Gangliosides Influence Experimental Influenza Virus Infection in Mice

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(Accepted 7 June 1989)

SUMMARY

Influenza virus infection in mice may be either stimulated or partially prevented by certain gangliosides, depending on the experimental conditions employed. When injected prior to virus infection gangliosides increased the mortality rate, whereas preincubation with the virus before infection had a protecting effect. Hybrid mice resistant to influenza virus became highly susceptible to infection after injection of a specific ganglioside whereas the corresponding antiganglioside antiserum protected virus-susceptible mice against infection by the virus. These results are discussed in the light of earlier findings that various gangliosides enhance non-specific binding of influenza virus, whereas gangliosides of the GT₁₁₄ and GD₁₁₄ type are able to act as specific virus receptors and to promote virus penetration.

INTRODUCTION

Virus attachment to the cell surface is the first step in virus–cell interaction resulting in the infection of the host cell. The attachment which leads to infection is a specific process involving certain determinants of virus attachment protein(s) and specific receptors on the host cell plasma membrane (Bukrinskaya, 1982).

It has been shown by some of our group that various mono-, di- and trisialogangliosides could serve as cellular attachment sites for influenza virus, whereas only one type of ganglioside, GT₁₁₄ (Svennerholm, 1977) and its immediate precursor, disialoganglioside GD₁₁₄, was shown to be able to induce virus penetration and subsequent delivery of the nucleocapsid to the cell nucleus (Bergelson et al., 1982). It was demonstrated that this effect of gangliosides GT₁₁₄ or GD₁₁₄ was due to their specific interaction with virus haemagglutinin (HA) which induces a rearrangement of the viral lipids resulting in the bilayer areas of the viral envelope becoming fluid to a significant degree. This in turn promotes fusion of the virus with target membranes (Slepushkin et al., 1988). Gangliosides of other types, e.g. gangliosides GM₁ and GD₁₁₄, promote only non-specific binding of influenza viruses to cells or liposomes (Slepushkin et al., 1986).

In this paper we show that mono-, di- and trisialogangliosides produce striking effects on experimental influenza virus infection in mice, stimulating infection when injected 1 h before virus infection and protecting mice from infection when preincubated with the virus.

METHODS

Virus. The R94 (H3N2) influenza virus strain pathogenic for mice was obtained by recombination of A/PR8/34 and A/Philippines/2/82 strains. HA and neuraminidase (NA) genes in the recombinant strain were derived from A/Philippines/2/82 and all the other genes are from A/PR8/34. Stock virus was prepared by allantoic inoculation of 9-day-old embryonated eggs with 10⁵ EID₅₀. The allantoic fluid was harvested after incubation of the eggs for 48 h at 37 ºC and stored at -40 ºC.

Animals. Mice of the CBA line susceptible to influenza virus and of the hybrid line [(CBA × C57BL/c) F1] resistant to influenza virus were used. The weight of mice was 12 to 15 g. Each experimental group consisted of 25
animals. The mice were infected intranasally with 0.5 or 5 LD_{50} of virus-containing allantoic fluid per mouse using light ether narcosis.

Antiganglioside sera. Antiganglioside sera were obtained by immunizing rabbits with G_{M1} ganglioside together with methylated bovine serum albumin and complete Freund's adjuvant. Antisera were used after one or two injections of ganglioside. Activity and specificity of the antisera were tested by immunodiffusion (Mikhaylov et al., 1981).

Gangliosides. Monosialoganglioside G_{M1}, disialoganglioside G_{D1a} and trisialoganglioside G_{T1b} were isolated from human brain as described (Dyatlovitskaya et al., 1980) and injected intranasally into mice using light ether narcosis.

Titration of influenza virus in mouse lungs. The lungs of five mice were washed with phosphate-buffered saline (PBS) containing penicillin (100 units/ml) and streptomycin (0.2 g/ml), homogenized and a 10% suspension in PBS was prepared. The suspension was centrifuged at 2000 r.p.m. for 15 min and the supernatant was used for allantoic inoculation of 10- to 11-day-old embryonated eggs. The results were recorded 48 h after incubation at 35 °C by titration of HA.

RESULTS

Mice of the CBA line were infected intranasally with 0.5 LD_{50} of the influenza virus, strain R94; gangliosides were injected intranasally 1 h before infection and then once a day for 2 days. One group of animals was infected with virus preincubated with gangliosides for 1 h at 37 °C. The results in Table 1 show that in the absence of gangliosides only 29% of the infected mice died on day 14 after infection. When 10 nmol of ganglioside G_{M1} was injected intranasally 1 h before infection and then once a day for 2 days (group 2), 70% of the animals died. With gangliosides G_{D1a} and G_{T1b} under the same conditions the mortality was 100% (groups 2 and 3). In the ganglioside-treated groups death occurred earlier than in the infected control group. This effect was more pronounced with ganglioside G_{T1b} than with G_{M1} or G_{D1a} (mean survival times 6-1 days and 8-2 to 9-2 days respectively). Gangliosides injected into uninfected mice did not provoke death which demonstrates that they were not toxic to the animals.

To show that the effect observed was due to virus infection, the amount of the virus in lung suspensions of the mice was determined by titration on embryonated eggs. As seen in Table 1, the EID_{50} value in lung tissue of mice of the ganglioside-pretreated group 2 is significantly higher than that of the non-pretreated group (group 1) suggesting that injection of gangliosides prior to virus infection provoked death as a consequence of enhanced multiplication of the virus in infected lungs. At the same time the antibody titres as determined by the HA inhibition test were nearly the same in G_{M1}-treated animals (group 2) and in the control group of infected mice (group 1).

Table 1. Effect of gangliosides on influenza virus infection of CBA mice

<table>
<thead>
<tr>
<th>Mouse group*</th>
<th>Inoculum</th>
<th>Mortality on day 14 (%)</th>
<th>Mean survival time (days)</th>
<th>EID_{50} in lung suspension (log_{10})</th>
<th>Antibody titre in HA inhibition test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virus</td>
<td>29</td>
<td>12.0</td>
<td>1.0 1.0 3.0</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Virus + G_{M1}†</td>
<td>70</td>
<td>9.2</td>
<td>2.0 6.0 6.0</td>
<td>1:640</td>
</tr>
<tr>
<td>3</td>
<td>Virus + G_{D1a}‡</td>
<td>100</td>
<td>8.2</td>
<td>2.0 4.0 6.0</td>
<td>ND§</td>
</tr>
<tr>
<td>4</td>
<td>Virus + G_{T1b}§</td>
<td>100</td>
<td>6.1</td>
<td>2.0 4.0 5.0</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Virus + G_{M1}§</td>
<td>18</td>
<td>14.0</td>
<td>1.0 1.0 1.0</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>G_{D1a}</td>
<td>0</td>
<td>14.0</td>
<td>– – – –</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>G_{M1}</td>
<td>0</td>
<td>14.0</td>
<td>– – – –</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>G_{T1b}</td>
<td>0</td>
<td>14.0</td>
<td>– – – –</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Each group containing 25 animals was observed daily until day 14, when the survivors were bled for sera. All animals in groups 1 to 5 received 0.5 LD_{50} of virus.
† Gangliosides (10 nmol/ml, 0.05 ml/mouse) were injected 1 h before infection and then once a day for 2 days.
‡ ND, Not determined.
§ The ganglioside (10 nmol/ml, 0.05 ml/mouse) was first mixed with the virus, incubated for 1 h at 37 °C and then injected into mice.
|| Gangliosides (10 nmol/ml, 0.05 ml/mouse) were injected intranasally once a day for 3 days.
Influence of gangliosides on infection

Fig. 1. Effect of different concentrations of gangliosides on experimental influenza virus infection. The mice were infected with 0.5 LD₅₀ of the virus and gangliosides (0.05 ml/mouse) G₉₁ (a), G₉₁₂ (b) and G₉₁₅ (c) were injected as described in Table 1 at 1.0 (●), 0.3 (□), 0.1 (■) or 0.03 (△) nmol/ml. Control mice were infected with virus but not with gangliosides (○).

Table 2. Effect of ganglioside G₁₅ on influenza virus infection of mice (line (CBA × C57BL/c) F₁) resistant to influenza virus

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Mortality on day 14 (%)</th>
<th>Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus*</td>
<td>0</td>
<td>14.0</td>
</tr>
<tr>
<td>G₁₅ + virus*</td>
<td>100</td>
<td>6.0</td>
</tr>
<tr>
<td>G₁₅</td>
<td>0</td>
<td>14.0</td>
</tr>
</tbody>
</table>

* Mice received 5 LD₅₀ per animal.

To determine whether the effect of gangliosides was dependent on their concentration, the infected mice received G₉₁, G₉₁₂, and G₉₁₅ at concentrations of 0.003, 0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 nmol/ml. Fig. 1 shows that the lethal effect is enhanced even by very low doses of gangliosides such as 0.03 nmol/ml.

Effect of gangliosides on infection of virus-resistant mice

As gangliosides added exogenously are known to be able to function as cellular receptors for influenza virus in NA-treated cells (Bergelson et al., 1982), the possibility arises that gangliosides injected into the respiratory tract of infected mice are inserted into the plasma membrane of epithelial cells and thus may serve as additional attachment sites or receptors for influenza virus. If so, they could be expected to provoke infection in resistant animals, provided the cause of the resistance is a lack of receptors.

To explore this possibility mice of the hybrid line [(CBA × C57BL/c) F₁], resistant to influenza virus infection, were treated intranasally with ganglioside G₁₅ (10 nmol/ml) and after 1 h, 5 LD₅₀ of influenza virus was injected intranasally. As shown in Table 2, 100% of ganglioside-pretreated animals died on day 6 after infection whereas no death was registered in the group of animals infected without ganglioside at the same virus concentration. Therefore, mice resistant to influenza infection became susceptible after the injection of gangliosides into the respiratory tract.

Effect of virus pretreatment with gangliosides on infection

As gangliosides are able to bind influenza virus particles to the surface of cells (Bergelson et al., 1982) or liposomes (Slepushkin et al., 1988) they may be expected to compete with natural
Table 3. Effect of antiserum* on the action of ganglioside GM₁ in an influenza virus infection of CBA mice

<table>
<thead>
<tr>
<th>Mouse group</th>
<th>Inoculum</th>
<th>Mortality on day 14 (%)</th>
<th>Mean survival time (days)</th>
<th>Antibody titre in HA inhibition test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virus†</td>
<td>75</td>
<td>9.5</td>
<td>1:640</td>
</tr>
<tr>
<td>2</td>
<td>Antiserum</td>
<td>0</td>
<td>14.0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Antiserum + virus</td>
<td>11</td>
<td>13.5</td>
<td>1:320</td>
</tr>
</tbody>
</table>

* All animals in groups 1 and 3 received 5 LD₅₀ of virus/animal.

receptors inhibiting virus adsorption on the cell surface. To test this possibility, the virus was pretreated with GM₁ for 1 h at 37 °C and the mixture then was injected intranasally into mice of the susceptible line (CBA). A striking result was obtained; only 18% of mice died and death occurred as late as day 14 after infection whereas the same ganglioside injected 1 h before infection and on the second and third days after infection provoked the death of 70% of mice on day 9 after infection (Table 1).

**Effect on infection of antibodies against gangliosides**

If gangliosides are essential for influenza virus infection in mice, antisera against gangliosides injected into the respiratory tract of mice should block the binding of virus to the cell and suppress infection. Rabbit antiserum against GM₁ (titre 1:160) was diluted 10-fold and injected intranasally into mice simultaneously with the virus. As shown in Table 3, the injection of antiserum sharply decreased the percentage of dead animals from 75% in the infected control group to 11% in the group that received antiserum. The mean survival time increased from 9.5 to 13.5 days in the presence of antiserum. This result suggests that antibodies to gangliosides may inhibit virus infection by blocking cellular surface receptors.

**DISCUSSION**

Our data show that gangliosides can produce strikingly different effects on influenza virus infection in mice. When injected intranasally 1 h before virus infection, they strongly stimulated infection increasing the mortality rate from 29% to between 70 and 100%. The virus titre in lungs of infected mice that received gangliosides was higher than that in lungs of control infected mice. The mono-, di- and trisialogangliosides tested were all active stimulators of infection, but the trisialoganglioside GT₁b produced the strongest effect, increasing the mortality rate up to 100% and reducing the mean survival time to 6 days. This is in accordance with our previous finding that many different gangliosides may enhance the binding of influenza virus to the cell surface when the cells had been pretreated with gangliosides before infection but only GT₁b was capable of inducing virus penetration into the cells (Bergelson et al., 1982). The correlation with previous results suggests that gangliosides injected into the respiratory tract of mice prior to virus infection are incorporated into the plasma membrane of epithelial cells, probably of lung alveoli, and thus increase the numbers of virus particles binding to the cells. The high activity of very low concentrations of gangliosides and the dose response of the effect support the suggestion of a specific role of gangliosides in the initiation of influenza virus infection.

When gangliosides were injected into the respiratory tract of hybrid mice resistant to influenza virus, the animals became highly susceptible to infection and all the mice died by day 6 after infection. Therefore it seems likely that the virus resistance of these mice is due to the lack of specific receptors on the surface of respiratory tract cells and that this type of resistance to the virus can be bypassed by injecting gangliosides prior to virus infection. As reported earlier, exogenously added gangliosides could restore the sensitivity of ascites carcinoma cells and tissue culture cells to influenza virus after destroying cellular receptors by NA (Bergelson et al., 1982; Herrler & Klenk, 1987).

An opposite effect on influenza infection was observed when the virus was pretreated with ganglioside GM₁ prior to infection. We believe that in this case gangliosides block specific
attachment sites of the virus and thereby prevent its binding to native cellular receptors. However even the limited reproduction of the ganglioside-pretreated virus appeared to be sufficient to stimulate the formation of antibodies.

REFERENCES


(Received 11 January 1989)