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Hemagglutination-Inhibition: Rapid Assay for Neuraminic Acid-Containing Viruses

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Influenza virus particles bind rapidly to vesicular stomatitis, Sindbis, or Rauscher murine leukemia virus particles, forming mixed aggregates demonstrable by electron microscopy. The normal hemagglutinating property of influenza virus is inhibited by these viruses, providing a rapid quantitative assay. Prior treatment with neuraminidase blocks the ability of other viruses to inhibit influenza virus hemagglutination.

Since the discovery of hemagglutination by influenza virus in 1941 (5, 15), this phenomenon has been useful in providing a rapid and convenient means of quantitation of virus particles. In addition, hemagglutination inhibition (HI) by antiviral antibody has provided an excellent method for quantitation of antibody titers, and for analysis of antigenic differences among virus strains. Whereas the hemagglutinating property of influenza, parainfluenza and certain other viruses can be observed without difficulty, in many other groups of viruses hemagglutination requires rather precise control of temperature, pH, and other variables in order to obtain reproducible results, and very low titers are observed with many virus strains. Thus a rapid, simple assay is unavailable for many viruses.

Most enveloped viruses which form by budding at the cell surface contain neuraminic acid as a component of glycoproteins and glycolipids (1, 10, 12, 16, 17), with the exception of myxo- and paramyxoviruses which contain neuraminidases and therefore lack neuraminic acid (9, 8, 11). The latter viruses bind specifically to neuraminic acid-containing receptors on cell surfaces (3, 7). Recently it has been found that temperature-sensitive mutants of influenza virus which lack neuraminidase do contain neuraminic acid in progeny virus particles, which form extensive aggregates (P. Palese, K. Tobita, M. Ueda, and R. W. Compans, Virology, in press). Disaggregation occurs upon treatment with neuraminidase, indicating that neuraminic acid in virions is serving as the attachment site for adjacent virions. It therefore seemed likely that neuraminic acid-containing components of other viruses might also serve as receptors for influenza virus hemagglutinin.

The WSN strain of influenza virus and the parainfluenza virus SV5 were grown in MDBK cells (2), purified as described previously (13), and dialyzed against 0.01 M phosphate buffer, pH 7.2. Vesicular stomatitis virus (VSV) (Indiana, New Jersey, and Cocal strains) and Sindbis virus were grown in BHK21-F cell monolayers and purified by the same procedure. Rauscher murine leukemia virus was purified by the same procedure from culture media of infected BALB-3T3 or JLS-V9 cells. Determination of protein concentrations of purified viruses was done by the Lowry procedure (14).

Aggregation of influenza virions with neuraminic acid-containing virions of other major groups could readily be observed by electron microscopy. Figure 1 shows an aggregate of influenza and VSV particles, and similar aggregates were observed upon mixing influenza virions with Sindbis or Rauscher leukemia virions (Fig. 2). The size of the aggregates increased with time, and aggregates containing hundreds of particles were frequently observed.

It seemed likely that the binding of heterologous virions to surfaces of influenza virions would inhibit hemagglutination by the latter, providing the basis for a rapid assay for neuraminic acid-containing viruses, many of which hemagglutinate only under very precise conditions (4, 6, 18, 19). The use of HI to measure the time course of appearance of VSV in BHK21-F cell cultures after single-cycle infection is shown in Fig. 3. The rise in HI titer parallels the rise in infectious virus, which was determined separately by plaque assay on BHK21-F cell monolayers. Reading the HI titer as the reciprocal of the highest dilution causing detectable inhibition of hemagglutination, a maximal HI titer of 1,024 U/ml was reached at 10 h, whereas control cells incubated for this length of time showed little or no HI activity in the culture medium.

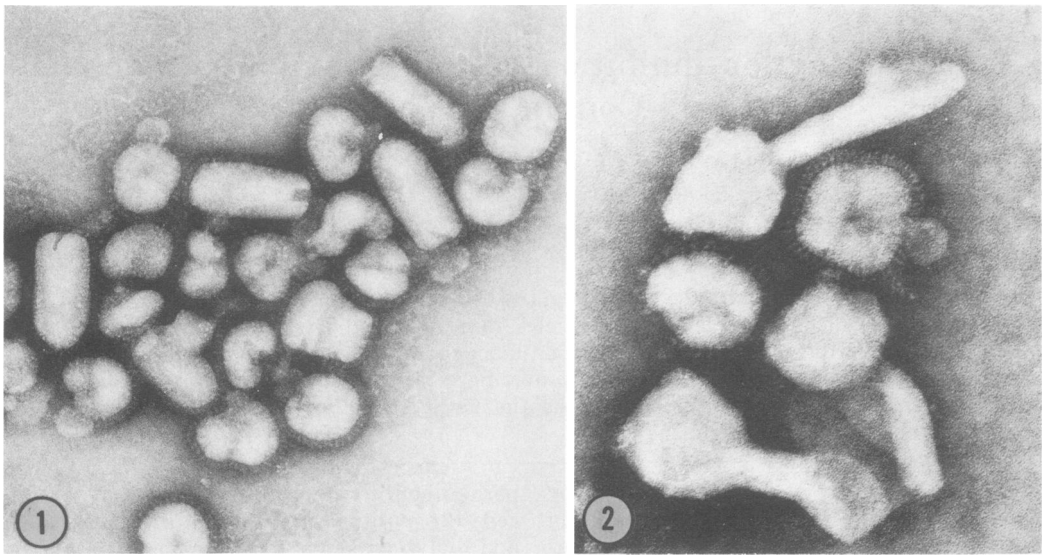

FIG. 1. *Aggregate of bullet-shaped VSV (Indiana) and roughly spherical influenza virions observed 10 min after mixing samples of purified virus particles. Sodium phosphotungstate stain. Magnification: ×85,000.*

FIG. 2. *Aggregate of two spike-covered influenza virions and three Rauscher murine leukemia virus particles. Sodium phosphotungstate stain. Magnification: ×120,000.*

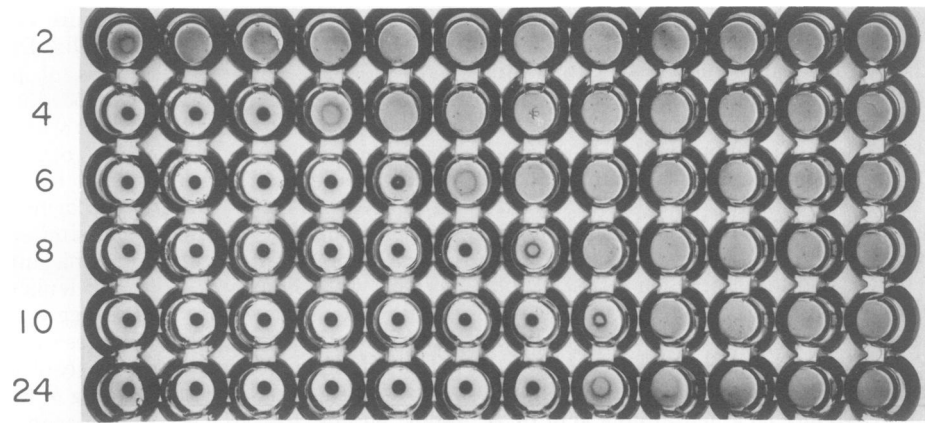

FIG. 3. *Assay of growth of VSV (Indiana strain) in BHK21-F cell cultures by HI. Cells were infected at a multiplicity of 50 PFU/cell, and after 1 h the inoculum was removed and replaced with reinforced Eagle medium. At the indicated times (hours postinfection), culture medium was harvested and portions were diluted serially (twofold) in phosphate-buffered saline in a Lindbro microtiter plate. To 50 μliters of each dilution was added 50 μliters of purified influenza virus, previously diluted in phosphate-buffered saline to a concentration of 12 hemagglutinating U/ml. The hemagglutination titer of the influenza virus was determined in a microtiter plate by serially diluting virus in phosphate-buffered saline and adding 100 μliters of a 0.36% chicken red blood cell suspension to 100 μliters of each dilution. Titers were read after 60 min, and the highest dilution showing marked or complete agglutination (2 or 3+) was considered as the end point. Mixtures of VSV and influenza virus were held for 30 min at 20 C, and 100 μliters of a 0.36% suspension of chicken red blood cells in phosphate-buffered saline was added. The titer was read after 60 min at 20 C, and the highest dilution causing detectable inhibition of hemagglutination was defined as containing 1 HI U/ml.*

There are approximately 10^7 PFU of VSV per HI unit under the conditions described for Fig. 3. Thus it is feasible to determine relative amounts of VSV in culture media by HI titrations without plaque assay, concentration, or purification of virus.

Enveloped viruses of several major groups were assayed for HI titers as described in Fig. 3,

and possessed activities comparable to that of VSV. Using purified virions, HI titers of Sindbis, Rauscher leukemia virus, and the Cocal, New Jersey, and Indiana strains of VSV were determined and all fell in the range of 2,000 to 10,000 HI U per mg of virus protein. In contrast, the parainfluenza virus SV5, which contains no neuraminic acid on its surface, is devoid of HI activity.

To determine conditions for obtaining maximal sensitivity of the assay, virus mixtures were held at 4 or 20 C for intervals from 15 min to 2 h before addition of red blood cells. With VSV the maximal titer was reached after 30 min at 20 C, whereas Sindbis virus required a 2-h preincubation at 20 C and Rauscher leukemia virus, 2 h at 4 C for maximal titers, which were two- or four-fold higher than those obtained by incubation for 30 min at 20 C. The WSN strain of influenza virus possesses low neuraminidase activity, which accounts for its lack of interference with the assay when virus mixtures or virus-red cell suspensions were incubated at 20 C.

To further demonstrate that neuraminic acid on the surfaces of virus particles is required for HI activity, virions were treated with neuraminidase (protease free, Behringwerke). Purified VSV (Indiana) was incubated for 2 h with 5 U of neuraminidase per ml. Measurement of HI activity was done at 4 C to prevent interference by neuraminidase. The HI titer of a control sample was 2,048 U/ml, whereas the titer of the neuraminidase-treated sample was 8 U/ml. Neuraminidase treatment of Sindbis and Rauscher leukemia virus produced similar inhibition of HI activity.

The present results demonstrate that inhibition of influenza virus hemagglutination is a potentially useful quantitative assay for neuraminic acid-containing viruses of several major groups. Measurement of virus titers in cell culture fluids, or fractions from purification procedures by this assay, obviously requires suitable control experiments since many neuraminic acid-containing substances act as inhibitors of influenza virus hemagglutination. In this regard, 2 percent calf serum or fetal calf serum, or 15% sucrose or potassium tartrate, had no inhibitory effect under the conditions described for Fig. 3.

Preliminary results indicate that blocking of HI by specific antibody may provide a useful method for determination of antibody titers, and for identification of serological specificity of viral surface antigens.

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