Lack of Characteristic Hexagonal Surface Structure on a Newly Isolated Influenza C Virus

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The hexagonal surface structure believed to be characteristic for influenza C virus was not initially observed for a newly isolated virus of this type. After a few additional egg passages of the virus, however, the hexagonal structure could be readily seen on many virions.

Influenza C viruses are rarely isolated, probably because most infections with this virus are mild or subclinical (7). Attempts at isolation may also be unsuccessful, since growth of influenza C from diagnostic specimens has not been demonstrated to occur in tissue culture systems and the virus grows erratically in the allantoic cavity of hen eggs (6, 8). Presence of influenza C in the amniotic cavity of eggs might often be missed, since the virus does not agglutinate guinea pig erythrocytes and since it can elute rapidly from human group O or chicken erythrocytes when left at ambient temperatures (3, 4, 6, 8). Because influenza C is so rarely isolated, its properties have been determined primarily by studying only a few strains.

Recently, we received a sample of amniotic fluid containing the second passage of an unidentified hemagglutinating agent suspected of being an influenza virus. After preparing fresh material by making two additional amniotic passages, we found the agent to have the following properties characteristic of influenza C virus: (i) ability to agglutinate chicken, but not guinea pig, erythrocytes; (ii) complete elution from chicken erythrocytes when warmed to 35°C for about 5 min; and (iii) lack of hemagglutination inhibition by antisera to current influenza A and B strains.

In an attempt to rapidly confirm whether the agent might be an influenza C strain, we examined it by electron microscopy to determine whether virus-like particles were present and, if so, whether they possessed the hexagonal surface structure which has been seen on influenza C, but not on influenza A and B, viruses (1, 2, 5, 9). Although pleomorphic and filamentous particles typical of influenza virus were seen, no hexagonal surface structures were evi-

dent (Fig. 1A). Subsequent antigenic analysis, however, confirmed that the agent was indeed an influenza C virus. Since all previous reports of a hexagonal surface structure on influenza C viruses related to laboratory-adapted viruses, we decided to continue passaging the new isolate and to examine the morphology of virus in harvests after each passage. After two additional amniotic passages, pleomorphic and filamentous virions with a hexagonal structure on their surface were seen (Fig. 1B). To ensure that our previous inability to see this structure on virus particles having only four egg passages was not a staining artifact, we prepared new batches of virus with a total of four and eight amniotic passages, which were stained and examined at the same time. On the second of two occasions when this was done, three samples each of viruses having four or eight passages were examined in double-blind fashion. Although similar amounts of virus were usually present in the different samples, we consistently found that particles with a hexagonal structure on their surface were extremely hard to find in virus preparations having only four passages and that, even when present, the hexagonal structure was largely obscured (Fig. 1C). In contrast, particles with clear hexagonal structures on their surface were always found in virus preparations having six to eight amniotic passages.

Influenza C viruses propagated in eggs are known to possess three glycosylated polypeptides (1, 4). Although the relationship between these polypeptides is not known, they may be subunits of two envelope glycoproteins. One of these might be attached to the virion surface in a hexagonal arrangement; visualization of this structure could depend on the quantity of the second glycoprotein on the virion surface (5). This is one possible explanation for the morphological heterogeneity of influenza C virions.

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FIG. 1. (A) Influenza C virus from fourth egg passage. Filamentous and pleomorphic particles are present, but no hexagonal surface structures are evident. Bar = 100 nm. (B) Influenza C virus from eighth egg passage. Filamentous and pleomorphic particles both show characteristic hexagonal surface structure. Bar = 100 nm. (C) Virus filament from fourth egg passage. Hexagonal surface structure is discernible, but largely obscured. Bar = 100 nm. Samples were mounted on carbon-coated Formvar grids, negatively stained with 2% sodium phosphotungstate, pH 6.5, and examined with a Philips 201 electron microscope.

Whatever the explanation, our observations suggest that the hexagonal structure on influenza C viruses may be consistently observed only after the virus has been laboratory adapted. Therefore, morphology is an unreliable property to use for identifying any new isolates of the virus.

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