Inhibition of BK Virus Haemagglutination by Gangliosides

By LAURA SINIBALDI, DONATELLA VITI, PAOLA GOLDONI, GIOVANNI CAVALLO, CECILIA CARONI AND NICOLA ORSI

Istituto di Microbiologia, Facoltà di Medicina e Chirurgia, Università ‘La Sapienza’ di Roma, Rome and 1 Wellcome Italia Research Laboratories, Pomezia, Rome, Italy

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SUMMARY

The effect of gangliosides extracted from human group O Rh+ erythrocytes on haemagglutination by BK virus was investigated. Experiments were performed on both ganglioside mixtures and isolated fractions separated by column chromatography and characterized by thin-layer chromatography. These results were compared with those obtained with standard preparations of gangliosides, and the inhibiting activity was shown to be confined mainly to gangliosides with a RF lower than GM1. It was also observed that the insertion of gangliosides in liposomes increased the haemagglutination-inhibiting activity and that ganglioside coating restored the ability of glycosidase-treated human red blood cells to agglutinate.

Gangliosides are important components of cell membranes, capable of determining receptor specificity towards different types of biological molecules and viruses. Some enveloped RNA viruses have been shown to bind to gangliosides that are effective as receptors (Blough & Tiffany, 1971; Haywood, 1974a, b; Sharom et al., 1976; Holmgren et al., 1980; Markwell et al., 1981, 1984; Umeda et al., 1984; Suzuki et al., 1985; Sinibaldi et al., 1985; Superti et al., 1986), and can antagonize viral infectivity and haemagglutination (HA). This phenomenon probably occurs with a wide variety of viruses and among these BK virus seemed to be of considerable interest. In fact, BK virus, a member of the papovavirus group, isolated from human cells (Gardner et al., 1971) was known to bind to human erythrocytes (or to human cells), and to induce HA specifically. While the nature of the cell membrane components involved in these interactions was not totally understood, it was possible to assume that gangliosides played a major role in securing the attachment of the virus to the target cells. Accordingly, we decided to check whether gangliosides extracted from human erythrocytes (group O Rh+) were able to inhibit specifically the HA induced by BK virus.

The results obtained have been compared with those given by standard preparations of gangliosides in order to identify the type of compound responsible for this inhibition. All these preparations have been tested for their haemagglutination-inhibiting (HI) activity after inclusion in liposomes.

The haemagglutinating antigen used was obtained by cultivation of BK virus (Gardner prototype strain) on Vero cells (De Stasio et al., 1980). HA titres of 256 to 512 were regularly obtained.

Group O Rh+ human red blood cells (RBC) were washed three times in phosphate-buffered saline (PBS), and gangliosides were extracted from 1 g of pelleted stromata as described in Table 1.

The ‘crude ganglioside’ mixture was then submitted to column chromatography on a dry silica gel Kieselgel 60 (230 to 400 mesh, Merck) according to Svennerholm (1957). Five fractions were obtained by using different solvent systems: chloroform–methanol (2:1, v/v), fraction 1; chloroform–methanol–H₂O (65:25:2, by vol.), fraction 2; chloroform–methanol–H₂O (65:25:4, by vol.), fraction 3; chloroform–methanol–H₂O (60:32:7, by vol.), fraction 4;
Table 1. *Haemagglutination-inhibiting titre of standard gangliosides and ganglioside-liposomes against BK virus*

<table>
<thead>
<tr>
<th>HI titre</th>
<th>Ganglioside preparation (700 μg/ml)</th>
<th>Ganglioside liposome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control liposomes</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Lactoceramide (Wellcome)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Asialo-GM1 (Wellcome)</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>GM1 (Wellcome)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>GM2 (Wellcome)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>GM3 (Wellcome)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>GD1a + GT (Wellcome)</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>Type II (Sigma)</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>Fraction 3 (from human O group Rh+)</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Fraction 4 (from human O group Rh+)</td>
<td>32</td>
<td>64</td>
</tr>
</tbody>
</table>

* Gangliosides were extracted from RBC as follows. Stromata preparations were obtained by adding 8 l cold H₂O to 200 ml pelleted RBC and storing the suspension overnight at 4 °C. After centrifugation at 8000 g for 20 min, the pellet was separated and washed three times in H₂O. Gangliosides were extracted from 1 g pelleted stromata by adding 0.01 m-potassium phosphate buffer pH 6-8 and tetrahydrofuran according to Tettamanti et al. (1973). After extraction the supernatants were collected and evaporated. Standard preparations of ganglioside were: gangliosides Type II from bovine brain (Sigma), lactoceramide, asialo-GM1, GM1, GM2, GM3, GD1a and GT (Wellcome Italia Research Laboratories), GD1b, GD3, GM2, GM3, (Trans-Bussan Geneve, Switzerland), GM1, GT1b (Supelco, Bellafonti, Pa., U.S.A.). TLC of either crude ganglioside mixture or purified gangliosides was carried out according to Ando et al. (1978). The colouration was that proposed by Svennerholm (1957). GD1b, GD3, GM3, GM2 (Trans-Bussan) and GM1, GT1b (Supelco) were used as the ganglioside standards.

chloroform–methanol–H₂O (55:45:10, by vol.), fraction 5. Each fraction was concentrated under vacuum and lyophilized. The amount of gangliosides in each fraction (determined from the content of sialic acid) was established according to Aminoff (1959).

Liposome preparations were made up as follows. L-α₂-Phosphatidylcholine (2.8 mg, Sigma), cholesterol (1.4 mg, Sigma), and gangliosides (from 0.112 μg up to 1.4 mg) were dissolved in 1 ml chloroform–methanol (1:1, v/v) and dried under a nitrogen stream. Vesicles were generated by sonication of the dry lipids for 10 min in 1 ml PBS pH 7.4. The dispersion was then centrifuged at 130000 g for 60 min according to Roche et al. (1978).

For enzymic treatment of human RBC a 4% suspension of RBC in PBS was treated as in the legend to Fig. 1. The HA and HI tests were carried out as described by De Stasio et al. (1980). The 50% endpoint was obtained by interpolation and was the dilution half-way between that showing no HA and the one with complete HA.

The inhibiting activity of human group O RBC gangliosides towards BK virus HA was first tested by using the crude ganglioside mixture obtained as described and the inhibiting titre found was 32. In order to eliminate the lipids and to separate the different ganglioside families, these preparations were subsequently submitted to chromatography on a dry silica gel Kieselgel 60.

Five fractions were obtained. They were tested for HI activity and analysed by thin-layer chromatography (TLC). Only fraction 5, containing a mixture of GM3, GD1a and GD3, inhibited to a significant extent the HA induced by BK virus (1:64). Fraction 1 and 2 (containing lipids and cerebrosides), fraction 3 (containing GM3) and fraction 4 (containing GM2 and GM3), on the contrary, lacked any HI activity.

The components of fraction 5 were then separated by means of a second run of Kieselgel chromatography. The four fractions obtained were checked for their HI activity and analysed by TLC. Only fractions 3 and 4 showed an HI activity related to the presence of GM3 and GD3 (fraction 3) and GD1a and GD3 (fraction 4). These results indicated that the HI activity was present in gangliosides with a Rf lower than GM1.

This was confirmed in experiments carried out with standard preparations of gangliosides (Table 1).
In agreement with the results obtained with gangliosides extracted from human erythrocytes, the HI activity was confined to GD1α + GT.

While these competition experiments showed the ability of free gangliosides of the GD1α and GT type to inhibit specifically the attachment of BK virus to the target cell, they did not prove conclusively that these compounds were indeed recognized by the virus within the cell membrane. In fact, early experiments from this and other laboratories (Sinibaldi et al., 1985; Haywood et al., 1974a, b; Sharom et al., 1976) had shown that the HI activity may be reduced to a considerable extent once the macromolecule is inserted into a membrane structure. Accordingly, we decided to test the HI ability of gangliosides Type II, GD1α + GT, fraction 3 and fraction 4 after their inclusion in liposome vesicles, a structure that mimics the cell membrane. The data reported in Table 1 show only a slight increase in HI activity.

Successive experiments were carried out by treating RBC with neuraminidase which is known to remove the sialic acid from membrane glycolipids and glycoproteins and with β-galactosidase which hydrolyses the terminal galactose. After the enzymic treatment, RBC were incubated with 30 or 60 µg/ml of standard Type II gangliosides and crude ganglioside mixture obtained from human RBC, in order to verify an eventual restoration of susceptibility to HA. The experiments were performed in parallel with gangliosides inserted in liposomes.

In Fig. 1 and 2 the results obtained by the addition of the two types of gangliosides to RBC treated with neuraminidase and neuraminidase + β-galactosidase are reported. It appears from these that RBC susceptibility to BK virus HA decreases after treatment with neuraminidase and
is abolished by the action of neuraminidase + β-galactosidase. The addition of gangliosides, alone or inserted in liposomes, restored gradually RBC susceptibility with an effect which was earlier and stronger in the case of gangliosides Type II.

It can be concluded, therefore, that even in the case of BK virus, gangliosides are involved in the binding of virus to RBC. The role of gangliosides in the receptor for virus is demonstrated by the fact that treatment of RBC with neuraminidase and neuraminidase + β-galactosidase makes them resistant to agglutination by BK virus but this ability is restored to almost 100% by cell coating with purified gangliosides. The same purified gangliosides are capable of inhibiting BK virus-induced HA when added to RBC–virus mixtures and this property is characteristic of polysialylated gangliosides with a \( R_f \) lower than that of GM1. Moreover, this inhibiting activity is enhanced when gangliosides are inserted in liposomes, i.e. when the hydrophobic portion of ganglioside (ceramide) is inserted in lipid vesicles, whereas the hydrophilic portion (containing glycides) is exposed on the outside. This underlines the role that the glycicidic moiety of gangliosides plays in the early interaction of the cellular receptor with BK virus recognition unit, and is in agreement with previous observations (De Stasio et al., 1980; Seganti et al., 1980, 1981a, b) that in a serum \( \alpha_2 \)-macroglobulin (glycoprotein) the non-antibody HI activity towards BK virus relates to the integrity of the glycidic part.

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REFERENCES


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