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ANALYSIS OF CELLULAR RECEPTORS FOR HUMAN CORONAVIRUS OC43

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ABSTRACT

Bovine coronavirus (BCV), human coronavirus OC43 (HCV-OC43) and hemagglutinating encephalomyelitis virus (HEV) are serologically related viruses that all have hemagglutinating activity. The receptor determinant for attachment to erythrocytes has been shown to be N-acetyl-9-O-acetylneuraminic acid ($Neu5,9Ac_2$). We compared the ability of the three coronaviruses to recognize 9-O-acetylated sialic acid and found that they all bind to $Neu5,9Ac_2$ attached to galactose in either A2,3 or A2,6-linkage. There are, however, some differences in the minimum amount of sialic acid that is required on the cell surface for agglutination by these viruses. Evidence is presented that HCV-OC43 uses $Neu5,9Ac_2$ as a receptor determinant not only for agglutination of erythrocytes but also for attachment to and infection of a cultured cell line, MDCK I cells.

INTRODUCTION

Bovine coronavirus (BCV) is known to use 9-O-acetylated sialic acid as a receptor determinant for attachment to cells[1,2]. In the case of erythrocytes, binding of virus results in the agglutination of cells. Studies with MDCK cells have shown that binding to 9-O-acetylated sialic acid is the initial step in the infection of cultured cells[3]. Two other coronaviruses, human coronavirus OC43 (HCV-OC43) and hemagglutinating encephalomyelitis virus (HEV) that are serologically related to BCV, are also potent hemagglutinating agents. Like BCV, HEV and HCV-OC43 require $Neu5,9Ac_2$ on the cell surface for the agglutination of erythrocytes[1,2].

We analyzed whether BCV, HEV, and HCV-OC43 differ in their ability to recognize $Neu5,9Ac_2$ present in different linkage types. Furthermore, evidence is presented that HCV-OC43 uses 9-O-acetylated sialic acid as a receptor determinant for infection of cells.

Corona- and Related Viruses, Edited by P. J. Talbot and G. A. Levy
Plenum Press, New York, 1995

MATERIALS AND METHODS

Cells. MDCK I cells, a subline of Madin-Darby canine kidney cells, were grown in minimum essential medium containing 10% fetal calf serum.

Virus. The different strains of coronaviruses were grown in MDCK I cells as described previously[2].

Resialylation of cells. Erythrocytes and MDCK I cells were treated with neuraminidase and resialylated to contain Neu5,9Ac_2 attached to galactose in either A2,3 or A2,6-linkage as described previously[2,3].

Hemagglutination assay. Hemagglutination titration was performed as described previously[2].

RESULTS AND DISCUSSION

Sialic acids are terminal sugars of oligosaccharides present on many glycoproteins and glycolipids. They are usually attached to galactose. Two common linkage types are SiaA2,3Gal found on O-linked oligosaccahrides and SiaA2,6Gal present on N-linked oligosaccharides.

By using CMP-activated Neu5,9Ac_2 and sialyltransferases specific for either linkage type, erythrocytes were modified to contain either A2,3-linked or A2,6-linked Neu5,9Ac_2 (Fig. 1). By varying the amount of CMP-Neu5,9Ac_2, batches of erythrocytes were obtained that differed in the amount of 9-O-acetylated sialic acid present on the cell surface. The erythrocytes were analyzed whether they are agglutinated or not by BCV, HEV, or HCV-OC43. As shown in Table 1, human coronavirus OC43 was most efficient in recognizing A2,6-linked Neu5,9Ac_2.

This virus was able to agglutinate erythrocytes that had been resialylated in the presence of as little as 0.5 nm of CMP-Neu5,9Ac_2, whereas HEV and BCV required a minimum amount of 4 nmol. With respect to the A2,3-linkage, the differences between

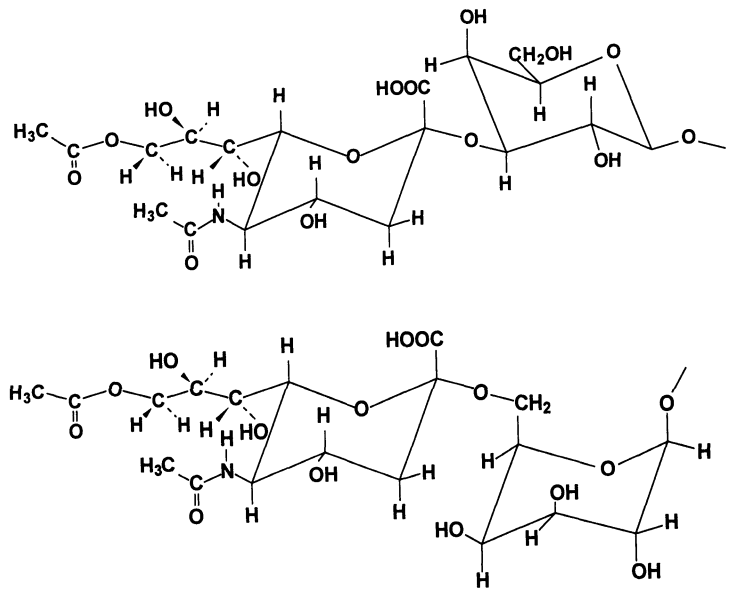

Figure 1. Structure of Neu5,9Ac_2 attached to galactose in A2,3-linkage (top) and A2,6-linkage (bottom).

Table 1. Comparison of the efficiency of HCV-OC43, BCV and HEV in recognizing 9-O-acetylated sialic acid as a receptor determinant on erythrocytes

		HA-activity (HA units/ml)		
Linkage Type	CMP-sialic acid	BCV	OC43	HEV
α2,3	4	256	256	128
	2	128	256	32
	1	32	< 2	< 2
	0 5	< 2	< 2	< 2
α2,6	8	64	256	256
	4	32	256	64
	2	2	< 2	64
	1	< 2	< 2	32
	0 5	< 2	< 2	5

the three strains of coronaviruses were less pronounced In this case BCV was more efficient than the two other strains agglutinating erythrocytes that had been resialylated in the presence of 1 nmol of CMP-activated sialic acid The hemagglutinating activity of HEV and HCV-OC43 was detectable only when 2 nmol or more CMP-Neu5,9Ac_2 was used for resialylation of the cells Thus, BCV, HEV, and HCV-OC43 are able to recognize 9-O-acetylated sialic acid both A2,3-linked and A2,6-linked to galactose, but with different efficiency

The role of Neu5,9Ac_2 as a receptor determinant for coronavirus in the infection of cultured cells has been demonstrated so far only with BCV[3] We analyzed whether HCV-OC43 also uses Neu5,9Ac_2 for binding to and infection of cultured cells For this purpose, MDCK I cells were treated with neuraminidase to remove Neu5,9Ac_2 from the cell surface thus inactivating endogenous receptors

As shown in Table 2, desialylated cells were resistant to infection by HCV-OC43 In contrast to untreated cells, no release of virus was detectable by hemagglutination titration in the supernatant of neuraminidase-treated cells If the asialo-cells were resialylated to contain A2,6-linked Neu5,9Ac_2 on the cell surface, the cells became susceptible to infection (Table 1) The virus yield obtained from the resialylated cells was lower than in the case of untreated cells indicating that the resialylated cells are infected less efficiently than the control cells One reason for this is that the supply of CMP-Neu5,9Ac_2 is limited and, therefore, only a limited amount could be applied in these experiments Another reason is that the resialylation time had to be kept short to avoid the regeneration of endogenous receptors The result shown in Table 2 indicates that not only BCV, but also HCV-OC43 uses 9-O-acetylated sialic acid as a receptor determinant for attachment to and infection of cultured cells

Table 2. Effect of neuraminidase treatment and resialylation (α2,6) on the susceptibility of MDCK I cells to infection by HCV-OC43

Treatment of cells	Virus yield (HA units/ml)
None	128
Desialylated	< 2
Resialylated, Neu5,9Ac2	16

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