DIFFERENTIAL REACTIVITY OF BOVINE CORONAVIRUS (BCV) AND INFLUENZA C VIRUS WITH N-ACETYL-9-O-ACETYLNEURAMINIC ACID (NEU5,9AC_2)-CONTAINING RECEPTORS

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INTRODUCTION

Only little information is available about the initial events in the infectious cycle of coronaviruses. Attachment of strain A59 of mouse hepatitis virus to the cell surface has been shown to be mediated by a protein of about 110 kDalton (Boyle et al., 1988). Recently, bovine coronavirus (BCV) and human coronavirus OC43 (HCV-OC43) have been shown to recognize receptors on erythrocytes similar to influenza C virus. The evidence is based on the finding that both influenza C virus and BCV contain a sialate O-acetylesterase, which functions as a receptor-destroying enzyme (Vlasak et al, 1988). Incubation of chicken red blood cells with the esterase of either virus renders the erythrocytes resistant to agglutination by both viruses as well as by HCV-OC43. This result indicates that N-acetyl-9-O-acetylneuraminic acid (Neu5,9Ac_2) is a receptor determinant for attachment of BCV and HCV-OC43 to erythrocytes as has been shown previously for influenza C virus (Rogers et al., 1986). Here we confirm the importance of Neu5,9Ac_2 for the binding of BCV to the cell surface by resialylation of erythrocytes. We present evidence that strain Johannesburg/1/66 of influenza C virus is more efficient in recognizing Neu5,9Ac_2-containing receptors than BCV.

RESULTS

There is a great variation among erythrocytes from different species as far as the presence of 9-O-acetylated sialic acids on the cell surface is concerned (Shukla and Schauer, 1982). While red blood cells from mice and rats are very rich in Neu5,9Ac_2 (50% and more of the total content of sialic acid), chemical analysis has failed to detect any O-acetylated sialic acid on cells from some other species such as human erythrocytes. In the case of chicken red blood cells the presence of Neu5,9Ac_2 has been shown to be a developmental marker (Herrler et al., 1987). Chemical analysis has revealed that this type of sialic acid is present on erythrocytes from adult chicken, but not on cells from one-day-old chicken. In order to obtain information about the hemagglutinating

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Table 1. Agglutination of red blood cells by bovine coronavirus (BCV) and influenza A (FPV/Rostock/34(H7N1)) and C virus (JHB/1/66).

erythrocytes	hemagglutination titer (HA-units/ml) BCV	JHB/1/66	FPV/Rostock
chicken, adult	64	512	512
chicken, 1-day-old	<2	<2	256
human	<2	<2	1024

activity of BCV, a hemagglutination assay was performed with erythrocytes from adult and one-day-old chicken as well as with human red blood cells. As shown in Table 1, only influenza A virus is able to agglutinate cells from all three sources. HA-titers of BCV and influenza C virus (strain JHB/1/66) were only obtained with erythrocytes from adult chicken, while cells from one-day-old chicken and human erythrocytes were resistant to agglutination. Considering the type of sialic acid present on the red blood cells (see above), the result from Table 1 is compatible with Neu5,9Ac_2 being a receptor determinant for attachment of BCV to erythrocytes.

With influenza C virus direct evidence for the importance of Neu5,9Ac_2 as an essential part of the receptors on red blood cells was obtained by resialylation of human erythrocytes (Rogers et al., 1986). The same approach was applied to BCV. Human red blood cells were incubated with neuraminidase from Vibrio cholerae to release most of the native sialic acids. The asialo-cells were resialylated using purified rat liver Galβ1,4GlcNAc α2,6-

Table 2. Generation of receptors for bovine coronavirus (BCV) and influenza C virus (JHB/1/66) by resialylation of human erythrocytes.

type of sialic acid attached to cells	hemagglutination titer (HA-units/ml) BCV	JHB/1/66
none	<2	<2
Neu5Ac	<2	<2
Neu5,9A_2	128	128

Table 3. Comparison of the efficiency of bovine coronavirus (BCV) and influenza C virus (JHB/1/66) to utilize Neu5,9Ac$_2$ as a receptor determinant for agglutination of human red blood cells.

erythrocytes	hemagglutination titer (HA-units/ml) BCV	JHB/1/66
chicken, untreated	256	256
human, untreated	<2	<2
human, resialylated with Neu5,9Ac$_2$		
4 µM	264	512
2 µM	16	512
1 µM	<2	512

sialyltransferase (specifically attaching sialic acid to galactose in an α-2,6-linkage) (Sticker et al., 1988) and activated sialic acids (CMP-Neu5Ac and CMP-Neu5,9Ac$_2$). As shown in Table 2, neither BCV nor influenza C virus is able to agglutinate cells resialylated to contain Neu5Ac on the surface. High hemagglutination titers were obtained, however, following attachment of 9-O-acetylated sialic acids to cell surface glycoproteins of asialocells. This result provides direct evidence that agglutination of red blood cells by BCV requires the presence of Neu5,9Ac$_2$ on the cell surface.

The efficiency of BCV and influenza C virus to recognize Neu5,9Ac$_2$-containing receptors was compared by determining the amount of 9-O-acetylated sialic acid required for generation of virus receptors on red blood cells. Following treatment with sialidase from Vibrio cholerae, human erythrocytes were incubated with sialyltransferase and different amounts of CMP-Neu5,9Ac$_2$ at 37°C. After 3 h cells were washed with PBS and used as a 1% suspension to determine the hemagglutination titer of BCV and influenza C virus. As shown in Table 3, optimal titers of influenza C virus were already obtained, when red blood cells were incubated in the presence of 1 µM CMP-Neu5,9Ac$_2$. Hemagglutination by BCV was only detectable with cells incubated in the presence of 2 µM of activated sialic acid, optimal titers requiring 1 µM CMP-Neu5,9Ac$_2$ during the resialylation reaction. This result indicates that strain JHB/1/66 of influenza C virus is more efficient in recognizing Neu5,9Ac$_2$-containing receptors on erythrocytes.

In order to get further information about the receptor-binding activity of BCV, an artificial sialic acid analogue was analyzed for its ability to function as a receptor determinant. 9-acetamido-9-deoxy-N-acetylneuraminic acid is very similar to Neu5,9Ac$_2$, the only difference being that the acetyl residue is

Table 4. Comparison of the ability of bovine coronavirus (BCV) and influenza C virus (JHB/1/66) to agglutinate human erythrocytes resialylated to contain the sialic acid analogue 9-N-acetyl-Neu5Ac.

type of sialic acid attached to cells	hemagglutination titer (HA-units/ml)	
	BCV	JHB/1/66
9-O-acetyl-Neu5Ac	128	128
9-N-acetyl-Neu5Ac (9-acetamido-Neu5Ac)	<2	256

attached to position C-9 via a nitrogen rather than an oxygen atom. The activated form of this sialic acid analogue has been shown previously to be a suitable donor substrate for Galβ1-4GlcNAc α2,6-sialyltransferase from rat liver (Groβ et al., 1987, 1989). Following transfer to the surface of human erythrocytes, this synthetic sialic acid functions as a receptor determinant for influenza C virus (J.C.Paulson, personal communication). As shown in Table 4, cells resialylated to contain 9-acetamido-Neu5Ac were agglutinated by influenza C virus, but not by BCV. This result provides further evidence that BCV and influenza C virus differ in their ability to recognize sialic acid-containing receptors.

DISCUSSION

The results presented above confirm that attachment of coronavirus to erythrocytes requires the presence of Neu5,9Ac_2-containing receptors on the cell surface. The resialylation method enabled us to compare two viruses with respect to their efficiency in recognizing Neu5,9Ac_2-containing receptors. Strain JHB/1/66 of influenza C virus requires less 9-O-acetylated sialic acid on the surface of erythrocytes to cause hemagglutination than BCV. This result does not implicate that influenza C viruses in general have a higher affinity for their receptors compared to coronaviruses. Analysis of more strains of the corresponding virus families will probably show that there is some variation in the number of receptors required for attachment of virus to the cell surface. In the case of strain JHB/1/66 a mutant was isolated which is more efficient in recognizing Neu5,9Ac_2-containing receptors than the wild type (Szepanski et al., 1989). This mutant has a broader cell tropism compared to the parent virus. It is very well possible, that there is also variation in the receptor-binding activity of different strains of BCV. Adaptation of BCV to growth in MDBK cells has been described. This cell line has a low amount of 9-O-acetylated sialic acid on the cell surface as judged by its resistance to infection by influenza C virus. We expect that MDBK-adapted BCV requires less Neu5,9Ac_2 on the surface of erythrocytes in order to cause hemagglutination than our strain of BCV, which has been grown in MDCK I cells. Another difference between strain JHB/1/66 of influenza C virus and bovine coronavirus was evident when 9-acetamido-Neu5Ac was analyzed for its ability to serve as a recep-

tor determinant. In contrast to influenza C virus BCV was unable to agglutinate red blood cells sialylated with the synthetic sialic acid analogue. This result shows that the ester linkage between the acetyl residue and position C-9 of sialic acid is crucial for binding of BCV. Sialic acid with an acetyl residue attached via an amide-linkage is recognized as a receptor determinant only by influenza C virus but not by BCV. The use of synthetic sialic acid analogues should be very helpful in the future to characterize the interaction between coronaviruses and their receptors. The resialylation method described here for analysis of erythrocyte receptors should be applicable also to cultured cells and give an answer to the question whether Neu5,9Ac_2 is required for BCV and other coronaviruses to initiate an infection.

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