanov and R. S. Dreyzin [6] from patients with diseases of the respiratory tract.

As can be seen from table 2 both strains agglutinate the erythrocytes of rhesus monkeys in high titers. Variations in their titers in respect to both strains with the erythrocytes of various specimens were within the limits of 4 times, while erythrocytes of only 2 out of the 10 Grivet monkeys were agglutinated by strain No 167, and in a low titer. Consequently the sensitivity of erythrocytes of Grivet monkeys to strain No 167 is very diverse and the variations in titers lie between 0 and 1:16. Reference strain type 21 agglutinated all the series of erythrocytes of Grivet monkeys with a variation in titers of no more than 2 times.

Thus sharp differences were revealed in the agglutinating activity of reference strain type 21 and strain No 167 which was freshly isolated in the laboratory.

With the majority of types of adenoviruses which agglutinated rat erythrocytes the titers equaled 1:64--1:128, with type 24 they were 1:2048--1:4096, and only with two types were low titers of hemagglutinins observed - down to 1:16.

Table 1

Agglutinating activity of various serotypes of human adenoviruses

Серотип аденовируса	Эритроциты						
	обезьян		белых крыс	белых мышей	мышей в клетках кролика	морских свинок	чуж
	макаки рhesus	Grivet					
3	8-64	4096	0	8	0	0	0
7a	8-256	32	0	4	0	0	0
(l) 14 (эталонный штамм Dewit)	2-16	0	0	0	0	0	0
(m) 14 (штамм № 277)	32	0	0	0	0	0	0
20	2-4	0	0	0	0	0	0
(n) 21 (эталонный штамм № 1645)	64-256	64	0	0	0	0	0
(o) 21 (штамм № 167)	128-512	0	0	0	0	0	0
25	2-8	2-4	0	0	0	0	0
11	4096	4096	0	4	0	0	0
16	1024	1024	0	0	0	0	0
(p) 8 (эталонный штамм Trim)	0	0	128	64	64	64	0
(q) 8 (штамм H-ва)	0	0	64	32	32	32	0
(r) 8 (штамм P-ва)	0	0	32	16	16	16	0
(s) 8 (штамм T-ва)	0	0	128	64	64	64	0
(t) 8 (штамм Y-ва)	0	0	256	128	128	64	0
9	0	0	256	256	128	16	0
10	0	0	512	512	32	2	0
19	0	0	128	64	64	4	0
13	4	4	16	4	16	0	0
26	0	0	2-16	4	0	0	0
27	8	4	256	32	16	0	0
15	4	2	512	256	0	0	0
17	0	0	64-256	256	0	0	0
22	0	0	128	8	0	0	0
23	8	4	256	16	0	0	0
24	0	0	4096	256	0	0	0
1, 2, 4, 5, 6	0	0	...	0	0	0	0

(u) Обозначения: здесь в табл. 2-4: 0 - отсутствие геммагглютинации; ... - частичное геммагглютинации; цифры - обратные значения титров.

Key: (a) Serotype of adenoviruses; (b) Erythrocytes; (c) Monkeys; (d) rhesus; (e) Grivet; (f) white rats; (g) white mice; (h) human blood type O; (i) guinea pigs; (k) chickens; (l) 14 (Dewit reference strain); (m) 14 (strain No 277); (n) 21 (reference strain No 1645); (o) 21 (strain No 167); (p) 8 (Trim reference strain); (q) 8 (strain N); (r) 8 (strain R); (s) 8 (strain P); (t) 8 (strain U); (u) Legend: here and in tables 2-4: 0 - absence of hemagglutinins; ... - partial hemagglutination; figures - reverse values of titers.

Table 2

Agglutinating activity of adenovirus type 21 and the freshly isolated strain No 167 in respect to erythrocytes of different species of monkeys

Виды обезьяньих эритроцитов	Титры геммагглютининов									
	эритроцитами 10 отдельных особей одного									
	1	2	3	4	5	6	7	8	9	10
(d) <i>Macaca rhesus</i>										
(f) 21 штаммовый (штамм № 1645)	1:64	1:128	1:512	1:256	1:256	1:64	1:256	1:64	1:256	1:256
(g) Штамм № 167	1:128	1:256	1:512	1:512	1:512	1:128	1:512	1:128	1:512	1:512
(e) <i>Orizet</i>										
(f) 21 штаммовый (штамм № 1645)	1:64	1:64	1:64	1:32	1:32	1:64	1:32	1:64	1:64	1:64
(g) Штамм № 167	0	1:16	0	0	0	1:16	0	0	0	0

Key: (a) Suspension of adenoviruses of serotype; (b) Titers of hemagglutinins; (c) erythrocytes of 10 different specimens of monkeys; (d) Rhesus monkey; (e) Grivet; (f) 21 reference (strain No 1645); (g) Strain No 167.

Table 3

Agglutinating activity of erythrocytes of various specimens of guinea pigs with various serotypes of adenoviruses

Серотип	Температура при взаимодействии вируса с эритроцитами (в градусах)	Титры геммагглютининов в зависимости от особи									
		1	2	3	4	5	6	7	8	9	10
8	4	1:32	1:64	1:64	1:32	1:64	1:32	1:64	1:64	1:64	1:32
	20	1:32	1:64	1:64	1:32	1:64	1:32	1:64	1:64	1:64	1:32
9	37	1:32	1:64	1:64	1:32	1:64	1:32	1:64	1:64	1:64	1:32
	4	0	1:16	1:16	0	1:16	0	1:16	1:16	1:16	1:8
10	20	0	0	0	0	0	0	0	0	0	0
	37	0	0	0	0	0	0	0	0	0	0
19	4	0	1:2	1:2	0	1:2	0	1:2	1:2	1:4	0
	20	0	0	0	0	0	0	0	0	0	0
	37	0	0	0	0	0	0	0	0	0	0
	4	0	1:4	1:4	0	1:4	0	1:4	1:4	1:4	1:2
	20	0	0	0	0	0	0	0	0	0	0
	37	0	0	0	0	0	0	0	0	0	0

Key: (a) Serotype; (b) Temperature during interaction of virus with erythrocytes (in degrees); (c) Titers of hemagglutinins depending on the specimen.

Table 4

Additional division of adenoviruses of the first groups based on Rosen's classification ★

№ Ресур	Предложен- ный нами	Серотипы	Агглютинация с эритроцитами				
			обезья- ны	крысы	мыши	челове- ки	испан- ской сазмы
1-я группа	1-я, подгруппы: → a	3, 7, 14, 20, 21, 25, 28 ¹	+	-	-	-	-
2-я группа	2-я, подгруппы: → b → c → d	11, 16 8, 9, 10, 19 13, 26, 27 15, 17, 22, 23, 24 29, 30	-	+	+	+	+
3-я	Не изучалась	1, 2, 4, 5, 6	-	Час- тично	-	-	-
4-я	→ p	12 ² , 18	-	-	-	-	-

Key: (a) Based on Rosen; (b) Proposed by us; (c) Serotypes; (d) Agglutination with erythrocytes; (e) of monkeys; (f) rats; (g) mice; (h) man; (i) guinea pigs; (j) First group; (k) Second group; (l) 3rd; (m) 4th; (n) First, subgroups; (o) Second, subgroups; (p) Not studied; (q) partially.

The values of titers of hemagglutinins in the reaction with erythrocytes of different specimens of rats differed by 2 times and rarely by 4 times, but no more.

Adenoviruses of types 13, 15, 23, and 27 possess a weak hemagglutinating activity with erythrocytes from rhesus and Grivet monkeys.

Serotypes of adenoviruses of types 8-10, 13, 15, 17, 19, 22-24, 26, and 27 agglutinate the erythrocytes of white mice. In addition to this types 8, 9, 10, 13, 19, and 27 agglutinate the erythrocytes of human blood group 0.

It is necessary to note that the best results with erythrocytes of human blood group 0 for types 13, 19, and 27 were obtained at temperatures of 20 and 37°.

Very interesting results were obtained in the study of the agglutinating activity of adenoviruses with erythrocytes of guinea pigs. They were agglutinated only by types 8, 9, 10, and 19 (see Table 3). Type 8 adenovirus agglutinates guinea pig erythrocytes in high titers both at a temperature of 4 and 20° and at 37° regardless of the individual peculiarities of the different specimens.

In contrast to it type 9, which possesses a high agglutinating activity in respect to rat erythrocytes, agglutinated guinea pig erythrocytes, but not in all the series and only at 4°. The average titer of agglutinins was 1:8 - 1:16.

Adenoviruses of type 10 and 19 also agglutinated guinea pig erythrocytes, but not all the series and then in a low titer (1:2 - 1:4) and only at 4°.

* Legend to Table 4.

(q) 1. Dependence of titers of hemagglutinins of different serotypes on the individual peculiarities of the animal's erythrocytes.

(r) 2. Agglutination was revealed with rat erythrocytes in the presence of heterologous serum of type 6 [10].

(s) Legend: + positive HA; - absence of hemagglutination.

It is interesting to note that in viruses of types 8, 9, and 10, and also 10 and 19, a certain antigenic community was revealed in the hemagglutination inhibition reaction.

We encountered a reference to the agglutinating activity of types 8 and 9 in respect to guinea pig erythrocytes in the work by Rosen [9].

The agglutinating activity of adenoviruses, especially type 8, with guinea pig erythrocytes can serve as a differential feature in their identification. We used this for strains which were isolated during an outbreak of epidemic keratoconjunctivitis in Moscow in 1963-1964 [1].

The results obtained in the study of the spectrum of agglutination of erythrocytes, the dependence of titers of hemagglutinins on the individual peculiarities of erythrocytes in different specimens of the same species, and also data on the resistance of hemagglutinins of various serotypes to a number of physico-chemical factors [4] give us a basis to propose an additional division of adenoviruses of the first group according to Rosen's classification into 2 subgroups, and the second group - into 4 subgroups (see Table 4).

Conclusions

1. Of the adenovirus serotypes 8-10, 13, 15, 17, 19, 22-24, 26, and 27, which agglutinate rat erythrocytes, types 8, 9, 10, and 19 also agglutinate erythrocytes of human blood group O, mice, and guinea pigs, types 13, 26, and 27 - erythrocytes of human blood group O and mice, and serotypes 15, 17, 22, 23, and 24 - erythrocytes of mice.

2. In adenoviruses of types 3, 7, 14, 20, and 25, which agglutinate monkey erythrocytes, a dependence is observed in the titers of hemagglutinins on the individual peculiarities of rhesus monkey erythrocytes, and in type 21 - on the individual peculiari-

ties of Grivet monkey erythrocytes. In types 11 and 16 no variations were observed in the titers of hemagglutinins depending on the individual peculiarities of erythrocytes from individual specimens of monkeys.

3. The most sensitive for type 3 are erythrocytes of Grivet monkeys, and for types 7, 14, 20, 21, and 25 - rhesus monkeys.

Serotypes 11 and 16 agglutinate the erythrocytes of both species of monkeys equally well.

4. Differences were revealed in the agglutinating activity of reference strain 21 and the strain No 167 which was freshly isolated by us. Both agglutinated erythrocytes of the rhesus monkey; erythrocytes of Grivet monkeys were agglutinated only by the reference strain.

5. The hemagglutinating properties of adenoviruses, in particular type 8, which are manifested in reactions with guinea pig erythrocytes, may be used for differentiation into subgroups.

6. A more fractional division is proposed for serotypes of adenoviruses based on HA indices and the resistance of hemagglutinins to a number of physico-chemical factors. The first subgroup in Rosen's classification is divided into 2 subgroups - a and b, and the second group - into 4 subgroups - a, b, c, and d.

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