Amino acid sequence identity between the HA1 of influenza A (H3N2) viruses grown in mammalian and primary chick kidney cells

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Primary isolation of type A influenza (H3N2) virus in mammalian Madin Darby canine kidney (MDCK) cells results in a virus with haemagglutinin (HA) identical to that of the virus replicating in the infected individual, whereas similar isolation of virus in the embryonated egg results in the selection of variants with amino acid substitutions in the globular head region of the HA molecule. To determine whether other mammalian and avian host cells routinely used in laboratory isolation of influenza viruses also impose a selective pressure on the replicating virus population, the HA of viruses isolated in several different primary or continuous mammalian cells or avian cells has been characterized. The HAs of H3N2 viruses isolated in monkey kidney LLC-MK2 and primary guinea-pig kidney cell culture were antigenically identical to MDCK cell-grown virus isolated from the same patient. The deduced amino acid sequence over the region of HA1 encoding residues implicated in host cell-mediated sequence variation revealed that the HA sequences of viruses isolated and passaged in these mammalian cell types, and in a human lung continuous cell line (MRC-5), were identical to that of the virus present in the infected individual. In addition, isolation of virus in avian primary chick kidney (CK) cells yielded a predominant virus with HA identical to that of mammalian cell-grown virus and the virus present in the original clinical material. However, passage of CK cell-grown virus in chicken embryos (eggs) resulted in the predominance of viruses with amino acid substitutions in HA, a minority of which resulted in antigenic variation. Since CK cell culture is used in the development of live attenuated influenza vaccines, the sequence identity between CK cell-grown virus and the virus present in the infected individual is reassuring. Nevertheless, subsequent passage of virus strains in eggs, necessary for vaccine production, must be monitored closely.

Introduction

Use of polymerase chain reaction (PCR) technology to amplify the genome of very low amounts of influenza virus present in clinical samples has, for the first time, enabled the direct sequence analysis of human influenza viruses without their prior growth in embryonated eggs or cultured cells. For both type A H3N2 and type B influenza viruses, such analyses have provided the first direct evidence that viruses grown in the mammalian Madin Darby canine kidney (MDCK) cell line possess haemagglutinin (HA) identical in amino acid sequence to that of the virus present in the original clinical sample. On the other hand, viruses isolated from the same patients but grown in embryonated eggs possess amino acid substitutions in the HA molecule not seen in viruses present in the original samples (Katz et al., 1990; Robertson et al., 1990).

The changes which occur in the HA of egg-grown type A and B viruses are located around the receptor-binding pocket of the HA molecule explaining why, in addition to their presumptive selective advantage for the virus in eggs, these substitutions may also result in concomitant antigenic changes if they occur in antigenically relevant sites. Typically, two or three distinct egg-grown influenza viruses can be isolated from a single infected individual (Katz & Webster, 1988; Wang et al., 1989) or from the progeny of plaque-picked MDCK cell-grown virus subsequently passaged in eggs (Robertson et al., 1987). The predominant egg-grown virus isolated by limiting dilution of original clinical material in eggs is usually antigenically similar to MDCK cell-grown viruses from the same source, but nevertheless possesses one or more amino acid substitutions in HA1, whereas minor variants are both antigenically and molecularly distinct from virus isolated in MDCK cells.

In addition to mammalian MDCK cells and embryonated eggs, a number of different mammalian and avian primary or continuous cell culture systems are currently used for the primary isolation of influenza viruses from...
clinical isolates in laboratories world-wide. These include rhesus monkey and baboon kidney cells, human MRC-5 cells and avian primary chick kidney (CK) cells (Frank et al., 1979; Oxford et al., 1983; Maassab et al., 1985; Lathey et al., 1986). In most cases, the embryonated egg is used for further passage and the growth of large quantities of virus for serological or molecular analyses. However it has not been determined whether replication of influenza virus in these other host cell systems, like the egg, results in the selection of antigenic and structural variants of HA or whether, like MDCK systems, like the egg, results in the selection of antigenic variants of HA and neuraminidase of relevant epidemic strains (Maassab et al., 1985). Such viruses are under trial as live attenuated influenza vaccines (Wright et al., 1982; Murphy & Clements, 1989). Because potential reference and vaccine strains of virus may also be initially isolated in mammalian cells of different species, it is important to establish whether these mammalian cells behave like MDCK cells in their ability to provide a non-selective growth environment for human influenza viruses.

This question is of particular importance for virus isolated in primary CK cell culture, since this host cell system is used in the preparation of cold-adapted master virus strains and their reassortant viruses containing the HA and neuraminidase of relevant epidemic strains (Maassab et al., 1985). Such viruses are under trial as live attenuated influenza vaccines (Wright et al., 1982; Murphy & Clements, 1989). Because potential reference and vaccine strains of virus may also be initially isolated in mammalian cells of different species, it is important to establish whether these mammalian cells behave like MDCK cells in their ability to provide a non-selective growth environment for human influenza viruses.

The aim of this study, therefore, was to establish at both the antigenic and molecular levels whether the isolation of human type A H3N2 influenza viruses in different mammalian cell types or in primary CK cells leads to the amplification of variant influenza viruses selected on the basis of their enhanced capacity for growth in a particular host cell. The molecular basis of variation in the HA of viruses isolated in these different host cell types will provide the necessary insight to evaluate current virus isolation procedures and may also provide the basis for establishing a protocol for virus isolation which will minimize the risk of selecting host cell-selected variants from epidemic field strains of influenza viruses.

Methods

Virus isolation in different host cell types. Throat wash samples from patients with clinical symptoms of influenza were collected in PBS to which 5% BSA was added subsequently. Samples were stored in aliquots at −70 °C until use. Growth of virus in MDCK cell culture in the presence of 1 μg/ml TPCK trypsin or in the amniotic and/or allantoic cavity of embryonated eggs has been described previously (Katz & Webster, 1988). Typically, influenza viruses were obtained from MDCK cells infected with a 10−2 to 10−4 dilution of the original throat wash material. Viruses cloned in CK cells were isolated at 10−3.5 to 10−2 dilution of CK cell culture supernatant. Subsequent passage of CK cell-grown viruses in the allantoic cavity of eggs was performed at the highest dilution of CK cell supernatant (10−7 to 10−6) resulting in growth of virus in eggs.

Antigenic analyses. Haemagglutination titration and haemagglutination inhibition (HI) tests were performed in microtitre plates using 0.5% chicken erythrocytes. Mouse ascitic fluids were used as the source of monoclonal antibodies (MAbs) to the H3 HA of egg-grown influenza A/Hongkong/1/1968 (B49-4, B49-5, B88-1) and A/Mem/14/85 (E12-1, E12-2) viruses, and MDCK cell-grown A/Mem/12/85 (M12-3) virus. These antibodies have been used in other studies and are known to detect antigenic variants of HA of influenza A virus isolated in primary CK cells (Katz et al., 1988; Wang et al., 1989). The preparation of MAbs has been described previously by Kida et al. (1982).

PCR gene amplification and direct sequencing. Amplification of the H1 HA coding region of the HA gene of influenza viruses grown in different mammalian or avian host cells was achieved using primers M8 (5′-TTCCGCCCCACAAAACCTTCCGG-3′) and M5 (5′-ATGATGTCGCGAGTATTGCC-3′) which are complementary to virion RNA nucleotides 72 to 91 and 376 to 395, respectively, and primer M6 (5′-CCTGGGACATGCCCCATATG-3′) which is complementary to plus-strand DNA nucleotides 998 to 979. Primers M8 and M5 were used for both DNA synthesis and amplification by PCR. The methods used for cDNA synthesis, PCR amplification and subsequent purification of amplification products have been described previously (Saiki et al., 1988; Katz et al., 1990). Depending on the primers used, DNA products of either 623 (M5 and M6) or 930 (M8 and M6) nucleotides in length were amplified. Purified DNA products were sequenced by the dideoxynucleotide chain-termination method (Sanger et al., 1977) using modified T7 DNA polymerase (US Biochemical) and nested oligonucleotide primers which had been end-labelled with [γ-32P]ATP in the presence of T4 polynucleotide kinase (Bethesda

<table>
<thead>
<tr>
<th>Host cell</th>
<th>Species and organ of origin</th>
<th>Cell type</th>
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<tbody>
<tr>
<td>Mammalian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDCK</td>
<td>Canine kidney</td>
<td>Epithelial cell</td>
</tr>
<tr>
<td>LLC-MK2</td>
<td>Rhesus monkey kidney</td>
<td>Epithelial cell</td>
</tr>
<tr>
<td>MRC-5</td>
<td>Human foetal lung</td>
<td>Fibroblast-like cell</td>
</tr>
<tr>
<td>WI-38</td>
<td>Human embryonic lung</td>
<td>Primary epithelial cell</td>
</tr>
<tr>
<td>GPK</td>
<td>Guinea-pig kidney</td>
<td></td>
</tr>
<tr>
<td>Avian</td>
<td>Embryonated chicken egg</td>
<td>Amniotic and allantoic cavities</td>
</tr>
<tr>
<td>CK</td>
<td>Chicken kidney (1 day old)</td>
<td>Primary epithelial cell</td>
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<td>CK</td>
<td>Chicken kidney (1 day old)</td>
<td>Primary epithelial cell</td>
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Research Laboratories). A total of seven nested primers, five priming for positive sense DNA and two priming for negative sense DNA, were used to determine overlapping sequences of the DNA products obtained from multiple independent amplification reactions. The templates for PCR reactions were cDNA synthesized from viral RNA extracted either from undiluted tissue culture supernatants or dilutions (10^{-2} to 10^{-3}) of allantoic fluid to ensure that similar amounts of tissue culture-grown and egg-grown viruses were used for amplification of the HA gene.

Results

Antigenic and sequence analyses of A/Mem/14/85 viruses from original clinical samples isolated in different mammalian and avian host cells

To compare the HAs of H3N2 virus isolated from the same clinical material by culture in cells from different mammalian and avian species, an antigenic analysis was performed by HI assay on virus from a single clinical sample (A/Mem/14/85) isolated in some of the cell types listed in Table 1. The A/Mem/14/85 virus original patient sample and viruses isolated from it were chosen for study because the HA sequence of the predominant virus species replicating in this patient has been determined previously (Katz et al., 1990).

Viruses isolated and passaged in CK cells are usually passaged in the allantoic cavity of eggs prior to their antigenic characterization because virus titres in culture supernatants are often too low for use in antigenic analysis. Therefore, the antigenic analyses of CK-cell-grown viruses described in this study have also been performed on CK cell-grown viruses passaged once by limiting dilution in the allantoic cavity of eggs. The HI reactivity of three MAbs, representative of a larger panel, for viruses grown in three different mammalian cell types (LLC-MK2, MDCK and GPK) are shown in Table 2. The HA molecules of viruses isolated from each of these cell types were antigenically identical. The virus grown in primary CK cells and then passaged once in eggs was antigenically similar to the mammalian cell-grown viruses, as was the predominant egg-grown virus isolated by limiting dilution (clone 14-1-1). However, antigenic variation was observed among egg-grown viruses isolated from this patient as demonstrated by the failure of egg-grown clone 14-2-4 to react with MAb M12-3. The HA titre of A/Mem/14/85 virus grown only in the MRC-5 mammalian cell line was too low to enable antigenic analysis by HI assay.

We have demonstrated previously that the MDCK cell-grown virus isolated from this individual is identical in amino acid sequence over a critical region of HA1 to the virus present in the original clinical material and distinct from the sequence in the viruses isolated in eggs (Katz et al., 1990). To determine whether isolation of the virus present in the original clinical sample by growth in these other cell types resulted in sequence changes in the HA molecule, purified PCR amplification products of the gene segment encoding most of HA1 were sequenced directly to deduce the amino acid sequence of residues 120 to 300 in HA1 of the predominant virus replicating in a particular host cell. This region of HA encompasses the

Table 2. Antigenic phenotype of A/Mem/14/85 (H3N2) viruses isolated and passaged in different mammalian and avian host cells

<table>
<thead>
<tr>
<th>Source of virus†</th>
<th>MAb MDCK</th>
<th>LLC-MK2</th>
<th>GPK</th>
<th>Egg (clone 14-1-1)</th>
<th>Egg (clone 14-2-4)</th>
<th>Clone 14-1-1</th>
<th>Clone 14-2-4</th>
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<tbody>
<tr>
<td>Original throat wash</td>
<td>Asn</td>
<td>Asn</td>
<td>Thr</td>
<td>Asn</td>
<td>Asn</td>
<td>12800</td>
<td>800</td>
</tr>
<tr>
<td>MDCK</td>
<td>Asn</td>
<td>Asn</td>
<td>Thr</td>
<td>Asn</td>
<td>Asn</td>
<td>12800</td>
<td>800</td>
</tr>
<tr>
<td>LLC-MK2</td>
<td>Asn</td>
<td>Asn</td>
<td>Thr</td>
<td>Asn</td>
<td>Asn</td>
<td>12800</td>
<td>800</td>
</tr>
<tr>
<td>MRC-5</td>
<td>Asn</td>
<td>Asn</td>
<td>Thr</td>
<td>Asn</td>
<td>Asn</td>
<td>12800</td>
<td>800</td>
</tr>
<tr>
<td>GPK</td>
<td>Asn</td>
<td>Asn</td>
<td>Thr</td>
<td>Lys</td>
<td>Asn</td>
<td>12800</td>
<td>800</td>
</tr>
<tr>
<td>Egg</td>
<td>Asn</td>
<td>Asn</td>
<td>Ile‡</td>
<td>Asn</td>
<td>Asn</td>
<td>12800</td>
<td>800</td>
</tr>
<tr>
<td>(clone 14-1-1)</td>
<td></td>
<td></td>
<td></td>
<td>(clone 14-2-4)</td>
<td></td>
<td></td>
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<tr>
<td>(clone 14-2-4)</td>
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<table>
<thead>
<tr>
<th>Amino acid at HA1 residue number*</th>
<th>145</th>
<th>246</th>
<th>248</th>
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* The deduced amino acid sequence of HA1 residues 120 to 300 was determined. Only residues where substitutions occurred and/or which contributed to the glycosylation site at 246 to 248 are shown.
† Viruses were isolated directly from the original throat wash material or by primary growth and passage in one of these host cell types. CK-egg virus was isolated and passaged once in CK cells and was then passaged once in the allantoic cavity of eggs.
‡ Denotes loss of potential glycosylation site.
Table 4. HI reactivity of A/Mem/6/86 viruses cloned in eggs, CK or MDCK cells*

<table>
<thead>
<tr>
<th>Host cell used to isolate (and passage) virus clone</th>
<th>CK (egg)†</th>
<th>Egg</th>
<th>MDCK</th>
</tr>
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<tbody>
<tr>
<td>MAb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.1E</td>
<td>12800</td>
<td>3200</td>
<td>3200</td>
</tr>
<tr>
<td>9.1E</td>
<td>12800</td>
<td>3200</td>
<td>3200</td>
</tr>
<tr>
<td>11.1E</td>
<td>&lt;</td>
<td>1600</td>
<td>1600</td>
</tr>
<tr>
<td>18.1E</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>E8.2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>E12.1</td>
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<td></td>
<td></td>
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<tr>
<td>E20.1</td>
<td></td>
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<tr>
<td>M10.1</td>
<td></td>
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* The antigenic characterization of egg- and MDCK cell-grown A/Mem/6/86 clones has been described previously (Katz & Webster, 1988).
† Viruses were isolated and then cloned twice in primary CK cell culture followed by a single passage in the allantoic cavity of eggs.
‡ <, Titre of less than 100.

receptor-binding site and antigenic sites on the globular head of HA1 and includes all of the amino acid residues known to be affected by host cell-mediated selection of A/Mem/14/85 variants and most other H3N2 viruses. Amino acid residues in HA2 have not been implicated in host cell-mediated selection of variant viruses. For comparison, the previously determined sequence of MDCK cell-grown and egg-grown virus, and the virus present in the original throat wash (Katz et al., 1990) are given in Table 3 along with the new sequence information for viruses grown in other host cell types. The HA molecules of viruses isolated and passaged once or twice in each of the different mammalian cell types tested were identical in amino acid sequence to that of the virus present in the original throat wash material. Similarly the HA of virus isolated by passage of the throat wash sample in primary CK cells was identical in amino acid sequence over the region analysed to those of mammalian cell-grown viruses and to that of the virus present in the clinical sample. However, a single passage of the CK cell-grown virus in eggs resulted in the isolation of virus with an amino acid sequence in the HA identical to that of the predominant virus isolated by passage in eggs exclusively (clone 14-1-1). A single nucleotide substitution resulted in an amino acid substitution at residue 248 in HA1 (Thr→Ile) and the loss of the N-linked glycosylation site at residues 246 to 248 of HA1 (Asn-Ser-Thr→Asn-Ser-Ile). This substitution appeared to be antigenically silent as determined by the panel of MAbs used since both the egg-grown virus (clone 14-1-1) and the CK cell-grown virus passaged in eggs were antigenically identical to the mammalian cell-grown viruses. This substitution in HA1 was stable, since virus passaged a second and third time in the allantoic cavity of eggs retained this substitution (data not shown). These results indicate that none of the mammalian or avian cell culture types tested selected viruses with variation in the HA molecule. However, a single passage in eggs of CK cell-grown virus amplified a variant virus population typical of the most frequently isolated egg-grown virus recovered from this patient sample.

Antigenic and sequence analyses of viruses isolated in CK cells and passaged in eggs

The above results indicate that viruses isolated from clinical material and those cultivated from this source in CK cells are structurally identical in the region of HA1 examined, but that a subsequent single passage in eggs results in the selection of a virus population possessing an amino acid sequence change which distinguishes this virus from that present in the sample material as well as virus from this source grown in mammalian cells. In this case, the change in amino acid sequence did not result in any apparent alteration in the antigenicity of the HA of this CK cell- and egg-grown virus.

However, since the use of CK cell-grown virus as a live attenuated vaccine depends on its passage in eggs, we further examined the extent of antigenic and amino acid sequence heterogeneity which may exist in viruses isolated in CK cells and passaged subsequently in eggs. We have previously shown that the heterogeneity in HA of egg-grown influenza viruses isolated from different infected individuals can also be demonstrated in multiple clones from a single individual. Therefore we chose to examine multiple clones isolated in primary CK cells at limiting dilution of the original throat wash sample which were then passaged once in eggs. Since MDCK cell-grown and egg-grown clones of A/Mem/6/86 virus have been characterized previously (Katz & Webster, 1988), this virus was chosen for the analyses of viruses cloned in primary CK cells. The HI reactivity of viruses with four discriminating MAbs, representative of a larger panel used, are shown in Table 4. Of seven clones isolated in CK cells and passaged in eggs, five (71%) were antigenically similar to the virus grown in MDCK.
cells, one virus clone (9.1E) was antigenically identical to a variant isolated by passage in eggs alone (E12.1), and the remaining virus clone (6.1E) was not only antigenically distinct from all of the CK-egg-grown viruses and MDCK cell-grown virus, but also from any of the variants isolated after growth exclusively in eggs. These results indicate that antigenic variants can arise following passage of CK cell-grown virus in eggs, but that the most frequently isolated virus clone is antigenically identical to mammalian MDCK cell-grown viruses isolated from the same source.

To determine the amino acid sequence diversity in the HA of these viruses, the region of the HA gene encoding HA1 was amplified and sequenced for three A/Mem/6/86 virus clones grown in CK cells before (clones 9.1, 11.1 and 18.1) and after (clones 9.1E, 11.1E and 18.1E) a single passage in eggs. These sequences were compared with those of the predominant (uncloned) CK cell-grown A/Mem/6/86 virus before and after one or two passages in eggs. Differences in the deduced amino acid sequences in HA1 are given in Table 5 and are also compared with previously determined sequences of an MDCK cell-grown clone and virus clones isolated and passaged exclusively in eggs (Katz & Webster, 1988). The predominant (uncloned) A/Mem/6/86 virus grown in CK cells was identical in HA1 sequence to MDCK cell-grown virus. Two of the three clones isolated in CK cell culture (9.1 and 11.1) also shared this sequence in HA1. However, the third CK cell-grown clone (18.1) had an amino acid substitution at residue 238 (Lys→Arg). This substitution has not been observed in any other A/Mem/6/86 virus grown either in MDCK cells, CK cells or eggs.

After a single passage in eggs, clone 9.1E had acquired a single base mutation resulting in an amino acid substitution at residue 156 (Glu→Lys). This substitution at position 156 was also observed in the variant (E12.1) isolated directly in eggs which displayed an antigenic phenotype identical to that of 9.1E (Table 4) and therefore explained the altered antigenic phenotype in both these viruses. Clone 18.1 after passage in eggs retained the unique amino acid substitution at residue 238 (Lys→Arg). This substitution has not been observed in any other A/Mem/6/86 virus grown either in MDCK cells, CK cells or eggs.

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eggs (HA1 residues 1 to 300 were sequenced to confirm this). Similar results were obtained when the uncloned CK cell-grown virus was passaged once in eggs. However, after a second passage in eggs, the virus acquired an amino acid substitution at position 138 of HA1, indicating that virus with an HA1 sequence identical to MDCK cell- or CK cell-grown virus was not stable in eggs. In support of this, after a second passage in eggs, the HA titre of virus had increased 30-fold. The substitution at residue 138, however, did not result in any antigenic change in the HA (data not shown).

These results indicate that amino acid substitutions in HA1 that result in antigenic change occur only in a minority (two of seven) of viruses isolated in CK cells and then passaged in eggs. The predominant CK-egg-grown virus, although it may have possessed sequence changes in HA1, was nevertheless antigenically identical to mammalian cell-grown virus.

**Discussion**

This study has shown that cultivation of type A H3N2 influenza viruses from original clinical material in mammalian cell lines other than in MDCK cells results in the isolation of a predominant virus species with HA1 that is antigenically and structurally indistinguishable from that of MDCK cell-grown virus. These results reflect the structural similarity between influenza viruses grown in vitro in mammalian cell culture systems and the virus resulting from the replication in the respiratory epithelium of infected humans. This establishes the MDCK cell line frequently used for in vitro cultivation of influenza viruses as a valid representative of mammalian cell types as far as the isolation of influenza virus is concerned. Of the mammalian cell types used in this study, MDCK cells provided the most sensitive host cell system for the primary isolation of virus and best yields in subsequent passage of the viruses.

With only one exception, primary CK cells like the different mammalian cell lines tested, supported the replication of virus with HA identical in amino acid sequence to that of virus isolated directly from infected individuals. Clone 18.1 had a unique amino acid substitution at HA1 residue 238. This residue is not located in an antigenic site, nor is it in close proximity to amino acid residues involved in receptor binding. The isolation of this clone may reflect a low level of heterogeneity in the virus population present in the original临床 specimen (Robertson *et al.*, 1991). Nevertheless, the finding that CK cells represent an avian cell type that does not select influenza viruses with structural changes in the HA is reassuring considering the use of CK cells for the cold-adaptation of master strains of virus and generation of reassortant viruses in the preparation of live attenuated vaccines (Maassab, 1967; Cox *et al.*, 1979).

Only upon subsequent passage of virus in the allantoic cavity of eggs do variants with amino acid sequence changes in HA1 predominate. This difference between the isolation of influenza viruses in 1-day-old avian CK cell culture and in the allantoic cavity of 10- or 11-day-old embryonated eggs, may be a result of the heterogeneous cell types which are infected following inoculation of the allantoic cavity of eggs. Although the endodermal cells of the chorioallantoic membrane are primarily infected following intra-allantoic inoculation, virus may also replicate in cells of the amniotic membrane or the embryo's respiratory tract. It is possible that one or more of these cell types provides the selective environment for the replication of variant viruses in eggs not provided by the more homogeneous and differentiated population of cells making up primary CK cell culture. The fact that subsequent passage of cloned CK cell-grown virus in eggs results in the expansion of a viral population with sequence changes in the HA is not unexpected and also occurs when mammalian cell-grown viruses are passaged in eggs, as seen in earlier antigenic analyses (Katz *et al.*, 1987) and sequence analyses (Robertson *et al.*, 1987) of MDCK cell-grown viruses passaged further in eggs.

For both A/Mem/14/85 and A/Mem/6/86 viruses, the predominant virus after one passage of CK cell-grown virus in eggs was antigenically similar to the virus grown in mammalian cells. The frequency of isolation of this antigenically non-variant virus in eggs was similar whether the virus had been isolated from the original patient sample either in primary CK cells (71% as shown in this study), or by limiting dilution of the original sample inoculated directly into eggs (74%; Wang *et al.*, 1989). This suggests that isolation of virus from a low dilution of the original clinical material in either a mammalian cell type or primary CK cells prior to their adaptation in eggs may result in the predominance of an egg-grown virus which is at least antigenically identical if not structurally identical to the mammalian cell-grown virus and the virus present in the infected individual. In contrast, passage of the original clinical material at low dilution in eggs often results in the predominance of a faster growing minor antigenic variant.

The A/Mem/14/85 CK-egg-grown virus possessed the same substitution in HA1 (248 Thr-→Ile) observed in the most frequently isolated egg-grown variant from this patient (Wang *et al.*, 1989; Katz *et al.*, 1990) and resulted in the loss of a potential glycosylation site at HA1 residues 246 to 248. Although residue 248 has not been implicated directly in receptor binding, it is possible that the loss of a carbohydrate moiety at this site may enhance the binding of HA to sialic acid-bearing receptors.
Similarly, after one passage in eggs, the HA of CK cell-grown A/Mem/6/86 virus was antigenically identical to the MDCK cell-grown virus. However, this egg-passaged virus was also identical in amino acid sequence to its CK cell-grown and MDCK cell-grown counterparts. Of the clones isolated from the original sample and analysed in detail, only clone 11.1E represented this antigenic and structural phenotype. However, upon a second passage in eggs, the predominant virus acquired an amino acid substitution at residue 138 in HA1. This substitution did not lead to any antigenic alterations, as could be detected by our panel of MAbs, but did result in a higher titre of virus which was presumably better suited to growth in eggs. The substitution of Ala-Thr at position 138 has been previously observed in two 1985 H3N2 egg-grown variants (Wang et al., 1989). Residue 138 is generally conserved in human H3 HA s as it forms, together with residues 134 to 137, the right side of the receptor-binding pocket (Weis et al., 1988). In contrast to the selection of egg-grown viruses with a single amino acid substitution in HA, Rajakumar et al. (1990) have reported that limited passage in eggs of a type A (H1N1) virus resulted in a high titre virus which retained its sequence identity in HA1 with that of the virus present in the original clinical sample.

Based on these studies, the use of mammalian epithelial-like cells or primary CK cell culture in the primary isolation of human type A H3N2 viruses is essential in order to isolate unaltered the predominant virus species from the original clinical samples. Nevertheless, the effect of subsequent passage in eggs of the HA of these viruses needs to be monitored at all times since it has been shown that even a single amino acid substitution in egg-grown antigenic variants can substantially reduce the protective efficacy of the virus when used as an inactivated influenza vaccine (Katz & Webster, 1989; Wood et al., 1989).

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References


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