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RECEPTORS FOR BK VIRUS ON HUMAN ERYTHROCYTES

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Summary. — The chemical nature of BK virus receptors on human erythrocytes was investigated. Glycosidases, KIO_4, proteases, phospholipases and other substances acting specifically on different structures of plasma membranes were used to ascertain the role of surface cell components in the interaction with BK virus. The results obtained indicate an analogy between BK virus receptors on red cells and non-antibody serum inhibitors active against haemagglutination by the same virus.

Key words: BK virus; *Papovaviridae*; *haemagglutination*; *erythrocyte receptor*

Introduction

BK virus, a human papovavirus, agglutinates red blood cells (RBC) of different animal species such as the guinea pig, 1-day-old chick and man (0 group). Similarly to polyoma virus, the haemagglutinin of BK virus is tightly connected with the nucleocapsid and it has been shown to be resistant to ether, chloroform, β-propiolactone (Pitko *et al.*, 1975), pH variations and high salt concentrations (Kende *et al.*, 1979). The binding of this haemagglutinin to RBC takes place on neuraminidase-sensitive receptors (Mäntyjärvi *et al.*, 1972), the chemical nature of which has not yet been elucidated. It is, however, well known that sera of many animal species, including man, contain non-antibody inhibitors which compete with the recognition sites for the binding of BK virus on RBC and may thus inhibit haemagglutination. These inhibitors have been identified with glycoproteins of a high molecular weight (De Stasio *et al.*, 1980; Seganti *et al.*, 1980). As with other viruses, a significant structural analogy between serum inhibitors and RBC receptors has been demonstrated. We attempted to obtain additional information on the chemical determinant groups of RBC for BK virus and their similarities with serum inhibitors. Human group 0 RBC were treated with various enzymes and chemical substances specifically acting on different structures of plasma membranes. The results concerning the modified agglutinability were compared with those obtained with influenza A virus strain PR8 which interacts with similar RBC receptors (Mori *et al.*, 1962).

Materials and Methods

Haemagglutinating antigens. BK virus, Gardner's prototype strain, was propagated in Vero cell monolayers (De Stasio *et al.*, 1980). Influenza A virus strain PR8 was cultivated for 48 hr in the allantoic cavities of chick embryos.

Tests for virus receptors on RBC. Human 0 RBC washed in phosphate buffer saline (PBS) pH 7, at 1000 rev/min for 10 min at 4 °C and then suspended to a 4% concentration were subjected to various treatments. After each treatment, the RBC were washed again three times to remove traces of the reagents used and then suspended to 0.8% final concentration.

Neuraminidase. To a 4% suspension of RBC in PBS, various concentrations of neuraminidase (1 U/ml) from *Vibrio cholerae* (Behringwerke) were added. After 30 min in a water bath at 37 °C, the tubes were centrigufed at 1000 rev/min for 10 min and sialic acid in the supernatant was determined according to the procedure described by Aminoff (1959).

β-Galactosidase. Neuraminidase-treated RBC samples were incubated at 37 °C for 30 min with 30 U/ml β-galactosidase (Boehringer). Total neutral hexose released was estimated in the supernatants by the phenol-H_2SO_4 method using D-galactose as a standard (Dubois *et al.*, 1956).

Proteases. Trypsin (Difco), α-chymotrypsin (Sigma Chemical Co.) from bovine pancreas and papain (B.D.H.) from papaya juice were used. The enzymes were added at various concentrations to 4% RBC suspensions. After 60 min at 25 °C for trypsin and α-chymotrypsin treatments and at 37 °C for papin treatment, protein concentration was determined in the supernatants by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Phospholipases. Phospholipase A_2 from bee venom (EC3114), C from *Clostridium welchi* (EC3143), D from cabbage (EC3144) (Sigma Chemical Co.) were used. One ml of a 4% RBC suspension was incubated with several concentrations of phospholipase A_2 and C at 37 °C, and of phospholipase D at 25 °C for 60 min. The tubes were then centrigufed and each supernatant was assayed by thin layer chromatography to verify the lipid compounds released. Standard: phosphatidilcholine; migration buffer: $CHCl_3-CH_3OH-NH_3-H_2O$ (7 : 3 : 1 : 4); silica gel slides were stained with resublimed iodine.

All enzymatic treatments mentioned above were positive; the presence of released compounds in the supernatants was confirmed by testing according to the procedures reported.

Potassium periodate. To 0.5 ml of a 4% RBC suspension was added 0.5 ml of KIO_4 in PBS, to a final concentration of 1.5×10^{-3} M. After 60 min at 4 °C, the periodate was inactivated by adding 0.5 ml of a 20% glucose solution in PBS.

Concanavalin A (Con A) and glutaraldehyde. RBC suspensions were kept with various concentrations of Con A and glutaraldehyde (Sigma Chemical Co.) for 60 min at 4 °C.

Haemagglutination (HA) tests for PR8 and BK viruses were carried out as described (De-Stasio *et al.*, 1980). The HA titre of the viruses was calculated as the reciprocal of the highest dilution of haemagglutinating antigen which gave a 50% of HA.

Results

The action of specific reagents for carbohydrates was studied with the use of (*1*) neuraminidase, which releases terminal sialic acid from membrane glycoproteins and glycolipids; (*2*) β-galactosidase because galactose is usually located on the terminal non-reducing portion of membrane polysaccharide chains; and (*3*) KIO_4 for its oxidizing action on alcoholic groups of glycids, as well as its capacity of reacting with protein components such as serine and other amino acids.

The data summarized in Table 1 show that receptors for both viruses were highly affected by treatment with neuraminidase which strongly reduced the agglutinability by BK virus and eliminated that by PR8 virus. As to β-galactosidase, the action of this enzyme on RBC previously treated with neuraminidase caused no modification. KIO_4 caused a decrease in the agglutinability of RBC, which was higher towards BK virus.

Table 1. Effects of various reagents on the agglutinability of human RBC by BK and PR8 viruses

Substance	Concentration μg/ml	HA titre BK virus	HA titre PR8 virus
None	—	100	100
Neuraminidase	1.1	100	75
	2.2	75	50
	4.5	70	25
	9	12	0
	18	6	0
Neuraminidase + β-galactosidase	18		
	92	12	—
Neuraminidase + β-galactosidase	4.5		
	92	—	25
KIO_4	345	25	50
Trypsin	1×10^3	100	50
	1.5×10^3	100	50
	3×10^3	65	35
α-Chymotrypsin	3×10^3	100	100
Papain	0.2×10^3	65	40
Phospholipase A_2	5	40	75
	10	40	50
Phospholipase C	0.5	150	130
	5	170	140
Phospholipase D	10	50	65
	50	50	65
Concanavalin A	12.5	150	200
	25	200	800
Glutaraldehyde	10	400	800

The role of surface protein components in the interaction between RBC and virus was investigated by means of various proteolytic enzymes such as trypsin, α-chymotrypsin and papain which act with different specificity. As a consequence of the action of these enzymes, RBC showed a decreased agglutinability, more marked with PR8 virus.

Another series of tests was carried out with A_2, C and D phospholipases which act on the surface phospholipids by causing the cleavage of different substitutes of glycerol in the various positions. These experiments concerning the role of the lipid components of RBC membranes in the binding with virus showed a slight reduction in the agglutinability after treatment with A_2 and D phospholipases and a slight increase due to C phospholipase.

To obtain additional information on the chemical nature of the receptor under investigation, RBC were treated with two different substances, namely Con A which binds to RBC surface due to its specificity for D-mannose contained in glycoproteins and glycolipids, and glutaraldehyde that blocks the membrane structures by hindering any type of aggregation. Both these treatments caused an increase in agglutinability, proportionally to the concentrations used.

Discussion

The present data concerning the action of reagents for carbohydrates on the agglutinability of RBC by BK virus clearly demonstrated the primary

role of sialic acid in the receptor, although RBC were still slightly agglutinated even at the highest concentrations of neuraminidase used. Our results are in agreement with data reported by other authors showing the existence of one type of creceptor shared by papovavirus and influenza virus (Mori *et al.*, 1962; Mäntyjärvi *et al.*, 1972). The highest sensitivity to neuraminidase and KIO_4 of BK virus receptors indicates, however, that the receptor sites for BK and influenza viruses are not identical.

As demonstrated for polyoma virus (Meager and Hughes, 1977), receptors for BK virus are weakly affected by the action of proteolytic enzymes. The slight reduction in the agglutinability observed with the highest concentrations of trypsin and papain was probably due to the removal of glycopeptides and the subsequent loss of polysaccharide fractions active in the virus-RBC interaction.

As to the action of phospholipases, the small increase in agglutinability after treatment with phospholipase C can be explained with a reduction in the polarity of phospholipid molecule.

Glutaraldehyde treatment of human 0 RBC produced a stabilization of cell surface components with a subsequent enhancement of the agglutinability of RBC by BK virus, which is in agreement with the results obtained by Wolff *et al.* (1977) with glutaraldehyde-fixed goose erythrocytes in arbovirus haemagglutination.

The increased agglutinability of RBC observed after treatment with Con A can be due to different mechanisms. This phenomenon could be due to a linkage between the viral envelope and Con A, thus acting as a bridge between virus and RBC membrane. A second explanation might be a reduced mobility of many components of cell membrane (Yahara and Edelman, 1972), i.e. a mechanism of action similar to that hypothesized for glutaraldehyde.

Our result obtained with Con A, on the other hand, is not in agreement with the observations made by Lomberg-Holm (1975) who reported a lower attachment of poliovirus to Con A-treated cells.

Many of these results concerning the action of different reagents on RBC receptors for BK virus are in accordance with similar findings on serum inhibitors of haemagglutination by BK virus (De Stasio *et al.*, 1980; Seganti *et al.*, 1980). It can be concluded that receptor sites for BK virus haemagglutinin, which are present on susceptible RBC, show very similar chemical characteristics to the active groups of non-antibody serum inhibitors active towards BK virus haemagglutination. A study of both RBC receptors and serum inhibitors thus represents useful and complementary ways of approach to the understanding of the mechanism of early virus-cell interactions.

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