N-ACETYLNEURAMINIC ACID PLAYS A CRITICAL ROLE FOR THE HAEMAGGLUTINATING ACTIVITY OF AVIAN INFECTIOUS BRONCHITIS VIRUS AND PORCINE TRANSMISSIBLE GASTROENTERITIS VIRUS

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ABSTRACT

Porcine transmissible gastroenteritis virus (TGEV) was found to resemble avian infectious bronchitis virus (IBV) in its interaction with erythrocytes. Inactivation of the receptors on erythrocytes by neuraminidase treatment and restoration of receptors by reattaching N-acetylneuraminic acid (Neu5Ac) to cell surface components indicated that α2,3-linked Neu5Ac serves as a receptor determinant for TGEV as has been reported recently for IBV (1). Similar to IBV, the haemagglutinating activity of TGEV is evident only after pretreatment of virus with neuraminidase indicating that inhibitors on the virion surface have to be inactivated in order to induce the HA-activity of these viruses. A model is presented to explain why the HA-activity of untreated virus is masked and how neuraminidase treatment results in the unmasking of this activity.

INTRODUCTION

Only a few members of the family Coronaviridae are efficient in agglutinating red blood cells: human coronavirus OC43, bovine coronavirus, porcine haemagglutinating

encephalomyelitis virus, and some strains of murine coronaviruses. Each of these viruses contains an HE protein, which is not found in other coronaviruses (2). As the HE protein has haemagglutinating (HA) activity (3), the presence or absence of this protein seemed to account for the difference in the agglutinating ability of coronaviruses. However, HE protein is only able to agglutinate mouse and rat erythrocytes, which contain a large amount of N-acetyl-9-O-acetylneuramininc acid, the receptor determinant recognized by BCV. Chicken erythrocytes, which are less rich in 9-O-acetylated sialic acid, are not agglutinated by HE protein, though they are agglutinated by BCV (4). Recently it has been shown that the S protein is able to agglutinate chicken erythrocytes using the same receptor determinant as BCV and HE protein (4). As the HA-activity of S parallels that of the intact virus, it is the actual haemagglutinin of BCV.

An S protein is present on all coronaviruses. Therefore, the question remains, why viruses lacking an HE protein are very poor haemagglutinins or even devoid of agglutinating activity. An answer may come from findings obtained with infectious bronchitis virus, which acquires HA-activity after enzymatic pretreatment of the virus (5). This virus uses $\alpha2,3$-linked N-acetylneuraminic acid (Neu5Ac) as a receptor determinant for attachment to erythrocytes (1). The HA-activity is evident only after treatment of the virus with neuraminidase. Here we show that TGEV resembles IBV in this respect : (i) the haemagglutinating activity is induced by neuraminidase treatment of the virus; (ii) $\alpha2,3$-linked Neu5Ac serves as a receptor determinant.

MATERIALS AND METHODS

Viruses and Cells. Strain M41 of IBV was grown in embryonated chicken eggs (6). The Purdue strain of TGEV was grown in LLC-PK1 cells.

Haemagglutination Assay. The haemagglutinating activity was determined according to published procedures (7).

Neuraminidase treatment. Viruses or cells were treated with neuraminidase as described recently (1).

Resialylation of erythrocytes. Erythrocytes were resialylated to contain $\alpha2,3$-linked Neu5Ac as described (1).

RESULTS AND DISCUSSION

TGEV has been reported to be a poor haemaggluinating agent (8). Significant HA-titres have been obtained only with virus preparations which had been concentrated by

ultracentrifugation. Based on the results obtained with IBV, we analyzed whether the HA-activity of TGEV can be enhanced by neuraminidase treatment. As shown in Table 1, TGEV behaved in the same way as IBV. Purified virus was unable to agglutinate chicken erythrocytes. High haemagglutination titres were observed, however, when the virus was pretreated with neuraminidase. Both the enzyme from *Vibrio cholerae* and Newcastle disease virus were effective. Among the two common linkage types of sialic acid, Neu5Acα2,3Gal and Neu5Acα2,6Gal, the viral neuraminidase has a preference for the cleavage of the former linkage type. This result indicates that α2,3-linked sialic acid has to be removed from the viral surface in order to induce the haemagglutinating activity of both coronaviruses.

Table 1. Induction of the haemagglutinating activity of IBV and TGEV by neuraminidase treatment.

| pretreatment of virus | haemagglutinating activity (HA-units/ml) | |
	IBV	TGEV
none	< 2	< 2
VC-neuraminidase	256	512
NDV-neuraminidase	256	512

Purified virus preparations were treated with neuraminidase from *Vibrio cholerae* (VC) or Newastle disease virus (NDV) and analyzed for their ability to agglutinate chicken erythrocytes.

The similarity between IBV and TGEV in the induction of the HA-activity suggested that TGEV may use the same type of receptors for attachment to erythrocytes. In fact, asialo cells obtained by treatment with neuraminidase from *Vibrio cholerae* were resistant to agglutination by both IBV and TGEV (Table 2) indicating a crucial role of sialic acid in the interaction of both viruses with erythrocytes. This conclusion was confirmed by the finding that receptors for TGEV can be restored by resialylation of asialo cells. Following attachment of Neu5Ac in an α2,3-linkage to the surface of erythrocytes, the

Table 2. Inactivation of receptors for TGEV on chicken erythrocytes by neuraminidase treatment and restoration of receptors by resialylation of the cells.

| erythrocytes | haemagglutinating activity (HA-units/ml) | |
	IBV-NA	TGEV-NA
control	512	256
asialo	< 2	< 2
resialylated Neu5Acα2,3Galß1,3GalNAc	256	64

IBV and TGEV had been pretreated with neurminidase to induce the HA-activity of bothe viruses. Asialo cells were obtained by treatment of chicken erythrocytes with neuraminidase from *Vibrio cholerae*. Resialylated cells were obtained by incubation of asialo cells with sialyltransferase and CMP-Neu5Ac.

cells became susceptible to agglutination by TGEV (Table 2) as has been reported recently for IBV (1).

The results show that not only coronaviruses of the BCV serogroup are potent haemagglutinating agents (BCV, HCV-OC43, HEV and some strains of murine coronaviruses), but also viruses of other serogroups (TGEV and IBV). Future work has to show whether haemagglutinating activity can be induced with human coronavirus 229E, feline infectious coronavirus and canine coronavirus. There are some similarities in the HA-activity of the BCV serogroup on one side and of IBV/TGEV on the other side. For both groups of viruses, the S protein is the haemagglutinin and sialic acid serves as receptor determinant. The difference is in the type of sialic acid recognized - N-acetyl-9-O-acetylneuraminic acid in the case of BCV and Neu5Ac in the case of IBV/TGEV - and in the presence or absence, respectively, of a receptor-destroying enzyme on the virus particle. A virus using sialic acid as a receptor determinant faces the problem that such a common sugar is present not only on the cellular receptors but also on a variety of other glycoproteins and glycoplipids. These glycoconjugates may act as inhibitors, because they prevent the virus from finding sialic acid on the target cell. Viruses like BCV contain a receptor-destroying enzyme and are, therefore, able to inactivate such inhibitors. In the case of IBV and TGEV, which lack a comparable enzyme, the inhibitors have to be inactivated by exogenous enzyme.
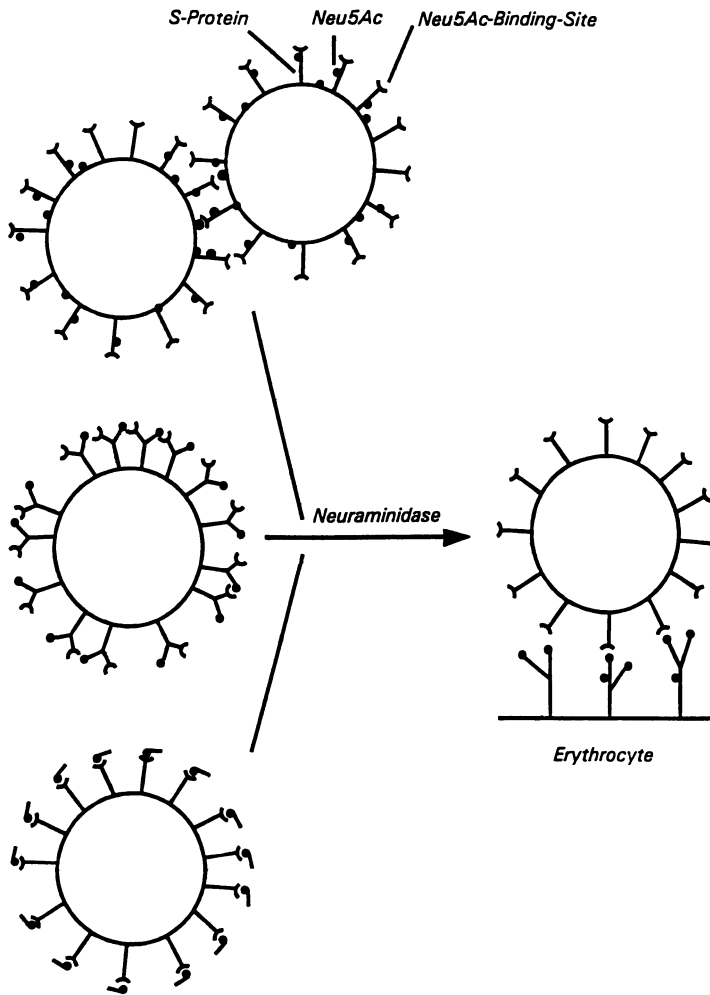
Figure 1. Schematic illustration of different possibilities to explain the lack of HA-activity of untreated IBV and TGEV and the induction of the activity by neuraminidase treatment.

There are several ways to explain how the inhibition of the HA-activity may occur (Fig. 1). The inhibitory sialic acid may be part of a viral component, e.g. of the S protein, and this may result in aggregate formation, because the virions can attach to each other. This possibility appears unlikely, because there is no evidence that IBV or TGEV have a greater tendency to form aggregates than BCV. Another possibility is that the inhibition is due to a viral component of the same virus particle, i.e. the sialic acid binding-site of the S protein binds to a sialic acid residue of a neighbouring glycoprotein. However, the glycoproteins M and S of egg-grown IBV have been shown to contain only oligosaccharides of the mannose-rich type which are sensitive to endo H treatment. Therefore, no sialic acid is expected to be present on the glycoproteins of IBV (9). For this reason, a third possibility appears to be the most likely explanation. Cellular compounds containing $\alpha2,3$-linked sialic acid may be attached to the S protein and prevent it from binding to erythrocyte receptors. Future work has to show whether this explanation is correct. It remains also to be shown whether the sialic acid binding activity plays a role in the infectious cycle. Aminopeptidase has been shown to serve as a receptor for TGEV (10). Whether the binding to sialic acid may have a supporting function is not known at present. However, the fact that this binding activity is conserved in different coronaviruses suggests that it is important for these viruses.

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