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Inhibition of Virus Release by Antibodies to Surface Antigens of Influenza Viruses

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When influenza virus was mixed with antisera to its surface subunits before inoculation of cell cultures, anti-hemagglutinin antibodies neutralized infectivity but anti-neuraminidase did not. When the antisera were added after infection of cell cultures, anti-hemagglutinin and anti-neuraminidase antibodies were equally effective in reducing virus titers in culture fluids. Decreased virus titers were not due to interference of antibody with assay and were not accompanied by a reduction in the synthesis of hemagglutinin and neuraminidase subunits. Both antisera also effectively prevented in vitro virus spread. Inhibition of virus release by neuraminidase antibody appeared unrelated to its antienzyme property. Hydrolysis of *N*-acetyl neuraminic acid residues of infected host cells proceeded unimpaired in the presence of subunit antisera. Anti-hemagglutinin and anti-neuraminidase antibodies may act to prevent virus release by binding newly formed virus subunits to each other and to anti-genically altered cell membranes.

The surface of the influenza virus contains two virus-coded antigenic structures, the hemagglutinin and the enzyme, neuraminidase. Antibodies to the hemagglutinin neutralize virus infectivity. Antibodies to the neuraminidase do not, but the continued presence of neuraminidase antibody significantly inhibits release of virus into the culture medium (2, 3, 7, 12, 13, 15).

It has been suggested that neuraminidase antibodies may prevent virus release by interfering with an essential enzyme function (3, 7, 12, 13, 15). Neuraminic acid residues are known to be lost from infected cell membranes during virus replication (8). An opposing view is that antibodies react with the enzyme as an antigenic constituent of the virus surface, thereby preventing release through binding newly formed virions to each other and to the antigenically altered cell membrane (2).

In this paper we present evidence in support of the latter view. We demonstrate that neuraminidase antibody has no influence on the loss of neuraminic acid residues from infected cells. In addition, we show that hemagglutinin antibodies are equally as effective as neuraminidase antibodies in preventing virus release.

MATERIALS AND METHODS

Viruses. Influenza strains B/Lee/40 and A/Aichi/2/68 (H3N2) were grown in 10- or 11-day-old embryonated eggs and stored at −70 C.

Antisera. B/Lee subunit antisera were prepared in rabbits with virus grown in calf kidney cell cultures. The neuraminidase antiserum was prepared with neuraminidase subunits isolated by electrophoresis of sodium dodecyl sulfate (SDS)-disrupted virus particles (9). The hemagglutinin antiserum was prepared with sucrose gradient purified virus suspensions which had been heated at 56 C for 30 min to destroy the neuraminidase antigens. Two intramuscular injections of antigen emulsified in Freund complete adjuvant were given 40 days apart. An intravenous injection of aqueous antigen was given simultaneously with the second adjuvant injection. Rabbits were bled 8 to 10 days later. A/Hong Kong subunit antisera were prepared at the World Health Organization (WHO) International Influenza Center for the Americas, Atlanta, Georgia, by intramuscular injection of chickens with antigens emulsified with Freund complete adjuvant. H3 and N2 subunits were isolated from recombinant viruses A/Aichi/2/68 (H3)-Bel/45 (N1) and A/NWS-33 (HO)-Hong Kong/8/68 (N2), respectively, by electrophoretic separation from SDS-disrupted virus (5). Rabbit and chicken antisera and normal sera were treated overnight at 37 C with receptor-destroying enzyme (RDE) to destroy nonspecific viral inhibitors (11). Excess RDE was destroyed by heating at 56 C for 1 h. Sera were dialyzed at 4 C for 48 h against frequent changes of phosphate-buffered saline (pH 7.2). Sera thus treated were shown to be free of residual RDE by the neuraminidase assay.

Chick kidney cell cultures. Kidney cells from 17-day-old embryos were grown in monolayers in Eagle minimal essential medium containing 0.12% sodium bicarbonate and 5% calf serum. With the exception of the cells for the tube neutralization tests, all cultures were grown in 150-ml milk dilution

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bottles. Average cell count per bottle after 48 to 72 h at 37 C was 2.2×10^6. Monolayers were washed twice with serum-free Eagle medium before virus inoculation. Virus was adsorbed at 37 C for 1 h. Residual inoculum was removed by washing monolayers twice with serum-free Eagle medium. The final volume of the medium was 10 ml.

Virus infectivity. Ten- to 11-day-old embryonated eggs were inoculated with 10-fold serial dilutions of virus. Allantoic fluids were tested for the presence of hemagglutinin after 72 h at 37 C.

Chick kidney (CK) cell culture tubes were inoculated with 10-fold serial dilutions of virus. After 5 days at 37 C, monolayers were tested for presence of virus by adding 0.2 ml of 0.4% guinea pig erythrocytes to each tube and reading for hemadsorption. The egg infectious dose (EID_{50}) and CK cell infectious dose ($CKID_{50}$) were essentially the same for B/Lee. EID_{50} titers consistently averaged $10^{1.5}$ higher than $CKID_{50}$ titers for A/Aichi.

Assay for hemagglutin (HA) and for hemagglutination (HI) antibodies were performed by microtiter as previously described (4).

Antibody blocking test for hemagglutinins. Two volumes of test samples in normal saline were mixed with one volume of 1% sodium deoxycholate (DOC) and left at room temperature for 4 h. Five volumes of saline were added to give a final dilution of 1:4 with respect to the test sample. One volume (0.025 ml) of the mixture was added to equal volumes of twofold serial dilutions of hemagglutinin antiserum including two dilutions beyond the known HI end point. After 1 h at room temperature, four HA units of reference virus (0.025 ml) and 1% chick erythrocytes (0.025 ml) were added. Tests were read for reduction in HI titer. The hemagglutinin equivalent was calculated by using as reference the corresponding reduction in HI titer caused by a DOC-treated virus suspension of known HA content in the same test.

Neuraminidase. Enzyme activity of virus preparations was determined according to the procedure recommended by the WHO (1). Virus dilutions were incubated with fetuin for 18 h at 37 C. Neuraminidase assay of released or cell-associated antigen in the presence of hemagglutinin antisera was performed by adding 0.025 ml of SDS to each 0.1-ml test sample and diluting in saline containing 0.1% SDS.

NI. Neuraminidase inhibition tests were performed according to the procedure recommended by the WHO (1).

Protein. Protein concentrations were determined by the method of Lowry et al. (10) with optical density (OD) readings at 750 nm.

NANA. Cell sonic extracts in saline were heated at 80 C for 1 h in 0.1 N H_2SO_4 to release bound *N*-acetyl neuraminic acid (NANA) (14). Free NANA was assayed by the thiobarbituric acid method. The NANA chromophore was extracted with *n*-butanol containing 5% HCl. OD readings were made at 549 nm. This procedure when applied to whole cells is not specific for NANA since other substances, chiefly 2-deoxyribose, may interfere. From hydrolysis and assay of purified calf thymus DNA in amounts equivalent to that in CK cells, the 2-deoxyribose chromophore was calculated to constitute approximately 12% of the OD_{549} values of hydrolyzed normal CK cells. Because of this, specific units were not assigned for NANA; rather, a percentage change was calculated in terms of OD_{549} (thiobarbituric acid)/OD_{750} (Lowry protein) as follows:

$$100\left(\frac{OD_{549}}{OD_{750}}\text{ infected cells}/\frac{OD_{549}}{OD_{750}}\text{ control cells}\right) - 100 = \%\text{ change}$$

Any change in the OD_{549}/unit of protein between infected and uninfected cells was assumed to reflect a change in the number of cell-bound NANA residues. That this assumption was correct was supported by the observation that noninfectious SDS-disrupted B/Lee virus caused a decrease in OD_{549} which was similar to that observed under the same conditions with infected cells.

Attempts were made to avoid interference by the chromophore of 2-deoxyribose by studying isolated cell surface membranes, but membrane yields from CK cells were unsatisfactory.

Neutralization: (i) Preinfection. Twofold serial dilutions of serum were mixed with equal volumes of virus suspensions containing 300 to 1,000 $CKID_{50}$/0.1 ml. After 1 h at room temperature, 0.2 ml of the serum-virus mixtures were inoculated into three tubes each of CK cell cultures. Tubes were incubated for 2 h at 37 C and washed twice with serum-free Eagle medium to remove residual inoculum. A final volume of 2 ml of Eagle medium was added to each tube, and tests were incubated at 37 C for 3 days. Supernatant fluids were assayed for hemagglutinins by mixing with an equal volume of a 0.4% suspension of guinea pig erythrocytes. Cell-associated hemagglutinins were assayed by adding 0.2 ml of 0.4% guinea pig erythrocytes to each tube and reading for hemadsorption.

(ii) Postinfection. CK cell cultures were inoculated with a multiplicity of virus of approximately 0.01 EID_{50}/cell, and adsorption of virus was allowed to proceed at 37 C for 2 h. Cells were washed twice with serum-free Eagle medium. To each of three tubes containing 1.8 ml of medium was added 0.2 ml of twofold serial dilutions of subunit antisera. Cultures were incubated for 3 days at 37 C. Supernatant fluid and monolayers were assayed for virus growth as described previously for the preinfection neutralization test.

Assay of released and cell-associated virus: (i) Released virus. Medium from triplicate bottles of CK cells was pooled and clarified by centrifugation at 1,400 $\times$ *g* for 30 min. The clarified medium was decanted from the pelleted cell debris. One milliliter of the clarified medium was reserved for infectivity titrations. The remainder was centrifuged at 70,000 $\times$ *g* for 1 h. The resulting small pellet was suspended in a volume of saline equivalent to one-twentieth the initial volume of medium and subjected to sonic treatment for 1 min with a probe powered by an ultrasonic generator (MEL Equipment Co. Ltd.) at an output of 1.5 amp.

(ii) Cell-associated virus. Cells were harvested by disrupting the monolayer with glass beads in a small amount of cold saline. Cell harvests and saline wash-

ings were combined with the cell debris recovered from the initial low-speed clarification of the medium described above. Cells were washed twice by centrifugation at 60,000 × *g* and suspension in saline. The final pellet was suspended in saline to one-twentieth the original volume of medium. Cells were disrupted by sonic treatment as described above.

RESULTS

Neutralization tests. In the preinfection (conventional) neutralization tests in which virus and antiserum were mixed before inoculation of CK cell cultures, neither B/Lee nor A/Aichi neuraminidase antiserum had any substantial effect on virus infection (Table 1). Although HA activity was absent after 3 days in fluids from cultures that received the lowest antiserum dilution (1:20), all cultures were shown by hemadsorption to have been infected.

In the postinfection neutralization test, when antiserum was added immediately after adsorption of virus to CK cells, HA activity in culture fluids was inhibited by antibody to B/Lee and A/Aichi neuraminidase as well as hemagglutinin antisera (Table 2). Although neuraminidase antisera were highly effective in inhibiting HA in culture fluids, its presence had no effect on hemadsorption. Hemagglutinin antisera at the lowest dilution (1:80) decreased the intensity of hemadsorption but did not prevent it. Subunit antisera under these conditions also had no effect on the development of the cytopathogenic effects characteristic of B/Lee. A/Aichi did not cause cytopathogenic changes.

Effect of subunit antisera on virus release. CK cell monolayers were grown in bottles and inoculated with virus at multiplicities of approximately 1 EID_{50}/CK cell for B/Lee and 10 for A/Aichi. After a 1-h adsorption period followed by two washings to remove excess inoculum, the appropriate RDE-treated antiserum was added. The final dilution (1:100) was the same for each serum, so that all test systems had similar protein concentrations. The effective antibody concentrations were 256 HI units/ml and 800 NI units/ml for B/Lee hemagglutinin and neuraminidase antisera, respectively, and 512 HI units/ml and 500 NI units/ml for A/Aichi. Three separate experiments were performed for each virus-serum combination.

Bottles were incubated at 37 C for 20 h, and infectivity titers of culture fluids were determined. Culture fluids and cells were examined separately for released and cell-associated virus by assaying for the virus surface antigen heterologous for antibodies in the culture fluids, i.e., hemagglutinins were assayed in those cultures containing neuraminidase antibodies and vice versa. Hemagglutinin titers were determined by antibody blocking tests with test samples which had been treated with DOC to disrupt the virus. Neuraminidase titers were determined by the thiobarbituric acid procedure with test samples which had been treated with SDS. Control experiments showed that under these conditions the presence of antibody to one surface antigen (H or N) did not interfere with assay of the remaining antigen.

In fluids from B/Lee-infected cultures (Table 3), infectious virus recovered in the presence of neuraminidase antibody was approximately 10^4 less than that in the presence of control sera. Hemagglutinins released into the medium under the same conditions were reduced by more than 90%. B/Lee hemagglutinin antisera caused a similar reduction in released neuraminidase. Neither the neuraminidase nor the hemagglutinin antisera had any appreciable effect on the titers of cell-associated virus subunits. Although direct hemagglutinin titers were reduced in the presence of neuraminidase antisera, the indirect hemagglutinin titers which are

TABLE 1. *Preinfection neutralizing titers of subunit antisera: exposure of virus to serum before inoculation of chick kidney cell cultures*

Virus	Serum	Neutralization titer[a] as determined by	
		HA	HAd
B/Lee[b]	Antineuraminidase	20	<20
	Antihemagglutinin	320	160
	Normal rabbit	<20	<20
A/Aichi[b]	Antineuraminidase	20	<20
	Antihemagglutinin	640	320
	Normal chicken	<20	<20

[a] Titer based on initial serum dilution inhibiting HA or hemadsorption (HAd).
[b] 300 to 1,000 $TCID_{50}$.

TABLE 2. *Postinfection neutralizing titers of subunit antisera: exposure of virus to serum after infection of chick kidney cell cultures*

Virus	Serum	Neutralization titer[a] as determined by	
		HA	HAd
B/Lee[b]	Antineuraminidase	3,840	<80
	Antihemagglutinin	2,560	<80
	Normal rabbit	<80	<80
A/Aichi[b]	Antineuraminidase	640	<80
	Antihemagglutinin	3,840	<80
	Normal chicken	<80	<80

[a] Titer based on final serum dilution in culture fluid inhibiting HA or HAd.
[b] Inoculated with EID_{50}/cell of 0.1 to 0.01.

not influenced by immunoaggregation showed no reduction. The findings with A/Aichi neuraminidase and hemagglutinin subunit antisera with respect to released and cell-associated subunits were similar to that seen with B/Lee. Both antisera substantially inhibited virus release yet had little or no influence on the quantity of cell-associated hemagglutinin and neuraminidase (Table 4).

Effect of subunit antisera on neuraminidase synthesis. More precise evidence that the presence of subunit antisera did not influence synthesis of virus subunits was obtained from one-step growth curves with B/Lee and A/Aichi viruses incubated in the presence of their respective hemagglutinin antisera. Infected cell monolayers were prepared exactly as for the experiments described in Tables 3 and 4. Triplicate bottles of monolayers were removed from incubation and frozen at 0, 5, 11, 18, and 23 h after incubation. Combined cells and culture fluids were harvested and viruses were concentrated by centrifugation at 70,000 × *g* for 1 h. Concentrates were sonically treated, treated with SDS, and assayed for neuraminidase as previously described.

Peak neuraminidase titers were obtained at 11 h for B/Lee and A/Aichi, and these were of virtually identical activity whether in the presence of anti-hemagglutinin or control sera.

Effect of subunit antisera on virus spread. Neuraminidase and hemagglutinin subunit antisera were also equally efficient in preventing secondary virus spread. CK cell cultures were inoculated with 0.001 EID_{50}/cell of B/Lee virus followed by a 1:100 dilution of the appropriate antiserum. Cultures treated with antisera yielded at least 97% less cell-associated hemagglutinin and neuraminidase than did cultures treated with normal serum (Table 5).

TABLE 3. *Virus release from chick kidney cells infected with B/Lee[a] and incubated in the presence of subunit antisera[b]*

Serum	Virus titers						
	Fluids[c]				Cells[c]		
	Infectivity (EID_{50})	Hemagglutinin		Neuraminidase[d]	Hemagglutinin		Neuraminidase[d]
		Direct	Indirect[e]		Direct	Indirect[e]	
Antineuraminidase	$10^{0.4}$	8	≤32		128	1,160	
Antihemagglutinin	10^{0}			5			320
Normal rabbit	$10^{4.3}$	398	322	120	1,290	1,170	320

[a] One EID_{50}/cell.
[b] Final dilution of 1:100 for each serum.
[c] One-twentieth the original volume.
[d] Highest dilution of SDS-treated sample yielding an OD at 549 nm of ≥0.200 after overnight incubation with fetuin.
[e] Calculated from HI antibody blocking titers of DOC-treated samples.

TABLE 4. *Virus release from chick kidney cells infected with A/Aichi[a] and incubated in the presence of subunit antisera[b]*

Serum	Virus titers						
	Fluids[c]				Cells[c]		
	Infectivity (EID_{50})	Hemagglutinin		Neuraminidase[d]	Hemagglutinin		Neuraminidase[d]
		Direct	Indirect[e]		Direct	Indirect[e]	
Antineuraminidase	$10^{1.0}$	<2	≤8		64	192	
Antihemagglutinin	10^{0}			<5			640
Normal chicken	$10^{7.3}$	16	28	20	256	256	640

[a] Ten EID_{50}/cell.
[b] Final dilution of 1:100 for each serum.
[c] One-twentieth the original volume.
[d] Highest dilution of SDS-treated sample yielding an OD at 549 of ≥0.200 after overnight incubation with fetuin.
[e] Calculated from HI antibody-blocking titers of DOC-treated samples.

Effect of subunit antisera on loss of NANA from infected CK cells. The hypothesis that neuraminidase antibody prevented virus release through specific inhibition of enzyme activity was examined by assaying infected CK cells for changes in NANA content in the presence of subunit antisera. Cell sonic extracts from experiments described in Tables 3 and 4 were hydrolyzed by mild acid treatment and assayed for NANA by the thiobarbituric acid method. Neither B/Lee nor A/Aichi hemagglutinin nor neuraminidase subunit antisera had any inhibitory effect (Table 6). The average loss of NANA for all infected cells was similar regardless of the serum-virus combination.

The percent change in OD_{549} expressed as NANA in the above experiments was shown to be consistent with the action of virus neuraminidase. In parallel experiments, cell monolayers were incubated for 20 h with SDS-disrupted (noninfectious) B/Lee virus adjusted to contain approximately the same neuraminidase activity as the 20-h harvests of B/Lee-infected CK monolayers. The mean percent change in NANA content for three experiments was −30.1% (SD = 8.3%), a value similar to those in Table 6.

The major loss of NANA from infected cell monolayers (Table 6) was shown to have occurred after the addition of subunit antisera. Cell monolayers inoculated with B/Lee and A/Aichi viruses of 1 EID_{50}/CK cell and 10 EID_{50}/CK cell, respectively, were harvested after the 1-h virus adsorption period and before the addition of antisera. The mean percent changes in NANA content, based on three experiments, were −8% (SD = 2%) for B/Lee and −8% (SD = 5%) for A/Aichi.

The failure of neuraminidase antisera to inhibit loss of NANA from infected CK cells (Table 6) was not related to the ability of the antisera to inhibit loss from virus-treated noninfected CK cells. A/Aichi virus which had been rendered noninfectious by treatment with ultraviolet light was added to CK cell monolayers in the amount of 50 EID_{50}/cell (calculated from pre-ultraviolet treatment infectivity titrations). A/Aichi neuraminidase antiserum was added simultaneously with the virus to the CK monolayer to yield a final concentration of 500 NI units/ml, the same concentration as used in experiments described in Table 6. Controls consisted of cell monolayers to which had been added ultraviolet-treated virus plus normal sera or normal allantoic fluid plus normal sera. Cells were harvested after 4 h at 37 C. In four experiments the mean NANA loss in the presence of normal sera was −27% (SD = 7%). The mean NANA loss in the presence of A/Aichi neuraminidase antisera was −4% (SD = 4%).

TABLE 5. *Inhibition of secondary virus spread in chick kidney cells infected with low multiplicities of B/Lee and incubated in presence of subunit antisera*[a]

Virus inoculum (EID_{50}/cell)	Serum	Virus titers[b]		
		Fluids (infectivity EID_{50})	Cells[c]	
			Hemagglutinin indirect[d]	Neuraminidase[e]
0.001	Antineuraminidase	10^{9}	≤32	
	Antihemagglutinin	10^{9}		<5
	Normal rabbit	$10^{8.5}$	1,024	320
1.0	Antineuraminidase	10^{9}	1,024	
	Antihemagglutinin	10^{9}		320
	Normal rabbit	$10^{8.5}$	1,024	320

[a] Final dilution of 1:100 for each serum.
[b] Titers determined after 48 h at 37 C.
[c] One-twentieth the original volume.
[d] Calculated from HI antibody-blocking titers for DOC-treated samples.
[e] Highest dilution of SDS-treated sample yielding an OD at 549 of ≥0.200 after overnight incubation with fetuin.

TABLE 6. *Mean change in NANA/unit protein of chick kidney cells infected with virus and incubated in presence of subunit antisera*[a]

Serum	NANA change (%)[b]			
	B/Lee[c]		A/Aichi[d]	
	$\bar{X}$	SD	$\bar{X}$	SD
Antineuraminidase	−30.4	7.9	−29.2	9.3
Antihemagglutinin	−25.1	9.2	−38.8	0.6
Normal	−31.2	7.9	−30.3	4.6
Normal (no virus)	None	None	None	None

[a] Final dilution of 1:100 for each serum.
[b] Calculated as 100 (OD_{549}/OD_{750} infected cells per OD_{549}/OD_{750} control cells) −100 = % change.
[c] One EID_{50}/cell.
[d] Ten EID_{50}/cell.

DISCUSSION

Our results with the conventional (preinfection) neutralization test are consistent with the accepted view that neuraminidase antibodies, unlike hemagglutinin antibodies, do not substantially neutralize virus infectivity. However, if neuraminidase subunit antiserum is added after virus infection (postinfection neutralization test), it is highly efficient in preventing release of virus into infected culture fluids (2, 3,

7, 12, 13, 15). We have also shown that under these conditions hemagglutinin antisera is as efficient as neuraminidase antisera in preventing virus release. It was unlikely that this effect with either antisera resulted from direct interference of antibody with antigen assay or from indirect interference of antibody by formation of virus aggregates in the medium. Any such interference was avoided, first, by disrupting the virus with detergents and, second, by assaying for the antigen heterologous for the antiserum in the medium. When these procedures were used, only trace amounts of virus were detected in culture fluids.

In our procedure for assay of released and cell-associated virus, the possibility cannot be dismissed that immunoaggregates may have been removed from culture fluids during the initial clarification step, that is, when fluids were centrifuged at low speed to remove cellular debris. If this had occurred, virus aggregates would have been scored as cell-associated virus subunits. Control experiments with B/Lee virus and neuraminidase antiserum mixtures showed that virus aggregates which may have formed under these conditions were not sedimentable at low-speed centrifugation. The size of such aggregates, however, may bear little relation to those formed in the antigen-saturated microenvironment of the infected cell surface.

There was no evidence that the reduction of virus in the culture fluid was associated with a reduction in synthesis of virus subunits, as has been suggested by others (13). Our initial observations were confirmed by determining one-step growth curves with B/Lee and A/Aichi virus in the presence of hemagglutinin antisera. Neuraminidase, which could be measured more accurately than hemagglutinin, was shown to be produced at virtually the same rate and in the same quantity with or without the presence of subunit antisera. From these experiments, it seems reasonable to conclude that the absence of virus in fluids from cultures treated with neuraminidase and hemagglutinin antisera was related directly to inhibition of virus at the cell surface.

Inhibition of virus release was not associated with inhibition of NANA hydrolysis. Loss of NANA from infected cells was essentially the same whether in the presence or absence of subunit antisera. It is possible that certain essential NANA residues on the cell surface were hydrolyzed by the virus inoculum before or after antibody was added, but the time at which NANA was lost is not crucial. The important observation is that, within the limits of the sensitivity of the assay system, the total loss of NANA was the same in the presence of antisera as in its absence, yet virus was not released. Neuraminidase antibody added simultaneously with noninfectious virus was shown to completely inhibit loss of NANA from chick cells. Although under these conditions some indirect inhibition by immunoaggregation may have been possible, these results strongly suggest that the failure of antibody to prevent NANA hydrolysis is unique for the emerging virus. Neuraminidase may act when the subunits are being assembled into complete virions at the cell membrane and is therefore unavailable to antibody, or NANA residues may be presented to the newly assembled enzyme in such a way that antibody cannot interfere (2).

Neuraminidase antibodies may function by interfering with some yet unknown process essential for virus assembly or release. More likely, it may bind newly formed virions to each other and to antigenically altered cell surfaces. This interpretation is strengthened by our findings that virus release can be prevented by hemagglutinin as well as neuraminidase antibodies. It is also consistent with the report of Becht et al. (2) that, in the presence of monovalent (Fab) neuraminidase antibodies, the release of virus proceeds largely undisturbed. Inhibition by bivalent antibodies has also been observed by electron microscopy to be associated with the formation of clusters and aggregates of virus particles at the cell surface (2, 3, 6, 12). The essential findings reported by most investigators (2, 3, 6, 7, 12, 13, 15) are explainable by neuraminidase antibody reacting with the enzyme as an antigen constituent of the virus surface. Differences in findings among investigators may reflect differences in virus or serum concentrations used.

Our findings do not imply that neuraminidase has no role in virus maturation or release. Its role is still unclear. Our data do suggest, however, that inhibition of virus release by neuraminidase antibody is probably unrelated to its antienzyme property.

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