Intraepidemic variants of influenza virus H3 hemagglutinin differing in the number of carbohydrate side chains

Brief Report


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Summary. During the epidemic outbreak in the region of Greifswald in the winter 1974/75, we found influenza virus variants which showed differences in the electrophoretic mobility of HA. Among the 25 isolates 13 were of slower and 12 of higher mobility. HA1 of 6 isolates was studied by determining the number of the carbohydrate side chains and by direct sequencing of vRNA. Evidence is presented that variants showing a slower electrophoretic mobility of HA1 had consistently acquired a seventh carbohydrate side chain at Asn 126 in epitope A. All the isolates differed from the reference strain A/Port Chalmers/1/73 by the loss of the oligosaccharide at Asn 81. The field strain A/Dresden/3/71 possessed only 5 oligosaccharides in HA1. These results suggest that changes in glycosylation are an important mechanism in the structural variation underlying antigenic drift of HA.

* It is well documented that most of the epidemiologically important amino acid substitutions in influenza viruses occur in their hemagglutinin. They are mainly located in epitopes which are involved in the process of selection of new strains due to the antibody pressure in the population [1, 16]. In this respect variations in the number and location of glycosylation sites as well as in the structure of the carbohydrate side chains of the hemagglutinin should be studied more intensively. The number, structure and location of the oligosaccharide side chains may significantly modulate the biological activities of hemagglutinin, not only by interfering with antibody binding but also by receptor binding, proteolytic activation, and trimer assembly [7, 12].
In order to contribute to the question of the epidemiological relevance of variations in the glycosylation patterns of influenza virus hemagglutinin, we studied a collection of H3N2 strains of influenza viruses isolated in the Greifswald area during an epidemic in 1974/75. These strains were chosen because we had previously analyzed their protein and nucleic acid variations [4, 8, 11]. In these previous investigations we found differences in the electrophoretic mobility of HA by SDS-PAGE which indicated probable variations at carbohydrate attachment sites. In the present investigation, we provide evidence of the cocirculation of different virus variants within a given epidemic period. Analyses of their carbohydrates and nucleotide sequences revealed that the hemagglutinin of the field isolates differs in its glycosylation pattern.

Of the 70 field virus strains in the Greifswald epidemic between December 1974 and March 1975 25 strains were subjected to SDS-PAGE analysis. 13 strains exhibited HA with a slower mobility, while the rest exhibited a faster mobility during electrophoresis. Only three of the eleven isolates of December 1974 exhibited more slowly migrating HA, as opposed to 8 of the 10 isolates of February 1975 (not shown). In respect of the number, structure, and location of the oligosaccharide side chains, we investigated 7 epidemic strains (Table I), 6 from Greifswald, of which 2 were isolated at the same time and place, compared with one from Dresden, which was isolated in November 1971 during an H3 epidemic, but its HA had faster electrophoretical mobility than that found for the Greifswald isolates. Viral proteins of these selected virus strains which were propagated in MDCK cells in the presence of trypsin, were immunoprecipitated and analyzed by SDS-PAGE. The different mobilities reside in the subunit HA1 of all isolates investigated so far, including the isolate A/Dresden/3/71 (Fig. 1).

Partial enzymatic removal of carbohydrate side chains by N-acetyl-glucosaminidase H from viral glycoproteins reveals the total number of carbohydrate side chains on the hemagglutinin [6]. By this means, HA1 of the isolates A/Greifswald/6/74, A/Greifswald/38/74, A/Greifswald/7/75, and A/Greifswald/37/75 contains 6 and HA1 of the isolates A/Greifswald/23/74 and A/Greifswald/5/75 contains 7 carbohydrates. HA1 of the isolate A/Dresden/3/71 contains only 5 carbohydrate side chains. By comparative analysis with virus grown in the absence and in the presence of the trimming inhibitor N-methyl-1-deoxynojirimycin (MdN), oligomannosidic side chains can be distinguished from complex ones. In HA1, all of these isolates possessed 2 oligosaccharide side chains of the mannose-rich type, and between 3 to 5 of the complex type. One example is given for HA1 of the influenza virus A/Greifswald/6/74 which possessed 6 carbohydrates (Fig. 2), two of which are of the mannose-rich type (−MdN) and 4 are of the complex type (+MdN). The results obtained for the 8 H3 hemagglutinins analyzed here are summarized in Table 1.

Nucleotide sequence analyses of the Greifswald isolates revealed 19 point mutations in HA1 (not shown), which are responsible for 10 amino acid exchanges, when the influenza virus A/Port Chalmers/1/73 (H3N2 recombinant MRC11) is used as the reference strain (Table 2). 9 out of 10 amino acid