Inactivation of inhibitors by the receptor-destroying enzyme of influenza C virus

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The importance of the receptor-destroying enzyme of influenza C virus for inactivation of inhibitors was analysed. Using three different inhibitors (rat serum, bovine submaxillary mucin and bovine brain gangliosides) inhibition of virus infection was observed only at an inhibitor concentration that was about 100-fold higher than the maximum concentration of inhibitor that could be inactivated by the receptor-destroying enzyme of a given amount of virus. From our data and other observations we conclude that the receptor-destroying enzyme is not required to inactivate inhibitors.

Among enveloped viruses, members of various families are able to use sialic acid as a receptor determinant for attachment to cells. Interestingly, all these viruses contain a receptor-destroying enzyme. Viruses of the genus Paramyxovirus as well as influenza A and B viruses recognize N-acetylneuraminic acid (Neu5Ac) and inactivate their receptors by a sialidase (neuraminidase) that releases terminal sialic acid from glycoconjugates (Klenk et al., 1955). Influenza C virus and several coronaviruses require a different type of sialic acid for binding to cells, N-acetyl-9-O-acetyleneuraminic acid (Herrler & Klenk, 1987b; Rogers et al., 1986; Vlasak et al., 1988a; Schultzze & Herrler, 1992). Their receptor-destroying enzyme is an acetylecterase that releases the 9-O-acetyl group from the receptor determinant (Herrler et al., 1985c; Vlasak et al., 1988a).

The importance of these enzymes is not completely clear. In the case of influenza A viruses evidence has been presented indicating a role in virus maturation by preventing the formation of virus aggregates and by facilitating the release of virions from the infected cell (Liu et al., 1995). Some evidence also suggests a role of the receptor-destroying enzyme during the initiation of infection either by promoting fusion activity (Huang et al., 1980, 1985) or by releasing sialic acids from oligosaccharides near the receptor-binding site that may interfere with the binding to cellular receptors (Ohuchi et al., 1995). In the case of influenza C virus, the importance of the receptor-destroying enzyme has been studied using sialic acid analogues. Some of these artificial sialic acids can function as receptor determinants but are resistant to the receptor-destroying enzyme (Herrler et al., 1992). With this approach, it has been shown that the acetylecterase is required to keep the virus surface free of receptor determinants, which otherwise would result in a decrease of the infectious virus titre due to aggregate formation (Höfling et al., 1996). Studies with sialic acid analogues as well as with enzyme inhibitors have indicated that the acetylecterase of influenza C virus and bovine coronavirus may also play a role during virus entry (Vlasak et al., 1988b, 1989; Brossmer et al., 1993; Strobl & Vlasak, 1993).

In addition to the role during virus entry and virus maturation, it has been suggested that the receptor-destroying enzyme may be important for the in vivo infection (for a review see Herrler et al., 1995). The primary target cells of the viruses mentioned above are the epithelial cells of the respiratory tract. Some coronaviruses may proceed to the epithelium of the intestinal tract. Both the respiratory and the intestinal epithelium are covered by a layer of mucous substances. Mucins are very rich in sialic acid and are known to be haemagglutination-inhibitors for viruses that interact with sialic acids on erythrocytes. The inhibitory activity of mucins might prevent viruses from binding to sialylated receptors on cells of the respiratory or intestinal epithelium. It has been proposed that the receptor-destroying activity enables viruses to inactivate these competitive inhibitors thus allowing access to the target cells. However, no experimental evidence has been provided to substantiate this possibility.

We have tested this concept for influenza C virus by comparing the amount of inhibitor that is required for inhibition of virus infection with the amount that can be inactivated by the receptor-destroying enzyme. Three different inhibitors were included in this study. Rat serum has long been known to be a very potent haemagglutination-inhibitor. Most of the inhibitory activity is accounted for by α1-macroglobulin and murinoglobulin (Herrler et al., 1985b; Kitame et al., 1985). Another inhibitor is bovine submaxillary mucin (BSM), which is a rich source of 9-O-acetylated sialic acid and has been reported to prevent influenza C virus from agglutinating erythrocytes (Herrler et al., 1985c). Glycolipids were also included in this study. Bovine brain gangliosides (BBG) can

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function as receptors for influenza C virus when they are incorporated into the plasma membrane (Herrler & Klenk, 1987b). When they are present in liposomes, they act as haemagglutination-inhibitors (Herrler & Klenk, 1987a). Among these inhibitors, only mucins might have relevance for natural infections with influenza C virus. However, human mucins from the respiratory tract have not been analysed for haemagglutination-inhibition activity. The ability of the receptor-destroying enzyme of influenza C virus to inactivate the three inhibitors mentioned above was first analysed in a haemagglutination assay. Influenza C virus strain Johannesburg/1/66 was grown in 8-day-old embryonated chicken eggs. After incubation for 3 days at 33 °C, the allantoic fluid was harvested, clarified by low speed centrifugation (3700 g, 20 min, 4 °C) and stored at —80 °C. The amount of virus was determined by haemagglutination titration in a microtitre assay as previously described (Herrler et al., 1985a). The reciprocal value of the highest virus dilution causing complete agglutination of chicken erythrocytes was used as a measure of the haemagglutinating activity expressed in HA units/ml. A virus suspension containing 16 HA units/ml was incubated with different dilutions of either of the three inhibitors for 20 min at 4 °C. Half of the sample was then transferred to a 37 °C water-bath, whereas the other half was kept at 4 °C. After an incubation time of 1 h, the samples were put on ice and the HA titre was determined. As shown in Table 1, rat serum was a very efficient haemagglutination-inhibitor; a 1:100 dilution completely prevented influenza C virus from agglutinating the cells. Incubation at 37 °C inactivated the inhibitors present in the 1:1000 dilution as shown by the positive agglutination reaction (16 HA units/ml). The 1:100 dilution resulted in a complete inhibition of haemagglutination irrespective of the incubation temperature indicating that the viral acetylesterase was unable to inactivate the amount of inhibitors present in this dilution. With BSM, complete inhibition was obtained with as little as 10 µg/ml. At that concentration, the inhibitory activity was lost after incubation at 37 °C. The virus was, however, unable to inactivate BSM at a tenfold higher concentration (100 µg/ml). Liposomes containing BBG at a concentration of 5 mg/ml completely prevented influenza C virus from agglutinating erythrocytes. In a 1:10 dilution the liposomes caused a partial inhibition (4 compared to 16 HA units/ml of the control virus). At the amount of virus used for this experiment, influenza C virus was unable to inactivate the glycolipid inhibitors except for a slight effect on the 1:10 diluted sample resulting in an increase of the HA titre from 4 to 8 HA units/ml. This is in accordance with a previous report which showed that 9-O-acetylated sialic acids present on gangliosides are only poor substrates for the acetylesterase of influenza C virus (Schauer et al., 1988).

The three haemagglutination-inhibitors were also analysed for their ability to prevent influenza C virus infection. In order to obtain a high sensitivity of our assay, the amount of influenza C virus was lowered to 2 HA units/ml of the control virus. At that concentration, the inhibitory activity was lost after incubation at 37 °C. The virus was, however, unable to inactivate the glycolipid inhibitors except for a slight effect on the 1:10 diluted sample resulting in an increase of the HA titre from 4 to 8 HA units/ml. This is in accordance with a previous report which showed that 9-O-acetylated sialic acids present on gangliosides are only poor substrates for the acetylesterase of influenza C virus (Schauer et al., 1988).

As shown in Table 2, a 1:10 dilution of rat serum resulted in a more than 10-fold reduction of the virus yield and complete inhibition of the influenza C virus infection was observed with undiluted serum. A 1:100 or

<p>| Table 1. Haemagglutination activity of influenza C virus after incubation at 4 °C or 37 °C with different dilutions of three haemagglutination-inhibitors: rat serum, bovine submandibulary mucin or bovine brain gangliosides (BBG) |</p>
<table>
<thead>
<tr>
<th>Inhibitor dilution</th>
<th>Rat serum (4 °C)</th>
<th>Mucin (10 mg/ml) (37 °C)</th>
<th>BBG (5 mg/ml) (4 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>1:10</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>1:100</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>2</td>
</tr>
<tr>
<td>1:1000</td>
<td>&lt; 2</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>No inhibitor</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

<p>| Table 2. Virus yield after infection of MDCK I cells by influenza C virus that had been preincubated at 4 °C or 37 °C with different dilutions of three inhibitors: rat serum, bovine submandibulary mucin or bovine brain gangliosides (BBG) |</p>
<table>
<thead>
<tr>
<th>Inhibitor dilution</th>
<th>Rat serum (4 °C)</th>
<th>Mucin (10 mg/ml) (37 °C)</th>
<th>BBG (5 mg/ml) (4 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
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<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>1:10</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>1:100</td>
<td>32</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>1:1000</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>No inhibitor</td>
<td>128</td>
<td>128</td>
<td>128</td>
</tr>
</tbody>
</table>

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enzymes appear to be necessary only for enveloped viruses. Above are nonenveloped viruses. Receptor-destroying sialic acid as a receptor-determinant. The viruses mentioned potential inhibitors are not really a problem for viruses that use infect man or animals. Thus, soluble sialoglycoconjugates as unable to inactivate potential inhibitors, they successfully neuraminidase or an acetylesterase. Though these viruses are determinant for binding to cells, but none of them contains a been reported to use N-acetylenuraminic acid as a receptor determinant for binding to cells, but none of them contains a neuraminidase or an acetylesterase. Though these viruses are unable to inactivate potential inhibitors, they successfully infect man or animals. Thus, soluble sialoglycoconjugates as potential inhibitors are not really a problem for viruses that use sialic acid as a receptor-determinant. The viruses mentioned above are nonenveloped viruses. Receptor-destroying enzymes appear to be necessary only for enveloped viruses that recognize sialic acid. In contrast to nonenveloped viruses, they contain glycoproteins that are inserted into the lipid envelope. As described in the introductory section, sialic acid present on these surface proteins may interfere with both virus entry and virus maturation. Therefore, the function of the receptor-destroying enzyme is to keep the virus surface free from receptor determinants but not to inactivate soluble inhibitors.

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References


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