STRUCTURE OF THE HEMAGGLUTININ GENE OF INFLUENZA VIRUS DURING SERIAL PASSAGE THROUGH CHICK EMBRYOS

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Hemagglutinin (HA), the most important protective antigen of influenza virus, possesses unique variability. This is clearly revealed by comparison of the primary structure of the corresponding genes in natural isolates, belonging to what are called drift variants of one serological subtype of influenza virus [5]. A change in the structure both of HA itself, and of the gene coding it, has also been found when influenza viruses are cultured under experimental conditions in the presence of antibodies [10], in a model of persistent influenzal infection in cell cultures [4], and during adaptation of the virus to a foreign host [1]. However, the fate of this gene during serial productive passages of the virus through chick embryos or cell cultures remains unclear. There is indirect evidence that the properties of HA during these procedures can vary [14, 15], but we have no information on the primary struc-

Scheme 1. Nucleotide sequence of positive chain of hemagglutinin gene of influenza A/USSR/2/85 virus at the 9th passage through chick embryos.

AGCAAAAGCA	GGGGATAATT	CTATTAACCA	TGAAGACTAT	CATTGCTTTG	100
AGCTACATTT	TCTGTCTGGT	TTTCACCCAA	AAACTTCCCG	GAAATGACAA	
CAGCACAGCA	ACGCTGTGCC	TGGGACACCA	TGCAGTGCCA	AACGGAACGC	
TAGTGAAAAC	AATCACAAAT	GATCAGATTG	AAGTGACTAA	TGCTACTGAG	200
CTGGTTCAGA	GTTCCTCAAC	AGGCAGAATA	TGCGACAGTC	CTCACCGAAT	
CCTTGATGGA	AAAAACTGCA	CACTGATAGA	TGCTCTATTG	GGAGACCCTC	300
ATTGTGATGG	CTTCCAAAAT	GAGAAATGGG	ACCTTTTTGT	TGAACGCAGC	
AAAGCTTTCA	GTAACTGTTA	CCCTTATGAT	GTGCCGGATT	ATGCCTCCCT	400
TAGGTCACTA	GTTGCCTCAT	CAGGCACCCT	GGAGTTTATC	AATGAAGGCT	
TCAATTGGAC	TGGAGTCACT	CAGAGTGGGG	GAAGCTATGC	TTGCAAAAGG	500
GGATCTGTTA	ACAGTTTCTT	CAGTAGATTG	AATTGGTTGT	ACAAATCAGA	
AAGCAAATAT	CCAGCGCTGA	ACGTGACTAT	GCCAAACAAT	GGCAAATTTG	600
ACAAATTGTA	CATTTGGGGG	GTTCACCACC	CGAGCACGGA	CAAAGAACAA	
ACCAACCTAT	ATGTTCGAGG	ATCAGGGAGA	GTCACAGTCT	CTACCAAGAG	700
AAGCCAGCAA	ACTGTAATCC	CGAATATCGG	GTCTAGACCC	TGGGTAAGGG	
GTCAGTCTAG	TAGAATAAGT	ATCTATTGGA	CAATAGTAAA	ACCGGGAGAC	800
ATACTGTTGA	TTAATAGCAC	TGGGAACCTA	ATTGCTCCTC	GGGGTTACTT	
CAAAATACGC	ACTGGGAAAA	GCTCAATAAT	GAGGTCAGAT	GCACCTATTG	900
GCACCTGCAG	TTCTGAATGC	ATCACTCCAA	ATGGAAGCAT	TCCCAATGAC	
AAACCCTTTC	AAAATGTAAA	CAAGATCACA	TATGGGGCAT	GTCCCAGGTA	1000
TGTTAAACAA	AACACTCTGA	AATTGGCAAC	AGGGATGCGG	AATGTACCAG	
AGAAACAAAC	TAGAGGCATA	TTCGGCGCAA	TAGCAGGTTT	CATAGAAAAT	1100
GGTTGGGAGG	GAATGGTAGA	CGGTTGGTAC	GGTTTCAGGC	ATCAAAATTC	
TGAGGGCACA	GGACAAGCAG	CAGATCTTAA	AAGCACTCAA	GCAGCAATCG	1200
ACCAAATCAA	CGGGAAACTG	AATAGGTTAA	TCGAGAAAAC	GAACGAGAAA	
TTCCATCAAA	TCGAAAAGGA	ATTCTCAGAA	GTAGAAGGGA	GAATTCAGGA	1300
CCTCGAGAAA	TATGTTGAAG	ACACTAAAAT	AGATCTCTGG	TCTTACAACG	
CGGAGCTTCT	TGTCGCCCTG	GAGAACCAAC	ATACAATTGA	TCTGACTGAC	1400
TCAGAAATGA	ACAAACTGTT	TGAAAAAACA	AGGAAGCAAC	TGAGGGAAAA	
TGCTGAGGAC	ATGGGCAATG	GTTGCTTCAA	AATATACCAC	AAATGTGACA	1500
ATGCTTGCAT	AGGATCAATC	AGAAATGGAA	CTTATGACCA	TGATGTATAC	
AGAGACGAAG	CATTAAACAA	CCGGTTTCAG	ATCAAAGGTG	TTGAGCTGAA	1600
GTCAGGATAC	AAAGACTGGA	TCCTATGGAT	TTCCTTTGCC	ATATCATGCT	
TTTTGCTTTG	TGTTGTTTTG	CTGGGGTTCA	TCATGTGGGC	CTGCCAAAAA	1700
GGCAACATTA	GGTGCAACAT	TTGCATTTGA	GTGCATTAAT	TAAAAACACC	
CTTGTTTCTA	CT				1762

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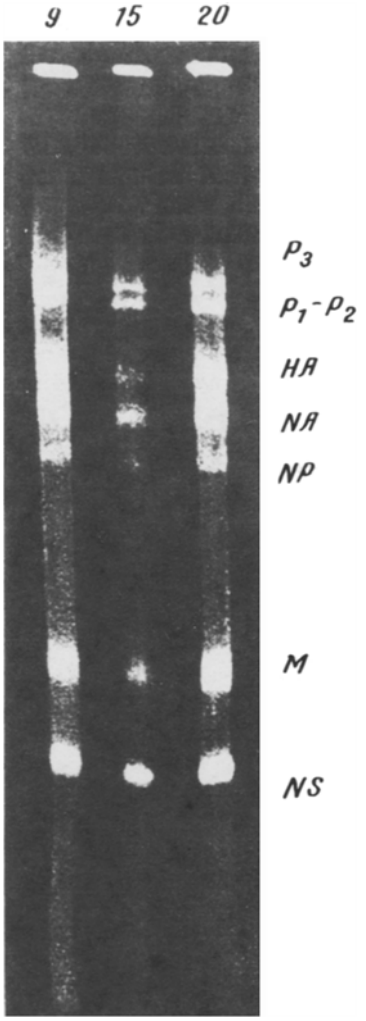

Fig. 1. Electrophoresis of virion RNA of influenza viruses A/USSR/2/85, after 9, 15, and 20 passages through chick embryos. Conditions of electrophoresis: 2.8% PAG with 6 M urea, duration 24 h, staining with ethidium bromide.

ture of HA in variants of the influenza virus obtained by different passages. This is a problem not only of considerable theoretical importance, but also of practical interest, since repeated passage of influenza virus through chick embryos remains one of the methods used to prepare strains for living influenza vaccines.

This paper gives information on sequencing of the HA gene of variants of influenza virus A/USSR/2/85 (H3N2) at different levels of passage through chick embryos.

EXPERIMENTAL METHOD AND RESULTS

In the first stage of the investigations RNA of passage variants of influenza virus A/USSR/2/85 (virus from the 9th passage was used as the original virus for subsequent passage through chick embryos at the 15th and 20th passage levels) was analyzed by electrophoresis in 2.8% polyacrylamide gel (PAG), containing 6M urea. It will be clear from Fig. 1 that repeated passage of the virus through chick embryos did not lead to any change in electrophoretic mobility of the gene coding for HA. Mobility of the genes coding for polymerases P_3, P_1, and P_2, for neuraminidase, and nucleoprotein proteins likewise was unchanged. In the next stage of the investigation the primary structure of the HA gene of variants of influenza virus A/USSR/2/85, after undergoing 9, 15, and 20 passages through chick embryos, was analyzed. For this purpose the following primer oligonucleotides were synthesized: 1)ATAATTCTATTAATCATGAA (15-34), 2) GACTAATGCTACTGA (185-199), 3) TTGAACGCAGCAAAGC (339-355), 4) GTGCTGAACGTGAC (564-577),

5) AAACCGGGAGACAT (789-802), 6) GTAAACAAGATCACATA (966-982), 7) GAGAAATTCCATCAAATC (1245-1262), 8) TACCACAAATGTGACAA (1485-1501). The nucleotide residues are numbered from the 5'-end of the positive chain of the HA gene. Since oligonucleotide 4 did not guarantee effective priming because of replacement of nucleotide 565: T → C in the HA of this virus, primer 9 — GTTCACCAC-CCGAGCAC (621-637) — was synthesized. The choice of primers was made by computer analysis of all primary structures of influenza A viruses so far published.

To obtain DNA copies with the HA gene the reaction with reverse transcriptase was carried out, using total virion RNA as the template; to initiate synthesis of these copies, primers radioactively labeled at the 5'-end were included in the reaction [7]. Their position on the genome was such that regions of the nucleotide sequence identified from neighboring primers, overlapped. The labeled DNA copies obtained as a result were sequenced [11], with the introduction of certain modifications of the basic method [3, 8].

As a result the primary structure of the HA gene of three variants of influenza virus A/USSR/2/85 was established at the 9, 15, and 20 passage stage. Scheme 1 shows the nucleotide sequence of the HA gene of the virus at the 9th passage. HA genes of viruses at the stages of the 15th and 20th passages do not differ from it in any single nucleotide.

Thus sequencing of the HA genes of passage variants of influenza virus A/USSR/2/85 confirmed data obtained previously in a study of influenza virus A/Leningrad/337/76 (H3N2) by the oligonucleotide mapping method. During serial passages of the virus, no changes likewise were found in the structure of the HA gene. Thus repeated passage of influenza virus through chick embryos is not reflected in structure or, correspondingly, in the immunochemical properties of its HA, evidence that the living vaccine, attenuated by passage, is equivalent with respect to this gene to its epidemic prototype. However, it cannot be concluded from these data that no changes take place in the HA gene in the very first stages of adaptation of the epidemic virus to the chick embryo (1st-3rd passages).

Since serial passage of influenza virus through chick embryos leads to attenuation of the agent [6], the results obtained contradict views on the exclusive role of HA in the determination of its virulence [9]. Changes in other genes can evidently have a much more significant influence on the level of virulence of the influenza virus than is stated in [12, 13].

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