Comparative Electrophoretic Study of Polypeptides of Influenza A/H3N2 Viruses Isolated in Circumscribed Geographical Areas

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SUMMARY

Two distinct groups of influenza A/H3N2 viruses, closely related to A/Bangkok/1/79 and to A/Belgium/2/81, have been chosen from viruses isolated in Italy during 1981 to 1983 with the aim of analysing the biochemical composition of their polypeptides. The strains of each group have shown differences in electrophoretic migration rates in one or more proteins in comparison to the prototype viruses. Polypeptide mobility variations among isolates from circumscribed geographical areas and from single outbreaks have also been observed. In particular, there was a high degree of variability in the NS1 protein. The detection of biochemical differences among identical antigenic variants, probably the result of point mutations in polypeptide sequences or of genetic reassortment among different co-circulating human viruses, is a further expression of the peculiar ability of the influenza A virus to exhibit variation in internal proteins during its circulation.

It is well known that a variety of influenza strains, differing not only in the antigenic specificity of the haemagglutinin, but also in a number of other genes coding for the internal proteins, can circulate simultaneously in widespread epidemics.

The mechanisms of emergence of epidemic influenza strains are still obscure. Nevertheless, it appears that, besides the variability of the haemagglutinin, changes in other proteins can also play an important role in this process, since variation in polypeptide composition might be associated with the appearance of more virulent strains. Early reports have already described marked heterogeneity in the electrophoretic mobility of proteins from field isolates belonging to different subtypes or to different antigenic variants of influenza A virus. Less is known about the biochemical differences among the strains serologically related to a single antigenic variant. For this purpose we have conducted a comparative electrophoretic analysis, in high-resolution SDS-polyacrylamide gels, of the structural and non-structural polypeptides of viruses belonging to the same antigenic variant.

One-hundred and eighteen viruses were chosen from those circulating in Italy in recent influenza outbreaks characterized by the simultaneous circulation of a large number of antigenically different A/H3N2 strains. With the aim of correlating the virus variability to the extent of its activity, we selected strains recovered from sporadic and epidemic cases and from localized outbreaks.

Throat swab material, from cases clinically diagnosed as influenza, were inoculated into the amniotic cavity of embryonated hens' eggs and passaged once or twice in the allantoic cavity. The viruses were serologically identified [by haemagglutination inhibition (HI) test] as being similar to A/Bangkok/1/79 (38 viruses) and to A/Belgium/2/81 (80 viruses). The HI analysis was performed according to standard procedures (Concepts and Procedures for Laboratory-based Influenza Surveillance, 1982) using post-infection ferret sera. Vero cells were infected with allantoic fluid containing approx. 10 p.f.u./cell and incubated in Gey's medium for 18 h before pulse-labelling with 2 μCi [35S]methionine (sp. act. approx. 1265 Ci/mmol; Amersham) per 16 mm diam. tissue culture plate in 0-05 ml Gey's medium (Oxford et al., 1978, 1980). The labelled
polypeptides were analysed by single-dimension, high-resolution SDS–polyacrylamide gel electrophoresis (SDS–PAGE) as previously described (Oxford et al., 1978, 1980). The identification of bands corresponding to the M and NS1 proteins was performed by labelling the infected cells at different times after infection, since the NS1 protein, unlike the M, is an early product of infection.

The results of the electrophoretic comparison of the structural and non-structural virus-specific polypeptides of the 118 influenza A/H3N2 isolates with corresponding proteins of the reference strains are shown in Table 1.

Twenty-one (55 °/o) of the 38 isolates related to A/Bangkok/1/79 virus show an electrophoretic pattern identical to that of the reference strain, whereas 17 (44.7 °) revealed clear mobility differences in one or more polypeptides. All of the viruses antigenically related to A/Belgium/2/81 appeared different from the prototype virus in at least one protein. Within each antigenic group of isolates, each protein shows a different rate of electrophoretic variability.

The most frequent differences were those detected in the NS1 polypeptide: 42°/o in the strains antigenically related to A/Bangkok/1/79 and 100°/o in those related to A/Belgium/2/81. The implication of this variability for the biological role of NS1 in viral replication is unknown, since no function has yet been ascribed with certainty to it. In contrast, the lowest degree of migration rate difference has been observed in the HA protein (5.3°/o and 1.2°/o respectively).

### Table 1. Electrophoretic analysis of the polypeptides of influenza H3N2 viruses in comparison to the corresponding proteins of the antigenic prototypes

<table>
<thead>
<tr>
<th>A/Bangkok/1/79</th>
<th>A/Roma/1/81†</th>
<th>A/Firenze/1/81 (NS1)</th>
<th>A/Verona/2/82 (NP,M,NS1)</th>
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<tr>
<td>1/81†</td>
<td>2/81†</td>
<td>3/81†</td>
<td>4/81†</td>
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<td>5/81†</td>
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<td>13/81†</td>
<td>14/81†</td>
<td>15/81†</td>
<td>16/81†</td>
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</tbody>
</table>

**Total 38 viruses:** differences 2 HA (5.3°), 2 NP (5.3°), 9 M (23.7°), 16 NS1 (42.1°)

<table>
<thead>
<tr>
<th>A/Belgium/2/81</th>
<th>A/Roma/1/83 (HA,NS1)†</th>
<th>A/Firenze/1/83 (NP,NS1)†</th>
<th>A/Milano/1/83 (M,NS1)</th>
<th>A/Parma/1/83 (NS1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/83 (NS1)</td>
<td>3/83 (NS1)†</td>
<td>4/83 (NS1)†</td>
<td>5/83 (NS1)†</td>
<td>6/83 (NS1)†</td>
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<td>7/83 (NS1)</td>
<td>8/83 (NS1)†</td>
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<td>14/83 (NS1)†</td>
<td>15/83 (NS1)†</td>
<td>16/83 (NS1)†</td>
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**Total 80 viruses:** differences 1 HA (1.2°), 16 NP (20°), 16 M (20°), 80 NS1 (100°)

* Viruses showing differences from the others isolated in the same town.
† Viruses isolated from an outbreak in the same community.
Fig. 1. Electrophoretic analysis of polypeptides of some representative isolates in comparison to the corresponding proteins of the antigenically related prototypes. (a, b) Isolates related to A/Bangkok/1/79 (BGK): PR/17, A/Parma/17/83; TS/4, A/Trieste/4/83; TS/14, A/Trieste/14/83; FI/3, A/Firenze/3/83; PV/4, A/Pavia/4/83; CC, uninfected control cells; VR/1, A/Verona/1/81; VR/2, A/Verona/2/81; TS/2, A/Trieste/2/81; CA/1, A/Cagliari/1/81; PV/5, A/Pavia/5/81; PA/1, A/Palermo/1/81. (c) Isolates related to A/Belgium/2/81 (BELG): RM/1, A/Roma/1/83; RM/6, A/Roma/6/83; RM/27, A/Roma/27/83; MI/2, A/Milano/2/83. Proteins marked are uncleaved haemagglutinin (HA), nucleoprotein (NP), matrix protein (M) migrating as a doublet or a triplet, and non-structural protein (NS1) migrating as a doublet in some viruses (see text). The polymerase polypeptides P1, P2, P3 are not resolved in this gel system.

The same small proportion of variations has been detected in the NP protein of the isolates related to A/Bangkok/1/79 (5.3%), whereas the NP protein of the isolates related to A/Belgium/2/81 showed a higher value of variability (20%). The percentage of migrational changes in M protein was 23.7% for the isolates similar to A/Bangkok/1/79 virus and 20% for those related to A/Belgium. Many of the viruses under study induced M and NS1 polypeptides migrating as a doublet or a triplet, as previously described by others (Hugentobler et al., 1981; Oxford et al., 1981), as shown in Fig. 1.

In addition to the mobility differences listed in Table 1, further strain heterogeneity, mainly in NS1 mobility, was found among isolates within each antigenic group. Particularly interesting was the detection of the electrophoretic differences among viruses (marked with an asterisk in Table 1) simultaneously circulating in individual towns and isolated from sporadic or epidemic cases and sometimes from outbreaks occurring in communities.

Among the viruses related to A/Bangkok/1/79, most viruses from Roma, including 18 strains isolated during an outbreak in the same community, showed an essentially identical electrophoretic pattern. Only two strains, both from sporadic cases (A/Roma/19/81, A/Roma/25/81), were distinguishable from the others by the faster migrating HA of the former virus and the slower NS1 and faster M of the latter (data not shown). Detectable mobility changes were also observed in the NP and NS1 polypeptides of two strains (A/TS/4 and A/TS/14), isolated in Trieste in 1983 (Fig. 1a) and in the HA, M and NS1 of two viruses (A/VR/1 and A/VR/2) isolated in Verona in 1981 (Fig. 1b).

More frequent mobility differences were detected among viruses related to A/Belgium/2/81, which were all isolated in 1983. Six of the 19 strains isolated in Roma induced differently migrating HA, NP, M and NS1 polypeptides, as shown in Fig. 1c). Similarly, electrophoretic mobility differences were observed among the 14 viruses from Firenze. The patterns of all these strains were identical with regards to HA and M proteins; nevertheless, considerable variation in virus-induced NS1 was observed in seven viruses, four of which (A/FI/1, A/FI/2, A/FI/4 and A/FI/5) were collected (with another two viruses, A/FI/3 and A/FI/6) from a circumscribed outbreak in a community of military recruits in February 1983 (data not shown). Three out of 12
strains isolated in Perugia showed different mobility of the NS1 polypeptides: A/Perugia/1/83 and A/Perugia/2/83 were collected from clinical sporadic cases, whereas the other (A/Perugia/7/83) was isolated from an outbreak in a community of elderly people. Less frequent differences were noted among 12 viruses isolated in Trieste; only a single virus (A/TS/1/83) showed a slower NS1 and a faster NP protein. In contrast, no detectable change was observed among the viruses from Milano (11 strains) and among those from Parma (10 viruses).

The reproducibility of these electrophoretic results has been tested. No significant variation was detected when the analysis was performed with different preparations of the same virus or when the same virus preparation was tested at different times with freshly prepared gels.

The biochemical differences in structural and non-structural virus-specific proteins detected in the present study might be considered as a consequence of minor mutation-induced changes in polypeptide sequences (Young et al., 1979). Detailed antigenic analysis using monoclonal antibodies has shown, for example, that variation in antigenicity occurs in the NS1 and NP proteins, indicating that antigenic drift does occur in these polypeptides (Brown et al., 1983; van Wyke et al., 1980; Schild et al., 1979). These changes in molecular structure of the protein might be correlated with the electrophoretic migration rate differences described in this paper, since others (De Jong et al., 1978) have shown that a single amino acid substitution can affect the electrophoretic mobility of proteins in high-resolution SDS gel systems. Alternatively, the electrophoretic heterogeneity could be the consequence of inter- or intratypic genetic reassortment between different co-circulating influenza viruses (Young & Palese, 1979; Bean et al., 1980).

The results of biochemical analyses of viruses isolated from single outbreaks in geographically circumscribed epidemics are of interest in comparison to those isolated in widely dispersed areas, because the opportunity of introduction of new viruses from the outside after the beginning of the outbreak would have been reduced. In contrast to the biochemical homogeneity of the viruses isolated in a closed community in Roma, isolates from a community of military recruits in Firenze were biochemically distinguishable, probably because of the more frequent opportunities of exchanges with the outside allowing introduction of different viruses during the outbreak. This hypothesis is supported by the simultaneous detection of strains, biochemically identical to some of these viruses, from sporadic cases in the same town. It follows that it is less likely that the observed variations occurred by sporadic mutations during the evolution of the epidemic. The outbreak in a community of elderly people, in Perugia, can be considered an intermediate epidemiological situation. Only one strain was found to be electrophoretically different from the others, probably as a result of a random mutation selected during the epidemic. All these findings suggest that the frequency of variation correlates with the particular epidemiological circumstances of the outbreak.

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REFERENCES


Short communication


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