Viral Aggregation Resulting in the Failure to Correctly Identify an Unknown Rhinovirus

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Received for publication 4 June 1970

A seed lot of strain SF 1684 of rhinovirus type 2 prepared in human embryonic lung cells (WI-38) contained aggregates which interfered with its neutralization by homotypic or homologous antisera. The same virus showed no evidence of aggregation at five other passage levels studied. Virus in the seed lot was not identified correctly, and the titer of homologous antiserum was mistakenly considered to be low as a result of neutralization tests conducted with the aggregated virus. Filtration and a more easily effected treatment with sodium deoxycholate (1%) disaggregated the virus and restored its susceptibility to neutralization by homologous and homotypic antiserum.

The abundance of rhinovirus (RV) serotypes, 89 thus far, and the existence of antigenic variants (5, 7) present a problem in identifying unknown RV. This report concerns an additional technical problem, the formation of aggregates of an unidentified RV at only one cell culture passage level. These aggregates resulted in the failure of the virus to be identified and led to confusion in the titration of the homologous antiserum. Aggregation as a cause of neutralization failure has been recognized to occur with many different viruses (8); however, the occurrence of aggregation at a single passage level has not been emphasized. A method of treatment of unknown isolates with sodium deoxycholate (DOC) before neutralization testing is suggested to avoid this difficulty in the future.

MATERIALS AND METHODS

Virus. SF 1684 was isolated in human embryonic lung cells (WI-38) during the course of a longitudinal study of acute respiratory disease in employees of an insurance company (3). A third passage of the virus in WI-38 cells was initially judged to show no inhibition by antiserum pools which contained 20 units of antiserum to type 2 RV. These tests were conducted prior to an understanding of the full importance of the cytopathy of some hyperimmune RV antisera then available (2). Cell degeneration caused by the serum was mistaken for viral cytopathic effect (CPE), leading to the incorrect conclusion that SF 1684 had not been neutralized by RV type 2 antiserum. SF 1684 was not inhibited by antiserum for the remainder of the then 55 known prototypes and was considered "untyped."

SF 1684 was purified by triple terminal dilutions, and a 50-ml pool was made of the sixth passage which was grown in WI-38 cell culture tubes. Virus harvested from individual tubes was combined and redistributed into sealed glass ampoules. By using previously reported methods (4), samples of this pool were used to verify that SF 1684 had the physical and biological characteristics of an RV and for neutralization testing against individual antisera. SF 1684 virus from the pool was not completely neutralized by 20 units of RV type 2 antiserum which had a homologous titer of 512 (30) nor was it neutralized by antisera for other RV prototypes. [Titters of antisera are expressed as reciprocals of the initial dilutions of serum. Numbers in parentheses represent virus concentration (TCID50/0.1 ml) used in the neutralization test.] Antiserum to SF 1684 was prepared in guinea pigs from a fifth WI-38, second HeLa (WI 5 HeLa 2) passage. When this antiserum was tested against virus from the seed pool, it was found to have an unaccountably low titer, 64 (100).

Samples of the seed pool were submitted to the Rhinovirus Reference Center at Ohio State University where they were passed into HeLa cells before being tested against other RV. A sixth WI-38, third HeLa (WI 6 HeLa 3) passage was neutralized by a 1,280 (1,000) dilution of RV type 2 antiserum which had a homologous titer of 1,280 (300) (R. M. Conant, personal communication).

Neutralization tests. Tests were performed in WI-38 (HEM Research, Rockville, Md.) or HeLa (obtained from V. V. Hamparian, Children's Hospital, Columbus, Ohio) cell culture tubes, or in both, with guinea pig antiserum for SF 1684 and bovine (prepared by Abbott Laboratories, North Chicago, Ill.) and guinea pig (kindly supplied by R. M. Conant, Children's Hospital, Columbus, Ohio) antisera for RV type 2 (HGP). Twofold dilutions of heat-inactivated (56 C for 30 min) serum were incubated for 2 hr at room
temperature with equal volumes of virus in a dilution calculated to give a concentration of 100 TCID₅₀/0.1 ml. End points were read as the serum dilutions which produced 50% or greater suppression of CPE at the time that a simultaneously conducted virus titration was completed.

Membrane filtration. Samples of the SF 1684 seed were passed through 0.22- and 0.05-µm filters or 0.45- and 0.05-µm filters by using syringe-adapted filter holders (Millipore Corp., Bedford, Mass.).

Treatment with DOC and trypsin. One volume of undiluted SF 1684 virus seed was exposed to 9 volumes of DOC (1% in distilled water) or to 9 volumes of a mixture of trypsin (0.01%) and DOC (1%) in distilled water. Both mixtures were incubated at 37 C for two time periods, 5 and 30 min. Preparations were briefly mixed on a Vortex Junior mixer before incubation. This procedure was modified from that reported by Black et al. (1) to prepare nonaggregated harvests of simian virus 40 for biochemical analysis.

RESULTS

Neutralization of samples of different passage levels of SF 1684. After the correct identification of SF 1684 became known, several passage levels of the virus were tested for neutralization by homologous and homotypic antiserum. All samples of SF 1684 except those of the original seed pool were neutralized by homologous antiserum at, or close to, the dilutions expected (Table 1). The seventh passage represented virus that had broken through in a neutralization test employing the non-neutralizable seed lot. Another sixth passage, prepared from the terminally diluted material that had been used to make the original seed, was neutralized by homologous antisera at the appropriate dilution.

Neutralization of SF 1684 by homologous antiserum was more efficient than was neutralization by antiserum prepared against the prototype strain (HGP). With guinea pig antiserum, the homologous titer with HGP virus was 1,920 to 2,048 (300), whereas SF 1684 was neutralized by this antiserum at dilutions of 512 (30) and 256 (100). HGP and SF 1684 viruses were neutralized by similar dilutions [1,024 to 2,048 (100)] of SF 1684 antiserum.

Filtration. Samples of the seed pool which had been passed through 0.45- and 0.05-µm or 0.22- and 0.05-µm filters were neutralized by homologous antiserum dilutions similar or equal to those measured by using other passage levels of SF 1684 virus (Table 2).

DOC and trypsin exposure. Treatment with DOC (1%) and trypsin (0.01%) or DOC (1%) alone for as little as 5 min resulted in the susceptibility of seed pool virus to homologous antiserum at dilutions comparable to those inhibiting nonaggregated passages (Table 3). Infectivity titers of SF 1684 in WI-38 cell culture tubes were not appreciably altered by exposure to DOC or DOC and trypsin. DOC (1%) was toxic for WI-38 cells so that no less than 10⁻² dilutions of virus DOC mixtures were used in performing neutralization tests.

DISCUSSION

It has long been recognized that specimens prepared from different viruses contained a proportion of virions that were not neutralized by

| Table 1. Results of neutralization tests performed with several passage levels of strain SF 1684 of type 2 rhinovirus |
|---------------------------------------------------------------|
| | | | | |
| Virus | Passage level | Antisera | | |
| | WI-38 | HeLa | HGP (bovine) | HGP (guinea pig) | SF 1684 (guinea pig) |
| HGP | 1,536a (10)b | 1,920 (300) | 2,048 (300) | 1,024 (100) |
| SF 1684 | 2 | 64 (30) | 512 (30) | 2,048 (100) |
| SF 1684a | 5 | 256 (100) | 2,048 (100) | |
| SF 1684d | 6 | 20 (100) | | |
| SF 1684d | 7 | | | |
| SF 1684f | 6 | | | |

a Reciprocal of initial serum neutralizing titer.

b Virus concentration, TCID₅₀/0.1 ml.

c Terminally diluted × 3; antigen used to produce SF 1684 antiserum.

d Virus seed sent to Ohio State University.

* Specimen prepared from original fifth passage for this test.
TABLE 2. Homologous neutralization tests performed with rhinovirus type 2 (strain SF 1684) before and after filtration

| Virusa | Antiserum (SF 1684, guinea pig) |
|--------|---------------------------------|
| Vial no. 1, unfiltered | 128b (3)c |
| Vial no. 1, unfiltered | 32 (30) |
| Vial no. 1, unfiltered | 64 (100) |
| Vial no. 2, filtered with 0.45- and 0.05-μm filters | 512 (30) |
| Vial no. 3, unfiltered | 64 (100) |
| Vial no. 3, filtered with 0.22- and 0.05-μm filters | 2,048 (100) |

a SF 1684 (sixth passage).
b Reciprocal of initial serum neutralizing titer.
c Virus concentration, TCID50/0.1 ml.

TABLE 3. Homologous neutralization tests performed with rhinovirus type 2 (strain SF 1684) before and after treatment with DOC and trypsin

| Virusa | Antiserum (SF 1684, guinea pig) |
|--------|---------------------------------|
| Vial no. 4, untreated | 256b (30)c |
| Vial no. 4, 30-min treatment with DOC and trypsin | 2,048 (10) |
| Vial no. 4, 5-min treatment with DOC and trypsin | 2,048 (30) |
| Vial no. 4, 30-min treatment with DOC | 1,024 (30) |
| Vial no. 4, 5-min treatment with DOC | 2,048 (100) |

a SF 1684 (sixth passage).
b Reciprocal of initial serum-neutralizing titer.
c Virus concentration, TCID50/0.1 ml.

Homologous antisera. Wallis and Melnick (8) demonstrated that the resistant portion of virions was due to the presence of viral aggregates which could be partially removed by trypsinization and entirely removed by filtration through membranes with pore diameters of approximately twice the diameter of the virion. Sonic treatment and centrifugation were presumably not effective in disassociating viral aggregates since virus specimens were not neutralized after these forms of treatment.

The present report describes what is assumed to be another example of viral aggregation leading to resistance to neutralization. In this instance, significant aggregation occurred at only one passage level of one lot of the virus in question. Preparation of a monodispersed sample of virus by filtration or treatment with DOC with or without trypsin resulted in neutralization of the virus at the expected dilution of antisera. Since DOC through its detergent action (6) is known to disrupt both hydrophobic and weak to moderately strong electrostatic bonds, its effectiveness in these cases suggests that aggregation of the virions may have resulted from the formation of such bonds between the virions and membrane fragments or other components released from cell disruption. Black et al. have proposed that the membrane-like structures associated with aggregates of simian virus 40 are probably lipoprotein in nature (1).

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